ASIC 2008

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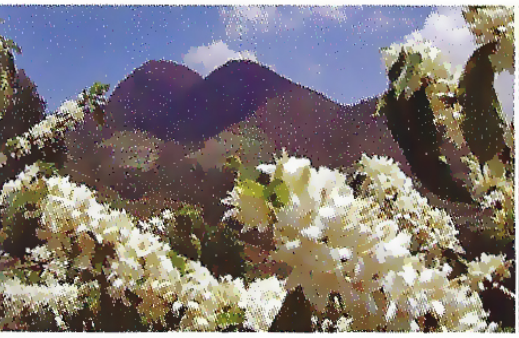

ASIC

ASSOCIATION FOR SCIENCE AND INFORMATION ON COFFEE
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VOLUME 1

22nd International Conference on Coffee Science
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22ème Colloque Scientifique International sur le Café
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Can Coffee Science Blend Quality, Price and Pleasure to Increase Consumption?

C.H.J. BRANDO

SUMMARY

Coffee science can help blend quality, price and pleasure to increase consumption in many different ways: consumer education; purity and quality measurement; technology in the fields of production, post-harvesting processing, logistics manufacturing, preparation and coffee dispensing; coffee and health; and sustainability. Coffee marketing is the driving force that blends all coffee science fields together to produce affordable coffee qualities that are consumed in a guiltless way.

FULL TEXT

The usual equation, the usual perception goes that more coffee quality means more pleasure to consumers. As a result consumers are then willing to consume more coffee and/or to pay a higher price for their coffee. In this process we have what is called value addition.

This equation, this perception – more quality brings more pleasure which in turn brings more consumption and/or higher price – holds true for many goods and services, for example: coffee, wine, cheese, cars, houses, a hair cut, a music show, etc. This perception holds specially well for luxury goods. This is the case of specialty and high quality coffees: the consumer is keener on the quality attributes of the product and therefore prepared to pay more for them.

However, if consumers cannot evaluate quality properly or if there are different definitions or concepts of quality for different consumer segments, this association becomes blurred. More pleasure may not necessarily derive from more price if pleasure is not associated with quality. The value addition process may then not work.

The key to associate quality, pleasure and price is consumer education which is easier to accomplish in the case of luxury goods and with higher income, better educated consumers, although it is required for all consumer segments. Consumer education is a very important component of programs to increase coffee consumption. This brings us as to the first role of coffee science in the process of promoting coffee consumption: consumer education at all levels, for all income groups. Coffee science supports consumer education in many different ways.

One key support area today is to positively influence consumers' perceptions about the impacts of coffee drinking on health, as done by the ISIC sponsored, ICO supported Positively Coffee Program and the Health Care Professions - Coffee Education Program. There are also other coffee and health programs, like the ones in Brazil and other producing countries. Another important area where coffee science can help is sensory analysis techniques, methods and equipment as well as systems to evaluate quality, to make quality concepts simple for consumers to understand, and to facilitate their dissemination. A third area is coffee marketing. Marketing is where science meets art. Marketing is a science and coffee marketing is treated as a science in the ICO Guide to Promote Coffee Consumption

that our company, P&A Marketing International, prepared for the ICO. The ICO Guide describes coffee specific marketing tools like seals and labels, competitions, auctions, festivals, certification, sampling at retail and consumption outlets, and other instruments.

Our first "Science Blender" is consumer education which, it is important to repeat, is easier to achieve for higher income, better educated segments of the population. Therefore the first way coffee science can help blend quality, pleasure and price to increase consumption is through consumer education.

Insufficient or inadequate consumer education may cause a flaw whereby more pleasure is not fully associated with more quality. This is what I call the specialty coffee paradox. A flaw in consumer education creates value addition at one end of the market only. This creates a special challenge for coffee marketing and trading and it involves the sustainability of the grower and the business itself.

The paradox is that the volume of specialty coffee sold by producers to importers is growing slowly but the volume of specialty coffee purchased by consumers in importing countries is growing fast. What is the reason for this paradox? The explanation lies on different definitions of specialty coffee.

Producers are told by buyers, traders and importers that specialty coffees are defined according to cup quality, e.g., coffees that reach 80 or 90 points at SCAA's scale. However, for consumers the definition of specialty coffee is associated with the way coffee is sold or presented. To many consumers all espresso beverages, all coffee sold in coffee shops and new coffee drinks are all considered specialty coffees and these consumers are prepared to pay more for them, even if their quality is not above SCAA 80, as it is often the case. The problem caused by the paradox is that value addition is concentrated at the consumers' end, at the expense of the grower.

There is high value addition and big growth on the consumers' side of the market but low value addition and small growth on the producers' side. There is in fact a transformation of commercial coffees into "specialty" coffees. How can this problem be solved? This may be a market imperfection and not really a marketing problem or scientific problem, but it is a challenge for marketing science and coffee science to help value addition to reach the grower and to make the coffee market more sustainable.

Some questions are in order as one moves from luxury to mass markets. Does the consumer want quality or pleasure? He obviously wants pleasure. He may want quality too, if he perceives that quality brings pleasure, that quality is associated with pleasure. Is quality an indicator for pleasure? I believe the answer is "yes" only if there is strong consumer education. Otherwise the answer must be "not necessarily". Can one have pleasure from low-quality coffee? Unfortunately, or fortunately, depending on one's point of view, the answer is "yes" and many consumers have pleasure from drinking low-quality coffee. What is the best coffee? It is the coffee the consumer likes! If the consumer has not been told, taught or educated otherwise, the best coffee is the one he or she is used to drink. It is a hard fact that most consumers do not drink what connoisseurs call quality coffee.

Consumers associate pleasure and the pleasure they perceive with price. Quality may or may not be a part of the equation. Consumers' expectations play a great role in their perception of pleasure and in their willingness to pay for coffee. Quality may have to be treated as an incremental value. Even in cases where consumers are educated to perceive quality, such quality may not be affordable.

At this juncture it is interesting to analyze the roles of the purity seal and quality labels used in Brazilian programs to promote consumption created by the Brazilian Coffee Roasters Association (ABIC).

ABIC's Purity Seal conveys the same, common message, that "coffee is free from impurities", in regards to all coffee qualities that comply with its purity standards. The Purity Seal is applicable to all quality and price ranges. It implies no quality judgment but, interestingly enough, it was eventually perceived as a quality seal by most consumers who associated the purity seal with quality. This perception was so strong that at one point ABIC had to have a campaign to help segment the market according to qualities. Its slogan was "purity is a right, quality is a choice".

A transparency in labeling strategy, like a purity seal, is a proven recipe to increase coffee consumption because its concept is easy to communicate and compliance is simple to measure and to enforce. The easier the more effective. A coffee quality program is much more difficult to communicate but it does create the basis for market segmentation according to quality categories.

The quality categories used in ABIC's Coffee Quality Program (PQC) are: traditional, superior and gourmet. In this case there is a quality judgment which causes consumer education to be both more complex and necessary. On the other hand, the quality categories are a wonderful instrument to associate quality and price, to segment markets and to add value to coffee. Each category indicates the best affordable quality the consumer can buy. The association between quality and price is clearly spelled out buy a quality labeling strategy though it poses much greater challenges as a strategy to promote coffee consumption.

Coffee science can assist and facilitate transparency and quality labeling in order to support the promotion of coffee consumption. Coffee science can contribute with technology to measure purity, be it by chemical means, imagery, or other techniques. Coffee science can help to develop procedures for cupping standards and panels. Coffee science can assist with methodologies of certification. Finally, it can help to develop equipments to measure quality, like the electronic nose and tongue.

The second "Science Blender", that mixes quality, pleasure and price to promote consumption, is purity and quality measurement.

Let's now move to a new dimension: practical pleasure and consistent quality. This is coffee for a fast moving, time constrained new generation. This is coffee prepared by machines for home use, espresso and filter, which have become affordable in traditional markets but are still expensive in producing countries and emerging markets. This is the on-going coffee revolution. At a different level, practical pleasure and consistent quality are also provided by freeze-dried soluble coffee, whose quality is superior to spray-dried products. This is happening now in traditional markets and developed producing markets. It is also the future in emerging markets. Finally, ready-to-drink (RTD) coffee beverages, which are cold and sweet, are very attractive to youngsters. This will be the next coffee revolution.

It is rather obvious how the pursuit of practical pleasure and consistent quality have been helped by coffees science: applied research and technology in roasting, soluble making, packing, single doses (pods, capsules, etc), coffee preparation, new coffee beverages, and new ways to prepare and serve traditional coffee beverages. This is clearly a science-supported revolution that creates coffee drinks for a multi-task generation.

The third "Science Blender" is manufacturing, preparation and packing technology.

Let's now move again, to yet another dimension, the social dimension of coffee drinking: out-of-home consumption. We had major strides in this field in the past 10 to 15 years. There was a coffee shop explosion and coffee was repositioned in restaurants from something offered for free at the end of a meal to a quality, high class beverage that closes the meal with a golden key. At the same time a new generation of office machines was developed both for filter and espresso, with vending machines becoming more popular in some markets. The social dimension is the magic coffee moment, the "coffee break", an expression that the Pan American Coffee Bureau coined decades ago, the "third way" of Starbucks, and "reloading" or "relaxing", antagonistic concepts as they are but identified as consumption drivers in different countries.

The fourth "Science Blender" is therefore coffee dispensing technology that supports out-of-home consumption and helps to create share of mind and consciousness about coffee and coffee products.

All concepts developed above can be brought together in a model of how consumption develops: the Coffee Consumption Ladder. This is a model to understand tendencies, to predict market moves and to promote coffee consumption. Models are always a simplification of reality, often an over-simplification, but they help understand reality better. The ladder has been conceived and designed to explain how new consumers enter the coffee market and how new and existing consumers move inside it.

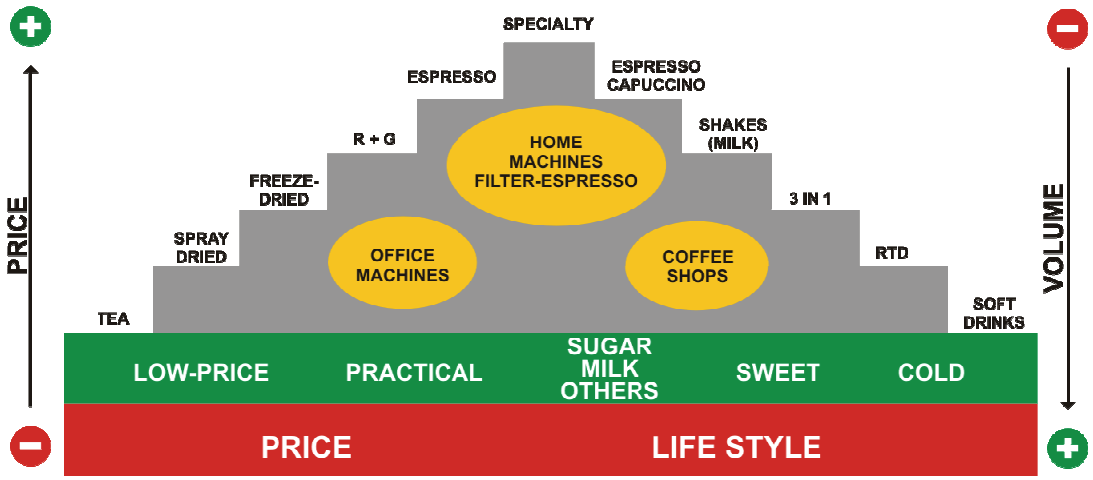

Figure 1. The coffee consumption ladder

Consumers in emerging markets that are predominantly tea-drinking tend to enter the coffee market and to start climbing the ladder on it is left-hand side. As practical to prepare as tea and low-cost, spray-dried soluble coffee is often the entry door into the coffee market for tea drinkers. After consumers acquire the habit of coffee drinking they may seek products with a more intense coffee flavor and start climbing the ladder, even switching from soluble to roast and ground coffee and eventually to espresso or specialty beverages. Low-price and practical preparation are strong consumption drivers in this market that also relies strongly on low-cost home brewing systems and machines that dispense coffee in offices.

Young consumers may prefer to enter the coffee market at the opposite side of the ladder and move from soft drinks, juices, natural or not, and isotonic beverages to ready-to-drink (RTD) coffee beverages or "3 in 1" (soluble coffee, non-dairy cream and sugar, flavored or not).

After the taste for coffee is acquired, the tendency is again for them to climb the ladder and move to products with a more intense coffee flavor though often preserving milk as a base or mixer, for example in cappuccinos. The consumption drivers on this side of the ladder, in this market, are life styles and cold and sweet coffee and milk beverages, often sold in coffee shops but also prepared in more sophisticated home brewing equipment where pods and capsules progressively play a stronger role.

Consumers face higher prices as they climb the ladder and consume products with a higher coffee content. As a result, the volumes consumed tend to be smaller for coffee products at the top of the ladder. There is a strong temptation to say that coffee quality increases as one goes up the ladder. However, as it will be explained later in this paper, quality is a relative concept related to consumers' perceptions, product affordability and willingness to pay.

It is a worth to repeat that although this is a simplified version of reality, it is a good instrument to understand tendencies, to predict and anticipate market moves, to create and introduce new products, and to learn when, how and why to intervene with branding and institutional programs to help promote consumption.

Where is coffee science in the coffee consumption ladder? It is everywhere, as presented before, helping the ladder to expand and creating the setting for the consumption drivers to act. Coffee science is the "blender", as coffee people would call it and as stated in the title of the presentation, or it is the "catalyzer", as scientists would probably prefer to call it. But coffee science does not stop here. It is also below, under the ladder, because it serves as its foundations.

Besides the roles mentioned earlier, coffee science has many other very important roles as the foundations and the pillars that sustain the ladder. These are the roles of coffee science in coffee production, post-harvest processing, logistics, storage, transport and so many other fields that create the basis for production, processing, trading, transport, etc. before coffee becomes a consumer product. It is here that the concepts of efficiency and quality come into play in production, processing and logistics in order to create coffees of affordable quality at origin, in producing countries.

This wider role of coffee science covers the fields of production, post-harvest processing, logistics and several others. The objectives are to lower costs, to improve quality and to create the basis for a different equation: affordable price plus affordable quality results in affordable pleasure and mass consumption. This is a very, very important role. This is where a lot of coffee science enters the consumption equation: coffee genomics and biochemistry; breeding and diversity; breeding for disease resistance; agronomy and ecophysiology; pathology management and plant protection; coffee chemistry; quality and coffee aroma and flavor chemistry; and others.

The fifth "Science Blender" is the scientific means to create affordable pleasure and mass, not niche, consumption.

Coffee science is permanently working to achieve more quality with less cost. This is critical to promote mass consumption in the mid-to-low-income environments of producing countries and emerging markets. It is critical to reach the largest number of consumers possible and to make the pleasures of coffee accessible to and affordable by the masses. However, very important, this cannot be achieved at the expense of growers' economic sustainability. It is not a matter of simply cutting costs, but of increasing efficiency with positive returns to

growers. This is perhaps the greatest challenge for coffee science today. We must offer affordable pleasure to consumers, but not at growers' expenses.
Long-standing paradigms must be challenged. For example, is selective hand picking the right model for the 21st century? Does it not mean condemning small coffee growers and hired labor to poverty? Numbers do not seem to match. A person can only pick a maximum volume of coffee per day. So there is a limit to pickers' remuneration but there is no limit to costs of living increases. Added-value markets are volume niches for growers and are not growing. The key to mass consumption is affordable pleasure and prices. Do the numbers match? Alternatives to selective hand picking is a critical subject for coffee science to study.

The last issue to be addressed is affordable guiltless pleasure, with emphasis on guiltless. Pleasure without guilt means drinking coffee that provides fair returns to growers. This is where sustainability really lies. There will be no coffee if there are no growers. Another dimension of guiltless pleasure is to clarify health based concerns and restrictions to coffee drinking. This can only be done by means of research on coffee chemistry and the physiological effects of coffee drinking, and studies of the beneficial effects of coffee on human health. Not only do we need these studies but also the dissemination of their positive results.

The sixth and last "Science Blender" is therefore guiltless consumption, to be achieved by ensuring economic sustainability for the grower and disseminating the benefits of coffee drinking to consumers.

The six science blenders described above show that, yes, coffee science can blend quality, pleasure and price to increase consumption. Consumer education causes value addition and more consumption too. Purity and quality measurement support transparency and quality labeling strategies that promote consumption. Manufacturing, preparation and packing technology ensure practical pleasure, consistent quality and greater consumption. Coffee dispensing technology supports and enlarges out-of-home consumption. Finally, affordable pleasure (affordable price and affordable quality) and guiltless consumption (benefits of coffee drinking and fair returns to growers) are key paths to mass consumption.

How to communicate all this to consumers? The answer is coffee marketing. As stated before, marketing is where science and art come together. There are many on going coffee marketing initiatives. There are institutional programs with the major example of Brazil whose domestic coffee consumption rose from 6.5 to nearly 18.0 million bags in 20 years. There is the ICO Guide to Promote Coffee Consumption that created the foundations for other producing-country initiatives: India, Mexico, El Salvador, Colombia, Costa Rica and other countries. Very important, there are also huge efforts devoted to brand promotion by coffee companies around the world. Hundreds of millions of dollars are spent every year on marketing and promotion of coffee brands.

Since the focus has now shifted to consumers, the internet offers wonderful opportunities to reach youngsters, tomorrow's consumers, and to promote coffee consumption at a modest cost. The internet is the medium of young consumers, of the multi-task generation. Social networks abound in the internet today: Peabirus in Brazil, blogs elsewhere, Flickr, YouTube, Facebook, orkut, myspace.com, twitter, etc. Coffee is present in most social networks.

The International Coffee Organization has created a social network dedicated to coffee. Its objective is to "blend" all ideas and initiatives that have a coffee focus in order to bring all coffee related subjects and gadgets in other networks together in an exclusive network created for coffee lovers. This is the ICO CoffeeClub Network that is now in operation. Readers are

welcome to participate: enter the network at www.coffeeclubnetwork.com, navigate, join a community or create their own, and have fun!
The CoffeeClub Network has a very special community, the Coffee Researchers' Forum, whose aim is to discuss agronomy, technology, coffee science and innovation. It is a place to exchange ideas and projects and to promote innovation.

In summary, yes, coffee science can blend quality, price and pleasure to increase consumption. The big "coffee science blender" shows us that consumption in equal to affordable quality plus affordable price plus guiltless pleasure. Coffee science is the foundation and the blender, the big catalyzer. Coffee marketing – surveys, instruments, tools, strategies, activities and programs – is the driving force moving the blender.

Coffee: What We Can Learn From the Social Aspects of Coffee Drinking to Increase Consumption?

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InterScience, São Paulo, Brazil

SUMMARY

The consumption of coffee in Brazil has been stimulated by different actions taken by the sector (ABIC) and institutions related to the Ministry of Agriculture. These actions have been developed for almost fifteen years, and had, as one of the major supporters, ABIC that introduced a quality certification in order to warrant a greater pleasure in the consumption of the beverage. Another relevant point in Brazil besides the quality issue, is the introduction of new kinds of coffee (decaf, Arabic, etc) and the creation of pleasant public places, that stimulated the consumption (thanks also to the efforts of ASSISCAFE). But we can't forget the effort undertaken to shift a paradigm revealed in researches that coffee is bad for health, with strong medical influence. In this case was also implemented an educational project focusing the medical community, and today, after five years, researches show a process of reduction in prejudice. What about the future? Brazilian Consumer has became used to options of quality, pleasant public places, prestigious brands, wide variety of products and different ways of preparations, now the question is how to incentive the consumption? As far as we concern, new products, new packages and, specially, a frequent communication of the benefits, the pleasure of the consumption with friends and relatives, and the sensorial pleasure given by coffee, have to be strong drivers of the consumption. In order to point out possible routes to encourage consumption, we have identified some major movements:

THE RECOVERY OF COFFEE'S PRESTIGE IN THE DOMESTIC MARKET

"Brazil is currently the world's greatest coffee producer responsible for 30% of the international coffee industry. Such volume is equivalent to the total produced by the other six largest coffee producing nations. It is also the second consumer market positioned only after the United States".

Although Brazil has had an important role in coffee production since the 18th century becoming one of the world's largest exporters, the same is not true for the domestic consumer market which offered products with doubtful brand and quality. Nevertheless, it was able to reach high penetration in Brazilian households as coffee is one of the main beverages for the Brazilian family's morning meal. The coffee marketed in Brazil was rejected by exports standards (www.abic.com.br).

In 1988, a usage habit survey with the Brazilian coffee consumer was conducted pointing out that 67% of Brazilians believed that 'pure coffee was only the one exported – the one for domestic consumption, was, unfortunately, always forged'. At that moment, ABIC (Brazilian Association of the Coffee Industry) saw an opportunity to revert such perception by launching the Purity Stamp (*Selo de Pureza*).

"While implementing the program, more than 30% of surveyed coffee brands outmaneuvered legislation, either with impurities above tolerance levels or by mixing in other substances" (www.abic.com.br).

After the launch of the Stamp of Quality, many brands were certified and Brazilians were able, for the first time, in one of the major coffee markets, to drink coffee from beans of better quality. Today, according to ABIC, less than 5% of brands are impure or adulterated and such brands represent only 1% of the coffee volume marketed in Brazil.

With such project of quality, other factors came about which contributed for encouraging consumption:

a) Market offer of different types of coffee: decaffeinated, Arabic, with flavors, etc.
b) Various options of coffee machines for both ground coffee and coffee beans
c) Places with pleasant atmosphere to drink coffee
d) The rise of coffee as a "gourmet" concept
e) Ease of finding other coffee versions to buy in retail stores besides the traditional ground coffee (beans, decaffeinated, with flavors).

On the other hand, we note a Brazilian consumer market with high consumption penetration (around 93% of all consumers), which already comes as a great challenge:

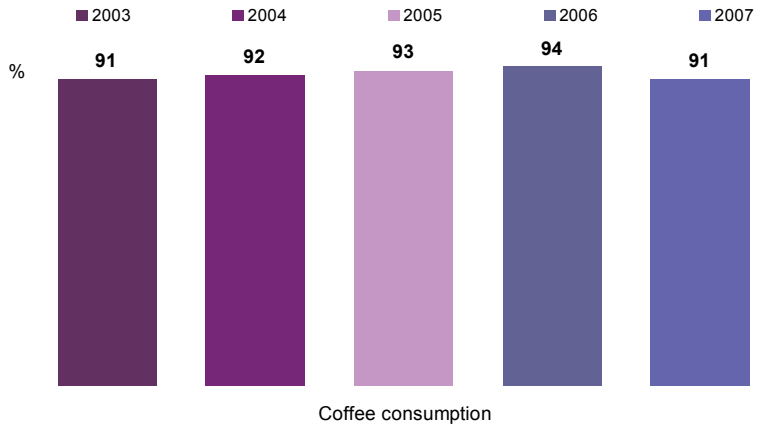

Figure 1.

Source: TNS Interscience (the sample of the study, "Consumption Trends", depicted in the graph above and margin of error of 6.8%, conducted annually for ABIC, is obtained in the following regions:

- Southeast, represented by São Paulo, Rio de Janeiro, Belo Horizonte, Juiz de Fora and Sorocaba
- South: Curitiba, Porto Alegre and Joinville
- North/Northeast; Belém, Salvador and Campina Grande
- Center-West: Goiânia and Brasília
- + 4 rural cities with less than 10 thousand inhabitants each

Around 2,500 consumers take part in the study.

Habits from childhood is one of the factors which encourages consumption and, for that reason, one must remember the powerful milk and chocolate milk industries competing for the same consumer; attention to be given to organoleptic aspects (flavor and aroma) as a way to encourage consumption:

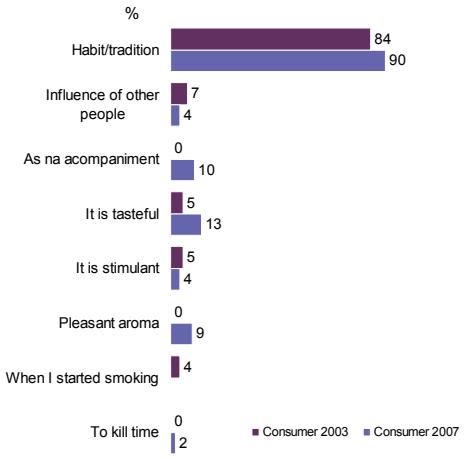

Figure 2. ***"My mom used to give me when I was very young, for breakfast…" (Youngster, age 17, SEC BC, São Paulo, research from 2007).***

The quality and options of coffee have been changing consumer habit in relation to places to drink coffee:

Table 1.

	2003 %	2007 %
• At home	95	98
• Outside home	17	**26**

Learning # 1

The movement of bringing more value to the product and its different drinking possibilities encourage at least consumption intensity and, possibly, the initiation of new consumers.

Not to lose its organoleptic characteristics in order to keep encouraging the "appetite appeal"

Not to lose the acquired quality standard in order to not lose consumers to other beverages with strong healthiness image, as natural juice, teas and even water and milk.

THE MOVEMENT BROUGHT BY THE PREJUDICE THAT COFFEE IS BAD FOR OUR HEALTH REINFORCED BY THE MEDICAL COMMUNITY

Although some movements made progress towards the recuperation of the coffee image in Brazil, the paradigm was kept (and still is, although at a lower intensity) among consumers and the medical community that coffee was a villain, was unhealthy. For this particular aspect, a research was conducted among health professionals (cardiologists, general practitioners, pediatricians, geriatricians, nurses, nutritionists) focusing on identifying their stand in relation to coffee consumption.

Table 2. Study samples from 2004 to 2006 were similar and were obtained proportionally to regions of Brazil, by TNS Interscience

Medical sample	2004 Realizada N.A.	2005 Realizada N.A.	2006 Realizada N.A.
• Nutritionist	58	58	24
• General Practitioner	51	50	48
• Other Specializations	40	40	9
• Cardiologist	33	33	40
• Gynecologistas	33	34	29
• Nurse	30	30	31
• Psychiatrist	30	30	20
• Neurologist	27	27	29
• Pediatrician	22	22	30
• Dentist	20	21	30
• Geriatrics	20	21	30
• Personal Trainers	20	21	30
• Total	344	345	350

After the research, communication efforts were carried out as an educational work among the medical community, with specific topics as Coffee and the Heart, Coffee and the Cardiovascular System, Coffee and the Brain, Coffee and Children, Coffee, Pregnancy and Breast Feeding. After a year, a new research was conducted with the same group of professionals and results exceeded expectations, since many health professionals were not aware of the benefits derived from coffee (The Program Coffee is Health", was managed by Professor Darcy Roberto Lima, MD, PhD from the London University);

Thus, it started to change the opinion of this community in terms of the influence of coffee in our health: there is a 23% rise in positive perception and 15% drop in negative influence:

Due to efforts applied, in a single year only, behavioral change of the medical community was observed when asked about the positive influence of coffee in our health, strongly reflecting the content from the adopted campaign:

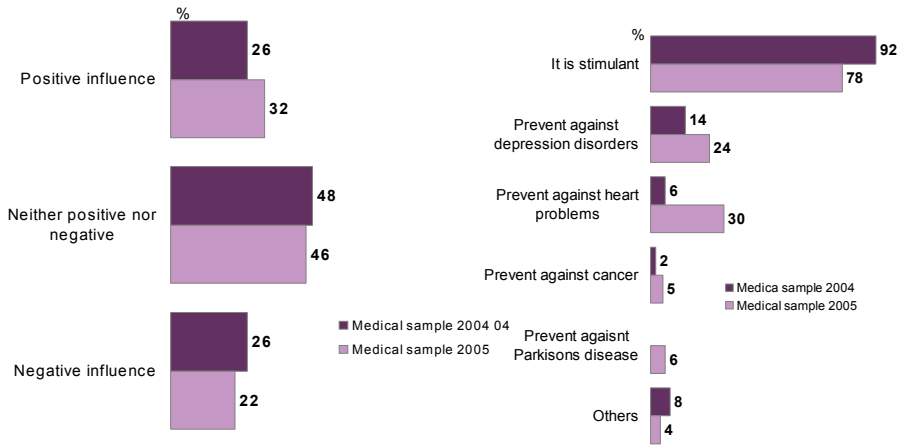

Figure 3.

Scientific studies and the single experience with patients have been showing the positive influence of coffee on our health:

Table 3.

What is your basis to believe that Coffee is a positive influence to health?	Total %	
	2004 %	2006 %
• Personal experience	64	56
• Scientific studies	53	50
• Experience with patients	32	41
• Others	3	4

And even those who believe that coffee has a negative influence on our heath, there is a loss of importance (except for those doctors who associate it right away with ulcer) in many aspects and we can see the decrease of suggestion to patients "limit"coffee consumption:

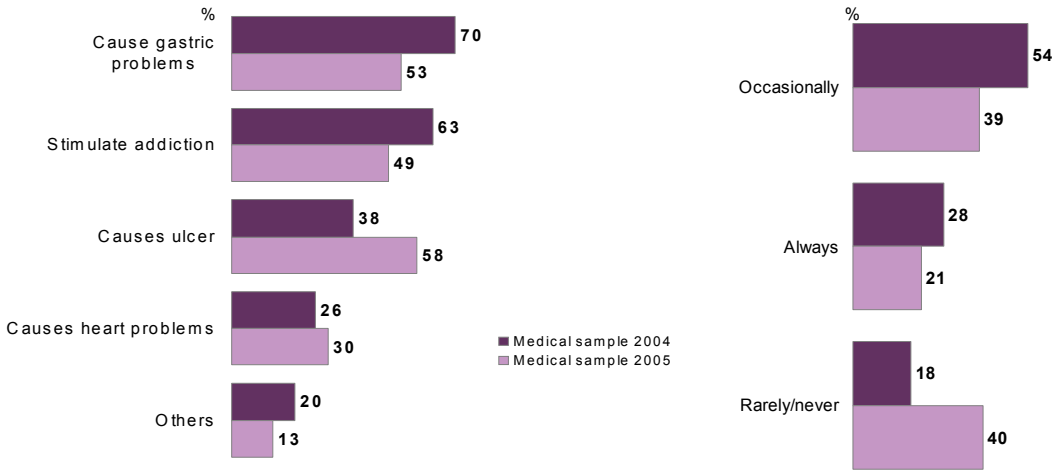

Figure 4.

After efforts brought about from the Program Coffee and Health, we also note changes in number of cups prescribed (increased percentage of number of cups recommended a day) or even (3 per day).

Table 4.

What is your recommendation for daily Coffee consumption?	Total %	
	2004 %	2006 %
• 1 cup a day	26	34
• 2 to 3 cups a day	48	66
• 4 cups a day	26	-

The surveyed medical community (and we believe that the population as a whole) believes that the main content of coffee is caffeine. They are totally oblivious of the remaining content. According to Dr. Aldir Roberto Lima, "Coffee is not a medication, but the scientific-medical community already considers the plant to be nutraceutical (nutritional and

pharmaceutical). Such is true due to the fact that coffee not only contains caffeine, but also potassium, zinc, iron, magnesium and many other minerals – but in small amounts. The coffee bean also contains amino acids, proteins, lipids, besides sugar and polysaccharides. But the major secret: it contains a large amount of plyphenol antioxidants, called chlorogenic acids. While the beans are roasted, these chlorogenic acids form new bioactive compounds: the quinic acids. It is at this stage that proteins, amino acids, lipids and sugars form nearly one thousand volatile compounds which are responsible for the particular coffee aroma".

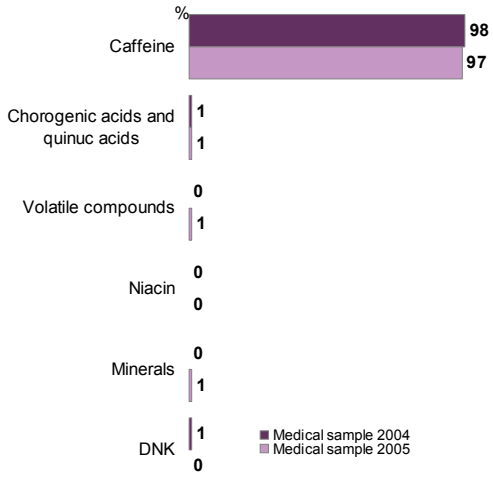

Figure 5.

Learning # 2

Educational campaigns about benefits of coffee are welcome mainly in the medical community, a strong influence of changes in food habit, for themselves and also in terms of their recommendation. Hence, keeping the campaign is a learning process.

THE MOVEMENTS WHICH ARE UNDERLYING CONSUMPTION, MAINLY THE HEDONISTS TEND TO ESTABLISH THEMSELVES AND INFLUENCE BEHAVIORS:

Taking into consideration all catastrophic indicators which we have to live with in the modern world, mainly in the western world, the entire effort to encourage consumption is based on the truth that 'you deserve it' (a better car, one which is bigger, more powerful; an alluring perfume; an unforgettable trip, etc..) which the coffee shall absorb.

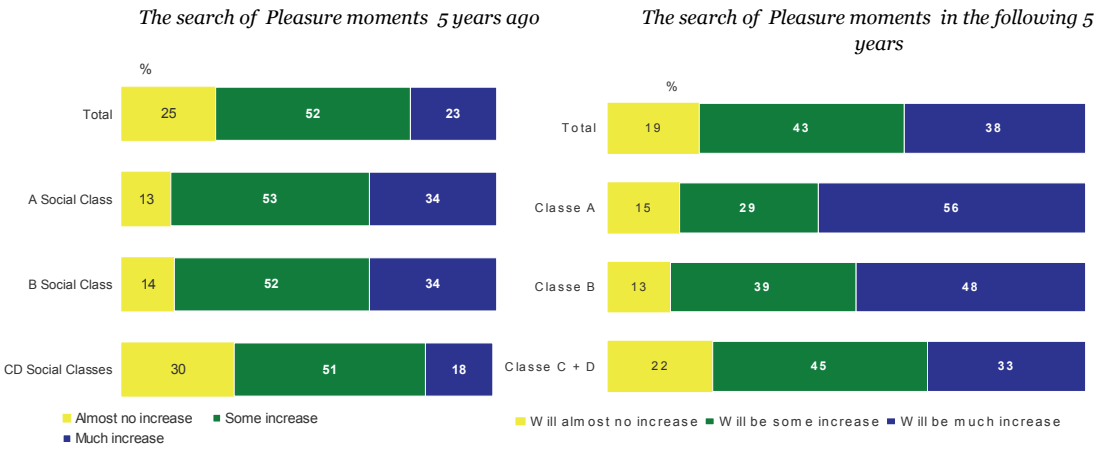

Figure 6.

And major trends are noted (research on usage trends carried out in 2007 by TNS InterScience):

- **Valuing Pleasure**

- Consumer have greater level of information and involvement with products purchesed

- Greater demand for custommization

- Valuing authenticity and originality of products and brands

- Demand for convenience

- Social environmental responsability

Figure 7.

Learning # 3

a) Brands which respect consumers, which offer convenience and do not harm the environment (attention to the start of the sustainability movement) will undoubtedly have strong support;

b) Offer products with different range of flavors, combination/ blend and create places where consumers are able to not only enjoy the coffee within its various options, but also relax and enjoy the moment, feeling no guilt – all shall maintain their status of major drivers for drinking not at home. It shall be the combination of personal pleasure with the organoleptic pleasure.

> **"***I enjoy going out with my friends and after going shopping, stop at a café and have a cup of coffee ... it is a total moment of pleasure..." (Woman, age 20, SEC, AB, from São Paulo, research from2007).*
>
> *"Hum… that great smell of coffee... "(man, age 35, SEC C, Rio de Janeiro, research from 2007)*

c) Coffee may bring to itself such movement: "you deserve it".

WHAT ABOUT THE FUTURE: MOVEMENTS TO ENCOURAGE COFFEE CONSUMPTION ?

Brazilian Consumer has became usedto options of quality, pleasant public places, prestigious brands, wide variety of products and different ways of preparation, now the question is how to incentive the consumption?

There are many ways to induce the consumption, asfollowing:

a. Quality coffee encourages and maintains the intensity of consumption
b. A habit acquired from childhood ensures the presence of coffee in the market
c. Coffee which triggers smell and taste buds encourages consumption
d. A knowledgeable medical community encourages their own consumption as well as explains to patients the benefits of coffee
e. Coffee must absorb the pleasure associated with hedonism ("you deserve it")
f. And new possibilities and options of individual coffee packets or sachets to drink not at home may broaden significantly product consumption
g. A frequent communication of the benefits

Source: all data mentioned above are from researches from TNS InterScience, São Paulo, Brazil.

Globalized Market and the Agro-Industrial System of the Coffee

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SUMMARY

The coffee is an agribusiness that was born globalized. Its produced in the hemisphere south and consumed by the hemisphere north, the coffee production always be directional to take care of the palate of the consumers, either in the field, the great cities, in developed countries or in developing countries. The entrance of new players in this market affected in significant way this. Its consumption is affected by some variables raised and commented in this paper, where if it notices a trend of growth of production and consumption of special coffees.

INTRODUCTION

The coffee is an agribusiness that was born globalized. Its produced in the hemisphere south and consumed by the hemisphere north, the coffee production always be directional to take care of the palate of the consumers, either in the field, the great cities, in developed countries or in developing countries. Differently of sugar cane, cacao and other products, where the production process intervenes little with the final product quality, the intrinsic quality of the coffee always depended on the process of production and the cultural treatments in the farming and the after-harvest, allowing distinguishing of prices between the different suppliers of the product. In the cafeterias of the consumers countries, the quality of the coffee not only depends on as coffee "is taken off", but it is related to the cares implemented throughout all the process of production since the farming, passing for the drying, bagging until the transport. At last, all the agro-industrial system (SAG) contributes for the final product quality.

Since 90´s, with the valuation of the special calls coffees, the fine coordination of all the agro-industrial system gained relevance. Efforts associates to the production of attributes of quality in the agricultural phase can be lost if it will not have adjusted treatment in the posterior phases of the system. The base, therefore, of the competitiveness of the coffee agribusiness walked for two routes: aggregation of recurrent value of the intrinsic quality of the product and profits of productivity, recurrent of innovation and reduction of cost. Brazil, as leader world-wide, is detached as inductive of innovation in the productive chain and as great competitor with focus in cost. From 90´s, however, with the growth of the market of special coffees and the entrance of new competitors, the SAG of the coffee of Brazil passes to confront with some questions that make to reflect on its positioning and its strategies.

The objective of this paper is to argue the main changes that occur in the SAG of the coffee from the decade of 90 and which the consequences of such changes with regard to the positioning of Brazil in the world-wide market.

The first part of the paper approaches the dynamics of the world-wide of the SAG coffee in view of new competitive environments and institutional market. Second it argues as such changes are impacting the SAG of the coffee, exploring in each one of its main segments. The

third part argues occurred changes in strict coordinated food system. Finally, some strategic considerations are tacked.

GLOBALIZATION AND DYNAMIC OF COFFEE SAG

The international production of coffee in the period from 1975 to 2008 presents a growth trend, as can to be observed in the annual data and also in the movable average of 4 periods of this production, carried through with the intention to minimize the seasonality of this production (Figure 1). On average, to each year 1.58 million bags of coffee in this production are increased, as it shows the linear regression.

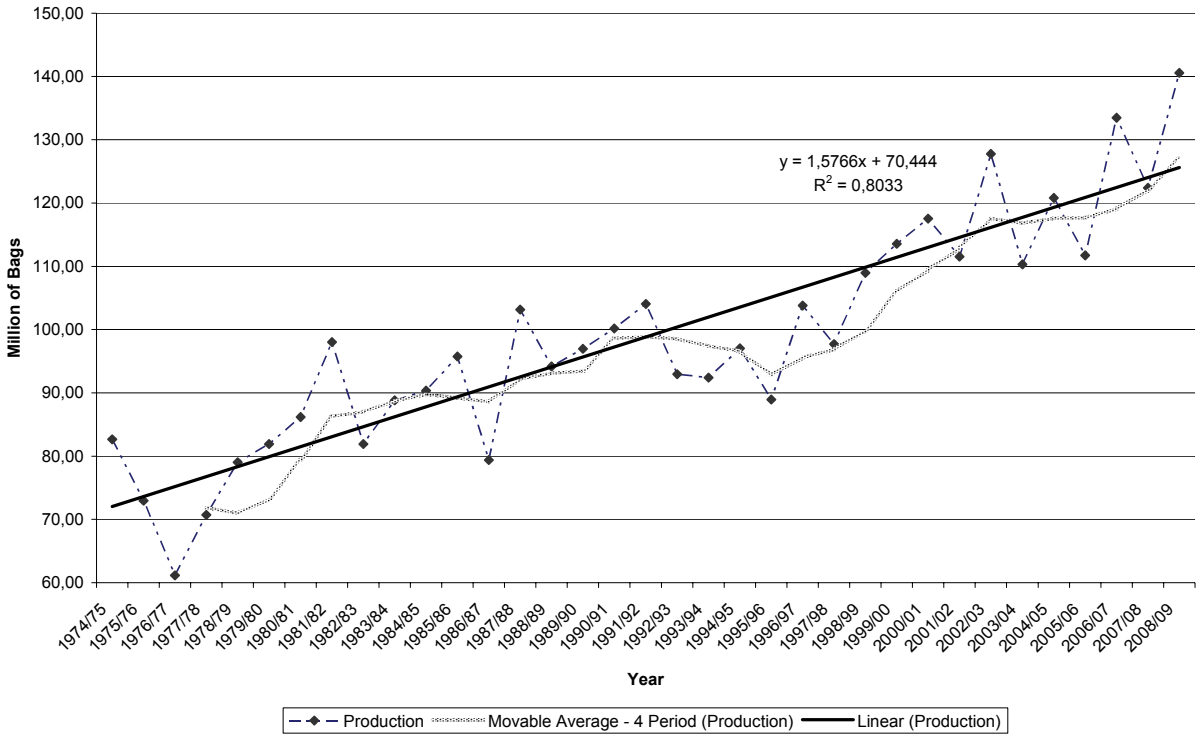

Figure 1. Evolution in the world production of Coffee in the period of 1974 to 2008, in millions of bags. Source: USDA (2008).

The trend observed from 90´s was mainly stimulated by the rise of the prices of this commodity in the international market (Figure 2). The high prices had elapsed of two factors: i. institutional factors – end of the regulation –, at the beginning of decade of 1990, that it reflected in it I discourage of the production leading to the eradication them farmings in some producing regions; e II. Climatic factors - frost in the Brazilian farmings in 1994. This period finished if becoming a landmark in the SAG it coffee in function them new competitive and institutional environments that if they install from now on.

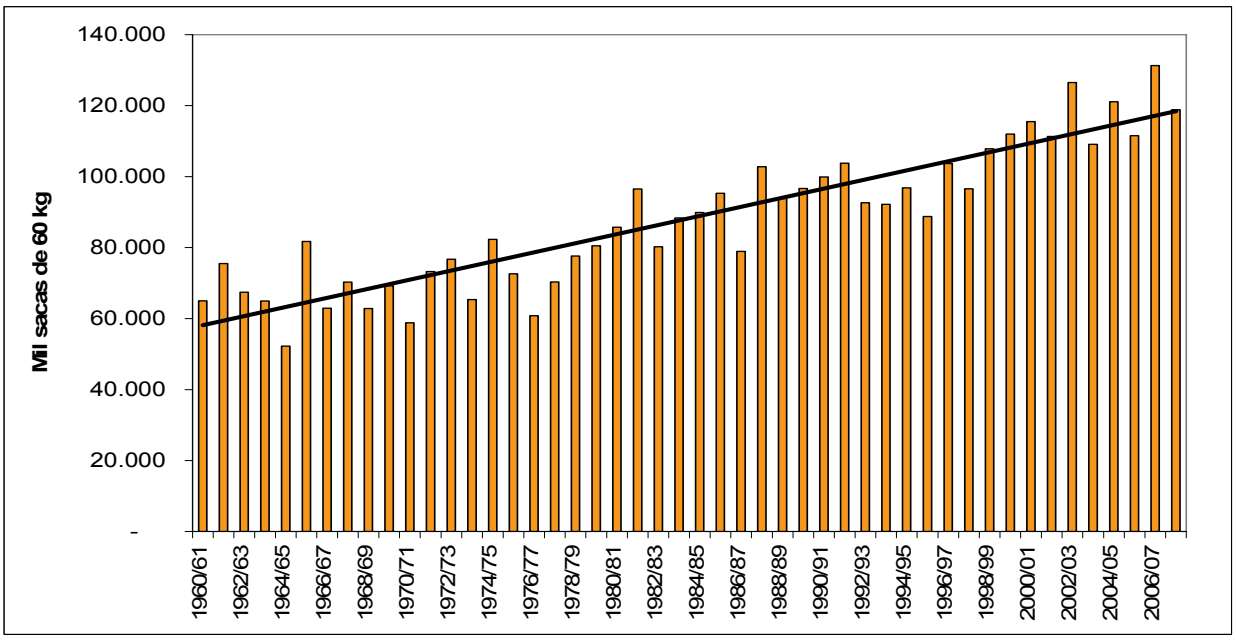

Figure 2. Evolution of the world-wide production of coffee (in a thousand 60 bags of kg). Source: USDA

Competitive Environment

In competitive environment terms we need to detach the entrance of a new competitor in the market: the Vietnam. The impact of this producer in the market coffee not yet completely was absorbed and understood for the agents of this market. From a residual participation in the decade of 1980, the Vietnam blunts as second bigger producer of world-wide coffee in years 2000. In decade 1980, the Vietnam on average produced 377 thousand bags of 60 kg, in the period from 2000/01 to 2007/08 this country produce 14.8 million bags of 60 kg (Figure 3).

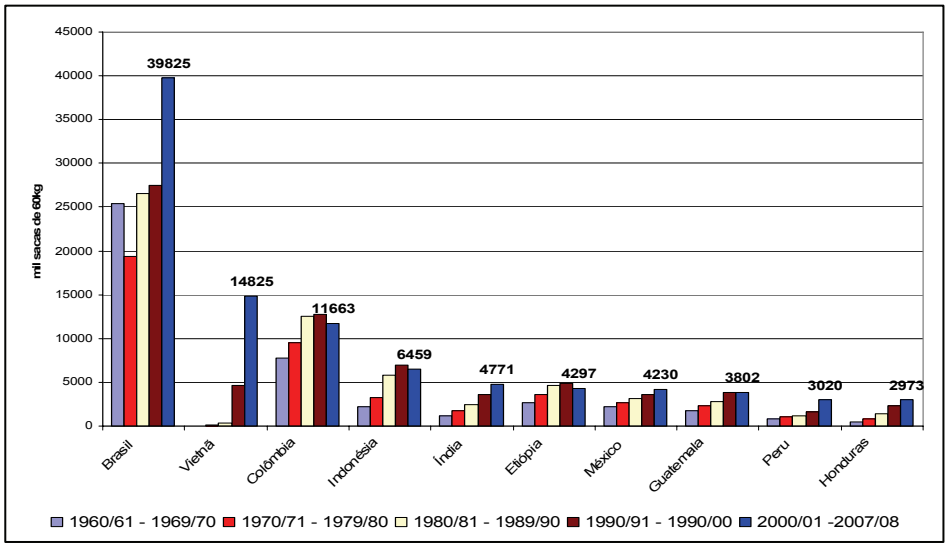

Figure 3. Average production of the main producing countries (in a thousand 60 bags of kg). Source: ICO.

One of the effects of the entrance of the Vietnam in the world-wide market was the growth of the participation of the Robusta species in the total of produced coffee. As if it observes in Figure 4, in 60's, the Robusta species represented only 19% of the total of coffee produced in

the world. In the half of 2000's this species already was responsible for 38% of the produced total.

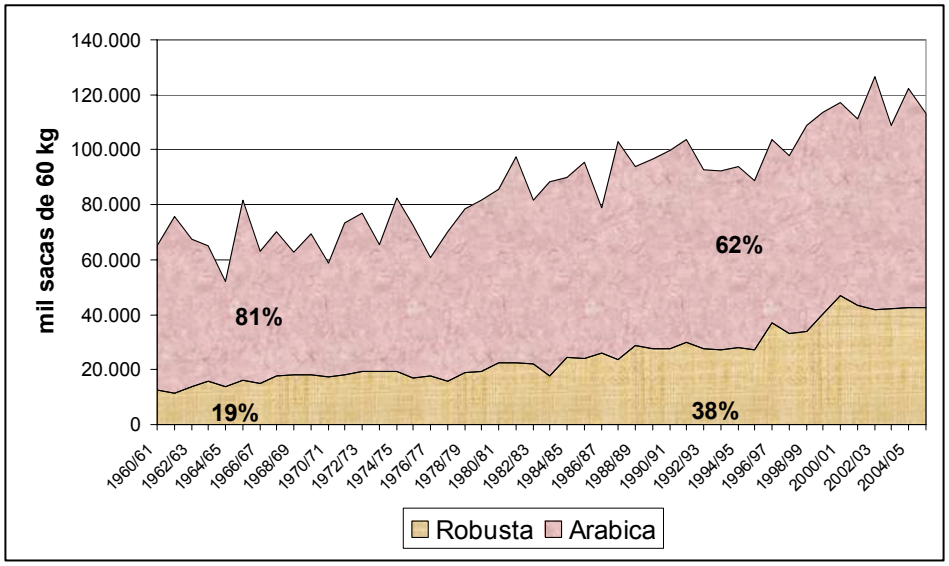

Figure 4. World-wide production of Arabica and Robustaa coffee. Source: ICO.

We cannot leave to detach that Brazil also had important participation in the growth of the Robusta species in this period, as can be observed in Figure 5. Its participation passes of 15% in the decade of 1990 to 29% in 2000's and the increase of the production occurs particularly in the state of the Espirito Santo.

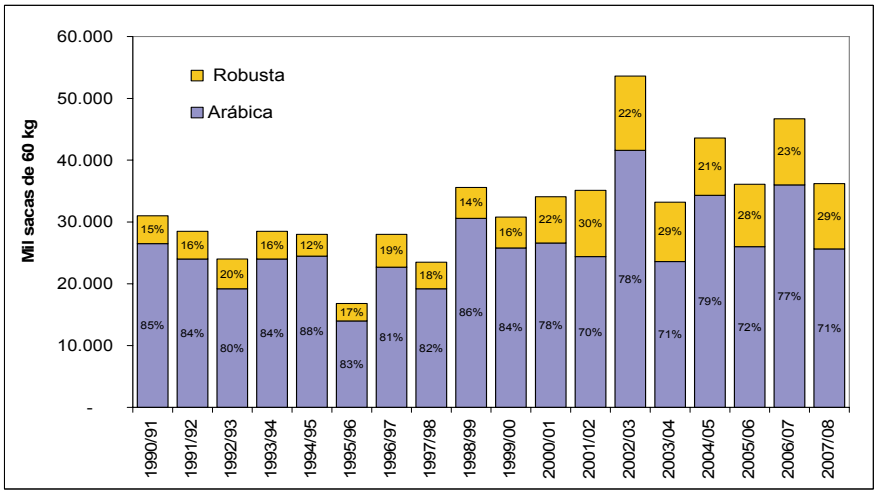

Figure 5. Brazilian production of coffee between harvests 1990/91 and 2007/08. Source: ICO

The growth of the coffee, mainly in the Robustaa species, tends to change the relative one of prices of the coffee between the two species, once these are substitute goods in the production and in the consumption and the Robustaa species it suffers a discount with regard to the Arabica. The differential of price of the Robustaa coffee can be explained as well as for the productivity – bigger than Arabica – and also because the inferior costs. In the Vietnam could be add another advantage, that is the cost of workers. Figure 6 presents the daily prices of the coffee Arabia and Robustaa, in the respective Stock markets of Future, deflationed, since the beginning of the decade of 1990.

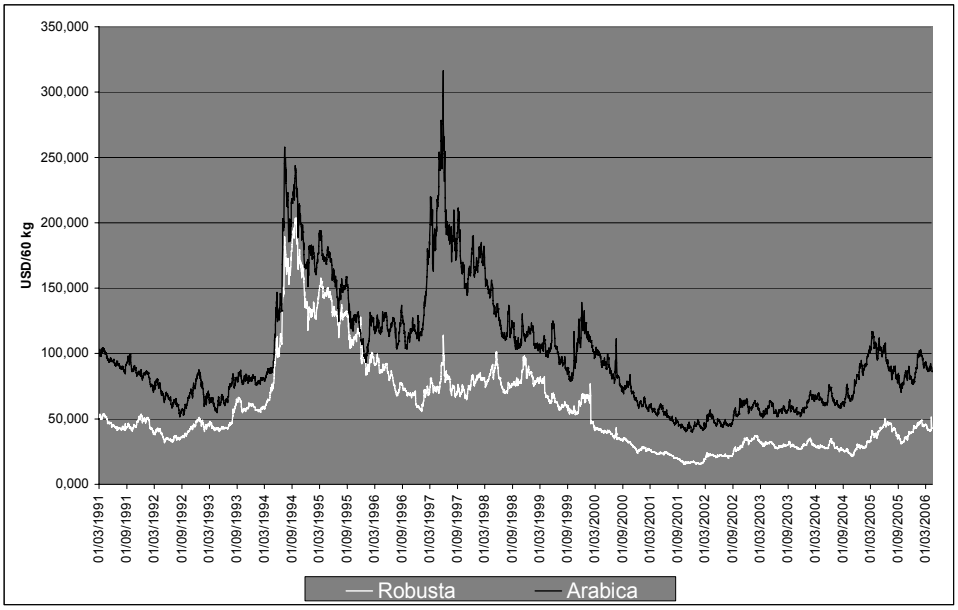

Figure 6. Closing Price of Arabica and Robusta coffee in the Stock market of New York and London Stock market, 1st contract, in constant values of 1982 for bag of 60 kg. Source: CIC (2008).

One of the effects of this new dynamics is the preference of the Robustaa coffee for the great mills when the differential of prices of the Arabica coffee if becomes greater. That is, it is observed that from the decade of 1990, as one form to use the advantage of the growth of supply of Robusta coffee, the great mills had developed technology – the vaporization – that allows to the increase of the use of the Robusta coffee in its blends. The vaporization uses the same equipment of the descaffeination that, when reducing the harshness of the palate of this raw material, neutralizes its impact in the beverage[1].

In this direction, Brazil has acted as inductive of technological changes in the SAG of the coffee, in a comfortable situation, in terms of cost of production with regard to its producing competitors of Arabica, that sees ahead a challenge, as for the Arabica coffee commodity: to compete in the production of Arabica with Robusta coffee of low cost of production.

As can be observe in Figure 7, the world average productivity of the production of coffee in the period from 1985 to 1995 was 8.8 bags for hectare and the Brazilian one was 10.6 bags for hectare. From 1996 to 2005, the world one passed to 10.9 bags for hectares and the Brazilian for 15.8 bags for hectare. Therefore, Brazil is the direct responsible for the increment of the productivity of the land in the world-wide SAG of the coffee[2]. If it is considered average data, one does not catch the real changes in innovation terms, that have be got in some areas from middle of the decade of 1990. In the new Brazilian areas of production, where the properties are greater that 500 hectares, the intensive use of technology is evidenced, whose prominences are the ferti-irrigation and mechanization. The scale of production, allied to the configuration of the plain areas of the Brazilian Cerrado, has favored mechanized harvest and raised the average productivity. In some Brazilian regions, as in the bahian Cerrado, the producers harvest more than 100 bags for hectare.

[1]Maria Sylvia Macchione Saes e Douglas Nakazone. El estudio de competitividad de cadenas integradas en el Brasil. Ensayos sobre Economia Cafetera. Bogotá, n. 19, pp. 11-68. 2003.
[2]The variability from one year to another is because to the bienality of the Brazilian production

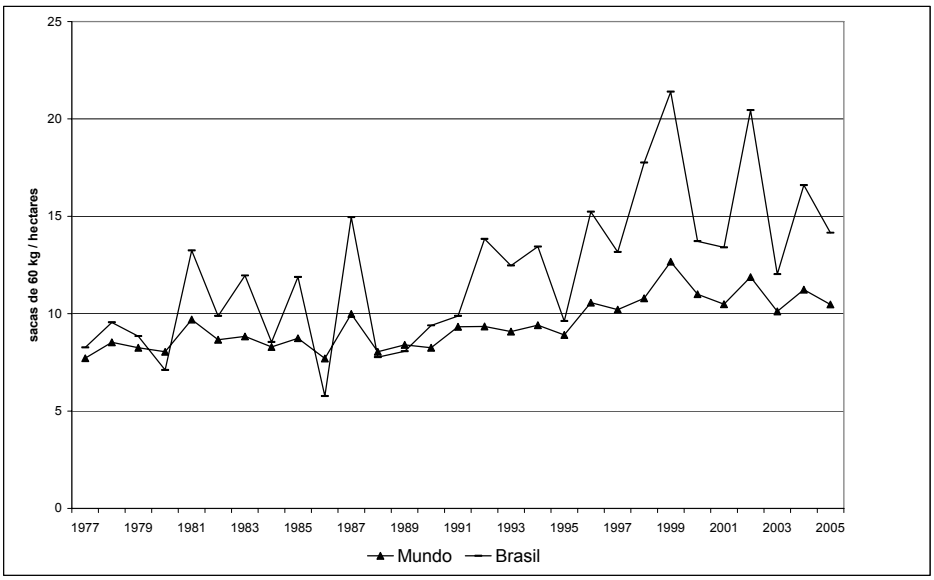

Figure 7. World-wide and Brazilian productivity in the coffee production (bags for hectare). Source: FAO (área colhida) e ICO (produção), CIC, 2008.

Institutional Environment and Consume

Understanding Institutional Environment as the rules created for the society whose structure affects the strategy and the performance of the organizations, we can consider its two natures: the formal and informal institutions[3]. The formal one is characterized for laws, contracts or regulations established for the society. The informal ones are those that appear spontaneously in the society, are rules created for the continuous interaction between the individuals, as religious customs, codes and codes of behavior.

In formal institutional environment terms it is not necessary to stand out that the market of coffee after more than 100 years of regulation has lived since 90´s the free market. The International Organization of Café (ICO) started to have a performance of promotion of the coffee in the international market and of accomplishment of specific programs being aimed at the support and product quality in diverse productive regions. Since the end of the International Agreements of the Coffee, in 1989, the prices reflect the international stock markets and the balance of offers and demand of commodity.

However, the institutional factor of bigger prominence from 90´s is, without any doubt, the informal one, that is, the one that it relates to the changes in the habits of the consumers and, therefore brought impacts in all the productive chain. The great change is the valuation for the consumer to special coffees market that is an ample specter of differentiation possibility. That is, the differentiation can be related to the attributes of the variety of the beans; passing for production processes (organic, shading, familiar, *Fair Trade*[4]); production place (origin, *estate coffee*[5]); improvement forms (natural coffee, peeled, cherry); quality of the beverage (that it takes in account aroma, flavor, body, acidity); industrialization process (perfumed, decaffeinated); type of preparation (espresso, cappuccino); until the place of sales or consumption (cafeteria, consumption in the home).

[3]Douglass North. Institutions, Institutional Change, and Economic Performance, Cambridge, Cambridge University Press, 1990.
[4]O café *Fair Trade* é comprado diretamente de cooperativas de pequenos produtores que garantem um preço mínimo pré-estabelecido por contrato. Esse mercado será abordado no próximo item.
[5]Café de origem de uma fazenda.

It is needed to stand out that although it is a new movement, the differentiation for origin in the coffee already was known, for example the coffee of Colombia or Costa Rica, well-known ones. However, until 80´s the chain as a whole presented few attributes of differentiation. Even in the sales points, the trade marks brought few variations between them, conferring one limited to be able in the formation of the prices[6]. The demand studies indicate that each trade mark has little possibility to determine prices, therefore the consumers have alternatives of products with other marks to its disposal.

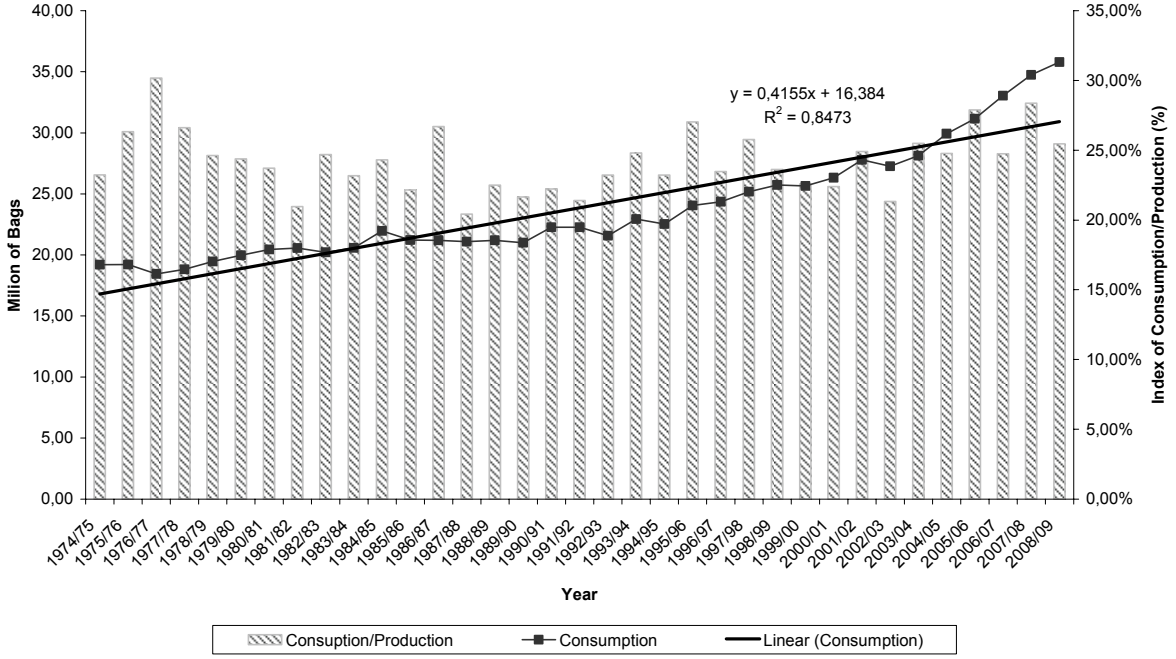

Figure 8. Consumption of coffee in the period of 1975 the 2008, in millions of bags. Source: USDA (2008).

It is observed in Figure 8, that the consumption of coffee in the period from 1975 to 2008, presented trend of high, with average increment in this period of 0.42 million bags per year. In this exactly period the relation between consumption and production go to 20.43% to 30.17%, with 24% in the average. As a variable that indicate the consumption of coffee of each countries, can be observed the importations of the countries that is important consumers but are not producers (Figure 9) and the domestic consumption in the case of the consumer and producer countries of this product (Figure 10).

[6]Um estudo realizado por Nevin (*apud* Pindyck and Rubinfeld, 2002, p. 426-427), com as marcas Hills Brothers, Maxwell House e Chase & Sanborn, verificou que as elasticidades preços da demanda foram 7.1, -8.9 e -5.6, respectivamente.

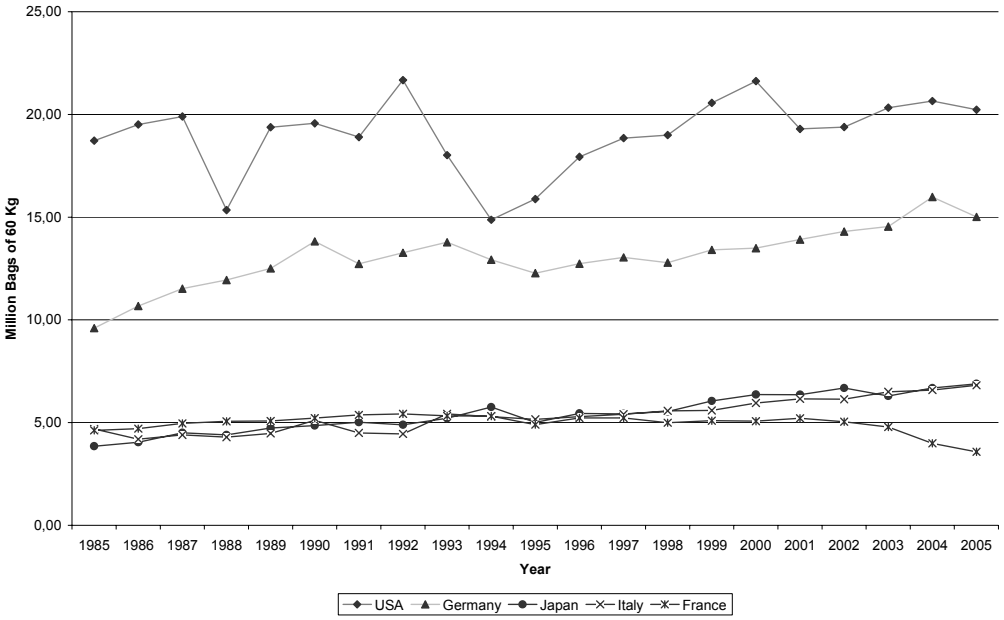

Figure 9. Evolution of the five greater importers of coffee in green grain in the period of 1985 the 2005. Source: FAO (2008).

It is observed in this graph that the five main world-wide importers of coffee are, respectively, U.S.A., Germany, Japan, Italy and France. The two great importers (U.S.A. and Germany) had presented, respectively, a trend of constancy and higher its importation. The domestic consumption of integrant the producing countries of coffee and of the ICO can be seen in Figure 10.

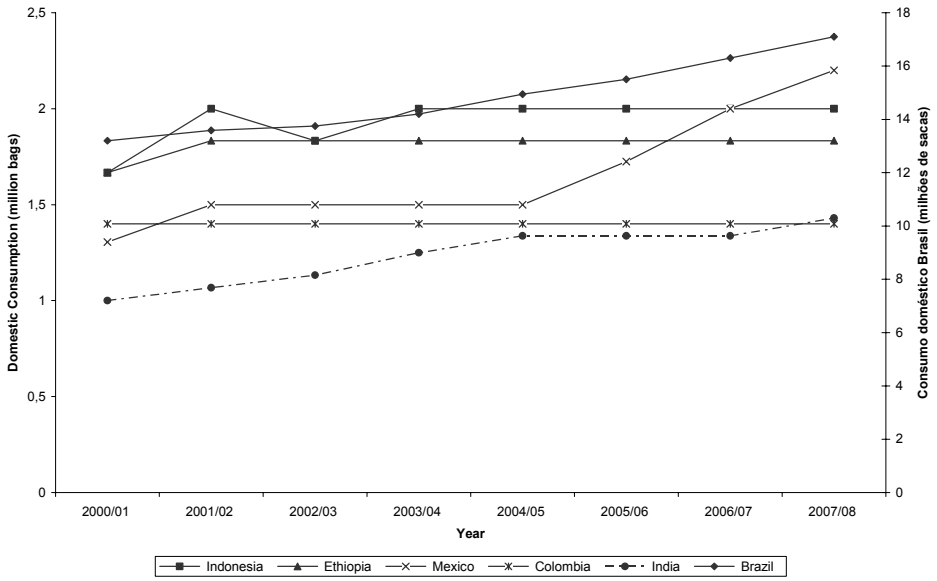

Figure 10. Domestic consumption of the six integrant consuming greaters of coffee of the OIC in the period of harvests of 2000/01 the 2007/08. Source: OIC (2008).

It is observed that Brazil leads the consumption between the six major consumers countries, presenting trend of high in the studied period, being followed, but with inferior amounts, for Mexico.

Fifty years ago, the world consumption per capita was, in the average, about 1 kilo/year. USA is the greater consumer in amount, followed for Brazil, Germany, Italy and Japan. Figure 11 shows the per capita consumption of these countries, in the period from 1998 to 2003 (Cunha, 2006).

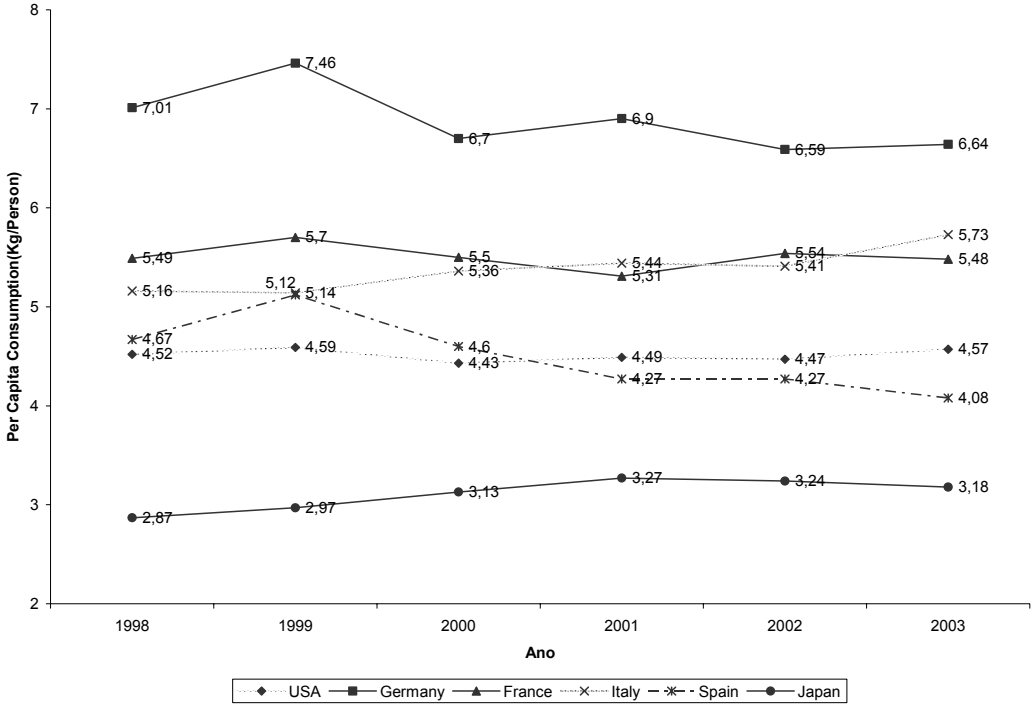

Figure 11. Per capita consumption of the main consumers of coffee in the period of 1998 the 2003. Source: Cunha (2006).

It is observed in this graph that the American per capita consumption presented a light rise in the studied period and that the German consumption if kept with a small constancy. With regard to the national per capita consumption, it is observed that in the period of 1990 the 2007 presented a trend of high, as can be seen in Figure 12. In this related period can be seen that an average annual addition of 0.12 kg of green coffee or 0.10 kg of toasted coffee, as it can be seen in the two regressions presented in this graph, both with high degree of explanation power (R^2 up to 0.95).

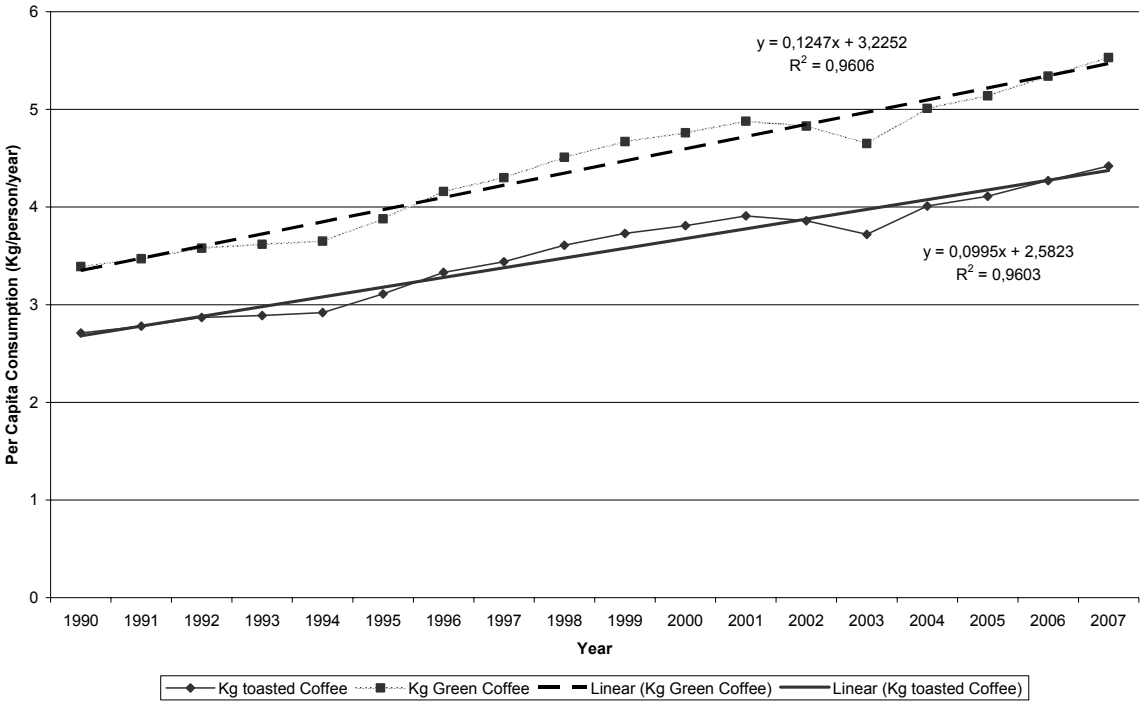

Figure 12. Evolution of the Brazilian per capita consumption in the period of 1990 the 207, in kg of green coffee and kg of toasted coffee. Source: ABIC (2008).

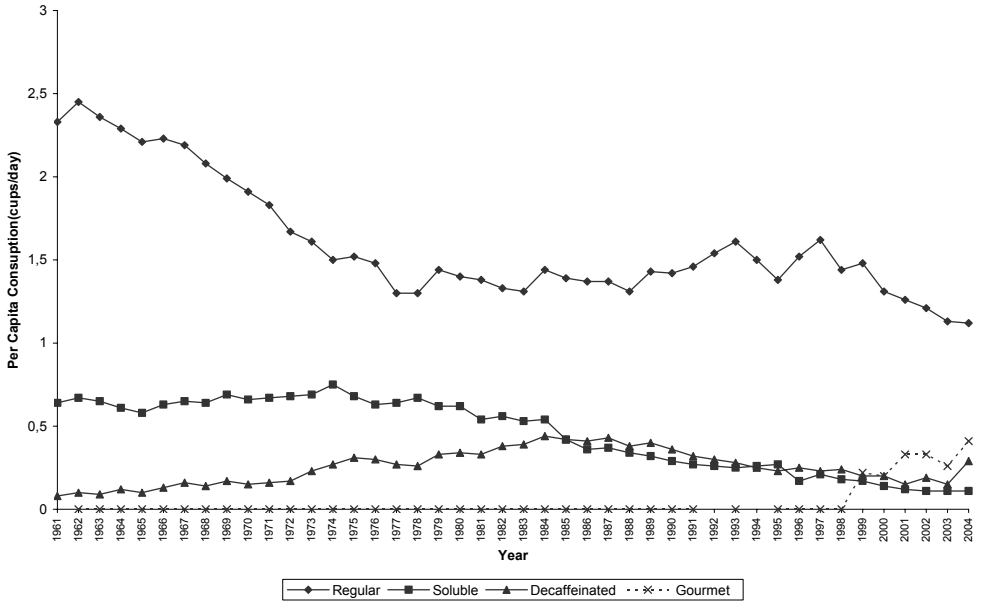

Figure 13. Per capita consumption of different types of coffees in U.S.A. in the period of 1961 the 2004. Source: USDA (2008).

It is observed in Figure 13 that the per capita consumption of conventional coffee comes declining in the analyzed period while the special "coffees" had presented an increase of consumption in the same period, being accented in the most recent years in the case of the decaffeinated gourmet and declining a little in the case of the soluble one.

Demand and Production Elasticity-price Studies

The elasticity-price of the coffee demand depends on the time series that is taken in account. The demand is more elastic in the long term period that in short term, as expected in the theory. In the traditional consumer countries of coffee, with steady or declining population, it has some indications that these ones are reaching the saturation point. In this point a collapse of prices would not in such a way increase the consumption in the short or long term. Increase in the quality in cup, however, can be a factor that would increase the consumption. The quality criterion includes content of caffeine, taste and aroma (ICO, 2004).

The inelasticity in supply and demand can directs the prices to top, following production shocks, but in reply in the long run this higher price increases the production with the ingression of new incoming and stimulating the rehabilitation of farms. This directs the prices for low until they are bellow marginal cost in the long run (Lewin et al., 2004).

This is complemented by a estimate of the FAO that this product has a elasticity-price of demand for industrialized countries of -0.2, but that this elasticity is not linear. So a great increase of international prices reduces the coffee demand more than proportionally to a price reduction that increases this demand (OXFAM, 2002).

With regard to the two most important consumers of coffee (U.S.A. and Germany), this study of the FAO shows that the demand elasticity-price is of -0.12 for Germany and -0.20 for U.S.A. (FAO, 2002). These estimated values in U.S.A. are not so different from the ons found in 70´s (- 0.18) (Parik, 1973), indicating that this can not vary very much throughout the time. About the elasticity of supply, these can vary from 0.5 to 2.0, depending on the country, being that in the long run this can be superior. Amongst the diverse estimated elasticities in this study it is distinguished that the inferior (0.50) was presented by the Columbian producers, the superior (2.0) for the producers of El Salvador and the Brazilian producers had presented an elasticity of 0.60 (Bohman and Jarvis, 1999).

These numbers are complemented by study of the World Bank in 1990 (Akiyawa and Varangis, 1990), which presents that the elasticity of price for new plantations in the two main agents at that moment, of 1.02 in time T-1 and 2.34 in the time t-4 for Brazil and 1.68 for Colombia in time T-1.

More recent data show that this elasticity of world supply reduced for 0.25 (Hallam, 2003) and the demand elasticity increased for -0.51 (Galindo, 2007). In the case of special coffees, these numbers are a little different as can be observed in the case of the shading coffee, which presented an demand elasticity varying from -4.4 to -0.5 and elasticity of supply from 2 to 0.5 (Larson, 2003).

The growth of the market of differentiated coffees has led to the reversion of the trend of fall of the per capita consumption, in the import countries, mainly in U.S.A. (Figure 14). The inflection of the market could be attributes to the coffee places that, with sophisticated style and differentiated drinks, had attracted the young public.

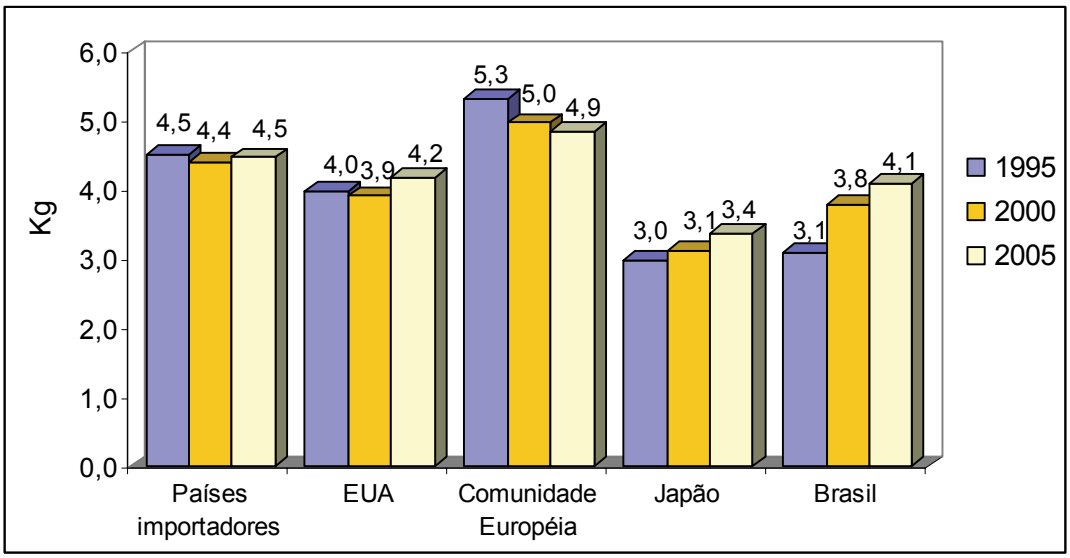

Figure 144. Per capita consumption in the years of 1995, 2000 and 2005 in the import countries, States Units, European Community, Japan and Brazil (in kilograms). Source: OIC e ABIC (para o Brasil).

One example of this is Starbucks Coffee Co. Established in Seattle, in US, in a little more than 15 years of existence its has almost 15 thousand points of sales and its invoicing in 2006 reached 7.8 billion dollar. Its growth induced the entrance of companies as the Second Cup, that has strong presence in the American market and is the second bigger company in this segment in the Canadian market. Other companies, such as McDonald's, had been also stimulated to introduce the item coffee espresso in its store, in some countries. The phenomenon of the special sales of coffees in the cafeterias reflected in the toasted market of great multinationals that had introduced segments in coffees special, such as of Nestlé.

In accordance with the Specialty Coffee Association of America (SCA), the market of special coffees grew from US$ 7.5 billion in 1999 to 12.3 billion in 2006 (CIC, 2008). In US, estimates of SCA[7] indicate that the daily consumption of coffee between the young from 18 to 24 grew in 2000´s. In 2007, 37 percent of the interviewed young had affirmed to daily consume the product, in 2004 was 16 percent. The number of adults who consume this product daily increased from 49 percent to 57 percent in this period. On account of this, the Department of the Agriculture of this country passed, since 1999, to follow the coffee consumption gourmet[8], that reflect in the growth of the global market in the United States, after almost four decades of retraction, as Figure 15.

[7]Based in a national annual *survey* by phone.

[8]The term coffee gourmet is used in the market of special as indicating of coffees of superior quality. It is related the intrinsic characteristics of the green beans that intervene with the final quality of the beverage as aroma, flavor, body, acidity and residual flavor. In a general, mentions prepared coffees to it type 3 for better (numbers of defects in a sample of 300 grams), of aspect very good uniform, with strict soft/soft beverage (punctuation higher than can be received for a coffee in the beverage classification).

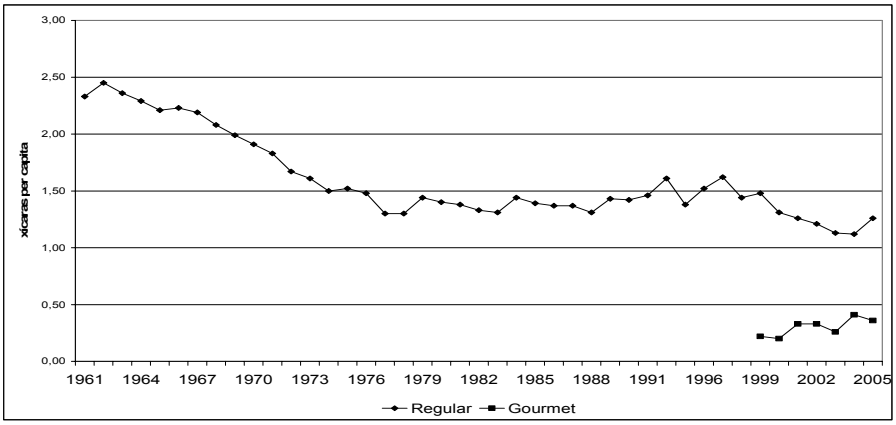

Figure 15. Domestic consumption of coffee in U.S.A. – Regular and Gourmet. Source: CIC (2008).

Parallel to boom of the cafeterias and the growth of the invoicing of the companies of coffee processing, three other movements had appeared in the developed countries gives a new connotation for the coffees special. Their appeals are, however, distinct: a movement worried about the impoverishment of the coffee producers, another one looking for to preserve the environment and third, with interest in the healthful food consumption. All had finished being aggregate in the market of sustainable coffees. The advance of these movements, that each time more are joined together because the similar style and philosophy of life, reflected in the global market, imposing to the processing and the retailers a special attention in terms of marketing strategy. Starbucks, for example, in 2005, bought 10 percent of the global supply of certified coffee Fair Trade, what it correspond to 11.5 million pounds[9].

Although the growth trend, the market of sustainable coffees is still small and occupies, in 2000, less of one percent of the world-wide total in volume terms (0.29 percent) and a little more than 1 percent in value terms (Table 1). In the period between 1999 and 2004, the segment of special coffees grew to a tax of about 10% per year in diverse countries of the Europe and Japan (Giovannucci, 2001). Only with regard to the certified coffee Fair Trade: in 2006, 52 thousand tons had been negotiated, in 1999 was 11.9, in accordance with FLO (Fair Trade Labeling Organizations International)[10].

[9] http://www.starbucks.com/aboutus/StarbucksAndFairTrade.pdf
[10] http://www.fairtrade.net/

Table 1. Market of sustainable coffees. Source: Ponte, 2004, p. 42.

	Volume		Valor no Varejo	
	Thousand Bags (60 kg)	% of total in Coffee Market	Million US$	% of total in Coffee Market
Total of the market of Coffee	111,545.5	100	49,257.00	100
Total coffee of sustainable coffee	318.2	0.29	565.00	1.15
Soluble Coffee Certificated	272.7	0.24	490.00	0.99
Total of Organic Coffee	160.6	0.14	286.00	0.58
Organic Coffee Certificated	125.0	0.11	223.00	0.45
***Fair Trade* certificated**	220.5	0.20	393.00	0.80
Total of Shade Coffee	17.4	0.02	30.50	0.06
Shade Coffee Certificated	9.1	0.0	16.20	0.0

THE NEW MARKET AND ITS IMPACT ON COFFEE SAG

Taking the main presented trends, this item has as a objective to explore as such changes impact the SAG of the coffee, in each one of its main productive segments. The SAG of the coffee includes the following segments (Figure 16): I. suppliers of inputs, machines and equipment; II. Agricultural production (producers of Arabica and Robusta coffee); III. Processing (companies of toasting, milling and soluble); iv. Distribution (Cooperative, exporting and traders); v. International purchasers (companies of international toasting and soluble milling and dealers); vi. National and international retail (supermarkets, small retail, store of coffee, bars and restaurants).

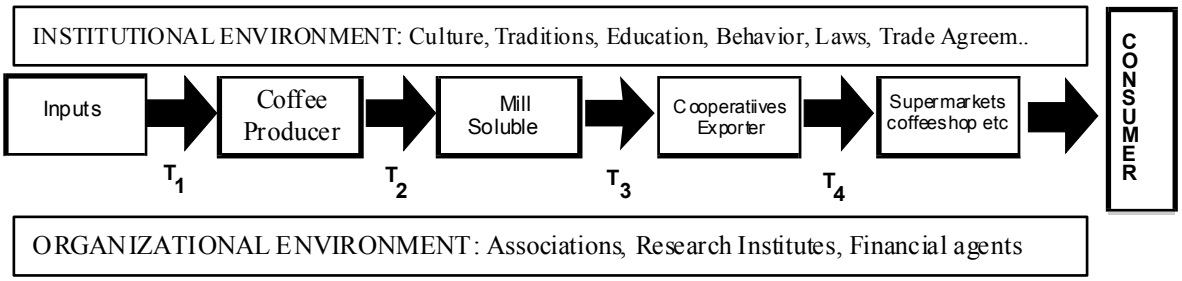

Figure 16. Simplified project of the SAG of the Coffee (SAG Generic Coffee).

The Input Segment

As already observed, a prominence fact that is directly related to the segment of inputs is the increment of the productivity in last the 20 years. As it was also noticed, Brazil has been the main inductor of this change with prominence for the ferti-irrigation in the new areas of production - particularly the *Cerrado*. The continuity of the improvement programs, the development of adequate harvester to the plain regions, is essential factors for the profit of productivity of the coffee plants.

The Processing Segment

In 90´s, the industry of toasting of coffee in the developed countries concentrates, starting to present important changes of leadership. In 1978, the Herfindahl-Hirshman index[11] in the average was of 543, while in 1999 it was of 1299. This means that the industry passed, of disconcentrated in 70´s, for moderately concentrated in the end of 90´s.

A distinction fits here between the companies of toasting and soluble, since they possess an important technological difference between itself. The industry of soluble coffee demands a sophisticated plant and sufficiently intensive capital, what it determines that the structure of the industry is concentrated, being, consequently, impossible the existence of companies of small and medium size in this sector.

In the case of the toasted industry, in contrast, in the sector can coexist small mills and large companies, once that the requirement of capital for this business is well lesser. This explains the difference of margin between the two industries. The industry of toasted coffee, although concentrated, coexists with a competitive fringe. In the US, for example, they estimated that it has about 1900 mills of medium and small size, they have less than 20 percent of domestic market (Daviron and Bridge, 2003), while the three biggest have more than 80 percent of the market, in volume terms: Folgers, with 38 percent; Maxwell House, 33 percent and Sara Lee, 10 percent.

The processing has its geographic distribution affected by many variables. In first place the possibility of trademarks with local importance, represented for small mills. Second the existence of tariff scheduling that makes it difficult the commercialization of the toasted coffee processed in countries such as Brazil, to supply the European market. Third a great relevance of the trademarks associates the products with differentiated attributes of quality.

The Retailer Segment

Cankorel (2000) when analyzing the market of coffee in 14 countries, that represent 77 percent of the world-wide importations of coffee, between 1977 and 1999[12], verifies the increase of the margin between the international prices and the prices in the retail of the consumer markets for eleven of the studied countries, especially Japan, the United Kingdom, U.S.A. and Italy. Only with respect to three countries, Germany, France and Finland, the results of the regressions point a weak relation between margin and time. The conclusion is similar of Morisset (1997), admitting that the joined result is the consequence of the transformations of the structures of market of the industry, that have suffered in some levels strong process from concentration. This dynamics, in accordance with Cankorel, allows that the great multinational companies be able to have market power, widening the differential of its margins[13].

[11]The index of Herfindahl-Hirschman is calculated from the addition of the squares of the participation of each firm in relation to the total size of the industry and takes in account all the firms of the industry. It is considered disconcentrated the industry with index up to 1.000, moderately concentrated the one that goes of 1.000 up to 1.800 and extremely concentrated the ones that have index above of 1.800.

[12]The used data, supplied for the ICO (International Organization of the Coffee), had been the price of the coffee toasted in the retail in each country and the Index of Composed Price, to represent the price of the green coffee in the international market. The Index of Composed Price, calculated for the ICO, is a weighed mean of four groups of coffees: Soft Colombians (15 percent), other Soft ones (30 percent), Natural Brazilians (20 percent) and Robust (35 percent).

[13]In accordance with Leibtag et al. (2007), the three bigger companies in the United States adopt one politics of prices coordinated, not following brusque alterations in the prices of the raw material. This means that an

Figura 17, presents the evolution of the prices of the green coffee (paid prices to the producer) and retail in the market of the United States. From 1997 to 1999, when the paid prices to the producers had increased due the climatic problems in the producing regions, the prices in the retail had gone up in lesser ratio. In compensation, in the period of low price, the margin of the retail had raised. Considering that, with the growth of the production of coffee in different regions of the world, the companies can acquire coffees of some origins, these can be used the raw material of lesser value at the moment of the purchase. This is possible because the harvests occur in distinct periods.

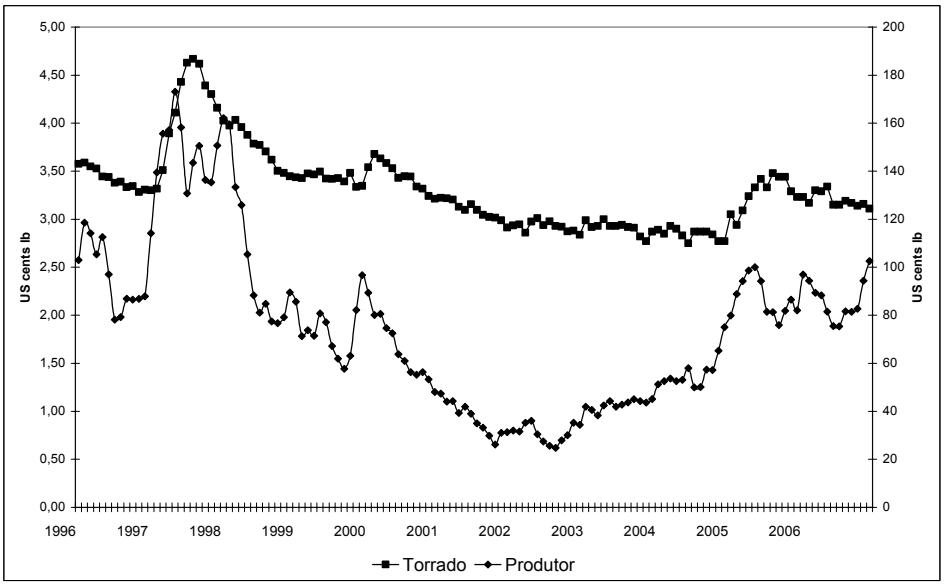

Figure 17. Paid prices to the Brazilian producer of Arabica coffee and torrado in the retail U.S.A. (US cents for lb). Source: OIC (2008).

Recent studies on the Coffee SAG

It has a great expansion of efforts of private regulation in the coffee sector. The five bigger certifications of third part of this are Organic, Fair Trade, Rainforest Alliance, Utz Kapeh and initiatives of coffee shading. All they with structures of governance, ambient, social standards and proper positions of marketing (Raynolds et al., 2007).

With the trend each time bigger of differentiation of the conventional coffee, the willingness to pay of the consumers for coffees special increases. In the case of organic coffee this differential of price can arrive 53.80% in conventional retail establishment or 96.20% in establishments specialized in organic (Cunha, 2006).

The same can be seen for American consumers interviewed how much was its willingness to pay (WTP) for different types of special coffees (Figure 18). It is observed that the coffees Fair Trade are more valued by these consumers, being the average WTP 21.64 cents of pounds for this type of coffee, in detriment the 20.02 cents for the organic coffee and 16.25 to shading on a price of US$ 6.50 for pound to the conventional one (Loureiro and Lotade, 2005).

increase of 10 percent in the price of the green coffee corresponds to an increment of only 3 percent in the prices of the retail.

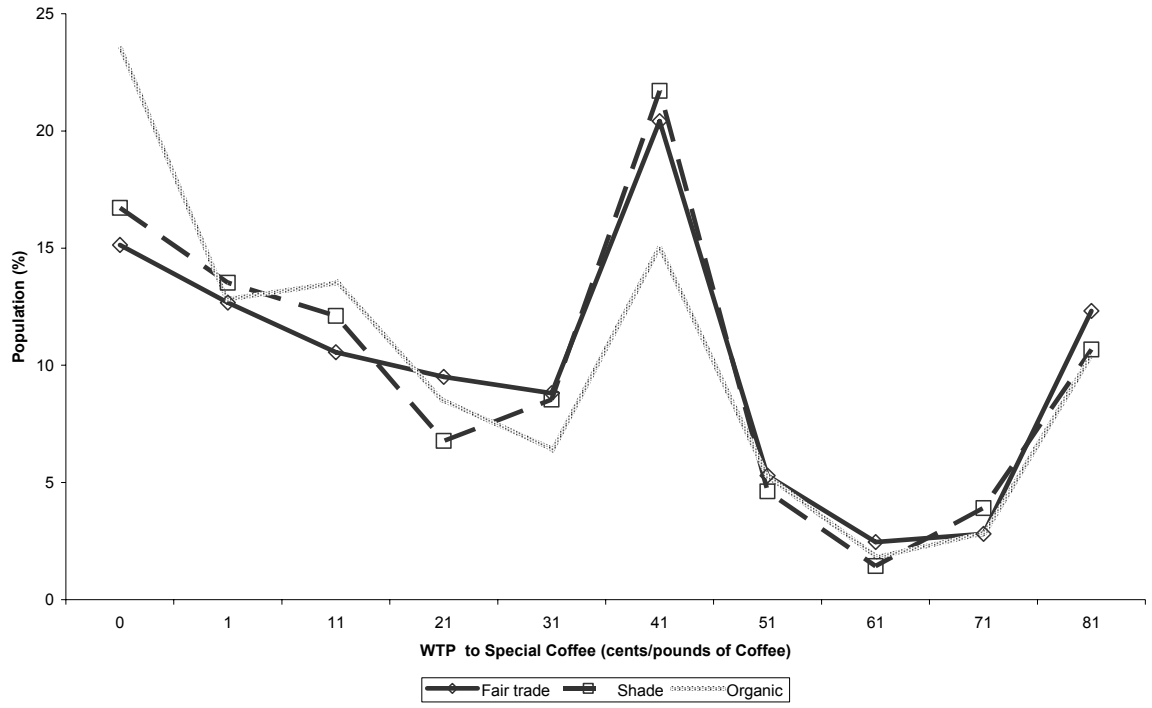

Figure 18. Distribution of the Willingness to Pay (WTP) of American consumers for different types of special coffees. Source: Loureiro and Lotade (2005).

This premium also can be observed to the English consumers, once these has an intention give a premium of more than 11% add to the price to the coffees that possess "green" characteristics (Galarraga and Markandya, 2004).

In the case of the Canadian consumers it is observed that the Fair Trade coffee presented an elasticity of demand of -0.42 (Arnot et al., 2006).

For the producer's side of special coffees it also has benefits, because for the producers of Fair Trade coffee it has one better distribution of the market benefits and a guarantee of minimum price of the product, which can arrive until the double of the received one for the conventional (Taylor, 2005).

With the increase of the requirement of quality for the coffee by the final consumers the sector of special coffees is using more contracts of long run, thus to identify the sources of the rank material, a way to deliver demanded quality (Bitzer et al., 2008).

These new requirements normally mean a set new of standards of quality, rastreability and environment, involving a bigger degree of coordination, rastreability and monitoring between different agents in the chain of this commodity. Codes, certifications and labels are all tools to codify the information and to increase the confidence of the consumer. These reduce the cost of monitoring for the purchaser and make possible that the suppliers demonstrate its abilities and standards of production (Muradian and Pelupessy, 2005).

Each time more multinationals companies, that already have tradition or is just new in the sector of the coffee, are focusing in activities and initiatives that objectify a supply of coffee of better quality and payment of bigger prices for the producers. For example Starbucks and the Procter and Gamble (P&G), companies where this niche of market adjusts with its strategies, are buying increasing amounts of coffee Fair Trade. Bigger Companies such as Kraft and Sara Lee/Douwe Egberts (SLDE) already had initiated the purchase of some coffees

certified, paying a prize for coffees of high-quality and activities of certification, while Nestlé, which has production in developing countries, directly looks for coffee in farms in a considerably high price if compared with that these would receive from another form (Kolk, 2005).

Strictly Coordinated Food System:

Beyond the description of the agro-industrial system (SAG) of the conventional and generic coffee (Figure 16), it is important to know that several Strictly Coordinated Food System (sub-SAG) exists (Zylbersztajn and Farina, 1999) inside of this structure. An example of this Sub-SAG is the Strictly Coordinated Food System of the organic coffee, that is presented in Figure 19 below.

In Figure 20 a generic structure for the special coffee is elaborated, which is integrated in the SAG of the generic coffee (Figure 21).

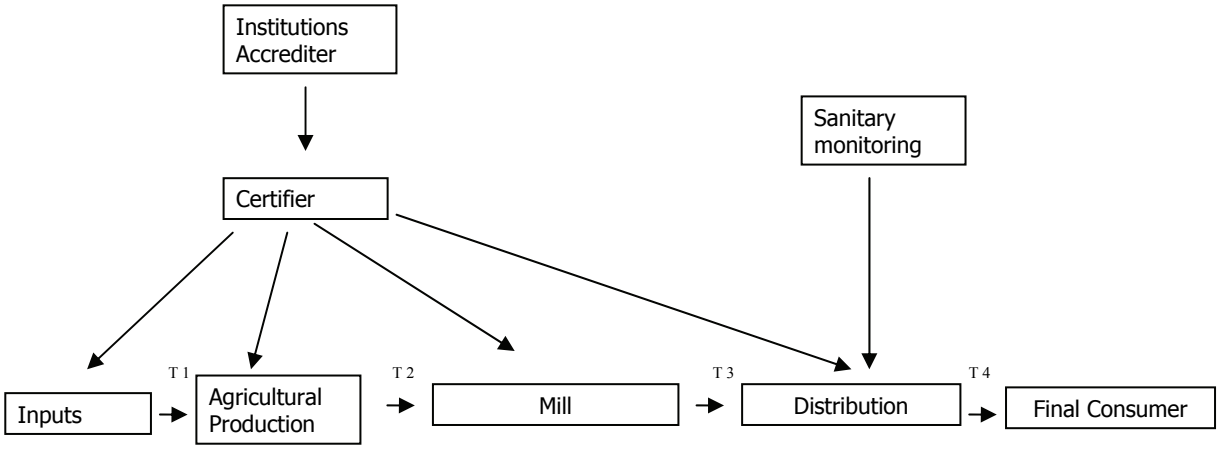

Figure 19. Strictly Coordinated Food System to Organic Coffee. Source: Cunha, 2006.

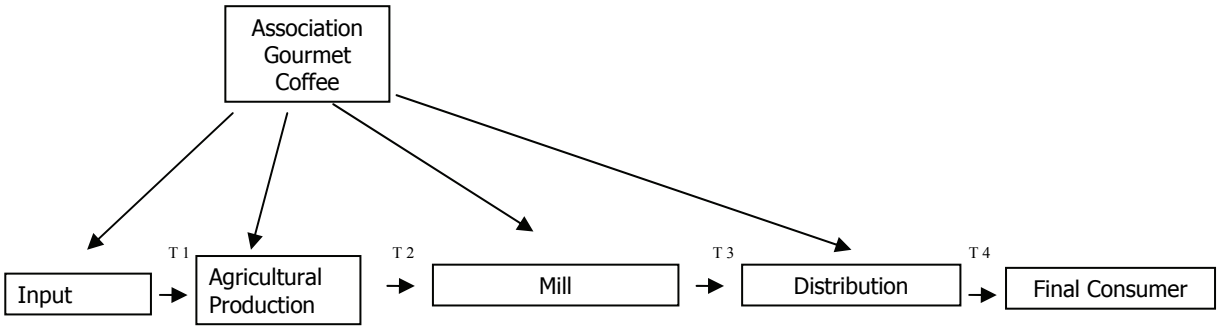

Figure 20. Strictly Coordinated Food System to Gourmet Coffee. Source: Authors.

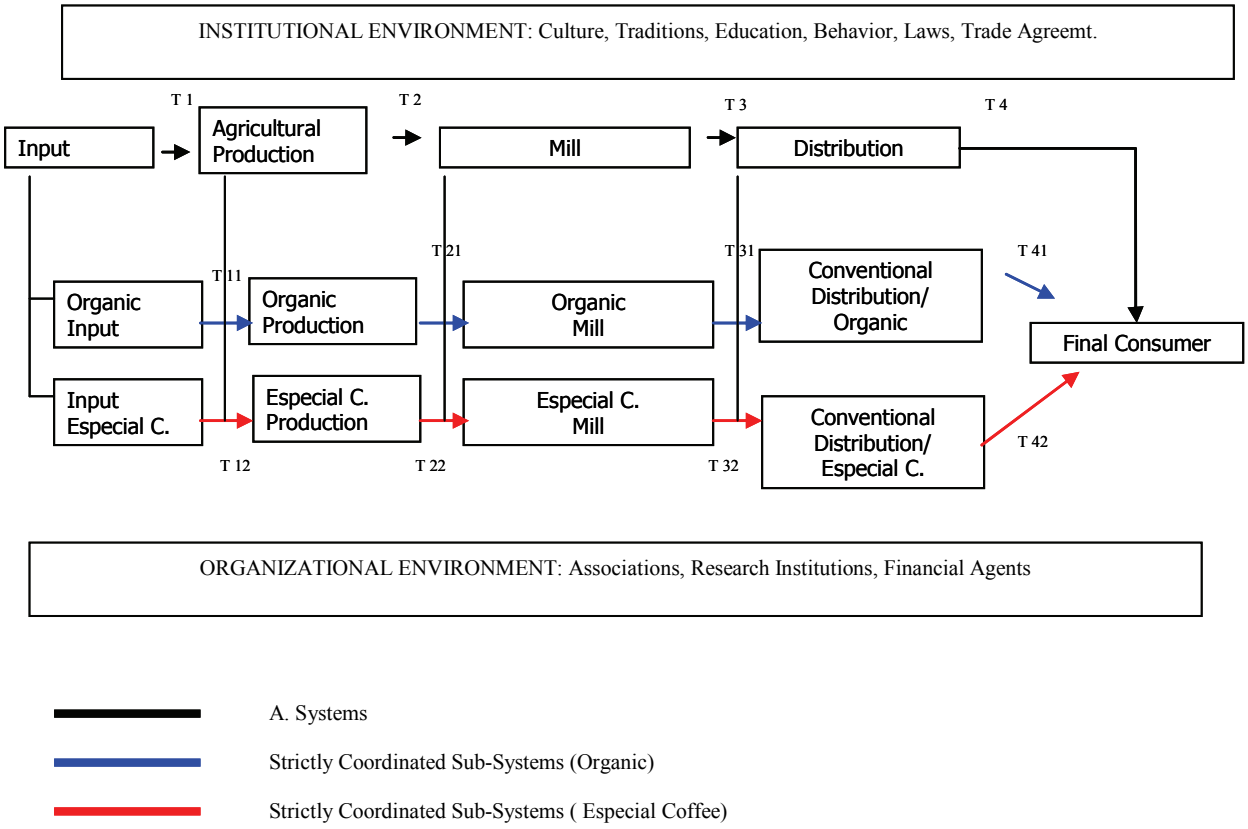

Figure 21. Generic SAG of Coffee and its Foods Systems. Source: Authors.

CONCLUSION

The market of coffees no more can be explained by means of typical instruments of market. The price pure system is just efficient to coordinate the productive chain of coffees of the commodity coffee. As this product is becoming no more a commodity, introducing new practical demanded by the markets such as: rastreability, sustainability, Fair Trade, among others, the market mechanisms become insufficient, being necessary the adoption of contractual mechanisms.

Such comment is coherent with the theory that suggests that the market mechanism has a cost to work, and such cost increases when investments in specific assets are become fulfilled.

It fits to detach, like conclusion, that the generation of value in the agro-industrial system of the coffee demands adaptations of all the involved actors, from the agricultural producer to the supermarket. Such adaptations means costs that must be covered by the value received. The environmental adequacy and the adoption of the practice of certification represent the internalization of these costs. In case that the observed specific arrangements are undo, the producers will lose, because the product will be equalized to the commodity coffee.

Generation of value comes be followed to increase of costs. The problem does not stop in this point, so to the new observed productive arrangements persist in the time, distributive mechanisms must be generated. That is, the systems strict coordinated food system of coffees special create value and distribute value between the agents who cooperate in the production.

Strategies that fail in the value generation, introducing attributes that the consumers do not give any premium. Or, if they fail in the distribution of the generated value, what includes mechanism of sharing of risks, its will be candidates to failure.

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Coffee Gives Birth to Art: Portinari, from the Coffee Plantation to the United Nations*

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SUMMARY

This presentation addresses the work and life of the Brazilian painter Candido Portinari (1903-1962), deeply rooted in the coffee plantation. It also aims at sheding some light on a mistery: how this humble coffee boy turns out to become one of the most revered painters of his times, whose work shines brilliantly all over Brazil and abroad, culminating with the monumental murals "War" and "Peace" that ennoble the main entrance hall of the United Nations General Assembly Room.

INTRODUCTION

Candido Portinari (1903-1962) was born and grew up on the vast coffee plantations of Brodowski, in the State of Sao Paulo. He was the second of the twelve children of Baptista and Domenica Portinari, who came to Brazil during the great wave of Italian immigration which, between 1875 and 1900 alone, brought one million immigrants to Brazil.

Portinari's biographer, the Brazilian writer Antonio Callado, once said that Portinari *"laboured in his paintings' plantation as his parents laboured in the coffee fields: from sun to sun..."*.

The great Brazilian poet Carlos Drummond de Andrade, in his poem "The Hand", recalls the painter's first encounter with Art:

...Between the coffee plantation and the dream the kid paints
a golden star on the chapel wall,
and nothing else will resist that painting hand.

Figure 1.

Throughout his life Portinari never forgot his coffee roots, and this theme was the subject of more than 50 of his paintings. Although he left the small village of Brodowski when he was only 15, his childhood, his family and countrymen were always on his mind, and he portrayed them unrelentlessly up to his last day.

In this presentation we show Portinari's work and his rise to the glory of becoming the painter that inspired the Louvre Chief-Curator Rene Huyghe to declare (1946):

...Portinari is one of the greatest painters of our time. His strength is tremendous.
The morning I saw his paintings, I had such an emotional shock that
I left the Galerie Charpentier suffering from a veritable case of nervous exhaustion.
All afternoon I was unable to work. I felt so tired.

THE PORTINARI PROJECT

The Portinari Project is dedicated to preserving the painter's memory, serving as guardian of the collection of documents related to his work, life and times. At the same time, the Project endeavors to make this content available to the widest possible audience, in Brazil and abroad. It also aims to use its collection to encourage activities that stem from the social and human values that pervade Portinari's work, transforming them into instruments for building a more humane, just and fraternal world.

The Portinari Project was founded in 1979 in the midst of sweeping social changes in Brazil. After fifteen years of military rule, the country was awaking to political freedom: amnesty, easing of censorship and the promise of elections.

Many initiatives dedicated to reviving cultural identity sprang up, among them the Portinari Project. Seventeen years after the death of Candido Portinari (1903-1962), his works had never been cataloged; books addressing his work, life and era were out of print; no one knew the whereabouts of the majority of his works; and his name was fading from memory. At that time, *O Globo* newspaper published an article with the headline *Portinari, o Pintor. Um*

Famoso Desconhecido (Portinari, the Painter. Famous but Unknown). Reporter Elias Fajardo da Fonseca inquired: *Over 95% of the works of Brazil's greatest painter are hidden from public sight. What happened to the work of a man who throughout his entire life gave emotional expression to the Brazilian soul, life and people?*

The Portinari Project was a response to this question.

Initially the Portinari Project sought to locate, catalog and research the painter's oeuvre and offer this content in a comprehensive socio-cultural action that would contribute to a better understanding of Brazil's historical and cultural development. The Portinari Project searched not only for the works themselves, but also for documents related to the painter's work, life and era: clippings from periodicals, letters, interviews, period photographs, films and recordings, books, monographs, assorted texts etc.

Born in the scientific community of mathematicians, physicists, computer scientists, engineers, etc. at the Pontifical Catholic University of Rio de Janeiro (PUC-Rio), the Portinari Project has incorporated science and technology into its daily activities since the beginning. The Project has consistently sought to adapt and create multidisciplinary methodologies, procedures and techniques that would not only serve its own objectives, but also assist other researchers and institutions involved in similar projects. Since its creation in 1979, the Portinari Project has cataloged over 5,400 paintings, drawings and engravings attributed to the painter and over 30 thousand documents related to his work, life and times. The Oral History Program alone recorded 74 interviews, a total of 130 hours recorded, with figures such as Carlos Drummond de Andrade, Oscar Niemeyer, Lucio Costa, Luis Carlos Prestes, Afonso Arinos, Raul Bopp, Antonio Callado, Clarival do Prado Valladares, Celso Antonio, Radames Gnatalli, Francisco Mignone, Jose Olympio, Carlos Scliar, Maria Clara Machado, Alfredo Ceschiatti, Enrico Bianco, Augusto Rodrigues, Jose Paulo Moreira da Fonseca and Pietro Maria Bardi. These testimonies along with those of the painter's friends and family have enriched our understanding of the artist and his generation.

The Project's documental archive comprises over 9,000 pieces of correspondence and more than 12,000 clippings from periodicals, 1,200 historical photographs, films, books, monographs, texts and assorted *memorabilia,* together forming a detailed roadmap to four very important decades of Brazilian culture. Today, the Portinari Project's collection is one of the most important multimedia archives on Brazilian history and culture from the 1920s to the 1960s. It includes texts, still and moving images (both color and black-and-white) and audio (oral history and music programs), as well as film and video footage. It lends itself to a full gamut of applications, from research in the arts and social and human sciences to the creation of cultural resources for everyone from primary school students to specialists. To offer this vast archive to the greatest number of people possible, the Portinari Project makes it available on its website at http://www.portinari.org.br.

In 2004, following 25 years of research, the Portinari Project published the Catalogue *Raisonne* “Candido Portinari − Complete Work”, the only one of its type in the southern hemisphere.

Figure 2. President Lula presents the *Catalogue Raisonne* to the President of France, Paris, July, 2005.

It not only received four of Brazil's most important awards, but the Brazilian President chose it as the country's official gift for state visits. In this context, President Lula presented the *Catalogue* to the Prime-Minister of Japan, the President of France, the President of Chile, the Queen of England, Pope Benedict XVI and the US Secretary of State, Condoleezza Rice.

The Effects of Coffee Consumption on Liver Function and Disease

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SUMMARY

Coffee is the second most valuable commodity in the world and is consumed by more than 50% of North Americans on a daily basis. Given its ubiquity, it is not surprising that the relationship between coffee consumption and human health and disease has received much attention. Over the last two decades numerous studies have focused on the association between coffee drinking and liver complaints extending from derangement of biochemical parameters to the development of, or mortality from hepatocellular carcinoma (hepatoma). In 1980 Arnesen *et al*[1] reported an inverse relationship between coffee consumption and serum γ-glutamyl transferase levels. Subsequently investigators across different continents have also addressed this issue, with similar results. Moreover, studies by Tanaka *et al*[2], Honjo *et al*[3] and Klatsky *et al*[4] have reported that the consumption of coffee was associated with lower alanine and aspartate aminotransferase levels, with Klatsky *et al* suggesting that the effect was maximal amongst those consuming the highest amounts of alcohol. Consumption of coffee may also afford some degree of protection from the development of liver cirrhosis and hepatocellular carcinoma. Incremental increases in daily coffee intake were shown to be associated with decreasing odds ratios for liver cirrhosis in several studies. The relationship between coffee and hepatocellular carcinoma development (and also mortality as a result of this diagnosis) has, perhaps, received the most interest in the research arena. Most groups have focused on hepatoma related to alcoholic liver disease or viral hepatitis, with a number of studies suggesting a decreased hepatoma risk with higher levels of coffee intake. Ohfuji *et al*[5] examined the relationship between HCV-related hepatoma and coffee consumption, reporting that intake of at least one cup per day significantly reduced the risk of hepatocellular carcinoma. Furthermore, Kurozawa *et al*[6] documented a significant reduction in the risk of hepatoma-related mortality associated with coffee consumption at this level. Despite the multitude of studies, meta-analyses and review articles addressing the putative hepatoprotective effects of coffee, the underlying mechanism(s) remain to be elucidated. It is possible that this difficulty may extend from the fact that coffee is a complex blend of a vast number of different chemicals, any number of which may play a role in this effect. A number of hypotheses have been proposed, however evidence exists both supporting and refuting each of these. It is evident from the wealth of literature relating to this subject, that the consumption of coffee affords some degree of hepatoprotection. However, several important questions remain to be answered, namely, the underlying mechanism(s) and the "dose" of coffee required for an individual to obtain these well described, beneficial effects.

COFFEE CONSUMPTION AND LIVER ENZYME LEVELS

When liver function tests are checked, there are two groups of tests which are of interest. The first is a group of enzymes which reflect levels of liver inflammation and includes aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT) and alkaline phosphatise (ALP). Despite being described as "liver function tests", this group provides little information about liver function. The second group give a much more

useful, albeit less than perfect assessment of how the liver is functioning. These tests of liver synthetic function include the albumin and bilirubin level together with the prothrombin time.

A number of studies have focused on the effects of coffee consumption on the level of liver enzymes. In the study carried out by Honjo *et al*, the coffee consumption of Japanese male subjects was examined. Approximately 5% of the population studied had evidence of elevated liver enzyme levels. However, when the subjects were subdivided according to their level of daily coffee consumption a clear relationship was evident with those consuming more than five cups per day having approximately a 40% reduction in the odds ratio for elevated liver enzymes compared to those not consuming coffee (1 V 0.61 (0.42 – 0.88)). A number of observers have suggested that the potential beneficial effects of coffee consumption on the liver may simply reflect the choice of those with live disease to consume less coffee in an attempt to minimise the potential side effects of coffee/caffeine. In an effort to address this, Honjo *et al* repeated their analysis, after first excluding all individuals with elevated AST/ALT levels. Their results demonstrated that, even in this group, increasing coffee consumption was associated with a significant reduction in AST and ALT enzyme levels.

Perhaps the most high profile study to examine this the relationship between coffee consumption and liver enzyme levels was published in 2005 by Ruhl and Everhart. In light of previous studies suggesting that coffee's hepatoprotective effect is most pronounced in those at the highest risk of liver disease this group only examined such individuals. Almost 6000 subjects were recruited, including those consuming excessive quantities of alcohol, those with a history of viral hepatitis, those with elevated transferring saturations or abnormal glucose status and those individuals with a high BMI and waist-to-hip ratio. Subdivision of the subjects according to their daily levels of coffee and caffeine consumption resulted in a significant trend towards a reduced odds ratio for elevated ALT activity with both increasing coffee and caffeine intake. The results may support the hypothesis that caffeine is the active component in coffee underlying this effect given that the reduction in odds ratio was greater for caffeine than observed with coffee (which also translated to a more significant p value (0.034 for coffee compared with <0.001 for caffeine intake).

In the next part of the study, the investigators subdivided the subjects according to which of the 5 risk factors for liver disease was evident and re-examine the relationship between coffee/caffeine and elevated ALT activity. Interestingly, whilst the odds ratio for elevated ALT was reduced in the case of each of the 5 risk factors and for both coffee and caffeine consumption, following adjusted, multivariate analysis the relationship between caffeine and elevated enzyme activity was only significant in the case of those individuals with elevated BMI and waist-to-hip ratio. The relationship between coffee consumption and ALT level did not achieve statistical significance for subjects in any of the 5 risk factor groups.

The results demonstrated by Honjo *et al* and Ruhl and Everhart are mirrored by a number of other studies in the literature, including those of Klatsky *et al*. This group followed a large number of subjects examining the effect of coffee drinking on the development of liver cirrhosis. However, in addition to this, at specific time points they had liver enzyme data on over 37000 cases with respect to AST and approaching 70000 cases for ALT. As in other studies, these investigators analysed the relationship between increasing daily coffee consumption and the risk of having either elevation of AST or ALT. The results supported the hepatoprotection hypothesis, demonstrating a reduction in the odds ratio for elevated AST/ALT levels with increasing daily exposure to coffee. Moreover, when the subjects were categorised according to their levels of daily alcohol consumption, the maximal reduction in odds of elevated AST/ALT with increasing coffee drinking was seen in those using the highest amounts of alcohol.

COFFEE CONSUMPTION AND CHRONIC LIVER DISEASE/CIRRHOSIS

The liver releases a number of mediators in response to insult/injury. These mediators can help in the process of regeneration of hepatocytes, which, over time and depending upon the amount/degree of damage may result in repair of liver architecture and function. The release of these mediators also leads to recruitment of inflammatory cells to the site of injury. The combination of inflammatory cell recruitment and differentiation of cells within the liver results both in increasing deposition of extracellular matrix (ECM) components together with a change in the composition of the connective tissue. As the insult continues, more connective tissue is deposited resulting in increasing degrees of fibrosis, ultimately leading to cirrhosis.

In the liver the main cell responsible for deposition of fibrotic material is the hepatic stellate cell (HSC). These cells normal reside in the space of Disse in the quiescent liver; however, they differentiate in response to the mediators released as a consequence of injury. The migration of HSCs to the site of injury results in an increase in the amount of extracellular matrix, partly due to increased expression, but also due to inhibition of ECM breakdown possibly due to upregulation of TIMPs (tissue inhibitors of metalloproteinase). This pro-fibrogenic process appears to be triggered in response to tissue growth factor β1 (TGFβ1).

As with the relationship between coffee consumption and liver enzyme levels, a number of studies have appeared in the literature of recent years focusing on the effect of coffee drinking on the development of chronic liver disease or cirrhosis. Work from Gallus *et al* has shown that those drinking coffee have a 50% reduction in their odds ratio for developing liver cirrhosis compared with those abstaining from coffee. When the level of coffee consumption was categorised according to increasing daily intake or increasing duration of coffee drinking a stepwise reduction in the odds ratio was evident with those with the greatest exposure having the maximal reduction in risk. Next, the investigators divided the subjects according to their risk factors for liver disease. In the case of BMI, as anticipated, those with the highest risk of liver disease*, i.e.,* those with an elevated BMI demonstrated a greater reduction in the risk of cirrhosis than those with a lower BMI. However, in the other two groups, namely alcohol intake and history of hepatitis, the maximal benefit of coffee consumption appeared to be derived from those with the lowest alcohol consumption or without a history of hepatitis. These groups would represent those at a lower risk of developing chronic liver disease; this would be in contrast to results from other groups, which had stated that those with the greatest risk of liver disease were more likely to derive benefit from coffee consumption.

As was the case with liver enzymes and coffee consumption, Ruhl and Everhart have also addressed this issue. This group for subjects for approaching a twenty-year period. They reported a reduction in the cumulative risk of chronic liver disease at 20 years with increasing levels of coffee drinking. Indeed those consuming more than two cups of coffee daily had almost a 40% reduction in the risk compared with those drinking less than a cup of coffee daily. The results were supported by a reduction in the hazard ratio for liver disease with increasing coffee consumption levels. Interestingly, when the cases were divided into those with the high or low risk of liver disease, only those at high risk had a reduction in the hazard ratio for liver disease with an increased level of coffee exposure; this trend achieved statistical significance, in contrast to the situation for those considered at low risk of liver disease.

In an effort to identify the active compound with coffee responsible for this effect, Ruhl and Everhart examined the relationships between a number of different coffees and caffeine containing foodstuffs and risk for developing cirrhosis. Whilst, the combined result for tea and coffee showed a significant reduction in the risk, when these were examined individually, only coffee was found to be significantly associated. A role for caffeine was suggested by

results showing no significant reduction in the risk with consumption of decaffeinated coffee, whereas increasing daily intake of caffeine was associated with a stepwise reduction in the risk of the developing chronic liver disease.

Whilst the majority of studies focused on the potential for coffee consumption to reduce the risk of developing chronic liver disease, a Norwegian group chose to focus on the relationship between the beverage on mortality from liver disease. During 17 years of follow up, the total number of deaths from all causes in the studied cohort was 4207. Fifty-three had the diagnosis of cirrhosis mentioned on the death certificate; of these, 36 had alcoholic cirrhosis. The adjusted relative risk of liver associated with an increase of two cups of coffee, was 0.6 (95% confidence interval, 0.5-0.8). For alcoholic cirrhosis the results were identical. When considering cirrhosis as the underlying cause of death a stronger relationship was evident.

COFFEE CONSUMPTION AND HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (hepatoma/HCC) is the malignant tumour of the liver parenchymal cell. It is the most common primary liver tumour and usually develops on the background of liver cirrhosis, although in up to 10% of cases the tumour may develop in a non-cirrhotic liver. Given that HCC usually develops in a cirrhotic liver, the risk factors for developing cirrhosis are also those for HCC. It should be noted that some aetiologies of cirrhosis appear to be associated with a more significant risk of developing HCC, namely viral hepatitis, alcohol and haemochromatosis. It is the eighth most commonly occurring tumour worldwide and represents the third most frequent cause of cancer death. However, there is wide variation in the prevalence of HCC, being most common in Asia and Africa and being less frequently seen in the West, although its incidence is rising in these countries.

In the last number of years, research groups have moved from studies examining the relationship between coffee and liver enzyme levels or chronic liver disease, towards those focusing on the effect of consumption of this beverage on the development of hepatocellular carcinoma. Rather than dealing with the each of these studies in turn, the two meta-analyses published in recent years with be the focus of this section.

Bravi *et al* carried out a meta-analysis of 10 studies, incorporating 2260 cases and almost 240,000 control subjects. The meta-analysis examined the risk of developing HCC amongst coffee drinkers compared with abstainers, and also the effect of an increasing levels of coffee consumption on HCC development. The analysis showed that, compared with non-drinkers, those consuming coffee had a 41% reduction in the risk of developing primary liver cancer. The analysis was then repeated after the cases were categorised according to their level of daily coffee consumption – either low/moderate or high. With respect to those drinking low or moderate levels of coffee, compared with non-drinkers there was a 30% reduction in liver cancer risk. This increased to a 55% reduction in relative risk when heavy coffee drinkers were compared with abstainers.

The second meta-analysis, carried out by Larsson and Wolk examine the same set of studies, however they focused upon the effect of an increment in coffee consumption of two cups per day. The summary relative risk for the development of hepatoma with a two cup per day increase was 0.57 (95% confidence interval 0.49-0.67). In the case of four of the studies, the initial investigators had divided the subjects according to their prior history of liver disease. Meta-analysis of these studies, again comparing a two-cup/day increment, showed a relative risk of HCC of 0.69 (0.55-0.87) amongst those with no previous history of liver disease. This compares with a value of 0.56 (0.35-0.91) for those with previous liver disease. Whilst there is a 44% reduction in the risk of HCC amongst those with prior liver disease, compared to

only a 31% reduction in the risk for those without, suggesting that those with previous liver disease (and therefore possibly a higher risk of developing HCC) might have derived more benefit from the effects of coffee consumption) this difference did not achieve statistical significance.

POTENTIAL MECHANISMS UNDERLYING THE HEPATOPROTECTIVE EFFECT OF COFFEE

It is difficult to identify exactly which component of coffee might lie behind the observed benefits of coffee drinking on liver function and disease. One reason for this is the large number of potential candidates. Many studies have an attempted to answer this question, focusing on a variety of coffee constituents, including caffeine, chlorogenic acid and the diterpenes, kahweol and cafestol (K&C).

Lee *et al* examined the effect of the diterpenes on carbon-tetrachloride (CCL_4)-induced liver damage in a murine model. Following CCL_4 treatment a huge rise in AST and ALT levels was induced, as were increased markers of lipid peroxidation (a marker of oxidative stress). However treatment with increasing concentrations of K&C resulted in reductions in both levels of transaminases and also lipid peroxidation levels. In the next part of the study, the investigators attempted to elucidate the underlying mechanism, demonstrating both induction of Phase II detoxifying enzymes and also the inhibition of the activity of the Phase I activating enzyme CYP2E1 in response to treatment of the mouse model with diterpenes.

Work by Higgins *et al* also focused on K&C as potential candidates. These investigators hypothesed that the diterpenes may exert their effect via an increase in the transcription of protective enzymes mediated through the transcription factor Nrf2. Using wild type and Nrf2-knock out models, Higgins and colleagues demonstrated increasing expression of a number of protective enzymes, including oxidoreductase, in response to coffee- and K&C- containing diets. This group also revealed that pre-treatment of the model with K&C resulted in protection against oxidative damage resulting from exposure to the toxic electrophile, acrolein.

Finally, more recent work by Gressner *et al* has suggested mechanisms whereby caffeine may protect against liver fibrosis. As already mentioned, the major cytokine behind liver fibrosis is thought to be TGFβ1. Studies have suggested that this may induce its effect via another growth factor downstream, namely connective tissue growth factor (CTGF). Indeed studies using CTGF-knock models have shown attenuated levels of liver fibrosis. Using a rat hepatocyte model this group first investigated the effects of caffeine on CTGF expression. Initial results successfully confirmed that treatment with TGFβ1 induced expression of CTGF. Subsequent studies illustrated that CTGF expression was reduced by cyclic AMP and also caffeine; moreover, co-treatment with both cAMP and caffeine almost completely abolished CTGF expression.

Gressner and co-investigators next attempted to elucidate this mechanism whereby caffeine could exert this effect on CTGF expression. Earlier studies had suggested that the proteins SMAD2 and 3, together with the PPARγ receptor may play a role in the pathway between TGFβ1 and CTGF. This group carried out further studies illustrating increased levels of SMAD2 and phosphorylated (p-) SMAD3 in response to TGFβ1 and also that caffeine treatment resulted in reduction in their levels. With respect to the PPARγ receptor, caffeine induced its expression, which subsequently allowed for an increased effect of the known PPARγ receptor inhibitory ligand, 15-PGJ_2 leading to a demonstrable reduction in levels of

CTGF expression. In summary, the results from this group show that caffeine strongly down-modulates TGFβ1 -induced CTGF expression in hepatocytes by stimulation of degradation of the TGFβ1 effector SMAD 2, inhibition of SMAD3 phosphorylation and up-regulation of the PPARγ-receptor. The authors suggested that the use of caffeine in anti-fibrotic trials in chronic liver disease might be of interest.

CONCLUSION

In recent years a number of studies have been published supporting a beneficial effect of coffee consumption on the development of a spectrum of liver diseases, ranging from elevation of liver enzymes to the development of heaptocellular carcinoma. Although the constituents of coffee that might cause these protective effects have not been definitively identified, studies have suggested a number of candidates and have begun the quest to elucidate the underlying mechanisms.

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Coffee and Its Mechanisms of Cell Protection

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SUMMARY

Coffee is one of the most popular beverages worldwide, with annual production numbers approaching 7 million tons in 2007. Consumers value its characteristic and desirable flavour and stimulating properties, as well as its typical forms of preparation and brewing. Moreover, physiological and long term health effects of coffee consumption, in the past often suspected to be disadvantageous, nowadays are subject to growing appraisal as being beneficial rather than detrimental. This notion finds support from many experimental findings, controlled human studies and epidemiological observations. Coffee represents an extremely complex mixture of many constituents, with significant amounts of chlorogenic acids and other phenolics, coffeine and the manifold compounds generated or modified during the roasting process, including low to high molecular weight browning products, and other products of the Maillard reaction. Epidemiologic evidence argues for inverse relationships of coffee consumption with risk of several chronic diseases, including some types of cancer, type II diabetes, inflammatory diseases and Alzheimer's disease. One of the hallmarks of cell protection conferred by coffee is its remarkable antioxidant acitvity. Many coffee constituents, including polyphenols, chlorogenic and other phenolic acids and melanoidins have been demonstrated to exert radical scavening and antioxidant effectiveness in experimental model systems, including a wide spectrum of human cells. Reactive oxygen species (ROS) are continuously generated from normal cellular metabolism and are formed in excess at specific situations, such as exposure to prooxidants or during inflammatory response. ROS attack lipids, proteins and DNA. This may result in cellular injury or DNA damage, the latter being fixed into mutations, if not repaired before cell division. Experimental evidence is accumulating that coffee protects cells against detrimental effects exerted by ROS as well as by certain genotoxic carcinogens. A key chromophore of the Maillard reaction was found to potently inhibit the growth of human tumor cells by interfering with the microtubule skeleton and by inhibition of a transcription factor that drives cell proliferation (Elk-1), downstream of the MAP kinase cascade. The coffee diterpenoids, cafestol and kahweol, were shown to prevent genotoxic effects of some dietary carcinogens in human cells. The underlying mechanisms of these protective effects of coffee constituents are not yet fully understood. However, it appears that cellular defense pathways are effectively activated, in addition to direct antigenotoxic and ROS scavenging activity. This applies especially to a spectrum of commonly regulated, antioxidant-response-element (ARE-2) driven gene products, including glutathione-S-transferase (GST), NAD(P)H: quinone oxidoreductase, UDP-glucuronyltransferase, gamma glutamate cysteine ligase and hemoxigenase-1. The transcription of these ARE-driven genes is regulated, at least in part, by the transcription factor Nrf2, normally sequestered in the cytoplasm by binding to the protein Keap1. ARE activation signals induce dissociation of Nrf2 from Keap1, leading to its nuclear

translocation. Binding of Nrf2 to ARE sites in cognate promoter regions triggers coordinate upregulation of cellular detoxification enzymes. In support of this perception, numerous studies have shown Nrf2 to protect many cell types and tissues, supposedly by upregulating ARE driven genes cooperatively with cell type-specific genes to ascertain effective detoxication throughout the organism and its tissues. Accordingly, human intervention studies have shown induction of detoxifying enzymes such as GST and protection of human lymphocytes from genotoxins (ex-vivo) by consumption of coffee.

INTRODUCTION

Coffee is one of the most popular beverages worldwide, with an annual production number approaching 7 million tons in 2007 (FAS USDA, 2008). Consumers value its characteristic and desirable flavour and stimulating properties as well as its typical forms of preparation and brewing.

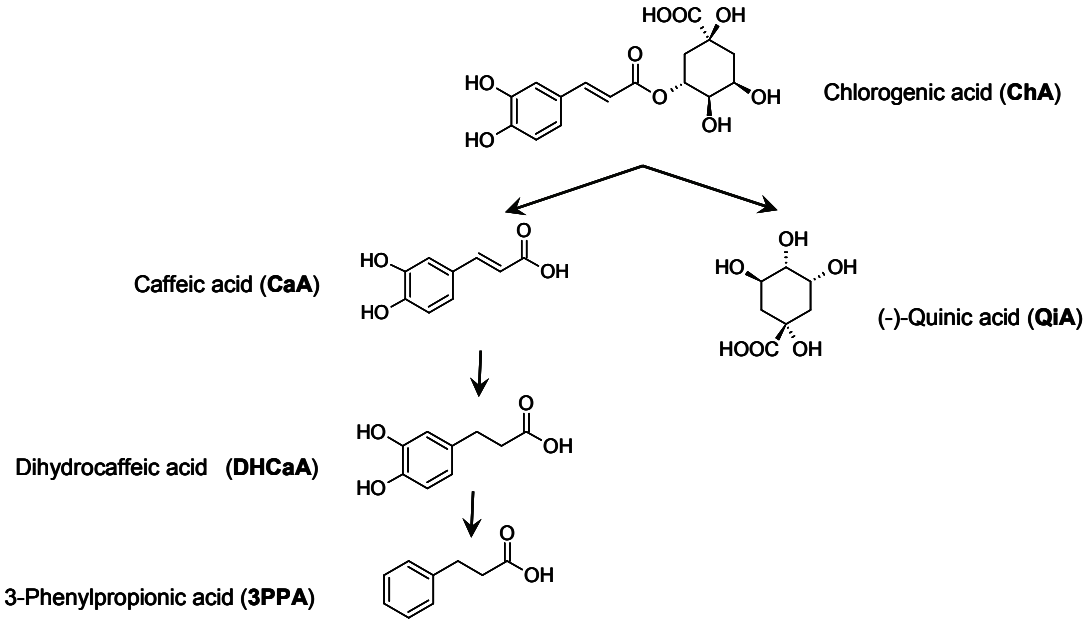
Moreover, physiological and long term health effects of coffee consumption, in the past often suspected to be disadvantageous, nowadays are subject to growing appraisal as being beneficial rather than detrimental. This notion finds support from many experimental findings and controlled human studies. Epidemiological evidence argue for inverse relationship of coffee consumption with the risk of several chronic diseases (Ranheim and Halvorsen, 2005), including some types of cancer (Oba et al., 2006; Ganmaa et al., 2008; Shimazu et al., 2005), type II diabetes (Bidel et al., 2008; Lopez-Garcia et al., 2006; Atanasov et al., 2006), inflammatory diseases (Lopez-Garcia et al., 2006; Andersen et al., 2006) and Alzheimer`s disease (Barranco Quitana et al., 2007).

Roasted coffee as well as coffee beverages represent a complex mixture of many constituents of potential biological activity. Groups of compounds, already under focus for being beneficial to human health, are plant derived phenolics, including chlorogenic acids, the diterpenoids cafestol and kahweol and compounds formed during the roasting process, including high- and low molecular weight browning products and the *N*-methylpyridinium ion (NMP) (Figure 1).

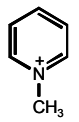
ANTIOXIDANT ACTIVITY

One of the hallmarks of cell protection conferred by coffee is its remarkable antioxidant activity, preventing cells from oxidative stress by scavenging reactive oxidant species (ROS). Many diseases are associated with an imbalanced oxidative status like arteriosclerosis, diabetes and cancer. ROS are continuously generated during cellular metabolism, predominantly during cytochrome P450 myeloperoxidase, xanthine oxidase, prosta-glandinsynthetase, oxidase and flavoprotein mediated reactions and as a consequence of inflammatory response (Halliwell and Gutheridge, 1999).

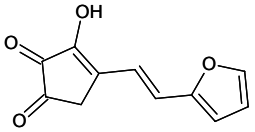
Chlorogenic acid and degradation products (adapted from [13])



Maillard reaction products

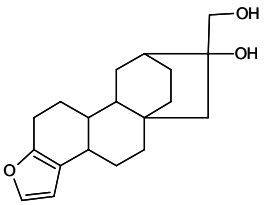


N-Methylpyridinium ion

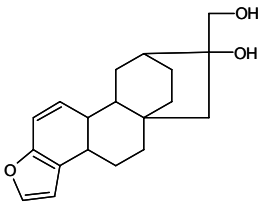


3-hydroxy-4-[(E)-(2-furyl)methylidene]methyl-3-cyclopenten-1,2-dione
(1)

Diterpenes



Cafestol



Kahweol

Figure 1. Coffee related stuctures with biological activity.

One of the most relevant reactive oxygen species formed in biological systems is the superoxide radical anion which is detoxified by superoxide dismutase and catalase via formation of dihydrogenperoxide to form water and oxygen. The highly reactive superoxide radical anion is formed via the so called "Fenton-"and "Haber-Weiss" reactions in the presence of Fe^{2+}. ROS are able to oxidize biomolecules like proteins, lipids and the DNA. Malondialdehyde, formed during lipid peroxidation, proteincarbonyls and 8-oxo-guanine,

isolated from DNA, are used as biomarkers for oxidative damage in biological systems (Sies, 1985; Kelly et al., 1998). The unstable 8-oxo-guanine is further degraded to formamidopyrimidine, a substitute recognized by the DNA repair enzyme formamidopyrimidine-DNA-glycosylase. ROS induced DNA damage -if not repaired before cell division- will be fixed into a mutation.

Direct antioxidant activity of coffee has been demonstrated in different model systems (Daglia et al., 2000; Del Castillo et al., 2005; Delgardo-Andrade et al., 2005). Coffee has been found to have higher antioxidative activity as compared to cocoa and tea (Richelle et al., 2001). This antioxidant activity is at least in part correlated to the amount of chlorogenic acids remaining in coffee beans after roasting (Borrelli et al., 2002). Chlorogenic acids are the main components of the phenolic fraction of coffee beans (Farah and Donangelo, 2006). As reported, dark roasting of coffee resulting in the formation of high molecular mass compounds lowers total antioxidant activity (Charurin et al., 2002), but also melanoidins have significant antioxidant activity (Delgardo-Andrade et al., 2005; Farah and Donangelo, 2006; Del Castillo et al., 2002).

Chlorogenic acid (ChA, 5-caffeoylquinic acid), caffeic acid (CaA) and some of their degradation products show significant antioxidant capacity (Table 1) as measured by the TEAC-test (Bellion et al., 2008; Schaefer et al., 2006). Similar results were obtained with other (Gómez-Ruiz et al., 2007). I*n vitro* studies in mammalian cells confirm these findings. In Caco-2 coloncarcinoma cells, ChA, CaA, quinic acid (QiA) and Dihydrocaffeic Acid (DHCaA) are able to reduce intracellular ROS-levels at concentrations in the µM-range as measured by the dichlorofluorescein (DCF) assay (Figure 2) (Bellion et al., 2008; Schaefer et al., 2006). In proband studies, coffee consumption enhanced plasma antioxidant capacity (Natella et al., 2002). With CaA, oxidative DNA damage induced by the redox cycler menadione in Caco-2 cells has been reduced at 1-30 µM concentration (Schaefer et al., 2006).

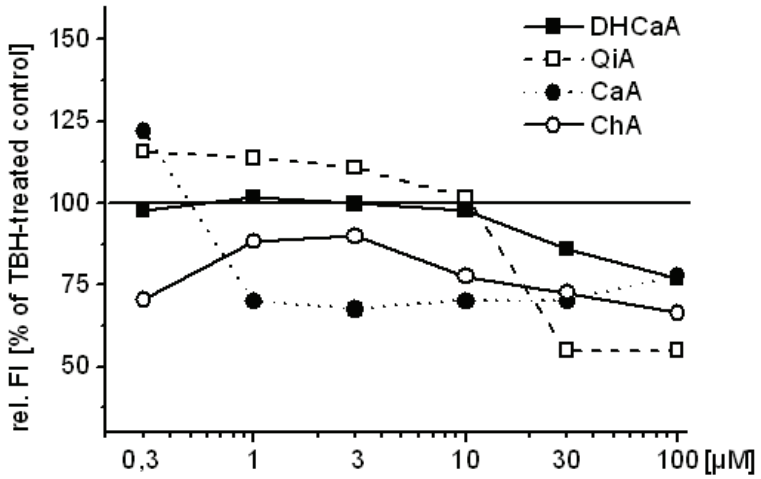


Figure 2: Modulation of tert-butylhydroperoxide (TBH) induced ROS-levels in Caco-2 cells after incubation with coffee constituents and degradation products, measured with DCF-Assay as described in Bellion et al. (2008) and Schaefer et al. (2006). Briefly, cells were treated with antioxidants for 24 h in 96 well plates. After 30 min incubation with DCF-FA, TBH treatment was performed for 30 min while fluorescence was measured at 485/528nm (ex/em). Fluorescence increase (FI) was calculated as difference between the measurements at t = 0 and 30 min normalized to the value for t = 0 min.

Table 1. Antioxidant capacitiy of some coffee constituents and degradation products as measured by TEAC-Test (adapted from Bellion et al., 2008).

Coffee ingredients / Degradation products	TEAC [mM Trolox]
ChA	1.1 ± 0.2
QiA	0.0 ± 0.0
CaA	1.2 ± 0.2
DHCaA	1.2 ± 0.2
3PPA	0.0 ± 0.0

Briefly, decolorization of ABTS$^{\bullet}$-radical by antioxidants was measured after 6 min incubation at 734nm. TEAC values were calculated as quotient between the slopes for antioxidants vs. Trolox decolorization. Chlorogenic Acid (ChA), (-)-Quinic Acid (QiA), Caffeic Acid (CaA), Dihydrocaffeic acid (DHCaA), 3-Phenylpropionic Acid (3PPA).

INHIBITION OF TUMOR CELL GROWTH

Maillard reaction products, characterizing colour and flavour of coffee are formed during the roasting process. Selected compounds, including key chromophores were tested for their ability to modulate tumour cell growth *in vitro* in the large cell lung carcinoma cell line LXFL 529 L. From all compounds tested, 3-hydroxy-4-[(E)-(2-furyl)methylidene]methyl-3-cyclopenten-1,2-dione (1), a key chromophore of the Maillard reaction had the highest growth inhibitory activity (Marko et al., 2003). (1) was also tested in cell lines originating from the gastrointestinal tract as target tissues for coffee related compounds. In the gastric carcinoma cell line GXF251L and the colorectal carcinoma cells CXF94L (1) appeared to have similar or higher growth inhibitory activity as compared to the lung carcinoma cells. As potential mechanisms behind these growth inhibitory effects, (1) has been found to interfere with the extracellular-signal-regulated/mitogen-activated protein kinase (ERK/MAPK) pathway crucial for the regulation of cell proliferation and the induction of apoptosis (Marko et al., 2002). ERK1/2 represent key elements of this pathway, mediating activating signals to the cell nucleus. The phosphorylation of ERK1/2, as a measure for their activity, was potently suppressed by the incubation of cells with (1) (Figure 3).

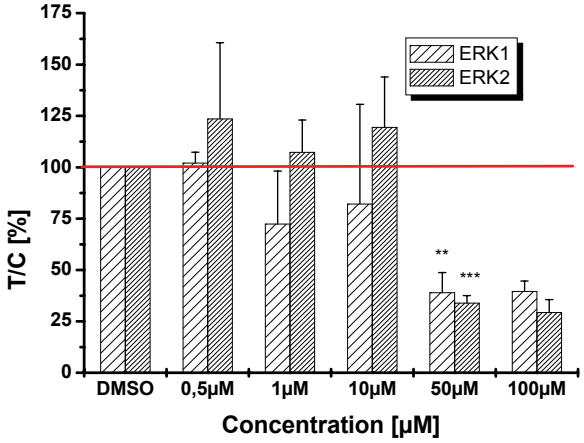


Figure 3. Phosphorylation status of ERK1/2 determined by Western blot analysis. A431 cells were incubated for 30 minutes with (1) and stimulated with epidermal growth factor (EGF) for further 15 minutes. The data were calculated as phosphorylated ERK1/2 over control cells x 100 [T/C]. Values are given as the mean ± SD of three independent experiments (Marko et al., 2002).

Furthermore, (1) has been found to potently inhibit Elk-1 phosphorylation, a potential cellular target of ERK1/2, using a luciferase based reporter gene approach. Thus, on the level of ERK1/2 effective inhibition of the MAPK cascade is achieved by (1). So far the direct target of (1) within that signaling cascade has not been identified. The upstream located epidermal growth factor receptor (EGFR) was not affected (data not shown). However, the MAPK cascade is connected with several other signalling pathways in a complex network pattern which might at least contribute to the inhibitory effect on ERK1/2 activity.

In accordance with the suppression of ERK1/2 activity, the cells accumulated in the G_1-phase of the cell cycle. After 24 h of incubation, the activation of caspase-3 activity and DNA fragmentation were observed as characteristic features for the onset of apoptosis (Marko et al., 2002). However, at higher substance concentrations, (1) was found to interfere with microtubule assembly, raising the question whether the compound possesses genotoxic and mutagenic properties e.g. by interference with nuclear spindle formation during mitosis. In a respective micronucleus assay with V79 cells no induction of micronucleus formation was observed up to a concentration of 1 µM for 24h. At higher substance concentrations a substantial decrease in the cell division rate and loss of cell viability prohibited further testing. In the hprt-assay (V79 cells) (1) did not affect the mutation rate in the *hprt* locus up to 30 µM for 24 h. Thus, it is likely to assume that due to the effective suppression of cell proliferation and the onset of apoptosis the potential interference of (1) with microtubule assembly is not to be associated with genotoxic and mutagenic properties, at least in V79 cells, lacking substantial expression of xenobiotic metabolising systems.

In summary, so far several Maillard reaction products were identified as potent modulators of tumour cell growth *in vitro*, raising the question whether normal cells are targeted as well. Potentially genotoxic and mutagenic properties, such as the interference with microtubule assembly appear not to be of toxicological relevance at least in V79 cells, but demands further investigations with respect to cell systems competent in xenobiotic metabolism, especially in non-transformed human cells. The interference of Maillard compounds with key enzymes of cell growth associated signalling cascades might be of interest in terms of chemoprevention.

ANTIMUTAGENICITY

Preventive effectiveness towards antimutagenicity and stimulation of cellular defence mechanisms of the two coffee related diterpenes cafestol and kahweol were investigated *in vitro* and *in vivo*. The kahweol/cafestol content in coffee brews strongly depends on the way of preparation. Low contents were found in filtered coffee, while Scandinavian boiling, Turkish/Greek preparation and French press resulted in higher contents up to 8 mg/100ml beverage (Gross et al., 1997; Ranheim and Halvorsen, 2005).

In the human liver carcinoma cell line HepG2, cafestol and kahweol prevent from genotoxicity induced by two dietary carcinogens, the heterocyclic aromatic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and N-nitrosodimethylamine (Majer et al., 2005). Similar DNA protective observations by coffee were reported for N-methyl-N-nitro-N-nitrosoguanidine or gamma irradiated mouse lymphoma cells (Abraham and Stopper, 2004; Abraham et al., 2004). Chlorogenic acid seems to contribute to this antigenotoxic activity (Abraham et al., 2007). The underlying mechanisms of these protective effects are not yet fully understood. However, it appears that cellular defense pathways are effectively activated. Application of kahweol and cafestol or turkish coffee to rats resulted in an elevated O^6-methylguanine-DNA methyltransferase (MGMT) activity in the liver of the animals (Huber et al., 2003). MGMT is a DNA repair enzyme and repairs mutagenic lesions at O^6 of guanine.

A group of enzymes involved in defence mechanisms is regulated via the antioxidant responsive element (ARE) pathway and transcription factor Nrf2. These enzymes include Glutathione-S-transferase (GST), NADPH-quinone oxidoreductase-1 (NQO1), UDP-glucuronosyl-transferase (UDP-GT), γ-glutamyl cysteine ligase (γ-GCL) and heme oxygenase-1 (HO-1). Nrf2 therefore is in focus as a promising target for cancer chemoprevention (Yu and Kensler, 2005). In rats, feeding of kahweol and cafestol significantly increased glutathione-S-transferase activity in liver, kidney, lung and colon (Huber et al., 2002). The Maillard reaction product *N*-methylpyridinium ion (NMP) (Stadler et al., 2002) was identified as a biologically active compound using activity guided fractionation of coffee in combination with *in vitro*- and *in vivo* techniques. Application of coffee beverage and NMP to rats over a period of 15 days enhanced UDP-GT activity in the livers of the animals 1.4 resp. 1.7-fold (Somoza et al., 2003).

In PhIP treated animals a significant dose dependent decrease of PhIP-associated DNA adducts was observed (Huber et al., 2004). Repeated application of turkish coffee to rats enhances glutathione levels and γ-glutamyl-cysteine-synthetase (γ-GCS) as well as GST and UDP-GT activities in liver (Huber et al., 2003). These results are confirmed by studies investigating Nrf2 dependent enzyme induction on the gene level. In mice fed with 3-6% coffee in the diet for 5 days, increased levels of mRNA for NQO1, the GST isoenzyme A1, UDP-GT isoenzyme 1A6 and the catalytic subunit of γ-GCL were found. Enzyme expression was lower in Nrf2-deficient mice (Higgins et al., 2008), confirming the impact of Nrf2 in the regulation of these enzymes.

HUMAN STUDIES

Results from *in vitro* and animal studies were confirmed by human *ex vivo* studies. Blood from volunteers taken before and after coffee consumption was treated with dihydrogenperoxide or the mutagenic heterocyclic aromatic amine Trp-P-2 (Bichler et al., 2007). After coffee consumption, induction of DNA damage with H_2O_2 and Trp-P-2 was significantly lower as compared to blood cells from the same probands taken before coffee uptake. In addition, superoxide dismutase activity after coffee consumption was significantly elevated. A similar protective activity of coffee consumption was also reported in a controlled human intervention trial in lymphocytes from coffee consumers after *ex vivo* treatment with activated benzo[a]pyrene (Steinkellner et al., 2005).

Experimental findings are supported by epidemiological studies: Coffee consumption seems to be inversely associated with markers of inflammation and endothelial dysfunction, as reported by Lopez-Garcia et al. (2006) with 730 healthy and 663 type II diabetic women. A weak inverse association between caffeine containing beverages and the risk of postmenopausal breast cancer was observed in a 22-year follow up study with 86,000 female participants (5,272 breast cancer cases) between 1980 and 2002 (Oba et al., 2006). An inverse association has also been observed between coffee consumption and the risk of liver cancer in Japanese people with history of liver disease (Ganmaa et al., 2008).

CONCLUSION

Groups of coffee components found to be biologically active are polyphenols, diterpenes and Maillard reaction products. Experimental *in vitro, in vivo* and in part human studies evidenced that these components are able to protect cells from oxidative stress and protect cellular DNA against damage induced by genotoxic agents.

Besides antioxidant activity, coffee components modulate signalling pathways relevant for cell growth and cellular detoxification processes, as demonstrated *in vitro* and *in vivo*.
Future research will focus on the identification of further coffee related biologically active components. Characterization of their protective potency will also encompass their ability to modulate cellular signalling pathways. Appropriate techniques including gene expression, protein expression and enzyme activity measurements should finally provide a basis to improve coffee products in terms of health benefits.

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Caffeine and Coffee as Therapeutics Against Alzheimer's Disease

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SUMMARY

These studies utilized a transgenic mouse model for Alzheimer's Disease (AD) in well-controlled studies to determine if caffeine and/or coffee have beneficial actions to protect against or reverse AD-like cognitive impairment and AD pathology. AD mice given caffeine in their drinking water from young adulthood into older age showed protection against memory impairment and lower brain levels of the abnormal protein (β-amyloid; Aβ) thought to cause the disease. Moreover, "aged" cognitively-impaired AD mice exhibited memory restoration and lower brain β-amyloid levels following only 1-2 months of treatment. In acute studies, one oral caffeine treatment significantly reduced plasma Aβ levels. "Caffeinated" coffee provided this same beneficial effect, but not "decaffeinated" coffee, suggesting caffeine is critical to the reduction in Aβ levels. Caffeine appears to provide its disease-modifying effects through multiple mechanisms, including a direct suppression of Aβ production. These results indicate a surprising ability of moderate caffeine intake (the human equivalent of 500 mg caffeine or 5 cups of coffee per day) to protect against or treat AD in a mouse model for the disease. On the basis of these promising results, clinical trials involving caffeine and caffeinated coffee administration are in progress.

INTRODUCTION

Among the diseases associated with aging, Alzheimer's Disease (AD) has continued to bewilder researchers both in terms of identifying a definitive pathogenic mechanism and providing an effective therapeutic to prevent, slow, or reverse the disease. Over a course of 2-10 years, the disease progresses from a modest lost of short-term memory to a tragic loss of all cognitive functionality and intellect. Although multiple mechanisms of AD pathogenesis have been proposed, the "Amyloid Cascade" hypothesis is the most prevalent one, being based on brain accumulation and deposition of an abnormal 40-42 amino acid protein called β-amyloid (Aβ) to form the core of neuritic plaques, a primary pathologic feature of AD (Figure 1). In the United States, the prevalence of AD in the aging population raises from 10% of individuals between 65-74 years of age to over 50% of individuals 85 or older; 5,000,000 people in the US currently suffer from the disease. With no effective treatment and an ever-increasing number of individuals affected world-wide, AD has become a target for synthetic drug development by the pharmaceutical industry. In this regard, there are four synthetic AD drugs on the US market – however, they only address the symptoms of AD and not its cause. Three of these drugs are acetylcholinesterase inhibitors (to increase brain cholinergic transmission), while the remaining drug is a partial NMDA receptor antagonists (to suppress possible over-activity of brain glutaminergic systems).

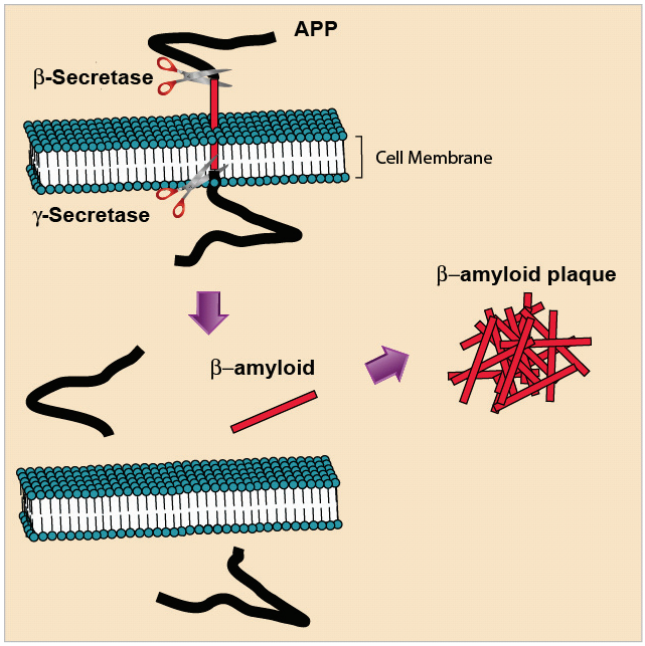

Figure 1. The production of β-amyloid (Aβ) protein from a much larger precursor protein, amyloid precursor protein (APP), which traverses the cell membrane of neurons. Two enzymes, β- and γ-secretase, are required for this abnormal cleavage of APP. Resultant Aβ then aggregates to form the core of neuritic plaques, which become heavily concentrated in cognitive areas of the brain such as the cerebral cortex and hippocampus in Alzheimer's Disease.

The premise that caffeine/coffee may provide a safe, effective, readily available, and inexpensive therapeutic against AD is underscored by a growing body of epidemiological literature. Several cross-sectional human studies have reported that caffeine/coffee consumption in both young and aged normal adults is associated with better cognitive performance (Jarvis,1993; Hameleers et al., 2000). Caffeine/coffee has been shown to improve information processing in the elderly, as well as in young adults (Lorist et al, 1995). In a 10-year prospective study involving aging men, Van Gelder et al. (2007) found that coffee consumption was linked to slower cognitive decline, particularly with modest coffee consumption of 3 cups per day (approximately 300 mg caffeine). More recently, women with higher coffee consumption over a four year period showed less cognitive decline than those consuming little or no coffee (Ritchie et al., 2007) and mid-life coffee consumption has been linked to a 65% decreased risk of AD (Eckelinen et al., 2008). Most convincing of the epidemiologic studies has been Maia and de Mendonca (2002), which reported that Alzheimer's patients consumed markedly less caffeine during the 20 years preceding diagnosis of AD compared with age-matched individuals without AD. Although these epidemiologic studies collectively suggest that chronic caffeine/coffee intake delays or reduces risk of AD, the extent to which caffeine/coffee protects against AD is problematic to assess in humans because: 1) retrospective studies are based on recall and cannot unequivocally isolate caffeine/coffee intake from other factors affecting cognition over years, and 2) controlled longitudinal studies involving caffeine/coffee administration over decades are impractical. Moreover, the potential for caffeine/coffee to treat established cases of AD has not been reported to date in the scientific literature.

The aforementioned issues concerning epidemiologic, longitudinal, and controlled studies in humans to evaluate the therapeutic potential of caffeine/coffee against AD are at least partially addressed by transgenic mouse models for AD, which have been developed over the

recent years. In these models, mutant human genes that appear to cause the genetic (familial) form of AD are inserted into mouse fertilized eggs. One such mutation is the "Swedish" mutation, having a double amino acid substitution at the β-secretase cleavage site of APP, making such cleavage much more likely and, thus, greatly increasing production of human Aβ. In the brains of these transgenic mice, the human Aβ that is produced then aggregates into the Aβ cores of neuritic plaques, as in human brains. During the process of Aβ aggregation, these "APPsw" mice become cognitively impaired in a variety of cognitive domains (Arendash et al., 2004; Arendash et al., 2004; submitted for publication), similar to human AD patients. Although these APPsw mice are only a model for the Aβ deposition of AD (and not the full pathologic complexity of the disease), they do model what is thought to be the primary disease mechanism – Aβ production and aggregation in the brain. As such, highly controlled studies can be done by utilizing these mice to develop insight into what therapeutics could protect against or treat AD in humans. This chapter provides a brief overview of the studies my collaborators and I have performed with AD transgenic mice to evaluate the potential of caffeine/coffee for both protection and treatment against AD in humans.

CAFFEINE TO PROTECT AGAINST ALZHEIMER'S DISEASE

Our initial studies were designed to determine if caffeine could protect against the development of Aβ neuropathology and cognitive impairment in APPsw transgenic mice. In those studies, which are detailed in Arendash et al. (2006), we put caffeine into the drinking water of APPsw transgenic (Tg) mice between 4 and 9 months of age. The resultant amount of caffeine intake was the human equivalent of 500 mg or 5 cups of coffee per day. Cognitive testing during the final weeks of treatment revealed surprisingly better performance of Tg mice being given caffeine compare to control Tg mice given non-caffeinated water. In caffeine-treated Tg mice, cognitive domains of spatial learning/reference memory, working memory, and recognition/identification were in fact at or near the performance level of non-transgenic control mice, which were being given water. Thus, long-term caffeine treatment protected AD mice from cognitive impairment. It is noteworthy that this cognitive protection is unlikely to involve the well-known stimulant effects of caffeine to increase alertness/focus since we have administered caffeine in drinking water life-long to normal (non-transgenic) mice and find that their cognitive performance is not improved when tested in older age (Arendash et al., submitted for publication).

Accompanying the cognitive protection provided by long-term caffeine treatment to Tg mice were significant 32-37% reductions in their hippocampal (brain) levels of Aβ compared to untreated Tg mice (Arendash et al., 2006). Importantly, the mechanism for this caffeine-induced reduction in brain Aβ involves suppression of Aβ production because both β- and γ-secretase levels were reduced in the hippocampus of caffeine-treated Tg mice (Arendash et al., 2006). Thus, caffeine can directly affect AD pathogenesis by suppressing levels of both enzymes needed for that production. *Parenthetically, there is currently no AD drug under development by the pharmaceutical industry that has caffeine's profound ability to reduce <u>both</u> Aβ-producing enzymes.* As depicted in Figure 2, "unmodified" brain Aβ production and aggregation during aging in Tg mice inevitably results in memory impairment. By contrast, long-term ("life-long") caffeine intake to Tg mice reduces brain Aβ production/aggregation, thus protecting against development of Aβ-induced memory impairment (Figure 2).

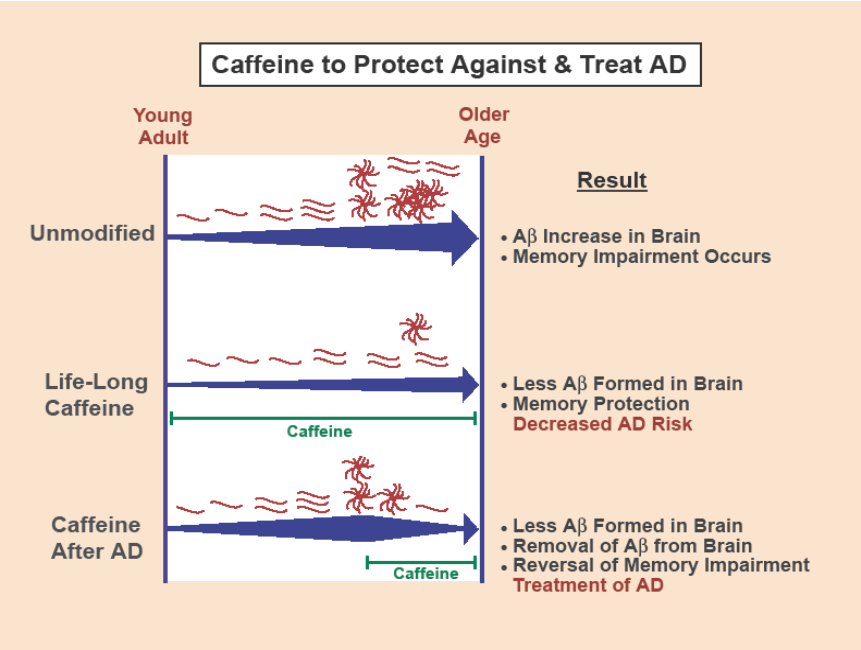

Figure 2. Protective effects of caffeine intake on Aβ production/aggregation and memory in Alzheimer's Tg mice when caffeine is started during young adulthood (life-long caffeine) or after development of AD pathology/memory impairment.

CAFFEINE TO TREAT ALZHEIMER'S DISEASE

To determine if caffeine treatment has the ability to reduce memory impairment and Aβ neuropathology, caffeine treatment in drinking water was administered for 1-2 months to "aged" Tg mice already exhibiting both of these AD characteristics. The dose was identical to that utilized in our prior protection-based study (e.g., human equivalent of 500 mg caffeine or 5 cups of coffee per day). Prior to caffeine treatment, aged Tg mice were substantially impaired in a working memory task, the radial arm water maze. Following caffeine treatment, however, these same mice exhibited a restoration of working memory to the level of normal, aged mice (Arendash et al., submitted for publication). In these same aged Tg mice, which had pre-existing and substantial brain Aβ deposition prior to caffeine treatment, reductions of 40% and above for both soluble and deposited (insoluble) brain Aβ were evident following caffeine treatment (Arendash et al., submitted for publication). This amazing caffeine-induced cognitive restoration is likely due to caffeine's aforementioned ability to reduce Aβ production by reducing both β- and γ-secretase (Arendash et al., 2006). We have further extended these mechanistic findings by showing that 1) caffeine administration to Tg mice was able to reduce the Raf-1/NFκB inflammatory pathway, which stimulates brain β-secretase, and 2) caffeine suppresses levels of GSK-3α (a stimulator of γ-secretase, in neuronal cell cultures. The effects of caffeine on established AD characteristics are depicted in Figure 2 (Caffeine after AD), which shows caffeine-induced suppression of Aβ production leading to removal of Aβ from the brain, resulting in reversal of cognitive impairment. This study, performed in aged AD mice, provides the first evidence that caffeine treatment can reverse cognitive impairment and Alzheimer's neuropathology in a model for the disease.

ACUTE EFFECTS OF CAFFEINE/COFFEE ON BLOOD AB LEVELS IN AD MICE AND HUMANS

Following formation of monomeric Aβ in the brain, this newly-formed "soluble" Aβ can either aggregate to form the core of neuritic plaques or enter the vascular system in the brain.

Therefore, blood levels of Aβ may be indicative of caffeine's therapeutic actions on brain levels of Aβ. In view of our findings that caffeine decreases Aβ production in AD transgenic mice through suppression of both β- and γ-secretase (Arendash et al., 2006), we have hypothesize that ensuing lower brain levels of soluble Aβ will result in lower plasma Aβ levels. This hypothesis is supported by the finding that Aβ is rapidly produced and cleared from the brain (Bateman et al., 2006). Moreover, following almost 2 months of caffeine administration to "aged" Tg transgenic mice (see preceding section), we have found that higher plasma caffeine levels were strongly associated with lower plasma Aβ levels in individual Tg mice ($r = -0.761$; $p = 0.011$).

In view of the above, we determined the effects of acute caffeine or coffee administration on plasma and brain Aβ levels in AD transgenic mice (Cao et al., submitted for publication). Acute (single treatment) administration of caffeine (1.5 mg/0.2 cc) or saline vehicle was given by i.p. injection or gavage to APPsw transgenic (Tg) mice in young adulthood (3 months old) or in older age (14 months old). At 3 hours following treatment, plasma Aβ levels in 3M old Tg mice were substantially reduced by 41%. Even the older 14M old Tg mice showed significant reductions in plasma Aβ following acute caffeine treatment. We have since repeated these studies in additional Tg mice, acutely administering caffeinated or de-caffeinated coffee (i.p.) instead of caffeine. Although caffeinated coffee provided the same beneficial reduction in plasma Aβ levels as caffeine, de-caffeinated coffee had no effect. Thus, caffeine is the likely coffee constituent that is critical for decreasing brain and plasma Aβ levels.

In a separate group of 3M old Tg mice, measurement of brain interstitial fluid (ISF) levels of Aβ through *in vivo* microdialysis was performed both before and following caffeine administration (1 mg, i.p.). This single caffeine treatment significantly lowered brain ISF Aβ levels by 32% at 2-3 hours post-treatment, compared to the basal ISF Aβ levels in each mouse (Cao et al., submitted for publication). Follow-up studies showed that this rapid suppression of brain Aβ levels by caffeine was not due to increased Aβ elimination from the brain, but rather was due to decreased Aβ production. These studies collectively indicate that plasma Aβ can be a viable biomarker for the efficacy of anti-Aβ therapeutics such as caffeine. It is in that context that we have embarked on acute caffeine administration studies in humans. Similar to our AD mouse studies, we find that acute oral caffeine administration (400 mg) to aged humans induces an immediate decrease in blood Aβ levels in approximately half of subjects. In both young adult and aged humans, we find that blood Aβ levels continue to be modified many hours following caffeine or coffee administration. These initial clinical studies are consistent with caffeine/coffee having an immediate and direct effect on what could be the primary pathogenic mechanism of AD. Additional clinical studies are in progress to determine more long-term effects of caffeine and coffee administration to aged humans and AD patients.

DISCUSSION

The results of our studies indicate a surprising ability of moderate caffeine intake to protect against or reverse AD-like cognitive impairment and Aβ neuropathology in an established mouse model for AD. The equivalent amount of daily caffeine intake that our studies suggest is required for human therapeutic benefit against AD is approximately 500 mg. The average US intake of caffeine is only around 150 mg/day and decreases during aging – thus, average caffeine intake appears to be significantly below levels necessary for benefits against AD. An 8 oz. cup of coffee contains 100 mg caffeine, while a similar serving of tea or soft drinks contain only 35 and 25 mg caffeine, respectively. As such, only five cups of coffee per day (a moderate consumption level) are needed to reach the 500 mg level, while many more servings of tea or soft drinks would be required. Moreover, given coffee's significant content of

antioxidants and other beneficial phytochemicals, this source of caffeine is clearly preferable over essentially any other source.

The effects of "chronic" caffeine intake on health have been reported in numerous retrospective and prospective studies. A comprehensive review of the literature (Nawrot et al., 2003) found that for healthy adults, moderate daily caffeine intake poses no adverse effects on the cardiovascular system, bone status and calcium balance, or incidence of cancer. Prospective studies have not found significant associations between coffee consumption and the risk of coronary heart disease (Myers and Basinski,1992; Kawachi et al., 1994). Contrary to public belief, caffeine intake of 500-600 mg daily does not increase the risk, frequency, or severity of cardiac arrhythmias (Myers, 1991; Frost and Vestergaard, 2005). Indeed, there is a growing list of age-related diseases wherein habitual caffeine intake of 400-600 mg (4-6 cups of coffee) daily reduces the risk. For example, strong data from a number of prospective studies show that both caffeine and coffee reduce the risk of Type 2 Diabetes Mellitus by 35-79% (Higdon and Frei,, 2006). Both retrospective and prospective studies report a reduced risk of Parkinson's disease with habitual caffeine/coffee intake (Ross et al., 2000; Hernan et al., 2002). Risk of liver cirrhosis is significantly reduced by both caffeine and coffee (Ruhl and Everhart, 2005). Thus, far from posing a threat to human health during aging, habitual caffeine/coffee intake provides a number of benefits.

Caffeine's beneficial effects against cognitive impairment and AD neuropathology probably involve first and foremost the unique ability of caffeine to suppress both enzymes needed for brain Aβ generation, namely β-secretase and γ-secretase. Nonetheless, multiple other mechanisms are probably contributing to the therapeutic actions of caffeine in AD animal models, including caffeine's ability to act as: 1) a strong anti-inflammatory agent, 2) an anti-oxidant, 3) a mitochondrial activator, and 4) a stimulator of both neuronal activity and glucose utilization. Although adenosine receptor antagonism should certainly be included in this list, caffeine's actions almost certainly do not involve simply the increase in alertness provided by such receptor blockade. Indeed, if this were a primary mechanism of caffeine's cognitive benefit, normal mice should have benefited cognitively from a lifetime of caffeine administration – but this was not the case (Arendash, et al., submitted for publication).

Finally, it should be underscored that acute effects of caffeine or coffee administration on plasma Aβ levels clearly occur in both AD mouse models and humans. This suggests not only that these mouse models are providing valuable insight into development of therapeutics against AD, but also that blood levels of Aβ could be a monitor for anti-Aβ therapeutic effects occurring in the brain, such as with caffeine. An exciting period of clinical research with caffeine and coffee against AD is on the horizon. This research may finally show that a safe and readily available natural agent – caffeine – provides the first effective therapeutic against Alzheimer's Disease.

CONCLUSIONS

These studies utilized AD transgenic mice to demonstrate that: 1) Caffeine *protects* Alzheimer's mice against memory loss through mechanisms that directly affect the disease process, 2) Caffeine *reverses* memory loss and Alzheimer's pathology in "aged" AD mice, and 3) Caffeine and/or caffeinated coffee both affect plasma levels of Aβ in AD mice and humans. Our results warrant the clinical trials in progress with caffeine and caffeinated coffee as safe, inexpensive, and effective therapeutics against Alzheimer's Disease.

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Coffee and Health in Brazil Program

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SUMMARY

Objective: Activities and actions related to disseminating the benefits of consuming coffee moderately every day for human health represent one of the most import pillars for the continuous increase of coffee consumption in Brazil. Positive communication directed to the public at large and to the medical community form the basis of the Coffee and Health Program in Brazil, and contribute to improve public awareness about coffee.
Contents: Promoting benefits of coffee consumption to human health in Brazil began in 1994, as an initiative of ABIC (Brazilian Roasters Association) to support a TV communication campaign called "Minuto do Café" ["Coffee Minute"], which had Darcy Lima, M.D. stressing the relationship between coffee and memory, concentration, and alertness to intellectual activities among other benefits. The campaign was presented in a series of four films, each of them covering a specific subject. The Coffee and Health Program was created recently, with the support of the Ministry of Agriculture and Food Supply, and includes:

- Creating the website www.cafeesaude.com.br
- Supporting the Coffee and Heart Project, developed by INCOR – Instituto do Coração, do Hospital das Clinicas da Faculdade de Medicina de São Paulo [INCOR – Heart Institute, of the University of São Paulo Medical College General Hospital]
- Preparing nine Medical Letters, addressed to 120,000 physicians and professionals of the health area, containing updated information on coffee medical and scientific researches;
- Preparing and distributing 5 million "Coffee and Health" folders, through Brazilian coffee industries, organizations, cooperatives, associations and in events;
- Educational campaigns addressed to the public at large, including the campaign carried out during the Pan American Games PAN RIO 2007, on the theme "Coffee is also Health";
- Talks and lectures for the medical community, on TV and on the Internet, through the Medical Connection network;
- Research with physicians to determine changes in coffee perception before and after the Coffee and Health Program.

Resources: Power Point presentation and films in DVD to show activities, music and other elements of the campaigns.

COFFEE AND HEALTH PROGRAM IN BRAZIL

Here, as in other countries, results of researches, surveys and scientific studies on coffee demonstrate the benefits of its daily moderate consumption to human health, and have an important role in reducing prejudice that inhibit coffee consumption. Furthermore, they position coffee as a traditional, natural and healthy drink, the consumption of which can compete with many other modern products placed on the market almost every week that appeal to healthiness and divide consumers' preference. However, the truth is that none of those products have resisted so much time and participate in such a significant way on the table of homes, restaurants and offices, as our tasty and healthy coffee.

The Coffee and Health in Brazil Program has been developed in a productive partnership between Government and private enterprise. With its actions that began in 1994, an initiative of the Brazilian Roasters Association (ABIC), the program gained strength and dimension since 2003, with the support and funds from the Ministry of Agriculture and from the other sectors that form the coffee agribusiness – from growers and their cooperatives to the soluble coffee industry, to coffee exporters and to EMBRAPA.

ABIC initiatives - 1994

In 1994, the Board of Directors of ABIC received Dr. Darcy Lima's visit, a physician and researcher, who was already carrying out researches on coffee and was looking for a form to disclose their important results, mainly referring to attention, memory, awareness improvement and to how coffee can help prevent drug and alcohol consumption, since it is an opiate antagonist.

It was then that ABIC decided to adopt a work plan that can be seen as the beginning of the program on Coffee and Health in Brazil. ABIC's objectives were to:

- Support Dr. Darcy Lima's researches
- Begin the consumer education program on coffee benefits
- Clarify press articles containing negative messages about coffee
- Produce positive communication about coffee

and to **Demystify prejudices against coffee**

NATIONAL COFFEE AND HEALTH CAMPAIGN ON TV

- A major challenge
- 4 short films - "The coffee minute"

One of the first actions was to develop an educational campaign on TV broadcasted Brazilwide during 1995. A major challenge, since the theme was still totally unknown. As part of the strategy, a series of four short films with the title "The Coffee Minute" were produced and presented by a TV star, bringing Dr. Darcy Lima's testimony on the benefits of coffee to health. This is how this campaign was developed.

The campaign was not accepted unanimously. There was a request to interrupt it filed by a physician in the *Conselho de Auto Regulamentação de Publicidade* [Brazilian Self-Regulating Advertising Board] (CONAR). Dr. Darcy Lima and ABIC answered the questions raised and

the campaign was approved and maintained. Today, the same physician who criticized the campaign at that time writes articles about coffee benefits to human health.

INFORMATIVE AND EDUCATIONAL MATERIALS – 1995/1996

- Production of folders
- Strategy – …….

Other initiatives followed after the campaign, such as the production of informative and educational printed materials, which were distributed to ABIC members.

- The strategy was to use the coffee industry national network – over 1,500 companies – to disseminate information and knowledge about coffee and health.

Marketing Integrated Program (PIM) - 2003

- Joint initiative of MAPA – CDPC – Private Sector
- Coffee and Health - a main focus of attention
- Coffee and Health Annual Budget = 10% of PIM
- US$ 300,000 / year

Coffee and Health has always been a main focus of attention. The annual budget for this theme was defined by its members as being 10 per cent of PIM's total financial resources, which represented approximately US$300,000 a year.

Integrated Marketing Program (PIM)

- Stimulating conscientious coffee consumption
- Coffee and Health
- Promoting coffee quality
- Researches

Some years after, in 2003, as the theme Coffee and Health gained more importance, a large opportunity arose to include it in the Marketing Integrated Program (PIM) of the Ministry of Agriculture, Livestock and Food Supply, through the Coffee Policy Decision-Making Council (CDPC) together with private initiative. PIM is the program that concentrates the main promotion and advertising actions of Brazilian Coffees, with initiatives in all the sectors of the agribusiness, from coffee growers to exporters and industry, with the institutional support of the Ministry of Agriculture and funds from FUNCAFE. Its coordinator is Mr. Lucas Tadeu Ferreira, who is here today. Some of the objectives of this program:

FILM – National TV and Movie Theater Network - COFFEE. The Rhythm of Brazil

The idea was to begin with efforts in the Brazilian domestic market to expand coffee consumption starting with modern, attractive messages that demonstrate some of the typical characteristics of coffee consumption, such as happiness, energy, associated to youngsters, children and senior citizens and a healthy life. The film "Coffee. The rhythm of Brazil" was aired on national TV and shown in hundreds of movie theaters. Let's see this delightful film: ….

COFFEE AND HEALTH PROGRAM

The Coffee and Health in Brazil Program, as well as the International Coffee Organization (ICO) Program, have two main directions:

- Consumer Education - Demystifying prejudice against and preconceived ideas about coffee that inhibit coffee consumption, through positive communication, disclosing the results of medical and scientific researches, clarifying myths and truths about coffee, stimulating the conscious consumption of the product.
- Knowledge dissemination and updating for the medical community, distribution updated information of progress in knowledge about the benefits of coffee consumption for human health, as well as stimulating new researches to expand this knowledge. Each one of these directions has specific actions, media and projects. We are going to show you what is being done, including using the produced films, which is much better than just listening to me.

COFFEE AND HEALTH PROGRAM

- Actions for the medical community
  * Coffee perception survey among physicians
  * Web/TV - Medical Connection
  * Newsletters for Physicians
  * Website - cafeesaude.com.br
  * Talks and events for Physicians

For the medical community, the Coffee and Health Program develops the following actions:

Medical Connection is a modern communication system using the Internet and satellite, which distributed information directly to the network accredited Universities, medical schools, hospitals and physicians' offices. Its programs were created with specialists and physicians, and sixteen programs were produced until now, with specific contents, which are constantly watched by the medical community. Let's see a brief presentation of Medical Connection on the theme Coffee and Health.

Medical Newsletters *[Cartas Médicas]* were created with the support of a committee of physicians and specialists, using the appropriate medical language for the medical community, and distributed in the whole Brazilian territory. Each copy with ___ pages contains different themes, contributing to physicians' essential information and knowledge updating. This has helped a lot to change their perception about coffee. In the papers of this event, you will find the complete set of the nine Medical Newsletters that were produced until now, and that were especially printed for those attending this Conference.

COFFEE AND HEALTH PROGRAM

- Creation of the Coffee and Health Research Nucleus – EMBRAPA
- COFFEE AND THE HEART PROJECT - EMBRAPA/INCOR

COFFEE AND HEALTH PROGRAM

- Consumer Education and Positive Communication
  * Features for TV and events

* Website - cafeesaude.com.br
* BlogSpot - coffeendhealth.blogspot.com
* Educational games for children and youngsters
* Articles and reports for the press, interviews, TV and radio (PR)
* Talks, events, Coffee Ombudsman
* COFFEE and SCHOOL MEALS, HEALTH in SCHOOLS Program
* Campaigns to inform the public at large (RIO PAN 2007)
* Coffee music theme - Coffee is also health

Chlorogenic Acids: from Coffee Plant to Human Body

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SUMMARY

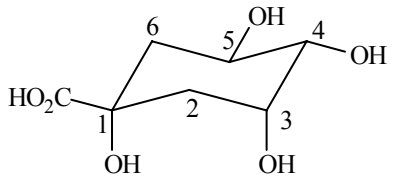
Chlorogenic acids (CGA) are the main components of the phenolic fraction of green coffee seeds, with levels commonly ranging from 6-9 g% (dry matter basis). CGA classes include caffeoylquinic acids, dicaffeoylquinic acids, feruloylquinic acids, *p*-coumaroylquinic acids, and mixed diesters, with more than 80 major and minor compounds and derivatives observed in green and roasted coffee. During roasting, CGA may be isomerized, hydrolyzed, epimerized and degraded into low molecular weight compounds. A fraction of CGA may also be transformed into quinolactones and, along with other compounds, incorporated into the backbone of melanoidins. Both CGA and their lactones play an important role in the formation of roasted coffee flavor and have a marked influence in determining cup quality. It is known that the major CGA compounds are absorbed by humans and may potentially exert a number of beneficial health properties largely explained by their antioxidant activity. Their lactones have also been studied for their normalizing effects on hyperglicemia, and potential brain functions involving the μ-opioid and adenosine receptors. In this review, the chemical characteristics of CGA, their distribution in coffee seeds, the influence of genetic, physiological, environmental factors and processing on their composition, their impact on cup quality, bioavailability in humans and potential biopharmacological properties will be briefly approached.

CHEMICAL CHARACTERISTICS OF CHLOROGENIC ACIDS

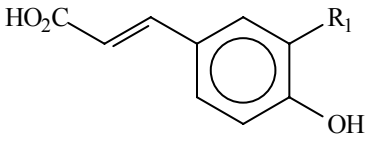
Chlorogenic acids (CGA) have been studied for more than a century as the main components of the phenolic fraction of green coffee seeds (Clifford, 1979). They include different classes of compounds formed by the esterification of one molecule of (-)-quinic acid and one to three molecules of *trans*-hydroxy-cinnamic acids (Trugo, 1984). Using the preferred IUPAC numbering system (Clifford, 1985; 2000), quinic acid (1L-1(OH), 3,4/5-tetrahydroxy-cyclohexane carboxylic acid) has axial hydroxyl groups on carbons 1 and 3, and equatorial hydroxyls on carbons 4 and 5. Esters of this acid are usually formed on carbon 5, but also on carbons 3 and 4, and less commonly on carbon 1 (Farah et al., 2005; Perrone et al., 2008). Hydroxy-cinnamic acids are *trans*-phenyl-3-propenoic acids with different substitutions in the aromatic ring. Caffeic acid (3,4-dihydroxy-cinnamic acid) is the most common hydroxy-cinnamic acid in coffee, followed by ferulic acid (3-metoxy-4-hydroxy-cinnamic acid) and *p*-coumaric acid (4-hydroxy-cinnamic acid) (Clifford, 2003). The main CGA classes found in green coffee seeds are caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), feruloylquinic acids (FQA) and *p*-coumaroylquinic acids (*p*CoQA), with three major isomers each, and caffeolyl-feruloyl-quinic acids (CFAQ), with six isomers (Clifford, 2003). These compounds are presented in Figure 1, according to their chemical identity, number and position of acyl residues.

Most recently, trace amounts of diferuloylquinic acids, dimethoxycinamoylquinic acids, caffeoyl-dimethoxycinamoylquinic acids and feruloyl-dimethoxycinamoylquinic acids were identified in *C. canephora* Robusta (Clifford et al., 2006). Other cinnamoyl conjugates found in coffee include amides with aromatic amino acids (eg. N_β –caffeoyl-L-tryptophan; N_β–*p*-coumaroyl-L-tryptophan; N_β –caffeoyl-L-tyrosine) and glycosides. Also, CGA-caffeine complexes have been described (Correia et al., 1995; Murata et al., 1995; Schrader et al., 1996; Clifford, 1997; Clifford, 1999).

A)



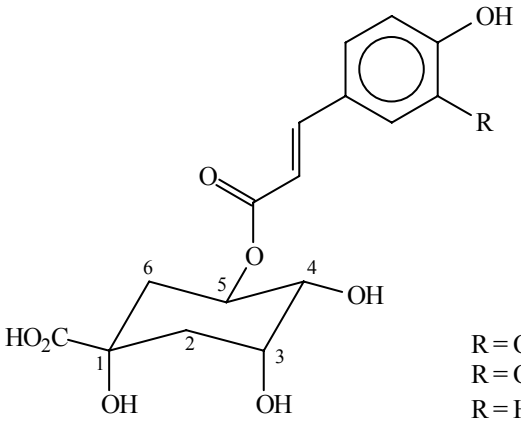
Quinic acid



$R_1 = OH$ CA
$R_1 = OCH_3$ FA
$R_1 = H$ *p*CoA

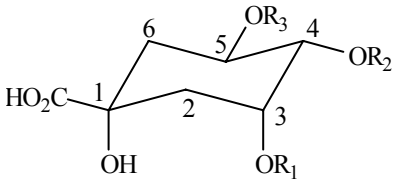
Hydroxy-cinnamic acids

B)



R = OH 5-CQA
$R = OCH_3$ 5-FQA
R = H 5-*p*CoQA

C)



$R_1 = CA, R_2 = CA, R_3 = H$ 3,4-diCQA
$R_1 = CA, R_2 = H, R_3 = CA$ 3,5-diCQA
$R_1 = H, R_2 = CA, R_3 = CA$ 4,5-diCQA

$R_1 = FA, R_2 = CA, R_3 = H$ 3F,4CQA
$R_1 = CA, R_2 = FA, R_3 = H$ 3C,4FQA
$R_1 = FA, R_2 = H, R_3 = CA$ 3F,5CQA
$R_1 = CA, R_2 = H, R_3 = FA$ 3C,5FQA
$R_1 = H, R_2 = FA, R_3 = CA$ 4F,5CQA
$R_1 = H, R_2 = CA, R_3 = FA$ 4C,5FQA

Figure 1. Chlorogenic acids and related compounds. (A) Basic compounds; (B) monoesters of quinic acid with hydroxyl-cinnamic acids (example of 5-isomers); (C) di-esters of quinic acid with caffeic acid, and mixed esters.

BIOSYNTHESIS AND RELEVANCE OF CHLOROGENIC ACIDS FOR PLANT PHYSIOLOGY

CGA are products of the phenylpropanoid pathway, one branch of the phenolic metabolism in higher plants that is induced in response to environmental stress conditions such as infection by microbial pathogens, mechanical wounding, and excessive UV or high visible light levels (Hermann, 1995; Haard and Chism, 1996). Plant phenolic acids are synthesized from phenylalanine and tyrosine via the shikimic acid pathway, which converts simple carbohydrate precursors, derived from glycolysis and the pentose phosphate shunt (phospho-enol-pyruvate and D-erythrose-4-phosphate), into aromatic amino acids. The parent *trans*-cinnamic acid is formed from L-phenylalanine by the action of phenylalanine ammonia-lyase, a key enzyme in the biosynthesis of phenolic compounds that is activated in response to different stress conditions. Quinic acid is synthesized from 3-dehydroquinate, an intermediate metabolite of the shikimic acid pathway. Hydroxy-cinnamic acids may be synthesized from cinnamic acid by hydroxylation or alternatively from tyrosine by the action of tyrosine amino-lyase (Farah and Donangelo, 2006).

The final steps of biosynthesis of the 5-monoacyl CGA (5-CQA, 5-FQA and 5-*p*-CoQA) have been studied in several plants as well as coffee, and appear to include binding of *trans*-cinnamic acid to coenzyme A (CoA) by a CoA lyase, followed by transfer to quinic acid by a cinnamoyl transferase (Gross, 1981). The origin of the CGA with acyl groups in positions 3- and 4- is unclear, although the possibility of acyl migration has been considered (Gross, 1981). Once formed, phenolic acids and CGA can be substrate of enzymes such as polyphenol oxidase (Mazzafera and Robinson, 2000) and peroxidase (Takahama, 2004), leading to polymerization products such as insoluble brown pigments and lignin that contribute to the plant defense mechanisms and to the synthesis of plant cell wall constituents.

Besides the recognition that CGA play an important role in plant stress adaptation, there are indications that these compounds may have specific physiological functions in the coffee plant, consistent with the relatively high levels of CGA usually found in coffee seeds (Clifford, 1987; Leloup, 1995). CGA synthesis in the coffee plant may contribute to the control of seed germination and cell growth, through regulation of the levels of indolacetic acid – a plant growth hormone of physiological significance during the formation and germination of the seeds – and possibly also through other still unknown mechanisms (Clifford, 1985).

CGA are found in the surface of coffee seeds, in association with the cuticular wax, and in the cytoplasm adjacent to the parenchyma cell walls of the endosperm (Clifford, 1987), but there seem to be no reports whether the distribution of CGA differs in these two locations. According to Horman and Viani (1971) and Zeller and Saleeb (1977), part of the cell wall CGA may be associated with caffeine, as a 1:1 or 2:1 molar complex. Although CGA are mostly found in the coffee seeds, they have also been found in the coffee tree leaves and in the fruit pulp (Clifford and Ramirez-Martinez, 1991).

CHLOROGENIC ACIDS CONTENTS IN GREEN COFFEE SEEDS

Despite the large distribution of some CGA in the plant kingdom, green coffee is known as one of the main food sources of CGA, with contents being equaled or exceeded only by the green leaves of *Ilex paraguariensis* (Yerba Maté) (Clifford, 1997; 1999; Clifford and Ramirez-Martinez, 1990; Mazzafera, 1997; Marques and Farah, 2008; Marques and Farah, 2009a; Marques and Farah, 2009b).

Total CGA content of green coffee seeds may vary according to genetics – species and cultivar –, degree of maturation, and, less importantly, agricultural practices, climate and soil (Clifford, 1985; Perrone et al., 2008; Guerrero et al., 2001; Camacho-Cristóbal et al., 2002; Farah et al., 2006a). The diversity of methodology employed in the analysis of CGA is another important factor in establishing levels, since there may be a certain discrepancy between results obtained by high resolution chromatographic methods and those obtained by less sophisticated ones.

In general, the values described in the literature for total CGA in regular green coffee seeds, on dry matter basis (dm), may vary from 4 to 8.4 g % for *Coffea arabica*, with 5-6 g% being a more common range, and from 7 to 14.4 g% for *Coffea canephora*, with 7-9 g% being more usual contents. Some hybrids present intermediate levels (Clifford, 1985; Farah et al., 2005; Perrone et al., 2008; Farah et al., 2006a; Ferreira et al., 1971; Roffi et al., 1971; Chassevent et al., 1973; Clifford and Wight, 1976; Rees and Theaker, 1977; Van der Stegen and Van Duijn, 1980; Trugo and Macrae, 1984a; Tono et al., 1989; Ky et al., 2001; Clifford et al. 1989). A low CGA content (1.2 g%, dm) was found in seeds of *Coffea pseudozanguebariae*, a caffeine-free species native of East Africa (Clifford and Ramirez-Martinez, 1991). Such low content has been also observed in some other low-caffeine or caffeine-free species from Africa (Clifford, 1985).

Despite the discrepancy caused by the use of different analytical methods, the large variation in CGA content and isomers distribution, along with qualitative differences (presence and absence of some isomers) make the CGA content a potential criterion on coffee genotype selection (Correia et al., 1995; Guerrero et al., 2001; Ky et al., 2001; Clifford et al., 1989; Duarte et al., 2009a). Likewise, the correlation between the patterns of minor CGA-like compounds with the geographical origin of the seeds has been attributed to genetic factors rather than to agricultural practices (Correia et al., 1995; Duarte et al., 2009a; Clifford and Jarvis, 1988). However, as stated before, small variations in CGA content of coffee seeds may be observed due to weather and agricultural practices. Severe weather conditions such as cold, high UV and visible light levels and water stress conditions tend to increase the contents of phenolic compounds not only in coffee plant and seeds but also in other plants (Douglas, 1996; Grace et al., 1998; Materska and Perucka, 2005; Pennycooke et al., 2005). The use of nitrogen-rich fertilizers (Malta et al., 2003), zinc-rich fertilizers (Perrone et al., 2009a), and situations of boron deficiency (Camacho-Cristóbal et al., 2002) have also shown to increase the content of total CGA in coffee seeds and other plants.

Table 1 presents the contents of the three main CGA classes in samples of green coffee seeds, obtained by chromatographic analytical methods. Considering the nine main isomers of CGA: 5-CQA; 4-CQA and 3-CQA; 3,5-diCQA, 4,5-diCQA and 3,4-diCQA, 5-FQA, 4-FQA and 3-FQA, in order of abundance in green coffee seeds, 5-CQA alone is responsible for about 56-62 % of total CGA. Keeping in mind that the amount of 4- isomers usually equals or slightly exceeds that of 3- isomers, 3-CQA and 4-CQA account for up to 10% each of total CGA. DiCQA isomers account for about 15-20% of total CGA in green coffee seeds and FQA isomers, for 5-13% of total CGA. *p*-CoQA isomers, CFQA isomers and the recently identified diferuloylquinic acids and dimetoxycinnamoylquinic acid derivatives account together for the remaining percentage (Clifford, 1985; Farah et al., 2005; Clifford, 2003; Cliffordet al., 2006; Schraderet al., 1996; Farah et al., 2006a; Clifford and Wight, 1976; Trugo and Macrae, 1984a; Ky et al., 1997; Farah et al., 2001; Farah, 2004).

Small amounts of caffeic, ferulic, *p*-coumaric and quinic acids may be found in green coffee seeds in their free form (Clifford, 1985; 1987). Despite the reports in the occurrence of complexes other than CGA-caffeine, such as caffeoyl-tryptophan (Murata et al., 1995;

Schrader et al., 1996), *p*-coumaroyl-tryptophan (Murata et al., 1995; Clifford, 1997), and caffeoyl-tyrosine (Correia et al., 1995; Clifford, 1997) quantitative data on these complexes are not available. Even though CGA lactones and volatile phenols are mostly produced during roasting of the seeds (see below), the presence of trace amounts of some lactones, attributed to primary processing (for example, drying of the seeds) and small amounts of free volatile phenols have been reported in raw coffee seeds (Farah et al., 2005; Perrone et al., 2008; Schrader et al., 1996; Toci and Farah, 2008). CGA-protein complexes have also been identified in immature coffee seeds (Griffin and Stonier, 1975).

CGA composition of coffee seeds varies considerably during fruit maturation. Allowing for variations between species and cultivars (Ohiokpehai et al., 1982), a sigmoidal increase in total CQA is initially observed, mostly in parallel with the total dry matter gain (Clifford and Kasi, 1987). The ratio CQA/diCQA appears to increase with maturation until ripeness of the fruit (Clifford and Kasi, 1987; Menezes, 1994a), probably due to hydrolysis of diCQA into mono-esters. At a certain stage before ripeness, CQA content starts to drop, according to Montavón et al. (2003), due to oxidation. From this point on, an inverse association between the levels of CQA and coffee fruits maturation is observed (Menezes, 1994b). Farah et al. (2007) reported a reduction not only in CQA levels, but also in FQA and diCQA levels, at this last stage of maturation. The authors reported a total of 8.7 g % of CGA (dm) for immature *C. arabica* seeds (from dark green fruits), while seeds of over-ripe fruits presented levels as low as 1.3 g %. These results were recently confirmed by Toci and Farah (unpublished data), using three additional sets of defective green, ripe and over-ripe fruits. Montavón et al. (2003) suggested that unripe seeds are more sensitive towards oxidation than ripe seeds and that the lower sensitivity of ripe seeds occurs because the defense mechanisms against oxidative stress become more efficient during maturation. Alternatively, according to the same authors, mature seeds may contain lower polyphenol oxidase and peroxidase activities than immature seeds. This hypothesis is in agreement with the reduction in polyphenol oxidase activity observed at later stages of maturation (Mazzafera, and Robinson, 2000; Arcila-Pulgarin and Valencia-Aristizabal, 1975).

CHANGES IN CHLOROGENIC ACIDS COMPOSITION OF COFFEE SEEDS DURING PROCESSING

Dry and wet post-harvesting processing

Comparing both post-harvesting methods, wet processed seeds tend to present a slightly but significantly higher content of CGA (Balyaya and Clifford, 1995; Leloup et al., 2004; Duarte et al., 2009b). This is actually a relative increase caused mostly by to the loss of other compounds with higher solubility in water during soaking of the seeds (wet method) and also due to a possible loss of CGA during sun drying in dry processed seeds. Semi-dry or naturally processed seeds should contain intermediate values, but more similar to dry method, since here the soaking step of the wet method does not occur. The variations in the seeds chemical composition will depend on the variations in the methods such as soaking period, sun intensity, humidity and duration of the exposure of seeds surfaces to sun heat.

Decaffeination

Moreira et al. (2005) measured the contents of CGA in ground and instant, light and dark roasted, regular and decaffeinated Brazilian commercial coffee samples, and observed lower CGA contents in all decaffeinated samples, compared to non-decaffeinated ones. An average loss of 10% was observed by Farah et al. (2006a) in CGA contents of water-decaffeinated and roasted Arabica coffee samples, in comparison with non-decaffeinated samples roasted in the

same conditions. On the other hand, a 7% average increase in CGA lactones content was observed in the same samples (Table 2). Higher CGA losses may be observed in green and roasted seeds decaffeinated with dichloromethane.

Roasting

In addition to their relevance for plant physiology, CGA take part in the generation of color, flavor and aroma of coffee during roasting (Farah and Donangelo, 2006; Trugo and Macrae, 1984a; Montavón et al., 2003.). Due to their thermal instability, CGA may be almost completely degraded into phenol derivatives when submitted to intense roasting conditions. During roasting, part of CGA is isomerized, part is epimerized, part is transformed into quinolactones due to dehydration and formation of an intramolecular bond and part is hydrolyzed and degraded into low molecular weight compounds (Figure 2) (Trugo, 1984; Clifford, 2000; Farah et al., 2005; Perrone et al., 2008; Leloup et al., 1995; Trugo and Macrae, 1984a). CGA also participate in the formation of polymeric material like melanoidins (Menezes, 1994a ; Steinhart and Luger, 1997). Drastic roasting conditions may produce losses of up to 95% of CGA (Trugo, 1984; Farah et al., 2005), with 8-10% being lost for every 1% loss of dry matter (Clifford, 2000; 1997; 1999). Total CGA content in commercial roasted coffee ranges from about 0.3 to 7 g% (Table 2) (Duarte and Farah, 2009b), depending on the type of processing, roasting degree, blend and analytical conditions. CGA contents in light or medium roasted coffees still stand out when compared to most food sources of CGA and medicinal plants (Clifford, 2000; Marques and Farah, 2008; Farah et al., 2001; Farah, 2004). Even dark roast coffees are among the highest edible sources of CGA in nature (Farah, 2004; Marques and Farah, 2009a). While coffee abstainers may typically ingest less than 100 mg of CGA/day, modest and heavy coffee drinkers intake may range from 0.1 to 2g (Clifford, 2000; 1997; Del Castillo et al., 2002).

In relation to changes in CGA individual classes and isomers, considering weight loss during roasting, at the beginning of the roasting process (6-9% weight loss), isomerization of CGA occurs. The levels of substitutes in the 5- position of the quinic acid decrease substantially while levels of substitutes in the 3- and 4- positions increase in some cases to almost double their original levels. (Trugo and Macrae, 1984a; Leloup et al., 1995; Farah et al., 2005; 2006b; Perrone et al., 2008). According to Leloup et al. (1995), at this roasting stage, diCQA may be partially hydrolyzed into monoesters and caffeic acid, which may be later hydrolyzed, decarboxylated and degraded to a range of simple phenols. Levels of volatile phenols increase along the whole process (Griffin and Stonier, 1975). The formation of chlorogenic acid lactones occurs after 6 to 7% weight loss (Hucke and Maier, 1985; Farah et al., 2005). About 7% of CGA in regular Arabica coffee and 6% in Robusta coffee seem to be transformed into 1,5-γ-quinolactones during the roasting process (Farah et al., 2005). Caffeoylquinic-1,5-γ-lactones (CQL) are the main lactones in coffee. 3-CQL and 4-CQL are expected to be the major 1,5-γ-quinolactones, since caffeoylquinic acids are the main CGA and only those CGA isomers that lack a substitute in the 5-position are able to form a 1,5-γ-quinolactone. Lactone formation of 3-CQA is favored relative to 4-CQA because of steric hindrance of the ester group in axial position of the equatorial conformer (Farah et al., 2005). Lactones derived from FQA, diCQA and *p*-CoQA, in order of relevance (Table 2) and other minor quinides, have also been identified in roasted coffee (Farah et al., 2005; 2006a; Perrone et al., 2008; Scholz and Maier, 1990; Scholz-Bottcher et al., 1991; Flores-Parra et al., 1989).

Table 1. Chlorogenic acids content in green coffee seeds, expressed in g% [a], dry matter basis.

Samples	CQA	FQA	diCQA	Total [b] CGA	References
C. arabica	5.76	0.25	0.87	6.88	Trugo and Macrae, 1984a
C. arabica cv.Caturra	4.63	0.33	0.66	5.62	Clifford and Ramirez-Martinez, 1990
C. arabica cv. Bourbon	4.77	0.34	0.56	5.67	Clifford and Ramirez-Martinez, 1990
Wild *C. arabica* (average)	3.26	0.19	0.60	4.10	Ky et al., 2001
C. *arabica* (Angola)	4.30	0.57	1.23	6.10	Correia et al., 1995
C. *arabica* (Angola)	4.84	0.28	0.53	5.65	Correia et al., 1995
C. arabica (Angola)	5.67	0.79	1.39	7.85	Correia et al., 1995
C. arabica cv. Boubon (Brazil)	4.20	0.28	0.77	5.25	Farah et al., 2005
C. arabica cv. Yellow Bourbon (Brazil)	6.30	0.29	0.63	7.22	Perrone et al., unpublished
C. arabica cv. Yellow Bourbon (Brazil)	5.04	0.30	0.79	6.14	Duarte et al., 2009b
C. arabica cv. Longberry (Ethiopia)	4.60	0.29	0.84	5.73	Farah et al., 2005
C. arabica cv. Red Catuai (Brazil)	4.27	0.28	0.89	5.44	Duarte et al., 2009b
C. arabica cv. Red Catuai (Brazil)	4.74	0.19	0.51	5.44	Perrone et al., 2008
C. arabica cv. Mundo Novo (Brazil)	5.08	0.23	0.85	6.16	Perrone et al., 2008
C. arabica cv. Rubi (Catuai x Novo Mundo)(Brazil)	4.28	0.25	0.81	5.34	Duarte et al., 2009b
C. arabica cv. Topasio (Catuai x Novo Mundo (Brazil)	4.26	0.24	0.93	5.42	Duarte et al., 2009b
C. canephora cv Robusta	6.82	0.60	1.37	8.80	Trugo and Macrae, 1984a
C. canephora cv Robusta	5.33	0.79	1.05	7.17	Clifford and Ramirez-Martinez, 1990
C. canephora cv Robusta (Angola)	3.43	0.54	1.20	6.08	Correia et al., 1995
C. canephora cv Robusta (Angola)	4.97	0.75	1.46	7.18	Correia et al., 1995
C. canephora cv Robusta (Colombia)	4.32	0.61	1.48	6.41	Duarte and Farah, 2009b (average of 4 samples)
C. canephora cv. Conillon (Brasil)	7.42	0.95	1.09	9.47	Farah et al., 2001
C. canephora cv. Conillon (Brasil)	6.45	0.61	1.30	8.36	Perrone et al., 2008
C. canephora cv. Conillon (Brasil)	8.28	0.63	1.25	10.16	Perrone et al., 2008
Wild *C. canephora (average)*	7.66	1.43	2.31	11.30	Ky et al., 2001
C. canephora cv. Robusta (Uganda)	5.77	0.47	1.34	7.58	Farah et al., 2005
Timor hybrid (*C.arabica x C. canephora*)	4.71	0.33	0.58	5.62	Clifford and Ramirez-Martinez, 1990
Catimor (Timor hybrid x *C. arabica*)	5.51	0.35	0.45	6.31	Clifford and Ramirez-Martinez, 1990
C. liberica cv. Dewevrei	5.39	0.48	1.10	6.97	Ky et al., 1997

[a]CQA- caffeoyilquinic acid; FQA – feruloylquinic acid; diCQA dicaffeoylquinic acid. Total CGA – total chlorogenic acids. Units may have been changed for consistency. [b]Sum of CQA, FQA and diCQA. Minor CGA compounds usually add 2% on these total CGA values.

From about 9-15% weight loss, a similar degradation rate of 3-, 4- and 5-isomers and different classes is observed. The content of CGA lactones increases until about 14-15% weight loss, i.e., light medium roast, reaching average levels of 398 and 424 mg% (dm) for Arabica and Robusta coffees, respectively, and decreasing gradually thereafter (Farah et al., 2005; Bennat et al., 1994). Farah et al., (2005) reported maximum 3-CQL contents of 230 and 254 mg%

(dw), during roasting of Arabica and Robusta, respectively. The contents of the second major lactone (4-CQL), were 116 and 139 mg%, respectively. Average lactones levels of 210 and 100 mg% (dm) were reported for commercial regular ground coffee (Schrader et al., 1996) (Table 2). A range of 47 to 214 mg% of total CQL was observed in nineteen Brazilian ground roasted coffee samples (Duarte and Farah, 2009b).

Due to analytical difficulties, the prediction of CGA content during coffee roasting, especially in industry, could be of interest. Knowing the CGA content of raw seeds and the roasting conditions such as time and temperature, modeling CGA loss is possible, considering that CGA thermal degradation follows first order Arrhenius-complied kinetics. However, distinct models for *C. arabica* and *C. canephora* should be developed, due to differences in the chemical composition and cell wall structure between these species (Perrone *et al*., unpublished).

From about 15-20% weight loss, a similar degradation rate occurs for all CGA isomers and lactones. At the end of the roasting process, CGA contents may vary, for example, from about 6 g% and 8 g% (w/w) in very light roasted Arabica and Robusta coffees, respectively, to 0.04 g% and 0.02g% in very dark roasted seeds, respectively (Farah et al., 2005; Perrone et al., 2008).

The speed at which coffee is roasted also affects the content of CGA in the coffee seeds. The faster the roasting speed, the lower is CGA loss. Average losses of CGA in coffees roasted to medium degree in a fluidized bed type roaster ranged from 13% at fast speed to 28% at slow speed (Toci et al., 2009a). However, before choosing the roasting speed, other factors must be considered such as flavor development and acrylamide formation (Toci et al., 2009a; Soares et al., 2009).

Due to analytical difficulties, the prediction of CGA content during coffee roasting, especially in industry, could be of interest. Knowing the CGA content of raw seeds and the roasting conditions such as time and temperature, modeling CGA loss is possible, considering that CGA thermal degradation follows first order Arrhenius-complied kinetics. However, distinct models for *C. arabica* and *C. canephora* should be developed, due to differences in the chemical composition and cell wall structure between these species (Perrone et al., unpublished).

Brewing

The content of CGA in the beverage depends on the blend, the roasting degree, the coffee grind, the proportion of coffee to water, the brewing method, the water temperature and length of time during which coffee is in contact with water. However, it is possible to say that domestic brewing substantially extracts CGA (80-100%) and CGL from roasted coffee (Clifford, 2000; 1997; Farah, unpublished). Higher temperatures under 100 °C result in greater extraction of CGA (Clifford, 1987; Trugo and Macrae, 1984a). Extraction rate increases over the first 10 min commonly employed in domestic brewing. The highest extraction rate of CGA usually occurs in the first 2 min at 93 °C, increasing less rapidly thereafter (Clifford, 1987; Merrit and Proctor., 1959). Domestic extraction will result in 35-400 mg of CGA per 100 ml cup, depending on the factors referred to above (Perrone et al., 2008; Clifford, 1987; Farah, 2004; Moura-Nunes, 2008). Keeping coffee brews at elevated temperatures reduces the contents of both CQA and CQL in the brew (Schrader et al., 1996; Bennat et al., 1994). In *espresso* coffees, generally CGA content is higher not only due to the common use of lighter coffees but because this method tends to extract these compounds

more efficiently. Average total CGA contents reported for espresso coffees is 430-500 mg (Petracco, 2005).

Table 2. Chlorogenic acids and quinolactones in ground roasted and instant coffee, expressed in g% [a], dry matter basis.

Coffee Samples	CQA	FQA	Di CQA	Total CGA	CQL	FQL	Di CQL	CoQL	Total CGL	References
Ground Roasted Coffee										
Commercial	2.70				0.26					Bennat et al., 1994,
	2.64				0.34					
	1.94				0.23					
	0.81				0.17					
	1.00				0.16					
Commercial (average)	2.26	0.21	0.19	2.66	0.31					Schrader et al., 1996
Comercial	1.25	0.22	0.19	1.66						Monteiro and Trugo, 2005
	1.29	0.19	0.24	1.72						
	0.85	0.12	0.09	1.06						
	0.38	0.06	0.03	0.47						
Comercial (average)	0.96	0.09	0.06	1.08	0.19				0.19	Duarte and Farah, 2009b
Comercial	0.23	0.02	0.01	0.27	0.06				0.06	
	1.47	0.08	0.12	1.70	0.27				0.27	
	1.74	0.10	0.14	1.97	0.33				0.33	
Comercial decaffeinated	1.42	0.09	0.07	1.58	0.11				0.11	
C. arabica [c] cv. Bourbon (Brazil)	2.15	0.17	0.14	2.46	0.36	0.04	0.01	0.01	0.41	Farah et al., 2005
C. arabica [c] cv. Yellow Bourbon (Brazil)	1.80	0.13	0.08	2.02	0.21	0.03	0.00	------	0.24	Perrone et al., unpublished
C. arabica [c] cv. Mundo Novo (Brazil)	1.87	0.16	0.13	2.16	0.30	0.06	0.01	0.01	0.38	Perrone et al., 2008
C. arabica [c] cv. Red Catuai (Brazil)	2.24	0.15	0.14	2.53	0.35	0.06	0.01	0.01	0.43	
C.arabica [c] cv.Longberry (Ethiopia)	1.65	0.15	0.13	1.93	0.33	0.04	0.01	0.01	0.38	Farah et al., 2005
C. arabica	3.23				0.32					Bennat et al., 1994
C.arabica [c] *Decaffeinated* (average)				1.80	0.38	0.06	0.01		0.45	Farah et al., 2006b
C.canephora [c] cv. Robusta (Uganda)	2.76	0.34	0.23	3.33	0.39	0.03	0.03		0.45	Farah et al., 2005

C.canephora [c] cv. Conillon (Brazil)	2.08	0.27	0.14	2.50	0.34	0.08	0.01		0.43	Perrone *et al*., unpublished
C.canephora [c] cv. Conillon (Brazil)	3.14	0.58	0.39	4.11	0.40	0.19	0.02	0.01	0.62	Perrone et al., 2008
Instant Coffee										
Regular (England)	5.28	1.16	0,53	6.97						Trugo and Macrae, 1984a
Regular (Brazil)	0.63	0.06	0.03	0.72						Nogueira andTrugo, 2003
	2.41	0.27	0.09	2.77						
	1.30	0.14	0.04	1.48						
	2.71	0.43	0.63	3.33	0.19					Duarte and Farah, 2009b
Average Regular (Brazil)	2.31	0.37	0.48	3.19	0.21					
Decaffeinated (Brazil)	4.73	0.84	0.28	5.85						Nogueira andTrugo, 2003
	3.33	0.60	0.17	4.10						
	4.42	0.63	1.60	6.66	0.43					Duarte and Farah, 2009b
Average Decaffeinated (Brazil)	4.41	0.67	0.95	6.05	0.43					

[a] Units may have been changed for consistency. [b] commercial samples' values are expressed on a wet basis [c]Laboratory roasted – weight loss after roasting 13-16% (light to medium roast).

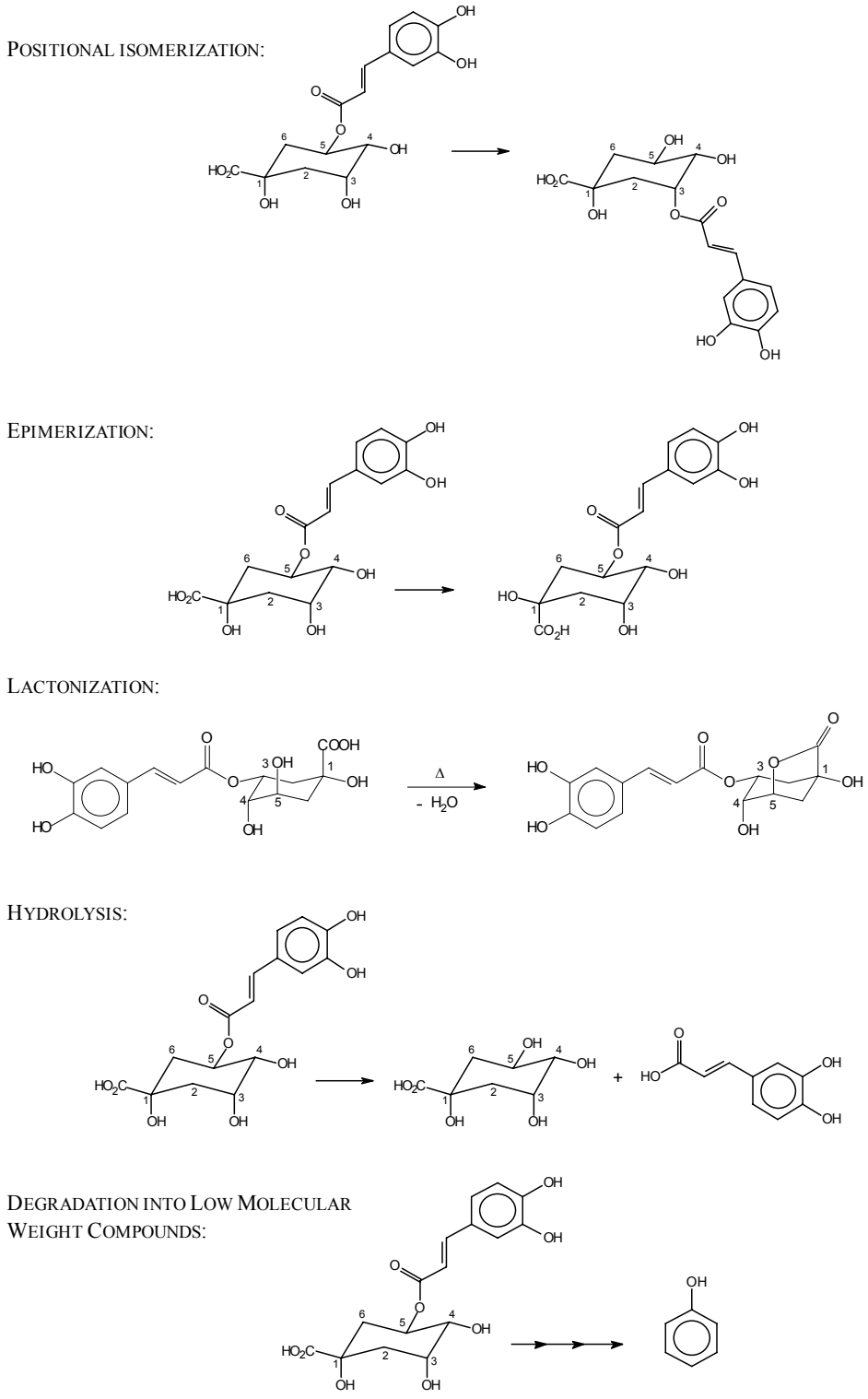


Figure 2. Examples of changes in chlorogenic acid molecules (represented by 5-CQA and 3-CQA) during coffee roasting.

Instant coffee processing

A large variation in the contents of total CGA was observed upon analyses of thirteen commercial instant coffee samples from England (3.6-10.7 g%, dw – (Trugo and Macrae, 1984a)). In two latter studies, ranges of 0.6-5.9 g% (n = 9) (Nogueira and Trugo, 2003) and

1.4-4.4 g% (dw) (n = 11) were observed in Brazilian commercial instant coffee samples (Duarte and Farah, 2009b) (Table 2). In these samples, 5-CQA alone accounted for about 30% of total CGA; CQA for 70%; FQA for 20% and diCQA for 10%. The large difference in CGA levels has been attributed to the use of different blends and roasting degrees, whereas the low percentage of diCQA has been attributed to loss during processing (Trugo and Macrae, 1984a; Nogueira and Trugo, 2003). Bennat et al. (1994) also performed analyses of CQA and lactones in instant coffee and attributed the low content of lactones to hydrolysis during processing (Duarte and Farah, 2009b).

Special types of processing

Steam-treated coffee

Coffee may be steam-treated prior to roasting as a means to make coffee "less irritable" to the stomach, and therefore acceptable to persons with stomach problems (Steinhart and Luger, 1997). Degradation of CGA during steaming of green seeds has been reported as a consequence of increased water uptake (Maier, 1994).

Monsooned coffee

This is a specialty coffee of India, which undergoes a natural process of curing dry raw Arabica and Robusta coffee seeds by exposing them to moist monsoon winds prevailing in the costal regions of Mangalore and Tellichery. Variyar et al. (2003) observed a substantial decrease in CGA levels of this type of specialty coffee, accompanied by an increase in the levels of free caffeic acid, which was attributed to hydrolysis of CGA during the process. Balyaya and Clifford (1995) also observed higher levels of free caffeic acid in monsooned coffee seeds.

Irradiation

This treatment has been applied to green seeds as a method to control microbiological contamination and insect infestation. While Deshpande and Aguilar (1975) did not observe differences in CGA and caffeic acid contents of Arabica and Robusta seeds treated with gamma irradiation, Variyar et al. (2003) observed a substantial decrease in CGA contents of irradiated seeds in comparison with non-irradiated ones. The decrease in CGA contents was not accompanied by an increase in caffeic acid levels, which was attributed to degradation of CGA, and not to hydrolysis, as it seems to be the case with monsooned seeds. Gamma-irradiation has been reported to reduce the contents of cinnamic acids in food products in general (Clifford, 2000).

CHLOROGENIC ACIDS COMPOSITION AND COFFEE QUALITY

CGA are known to be important determinants of coffee flavor. They contribute to the final acidity (Trugo and Macrae, 1984a) and confer astringency (Clifford and Wight 1976; Variyar et al., 2003; Carelli et., 1974) and bitterness to the beverage (Trugo, 1984). As a result of Maillard & Strecker´s reactions, bitterness increases during roasting due to release of caffeic acid and formation of lactones and other phenol derivatives responsible for flavor and aroma (Variyar et al., 2003; Ginz and Enhelhardt, 1995).

The relationship of CGA with coffee cup quality is still unclear and somehow controversial. Ohiokpehai (1982) reported that the addition of diCQA conferred a disagreeable flavor to coffee beverage, which disappeared on subsequent addition of CQA. However, according to

Silva (1999), total CGA levels present an inverse association with coffee quality, with higher CGA content being observed in lower quality samples. Considering that CQA accounts for at least 60% of CGA contents in roasted coffee, higher levels of CQA would be more likely to be associated with poor cup quality. Farah et al. (2006b) also observed a strong association between the levels of CQA and FQA and low cup quality. The decrease in quality was accompanied by an increase in color intensity of raw seeds that was attributed indirectly to the presence of reactive *o*-quinones formed by the action of the enzyme polyphenol oxidase over these compounds. Therefore, products of CGA oxidation, and not CGA themselves, may be associated with cup quality decrease. These data, as well as the data obtained by Silva (1999), are in agreement with reports according to which *o*-quinones are responsible for the inhibition of the activity of polyphenol oxidase (Mazzafera and Robinson, 2000). Coffee cup quality is known to be directly related to polyphenol oxidase activity and 5-CQA levels in seeds from mature coffee fruits seem to be inversely associated with polyphenol oxidase activity in coffee seeds (Silva, 1999; Amorim and Silva 1968; Carvalho et al., 1994; Mazzafera, 1999). Moreover, Amorim et al. (1975) had already associated oxidation products of phenolic compounds as a possible cause for Rio-off-flavor (a negative phenolic note responsible for loss on cup quality), strengthening the hypothesis of association between high levels of CQA and FQA with low cup quality.

Amorin (1975) observed that 5-CQA levels above usual levels for a determined species contributed to a decrease in quality. Taking into account the well-known importance of CGA for flavor and aroma formation, there appears to be a limiting CGA level over which cup quality decreases. Lower CGA levels also appear to explain the superiority of *C. arabica* cup quality when compared to *C. canephora*. The large difference in CGA contents of these two species has been considered one of the factors responsible for flavor differences between the two species (Ky et al., 2001; Trugo and Macrae, 1984b; Bertrand et al., 2003).

The presence of defective coffee seeds is also relevant in establishing coffee quality. The CGA content of most defective seeds, excluding physical defects (bored, broken, etc.) and defects of extraneous matter (husks, twigs, stones, etc.), vary according to the degree of maturation of the fruit that generates the respective defect (Toci and Farah, 2008; Farah et al., 2006b). The main defects occur due to strip-picking of immature and over-ripe fruits along with ripe (cherry) fruits. The four most common defects in coffee that may considerably affect cup quality are immature seeds (originated from immature fruits), immature-black seeds (immature seeds with oxidized skin), black seeds (from over-ripened fruits) and sour seeds (from fruits fermented on the ground or due to improper processing conditions). Comparing immature and immature-black seeds with good quality seeds, Mazzafera (1999) observed that the contents of total phenolic substances and 5-CQA were higher in immature and immature–black defective seeds. Lower levels of 5-CQA in black defective seeds were found when compared with good quality and immature defective seeds (Farah, 2004; Farah et al., 2007; Franca et al., 2004). After analysis of eight CGA isomers in defective coffee seeds, Farah et al. (2007; 2006b) also observed that immature and immature-black defective seeds contained significantly higher levels of all CGA isomers, particularly CQA and FQA, compared to healthy and black defective seeds. 5-CQA was the main CGA in all defective seeds (50-75% of total CGA), varying from 5,6 g% (dry weight) in dark green immature seeds to 0,6 g% (dw) in black defective seeds, whereas good quality seeds contained about 4 g% (dw). CGA isomers distribution within each class was similar in good quality seeds, immature and immature-black defective seeds. However, sour and black defective seeds showed an increase of up to 25% in the isomers 3-CQA, 4-CQA and 4-FQA, in comparison with those isomers in good quality seeds, probably due to the occurrence of isomerization of 5-CQA and 5-FQA, and possibly hydrolysis of diCQA during the fermentative process (in the case of sour defects) or aging (in the case of black defects).

In summary, *0*-quinones and other oxidation products of CQA and FQA by PPO and peroxidase may contribute to poor quality and Rio off-flavor. Moreover, although CGA are important to flavor, high contents of CQA and FQA may be poor quality determinants, especially when coffee beans are not well taken care of. A strong contribution of sour beans (oxidized beans) to undesirable flavor due to CGA oxidation products is suggested and should be investigated.

CHLOROGENIC ACIDS AND HEALTH

In the last few years, a series of epidemiological and clinical studies have associated moderate coffee consumption, independently of caffeine, with health benefits such as reduction in the relative risk of development of hypertension (Periti et al., 1987), coronary heart disease (Bonita et al., 2007), type 2 diabetes (Rosengreen et al., 2004; Agardh et al., 2004; Salazar-Martinez et al., 2004; van Dam, 2006), Alzheimer's disease (Lindsay et al. 2002), colon cancer (Rosenberg et al., 1989; Tavani et al., 1997; Cavin et al., 2002), hepatic cirroses and liver cancer (Corrao et al., 2001; Klatsky et al., 2006; Tverdal and Skurtveit, 2003; Ranheim and Halvorsen, 2005; Larsson and Wolk, 2007), alcoolism, depression and suicides (Flores et al., 2000; Reich et al., 2008). *In vitro* and animal studies have mainly attributed such beneficial properties of coffee to its antioxidant capacity and other mechanisms involving CGA compounds (Cavin et al., 2002; Hemmerle et al., 1997; Herling et al., 1998; Laranjinha et al., 1994; Kono et al., 1997; Nakatani et al., 2000; Johnston et al., 2003; Natella et al., 2002; Aruoma, 1999; Huang et al., 1985; Arion et al., 1997; Gerin and Van Schaftingen, 2002; Shearer et al., 2007).

Chlorogenic acids are known to have similar antioxidant activity to ascorbic acid (Nakatani et al., 2000). They are able to quelate transition metals such as Fe^{2+}, to scavenge free radicals and interrupt free radical chain reactions. In addition, they have been able to prevent LDL oxidation and DNA damage *in vitro* (Cavin et al., 2002; Laranjinha et al., 1994). Because of the high coffee consumption worldwide and its expressive CGA contents, coffee became an important source of antioxidant compounds for many countries such as Brazil, USA and North European countries (Farah, 2004). In fact, studies performed in Denmark and the USA showed that coffee is the most important contributor to antioxidant compounds intake in their diets (Svilaas et al., 2004; Wattenberg et al., 1980).

Long before the epidemiological studies involving coffee consumption, the anti-mutagenic property of CGA and their metabolites had been demonstrated by a series of studies (Wattenberg et al., 1980; Wattenberg, 1983; Stich et al., 1982; Wood et al., 1982; Mori et al., 1986; Namiki, 1990). Recent studies have confirmed this property and elucidated a few mechanisms involved, among which are typical antioxidant mechanisms as well as inactivation of reactive compounds and metabolic pathway changes (Cavin et al., 2002; Mori et al., 1996; Pannala et al., 1998; Lo and Chung, 1999; Kasai et al., 2000). Additional biopharmacological properties have been attributed to different caffeoylquinic and dicaffeoylquinic acids, for instance, antiviral activity against adenovirus and herpes virus (Chiang et al., 2002); antibacterial activity against Enterobacteria (Almeida et al., 2006; Furuhata et al., 2002; Dogasaki et al., 2002) and *Streptococcus mutans*, the main causative agent of caries (Daglia et al., 2002; Almeida et al., 2007; Antonio et al., 2009); hepatoprotective activity in injured liver experimental model (Basnet et al., 1996) and in hepatocites (Gebhardt and Fausel, 1997); imuno-stimulating activity (Tatefuji et al., 1996); and hipotensive activity (Suzuki et al., 2002; Huang et al., 2004; Kozuma et al., 2005). Isolated dicafeoylquinic acids and synthetic dicaffeoylquinic acid derivatives also have inhibited the replication of HIV-1 in cells (Robinson et al., 1996a; 1996b; Mc Dougall et al.,

1998; Kyng et al., 1999; Zhu et al., 1999), which raises the possibility of production of new coffee based anti HIV drugs. Because only a few chlorogenic acid compounds are commercially available or synthesized in laboratories, studies on biological properties of FQA and *p*-CoQA are scarce.

CGL have also exhibited hypoglicemic activity in rats (Shearer et al., 2007; 2003) as well as the capacity to bind to brain μ-opioid receptors (De Paulis and Martin, 2003). This μ-opioid antagonistic property raises the possibility of using coffee to fight certain types of drug addiction. In addition, CGL were shown to be capable of indirectly counteracting the effect of caffeine *in vitro* (De Paulis et al., 2002).

BIOAVAILABILITY OF CHLOROGENIC ACIDS FROM COFFEE

Until recently, only a few studies on CGA absorption and bioavailability were available in the literature, especially those involving humans. Moreover, most of the data available was related to 5-CQA only. Due to analytical difficulties, after 5-CQA or coffee consumption, only caffeic acid and/or very small amounts of 5-CQA were identified in plasma or serum of animals and humans (Choudhury et al., 1999; Bourne and Rice-Evans, 1999; Azuma et al., 2000; Rice-Evans et al., 2000; Cremin et al., 2001; Nardini et al., 2002; Bugianesi et al., 2004; Wittemer et al., 2005; Lafay et al., 2006). In addition to CGA natural instability in biological matrixes and their affinity for other components in plasma and serum such as proteins (Muralidhara and Prakashla, 1995) and lipoproteins (Natella et al., 2007), most phenolic compounds absorbed in humans are conjugated with sulfate and glucuronic acid for circulation and excretion in the human body, and this increases the analytical difficulties. Moreover, these compounds and primary metabolites seem to be further and rapidly metabolized to low molecular weight compounds that are difficult to analyze. As a consequence, it had been estimated in the past that CGA were poorly absorbed (Bourne and Rice-Evans, 1998; Spencer et al., 1999; Dupas et al., 2006). Today, it is known that the absorption of coffee CGA is much higher and variable than what it was initially thought, and that some individuals may apparently absorb more than 70% of the ingested amount of these compounds (Farah et al., 2008).

Digestion of chlorogenic acids

CGA are stable in artificial and natural saliva and gastric fluids. No hydrolysis has been observed due to the action of saliva and gastric fluid ionic forms or esterases (Farah, 2004; Rice-Evans et al., 2000; Dupas et al., 2006; Lambert et al., 1999; Olthof et al., 2001; Rechner et al., 2001; Farah et al., 2007a; 2007b). In a study using human intestinal fluids, however, part of 5-CQA was isomerized and part hydrolyzed, with losses of up to ~ 60%. Nevertheless, 5-CQA was less susceptible to hydrolysis and isomerization when naturally present in the coffee matrix (maximum 5-CQA loss of 10% after 8h incubation) than when isolated. Maximum loss of total CGA present in coffee was 8% after 8h incubation (Farah et al., 2007a; 2007b). A considerable variation was observed in the effects of a number of human intestinal fluid samples on CGA during *ex vivo* digestion, probably due to different concentrations of ionic forms and/or the presence of a small amount of esterases able to hydrolyze CGA in some individuals (Farah et al., 2007a; 2007b). Although Andreason et al. (Andreason et al., 2001) have identified esterases with the ability to hydrolyze CGA in the small intestine mucosa of rats, Plumb et al. (1999) did not find esterase activity during incubation of 5-CQA with human intestine and liver extracts. Therefore, on average, even after 8 h digestion (which is an overestimation of digestion time), at least 90% of CGA from coffee seem to be available for absorption in the gastrointestinal tract.

Absorption, metabolism and bioavailability of chlorogenic acids

As explained earlier, until recently, animal and human studies failed to detect intact CGA in plasma or serum after coffee consumption, although urinary metabolites had been identified in urine. Therefore, it was generally concluded that only very small amounts of intact CGA were absorbed by humans and that most of the amount ingested was degraded during digestion or excreted through fesses. Caffeic acid, a primary metabolite of CQA and diCQA, has been detected in rat and human plasma and urine, especially in conjugated forms (Azuma et al., 2000; Rice-Evans et al., 2000; Cremin et al., 2001; Nardini et al., 2002; Wittemer et al., 2005; Rechner et al., 2001; Olthoff et al., 2003). The presence of small amounts of free and conjugated forms of 5-CQA in murine plasma has been reported after 5-CQA intraperitoneal administration (Azuma et al., 2000). Lafay et al. (2006) identified 5-CQA and caffeic acid in murine plasma 1.5 h after the consumption of a diet supplemented with 5-CQA.

Recently, after the administration of a roasted coffee brew containing 3.4 mmols of CGA (Monteiro et al., 2007), in addition to caffeic acid, three CQA and three diCQA isomers were identified in the plasma of 6 subjects, while in one subject two FQA isomers were also identified. More recently, the same CQA and diCQA isomers were identified in the plasma of 10 subjects after consumption of 0.4 g of a green coffee extract (Farah et al., 2008) containing 451 μmols (170 mg) of CGA. In addition to CGA compounds, caffeic, ferulic and isoferulic acids were also identified in plasma. Results of these two studies indicated that a small percentage of the ingested CGA is absorbed in the stomach and that a large amount is absorbed throughout the small intestine, where these compounds are conjugated with sulfate and glucuronic acid. These results also showed that CGA isomers are absorbed and/or metabolized by humans at different rates. Considering results from a study using HepG2 hepatoma cells (Farah and Trugo, 2007), it appears that FQA and isomers with the acyl moiety liked to the 3- and 4-positions of the quinic acid moiety would be preferentially absorbed by these cells, while the remaining major CGA compounds in coffee would preferentially circulate in plasma.

In a study using ileostomized patients (Olthoff et al., 2003), after the consumption of 5-CQA, an average of 67% was recovered in the ileostomy fluids. It was estimated therefore, that about 33% of the ingested 5-CQA had been absorbed by these patients. However, it had not been considered that part of CGA could have been lost during digestion. Recently, after the consumption of 451 μmols of CGA through a green coffee extract, 7.8-72.1% of CGA were recovered in plasma during 8 hours after the extract consumption, with average recovery of 33%. Therefore, because most of CGA ingested is not degraded in the digestive system, the mean estimated apparent bioavailability of 33% of roasted coffee CGA was probably correct (Farah et al., 2008).

The same CQA and diCQA isomers identified in plasma after coffee consumption have been identified in addition to other cinnamates, in human digestive fluids (in a range of 3.5-40 μmol/L), in conjugated and free forms, after 12 h fasting. This finding corroborates their circulation in the blood stream and, therefore, that these compounds are bioavailable in humans. In addition, it was observed that the concentrations of free forms (< 8μmol/L) were in the order of magnitude of plasma CGA concentration after coffee consumption (Monteiro et al., 2007). Additionally, this finding indicates that digestive fluids are a major excretion route for intact CGA and evidences the occurrence of recycling through entero-hepatic circulation of CGA in humans. In 1957, Booth et al. had already suggested that 5-CQA was kept in the liver as a way to store caffeic acid for the human body, which would be slowly released into the blood stream. Apparently, they only did not know about the potential value of CGA themselves as bioactive compounds in humans.

Although other phenolic compounds have also been previously identified in human bile (Code, 1968), no literature data was found for identification of such compounds in gastric fluids. However, Farah and Farah et al. (2004; 2007a; 2007b; 2009) found no significant difference between the amounts and profile of CGA in gastric and enteric fluids, suggesting that CGA are equally distributed in the gastrointestinal fluids. Another possibility is that such lack of difference between bile and gastric fluid might be caused by the large inter-individual differences observed in CGA concentrations among the fluids. On the other hand, significant differences in profile and concentration were observed between the compositions of saliva and gastrointestinal fluids.

Differently from digestive fluids, urine is not a major excretion route for intact CGA in humans. Only very low amounts of 4-CQA and 5-CQA have been identified in human urine comparing to the amount of metabolites (Farah et al. 2008; Monteiro et al., 2007; Booth et al., 1957). On the other hand, more than thirty different metabolites have been identified in human urine after CGA or coffee consumption, with hipuric, ferulic, isoferulic, dehydroferulic, dehydrocaffeic, galic, sinapic, vanillic, *p*-hydroxybenzoic and *m*-hydroxyhipuric acids being considered, among others, as major metabolites of CGA (Farah et al. 2008; Rechner et al., 2001; Olthoff et al., 2003; Farah and Trugo, 2007; Gonthier et al., 2003).

The action of esterases from large intestine bacteria on CGA isomers which are not absorbed in the stomach and small intestine has been reported (Rechner et al., 2001; Andreason et al., 2001; Olthoff et al., 2003; Gonthier et al., 2003; Couteau et al., 2001; Hodgson et al., 2004; Rechner and Kroner, 2005). These esterases appear to be able to release the cinnamic acid from the CGA molecule, which may be absorbed and metabolized into other compounds such as ferulic and isoferulic acids and derivatives (Couteau et al., 2001). However, no significant increase was observed in caffeic and ferulic acids concentrations in human plasma 4-8 h after coffee ingestion (comparing to 0-4 h), which would be the time when CGA would reach the large intestine (Farah et al., 2008). Other bacteria seem to be able to break glucuronated forms present in digestive fluids, allowing for recycling.

Bioavailability of chlorogenic acids lactones

After ingestion of roasted coffee brew containing significant amounts of 3-CQL (0.9 mmol) and 4-CQL (1.0 mmol) as well as CGA, only CQA and not CQL isomers were observed in human plasma (Monteiro et al., 2007). Synthetic lactones of FQA and *p*-CoQA were recently incubated for 2-8 h with human gastrointestinal fluids and losses from 40-100% were observed (Monteiro, de Paulis and Farah, unpublished results).

Therefore, despite the potential use of coffee lactones for health purposes, it is very likely that once these lactones are consumed in the beverage, a major part of them returns to their chlorogenic acid form during digestion or metabolism, indirectly increasing the chlorogenic acids intake (Farah, 2004; Farah et al., 2007a; 2007b), and part of them is degraded. The same type of behavior was observed when a synthetic CGA lactone (3,4-diferuloylquinic-1,5-γ-quinolactone - 3,4-diFQL) was administered directly into the stomach of rats (Farah et al., in press). However, the incubation of 3,4-diFQL with various human gastrointestinal fluid samples of different pH did not cause changes in the molecule. A thorough investigation regarding the bioavailability of lactones in humans should still be performed.

CONCLUDING REMARKS

The high levels of chlorogenic acids and related compounds in coffee seeds reflect their physiological importance for coffee plant. Genetic factors such as species and variety, degree of maturation, and - to some extent - environmental conditions and agricultural practices, are important determinants of the composition of chlorogenic acids in green coffee seeds, and will also affect the composition of the final beverage. It is known that processing, especially roasting, modifies dramatically the chlorogenic acids composition of coffee, producing flavor, bioactive and color compounds characteristic of coffee beverage, although more studies on the influence of chlorogenic acids on cup quality are necessary.

Despite the considerable published information on 5-caffeoylquinic acid content in coffee, less is known on the composition and biological properties of other chlorogenic acid isomers and derived components such as lactones. The lack of commercial standards for most of these compounds and the similarity of their chemical structures require highly efficient chromatographic separation methods coupled to mass spectrometry for proper identification and makes difficult the performance of biological studies involving all isomers.

Studies have demonstrated that chlorogenic acids are bioavailable and potentially beneficial to humans. However, considering the large variation in the chlorogenic acids concentration in coffee beverage; the large inter-individual variability in their metabolism in humans and that the exact amount of ingested chlorogenic acids necessary to promote each of their potential benefits to human health is still unknown, more studies are needed in order to establish a daily dietary recommendation for these compounds as well as the number of coffee cups necessary to promote such benefits in both short and long terms.

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Chlorogenic Acids from Green Coffee Are Highly Bioavailable in Humans

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SUMMARY

This study evaluated for the first time the pharmacokinetics and apparent bioavailability of chlorogenic acid compounds (CGA) in 10 adult subjects, after the acute consumption of a green coffee extract. On average, 33.1 ± 23.1% of the ingested cinnamic acid moieties were recovered in plasma, including metabolites, with peak levels observed from 0.5 to 8 h after treatment. CGA and metabolites identified in urine after treatment were 4-CQA, 5-CQA and sinapic, *p*-hydroxybenzoic, gallic, vanillic, dihydrocaffeic, caffeic, ferulic, isoferulic, and *p*-coumaric acids, totaling 5.5 ± 10.6% urinary recovery of the ingested cinnamic and quinic acid moiteties. Our results show that the major CGA compounds present in green coffee are highly absorbed and metabolized in humans.

INTRODUCTION

Chlorogenic acids (CGA) are phenolic compounds with beneficial biological properties. They are formed by the esterification of hydroxycinnamic acids such as caffeic, ferulic and *p*-coumaric acids, with quinic acid. Caffeoylquinic (CQA) and dicaffeoylquinic (diCQA) acids are the main CGA in coffee, being responsible for about 90% of CGA composition of the green seeds, while feruloylquinic acids, *p*-coumaroylquinic acids and mixed esters account for the remaining amount of CGA (Farah and Donangelo, 2006). Because green coffee is a major source of CGA (5-12g/100 g) (Farah and Donangelo, 2006), it has been used for the production of nutraceuticals.

Recent studies demonstrated that the consumption of green coffee extracts produced antihypertensive effect in rats and humans (Suzuki et al., 2002; Kozuma et al., 2005), improvement in human vasoreactivity (Ochiai et al., 2004), inhibitory effect on fat accumulation and body weight in mice and humans (Shimoda et al., 2006; Dellalibera et al., 2006) and modulation of glucose metabolism in humans (Blum et al., 2007). Such biological effects have been attributed to the CGA present in green coffee.

Data on the bioavailability of CGA from green coffee in humans are inexistent, although they are necessary in order to support these pharmacological studies and help us to better understand the physiology and mechanisms involved in their absorption. This study evaluated the pharmacokinetic profile and apparent bioavailability of CGA isomers in humans after the acute ingestion of a green coffee extract.

MATERIAL AND METHODS

Ten healthy non-smoker subjects, five male and five female (22-55 y of age), were recruited. After a 48 h low phenolic diet and overnight fasting, 2 capsules of a hydro-alcoholic decaffeinated green coffee extract produced from *C. canephora* cv. Pierre beans (SVETOL®) and containing 167.3 mg of CGA (496 μmol of hydroxycinnamates) were offered to subjects. Sequential blood draws were obtained 0.5; 1; 2; 3; 4; 5; 6; 7 and 8 h after the capsules consumption. Urine samples were also collected at baseline interval (minus 2 to 0 h) and at intervals of 0-2 h; 2-4 h; 4-6 h and 6-8 h after coffee consumption. Total urine volume was measured for each collection period. Every hour, starting one hour after green coffee extract consumption, subjects ate a CGA-free snack composed of white bread (25 g) with pasteurized cheese (15 g) and 100 mL of a saline solution, until the end of blood draws.

Analyses of CGA (and related compounds) in the green coffee extract, plasma and urine were performed by HPLC and LC-DAD-MS gradient systems as described in details by Farah *et al*. (Farah et al., 2005; 2006) and Monteiro et al. (2007). Results of CGA and phenolic acids in urine were normalized by creatinine excretion (Monteiro et al., 2007). Molar ratios of specific CGA compounds were calculated in green coffee extract as ratios of total amounts and, in plasma, as ratios of the corresponding area under the curve (AUC). Pharmacokinetics and apparent bioavailability calculations were performed using plasma AUC of cinnamic acids, and of individual and total CQA, diCQA and CGA compounds, associated with the estimation of total blood volume performed according to Frenkel et al. (1972) adapted.

Urinary recovery calculations were made considering the total number of equivalent moieties of cinnamic and quinic acids consumed in the green coffee extract and the total number of phenolic acid moieties recovered in urine, as a percentage.

Results were presented as means with corresponding standard deviation. Associations between plasma AUC or C_{max} and urinary excretion of specific compounds were tested by nonparametric correlation using GraphPad Prism. Differences were considered significant when $p \leq 0.05$.

The present study obtained approval by the Ethical Committee of the UFRJ University Hospital (Rio de Janeiro, Brazil).

RESULTS AND DISCUSSION

Although subjects were on a low-phenolic diet for two days prior to the study, and although they were fasting for 10-12 h when baseline blood and urine were collected, all subjects presented phenolic compounds in their baseline plasma and/or urine. This is in agreement with the fact that CGA and other phenolic compounds have been observed in saliva, gastrointestinal fluids and urine of fasting subjects (Farah, 2004; Farah et al., 2006; Cremin et al., 2001) and corroborates the hypothesis of storage and recycling of these compounds through excretion and re-absorption suggested by Baer-Dubowska and Szaefer, and Farah et al. (2006) and Baer-Dubowska and Szaefer (1998).

After green coffee extract consumption, 6 major CGA compounds (3-CQA, 4-CQA, 5-CQA, 3,4-diCQA, 3,5-diCQA and 4,5-diCQA) and caffeic acid were identified in the plasma of all subjects, as previously observed after roasted coffee consumption (Monteiro et al., 2007), accounting, on average, for 79.7% and 6.6% of total phenolic compounds in plasma, respectively. In addition, ferulic, isoferulic and *p*-coumaric acids were identified in the plasma

of different subjects, contributing, on average, to 6.2%, 6.1% and 1.4% of total phenolic compounds in plasma.

There was a large inter-individual variation in the pharmacokinetic profile of all CGA compounds and phenolic acids in plasma and urine after green coffee extract consumption. This variability may be attributed to inter-individual differences in digestive transit time, preferential site of absorption, and metabolism of cinnamates as reported in the literature for other phenolic compounds and for chlorogenic acids from roasted coffee (Monteiro et al., 2007; Farah et al., 2006; Manach et al., 2004).

In contrast to the pharmacokinetic profile of flavonoids such as catechin (Masukawa et al., 2006) and isoflavones (Pascual-Teresa et al., 2006), various plasma concentration peaks were observed from 0.5 up to 8 h after the green coffee extract consumption (Figure 1), suggesting a complex and dynamic process of absorption and metabolism. This irregular pharmacokinetic pattern makes the calculation of the CGA compounds' half-life difficult. Longer duration studies would be necessary for this measurement.

Considering that a liquid food may take up to 1 h to reach the small intestine (Minami and McCallum, 1984; Moore et al., 1981), and that preliminary tests showed that the extract capsules take less than 15 min to solubilize in a simulated gastric condition (data not shown), the initial plasma concentration peaks confirm an early absorption of CGA in the stomach and jejunum followed by absorption along the small intestine (Monteiroet al., 2007; Lafay et al., 2006; Konishi et al., 2006; Spencer et al., 1999; Lafay et al., 2006). The later plasma peaks (> 5 h) in some subjects may indicate absorption through the large intestine (Dupont et al., 2002) and/or recycling through digestive fluids (Farah et al., 2006).

CGA C_{max} and T_{max} varied largely among the subjects; C_{max} of total CQA varied from 0.6 to 16.9 μmol/L, C_{max} of total diCQA varied from 0.3 to 22.8 μmol/L, whereas C_{max} of total CGA varied from 1.2 to 39.7 μmol/L, with mean concentrations of 8.2, 6.6 and 14.8 μmol/L, respectively. T_{max} for total CQA, total diCQA and total CGA varied considerably among the subjects (from 0.5 to 8 h), with mean values of 3.3; 3.2 and 3.1 h, respectively.

Regarding individual compounds, 5-CQA was the major CGA identified in the plasma of all subjects at all time points after green coffee extract consumption. Considering mean values of plasma AUC, 5-CQA, 4-CQA and 3-CQA contributed with 31.3%, 7.5% and 5.2% of AUC of total phenolic compounds in plasma.

In the present study, the molar ratio diCQA:CQA in plasma was 6.2 x higher than in the green coffee extract. This supports previous evidence of differential mechanisms of absorption and/or metabolism for CQA and diCQA (Monteiro et al., 2007), with favored tissue uptake of CQA compared to diCQA and/or favored diCQA absorption that could be related to the higher lipophilicity of diCQA compared to CQA, possibly favoring diCQA diffusion through the intestinal mucosa cells. Moreover, despite similar amounts of FQA and diCQA in the extract, FQA was not detected in the plasma of any subject, supporting evidence of a poor absorption of these compounds in comparison with CQA and diCQA and/or of a rapid uptake by organs such as liver (Booth et al., 1957; Farah and Trugo, 2006) and adipose tissue.

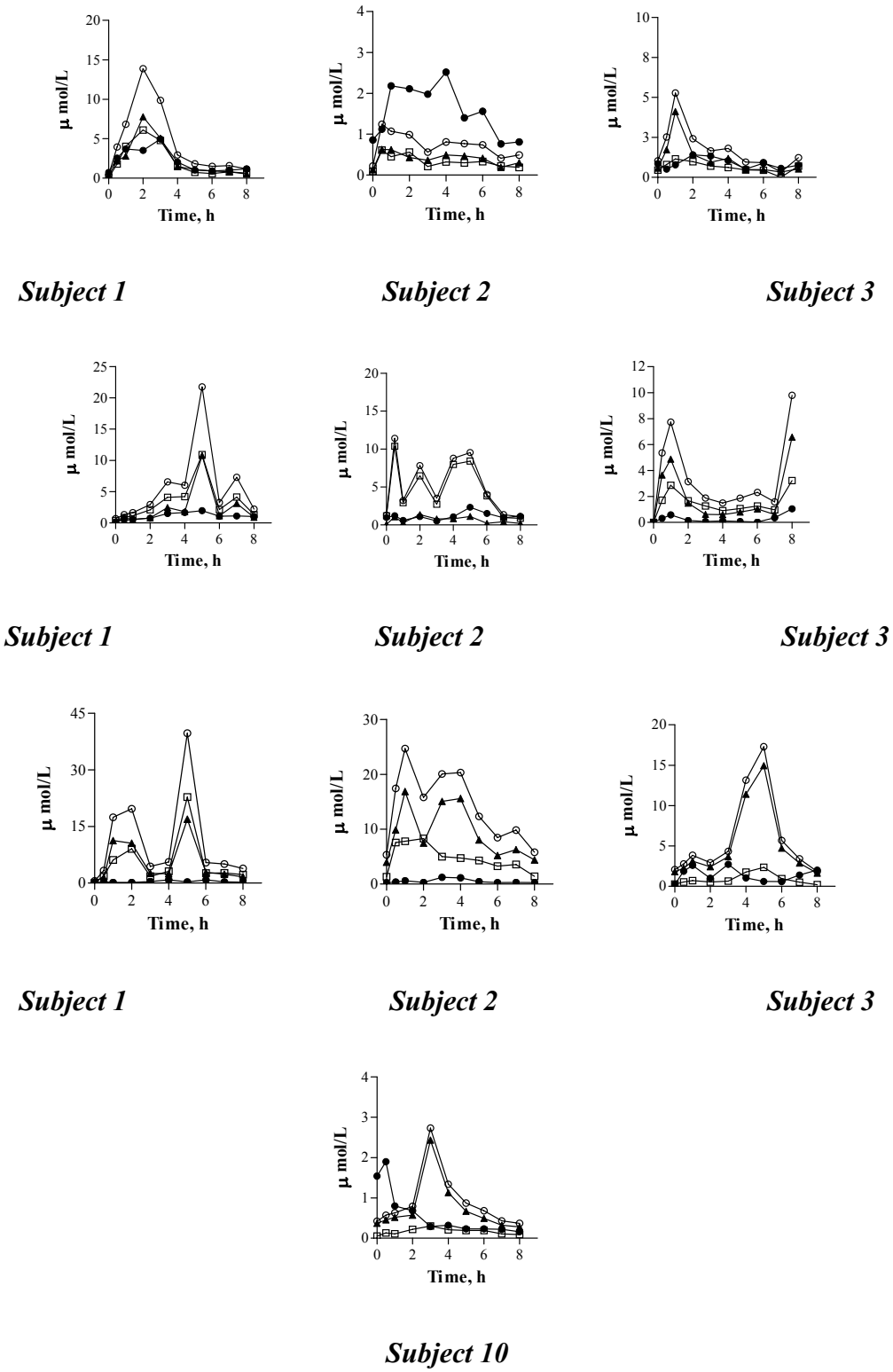

Figure 1. Pharmacokinetic profile of total chlorogenic acids (—○—), caffeoylquinic acids (—▲—), dicaffeoylquinic acids (—□—) and total cinnamic acids (—●—) in plasma samples of the individual subjects during 8 h after decaffeinated green coffee extract consumption.

Another possible explanation would be demethylation of the ferulic acid moiety of the ester and conversion of FQA into CQA, since the only difference between both classes of

compounds is the substitution of a methoxyl group by a hydroxyl group in the number 3 position of the aromatic ring of the cinnamic acid moiety (Figure 2).

Regarding individual isomers, the mean molar ratio of 5-CQA:4-CQA:3-CQA in green coffee was 1.2:1.0:1.1, while in plasma it was 6.0:1.4:1.0. The higher mean molar ratio of 5-CQA in plasma comparing to 4-CQA and 3-CQA increases evidence of preferential absorption of 5-CQA compared to 4-CQA and 3-CQA isomers, or of rapid metabolization and/or uptake of 3-CQA and 4-CQA by organs such as liver (Farah and Trugo, 2006; Monteiro et al., 2007) and adipose tissue. In the same way, the molar ratios 3,4-diCQA:4,5diCQA:3,5diCQA were 1.0:1.6:1.7 in green coffee and 1.7:1.4:1.0 in plasma. The higher molar ratios of 3,4-diCQA related to 4,5diCQA and 3,5diCQA in plasma than in the green coffee extract also suggests mechanisms favoring plasma levels of 3,5-diCQA as described for 5-CQA.

Although the amount of CGA offered through decaffeinated green coffee in the present study was 7 x lower than the amount offered through decaffeinated roasted coffee in our previous study (Monteiro et al., 2007), the average AUC of total CGA in the present study up to 4 h after treatment (duration of our previous study) was 1.4 x higher than the respective AUC in our previous study using roasted coffee. Even though both studies cannot be compared because of various differences, including subjects, the influence of the matrix in which CGA is consumed deserves investigation.

The urinary excretion of phenolic compounds increased after green coffee consumption. Sinapic, gallic, *p*-hydroxybenzoic, and dihydrocaffeic acids were the major phenolic compounds identified in urine after the extract consumption, followed by small amounts of cinnamates (4-CQA, 5-CQA, caffeic, ferulic, isoferulic and p-coumaric), vanillic and syringic acids. The 4 major urinary phenolic compounds are probably preferential metabolic products of the cinnamates identified in plasma, and vanillic and siringic acids are probably derived from a secondary metabolic pathway. Protocatechuic, dihydroferulic, benzoic and hippuric acids, which were identified in studies evaluating the urinary products of cinnamates from different food sources (Olthof et al., 2003; Rechner et al., 2001), were not identified in our study although they are consistent with the metabolic pathway presented in Figure 2.

The apparent bioavailability of CGA from the green coffee extract varied from 7.8 to 72.1% among the subjects, with mean of 33 ± 23%. This is the first study investigating the bioavailability of CGA as a family of compounds and also the first study evaluating the bioavailablity of CGA using plasma AUC values. The mean apparent bioavailability of 5-CQA, the only CGA evaluated in previous bioavailability studies was also 33 ± 27%. This result is in contrast with the very low absorption of 5-CQA estimated in rat and human studies (down to 0.1%) (Spencer et al., 1999; Dupas et al., 2006; Bourne and Rice-Evans, 1998). On the other hand, our results are in agreement with 33 ± 17% absorption estimated by Olthof et al. (2001), who analyzed ileostomy fluids from colostomized subjects during 24 h after the consumption of 2.8 mmol of isolated 5-CQA. The results by Olthof et al. were considered an overestimation (Nardini et al., 2002) because the amount of 5-CQA possibly lost during digestion was not taken into account, and because previous studies (Nardini et al., 2002; Azuma et al., 2000; Cremin et al., 2001) had been unable to identify intact chlorogenic acids in plasma. However, the results obtained by Olthoff et al. are actually very close to those in the present study and their estimate was probably correct because only a small portion of 5-CQA seems to be hydrolyzed in the digestive tract (Monteiro et al., 2007; Farah et al., 2006) and only a minor fraction is absorbed in the form of caffeic acid (Baer-Dubowska and Szaefer, 1998; present study).

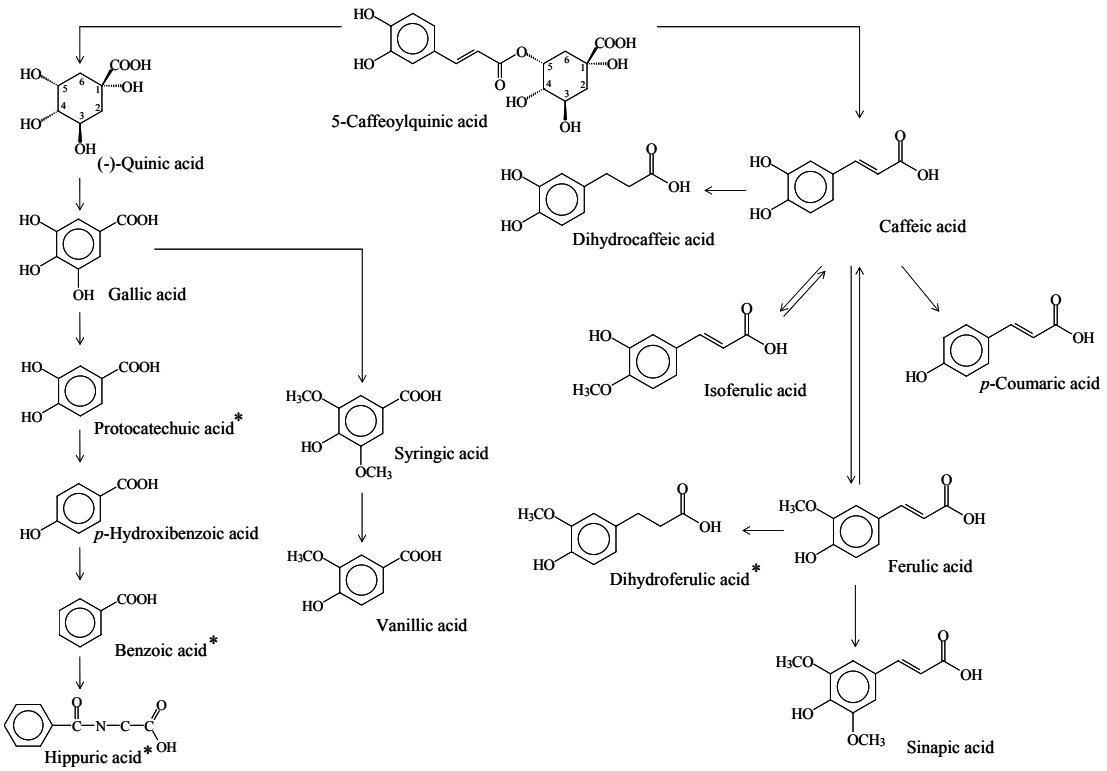

Figure 2. Proposed simplified scheme of metabolization of CQA to caffeic and quinic acids and other phenolic acids. *Not identified in the present study but possible intermediate or final metabolite according to the literature (Booth et al., 1957; Rechner et al., 2001). ***The authors adopted the IUPAC numbering system for chlorogenic acids.***

CONCLUSION

The present study confirms that CQA and diCQA isomers, which are major CGA compounds from coffee, are diferentially absorbed and/or metabolized throughout the human gastrointestinal tract. This study also increases evidence that urine is not a major excretion pathway of intact CGA compounds and their metabolites, and identifies sinapic, gallic, *p*-hydroxybenzoic, and dihydrocaffeic acids as major urinary metabolites of CGA in humans. In addition, this study shows that the main CGA compounds present in green coffee matrix are highly bioavailable in humans. A large inter-individual variation clearly exists in CGA absorption and/or metabolism in humans and requires further investigation regarding differences in genetic polymorphisms.

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Metabolism of Chlorogenic Acids After Coffee Ingestion in Ileostomy and Healthy Subjects

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SUMMARY

Chlorogenic acids are bioactive compounds found in coffee. Powerful antioxidants, they are becoming of great interest for their potential health promoting effects, possibly partly responsible for the positive epidemiology from coffee. However, human bioavailability of these compounds is not well known. We investigated via an ileostomy and a human clinical trial the metabolism of chlorogenic acids after coffee consumption. The ileostomy model was used to better understand small intestinal metabolism of those compounds via mass spectrum analysis of urinary and ileal excretion of chlorogenic acids. A healthy human model not only provided key information on plasma appearance of coffee bioactives but also highlighted the possible role of colonic metabolism and absorption of chlorogenic acids. Different extraction of fluids with or without enzymatic cleavage provided us with data on the types of metabolites obtained (parent compounds or smaller metabolites) and their possible conjugation as sulphates or glucuronides. After MS analysis, we observed that chlorogenic acids are most likely not absorbed as parent compounds but first metabolized to smaller phenolics prior to absorption. Indeed, caffeic and ferulic acid derivatives were detected in plasma or urine after coffee ingestion. Finally, we observed differences in urinary excretion or plasma appearance of certain of those phenolics in ileostomized compared to healthy humans. This would suggest not only that the small intestine is a key site of absorption of phenolic acids but also that the colon and the microflora play a major role in the metabolism of chlorogenic acids.

INTRODUCTION

Coffee contains phenolic compounds called hydroxycinnamates, which consist principally of chlorogenic acids, a family of trans-cinnamic acids conjugated with quinic acid. The main chlorogenic acid in coffee is 5-caffeoylquinic acid (5-CQA) (Clifford, 2000), although other caffeoylquinic (CQAs), feruloylquinic (FQAs) and di-caffeoylquinic (diCQAs) acids are present in significant quantities. Derivatives and phenolic metabolites of chlorogenic acids, like caffeic (CA) or ferulic (FA) acid have been studied for potential bioefficacy. However, there is a lack of data on the metabolism and biovailailability of the parent compounds and how they are being metabolized in humans. To investigate as precisely as possible the metabolism of those molecules, we ran 2 human studies.

The first study was performed with ileostomy volunteers to appreciate small intestinal metabolism of chlorogenic acids. Ileostomy subjects provide a valuable model for estimating the absorption of dietary antioxidants. By measuring the amount of a compound or metabolite

present in ileal fluid and excreted in urine we are able to estimate how much has disappeared in the small intestine and how much has been excreted by the body.

The second bioavailability study described here involved healthy volunteer in order to investigate if the colon may also play a role in metabolizing coffee bioactives.

STUDY DESIGN, MATERIAL AND METHODS

Ileostomy study

The aim of the study was to monitor the absorption of coffee chlorogenic acids by examining the compounds in ileal fluid not absorbed in the small intestine of ileostomy volunteers. An ileostomy is a surgically created opening in the abdominal wall, by which the end of the ileum is brought through to form a stoma, usually on the lower right side of the abdomen. The terminal ileum is pulled through the abdominal wall and a segment is turned back and sutured to the skin, leaving a smooth, rounded, everted ileum as the end of the ileostomy. The stoma is usually placed in the right lower quadrant and located on a flat surface. The colon and rectum are removed, suppressing their normal functions.

The study design was based on a single administration of coffee to five volunteers (3 males and 2 females) who have had their colon surgically removed. Samples of ileal fluid and urine were then collected at 0-2 h, 2-5 h, 5-8 h and 8-24 h following the ingestion of coffee. The volunteers were asked to follow a low polyphenol diet for 36 hours prior the beginning of the study and coffee samples were prepared by adding 200 mL of boiled distilled water to powdered instant coffee (145mg total chlorogenic acids). Subjects ingested the beverage within 5 min.

Ileal fluid was extracted using a solution of 1% formic acid in 50% aqueous methanol containing 20 mM of sodium diethyldithiocarbamate. Samples were homogenized, centrifuged, and the supernatant transfered to a clean tube. The pellet was extracted again using the same procedure. Pooled supernatant were then dried using a heated rotary evaporator. The residues were re-suspended in mobile phase containing 10% methanol, centrifuged, and finally filtered.

Chlorogenic acids and metabolites recovered in ileal fluid extracts were analysed using a Surveyor HPLC coupled to a LCQ Duo ion trap mass spectrometer. Five µL of extracted ileal fluid samples were injected into the Surveyor HPLC system. Following separation, hydroxycinnamates and metabolites were detected and quantified using PDA monitored at 325 nm and SIM, respectively. Identification of the parent compounds and metabolites was confirmed by CRM, collision energy 35%. Mean data were expressed as µmoles ± SE (n = 5) of 5-CQA equivalent for the CQAs, FQAs, di-CQAs, ρ-CoQAs, as well as the conjugated molecules, and as µmoles ± SE (n = 5) of 3-CQAL and 4-CQAL for the respective lactones quantified as aglycone and as conjugate.

Urine samples were defrosted and centrifuged. Urinary excretion of conjugated chlorogenic acids were analysed using a Surveyor HPLC coupled to a LCQ Duo ion trap mass spectrometer, as previously described. Twenty µL of samples were injected into the Surveyor HPLC system. Following separation, hydroxycinnamates and metabolites were detected and quantified using SIM. Identification of compounds was confirmed by CRM, collision energy 35%, as well as co-chromatography with sulphated and glucuronidated standards of phenolic acids (supplied by NRC). The chlorogenic acids excreted in urine samples were quantified on

SIM traces, and the values were expressed in μmoles ± SE equivalence of the corresponding standard.

Human clinical study

The primary objective was to investigate plasma bioavailability of coffee chlorogenic acids by measuring AUC over 12 h after ingestion of phenolic acids derived from chlorogenic acids (caffeic acid (CA), ferulic acid (FA), dihydroferulic acid (DHFA), dihydrocaffeic acid (DHCA), isoferulic acid (iFA)) in the blood (breakdown of chlorogenic acids after full enzymatic deconjugation). The study design was based on a single administration of coffee to nine healthy volunteers (330 mg total chlorogenic acids) and plasma collected at several time points over 12 h after ingestion.

Plasma samples were extracted via protein precipitation using ethanol, homogenized and finally centrifuged. The supernatant was transferred into a clean tube and the pellet extracted 2 more times again using the same procedure. Pooled supernatants were then dried under nitrogen flow at room temperature for ~2 h. The dried residue was dissolved in sodium phosphate buffer containing 1000 units of β-glucuronidase + 60 units of sulfatase + 0.1 units of chlorogenate esterase. Incubation followed for 1 h in a thermomixer. At the end of incubation, HCl and NaCl were added to stop the reaction. This mixture was then extracted 4 times by adding ethyl acetate, homogenized and centrifuged. Pooled upper phases were dried under nitrogen flow and the dried residue resuspended in water/acetonitrile containing 0.1% formic acid and finally filtered.

Analyses were performed on an Agilent 1100 quaternary pump LC system coupled to a 3200 Q TRAP mass spectrometer instrument equipped with a TurboIonSpray® ionisation source. HPLC analyses were run on a Zorbax SB-C18 reverse phase column (2.1 x 50 mm, 1.8 μm). Following separation, MS/MS detection was realized using the selected reaction monitoring (SRM) acquisition mode. The two most intense fragment ions of each compound were selected using a constant dwell times of 50 ms, resulting in a total scan time of 0.7 sec (including a 5 ms pause time between each SRM). Quantitative analysis was performed using the most intense SRM signal (SRM1) whereas the second SRM transition (SRM2) was used for analyte confirmation based on appropriate area ratio calculated from standard solutions. Data processing was performed using Analyst 1.4.2 software.

RESULTS

Ileostomy study

The recovery of the parent compounds was found to be an overall 55% of the amount ingested for the five volunteers, ranging from 35% to 72%. The compounds that appeared to be the least absorbed are the FQAs, with a recovery value of 75%, followed by 59% of the CQAs recovered in ileal fluid extracts. p-CoQA and di-CQAs acids were the least recovered, with percentages of 45% and 47%, respectively (Table 1).

Overall, 3-CQA and 3-FQA were recovered in higher but not statistically significant proportion in ileal fluid samples (74% and 87%, respectively) than their 4- (55% and 74%, respectively) and 5- (53% and 68%, respectively) counterparts. A small proportion of the CQALs were present in ileal fluid (6.5%). However, 58% was recovered mainly as sulfates (55%) and glucuronides (3%), leaving a potential 36% absorbed. Small amounts of sulfates and glucuronides of both CQAs and FQAs were also recovered (3.7% and 8.5% of the CQAs and FQAs ingested, respectively). Free and sulfated forms of CA and FA were also detected

in ileal fluid. The percentages unaccounted for, could represent the amount of compounds potentially absorbed by the human body and ranged from 17% for the FQAs, 36% and 37% for the CQALs and CQAs, and 53% and 55% respectively for the diCQAs and p-CoQA. The average recovery of chlorogenic acids and conjugated compounds in the ileal fluid extracts of the five volunteers was 71%, resulting in a combined potential absorption of the chlorogenic acids contained in instant coffee of 29% (Table 1).

Fifteen metabolites could be identified and quantified in urine samples. The only metabolites excreted in urine and initially present in the coffee were the three FQAs, representing 0.8% of the total amount of chlorogenic acids ingested, and accounting for 6.8%, 5.8% and 5.9% of the respective 3-, 4-, and 5-FQAs. FA sulfate appears to be the metabolite being excreted in highest amount, accounting for 2.6% of the amount ingested, followed by sulfated CA (1.8%). Also detected were sulfated 3-CQA and 4-CQA lactones (2.5% ingested dose). Small amounts of chlorogenic acid metabolites could also be quantified as sulphated DHCA (0.8%) and DHFA (0.2%), dehydrogenated molecules of CA and FA, and sulphated (0.1%) and glucuronidated iFA (1.0%), an isomer of FA.

Table 1. Chlorogenic acid parent compounds and metabolites recovered in ileal fluid collected from 5 volunteers over 24h following consumption of 200 mL of instant coffee (3.4 g solids). Data are expressed as percentage of the ingested dose.

Parent Compounds	% ingested dose recovered in ileal fluid	Parent Compounds	% ingested dose recovered in ileal fluid
3-CQA	74.0	3,4-diCQA	51.0
4-CQA	55.1	3,5-diCQA	40.3
5-CQA	52.8	4,5-diCQA	46.3
Total CQAs	59.2	Total di-CQAs	46.9
3-FQA	86.7	**Total parent compounds**	**55.2**
4-FQA	73.8		
5-FQA	67.6	**Metabolites**	
Total FQAs	74.9	Total CQA metabolites	3.7
3-CQAL	4.6	Total FQA metabolites	8.5
4-CQAL	8.9	Total CQAL metabolites	57.5
total CQAL	6.5	**Total metabolites**	**16.0**
4-pCoQA	40.3		
5-pCoQA	48.4	**Total parent compounds**	**71.2**
total pCoQA	45.0	**and metabolites**	

Human clinical study

The overall comparison of plasma coffee phenolics pharmacokinetic curves show 2 groups of metabolites. Both groups show biphasic curves with a first peak at ~1 h after ingestion and second 8-12 h after ingestion. The first group: CA, FA and iFA show a Cmax within the first hour after ingestion (Figure 1). The second group: DHCA and DHFA show the maximum concentration in plasma 8-12 h after ingestion (Figure 2).

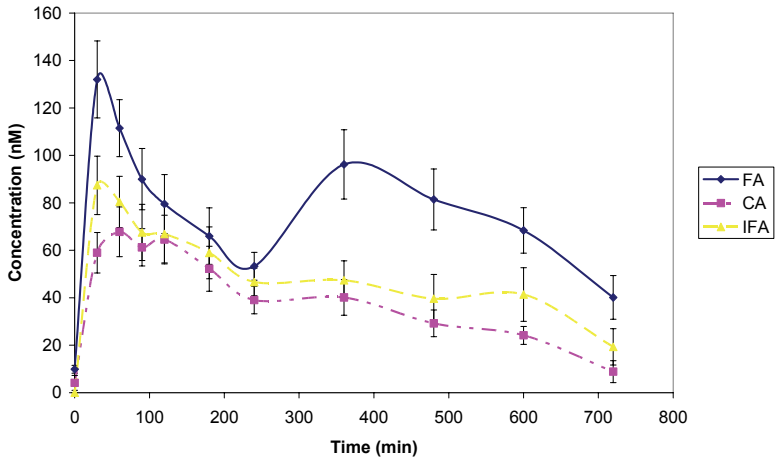

Figure 1. Mean of plasma kinetics of intestinal metabolites CA, FA and iFA in healthy volunteers after ingestion of 400mL of instant coffee.

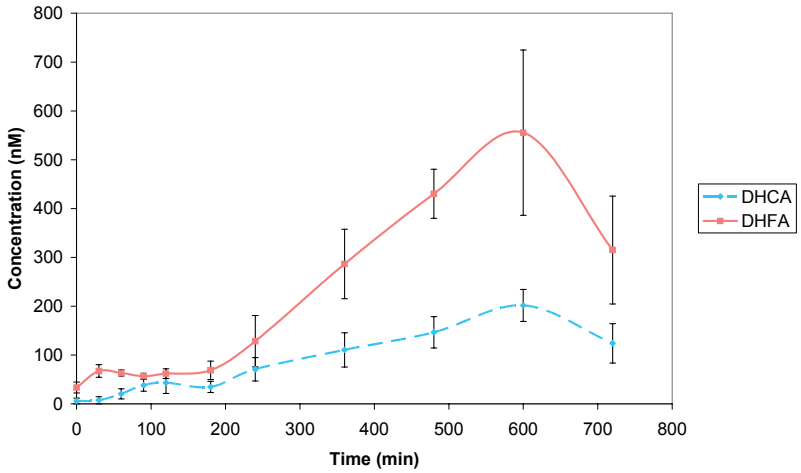

Figure 2. Mean plasma kinetics of colonic metabolites DHCA and DHFA in healthy volunteers after ingestion of 400mL of instant coffee.

Calculation of AUC was done over the first 12 h after ingestion despite the fact that we have a time point at 24 h. Statistically, extrapolating kinetic curves between 12 and 24 h is not feasible in the present case as there are not enough points in the "descent phase" of the kinetic curve. The 24h time point is only used to assess if plasma concentration of the compounds of interest are back to baseline but is not used in statistical analysis of AUC. Indeed, while some subjects showed levels close to baseline by 12 h for the second group of metabolites, other subjects failed to even show a Tmax as maximum plasmatic concentration of DHFA or DHCA was not even reach by 12 h. Therefore, it is difficult to fully comprehend the "descent" phase of those compounds and the extrapolation 12-24 h is impossible to do statistically. Therefore, we lacked the estimation of Tmax and Cmax for many subjects with respect to colonic metabolites.

Great interindividual variations were observed for Cmax and Tmax of small intestinal metabolites. Whether it is caffeic, ferulic or isoferulic acid, we observed Cmax varying between ~50 and ~150 nM with a Tmax varying between 30 and 240 min after ingestion.

DISCUSSION

With respect to the ileostomy trial, the total amount of hydroxycinnamates recovered in ileal fluid accounted for 71% of the amount ingested from a single cup of instant coffee (385 μmoles), leaving a potential 29% for absorption. These results are in agreement with previous, where seven ileostomy subjects were fed 2.8 mmoles of chlorogenic acid (equivalent to 7 times the dose given in this study), and recovery of chlorogenic acid in ileal fluid accounted for 67% (Olthof et al., 2001). A net absorption of chlorogenic acid of 8 % was observed, when 50 μM were loaded onto a jejunal and ileal segment of rat intestine (amount equivalent to three times as much as that given in this study, based on the weights of humans and rats) (Lafay et al., 2006).

Of the 71% recovered in ileal effluent, 55% comprised of non-metabolised compounds. The recovery of caffeic and ferulic acids accounted for 6.5% of the ingested amount, implying that a certain degree of hydrolysis of chlorogenic acids must have occurred prior absorption. The majority of conjugated metabolites recovered in ileal fluid comprised of sulfates (77%), with only 7% as glucuronidates, and 16% as free ferulic and caffeic acids. Those results are in keeping with the literature, suggesting the human small intestine epithelium as a site for phase II enzyme reactions, leading to sulfation, glucuronidation and / or O-methylation (Lin et al., 1999; Chen et al., 2003).

Although a phenomenon of efflux could be observed for the CQAs, FQAs and CQALs, no conjugates of di-CQAs or ρ-CoQAs could be identified, which could be explained by either a lack of conjugation occurring in the upper intestinal walls for those molecules, or by amounts that were below the limit of detection (1 ng of 5-CQA). The presence of caffeic and ferulic acids in their free and sulfated forms could be explained by i) an hydrolysis of the di-caffeoylquinic acids in either the stomach and / or small intestine, resulting in a molecule of CQA and a molecule of caffeic acid being produced; and / or ii) the hydrolysis of CQA and / or FQA releasing the simple phenolic acids. The presence of a higher proportion of free and conjugated caffeic acid compared to ferulic acid may suggest that the caffeoylquinic acids undergo a more extensive metabolism in the upper small intestine than the feruloylquinic acids. This may explain the higher percentage of intact FQAs recovered in ileal fluid, compared to the CQAs. Alternatively, it may suggest that the di-caffeoylquinic acids are not absorbed intact in the small intestine, but primarily hydrolysed to release molecules of caffeic acid. This may explain the lower recovery in ileal effluent of dicaffeoylquinic acids. Despite an apparent higher absorption of the CQAs in the small intestine compared to the FQAs, only traces of feruloylquinic acids but no CQAs could be detected in the urine of the five ileostomy volunteers. CQAs may then be metabolized to smaller by products and/or excreted more favourably in the bile compared to FQAs. In addition, 3-FQA appeared to be excreted to a larger extent compared to 4- and 5-FQA, despite being recovered in greater proportion in ileal fluid. This suggests that the chlorogenic acids might undergo an isomerisation in vivo, prior either further metabolism or excretion (Mateos et al., 2006). The urinary excretion of chlorogenic acids in the form of mainly sulfated ferulic and caffeic acids suggest that part of the hydroxycinnamic acids ingested from instant coffee are absorbed in the small intestine and extensively metabolised through hydrolysis, sulfation and methylation. The presence of low but detectable levels of DHCA and DHFA sulphate in urine may suggest that CA and / or FA has undergone microbial transformation. This suggests that despite the absence of colon, ileostomy volunteers have preserved a certain microflora that have moved up to the small intestine.

In this study, the recoveries of chlorogenic acids in ileal effluent and metabolites excreted in urine of the five ileostomy volunteers did not account for the entire amounts initially ingested

from the coffee beverage. The amount recovered in ileal fluid and urine averaged 79% of that found in coffee, ranging from 60% to 93%. This may be explained by i) stores of compounds in various tissues of the body and / or ii) further metabolism of compounds that have not been identified by LC-MS.

Before the present clinical trial in healthy volunteers, only 2 other reports have investigated CGA appearance in plasma after coffee ingestion. The first one reported data over 2 h (Nardini et al., 2002) and the other over 4 h (Monteiro et al., 2007) after coffee ingestion. Therefore, detection of what appeared to be major colonic metabolites (appearing 8-10 h after ingestion) has not been fully investigated. In addition, Nardini et al. measured only appearance of caffeic acid at 1 and 2 h after ingestion. Monteiro et al. investigated parent compounds (CQA, FQA and di-CQA) after giving a much higher dose than in the present study (1300 mg vs 330 mg total CGA). While both Nardini and Monteiro reported the presence of caffeic acid in plasma after coffee ingestion, the present study has additionally identified the presence of FA and iFA that follow similar kinetics than CA (maximum plasma appearance 1-2 h after ingestion). Therefore, those metabolites are more likely to be absorbed at the level of the small intestine and metabolized by the small intestine or the liver. We did not detect 5-CQA or any other parent compounds like Monteiro did mostly because we used a full enzymatic cleavage method. This included the use of an esterase that cleaved all possible CQA, FQA or di-CQA present in plasma to release only CA and FA. Therefore, we detected CA, FA and iFA but the hypothesis that parent compounds may be absorbed as such cannot be excluded from the data we obtained. Additionally, the presence of FQA in urine of the ileostomy patients described above also reinforces the idea that plasma kinetics should be further studied without esterase cleavage and greater method development to detect the parent compounds in addition to the metabolites already described.

A major finding in the present study is the detection of colonic metabolites, namely DHFA and DHCA in significant concentration (Cmax > 1 μmol in some subjects for DHFA). This important piece of information could not be obtained by the ileostomy trial as subjects involved in that study lacked a colon and therefore a proper microflora. Some sulfated DHCA was detected in ileostomy volunteers but the levels were much lower than in healthy subjects. This finding reinforces the idea that a colon and a microflora are essential to produce significant levels of dehydro- forms of phenolic acid. On the other hand, we did not obtain complete kinetics of those dehydro- metabolites for most healthy subjects as after 12h after ingestion, Tmax was still not reached for some subjects or was not back to baseline for others. To our knowledge, only one other report showed the presence of DHCA and DHFA in plasma after CGA consumption from artichoke leaf extract (Wittemer et al., 2005). The authors also found significant amounts of DHCA appearing in plasma and both colonic metabolites showed a late Tmax at about 6 h. In the present study, Tmax for DHFA and DHCA was estimated ~10-12 h after ingestion, much later than what has been reported by Wittemer et al. Thus, the matrix and form of delivery of the chlorogenic acids modulates pharmacokinetics parameters (especially Tmax). Ingestion of chlorogenic acids with food (breakfast given with beverage in the present study) or without (Wittemer et al.) may also contribute to explaining those differences. Indeed, adding a food source may interefere with gastric emptying and gut transit time so that Tmax may be significantly shorter or longer, depending on the food source. This hypothesis will certainly deserve further investigation.

CONCLUSIONS

Throughout the details of those ileostomy and clinical trials, we identified several key points. The small intestine is a key site of metabolism, cleavage, conjugation, efflux and absorption of chlorogenic and phenolic acids. The extent and type of conjugation depends on the

individual as reflected in the ileostomy trial. Time and concentration of phenolics appearing in the blood also vary between individuals. In the clinical trial with healthy subjects we also identified the colon as another key site of metabolism for coffee chlorogenic and phenolic acids. Major variability in the production of those colonic phenolics underlies the key role of the microflora in modulating the bioavailability of coffee bioactives.

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Antioxidative Activity of Coffee Extracts Depending on Roasting and Extraction Conditions

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SUMMARY

Coffee exhibits significant antioxidative activity that is essentially derived from coffee polyphenols (i.e. chlorogenic acids). Upon roasting, chlorogenic acids (CGAs) are for a great part transformed through *Maillard*-type reactions into more complex macromolecules (i.e. melanoidins), which preserve their antioxidative activity. The level and characteristics of extracted antioxidant species present in a coffee beverage was investigated in lab-scale systems. Green *Robusta* coffee was from Vietnam. Three roast levels (i.e. light, medium, dark) were prepared in a Neotec roaster. The ground coffees (~500 µm) were sequentially extracted at 100 and 180 °C using a Dionex extractor. The extracts were further analyzed for their total phenol (TPs) content using *Folin-Ciocalteu* assay and for their antioxidative capacity using ABTS radical scavenging assays (e.g. $ABTS^{\bullet+}$). The relative contributions of CGAs and melanoidins in coffee beverages were investigated with regard to roasting and extraction conditions. Coffee is rich in phenolic species exhibiting antioxidative activity. They are for a great part readily extractable at 100 °C (~290µmol/g GC d.b.), but additional amounts can be extracted at 180 °C (126-217 µmol/g GC d.b.). Roasting leads to a sharp decrease of the free phenols (i.e. CGAs) while new antioxidant species are generated. While in green coffee extracts free phenols account for above 60% of the antioxidative activity, their contribution decreases to below 20% in roasted coffees. The remaining part of the antioxidative capacity is most likely brought along by bound phenols (i.e. melanoidins) generated during roasting. Non-phenolic antioxidants generated during roasting could also contribute to a minor part of the overall antioxidative capacity.

INTRODUCTION

For many consumers, coffee firstly represents a pleasurable experience appealing to the senses by its unique aroma balance, taste and mouthfeel. In the last decade, there has been increasing scientific evidence showing that moderate coffee consumption (i.e. 3-5cups/day) positively impacts human health. Epidemiological and clinical studies suggested that coffee consumption may be associated with a reduced risk of chronic diseases (e.g. cancer, cardiovascular disease, inflammatory disease, type 2 diabetes, Parkinson). Coffee phenolics (i.e. chlorogenic acids, CGAs) and related substances (e.g. *Maillard* reaction products, melanoidins) have been proposed as being key players in these protective effects of coffee. Depending on the protective effect, various action mechanisms have been proposed for phenolic and associated substances such as free radical scavenging effect, induction of detoxifying enzymes, and regulation of glucose metabolism (Hoelzl et al., 2006; Coughlin, 2006; Bidel and Tuomilehto, 2006).

Coffee is one of the richest sources of CGAs (7-10%). Upon roasting, CGAs are almost entirely transformed during non-enzymatic browning reactions, being essentially incorporated into more complex macromolecules (i.e. melanoidins). The incorporation of CGAs in melanoidins has recently been studied. Different incorporation mechanisms of CGA moieties

(i.e. cinnamic acid, quinic acid) were proposed including ester (Leloup et al., 1995), non-ester (Nunes and Combra, 2007; Bekedamet al., 2008) or ionic (Delgado-Andrade et al., 2005) bonds.

CGAs through their phenolic moiety are powerful antioxidants *in vitro* (Pannala et al., 2001). During roasting, despite profound chemical changes affecting CGAs, the antioxidative properties are preserved and most likely transferred into melanoidins (Charurin et al., 2002; Borrelli et al., 2002). The antioxidative activity of coffee brews has been characterized using various assays for the effect of brewing procedure, coffee origin and roasting level. Brewing methods (i.e. filter, Italian, Espresso) did not impact the antioxidative activity (Parras et al., 2007). *Robusta* brews exhibited slightly higher antioxidative activity than *Arabica* (Daglia et al., 2000). Brews from lighter roast presented higher antioxidative activity compared to brews prepared from darker roast as measured by $ABTS^{\bullet+}$ radical scavenging assay (Parras et al., 2007; Del Castillo et al., 2002; Gomez-Ruiz et al., 2008). Electron spin resonance using different stabilized radicals further demonstrated that upon roasting the contribution of CGAs to antioxidative activity decreased, while that of the high molecular weight melanoidin increased (Cämmerer and Kroh, 2006; Bekedam et al., 2008).

High molecular weight molecules such as melanoidins are only partially extracted at moderate temperature as upon brewing (Clarke and Macrae, 1987). The objective of this work was thus to evaluate for different roast levels the additional extraction of antioxidant species in the temperature range of 100-180 °C. The antioxidative activity was assessed using $ABTS^{\bullet+}$ assay. The contribution of the major CGAs and melanoidins to the antioxidative activity was estimated.

MATERIAL AND METHODS

Chemicals

All reagents and solvents were of analytical grade. Chlorogenic acids (i.e. 3-CQA, 4-CQA, 5-CQA, 3,5diCQA, 3,4diCQA, 4,5diCQA) were supplied by Chengdu Biopurify Phytochemicals Ltd (Chendu, China). 3-FQA, 4-FQA and 5-FQA were synthesized as previously described (Huyinh-Ba, 1995). All other chemicals were purchased from Sigma-Aldrich (Germany).

Green and Roasted Coffees

Green *Robusta* coffee was from Vietnam. 3 kg-batches of green beans were roasted in a Probat RT3 roaster for 8 minutes. Roasting temperatures were respectively 280, 285 °C and 300 °C to produce light-, medium- and dark-roasted coffees. Organic losses (OL_{RG}) expressed on green coffee d.b. were calculated from initial and final coffee weights (i.e. w_{GC}, w_{RG}) and humidities (i.e. H_2O_{GC}, H_2O_{RG}) as follows:

$$OL_{RG}(\%) = \frac{\left(w_{GC}x(100 - H_2O_{GC})\right) - \left(w_{RG}x(100 - H_2O_{RG})\right)}{\left(w_{GC}x(100 - H_2O_{GC})\right)}x100$$

Roasted coffees were further ground on a Ditting grinder to an average grinding size of ~600 µm, whereas the softer green coffee had to be ground on a Retsch Ultra Centrifugal Mill ZM 200 using a 2 mm grid. Its average grinding size was ~1000 µm.

Coffee Extraction

Green / roasted coffees were extracted using a bench-scale Dionex extractor ASE200. A two-step extraction procedure was applied consisting firstly in an extraction step at moderate temperature (i.e. 100 °C/10 min) followed by a second extraction step at high temperature (i.e. 180 °C/10 min). The extraction procedure applied to 3 g (W_{RG}) of coffee was repeated ~20 times (n) to gather enough material for further analyses. The extract weight ($W_{Extract}$) and concentration ($C_{Extract}$) were evaluated. The extracts were then freeze dried. Extraction yield of each extraction step was further calulated on green coffee d.b. using the following equation:

$$Y_{GC}(\%) = \frac{W_{Extract} x C_{Extract} x(100 - OL_{RG})}{nx W_{R\&G} x(100 - H_2O_{GC})} x100$$

Total Phenol content by *Folin-Ciocalteu* Assay

Coffee solutions were prepared from the freeze-dried samples at different concentrations in the range of 0.1-1 mg/mL. On each sample, interfering compounds were recovered through a cleaning step using SPE HLB cartridge (Waters) and further assayed. 300 µL of sample or standard were added to 1.5 mL of *Folin-Ciocalteu*'s phenol reagent (0.2N). The mixture was incubated for 2 min at room temperature. 1.2 mL of sodium carbonate solution at 75 g/L were then added. The mixture was vortexed and incubated during 15 min at 50 °C in a water bath. The absorbance was finally measured at 760 nm and corrected for the absorbance of the interfering material (Georgé et al., 2005). Total phenol (TPs) content was expressed in µmol of 3-CQA equivalent. The different CGAs isomers were also assayed and the relative response to 3CQA was calculated for each isomer.

CGAs content by HPLC

Freeze-dried extracts were dissolved in methanol/water (80/20) at concentrations between 0.04 and 0.3 $mg.mL^{-1}$ depending on the sample. The separation of CGAs was performed by reverse phase chromatography followed by UV detection as previously described (Leloup et al., 1995). The identification and quantification of main CGAs were performed using the commercial standards.

$ABTS^{\bullet+}$ Radical Cation Assay

The freeze-dried extracts (125 mg) were dissolved in MilliQ water (25 mL) and further diluted 50times. Standard of the different chlorogenic acid isomers were prepared in the concentration range 0-50 µg/mL.

A stable stock solution of $ABTS^{\bullet+}$ was produced by reacting a 7 mM aqueous solution of ABTS with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature overnight before use. The day of the analysis the stock $ABTS^{\bullet+}$ solution was diluted in ethanol to reach an absorbance of 1.2 at 734 nm.

$ABTS^{\bullet+}$ solution was used as mobile phase at a flow rate 1mL/min. Direct flow injection of the coffee solutions or standards was carried out through an HPLC injection valve. A reaction coil allowed the reaction to occur for ~1.5 minutes (Pellegrini et al., 2003).

The height of the decolorization peak was measured at 734 nm. The antioxidant capacity was evaluated as chlorogenic acid equivalent using the calibration curve established for 3CQA.

The method accuracy was ± 4%. The different CGAs isomers were also assayed and the relative response to 3CQA was calculated for each isomer.

RESULTS AND DISCUSSION

Extraction of coffee solids

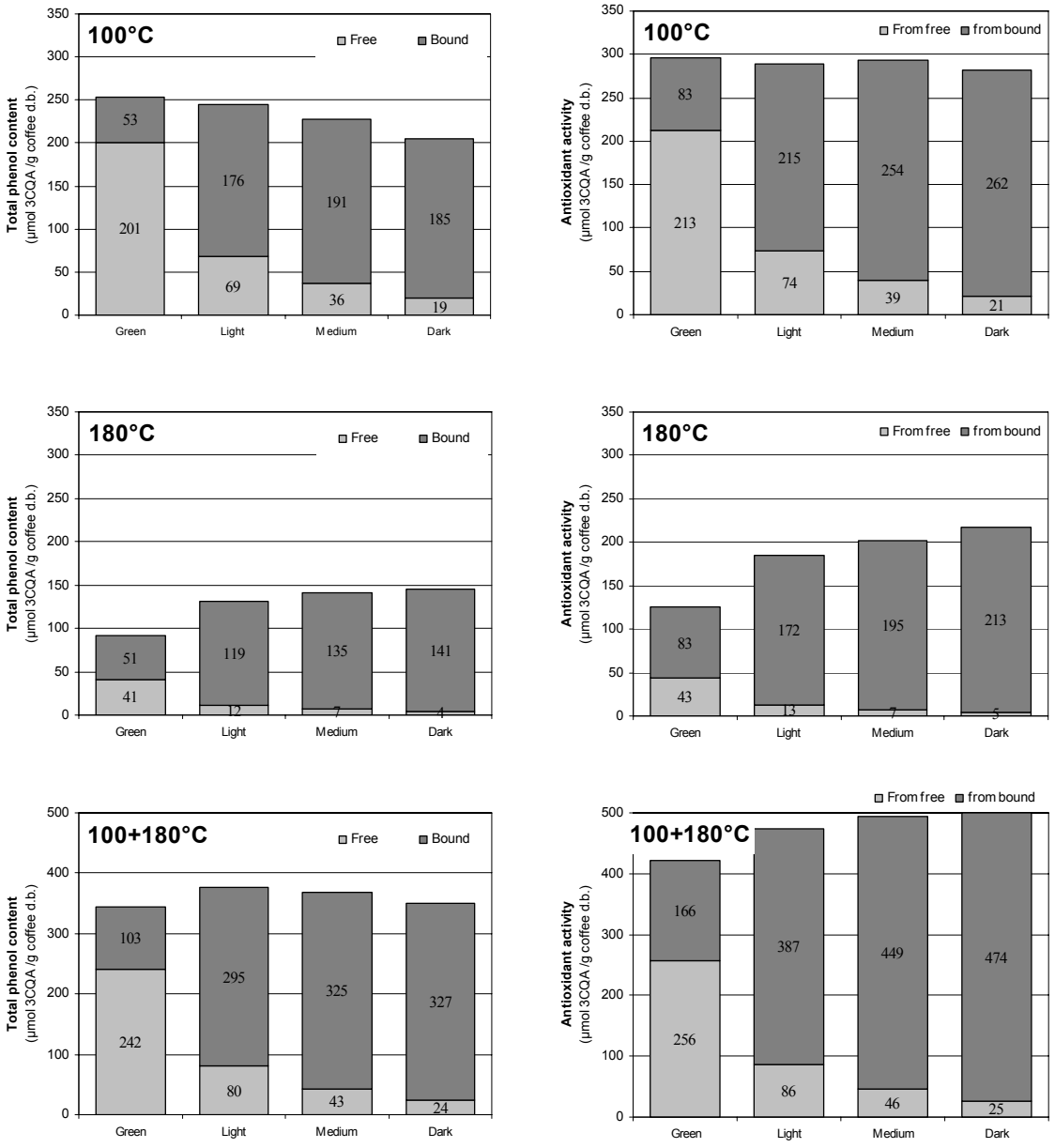
Table 1 presents the extraction level obtained from the two-step extraction procedure. The results are expressed on coffee as such and further recalculated on dry green coffee basis. The cumulated extraction level expressed on dry green coffee significantly increases from ~44% for green coffee to about 51-53% when roasted. The contribution of each extraction step is approximately half/half. The extraction level expressed on dry green coffee remains almost constant for roasted coffees. Low molecular weight compounds are essentially extracted during the 100 °C extraction step, whereas larger molecules including carbohydrates and melanoidins are extracted during the 180 °C step (Leloup and Liardon, 1993; Clarke and Macrae, 1985). The expression of extraction yield and further that of extracted compounds on dry green coffee is the only acceptable way to assess the fate of coffee solids during roasting and extraction. All further results will be assessed on green coffee dry basis.

Table 1. Organic losses and extraction level reached in the two-step extraction procedure. Results expressed on coffee as such and calculated on a green coffee dry basis.

Coffee	H_2O (%)	OL (% d.b.)	$Y_{RG/1o0}$ (%)	$Y_{GC/100}$ (% d.b.)	$Y_{RG/180}$ (%)	$Y_{GC/180}$ (% d.b.)	$YR_{C/100+180}$ (%)	$Y_{GC/100+180}$ (% d.b.)
Green	11.6	0.0	20.2	22.8	18.6	21.1	38.8	43.9
Light	2.2	6.8	24.8	23.6	29.0	27.6	53.8	51.3
Medium	1.9	8.0	26.6	24.9	29.1	27.2	55.7	52.2
Dark	1.9	9.5	27.4	25.3	29.9	27.6	57.3	52.9

Table 2. Composition in phenolic compounds in coffee extracts prepared with the two-step extraction procedure. CGAs are evaluated by liquid chromatography and TPs measured with the *Folin-Ciocalteu* assay expressed in µmol of 3CQA. The response of CGAs standard in *Folin* assay is also expressed in µmol of 3CQA.

	Coffee	3CQA	4CQA	5CQA	3FQA	4FQA	5FQA	3,4diCQA	3,5diCQA	4,5diCQA	Total CGAs	Total TPs	Free TPs	Bound TPs
		(µmol/g GC d.b.)										(µmol ~3CQA/g GC d.b.)		
100°C	Green	106.8	18.2	17.7	2.2	3.4	18.9	8.1	6.0	8.8	190	254	201	53
	Light	24.7	12.7	14.9	2.8	3.4	6.5	1.7	0.9	1.6	69	245	69	176
	Medium	12.1	7.1	8.6	1.8	2.3	3.7	0.7	0.4	0.6	37	227	36	191
	Dark	6.0	3.9	4.8	1.3	1.5	2.1	0.3	0.2	0.3	20	205	19	185
180°C	Green	15.5	5.6	6.6	0.7	0.9	2.3	2.2	1.4	2.1	37	91	41	51
	Light	2.9	2.0	2.6	2.0	0.6	0.8	0.4	0.2	0.4	12	131	12	119
	Medium	1.7	1.3	1.7	0.4	0.5	0.5	0.2	0.1	0.2	7	141	7	135
	Dark	1.1	0.8	1.1	0.3	0.4	0.4	0.1	0.1	0.1	4	145	4	141
100+180°C	Green	122.3	23.8	24.3	2.9	4.3	21.3	10.3	7.4	11.0	228	345	242	103
	Light	27.6	14.8	17.5	4.8	4.1	7.3	2.1	1.1	1.9	81	376	80	295
	Medium	13.9	8.4	10.3	2.3	2.8	4.2	0.9	0.5	0.8	44	368	43	325
	Dark	7.1	4.7	5.9	1.6	1.9	2.5	0.4	0.2	0.3	25	350	24	327
Response ~3CQA		1.00	1.18	0.87	0.74	0.69	0.65	1.72	1.81	1.83				



(a) Total phenolic compounds measured by *Folin* **(b)** Antioxidative activity measured by $ABTS^{\bullet+}$

Figure 1. (a) TPs and (b) antioxidative activity extracted at 100 and 180 °C from *Robusta* coffee roasted at different levels measured by *Folin-Ciocalteu* assay and $ABTS^{\bullet+}$, respectively. The distribution between free (i.e. CGAs) and bound (i.e. melanoidins) phenols expressed in µmol 3CQA were calculated from CGAs composition and their reactivity in the respective assays.

The extracts were characterized for their phenolic compounds that are the main antioxidant species in coffee. Table 2 presents the levels of extracted TPs and CGAs recovered from the two-step extraction procedure. The TPs level in the cumulated extracts is rather constant 360 ± 15 µmol 3CQA/g GC d.b. for all roasts. Most of the TPs (i.e. 58-74%) are already recovered at 100 °C. The TPs extractability at 100 °C decreases with roasting (i.e. 254 to 205 µmol/g GC d.b.), whereas a reverse trend is observed at 180 °C (i.e. 91 to 145 µmol/g GC d.b.).

For each extract, the balance in free (i.e. CGAs) and bound (i.e. melanoidins) phenols was calculated from the CGAs composition and their reactivity in the *Folin* assay. Figure 1a presents the TPs distribution between free and bound species expressed in µmol of 3CQA. In green coffee the TPs are essentially composed of free phenols (i.e. 70%), mostly extracted at 100 °C. A small proportion of bound phenols (i.e. 30%) are extracted and possibly generated during both extraction steps. In roasted coffee, the proportion of free species decreases below 20%, while that of bound phenols increases. The bound phenols are generated during roasting[4], and extracted for 60% at 100 °C and 40% at 180 °C.

Antioxidant activity of coffee extracts

Antioxidants can deactivate radicals by two major mechanisms: Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET). Thermodynamically, a compound with a redox potential lower than 0.68V, such as many phenolic compounds can reduce $ABTS^{\bullet+}$ (Huang et al., 2005). The antioxidative activity of coffee extracts was thus assessed using the $ABTS^{\bullet+}$ radical scavenging assay. This method however has no direct physiological significance.

The CGAs standards were assessed for their antioxidative activity. Table 3 presents the antioxidative capacity of each CGA isomer relatively to that of the main chlorogenic acid 3CQA (CAS numbering). The antioxidative activity of CGAs decreases with the presence of a methoxy group as in FQA (i.e. ~0.85), and is almost doubled in presence of two phenolic moieties as in diCQA (i.e. ~1.99). For the first time the relative antioxidative activity of coffee CGAs isomers was evaluated with accuracy. These results differed from on-line HPLC evaluation reported by Stalmach et al. (2006), but presented similar trends with reported observations on ferulic acid (Rice-Evans et al., 1996) and bioactivity of diCQA (Yoshimoto et al., 2002). In view of allowing a better comparison with data from the literature, the antioxidative activity of 3CQA was compared to that of Trolox. A 3CQA concentration of 1.15 ± 0.02 mM was equivalent to that of 1mM of Trolox (TEAC value) as measured by the $ABTS^{\bullet+}$ radical scavenging assay. This agrees with the factor previously reported (Del Castillo et al., 2002).

Table 3. Relative antioxidative activity of CGAs isomers measured by $ABTS^{\bullet+}$ assay and calculated versus 3CQA (CAS numbering).

CGAs isomer	3CQA	4CQA	5CQA	3FQA	4FQA	5FQA	3,4diCQA	3,5diCQA	4,5diCQA
Relative antioxidant activity	1.00	1.19	0.98	0.85	0.81	0.89	1.93	2.03	2.02

Table 4 presents the antioxidative activity of coffee extracts obtained at 100 and 180°C expressed in µmol of 3CQA. The antioxidative activity recovered at 100 °C was fairly constant with the roasting level ~290µmol/g GC d.b. That recovered at 180 °C increased from 126µmol/g GC d.b. in green coffee to 217 µmol/g GC d.b. in darker roast. The range of total antioxidative activity was 422-500 µmol/g GC d.b., from which 60-70% present in the 100 °C extract and 30-40% in the 180 °C extract. When recalculated on either roasted coffee or coffee extract, the present results were in line with previous observations (Delgado-Andrade et al., 2005; Del Castillo et al., 2002; Cämmerer and Kroh, 2006).

For each extract, the contribution of free (i.e. CGAs) and bound (i.e. melanoidins) phenols to the antioxidative activity was calculated from the CGAs composition and their reactivity in the $ABTS^{\bullet+}$ assay (Table 4, Figure 1b). In green coffee the antioxidative activity essentially results from CGAs (i.e. 60%), mostly extracted at 100 °C. The remaining antioxidative activity is provided by bound species, equally brought along by the extracts at 100 and 180 °C. In roasted coffee, the contribution of CGAs to the antioxidative activity decreases

below 20%, while that of bound species increases. The antioxidant activity imparted by the bound species is 55% at 100 °C and 45% at 180 °C.

Table 4. Composition in phenolic compounds in coffee extracts prepared with the two-step extraction procedure. CGAs are evaluated by liquid chromatography and TPs measured with the *Folin-Ciocalteu* assay expressed in μmol of 3CQA. The response of CGAs standard in *Folin* assay is also expressed in μmol of 3CQA.

	100°C			180°C			100+180°C		
Coffee	Total	From free	From bound	Total	From free	From bound	Total	From free	From bound
	(Antioxidant activity in μmol of 3CQA/g GC d.b.)								
Green	296	213	83	126	43	83	422	256	166
Light	289	74	215	185	13	172	473	86	387
Medium	293	39	254	202	7	195	495	46	449
Dark	283	21	262	217	5	213	500	25	474

The correlation between the antioxidative activity measured by $ABTS^{\bullet+}$ and the TPs evaluated by *Folin-Ciocalteu* in coffee extracts is shown in Figure 2 for free and bound phenolic species. A very good correlation is found for both free and bound phenolic species (i.e. R^2~1,R^2~0.95). However, in the case of bound phenolic species the coefficient of 1.38 could indicate either that bound phenolic species have higher reactivity compared to free species or most likely that other antioxidant species are generated during roasting and extraction.

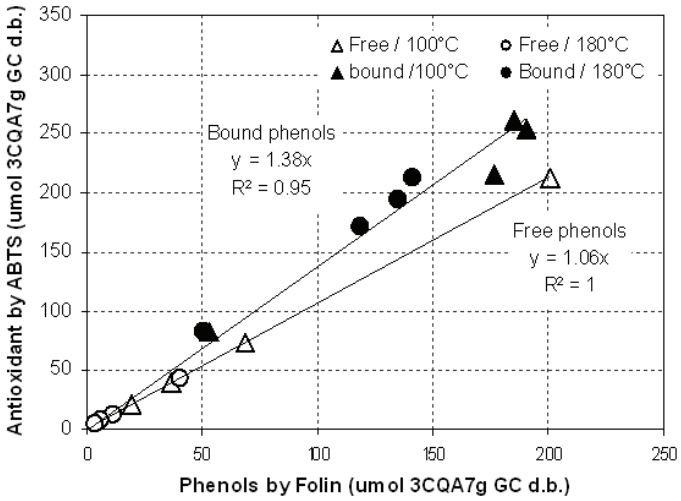


Figure 2. Correlation between antioxidative activity measured by $ABTS^{\bullet+}$ and phenols evaluated by *Folin-Ciocalteu*. The correlation is investigated for the free and bound species. All values expressed in μmol 3CQA/g GC d.b.

In conclusion, coffee is rich in phenolic species exhibiting antioxidative activity. They are for a great part readily extractable at 100 °C (~290 μmol/g GC d.b.), but additional amount can be extracted at 180 °C (126-217 μmol/g GC d.b.). Roasting results in a sharp decrease of the free phenols while new antioxidant species are generated. Whereas in green coffee extracts free phenols account for above 60% of the antioxidative activity, their contribution decreases to below 20% in roasted coffees. The remaining part of the antioxidative capacity is brought along by bound phenols (i.e. melanoidins) generated during roasting. Non-phenolic antioxidants generated during roasting could also contribute to a minor part of the overall antioxidative capacity.

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Plasma Antioxidant Capacity but not Endogenous Non-Enzymatic Antioxidant Levels Increases After Acute Coffee Intake

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SUMMARY

Coffee is one of the most widely consumed beverages in the world and a significant source of phenolic compounds with antioxidant properties. This work aimed at investigating the effect of acute coffee intake on human plasma antioxidant capacity, evaluating the contribution of plasma endogenous nonenzymatic components to the observed responses. In a cross-over design, eleven subjects consumed, after overnight fasting, a standardized meal with or without instant coffee. Blood was drawn before and 90 min after each treatment. At both time points, the following analyses were performed: plasma antioxidant capacity (measured by FRAP – *Ferric Reducing Ability of Plasma* – and TRAP – *Total Radical-trapping Antioxidant Parameter*), vitamins C and E, albumin, bilirubin and uric acid. FRAP and TRAP percent responses after coffee ingestion (increases of 2.2% and 6.1%, respectively) were higher ($p < 0.05$) than those observed after water ingestion (decreases of 2.3% and 1.7%, respectively). FRAP and TRAP net percent variations (4.5% and 7.8%, respectively) were not statistically different. After treatments, FRAP and TRAP responses were not correlated with levels of endogenous compounds, which remained unchanged. These results indicate that endogenous plasma antioxidant components do not contribute to the coffee-induced acute antioxidant plasma response.

INTRODUCTION

Coffee is a valuable primary product in world trade and is among the most consumed beverages worldwide. Therefore, during the last decades, the consequences of coffee consumption on human health have been extensively studied. Reduction in the risk of several human chronic diseases, such as liver and colon cancers, type 2 *Diabetes mellitus*, and Parkinson and Alzheimer diseases, was associated with coffee consumption (Higdon and Frei, 2006). The beneficial health effects of coffee are possibly explained by the fact that coffee is a significant source of phenolic compounds with antioxidant properties (Farah and Donangelo, 2006).

In vitro studies have shown that coffee has a remarkable iron-reducing activity (Moreira et al., 2005) and the highest antioxidant capacity (AC) when compared to other commonly consumed beverages also rich in phenolic compounds such as red wine and green tea (Pellegrini et al., 2003). Despite the promising *in vitro* findings, only few studies evaluated the plasma antioxidant response to coffee consumption in humans. One study reported that plasma AC, measured by the TRAP assay and the Crocin test, significantly increased after acute coffee administration (Natella et al., 2002). As this increase was accompanied by enhanced levels of uric acid in plasma, the authors hypothesized that the response could be

linked to both the effect of coffee on plasma uric acid and to direct absorption of coffee phenolic compounds. More recently, another *in vivo* study observed a significant increase in plasma AC measured by the FRAP assay after acute coffee consumption but found that albumin, bilirubin and uric acid levels in plasma were not significantly altered (Ribeiro-Alves, 2006). Therefore, the contribution of plasma endogenous antioxidant components to the plasma antioxidant capacity in response to coffee intake in humans is still unclear.

Besides that, the literature recommends the simultaneous use of assays based on the two major mechanisms of free radical deactivation, *Hydrogen Atom Transfer* (HAT) and *Single Electron Transfer* (SET) (Prior et al., 2005). The use of a single assay does not seem to be adequate to reflect the total antioxidant capacity of a complex biological sample such as plasma. FRAP and TRAP are both hydrophilic assays, based on SET and HAT mechanisms, respectively (Huang et al., 2005), and were previously shown as suitable for the assessment of plasma AC after coffee consumption (Natella et al., 2002; Ribeiro-Alves, 2006), although they have not yet been tested simultaneously.

The objectives of this study were to assess plasma AC in response to acute coffee consumption, using both the TRAP and FRAP assays, and to evaluate the contribution of endogenous plasma nonenzymatic antioxidant components to the observed response.

SUBJECTS AND METHODS

Subjects

Eleven healthy subjects (4 men and 7 women) aged 22-57 years were recruited at the Universidade Federal do Rio de Janeiro (UFRJ). Subjects were instructed to avoid the consumption of foods and beverages containing phenolic compounds and caffeine for 2 days prior to the experiment. Throughout the remaining days of the study, subjects were advised to maintain their usual diet. The study protocol was approved by the Ethical Committee of Clementino Fraga Filho Hospital at UFRJ, and written informed consent was obtained from each subject.

Study protocol

Test (coffee beverage) and control (water) treatments were done in a randomized crossover design with a 7d interval between tests. After an overnight (10 h) fast, a venous blood sample was taken at time 0 (baseline). Immediately after baseline blood collection, a standard amount of 200 mL of water or freshly prepared instant coffee beverage was offered to each subject, together with two slices of white bread (50 g). The coffee beverage (4% w/v) was prepared by mixing 8g of instant coffee (100% Arabica, organic) with 200 mL of boiling water. Aspartame was used as sweetener. A subsequent blood collection was performed 90min after the consumption of water or coffee. Blood samples were collected into heparinized tubes (3mL), Na_2EDTA tubes (5 mL) and tubes without anticoagulant (2mL). Plasma and serum samples were obtained by centrifugation at 1600*g* for 10 min. Plasma samples for FRAP and TRAP assays were kept on ice and analyzed up to 4 h after collection. Plasma aliquot for vitamin C analysis was immediately stabilized with an equal volume of MPA 10% (w/v). Aliquots for vitamin C and vitamin E analyses were stored in liquid nitrogen. Serum aliquots for albumin, bilirubin and uric acid were stored at -20 ºC. In order to avoid exposure to light, tubes were covered with aluminum foil prior to blood processing and storage.

Plasma antioxidant capacity

The FRAP assays were performed according to Benzie and Strain (1996). The TRAP assays were performed according to Bartosz et al. (1998).

Plasma and serum components

Serum albumin, bilirubin and uric acid were measured by commercial kits (Bioclin, Quibasa, Brazil). Plasma vitamin E (α- and γ-tocopherol) was determined by HPLC-UV as described by Hess et al. (1991). Plasma vitamin C was analyzed spectrophotometrically using an adaptation of the method reported by Wey et al. (1996).

Coffee analysis

Chlorogenic acids in the coffee beverages were analyzed by LC-MS according to Perrone et al. (2008), and caffeine according to Trugo et al. (1983).

Statistics

Data are presented as mean ± SD. For each variable, comparisons considering treatments and time points were done by Wilcoxon matched pairs test, using Prism 4 for Windows (version 4.00, GraphPad Software Inc.). Correlation analysis was performed using Statistica (version 6.0, StatSoft Inc.). Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

At baseline, plasma FRAP and TRAP values showed a strong positive correlation ($r = 0.88$, $p < 0.001$) with each other, indicating that both assays similarly assessed the radical-scavenging and/or reducing capacity of biological antioxidants present in plasma. Also at baseline, correlation coefficients between each AC assay and the evaluated plasma endogenous components were calculated. At baseline, FRAP and TRAP values were correlated ($r = 0.89$, $p < 0.001$). FRAP was correlated with bilirubin ($r = 0.48$, $p < 0.001$), and FRAP and TRAP were correlated with uric acid, α-tocopherol and γ-tocopherol ($r > 0.75$, $p < 0.001$). These results are consistent with the significant contributions of uric acid, α-tocopherol and bilirubin to the FRAP value of fresh plasma (Benzie and Strain, 1996), and with the contribution of uric acid and tocopherols to plasma TRAP values (Wayner et al., 1987). Table 1 presents serum and plasma concentrations of the evaluated endogenous nonenzymatic components of the subjects before and after water and coffee treatments. No significant difference between treatments and time points was observed for any of the components. The unchanged levels of vitamin C and bilirubin after coffee consumption are in agreement with previous studies (Natella et al., 2002; Ribeiro-Alves, 2006), as also the unchanged levels of uric acid (Ribeiro-Alves, 2006).

Table 1. Endogenous components in serum/plasma at baseline and after treatments[a,b].

	Water			Coffee	
	Baseline	90 min		Baseline	90 min
Serum					
Uric acid (mg/dL)	3.9 ± 1.7	3.8 ± 1.7		4.0 ± 1.4	3.9 ± 1.3
Albumin (g/dL)	3.9 ± 0.8	3.9 ± 0.6		4.1 ± 0.7	4.4 ± 0.6
Bilirubin (mg/dL)	0.5 ± 0.3	0.5 ± 0.3		0.6 ± 0.4	0.6 ± 0.4
Plasma					
Vitamin C (μmol/L)	22.0 ± 7.2	20.9 ± 6.8		24.7 ± 10.3	24.1 ± 8.7
α-tocopherol (μmol/L)	23.0 ± 6.7	22.4 ± 5.8		23.6 ± 5.8	23.5 ± 6.4
γ-tocopherol (μmol/L)	3.9 ± 1.7	3.8 ± 1.7		4.0 ± 1.4	3.9 ± 1.3

[a]Mean ± standard deviation; nine subjects. [b]No significant differences between treatments and time points for each component (Wilcoxon matched pairs test).

Plasma AC responses, expressed as absolute values (Table 2), decreased after water consumption ($p < 0.04$), and increased after coffee consumption ($p < 0.04$), when measured by FRAP and TRAP.

Table 2. Plasma FRAP and TRAP at baseline and after treatments[a,b].

	FRAP	TRAP
Water		
Baseline	993 ± 78	398 ± 37
90 min	968 ± 75	391 ± 36
Variation	-25.1 ± 14.6[a]	-6.9 ± 5.9[a]
Coffee		
Baseline	980 ± 60	380 ± 26
90 min	997 ± 56	403 ± 31
Variation	17.6 ± 11.7[b]	23.6 ± 12.5 [b]

[a]Mean ± standard error; eleven subjects. [b]Different letters in the same column means significant difference (Wilcoxon matched pairs test, p < 0.04)

The percent responses were also significantly higher ($p < 0.05$) after coffee consumption (increase of 2.2 and 6.1%, respectively) when compared to those after water consumption (decrease of 2.3% and 1.7%, respectively). Natella et al. (2002) also observed an increase in TRAP values 1-2 h after coffee consumption, although to a lower extent (4-5.5%). The higher percent response in our study compared to that of Natella et al. (2002) may be due to differences in concentration and composition of the coffee beverage tested and to differences in the physiological conditions of the subjects. In our study, the coffee beverage contained 240 mg of caffeine and 284 mg of total chlorogenic acids, whereas in the study by Natella et al. (2002) it contained 181 mg of caffeine and 161 mg of chlorogenic acids measured as total phenolic compounds. It should also be considered that there is a large inter-individual variability in digestive transit time, preferential site of absorption and metabolism of coffee components, thus resulting in different time patterns of plasma concentration peaks after coffee consumption (Monteiro et al., 2007).

The observed decrease in FRAP and TRAP values after water (and bread) consumption suggests a metabolic response to food intake that contributes to reduction in plasma AC. A study conducted in 2007 reported a decline in plasma AC after the consumption of a meal containing macronutrients (carbohydrate, fat and protein) but with no antioxidants (Prior et

al., 2007). In the present study, the control (water) treatment was used to properly isolate the effect of coffee consumption from that of meal consumption, with the only difference between treatments being the ingestion of the instant coffee. Therefore, to accurately reflect the contribution of coffee ingestion to plasma AC, the paired difference between the coffee and water percent responses, that is the net percent variation, was calculated and compared between assays (Figure 1). Using this approach, FRAP and TRAP net percent increases (4.5% and 7.8%, respectively) were not statistically different. This result indicates that both assays were able to detect similarly, and in a complementary manner, the increase in plasma AC after coffee consumption. This is probably explained by the fact that TRAP assay monitors the beginning of lipid peroxidation and because this essay is sensitive to almost all known chain-breaking antioxidants (Huang et al., 2005). Moreover, FRAP determines antioxidants reducing power, reflecting the ability of compounds to modulate redox tone in plasma (Prior et al., 2005). Thus, in combination, these two assays can be very useful in distinguishing the activity of different antioxidants with their own dominant mechanisms (Prior et al., 2005).

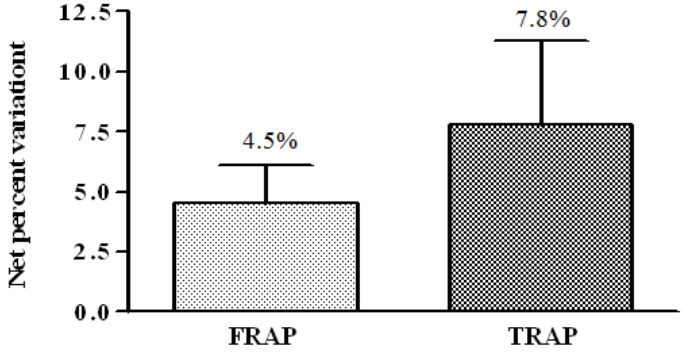

Figure 1. Net percent variation in plasma FRAP and TRAP after coffee consumption.

In the present study, FRAP and TRAP absolute and percent responses after coffee consumption did not correlate with levels of the evaluated endogenous antioxidant components. These results indicate that the endogenous plasma/serum antioxidants did not contribute to the observed increase in plasma AC after coffee ingestion and, therefore, this increase was mainly due to coffee constituents and/or their metabolites. Chlorogenic acids, the main phenolic compounds in coffee, known to have strong antioxidant activity *in vitro* (Moreira et al., 2005) and to be absorbed and metabolized by humans (Monteiro et al., 2007), are probably major contributors to the plasma AC increase after coffee intake. The enhancement in plasma AC after acute ingestion of other phenolic-rich beverages (tea, beer, wine and cocoa) has also been related to phenolic compounds (Leenen et al., 2000; Ghiselli et al., 2000; Otaolaurruchi et al., 2007; Modun et al., 2008; Serafini et al., 2003). Besides phenolic compounds, in the present study, caffeine and its metabolites could also have contributed to the increase in plasma AC measured by FRAP and TRAP assays after coffee consumption. *In vitro* studies have shown that caffeine inhibited peroxidation of rat liver microssomes at milimolar concentrations (Devasagayam et al., 1996) and that the main caffeine metabolites, 1-methylxanthine and 1-methylurate, are effective antioxidants at physiologically relevant concentrations, equivalent to plasma ascorbic acid and uric acid, respectively (Lee, 2000). Ribeiro-Alves et al. 2005, demonstrated in young women that the acute consumption of regular coffee produced a higher increase in plasma FRAP than the corresponding decaffeinated beverage in spite of similar chlorogenic acid content, suggesting that caffeine may contribute to the increase in plasma AC after coffee ingestion.

CONCLUSION

For the first time, two antioxidant capacity assays based on different mechanisms were used simultaneously to assess the human plasma antioxidant capacity response to acute coffee consumption. Our results showed that both FRAP and TRAP assays are able to detect, similarly and in a complementary manner, the increase in plasma AC after coffee consumption and that this increase is not related to endogenous non-enzymatic plasma antioxidants, indicating that coffee components are probably the main contributors to this response.

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Immunostimulatory Properties of Coffee Mannans

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SUMMARY

In this study, the immunostimulatory properties of coffee infusion mannans, isolated and purified from two coffee infusions prepared from coffee beans submitted to different degrees of roasting, and the mannans isolated from coffee residue and further acetylated, were evaluated. The mannans from both coffee infusions and coffee residue were shown to stimulate murine B- and T-lymphocytes, as evaluated by the in vitro expression of the surface lymphocyte activation marker CD69, more marked on B than on T lymphocytes, without a noticed proliferative effect. Similar activity to that of *Aloe vera* mannans from a commercial source used for comparison was obtained. However, no activity were observed for locust bean mannans, which shows that coffee mannans have immunostimulatory properties whose properties seem to be related to its chemical structure that are necessary to be understand in order to be accurately exploited.

INTRODUCTION

A large number of plants derived polysaccharides with immunostimulatory activity have already been reported. Polysaccharides can exhibit immunological and anti-tumural, immunostimulatory, anti-complementary, anti- inflammatory, anti-coagulant, and fibriogenic activities. Among these are the mannans, namely the mannans of *Aloe vera*, whose immunostimulatory properties have been claimed.

Coffee infusions account for, approximately, 25% of polysaccharides in its dry matter, 70% of which are galactomannans (Nunes and Coimbra, 2001). These coffee mannans are acetylated polysaccharides, containing single D-galactopyranose and L-arabinofuranose residues as side chains in a backbone that can have β-(1→4)-linked D-glucopyranose residues interspersed in the β-(1→4)-linked D-mannopyranose residues (Gniechwitz et al., 2007). These structural features are similar to those reported for the bioactive acetylated mannans of *Aloe vera* (Gniechwitz et al., 2007).

The coffee residue resultant from the coffee beverage preparation is composed by the materials that were not able to be extracted by the hot water. From the known polysaccharide composition of the roasted coffee beans (Redgwell et al., 2002; Oosterveldet al., 2003) this residue could be a good source of valuable polysaccharides, and especially of mannans. Nevertheless, the use of the coffee residue as a source of polysaccharides has been hampered due to their insolubility. The acetylation of coffee residue polysaccharides presents a real possibility to render them soluble in water. The utilisation of coffee residue as a source of polysaccharide with immunostimulatory activity and the consequent valorisation of this by

product is a possibility keen to be exploited. In this work, the immunostimulatory activity of coffee infusion mannans isolated and purified from two coffee infusions prepared from roasted coffee beans, and mannans prepared from coffee residue, was studied (Simões et al., submitted for publication). The results were compared with the activity of the mannan from *Aloe vera* and the galactomannans of locust bean gum.

MATERIAL AND METHODS

Coffee infusion samples

Coffee infusions were prepared from Arabica Brazil coffee from two degrees of roast: a light roast, with 5% matter loss on a dry weight basis (DR 5%), and a dark roast, with 10% dry matter loss (DR 10%), as described by Nunes and Coimbra (2001, 2002). Briefly, each ground and defatted coffee was extracted with water (50 g/L) at 80 °C, 20 min, filtered, concentrated, dialysed, frozen, and freeze-dried, giving the High Molecular Weight Material (HMWM). The mannan rich fractions were obtained from the HMWM as described by Nunes et al. (2005, 2006). Briefly, the HMWM was gradually precipitated in ethanol and the fraction insoluble in 50% ethanol (Et50) was recovered and further purified by anion exchange chromatography on Q-Sepharose. The material recovered on the nonretained fraction were further purified by phenylboronic acid (PBA) affinity chromatography, giving origin to fractions CI5 and CI10, for the light and dark roasted coffee infusion, respectively.

Coffee residue samples

Coffee residue mannans were prepared from espresso coffee residue obtained by a commercial batch of Buondi coffee. The coffee residue was extracted with 4 M NaOH (1 g/L) at 25 °C for 2 h, under an inert atmosphere (N_2) with O_2 free solutions containing 0.02 M $NaBH_4$. After each extraction step the mixture was filtered, concentrated under reduced pressure and dialysed for 3 days, with several changes of distilled water. The alkali extract was previously acidified to pH 5.0 with glacial acetic acid. After dialysis, the extract was centrifuged and the precipitate was recovered and freeze-dried.

In order to solubilise the mannans present in the precipitate, the polyssacharides were acetylated (CR1) according to the methodology used by Biswas et al. (2005) for starch and cellulose. The material present in the residue was separated from CR1 and submitted to a new acetylation procedure. The acetylated material recovered in the second water extraction was named CR2.

Aloe vera and locust bean gum samples

The high molecular weight material from *Aloe vera* (AV), was obtained by dialysis from a commercial capsule containing 405.5 mg of powder (Molo-Cure, USA).

The galactomannans from locust bean gum (LBG) were purified from a commercial sample (HG M200- INDAL) by solubilisation in water 0.5% at room temperature during 1 h and then at 90 °C during 30 min. Finally it was done a precipitation in ethanol 80% (12 h/ 4 °C) followed by filtration (Tavares et al., 2005).

Sugar analysis

Neutral sugar was released from polysaccharides by treatment with 11 M H_2SO_4 (10 mg/mL) during 3 h at room temperature with occasional stirring followed by hydrolysis for 2.5 h with 1 M sulfuric acid at 100ºC. The sugars were than derivatised to their alditol acetates and analysed by gas chromatography (GC-FID), as previously described (Nunes and Coimbra, 2001; 2002).

Acetylation of coffee residue mannans

The determination of the degree of acetylation was performed by saponification with NaOH and analysis of the released acetic acid by solid phase micro extraction and gas chromatography (SPME-GC) according to the method developed by Nunes et al. (2006).

Immustimulatory activity assays

The polysaccharide solutions (ca 1 mg/mL) were prepared as described by Dourado et al. (2004) by dispersing the polysaccharides in endotoxin-free Phosphate Buffered Saline (PBS). The samples were then sterilized by filtering through a 0.22 μm filter (Whatman) and aliquots were collected and assayed for total carbohydrates by the phenol-H_2SO_4 method. For removal of any contaminant bacterial lipopolysaccharide (LPS) the filtered solutions were passed under sterile conditions through a 5 mL Detoxigel column (Pierce), previously washed several times with endotoxin-free water (25 mL), 1% (w/v) deoxicolic acid solution (25 mL), and then equilibrated with PBS. The solutions were finally concentrated and total carbohydrates were also determined before and following column passage by the phenol-H_2SO_4 method. No losses were detected following column passage.

Male C57BL/6 mice (6-8 weeks old) were purchase from Charles River (Barcelona, Spain), and were kept at the animal facilities of the Institute Abel Salazar, under specific pathogen-free conditions until used.

Spleen cells were obtained by gently teasing the organ in RPMI-1640 medium (Sigma, St. Louis, USA) supplemented with penicillin (100 IU/mL), streptomycin (50 μg/mL), 2-mercaptoethanol (0.05 M) and 10% of fetal bovine serum (Sigma, St. Louis, USA) (RPMI). Mononuclear cell suspensions were distributed on 96-well plates (10^6cells/well) and cultured for 6h at 37 °C, in 95% humidified atmosphere containing 5% CO_2. Plated cells were stimulated with RPMI medium alone (negative control), 5μg/mL of LPS from Salmonella abortus equi (Sigma, St. Louis) (positive control) or with 12.5- 100 μg/mL of coffee beverage samples (CI5 and CI10), coffee residue samples (CR1 and CR2) and *Aloe vera* extract (AV).
For cytometry analysis, C57BL/6 mice spleen cells were resuspended in Balanced Salt Solution (BSS) supplemented with 10 mM of sodium azide and 1% Bovine serum albumin (BSA). The following monoclonal antibodies were used for immunofluorescence cytometric analysis in a FACScan: FITC-conjugated rat anti-mouse B220, FITC-conjugated rat anti-mouse CD8, FITC-conjugated rat anti-mouse CD4 and phycoeritrin-conjugated hamster anti-mouse early activation marker (CD69). CELLQUEST software was used to process the gathered data. Dead cells were excluded by propidium iodide incorporation.

Statistical Analysis

The results obtained were analysed by one-way ANOVA in order to detect significantly ($p = 0.05$) difference between the means. Significantly different means relative to control were assign after post-hoc analysis using the Dunnet method.

RESULTS AND DISCUSSION

Chemical characterisation of coffee infusion mannans

The coffee infusions mannans were purified from two coffee infusions with two different degrees of roast using a methodology that was shown to be specific for the isolation of relatively high substituted galactomannans (Redgwell et al., 2002). The purified coffee infusion galactomannans accounted for 12% and 8.3% of its high molecular weight material (HMWM) for the light and dark roasted coffee, corresponding to 1.0% and 0.66%, respectively, of the whole roasted coffee. This means that for 150 mL of a typical coffee infusion prepared with a proportion of 50 g of ground roasted coffee per 1 L of water, the amount of acetylated galactomannans is approximately 77 mg for the light roasted coffee and 50 mg for the dark. The sugar composition of these purified galactomannan-rich fractions is presented in Table 1.

Table 1. Sugar composition and acetylation of mannan-rich samples used.

	Sugar composition (% mol)				Total sugars
Sample	Ara	Man	Gal	Glc	(%)
CI5	2	89	7	1	76
CI10	1	92	5	1	63
AV	4	41	5	41	34
LBG	1	77	20	3	86

CI- Coffee infusion; CR- coffee residue; AV- Aloe vera; LBG- Locust bean gum. n.d.- not determined.

Both galactomannans presented an equal abundance of acetyl groups that accounted for 8% on a basis in relation to the sugar residues. These structural features of the coffee infusion galactomannans show resemblance with the immunostimulatory polysaccharide acemannan of *Aloe vera*, although this has been classified as an acetylated glucomannan, having a higher percentage of acetyl groups (0.91 acetyl groups per sugar residue (McAnalley)).

Immunostimulatory properties of coffee infusion mannans

The *in vitro* immunostimulatory properties of coffee infusions acetylated mannans were tested and compared with a commercial extract of *Aloe vera* (AV) bioactive mannans. Additionally, locust bean gum (LBG) galactomannans was used as a different source of mannans, working as a control sample. Table 2 shows the *in vitro* lymphocyte stimulatory effect of CI5 and CI10 samples evaluated by flow cytometric analysis of the expression of the early activation marker CD69 on the surface of C57BL/6 mice spleen B- and T-cells. B-lymphocytes were activated by both coffee infusion mannans, as well as by AV, but not LBG (Figure 1). The stimulatory effect observed of 89% for CI5 and 74% for CI10 was similar to that obtained for AV (74%) and significantly different from that observed for the LBG galactomannan (5%). LBG showed a value not significantly different from PBS blank test (4%).

Table 2. *In vitro* lymphocyte stimulatory effect (%) of galactomannans of coffee infusion (CI5 and CI10), coffee residue (CR1 and CR2), *Aloe vera* (AV) and locust bean gum (LBG).

	B220	CD4	CD8
CI5	89±1**	40±3**	35±1**
C10	74±1**	39±3**	52±5**
AV	74±7**	46±7**	58±5**
LBG	5±2	4±1	4±2
PBS	4±1	8±1	6±1
LPS	98±1**	14±2**	13±5*
CR1	79±2**	13±4*	12±4*
CR2	28±5**	18±6*	21±8**

** $p < 0.05$, ** $p < 0.01$, compared with control (PBS).*

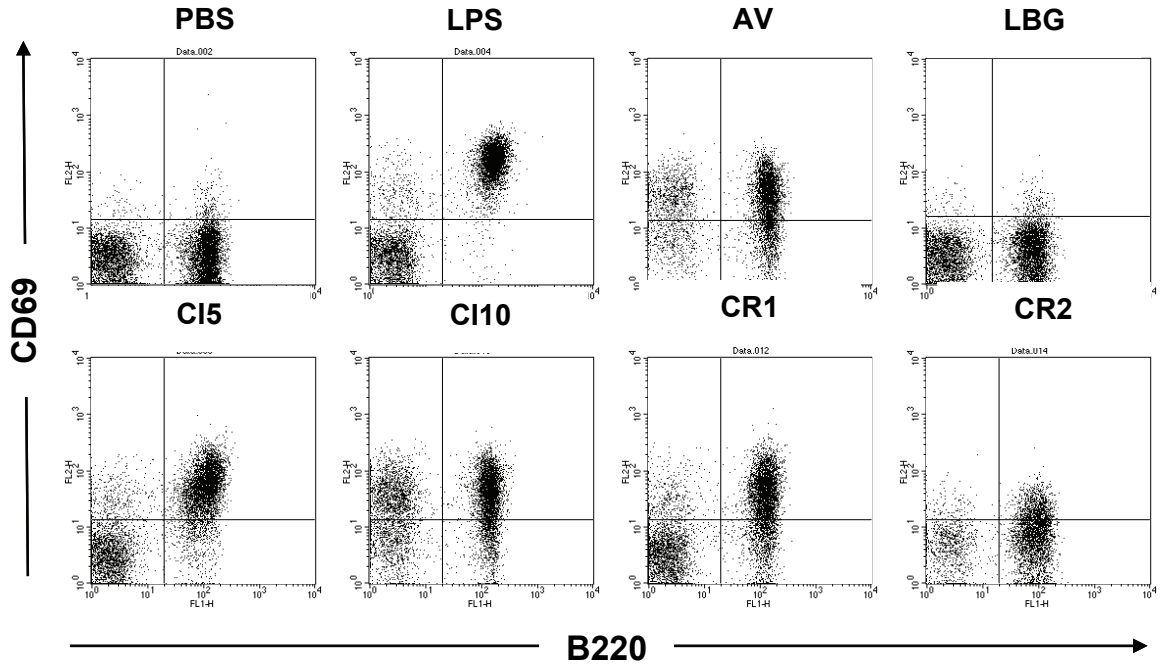

Figure 1. Typical example of dot plots showing CD69 expression on the surface of B cells (B220) stimulatory effect of galactomannans of coffee infusion (CI5 and CI10), coffee residue (CR1 and CR2), *Aloe vera* (AV) and locust bean gum (LBG).

The flow cytometric analysis of CD69 expression on the surface of C57/BL6 mice $CD4^+$ and $CD8^+$ T- cells in spleen mononuclear cell cultures (Table 2) also shows a stimulatory effect on theses lymphocyte population by coffee infusion mannans. On $CD4^+$ cells, the maximum observed activation was 39-40% for both coffee infusions samples, values comparable to that obtained for AV (59%). For $CD8^+$ cells, a maximum activation of 35% and 52% was obtained for CI5 and CI10 samples, respectively (Figure 1), which are values comparable to that obtained for AV (47%). LBG mannan extract did not show stimulatory activity for $CD4^+$ or $CD8^+$ T-cells in spleen mononuclear cell cultures (Table 2).

Extraction and chemical characterisation of mannans from coffee residue

Mannose accounts for 57% of the sugar composition of coffee residue (Table 3). Galactose (26%), glucose (11%), and arabinose (6%) are also present. This sugar composition is similar to that observed for the cell wall material (Redgwell et al., 2002) and for the whole roasted coffee (Oosterveld et al., 2003) with a medium degree of roast, showing that mannans are the major polysaccharides of coffee residue.

Table 3. Sugar composition of coffee residue and fractions from the extraction with 4M NaOH (NaOH sn, NaOH ppt and cellulose residue).

Sample	Yield	Sugar composition (% mol)				Total sugars
	(%)	Ara	Man	Gal	Glc	(%)
Coffee residue	-	6	57	26	11	35
NaOH sn	1.4	12	14	69	3	40
NaOH ppt	4.6	2	87	8	2	73
CR1	8.4*	6	69	23	2	55
CR2	3.7*	1	90	6	2	44
Cellulose residue	49.1	5	60	20	14	54

**Yield based on the NaOH ppt extract.*

In order to extract and purify the mannans, the coffee residue was sequentially extracted with several aqueous solvents. The best solvent for extraction was the 4 M NaOH. Upon neutralisation and dialysis, the precipitate (NaOH ppt) was separated from the supernatant (NaOH sn). The sugar composition of NaOH ppt (Table 3) showed to be very different from the one observed for NaOH sn fraction, which was rich in galactose and arabinose (Table 3), characteristic of arabinogalactans. However, a large proportion of mannans remain in the coffee residue (Table 3). Polysaccharides are linked together by many intermolecular hydrogen bonds which, due to their abundance, are responsible for their low solubility in water. The insolubility increases with the increase of the degree of polymerisation and decreases with the increase of the number of branching residues or acetyl groups in the polymers. Chemical acetylation is a process that can be used to promote the solubilisation of polysaccharides by functionalization of hydroxyl groups and, consequently, decreasing the extent of polymeric intermolecular hydrogen bonds. The acetylation procedure performed on the 4 M NaOH ppt allowed to obtain a yield of 8.4% (CR1).

The main sugar residue present in fraction CR1 was mannose. However, this fraction contains a large amount of galactose (23%) and arabinose (6%). This sugar composition suggests the presence of mannans but, due to the amount of galactose residues, the presence of arabinogalactans cannot be excluded. The amount of acetyl groups, on a molar basis in relation to the sugar residues, was 84%, which shows that the acetylation was performed efficiently. With the purpose of increasing the yield of soluble mannans from coffee residue and trying to obtain a pure mannan, another acetylation procedure was performed on the resultant residue, allowing to obtain CR2 with an additional yield of 3.7%. The CR2 fractions had a sugar composition similar to those obtained for coffee infusions mannans (Table 3). The amount of acetyl groups, on a molar basis in relation to the sugar residues, was 94%, a value comparable to that observed for CR1.

Immunostimulatory properties of acetylated mannans from coffee residue

The *in vitro* lymphocyte stimulatory effect of the chemically acetylated coffee residue mannans is also shown in Table 2. The B-lymphocytes were activated by the chemically acetylated coffee residue mannans with a maximum stimulatory effect observed of 79% and 26% for CR1 and CR2, respectively. These values were statistically different from those observed for the negative control (PBS, 4%) and LBG (5%) and the value for CR1 was similar to the one obtained for coffee infusions and AV. The flow cytometric analysis of CD69 expression on the surface of C57/BL6 mice $CD4^+$ and $CD8^+$ T- cells in spleen mononuclear cell cultures also shows a stimulatory effect on theses lymphocyte population by coffee residue chemically acetylated mannans. On $CD4^+$ cells, the maximum observed activation was 13-18% for chemically acetylated coffee residue mannans, values lower than those obtained for AV (59%) and for coffee infusions (39-40%). For $CD8^+$ cells, a maximum activation of 17% and 21% was obtained for CR1 and CR2, respectively. These values, although significantly different from those observed for LB (4%), were lower than that obtained for AV (47%) and coffee infusions mannans (35-52%).

CONCLUDING REMARKS

This work allowed to obtain the following conclusions:

1. The mannans from coffee infusions induce the *in vitro* expression of surface lymphocyte activation markers on murine B- and T- lymphocytes.
2. The observed lymphocyte stimulatory effect was more marked on B- than on T-cells.
3. Coffee mannan-rich fractions showed similar activity to those of *Aloe vera*.
4. The mannans of locust bean gum do not show any immunostimulatory activity.
5. The acetylated mannans from coffee residue also induce the *in vitro* expression of surface lymphocyte activation markers on murine B- and T- lymphocytes.
6. The occurrence of acetyl groups in the polysaccharide can be important for their immunostimulatory properties.

Studies to establish structure-function relationships are required in order to understand the specific responses to the different polysaccharide structures.

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Species, Roasting Degree and Decaffeination Influence the Inhibitory Effect of Coffee on the Growth of *Streptococcus mutans*

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SUMMARY

The present study aimed at identifying natural compounds that contribute to coffee's antibacterial activity against *Streptococcus mutans* and to investigate the influence of species, roasting and decaffeination on such activity. Selected coffee chemical compounds and aqueous extracts of green and roasted regular and decaffeinated *C. arabica* and *C. canephora* beans at 10% were tested. Minimal inhibitory concentration (MIC), biofilm inhibition and biofilm reduction results were correlated with the concentration of coffee compounds in the extracts. 5-caffeoylquinic acid, trigonelline and caffeic acid solutions showed bacteriostatic activity. All tested coffee extracts roasted for 6 and 7 min showed cell-growth inhibition. At 8 min of roasting, only regular extracts showed inhibitory activity. Extracts from coffees roasted for 13 and 15 min did not exhibit antibacterial activity at tested concentrations. An inverse correlation was found between bacterial colony-forming units and roasting degree. Only regular *C. canephora* extracts showed biofilm formation inhibition (up to about 40%). The joint effect of chlorogenic acids, trigonelline and caffeine or other compounds removed by decaffeination seems to be one of the causes for coffee antibacterial activity against *S. mutans*.

INTRODUCTION

Coffee is one of the most popular and widely consumed beverages throughout the world. Recently, the scientific and popular interest concerning its effects on health has increased due to beneficial pharmacological properties demonstrated in clinical and epidemiological studies such as anti-inflammatory, antifungal and immunostimulant (Kendrick and Day, 2007; Ohshima et al., 2003; Nair et al., 2002; Robinson et al., 1996). The *in vitro* antibacterial activity against gram-positive and gram-negative bacteria has also been reported (Daglia et al., 1994; 1998; 2007; Almeida et al., 2004; 2006). This activity seems to change according to the chemical composition of coffee (Daglia et al., 1998), which may be influenced by species and processing such as roasting and decaffeination (Farah et al., 2005; 2006). According to Daglia et al. (1998), the greatest activity against Staphylococcus aureus and Streptococcus mutans was observed in fractions containing compounds with low molecular weight (< 200 Da) and weak acidic properties. Furthermore, a few natural active substances in coffee of low molecular weight such as trigonelline, caffeic acid and 5-caffeoylquinic acid (5-CQA) have shown activity against the growth of *Legionella pneumophila* (Furuhata et al., 2002), enterobacteria (Almeida et al., 2006) and *S. mutans* (Almeida et al., 2004).

In view of the persistent need for new strategies for caries control, the aim of the present study was to identify natural compounds that contribute to coffee's antibacterial activity

against *Streptococcus mutans* and to investigate the influence of species, roasting and decaffeination on such activity.

MATERIALS AND METHODS

Coffee Chemical Compounds and Aqueous Extracts

Solutions at 1mg/mL of 5-CQA,, caffeic acid, ferulic acid, trigonelline, nicotinic acid and caffeine were prepared using Milli-Q purified water. Two groups of coffee were tested: Regular *C. arabica* cv. Yellow Bourbon and *C. canephora* cv. Conillon beans - harvested, respectively, in Minas Gerais and Espírito Santo, Brazil (GROUP 1) and *C. arabica* harvested in São Paulo and a second sample of *C. canephora* cv. Conillon harvested in Espírito Santo were used in both regular and decaffeinated forms (GROUP 2). Samples were roasted in a commercial spouted bed roaster, operating at a max. temperature of 220 ºC, for 6, 7, 8, 13 and 15 min. The Roast Color Classification System, percent weight loss values (Farah et al., 2005; 2006) and the instrumental color were used as tools for roasting degree determination. Green and roasted coffee beans were ground in a laboratory-scale mill to pass through a 0.46 mm sieve.

Coffee extracts at 10% were obtained by a coffee brewing procedure commonly used in Brazil, percolating 100 mL of pre-boiling (95 °C) Milli-Q purified water through 10 g of green or roasted coffee samples. Aliquots of the filtered extracts were reserved for chromatographic analyses.

Chemical Characterization of Coffee Extracts

The pH of the extracts was obtained by means of a pH meter. The concentration of chlorogenic acids (CGA), including 5-CQA, their respective 1,5-quinolactones(CGL) and caffeic acid, ferulic acid, caffeine, trigonelline, nicotinic acid and sucrose were determined by a LC-DAD-MS according to Farah et al. (2006) and Perrone et al. (2008).

Bacterial Strains and Culture

S. mutans ATCC 25175 were kept at –20 ºC in TSB with 20% glycerol and activated by transfer into blood agar, and incubation at 36.5 ± 1.0 ºC, in anaerobic conditions, for 48 h..Bacterial cells were suspended, according to the McFarland protocol (McFarland, 1907), in saline solution to produce a suspension of about 1.5 x 10^8 CFU/mL. 300 µL of this suspension was mixed with 9.7 mL of Mueller-Hinton bacterial broth medium, resulting in an inoculum with 4-5 x 10^6 CFU/mL.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

MIC was evaluated by the dilution method in Mueller-Hinton broth medium according to NCCLS guidelines (1999), with concentrations ranging from 1 to 10 mg/mL of the 10% coffee extracts. The concentrations varied from 1 to 1000 µg/mL for the standard solutions. The MBC determination was used to assess if the inhibitory effect observed in MIC determinations was through a bactericidal action. The experiments were performed three times in duplicate. Samples from tubes where the MIC results showed no bacterial growth were removed with a loop, inoculated onto a blood agar plate, and incubated at 36.5 ± 1.0 ºC, for 48 h in a candle jar. In the cases where the referred samples showed no bactericidal

activity, the number of CFU on that plate was calculated at the inhibitory concentrations. The controls included an inoculated Mueller-Hinton Broth medium without the test compounds.

Biofilm Susceptibility Assay

All 6 min roasted coffee extracts were tested. The effect of 5-CQA, trigonelline and selected extracts on *S. mutans* biofilm formation was examined by microdilution method according to Wei et al. (2006). Two-fold serial dilutions of the 10% selected coffee extracts were prepared in 96-well polystyrene tissue culture plates with final concentrations of 1.25, 2.5, 5.0, 50 and 90 mg/mL. The selected standard solutions were tested at the concentration of 0.8 mg/mL. Chlorhexidine (0.05%, w/v) was used as the positive control; the medium without the tested agents was used as the non-treated control and the medium alone as the blank control. The absorbance at 570 nm (A_{570}) was determined using a microplate reader. The percentage of inhibition was calculated using the equation (1- A_{570} of the test /A_{570} of non-treated control) × 100. To examine the effects of the selected chemical compounds and coffee extracts on the one day developed biofilm, the samples were prepared according to the methods introduced by Wei et al. (2006). The biofilm assays were performed in quadruplicate.

Statistical Analysis

The association between chemical composition and number of colonies formed on the plate surface results was investigated using Pearson correlation analysis. CFU and biofilm results were analyzed using Student's *t* test. SSPS software, version 11.0 was used for all statistical analysis. A 5% significance level was considered.

Results and Discussion

5-CQA, caffeic acid and trigonelline were able to inhibit the growth of *S. mutans,* with MIC of 0.8 mg/mL. No bactericidal activity was observed at this concentration. Nicotinic acid, caffeine and ferulic acid did not show bacteriostatic activity against *S. mutans* at tested concentrations. Although it is possible that at higher concentrations nicotinic acid, caffeine and ferulic acid would show bacteriostatic activity and 5-CQA, caffeic acid and trigonelline would show bactericidal activity, we opted for testing concentrations that would mimic those found in coffee or other food sources.

The number of CFU from the tube containing trigonelline (0.1×10^2) was significantly lower than those containing 5-CQA and caffeic acid, (0.3×10^2 and 0.5×10^2, respectively) ($p<0.01$), evidencing a stronger antibacterial activity of trigonelline against *S. mutans* compared with those of the tested phenolic compounds. Among the phenolic compounds, 5-CQA showed the highest inhibitory performance. These results are in accordance with previous studies investigating the antibacterial activity of coffee compounds against *S. mutans* and other bacteria (Almeida et al., 2004; 2006). Almeida et al. (2006) and Almeida (2004) described antibacterial effects of trigonelline, 5-CQA and caffeic acid on the growth of Enterobacteria and *S. mutans*, respectively, using the agar diffusion method. Despite the inhibitory effect of 5-CQA and trigonelline on *S. mutans* cell-growth, these compounds did not exert inhibition or reduction of the bacteria biofilm formation at the tested concentration. This is not surprising, given that biofilm is less susceptible to antimicrobial agents compared with planktonic cells (Wei et al., 2006).

In GROUP 1 both species inhibited *S. mutans* growth when roasted for 6, 7 and 8 min, with MIC of 5.0 mg/mL. The extracts obtained from green beans and at 13 and 15 min of roasting did not show inhibitory activity at tested concentrations. Inoculation of positive tubes onto a

blood agar plate showed that at tested concentrations (5mg/mL and 10mg/mL), the lighter the roasting degree the higher was the inhibitory activity (Table 1). Daglia et al. (1998) observed higher antibacterial activity in acidic fractions of coffee extracts. In our study, samples showing higher inhibitory activity (lighter samples) also showed lower pH compared to darker samples (Table 2).

Table 1. Number of colony-forming units (CFU) for coffee extracts from GROUPS 1 and 2 with inhibitory activity against *S. mutans*.

Roasting time (min)	Roasting degree	Concentration (mg/mL)	CFU[a]
C. canephora (Group 1)			
6	very light	10	*1.85*
		5	*2.48*
7	Very light	10	*2.28*
		5	*2.90*
8	moderately light	10	*3.04*
		5	*2.31*
C. arabica (Group 1)			
6	moderately light	10	*1.70*
		5	*2.36*
7	moderately dark	10	*2.20*
		5	*2.85*
8	Dark	10	*2.95*
		5	*3.26*
C. canephora (Group 2)			
6	Very light	10	*2.26*
		5	*2.81*
7	Very light	10	*2.65*
		5	*3.18*
8	medium	10	*3.00*
		5	*3.48*
C. arabica (Group 2)			
6	Very light	10	*2.08*
		5	*2.78*
7	Very light	10	*2.60*
		5	*2.99*
8	Very light	10	*3.11*
		5	*3.30*
Decaffeinated *C. canephora* (Group 2)			
6	Very light	10	*2.38*
		5	*2.89*
7	Very light	10	*3.04*
		5	*3.97*
Decaffeinated *C. arabica* (Group 2)			
6	Very light	10	*2.36*
		5	*2.85*
7	Very light	10	*2.78*
		5	*3.96*

[a] *Results are shown in log_{10} scale for better comparison.*

Table 2. Roasting time and degree, pH, sucrose, caffeine, trigonelline, nicotinic acid, 5-CQA, total of CGA and total of CGL contents in green and roasted coffee extracts from Groups 1 and 2.[a]

Roasting Time	Roasting degree	pH[b]	Sucrose	Caffeine	Trigonelline	Nicotinic acid	5-CQA	Total CGA	Total CGL
C. canephora (Group 1)									
-	Green	5.3	4770.1	1031.2	698.7	ND[c]	3588.9	7026.8	ND
6	very light	4.9	74.7	1111.1	536.3	8.8	946.3	2451.6	289.1
7	very light	5.1	35.3	1100.2	430.0	13.1	514.1	1417.5	199.4
8	moderately light	5.1	20.5	997.2	317.0	18.9	218.1	633.8	106.8
13	moderately dark	5.2	11.1	971.1	72.0	24.2	18.2	59.4	15.4
15	Dark	5.2	12.4	955.8	24.3	23.6	7.2	23.2	5.4
C. arabica (Group 1)									
-	Green	6.0	10213.7	513.6	850.5	ND[c]	2132.5	3202.9	ND
6	moderately light	5.0	112.3	540.2	641.3	6.5	727.0	1925.2	74.9
7	moderately dark	5.2	41.8	519.2	320.6	18.3	224.2	640.5	46.1
8	Dark	5.3	31.2	496.7	169.1	20.2	92.7	283.2	21.2
13	very dark	5.5	21.2	413.6	25.9	19.2	8.9	28.7	3.3
15	very dark	5.6	20.2	387.2	10.3	16.8	5.9	19.1	2.9
C. canephora (Group 2)									
-	Green	5.1	4086.7	666.7	557.9	ND[c]	1527.5	3143.9	5.4
6	very light	5.3	424.2	759.6	508.2	2.9	1118.6	2886.3	184.5
7	very light	5.5	122.2	730.1	456.1	6.3	853.4	2317.9	293.8
8	medium	5.7	37.3	750.6	226.1	14.0	220.1	692.3	130.5
13	moderately dark	5.9	24.4	719.7	23.4	21.0	6.8	27.8	12.1
15	Dark	6.0	21.0	599.4	23.5	18.5	11.0	40.6	5.1
C. arabica (Group 2)									
-	green	5.6	4603.8	357.1	958.8	ND[c]	1499.9	2987.1	ND
6	very light	5.3	725.7	485.9	652.9	5.0	1098.4	2738.2	169.5
7	Light	5.6	228.9	476.2	523.3	11.7	709.7	1826.9	150.8
8	moderately light	5.7	61.7	505.4	335.4	39.8	429.1	1148.1	124.4

13	moderately dark	5.8	27.6	524.6	28.3	32.6	10.6	38.4	11.5
15	Dark	5.9	20.6	536.9	21.1	33.4	15.0	51.9	9.6
Decaffeinated *C. canephora* (Group 2)									
-	green	5.0	2451.1	18.4	712.3	ND[c]	1678.1	5394.8	22.4
6	very light	5.3	273.0	20.9	687.6	3.7	1470.3	3973.7	361.1
7	very light	5.5	98.5	18.2	578.8	8.6	692.8	1955.8	231.3
8	Light	5.5	82.2	17.9	520.0	8.4	608.4	1740.2	194.8
13	moderately light	5.7	38.2	17.1	386.0	13.2	185.6	583.8	85.2
15	light medium	5.8	39.1	13.9	237.7	21.7	75.2	240.2	27.6
Decaffeinated *C. arabica* (Group 2)									
-	green	5.0	4002.5	16.0	1001.2	ND[c]	2359.2	7535.2	21.2
6	very light	5.1	1116.6	14.6	852.3	2.2	2188.6	5944.2	291.8
7	very light	5.3	475.4	14.5	803.5	4.0	2010.4	5237.3	455.3
8	very light	5.5	331.1	16.4	774.2	5.4	1479.8	3894.4	332.7
13	light medium	5.7	52.8	13.1	404.1	15.6	148.6	497.5	122.4
15	medium	5.8	47.2	13.2	198.2	30.1	76.4	244.3	29.5

[a]Results are shown as mean of triplicate of analysis, expressed in μg/mL. Analyses' coefficient of variation was < 5%. [b]Means of determinations in duplicate. [c]ND = not detected.; CGA= chlorogenic acids, 5-CQA = 5-caffeoylquinic acid

Like 5-CQA, other CQA and diCQA isomers are also formed from caffeic and quinic acid, and it is very possible that all these compounds, which together are responsible for more than 90% of the chlorogenic acids content in the coffee extracts, exert antibacterial activity. On the other hand, since ferulic acid did not show activity against *S. mutans*, it is possible that feruloylquinic acids and their lactones do not exert such activity. The mechanisms of growth inhibition of chlorogenic acids and trigonelline are not clear, but according to Cowan (1999) the causes for phenolic toxicity to microorganisms may include enzymatic inhibition by oxidized compounds, possibly through reaction with sulfidryl groups or also through more nonspecific interactions with proteins.

Although the contents of 5-CQA and trigonelline (Table 2) in the coffee extracts were similar to those of the standard solutions with bacteriostatic activity, other non-bacteriostatic compounds such as polysaccharides, aminoacids, etc. that are also part of coffee composition may have contributed to increase the MIC of coffee extract compared to those of the isolated compounds.

Despite caffeic acid inhibitory activity, the amount of this phenolic acid in its non-esterified form in coffee is generally very low, especially in the roasted beans (Farah et al., 2005). In the present study, we only identified caffeic acid in the green coffee extracts. Although the high concentrations of chlorogenic acids, trigonelline (Table 2) and caffeic acid were found in the green coffee extracts, sucrose concentration in these extracts was quite high (Table 2). The majority of the oral microbiota depends on sugar as energy source (McNeill and Hamilton, 2004). Although glucose is the preferred sugar for bacterial growth (Wen et al., 2001), bacteria may use a large variety of sugars such as sucrose, maltose, lactose, etc. as alternative sources of energy (Wen et al., 2001; Iwami et al., 2000). Therefore, it is most likely that sucrose has promoted the growth of the referred bacteria, counteracting the inhibitory effect of trigonelline and chlorogenic acids in the green coffee extracts.

The present results are in accordance with Daglia et al. (1998; 2007) who did not observe inhibitory activity in green coffee extracts. However, one of these studies (Daglia et al., 2007) reported that due to the lack of antibacterial activity of green coffee, along with a positive activity of dark roasted coffee, the compounds with inhibitory activity could not be naturally present in coffee, being formed by Maillard reaction during roasting process. The study went further identifying α-dicarbonyl compounds as the main agents with inhibitory activity (Daglia et al., 2007). Nevertheless, such compounds are present in very low concentrations in commonly consumed aqueous coffee extracts (Daglia et al., 2007). Furthermore, even though the medium roasted coffee extract evaluated by Daglia et al. (2007) was about six times more concentrated than the light roasted extracts used in the present study, all extracts exhibited similar MIC results. This indicates that the antibacterial effect of the referred α- dicarbonyl compounds is lower than those of natural coffee compounds such as 5-CQA, trigonelline, caffeic acid and possibly other compounds that are more abundant in lighter roasted coffees.

Regarding differences between both investigated species from GROUP 1, *C. arabica* cv. Yellow Bourbon extracts showed similar inhibitory activity on the growth of *S. mutans* when compared to *C. canephora* cv. Conillon. No significant difference was observed between the numbers of CFU of both species (Table 1)**.** In the biofilm assays, on the other hand, while *C. arabica* cv. Yellow Bourbon extract did not inhibit the biofilm formation at tested concentrations, *C. canephora* cv. Conillon showed an inhibition of 39.6% at 90 mg/mL, a concentration similar to that commonly used in Brazil and other countries for coffee preparation (100 mg/mL). It is possible that the anti-adhesive property reported for coffee (Daglia et al., 2002) is related to this activity. Considering that the contribution of caffeine for the anti-adhesive property of coffee has previously been discarded (Daglia et al., 2002), the

possible involvement of chlorogenic acids and their lactones in such activity should be investigated, since in the amount of both classes of compounds in the active *C. canephora* extract (6 min roasted) was about 27% and 73% higher than in *C. arabica,* respectively.

The concentration for inhibition of biofilm formation was higher than that of MIC, as expected since in a biofilm bacteria are invariably less susceptible to antimicrobial agents than their planktonic counterparts (Wilson, 1996). The *C. canephora* extract of GROUP 1 reduced only 6.6% of the mature biofilm at 90 mg/mL, while *C. arabica* did not show reduction at tested concentrations. Nonetheless, the inhibition of early biofilm formation is more important than the reduction of mature biofilm because once its formation of biofilm is inhibited, mature biofilm existence may be prevented.

All regular coffee extracts roasted for 6, 7 and 8 min from GROUP 2 inhibited the growth of *S. mutans,* with a MIC of 5 mg/mL. No activity was observed in extracts roasted for 13 and 15 min. No significant difference was observed between the numbers of CFU of both species (Table 1). Also, as in GROUP 1, regular *C. canephora* (GROUP 2) showed biofilm formation inhibition (18.3% inhibition at 90 mg/mL), while regular *C. arabica* did not exhibit inhibitory activity at tested concentrations. Differently from the regular samples, extracts from decaffeinated beans roasted for 8 min did not show inhibitory activity. Only decaffeinated extracts prepared from beans roasted for 6 and 7 min inhibited the bacterial growth (MIC of 5mg/mL). Additionally, none of the decaffeinated extracts inhibited the biofilm formation. Although in the present study caffeine alone did not exert inhibitory activity on the growth of *S. mutans*, studies performed by Daglia et al. (2007) and Almeida et al. (2004) observed that at concentrations of 5.0 and 2.0 mg/mL, respectively, caffeine provided antimicrobial activity against S. *mutans*. These authors also showed that caffeine was able to enhance the inhibitory effect of other natural coffee compounds. This could explain why decaffeinated coffee roasted for 8 min did not show inhibitory effect. Furthermore, other possibly inhibitory compounds may have been washed out by water and dichloromethane during decaffeination process. As in regular samples, a significant inverse correlation was found between roasting degree and inhibitory activity among decaffeinated samples (Table 1). GROUP 2 samples did not show biofilm reduction at tested concentrations.

Regular samples from GROUP 1 showed a tendency ($p = 0.08$) to exhibit better antibacterial performance than those from GROUP 2. Additionally, regular *C. canephora* samples from GROUP 1 showed better inhibitory performance of biofilm formation than those from GROUP 2. This could probably be explained by the inferior quality of samples from GROUP 2, which contained a higher number of fermented defective beans such as black and sour (Farah et al., 2006), and therefore a higher initial counting of microorganisms.

In conclusion, this study showed that 5-CQA, trigonelline, and caffeic acid from coffee exert inhibitory activity against the growth of *S. mutans*; that such activity is enhanced by the presence of caffeine and/or other compounds extracted by decaffeination; that *C. canephora* extracts seems to exert better performance in relation to inhibition of biofilm formation than those of *C. arabica* and that roasting degree is inversely associated with antibacterial activity against *S. mutans*.

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β-Carbolines Contents in Instant Coffee and Coffee Substitute Beverages

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SUMMARY

Norharman and harman are two β-carbolines present in coffee that may be partially responsible for the prevention of Parkinson's disease and reduced suicide risk associated to moderate coffee consumption. Several persons, for diverse reasons, avoid caffeine ingestion, and prefer instant cereal blends, frequently with reduced coffee amounts. Also due to their easier and faster preparation, these instant coffee/cereal beverages are consumed worldwide. The aim of this work was to study the β-carbolines contents in commercial instant beverages, containing different blends of coffee species, chicory, barley, malt and rye while comparing with classical coffee brews. The compounds were analysed by SPE/HPLC/FLD. Generally, the highest values of both compounds were found in 100% instant coffee blends. The lowest levels were detected in 100% barley samples. β-Carbolines contents increased proportionally with the amount of coffee present in the mixtures. Except for barley, the amounts provided by a 2 g portion of all analysed samples were higher than those expected for a standard arabica espresso. These results also show that robusta coffee is the main responsible for the β-carbolines intake through this kind of beverages. For that reason, the addition of some robusta coffee to the blend would improve the above mentioned beneficial health effects of the brew.

INTRODUCTION

Moderate coffee consumption has been recently associated with a lower risk of suicide and a reduction in the incidence of Parkinson's disease (Higdon and Frei, 2006). Two neuroactive β-carbolines, norharman (NH) and harman (H), formed during coffee roasting, might be partially responsible for these effects due to their competitive and reversible inhibitive action on monoamino oxidases A (NH and H) and B (NH) (Herraiz and Chaparro, 2006). The β-carbolines amount ingested through coffee brews depends essentially on coffee species used to prepare the blends, due to a higher content (2-3 times) in robusta coffee, comparing with arabica one (Alves et al., 2007).

Robusta coffees are widely used to produce instant coffee blends. Instant coffee, the dried soluble portion of roasted coffee, is appreciated by several coffee consumers, due to its simple preparation, instantly dissolving in hot water (Clarke, 1987). Several consumers, however, and for diverse reasons, avoid caffeine consumption, and prefer instant cereal blends, frequently with reduced coffee amounts. Coffee substitutes are prepared from many plants, mainly from their seeds and roots. On roasting, these seeds and roots generally tend to take on a coffee-like colour and give a high percentage of water-soluble extract, which sometimes has a flavour similar to coffee. The more consumed substitutes are essentially composed of chicory roots, malt, barley and rye. Malt coffee is made from germinated cereals, especially from barley. Blending of coffee substitutes often improves the overall coffee-like flavour (Maier, 1987; Fadel et al., 2008).

Instant coffee β-carbolines content was already described by Herraiz (2002), with mean amounts of 3.0 μg/g, for NH and 0.7 μg/g, for H. Despite being reported for roasted chicory roots by Proliac et al. (1976) (about 10 μg/g), no data about β-carbolines levels of instant coffee substitutes was found in literature.

The aim of this work was to evaluate β-carbolines levels in commercial instant beverages containing different blends of coffee species, chicory, barley, malt and rye, while comparing with classical coffee brews.

MATERIAL AND METHODS

Reagents and Standards

Harman and norharman were obtained from Aldrich (Steinheim, Germany) and Sigma (St. Louis, MO), respectively. Internal standard harmaline and trifluoroacetic acid were purchased from Fluka (Neu-Ulm, Germany). Acetonitrile Chromasolv and semicarbazide were from Sigma-Aldrich (Steinheim, Germany), and sodium formate was from Merck (Darmstadt, Germany). All other chemicals were of analytical grade. HPLC water was purified with a "Seral" system (SeralPur Pro 90 CN). All instant beverages were prepared with deionised water (Amberlite MD20). The solution of harmaline (IS) was prepared with 1% of ascorbic acid.

Samples

Commercial brands of coffee and coffee substitutes of instant preparation were obtained from local supermarkets: 100% coffees (n = 8), coffee substitutes with coffee (n = 17) and coffee substitutes without coffee (n = 23).

Sample preparation

A 2 g, exactly weighted, of instant ground coffee was spiked with harmaline solution (internal standard), being the volume completed with hot water (90 °C) to 25 ml. The mixture was well homogenized, centrifuged and 2.5 ml aliquots taken in duplicate from the supernatant.

Compounds extraction

The compounds extraction was achieved following a previously published procedure by Herraiz (2002). Briefly, the aliquots, diluted (1:1) with 0.1 M HCl and spiked with semicarbazide and ascorbic acid, were loaded into SPE cartridges (Bond Elut PRS, 500 mg, 3 mL; Varian) previously conditioned with methanol and 0.1 M HCl. The columns were washed with HPLC water and rinsed with 3 ml of 0.4 M K_2HPO_4 (pH 9.1). β-Carbolines elution was achieved with 3 ml of methanol + 0.2 M K_2HPO_4 (pH 9.1) (1:1). All washing and elution solutions contained ascorbic acid.

Chromatographic analysis

Chromatographic separation was achieved on a reversed phase Teknokroma Tracer Excel ODSA (5 μm; 250 x 4 mm) column (Spain) with a gradient solvent system (0.03 M formate buffer + 0.025 M trifluoroacetic acid (pH 3.0) (A) and acetonitrile (B)) (Alves et al., 2007).

Quantification was performed on the basis of the internal standard method using fluorescence detection. The detector was programmed with the wavelengths of excitation and emission

being, respectively, 300 and 440 nm, for NH and H, and 267 and 477 nm, for harmaline (Alves et al., 2007).

Statistical Analysis

Data are reported as mean ± standard deviation. Data were analyzed by the one-way ANOVA and Student's *t* tests. All analyses were carried out with Microsoft Excel statistical software (Microsoft Office Excel 2003, Microsoft Corp., Redmond, WA).

RESULTS AND DISCUSSION

The analysed samples can be grouped according to their composition, as described in Figure 1. The results are reported for the powder, in μg/g, due to the great variability in the preparation mode and amounts used by consumers.

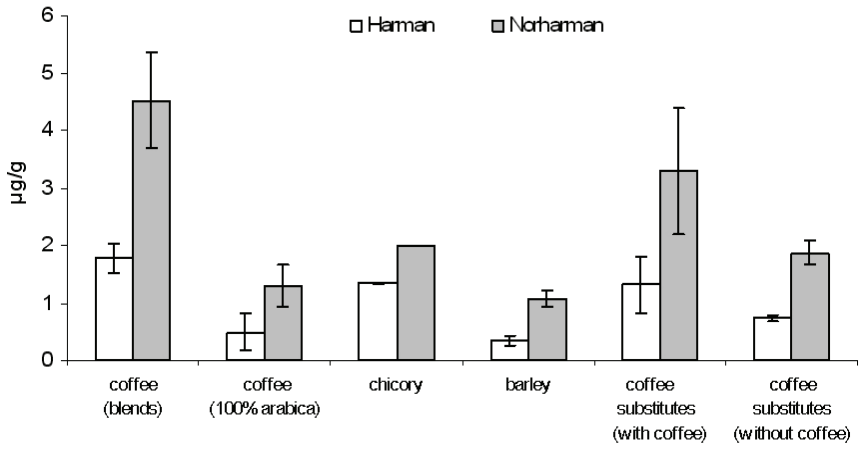

Figure 1. Harman and norharman contents of commercial instant coffee and coffee substitutes.

Regarding all the instant coffees, some variability was found in the β-carbolines content. Mean amounts of 1.46 ± 0.62 and 3.75 ± 1.59 μg/g were found for H and NH, respectively. Within this group significant differences ($p < 0.05$) were detected between the samples labelled as blends and the 100% arabica ones, with approximately three times lower β-carbolines levels for the last. In spite of this distinction, no significant differences ($p > 0.05$) were found between the mean NH/H ratio of both blends and 100% arabicas (approximately 2.7).

The 100% chicory sample presented high amounts of both β-carbolines, but still lower than those of instant coffee blends (although higher than 100% arabica ones). The NH/H ratio for chicory was 1.5, the lowest of all samples analysed.

Instant barley presented the smallest content ($p < 0.05$) of both compounds. However, the mean NH/H ratio found for instant barley was the highest: 3.3.

Regarding the blends of coffee and coffee substitutes (chicory and/or barley), it is possible to observe a great variability in the samples composition. They are disposed in Figure 2 according to their coffee percentage: 20%, 33%, 40% and 50%.

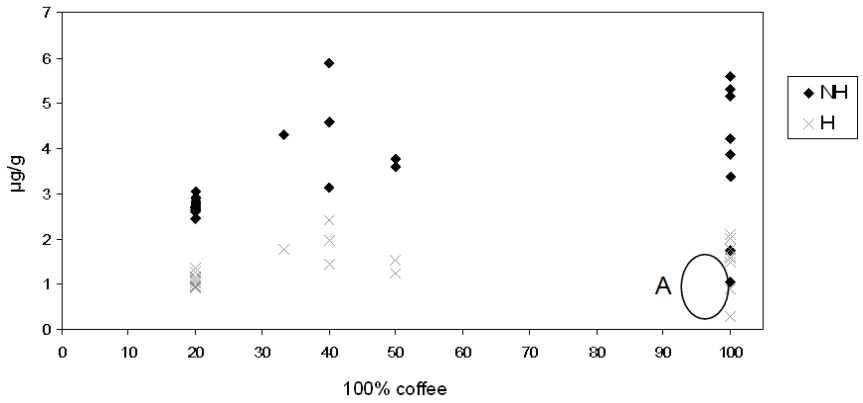

Figure 2. Harman and norharman contents in blends of coffee substitutes with different coffee percentages, comparatively with 100% coffee samples (A = arabica).

The 50% coffee blends showed intermediate amounts of β-carbolines when compared with 100% coffee blends and 100% arabica coffees. Comparing with the 100% chicory sample, 50% coffee ones contained similar H levels ($p > 0.05$) and higher ($p < 0.05$) NH amounts.

No significant differences ($p > 0.05$) were found between samples with 40 and 33% of coffee, both presenting higher amounts than 50% coffee ones, probably due to the coffee species (arabica or robusta) used by the several brands.

The 20% coffee samples showed statistically lower β-carbolines levels ($p < 0.05$) than all mixtures containing coffee (33-100%), except when compared with 100% arabica coffee ones, with lower amounts for this last case.

In a general way, β-carbolines content is proportional to the coffee percentage of the sample, showing that coffee (namely robusta) is the main contributor to the β-carbolines content of the brew. No significant differences ($p > 0.05$) were found between the NH/H ratios of the subgroups (50%, 40%, 33% and 20%) and the 100% coffee samples (mean value of 2.5).

Several options of 100% coffee substitutes blends are also available in the market, mainly with mixtures of barley (25%), chicory (35%), rye (5%) and malt (35%). Two samples without malt in their composition were also analysed. However, in these last cases quantitative composition was not labelled.

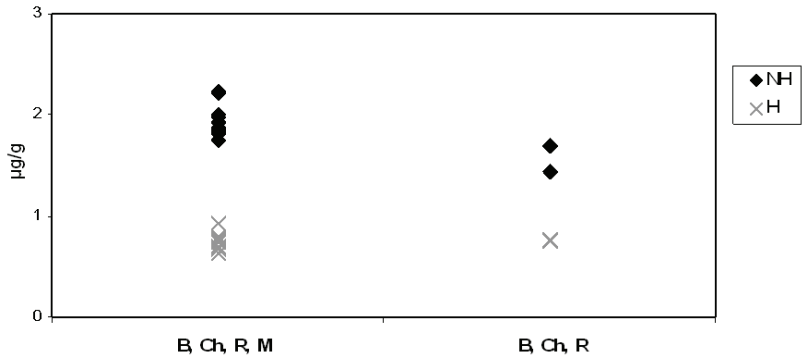

Figure 3. Harman and norharman contents in blends of coffee substitutes without coffee (B: barley, Ch: chicory, R: rye, M: malt).

The mean values of all substitute blends were 0.74 ± 0.06 µg/g, for H, and 1.87 ± 0.21 µg/g, for NH (mean NH/H ratio of 2.6). Samples without malt contained lower NH amounts, when

compared with mixtures with malt. β-Carbolines contents of substitute blends were lower ($p < 0.05$) than samples containing coffee, except when compared with 100% arabica ones, with similar H amounts ($p > 0.05$). Concerning NH, only the samples without malt presented no significant differences ($p > 0.05$) when compared with 100% arabica coffees, while others show significantly higher ($p < 0.05$) amounts.

CONCLUSION

The highest contents of NH and H were found in 100% instant coffees not labelled as 100% arabica. For pure arabicas the amounts were lower, showing that robusta was present in high amounts in the blends. The lowest levels were detected in samples of 100% barley, and β-carboline contents increased proportionally with coffee content in the mixtures. The NH/H ratio was also different between the samples, being 1.5 for 100% chicory, 3.3 for 100% barley and approximately 2.6 for the remaining samples.

Except for barley, the amounts provided by a 2 g portion of all samples analysed were higher than those expected for a standard arabica espresso. The β-carbolines levels in instant coffees are similar to those of espresso coffee blends (30 ml) and press-pot (60 ml), but lower than those of mocha (60 ml) or filter (120 ml), for the described volumes. For the instant coffee substitutes, however, the levels were significantly lower ($p < 0.05$).

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Redox Potential of Roasted Coffee Aqueous Extracts†

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†In memory of Dr. Ernesto Illy (July 18, 1925 - February 3, 2008)

SUMMARY

Redox potential of roasted coffee aqueous extract was determined under different experimental conditions. Results showed that the extraction and analysis procedure strongly influence redox potential values. In particular, coffee extract obtained and analyzed in anaerobic atmosphere shows highly reproducible negative values whereas coffee extracted and analyzed under ordinary atmosphere or under nitrogen flushing shows positive values affected by a large standard deviation and a substantial irreproducibility. The presence of oxygen during extraction and subsequent analysis remarkably affects the redox potential leading to different and possibly uncorrected interpretations of experimental data. Several roasted and ground coffee samples differing in species, origin and roasting degree have been extracted and analyzed under anaerobic conditions. Experimental results put in evidence that roasted coffee confers a high reducing power to its aqueous extracts. The relevant role played by roasting degree in the high reducing power of the roasted coffee aqueous extracts is also evidenced. The comparison between *Coffea arabica* L. and *Coffea canephora* Pierre is particularly informative on the chemical origin of the reducing power. As a matter of fact, in view of the chemical differences between the two species, the redox potential seems to be mainly determined by the non-enzymatic browning compounds originated by roasting.

INTRODUCTION

Experimental evidence has demonstrated that coffee beverages possess high antioxidant properties (Daglia et al., 2000; Daglia et al., 2004, Nicoli et al., 1997; Anese and Nicoli, 2003; Bekedam, 2008). These antioxidant properties are attributed in part to chlorogenic acids and polyphenolic compounds present in green coffee and in part to Maillard reaction products (MPRs) formed during roasting. Green coffee contains different amounts of phenolics depending on coffee species, however most of this natural antioxidant stock is partially degraded and incorporated into melanoidins during roasting and the overall antioxidant capacity is counter-balanced by the arising of MPRs in roasted coffee.

Several methodologies have been used in the past years to assess the antioxidant properties of foods and most of them have been applied also to green and roasted coffee. It is well known that each method is appropriate to describe only few aspects of those different and complex mechanisms through which antioxidants act. One of the interesting way of estimating the antioxidant capacity of a product is the measurement of its redox potential, in fact this value can give information about the oxidative state of the product (Anese and Nicoli, 2001; Nicoli et al., 2004; Brainina et al., 2007). Rigorously the redox potential applies to systems that are reversible and have reached equilibrium, and is usually defined with respect to a single charge carrier such as the ferrous/ferric ion couple. In this case the redox potential is determined according to the Nerst equation. With many species present, the redox potential can respond to the most active substances or represent a steady state “mixed potential”, where the sum of

the anodic and cathodic currents is zero (Tomlinson and Kilmartin, 1997; Kilmartin and Zou, 2001). However, in view of its practical aspects, redox potential determination was used in several studies on different products including wine, tea, tomato paste, beer and coffee brews to assess the influence of processing, storage conditions, and others factors on the reducing properties of these foods (Tomlinson and Kilmartin, 1997; Kilmartin and Zou, 2001; Manzocco et al., 1998; Anese et al., 1999; Andueza et al., 2004).

The aim of this study was to set up a procedure suitable for the redox potential determination of coffee aqueous extracts and then to evaluate through this analysis the influence of raw material and roasting process on the overall antioxidant properties of these extracts.

MATERIALS AND METHODS

Materials

Coffee was obtained as green beans directly from its point of origin. Five coffee samples, *Coffea arabica* from Guatemala (Guate), Brazil (Bra), Ethiopia (Eth), and India (Ind) and *Coffea canephora* from India (H) were used. 100% *Coffea arabica* L. blend was provided by illycaffé S.p.A. Coffee beans were roasted at 220 °C to give respectively, light-, medium-, and dark-roasted samples. Weight loss during roasting are shown in Table 2. Samples were ground (Faema coffee grinder, Italy), extracted and analyzed 2 hours after roasting.

A green tea sample (Vert du Japan sencha natural leaf), one black tea sample (Hassan mokalbari) were provided by Dammann (France) and one roasted barley sample (Coop, Italia) was purchased from the market.

Preparation of aqueous extracts

Coffee aqueous extracts were obtained by solid-liquid extraction with deionized water at 25 °C for 3 min. The ratio between coffee powder and water was 1:10 (w/w). After extraction samples were filtered through Whatman No.4 filter paper and then immediately analyzed (within 30 s). Extraction procedures and subsequent analysis were carried out in a glove box saturated with nitrogen. The residual oxygen present in the glove box was measured with a Hewlett-Packard PSeries gas-chromatograph equipped with a filament detector, a Molsieve 5A 10M column and a PPQ 8M column. The operating conditions were as follows: column temperature 75 and 90 °C respectively; carrier gas Helium. The chromatograms were recorded and analyzed using a EZChrom 200 Vers. 4,5 MTI Analytical Instrument integrator. The residual oxygen measured inside the glove box was less then 1,5% (vol/vol).

Water used for extraction was previously de-aerated by nitrogen flushing. The de-aeration has been followed by using a dissolved oxygen meter equipped with a Clarke electrode (Amel Instruments, Italy).

In order to assess the influence of atmospheric oxygen on the extraction of antioxidant compounds from coffee powder some solid-liquid extractions were carried out in ordinary atmosphere.

Tea and roasted barley were extracted with the same protocol as roasted coffee powder.

Redox Potential Determination

Redox potential determinations were carried out according to the methodology proposed by Manzocco et al. (1998) with some modifications. Measurements were made with ORP combined electrode (SenTix WTW, Germany). The latter consists of a platinum indicating electrode and a silver/silver chloride reference electrode, connected to a pH/mV meters (inoLab WTW, Germany). Calibration was performed against redox standard solutions having a redox potential value of 200mV at 25 °C (Reagecon, Shannon, Co.Clare, Ireland) and 475 mV at 25 °C (Hamilton Bonaduz AG, Swiss). ORP electrode was placed in a 110 mL flask containing a 100-mL volume of each sample. The redox potential was recorded for at least 30 min at 25 °C.

All following reported redox potential values are referred to the silver/silver chloride reference electrode and are expressed in mV units.

According to Dikanovic-Lucan and Palic (1995), it was introduced rH unit, that represents the oxidation/reduction capacity of a chemical system.

Characterization of aqueous extracts

Radical scavenging activity

Radical scavenging activity of aqueous coffee extract was evaluated by the DDPH and TEAC assays. The two determinations were carried out according to methodologies proposed respectively by Brand-Williams et al. (1995) and modified by Manzocco et al. (1998) and by Re et al. (1999). Results were expressed in terms of kinetic constant of discoloration of the radical DPPH and in terms of Trolox equivalent in the TEAC assay.

Soluble solids content

Soluble solids content determination of aqueous extract were assessed by a Moisture Analyzer (MA150, Sartorius Mechatronics, Germany).

pH measurement

The pH was measured at 25 °C using a pH meter (inoLab WTW, Germany) equipped with a combination of glass electrode and a temperature probe.

Caffeine, trigonelline and organic acid determination

Samples were centrifuged for 5 min at 5000 rpm and filtrated with a hydrophobic PTFE membrane filter (Whatman, USA). Diluted samples were analysed by means of HPLC. A 1100 HPLC system (Agilent, Waldbronn, Germany) was used, consisting of degasser, quaternary pump, column thermostat and diode array detector (DAD) operating at 254 nm, 272 nm and 324 nm.

Molecular weight determination

Molecular weight determination was performed by means of High Performance Size Exclusion Chromatography (HPSEC) coupled with Multi Angle Light Scattering (MALS), Interferometric Refractive Index (IRI) and UV detection (220 nm). Isocratic elution with

0.9% NaCl and a set of TSK-GEL PWxl columns (Tosoh Bioscience, Germany) maintained at a temperature of 40 °C were used.

Statistical Analysis

Results reported here are the average of at least three measurements. One-way analysis of variance was determined using the Tukey- Krammer test (XLSTAT-Pro7.5.2). Difference between means were considered to be significant at $P < 0,05$.

RESULTS AND DISCUSSION

The strong dependence of the redox potential on levels of dissolved O_2 has long been recognized and the decrease in potential when products like wine are stored in closed systems has been associated with a decline in levels of O2 (Kilmartin and Zou, 2001). Coffee extraction as usually performed to get coffee brews cannot ensure the absence of dissolved O_2 in the resulting aqueous extracts.

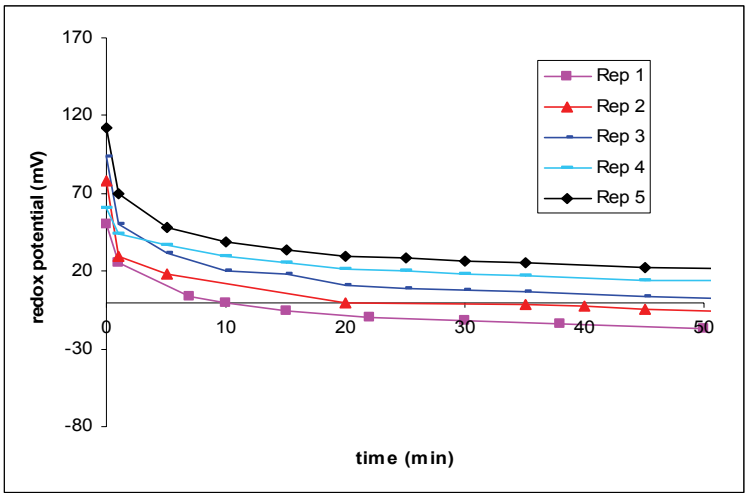

Figure 1. Redox potential (mV) as a function of time of aqueous extracts obtained from the same medium roasted Arabica coffee blend samples in ordinary atmosphere.

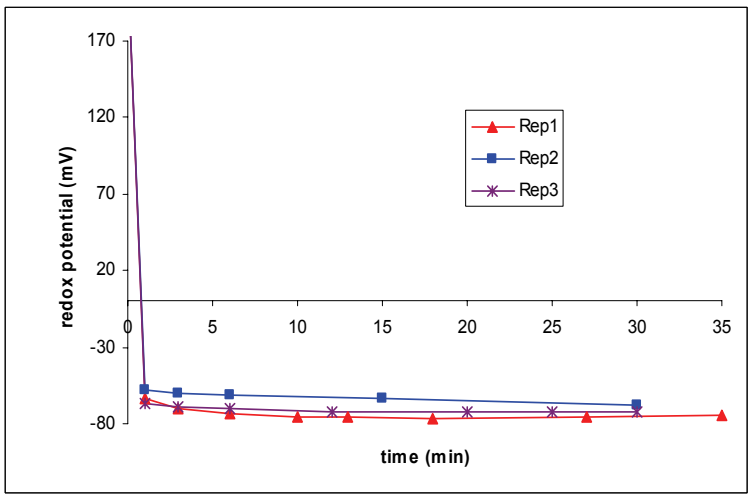

Figure 2. Redox potential (mV) as a function of time of aqueous extracts obtained from the same medium roasted Arabica coffee blend samples in anaerobic environment.

Preliminary redox potential measurements carried out in ordinary atmosphere, highlighted that nitrogen flushing alone is not a sufficient tool to avoid oxygen presence in the extract. As a consequence, as it can be seen in Figure 1, in these experiments a wide range of different data were determined on aqueous extracts obtained from the same sample of roasted coffee, meaning that the procedure was quite a little consistent and that end points of the measurement were not easy to establish. On the contrary, Figure 2 shows data obtained from the same sample of roasted coffee extracted and analyzed in an anaerobic environment; in this case the analysis shows an excellent reproducibility and the end point can be determined after 30 min.

It has long been known the highly antioxidant activity of coffee brews and a multitude of studies account besides the chain-breaking activity (Tubaro et al., 1996; Bressa et al., 1996), metal sequestering capacity (Morales and Babbel, 2002; Takenaka et al., 2005) and hydroperoxides reduction (Homma and Murata, 1995) also a highly oxygen scavenging properties of molecules extracted from roasted coffee (Hayase et al., 1989; Yen and Hsieh, 1995). The awareness of this reactivity of roasted coffee compounds toward atmospheric oxygen in addition to the findings of preliminary tests have determined in this study the choice to carry out the whole experimental procedure in an anaerobic environment. As a consequence, the extraction and measurement protocol have been set-up to be run inside the glove box.

A crucial point very important in the attempt to standardize the experimental procedure was the sample preparation. In the case of roasted and ground coffee, a plethora of extraction conditions can be used: from traditional home and professional preparations to typical laboratory approach. In the present paper, the extraction conditions (method, water temperature, and extraction time) have been selected with the aim to ensure a reasonable compromise between protocol reproducibility and possibility to perform the extraction in a glove box. The more evident consequence of this compromise, e.g. the low water temperature, renders the aqueous extracts of the present study, organoleptically different from “regular” coffee brews. From a chemical point of view, however, the chosen extraction conditions permit to solubilize in significant amount water soluble compounds typically present in coffee brews (in the case of pure *Coffea arabica* blend at medium roasting: caffeine = 750 mg/L, trigonelline = 250 mg/L) including the naturally occurring phenolics, such as chlorogenic acids (3-CQA =190 mg/L; 4-CQA = 235 mg/L; 5-CQA = 390 mg/L) as well as the brown polymers (Mw 30.000 – 40.000) as additionally witnessed by pH and color (UV absorption) of the obtained aqueous extracts. Moreover, in order to assess the suitability of the established protocol, a comparison between the selected extraction conditions and the typical laboratory approach (extraction of coffee powder with hot water) was performed in terms of extraction process efficiency and extracts’ antioxidant power (see Table 1).

Table 1. Chemical properties comparison between aqueous extracts obtained from medium roasted Arabica coffee blend with cold water (25 °C) and hot water (100 °C).

Extraction condition	s.s.	pH	Radical scavenging activity	
	%		DPPH assay(Equivalent s.s.(mg) to obtain k=0,008189	TEAC assay (mmol Trolox/g s.s.)
Cold extract 25 °C x 3 min	1.80 ± 0.1	5.29 ± 0.05	0.012	0.461
Hot extract 100	2.19 ± 0.1	5.18 ± 0.06	0.001	0.952

°C x 3 min				

As expected, coffee extract obtained with cold water shows a lower concentration of soluble solids; however compounds extracted with cold water still exhibit antioxidant properties, expressed as radical scavenging activities. In the light of these results the protocol of cold water extraction was considered suitable for the purpose of the present investigation.

Redox potential determination was used to investigate the effects of some aspects related to raw material and to production process on the overall antioxidant capacity of aqueous coffee brews. As concerns raw material two factors were considered: species (*Coffea arabica vs Coffea canephora var. robusta*) and different origins within each species; while regarding the production process only one aspect was taken into account, the roasting degree.

The soluble solids content (% w/w) and pH values of freshly prepared light-, medium-, dark-roasted coffee aqueous extracts are shown in Table 2.

Table 2. Soluble solids content (% w/w) and pH of aqueous extract obtained from coffee roasted at different degrees (expressed in terms of weight loss, %).

Roasting degree	Weight loss (%)	Coffee extract s.s. (% w/w)	pH
light	13.2	1.68 ± 0.1	5.05 ± 0.02
medium	15.0	1.80 ± 0.1	5.29 ± 0.05
dark	18.1	1.93 ± 0.1	5.76 ± 0.02

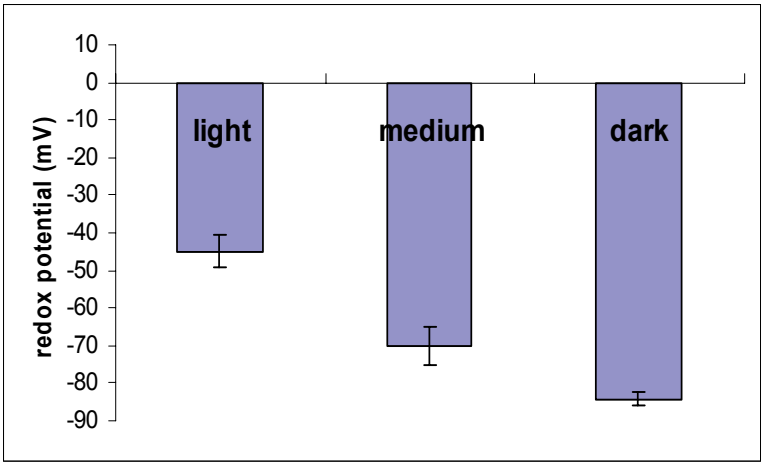

Figure 2. Redox potential values (mV) of coffee extracts depending on roasting degree (light, medium, dark).

Four pure Arabica coffees (BRA, GUATE, IND and ETH) and one pure Robusta coffee (H) were roasted at the same degree corresponding to a total weight loss of 15.0 ± 0.5%. Redox potential determination was carried out on the corresponding cold extracts and results were analyzed through ANOVA. No differences were found among samples from different species roasted at the same roasting degree ($p > 0,05$). Redox potential values of these samples were -70 ± 5 mV. The level of phenolic antioxidants in the Robusta coffee is expected to be higher than that of Arabica coffee (Farah and Donangelo, 2006), in both cases, however, these compounds are degraded upon roasting (Illy and Viani, 1995) and incorporated in coffee melanoidins (brown polymers) (Bekedam, 2008). The redox potential value determined on

both Robusta and Arabica samples, suggest that possible differences in the amount of redox potential active compounds in the starting green coffee beans are levelled upon roasting. Moreover, previous studies on redox potential of wines report that the electrode materials are preferential catalysts for different species in wines (Kilmartin and Zou, 2001) and therefore it cannot be excluded a *priori* that by using different electrode material, possible differences among samples could be detected.

On the other side significant differences in redox potential values ($p < 0,05$) were found among samples belonging to the same specie and country of origin, but roasted at different levels (Figure 3). These differences among roasting degrees remain statistically significant even if the amount of soluble solids in the aqueous extracts is taken into account. In fact, the normalization of ORP values with the concentration of soluble solids ($mV/g_{s.s.}$) in the extracts does not modify the results of the ANOVA and the trend of the values, meaning that the reducing power measured on the extracts depends not only by the amount of extracted molecules, but also by their chemical nature.

Results reported in Figure 3 are in agreement with those reported by Anese & Nicoli, 2003; in fact reducing power increases with increasing intensity of heat treatment. However it must be pointed out that there are some discrepancies on redox values, being ORP values obtained in the present study more reducing than values obtained in the previous work. This difference may be probably attributed to the avoidance of the contact among atmospheric oxygen and highly reactive roasted coffee compounds in the extraction phases. In this way the whole reducing capacity of the system can be preserved from any partial oxidation due to the chemical reaction with oxygen molecule.

In addition to coffee, several other systems are recognized to exhibit antioxidant properties, such as green and black tea (Manzocco et al., 1998; Yen and Chen., 1995, Cao et al., 1996) and roasted barley (Duh et al., 2001). In tea extracts the antioxidant activity is mainly associated with the polyphenol content and in particular lower antioxidant properties of black tea are attributed to the enzymatic oxidation of polyphenols, which is the basis of black tea manufacture. Instead, antioxidant activity of roasted barley is attributed both to the catechin, lutein and tocopherols content and, as well as in roasted coffee, to the Maillard reaction products generated during roasting. Hence, redox potential was determined on each extract in order to compare these different matrixes.

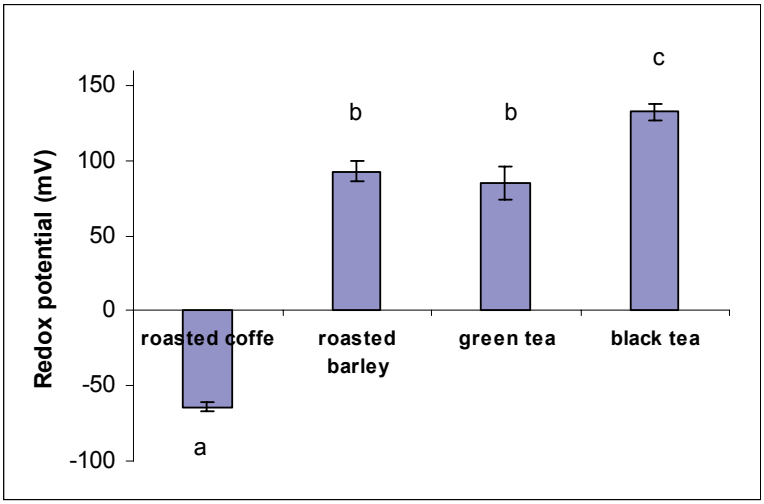

Figure 3. Redox potential values (mV) of aqueous extract from different matrixes: medium roasted Arabica coffee blend, roasted barley, green tea and black tea. Redox

potential values are presented as the mean ± SD (n = 3). Means with different letters are significantly different (p < 0,05).

Figure 4 shows that redox potentials of coffee extract is significantly different if compared to the other extracts. In particular it results more reducing. Roasted barley and green tea extracts show comparable redox value, while redox values obtained from black tea extract resulted to be the most positive. However it must be taken into account that extracts obtained from different matrixes exhibit quite different yields in the amount of soluble solids and different pH values. So, according to Dikanovic-Lucan and Palic (1995), it was decided to consider also these two parameters for a meaningful comparison in terms of reducing potential.

The pH dependence of the redox potential has been accounted for by past researchers (Kilmartin and Zou, 2001; Tomlinson and Kilmartin, 1997, Dikanovic-Lucan and Palic, 1995) through the use of a measure known as the "rH", a function of the electrode potential on the hydrogen scale (Eh):

$$rH = \frac{Eh + 0{,}06\,pH}{0{,}03}$$

Moreover the rH values were normalized with the concentration of soluble solids (rH/g s.s.) and results are reported in Figure 5.

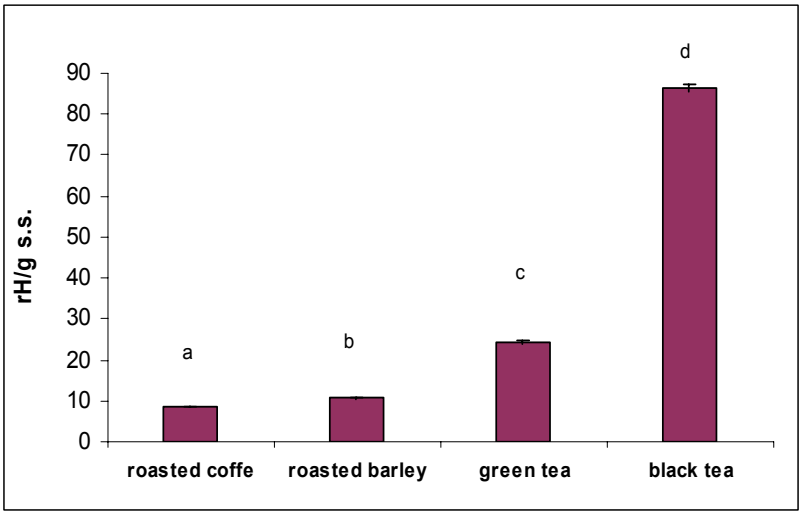

Figure 4. Normalized rH values through soluble solids content (rH/g s.s.) of aqueous extract obtained from medium roasted Arabica coffee blend, roasted barley, green tea and black tea. Redox potential values are presented as the mean ± SD (n = 3). Means with different letters are significantly different (p < 0,05).

As shown, significant differences among all these four extracts were obtained ($p < 0{,}05$). After the inclusion of the pH and the extracts' concentration in the calculation of the reducing properties, roasted coffee extract still continues to exhibit the most reducing power, it is then followed by roasted barley, green tea and black tea extracts.

CONCLUSIONS

From a practical standpoint, the results reported here suggest that the redox potential determination on matrixes which require the extraction phase and at the same time which are highly reactive to atmospheric oxygen needs a careful protocol of the analysis. In this

situation in fact, an anaerobic environment is required in order to avoid oxygen interference during the analysis.

These cautions allow the method to be suitable for the analysis of roasted coffee reducing power, in which the antioxidant effect promoted by roasting seems to prevail on the antioxidant effect due to polyphenolic content originally present in the green coffee. This important point will be the subject of further investigation. Reducing power of extract from roasted coffee were remarkably affected by the roasting degree and in particular higher roasting level determined an increase of reducing power in the coffee extracts. In terms of redox potential measured under experimental conditions of the present work, coffee shows a strong reducing power in comparison with other matrices used to prepare hot beverages.

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Tocopherols in Espresso Coffee

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SUMMARY

Tocopherols, globally named as vitamin E, are important biological and technological antioxidants. Despite being present in coffee beans, information dealing with their content in coffee brews is almost inexistent. Therefore, the aim of this work was to evaluate the contribution of espresso coffee (EC) to this vitamin ingestion, and to ascertain the influence of several technological parameters: coffee species, roast degree and brew volume. The compounds were liquid-liquid extracted from the beverage and analysed by NP-HPLC/DAD/FLD. Average amounts of 1.39 ± 0.40 μg/beverage of α-tocopherol were found in commercial 30 ml ECs, but the main vitamer was β-tocopherol, with 2.01 ± 0.57 μg/beverage. No other isomers were detected. The amounts found were significantly higher ($p < 0.05$) in pure arabica ECs, comparatively with 100% robusta ones. Generally, the extraction percentages into the brew represent only about 1%. However, a slight increase in the extraction efficiency was observed in the medium-dark roasted samples, when compared with the lightest ones. For darker roasts, some loss was observed. The brew volume was associated with a slightly rise in the tocopherols content for robusta brews. However, for arabica, the 20 ml EC already contained 96% of the tocopherols found in the longest brew. Being EC an aqueous beverage, its contribution to the recommended daily ingestion (15 mg) of vitamin E is small. However, these results show that it is possible to improve the EC chemical quality in terms of tocopherols contents.

INTRODUCTION

Coffee beverages are increasingly popular worldwide, due to its organoleptic characteristics and stimulating effects. One of the most relevant properties found in coffee brews is their antioxidant activity (Svilaas et al., 2004). Besides other main antioxidants, as melanoidins and phenolic acids, roasted coffee beans also contain tocopherols (Speer and Kölling-Speer, 2006).

Tocopherols, a group of naturally occurring lipidic compounds with a chromanol ring core and a 16-carbon phytyl saturated side chain, are classified according to their substitution pattern of methyl groups at the head group (α-, β-, γ- and δ-). They are globally named as vitamin E, and recognized as important biological and technological antioxidants. Among them, a special reference to the α-tocopherol isomer is compulsory, due to its association with the delaying of several degenerative diseases (cardiovascular, inflammatory, neurological disorders, cancer, cataract, and others) and preservation of the immune system.

Vitamin E is known to be present in coffee beans (Speer and Kölling-Speer, 2006) but information dealing with its content in coffee brews is almost inexistent (Ogawa et al., 1989). From all the coffee brews, espresso coffee (EC) is the most popular one in Portugal. Therefore, this work aimed to quantify the tocopherol levels of commercial ECs and to

ascertain the influence of several technological parameters in their final brew content, namely, coffee species, roast degree and brew length.

MATERIAL AND METHODS

Coffee samples

Commercial caffeinated (n = 16) roasted beans were obtained in local supermarkets and cafeterias. Green arabica (n = 8) and robusta (n = 8) bean samples were kindly supplied by a local industrial importer and roaster of coffee. The arabica samples were from Hawaii, Costa Rica, Jamaica, Colombia, Ethiopia, Honduras (n = 2) and Brazil, and robusta ones from India (n = 2), Uganda (n = 2), Cameroon (n = 2), Ivory Coast and Indonesia. All samples were submitted to a standard roast procedure (210 °C, 10 min), with an industrial Probat roaster. Additionally, two arabica samples (Honduras and Brazil) and two robustas (Uganda and Ivory Coast), were separately roasted, at 210 °C, for different heat exposure lengths to achieve three different roast degrees (light, medium and dark). All coffee beans were mechanically powdered in the integrated grinder of an Espresso Professional Philips HL3854/A and espresso coffees (6.5 g) were prepared in the same machine. Each brew was prepared and analyzed in duplicate.

Sample preparation and analysis

Tocopherol contents of coffee powder were analysed according to the methodology reported by Alves et al. (in press). For coffee brews, a 5 ml aliquot was spiked with BHT (1%), methanol, and tocol as internal standard. The extraction was performed twice with n-hexane:acethyl acetate (90:10), the organic phases combined, taken to dryness under a nitrogen stream, and the residue re-suspended in n-hexane. Interferents were extracted with dimethylformamide, while the n-hexane layer was injected into the HPLC system. The tocopherols separation was achieved on an Inertsil 5 SI normal phase column (5 μm, 250 x 3 mm) from Varian (Middelburg, The Netherlands), operating at constant room temperature (21 °C), with a mobile phase of n-hexane and 1,4-dioxane (97:3 v/v) eluted at a flow rate of 0.7 ml/min. The effluent was monitored with the DAD connected in series with the fluorescence detector programmed at the excitation and emission wavelengths of 290 and 330 nm, respectively.

RESULTS AND DISCUSSION

A great variability was found in the tocopherol contents of commercial roasted coffee blends extracted as standard espressos (Figure 1). The major isomer was β-tocopherol, with 2.01± 0.57 μg/30 ml. Average amounts of 1.39 ± 0.40 μg/30 ml were also found for α-tocopherol. No other vitamers were detected. Generally, and taking into account the total amounts in the coffee cakes, the extraction efficiency into the brew represent only about 1%. The amounts for each vitamer individually are detailed in Figure 1, for the 16 commercial samples analysed.

In order to understand if the coffee species, arabica and robusta, contributed to the variability observed in the commercial samples, both types of coffee were analyzed separately, eight different samples in each case. In order to eliminate possible variations due to roast, the samples were supplied as green beans, and submitted to a similar roast procedure.

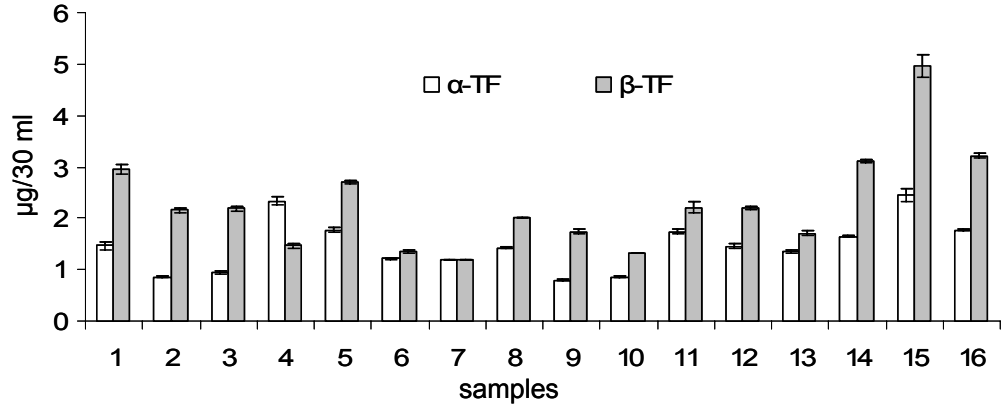

Figure 1. Tocopherols content in espresso coffees from commercial samples.

Figure 2 shows the influence of each coffee species (arabica-A and robusta-R) to the EC tocopherol contents. Significantly higher amounts ($p < 0.05$) of α- and β-tocopherol were found in arabica samples. Still in arabicas, β-tocopherol was always present in superior quantities ($p < 0.05$). Robusta ECs showed lower tocopherol amounts ($p < 0.05$), especially for β-tocopherol. These differences observed between arabica and robusta samples can, therefore, justify the above mentioned variability observed in the commercial ECs due to the different proportions of species used by each brand to prepare the blends. In fact, the addition of some robusta coffee to the blend is a very common procedure in Portugal, in order to increase the body and improve the EC foam, together with some cost reduction.

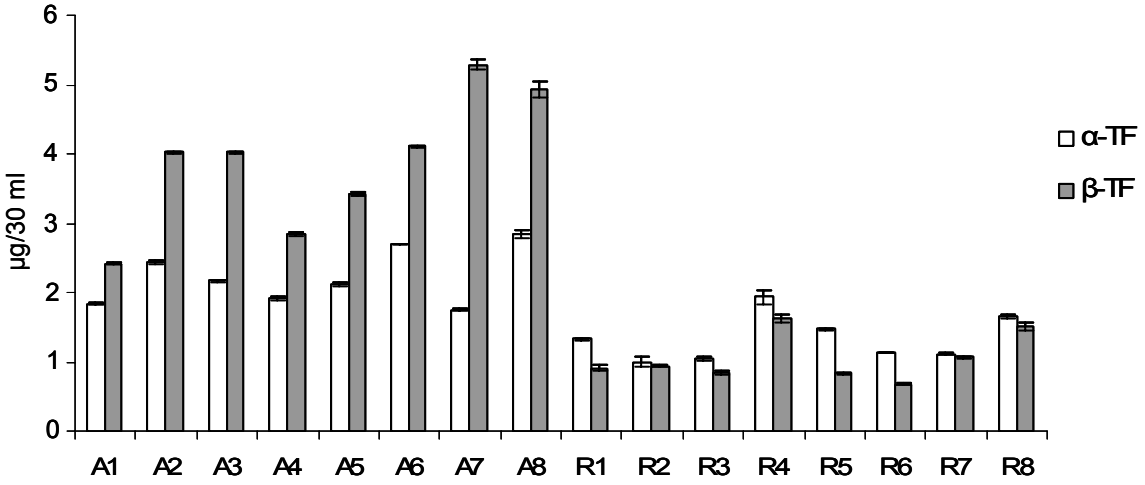

Figure 2. Tocopherol contents in espresso coffees prepared with arabica or robusta roasted beans.

Despite considering 30 ml as a standard espresso, the consumer's preferences are variable, with some preferring short espressos, while others demand for a full cup. Therefore, for two arabica and robusta samples, espressos of different lengths were prepared (20, 30, 50 and 70 ml). The brew volume was directly related with a slightly rise in the tocopherols content for robusta brews (61-100%). However, for the arabica ones, 96% of the tocopherols where already present in the 20 ml EC when compared with the longest brew (70 ml). Apparently, these compounds are easily extracted in the beginning of the percolation process, a situation

explained by their lipidic nature and higher pressure during this initial step.

Regarding the influence of the roast degree, an increase in the extraction efficiency was observed in the medium-dark roasted samples, when compared with the lightest ones. The higher oil availability in the medium roasted samples might contribute to their increased extractability. For darker roasts, however, a reduction in their vitamin E contents is observed, certainly due to their chemical loss.

CONCLUSION

The variability observed in the commercial ECs seems to be largely related with the coffee species constituting the blend. However, differences in the roast degree should also be considered. Tocopherols amount ingested through EC is also dependent on the brew length preferred by consumers. Generally, being EC an aqueous beverage, its contribution to the recommended daily ingestion (15 mg) of vitamin E is small. However, these results show that it is possible to improve the EC chemical quality in terms of tocopherols, by using preferably fresh arabica medium roasted beans to prepare the brew.

ACKNOWLEDGMENTS

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Kinetic Analysis and Mechanism on The Inhibition of Chlorogenic Acids Against Porcine Pancreas α-Amylase

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SUMMARY

Chlorogenic acids (CQAs) are a family of esters in the form of one or more residues of a hydroxycinnamic acid (usually caffeic, ferulic, or *p*-coumaric acid) and quinic acid (QA). Green coffee beans are known to contain at least 30 kinds of CQAs, and their contents are in the range from 6 to 10% in the dry weight of the beans. α-Amylase inhibitors are effective to the prevention of diabetes and obesity in order to control elevation of plasma blood glucose levels by delaying postprandial carbohydrate digestion and absorption. It has been reported that 5-caffeoylquinic acid (5-CQA) inhibits α-amylase activity. However the inhibition mechanism by 5-CQA has not yet been revealed. The aim of the present study is to clarify the inhibition mechanism of CQAs against porcine pancreas α-amylase (PPA). PPA activity was determined by measuring *p*-nitrophenol liberated in the enzyme-catalyzed hydrolysis of *p*-nitrophenyl-α-D-maltoside (G_2-*p*NP) at pH 6.9 and 30 ^{o}C. Inhibition of PPA activity by various CQAs such as caffeic acid (CA), ferulic acid (FA), QA, 5-feruloylquinic acid (5-FQA), 4, 5-dicaffeoylquinic acid (4, 5-diCQA), and 5-CQA was examined. The inhibitor concentrations (IC_{50}), at which 50% of PPA activity is inhibited, of CA, FA, QA, 5-FQA, 4, 5-diCQA, and 5-CQA were determined to be 330, 4400, 4200, 880, 25, and 77 μM, respectively. By comparing the IC_{50} values, the inhibition potencies of CQAs were evaluated to be in the order of 4, 5-diCQA > 5-CQA > CA > 5-FQA > QA > FA. The IC_{50} values of 5-CQA and 5-FQA were 1/5 of the values of CA and FA, respectively, suggesting that the QA moiety enhances 5 times the inhibitory activity of CA and FA. On the other hand, the IC_{50} values of 5-CQA and CA were 1/10 of those of 5-FQA and FA, respectively, suggesting that the catechol structure of CA may play a significant role in enhancing the inhibitory activity. The inhibition model of 5-CQA, 5-FQA, 4, 5-diCQA, and CA was investigated by kinetic analysis using Hanes-Woolf plots, and the inhibitor constants K_i and K_i' that correspond to the dissociation constant K_d of the EI complex into the inhibitor (I) plus enzyme (E) and the K_d of the ESI complex into I plus ES were determined. It was indicated that 5-CQA, 5-FQA, and CA showed mixed-type inhibition with $K_i > K_i$', and that 4, 5-diCQA did mixed-type inhibition with $K_i < K_i$'. It is suggested that 5-CQA, 5-FQA, and CA bind to ES stronger than E and conversely 4, 5-diCQA binds to E stronger than ES. The results presented here provide insights into the molecular mechanism for the inhibition of CQAs against PPA and some hints for development of α-amylase inhibitors useful for prevention of diabetes and obesity.

INTRODUCTION

Chlorogenic acids (CQAs) are a family of esters in the form of one or more residues of hydroxycinnamic acid (usually caffeic, ferulic or *p*-coumaric) and quinic acid (QA) (IUPAC, 1976). CQAs are widely conteined in plants. Green coffee beans are known to content at least 30 kinds of CQAs, the total contents are in the range from 6 to 10% in the dry weight of the

beans (Clifford et al., 2006). 5-Caffeoylquinic acid (5-CQA), which the structure is shown in Figure 1, is the highest contents in green coffee beans, and the content is over 50% (Clifford, 1999). α-Amylase inhibitors are effective to the prevention of diabetes and obesity in order to control elevation of plasma blood glucose levels by delaying postprandial carbohydrate digestion and absorption. It has been reported that porcine pancreatic α-amylase (PPA) inhibition effect and the inhibition mechanism by various substances such as acarbose (Al Kazaz et al., 1996), α-amylase inhibitor from kidney bean (Koukiekolo et al., 1999), 0.19 α-amylase inhibitor from wheat kernel (Oneda et al., 2004). It has been reported that 5-CQA inhibits α-amylase activity (Rohn et al., 2002). However the inhibition mechanism by 5-CQA has not yet been revealed. The aim of the present study is to clarify the inhibition mechanism of CQAs against PPA.

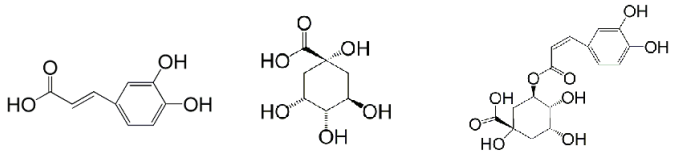

Fig. 1 Chemical structures of 5-CQA, CA and QA

Figure 1.

MATERIALS AND METHODS

Inhibition of PPA activity by CQAs

PPA activity was determined by measuring *p*-nitrophenol liberated in the enzyme-catalyzed hydrolysis of *p*-Nitrophenyl-α-D-maltoside at pH 6.9 and 30^{o}C. Inhibition of PPA activity by various CQAs such as Caffeic acid (CA), Ferulic acid (FA), QA, 5-feruloylquinic acid (5-FQA), 4, 5-dicaffeoylquinic acid (4, 5-diCQA), and 5-CQA was examined.

Determination of the PPA inhibition model by 5-CQA, 5-FQA, 4, 5-diCQA and CA

The inhibition model of 5-CQA, 5-FQA, 4, 5-diCQA, and CA was investigated by kinetic analysis using Hanes-Woolf plots, and the inhibitor constants K_i and K_i' that correspond to the dissociation constant K_d of the EI complex into the I plus E and the K_d of the ESI complex into I plus ES were determined.

RESULTS

Inhibition of PPA activity by 5-CQA, 5-FQA, 4, 5-diCQA, CA, QA and FA

Table 1 shows the inhibitor concentrations (IC_{50}), at which 50% of PPA activity is inhibited, of CA, FA, QA, 5-FQA, 4, 5-diCQA, and 5-CQA.

Determination of the PPA inhibition model by 5-CQA, 5-FQA, 4, 5-diCQA and CA

The inhibition model of 5-CQA, 5-FQA, 4, 5-diCQA, and CA was investigated by kinetic analysis using Hanes-Woolf plots. It was indicated that 5-CQA, 5-FQA, 4, 5-diCQA and CA showed mixed-type inhibition. As shown in Table 2, the inhibition constants K_i and K_i' of CQAs were determined.

Table 1. IC_{50} values of CQAs

Inhibitor	IC_{50} (μM)
5-CQA	76
4, 5-diCQA	23
5-FQA	880
CA	330
FA	4400
QA	4200

Table 2. Inhibition constants of CQAs

Inhibitor	K_i (μM)	K_i' (μM)
4, 5-diCQA	18	23
5-CQA	300	30
5-FQA	2380	1150
CA	1460	180

DISCUSSION

The IC_{50} of CA, FA, QA, 5-FQA, 4, 5-diCQA, and 5-CQA were determined to be 330, 4400, 4200, 880, 25, and 77 μM, respectively. By comparing the IC_{50} values, the inhibition potencies of CQAs were evaluated to be in the order of 4, 5-diCQA > 5-CQA > CA > 5-FQA > QA > FA. The inhibition model of 5-CQA, 5-FQA, 4, 5-diCQA, and CA was investigated by kinetic analysis using Hanes-Woolf plots. It was indicated that 5-CQA, 5-FQA, and CA showed mixed-type inhibition with $K_i > K_i$', and that 4, 5-diCQA did mixed-type inhibition with $K_i < K_i$'. It is suggested that 5-CQA, 5-FQA, and CA bind to ES stronger than E and conversely 4, 5-diCQA binds to E stronger than ES.

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Compounds Related to Antioxidant Activity in Coffee with Different Roasting Degrees

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SUMMARY

Coffee beverages, prepared from roasted coffee beans, are widely consumed throughout the world for their physiological effects and attractive aroma and taste. These beverages are rich in biologically active substances such as chlorogenic acids, caffeine and high molecular weight products (melanoidins) originated from Maillard reactions. Roasted coffee is a complex system, and the relative contribution of its different components to antioxidant activity (AA) still requires research. This work evaluated the roasting process influence on AA in both arabica and robusta coffees, using different roasting degrees (from light to dark roast). AA content was evaluated through Folin-Ciocalteau, FRAP and TEAC methods. Caffeine, 5-cafeoilquinic acid (5-CQA) and the formation of high molecular weight compounds (melanoidins) with roasting process variations were monitored. The results were submitted to a Principal Component Analyses, where CP1 was associated to both the antioxidants methods and caffeine and CP2 to the roasting intensity. Results of Folin-Ciocalteau and TEAC methods showed high correlation. Samples of Robusta coffee had shown greater AA, due to the highest content of caffeine of this species. High molecular weight compounds originated on roasting process and 5-CQA (heat unstable), had shown a negative correlation, and low significance for the CP1, however it allowed discriminating the samples according to light, medium and dark roast degrees (CP2). Despite the fact that these compounds had presented AA, the 5-CQA reduction occurred proportionally to the melanoidins increase; thus, the product AA was not directly correlated to the concentration of these components. However, when high intensity of roasting is achieved, the polyphenol degradation was not balanced by melanoidins formation. On the studied conditions, the AA of the coffee was less affected by the roasting process than for its species.

INTRODUCTION

Coffee contains several phenolic components, especially chlorogenic acids and its degradation products (caffeic, ferulic and coumaric acids). One of its isomers, 5-caffeoylquinic acid, has been reported as powerful antioxidant (Pulido et al., 2003) but is not the only one responsible for this property. Furthermore, some other compounds such as caffeine, hydroxycinnamic acids or high molecular substances developed along Maillard reactions (like melanoidins) had show antioxidant activity (AA) (Daglia et al., 2000; Delgado-Andrade et al., 2005). Volatile compounds derived from Maillard reactions have also been studied as a new source of natural antioxidants (Yanagimoto et al., 2004).

However, differences on raw material and processing could modify this characteristic. Daglia et al. (2000) reported that *Coffea canephora* had higher antioxidant capacity than *Coffea arabica.* In relation to roasting degree influence discordant results were obtained in several

studies. Discrepancies could be due to the use of different methods to test AA or to a lack of a standard definition of roasting degree.

Despite of many studies concerning the roasted coffee antioxidant activity, little is known about the antioxidants compounds that are developed or destroyed during the roasting process. Thus, this work aimed to evaluate the behavior of the principal compounds described as antioxidants, such 5-CQA, caffeine and melanoidins, during the roasting process of arabica and robusta coffees and correlate this results with AA.

MATERIAL AND METHODS

Material and samples preparation

The samples were manufactured by Companhia Iguaçu de Café Solúvel (Cornélio Procópio-PR, Brazil). The coffee beans (arabica and robusta) were roasted in a pilot roaster (Rayar), for 7-10 minutes, at 215- 225 °C. The color samples ranged from very light to very dark roast. The process was carried out in triplicate. The samples were dissolved in boiled water and filtered, being the filtered used for AA determination.

Antioxidant Activity Assays (AA)

Antioxidant activities of the samples were estimated by ABTS and FRAP tests, following the procedure described by Sánchez-Gonzalez et al. (2005). The results were expressed as Trolox equivalent antioxidant capacity (TEAC- μMol/L Trolox/μg.mL^{-1} coffee). Total fenolics were determined photometrically according to the Folin- Ciocalteau procedure (Singleton et al., 1999). Gallic acid was used as standard and the results were expressed as gallic acid equivalent (mMol/L gallic acid/mg.mL^{-1} coffee).

Determination of 5-CQA, caffeine and melanoidins

5-CQA and caffeine were analyzed by HPLC according to Alves et al. (2006). Melanoidins were obtained from coffee samples by membrane dialysis with a molecular weight cutoff of 12-14 kDa (Bekedam et al., 2006).

Statistical Analysis

Antioxidant activity and chemical composition results were submitted to Principal Components Analysis by "Multivariate Exploratory Techniques"– "Principal Components & Classification Analysis" procedure, using the Statistica 7.0 (STATSOFT, São Caetano do Sul, Brazil).

RESULTS AND DISCUSSION

The samples with different roasting degree and raw material (*Coffea arabica* and *Coffea canephora)* were evaluated by ABTS (TEAC) and FRAP tests and Folin-Ciocalteau method (Table 1). Coffee compounds usually related to antioxidant capacity as caffeine, 5-CQA and melanoidins were estimated.

Table 1. 5-CQA, caffeine, melanoidins and AA for arabica (A) and robusta (R) species with different roasting degrees (very dark to very light).

Samples	Melanoidins g/100g	Caffeine g/100g	5-CQA g/100g	ABTS µMol Trolox/µg/mL	FRAP µMol Trolox/µg/mL	Folin mMol G.A./mg/mL
A- VD	24,72	5,22	0,78	1,17	0,50	0,89
A-D	22,15	4,91	2,29	1,25	0,55	1,08
A-M	20,20	5,23	4,02	1,47	0,53	1,23
A-L	17,24	4,92	4,81	1,33	0,52	1,27
A-VL	15,75	4,84	6,29	1,44	0,55	1,17
R-VD	23,39	6,93	0,20	1,45	0,64	1,10
R-D	22,67	8,01	0,80	1,62	0,72	1,23
R-M	20,10	7,21	1,86	1,75	0,62	1,44
R-L	20,32	7,76	3,62	1,78	0,67	1,38
R-VL	17,33	8,57	5,74	1,93	0,66	1,66

Results represent the averages of process conditions in triplicate, each analysed twice. ***VD****: very dark;* ***D****: dark;* ***M:*** *medium;* ***L****:light;* ***VL****: very light.*

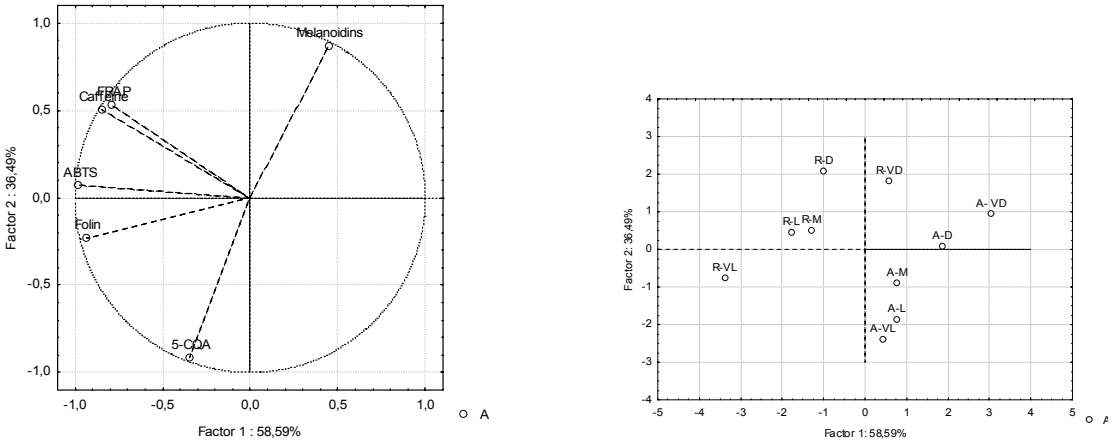
The AA variation was less significant than the difference among the contents of the bioactive compounds during the process. AA shows low dependence on roasting process, but it was possible to notice a directly inverse correlation for roasting and AA.

It can be observed that PC1 and PC2 which showed eigenvalues of 3.52 and 2.19, respectively, could explain 95.08% of experimental dates variance. PC1 was characterized by AA and caffeine content with PC2 mainly correlated to 5-CQA and melanoidins (Figure 1-A). A high correlation was demonstrated between Folin and ABTS results (r = 0,91); however minor correlation was noticed between FRAP and others methods (ABTS (TEAC), Folin). Amongst the chemical compounds, caffeine was the only parameter that showed correlation to antioxidant determination methods (r between 0,67 and 0,93). A similar behavior was found studying soluble coffee, indicating that AA did not have direct dependence on roasting degree. It was also pointed that caffeine, being heating stable on the process conditions applied, was the component that showed better correlation to AA (Vignoli, Bassoli and Benassi, 2007). Lopez-Galilea; De Pena e Cid (2007), studying the influence of brewing procedure on AA, reported a significative correlation between caffeine and the AA evaluated by DPPH (r: 0,826) and redox potential (r: -0,844).

The PCA allowed to discriminate the samples by AA (PC1) or by the roasting degree (PC2) (Figure 1-B). Dark samples (arabica and robusta) were separated of light samples, characterized by their high 5-CQA content, as well as it was possible to separate arabica and robusta samples, distinguished by the greater AA and caffeine content.

Considering the samples with different roasting degrees for each species and following the degradation and generation of bioactive compounds, it was possible to notice that final AA is due to the contribution of different components. The caffeine presented, in all situations, important contribution to AA, and the raw material with higher caffeine level (the robusta coffee) resulted in a product with greater AA. Besides the degradation of bioactive components, the roasting process can generate other antioxidant compounds as melanoidins, allowing the endurance or relative increase in the AA. A more severe roasting process can however promote polyphenol degradation, that will not be balanced by generation of other compounds. Similar results were found by Sacchetti et al., (2008) in evaluation of roasting degree on AA. Brews originated of medium roasting beans showed a increasing on scavenge of free radicals comparing with green coffee. However, when dark samples were evaluated the AA was less due to the phenolic degradation not balanced by melanoidins production.

These findings allow to conclude that coffee brew produced from light roasting coffees, with a higher polyphenol content (here represented by 5-CQA), showed in general better antioxidant potential. Besides the raw material with higher caffeine level (the robusta coffee) resulted in a product with greater AA.



(A) **(B)**

Figure 1. Principal Component Analysis considering the antioxidant activity and levels of bioactive compounds: variable projection (A) and samples graph (B). Species: arabica (A) and robusta (R); roasting degree: (very light to very dark).

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The Effect of Arabinogalactan from Coffee Beans in an Allergic Mouse Model

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SUMMARY

Arabinogalactan (AG) is widely distributed in the extracellular matrixes from plant species and is frequently contained in coffee beans. Type II AG from coffee beans has a beta-(1→3)-linked galactan main chain with frequent arabinose and galactose residues containing side chains. We reported that AG from coffee beans enhanced T helper (Th) 1 immune responses (ASIC 2006). The immune responses are controlled by the balance of Th1 and Th2 responses. It is evident that the allergic reactions are characterized by Th2 responses. Therefore we have investigated the effect of AG from coffee beans on allergic dermatitis-like skin in mice. The ears of mice with oral administration of AG (2.5 mg/day) were induced dermatitis by sensitizing with 2, 4, 6-trinitrochlorobenzene (TNCB). In mice treated with AG, the level of total serum IgE was lower than that of control mice. In mice splenocytes stimulated by concanavalin A, the production of interferon-gamma significantly increased in mice treated with AG. Moreover, interleukin-4 in mice treated with AG also increased. Mast cells in the ear of mice treated with AG slightly decreased as compared to control mice in histological analysis. These results suggest that AG from coffee beans inhibits dermatitis sensitized with TNCB by enhancing Th1 responses, and that AG treatment could provide an effective activity for the allergic reactions.

INTRODUCTION

Coffee beans mainly contain polysaccharides such as arabinogalactan (AG), (galacto)mannan and cellulose (Bradbury, 2001). AG from coffee beans has a type II structure and accounts for about 17% of coffee bean (Redgwell et al., 2006). It was reported that polysaccharides contained in foods, such as Konjac glucomannan (Oomizu et al., 2006) and soy sauce polysaccharides (Matsushita et al., 2006), are involved in T helper (Th) 1 response, cell-mediated immunity, and/or Th2 response, humoral immunity. Allergic reactions result when the balance of Th1 and Th2 responses is disturbed and shifted to Th2 responses. We reported that AG from coffee beans could activate of mouse immunocytes, such as splenocytes, dendritic cells and macrophages and increase the production of Th1 cytokines *in vitro* and *in vivo* (Gotoda et al., 2006). In this study, to investigate whether AG from coffee beans show anti-allergic effects by activating Th1 responses, we measured serum level of IgE, and the number of mast cells and eosinophils in ear skin with a model of atopic dermatitis by 2, 4, 6-trinitrochlorobenzene (TNCB) application. Furthermore, we measured interferon (IFN)-gamma, interleukin (IL)-4 and IL-10 when splenocytes from mouse sensitized by TNCB were stimulated with mitogen.

MATERIALS AND METHODS

Preparation of AG from coffee beans

Isolation of AG from coffee beans was performed as previously described (Gotoda et al., 2006). AG from coffee beans was prepared according to the modified methods of Hashimoto (1971), Wolfrom and Patin (1960) and Bradbury and Halliday (1990). Briefly, the commercial quality (Indonesia AP-1) coffee beans were used. The powdered beans (100 g) were extracted in water at 121 °C and sediment was removed by centrifugation. The hot water extract was extracted with NaOH, and sequentially extracted with chloroform, ethyl acetate, diethyl ether. Then, in the aqueous layer, trypsin (Nacalai Tesque Inc. Kyoto, Japan) was added while stirring continuously. After 72 h, the mixed solution was filtered and the supernatant was dialyzed. Then, NaClO was added to the solution at 60 °C for the purpose of degrading polyphenolic compounds and ethanol (98%) was added and stirred constantly until it reached a final concentration of 75%. After centrifuge of that mixture, the supernatant was discarded and the precipitate was dialyzed in water for 2 weeks and freeze-dried.

Oral administration of AG and TNCB application

Oral administration of AG (2.5 mg/day) to each mouse was started from a month before the induction of dermatitis. AG was dissolved in water and given to mice *ad libitum*. Animals in control group were given water instead of AG.

Mice were sensitized by application of 50 µl of 5% TNCB (Tokyo chemical Industry, Tokyo, Japan) in acetone : olive oil = 4 : 1 on shaved abdominal skin for the second successive days (day -4 and -3). At 3 days after the last sensitization (day 0), 20 µl of 1% TNCB solution (acetone : olive oil = 1 : 4) was applied on both sides of each mouse right ear. Application of TNCB was performed repeatedly from day 0 to day 26 every 48 h.

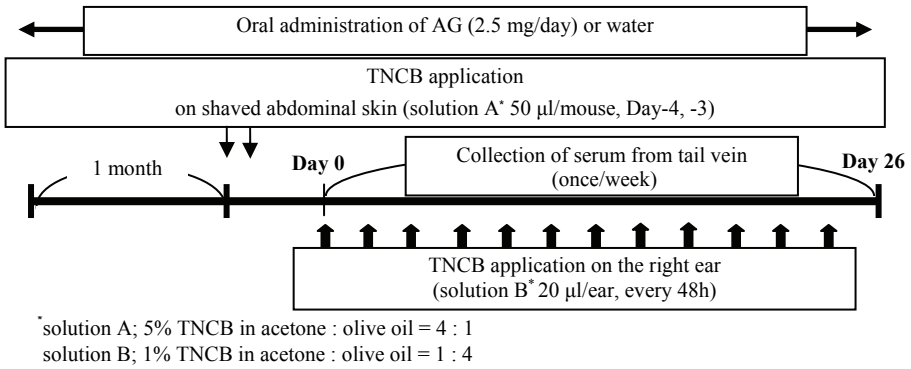

Figure 1. Experiment procedure.

Measurement of IgE

Serum was collected before and after starting elicitation once a week. Total IgE level in each serum was measured by ELISA Mouse IgE Kit (Morinaga institute of biological science Inc., Kanagawa, Japan).

Cytokine assay

At final day, each spleen was removed and homogenized with phosphate-buffered saline (PBS). Splenocytes were suspended in RPMI 1640 supplemented with 10% fetal bovine

serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 2×10^{-5} M of 2-mercaptoethanol in culture dishes for 1 h at 37 °C. After that, non-adherent cells were collected and replaced at densities of 30 x 10^5 cells/well in 24-well plates for cytokine assay. Splenocytes were stimulated with 5 μg/ml of concanavalin A (Con A) for 48 h at 37 °C in 5% CO_2. The supernatants were collected and used to cytokine assay. IFN-gamma, IL-4 and IL-10 were measured by each commercial ELISA kits (Biosource, California, USA).

Histological analysis

The removed both ears were fixed with 10% formalin and embedded in paraffin. Sections (4 μm) were stained with hematoxylin-eosin or toluidine blue. The number of mast cells or eosinophils were counted in several fields per a sample (mast cells; x400, eosinophils; x1,000).

RESULTS

Time course of serum IgE level in mice

Elicitation of dermatitis on mice skin by TNCB was performed until day 26. In mice treated with AG, the IgE level was lower than that of control group at final day (Figure 2.).

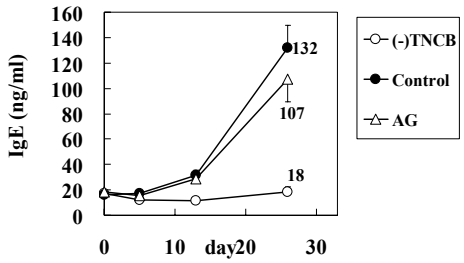

Figure 2. Time course of serum IgE level in TNCB(-), non-treated TNCB group mice, Control, water administrated group mice and AG, AG from coffee beans administrated group mice. Each serum was collected from tail vein once a week. Measurements were performed by ELISA. The values are the means ± SEM ((-)TNCB, n = 5, control, n = 10, AG, n = 9,).

Histological analysis of mast cells and eosinophils

Mast cells and eosinophils were counted in dermatitis-ear skin. In mice treated with AG, the number of mast cells were lower than that of control group (Figure. 3 (A)). Eosinophils did not change compared with control group (Figure 3 (B)).

Cytokine assay

The productions of IFN-gamma, IL-4 and IL-10 were measured by ELISA. As shown in Figure 4. (A), IFN-gamma that is known as one of Th1 cytokines significantly increased in AG group in comparison with control ($p < 0.05$). Moreover, IL-4 also increased compared with that of control group (Figure 4 (B)). However, IL-10 from AG group decreased in comparison with control group (data not shown).

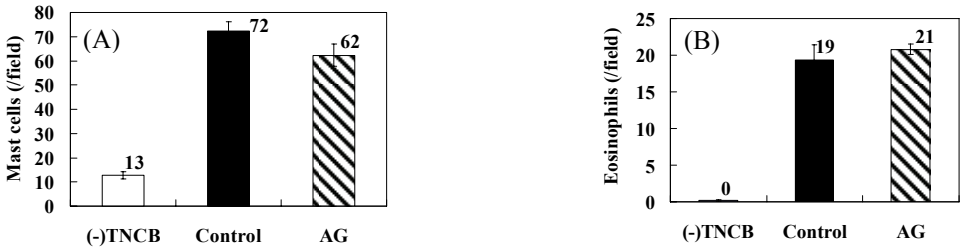

Figure 3. Histological analysis of mast cells (A) and eosinophils (B) in the ear tissues. Sections were stained by toluidine blue (mast cells) or hematoxylin-eosin (eosinophils). Values are the means ± SEM, (-)TNCB, n = 5, control, n = 10, AG, n = 9.

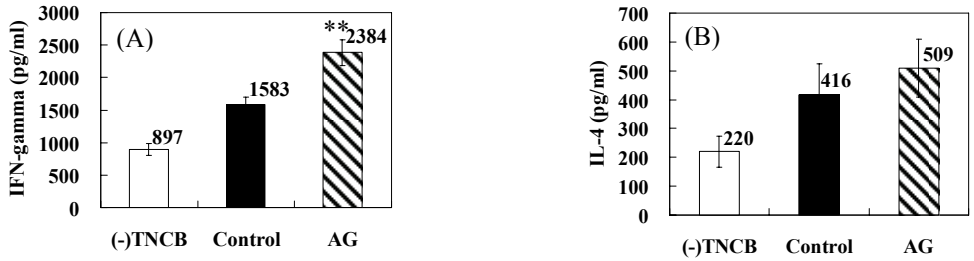

Figure 4. Cytokine, IFN-gamma (A) and IL-4 (B), production from mice splenocytes stimulated by Con A. Splenocytes were incubated with Con A (5 μg/ml) for 48 h. The values are the means ± SEM, n = 3. **, p < 0.05.

DISCUSSION

In this study, serum IgE level, mast cell numbers in the ear tissues and IL-10 decreased when AG from coffee beans was administered orally to mouse which induced dermatitis by sensitizing with TNCB. On the other hand, the productions of IFN-gamma and IL-4 were increased when splenocytes from mouse induced dermatitis were stimulated with Con A. It has been reported that IFN-gamma induces apoptosis to mature mast cells, critical mediators of allergic diseases. These results suggest that AG from coffee beans inhibits dermatitis sensitized with TNCB by enhancing Th1 response, and that AG treatment could provide an effective activity for the allergic reactions. Further studies are needed to understand the immunoresponse through the mucosal immune system by AG from coffee beans.

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Influence of Roasting on the Levels of Serotonine Precursors – Tryptophan and 5-Hydroxy Tryptophan – in Arabica and Robusta Coffee

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SUMMARY

The influence of roasting on the levels of free and total tryptophan and of 5-hydroxytryptophan (5-HTP) in *Coffea arabica* L. (Arabica) and *Coffea canephora* Pierre var. *robusta* (Robusta) was investigated. Coffee grains were roasted at 171 °C/8 min, 171 °C/15 min, and 210 °C/15 min. Free and proteic tryptophan were extracted from the samples. The levels of tryptophan and 5-HTP were quantified by ion-pair HPLC and fluorimetric detection at 340 and 445 nm of excitation and emission, respectively, after derivatization with *o*-phthalaldehyde. The samples were also analyzed for moisture content, protein levels and color characteristics. Higher total and proteic tryptophan were found in Robusta green coffee, whereas free tryptophan was present at higher concentration in Arabica. Free tryptophan corresponded to 2 and 15% of the total tryptophan levels in Robusta and Arabica coffees, respectively. 5-HTP was not detected in the samples either before or after roasting. During roasting, free tryptophan was completely degraded, indicating its sensitivity to roasting. There was a significant decrease on proteic tryptophan, and the rate of loss was smaller for Arabica at every roasting condition.

INTRODUCTION

Coffee is one of the most widely consumed beverages throughout the world due to its pleasant taste and aroma and stimulant effect. Therefore, coffee plays a major social and economical role. It is the second major commodity commercialized worldwide, losing only to petroleum. It represents an important financial source for developing countries, involving a large number of workers in its production chain (Embrapa Café, 2007).

Recent studies have attributed beneficial health effects to coffee. Coffee is frequently recommended as a stimulant and to prevent hypertension; furthermore, it is a bronchodilator and a good source of potassium in the diet. Studies have also described the association of the daily moderate consumption of coffee with lower prevalence of cirrhosis (Klatsky et al., 2006); lower risk of diabetes type 2 (Van Dam et al., 2006), benefic effects with respect to mortality by coronary heart diseases, decreased prevalence of some types of cancer (Oba et al., 2006), inhibition of fat absorption and activation of lipid metabolism in the liver (Shimoda et al., 2006).

Several epidemiological studies have associated coffee intake with beneficial effects in the Central Nervous System (CNS). Associations of coffee intake with lower risk of suicides (Kawachi et al., 1996), lower incidence of Parkinson disease (Stark, 2006), prevention of depression (Flores et al., 2000), and decrease of alcohol consumption and cigarette smoking (Skog, 2006) have been described. The majority of the studies blame caffeine for these

effects. However, coffee has many other compounds, and, therefore, the possible functional role of other substances present in coffee must be investigated.

Serotonin is present in the gastrointestinal tract, in platelets, and also in the CNS. In the last, serotonin is a neurotransmitter involved in the control of appetite, sleep, humor, hallucinations, behavior and pain perception, among others. This compound is present in coffee grains; however it cannot affect the cerebral levels, as it cannot cross the hematoencephalic barrier (Goihl, 2006), because the majority is metabolized before getting to the blood.

Tryptophan and 5-hydroxytryptophan (5-HTP) are precursors of serotonin. These compounds, at high levels in the diet, could affect brain serotonin levels. High levels of non-protein tryptophan, which is easily absorbed, could increase its availability in the brain (Comai et al., 2006). 5-HTP has been an effective dietary supplement in the treatment of a variety of conditions. It has advantages over tryptophan, as its intestinal absorption does not require the presence of a transporter and it is not affected by the presence of other amino acids (Magnussen and Nielsen-Kudsk, 1980).

Very little information is available regarding the levels of serotonin precursors in coffee. Therefore, the objective of this study was determine the levels of free and total tryptophan and of 5-HTP in green coffee beans and to investigate the influence of roasting on their levels.

MATERIAL AND METHODS

Material

Samples of green coffee (*Coffea arabica* L. (Arabica) and *Coffea canephora* Pierre var. *robusta* (Robusta)) grains were kindly provided by Incofex Armazéns Gerais LTDA, Viçosa, MG, Brazil. The Arabica (Catuaí amarelo, hard beverage, 2007/2008) grains were from Viçosa, MG, and the Robusta (clean beverage, 2006/2007) were from Lajinha, MG. Samples from 3 different lots of each species were used. The standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The reagents were of analytical grade, except those used in HPLC which were chromatographic grade. Water was purified in Milli-Q (Millipore Corp., Milford, MA, USA).

Methods

Green coffee grains and coffee grains submitted to roasting at three different conditions: 171 °C/8 min, 171 °C/15min, and 210 °C/15 min, were ground and analyzed.

Methods of analysis

Free and proteic tryptophan were extracted from the samples with 50% methanol before and after hydrolysis with 4.2 *N* NaOH, respectively. The levels of tryptophan and 5-HTP were quantified by ion-pair HPLC and fluorimetric detection at 340 and 445 nm of excitation and emission, respectively, after derivatization with *o*-phthalaldehyde (Martins, 2008).

The samples were also analyzed for moisture content, protein levels and CIE L*a*b* color characteristics. The moisture content was determined by dehydration in an oven at 102 ± 2 ºC until a constant weigh was reached (Casal et al., 2005). The nitrogenous compounds and the levels of total protein were determined by the Micro-Kjeldahl method (AOAC, 1995). Roasted and ground coffee grains were analyzed for color characteristics using a ColorTec

PCM (Pittsford, EUA). The colorimeter was calibrated against a white. The color was characteristics were measured according to CIE L*, a*, b*. Based on these values, chroma and hue were calculated.

Statistical analysis

The analyses were performed in duplicate. The data were submitted to ANOVA and the means were compared by the Tukey test at 5% probability using SIGMA STAT 2.0 (Systat Software Inc, Richmond, CA, USA).

RESULTS AND DISCUSSION

The levels of moisture, protein, tryptophan and 5-HTP in green coffee grains are indicated on Table 1. Robusta had significantly higher moisture content compared to Arabica. Protein levels were in the range of 13 g/100 g dry weight basis. No significant difference was observed on protein levels among coffee varieties investigated.

5-HTP was not detected in any of the samples analyzed; however, tryptophan was present at high levels. Significantly higher total and proteic tryptophan were found in Robusta green coffee whereas free tryptophan was present at higher concentration in Arabica coffee. Free tryptophan corresponded to 2 and 15% of the total tryptophan levels in Robusta and Arabica coffees, respectively.

Table 1. Mean levels of moisture, protein, 5-hydroxytryptophan (5-HTP) and tryptophan of Arabica and Robusta green coffee.

Parameters	**Green coffee**	
	Arabica	**Robusta**
Moisture (g/100 g)	8.65 ± 0.21b	10.24 ± 0.16a
Protein (g/100 g, dwb[2])	13.92 ± 0.98	13.73 ± 0.74
5-HTP (mg/100 g, dwb[2])	nd[1]	nd[1]
Tryptophan		
Total (mg/100 g, dwb[2])	142.9 ± 2.9[b]	157.0 ± 4.5 [a]
Proteic (mg/100 g, dwb[2])	120.8 ± 3.3 [b]	154.0 ± 4.5 [a]
Non-proteic (mg/100 g, dwb[2])	22.08 ± 1.53[a]	3.09 ± 0.10 [b]
Proteic (mg/g protein)	8.69 ± 0.46 [b]	11.24 ± 0.57 [a]

Mean values (± standard deviation) with different letters (a,b) are significantly different (ANOVA, 5%). [1] Not detected (< 0.3 mg/100 g). [2] dwb = dry weight basis.

The levels of moisture, protein and total tryptophan in the roasted coffee samples are indicated on Table 2. No significant difference was observed for moisture and protein contents among degrees of roasting used. The stronger the degree of roasting, the lower the L and chroma values and the higher the hue angle.

During roasting, there was a significant decrease on total and free tryptophan. Free tryptophan was completely degraded, resulting in non detected levels in the roasted coffee. This result indicates its sensitivity to roasting. There was a significant decrease on proteic tryptophan levels, however, the rate of loss was smaller for Arabica at every roasting condition.

Based on these results, the samples of green coffee analyzed did not have 5-HTP, however the samples were observed to be good sources of free and proteic tryptophan. During roasting,

there was a complete degradation of free tryptophan and the levels of proteic tryptophan decreased with the intensity of the roasting process.

Table 2. Color characteristics, levels of moisture, protein and tryptophan in Arabica and Robusta coffee submitted to different roasting conditions.

Parameter	Coffee roasting		
	171 °C/8 min	171 °C/15 min	210 °C/15 min
CIE color			
L			
Arabica	27.97 ± 2.00aA	18.97 ± 0.78aB	12.74 ± 0.83bC
Robusta	28.84 ± 1.61aA	18.67 ± 1.32aB	14.53 ± 2.06aC
Chroma			
Arabica	27.90 ± 3.43aA	12.20 ± 1.46aB	2.64 ±1.24bC
Robusta	29.10 ± 2.90aA	11.67 ± 1.12aB	5.65 + 1.57aC
Hue			
Arabica	66.97 ± 5.28bB	57.59 ± 8.32aB	220 ± 52.2bA
Robusta	70.88 ± 4.03aC	56.53 ± 10.22aB	164 ± 25.3aA
Moisture content (g/100 g)			
Arabica	1.31 ± 0.38	1.40 ± 0.38	1.53 ± 0.49
Robusta	1.63 ± 0.33	1.45 ± 0.30	1.57 ± 0.38
Protein (g/100 g dwb)			
Arabica	13.37 ± 1.58	14.26 ± 1.64	14.67 ± 2.70
Robusta	14.10 ± 0.66	14.39 ± 1.27	14.78 ± 1.72
Total tryptophan (mg/100 g)			
Arabica	107.0 ± 9.1aA	65.84 ± 15.3aB	33.77 ± 8.39aC
Robusta	112.4 ± 10.9aA	72.80 ± 6.77aB	35.97 ± 4.23aC

Mean values (± standard deviation) with different letters in the same column and with different capital letters in the same line are significantly different by the ANOVA and Tukey tests, respectively (5%).

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Insights into the Chemistry of Antioxidant Compounds in Coffee

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SUMMARY

Coffee is among the major sources of polyphenols for human nutrition. Special extracts from coffee further enriching these polyphenols can now be used as functional food ingredients delivering antioxidants from natural sources and are commercially available now. As well available are the single standard compounds 3-, 4- and 5-CQA and the di-CQA (3,4-, 3,5- and 4,5-di-CQA). Accessing the chlorogenic acids profile of such extracts against dried coffee beverages (soluble coffee) a contribution of the single active principles can be calculated. Comparing these special extracts and soluble coffees the same trolox equivalent antioxidant capacity (TEAC) was measured, but the contribution of the free chlorogenic acid derivatives explains about 70% in the case of the functional food ingredients, whereas in coffee these compounds are more or less transformed or incorporated in higher molecular weight compounds depending on the roasting degree or the molecular weight resulting in only 10% of the antioxidant capacity explained by the CQA compound family.

ZUSAMMENFASSUNG

Kaffee ist eine Hauptquelle von Polyphenolen der menschlichen Ernährung. Spezielle chlorogensäurereiche Extrakte, die diese Antioxidantien aus Kaffee als funktionelle Inhalsstoffe angereichert enthalte,n sind nun kommerziell erhältlich. Ebenfalls erhältlich sind die Referenzsubstanzen der 3-, 4- und 5-CQA sowie die 3,4-, 3,5- and 4,5-di-CQA. Damit ist es nun möglich diese Komponenten in Kaffees und funktionellen Zutaten zu quantifizieren und Ihren Beitrag zur antioxidativen Aktivität (TEAC) zu bestimmen. In den funktionellen ingridients erklären die CQA-Derivate ca. 70% der antioxidativen Aktivität, dagegen haben diese Produkte in Kaffee während des Röstens bereits reagiert oder sind in höhermolekulare Komplexe integriert worden.

INTRODUCTION AND OBJECTIVES

Coffee is consumed all over the world and recent studies suggest potential health benefits from coffee consumption due to its antioxidant potential (Ovaskainen et al., 2008). During the last ASIC meetings many contributions dealt with the antioxidant capacity (DPPH-test) of the individual chlorogenic acids (mono- and di-esters of quinic acid with cis-hydroxycinnamic acid), their biosynthesis, function and correlation to maturity in plants. Moreover their absorption and metabolism in humans were studied and online evaluation techniques are available to compare the in vitro antioxidative potential (TEAC-FIA) of coffee beverages (Cassano et al., 2006).

The aim of this paper is to assess the profile of polyphenols of coffee responsible for the Trolox-equivalent antioxidant capacity, calculate the contribution of the single compound and comparing soluble coffees to special extracts rich in antioxidants extracted from coffee.

METHODS AND MATERIALS

Samples

Commercial soluble coffee of standard quality and German, French and Indian CQA-enriched extracts was supplied by the manufacturers.

Standard substance

The standards for mono- and di-CQA compounds (we use the IUPAC nomenclature herein) were bought from Phytolab (Vestenbergsgreuth, Germany).

Methods

Determination of the CQA, FQA, CoQA, DQA and their respective (mixed) di-compounds was performed by ESI-LC-MSMS in negative MRM using a gradient of 0.1% TFA in water and MeCN on XDB-C18 material (Agilent, Waldbronn). In parallel detection by DAD detector using the wavelength of 324 nm characteristic for the chlorogenic acid derivatives was monitored.

HPLC: Agilent 1200 RRLC consisting of degasser, binary high-pressure pump, column oven and diode array detector was used with a 150 x 4.6 mm XDB column an a gradient starting of 92/8 to 88/12 in 13 minutes followed by 85/15 at 17 minutes and a final mixture of 30 percent MeCN at 25 minutes, followed by wash and reconditioning at 1 ml/min.

Mass spectrometer: The QTRAP 3200 (Analyst 1.5, Applied Biosystems, Darmstadt) mass spectrometer was set to a current of 30 (exchangeable unit) collision gas was high, and spray voltage of - 4500 V with a source temperature of 500°C, gas 1 of 40 and gas 2 of 70 psi similar to the methods described (Clifford et al., 2006).

MRM transitions were optimized with the single compounds or coffee itself for the compounds not available and are summarized in Table 1.

Table 1. multiple-reaction-monitoring transitions in negative ESI-mode.

Substance (short symbol)	RT [min]	MRM [m/z]	DP [V]	CE [V]
3-caffeoyl quinic acid (**3CQA**)	6.3	353/179	-20	-30
5-caffeoyl quinic acid (**5CQA**)	10.4	353/191	-20	-40
4-caffeoyl quinic acid (**4CQA**)	12.5	353/173	-20	-40
3-feruloyl quinic acid (**3FQA**)	11.4	367/193	-15	-60
5-feruloyl quinic acid (**5FQA**)	18.8	367/191	-15	-60
4-feruloyl quinic acid (**4FQA**)	19.3	367/173	-15	-60
3,4-dicaffeoyl quinic acid (**34diCQA**)	24.6	515/353	-15	-60
3,5-dicaffeoyl quinic acid (**35diCQA**)	25.2	515/353	-15	-60
4,5-dicaffeoyl quinic acid (**45diCQA**)	25.8	515/353	-15	-60

TEAC-test was performed in ethanol after 6 min reaction with ABTS-radical (Re et al., 1999).

RESULTS

Recent advantages in the identification of the chlorogenic acids in coffee and the commercial availability of pure standard compounds made it possible to setup a method for the

quantification of the mono- and di-CQA in coffee by sensitive ESI-negative MRM quantification. Using the UV traces at 324 nm the common pratice of using 5-CQA as standard for the quantification of the mono-CQAs and applying a factor of 0.77 for the di-CQAs showed some error when using the gradient described here. In figure 1 the sensitivity of the UV signal and the respective MRM channels can be compared and the UV absorption response is given to compare the UV activity of the single compounds.

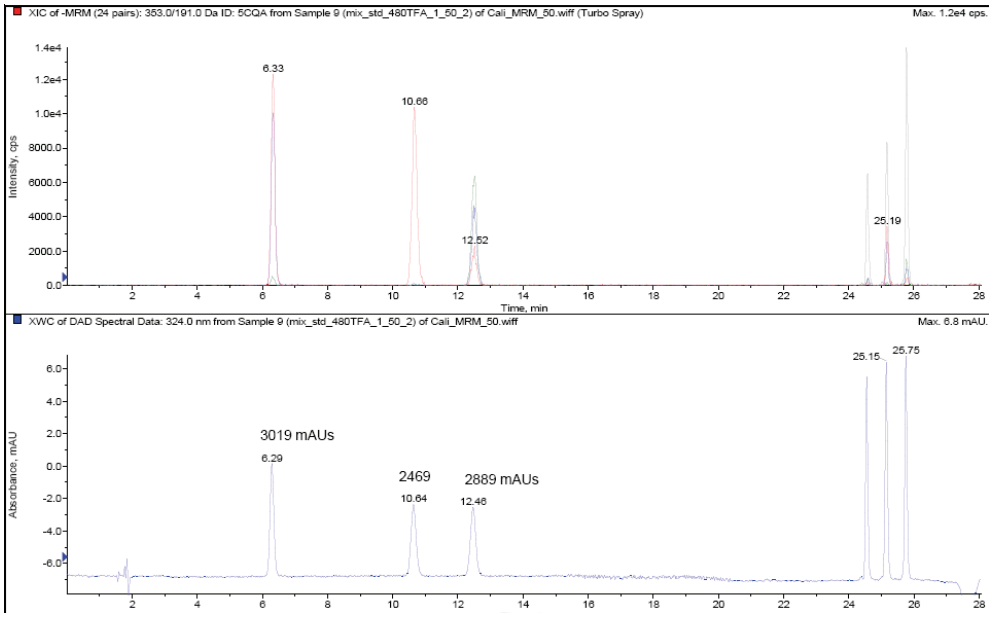

Figure 1. HPLC MRM vs. UV chromatogram and area for equal weight mix of the 3-, 5-, 4-CQA and 3,4-, 3,5-, 4,5-diCQA standard.

Applying the method to soluble coffees and the available chlorogenic acid rich extracts their CQA/FQA and di-CQA profile differed, showing a sum concentration of CQAs in the soluble coffee of 3.5 % compared to up to 45 % for CQA extracts. Using further MRM traces for the quantification of chlorogenic acid lactones CQL products from raw or roasted coffee could be differentiated and other compounds present were mono- and disaccharides, proteins and traces of caffeine (not reported here). Table 2 summarizes the results for different products.

Table 2. Results for CQA quantification using authentic standards in coffee extracts.

[g/100 g dm]	3-CQA	5-CQA	4-CQA	34-diCQA	35-diCQA	45-diCQA
GC (Ger)	9.1	14.0	9.7	1.8	1.1	1.5
GC (Ger)	8.9	13.9	9.7	1.6	1.0	1.3
GC (Ind)	4.8	26.1	6.7	4.5	3.0	4.8
RC (Ger)	9.1	12.0	9.7	1.8	1.0	1.6
SOL (Ger)	0.9	1.3	1.0	0.1	0.1	0.1
GC: green coffee CQA-rich extract *RC: roast coffee CQA-rich extract* *SOL: soluble coffee (standard consumer quality)*				*Ger: produced in Germany* *Ind: produced in India (extraction)*		

In Figure 2 the TEAC of the pure reference compounds are summarized. As expected mono-CQA show lower activity correlated to the number of hydroxyl groups present in the molecule.

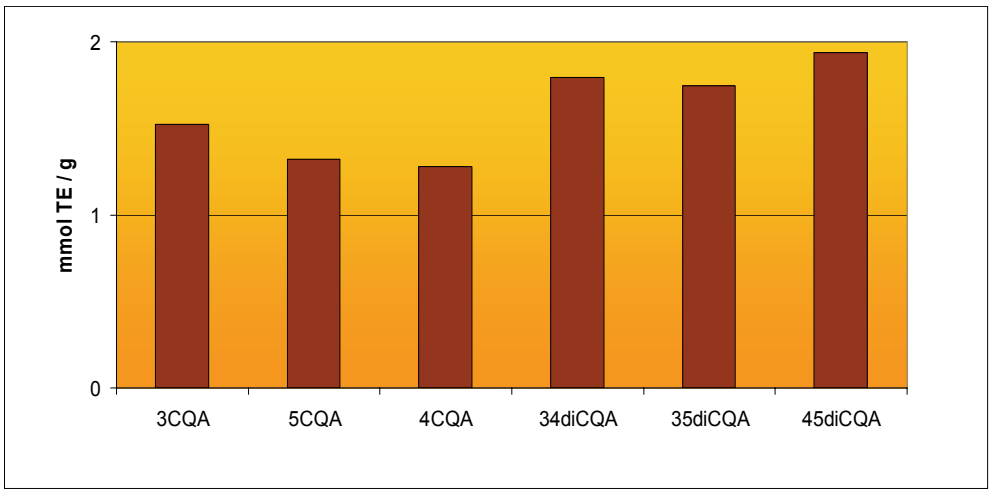

Figure 2. TEAC of single authentic standards.

Using this values the contribution of the single CQAs and the sum in coffee and polyphenol rich extracts can be compared and 70% of the TEAC can be explained by the sum of the CQA in the extracts from raw and light roast coffee, whereas only about 10% of the values can be explained in the soluble coffee. During the coffee production (especially the maillard reaction during and after the roasting) a great part of the coffee polyphenols get bound to the coffee matrix mostly via a non-covalent link to the melanoidins fraction and polymerization reactions.

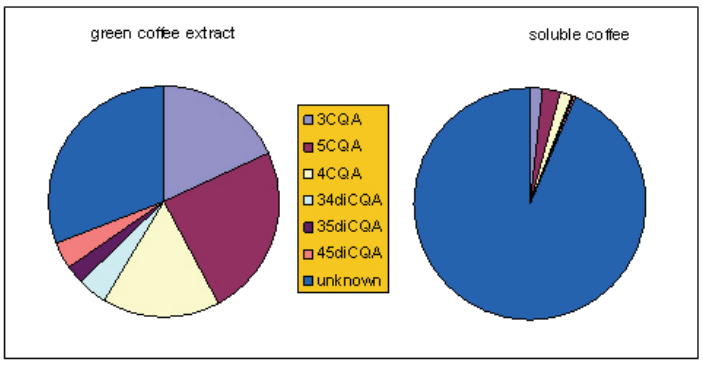

Figure 3. Contribution of individual compounds to the total TEAC of 0.76 mmol trolox/g extract.

CONCLUSIONS

Using the described LC-MSMS the major so far identified CQA-derivatives could be assessed and rated for their contribution to the total trolox equivalent of antioxidant capacity (TEAC) in coffee and CQA-rich coffee extracts. In these nutraceutical extracts from raw or roasted coffee the contribution of CQAs and di-CQAs to the TEAC is of approx. 70%, whereas in roast or soluble coffee less than 10% originate from CQA derivatives. MRP (maillard reaction products) or bound CQA should be responsible for the TEAC in roast coffee, whereas the high content of CQA and di-CQA in extracts may contribute to other functional activities like modifying the glycemic metabolism.

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Association of Coffee Intake with Diabetes Type 2, Dyslipidaemias, and Obesity in Adults from the Federal District, Brazil

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SUMMARY

Considering recent associations between habitual coffee consumption and human health, the objective of the current study was to verify the association of coffee intake with socio-behavioural parameters and the incidence of Diabetes mellitus type 2 (DM 2), dyslipidaemias and obesity in the Federal District population. This is a cross-sectional study with a sample size of 1072 individuals, interviewed by telephone. There was a significant association between the categories of coffee consumption and age ($p = 0.000$), tobacco use ($p = 0.000$), body mass index ($p = 0.002$), physical activities practised ($p = 0.010$) and hypertension ($p = 0.0000$). Coffee consumption was greatest among smokers, the sedentary and those with higher body mass index (BMI), and was not associated with dyslipidaemias. The conclusion of the project will provide the complete characterization of the association between coffee consumption and the health of Federal District's population.

INTRODUCTION

Much research showing the benefits of coffee consumption has been published (Dorea and Da Costa, 2005; van Dam and Hu, 2005; Bidel et al., 2008). Among these studies, the inverse association between coffee consumption and the risk of DM2 has been notable. There also exists evidence that caffeine increases thermogenesis, lipid oxidation and lipolysis, which would help in weight loss, both in obese and non-obese individuals (Greenberg et al., 2006). In other studies, there has been an effort to evaluation the relationship between habitual coffee consumption and serum lipids, with some showing a positive association between coffee consumption and higher cholesterol levels (Grundy, 1995; Thelle et al., 1983). However, the preparation method for (non-filtered) coffee was a fundamental factor in this increase, since non-filtering allows the diterpenes that raise cholesterol to be present (Ahola et al., 1991). Therefore, the objective of the present study was to verify the association of coffee intake with socio-behavioural parameters and the incidence of Diabetes mellitus type 2 (DM 2), dyslipidaemias and obesity in the Federal District population.

METHODS

This is a cross-sectional study with a sample size of 1072 individuals, drawn at random from the residential telephone directory of the Federal District and the list of mobile phone prefixes supplied by ANATEL. The sample included individuals of 18 years old or over who agreed to take part in the study. They completed a questionnaire requiring data about the frequency and quantity of their coffee intake; age; sex; tobacco use; physical activities; reports on diabetes type 2, personal and family incidence of hypercholesterolaemia and hypertriglyceridaemia; weight and height. The weight and height data were used to calculate the BMI (weight in

kg/height in m^2), using for the analysis of nutritional status the classification proposed by the World Health Organization (1997). Descriptive statistical analysis of the variables was carried out using the Epi Info software version 6.04d and SPSS. Fisher's exact test was used to verify the existence of significant relationships between coffee consumption groups (0, ≤ 200 and > 200 mL/day) and the other variables. The research project was approved by the Ethics in Research Committee of the Health Sciences Faculty at the University of Brasilia. The individuals participated in the research after being made familiar with the study's proposed terms and agreeing with the same.

RESULTS

The majority of individuals (82.5%) reported that they consume coffee regularly. Of those interviewed, 68% were female. There was no significant association between the groups of coffee intake and sex ($p = 0.131$), diabetes type 2 ($p = 0.230$), hypercholesterolaemia ($p = 0.303$) and hypertriglyceridaemia ($p = 0.496$). There was a significant association between the categories of coffee intake and age ($p = 0.000$), tobacco use ($p = 0.000$), BMI ($p = 0.002$), physical activity ($p = 0.010$) and hypertension ($p = 0.0000$). More than half of the smokers (54.5%) were in the group of higher coffee intake (> 200 ml/day). In the other intake groups, there was a higher percentage of non-smokers. Among the individuals with BMI higher than 30 kg/m², 39.8% were to be found in the group that consumed > 200 ml/day, while in the other groups there was a higher percentage of individuals with BMI considered normal. It can be observed that among the coffee consumers the highest percentages were of those who do not practise physical activities, and about 94% of individuals considered to be suffering from hypertension are regular coffee consumers.

DISCUSSION

There was a significant association of coffee intake with age, tobacco use, BMI, physical activity and hypertension. Several studies have shown an association of coffee intake with smoking (Miyake et al., 1999; Bree et al., 2001). Some authors confirmed that the association of consuming this drink with BMI has a J-form, being lower among the people who presented moderate intake and higher when the quantity of coffee consumed goes up (Kleemola et al., 2000). In contrast with findings in the current study, Sesso et al. (1999) verified that individuals that drink coffee with caffeine were more physically active. However, it is interesting to emphasise data presented by Bidel et al. (2008), indicating that, among obese and inactive people, the intake of coffee (≥ 7 cups/day) reduces by half the risk of DM 2. In relation to the association between coffee and hypertension, a meta-analysis provided support for the link between coffee intake and high arterial pressure (Jee et al., 1999). However, some authors have suggested that despite the association of coffee consumption with slight rises in arterial pressure, this relationship seems to play a minor role in the development of hypertension (Klag et al., 2002).

The study presented some limitations, such as self-reported diseases, which can lead to under-reporting. For this reason, clinical and laboratory confirmation in a sub-sample of this population will be an important step in the study. Since it is a cross-sectional study, causal relations between the analysed variables cannot be established.

Table 1. Association of coffee intake categories with socio-behavioural, clinical and anthropometric variables, and dyslipidaemias in adult population of the Federal District.

Parameters	Coffee intake (mL/day)			*p* value
	0 (N=188)	**≤ 200** (N=543)	**> 200** (N=341)	
Sex				
Men	66 (19%)	157 (46%)	117 (35%)	0.131
Women	122 (17%)	386 (53%)	224 (30%)	
Age (years)				
18 - 25	54 (28%)	99 (51%)	42 (21%)	0.000
26 - 35	45 (17%)	146 (57%)	68 (26%)	
36 - 55	63 (15%)	199 (48%)	155 (37%)	
> 55	25 (13%)	101 (50%)	75 (37%)	
Tobacco use				
Smokers	7 (5%)	53 (40%)	72 (55%)	0.000
Ex-smokers	25 (12%)	99 (48%)	81 (40%)	
Non-smokers	156 (21%)	391 (53%)	187 (26%)	
BMI (kg/m²)				
Not given	7 (16%)	19 (42%)	19 (42%)	0.002
<18.5	11 (27%)	23 (56%)	7 (17%)	
18.5 – 24.9	120 (21%)	298 (51%)	166 (28%)	
25 – 29.9	38 (12%)	162 (52%)	114 (36%)	
≥ 30	12 (14%)	41 (46%)	35 (40%)	
Physical activity				
Yes	119 (21%)	276 (48%)	177 (31%)	0.010
No	69 (14%)	267 (54%)	163 (32%)	
Type 2 Diabetes				
Yes	5 (11%)	19 (43%)	20 (46%)	0.230
No	182 (18%)	520 (51%)	316 (31%)	
Not given	1 (10%)	4 (40%)	5 (50%)	
Hypertension				
Yes	12(6%)	122 (61%)	66 (33%)	0.0000
No	173 (20%)	417 (48%)	271 (32%)	
Not given	3 (27%)	4 (36%)	4 (36%)	
Hypercholesterolaemia				
Yes	21 (13%)	84 (53%)	55 (34%)	0.303
No	164 (19%)	439 (50%)	275 (31%)	
Not given	3 (9%)	20 (59%)	11 (32%)	
Hypertriglyceridaemia				
Yes	13 (12%)	56 (53%)	37 (35%)	0.496
No	168 (18%)	461 (50%)	285 (31%)	
Not given	7 (14%)	26 (50%)	19 (36%)	

Notes: p value was obtained from Fisher's exact test and it is significant when p < 0.05.

CONCLUSIONS

The consumption of coffee was associated with undesirable habits, such as smoking and sedentary behaviour, with body mass index and hypertension. These variables should be considered as confounding factors in the necessary adjustment for analysis between coffee

and diabetes type 2. The consumption of coffee was not associated with hypertrigliceridaemia and hypercholesterolaemia, possibly since filtered coffee is more commonly consumed by this population. The conclusion of the study will make it possible to characterise completely the consumption of coffee and its associations with the health of the population in the Federal District.

ACKNOWLEDGMENTS

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Association of Coffee Intake with Depression, Lifestyle and Habits of the Adult Population in the Federal District, Brazil

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SUMMARY

Coffee is widely consumed throughout the world, and this has led to great interest in evaluating the effect of its consumption on health. The aim of this work was to verify the association of coffee intake with the risk of developing depression, with body mass index (BMI), and with socio-behavioural parameters, habits and lifestyle among the adult population of the Federal District. A significant difference was observed between the quantity of coffee consumed and age ($p = 0.000$), tobacco use ($p = 0.000$), physical activities practised ($p = 0.006$) and BMI ($p = 0.000$). The other variables did not present significant values. The conclusion of the study will allow the complete characterization of the association between coffee consumption and the health of the Federal District population.

INTRODUCTION

Coffee possesses a variety of bioactive compounds and can be considered a functional food (Dorea and Da Costa, 2005). Some studies indicate that the regular consumption of coffee can prevent the development of depression (Encarnação and Lima, 2003). Others have found a significant association between coffee intake and arterial pressure levels (Jee et al., 1999), BMI (weight in kg/height in m^2) (Rosengren et al., 2004), physical activities practised (Machado, 2006), and unhealthy habits and lifestyles, such as smoking and drinking (Salazar-Martinez et al., 2004). These factors may also be associated with depression, and the result is that there is much controversy in the literature on this point. This study, therefore, aimed to verify the association of coffee consumption with the risk of developing depression, and with BMI, socio-behavioural parameters, habits and lifestyle among the adult population of the Federal District.

MATERIAL AND METHODS

This is a cross-sectional study of 1070 adult individuals, randomly drawn from residential and mobile telephone directories supplied by ANATEL. A questionnaire was filled in, by telephone, after verbal consent. It included questions about coffee intake, symptoms of depression, number of years of study, age, sex, weight, height, tobacco use, alcoholic beverage intake and physical activity.

The BMI was classified according to methods proposed by the World Health Organization (1997). Questions asked to identify the risk of depression were based on the Hamilton Scale (Hamilton, 1960), and the classification was carried out in an arbitrary way by adding up the points, producing the categories of no risk (0), low risk (1-5 points), moderate risk (6-11 points) or severe risk (12 to 16 points). Coffee intake was measured by number of cups (50 mL) consumed, and grouped as: no consumption (0); ≤ 4 cups/day and > 4 cups/day. The Epi Info software, version 6.04d, was used for descriptive analyses. Fisher's exact test ($p < 0.5$) in

the SPSS software, version 13.0, was used to check significant differences between the consumption of coffee and each variable studied.

RESULTS

Of the studied population, more than 82% consumed coffee. The majority of those interviewed were female (68%). However, there was no statistical difference between the groups of coffee intake and the sex of the consumer (p = 0.124). The same thing was observed in terms of coffee intake groups and years of study (p = 0.217), alcohol intake (p = 0.274) and risk of depression (p = 0.081). There was a significant difference between coffee intake and age (p = 0.000), smoking (p = 0.000), physical activity (p = 0.006) and BMI (p = 0.000) (Table 1).

Table 1. Association of coffee intake (in cups per day) with socio-behavioural parameters, depression risk and BMI in adults from the Federal District, Brazil.

Parameters		Coffee intake in cups*								
		0		≤ 4		> 4		Total		P value
		n	%	n	%	n	%	n	%	
Number of subjects		188	18	542	50	340	32	1070	100	
Age (years)	< 35	96	22	232	54	103	24	431	100	
	35 - 54	67	16	198	47	157	37	422	100	0.000
	≥ 55	25	11	112	52	80	37	217	100	
Sex	Male	66	20	157	46	117	34	340	100	
	Female	122	17	385	53	223	30	730	100	0.124
Tobacco use	Smoker	7	5	53	40	72	55	132	100	
	Ex-smoker	25	12	98	48	81	40	204	100	0.000
	Non-smoker	156	21	391	53	187	26	734	100	
Alcoholic beverage										
Non-drinkers		141	19	375	51	225	30	741	100	0.274
Up to 200 mL/day		34	13	124	50	89	37	247	100	
More than 200 mL/day		13	16	43	52	26	32	82	100	
Years of study										
Under 8		44	16	130	47	100	37	274	100	
8 - 11		68	18	195	53	106	29	369	100	0.217
12 - 15		64	19	174	51	105	30	343	100	
16 - 19		9	16	29	52	18	32	56	100	
Illiterate		3	33	2	22	4	45	9	100	
Not given		0	0	12	63	7	37	19	100	
Physical activity	Yes	119	21	275	48	177	31	571	100	
	No	69	13	267	54	163	33	499	100	0.006
Depression risk	No risk	64	22	139	49	83	29	286	100	
	Low	84	17	258	51	160	32	502	100	0.081
	Moderate	29	13	114	50	84	37	227	100	
	High	11	21	31	56	13	23	55	100	
BMI (Kg/m^2)										
18.5 – 24.9		131	21	320	51	173	28	624	100	0.000
25 – 29.9		38	12	161	51	115	37	314	100	
≥ 30		12	13	41	47	35	40	88	100	
Not given		7	15	20	46	17	39	44	100	

***Note:** * cups are 50 mL each.*

It was thus verified that the number of consumers and the quantities of coffee consumed increase with age; among consumers of more than 4 cups/day there were more smokers (55%), while 53% of consumers of ≤ 4 cups and 21% of non-consumers were among the individuals who had never smoked; there is a higher percentage of sedentary individuals among the coffee consumers; in terms of nutritional status, there was a higher percentage of consumers of > 4 cups among the obese individuals (40%), while among non-consumers there was a higher percentage among the normal ones (21%) (Table 1).

DISCUSSION

The results show a greater percentage of non-consumers of coffee among young people. Among high consumers of coffee there was a greater percentage of smokers and overweight individuals. In addition, there were more coffee consumers found among the sedentary. Other studies have verified an increase in coffee intake with advancing age (Kleemola et al., 2000), among smokers (Grobbee et al., 1990) and higher BMI with an increase in coffee intake (Kleemola et al., 2000). In contrast to this study, some authors have reported that individuals who consume coffee are more physically active (Sesso et al., 1999). It was also observed that there was no statistical difference between the coffee intake groups and sex, years of study, intake of alcoholic beverages and risk of depression. More studies on this question are necessary.

It should be mentioned that some authors believe that endogenous peptides are important in determining mood. Blocking opioid receptors for long periods may be beneficial, as long as there are no important side effects. This blocking can be obtained from moderate daily coffee intake, leading to the hypothesis that coffee intake may be a preventive agent of depression (Encarnação and Lima, 2003). The univariate analysis is preliminary. A more robust evaluation taking into account behavioural aspects, lifestyle, coffee intake and depression will be made at the end of this study.

CONCLUSION

The results presented are still preliminary and show a significant difference between coffee intake groups and age, tobacco use, sedentary habits and BMI. There was no statistical difference between coffee intake groups and sex, years of study, drinking and depression risk. The conclusion of this study will make it possible to characterise completely, employing a multivariate analysis, the variables involved in the risk of depression and consumption of coffee and associations with the health of the population in the Federal District.

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Bioactive Compounds and Antioxidant Activity of Brazilian Roasted Coffees from the South of the Minas Gerais State, Brazil

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SUMMARY

At the present work four Arabica coffee (*Coffea arabica*) samples from South of Minas Gerais State − Brazil, that were previously classified as Soft, Hard, Rioysh and Rio, were submitted to light roasting (light brown beans) and chemically analyzed. When considering to the content of total phenolics, chlorogenic acids, caffeine and trigonelline significative differences were observed between the beverage quality types. The levels of total phenolic compounds, chlorogenic acids and trigoneline were higher in soft coffee; the proanthocyanidin levels were higher at Rio coffee and caffeine levels were higher in Hard coffee. Both coffee presented scavenger activities against DPPH$^{\cdot}$ radicals, however, the coffee antioxidant activity of Rio coffee was higher. The variations observed for caffeine, total phenols, proanthocyanidins, trigonelline and chlorogenic acids levels cannot explain the higher antioxidant activity observed for the Rio coffee (EC_{50} of 14,89 ± 4,40 mg mg^{-1} of DPPH$^{\cdot}$).

INTRODUCTION

Brazil is the world's largest coffee beans producer and 28 million 60-kilogram sacks of coffee were exported after 2007 harvest, which represent a 2.4% increase related to 2006. According to Brazilian Coffee Industry Association (ABIC), the internal sales will jump up from 16,3 millions of sacks in 2006 to 21 millions sack in 2010, so Brazil will become the largest world's consumer of the product, surpassing an estimate of 20 million sacks consumed by USA.

The drinking coffee quality is directly related to its chemical composition. Otherwise, its classification is entirely based on a sensorial analysis done by the cup test (Toledo and Barbosa, 1998). This can explain the reasons why chemical analyses are so primordial and essential to complement this classification.

By possessing bioactive compounds, coffee presents antioxidant activity (Anese and Nicoli, 2003). Research shows that coffee has many other bioactive compounds besides caffeine, like B3 vitamin, chlorogenic acids, quinides (formed by chlorogenic acid roasting) that are among hundreds of others, in majority volatile, that need to be studied (Morais et al., 2008).

Recently, many studies have indicated that free radicals and other oxidant compounds are responsible for ageing and degenerative diseases associated with ageing as cancer, cardiovascular diseases, cataract, immune system and cerebral malfunctions (Atoui et al., 2005).

The spectrophotometric method used to determine the antioxidant activity of organic compounds is based on the reduction of the free radical DPPH (1,1-diphenyl-2-picrilhydrazil). The free radical is converted to a DPPH-H form, showing fast absorbance decay at wave-

length of 515 nm. The DPPH is reduced by compounds present in coffee with antioxidant properties (AH) (Nebesny and Budryn, 2003).

MATERIAL AND METHODS

The coffee samples from Mundo Novo cultivar, sieve 17/18, previously classified as Soft, Hard, Rioysh and Rio were provided by COOPACAFÉ, from Perdões city, Minas Gerais State, Brazil. All samples were from 2001/2002 harvests and were sun dried in a cemented terrain. The beans roasting were processed in a Pinhalense brand, model TC-0 bench micro roaster, from 180 to 220 °C (mean temperature of 200 ± 20 °C). The light roasting point was reached after 6,0 ± 1,0 minute. The samples were ground and passed through 0,710 mm (24 mesh) sieves and stored in polyethylene bags under refrigeration at -10,0 ± 3,0 ^{o}C until analysis time (Morais et al., 2008).

The total phenolics were determined through a UV-Visible spectrophotometric assay using Folin-Ciocalteau method with some modifications (Morais et al., 2007; 2008).

The proanthocyanidin determination was obtained with vanillin method (Morais et al., 2007). Trigoneline, caffeine and chlorogenic acids levels were determined by High Performance Liquid Chromatography (HPLC) (Morais et al., 2008).

The antioxidant activity of coffee extracts were determined by the consumption of the stable free radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\cdot}$) (Morais et al., 2008, Yildirim et al., 2001).

RESULTS AND DISCUSSION

The total phenolic contents (Folin-Ciocalteu method), proanthocyanidins, chlorogenic acids, caffeine and trigonelline showed significant differences among the beverage types (Table 1). The Soft coffee type presented higher contents of total phenolics, chlorogenic acids and trigonelline and Rio coffee type is richer in proanthocyanidins.

Table 1. Bioactive compounds of coffee samples from the South of the Minas Gerais State.

Coffee beverages	BIOACTIVE COMPOUNDS (%)				
	Total Phenol	Proanthocyanidins	Chlorogenic acids	Caffeine	Trigonelline
Soft	44.72	2.75	3.55	1.31	1.22
Hard	36.72	2.85	3,05	1,38	1.19
Rio	42.52	4.60	2,75	1,31	0,56
Rioysh	35.80	3.17	2,96	1,28	0,78

The Hard coffee showed a higher caffeine level, but the differences among the coffee samples were not significant.

The reduction of [DPPH$^{\cdot}$] was followed monitoring the decrease in absorbance at 515 nm until the reaction reached a steady state. The sample required to decrease the initial DPPH$^{\cdot}$ by 50% (EC_{50}) was calculated graphically. Rio coffee extract presented the highest activity (Table 2).

Table 2. Antioxidant activity of coffee extracts from the South of the Minas Gerais State (~ 30 mg L^{-1} and 40 min of reaction).

Coffee	Inhibition of DPPH· (%)	$EC_{(50)}$ (ppm)	mg mg^{-1} DPPH·
Soft	67,63±8,58	19,92±5,01	0,93±0,25
Hard	77,46±7,45	16,89±3,28	0,87±0,14
Rio	72,74±9,54	14,89±4,40	0,72±0,21
Rioysh	65,46±5,58	18,35±4,42	0,91±0,22

The Figure 1 shows the time response curve for the radical scavenging activity of a Rio coffee in different concentrations.

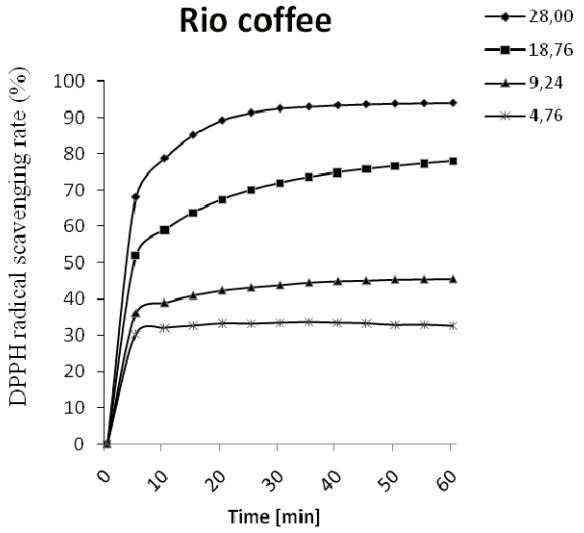

Figure 1. The time response curve for the radical scavenging activity of a Rio coffee in different concentrations.

All the samples showed a fast DPPH· consumption, and no significant DPPH· consumption was observed after 10 minutes of reaction, suggesting this is a fast kinetic reaction (Brand-Williams et al., 1995)

All the samples showed free radical scavenging activity. The amount needed for to reduce the initial DPPH· concentration by 50% (CE_{50}) was about 14,89 ± 4,40 to 19,92 ± 5,01. The Rio sample presented the higher scavenging activity.

CONCLUSION

The results of this study indicate the presence of compounds with antioxidant activity in coffee extract. The variations observed for caffeine, total phenols, proanthocyanidins, trigonelline and chlorogenic acids levels cannot explain the higher antioxidant activity observed for the Rio coffee (EC_{50} of 14,89 ± 4,40 mg mg^{-1} of DPPH·). The higher proanthocyanidins concentration presented by the coffees from the South of Minas Gerais could be responsible for their greater astringency.

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Profile and Levels of Bioactive Amines During Instant Coffee Processing

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SUMMARY

The influence of processing steps on the profile and levels of free bioactive amines in instant coffee was investigated. Samples were collected at an instant coffee processing plant on 3 different days, at different stages of processing, including green coffee beans, roasted coffee, concentrated coffee and instant coffee. Amines were determined by ion pair-HPLC and fluorimetric detection. Processing significantly affected the profile and levels of amines in instant coffee. Spermidine (SPD), spermine (SPM), putrescine (PUT), tyramine (TYM), serotonin (SRT) and tryptamine (TRM) were detected in the green coffee beans, however only low levels of SRT and SPD were detected in the roasted beans. After extraction, the levels of these amines increased. Instant coffee had only TYM and phenylethylamine (PHE). Total amine levels were higher in green beans (2.87 mg/100 g), decreasing to 0.18 mg/100 g on the roasted beans, increasing after extraction (1.02 mg/100 g) and decreasing afterwards (0.31 mg/100 g). Green coffee beans contained amine from 0.09 mg/100 g for tryptamine to 1.13 mg/100 g for putrescine. Phenylethylamine was only detected in instant coffee at 0.14 mg/100 g.

INTRODUCTION

Coffee is the most widely consumed beverages throughout the world due to its pleasant taste and aroma, stimulant effect and several beneficial health properties (Farah et al., 2006). Instant coffee is a very popular way to commercialize coffee. Even though it was invented in 1901, it was commercially available only in 1938. Its popularity and use has increased significantly because of the ease and speed of preparation and long shelf life (Nogueira and Trugo, 2003).

In recent years the number of studies on the composition of coffee has increased significantly, mainly concerning phenolic acids and nitrogenous compounds such as caffeine and trigonelline. Studies have also been undertaken on bioactive amines, which play important roles in plant development and also in human health. In plants, amines are required for growth, control intracellular pH, response to stress, and defense responses to pathogens, insects and predators. The profile and levels of bioactive amines in a product can be used as a quality index, reflecting the quality of the raw materials or the hygienic conditions prevalent during processing. With regard to human health, some amines are required for normal development and growth, response to stress, inhibition of lipid peroxidation, stabilization of membranes, maturation of the gastrointestinal tract, whereas others are vasoactive or psychoactive (Glória, 2005; Kalac and Krausova, 2005).

Amorim et al. (1977) were the first to study free amines in coffee. However, only 25 years later, further studies were undertaken. Green coffee was reported to contain mainly putrescine, spermidine and spermine. Other amines were also found in green coffee, among them serotonin, tyramine, histamine and cadaverine. When comparing amines in *Coffea arabica* L. and *Coffea canephora* var. robusta, the levels of putrescine were lower and of tyramine were higher in the latter. Besides the differences on amine levels among coffee varieties, there were also significant differences on the levels of amines on samples from different origins, cultivation practices, sensory quality of the beverage, coffee bean quality and also to the analytical methodology used in the extraction of the amines (Cirilo et al., 2003; Casal et al., 2004; 2005; Oliveira et al., 2005; Vasconcelos et al., 2007).

Silveira et al. (2007) determined the types and levels of amines in instant coffee. Total amine levels in the dry instant coffee varied from 0.28 to 2.76 mg/100 g. Overall, nine amines were detected: serotonin, cadaverine, tyramine, spermidine, putrescine, histamine, agmatine, phenyletylamine and spermine. Tyramine was present in every sample, followed by cadaverine and serotonin. Overall, serotonin was present at higher levels followed by cadaverine, tyramine and spermidine. The levels of amines varied among lots of the same brand and among brands.

During production of instant coffee, the grains are roasted, submitted to extraction under pressure at high temperatures (180 °C), and the extract is dehydrated by spray or freeze drying (Nogueira and Trugo, 2003). Therefore, low levels of amines would be expected in instant coffee, what does not agree with results reported by Silveira et al. (2007). It would be interesting to determine the source of amines during instant coffee production. The objective of this study was to investigate the influence of the processing steps on the profile and levels of free bioactive amines in instant coffee.

MATERIAL AND METHODS

Samples of coffee were collected at an instant coffee processing plant in three different days, at different stages of processing, including green coffee beans, roasted coffee, concentrated coffee and instant coffee.

The standards of bioactive amines were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The reagents were of analytical grade, except those used in HPLC analysis which were chromatographic grade. Water was purified in Milli-Q (Millipore Corp., Milford, MA, USA).

Determination of free bioactive amines

The free amines were extracted from the coffee samples (5 g) with 20 mL of 5% TCA and filtered through 0.45 μm pore diameter HAWP membrane. The amines were separated by ion pair-HPLC using μBondapak C18 column (300 x 3.9 mm i.d, 10 mm) (Waters, Milford, USA), and quantified after post column derivatization with *o*-phthalaldehyde and fluorimetric detection at 340 nm of excitation and 445 nm of emission (Cirilo et al., 2003). The amines were identified by comparison of the retention time of peaks in the sample in relation to standards and confirmed by the addition of the suspect amine to the sample. The concentrations of the amines were determined by direct interpolation in individual standard curves with $R^2 \geq 0.9926$. The limits of determination were 0.02 mg/100 g for spermidine, spermine, agmatine, putrescine, cadaverine, histamine, tyramine and phenylethylamine and 0.04 mg/100 g for serotonin and tryptamine.

Moisture content

The moisture content was determined in the samples using the Karl Fisher method (IAL, 1985).

Statistical analysis

All of the analyses were performed in duplicate. The data were submitted to analysis of variance and the means were compared by the Duncan test at 5% probability using SIGMA STAT 2.0 (Systat Software Inc, Richmond, CA, USA).

RESULTS AND DISCUSSION

Six amines were detected in green coffee: spermidine, spermine, putrescine, tyramine, serotonin and tryptamine. Putrescine was the prevalent amine, followed by serotonin, spermidine, tyramine, spermine, and tryptamine (Figures 1 and 2). The levels of amines in green coffee are similar to literature data, with small differences probably related to different origins, cultivation practices, coffee bean quality, and also to the analytical methodology used in the extraction of the amines.

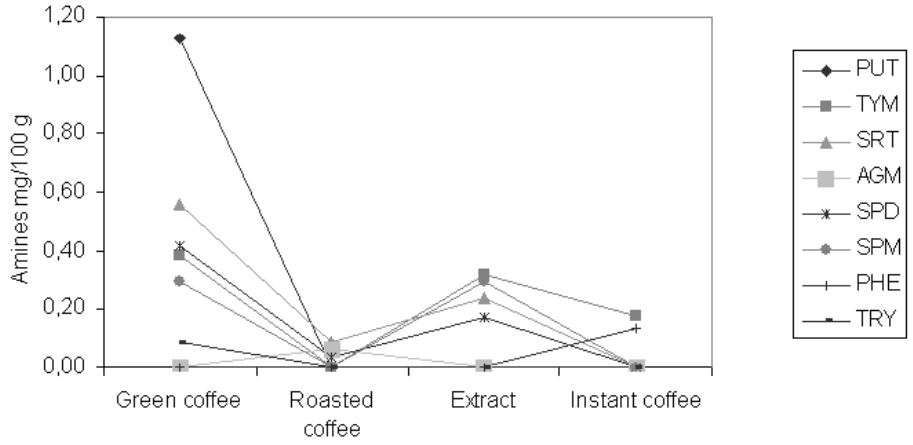

Figura 1. Influence of processing on profile and levels of bioactive amines in instant coffee production.

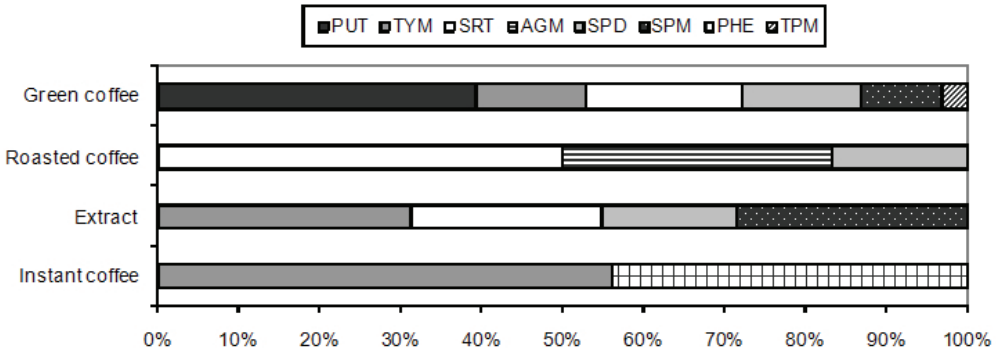

Figura 2. Contribution of each amine to total levels in process instant coffee step.

Processing significantly affected the profile and levels of amines in instant coffee. Total amine levels in green beans (2.87 mg/100 g), decreased to 0.18 mg/100 g upon roasting, increasing after extraction (1.02 mg/100 g) and decreasing afterwards (0.31 mg/100 g).

During roasting there was a significant decrease on the levels of amines and the presence of agmatine was observed, and only low levels of SRT and SPD were detected in the roasted beans. Based on these results, it can be concluded that the roasting temperatures used were capable of destroying some amines, whereas there was also formation of agmatine.

After extraction, the total levels of these amines increased. The presence of spermidine, serotonin, spermine and tyramine was observed. Tyramine and spermidine were the prevalent amines, followed by serotonin and spermidine. During this stage, there was loss of serotonin and agmatine, and formation of tyramine and spermine. Instant coffee had only tyramine and phenylethylamine. This last amine was only detected in instant coffee at 0.14 mg/100 g. No information was available on the effects of extraction and dehydration of coffee during instant coffee production; therefore, studies are necessary to investigate the changes on amines levels during these stages.

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Comparison of Chlorogenic Acids Contents in Coffee and Medicinal Plant Extracts

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SUMMARY

Chlorogenic acids (CGA) are mono- and di-esters of trans-cinamic and quinic acids found in almost every existing plant. Various pharmacological properties have been attributed to CGA compounds from medicinal plants. Green coffee has been reported as one of the main CGA food sources. However, comparing the CGA contents in coffee, medicinal plants and foods is difficult due to the lack of data on CGA content in most of these food products. In this study, the CGA composition of coffee extracts and medicinal plants commonly used in South America was investigated by HPLC and their contents were compared.

INTRODUCTION

Chlorogenic acids (CGA) are a family of phenolic compounds, generally mono- and diesteres, formed between hydroxycinnamic acids (mainly caffeic, ferulic and *p*-coumaric acids) and quinic acid. At least trace amounts of CGA are found in almost every existing plant. These antioxidant compounds are produced in response to environmental stress conditions such as infections by microbial pathogens, mechanical wounding, and excessive UV or visible light levels (Farah and Donangelo, 2006).

Wild plants have been receiving much attention as sources of biologically active substances (Dillard and German, 2000) and research on phytochemicals and their effects on human health have been intensified (Chen et al., 2007). As a consequence, pharmacological properties inherent to plants consumed as beverages believed to provide health benefits have been attributed to CGA, among other phenolic compounds (Yonathan et al., 2006; Choi et al., 2005). Some of the potential pharmacological properties of CGA observed in *in vitro* and *in vivo* studies were: hypoglycemic, hepatoprotective, anti-inflammatory, immunostimulant and antiviral activities (Monteiro et al., 2007).

Green coffee stands out as a major food source of CGA compounds (Farah and Donangelo, 2006). Moreover, even after the roasting process during which extensive losses may occur due to their thermolability, coffee still contains a significant amount of these compounds (Farah et al., 2005). Considering the high CGA contents in coffee as well as their potential beneficial effects to humans, the idea of coffee as a nutraceutical food has been highlighted (Farah, 2004). However, comparing CGA contents in coffee, medicinal plants and foods is difficult due to the lack of data on CGA content in most of these food products.

In this study, medicinal plants commonly consumed in South America were selected according to their popular use and their CGA composition was determined. Subsequently, CGA contents of coffee extracts prepared from seeds roasted to different degrees were determined and compared to those of the plant extracts.

MATERIAL AND METHODS

Samples

Fourteen samples of dried medicinal plants popularly used in South America were obtained from reliable commercial sources in Rio de Janeiro (Brazil): leaves of *Ilex paraguariensis* (green and toasted maté); *Baccharis genistelloides* ("carqueja"), *Camellia sinensis* (green and black fermented tea); *Melissa officinalis* (lemon balm); *Cymbopogon citratus* (lemon grass); *Cydonia oblonga* (quince); *Maytenus ilicifolia* ("espinheira santa"); *Annona muricata* ("graviola"); *Ginkgo biloba* (Maidenhair or Ginkgo); *Peumus boldus* (boldo) and *Syzygium cumini* (jambolan); seeds of *Pimpinella anisum* (anise); flowers of *Achyrocline satureioides* ("macela") and peels of *Erythrina velutina* ("mulungú"). Two samples of green *C. arabica* beans (classified by the Brazilian Association of Coffee Industry – ABIC – as Soft and Hard cup quality) and one sample of green *C. canephora* beans (cv. Conillon) were roasted in an electric roaster similar to a corn popper (CAEL Ltda, Brazil), at 200 °C, for 8, 12, 27 and 45 min, to obtain light, medium, dark and very dark roasting degrees, respectively, according to the Roast Color Classification System (Agtron-SCAA, USA).

CGA analysis

Medicinal plants and coffee extracts were prepared with aqueous methanol (40%), at 0.5%, and clarified with Carrez reagents, according to a modification of the method of Trugo and Macrae (1984). The duplicate extractions were analyzed in a gradient HPLC system using a UV detector operating at 325nm, according to Farah et al. (2005). In order to express the CGA contents per weight of dry matter, water content for each sample was determined according to the A.O.A.C. method (2002).

RESULTS AND DISCUSSION

A total of eight CGA compounds were identified in the investigated medicinal plants and coffee extracts: 3-, 4- and 5-caffeoylquinic acids, 4- and 5-feruloylquinic acids and 3,4-, 3,5- and 4,5-dicaffeoylquinic acids (Table 1).

Total CGA contents in medicinal plants varied from 0.60mg/100g dry matter basis (dm) (*S. cumini*) to 9.73g/100g dm (green *I. paraguariensis*). The highest CGA content among investigated plants were observed in *I. paraguariensis* (green and toasted), *B. genistelloides*, *P. anisum*, *A. satureioides*, *C. sinensis* (green and fermented), *M. officinalis* and *C. citratus*. Such CGA contents were also expressive when compared to other plants considered as good CGA sources in the literature, for example, *Lavandula officinalis, Hyssopus officinalis* (Zgorka and Glowniak, 2001) and *Bidens pilosa* (Chiang et al., 2004).

In general, CQA and diCQA isomers were the most prevalent and abundant CGA compounds, although diCQA isomers were not identified in *M. ilicifolia*. FQA isomers were only identified in four plants. The contribution of CQA and diCQA classes for total CGA content varied according to the type of plant, reaching 100% of CQA in *C. sinensis* and 69% of diCQA in *A. satureioides*. Regarding individual isomers, 5-CQA was the only CGA identified in all investigated plants and also the most abundant in the majority of them, which indicates the importance of 5-CQA to plant metabolism in nature (Farah and Donangelo, 2006)

Table 1. Chlorogenic acids content in methanolic extracts of dried medicinal plants.[a]

Samples	**3-CQA**	**4-CQA**	**5-CQA**	**4-FQA**	**5-FQA**	**3,4-diCQA**	**3,5-diCQA**	**4,5-diCQA**
Green I. Paraguariensis	2386.5±115.7	1337.9±21.8	1599.6±77.3	47.4±2.2	28.6±1.1	549.7±25.5	2332.9±115.2	1364.9±24.1
Toasted I. paraguariensis	316.3±16.6	442.7±0.6	670.9±35.3	23.5±1.2	27.4±1.6	81.9±1.6	145.0±3.3	242.8±21.8
B.genistelloides	229.0±9.9	180.1±1.7	362.8±1.0	5.65±0.2	4.4±0.2	120.3±5.9	301.2±3.2	194.0±2.9
P. anisum	16.1±0.7	24.1±0.9	87.1±4.8	2.02±0.1	3.84±0.3	18.3±0.8	18.5±0.8	19.2±0.4
A. satureioides	6.6±0.3	9.7±0.5	33.6±1.2	Nd	Tr	57.1±3.2	30.9±0.3	24.2±0.7
Black C. sinensis	26.5±1.4	63.5±0.1	49.5±1.7	Tr	Nd	Tr	Tr	Tr
Green C. sinensis	36.6±1.2	81.8±4.5	22.4±0.2	Tr	Tr	Tr	Tr	Tr
M. officinalis	10.6±0.3	3.5±0.2	17.0±0.8	Nd	Nd	16.8±0.8	Tr	45.5±1.5
C. citratus	4.8±0.2	3.0±0.1	44.9±0.3	3.8±0.1	0.9±0.1	1.7±0.1	4.7±0.2	3.5±0.1
C. oblonga	15.2±0.1	15.1±0.6	33.1±1.8	Tr	Tr	3.1±0.1	2.1±0.1	11.0±0.2
M. ilicifolia	7.1± 0.3	12.7± 0.4	60.6±2.2	Nd	Tr	Nd	Nd	Nd
E. velutina	1.3±0.1	1.2±0.1	5.5±0.2	Tr	Tr	3.0±0.1	Tr	Tr
A. muricata	3.6±0.1	0.5±0.1	3.3±0.2	Nd	Nd	Tr	Nd	Nd
G. biloba	Nd	Nd	4.0±0.2	Nd	Nd	Tr	Tr	Tr
P. boldo	Tr	Tr	1.8±0.1	Tr	Tr	Tr	Tr	Tr
S. cumini	Tr	Tr	0.6±0.1	Nd	Nd	Tr	Tr	Tr

[a]*Results are shown as the means of extractions in duplicates ± SD, expressed in mg per 100g. CQA = caffeoylquinic acid; FQA = feruloylquinic acid; diCQA = dicaffeoylquinic acid; Nd = not detected (below detection limit of 1.70 µg/mL); Tr = trace amount (between detection limit and quantification limit of 5.00 µg/mL).*

Total CGA contents in coffee varied from 0.40g/100g dm (*C. arabica* Hard, roasted to very dark degree) to 9.92 g/100 g dm (green *C. canephora*). Total CGA contents found in Conillon sample were approximately 1.6 times higher than those of the average arabica samples, as previously reported in the literature (Farah and Donangelo, 2006; Trugo and Macrae, 1984; Clifford, 1989). The lowest total CGA contents were found in very dark roasted coffees (0.39 to 0.64 g/100 g), while intermediate contents were found in light (3.34 to 6.15 g/100 g), medium (1.18 to 2.10 g/100 g) and dark roasted coffees (0.50 to 0.90 g/100 g), due to gradual losses that occur in the roasting process. CQA was the main CGA class found in coffee samples, representing about 82%, 82%, 74%, 71% and 67% of the average of total CGA in green, light, medium, dark and very dark coffees, respectively.

Green *C. canephora* and green *I. paraguariensis* showed similar total CGA contents, although large differences in individual CGA contents have been observed. For example, while 5-CQA was the most prevalent in coffee (57% of total CGA), the major CGA compound in green *I. paraguariensis* was 3-CQA (24% of total CGA). Total CGA contents in medium roasted coffees (1.18 g to 2.10 g/100 g, dm) were similar to those in toasted *I. paraguariensis* leaves (1.97 g/100 g dm) and *B. genestelloides* leaves (1.41g/100g dm).

Table 2. Chlorogenic acids contents in green and roasted coffee extracts. [a]

Roasting Degree	**3-CQA**	**4-CQA**	**5-CQA**	**4-FQA**	**5-FQA**	**3,4-diCQA**	**3,5-diCQA**	**4,5-diCQA**
C. arabica type Soft								
Green	0.54±0.03	0.58±0.02	3.50±0.09	0.07±0.01	0.18±0.01	0.27±0.01	0.31±0.01	0.33±0.00
Light	0.63±0.00	0.71±0.00	1.34±0.03	0.21±0.00	0.12±0.01	0.14±0.00	0.09±0.00	0.10±0.01
Medium	0.23±0.00	0.20±0.01	0.46±0.02	0.14±0.01	0.05±0.00	0.04±0.00	0.03±0.00	0.02±0.00
Dark	0.10±0.00	0.10±0.00	0.23±0.00	0.09±0.00	0.03±0.00	0.02±0.00	0.02±0.00	0.01±0.00
Very dark	0.10±0.00	0.10±0.00	0.25±0.00	0.09±0.00	0.03±0.00	0.03±0.00	0.02±0.00	Nd
C. arabica type Hard								
Green	0.77±0.02	0.90±0.02	3.84±0.13	0.09±0.00	0.19±0.00	0.26±0.01	0.22±0.00	0.28±0.01
Light	0.66±0.01	0.72±0.00	1.34±0.01	0.22±0.00	0.11±0.00	0.14±0.00	0.08±0.00	0.08±0.01
Medium	0.24±0.01	0.36±0.01	0.52±0.02	0.17±0.00	0.06±0.00	0.06±0.00	0.03±0.00	0.02±0.00
Dark	0.09±0.00	0.09±0.00	0.17±0.01	0.07±0.00	0.03±0.00	0.03±0.00	0.02±0.00	Nd
Very dark	0.07±0.00	0.06±0.00	0.15±0.00	0.07±0.00	0.02±0.00	0.02±0.00	0.01±0.00	Nd
C. canephora cv. Conillon								
Green	1.23±0.00	1.64±0.16	5.23±0.11	0.12±0.00	0.60±0.01	0.48±0.01	0.27±0.01	0.29±0.00
Light	1.21±0.05	1.89±0.05	2.04±0.13	0.32±0.01	0.27±0.01	0.16±0.00	0.13±0.00	0.12±0.00
Medium	0.33±0.00	0.38±0.01	0.78±0.01	0.31±0.02	0.17±0.01	0.06±0.00	0.05±0.00	0.03±0.00
Dark	0.14±0.00	0.14±0.00	0.33±0.01	0.16±0.00	0.08±0.01	0.03±0.00	0.02±0.00	0.01±0.00
Very dark	0.07±0.00	0.06±0.00	0.13±0.00	0.10±0.00	0.04±0.00	0.04±0.00	Nd	Nd

[a]*Results are shown as the means of extractions in duplicates ± SD, expressed in g per 100g. CQA = caffeoylquinic acid; FQA = feruloylquinic acid; diCQA = dicaffeoylquinic acid; Nd = not detected (below detection limit of 1.70 µg/mL).*

Our results show that green, light and medium roasted coffee seeds, green and toasted *I. paraguariensis* leaves, and *B. genestelloides* leaves stand out as major CGA food sources. These extracts should be thoroughly studied in relation to the pharmacological properties

attributed to CGA. Moreover, our results highlight coffee as a candidate food to be used for medicinal purposes.

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Comparison of Chlorogenic Acids Contents in Coffee Brews and Medicinal Plant Infusions Commonly Consumed in South America

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SUMMARY

Chlorogenic acids (CGA) are phenolic compounds considered as important active constituents of coffee and other plant material. However, lack of data on CGA composition in plants makes content comparisons very difficult. In this study, the CGA composition of dried medicinal plants commonly consumed in South America were investigated by HPLC analyses and compared to those of coffee brews.

INTRODUCTION

Phenolic compounds are secondary metabolites of higher plants found in a wide range of commonly consumed foods and beverages, which are associated with health benefits (Farah and Donangelo, 2006). Hydroxycinnamic acids such as caffeic, ferulic or *p*-coumaric acids form an important class of phenolic compounds in nature that occur naturally in free form or as a family of mono or diesters with (-)-quinic acid, collectively known as chlorogenic acids (CGA) (Pereira et al., 2003). Potentially beneficial properties to humans such as antioxidant, hypoglycemic, antiviral and hepatoprotective activities have been attributed to CGA *in vitro*, *in vivo* and epidemiological studies (Farah and Donangelo, 2006).

Green coffee is considered to be an excellent source of CGA (up to 14 g/100 g on dry matter basis) (Farah and Donangelo, 2006). Although extensive loss and changes may occur in CGA during the roasting process due to their thermolability, roasted coffee brews may still be considered as good CGA sources (Clifford, 2000). Therefore, coffee has been reported as a potential nutraceutical food (Farah, 2004).

CGA are also important active constituents of other food matrix, including plant materials consumed as beverages believed to provide health benefits. However, the lack of data on CGA composition in plants makes content comparisons very difficult.

In this study, the CGA composition of fourteen dried medicinal plants commonly consumed in South America were determined by organic solvent extraction followed by chromatographic analysis. Subsequently, infusions of plants showing the highest CGA contents were analyzed to properly evaluate the actual consumption of CGA through these beverages. Finally, CGA contents of coffee brews prepared from seeds roasted to different degrees were determined and compared to those of plant infusions.

MATERIAL AND METHODS

Samples

Fourteen dried medicinal plants popularly used in South America were obtained from reliable commercial sources in Rio de Janeiro (Brazil): leaves of *Ilex paraguariensis* (green and toasted maté); *Baccharis genistelloides* ("carqueja"), *Camellia sinensis* (green and black fermented tea); *Melissa officinalis* (lemon balm); *Cymbopogon citratus* (lemon grass); *Cydonia oblonga* (quince); *Maytenus ilicifolia* ("espinheira santa"); *Annona muricata* ("graviola"); *Ginkgo biloba* (Maidenhair or Ginkgo); *Peumus boldus* (boldo) and *Syzygium cumini* (jambolan); seeds of *Pimpinella anisum* (anise); flowers of *Achyrocline satureioides* ("macela") and peels of *Erythrina velutina* ("mulungú"). Following, three brands of each of the plants with highest CGA contents were purchased from different states in Brazil and used to prepare the infusions. Two samples of green *C. arabica* beans (cv. Bourbon Amarelo and Mundo Novo) and one sample of green *C. canephora* beans (cv. Conillon) were roasted in a spouted bed roaster at a maximum temperature of 230 ^{o}C for 6 to 9 min to obtain light, medium and dark roasted coffees according to the Roast Color Classification System, (Agtron-SCAA, USA).

CGA extractions and analyses

CGA organic solvent extractions were performed using a mixture of methanol/water, according to Trugo and Macrae (1984). "Home-made" type infusions of plants and coffee brews were prepared by pouring boiling water over ground-roast beans (0.5 and 10% w/v, respectively) followed by paper filtration, infusions and brews were then clarified with Carrez solutions (Ky et al., 1997). CGA analyses were performed in a gradient HPLC system, using a UV detector operating at 325 nm, according to Farah et al. (2005). In order to express the CGA contents per weight of dry matter, water content for each sample was determined according to the A.O.A.C. method (2000).

RESULTS AND DISCUSSION

A total of nine CGA compounds were identified in the investigated medicinal plants and coffee brews: 3-, 4- and 5-caffeoylquinic acids, 3-, 4- and 5-feruloylquinic acids and 3,4-, 3,5- and 4,5-dicaffeoylquinic acids. The highest CGA content among the fourteen investigated plants were observed in *I. paraguariensis* (green and toasted), *B. genistelloides*, *P. anisum*, *A. satureioides*, *C. sinensis* (green and fermented), *M. officinalis* and *C. citratus*.

Total CGA contents in plant infusions varied from 98 mg/200 mL cup (green *I. paraguariensis*) to 0.04 mg/200 mL (*M. officinalis*). Although, in general, samples of the same medicinal plants showed similar relative distribution of CGA compounds, a significant variation in the contents of individual isomers was observed for some of the plants, such as *C. sinensis* and *M. officinalis*.

In general, the highest CGA contents (Table 1) were observed in plants in which properties such as antioxidant, hepatoprotective, and antiviral have been observed in *in vitro* and animal studies (Bastos et al., 2007; Anderson and Fogh, 2001; Yam et al., 1997).

In coffee brews, CGA contents varied from 53 mg/ 200 mL cup (dark roasted *C. arabica*, cv. Mundo Novo) to 1405 mg/ 200 mL cup (green *C. canephora*), with intermediate contents found for light roasted (284 to 385 mg/ 200 mL cup) and medium roasted (127 to 162 mg/ 200 mL cup) coffee brews (Table 2).

Table 1 . Chlorogenic acids contents in plant infusions commonly consumed in South America[a].

Samples	**3-CQA**	**4-CQA**	**5-CQA**	**3-FQA**	**4-FQA**	**5-FQA**	**3,4-diCQA**	**3,5-diCQA**	**4,5-diCQA**
Green I. paraguariensis brand A	21.73±1.32	10.73±0.48	14.75±0.57	0.48±0.02	0.27±0.01	0.06±0.00	5.37±0.12	29.23±0.07	15.19±0.34
Green I. paraguariensis brand B	24.71±0.11	10.23±0.10	14.39±0.25	0.65±0.03	Tr	0.56±0.01	5.06±0.09	26.88±0.27	13.12±0.23
Green I. paraguariensis brand C	20.96±1.12	9.48±0.50	14.90±0.90	0.56±0.02	0.04±0.00	0.35±0.00	3.58±0.08	18.58±0.40	10.19±0.25
Toasted I. paraguariensis brand A	4.33±0.04	5.55±0.09	7.51±0.19	0.25±0.00	0.26±0.01	0.33±0.01	1.51±0.00	2.10±0.01	3.29±0.15
Toasted I. paraguariensis brand B	3.10±0.02	3.84±0.02	5.85±0.17	0.18±0.00	0.21±0.01	0.34±0.00	1.20±0.01	1.41±0.05	2.59±0.03
Toasted I. paraguariensis brand C	1.88±0.01	2.41±0.02	3.78±0.17	0.09±0.00	0.17±0.01	0.23±0.00	0.44±0.00	0.63±0.00	0.97±0.02
B. genistelloides brand A	1.96±0.02	1.57±0.04	3.23±0.04	0.09±0.00	Tr	0.09±0.00	1.20±0.05	3.20±0.05	2.28±0.06
B. genistelloides brand B	0.89±0.03	0.87±0.02	2.08±0.02	0.07±0.00	Tr	0.10±0.00	0.73±0.03	1.89±0.01	1.38±0.02
B. genistelloides brand C	0.12±0.01	0.27±0.01	1.08±0.03	0.09±0.00	0.02±0.00	0.04±0.00	0.70±0.01	1.59±0.04	0.56±0.01
A. satureioides brand A	0.12±0.01	0.14±0.00	0.69±0.03	Nd	Nd	Tr	0.94±0.02	1.06±0.03	0.53±0.01
A. satureioides brand B	0.11±0.00	0.13±0.01	0.52±0.02	Nd	Nd	Tr	0.75±0.03	1.12±0.03	0.39±0.01
A. satureioides brand C	0.05±0.00	0.03±0.00	0.16±0.01	Nd	Nd	Tr	0.14±0.00	0.57±0.02	0.27±0.02
P. anisum brand A	0.22±0.01	0.15±0.01	0.71±0.01	0.06±0.00	0.05±0.00	0.05±0.00	0.10±0.00	1.07±0.00	0.29±0.00
P. anisum brand B	0.24±0.01	0.14±0.01	0.92±0.01	0.02±0.00	0.03±0.00	0.12±0.00	Tr	0.91±0.02	Tr
P. anisum brand C	0.20±0.01	0.23±0.01	0.96±0.02	0.02±0.00	0.02±0.00	0.12±0.01	0.07±0.00	0.21±0.00	0.23±0.00
Black C. sinensis brand A	0.68±0.03	0.98±0.04	0.72±0.03	Tr	Tr	Nd	Nd	Nd	Nd
Black C. sinensis brand B	0.43±0.02	0.85±0.03	0.36±0.02	Tr	Tr	Nd	Nd	Nd	Nd
Black C. sinensis brand C	0.33±0.00	0.76±0.02	0.24±0.01	Tr	Tr	Nd	Nd	Nd	Nd
Green C. sinensis brand A	0.36±0.00	0.84±0.02	0.37±0.01	Tr	Tr	Tr	Nd	Nd	Nd
Green C. sinensis brand B	0.36±0.02	0.80±0.04	0.26±0.00	Tr	Tr	Tr	Nd	Nd	Nd
Green C. sinensis brand C	0.10±0.00	0.10±0.00	0.34±0.00	Tr	Tr	Tr	Nd	Nd	Nd
C. citratus brand A	0.04±0.00	0.04±0.00	0.52±0.02	0.15±0.00	Tr	0.03±0.00	0.30±0.01	0.24±0.01	0.02±0.00
C. citratus brand B	0.02±0.00	0.02±0.00	0.41±0.02	0.07±0.00	Tr	0.04±0.00	0.21±0.01	0.08±0.00	0.01±0.00
C. citratus brand C	0.02±0.00	0.02±0.00	0.30±0.00	0.13±0.00	Tr	0.04±0.00	Tr	0.08±0.00	0.03±0.00
M. officinalis brand A	1.08±0.03	0.77±0.02	0.98±0.00	0.03±0.00	Tr	Tr	0.35±0.01	2.15±0.09	0.72±0.02
M. officinalis brand B	0.03±0.00	0.01±0.00	0.11±0.00	Nd	Nd	Nd	0.07±0.00	Tr	0.57±0.00
M. officinalis brand C	Tr	Tr	0.02±0.00	Nd	Nd	Nd	0.01±0.00	0.01±0.00	Nd

[a]*Results are shown as the means of extractions in duplicates ± SD, expressed in mg per cup (200mL). CQA = caffeoylquinic acid; FQA = feruloylquinic acid; diCQA = dicaffeoylquinic acid; Nd = not detected (below detection limit of 1.70 µg/mL); Tr = trace amount (between detection limit and quantification limit of 5.00 µg/mL). Infusions were prepared at 0.5%.*

Table 2. Chlorogenic acids contents in coffee brews[a].

Roasting Degree	**3-CQA**	**4-CQA**	**5-CQA**	**3-FQA**	**4-FQA**	**5-FQA**	**3,4-diCQA**	**3,5-diCQA**	**4,5-diCQA**
C. arabica cv. Yellow Bourbon									
Green	109.3±1.3	145.4±2.8	639.6±10.6	11.8±1.2	11.3±1.3	44.6±6.3	13.4±1.7	16.5±2.3	9.7±2.4
Light	104.5±3.6	108.7±5.8	156.9±6.9	9.0±1.4	2.5±0.5	10.5±2.1	2.9±0.1	1.6±0.0	1.6±0.0
Medium	65.4±0.7	66.4±2.2	81.4±2.7	18.1±3.4	3.5±0.9	15.9±3.7	2.7±0.3	1.3±0.2	1.6±0.1
Dark	39.6±2.9	38.7±2.9	47.6±3.8	5.4±0.4	1.5±0.1	4.9±0.5	1.0±0.1	0.5±0.0	0.5±0.0
C. arabica cv. Mundo Novo									
Green	69.2±1.7	91.4±2.3	445.4±18.6	6.5±0.8	10.8±0.6	30.0±0.9	13.0±0.8	16.3±1.4	10.4±1.0
Light	77.0±0.0	81.4±0.5	113.6±1.0	7.6±0.3	2.2±0.6	10.0±1.5	3.2±0.0	1.9±0.0	1.7±0.2
Medium	43.8±0.5	44.7±0.7	56.6±0.0	5.5±0.5	1.0±0.0	5.4±0.1	1.3±0.2	0.6±0.1	0.6±0.0
Dark	14.6±0.6	14.4±0.6	17.4±0.8	2.6±0.3	0.5±0.0	1.9±0.1	0.4±0.1	0.1±0.0	0.1±0.0
C. canephora cv. Conillon									
Green	193.2±1.7	249.9±2.7	717.8±3.6	16.3±0.4	2.4±0.0	65.9±1.6	56.2±0.9	35.4±0.1	36.9±1.0
Light	60.2±0.4	69.3±0.6	102.8±1.1	13.3±0.4	0.8±0.0	23.0±1.2	2.8±0.1	1.5±0.1	1.9±0.1
Medium	26.6±0.9	29.7±0.8	43.6±1.7	7.3±0.3	0.6±0.0	12.3±0.7	0.8±0.1	0.4±0.0	0.8±0.1
Dark	11.4±0.3	13.5±0.1	19.2±0.1	4.5±0.1	0.3±0.0	7.8±0.1	0.2±0.0	0.2±0.0	0.1±0.0

[a]*Results are shown as the means of three replicates of extraction ± SD, expressed in mg per cup (200 mL). CQA = caffeoylquinic acid; FQA = feruloylquinic acid; diCQA = dicaffeoylquinic acid. Brews were prepared at 10% (w/v).*

Despite the decrease in CGA contents during the roasting process, roasted coffee brew still stood out as an excellent source of these compounds, followed by green *I. paraguariensis* infusion. Even thought green *I. paraguariensis* leaves possess a higher CGA content than roasted coffee beans per dry weight composition, the latter turned out to be a better source of CGA than green *I. paraguariensis* because coffee brews are more concentrated than plant infusions. Although green coffee is not usually consumed in the form of brews, from a pharmacological point of view, it may be approached as a potential therapeutic agent, along with green *I. paraguariensis* infusions, both being potentially useful in the production of nutraceuticals, cosmetics and plasters.

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Influence of Zinc Fertilization on Chlorogenic Acids and Antioxidant Activity of Coffee Seeds

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SUMMARY

In this study, we investigated the influence of zinc fertilization on the CGA levels and the antioxidant activity of coffee seeds. Coffee plants (*C. arabica*, cv. Catuaí Vermelho) were grown in Zn-deficient (control) and Zn-supplemented (treatment) soils. In the seeds obtained from the Zn-treated plants, the total CGA content was, on average, 4.2 g/100 g dwb, significantly higher ($p < 0.05$) than that of the control plants (3.6 g/100 g dwb). The antioxidant activity of the seeds was strongly correlated with total CGA content ($r = 0.84$, $p < 0.001$). The low CGA content found in the control plants suggest a metabolic response of the plant to the Zn deficiency condition, highlighting the importance of adequate Zn levels in soil for the development of coffee plant and seeds, and potentially, for consumers health.

INTRODUCTION

Zinc deficiency is one of the nutritional deficiencies that most dramatically interfere in plant growth and development. The coffee plant frequently shows deficiency in this micronutrient, especially when cultivated in soils with low Zn content, as those found in Minas Gerais, mainly at the *Cerrado* region (Martinez et al., 2003). Most of the observed symptoms of Zn deficiency are associated with disorders in the metabolism of auxins, mainly indolacetic acid (IAA), a phythormone responsible for plant growth (Malta et al., 2002). The mechanism by which Zn acts on the metabolism of auxins is still unclear. However, it is generally accepted that Zn is involved in the synthesis of tryptophan, an amino acid and precursor of IAA. Many studies have observed that Zn deficiency causes a lower rate of protein synthesis and tryptophan accumulation, which may lead to variations in IAA levels (Malta et al., 2002). Chlorogenic acids (CGA) are also involved in the control of IAA levels by the regulation of IAA oxidase activity. The compounds are a family of esters formed between certain *trans* cinnamic acids, such as caffeic (CA), ferulic (FA) and *p*-coumaric (*p*-CoA) acids, and (–)-quinic acid. The main subgroups of CGA isomers in coffee are the caffeoylquinic acids (CQA), feruloylquinic acids (FQA), dicaffeoylquinic acids (diCQA) (Clifford, 1999). Specific CGA compounds, mainly FQA isomers, are able to activate IAA oxidase and prevent IAA accumulation, whereas classes such as CQA and diCQA competitively inhibit this enzyme (Lee et al., 1982). In this study, we investigated the influence of zinc fertilization on the CGA levels and the antioxidant activity of coffee seeds.

MATERIALS AND METHODS

Coffee samples

Coffee plants (*C. arabica*, cv. Catuaí Vermelho) were grown in Zn-deficient (control) (n = 4) and Zn-supplemented (treatment) (n = 8) soils. Coffee fruits were harvested upon ripening and processed by the dry method.

Zinc analysis

Coffee leaves were dried at 70 ºC and ground. After digestion of the dried material with nitric and perchloric acids, zinc content was determined by atomic absorption spectrometry.

Chlorogenic acid analysis

CGA contents in the seeds were determined in triplicate by liquid chromatography-electronsray ionization–mass spectrometry (LC-ESI-MS) according to the method described by Perrone et al. (2008).

Antioxidant activity

The antioxidant activity of the CGA extract was evaluated in triplicate using the Ferric Reducing Antioxidant Power (FRAP) assay, according to the method reported by Benzie and Strain (1996) and modified by Moreira et al. (2005).

RESULTS AND DISCUSSION

Figure 1 shows the Zn content in the leaves and the total CGA contents in the seeds of both control and supplemented coffee plants. The leaves of the control plants showed Zn content consistent with a Zn-deficient state and significantly lower ($p < 0.05$) than the content found in the Zn-treatment plants, which reached adequate Zn levels (Martinez et al., 2003).

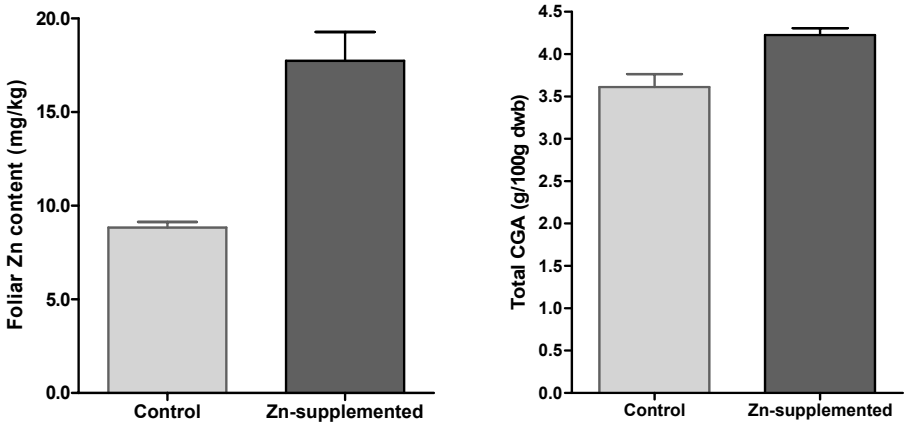

Figure 1. Zinc content in the leaves and total CGA content in the seeds of coffee plants cultivated in control (Zn-deficient) and Zn-supplemented soils.

On average, total CGA content in the seeds of the control plants was 3.6 g/100 g (dry weight basis - dwb), lower than the contents usually found in the literature for *C. arabica* samples (Farah and Donangelo, 2006). In the seeds obtained from the Zn-treated plants, the total CGA content was, on average, 4.2 g/100 g dwb, significantly higher ($p < 0.05$) than that of the control plants and in agreement with other results found in the literature (Farah and

Donangelo, 2006). This increase was mainly due to the increase in the levels of CQA and diCQA (Figure 2).

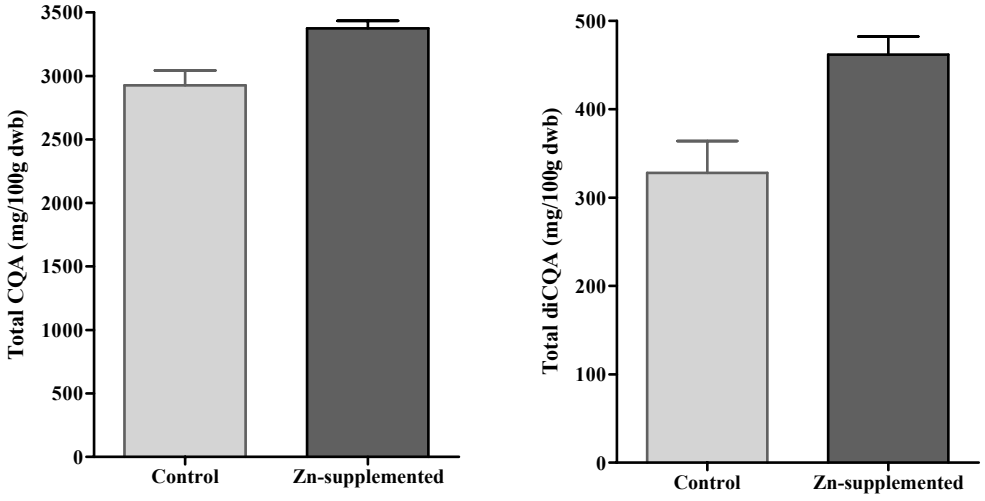

Figure 2. Total CQA and diCQA contents in the seeds obtained from coffee plants cultivated in control (Zn-deficient) and Zn-supplemented soils.

The antioxidant capacity of the seeds evaluated by the FRAP assay, was strongly correlated with the total CGA content (r = 0.84, $p < 0.001$) (Figure 3). A strong correlation between FRAP and the total CGA content, particularly that of the CQA isomers, has been reported in the literature (Moreira et al., 2005).

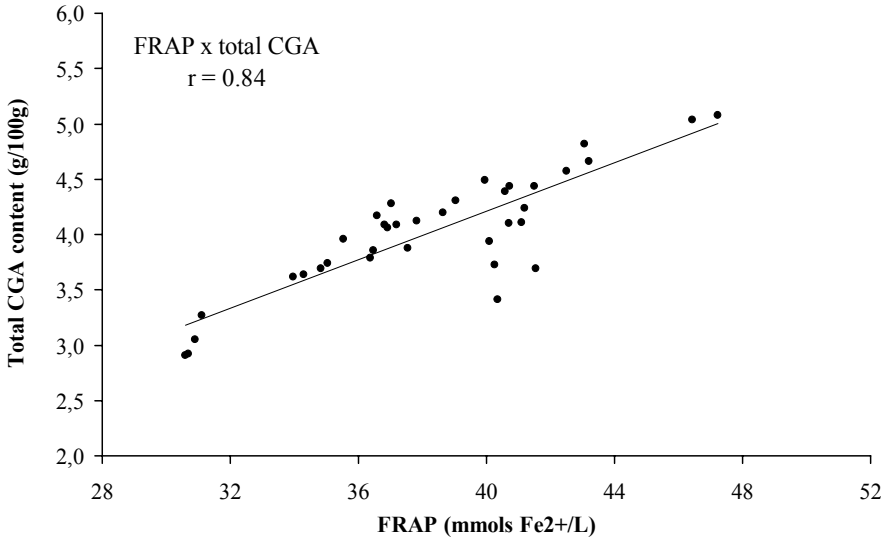

Figure 3. Correlation between total CGA content and the antioxidant capacity evaluated by the FRAP assay in coffee samples (*n* = 36).

The low content of CGA found in the control plants indicate a metabolic response of the plant to the Zn deficiency condition. Such condition would lead to tryptophan accumulation and, possibly, to variations in IAA levels. In order to counterbalance such variations, the plant would then decrease the biosynthesis of CGA.

Although further investigation is still necessary in order to fully understand the mechanisms by which Zn may influence CGA levels in coffee, our work demonstrated that adequate levels of Zn in the soil are very important for the development of coffee plants with high contents of antioxidant compounds, which may be beneficial for human health.

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Chlorogenic Acids and Lactones in Brazilian Commercial Coffees

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SUMMARY

Chlorogenic acids are a family of phenolic compounds responsible for 4-12% of coffee dry matter composition. These compounds are biologically active and contribute to coffee flavor. Cinnamoyl-1,5-γ-quinolactones (CGL), which are generated from CGA during the roasting process, are also biologically active and contribute to coffee bitterness. CGA and CGL contents in roasted coffee are highly dependent on the degree of roasting and data on this theme in the scientific literature is scarce. In the present work, we determined CGA and CGL distribution profiles in regular and decaffeinated commercial coffees available in the Brazilian market, the second world coffee market.

INTRODUCTION

After water, coffee is the most popular and most consumed beverage in the world (www.abic.com.br). Brazil is the first world producer and second consumer, with the annual average *per capita* consumption of 4.4 kg (www.abic.com.br). Quality improvement, marketing, increase in production of gourmet and special coffees, and reports on the positive effects of coffee on human health are important factors responsible for the increase in coffee consumption (www.abic.com.br). The decaffeinated coffee market has also expanded in the last few years due to the adverse effects of caffeine in some people, along with quality improvement, and nowadays decaffeinated coffee account for more than 10% of coffee market (Farah et al., 2006).

Chlorogenic acids (CGA), a family of phenolic compounds that generally accounts for 5-12% of dry matter composition, are known for their contribution to the final acidity, astringency and bitterness of the beverage (Farah et al., 2006; Perrone et al., 2008; Farah et al., 2005). Cinnamoyl-1,5-γ-quinolactones (CGL), which are generated from CGA during the roasting process, also contribute to coffee bitterness (Farah et al., 2005). Both CGA and CGL have been studied for their biological properties in humans, such as reduction of the relative risk of type 2 diabetes and changes on mood due to their action on brain receptors (Farah et al., 2005). CGA and CGL contents in coffee are highly dependent on the degree of roasting, being the light medium roast, on average, an optimum roasting degree to achieve the maximum amount of lactones and maintain a reasonable amount of CGA in the beans at the same time (Farah et al., 2006; 2005). In addition to losses during roasting, decaffeination process may also produce losses in chlorogenic acids (CGA) and their lactones (Farah et al., 2006).

Data on the chemical composition of Brazilian commercial coffees are scarce. In view of recent changes in Brazilian coffee industry practices produced by increase in education and quality control by the Brazilian Coffee Industry Association, information on CGA and CGL distribution profiles in regular and decaffeinated commercial coffees available in the Brazilian market is needed.

MATERIAL AND METHODS

Samples

Thirty samples, including nineteen ground-roast (five decaffeinated) and eleven instant (three decaffeinated) coffees, were bought from Rio de Janeiro and São Paulo markets.

Extractions and Chromatographic analyses

Coffee samples were extracted in duplicate by hot MilliQ water (90 ºC) and clarified with Carrez reagents (Farah et al., 2005; 2006). Extracts were analyzed in a gradient HPLC system, using a UV detector operating at 325nm, according to Farah et al. (2005).

Instrumental Color measurements

The determinations of instrumental color were conducted in a specific colorimeter for coffee (LEOGAP®, Probat Group).

Statistical analyses

The analyses were performed by GraphPad Prism® software, version 4.0 (San Diego, California, USA), using unpaired *t*- test for significant difference between two groups of means. Pearson correlation coefficient was calculated at a confidence level of 95%.

RESULTS AND DISCUSSION

Total CGA content in ground-roast coffees ranged from 0.27 to 1.98 g/100g, on wet basis (wb), whereas in instant coffees it ranged from 2.22 to 6.70 g/100 g (wb). As expected, instant coffee samples showed higher ($p < 0.001$) average total CGA content (3.70 g/100g of coffee) when compared with ground-roasted coffees (1.02 g/100 g of coffee) (Figure 1). This fact is mainly due to the higher content of soluble solids in instant coffee, among others factors such as species and varieties in blend formation (Smith, 1985).

Although in ground-roast coffees no difference was observed between the CGA contents of regular and decaffeinated coffees, in instant coffees, decaffeinated samples showed higher ($p < 0.001$) total CGA average content than in regular coffees. According to Smith (1985), after being decaffeinated, the green beans are usually darker and, as a consequence, a decrease in the duration of the roasting process to reach the same roasting degree as in regular coffee may be observed. This decrease in roasting time involves lower loss of CGA. The same significative difference was probably not observed in CGA content of decaffeinated ground-roasted coffees, due to large variability in CGA content among samples in this group.

3-caffeoyl-1,5-quinide (3-CQL) and 4-caffeoyl-1,5-quinide (4-CQL) were identified in all ground-roast coffees, being 3-CQL the major lactone, ranging from 47 to 214 mg/100 g. These results are in conformity with those previously obtained by Farah et al. (2005; 2006), for non-commercial coffee samples from Africa and Brazil. No difference was observed between total CQL contents of regular and decaffeinated samples.

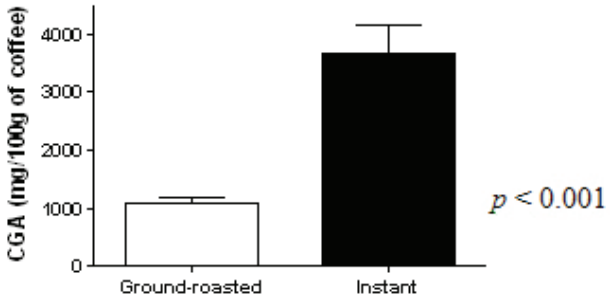

Figure 1. Comparison between average total CGA content of ground-roasted and instant coffees available in the Brazilian market (Rio de Janeiro and São Paulo) (n = 30).

Regarding instrumental color, total CGA content in ground-roast coffees was directly associated with the degree of luminosity (p = 0.003). In contrast, there was no correlation between instrumental color and total CGA content in instant coffee, probably due to the use of techniques for color and flavor modification commonly applied in this type of coffee. In addition, comparing the present colorimetric data with those obtained in the Brazilian market five years before (Nogueira and Trugo, 2003; Smith, 1985), it is possible to observe a tendency of lighter coffees production with a slightly higher CGA content along the years, probably due to the improvement in quality and the dissemination of the potential health effects of lighter coffees in the Brazilian coffee industry.

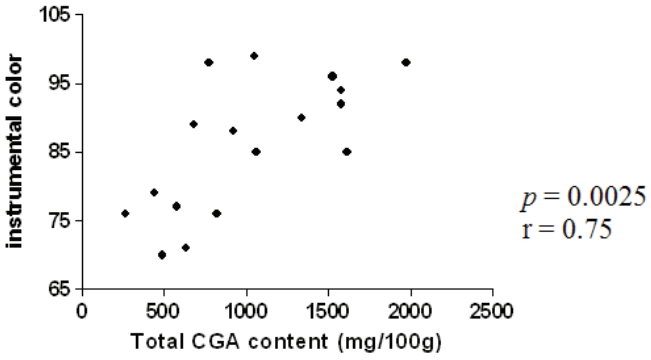

Figure 2. Pearson correlation between coffee instrumental color and total CGA content in ground-roast samples (n = 19).

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Contribution of Chlorogenic Acids and Melanoidins to Coffee Antioxidant Activity

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SUMMARY

In this study, we investigated the contribution of chlrogenic acids (CGA) and melanoidins to the antioxidant activity (AA) of coffee using three assays: FRAP, TEAC and TRAP. These three assays showed a strong correlation with each other ($R > 0.93$, $p < 0.001$) and with the CGA content of both whole and melanoidin-free coffee brews ($R > 0.70$, $p < 0.001$). In general, AA decreased with roasting and maximum AA was observed for melanoidin-free green and 6-min roasted whole coffee brew samples. The estimated contribution of CGA to the AA of the coffee brews ranged from 0.7% in the darker sample to 102.3% in the green sample. These results confirm the hypothesis that probably melanoidins, and not CGA, are the main compounds responsible for the AA of dark roasted coffees.

INTRODUCTION

Several studies have shown that chronic maladies such as cancer, cardiovascular, inflammatory and neurodegenerative diseases, and aging are associated with oxidative stress, a metabolic condition that causes cell degeneration (Beal, 1995). Antioxidant compounds present in fruits and vegetables appear to play a major role in the protection against oxidative stress (Pellegrini et al., 2003). Besides fruits and vegetables, plant beverages such as coffee contribute to the dietary intake of antioxidants (Daglia et al., 2004; Natella et al., 2002).

The antioxidant activity (AA) of coffee beverages has been assessed by several studies, using different methods (Daglia et al., 2004; Natella et al., 2002; del Castilo et al., 2002; Anese and Nicoli, 2003). It was shown that coffee has a high AA compared to other beverages, three times higher than red wine and five times higher than green tea (Pellegrini et al., 2003).

Coffee AA is usually associated to the content of chlorogenic acids (CGA), as well as melanoidins which are brown pigments formed during the roasting process (Daglia et al., 2004; del Castilo et al., 2002; Moreira et al., 2005). In the present study, we investigated the influence of roasting on the AA of whole and clarified (melanoidin-free) coffee brews, using three different assays: FRAP, TEAC and TRAP. We also estimated CGA contribution to the AA, using 5-caffeoylquinic acid (5-CQA) as a representative compound of the class.

MATERIALS AND METHODS

Coffee samples

Good cup quality samples of green *C. arabica*, cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho (Red Catuaí), *C. arabica* cv. Bourbon Amarelo (Yellow Bourbon) and *C. canephora*, cv. Conillon were obtained directly from producer in Guaxupé, Minas Gerais, Brazil.

Roasting and brewing

All coffee samples (100 grams) were roasted for 6, 7, 8, 9, 12 and 15 min in a commercial spouted bed roaster (i-Roast® Model 40009, Hearthware Products, USA) operating at a maximum temperature of 230 °C. After grinding, brews at 10% were prepared using the home-style percolation method with filter paper (Whatman, No. 1).

Clarification of brews

The clarification of whole brews for exclusion of high molecular weight components such as melanoidins was performed by mixing 500 μL of coffee brew with 500 μL of each Carrez solution in a 50 mL volumetric flask. The final volume was made up with water and the colloidal dispersion was filtered through filter paper (Whatman, No. 1).

Chlorogenic acid analysis

CGA contents in whole and clarified brews were determined by liquid chromatography–diode array detection–mass spectrometry, according to the method described by Perrone et al. (2008).

Antioxidant activity

The AA of the whole and clarified brews was measured using FRAP (Moreira et al., 2005), TEAC (del Castillo et al., 2002) and TRAP (Bartosz et al., 1998) assays. Additionally, to investigate the individual contribution of CGA to the AA, the commercial standard of 5-CQA was assayed as a representative of the class (Sigma-Aldrich).

RESULTS AND DISCUSSION

The responses measured by the three assays tested in this study were strongly correlated. AA was correlated with total CGA content both in whole and clarified brews (Table 1). This correlation was in agreement with the observed correlation between AA and 5-CQA standard ($R > 0.98$, $p < 0.001$).

Table 1. Pearson correlation coefficients between AA assays and chlorogenic acids content.

Variables	R	*p*	*n*
FRAP x TEAC	0.93	<0.001	28
FRAP x TRAP	0.93	<0.001	28
TEAC x TRAP	0.93	<0.001	28
FRAP x CGA	0.78	<0.001	28
TEAC x CGA	0.83	<0.001	28
TRAP x CGA	0.92	<0.001	28

In the clarified brews, FRAP, TEAC and TRAP values decreased with coffee roasting, with maximum activity observed for the green samples. Although the whole coffee brews also showed an inverse association between AA and roasting, the maximum activity was observed in the samples roasted for 6 min, and not in the green ones (Figure 2).

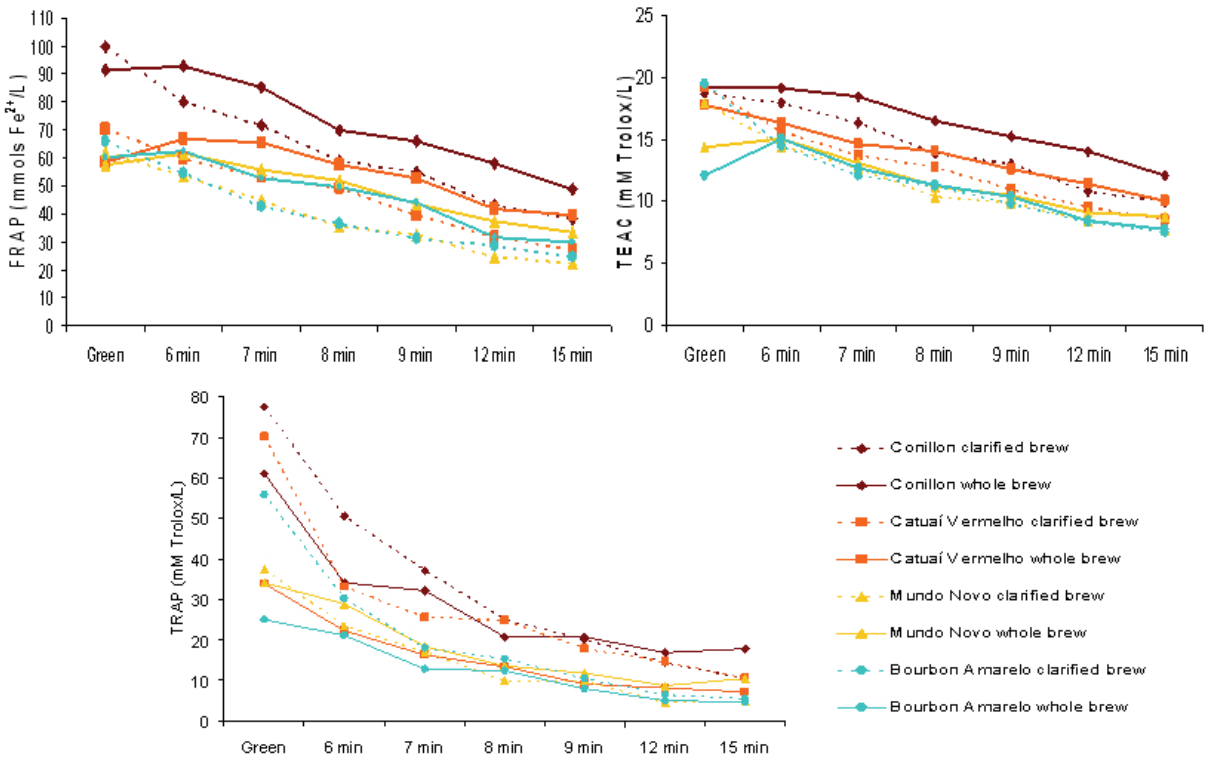

Figure 2. FRAP, TEAC and TRAP values in whole and clarified brews prepared from green and roasted coffee samples.

Moreover, FRAP and TEAC values were consistently higher in the whole brews compared to clarified brews, with the exception of the green samples, where this relation was inverted. The estimated contribution of CGA to the FRAP values of coffee brews was calculated using 5-CQA standard. The average contribution in the whole brews ranged from 0.7% in the darker samples to 102.3% in the green samples (Figure 3).

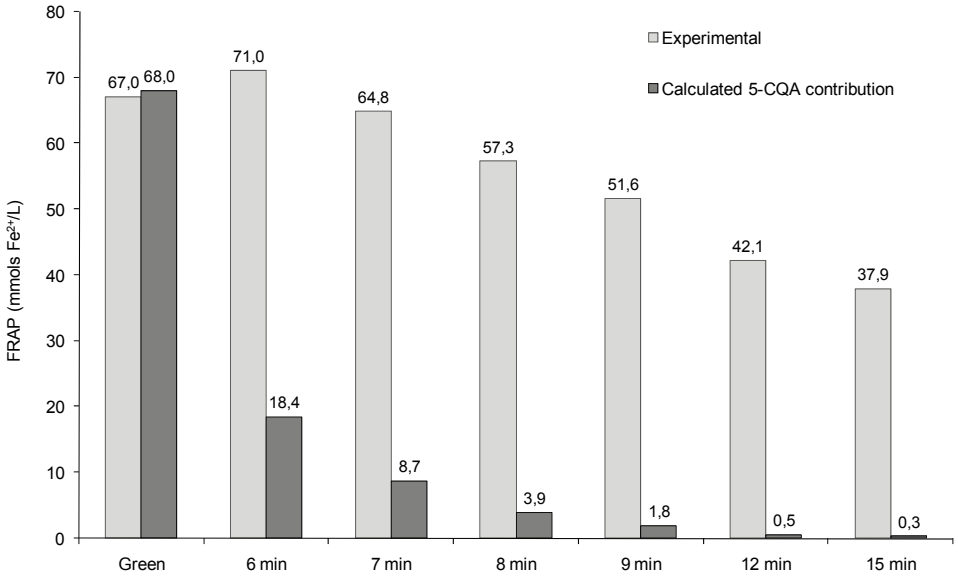

Figure 3. Experimental FRAP values and calculated contribution of 5-CQA to these responses in coffee brews.

It should be noted that this is only an estimate of CGA contribution to the observed AA of coffee brews, since the potentially different contribution of the various CGA compounds to

the AA of coffee was not considered. In addition, it is reasonable to think that the contribution of CGA in the coffee matrix to AA of coffee could be different from that obtained using isolated compounds. The very low calculated contribution of 5-CQA to the AA of the darker roasted coffee brews (up to only 5%), indicates that CGA are not the only contributors to coffee AA measured by FRAP, TEAC and TRAP, especially in coffees roasted from light medium to dark degree, and that the melanoidins formed during coffee roasting may play an important role in coffee AA, especially in dark roasted seeds, probably due to the CGA moieties incorporated into their backbones during roasting.

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3,4-Diferuloylquinideis Converted to Feruloylquinic Acid and Other Phenolic Compounds During Metabolism in Rats

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SUMMARY

Chlorogenic acids 1,5-γ-lactones, or quinides, are phenolic compounds formed from chlorogenic acids through the loss of a water molecule and formation of an intramolecular ester bond in the (-)-quinic acid moiety during coffee roasting. Diferuloylquinide (DIFEQ), a synthetic representative compound of this class, has previously exhibited hypoglycemic activity in rats but data on DIFEQ bioavailability is inexistent. In the present preliminary study, we investigated the presence of DIFEQ in rat plasma after oral administration. We also performed *in vitro* digestibility assays to examine DIFEQ digestibility in human gastrointestinal fluids.

INTRODUCTION

Chlorogenic acids are esters of one or two molecules of *trans*-cinnamic acids such as caffeic, ferulic and *p*-coumaric acids with (-)-quinic acid. Green coffee is a major food source of chlorogenic acids. The main chlorogenic acid classes in coffee are caffeoylquinic, feruloylquinic and dicaffeoylquinic acids. During the roasting of the green beans, part of these compounds (about 10%) is transformed into 1,5-γ-lactones or quinides, through the loss of a water molecule and formation of an intra-molecular ester bond in the (-)-quinic acid moiety (farah et al., 2005) (Figure 1). These lactones contribute with bitterness to coffee flavor (Ginz and Enhelhardt, 1995) and were shown to be biologically active in animal and cell culture studies (Wynne et al., 1987; De Paulis et al., 2002, 2004; Shearer et al., 2003). 3,4-Diferuloylquinide (DIFEQ), a synthetic representative compound of this class, has previously exhibited hypoglycemic activity in rats (Shearer et al., 2003). However, data on the bioavailability of lactones is unavailable in the literature. Therefore, in the present preliminary study, we investigated the presence of DIFEQ in rat plasma after oral administration.

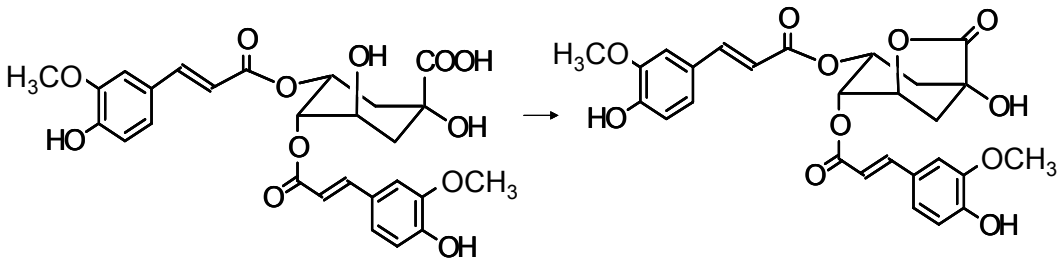

Figure 1. Example of a 1,5-γ-lactone formation represented by the formation of 3,4-diferuloylquinide (DIFEQ) from 3,4-diferuloylquinic acid.

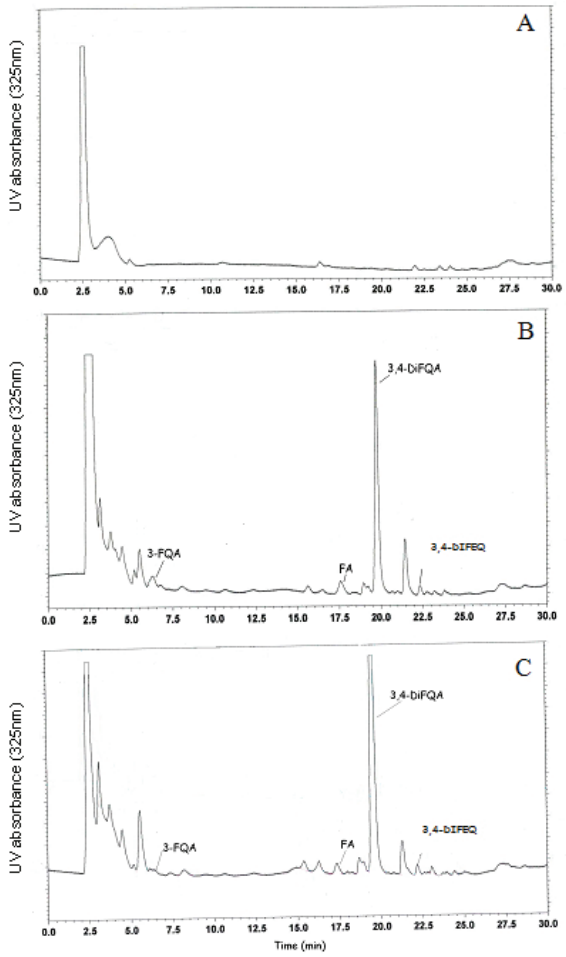

Figure 2. Example of a rat plasma chromatogram profile at 325 nm before (A), 20min (B) and 60min (C) after 3,4-DIFEQ administration. 3-FQA = 3-feruloylquinic acid; 3,4-diFQA = 3,4- diferuloylquinic acid; FA = ferulic acid.

MATERIALS AND METHODS

DIFEQ was diluted in saline solution and administered to 3 male Sprague Dawley rats through a gastric catheter. Blood draws were taken 20, 40 and 60 min after DIFEQ administration (110 mg/kg) as in Shearer et al. (2003) and plasma samples were analyzed by a HPLC-UV gradient system, according to Farah (2004) and Monteiro et al. (2007), adapted. Complementary digestibility assays were performed incubating DIFEQ for 0, 2, 4 and 8 h (37 °C, mild agitation) with human gastrointestinal fluid samples (pH 1.4, 7.2 and 7.8) collected during upper endoscopy diagnostic medical procedure, according to Farah et al. (2006).

RESULTS AND DISCUSSION

Results showed that the amount of DIFEQ in plasma decreased as time after DIFEQ administration increased. Sixty min after DIFEQ oral administration, only a small fraction of the total amount of the compound administered (about 10%, on average) was identified in plasma. The main DIFEQ metabolite was diferuloylquinic acid, its corresponding chlorogenic acid. Various other metabolites, including ferulic acid, isoferulic acid and 3-feruloylquic acid, were also identified (Figure 2), indicating that the metabolization of DIFEQ into diferuloylquinic acid and other compounds occurred either prior, during or after absorption.

Following, the digestibility of DIFEQ in human gastrointestinal fluid samples was evaluated. No changes were observed in the amount of DIFEQ incubated in the three samples of human gastrointestinal fluids up to 8h of incubation. Although results from this *ex vivo* digestion essay cannot be extrapolated to rats, they indicate that in humans, DIFEQ is most probably not converted into chlorogenic acids and metabolites due to the action of ionic forms and/or enzymes contained in the intestinal fluids. If lactones are converted back to chlorogenic acids in humans, this probably occurs during absorption and/or metabolism rather than during digestion.

Considering that monoacyl-lactones have shown instability in alkaline pH of cell culture media (Farah and Trugo, 2006), and that after ingestion of roasted coffee containing considerably high amounts of these lactones none of them was observed in plasma (Monteiro et al., 2007), the digestibility and bioavailability of monoacyl-lactones, which are more abundant in coffee than diacyl-lactones such as DIFEQ, should be investigated.

CONCLUSION

Together, our results suggest that the major compounds responsible for the hypoglycemic and other biological activities of DIFEQ observed *in vivo* might be their corresponding chlorogenic acid compounds and/or other phenolic metabolites, and that the conversion of diacyl-lactones into their acidic forms are not likely to occur in response to the effect of gastrointestinal fluids. The bioavailability of chlorogenic acid lactones in humans should still be investigated

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Caffeoylquinic and Dicaffeoylquinic Acids Are Excreted in Both Free and Bound Forms in Human Digestive Fluids

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SUMMARY

The aim of this study was to investigate the presence of conjugated forms of chlorogenic acid (CGA) isomers and their metabolites in human gastrointestinal digestive fluids from regular coffee drinkers and non-coffee drinkers. Three caffeoylquinic acid isomers, three dicaffeoylquinic acid isomers and caffeic acid (CA) were identified in all evaluated gastrointestinal digestive fluid samples. No significant differences were observed between the concentrations of CGA and CA of both groups, probably due to the large inter-individual variation in the results. Our results show that the major CGA isomers existing in coffee and other plants are excreted in gastrointestinal digestive fluids, in both conjugated and non-conjugated forms.

INTRODUCTION

Chlorogenic acids (CGA) are a family of phenolic compounds derived from the esterification of hydroxycinnamic acids such as caffeic (CA), ferulic (FA) and *p*-coumaric (*p*-CoA) acids with quinic acid. The major CGA groups in coffee are the caffeoylquinic acids (CQA); feruloylquinic acids (FQA) and dicaffeoylquinic acids (diCQA), with at least three isomers per group. Although CGA are present in almost every existing plant, coffee, which is largely consumed worldwide, is one of their main dietary sources. These compounds are potentially biopharmacologically active in humans and despite this, data on their absorption and metabolism, in humans, are scarce. Among CGA compounds present in coffee, 3-CQA, 4-CQA, 5-CQA, 4-FQA, 5-FQA, 3,4-diCQA, 3,5-diCQA and 4,5-diCQA have been identified in human plasma after roasted and green coffee consumption, demonstrating that they are absorbed and metabolized by humans (Monteiro et al., 2007; Farah et al., 2008). Farah et al. (2006) identified free forms of 3-CQA, 4-CQA, 5-CQA, CA, FA and *p*-CoA in human saliva and gastrointestinal digestive fluids after 12 h fasting, demonstrating that these compounds are excreted via digestive fluids and potentially recycled in the human body. In this study, we investigated the presence of conjugated forms of CGA compounds and metabolites in gastrointestinal fluids and tried to differentiate regular coffee drinkers from non-drinkers.

MATERIAL AND METHODS

Samples with pH 1.2-8.0 were obtained after 12 h fasting from 15 male and female adults during upper endoscopy diagnostic procedures. From these samples 7 were from regular coffee drinkers and 8 from non-coffee drinkers. Samples extraction followed the procedure reported by Monteiro et al. (2007), with the following modification: each sample was submitted to two types of treatments: addition of an enzymatic pool of *H. pomatia* containing β-glucuronidase and sulfatase activities for deconjugation of glucuronate and sulfated forms and no addition for non-conjugated forms. The extracts were analyzed by a HPLC-UV

gradient system. Data were treated by unpaired t-test to evaluate the influence of coffee consumption on CGA and metabolites concentration. Differences were considered significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

The six main CGA compounds (three caffeoylquinic acids and three dicaffeoylquinic acids) existing in coffee and other plants, as well as CA were identified in all gastrointestinal fluid samples, independently of the type of treatment used during extraction. These results confirm that CQA, the major CGA present in plants, are excreted in gastrointestinal fluids in their free forms (Farah et al., 2006). In addition, the present work identifies, for the first time, diCQA in human gastrointestinal fluids and reveals that most CGA in these fluids are found in conjugated forms.

5-CQA was the major isomer identified in all samples, with average concentrations of 10.7 ± 9.3 µmol/L, which represented about 61% of total CGA identified (Figure 1).

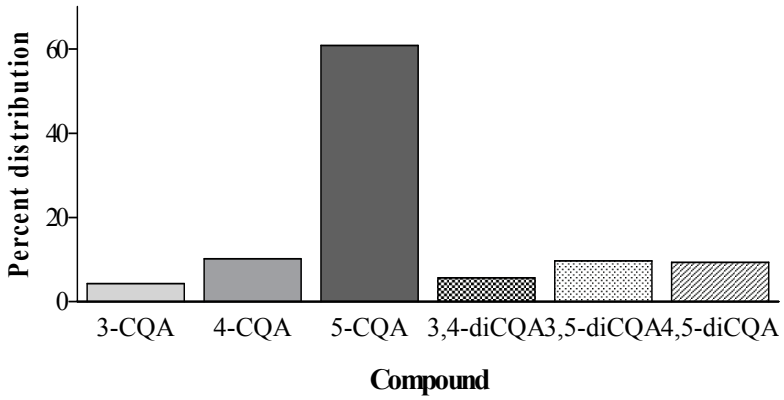

Figure 1. Average percent distribution of major isomers of CGA identified in all gastrointestinal fluid samples (n = 15).

A large inter-individual variation was observed in the concentration of CGA and CA (3.5–40.0 µmol/L and 0.1– 1.1 µmol/L, respectively) (Figure 2). These concentrations are higher than those previously observed in human plasma after coffee consumption (Monteiro et al., 2007; Farah et al., 2008). High concentrations of phenolic compounds compared to blood have been identified in bile (Code, 1968).

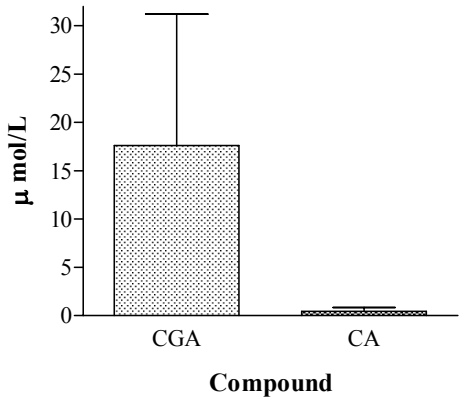

Figure 2. Average concentrations of CGA and CA in gastrointestinal fluid samples (n = 15).

A higher amount of glucuronate/sulfate forms was identified in all gastrointestinal fluid samples compared to non-conjugated forms. Concentrations of CGA, CQA, diCQA and CA in glucuronated/sulfate forms was 54%, 60%, 31% and 73% higher than those of non-conjugated forms, respectively (Figure 3).

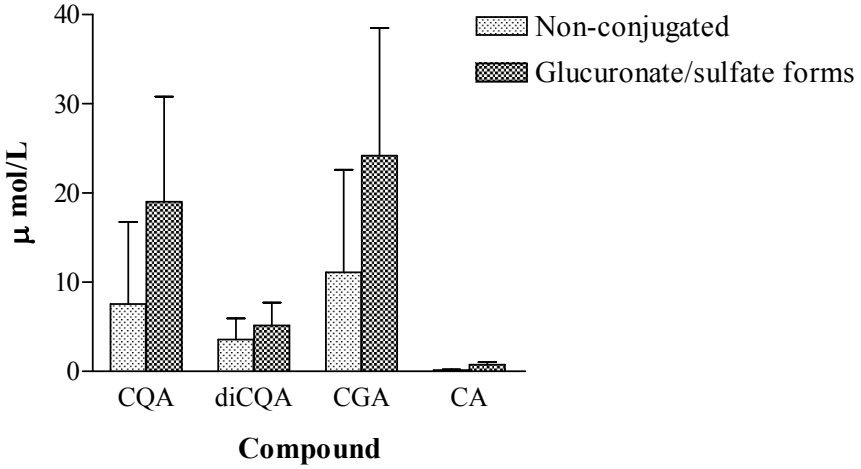

Figure 3. Average concentrations of non-conjugated and glucuronate/sulfate forms of CQA, diCQA, CGA, and CA in gastrointestinal fluid samples (n = 15).

Regarding the two subgroups of subjects, the average excretion of CGA was 19.4 ± 13.9 and 12.3 ± 10.2 µmol/L for regular coffee drinkers and non-coffee drinkers, respectively. For CA, the averages were 0.5 ± 0.3 and 0.6 ± 0.4 µmol/L, respectively (Figure 4). Despite of the higher average content in the coffee drinkers group, no significant difference was found between the concentrations of CA, total CGA or individual isomers of regular coffee drinkers and non-coffee drinkers, probably due to the large inter-individual variation observed. Therefore, a larger number of samples should be investigated in order to compare both groups.

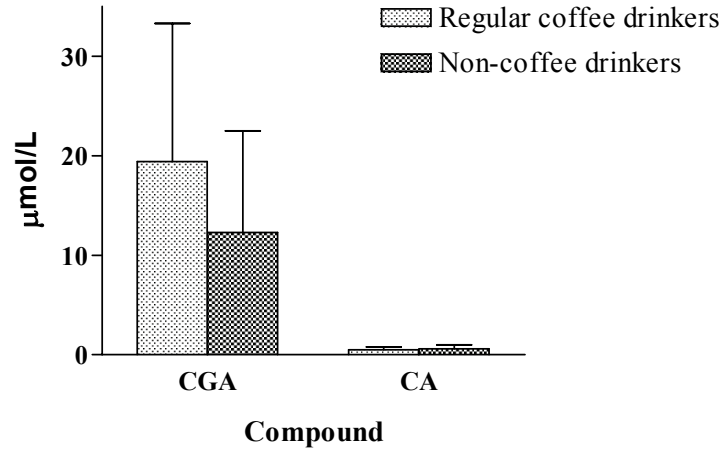

Figure 4. Average concentrations of CGA and CA in regular coffee drinkers and non-coffee drinkers (n=15).

CONCLUSION

Our results confirm that caffeoylquinic acids are excreted in gastrointestinal fluids in free form, being potentially available for re-absorption in the gastrointestinal tract. Additionally, they reveal the existence of bound and unbound diCQA compounds in these fluids and of higher concentrations of CGA in glucuronate/sulfated forms than in unbound forms.

ACKNOWLEDGEMENTS

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Influence of the Roasting Conditions on the Formation of Acrylamide in Brazilian Coffee: Preliminary Results

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SUMMARY

The present work reports a study concerning the formation of acrylamide in Brazilian coffee, using different roasting conditions. Temperature and roasting time were kept constant and the flow of hot air in a fluidized bed roaster was set to three different velocities. Acrylamide contents were determined using a MSPD-GC/MS method recently developed and applied for coffee samples. Results obtained so far clearly show that as the velocity of hot air increases, the content of acrylamide also increases in all samples analysed. More studies are being performed in order to determine the best conditions to roast coffee with the least possible formation of acrilamydes.

INTRODUCTION

In 2002, Swedish scientists reported the discovery of large amounts of acrylamide in starch-rich foods that had been cooked at high temperatures. Although the mechanism of acrylamide formation remains unclear, it is believed that this compound is a by-product of the Maillard reaction through a mechanism involving asparagine and glucose. Acrylamide has been demonstrated to cause cancer in animals and it is thought that its presence in foods may be potentially harmful to people's health (Tareke et al., 2002).

Since the Swedish discovery, a global effort has been undertaken to gather data involving this compound. According to several studies on the contribution of the nutritional habits to acrylamide intake, coffee contributes to about 40% of the total acrylamide exposure in Sweden and about 33% in Switzerland, making this beverage a significant contributor to acrylamide intake in dietary sources. The level of acrylamide in a cup of coffee depends not only on how concentrated it is but also the way the beans are roasted. Concentrations of acrylamide in instant and ground coffees are reported to be very similar − about 290 μg/kg − because this compound is a product of the roasting of the beans rather than of any subsequent processing Soares et al., 2006; Dybing et al., 2005; Granby et al., 2004).

Acrylamide levels in roasted coffee are dependent on the formation and elimination reactions that take place during the roasting process. The profile of acrylamide formation reflects this effect very clearly. Acrylamide formation reactions are dominant at the beginning of the roasting cycle, leading to increased levels at this stage, and then steeply decline toward the end of the roasting cycle. Therefore, generally speaking, light roasted coffees may contain relatively higher amounts of acrylamide than very dark roasted beans. Reports on commercial samples of roasted coffee showed lower levels of acrylamide for darker coffee roasts in comparison to lighter roasts, confirming these predictions. However, while lighter roasted coffees may contain higher amount of antioxidants such as chlorogenic acids (Farah et al.,

2005), darker roasts as a potential option to reduce acrylamide could generate other undesirable compounds like polycyclic aromatic hydrocarbons (PAH´s) (Helmut Guenther et al., 2007) and will possibly have a negative impact on taste/aroma and health. Consequently, to date, no practical solutions are available that would reduce acrylamide levels and concomitantly retain the quality characteristics of coffee, since the roasting step cannot be fundamentally changed (Helmut Guenther et al., 2007). Nonetheless, changes in the roasting parameters may appear as a way to manipulate the chemical composition of coffee.

The objective of this work was to study the roasting conditions that would lead to a decrease in the acrylamide formation. Different roasting conditions were set and some preliminary results of this study are presented, particularly the effect of the velocity of the hot air flow used in a fluidized bed roaster.

MATERIAL AND METHODS

Sampling

A total of 4 roasted coffee samples of different cup qualities were tested: Espresso (good quality) and 3 blends of *C. arabica* and C. *canephora*. Samples were roasted to reach dark medium roasting degree at three different velocities: fast (4 min, medium (8min) and slow (15 min) in a fluidized bed roaster (Lilla, SP, Brazil).

Acrylamide extraction

A 0.5 g aliquot of ground coffee and 2 g of C_{18} sorbent were placed in a glass mortar, spiked with 0.50 µg of IS (25 µl of the 10 mg l^{-1} IS solution) and blended together using a glass pestle to obtain the complete disruption and dispersion of the sample on the solid support. When blending was complete, the sample was transferred to an Isolute C18/Multimode 2 g/15 mL layered SPE column. A frit was placed on the top of the sample mixture before careful compression with a syringe plunger. The packed column was placed in a vacuum manifold and acrylamide was extracted with 4 ml of water holding the flow to permit a soaking step of 5 min. This water was collected until the limit of the frit to avoid drying of sorbents and a second aliquot of 4 ml of water was added observing again a 5 min soaking step. The second elution volume was completely collected to the same vial and the column was kept under vacuum aspiration during 5 minutes to collect all the water. The aqueous extracts obtained were derivatized with bromine and after the derivative's extraction with an organic solvent the total volume was reduced to 0.5 ml under a gentle stream of nitrogen. The extracts were then injected in the gas-chromatograph.

RESULTS AND DISCUSSION

As the velocity of hot air increased, acrylamide content also increased in all samples analysed, independently of the cup quality of the coffee beans (Figure 1). Probably, the higher acrylamide content in the fastest speed roast can be explained by the short period of time coffee beans are exposed to heat, and therefore, not allowing degradation of the compound.

To support these findings, more studies are being performed using different roasting conditions. The final objective is to determine and propose a new approach of coffee roasted in fluidized bed and similar type roasters to obtain coffee with appropriate organoleptic properties and small amounts of acrylamide.

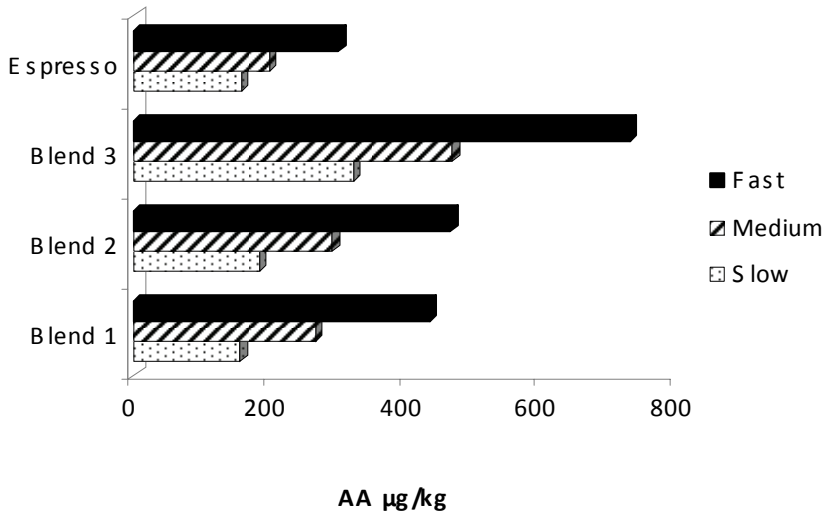

Figure 1. Concentration of acrylamide in the different coffee samples related to the hot air velocity inside the roaster

CONCLUSION

The change in roasting conditions, namely the hot air flow velocity, is a potential contributor to the preparation of coffee with less acrylamide. Other roasting parameters and samples are being tested and the conclusions will be reported shortly.

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Method for Quantification of Coffee-Derived Chlorogenic Acids and Their Metabolites in Human Plasma Using Two Analytical Platforms

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SUMMARY

Coffee is one of the most popular and widely consumed beverages. Recent data suggest that coffee consumption is beneficial for human health, e.g. reducing risk of diabetes or colon cancer. These effects might be explained by the high polyphenol content of coffee which contains considerable amounts of chlorogenic acids (CQA). Coffee beverages provide up to 10.9% total CQA on dry weight basis. Depending on roasting levels and origin of the beans, a serving of instant coffee will supply 65-125 mg total CQA. In order to exert biological effects CQA (or any active metabolites) have to be absorbed into the blood circulation and distributed to the target tissue. Currently, data on bioavailability of CQA from foods in humans are limited, most likely due to the lack of appropriate analytical methods. Here we describe a novel and very sensitive method for quantification of CQA metabolites and phenolic acids in human plasma using liquid chromatography coupled to either coulometric-array or tandem mass spectrometry detections. Key to the method is sample preparation for the very polar CQA. Using glucuronide and sulfate standards of caffeic, ferulic, isoferulic acids, and their dihydro- forms, we monitored the efficiency of enzymatic deconjugation. Quantification and detection limits will be presented; efficiency of enzymatic hydrolysis, extraction recoveries, variability and repeatability will be reported for both analytical platforms.

INTRODUCTION

Coffee contains phenolic compounds called hydroxycinnamates, which consist principally of CQA, a family of trans-cinnamic acids conjugated with quinic acid. The main CQA in coffee is 5-caffeoylquinic acid (5-CQA), although other caffeoylquinic (CQAs), feruloylquinic (FQAs) and di-caffeoylquinic (diCQAs) acids are present in significant quantities. Derivatives and phenolic metabolites of CQA, like caffeic (CA) or ferulic (FA) acids have been studied for potential bioefficacy. However, there is a lack of validated analytical methods to detect those compounds in biological matrices such as plasma. Here, we will describe the complete validation of detection of phenolic acids in plasma via LC-ESI-MS/MS and describe the first step of validating the same compounds via LC-Coularray.

STANDARD SOLUTIONS

Stock standard solutions were prepared individually by dissolving each analyte in methanol at a concentration of 100 μM. Further successive dilutions were made to prepare the spiking experiments and to optimise the LC-Coularray and LC-ESI-MS/MS parameters.

SAMPLE PREPARATION

An aliquot of 380 µL plasma sample was introduced in a 2 mL Eppendorf tube, in which 20 µL of IS1, solubilised in water/acetonitrile (95:5, v/v) containing 0.1% formic acid (to give a final concentration of 500 nM), was previously added. After adding three volumes of ethanol (1.2 mL), the vial was vortexed for 5 min at 2,700 rpm and centrifuged for an additional 5 min at 17,500 g at 4 °C. The ethanol supernatant fraction was poured into a clean 2 mL Eppendorf tube. The protein precipitation procedure was repeated twice by adding 1 volume (400 µL) of ethanol. The pooled ethanol phases were dried under a nitrogen stream at room temperature (~2 h), then reconstituted with 400 µL of 50 mM sodium phosphate buffer (pH 7.0) freshly prepared containing an enzymatic cocktail of 1,000 units of β-glucuronidase, 60 units of sulfatase and 0.1 unit of chlorogenate esterase. The sample was briefly vortexed (2,700 rpm) and incubated for 60 min at 37 °C in a thermomixer. At the end of incubation, 42 µl of 1 N HCl and 240 mg NaCl were added and briefly vortexed (2,700 rpm). A liquid-liquid extraction was realised 4 times by adding 800 µL of ethyl acetate, followed by a 5 min vortex at 1,600 rpm, centrifugation for 5 min at 3,000 g and collection of the ethyl acetate upper phases. The latter organic phases were dried under a nitrogen stream at room temperature (~30 min). The dried extract was reconstituted with 400 µL of methanol/water/acetonitrile (20/76/4, v:v:v) containing 0.08% formic acid and vortexed for 5 min (1,600 rpm). After centrifugation at 17,500 g for 5 min, the plasma extract was filtered on a 0.45 µm nylon membrane filter. An aliquot of 5 µL IS2 (to give a final concentration of 200 nM) was added to 95 µL filtered plasma extract in a vial and placed in the autosampler for analysis.

LC-COULARRAY

Analyses were performed on a Coularray detector model 5600A (ESA) fitted a solvent delivery module 582.

HPLC analyses were run using an HPLC from ESA with a Nucleosil C18 column (3 x 250 mm, 5 µm) fitted with a Nucleosil C18 precolumn (CC 8/3 100-5) (Macherey-Nagel).

Composition of phases were 75 mMol citric acid + 25mMol ammonium acetate (pH 2.66) for phase A and 500ml phase A + 500 ml acetonitrile for phase B.

The gradient program was: 0 min 20% B, 0-12 min 20% B, 12-26 min 25% B, 26-40 min 30% B, 40-45 min 60% B, 45-50min 100% B, 50-60min re-equilibration at 20% B; running at a constant flow rate of 0.5 mL/min. Injection volume was 50 uL and cell potentials used to detect compounds of interest were: -100, -10, 100, 200, 270, 450, 550 and 600mV.

LC-ESI-MSMS

Analyses were performed on an Agilent 1100 quaternary pump LC system (Agilent Technologies) coupled to a 3200 Q TRAP tandem mass spectrometer instrument equipped with a TurboIonSpray® ionisation source (Applied Biosystems). HPLC analyses were run on a Zorbax SB-C18 reverse phase column (2.1 x 50 mm, 1.8 µm) (Agilent Technologies, Basel, Switzerland). The mobile phases were constituted with solvent A: water containing 1% acetic acid, solvent B: methanol and solvent C: acetonitrile. The gradient program was: 0 min 100% A, 0-10 min 60% A (30% B and 10% C), 10-11 min 10% A (60% B and 30% C), 11-13 min held at 10% A (60% B and 30% C), 13-14 min back to 100% A, 14-19 min re-equilibration at 100% A; running at a constant flow rate of 0.3 mL/min. The injection volume was 25 µL and the LC column was thermostated at 40 °C. The LC flow was directed into the MS detector between 4 and 14 min using a VICI diverter (Valco Instrument Co. Inc., Houston, TX, USA).

MS tuning was performed in negative electrospray ionization (ESI) by infusing (Pump-11, Harvard Apparatus, Holliston, MA, USA) individual solution of each analyte (5 μg/mL in methanol) mixed with a HPLC flow made of solvents A, B and C (50/40/10, v:v:v; 0.3 mL/min) using a Tee-connector. Nitrogen was used for the nebuliser (GS1 and GS2) and curtain (CUR) gases at pressures of respectively 70, 20 and 10 psi. The interface heater was activated and the block source temperature was maintained at 600°C with a capillary voltage set at -4 kV. Nitrogen was also used as collision gas at a medium pressure selection. MS/MS detection was realized using the selected reaction monitoring (SRM) acquisition mode. The two most intense fragment ions of each compound were selected using a constant dwell times of 50 ms, resulting in a total scan time of 0.7 sec (including a 5 ms pause time between each SRM). Quantitative analyses were performed using the most intense SRM signal (SRM1) whereas the second transition (SRM2) was used for analyte confirmation based on appropriate area ratio calculated from standard solutions. Data processing was performed using Analyst 1.4.2 software (Applied Biosystems MDS/SCIEX).

RESULTS

LC-Coularray metabolite separation

Adequate separation of the targeted compounds was achieved from a mix of standards in buffer and from spiked plasma extracted samples. Optimization of potentials to correctly quantify metabolites was also realized to improve the overall sensitivity and selectivity of the method. This current detection method is under validation (recovery values, establishment of repeatability, intermediate reproducibility, LOD and LOQ).

LC-ESI-MSMS

Recovery experiments

This set of experiments was realised by spiking a human plasma sample at three concentration levels (50, 200 and 500 nM) of each analyte (free form, glucuronides, and sulfates), in six independent replicates; both at the right beginning of the sample preparation and prior to LC-ESI-MS/MS analysis. The recovery values were calculated from the area ratio of the former MS/MS trace (spiking experiments before sample preparation) against the latter one (spiking before analysis) multiply by 100. For the free aglycones, the recovery values for CA, FA, DHFA and 3-(4-hydroxyphenyl)propionic acid ranged between 103-118%, at the lowest concentration value with a CV below 14%. Besides these compounds, DHCA and IFA gave lower recovery values at respectively 50 and 65% (50 nM spiking level), with a good CV below 8%.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

These limits were calculated from three human subject submitted to a caloric restriction prior taking the blood. Very weak or almost no signals were observed on the non-spiked plasma sample, meaning that dietary restriction without phenolic compounds minimised the presence of endogenous compounds. LOD and LOQ were calculated from the non-spiked and spiked plasma samples at a concentration levels of 25 and 50 nM (n = 9 for each fortification level). After confirmation that no response (or very weak response) was observed on the non-spiked plasma sample, the signal-to-noise (S/N) ratio of each analyte was calculated from the Analyst software at both 25 and 50 nM spiking levels. LOD and LOQ values were then deduced to provide S/N ratio of 3 and 10, respectively. Thus, these calculated values were 3 and 9 nM (CA), 15 and 50 nM (DHCA), 3 and 11 nM (IFA), 1 and 3 nM (FA), and 4 and 12

nM (DHFA). The high values obtained for DHCA may suggest that relatively high ion suppression occur during the retention time of this analyte.

Repeatability and intermediate reproducibility

Accuracy and precision (within- and between-day) of the free aglycone chlorogenic acids were calculated from the analysis of six human plasma replicates fortified with all five analytes at each of the three specified fortification levels (50, 200 and 500 nM; 200 nM for the two ISs) and performed by the same operator on four separate occasions. Within-laboratory precision was obtained by following the same protocol but analyses were performed by two other operators in triplicate experiments on three separate occasions. Good accuracy was obtained for all compounds above 94% with a CV ranging from 5 up to 45%; with an acceptable iR values.

Accuracy and precision for measuring the conjugated metabolites were calculated from the analysis of three human plasma replicates fortified with in-house synthesized analytes at each of the three concentration levels mentioned above and performed by two different operators on three separate occasions. Repeatability (r) at the 95% confidence level was deduced from the within-day precision using an expansion factor of 2.77. Similarly, the intermediate reproducibility (iR) was calculated for each analyte from the within-laboratory results using the same expansion factor; applying robust statistics. Very good accuracy values were obtained from 98% up to 109%, with a CV ranging from 2 to 20%. Repeatability values ranged from 5.0-10.7 nM (at the lowest 50 nM spiking level). Intermediate reproducibility was also good for all targeted analytes with values ranging from 11.2-29.1 nM (lowest concentration level).

CONCLUSION

We have developed a sensitive method for extraction and detection of coffee chlorogenic acid metabolites from plasma samples quantified after LC-ESI-MSMS (full validation) and LC-Coularray (on going validation) analyses. Good separation of metabolites of interest with reproducibility for the LC-Coularray indicates that the validation of the detection method should be completed soon. Validated LC-ESI-MS/MS detection method showed excellent LOD and LOQ values, high recoveries of extraction as well as excellent repeatability and intermediate reproducibility values. We can therefore use the liquid-liquid extraction and LC-ESI-MS/MS detection methods described here to perform a clinical trial and fully validate the results obtained.

Prediction of Sensory Profiles of Brazilian Arabica Coffees Using Aroma Composition, Gas Chromatography and Partial Least Squares Regression Models

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SUMMARY

In this work, the volatile compounds of fifty-eight Brazilian Arabica roasted coffee samples were analyzed by solid phase microextraction and gas chromatography (SPME-GC) and the data were correlated with sensory evaluation by cuppers. The sensory attributes investigated were body, flavour, cleanliness and overall quality. Regression models (partial least squares) based on correlation of chromatographic profiles with each sensory attribute were developed. The ordered predictor selection (OPS) method was used for variable selection.

INTRODUCTION

The quality control that assures and certifies food and agricultural products has been widely studied and developed as an indispensable strategy for maintaining commercial competitiveness. The usual route to the development of these standards started with the definition of the quality parameters that could be measured. Measurements should be made by the best method available, scientifically validated, and fully correlated in the way that consumers will appreciate such standard of quality (Feria-Morales, 2002).

Commonly, the quality of coffee is evaluated according to criteria such as bean size, color, shape, cupping and presence of defects (Selmar et al., 2006; Agrestiet al., 2008). However, sensory profiling is still the most widespread technique employed to evaluate the final quality of coffee.

The correlation between flavour and sensory profiles using multivariate analysis becomes an excellent tool in the quality control of foods and agricultural products, being applied successfully in analyses of hazelnut, wine and juice (Alasalvar et al., 2003; Aznar et al., 2003; Moshonas and Shaw, 1997), among other products. The advantage of working with flavour in analytical measures is the clean-up step that is quick, simple and eliminates interferents.

The chemistry of coffee flavour is highly complex and is still not completely understood, but it contains important informations about the composition of the beans. Several efforts have been made to identify the main volatile compounds responsible for the real flavour of roasted coffee (Czerny and Grosch, 2000; Mayers and Grosch, 2001; Mayers et al., 1999). However,

the question of which volatiles are the most relevant contributors for the quality of coffee is still being elucidated (Agresti et al., 2008).

Because of all these reasons, in this work, the chromatographic profiles of Brazilian Arabica sound coffee volatiles were used to construct multivariate regression models for prediction of four sensory attributes determined by cuppers.

METHODS AND MATERIALS

Fifty eight Arabica green (not roasted) coffee samples from different origins were supplied by Instituto Agronômico de Campinas. Flat coffee beans were visually inspected, and those with defects were excluded. The roasting process was carried out in a gas fired drum roaster (Pinhalense S/A Máquinas Agrícolas) to the medium roast point. Roasted coffee samples were packed in films consisting of plastic (polystyrene and polyethylene) and aluminium and stored at -5 °C for a maximum period of 48 h before chromatographic analyses.

All samples were evaluated by two cuppers. The cup quality was assessed by flavor, body, cleanliness and overall quality using sample preparation according to Brazilian legislation (Normative instruction nº 8 from 11/06/2003, obtained from www.pr.gov.br/claspar/pdf/ cafebenef008_03.pdf). Thus, for the four sensory attributes selected for evaluation a 5-point scales were adopted, in such a way that each attribute, according to the degree of sensory magnitude perceived, were given corresponding scale points, e.g. the cleanliness classifications 'rio' (1) and 'strictly soft' (5) were defined as the extreme scores on the rating scale.

The analyses were performed on a G-6850 GC-FID system (Agilent, Wilmington, DE) fitted with a HP-5 capillary column (30 m x 0.25 mm x 0.25 μm). Helium (1 mL min^{-1}) was the carrier gas. The oven temperature was programmed as follows: 40 °C → 5 °C/min → 150 °C → 30 °C/min → 260 °C. The injection port was equipped with a 0.75 mm i.d. liner and the injector was maintained at 220 °C on splitless mode. Under these conditions, no sample carry-over was observed on blank runs conducted between extractions.

The SPME fibers coated with 65 μm thick polydimethylsiloxane /divinylbenzene (PDMS/DBV) and the manual holder were purchased from Supelco (Bellefonte, PA). The fibers were conditioned according to the SPME data sheet (T7941231, Supelco Co., Bellefonte, PA) in the gas chromatographer injector port.

Ground coffee (250 mg) and 2 ml of saturated aqueous sodium chloride solution were transferred to a septum-sealed glass sample vial (5 mL). After 10 min of sample / headspace equilibration under agitation of 900 rpm at 42.5 °C, the fibers were exposed to the sample headspace for 22 min. After sampling, the fiber was immediately exposed in the injection port of the gas chromatographer and the analytes thermally desorbed for 10 minutes at 220 °C. All analyses were carried out in triplicate.

The original chromatographic profiles were organized into a matrix format **X** (*I* x *J*), where each replicate represented one sample. Data analysis was carried out by Matlab 6.5 software (The MathWorks, Co., Natick, MA, USA) using the computational package PLS_Toolbox (Eigenvector Research, Inc. – PLS_Toolbox version 3.02.) (Wise et al., 2004).

The regression method used for data treatment was partial least squares (PLS) (Ferreira et al., 1999). PLS is a multivariate projection method used for modeling a relationship between a

matrix **X** of independent variables and a vector **y** of dependent variables (Figure 1). It can be presented as follows:

$$\mathbf{X} = \mathbf{S}\mathbf{L}^{\mathbf{T}} + \mathbf{E} \quad [1]$$

$$\mathbf{y} = \mathbf{S}\mathbf{q} + \mathbf{f} \quad [2]$$

where **X** represents the data matrix (chromatograms), vector **y** is the dependent variable (sensory analysis notes), **S** is the score matrix, $\mathbf{L}^{\mathbf{T}}$ denotes the transposed loadings matrix, **q** is a loading vector and **E** and **f** are the residuals.

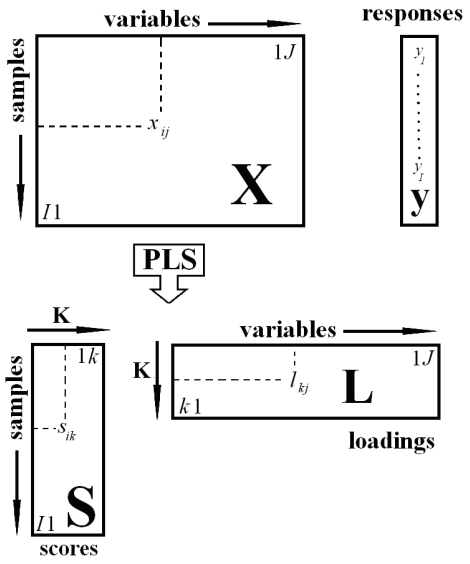

Figure 1. Schematic representation of PLS and the matrices X of independent variables and y of dependent variable. *k* is the number of latent variables.

In order to predict y_i for a new autoscaled chromatogram $\boldsymbol{x}_{i(as)}$, the following equation can be used:

$$\hat{y}_i = \bar{y} + \mathbf{x}_{i(as)}\mathbf{b} \quad [3]$$

where $\hat{y}_i$ is the predicted dependent value for the *i*th new sample, $\bar{y}$ denotes the mean of the dependent values for the calibration samples, and **b** is the computed vector of PLS regression coefficients:

$$\mathbf{b}=\mathbf{W}(\mathbf{L}^{\mathbf{T}}\mathbf{W})^{-1}\mathbf{q} \quad [4]$$

where the **W** is the matrix of loading weights.

The optimal model complexity, *i.e.*, the number of latent factors (*k*) in the PLS model can be determined by a cross-validation procedure. Leave-one-out cross-validation is performed by excluding one chromatogram at a time, the model is built and the estimated class ($\hat{y}_i$) for each sample is used to calculate the root mean square error of cross validation (RMSECV). The performance of the final PLS model is evaluated in terms of RMSECV (Equation 5), computed for different numbers of latent variables, and the correlation coefficient of cross-validation (r_{cv}) (Equation 6).

$$RMSECV_k = \sqrt{\frac{\sum_{i=1}^{I}(y_i - \hat{y}_i)^2}{I}} \quad [5]$$

In Equation 5, y_i is the measured response of the ith sample, $\hat{y}_i$ is a predicted response from the calibration equation obtained for the data without the ith sample and I is the number of samples in the calibration set. The optimal PLS model corresponds to the number of latent factors resulting in the lowest RMSECV.

The correlation coefficient between the estimated values in cross validation and the experimental values of the reference method is:

$$r_{cv} = \frac{\sum_{i=1}^{I}(\hat{y}_i - \bar{\hat{y}})(y_i - \bar{y})}{\left[\sum_{i=1}^{I}(\hat{y}_i - \bar{\hat{y}})\right]^{1/2}\left[\sum_{i=1}^{I}(y_i - \bar{y})\right]^{1/2}} \quad [6]$$

where $\bar{\hat{y}}$ is the mean estimated response.

Once the model has been internally validated and tested by an external data set, it can be used for the prediction of new samples. For the external validation set, the root mean square error of prediction (RMSEP) is used:

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{I}(y_i^p - \hat{y}_i^p)^2}{I_p}} \quad [7]$$

where I_p is the total sample number and $\hat{y}_i^p$ and y_i^p are the predicted and measured response values for the test set samples.

The chromatogram alignments were performed using a correlation optimized warping (COW) algorithm (Nielsen et al., 1998). For the alignments, the chromatogram with the best peak resolution was taken as reference vector. The chromatograms were divided into 10 regions and for each region, the segment length and the slack–parameter used were 10 and 1, respectively.

Each aligned profile was normalized to unit length, smoothed by the Savitzky-Golay algorithm, with a window size of 10 points followed by taking the first derivative (Savitzky and Golay, 1964) and, at last, the data were column-wise auto-scaled.

Variable selection was carried out by the OPS method (Teófilo et al., 2008). This method uses an intuitive vector formed by the combination of vectors such as the regression vector and correlation vector, among others. With this intuitive vector the independent variables are ordered based on their importance to the model. Then, the ordered variables are tested using increments over a previously defined window. The RMSECV and the correlation coefficient of cross-validation (r_{cv}) values are stored for each analyzed window. The best set of variables is indicated by the lowest RMSECV and the highest r_{cv}.

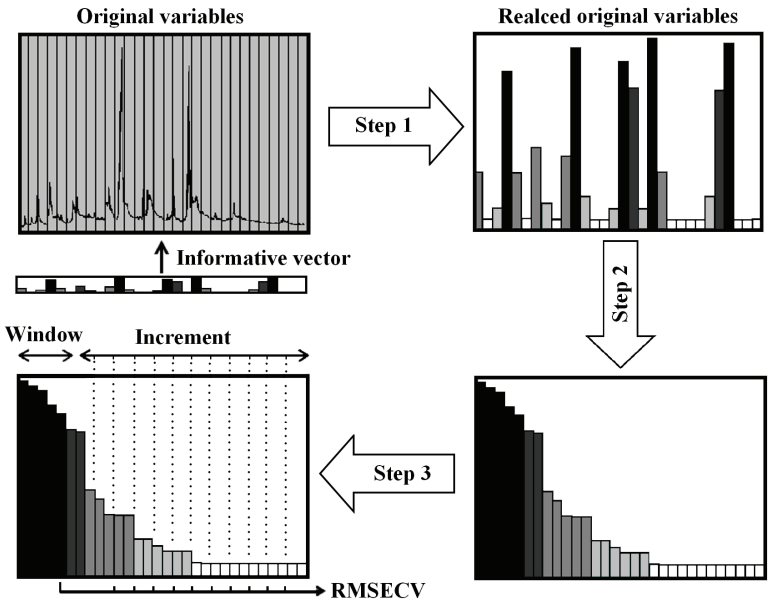

Figure 2. Schematic representation of the OPS method.

RESULTS AND DISCUSSIONS

To build the regression models for the four descriptive quantitative sensory analyses (flavor, body, cleanliness and overall quality), the mean values of the notes indicated by the two cuppers were used as the dependent variables (y) and 174 chromatograms referred to 58 Arabica coffee samples as independent variables (matrix **X**). Through a t-paired test (Gemperline, 2006), using the confidence limit of 95%, it was observed that the notes supplied by the cuppers for three attributes (body, cleanliness and overall quality) did not present significative difference. Only with the attribute flavor a higher reliable limit was necessary (99%).

From the pre-treated data matrix (174 x 20640), the baseline regions without chemical information had been removed, remaining only the chromatographic peaks. The variable selection for the construction of the models was carried out by the OPS method. In this way, from an initial set of 20640 variables, 1350 were selected for the construction of the flavour model (A), 1350 for body (B), 1550 for cleanliness (C) and 1350 for overall quality (D). These variables are indicated as vertical lines in Figure 3.

To form the calibration sets of each model, 48 samples (144 chromatograms) were randomly selected (Kennard and Stone, 1969). The 10 remaining samples, corresponding to 30 chromatograms were used to form the prediction sets. Leave-five-out cross-validation, was the method used to select the number of components in the models (15 replicates of five samples were left out at a time).

The number of latent variables, used to built the PLS models, was determined from the root mean square error of cross validation (RMSECV) values shown in cross-validation sets. The minimum RMSECV values shown for cross-validation sets were obtained for model when the number of latent variable used was as indicated in Table 1.

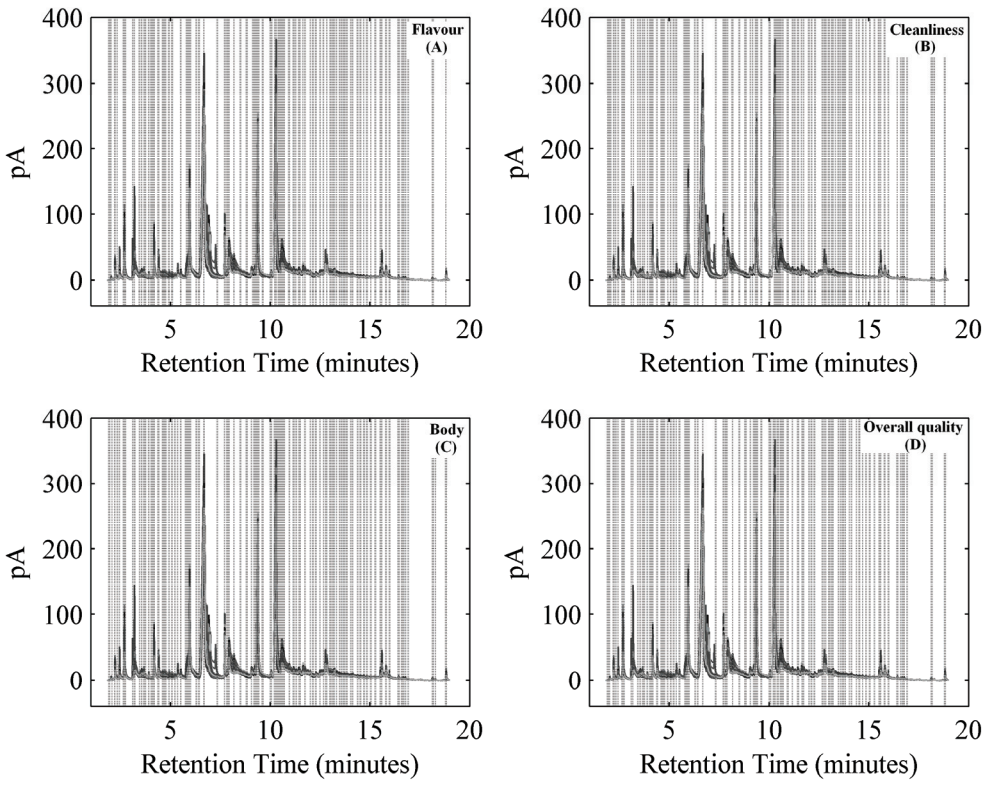

Figure 3. Regions of the chromatograms selected by the OPS method for the regression models. Flavour (A), Cleanliness (B), Body (C) and Overall quality (D).

Table 1. Latent variable numbers, RMSECV, r_{cv} and RMSEP for PLS models.

Model	N° LV[a]	RMSECV[b]	r_{cv}[c]	RMSEP[d]
Flavour	7	0.39 ± 0.05	0.89 ± 0.03	0.32
Body	7	0.18 ± 0.02	0.88 ± 0.03	0.22
Cleanliness	8	0.32 ± 0.04	0.91 ± 0.02	0.28
Overall quality	6	0.38 ± 0.05	0.91 ± 0.03	0.34

[a]Latent variable number; [b]Root mean square error of cross validation; [c]Cross validation correlation coefficient; [d]Root mean square error of prediction.

Using the indicated number of latent variables (Table 1), it was possible to describe 95 % and 52 % of the variance used in blocks **Y** and **X**, respectively, for all models. The models were validated by external validation, using a set of 30 replicates (10 samples). Figure 4 shows the measured *versus* the estimated notes (y) for each model. The RMSEP (root mean square error of prediction) values for all models were indicated in Table 1.

Due to the scale used to describe the sensory analysis notes (1 – 5 points), the relative errors tend to be higher for samples with low grades and decrease when the values of the notes became higher. Because of this, when calculating the cross-validation and prediction errors for each replicate, first the Hotelling T^2 statistics (Hotelling, 1933) was used. In this method, when the T^2 values of new samples are smaller than the upper limit, then the sample is declared compatible with the result.

The hatched horizontal line indicates confidence limit of 95% for T^2. In accordance with the plots indicated in Figure 5, the prediction values of the replicates of the 10 prediction samples of all the models were predicted correctly.

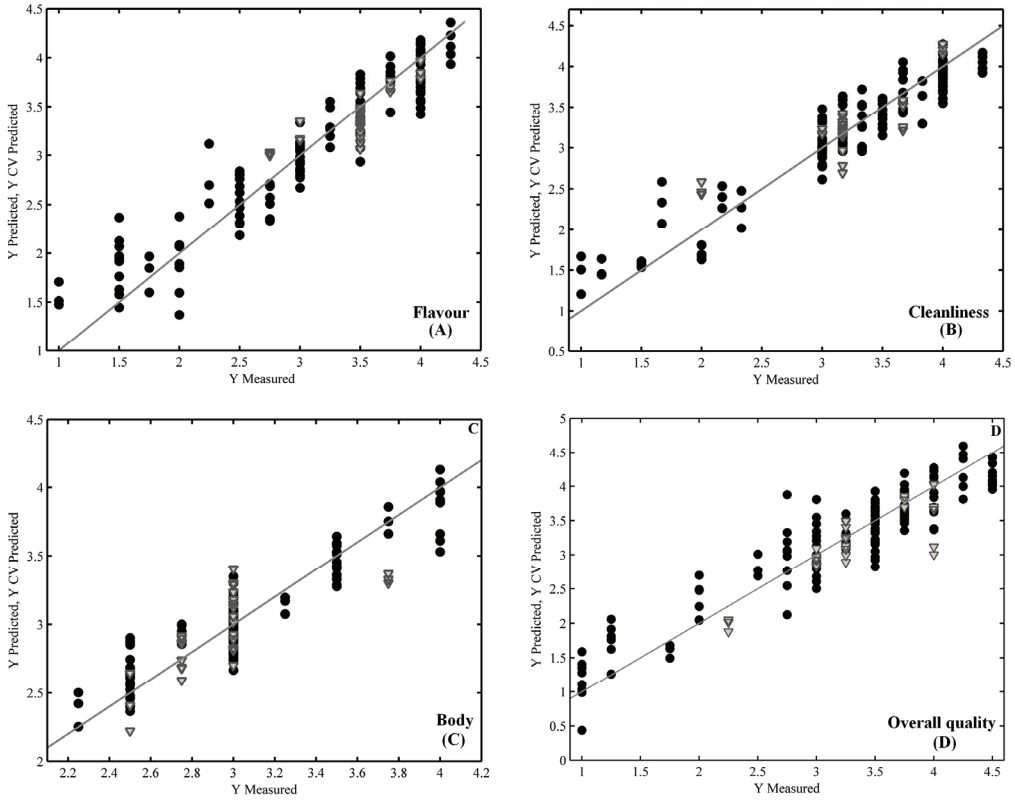

Figure 4. Plots of measured versus predicted notes in calibration (●) and prediction (▼) sets. Flavour (A), Cleanliness (B), Body (C) and Overall quality (D).

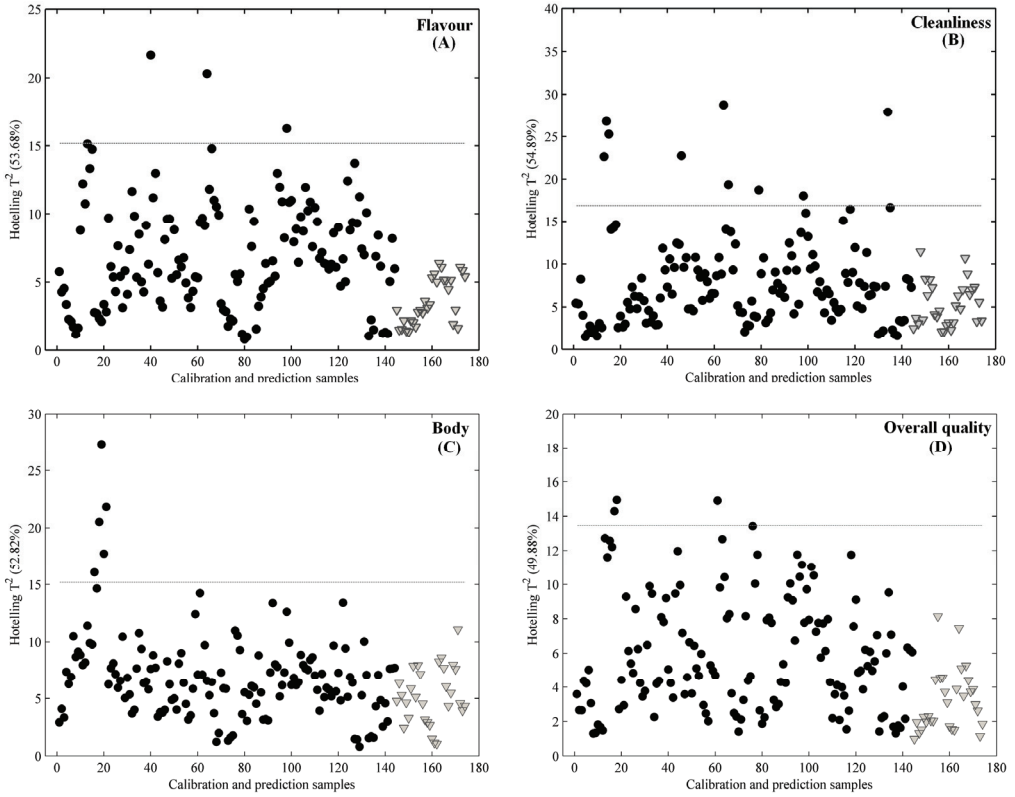

Figure 5. Hotelling T^2 (%) for calibration (●) and prediction (▼) samples.

Another way to predict errors in the models is described as followed: if the difference between the cuppers mean notes and the notes predicted by the regression model was higher

than two standard deviations obtained by the difference between the cuppers notes, the cross validation and predicted notes of the replicates were disregarded. The calibration and prediction errors for each model are indicated in Table 2.

Table 2. Number of replicates not well predicted during the cross-validation and prediction steps by the PLS models.

Models	Replicates not well predicted
Flavor	15
Body	0
Cleanliness	1
Overall quality	3

The flavor of coffee is composed of an extremely complex mixture of volatile compounds with different intensities, concentrations and odorific sensations. According to De Maria et al. (De Maria et al., 1999) the same compound could present a positive as well a negative flavor to the beverage depending of its concentration and synergic effects, combining in this sense with other compounds. Due to all these reasons, the majority of compounds detected with the SPME-GC-FID were selected by the OPS algorithm for the construction of the calibration models (Figure 3).

CONCLUSIONS

The PLS regression models using chromatographic profiles predicted well the notes conferred by the cuppers for flavor, body, cleanliness and overall quality of Brazilian Arabica coffees. From the results obtained in this study, the methodology proposed is a promising alternative tool for monitoring coffee beverage evaluation.

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Optimisation of Methodology for Identification of Aromatic Volatile Compounds in Roasted Coffee Beans

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SUMMARY

The methodology generally used for identification of coffee volatile compounds in roasted coffee beans shows some weaknesses, especially in the extraction and also in the selection of operating parameters for compound identification. The isolation and identification steps have been studied with regard to their efficiency in the qualitative and quantitative analysis. The coffee samples were extracted by Simultaneous Distillation Extraction (SDE) and identified by gas chromatography-mass spectrometry (GC-MS). This study developed an optimised method for identification of coffee volatile compounds. Instead of concentrating the extract, the extract was diluted. The parameters involved in GC-MS such as holding time, the plunger speed and flow rate of carrier gas were thoroughly investigated in this study. The extract was analysed in pulsed pressure splitless injection. A total of 83 compounds were identified in this study, which were mainly ketones, furans, pyrazines, pyridines, phenols and pyrroles.

INTRODUCTION

Besides its stimulatory effect, coffee is appreciated and consumed for its pleasing aroma which is a result of roasting (Grosch, 2001). At the start of roasting, moisture is reduced by heating until the beans reach a temperature of approximately 160 °C. Organic losses such as sucrose, chlorogenic acid and protein begin with evolution of carbon dioxide, swelling of the beans and reduction of their apparent density. Important chemical transformations occur and a large number of volatile substances that make up the aroma of roasted coffee are formed together with polymeric brown pigments, the melanoidins (Niani, 1992).

The aroma of a coffee is responsible for all the flavour attributes that are perceived by human senses of taste and smell. Therefore, aroma is the most important factor in identification of the quality of a coffee (Coffee Research Institute, 2006). Aroma is perceived by two different mechanisms – nasal and retronasal. Nasal perception can be done by smelling the coffee through the nose. As for the retronasal perception, it occurs when the coffee taken in the mouth releases volatile compounds which travel to odour receptor sites on the olfactory epithelium high up in the nasal cavity, and the resulting interaction triggers a specific response in the brain (Moulton et al., 1975).

It is not surprising, therefore, that numerous investigations have been carried out to identify the volatile compounds which evoke this pleasing aroma in coffee. The first research on coffee volatile compounds was done by Reichstein and Staudinger in the year 1920-1930. They isolated yellow-coloured oil from large quantities of roasted ground coffee and identified more than 29 volatile substances by the preparation of derivatives and measurements of physical constant. They pointed out that not a single compound was identified as the source of the coffee aroma. However, they emphasised that a highly diluted

aqueous solution of 2-furfurylthiol exhales ‘a pleasant note indicative of coffee’. Since 1963, there was generalisation of gas chromatography and progressive introduction of mass and NMR spectrometers which led to more advanced identification of volatile compounds in many beverages (Grosch, 2001).

Progress in instrumental analysis has shown that the volatile fraction of roasted coffee consists of a great multiplicity of compounds. More than 800 volatile compounds with wide variety of functional groups have been identified. The compounds that are present in the basic fraction of green coffee are pyridines, quinolines, aromatic amines, and alkylpyrazines. The neutral fraction mainly consists of furans, aromatic aldehydes, and alcohols. Subsequently, there are non-volatile compound of green coffee that can be considered as flavour precursors. They are carbohydrates, proteins, peptides and free amino acids, polyamines and tryptamines, lipids, phenolic acids, troginelline and various non-volatile acids. The perception of aroma depends upon both the concentration of the compound and its odour threshold. High concentration does not necessary contribute to the high odour threshold which makes up the coffee aroma (Parliament, 2000).

In the extraction of coffee volatile compounds, Likens-Nickerson Simultaneous Distillation Extraction (SDE) is the method of choice. This is because SDE provides the simultaneous condensation of the steam distillate and an immiscible organic solvent. In this apparatus, both liquid are continuously recycled which lead to the transfer of steam distillable-solvent soluble compounds from the aqueous phase to the solvent (Parliament, 1989). The extract is concentrated in a stream of nitrogen gas. The compounds are identified using Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS is a combination of two powerful analytical tools: gas chromatography for the highly efficient gas-phase separation of components in complex mixture and mass spectrometry for the confirmation of identity of these components and for the identification of unknowns (Niessen, 2001). However, the methods of qualitative and quantitative analysis of volatile compounds described above still presents several weaknesses and therefore need thorough optimisation. An attempt at optimisation of extraction and identification methodology will be presented in this paper.

MATERIALS AND METHODS

Whole, freshly roasted beans of arabica coffee of ‘Ernesto roast’ type were supplied by Zentveld’s Coffee, from a coffee plantation located in Newrybar in northern NSW (Australia). The sample was sealed and stored in a freezer at -18 °C until required for analyses. Then, the beans were ground using a household coffee grinder (MEZZO, made in P.R. China for ALDI Stores) of a capacity of 80 g with a stainless steel bowl and blades. Following sample weights were investigated: 10 g, 25 g and 50 g.

The distillation extraction process was taking place in a Likens & Nickerson apparatus (see Figure 1). A volume of 40 mL of purified dichloromethane and 3 boiling chips were put in the smaller (500 mL) flask. The coffee sample (10 g, 25 g or 50 g) and 1000 mL of purified water were placed in a 2 L sample flask. Both flasks were placed in a heating mantle during the extraction.A cold trap was then inserted in the top part of the extraction unit, as shown in Figure 1. The cold trap was filled with dry ice and 2 mL of ethanol. The solvent flask was heated first until it filled half of the U-tube in the Likens and Nickerson extractor. The sample flask was then heated at high temperature until water with sample boiled. While waiting for the sample flask to boil, the solvent flask had to be put in as low heat as possible to avoid the solvent from evaporating and moving to the sample flask. The water from the sample which contained the volatiles would flow into the u tube and be trapped by the solvent. The extraction process was timed from the moment when the sample started to boil. At the end of

the extraction process internal standard was added to the solvent flask. Any water that had entered the solvent flask was frozen out in by placing the flask in dry ice box and subsequently adding anhydrous sodium sulfate to the solvent.

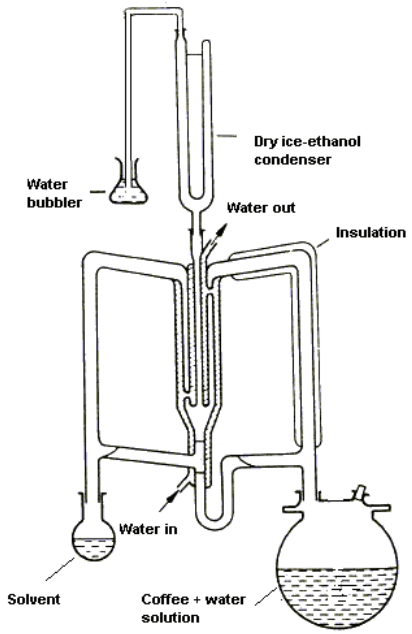


Figure 1. Schematic of Likens and Nickerson extraction apparatus.

The identification of the volatile compounds by GC-MS was performed with the help of Agilent model 6890 N (Agilent Technologies, Inc.Wilmington, DE, USA) apparatus. A fused silica capillary column with 5% phenylpolysiloxane as the non polar stationary phase was used. The dimensions of this column were 60 m x 220 µm x 0.25 µm. Splitless was the main injection mode used in the experiments. The temperature program is shown in Table 1. The GC/MS parameters studied are shown in Figure 2.

Table 1. Temperature program for splitless injection.

Oven ramp	°C/min	Next °C	Hold time (min)	Runtime
initial	0	30	1	1
ramp1	50	60	0	1.60
ramp2	3	250	10	74.93

The qualitative analysis was based on NIST107 spectral library in the GC-MS. The identified compounds were confirmed with Adams (2007) in terms of retention time. Tetradecane was used as internal standard for quantitation.The quantitative analysis was done by integrating the peaks of identified compounds and comparing them with those of the internal standard.

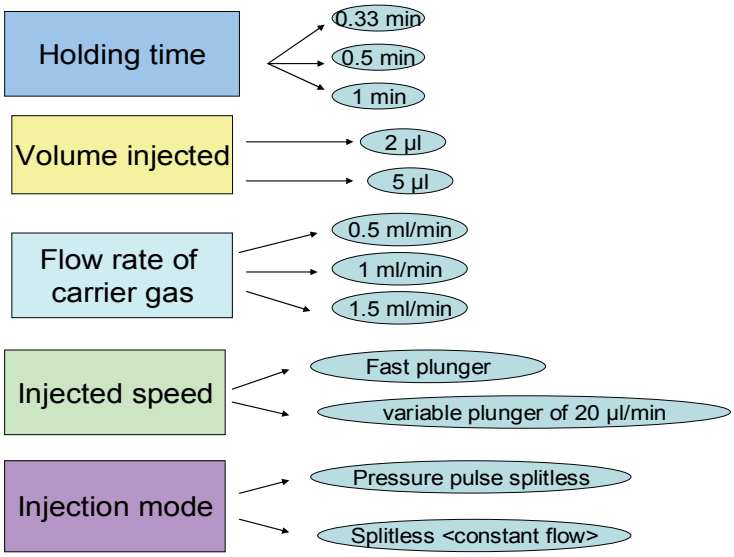


Figure 2. GC/MS parameters that were studied in the experiments.

RESULTS AND DISCUSSION

In the first experiment, the sample weight was 25 g and the solvent from the extraction was diluted to 50 mL. In the three subexperiments the holding time was set to 0.33, 0.5 and 1 minute respectively to determine the effect on the amount of analyte recovered. The results are shown in Figure 3.

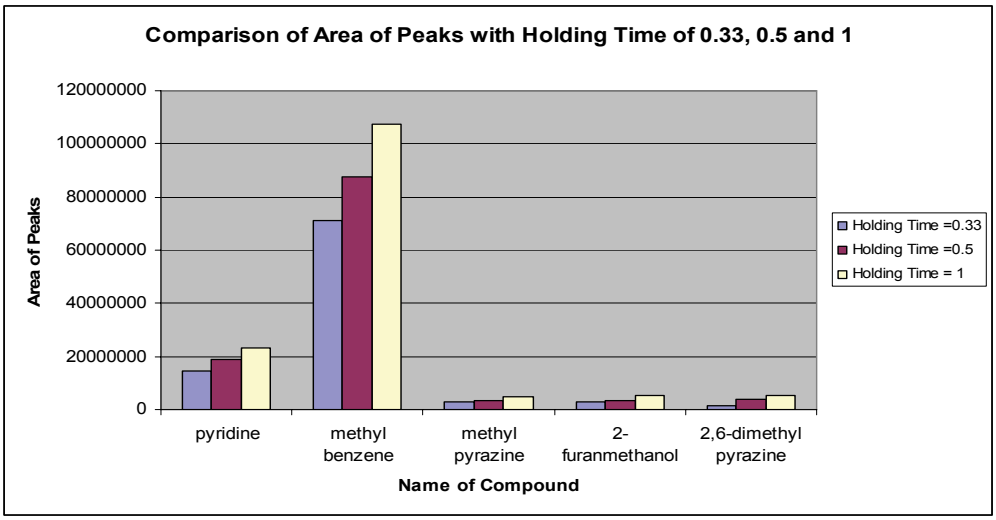
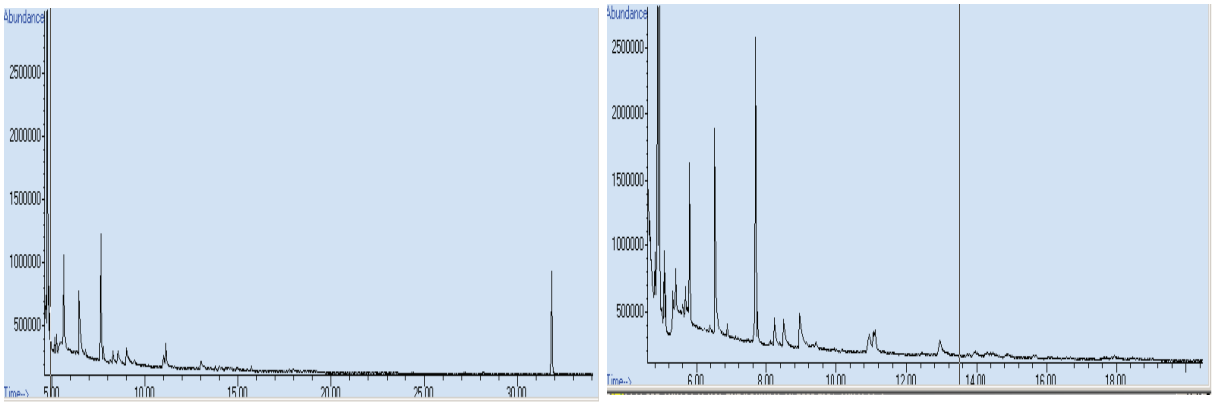


Figure 3. Comparison of key compounds appearing when varying holding time.

Figure 3 shows comparison of only 5 compounds due to the presence of some of the compounds in in very low quantity. The comparison shows that holding time of 1 minute was the best holding time used in analysis by the used of splitless injection. According to Grob (Grob, 2001), it is essential that most of the sample material be transferred from the vapourising chamber into the column for precision, accuracy and sensitivity. With incomplete transfer, different sample components are lost unequally, resulting in a distorted picture of the composition of the sample analysed. It may be deduced from this experiment that holding time of 0.33 and 0.5 minute did not allow the transfer of most of the sample injected.

In the second experiment 10 g of coffee sample were extracted for 2 hours using the Likens and Nickerson apparatus. The extract was diluted to 100 mL with purified dichloromethane and injected to GC-MS. The quantity injected was 2 µL and 5µL. The chromatograms are shown in Figure 4.



A) B)

Figure 4. Chromatograms showing effects of injecting different volumes of extract. A) 2 µL; B) 5 µL.

The results of the experiment show that smaller sample volume (2 µL) gave smaller peaks than 5 µL. As a result, 5 µL injection gave a more informative result than 2 µL injection in this experiment.

The third experiment was comparing the effects of varying the flow rate of carrier gas between 0.5-1.5 mL/min on some of the quantity of the key compounds. A 25 g coffee sample had been extracted and diluted with purified dichloromethane to 50 mL. The results are shown in Figure 5.

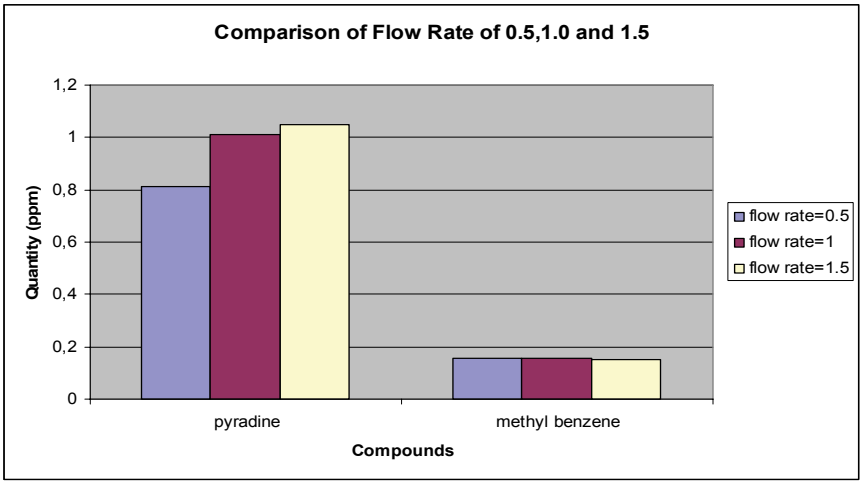


Figure 5. Comparison of the effects of different carrier gas flow rates on key compounds in coffee.

The fourth experiment compared speeds at which samples were injected. The two options were fast plunger and variable plunger speed of 20 µl/minute. The speed of 20 µl/minute is considered slow as compared to the fast plunger speed. The fast plunger injection speed minimises needle discrimination and reduces background interference that provides better result than the slow plunger speed (CTC Analytics, GC-PAL). The effects of the of the

injection speed are shown in Figure 6. Most of the compounds being compared showed that fast plunger injection gave higher quantity than variable plunger injection.

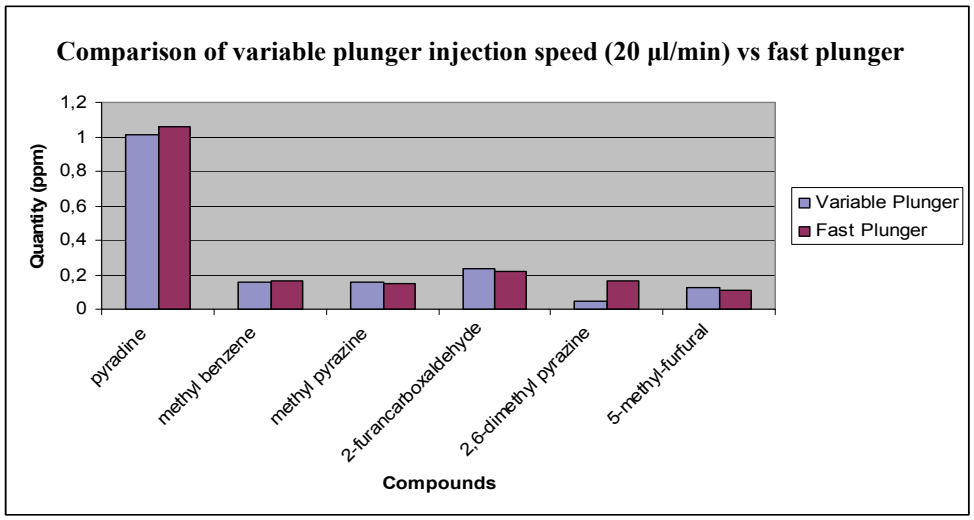


Figure 6. Comparison of the effects of different plunger speeds on key compounds in coffee.

The fifth experiment compared two splitless injection modes, constant flow injection and pulsed pressure injection. The pulsing effect maximises sample introduction into the column while narrowing the sample bandwidth. Morover, the sample has a very short residence time in the liner and thus losses of active compounds are minimised (Rosal et al.). The principle of pulsed injection is that the inlet pressure is increased above that suitable for chromatography and is reduced again when sample transfer is complete during splitless period. The results are shown in Figure 7. It appears that the quantity of compounds found was larger in the sample injected under constant flow. However, the number of compounds found was larger in the sample injected under pulsed pressure.

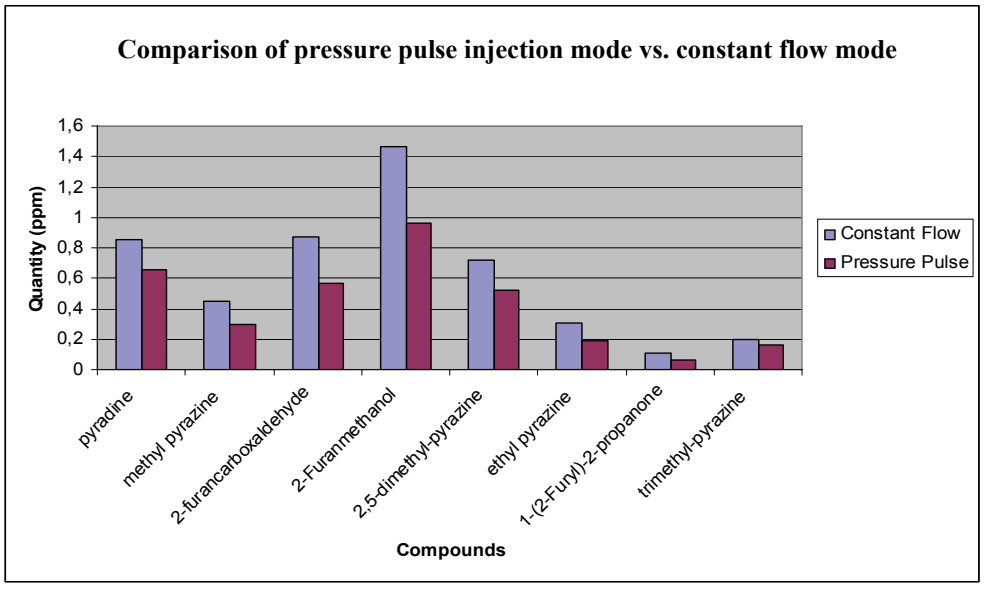


Figure 7. Comparison of the effects of injection modes on key compounds in coffee.

An additional sixth experiment was conducted to compare the effects of increasing the size of the extracted coffee sample. The extracts from the two extractions were diluted to 50 mL by

purified dichloromethane and the two extracts were injected under the same condition: 1 mL/minute flow rate of carrier gas, 1 minute holding time and fast plunger injection. The results (see Figure 8) show the quantities of the 6 compounds were higher in the 50 g sample than in the 25 g sample.

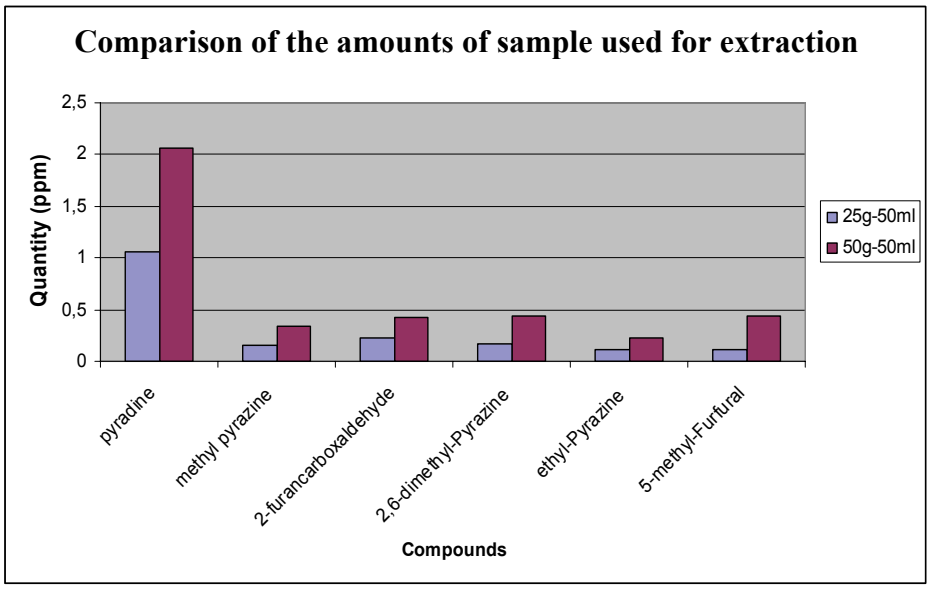


Figure 8. Effects of sample size on key compounds in coffee samples.

A total of 83 compounds were identified in this study. Among the compounds found in the roasted coffee samples were compounds originally present in green coffee (some lipid degradation products and the biologically derived aldehydes, acid and esters) but predominantly products of thermal reactions occurring during roasting. Among them were caramelisation products (furaneol), cyclic enolones (furanones), lipid degradation products (from lipid oxidation of unsaturated fatty acids such as hexanal, nonenal and other enals and dienals) products of degradation of phenolic acids and especially Maillard reaction products. Among the latter were the main aroma compounds such as pyridines, pyrazines, pyrroles and furans. Most of them are known as contributors to the coffee aroma.

Although the sensory analysis is still the main way to assess the quality of roasted coffee, the fact of optimising some of the key parameters of instrumental analysis provides a tool for better understanding of the composition of roasted coffee.

CONCLUSIONS

Six experiments were conducted to determine the parameters affecting the identification of volatile compounds present in roasted coffee. Five of them were related to the operating parameters of GC/MS. The sixth, additional experiment was dealing with the amount of sample to be extracted by SDE.

As a result of the experiments, the proposed optimised methodology for coffee extraction would use a 50 g sample extracted in purified dichloromethane. The volatile compounds would then be identified in GC-MS. The optimum program for a splitless injection into the GC-MS would be as follows:

- The longest holding time (1 min.) resulted in obtaining the highest quantity of each of the key compounds

- Injecting a volume of 5 µL gave a more informative result than 2 µL.
- The flow rate of 1.5 mL/minute was leading to higher amounts of analyte than 0.5 and 1 mL/minute.
- The sample injected with fast plunger gave higher quantity than the variable plunger speed injection.
- When comparing the constant flow vs. pulsed pressure it was found that the quantity of each compound was larger in the sample injected under constant flow. However, the number of compounds found was larger in the sample injected under pulsed pressure.

ACKNOWLEDGEMENTS

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New Volatile Compounds as Brazilian Defective Coffee Seeds' Markers

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SUMMARY

The addition of defective coffee seeds to healthy seeds is a common practice in Brazilian coffee industry. The absence of analytical techniques for identification of defects, especially in ground seeds, is a limiting factor to Brazilian coffee quality. In the present study, we confirmed volatile marker compounds previously identified in raw and roasted defects and identified new ones. Among 121 identified compounds in raw and roasted samples, the following compounds were identified as markers for defects in general: 2,3,5,6-tetramethylpyrazine, hexanoic acid and butyrolactone in raw samples and 3,5-trimethyl-6-ethylpyrazine, 3,5-dimethyl-2-butylpyrazine, β-linalool, and hexanoic acid in roasted samples. The compounds 2,3,5-trimethylpyrazine, 2-pentylfuran, 2-octen-2-one and 2-octenal were markers for raw black and sour seeds. 3-ethyl-2-methyl-1,3-hexadiene was marker for the raw black seeds, while 2-pentylfuran, 3-methylpiperidine and 2-pentylpiperidine were markers for the roasted black seeds. Finally, in the roasted sour seeds isoamyl-6-methylpyrazine and the 3-methyl-2-butylpyrazine were considered as markers.

INTRODUCTION

Defective coffee seeds are a limiting factor for Brazilian coffee quality. Because coffee fruits do not reach ripeness at the same time, and due to the harvesting methods used in Brazil, about 20% of coffee production (8 millions of bags) corresponds to defective seeds (Toci and Farah, 2008). These seeds are considered to be inappropriate for exportation. Consequently, they are incorporated to the internal market, causing a general concern about the amount of defective seeds incorporated to good quality seeds in industry.

Recently, there has been a growing effort aiming to characterize the chemical composition of defective coffee seeds in order to make possible the identification of large amounts of them in coffee blends. However, very little is known about their chemical composition, which is extremely variable. Particularly, the volatile composition is an aspect that varies considerably according to genetics, soil, climate, and agricultural practices (Smith, 1985).

Aroma is one of the main attributes of coffee beverage. It is the result of a complex balance of about 900 volatile compounds of distinct characteristics and intensities (Flament, 2002). In order to understand this flavour complexity, innumerous scientific investigations have been performed. Negative notes of coffee flavour have been attributed to 2-methyl-isobutanol, 2,4,6-trichloroanisole, geosmin, 2-methoxy-3-isopropylpyrazine and 2-methoxy-3-isobutylpyrazine (Bortoli and Fabian, 2001; Cantergiani et al., 1990; Spadone et al., 1990). However, most of these compounds were considered as derived from microbial contamination and not intrinsically from defective seeds. On the other hand, ethyl isobutanoate, isoamyl acetate, isobuthyl acetate, ethyl butanoate, 2-methyl-ethyl butanoate, n-hexyl acetate, 2-

acethylpyrazine and β-linalool have been exclusively observed in *stinker* coffee seeds when compared to healthy (non-defective) seeds (Guyot et al., 1982). Recently, Toci and Farah (2008) studied the volatile composition of defective coffee seeds compared to healthy seeds from the respective lots. Potential defective seeds' markers were identified. 2-methylpyrazine and 2-furylmethanol acetate were identified only in raw black-immature seeds and butyrolactone only in raw sour seeds, while benzaldehyde and 2,3,5,6-tetramethylpyrazine showed to be potential markers of raw defective seeds in general. In the roasted mixture of defective seeds (PVA), pyrazine, 2,3-butanediol *meso*, 2-methyl-5-(1-propenyl)pyrazine, hexanoic acid, 4-ethyl-guayacol and isopropyl *p*-cresol sulfide were also suggested as potential defective coffee seeds' markers in general.

The literature on quantification of volatile compounds in coffee using SPME is practically nonexistent. Most of the quantification performed uses the relative percentage (Toci and Farah, 2008; Ryan et al., 2004; Nebesny et al., 2007; López-Galilea et al., 2006). However, it is known that the mass detector response is different for the various compounds.

The aim of this study was to confirm previously identified volatile compounds as markers of Brazilian defective coffee seeds, and, for the first time, quantify these compounds. Additionally, we aimed at identifying new potential volatile markers for defective seeds.

MATERIAL AND METHODS

Samples

Three lots of raw and roasted immature, sour, black and healthy coffee seeds, harvested in three different farms of Guaxupé, South of Minas Gerais, Brazil, were used. Samples were roasted in a spouted bed roaster (IRoast, Gurnee, IL, USA) at 210 °C, for 6 min, to give a light-medium degree according to the Roast color Classification System (AGTRON – SCAA, USA, 1995).

Sample extraction and GC/MS Analysis

A SPME triple phase 50/30 μm fiber (divinylbenzene/carboxen/polydimethylsiloxane), was used for the extraction of volatiles from the coffee samples headspace and the analyses were carried out by a GC/MS, according Toci and Farah (2008).

Data elaboration

The mainlib NIST-2004 spectral library, the Kovats Index and the standards of 3-methylbutanal, pentanal, hexanal, heptanal, 1-penten-3-ol, pyridine, pyrazine, 3-methylbutanol, 2-methylpyrazine, 1-hexanol, benzaldehyde, 2,3-butanediol, 1H-pyrazol, benzyl alcohol, phenol, heptanoic acid, ethyl heptanoate, 1,6-hexanodiol, β-linalool and butyrolactone (Sigma-Aldrich, Steinheim, Germany) were used for peak identification.

Quantification

A semi-quantification was performed, using 13 calibration curves made of 5 concentration levels of representative compounds of the main volatile classes in coffee: 3-methyl-butanol, hexanal, 1-penten-3-ol, pyridine, pyrazine, 1-hexanol, benzaldehyde, 1H-pyrazol, phenol, heptanoic acid, etil heptanoate, 1,6-hexanodiol and butyrolactone (Sigma-Aldrich).

RESULTS AND DISCUSSION

In order to identify volatile markers in defective seeds, it is essential to use appropriate controls, since seeds of different origins may be differentiated by their volatile composition (Dirinck et al., 2001; Costa Freitas et al., 2001). Therefore, in addition to the individual defective beans (immature, sour and black), the respective beans were also used.

Raw samples

A total of 43 different volatile compounds were identified in the investigated raw coffee samples, being 8 of them found only in defective seeds (considered as markers) (Table 1). From these compounds, 2 were identified in our previous work (Toci and Farah, 2008), 1 was found in all defects and 5 were identified in specific defects. Alcohols comprised the main classes of compounds in raw healthy and defective seeds, as previously observed (Flament, 2002). In addition to alcohols, defective seeds were also rich in acids, aldehydes and cetones, indicating the occurrence of fermentative and oxidative processes.

Table 1. Volatile compounds identified exclusively in defective raw coffee samples*.

			Lot 1			Lot 2			Lot 3		
Compounds	**IK**	**ID**	**Black**	**Immature**	**Sour**	**Black**	**Immature**	**Sour**	**Black**	**Immature**	**Sour**
2-pentylfuran	1267	B	29448	--	4315	18886	--	7642	21338	t	--
2,3,5-trimethylpyrazine	1407	B	--	--	273	--	--	204	465	--	--
3-octen-2-one	1420	C	507	--	--	448	--	t	116	--	t
3-ethyl-2-methyl-1,3-hexadiene	1421	C	407	--	--	190	--	--	170	--	--
2-octenal	1438	C	974	--	--	698	--	18	223	--	t
2,3,5,6-tetramethylpyrazine	1480	B	793	t	1566	t	--	369	316	--	--
butyrolactone	1633	A	--	1700	4224	t	1316	1670	t	1632	1449
hexanoic acid	1857	C	1222	t	1008	1164	t	1176	1103	t	937

*IK – Index Kovats; ID – The reliability of the identification proposal is indicated by the following: A- mass spectrum and retention time agreed with standards; B – mass spectrum agreed with Nist and Kovats index agreed with the literature data; C – Mass spectrum agreed with 90% in the Nist virtual library. *Results are given in (µg/100g)*

The compounds 2,3,5,6-tetramethylpyrazine and butyrolactone were present in all 3 lots of raw defects (Table 1), confirming data from Toci and Farah (2008). Black and sour seeds were richer in 2,3,5,6-tetramethylpyrazine (316-1566 µg/100g) comparing to immature seeds and sour and immature seeds were richer in butyrolactone (1316-4224 µg/100g) compared to black seeds. The presence of pyrazines indicates that these seeds have been exposed to elevated temperatures, since their formation usually takes place during the roasting process. Regarding butyrolactone, the ability of many yeast strains and fungi to form high amounts of lactones have been reported in the literature (Berger et al., 1992), suggesting that this compound derives from microbial contamination.

The compound hexanoic acid was present in all defects (Table 1) and not in the healthy seeds. Therefore, we considered it as a new volatile marker for defects in general. The highest concentrations (937-1222 µg/100g) were found in black and sour seeds. Acids can be formed by fermentative processes derived from fungi, that may use triacylglicerols, alcohols, acids and xylose present in the fruit peel and pulp as raw material (Baruffaldi, 1975). The formation of these unwanted volatile compounds by fungi and bacteria fermentation is probably derived from lack of care during post-harvest processing of the seeds. In 1987, microscopic investigations of Brazilian coffee seeds classified as “Rio” (rich in defective beans (Farah et

al., 2006)) revealed that they were heavily infested with various fungi and bacteria, resulting in degradation of the cell structure (Garay et al., 1987; Dentan, 1987).
The compounds 2-pentylfuran, 2,3,5-trimethylpyrazine, 3-octen-2-one and 2-octenal were found in all black and sour seeds (Figure 1), being considered as new markers for these specific defects. It is known that the oxidation of lipids may generate furans (Clifford, 1985). In addition, the formation of volatile aldehydes and cetones has been previously attributed to self-oxidation of alcohols (Guyot et al., 1982; Dart and Nursten, 1985).

The compound 3-ethyl-2-methyl-1,3-hexadine was found in all black seeds (Figure 1), being considered as a new marker for this defect. It is known that the oxidation of lipids may generate some hydrocarbons (Akoh and Min, 2008). The lipids present in raw coffee, mainly in black seeds, are more susceptible to oxidation due to longer exposure to environmental circumstances than the other seeds.

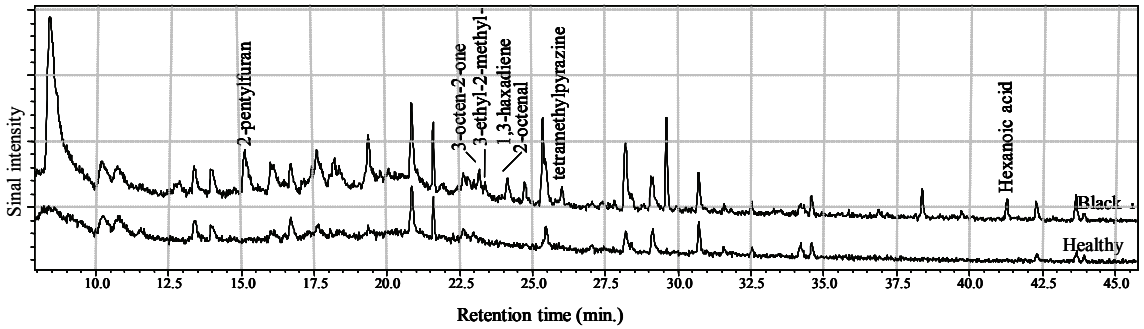

Figure 1. Juxtaposed chromatograms of black defective and healthy raw seeds.

Roasted samples

A total of 121 different compounds were identified in the investigated roasted coffee samples, being 9 found only in defective seeds (Table 2). From these compounds, 1 was found in all defects and confirmed results from our previous work (Toci and Farah, 2008), 3 were newly identified in all defects and 5 were identified in specific defects. From all identified compounds in healthy and defective seeds, pyrazines and furans were the major classes, with 31 and 22 compounds, respectively. In addition to pyrazines and furans, defective seeds were also rich in acids, aldehydes and cetones, like in the raw samples.

Table 2. Volatile compounds identified only in defective roasted coffee samples*.

			Lot 1			Lot 2			Lot 3		
Compounds	**IK**	**ID**	**Black**	**Immature**	**Sour**	**Black**	**Immature**	**Sour**	**Black**	**Immature**	**Sour**
2,3,5-trimethyl-6-ethylpyrazine	1520	C	t	898	t	t	410	367	440	911	375
3-methylpiperidine	1126	C	12552	--	--	--	--	--	13539	t	--
2-pentylpiperidine	1226	C	19652	--	--	--	--	--	35728	--	--
2-pentylfuran	1243	B	22545	--	--	16959	--	--	15226	--	--
3,7-dimethyl-1,6-octadien-3-ol (β-linalool)	1562	B	220	308	t	230	219	586	539	105	178
isoamyl-6-methylpyrazine	1630	C	--	--	1489	--	--	1441	--	--	2454
3-methyl-2-butyl-pyrazine	1636	C	--	--	1263	--	--	1417	--	--	1266
3,5-dimethyl-2-butylpyrazine	1666	C	526	370	760	253	467	724	280	479	18
hexanoic acid	1859	C	1882	1450	1303	2094	1257	1465	1504	t	1228

*IK – Index Kovats; ID – The reliability of the identification proposal is indicated by the following: A- mass spectrum and retention time agreed with standards; B – mass spectrum agreed with Nist and Kovats index agreed with the literature data; C – Mass spectrum agreed with 90% in the Nist virtual library. *Results are given in (µg/100g).*

Hexanoic acid, also identified by Toci and Farah (2008) in defective beans, was present in all 3 lots of defects (Table 1). No significant difference in hexanoic acid concentration was found among the defects. This compound may be formed for different reasons such as thermal degradation of sugars and esters, and autooxidation of aldehydes and cetones (Kawanda et al., 1985; May, 1985; Feldman et al., 1969).

2,3,5-trimethyl-6-ethylpyrazine, 3,5-dimethyl-2-butylpyrazine (apparently identified in coffee for the first time) and β-linalool, which were found in all defective coffee seeds, can be considered as new markers for roasted defective beans in general. Pyrazines are generated, during roasting, by Maillard reaction, degradation of Strecker or pyrolysis of hydroxy-amino acids (Maga, 1978; Baltes and Bochamann, 1987). β-linalool may be derived from the breakdown of carotenoids (Dart and Nursten, 1985); Merritt et al., 1985).

3-methylpiperidine and 2-pentylpiperidine (apparently also identified in coffee for the first time) and 2-pentylfuran were found only in black seeds (Figure 2). Therefore, they were considered as markers for roasted black seeds. The concentration of 2-pentylfuran (15226-22545 μg/100g) was lower in the roasted than in the raw seeds, with losses of 10-29%, suggesting degradation during roasting process. Although 2-pentylfuran was found in raw sour seeds, in roasted seeds, this compound was not identified, possibly due to complete degradation during roasting. 2-pentylfuran presents notes of earth and mold (Flament, 2002), and probably because of this, the black defective seed is considered to be one of the most detrimental defects for coffee quality (Teixeira and Gomes, 1970).

Isoamyl-6-methylpyrazine (apparently identified in coffee for the first time) and 3-methyl-2-butylpyrazine were found in all lots of sour seeds, with concentrations in the range of 1.3-2.5 mg/100g. These compounds were considered, therefore, as new markers for roasted sour seeds. It has been reported that 3-methyl-2-butylpyrazine, at a concentration of 250 μg/100 g, imparts an anise-like, liquorice flavor to a sugar syrup (Winter et al., 1976). However, no reports were found for the note imparted by a 1000 fold higher concentration.

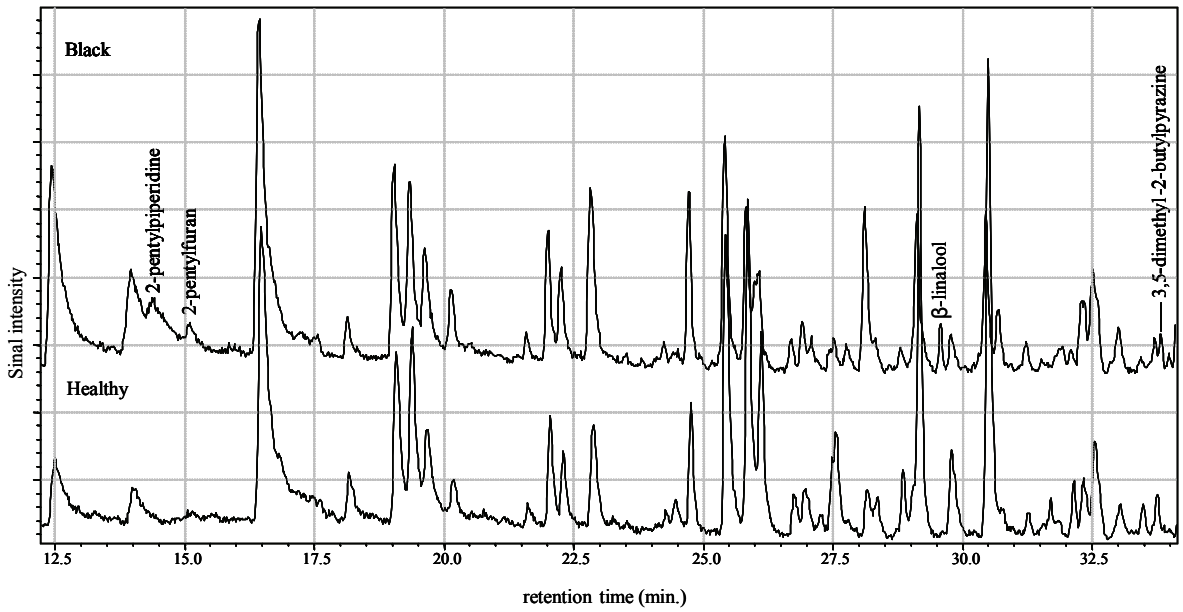

Figure 2. Juxtaposed chromatograms of black defective and healthy roasted seeds.

CONCLUSION

The present results confirm 2,3,5,6-tetramethylpyrazine and butyrolactone as marker compounds for intrinsic coffee defects in general, and indicate hexanoic acid (in raw and roasted defects), 3,5-trimethyl-6-ethylpyrazine, 3,5-dimethyl-2-butyl-pyrazine, and β-linalool (in roasted defects) as new volatile marker compounds for defects in general. New markers for specific defects were: 3-ethyl-2-methyl-1,3-hexadiene in raw black defects; 2-pentylfuran, 3-methylpiperidine and 2-pentylpiperidine in roasted black defects; 2,3,5-trimethylpyrazine, 2-pentylfuran, 2-octen-2-one, and 2-octenal in raw black and sour seeds; and isoamyl-6-methyl-pyrazine and 3-methyl-2-butylpyrazine in roasted sour seeds.

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Acidity in Coffee: Bridging the Gap Between Chemistry and Psychophysics†

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†In memory of Dr. Ernesto Illy (July 18, 1925 - February 3, 2008)

SUMMARY

Acidity is an important sensory attribute of coffee brews influenced by several factors including coffee variety and processing, country of origin, roasting degree, water composition and coffee brewing method. In spite of the relatively narrow pH range exhibited by *Coffea arabica* brews (mostly 4.80-5.80), the perceived acidity (PA) seems to cover more than one pH unit range, only. Although the sensory perception of acidity is linked to the concentration of H^+ ions, few authors observed a good linear correlation between pH and PA of coffee brews. Moreover, recent findings on perception transduction mechanisms of acid taste, put in evidence the role of the undissociated form of acid molecules as primarily responsible for acid perception. Therefore titratable acidity (TA) should be a more reliable way of linking perceived acidity to chemical properties, and a recent investigation on coffee brews seems to confirm PA - TA linear correlation. The present work tries to bridge the gap which seems to exist between PA and chemistry in coffee brews taking into the account possible source of error and setting up strictly controlled sensory protocols. For this purpose, both chemical determinations and sensory evaluations of acidity have been carried out. A panel of 7 judges has been trained with orthonasal viae closed by a noseclip, in order to eliminate possible interplay between coffee taste and aroma. Samples of *Coffea arabica* from different origin and one sample of *Coffea canephora* have been used to prepare brews by liquid/solid extraction. Each coffee sample has been roasted to medium roasting degree (16.5 + -0.2% of total weight loss). Simultaneously, pH determinations and titratable acidity have been carried out on the same samples subjected to sensory analysis. Human saliva has been used as neutralizing agent for coffee brews in order to determine the proper brew amount to be examined by judges. A very good correlation between sensory evaluation and chemical determinations has been found. The role played by coffee aroma (e.g. volatile acidity) in affecting subjective/objective acidity correlation is emphasized.

INTRODUCTION

Acidity of coffee brews has long been recognised as an outstanding sensory quality attribute (Vitzthum, 1976). Coffee lots can be purchased at a record high price due to pronounced perceived acidity or due to acidity-related sensorial properties. The chemical determination of coffee acidity is done through measurement of the pH of its brew. For example, when *Coffea arabica* is considered, the range of pH is between 4.80 and 5.80 (Dalla Rosa et al., 1986a; Dalla Rosa et al., 1986b; Petracco, 2001). Acidity is influenced by many factors, like species, cultivar, country of origin, and post-harvest processing (sundried as opposed to wet). Even one well defined coffee sample may be the origin of a number of coffee brews differing in acidity, depending on brewing method, coffee/water ratio (Peters, 1991), water composition used to brew the coffee (Sivetz, 1972), and the roasting method itself (Blanc, 1977).

Several studies have focused on the attempt to correlate chemical acidity of coffee brews with perceived acidity (PA), sometimes defined as sourness. Despite the fact that the sensory perception of acidity is somehow linked to proton concentration (Sivetz, 1972; Shallemberger 1996), many authors (Voilley et al., 1981; Maier, 1987; Ciurea and Voesgen, 1985; Wurziger and Drews, 1983a; 1983b) observed a low correlation between pH and PA. Psychophysical research on the mechanisms responsible for the sour sensation and the investigation of the relationship between H^+ concentration and PA started at the end of the 19^{th} century (Kahlenberg and Austin, 1900). The general conclusion of these studies over one century was that the sour taste of weak acids is not entirely dependant on the H^+ concentration. Moskowitz (1971) measured the PA of 24 organic acids without being able to find a clear relationship between PA and the physico-chemical properties of the acids. PA was not found to be quantitatively proportional to the acid strength (Ganzevles and Kroezer, 1987). Research on the perception transduction mechanisms of acid taste put in evidence the role of weak acids as responsible for acid perception (Norris et al., 1984; Ganzavles and Kroezer, 1987; Sowalsky and Noble, 1998). Furthermore, PA was found to correlate with the dissociation constant K_a for carboxylic acids and the titration with the associated salt did not change the PA (Ganzevles and Kroezer, 1987). Another finding was that when saliva interaction is present, PA is better predicted from titration results.

A study of the correlation between lipophilicity and PA showed that the penetration of the acid into the taste cell play a fundamental role in the process of perception. These findings were recently supported by neurophysiological results, which demonstrated how the undissociated form of the acid molecule is the major contributor for acid taste (Lyall et al., 2001). The undissociated molecule enters the taste receptor cell membrane and dissociates there, thus decreasing the intracellular pH. This mechanism seems to be more effective than the H^+ entering the taste receptor cell through ion channels even if taste cells contain a variety of conductances and ion exchangers that are pH modulated although their role in sensory transduction has not yet been fully determined (DeSimone and Lyall, 2006). Therefore titratable acidity (TA) should be a more reliable way of linking PA to chemical acidity.

As far as TA is regarded, the debate on the choice of the end point for titration is still open.

Early attempts (Voilley et al., 1981) to correlate physico-chemical analyses with organoleptic properties showed, on one hand, that pH was poorly correlated with perceived acidity, and, on the other hand, that the correlation between sour taste and titratable acidity (TA) up to pH 8.1 was very high. Several studies confirmed these findings, with some disagreement, however, about the titration pH end point. In particular, some authors suggested pH 7.0 as an end point (Ciurea and Voesgen, 1985; Wurziger and Drews, 1983a; 1983b), because at these conditions 99% of acids are in dissociated form, whereas Maier (1987), after analyzing 26 coffee extracts from roast and ground coffee and 36 different soluble coffees, suggested pH 6.0 to be preferred in comparison with other pH end points (pH 7.0 and pH 8.0).

The poor correlation between pH and perceived acidity, reported in previous studies, may indicate a non-linear relationship between titratable acidity and proton concentration. The linear correlation, however, has been found firstly by Cross (1980) with his studies about roast and ground *arabica* coffee during storage, and recently has been reported by Zehentbauer et al. (2004) in a detailed investigation aimed at studying the relationship between pH, titratable acidity and PA in coffee brews prepared from six different *arabica* and two *canephora* samples, which have been roasted under three different conditions. The linear correlation between pH and titratable acidity (to pH 7.0) has been obtained only in cases where the same coffee has followed different roasting conditions. When coffees differing in origin, variety or roasting conditions are compared, neither pH nor titratable acidity is

correlated with PA. Moreover, in the same study, the comparison between two different end points (pH 7.0 and pH 6.0) put in evidence in both cases very similar linear correlation between pH and titratable acidity.

The partial contradiction between coffee research on one hand and neurophysiological and psychophysical results on the other might be justified by the fact that the latter studies, when performed resorting to a human panel, are carried out on very simple tastant systems (stimulus in pure water) and under very well defined conditions for sensory analysis. It is well known that even under very strict experimental conditions, sensory analysis of taste mixtures is very challenging, and literature does not always provide concurring results (Stevens, 1996). The role of taste masking has been discussed also in the field of coffee beverages, and in particular, the diminution of the sour taste by the bitter taste has been reported (Maier et al., 1984).

Therefore, taking into account the complexity represented by coffee brews as a taste mixture and that, in this case, the ultimate scope of sensory analysis is to relate perception to chemical properties rather than understanding the perception/transduction process, it is not surprising to record the disagreement in correlating chemical acidity to perceived sourness, as discussed above.

Starting from this state of the art, the present work is aimed at bridging the gap which seems to exist between PA and chemical acidity in coffee brews. For this purpose both chemical determination and sensory evaluation of acidity have been performed on a set of coffees from different countries of origin, taking into account the possible sources of error and setting up strictly controlled sensory protocols.

MATERIALS AND METHODS

Coffee was obtained directly from its point of origin as green beans. Seven samples were used, six of which belonging to *Coffea arabica* (El Salvador, Ethiopia, Guatemala n° 1; Guatemala n° 2, India, Kenya) and one to *Coffea canephora* (Ivory Coast). Each sample (80 g) was roasted with a Probat laboratory roaster to a medium roasting degree (16.5 ± 0.2% of total weight loss).

In order to avoid any possible influence of the coffee maker, the coffee brews were prepared by solid-liquid extraction (infusion). 200 g of water (T = 95 °C) was poured into a beaker containing 20 g of coarse ground coffee powder. After one minute of infusion at rest, the suspension was stirred by means of a magnetic stirrer, and after a further three minutes it was filtered through a paper filter (Whatman n° 3).

Tap water, characterized originally by a total hardness of 18 °F, was boiled in order to completely remove temporary hardness and to avoid possible influence of alkalinity on coffee brew acidity.

pH has been measured by using a combination electrode (mod. SenTix 61, WTW, Germany) with a 2-points calibration procedure and a pHmeter (mod. InoLab Level 3, WTW, Germany). Potentiometric titrations were performed by adding a standard solution of NaOH (0.0986 N, Aldrich) by means of a microsyringe (Hamilton, UK) to 22.0 g of sample at 25 °C, under stirring . Two different titration end points were used: pH equals 6.0 and 7.0.

Stimulated whole saliva (SWS) pH of panellists was measured. SWS production was stimulated by chewing a piece of Parafilm®. For each panellist 10 ml were sampled,

recording production time and measuring pH. No correlation was found between the mean flow (ranging from 0.71 ml/min to 1.62 ml/min) and pH. Results are reported in Table 1.

Table 1. Panellist SWS flow and pH values.

subject	SWS flow (ml/min)	SWS pH
1	1.19	7.06
2	1.05	6.98
3	1.62	7.08
4	1.05	7.02
5	1.00	7.19
6	0.71	7.00

5 ml of saliva of one subject were titrated with *arabica* coffee brew (pH = 5.44). Figure 1 shows that about 6 volumes (30 ml) of coffee brew are necessary to form a system characterized by a pH value close to 6.

According to Lagerlof and Dawes (1984) the saliva volume in the mouth varies from 0.96 mL (in females) to 1.19 mL (in males). Therefore using 6 mL volume tasting spoon (which is the average volume of a coffee tasting spoon and 6 times the volume of saliva in the oral cavity) can ensure the panellists reproduce in their oral cavity a system covering the end-point pH range chosen in the present work.

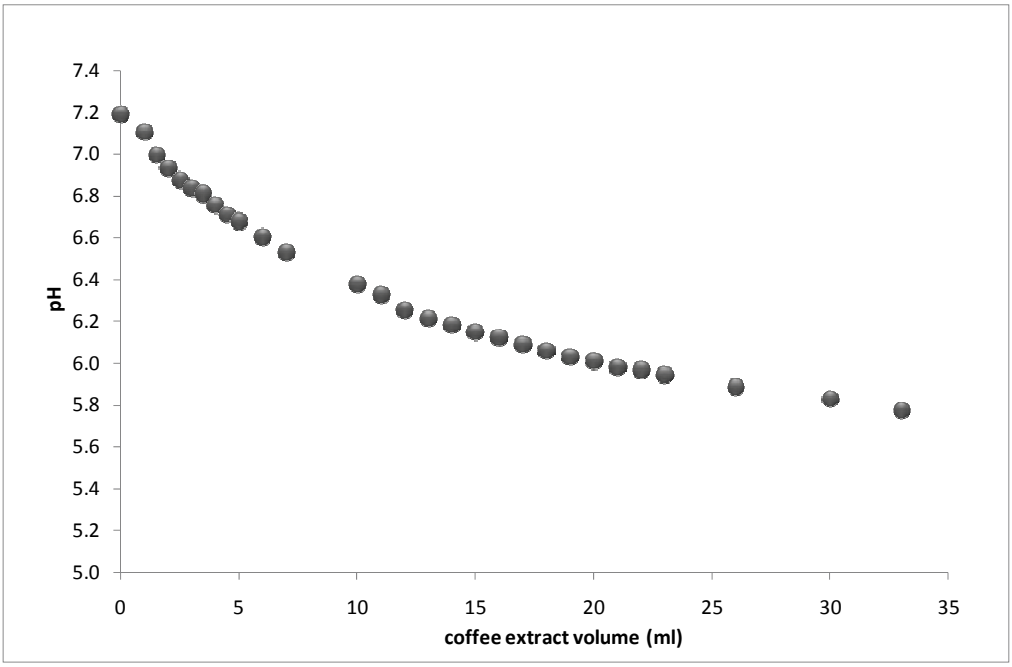

Figure 1. SWS's pH versus added coffee extract volume.

Often, when evaluating the response to a sensory stimulus, the cognitive process, which can influence the measurement process, is taken into consideration (Schifferstein, 1996). The cognitive variables can interfere while the response is being processed. The coffee world consists of many stereotypes which influence real perception, also in expert judges. For example, coffee from Kenya is known for its acidity, whereas all *robusta* coffees are defined as rather bitter and with low acidity. When performing a test on acidity of coffees from different origins, a panel of experts would certainly recognize the *robusta* coffee, and

therefore their judgments would be biased by preconceived ideas. Moreover the interaction between taste and olfaction, which distorts the perception, has been extensively studied and demonstrated (Prescott et al., 2004; Prescott, 1999). A typical case in everyday life is vanilla odour, which is defined as 'sweet', when it is well known that there are no taste receptor cells in the olfactory epithelium and therefore no sweet sensation can be directly elicited by the odour. These phenomena are probably the result of previous experiences with a particular flavour that lead to a form of learining. The single components are, in fact, coded in our memory in such a way that a future odour stimulus will also elicit a taste sensation (Prescott, 1999). From a neurophysiological point of view researchers have demonstrated the existence of multimodal neurons which respond to both olfactory and gustative stimuli (Rolls and Bayliss, 1994). Recent research showed that caramel aroma can decrease the acid sensation of citric acid (and increase the sweet sensation of sucrose) (Stevenson et al., 1999) demonstrating an interaction between taste and olfaction processes.

In this study we want to focus on the real acidity of the coffee, resulting from the acid stimulus in the taste receptor cell, without the enhancement of suppression due to the odour, besides the cognitive bias. On the basis of previous studies by Settle et al. (1988) we have tried to avoid intranasal stimulation which may confound a sour taste. For this reason a noseclip was used during the two sensory evaluation sessions. Therefore, samples have been evaluated with orthonasal viae closed. Panellists (2 female, 5 male) were asked to rinse their mouth for 1 minute with warm water before each evaluation consisting of 6.5-7.0 ml of sample. Samples were kept in the mouth for 15 seconds and subsequently expectorated. The perceived acidity was marked on a line scale (0-10). Panellists were instructed to rinse well between samples. Samples were presented according to Latin Square design with 2 replicates. In order to define the extremes of the intensity scale an alignment session was performed, in which 3 samples were prepared, consisting of a very dark roasted *arabica* coffee (total weight loss = 18.3%) representing barely perceivable acidity (lower end of scale), a very light roasted *arabica* coffee (total weight loss = 13.3 %) representing extremely acid (upper end of scale), and an intermediate value (total weight loss = 15.3 %).

Sensory data were analysed with SENPAQ 2.3 (QI Statistics 2005).

RESULTS AND DISCUSSION

In order to correlate titratable acidity of coffee brews with perceived sourness, several pH end points of titration have been proposed by previous studies, revealing some disagreement and debate on its choice. The end point equal to pH 7.0 has been more frequently used, since under these conditions 99% of coffee acids are in dissociated form (Ciurea and Voesgen, 1985; Wurziger and Drews, 1983a; 1983b; Da Porto, 1991), however, pH 8.1 and 6.0 were also taken into consideration. The latter was suggested as particularly appropriate in view of its vicinity to the pH of human saliva (Maier, 1987) In the present paper, a sws pH mean value of 7.06 $\pm$ 0.08 (range: 6.98-7.19) was measured suggesting that the choice of pH 7.0 as titration end point, seems to be appropriate also in terms of oral cavity environment.

Table 2 reports the whole set of chemical as well as sensory results. The pH range is that expected for similar coffee brews, being the pH of *Coffea canephora* brew significantly higher than that of *Coffea arabica* brews.

Table 2. Perceived acidity, pH, titratable acidity espressed in mEq/l at pH = 6.0 and pH = 7.0

Sample by origin	perceived acidity	LSD grouping	pH	mEq/l pH 6	mEq/l pH 7
Guatemala2	7.0±0.5	d	4.96±0.02	10.17±0.06	14.6±0.2
Guatemala1	5.6±0.6	c	5.09±0.09	7.3±0.7	11.6±0.5
El Salvador	5.6±0.7	c	5.01±0.02	9.8±0.3	14.5±0.3
Kenya	3.5±0.5	b	5.32±0.02	5.2±0.3	9.7±0.4
Ethiopia	3.5±0.6	b	5.342±0.003	4.55±0.03	8.92±0.06
India	2.5±0.4	b	5.374±0.008	4.3±0.1	8.5±0.4
Ivory Coast	0.5±0.2	a	5.838±0.003	0.9±0.2	5.1±0.9

A linear relationship between pH and titratable acidity exists (see Table 3) with a very high and identical correlation for both end point pH.

Cross adaptation effects, i.e. the gradual decline of sensitivity to a taste stimulus when it is applied constantly, can be present in PA experiments (Ganzevles and Kroezer,1987). Cross adaptation can affect the results if the design is not balanced, and should be taken into consideration by an appropriate DOE, a reasonable time interval between samples and ultimately by checking the PA of the panel against the presentation order of the sample.

Figure 2 shows the scores of the panel on PA in relation to the presentation order. If a cross adaptation effect is present, a linear regression will give a negative trend. This is not the case for our data that appear randomly distributed. The analysis was carried out for each panellist and both sessions. Thus we can be sure that no adaptation effect affected the results.

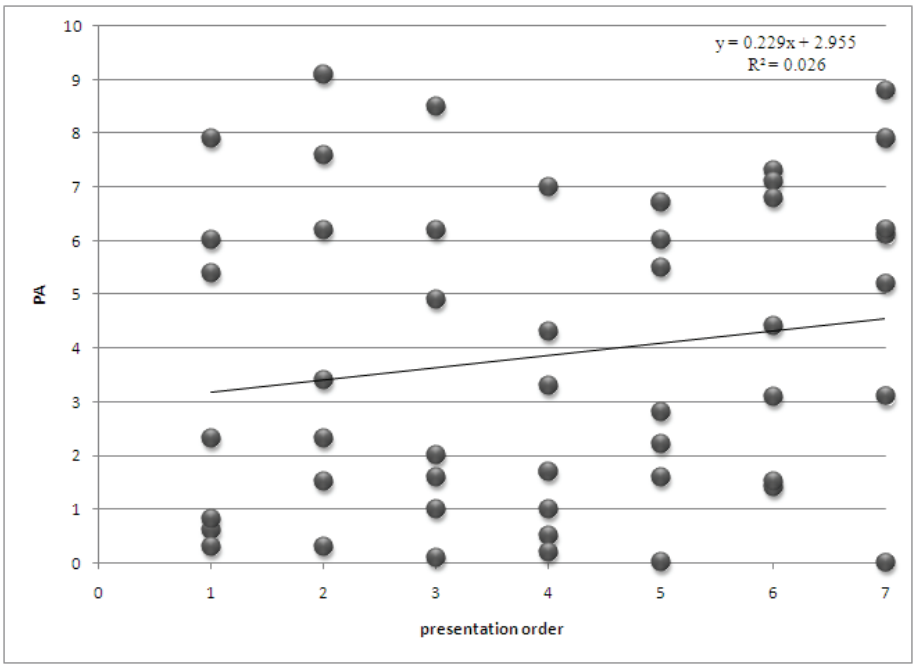

Figure 2. PA scores vs presentation order. If cross adaptation effects were present a negative trend would have been detected.

Data underwent panel monitoring and data cleaning procedure. Even though well trained, assessors are always prone to errors, which affect the overall performance. The replicated data

are used in a battery of tests that identifies any problem attributes so they can be removed for specific panellists, leaving only useful data representing the real characteristics of the products, thus maximizing the signal/noise ratio.

Sensitivity, agreement and reproducibility errors were monitored. No data was removed from the set, meaning that the agreement of the panel was high. The Analysis of Variance showed a significant effect of the product ($F_{(6,49)} = 22.43$; $p < 0.05$); furthermore, ANOVA showed that there is not assessor or replicate effect.

Table 2 shows that samples can be dived into 3 groups: coffee samples from Central America were perceived as most acid, both from a chemical and a sensory point of view. The extract from *robusta* coffee presents the highest pH value, as already reported in the literature (International Coffee Organization, 1991), and elicits the least acid sensation. The trend is clearly represented in Figure 3.

In order to estimate significant differences between samples, an LSD *post hoc* test was performed on the perceived acidity. Central American coffees belong to the same group (even if one sample from Guatemala is significantly sourer than the others). The extract from Kenyan coffee, traditionally considered as highly acidic, is statistically less sour than the Central American samples and not statistically more acid than the sample from India, when tasted without the possibility of using olfaction and therefore recognize it from its flavour. Besides a possible bias from cognitive processes, the interaction of taste and olfaction should probably play an important role. There is an associative learning of taste-odour qualities (Stevenson et al., 1999) and it could affect the perceived acidity of coffee brews. The perceived intensity of tastes can be enhanced by tasting flavoured solutions (Delwiche, 2004), especially when there is a logical association between taste and smell. This can be the case of coffee brews from different origins. Kenya, for example, is traditionally defined as a coffee with fruity, citrus and floral notes (International Coffee Organization, 1991). This could influence the sour perception of this coffee beverage.

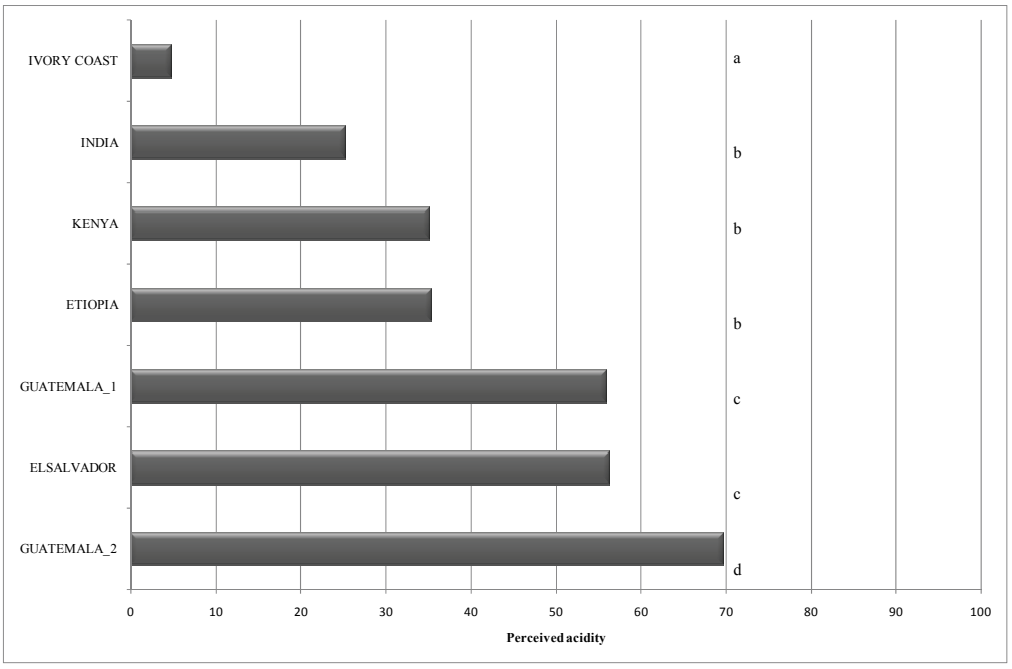

Figure 3. PA for the 7 coffee brews. The scale has been transformed from 0-10 to 0-100. The LSD (Least Significative Difference) from the ANOVA is 13.3. Same letter means no statistically significant difference.

Finally the correlation matrix between PA, pH and TA presents extremely high values for every pair, as shown in Table 3.

Table 3. Correlation matrix between PA, pH and TA

	PA	pH	TA (meq/l) pH 6	TA (meq/l) pH 7
PA	1			
pH	-0.97	1		
TA (meq/l) pH 6	0.97	-0.97	1	
TA (meq/l) pH 7	0.96	-0.97	0.999	1

The high correlation between PA and TA is in agreement with neurophysiological results and was to be expected, when the experiment is performed in controlled conditions. A possible reason for the high correlation between PA and pH, which might appear to contradict recent psychophysical results, is that those experiments were performed on a very simple tastant model (single acid).

Also, the correlation between PA and TA is exactly the same for both end points, in full agreement with Zehentbauer et al. (2004); hence, the previously reported better correlation between perceived sourness and titratable acidity to end point of pH 6.0 could not be confirmed.

Figure 3 reports PA as a function of coffee brews pH. The correlation is very high (0.95). The linear relationship and the high correlation between PA and pH is a hint towards the use of pH determination − a rapid, simple and robust method − for measuring coffee acidity, instead of obtaining it though titration, which is more cumbersome, or by a sensory evaluation which is time consuming and can lead to more noisy data.

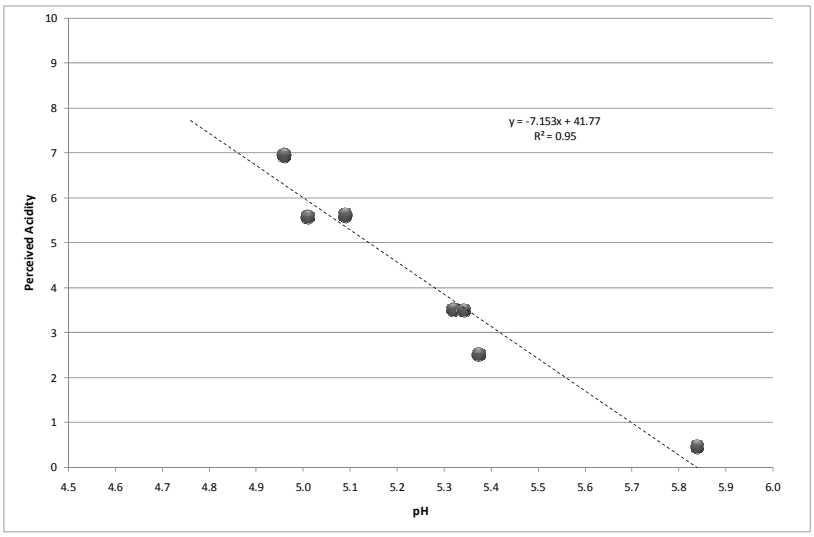

Figure 3. Perceived acidity as a function of coffee brews pH.

In an attempt to interpret the experimental data, it is necessary to introduce two different aspects not yet discussed, as far as coffee brews acidity is concerned. First of all, the buffer capacity of saliva must be kept into consideration. This is regulated by three major buffer systems: bicarbonate, phosphate, and protein. These systems have different ranges of maximal buffer capacity, the bicarbonate and phosphate systems having pK values of 6.1-6.3 and 6.8-7.0, respectively (Lenander-Lumikari and Loimaranta, 2000; Kivela et al., 1999). The

phosphate and protein buffer systems make a minor contribution to the total salivary buffer capacity, relative to the bicarbonate system, based on the following equilibrium:

$$HCO_3^- + H^+ = CO_2 + H_2O \quad [1]$$

When an acid is added to saliva, the bicarbonate releases carbonic acid as a weak acid. The carbonic acid is rapidly decomposed into water and carbon dioxide (thanks to the action of carbonic anhydrase), and then is liberated from the solution. Contrary to many buffering agents, this mechanism does not result in the accumulation of weak acid, but in the complete elimination of the acid. At pH = 6 or = 7, the bicarbonate is the dominating specie but when pH is higher than 7, the carbonate specie is no more negligible. Therefore, in our opinion, the titration end-point could be related not merely to the saliva pH, but also to its buffer capacity, and this may explain the high correlation observed at both end-point pH.

The second neglected aspect is the carbon dioxide (carbonic acid precursor) present in roasted and ground coffee and originated by the roasting process. Immediately after grinding, carbon dioxide content ranges from 4.0 to 8.6 mg/ g of coffee with an overall average of 5.7 mg/g (higher content in dark roasted). *robusta* coffee has higher carbon dioxide (average of 6.9 mg/g) than *arabica* (average of 4.6 mg/g) (Anderson et al., 2003). Thanks to the presence of the organic acids, the carbon dioxide solubilised by hot water is mostly (but not completely) present as bicarbonate in the coffee brews, and this can represent an additional source of weak acid and bicarbonate in saliva. In this regard, the pH is very important in determining the relative amount of ionic species of the bicarbonate system in the coffee brew-saliva system.

Assuming that the sour stimulus comes from a decrease of the intracellular pH in acid sensing taste receptor cells (DeSimone & Lyall, 2006), this decrease is not only due to undissociated acids and protons entering taste cells, but also by intracellular formation of carbonic acid through the carbonic anhydrase catalyzed hydration of CO_2 (Lyall et al., 2001; DeSimone & Lyall, 2006). The presence of carbon dioxide/bicarbonate system in coffee brews, as far as we know, has not yet been taken into consideration in discussing coffee acidity. On the other side, in the attempt to correlate perception (PA) and chemistry (pH or TA), very scarce attention has been given to both saliva and carbon dioxide/bicarbonate equilibrium (Spielman, 1990). For example, very recently, in a detailed investigation on acidity perception, tap water was used to prepared model solutions without details on its bicarbonate content (Lugaz et al., 2005).

Whether the carbon dioxide aqueous equilibrium may represent a key in bridging chemistry and psycophysics as far as coffee acidity perception is concerned, has to be proved with further focused studies.

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Variability and Association of Sensorial and Physical Quality Attributes of Forest Arabica Coffee (*Coffea Arabica* L.) Collections at Jima

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SUMMARY

Ethiopian Arabica coffee (*Coffea arabica* L.) diversity is expected in forest, semi-forest, garden and plantation coffee production systems. In the present study, presence of diversity was indicated among forest arabica coffee accessions and populations (*Yayu, Harenna, Bonga and Berhane Kontir*) for organoleptic and physical quality attributes under *ex situ* field conservation at *Jima*. The presence of variation among forest coffee populations, initiates further study on demarcation and characterization of forest coffee population according to ecological conditions and quality profile variation. Importance of considering aromatic intensity, aromatic quality, acidity, flavour and overall standard during coffee quality "*terrior*" formation and establishment of core collection was indicated in this study. Therefore, it was possible to indicate the importance of establishing core collections with respect to patented Ethiopian coffee origins and production systems, prior selection of suitable environment for *ex situ* field conservation and further analysis of Ethiopian coffee production systems for quality, disease reaction and bean biochemical composition for detailed mapping of genetic structure of Ethiopian coffee genetic resource.

INTRODUCTION

Coffee is the backbone of Ethiopia's economy, contributing the highest of all exports revenues (ITC, 2002) and accounted for about 35% of total exports of Ethiopia (IMF, 2007). Ethiopia is currently the third largest Arabica coffee producer after Brazil and Colombia, (ICO, 2007). The country's coffee production system is classified as forest, semi-forest, garden and plantation coffee production systems (Woldestadik and Kebede, 2000). Diversity of arabica coffee for different important traits is expected within these systems (Gole, 2002; Gole et al., 2001, Teketay and Tigneh, 1994). Moreover, Anthony et al. (2002) indicated the presence of high genetic diversity in southwest wild coffee population as compared to cultivated crops abroad. In addition to the existing production systems, good source of genetic variability is also expected from conserved coffee accessions for breeding program. Despite this fact, detailed study has not yet been made in Ethiopia to study the exiting diversity with in each system.

Labouisse et al. (2008) reviewed that change in land use policy, natural disasters, coffee price crises and expansion of new varieties are the main factors for coffee genetic erosion which is

estimated to be 0.6% per year in Ethiopia. It is apparent that study of genetic structure of coffee production systems and *ex situ* conserved coffee genetic resources for an important trait has paramount importance. Thus, the purpose of this study is to assess the diversity in *ex situ* conserved forest coffee populations for organoleptic and physical quality attributes and thereby to indicate phenotypic variability and possible conservation strategy.

MATERIALS AND METHODS

In the framework of the CoCE project (Conservation and Use of Wild Populations of *Coffea arabica* in the Montane Rainforests of Ethiopia) eight project regions were identified where *Yayu, Harenna, Bonga* and *Berhane Kontir* forest coffee populations are the target areas of the project (www.coffee.uni-bonn.de). Within each forest coffee population, three sites represented by five forest arabica coffee accessions were used for this study. A total of 60 forest coffee accessions were planted in 2004/2005 at Jima Agricultural Research Center (JARC) in single rows of 10 trees per accession. Samples were prepared from each accession separately and independently following the recommended wet processing procedure at JARC. Sample of disease resistant, high yielding and good quality coffee accessions improved through selection originated from *Kaffa* (improved) were used as check for clustering. Data on organoleptic and physical quality parameters were collected at JARC's coffee liquoring unit with well-trained coffee tasters of JARC panel and CIRAD (Centre de cooperation internationale en recherche agronomique pour le développement) experts in 2007/08. Aromatic intensity, aromatic quality, acidity, astringency, bitterness, flavor, and overall standard were recorded with 0 to 5 scales. Bean density (g/mm^3) was estimated as a ratio of bean volume (length x width x thickness) and hundred-bean weight. Above screen 14 were measured with electrical screener adjusted at 20 seconds of vibration. Mean values of attributes were computed for forest coffee population for further statistical analysis. SAS, 2000 and XLSTAT, 2008 statistical software packages were used for principal component analysis (PCA), cluster analysis and correlation tests.

RESULTS AND DISCUSSION

Mean performance

Mean performance of forest coffee collections for organoleptic and bean physical quality attributes is described in Table 1. High mean value (90.49%) of above screen 14 with acceptable range of export standard (> 80%) was recorded. Average mean values of accessions were recorded for aromatic intensity (2.67), aromatic quality (2.56), body (2.75), flavor (2.39), overall standard (2.61), and acidity (2.69). Relatively high coefficient of variation was obtained from astringency (41.16%), flavor (30.28%) and overall standard (22.50%). The result indicated existence of good potential in forest coffee population to improve coffee quality.

Table 1. Mean performance of forest coffee population.

Variable	Mean	C.V (%)	Range
Screen above 14(%)	90.49	6.11	88.25-94.84
Acidity	2.688	18.15	2.24-2.94
Aromatic intensity	2.669	15.01	2.47-2.76
Aromatic quality	2.560	17.05	2.34-2.83
Astringency	1.382	41.16	1.27-1.87
Body	2.746	12.91	2.54-3.00
Flavor	2.392	30.28	2.07-2.83
Overall standard	2.606	22.50	2.2-2.89

Association among quality parameters

Inherent expression of arabica coffee quality is influenced by environment, genetic and genetic x environment interaction (Leory et al., 2006). Expression of characters of crop plants is correlated due to genotypic and/or environmental factors. Direct observable phenotypic association of characters resulted from genotypic and/ or environmental correlations (Falconer and Mackay, 1996). Observable phenotypic association of organoleptic and physical quality attributes of forest coffee collections is described in Table 2.

In this study, strong positive phenotypic association was observed among aromatic intensity, aromatic quality, acidity, flavor, and overall standard. Yigzaw (2005) indicated positive association among good cup quality attributes. Agwanda (1999) identified flavor as an all round organoleptic attribute to be considered during selection to develop superior coffee genotypes. Attributes, which correlated strongly and positively with flavor, were considered as good cup quality attributes. Flavor revealed negative association with astringency and bitterness. Therefore, aromatic quality, aromatic intensity, and acidity were described as good cup quality attributes, whereas astringency and bitterness were considered as poor cup quality attributes. Physical quality parameters such as above screen 14 and estimated bean density showed weak positive association with good cup quality attributes. However, above screen 14 revealed weak negative association with poor cup quality attributes indicating indirect association of poor cup quality attributes with bean size and roasting.

Table 2. Correlation between attributes.

Variables	AI	AQ	AC	AS	BI	BO	FL	OVS	Above screen 14
AQ	0.840*								
AC	0.504*	0.530*							
AS	-0.240	-0.114	-0.198						
BI	-0.482*	-0.362*	-0.588*	0.594*					
BO	-0.149	0.010	-0.297*	0.487*	0.615*				
FL	0.507*	0.613*	0.694*	-0.128	-0.42*	0.101			
OVS	0.666*	0.746*	0.776*	-0.176	-0.522*	-0.047	0.862*		
Above screen 14	0.140	0.157	0.176	-0.013	-0.08	-0.049	0.110	0.190	
Bean density	0.060	0.091	0.069	0.075	0.023	0.186	0.186	0.033	0.080

**Significant at 0.05 probability level; AI=Aromatic intensity, AQ=Aromatic quality, AC=Acidity, AS=Astringency, BI=Bitterness, BO=Body, FL=Flavor, OVS=Overall standard*

Principal component analysis

The first two principal components CP1 and CP2 with eigenvalues of 4.28 and 1.88, respectively explained 61.64 % of the total variability in the forest coffee population for organoleptic and physical quality attributes. The first principal component (CP) explained the largest variability of 42.80% (Table 4). Percent variability explained by physical and organoleptic quality attributes was different. Aromatic intensity, aromatic quality, acidity, flavor and overall standard contributed 15.03%, 15.05%, 16.18%, 14.99%, and 19.28% to the variation in the first principal component, respectively. However, physical quality attributes contribution was negligible in the first two principal components. Moreover, the eigenvectors in the first principal component also explained the extent of variation caused by the attributes (Table 3).

According to Chahal and Gosal (2002) attributes with eigenvectors close to unity in the first principal component explains more variation as compared to other attributes. Therefore, aromatic intensity, aromatic quality, acidity, flavor, and overall standard showed eigenvector values close to unity (Table 3). Aromatic intensity, aromatic quality, acidity, flavor and overall standard associated strongly with the first principal component with correlation coefficient of 0.80, 0.80, 0.83, 0.80 and 0.90, respectively (Table 4). Thus, these attributes were identified as the main source of variation among forest coffee population. Therefore, attention should be given to these attributes during selection program and establishment of core collections.

Table 3. Eigenvectors, eigenvalues, percent variability, and cumulative variability of principal components.

	Eigenvectors	
Category	CP1	CP2
Aromatic intensity	**0.388**	0.081
Aromatic quality	**0.388**	0.230
Acidity	**0.402**	0.003
Astringency	-0.189	0.494
Bitterness	-0.354	0.389
Body	-0.141	0.606
Flavor	**0.387**	0.258
Overall standard	**0.439**	0.174
Above screen 14	0.107	0.073
Bean density (gm/mm^3)	0.041	0.276
Eigenvalue	4.280	1.883
Variability (%)	**42.804**	18.831
Cumulative (%)	42.804	61.635

Values in bold are eignvector values close to unity in CP1 (Principal Component1).

Association of forest coffee populations and attributes with principal component is indicated in Table 4. Forest coffee populations characterized according to their association with the attributes and principal components. Poor association of forest coffee populations with principal components indicated less association of specific forest coffee population with those attributes caused variation. This is explained in Table 5 where *Kaffa* improved, *Yayu, Berhane Kontir*, and *Harenna* showed poor association with the attributes except *Bonga* that had strong negative association with good cup quality attributes.

Even though most of forest coffee population showed poor association with quality attributes, they had positive correlation with good cup quality attributes. Therefore, poor association of populations with most of the attributes indicated the presence of forest coffee population x environment interaction which causes blending of coffee quality and finally leads to losses of the typical identity of Ethiopian coffee quality profiles. Secondly, *Harenna* and *Berhane Kontir* showed strong positive association with flavor and overall standard, respectively. This indicated potential of forest coffee population for further selection and development of good cup quality coffee accessions.

This study enabled to structure coffee quality performance of forest coffee population groups under *ex situ* conservation at Jima using the first two principal components that explained the highest variation. Three different groups of forest coffee population were identified according to their organoleptic quality attributes profile (Figure 1 and Table 5).

Kaffa improved, *Berhane Kontir*, and *Harenna* showed positive association with most of good cup quality attributes. Therefore, these forest coffee populations considered to be categorized in the same group sharing the same response to environment for quality expression. *Yayu*, which showed negative association with flavor and overall standard constituted the second group while the third group was characterized by negative association of *Bonga* with good cup quality attributes. The populations were not grouped according to their altitudinal range of their region of origin.

Table 4. Correlations between variables and factors.

Category	CP1	CP2
Quality attributes		
Aromatic intensity	0.80(15.03)	0.111(0.65)
Aromatic quality	0.80(15.05)	0.315(5.275)
Acidity	0.83(16.18)	0.004(0.001)
Astringency	-0.39(3.58)	0.678(24.38)
Bitterness	-0.73(12.56)	0.534(15.14)
Body	-0.29(1.99)	0.831(36.69)
Flavor	0.80(14.99)	0.354(6.65)
Overall standard	0.91(19.28)	0.239(3.04)
Above14	0.22(1.15)	0.100(0.53)
Density	0.08(0.16)	0.379(7.65)
Forest coffee population		
KAFFA improved	0.064	0.249
YAYU	0.070	-0.238
BONGA	-0.412	-0.198
BERHANE KONTIR	0.207	0.104
HARENNA	0.103	0.206

Values in parenthesis are percentage contribution of the variables to principal components.

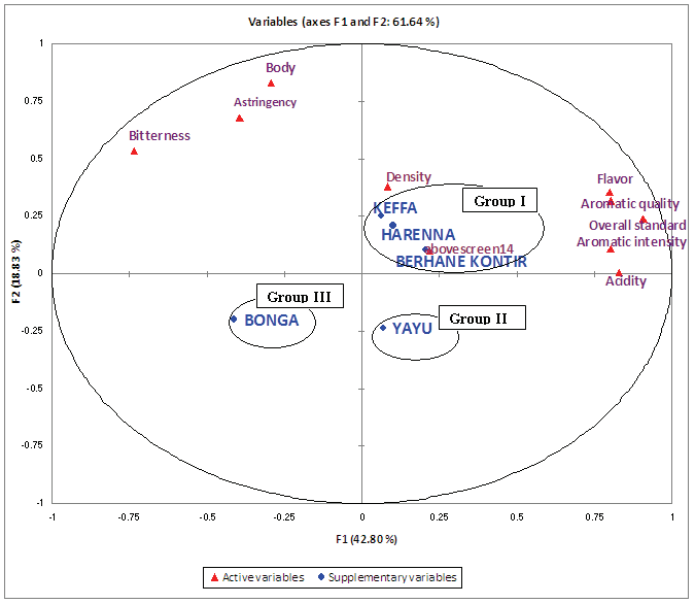

Figure 1. Association of forest coffee population with organoleptic and bean physical quality attributes.

Molecular level study of wild coffee population indicated presence of similarity between some regions and difference among regions (Tesfaye et al., 2006). Similar study indicated that wild coffee population at *Bonga* has been characterized differently with Inter-Simple Sequence Repeat (ISSR) molecular genetic analyses markers. Thus, ecological variation among coffee populations and genetics of the plant structured the population indicating the presence of quality attributes diversity among forest coffee populations. Moreover, response of forest coffee population to new environment was well observed where some population adapted poorly at Jima. This underlines the need for *in situ* conservation of coffee accessions.

Table 5. Correlation between germplasm groups and quality attributes.

Variables	KAFFA improved	YAYU	BONGA	BERHANE KONTIR	HARENNA
Aromatic intensity	0.049	0.102	-0.298*	0.118	0.054
Aromatic quality	0.136	0.086	-0.282*	0.180	-0.051
Acidity	0.115	0.163	-0.535*	0.144	0.170
Astringency	0.182	-0.032	-0.049	-0.135	0.125
Bitterness	0.118	-0.148	0.201	-0.041	-0.072
Body	0.155	-0.350*	0.032	0.067	0.174
Flavor	0.132	-0.211	-0.272*	0.165	0.252*
Overall standard	0.092	-0.082	-0.409*	0.266*	0.179
Above screen 14	0.171	0.171	-0.456*	0.390*	-0.190
Bean density (g/mm^3)	0.051	-0.045	-0.289*	0.061	0.247

**Significant at 0.05 probability level.*

Cluster analysis

Forest coffee population conserved at Jima was clustered according to their organoleptic quality performance. Aromatic intensity, aromatic quality, acidity, flavor, bitterness, astringency, overall standard were considered for clustering. Principal Component Analysis (PCA) indicated that most of organoleptic quality attributes caused variation among forest coffee populations conserved at *Jima*. Classification of forest coffee population (*Yayu, Harenna, Bonga* and *Berhane Kontir*) with agglomerative hierarchical clustering (AHC) of

determining similarity between individuals using Euclidean distance of Ward's aggregation method (Ward, 1963) was made considering organoleptic quality attributes.

Table 6. Clusters' mean value for organoleptic quality attributes.

Cluster	AI	AQ	AC	AS	BI	BO	FL	OVS
I	1.67c	1.67c	1.00d	2.00a	3.50a	3.67a	1.00c	1.00d
II	2.56b	2.51b	2.61bc	1.90a	1.38b	2.93b	2.28b	2.52b
III	2.47b	2.14bc	2.28c	0.89b	0.33c	2.28c	1.25c	1.86c
IV	2.64b	2.49b	2.76b	1.10b	0.18c	2.52c	2.38b	2.53b
V	3.01a	2.94a	3.08a	1.11b	0.29c	2.77b	3.11a	3.21a

Means followed by same letters are not significantly different by Tukey's test at P=0.05 probability level.
AI = Aromatic intensity, AQ = Aromatic quality, AC = Acidity, AS = Astringency, BI = Bitterness, BO = Body, FL = Flavor, OVS = Overall standard

Five cluster groups were identified with intra cluster and inter cluster variances of 34.93% and 65.07%, respectively. The variance decomposition for optimal classification with lower intra cluster variance indicated good quality of classification where almost homogenous individuals were grouped together.
Mean separation test indicated two different cluster groups (Cluster I and Cluster V) of distinct organoleptic quality characteristics. High mean values of aromatic intensity, aromatic quality, acidity, flavor and overall standard characterized cluster V while lowest mean values of these attributes characterized in turn cluster I (Table, 6).

Forest coffee population composed clusters with different percentage. As indicated in Table 7, accessions from Harenna, *Kaffa* improved and *Berhane Kontir* associated with high percentage with cluster II followed by cluster V. Average to high mean values of good organoleptic quality attributes characterized these two origins. Forest coffee population from *Bonga* showed high percentage in cluster III followed by cluster II. Those accessions from *Yayu* associated with high percentage of 33.33% in cluster IV and V

Table 7. Percentage of forest coffee population characterized clusters.

Forest coffee population	Cluster					
	I	II	III	IV	V	Total
Harenna	0	50	0	14.29	**35.71**	100
Bonga	**13.33**	26.67	33.33	13.33	13.33	100
Kaffa	0	**66.67**	0	0	**33.33**	100
Berhane Kontir	0	40	0	26.67	**33.33**	100
Yayu	0	26.67	6.67	33.33	**33.33**	100

Cluster I which was characterized by poor cup quality attributes was solely constituted of accessions from *Bonga*. Similarly, poor characteristics of cluster III for cup quality attributes particularly for flavor and overall standard was caused by accessions from *Bonga* and *Yayu*. Equal percentage (27.78 %) of *Harenna, Berhane Kontir* and *Yayu* contributed to good cup quality characteristics of Cluster V (Table 8).

Coffee accessions originated from old administrative region of *Kaffa* (improved) was used as check to identify forest coffee population groups that perform similar to the check. Accordingly, overall cluster composition of germplasm groups indicated that a high

percentage of *Kaffa* improved was observed in cluster II (66.67%) (Table 7). Similarly, 50 % and 40% of germplasm group *Harenna* and *Berhane Kontir*, respectively characterized this cluster. A similar result of coffee population structure was depicted by PCA (Fig. 1). Therefore, *Harenna* and *Berhane Kontir* had similar performance with *Kaffa* improved for organoleptic quality. However, other origins, particularly *Yayu* and *Bonga* showed some deviation as compared to performance of the check. Performance deviation of forest coffee populations at Jima indicated the presence of genetic x environment interaction for coffee quality.

Table 8. Percentage of germplasm groups characterized cluster.

Forest coffee population	Cluster				
	I	II	III	IV	V
Harenna	0	30.43	0	15.38	**27.78**
Bonga	**100**	17.39	83.33	15.38	11.11
Kaffa	0	8.7	0	0	5.56
Berhane Kontir	0	26.09	0	30.77	**27.78**
Yayu	0	17.39	16.67	38.46	**27.78**
Total	100	100	100	100	100

According to this study, association of forest coffee population with different percentage in all clusters of distinct characteristics indicated the presence of genetically different materials for quality within and between forest coffee populations. Therefore, genetic structure of forest coffee populations evaluated *ex situ* at *Jima* indicated the presence of genetically diverse coffee accessions and forest coffee populations for coffee quality. The result therefore puts future line of work towards demarcation of forest coffee areas targeting quality traits for quality mapping. Furthermore, core collection of coffee materials within geographically distant forest coffee populations considering cup quality and other important traits is an inevitable option to conserve Ethiopian special coffee quality profile.

This study also indicated that development of superior genotypes from forest population could be employed targeting those good cup quality attributes during selection. Similarly, in order to establish core collections for typical quality profile of an origin or forest coffee population, good cup quality attributes should be target of interest to represent existing diversity in a given population.

The study also points to the need of effective *in situ* conservation strategies, together with on going evaluation of organoleptic quality, morphological, disease and biochemical data under *ex situ* conditions.

Currently, *ex situ* conserved coffee genetic resource of Ethiopia is dwindling at alarming rate (Labouisse et al., 2008). In order to overcome such a problem, Jima Agricultural Research Center and Institute of Biodiversity Conservation in Ethiopia, who have already conserved 9976 accessions (Labouisse et al., 2008) in their *ex situ* field condition, needs paradigm shift in their conservation strategy and particularly towards establishment of core collections with respect to economically important traits.

ACKNOWLEDGMENT

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An Original Decision-Support Tool for Adding Value to Coffees

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SUMMARY

Producing countries are seeking to add value to their coffees and an original method has been developed to meet their requirements. It uses sensory categories based on descriptive profiles linked to different kinds of data. It culminates in zoning that facilitates product recognition and marketing. The method can be used to propose several country-scale scenarios. Scenarios are constructed taking into account technical, but also social, political and organizational constraints. Each of the proposals offers advantages in terms of clarity and ease of implementation, but they also have drawbacks, such as the exclusion of some producers. Other zoning possibilities can be imagined, depending on available data and the objectives fixed.

RÉSUMÉ

Une meilleure valorisation des cafés est recherchée dans les pays producteurs. La méthode originale des scénarios a été mise au point pour répondre aux demandes de ces pays. Cette méthode utilise des catégories sensorielles basées sur des profils descriptifs associés à des données de différentes natures. Elle aboutit à une zonification facilitant la reconnaissance et la commercialisation des produits. La méthode permet de proposer plusieurs scénarios à l'échelle d'un pays. La construction des scénarios prend en compte différentes contraintes techniques mais aussi sociales, politiques, organisationnelles. Chacune des propositions présente des avantages en termes de lisibilité, de facilité de mise en oeuvre mais aussi des inconvénients comme l'exclusion de certains producteurs. D'autres zonifications peuvent être imaginées en fonction des données disponibles et des objectifs fixés.

INTRODUCTION: CONTEXT

Market segmentation is currently being seen for many products, especially for coffee. Such segmentation corresponds to the joint requirements of consumers and producers. Consumers wish to procure a particular product for different reasons:

- A gourmet seeks a product capable of providing new taste sensations,
- A discerning consumer feels the need for information on the product used, its origin, its manufacture, its production conditions (*terroir* and traceability concepts),
- An unconventional consumer seeks to stand out from other consumers by not consuming products known to everyone.

Producers seek to make the most of the fruit of their labour, in order to stabilize and improve their income in a context of fluctuating commodity prices, or to diversify their activity.

The coffee market needs differentiated products providing certain guarantees which can be provided by geographical indications and quality labels, etc.

CIRAD is called upon to assist in characterizing coffees, by providing tools for adding value to differentiated products on both the general market and the specialty coffees market. The demand usually comes either from institutional partners in producing countries, or from agricultural organizations, such as cooperatives, whose members are directly involved.
A few examples indicate the reasons for such demand:

- The Dominican Republic is seeking to establish the renown of its coffee and return some coherence to designations,
- The island of Réunion is seeking to revive its coffee sector with a view to diversification and substitution for geranium growing, which has been experiencing productivity problems for several years,
- Laos is seeking international awareness and recognition of its products and their originality,
- Indonesia wants to protect its designations (Kintamani, Bali coffee) and prevent others from using them.

This demand is being met through projects with social, legal, commercial and technical components. The differentiation of coffees has to be established on a sound scientific basis.

The proposed approach makes it possible to tackle scientific issues associated with the product and provide decision-makers with a decision-support tool. It is based on the construction of descriptive sensory profiles which culminate in uniform coffee categories. A comparison with different types of data makes it possible to identify the main quality determinants. Those elements, which give coffees their characters, are of different kinds: the variety grown, the environment (such as height above sea level, soil properties, rainfall), and post-harvest processing procedures.

Irrespective of the objective expressed when a request for assistance is received, the initial stages are similar:

- Characterization of the product and its quality attributes,
- A study of coffee category determinants,
- Establishment of differentiated production zones.

This novel approach proposes different zoning scenarios to help decision-makers to choose the most appropriate way of setting in place signs of recognition or quality to add value to products.

MATERIALS AND METHODS

The first stage in the approach consists in getting to know the product and describing it. This initially involves rational sampling. The sampling plan is defined according to objectives, the production structure in the country considered, and prior knowledge of the variables affecting sensory quality.

Based on prior knowledge, the sampling protocol is designed to study the effect of chosen factors on sensory characteristics. Some factors are standardized to facilitate the study. For example:

- Height above sea level: several samples per altitude range,
- Variety: several samples per main variety,
- Harvesting year: replication over several years,
- Harvesting date: sampling during the harvest peak only,
- Post-harvest: a single standardized protocol or several compared protocols.

It is a matter of establishing a product profile using physical analysis techniques and sensory evaluation.

Physical analyses reveal the physical qualities of the product:

- Existence of foreign bodies,
- Bean colour,
- Bean integrity (existence or not of damaged beans),
- Distribution in the different grades.

Sensory attributes are scored during tasting sessions:

- Flavour intensity,
- Acidity,
- Bitterness,
- Sourness,
- Astringency,
- Fruitiness,
- Woodiness, etc.

This set of descriptive attributes is used to establish the product profile.

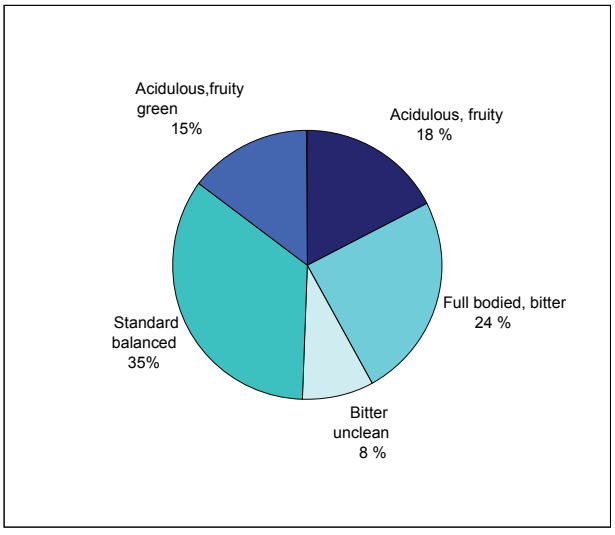

Figure 1. Example of coffee distribution in uniform groups on a country scale over two harvesting years.

The evaluation techniques are standardized. They are based on ISO standards where they exist. Sensory evaluation is carried out by a panel of at least five trained people. That minimum number of five panel members is necessary to statistically analyse scoring

coherence. These tasters belong to the local coffee supply chain. They need to receive full training enabling them to carry out a repeatable assessment of the coffee profiles to be tested. An appropriate training module has been developed by the CIRAD team. Training such a panel subsequently enables the supply chain to control coffee characteristics.

The quality of sensory evaluation is essential. It makes it possible to trace the profile of the coffee and will form the "hard core" of the subsequent statistical analyses. After checking the coherence of the sensory profile results by analyses of variance, sensory groups are created by a principal components analysis (PCA), then by classification (clustering and dynamic clouds). Analyses of variance reveal that each group has a particular physical and organoleptic profile.

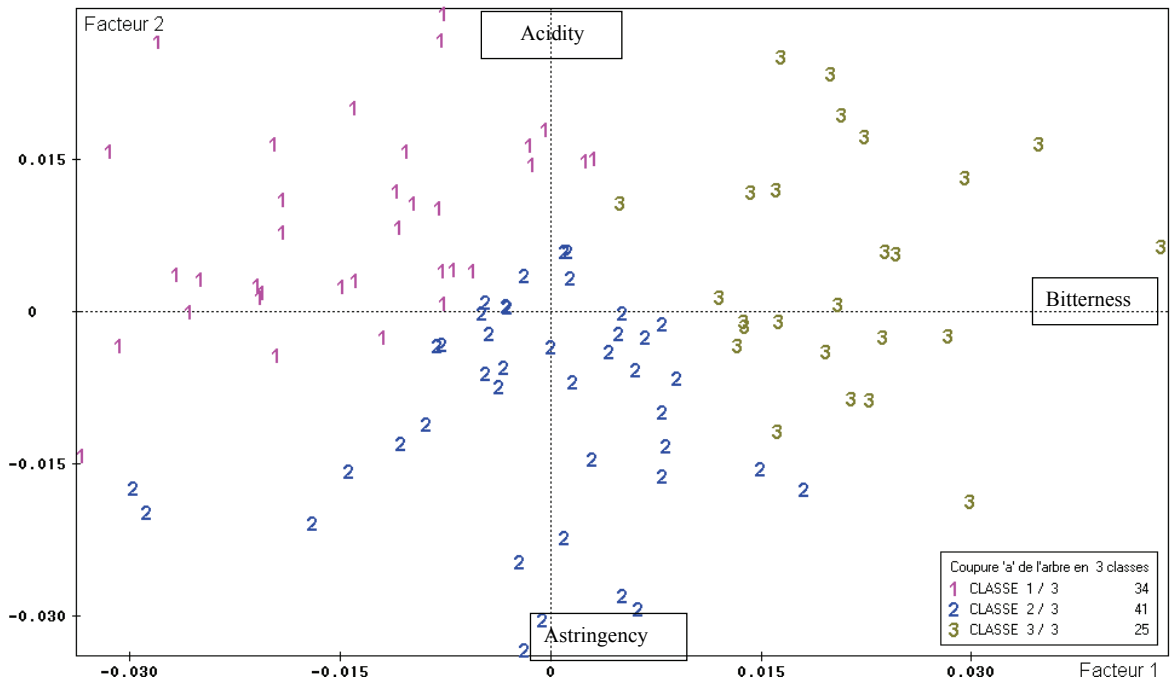

Figure 2. Example of sensory group representation on a PCA.

The second stage consists in studying determinants of the coffee categories or groups. This stage calls for major data gathering and statistical analyses. The sensory evaluation data, the "variables to be explained", are cross-checked against different types of data (environment, soil, post-harvest processing, etc.), "explanatory variables" from compiled databases.

The databases must be as far-reaching as possible to cover all the situations encountered in the zone in question (country, region, etc.). To achieve that objective, a survey has to be conducted, in addition to sampling, in order to gather data on the explanatory variables. Studies already undertaken show that coffee tree variety is important but it cannot be analysed separately from the cropping system (pruning, fertilization, etc.). The same applies for the height above sea level, which is linked to the environment – temperature, aspect and sunlight, etc.).

The plots or plantations from which the samples are collected must be clearly georeferenced. Precise data must be collected, in particular:

- Soil, with sampling and analysis (chemical composition and texture),
- Height above sea level,

- Aspect,
- Meteorology (temperatures, rainfall, sunlight, evapotranspiration, wind speed).

Cultural practices play an important role. The data required for the analyses concern:

- The harvest (yield, date, method, quality),
- The phytosanitary treatments carried out,
- Soil amendments applied by the producer (chemical and/or organic fertilizers),
- Pruning methods.

Post-harvest processing methods need to be accurately known:

- Individual or group processing,
- Dry processing (without pulping prior to drying) or wet processing (pulping),
- The time taken for each phase, in the case of comparison,
- Drying method,
- Storage method (parchment or green coffee).

All the data are entered in a single base in which information is provided on each variable for each sample.

The sensory groups created during the characterization phase will be compared to the explanatory variable data using appropriate statistical methods (Correlations, analyses of variance, Khi2). The Hierarchical Answer Tree method is used to class the influence of factors.

The third stage consists in determining differentiated production zones and creating scenarios. The geographical distribution of the sensory groups makes it possible to determine zones where coffees are uniform. Their boundaries depend on quality determinants. Based on privileged criteria (socio-economic, geographical, sensory, etc.), several types of zoning can be considered, which leads to several scenarios.

RESULTS AND DISCUSSION

Zoning scenarios are proposed in accordance with the results and depending on requirements. When establishing signs of recognition, specifications have to be respected for each scenario. Scenario construction takes into account different technical constraints, but also social, political and organizational restraints.

We propose 3 zoning constructions. The first proposal relies on associating the coffee with the image the producing country conjures up in consumer countries, based on its exotic charm, culture and traditions, its protected environment. The aim is to market all the coffees in the country under the same quality sign. This is the case encountered for small countries with a strong public image.

Specifications need to be drawn up so that the commercial and sensory quality of the product is guaranteed. Technical constraints are therefore imposed on producers wishing to use the quality sign created. In this case, the specifications do not entail any territorial restriction. No producer is excluded.

The second proposal is to establish groups of coffees based on precise knowledge of the environment, production techniques and the organization of local stakeholders in the supply chain. This territorial break-down leads to the definition of fairly extensive zones (e.g. a valley) in which the coffees are often more uniform with a more marked identity than in the previous scenario. Sensory characteristics play a not insubstantial role and are taken into account in the specifications corresponding to this type of scenario. These are more restrictive than in the global scenario. In this case, the specifications become restrictive for producers. A producer not located in the defined zone will not be able to claim the quality sign that is set in place. Social management of this scenario can prove tricky.

In the third proposal, zone construction is based on sensory data. Coffees are divided into clearly distinct sensory groups, with the product groups corresponding to restricted zones. The specifications will be based on the sensory characteristics of the product, which will exert strong technical and geographical constraints. In zoning terms, it is a difficult scenario to implement. It may not correspond to the current break-down (cooperative, administrative, catchment area, etc.). This scenario makes it possible to define the most differentiated coffees. It is particularly suited to small zones producing an original coffee earmarked for the niche markets in sufficient quantity (cooperative covering a village or set of villages).

Each of the proposals offers advantages in terms of clarity and ease of implementation... but also some drawbacks, such as the exclusion of certain producers. Other types of zoning can be imagined depending on the data available and the objectives fixed.

This method calls for considerable information gathering (sampling with a command of post-harvest processing, and a survey) and data validation. Some data are particularly difficult to obtain in a reliable manner (e.g. yields). The method therefore has to be adapted to the available data and, sometimes, variables that are generally correlated to missing variables (e.g. height above sea level and temperature) have to be used.

This method describes the potential of the coffee over a zone. For marketing, it is then necessary to take into account the variability associated with each producer and with the harvesting year. Samples can then be tested by the locally-trained sensory panel to validate the match between coffee products and the expected profile. This makes it possible to monitor product characteristics over time.

Differentiation of coffee categories can be confirmed by tasting sessions involving buyers, which enables traders and roasters to match market expectations with the proposed products.

CONCLUSION

Comparing the advantages and disadvantages of each type of zoning provides a decision-support tool. The choice of zoning type and specifications is left to stakeholders in the coffee supply chain of the country in question, and has to be determined in line with the recognition being sought. This original method provides a scientific basis for designing production zones, since it is based on statistical analyses. It can be used to define ***terroirs*** suited to quality coffee production, considering the coffee tree in its environment, along with cultural practices and farmer know-how.

This method is founded on sensory aspects, but other criteria can be taken into consideration to meet the demand for particular quality signs linked to the environment, such as organic agriculture, rain forest, etc.

Hyper Espresso Coffee Extraction: Adding Physics to Chemistry†

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†In memory of Dr. Ernesto Illy (July 18, 1925 - February 3, 2008)

SUMMARY

The traditional *espresso* extraction method results in a polyphasic beverage constituted by a foam layer of small bubbles with a particular tiger-tail pattern, on top of an emulsion of microscopic oil droplets in an aqueous multicomponent solution with dispersed gas bubbles and solid particles. Interfacial phenomena occurring during the extraction are extremely important in sensory properties so highly appreciated by the consumer. It is well known that variations in the preparation variables (e.g. raw material, water temperature and pressure, percolation time and/or beverage volume) can dramatically alter the "cup quality" not only in terms of taste and flavour (chemical composition) but also in terms of the characteristics of different phases present in the beverage (foam, emulsion, suspension and solution) and their sensory effects (visual aspects and mouthfeel) and then in the physical properties. The new *Hyper Espresso* coffee extraction method has been ideated and developed on one hand, to remarkably reduce the preparation variables, on the other hand to increase the sensory attributes related to the physical state of the beverage. This study reports on the comparison between *espresso* and *Hyper Espresso* coffee extraction methods as evinced by chemical and physico-chemical characterization of the resulting beverages. Extraction yields of several important chemical compounds together with relevant physical properties have been determined on both whole and fractionated beverages. The role of the heterophases in differentiating the two coffee extraction methods is emphasized.

INTRODUCTION

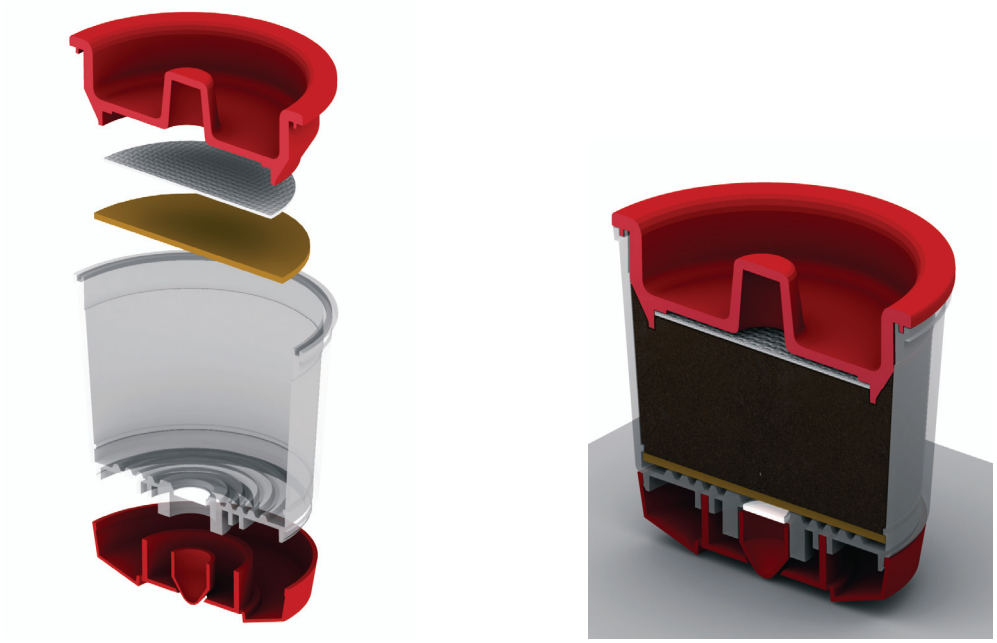
Traditional *espresso* brewing provides a peculiar and highly appreciated coffee beverage characterized by the coexistence of several colloidal phases: foam, emulsion, suspension. As far as foam, well known as *crema*, is concerned, the gas phase is mainly constituted by carbon dioxide generated during coffee roasting and entrapped within the cell structure, whereas the continuous phase is a microscopic oil droplets (90% < 10 microns) O/W emulsion in an aqueous solution of several solutes (including sugars, acids, protein-like material and caffeine) containing solids coffee cell wall fragments of 2-5 microns (Illy and Viani, 2005). The typical pure *Coffea arabica* regular *espresso* (30 mL cup volume percolated in 30 s) is an O/W emulsion of 0.2-0.3 % volume fraction, a suspension in which the dispersed phase is represented by about 150 mg solid coffee particles and a solution with total soluble solids concentration of 52.5 g/L (Illy and Viani, 2005). Foam and emulsion, in particular, are very important in ensuring the organoleptic properties which make *espresso* very different from coffee beverages prepared by other popular methods (Petracco, 1989). In facts, it is well known that the foam is a sort of quality marker of correct *espresso* coffee extraction (in terms of grinding, water temperature and pressure, brewing time, cup temperature etc.) detected simply by visual inspection, whereas both foam and emulsion play an extremely important role in the *espresso* mouthfeel (or *corpo*) being the emulsion particularly relevant in the

wetting of the oral cavity and then in the long-lasting after taste (Petracco, 1989; Navarini et al., 2004; Ferrari et al., 2007; Navarini et al., 2004). The suspension contribution to the *espresso* coffee sensory properties has not yet been the subject of detailed investigation, however, minute floating cell wall fragments have been found to be involved in the so called "tiger skin" effect in the foam visual aspect (Illy and Viani, 2005). Moreover, it may be hypothesized that larger cell wall fragments, in addition to leave a sediment in the empty cup, contribute to taste and mouthfeel being even particles of 2 microns in diameter perceived in the oral cavity (Engelen et al., 2005a; 2005b).

The complex colloidal system created in a regular *espresso* coffee by properly brewing, can be perturbed and altered by changing even one of the several factors constituting the set of preparation variables. It has been demonstrated that by varying the geometry of the cake made up of ground and compacted coffee (and then the percolation time), it is possible to obtain *espresso* coffee differing in the mouthfeel, and this difference has been largely attributed to the emulsion characteristics in terms of oil droplets sizes and their distribution (Petracco, 1989). Once fixed raw materials, it is necessary to resort to professional equipment as well as to skilled personnel to ensure full preparation reproducibility. The new *Hyper Espresso* coffee extraction method has been ideated and developed on one hand, to remarkably reduce the preparation variables (including coffee machine cleaning and hygiene-related practice), on the other hand to increase the sensory attributes related to the colloidal state of the beverage. This new method is based on a disposable extraction chamber containing an hermetically closed optimized ground and compacted coffee dose (capsule) and an innovative coffee machine which can be briefly described as an hot water injector. The capsule (see Figure 1A) is composed by five parts: the cover, the upper internal filter, the lower internal filter, the body, and the flow-conveyor. The coffee dose is located between the upper filter and the lower filter, as shown in Figure 1B. What is known as the traditional extraction chamber consists in an upper block, on which is inserted (like a bayonet) a filter-holder cup. The ground coffee portion is poured and compacted into this filter. The term filter was mentioned as inappropriate for indicating the perforated plate that constitutes the base of the cup containing the ground coffee bed. Actually, this plate does not act as a filter but as a supporting structure for the compacted coffee cake. In this case the classical shape of the coffee cake of a double shot *espresso* is cylindrical around 12 mm in height by 60 mm in width, the exact dimension depending on the machine (Illy and Viani, 2005). The shape of the coffee cake of the *Hyper Espresso* capsule (single shot *espresso*) is approximately cylindrical around 14.6 mm in height by 32.5 mm in width leading to height-to-diameter ratio of more than two times higher than that of the classical *espresso* cake shape.

In the *Hyper Espresso* capsule, the lower internal filter and the body represent the supporting structure for the compacted coffee cake. The central part of the body base is elastic and pre-perforated. When hot water is injected into the capsule by piercing the cover central part, the forthcoming aqueous extract is forced to remain into the capsule under pressure (infusion under pressure) up to an elastic deformation of the central part of the body base capable to permit the brew to reach the flow conveyor which, differently from the traditional *espresso* flow conveyors, actively contributes from a fluidodynamics point of view.

The present work is aimed at comparing *espresso* and *Hyper Espresso* coffee extraction methods as evinced by chemical and physico-chemical characterization of the resulting beverages. Important non-volatile chemical compounds and relevant physical properties have been determined on both whole and fractionated beverages. The role of the heterophases in differentiating the two coffee extraction methods is emphasized.



A B

Figure 1. *Hyper Espresso* capsule. A) Cut section showing the different parts. B) Cut section including coffee cake.

EXPERIMENTAL

Materials

Two different lots of medium roasting degree coffee beans (total weight loss: 16%; 100% *Coffea arabica* L. blend) were used. One lot has been industrially processed to obtain *Hyper Espresso* capsules whereas the second lot has been used to prepare traditional *espresso* coffee. In the latter, coffee beans were ground using a professional coffee grinder (mod. MC99, Faema S.p.A., Italy). Two different coffee machines have been used: a professional *espresso* coffee machine (mod. 2EE, La Marzocco s.r.l., Italy) and a professional *Hyper Espresso* coffee machine (mod. X2, illycaffè S.p.A., Italy). Traditional *espresso* coffee has been prepared under strictly controlled conditions as far as coffee dose (6.5 ± 1.5 g), water temperature (90 ± 5 °C), pressure (9 ± 2 bar), brewing time (30 ± 5 s) and cup volume (25 - 30 mL to select the appropriate beverage flow) are concerned. Compaction has been performed by using a dynamometric tamper (Macap s.r.l., Italy) which ensures 23 ± 1kg of constant dynamic pressure on the coffee portion. *Hyper Espresso* coffee has been prepared under the following conditions: coffee dose = 6.6 ± 0.2 g, water temperature = 93 ± 3 °C, pressure = 12 ± 1 bar, beverage flow = 1.2 ± 0.1 mL/s. Tap water (total hardness 19 French degrees, °F) has been treated by ion exchange softening up to a total hardness of 4 °F.

The whole beverage has been prepared in a wide range of cup volumes, from an unrealistic *ristretto* of 3 mL to a *lungo* of 42 mL. Beverage fractionation (in triplicate) has been performed in order to collect 10 fractions of 4.5 ± 1.5 mL in the course of brewing. Organic solvents were obtained from Carlo Erba Reagenti (Italy) and phosphoric acid "pro analysis" from Merck (Germany). The water used for analysis was doubly distilled.

For a preliminary assessment of assess the *Hyper Espresso* capsule emulsifying performances, sunflower oil (refractive index at 30 °C = 1.47392 and density 0.919 kg/m^3) and ultrapure water (refractive index at 30 °C = 1.33303) in variable amount for a total mass equal to 7 g

have been put in empty capsules subsequently closed. The capsules have been used to mimic *Hyper Espresso* coffee preparation. After "brewing" into a flat-bottomed glass cylindrical cell immediately submitted to analysis, residual sunflower oil in the exhausted capsule has been determined. The resulting "brews" are O/W emulsions with a volume fraction, Φ, ranging from 2 to 10%.

Methods

Chemical characterization has been performed by determining total solids concentration, caffeine, trigonelline, chlorogenic acids (cumulative 3-, 4- and 5-caffeoylquinic acids) and short-chain organic acids (oxalic, quinic, formic, malic, lactic, acetic, citric, fumaric and citraconic).

Total solids concentration was determined as described elsewhere (Navarini et al., 2004). For caffeine, trigonelline and caffeoylquinic acids (3-, 4-, and 5-) quantitative determination, samples were centrifuged for 5 min at 5000 rpm and filtrated with a hydrophobic PTFE membrane filter (Whatman, USA). Diluted samples were analysed with HPLC. A 1100 HPLC system (Agilent, Germany) was used, consisting of degasser, quaternary pump, column thermostat and diode array detector (DAD) operating at 254 nm, 272 nm and 324 nm. A Gemini C18 column, 5 micron 250 x 4.60 mm (Phenomenex, USA) and gradient elution (methanol and 1% phosphoric acid) were used. Organic acids were determined according to Colomban et al. (2006). In particular, coffee samples were centrifuged for 5 min at 5000 rpm, 1 mL of each sample was treated via solid phase extraction (SPE). Cleanup of samples was performed using Strata C18 and SAX (strong anion exchange) cartridges (Phenomenex, USA), a non polar retention mechanism was used to retain macromolecules and lipids, the ion exchange cartridge instead allowed the retention of ions of the organic acids at pH of coffee beverage. The organic acids retained were eluted using phosphoric acid or formic acid in the case of DAD or MS analysis, respectively. A series 1100 HPLC system (Agilent, Germany) was used, consisting of degasser, quaternary pump, column thermostat and diode array detector (DAD) operating at 210 nm. For structure confirmation the system was coupled to a QTRAP (Applied Biosystems) and enhanced MS spectra were recorded on peak apex in ESI negative mode. Source parameters were: Curtain gas (CUR): 40, Collision gas (CAD): high, IonSpray Voltage (IS) -3500 V, Temperature (TEM) 350 °C and Ion Source Gas 1 (GS1) 25 and Gas 2 (GS2) 50 respectively. The organic acids were separated isocratically using 1% phosphoric acid (pH 2.20) at 1 mL/min in case of DAD detection, in case of mass spectra same elution order was achieved with 0.1% formic acid on a Synergy Hydro-RP column at 30 °C (4u, 80A). Experimental data are expressed in mg/g coffee powder.

In order to ascertain possible remarkable differences in the oil content of the brews prepared according the two different extraction methods, several regular beverages (25 mL cup volume) were collected and freeze dried. Freeze dried samples (3-9 g) were Soxhlet extracted with n-pentane (temperature = 50 °C, time = 4 h). About 10 % of the oil originally present in the coffee dose have been found in the brew, independently on coffee extraction method.

Carbon dioxide content in roasted and ground coffee was determined by the method of Hinman (1993) in an apparatus shown and described in detail elsewhere (Anderson et al., 2003).

Colloidal characterization has been performed by Multiple Light Scattering (MLS) technique by using an optical sensor composed of a pulsed near infrared light source (λir = 880 nm) and two synchronous detectors (transmission and backscattering detectors) (Alfatest, Italy). The backscattering detector receives the light scattered by the sample at 135 °C from the incident

beam. The experimental set up and related physics are described in detail elsewhere (Abismail et al., 2000; Meunier et al., 1997; Bru et al., 2004; Snabre et al., 2004). Flat-bottomed 25 mL glass cylindrical cell (height 55 mm) have been used. Experimental data, expressed as diffuse reflectance, R, (in %) in ordinate and the height of the cell in abscissa have been converted into foam volume (mL), recorded after 70 sec after preparation, and droplet mean diameter. For the latter, the characteristic size of the backscattered spot light is representative of the photon transport mean free path, l* which, according to Mie theory as described by Bru et al. (2004), scales as droplet mean diameter, d, and the inverse of droplet volume fraction, Φ:

$$l^* = 2\,d/\,[3\,\Phi\,(1 - g)\,Qs] \qquad [1]$$

where g (d, λir, np, nf) and Qs(d, λir, np, nf) are optical parameters given by the Mie theory being np and nf the refractive indexes of droplet and continuous phase (water in the present case), respectively. Optical microscopy observation have been carried out to confirm droplet mean diameter measurements by MLS technique.

Difference between means were considered to be significant at $p < 0.05$. Foam volume measurements are affected by an average error of 0.18 ± 0.04 mL.

RESULTS AND DISCUSSION

In Figure 2, caffeine, trigonelline and chlorogenic acids (cumulative 3-, 4, and 5-caffeoylquinic acid) content are reported as a function of cup volume for both whole traditional *espresso* and fractionated beverages.

The trend as well as the absolute values of the experimental data are in good agreement with previous studies (Petracco, 1989; Nicoli et al., 1987). In all cases, the differences between the two different sampling procedures are within the experimental error in spite of some operative difficulties encountered to perform the beverage fractionation. In Figure 3, the experimental data of *Hyper Espresso* coffee beverages are reported. In Figure 4 caffeine, trigonelline and chlorogenic acids average content in the coffee beverages prepared by the two extraction methods are compared. Taking into the account the "lot-to-lot" natural variability, the content of the selected non-volatile analytes appears to be not affected significantly by the two extraction methods.

Table 1. Organic acid average content of whole beverages (cup volume range: 5-41 mL).

Acid	*Hyper Espresso* (mg/g)	Traditional *espresso* (mg/g)
oxalic	0.160 ± 0.048	0.168 ± 0.043
quinic	10.70 ± 2.65	10.25 ± 0.98
formic	1.19 ± 0.32	1.30 ± 0.29
malic	1.69 ± 0.19	1.53 ± 0.30
lactic	6.39 ± 2.91	8.42 ± 1.27
acetic	3.20 ± 0.95	3.08 ± 0.30
citric	10.91 ± 3.24	15.91 ± 3.14
fumaric	0.129 ± 0.037	0.149 ± 0.017
citraconic	0.201 ± 0.073	0.247 ± 0.022

The comparison between the organic acids determined on whole beverages (cup volume range: 5 - 41 mL) prepared by the two different extraction methods is reported in Table 1.

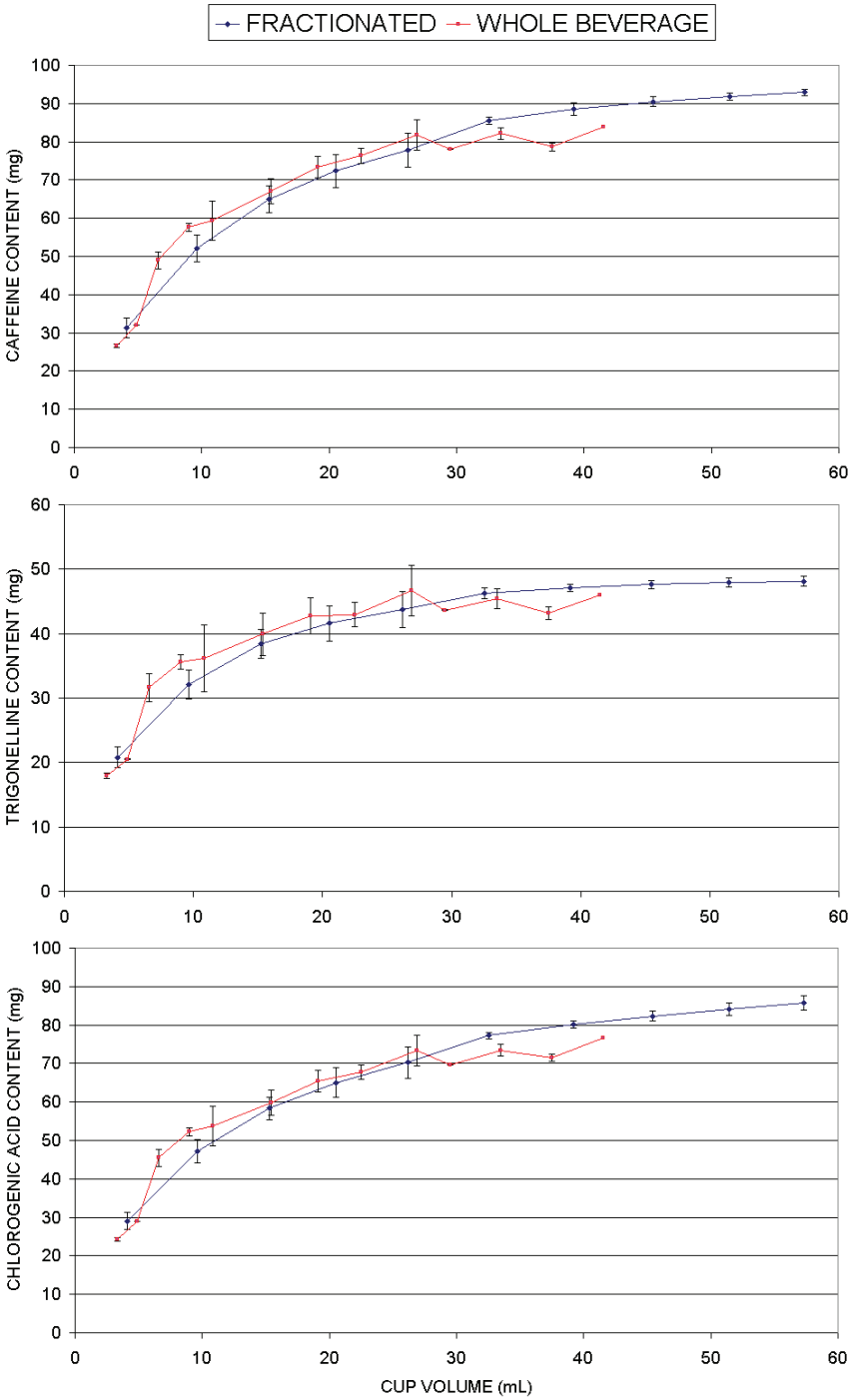


Figure 2. Traditional *espresso* method: caffeine, trigonelline and chlorogenic acids content as a function of cup volume

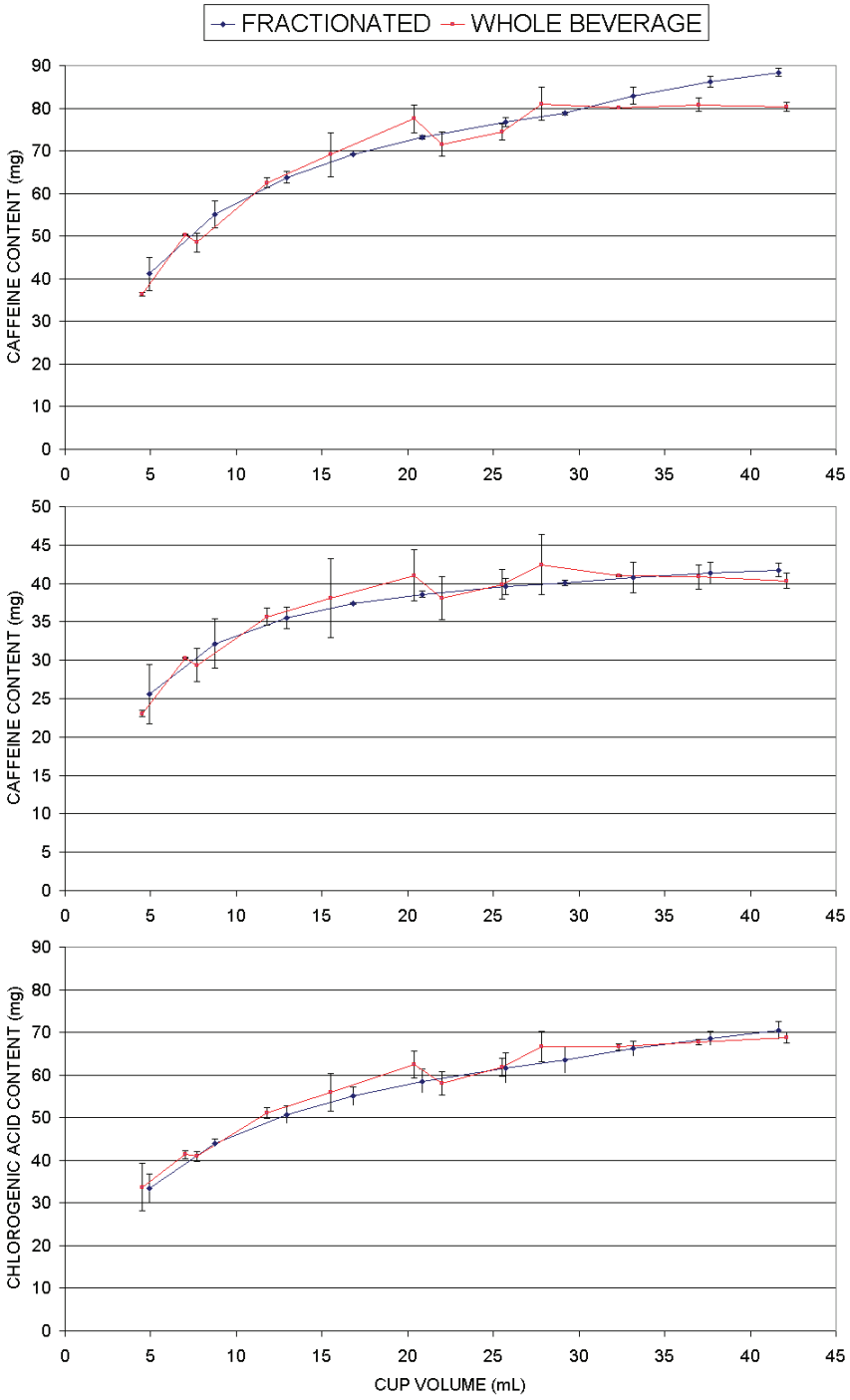


Figure 3. ***Hyper espresso*** **method: caffeine, trigonelline and chlorogenic acids content as a function of cup volume**

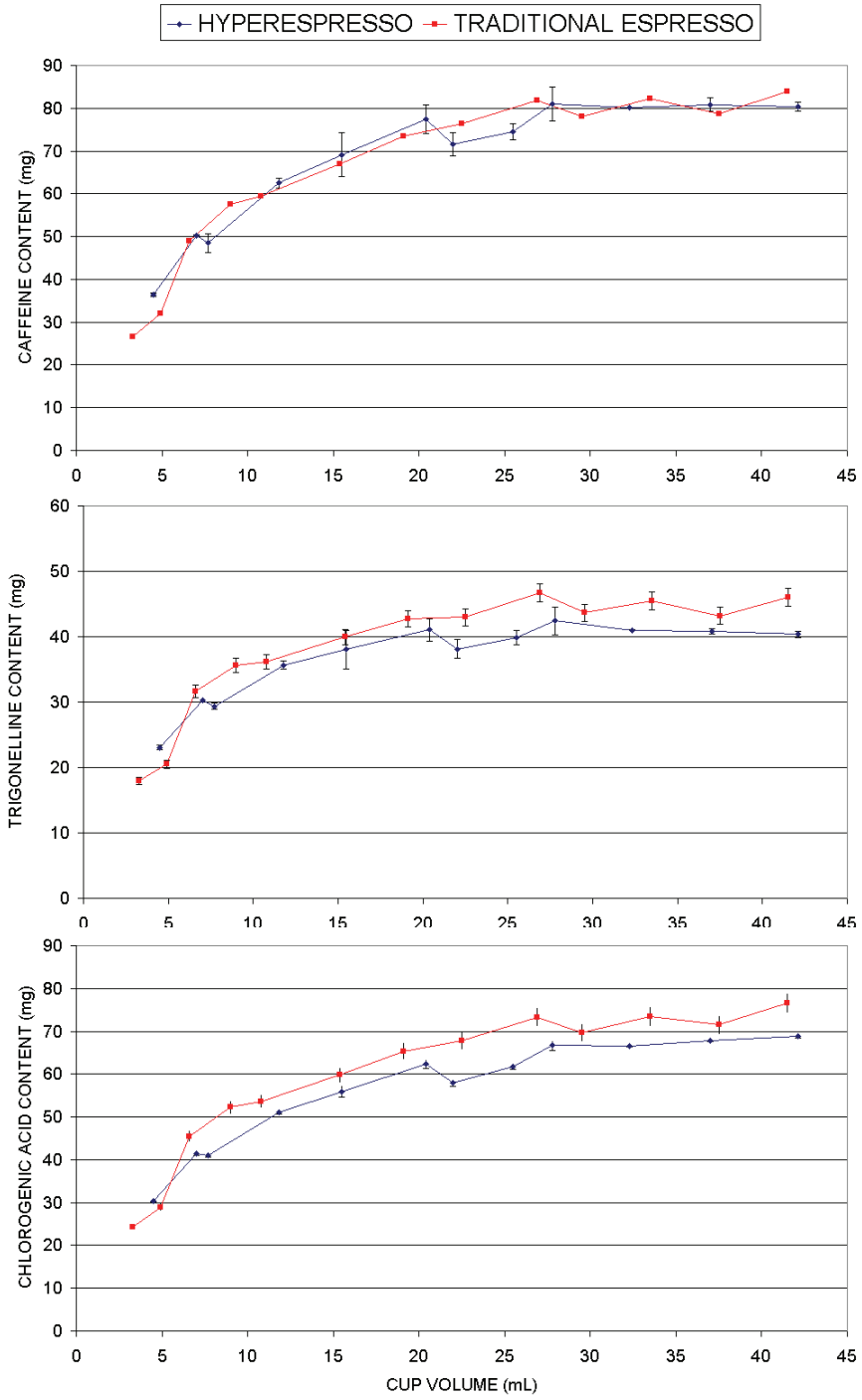


Figure 4. Caffeine, trigonelline and chlorogenic acids average content in the coffee beverages prepared by the two extraction methods

The easy extraction of the examined organic acids, thanks to their high water solubility (Van der Stegen and Van Duijn,1987) is fully confirmed considering that even the individual acid content in the unrealistic 5 mL *ristretto* beverage is not recognized as an outlier upon statistical data processing. Oxalic, quinic, malic, citric, fumaric and citraconic content is within the range of reported data whereas formic and acetic acids content is close to the lower value and this may be due to their volatility (Ginz et al., 2000). Lactic acid content is remarkably higher than that expected on the basis of literature data (Galli and Barbas, 2004; Balzer, 2001; Ginz et al., 2000). It has to be underlined that acetic and lactic acids share the same precursor derived by carbohydrate thermal degradation, in a competitive formation mechanism (Ginz et al., 2000). However, as far as we know, short-chain organic acid content

data determined by using *espresso* coffee extraction are not available in literature for a proper comparison.

In all cases, with the exception of citric acids the differences in the short-chain organic acids content are not statistically different ($p < 0.05$). These findings, further confirm that the influence of the two different preparation methods in the extraction of the selected chemical compounds is very weak, if any.

The visual inspection of regular traditional *espresso* and *Hyper Espresso* coffee beverages, however, reveals remarkable differences. In facts, in addition to an evident difference in the foam volume, particularly abundant in the *Hyper Espresso* coffee brew, the special visual pattern typical of pure *Coffea arabica* foam known as "tiger skin" or "tiger tail" is more pronounced in the traditional *espresso* preparation. In parallel, some solid particles settled at the cup bottom and normally expected for the traditional *espresso* brew (Petracco, 1989), are not present in the *Hyper Espresso* coffee beverages. These observations led to go into the colloidal characteristics of the two coffee beverages. In Figure 5 the *Hyper Espresso* coffee overall concentration (total solids) is reported as a function of cup volume.

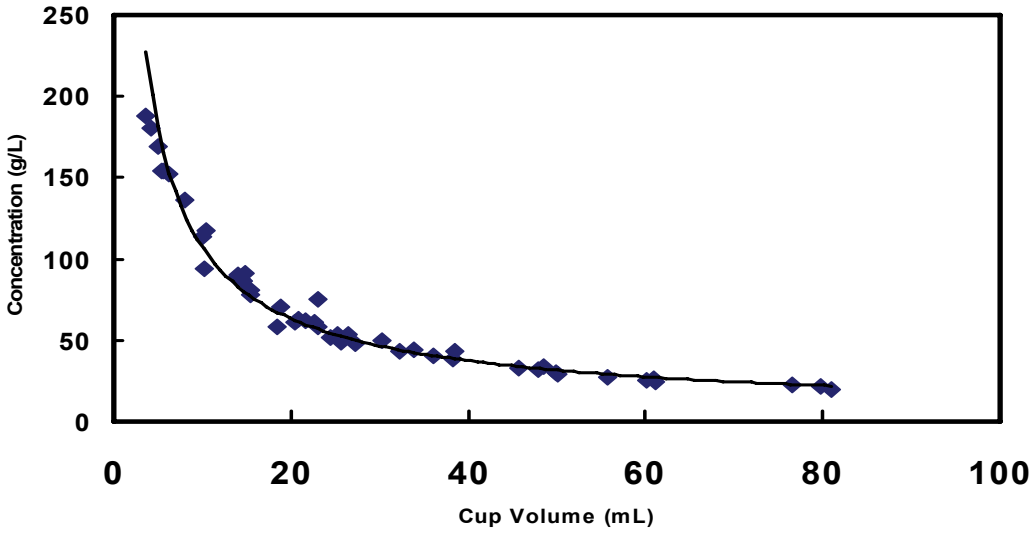


Figure 5. *Hyper Espresso* coffee concentration (total solids) as a function of cup volume.

The experimental data follow a power law ($R^2 = 0.98$) with an exponent equal to -0.76. Very similar trend has been reported for traditional *espresso*, being the exponent slightly different (-0.69) (Navarini et al., 2004). The overall concentration at 30 mL cup volume resulted to be 51.9 g/l and 46.6 g/l for traditional and *Hyper Espresso* coffee, respectively. The *Hyper Espresso* coffee concentration is close to that of a filtrated traditional *espresso*. As a matter of fact, a difference equal to 5.2 mg/mL has been found in comparing total solids and total solids of filtrate of a traditional *espresso* at 30 mL cup volume (Illy and Viani, 2005). The first consequence of changing the coffee cake geometry and of confining this coffee cake in a closed extraction chamber (e.g. the capsule) is to influence the amount of solid particles (floating and settled) in the beverage. In the traditional *espresso* method the energy of the water pressure is spent within the coffee cake (Illy and Viani, 2005) whereas in the *Hyper Espresso* method a part only of the energy involved in the coffee extraction is dissipated within the coffee cake, being the other part, spent in the elastic deformation of the central part of the base of the capsule body. This means that in order to ensure similar brewing time, different grinding level are used and then different coarse to fine particle size ratio, being lower in the traditional *espresso* method.

As far as the traditional *espresso* method is concerned, foam volume was shown to be highly correlated to carbon dioxide content in the roasted and ground coffee powder (Navarini et al., 2006). It has been hypothesized that during traditional *espresso* coffee brewing, the CO_2 water solubilisation at high pressure and temperature lead to a carbon dioxide supersaturation conditions into the beverage. The subsequent temperature and pressure drops causes the sudden release of dissolved gases, manly CO_2, as effervescence, resulting in the build up of the foam layer. The effervescence (and the related foam build up) terminates generally within 10-12 sec after preparation. In the *Hyper Espresso* method the effervescence is still evident at time higher than 12 sec after preparation and the concomitant foam build up results in a higher foam volume. In order to quantitatively compare the two extraction methods, in Table 2, foam volume at different cup volume is reported. For both coffee systems, the measured content of CO_2 is equal to 1.1 ± 0.1 mg/g coffee powder.

Table 2. Foam volume (mL) as a function of cup volume (mL).

Cup Volume (mL)	**Traditional *espresso* Foam Volume (mL)**	***Hyper Espresso* Foam Volume (mL)**
4.0 ± 0.1	1.37	1.98
7.2 ± 0.1	1.96	3.93
10.8 ± 0.1	2.56	5.28
17.9 ± 0.1	3.80	6.95

In the range of examined cup volumes, the *Hyper Espresso* method is more efficient than the traditional one in generating the foam layer. The assembly constituted by the lower internal filter, the perforated elastic central part of the capsule body base and the flow-conveyor create a turbulent environment which contributes in generating foam in excess to that merely derived from the carbon dioxide originally present in the coffee powder. Moreover, the headspace within the hermetically closed *Hyper Espresso* capsule is a source of extra gas phase available for foaming. In agreement with the visual inspection, MLS data revealed differences in the foam colour, particularly at low cup volume. In fact the traditional *espresso* method results in a foam colour very dark at 4 - 7.2 mL cup volumes (diffuse reflectance, R in the range 50 - 70%) whereas the foam colour of the *Hyper Espresso* brews in the same cup volume range is lighter (R higher than 70%) and similar to that of the foam at higher cup volumes. The foam persistency is higher in the *Hyper Espresso* brews, but this topic will be studied in a forthcoming paper.

The role played by the flow-conveyor in foaming has been preliminary assessed by comparing the foam volume measured in the presence and in the absence of this capsule part, at a cup volume close to 15 mL. Without the flow-conveyor a remarkable foam volume reduction has been detected (from 6.7 to 2.7 mL).

As far as the *Hyper Espresso* flow regime is concerned, it is possible to approximately evaluate the ratio of inertial effects to viscous effects, well known as the Reynolds number, Re and defined as:

$$Re = \rho VL / \eta \qquad [2]$$

where ρ and η are the fluid density and viscosity, respectively, V the fluid velocity and L the characteristic length (for flow in pipe this length is the pipe diameter when the cross-section is circular). The approximation mainly resides in the elastic deformation of the perforated central part of the body base during brewing and then in a variable “pipe diameter”. For a

fluid such as water at 90 °C (Perry and Green, 1984) with a velocity in the range 2.6 m/s - 31.8 m/s (assuming a flow equal to 1 mL/s and L in the range $2 \cdot 10^{-4}$ - $7 \cdot 10^{-4}$ m) the calculation leads to Re in the range 5580-19480. This range indicates that fully turbulent flow occurs during coffee preparation (Re > 3000). Under these conditions the flow is dominated by inertial effects producing random eddies, vortices and other flow fluctuations. Moreover a shear rate of the order 10^3 - 10^5 s^{-1} can be estimated (Skurtys & Aguilera, 2008).

In order to better understand the physics involved in the *Hyper Espresso* method, sunflower oil and ultrapure water, in variable amount, have been put in empty capsules subsequently closed. The capsules have been used to mimic *Hyper Espresso* coffee preparation and the resulting emulsions have been characterized. In other words, the capsule has been used as an emulsification chamber.

In Table 3, determined volume fraction, Φ, and diffuse reflectance, R, (in %) are reported.

Table 3. Oil-in-Water emulsions prepared by using *Hyper Espresso* capsule and characterized by MLS.

Volume fraction, Φ	Diffuse reflectance, R (%)
0.020	11.0
0.037	14.6
0.043	27.0
0.105	42.6

On the basis of determined R values, the droplet mean diameter, d, has been estimated (Abismail et al., 2000; Mengual et al., 1999; Bru et al., 2004). Depending on scatterers size and according to the theory, two different estimations may be obtained: d = 0.10 - 0.12 μm or d = 15 - 20 μm.

Lower d range is consistent with the optical microscopy observations and with the remarkable system stability observed (in spite of no added surfactants, several days at room temperature are not sufficient for complete phase separation).

According to Kolmogorov (1949) and Hinze (1955) in the emulsification process in turbulent flow (turbulent isotropic regime), two different regimes should be distinguished: turbulent inertial and turbulent viscous regimes, respectively. In the turbulent inertial regime, the maximum diameter, d_{max}, of the stable oil droplets, (those able to resist the disruptive forces of the flow) is related to the rate of energy dissipation, ε (which characterizes the intensity of the turbulent flow and is expressed as J/kg s or W/kg) and to the interfacial tension of the oil droplets, σ. According to Vankova et al (2007):

$$d_{max} = A\, \varepsilon^{-2/5}\, \sigma^{3/5} \rho_c^{-3/5} \qquad [3]$$

where, A is a constant of proportionality of the order of unity and ρ_c is the density of the continuous phase.

According to Kolmogorov's approach, the rate of energy dissipation per unit mass of the fluid (or average power density in the emulsification chamber) is defined as:

$$\varepsilon = pQ / \rho_c\, Vdiss \qquad [4]$$

where p is the applied pressure difference along the emulsification chamber, Q is the flow rate during emulsification and Vdiss is the effective volume in which the main turbulent dissipation of energy takes place. Differently from well know geometries like narrow gap emulsifiers and homogenizers described in literature (Vankova et al., 2007, Dickinson, 1992, Haque and Kinsella, 1989; Kowalewski et al., 2006, Tcholakova et al., 2003), the calculation of Vdiss of the *Hyper Espresso* capsule is not straightforward, since the system is not rigid. However, it may be hypothesized that the volume below the white central part of the capsule body base and the central part of the flow conveyor (see Figure 1B) is the space where the relevant part of energy dissipation occurs. Although the geometry of this space during coffee extraction has not yet been determined, this volume may by preliminary estimated to be in between $2.65 \cdot 10^{-8}$ and $2.65 \cdot 10^{-9}$ m^3. By assuming p equal to $6 \cdot 10^5$ Pa and Q equal to $8.8 \cdot 10^{-6}$ m^3/s and ρ_c the density of water at 90°C, it is possible to calculate ε in the range $2.0 \cdot 10^5$ to $2.0 \cdot 10^6$ J/kg s. By resorting to eq. 3 (σ equal to 5 mN/m (Walstra, 1987) a d_{max} in the range from 5.0 (low ε) to 2.0 μm (high ε) may be calculated.

These values, particularly that calculated by using high energy dissipation rate, are consistent with those experimentally determined through MLS and optical microscopy.

As far as traditional *espresso* coffee is concerned, a very high number of droplets with diameter below 0.5 μm has been reported to characterize a high body *espresso* brew in comparison with that of a low body one (Petracco, 1989). In view of the observed *Hyper Espresso* capsule emulsifying performance, it may be speculated that during *Hyper Espresso* coffee preparation, the emulsion formed by coffee oil (and stabilized by coffee natural surfactants) could be characterized by droplets size distribution compatible with a mouthfeel perception higher than that of a high body traditional *espresso* brew.

CONCLUSION

The present paper is the first attempt to compare the traditional *espresso* method and the new *Hyper Espresso* method from the beverage characteristics point of view. Some relevant chemical compounds have been selected to compare the extraction performances. In a wide range of cup volumes and independently on adopted sampling procedure, the experimental data showed that no significant differences can be traced by comparing the two *espresso* methods. However, the two methods permit to prepare coffee beverages differing from a colloidal point of view. By changing the coffee cake geometry and by confining this coffee cake in closed extraction chamber with excellent emulsifying performances it is possible to influence the different dispersed phases present in the *espresso* brew. Solids particles (floating and settled) are quantitatively lower in *Hyper Espresso* coffee beverage whereas the foam volume is quantitatively lower in the traditional *espresso*. The coffee oil droplets size distribution of *Hyper Espresso* coffee beverage should be remarkably different from that of traditional *espresso* brew, however, in the lack of further experimental evidences, this is just a speculation. The observed differences in the colloidal state of the two beverages are believed to remarkably influence the sensory properties other than visual aspect, and this will be the topic for further investigation.

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DOE Approach in Sensory Studies†

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SUMMARY

The sensory properties optimization, represent an inescapable step during the development of new food product. This step, however, is a complex process in which several factors are involved, including ingredients, physical properties, environmental conditions etc. By adopting the traditional "try and error" approach, a huge number of trials are necessary to achieve the target in terms of sensory properties; however, by using this approach, the influence of each single factor and the mutual interactions among the different factors on the final result, are not taken into consideration. Design of Experiment (DOE) is a technique widely used in many fields and it consists of a systematic variation of some input parameters (factors) and the observation of changes in a set of output variables (responses). In our study the effects of three controlled ingredients and one environmental factor on sensory properties of a coffee-based beverage model system, have been evaluated. These formulations and environmental factors were investigated at two or three levels each; the examined factors were: (1) amount of coffee extract, (2) amount of sugar, (3) texture of the beverage and (4) temperature of consumption. A trained panel provided intensity evaluations for typical tastes (sweet, bitter and sour) as well as aromatic and mouth-feel attributes. Fitting of the experimental data with a PLS regression allows to obtain a model able to explain the relative importance of the considered factors and how they influence the sensory properties of the model system; moreover, the model results to be satisfactorily predictive of the sensory

INTRODUCTION

Coffee is one of the most popular beverages around the world, however the term *coffee* may be referred to a huge variety of preparations with different physical, chemical and organoleptic characteristics.

In traditional preparations (domestic or professional) consumers can decide the type of preparation, the variety, origin and dose of coffee to use according to their own taste. In addition, they can decide whether to add milk, sugar (Narain et al., 2004) and to consume it at a certain temperature.

The consumption of a ready-to-drink (RTD) coffee is something different. The consumer is faced with a product that should ideally be drunk "as it is", in which all the ingredients are already contained in the beverage (Petracco, 2001).

Understanding the organoleptic characteristics desired by consumers and subsequently concocting a product to fit these characteristics are key elements in a product development step (Van Kleef et al., 2005).

Design Of Experiment (DOE) is a technique which allows to study a phenomenon through a carefully selected set of experiment, in which all relevant variables (called factors) are varied simultaneously.

The aim of this study was to analyse a type of coffee-based beverage as the model system: by DOE approach (Eriksson, 2000), the variations in taste caused by different coffee doses, the amount of added sugar, consumption temperature and the presence of a thickening agent have been investigated. Factors and their levels were chosen so as to mimic a range of plausible coffee-based RTD formulations.

To understand how the ingredients used in the formulation are involved in the determination of the organoleptic properties, the contribution of different factors has been examined and response surfaces for each sensory descriptor used in the evaluation of samples have been generated.

The first factor examined has been the amount of coffee exctract (express in grams of solids per litre of beverage). Three levels have been considered: 6,0 g/l, 10 g/l and 16 g/l.

The second factor has been the amount of sugar added. A wide range of consumers usually add sugar to their coffee (sucrose or intensive sweeteners), in order to modify the original taste of the beverage (Keast, 2008; Schiffman, 1985; Schifman, 2000). The influence of sugar over the sensory profile was studied by using 4 levels of sucrose added, starting from none (a black coffee) up to 75 g/l.

It was also decided to evaluate the influence of a thickening agent on sensory properties: it is well-known that a change in the rheological properties of a beverage does not influence only its textural properties (mouthfeel), but also the taste and flavour perception (Pangborn et al., 1973; Pangborn and Szczesniak, 1974; Kokini et al., 1982; Christensen, 1984; Cliff and Noble, 1990; Calvino et al., 1993).

The role of the thickening agent (Guar gum) was examined using two levels: absent or in a concentration of 0.09%.

The last factor analyzed was the temperature at which beverages were tested. Three different levels were analyzed; a typical temperature of a beverage kept in a refrigerator (4 °C), at room temperature (25 °C) and warm coffee (60 °C).

A full factorial design was created starting from these factors, for a total of 75 samples (72 possible combinations plus 3 replicates of a sample chosen as the centre point).

Table 1 reports the *factors* examined and *levels* studied.

Table 1. Factors studied and their levels.

Factor	Abbreviation	Unit of measurement	Levels
Coffee content	Cof	grams of soluble solids per litre of beverage	6; 10; 16
Sugar Content	Sug	grams of sucrose per litre of beverage	0; 20; 40; 75
Temperature	Temp	Celsius degrees	4; 25; 60
Texturizer	Tex	grams of Guar gum per litre of beverage	0; 0,009

EXPERIMENTAL

Materials and methods

Coffee extract was obtained starting from a single batch of roasted and ground pure *Coffea arabica* coffee blend produced by illycaffè S.p.A. (Trieste, Italy) in roasting grade N (medium). Coffee extract was prepared using a continuous counter-current extractor operating at atmospheric pressure.

Samples were prepared by diluting coffee extract with fresh deionized water in order to obtain beverages with the requested coffee solids content. Concentration of coffee solids in beverages was measured using a calibrated refractometer. Samples, prepared the same day of test, were kept at room temperature (25 °C), or stored in a refrigerator (T = 4 °C) whereas samples served at 60 °C were warmed up by using a microwave oven.

Guar gum (Cps 3500 – 200 Mesh) (Giusto Faravelli SpA, ITALY) and commercial crystalline sucrose were used.

Coffees were evaluated by judges in 12 sessions: in each session 6 (or 7) samples were described; each sample consisted of about 30 ml of beverage. Samples were presented in a white plastic glass coded with a 3 digit number.

A panel of 12 trained judges created the samples profile using Quantitative Descriptive Analysis (Meilgaard, 1999). Panellists were preliminary selected using rules described in the ASTM Special Technical Publication 758 "Guidelines for the Selection and Training of Sensory Panel Members"; moreover all panellists had received a specific training on the sensory evaluation of coffee beverages. In order to avoid a non-uniform response to bitterness of different samples. All panellists involved in this study were PROP tasters (Tepper, 2001).

Judges' performance were evaluated on the basis of their repeatability in the evaluation of a sample tested four times (10,0 g/l of coffee solids, 20 g/l of sucrose, temperature of 25 °C and without thickening agent).

A dedicated room built in compliance with the ISO standard (ISO 8589: Sensory analysis -- General guidance for the design of test rooms) was used.

A fully randomized presentation order has been modified in order to allow a sample sequence in which a warm sample could not follow a cold one (and vice versa).

Attributes used in the evaluation form were a series of *taste attributes* and *flavour attributes* developed on the base of ICO manual for Sensory Evaluation of coffee. For each attribute, the perceived intensity was scored on an unstructured 10,5 cm line with semantic anchors at the ends (none – very intense) and at intermediate points of scale (barely perceivable at 15% of scale, weak at 30%, medium at 50% and intense at 75%). Scores on line were then converted in a 1-9 scale.

Evaluation forms were created using FIZZ FORMS software (version 2.30B, BIOSYSTEMES, Couternon, France).

Software used in the elaboration data were MODDE (version 8.0, UMETRICS, Kinnelon, USA) and XLSTAT (XLSTAT version 2006, ADDINSOFT, NY, USA) and PANELCHECK

to measure panellists performance (PANELCHECK version 1.2.1, MATFORSK AS, Norway).

RESULTS AND DISCUSSION

Raw data from panellists was collected and a preliminary analysis was carried out in order to assess the panel performance. After this evaluation, 2 panellists were excluded from the final computation because their performances were not satisfying.

A further analysis was carried out on the attributes used in the form. This was done in order to exclude an attribute if it was not discriminating or if judges were not able to evaluate its intensity in an uniform way (significant judge effect; $p < 0.01$).

With these criteria, attributes used in data analysis were *taste attributes* (acidity, bitterness and sweetness) and *flavour attributes* (coffee note intensity, global aroma intensity, chocolate note and caramel note).

After these steps, the data from panel were aggregated and average scores have been used for the computation of the surface response model.

Table 2. Raw data descriptive statistics.

	Acidity	Bitterness	Sweetness	Coffee Note	Global Aroma	Chocolate	Caramel
Min	1,39	1,06	1,35	2,21	1,43	1,33	1,00
Max	7,25	7,22	8,15	7,32	7,50	4,66	4,50
Mean	3,59	3,58	4,11	4,59	5,44	2,93	2,57
Q(25%)	2,64	2,34	2,15	3,15	4,57	2,40	2,00
Q(75%)	4,28	4,48	6,30	5,86	6,50	3,37	3,20
Median	3,45	3,47	3,76	4,69	5,54	2,80	2,50
Std. Dev.	1,26	1,53	2,14	1,42	1,40	0,74	0,86
Min/Max	0,19	0,15	0,17	0,30	0,19	0,29	0,22
Number of observations	75	75	75	75	75	75	75

Regression model adopted was a linear model with interaction among factors: a quadratic model could not be adopted because factor *texture* was studied in 2 levels only. Moreover, sensory response to sucrose in water is linear within the concentration limit of 150 g/l (Schiffman et al., 1991); the perceived bitterness of caffeine also shows a linear response in the range of concentration used in this study (Drewnowski, 2001).

The model has been generated using a PLS regression with 3 components. The number of components was chosen using cross validation rules (Eriksson et al., 2000).

In Table 3, in which R^2, Q^2 and R^2 adjusted are reported for each component of the regression model, it is possible to examine the *fitting goodness* achieved with the generated model. R^2 is the correlation *coefficient*, giving an estimation about *goodness of fit*: it is a measurement of how well the regression model fits the raw data. Q^2 (based on the Prediction Residual Sum of Squares) is the *goodness* of the prediction, and estimates the predictive ability of the model. Commonly, a model has a good predictive ability when Q^2 is greater then 0,5 (Eriksson, 2000). R^2 adjusted is the response variation fraction explained by the model, adjusted by degrees of freedom.

Table 3. Goodness of fit.

		Comp 1	Comp 2	Comp 3
Total	R2	0,553	0,758	0,770
	R2adj	0,483	0,720	0,734
	Q2	0,505	0,705	0,709
Acidity	R2	0,767	0,771	0,774
	R2adj	0,730	0,735	0,738
	Q2	0,701	0,701	0,703
Bitterness	R2	0,741	0,803	0,808
	R2adj	0,701	0,772	0,778
	Q2	0,667	0,738	0,741
Sweetness	R2	0,576	0,855	0,865
	R2adj	0,510	0,833	0,844
	Q2	0,526	0,816	0,821
Coffee Note	R2	0,539	0,773	0,787
	R2adj	0,467	0,737	0,753
	Q2	0,526	0,741	0,741
Global Aroma	R2	0,178	0,743	0,745
	R2adj	0,050	0,703	0,706
	Q2	0,168	0,677	0,677
Chocolate	R2	0,640	0,697	0,701
	R2adj	0,584	0,650	0,654
	Q2	0,592	0,637	0,637
Caramel	R2	0,427	0,662	0,710
	R2adj	0,337	0,610	0,665
	Q2	0,355	0,582	0,616

The model generated using PLS regression is able to fit all attributes with a good approximation: all correlation coefficients are greater than 0,7; the predictive ability of the model is also satisfactory because the Q^2 index is greater than 0,6.

Once the model *goodness of fit* is verified, it is possible to proceed with the evaluation of the contribution of each factors examined in the sensory profile of the sample. Contribution of each factor can be evaluated by investigating the model coefficients; in Table 4 scaled and centred coefficients for each response are represented: in bold those with a statistical relevance for the model.

Figure 1 shows the sucrose impact on all sensory aspects investigated: its effect is not limited to a modification of taste attributes (Keast, 2002) but is also extended to a change in the perceived aroma. Similar effect has already been reported for other systems such as yoghurt or flavoured beverages (King, 2003; Hewson, 2008). The role played by sucrose is unequivocal respect to bitterness and acidity whereas its effect on aroma is more ambiguous because it enhances some notes (caramel) and reduces others like coffee and chocolate notes.

The model obtained for acidity is characterized by 3 terms: the concentration of coffee, the amount of sugar and their interaction. It is easy to understand the contribution of coffee (Woodman, 1985): it is the only source of acids in the model system and therefore perception of acidity in the beverage increases with coffee content. The sucrose plays a role of antagonist to coffee in the perception of acidity: by adding sucrose we obtain an immediate reduction of

perceived acidity, even if the pH doesn't change. Influence of this masking effect is not constant among different levels of sucrose: the effect is greater when the amount of sugar is greater (see Figure 1).

Table 4. Scaled and centred coefficients; in bold coefficients significant for the model ($p < 0.05$).

	Coeff. SC: Acidity	Coeff. SC: Bitterness	Coeff. SC: Sweetness	Coeff. SC: Coffee Note	Coeff. SC: Global Aroma	Coeff. SC: Chocolate	Coeff. SC: Caramel
Constant	**3,582**	**3,585**	**4,097**	**4,587**	**5,425**	**2,929**	**2,565**
Cof	**0,804**	**0,639**	**-0,341**	**1,214**	**1,114**	**0,512**	**-0,173**
Sug	**-0,704**	**-1,172**	**1,916**	**-0,218**	**0,334**	**-0,288**	**0,654**
Temp	-0,119	-0,151	**0,215**	-0,010	-0,025	-0,072	-0,050
Tex	0,005	-0,012	0,036	0,009	0,035	0,009	0,037
Cof*Sug	**-0,168**	-0,104	0,023	-0,126	**-0,260**	**-0,133**	**-0,208**
Cof*Temp	0,004	0,034	-0,080	-0,030	-0,057	-0,006	-0,032
Cof*Tex	0,052	0,019	0,031	0,071	0,112	0,043	0,058
Sug*Temp	0,029	0,067	-0,132	-0,041	-0,058	0,010	-0,010
Sug*Tex	-0,037	-0,081	0,151	0,008	0,058	-0,009	0,061
Temp*Tex	-0,033	-0,045	0,069	0,002	0,001	-0,019	-0,011

Others factors involved in this study do not seem to give their contribution to sensory profile of coffee. Temperature only seems to influence the acidity by reducing its intensity perception, but this influence is not statistically relevant.

In Figure 2 a graphic representation of acidic intensity with regards to coffee and sugar concentration is reported: this kind of visual results is easy to understand and gives an immediately idea about the factor influences on a response (in this graph, levels for Temperature and texturizer were respectively 25 °C and 0,009 g/l).

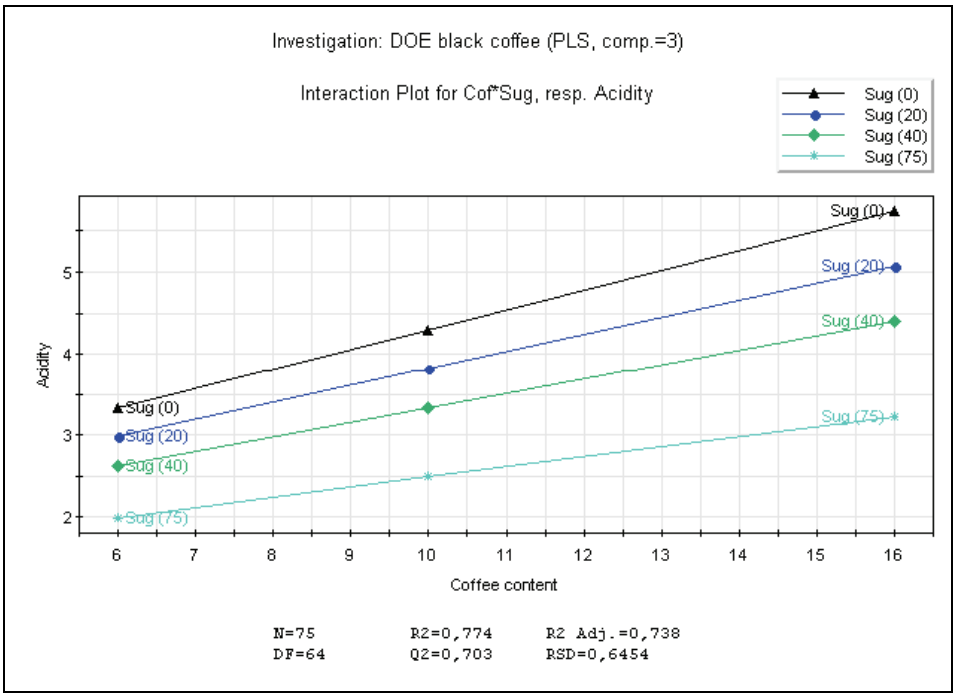

Figure 1. Coffee-sucrose interaction plot for acidity perceived.

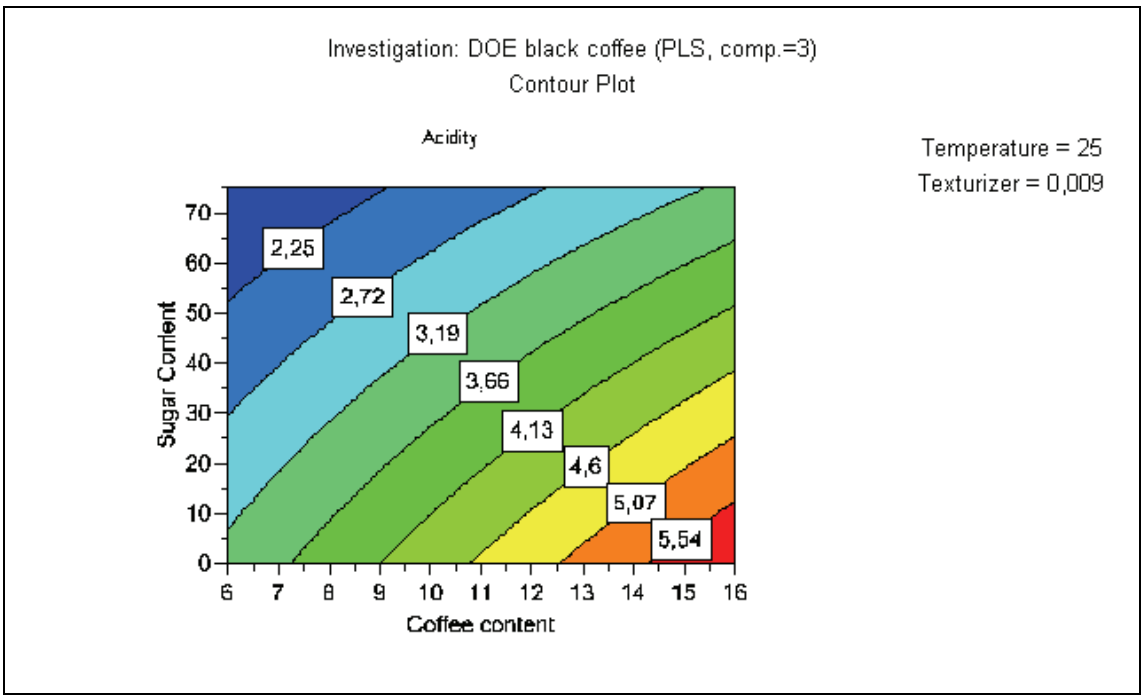

Figure 2. Acidity: contour plot.

The bitterness model has only two statistically relevant factors: the concentration of coffee and the amount of added sugar. Sugar addition reduces the bitterness perception in the sample and, more interesting, its effect is stronger than the effect showed in the acidity perception.

This fact is relevant since it allows us to understand that a cup of coffee with a certain acidity-bitterness balance changes this equilibrium once sweetened.

In the Figure 3 the influence of sucrose on the bitterness perception at different coffee concentrations is reported. Contour levels are more horizontal than in acidity contour plot because the effect of sucrose on bitterness is stronger. Lines are also less curved because coffee*sugar interaction term is not relevant.

Sweetness is clearly linked to the amount of sucrose present in the coffee and it is largely determined by this factor; however sweetness is also affected by the quantity of coffee and, more relevant, by the temperature of consumption.

Coffee reduces the perception of sweetness in the beverage like the sucrose influences the bitterness perception. Probably both perceptive and cognitive mechanisms are responsible for these influences.

More interesting is the effect of temperature. The lower the temperature of consumption is, the lower the sweetness perceived in agreement with Talavera (2005).

The effect of temperature is clearly visible in the Figure 4: a temperature increase leads to a stronger sweetness perception; this effect seems to be more relevant at low concentrations of sucrose (levels of coffee and texturizer have been chosen arbitrarily).

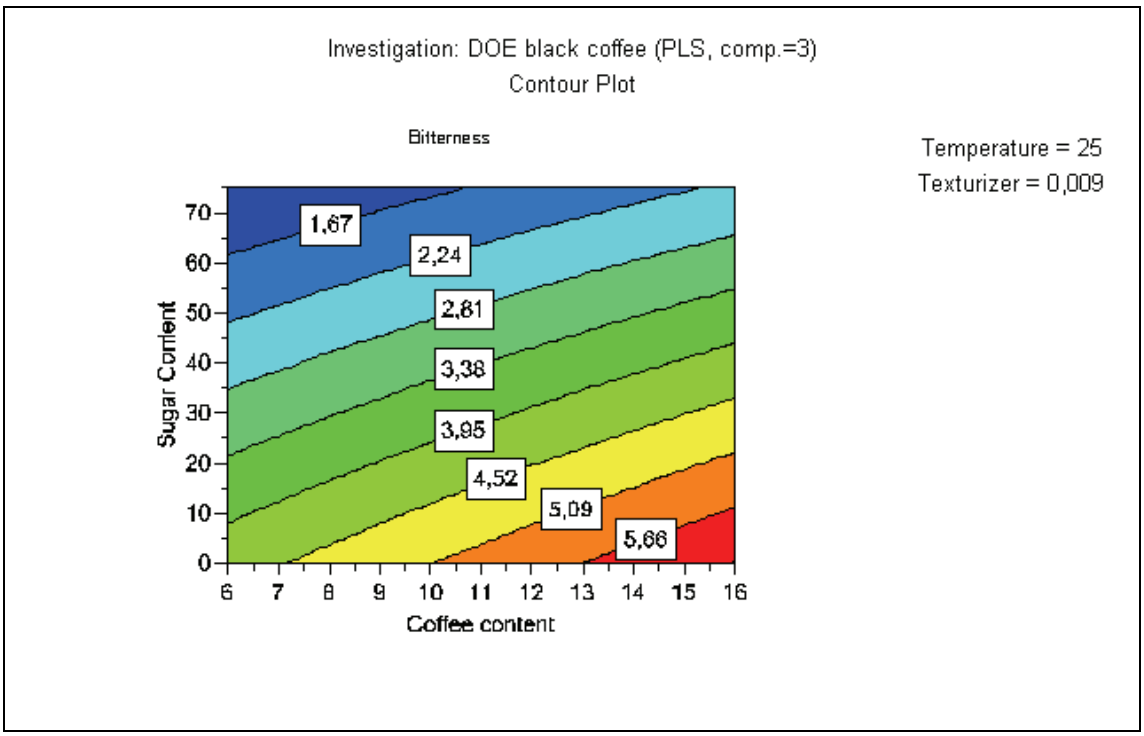

Figure 3. Bitterness: contour plot.

This fact strongly suggests that sample temperatures have to be carefully controlled. Moreover in a consumer test environmental, conditions should be controlled since an incorrect temperature of consumption could lead consumers to prefer a product on the basis of an altered sweetness perception.

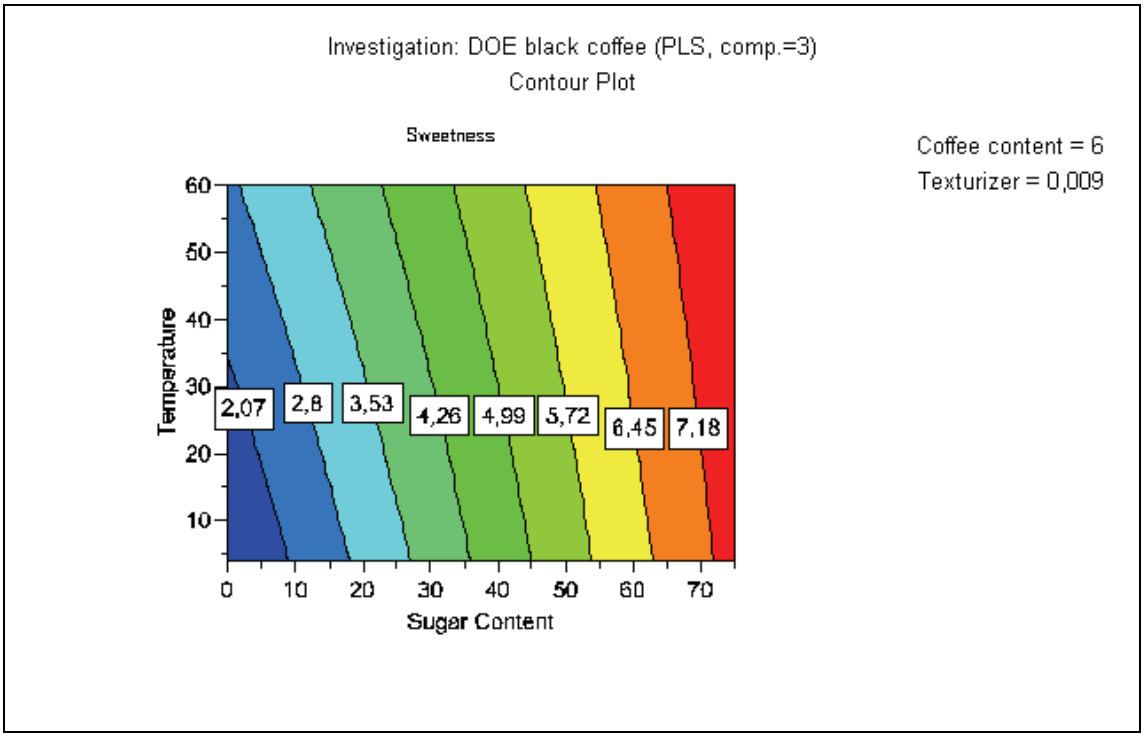

Figure 4. Sweetness: c ontour plot.

The flavour attributes studied were the coffee note, global aroma, chocolate and caramel (see Table 3). The coffee note is characterized by two factors: coffee concentration and sugar amount.

The coffee note is obviously strongly related to the coffee concentration: sugar is able to modulate its intensity with a limited reduction; PLS regression did not point out any significant interaction among factors nor contribute to the model from the factors examined.
For other three attributes examined, however, there is a significant interaction between coffee content and sugar.

Starting from the global aroma attribute, Table 3 shows that it is mainly related to the amount of coffee: it is obvious because, in the model studied, the coffee was the only ingredient with aromatic compounds. Sucrose however plays a role also in the global aroma perception because it increases when sucrose is added to the beverage.

The effect, however, is not uniform (for this reason the interaction term Cof*Sug is statistically significant): at low coffee concentration the presence of sugar brings an increase of global aroma perceived. Whereas at high coffee concentrations, the contribution of sucrose to global aroma does not seem relevant (Figure 5).

The chocolate attribute shows a different behavior: at low coffee concentrations the influence of sucrose is limited (addition of sucrose leads to a decrease of chocolate note perceived) while at high coffee concentrations becomes relevant. Also, the chocolate attribute is related to coffee concentration in the beverage.

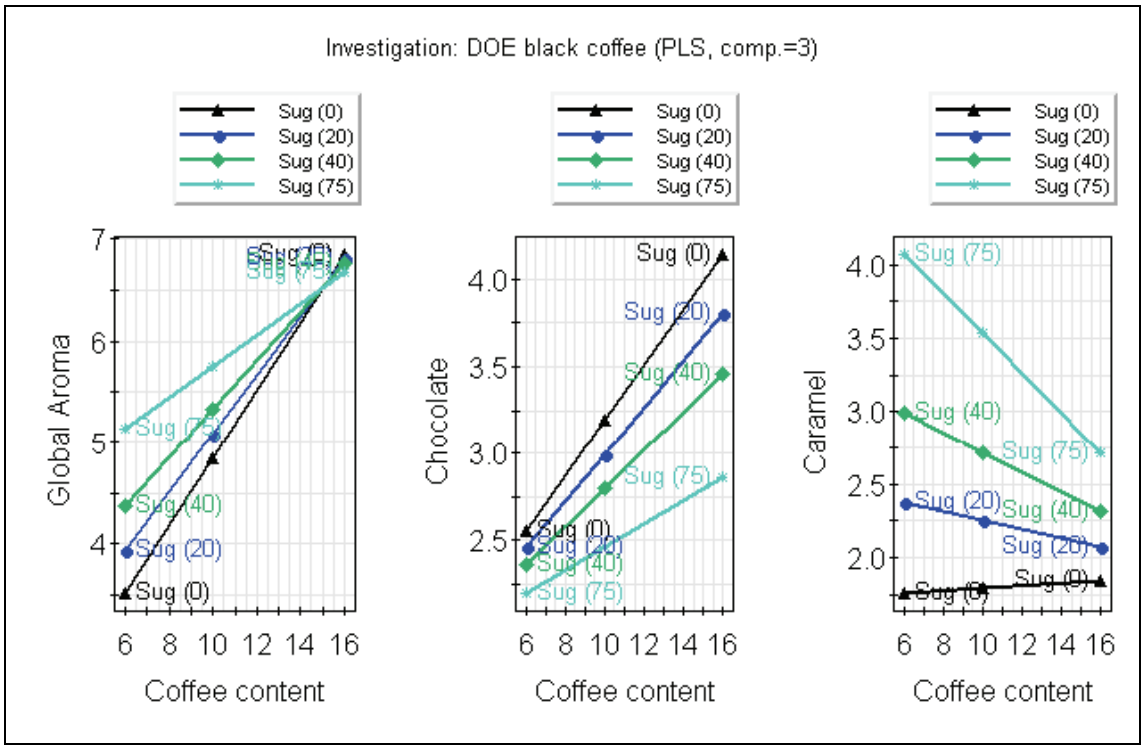

Figure 5. Coffee*Sucrose interaction for attributes Global Aroma, Chocolate and Caramel.

The last response examined was the caramel note: it is strongly related to the amount of sugar and inversely related to the concentration of coffee. There is however a strong interaction between coffee and sugar: in the samples without sucrose the caramel note increases when

coffee concentration change from 6 g/l to 16 g/l. While in samples with sucrose, the caramel note decreases when the coffee concentration is increased.

This fact is relevant because it shows how a non-aromatic compound, like sucrose, is able to modulate a range of flavour attributes (as well as taste); for this reason it is important to adopt an holistic approach in sensory evaluation. Techniques, like the one used in this study, allow to study the influence of each factor investigated on the overall sensory profile.

CONCLUSIONS

In the present study, a predictive model for the sensory properties of coffee-based beverage, based on sensory data, has been developed.

Sensory data used for the computation were consistent. The model obtained allows a good fitting for most important sensory characteristics, and its predictive ability has been found good enough to enable an estimation of sensory profile of new recipes

Another possible application is the opportunity to find out a range of possible formulations staring from a target sensory profile. In this way it is immediately clear if a desired combination of bitterness, sweetness and aromatic notes can be achieved or not by using a certain recipe.

Among factors studied, sucrose appeared particularly interesting because it affects the perception of acidity and bitterness as well as flavour notes.

Sucrose is able to reduce the acidity and bitterness perceived by judges and its effect is clearly noticeable either at low or high coffee concentration.

Sucrose has been found to be responsible for enhancing Global Aroma and Caramel note perceived at low coffee concentration.

Temperature effect is relevant for the perception of sweetness (Talavera et. al., 2005): sweetness perception increases when temperature rises from 4 °C to 60 °C.

The influence of the thickening agent on the organoleptic properties was not clearly perceivable: probably the level chosen in this study has been too slight in comparison to other factors examined and its influence should be investigated using a wider range of concentrations.

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Comparison of Different Extraction Methods to the Identification of Soluble Coffee Volatile Compounds

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SUMMARY

Gas chromatography coupled with mass spectrometry (GC-MS) can be cited as an important analytical tool when researching foodstuffs aroma profile. However, its success is dependent on a suitable sample preparation. The objective of this work was to compare five analytical extraction methods of soluble coffee volatile compounds: solvent extraction, simultaneous distillation-extraction (SDE), static headspace, dynamic headspace (purge-and-trap) and headspace solid-phase microextraction (HS-SPME). The mass spectrum library (NIST/02) was used in the volatile compounds qualification, quantification done by GC-MS, using external standards. Amongst the methods evaluated in this work, HS-SPME with a DVB/CAR/PDMS (bipolar) fiber provided the most representative aromatic profile. According to the presented results, it was concluded that this method can be conveniently used to characterize soluble coffees aroma profiles, with high sensitivity and repeatability.

INTRODUCTION

Sample preparation and introduction for gas chromatography-mass spectrometry (GC/MS) analysis are critical for successful analyses (Poliak et al., 2006).

The performance of GC/MS technique depends significantly on a homogeneous sample preparation stage, and samples must not be directly analyzed without previous treatment, as they may contain artifacts capable of masking the real values achieved. In order to avoid such problem, sample extraction and preparation procedures are used to concentrate compounds up to an adequate purity level, hence not compromising both chemical analysis result and equipment integrity.

Solvent extraction and concentration methods had been widely used to recover aromatic compounds in foods. However, low levels of these, combined with the high content of co-extracted matrix compounds, restrict its direct application.

The purge-and-trap technique involves the passing of a carrier gas through a liquid sample, followed by trapping of the analytes in an adsorbent material and desorption onto a GC, being used to analyze extremely low concentrations (ppb and ppt) of volatile organic compounds in aqueous matrices (Snow and Slack, 2002).

Solid-phase microextraction (SPME) is a modern, solvent-free sample preparation technique, commonly used in trace analysis. This technique has been developed to combine sampling and sample preparation in one step (Wardencki et al., 2004).

Some authors (Akiyama et al., 2003) described a SPME method and GC-FID/O analyzing volatile compounds released during the grinding of roasted coffee beans, with results comparable to the achieved by conventional techniques.

The objective of this work was to compare five analytical extraction methods of volatile compounds in soluble coffee samples using GC-MS: solvent extraction, SDE, static headspace, dynamic headspace and HS-SPME.

MATERIALS AND METHODS

Materials - Soluble coffee samples

The soluble coffee samples were supplied by Café Iguaçu.

Methods - Solvent Extraction

A 2.80 g of soluble coffee sample, 0.60 g of sodium chloride (anti-foaming agent) and 100 mL of pure water (Millipore water) were placed in 500mL round-bottom flask. The essential oil separator was connected with a reflux condenser and to a 500 mL round-bottom flask. A volume of 10 mL of water and 20 mL of solvent (diethyl ether / pentane 1:1) are placed in the essential oil separator. The flask was kept in an oil bath, heated above 140 °C and refluxed for more than 3 hours. Volatile organic compounds were distilled with water and extracted into the organic layer of the separator during distillation, being the temperature into the reflux condenser kept at 15 °C. After the distillation period, the organic layer was separated, washing the separator four times with 2 mL portions of diethyl ether. The organic layer combined with diethyl ether washings were dried for 2 hours, using anhydrous sodium sulfate. The solvent was evaporated at 85 °C in a heart shaped flask until a very small volume. The remainder was transferred to an automatic sampler (ALS) 2 mL vial and filled up to 0.5 mL with acetone.

Simultaneous Distillation-Extraction (SDE)

The sample preparation was identical to the described above, except the amount of water used of 200 mL. A 500 mL round flask with the sample was connected to one device connector. The flat-bottom flask with 50 mL of solvent (dichloromethane) was connected of the other connector (without heating). The flask with sample was kept at constant temperature (65 °C) under 80 Kpa vacuum for 2 hours. Temperature into the condensing system was kept at -6 °C. The organic layer was dried for 2 hours using anhydrous sodium sulfate. The concentration of the volatile compounds was identical to the solvent extraction (described previously).

Static Headspace

A 2.8 grams of soluble coffee sample were directly weighted in a 20 mL vial, closed immediately after with silicon septum, so that the composition present in each sample was kept unchanged. An automatic headspace sampler Agilent model 7694-E was used. The analysis conditions were as follows: carrier gas pressure 8.3 psi; vial pressure, 18.80 psi; sampling loop, 3 mL; vial temperature, 70 °C; sampling loop temperature, 100 °C; transfer line temperature, 120 °C; vial pressurization time, 0.20 min; injection time, 1.00 min; extraction time, 30 min.

Dynamic headspace (Purge-and-Trap)

Dynamic headspace targets the concentration of aroma compounds, using for this a trapping device with an adsorbent material (Tenax 150mg) followed then by desorption directly onto a GC. Sample preparation was identical to the static headspace sample preparation.

The vials were kept closed in a thermostatic oil bath at constant temperature of 60 °C. After reaching the equilibrium temperature into the vials, the analysis were carried in an enrichment desorption unit (EDU) by Airsense Analytics, under the following conditions: sampling time, 120 sec; sampling temperature, 35 °C; desorption time, 120 sec; desorption temperature, 230 °C; injection time, 60 sec; injection temperature, 230 °C; cleaning time, 90 sec; cleaning temperature, 250 °C; cooling time, 240 sec. After sampling, the adsorbent material was heated for thermal desorption of compounds and directly transferred to the chromatographic column.

Headspace solid-phase microextraction (HS-SPME)

Both SPME fibers and the manual holder were purchased from Supelco Co. (Bellefonte, PA). The following types of SPME fibers were evaluated: poly (dimethylsiloxane) (PDMS) with 100 μm thickness; divinylbenzene/carboxen/PDMS (DVB/CAR/PDMS) with 50/30 μm thickness; (PDMS/DVB) with 65 μm thickness and Carbowax/DVB (CW/DVB) with 70 μm thickness. Before headspace sampling, the SPME fiber was reconditioned according to the SPME data sheet (T794123M, Supelco Co., Bellefonte. The extraction was carried according to Bassoli, 2006.

Gas Chromatographic with Mass Spectrometry Detector (GC-MS)

A 6890N gas chromatograph coupled to a 5973 selective mass detector (Agilent) was used as described in Viegas and Bassoli, 2007, except in static and dynamic headspace, that the injector operated in split mode (split ratio 5:1). The quantitative determination used 39 external standards considered of aromatic impact in the consulted literature (Akiyama et al., 2003 and Schenker, 2000).

RESULTS AND DISCUSSION

The results achieved using SDE were worse than solvent extraction performance for the majority of evaluated compounds (Table 1). Compounds cited in literature (Akiyama *et. al*., 2003 and Schenker, 2000) as being important components for the aromatic quality in coffee, such as: pyrazine, methional, vanillin and isoeugenol, could not be detected by SDE. For the presented results, it can be seen that both solvent and SDE extraction methods had not presented satisfactory extraction performance.

The dynamic headspace method showed better extraction performance than static headspace. This result is in accordance with the literature (Amstalden et al., 2001) which reports that some important aromatic coffee compounds such as 2-furfurylthiol, methional and vanillin could not be detected by static headspace.

Comparing the different SPME fibers, it was observed a great difference on extraction sensitivity, being that the use of a PDMS fiber did not provide the desired effects because of its low efficiency in relation to polar compounds; this is in accordance with the literature (Bicchi et al., 2002 and Wardencki et al., 2004).

Table 1. Volatile compounds quantified (ppm)* for the five methods evaluated.

	Solvent		Headspace		Fibers - SPME			
Compounds	**Solvent extraction**	**SDE**	**Static headspace**	**Dynamic headspace**	**DVB/CAR/PDMS**	**CW/DVB**	**PDMS/DVB**	**PDMS**
2,3,5,6- tetra-methyl-pyridine	0,08	0	0	0,43	0,84	0,11	0,21	0,08
2-acetyl-3,5-dimethylpyrazine	0,09	0	0	0	0,64	0	0,1	0
5-methylfurfural	0,09	0	0,78	7,04	210,22	12,05	19,47	0
4-vinyl-guaiacol	0	0,79	0	5,88	51,82	39,88	37,92	3,82
acetoin	2,07	0	0	77,75	5,36	0,8	0	0
benzyl alcohol	0,39	0,04	0	0,04	0,95	0,42	1,33	0
phenyl-ethyl alcohol	1,4	0	0	1,11	3,8	1,7	1,38	0
furfuryl acetate	1,39	0	1,26	37,21	145,41	19,4	28,89	0
maltol	0,38	0	0	0	260,97	132,41	108,58	8,82
benzaldehyde	0,41	0	0	7,1	14,33	2,27	2,42	0,56
furfural	3,96	0	14,49	672,22	474,66	115,47	72	0
2-acetyl-pyridine	0,31	0	0	1,17	5,03	0,5	0,84	0,16
pyridine	2,77	0,27	6,06	101,56	48,78	1	2,38	0,78
4,5-methylthiazole	0	0,09	0	0,84	0,84	0,08	0,17	0,07
isovaleric acid	0,79	0,25	1,56	51,66	42,96	23,23	8,59	0,98
furaneol	1,29	1,04	0	5,89	69,38	47,73	32,14	2,45
isoeugenol	0	0	0	0,3	0,1	0,08	0,07	0,06
linalool	4,61	0,49	0	0,2	1	0,37	0,76	0,42
2,3-dimethyl-pyrazine	1,52	0	0,33	22,77	15,44	0,99	2,24	0,59
guaiacol	0,15	0,1	0	0	29,93	9,11	2,97	0
4-ethyl-guaiacol	2,43	1,54	0	9,47	15,72	9,07	9,33	2,23
4-methylthiazole	0	0	0	11,25	6,38	0,65	0,74	0,29
2,6-dimethyl-pyrazine	0,24	0	2,78	206,99	78,53	1,26	14,23	0,69
2,3,5-trimethyl-pyrazine	1,02	0	0,74	31,41	64,52	0	6,19	0
2-ethyl-3-methyl-pyrazine	0,53	0,03	0,87	19,48	31,28	1,17	4,56	0,3
2-furfurylthiol	0,23	0	0	0	0,34	0	0	0
methional	0	0	0	0	0,77	0	0	0
2,3-diethyl-5-methyl-pyrazine	0,08	0	0	3	8,57	0,83	2,36	0,57
2-Isobutyl-3-methoxy-pyrazine	0	0	0	0	0,31	0	0	0
5-hydroxy-methyl-furfural	11,29	0	0	0	138,21	118,65	114,78	12,05
2,3-butanedione	0	0,21	8,06	49,6	13,28	1,21	1,08	0
2,3-pentanedione	0	0,02	4,31	86,91	17,05	1,33	1,91	0
pyrazine	0	0	7,58	78,54	20,66	2,8	2,98	1,82
2,5-dimethyl-pyrazine	1,92	0	10,27	119,89	79,16	6,14	12,82	4,5
acetic acid	10,05	9,43	238,84	2283,91	1387,75	1292,53	221,21	53,11
vanillin	0	0	0	0	3,37	2,86	2,72	0,43
propanoic acid	21,77	21,35	112,6	360,7	133,56	106,46	39,17	23,63
disulfide dimethyl	0,1	0,12	0,55	7,07	2,38	0,13	0,4	0,11
3-methyl-butanal	0,59	0,75	9,05	343,06	18,03	2,03	2,77	0,15

**mean values of triplicate analysis, being the relative standard deviation value (RSD) for each compound smaller than 10%.*

The DVB/CAR/PDMS fiber (bi-polar) showed the best extraction performance, capable to detect and quantify all the 39 evaluated compounds, thus the choice to extract the volatile compounds in soluble coffee samples.

Amongst the five methods evaluated in this work, HS-SPME with a DVB/CAR/PDMS (bipolar) fiber provided the most representative aromatic profile, quantifying the 39 impact volatile compounds, with high sensitivity and repeatability.

Nevertheless, the dynamic headspace presented greater sensitivity than HS-SPME for some compounds, such as 2,3-butanedione, 2,3-pentanedione, acetic acid and 3-methyl-butanal, and can be used as complementary technique.

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Discrimination of Brazilian Arabica Coffees According to Overall Quality Using Aroma Composition, Solid Phase Microextraction (SPME) and Partial Least Squares - Discriminant Analysis (PLS-DA)

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SUMMARY

Eleven samples with high overall quality, and nine coffees samples with low overall quality were investigated by chemometric methods in order to get insight into which peaks of gas chromatographic analysis could be responsible for the discrimination of the Brazilian Arabica coffee samples according to their overall quality.

INTRODUCTION

Flavor can be considered a "fingerprint" of products since it plays an important role in sensory analyses (Cuevas-Glory et al., 2007). The flavor of coffee is composed of an extremely complex mixture of volatile compounds that presents different concentrations and intensities. Each functional class found in roasted coffee flavor shows different compounds and derivatives with distinct sensorial attributes and odorific impact.

Gas chromatography has been established as one of the most important analytical technique used to analyze and separate volatile fractions of different products. Coupled with gas chromatography, solid phase microextraction (SPME) has been shown to be an excellent sampling method, allowing simultaneous extraction and concentration of analytes from sample matrices (Pawliszyn, 1999).

Looking for characteristic volatile compounds that could be responsible for the discrimination of different overall quality Arabica coffee, chromatographic profiles and Partial least squares-discriminant analysis (PLS-DA) (Barker and Rayens, 2003) data treatments were carried out in this work.

METHODS AND MATERIALS

Twenty Arabica green (not roasted) coffee samples from different origins were supplied by Instituto Agronômico de Campinas. Eleven samples were classified by cuppers as of high overall quality (soft and extrictly softy), while the other nine were classified as of low overall quality (rio). The roasting process was carried out in a gas faired drum roaster (Pinhalense S/A Máquinas Agrícolas) to the medium roast point. Roasted coffee samples were packed in

films consisting of plastic (polystyrene and polyethylene) and aluminium, to avoid aroma losses and contamination by external substances, and stored at -5 °C. The samples were analyzed immediately.

The SPME fibers coated with 65 μm thick polydimethylsiloxane /divinylbenzene (PDMS/DBV) and the manual holder were purchased from Supelco (Bellefonte, PA). The fibers were conditioned according to the SPME data Sheet (T7941231, Supelco Co., Bellefonte, PA) in the GC injector port. The analyses were performed on a G-6850 GC-FID system (Agilent, Wilmington, DE) fitted with a HP-5 capillary column (30 m x 0.25 mm x 0.25 μm). Helium (1 mL min^{-1}) was the carrier gas. The oven temperature was programmed as follows: 40 °C → 5 °C / min → 150 °C → 30 °C / min → 260 °C. The injection port was equipped with a 0.75 mm i.d. liner and the injector was maintained at 220 °C on splitless mode. Under these conditions, no sample carry-over was observed on blank runs conducted between extractions.

Identification of the extracted analytes was performed in a HP-5890 gas chromatographer (Hewlett-Packard, Wilmington, DE, USA) equipped with a HP-5973 mass-selective detector fitted with the same column and operated under the same conditions as the GC-FID. GC-MS data treatment was carried out using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) v. 2.61 software and the NIST Mass Spectral Search Program v. 1.6d (Washington, DC, USA), as well as using comparisons with previous reports on the volatile compounds of coffee (Zambonin et al., 2005; Ryan et al., 2004; Ribeiro et al., 2008).

Ground coffee (250 mg) and 2 ml of saturated aqueous sodium chloride solution were transferred to a septum-sealed glass sample vial (5 mL). After 10 min of sample / headspace equilibration under agitation of 900 rpm at 42.5 °C, the fibers were exposed to the sample headspace for 22 min. After sampling, the fiber was immediately exposed in the injection port of the GC and the analytes thermally desorbed for 10 minutes at 220 °C. All analyses were carried out in triplicate.

The original chromatographic profiles (not peak areas) were organized into a matrix format **X** (*I* x *J*), where each replicate represented one sample. Data analysis was carried out by Matlab 6.5 software (The MathWorks, Co., Natick, MA, USA) using the computational package PLS_Toolbox (Eigenvector Research, Inc. – PLS_Toolbox version 3.02.) (Wise et al., 2004).

PLS-DA is a multivariate classification method where the model is built between the matrix **X** (chromatograms) and the matrix of known classes **Y** (Barker and Rayens, 2003). In PLS-DA each class is described by one column in **Y**, with values 1 or 0 depending into which class an object belongs.

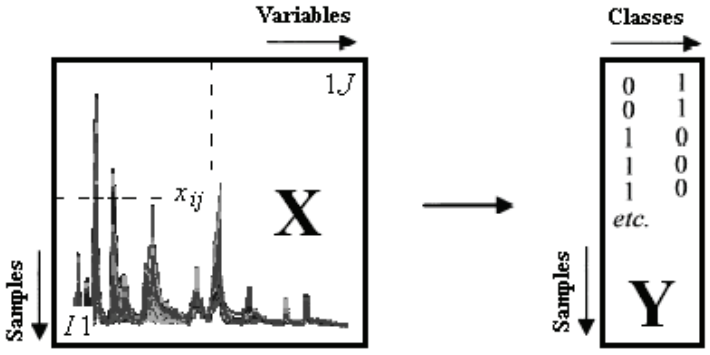

Figure 1. Schematic representation of the PLS-DA.

The chromatogram were aligned using the correlation optimized warping (COW) algorithm (Nielsen et al., 1998) obtained from www.models.kvl.dk/source/. To perform the alignments, the chromatograms were divided into 10 regions and for each region, the segment length and the slack-parameter used were 10 and 1, respectively, and the chromatogram with the best peak resolution was taken as reference vector.

Each aligned profile was normalized to unit length, smoothed by the Savitzky-Golay algorithm, with a window size of 10 points followed by taking the first derivative (Savitzky and Golay, 1964) and, at last, the data was column-wise auto-scaled. Variable selection was carried out by the ordered predictors selection method (OPS) (Teófilo et al., 2008).

RESULTS AND DISCUSSIONS

In order to get insight into which chromatographic peaks could be responsible for the discrimination of the Arabica coffee samples according to their overall quality, eleven samples with high overall quality (class one), and 9 samples with low overall quality (class two) have been investigated. The OPS method was applied to the pre-treated data from the twenty samples, to identify the main peaks involved in the discrimination of the two classes. The thirteen selected peaks are indicated as vertical lines in Figure 2.

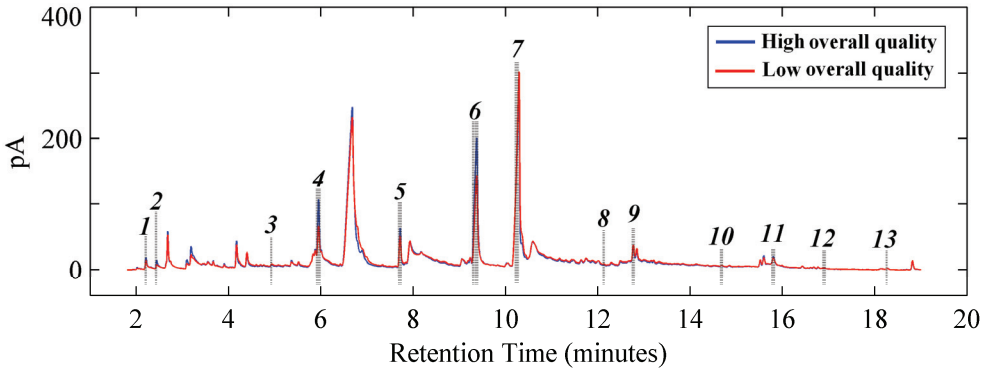

Figure 2. Peaks selected by OPS and used in PLS-DA.

Partial least squares-discriminant analysis (PLS-DA) was applied to the pre-selected peaks and, from the scores plot shown in Figure 3A, two distinct groups can be visualized. Coffee samples with low overall quality are located on the left side, with negative scores in LV1 (51.28 % of information), well separated from samples with high overall quality on the right side, with positive scores.

From the loadings plot of LV1 (Figure 3B) it can be seen that the higher the concentration of 3-methypropanal **(1),** 2-methylfuran **(2)**, furfural **(4)**, furfuryl formate **(5)**, 5-methyl-2-furancarboxaldehyde **(6)** and 4-ethylguaiacol **(13)** are related to the higher the quality of the arabica coffee (positive loadings). On the other hand, 3-methylthiophen **(3)**, 2-furanmethanol acetate **(7)** and 2-ethyl-3,6-dimethylpyrazine **(9)** tend to be more concentrated in the beverages of the lowest quality (negative loadings). Further studies are being carried out to identify the compounds **8**, **10**, **11** and **12**.

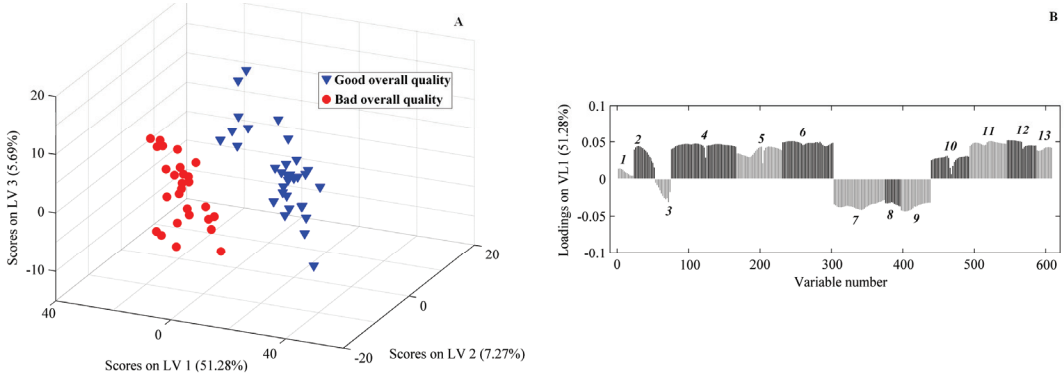

Figure 3. LV1 x LV2 x LV3 scores plot (A) plot for the PDMS/DVB fiber - High overall quality coffee samples (▼) and samples with low overall quality (●). Loadings of LV1 for the variables selected by OPS (B) - Numbers represent important peaks for high and low overall quality.

CONCLUSIONS

According to the PLS-DA on chromatographic profiles of different quality samples, compounds 3-methypropanal, 2-methylfuran, furfural, furfuryl formate, 5-methyl-2-furancarboxyaldehyde, 4-ethylguaiacol, 3-methylthiophene, 2-furanmethanol acetate, 2-ethyl-3,6-dimethylpyrazine and other four not identified compounds can be considered as possible markers for the coffee beverage overall quality.

ACKNOWLEDGMENTS

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Simultaneous Multiple Response Optimization of Microextraction Conditions Using Principal Component Analysis and Response Surface Methodology to Coffee Volatile Extraction

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SUMMARY

Principal component analysis (PCA) and response surface methodology (RSM) were applied to find the optimal headspace-solid-phase micro-extraction (HS-SPME) conditions for simultaneous optimization of multiple responses. The initial analyzed responses consisted of 57 peak areas of volatile compounds of low and high molecular weight from roasted Arabica coffee obtained from a gas chromatographic system with flame ionization detector (GC-FID). Due to the large amount of responses to be treated, it is impossible to find out one single experimental condition at which the goals of all responses are fulfilled. Therefore, the strategy proposed in this work consisted in the compression of several correlated peak areas into one component through PCA and to use it as the response in a central composite design (CCD), to identify an optimal factor combination that reflects a compromise between partially conflicting goals.

INTRODUCTION

Optimization procedures are generally carried out by selecting an objective function, which includes the most important factors affecting the analytical process and investigating the relationship between responses and factors by response surface methodology (Barros Neto et al., 2002). However, the use of several responses makes the statistical analysis rather complicated when no treatment with simultaneous responses is used. So, methodologies for multiple responses are necessary in order to make feasible the complex analysis.

In a great number of situations, some of the responses are highly correlated, providing redundant information, a fact that makes the great difference when multiple responses have to be simultaneously optimized. In this case, the several correlated responses can be compacted *a priori* in one component by applying PCA (Ferreira et al., 1999) and this resultant component is then used as response in a central composite design (CCD).

METHODS AND MATERIALS

In this study, one Arabica green coffee sample was used. The roasting process was carried out in a gas faired drum roaster (Pinhalense S/A Máquinas Agrícolas) to the medium roast point. The roasted coffee sample was analyzed immediately.

The chromatographic analyses were performed on a G-6850 GC-FID system (Agilent, Wilmington, DE) fitted with a HP-5 capillary column (30 m×0.25 mm×0.25 µm). Helium (1 mL min^{-1}) was the carrier gas. The oven temperature was programmed as follows: 40 °C → 5 °C/ min → 150 °C → 30 °C/ min → 260 °C. The injection port was equipped with a 0.75 mm i.d. liner and the injector was maintained at 220 °C in the splitless mode. The volatiles extraction was carried out using a polydimethylsiloxane /divinylbenzene (PDMS/DVB) fiber with 65 µm of thickness purchased from Supelco (Bellefonte, PA). All assays were carried out using 250 mg of ground Arabica roasted coffee and 2 mL of saturated aqueous sodium chloride solution transferred to a septum-sealed glass sample vial (5 mL). The experimental conditions of the assays were those indicated by the experimental design.

A central composite design (CCD) with three independent variables was the protocol chosen for carrying out the RSM. The design consisted of a total of 18 experiments: 8 in the factorial points, 6 in the axial points and 4 central points. The factorial points levels of independent variables investigated were: bath temperature (*T* – 30-50 ºC), pre-equilibrium time (*PET* – 5-15 min), extraction time (*Ext* – 10-20 min.). These ranges were selected based on prior knowledge about the system under study. All experiments were performed in random order to minimize the effects of uncontrolled factors that may introduce a bias on the measurements.

The data analysis was carried out using Matlab 6.5 (The MathWorks, Co., Natick, MA, USA), Microsoft Excel™ 2003 (The Microsoft, Co, USA) and Statistica 6.0 (The StatSoft, Inc., Tulsa, OK, USA). The algorithms for PCA were made in-house and the experimental design calculations were performed using the spreadsheets presented by Teófilo and Ferreira (2006) and are available at http://lqta.iqm.unicamp.br.

RESULTS AND DISCUSSIONS

As the PCA method groups correlated variables, we can expect that peaks with similar responses, independent of the experimental condition variations of the system, are highly correlated. In this way, observing the correlation (Figure 1A) among multiple responses, the use of PCA can be recommended and the peak areas are replaced by the scores of the first principal component. It was observed a direct correlation between two subsets (red regions indicated in Figure 1A).

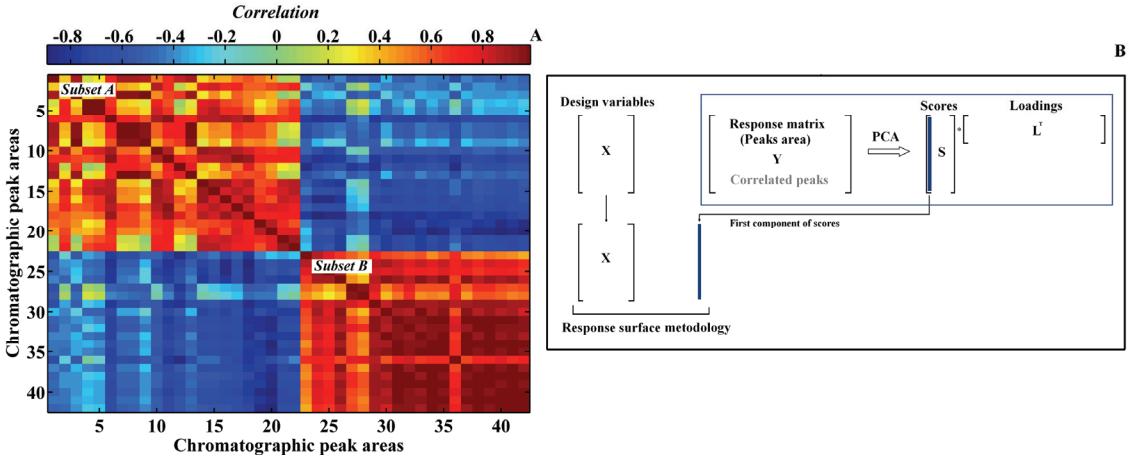

Figure 1. Correlation map of peak areas (A) and scheme showing the calculations used for MRO using PCA and RSM (B).

The first subset (subset *A*) was formed by 24 peaks distributed between the beginning of the chromatographic run up to 8 minutes, and the second subset (subset *B*), formed by 33 peaks with retention times between 8 and 19 minutes (end of the chromatographic run). Among peak areas of subsets A and B negative correlations (blue regions in Figure 1A) were observed. Hence, the multiple response analysis using PCA were performed separately for each subset, in order to obtain higher explained variance in first component for both subsets.

The first PCA components obtained using auto-scaled data from the subsets *A* and *B* explained 64.51 and 81.98 % of the data variance, respectively. Besides, these components showed satisfactory correlation with all peak areas in their respective subsets. The mean correlations (± standard deviation) between the peak areas from subset *A* and the respective PC1 scores was 0.80 (± 0.10), while for subset *B* the mean correlation was 0.90 (± 0.12).

For the statistical analysis, two independent linear models were built. Table 1 presents the variables coefficient for each analysis. It can be noticed for the subset *A* that only the linear effect of the temperature (*T*) was significant and negative. On the other hand, for the subset *B*, positive linear effects of the *T* and *Ext* were significant.

Table 1. Statistical analysis of the model coded coefficients for the subsets *A* and *B*.

	Subset A					***Subset B***			
	Coefficients	Error	t (3)	*p*		Coefficients	Error	t (3)	*p*
Intercept	0	0.48	$1x\ 10^{-15}$	1		$1.1\ x\ 10^{-15}$	0.12	$9x10^{-15}$	1
T	**-3.41*	0.55	6.24	0.008		* 3.91	0.14	28.88	$9.1\ x\ 10^{-5}$
PET	0.45	0.55	0.83	0.468		0.12	0.14	0.91	0.4285
Ext	-1.09	0.55	2.00	0.139		* 2.15	0.14	15.88	0.0005
T×PET	-0.27	0.71	0.38	0.728		0.26	0.18	1.47	0.2381
T×Ext	1.82	0.71	2.55	0.084		0.37	0.18	2.08	0.1287
PET×Ext	-0.98	0.71	1.37	0.265		0.02	0.18	0.14	0.8982

**Significant coefficients using significance level of 0.05 and three degree of freedom for the test t using the pure error.*

Analyses of variance (ANOVA) indicate that both regression models are significant ($p < 0.05$) and the lack-of-fit is not significant ($p > 0.05$). These results suggest that the response surfaces methodology can be applied to obtain the optimum volatile extraction conditions (Table 2).

Table 2. ANOVA for the two linear models built.

	Subset A					***Subset B***				
V. source	SS	D.f.	MS	F	*p*	SS	Df	MS	F	*p*
Regression	212.94	6	35.49	13.78	0.0002	274.30	6	45.72	113.48	$3x10^{-9}$
Residues	28.33	11	2.58			4.43	11	0.40		
Lack-of-fit	13.23	8	1.65	0.33	0.9074	3.84	8	0.48	1.49	0.41
Pure error	15.08	3	5.03			0.96	3	0.32		
Total SS	241.27	17				278.73	17			
R	0.99					0.99				

V. source, variation source; SS, sum of squares; D.f., degree of freedom; MS, mean square.

The results of the surface responses indicate two extreme conditions and demonstrate how it is possible to dislocate the sorption equilibrium of the system for more efficient extraction of desired compounds. In the first surface response (Figure 2A), constructed with information about light volatile compounds, the optimum responses are obtained at low temperatures. In this case, low temperatures make that heavier compounds appear in smaller concentrations in the headspace whilst the concentration of the lightest compounds increases, what greatly facilitates their extraction. On the other hand, the surface response described by Figure 2B shows the optimum responses at high temperatures and that the extraction time gives priority for heavy volatile compounds. This surface, formed basically by heavier compounds information, indicates that higher temperatures are required to drive the sorption equilibrium in a way to enhance the concentration of heavy compounds in the headspace.

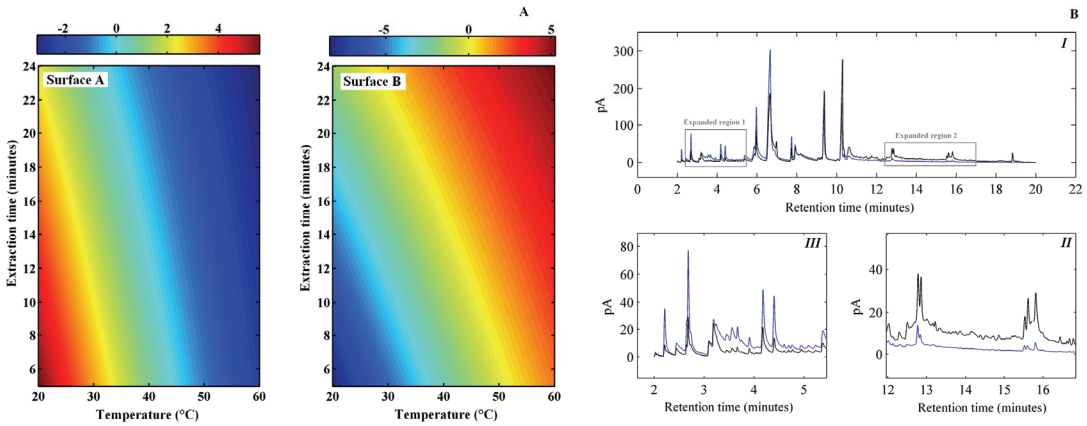

Figure 2. Response surfaces for the two subset scores (A). Typical full chromatograms (B-*I*), regions corresponding to expanded region 1 (B-*II*) and expanded region 2 (B-*III*).

The extraction time also presented significant effect for surface B. In accordance with the results, larger extraction time increases the sorption of heavy compounds onto the fiber. The compounds extraction in equilibrium through the PDMS/DVB fiber can be described by the Langmuir adsorption isotherm (limited number of active sites -porous). Due to the fact of sorption being a competitive process in which molecules with low affinity for the SPME fiber could be substituted by those of high affinity, a higher extraction time makes that the light molecules (more volatile) are quickly adhered the surface of the fiber, so they are gradually substituted by heavy molecules with stronger affinity (Pawliszyn, 1999).

CONCLUSION

The use of PCA for data compression prior to building the surface responses was of great importance to define the optimum chromatographic conditions for extraction of volatile compounds from roasted coffee beans. With this strategy, multiple responses were simultaneously handled without the necessity to use more complex methodologies. The high correlation among the chromatographic peak areas independent of the experimental conditions, make possible the use of the first PCA component as an analytical response.

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Evaluation of Physical, Chemical and Sensorial Characteristics of Arabica and *Canephora* (Robusta) Coffee Blends

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SUMMARY

The objective of this study was to produce arabica and robusta coffee blends. Optimized roasted was previously done for pure coffees (arabica 215 °C/17.5' and robusta 225 °C/24'). The blends formulation varied from 10 to 50% coffee robusta addition. Pure arabica and robusta coffees were also analyzed, resulting in 7 samples. The samples were analyzed regarding chemical characteristics (moisture, acidity, soluble solids, reducing sugars, pH and caffeine), physical characteristics (density, roasting degree, reflectance and instrumentally measured color CIELab - L* a* b*), sensory analysis and principal component analysis using electronic tongue. Physical and chemical results indicated that the addition of robusta coffee promotes increase of pH, density, caffeine and soluble solids contents, thus decreasing moisture, acidity and beverage sweetness. Sensory analysis results indicated that the addition of 30 to 50% robusta changed superior quality coffee to traditional, under a gradual quality reduction.

INTRODUCTION

The main characteristics of coffee beverages defined for taste are: acidity, bitterness, sweetness, astringency and body (Ko, 2000; Clarke, 1986; Clifford, 1985). Blend can be defined as the art of combining coffees with supplementary characteristics – acidity with sweetness; very full-bodied with little full-bodied; clear roast and dark roast (Relvas et al., 1997). A digital characterization of aromas using an electronic nose to assort 6 arabica and robusta coffee varieties was performed by Dirinck et al., (2002). With support of principal component analysis (PCA) and based on aroma phenolic compounds, the authors have concluded that electronic nose presented good discernment between arabica and robusta. Electronic tongue is another tool that has shown high sensitivity and results quite propitious to discern several types of beverage, including for global quality analysis on coffee (Relvas et al., 1997; Correa et al., 2004). Importantly, coffees used in blends must be roasted separately in order to completely develop their best sensorial properties, since they have different chemical composition, size and moisture.

The purpose of this paper was to produce and perform physical, chemical, sensorial assessment of arabica and robusta coffee blends, as well as through electronic tongue.

MATERIAL AND METHODS

In order to produce blends, optimized roasting processes were performed initially to pure coffees (Arabica coffee: 215 °C/17.5 min and robusta coffee: 225 °C/24 min). The samples were prepared in a PROBAT-WERKE pilot roaster type PRE 1 Z, and a PROBAT-WERKE

grinder type 55 LM 500. The blends formulation has complied with the following composition: 50% to 100% Arabica coffee against 50% to 0% robusta coffee. The quantitative descriptive sensory analysis of coffee was conducted by a selected and qualified team, composed of eight panelists using a non-structured scale of 0 to 10cm to evaluate powder aroma, characteristic flavor, taste (typical, caramel, chocolate, toasted bread and citric fruits), sweetness, acidity, bitterness, residual taste, beverage body, astringency and global quality of the coffee. The analysis was individually conducted and repeated in red light booths equipped with computerized system (Compusense Five) to collect data. An analysis of variance (ANOVA) was applied, followed by an average comparison test (Tukey) of data obtained from mixtures distributed into randomized complete blocks study. The electronic tongue analysis was composed of special polymers, which conduct electricity (polyaniline derivative) and are sensitive to substances that determine beverages' taste, below sensorial perception threshold of basic tastes. The principal component analysis (PCA) was used to provide samples statistic correlation.

RESULTS AND DISCUSSIONS

Physical and chemical characteristics assessment

Powder density values have increased as robusta coffee percentage was also increased in blend. Otherwise, L* value from pure arabica coffee was significantly lower than L* value from pure robusta coffee, and b* values observed increased as robusta coffee percentage was raised in blend. pH values have shown that the higher robusta percentage in blend, the greater is its pH, and pH value of pure arabica coffee (sample 1) was significantly lower than pH value of pure robusta coffee (sample 7). However, total acidity values inversely correlate with pH values. The results are consistent with literature (Clifford, 1986; Clarke, 1986; Mendes, 1999), which demonstrated that an increase in robusta coffee percentage in blend tends to reduce coffee acidity and, thus increase beverage pH. As expected, caffeine contents raised with increment of robusta percentage in blend. Better quality of arabica coffee suggests a lower quantity of caffeine (Clifford, 1975). The range obtained of 1.4% (arabica coffee) to 2.2 (robusta coffee) was consistent with Ordinance no. 377 of ANVISA [Brazilian Health Authority] (Brasil, 1999), which establishes minimum content of caffeine as 0.7%. Moisture has reduced as robusta percentage was increased in blend, and pure robusta coffee has shown moisture significantly lower than moisture from pure arabica coffee. As per Illy and Viani (1998), moisture range found for both robusta coffee and arabica coffee was from 0 to 5%. Regarding soluble solids data, it was concluded that the higher robusta percentage in blend, the higher is soluble solids percentage. The results are consistent with the literature (Mendes, 1999). In relation to sugars quantity, values show that robusta addition in blend has diminished the quantity of reducing sugars. The superior quality of arabica coffee suggests a higher quantity of sugars (Clifford, 1975). Such trend was also observed by Sabbagh et al. (1977), in which values of reducing sugars achieved were from 0.84 to 1.31% for robusta coffee and from 0.89 to 1.36 for arabica coffee. Illy and Viani (1998) obtained reducing sugar values of 0.3% both for roasted arabica coffee and roasted robusta coffee. Values obtained from our paper range from 1.47 (pure robusta) and 2.65% (pure arabica) of reducing sugars and they are substantially different. Reflectance results have presented much variation, and roasting degree by visual comparison has not shown significant difference between samples, which demonstrates the difficulty of visual perception of color difference between the samples.

Sensorial characteristics assessment

Sensory results on: powder flavor characteristics (1), typical aroma (2), typical taste (3), caramel taste (4), chocolate taste (5), toasted bread taste (6), citrus fruits taste (7), sweetness (8), acidity (9), bitterness (10), residual taste (11), body (12), astringency (13), and global quality (14) were assessed. Pure arabica coffee (sample 1) has shown grades significantly higher for powder flavor, typical aroma, typical taste, caramel, chocolate and citrus fruits taste, sweetness, acidity, residual taste and global quality; as well as grades substantially lower to bitterness compared with pure robusta coffee (sample 7). The results achieved show that blends samples have exhibited sensorial characteristics resulting in global quality with averages ranging from 4.8 to 6.1, corresponding to quality between "regular and good" in quality scale. According with rating system from Technical Rule for Identity and Quality Determination of Coffee in Grains and Milled and Roasted Coffee (Governo Do Estado De São Paulo, 2007; 2004), samples 4, 5, and 6 were rated as "Traditional" coffees, and samples 1, 2, and 3 were rated as "Superior" coffees. Sample 7 (100% robusta) was rated as below minimum acceptable limit in global quality scale. It is important to note that robusta coffee used was a selected coffee from which most defects were removed. Through this process, coffee blends of regular quality were obtained, even with 50% of robusta. Decrease of grades given to beverage with robusta percentage increase could be observed. Addition of robusta in blend also reduced sweetness, acidity, and increased bitterness; however quality was considered irregular, yet with 50% of robusta. Coffee mixtures ranged from 10% to 50% of robusta in blend with arabica, and grades obtained for global quality of the beverage were above minimum acceptable limit (4.5) in sensorial quality scale.

Electronic tongue answers assessment

The arrangement of samples of arabica coffee, robusta coffee and their blends was assessed, as per principal component analysis (PCA) from data obtained through electrical capacitance measurement of 10 sensorial units from electronic tongue. Such data comprise typical digital impression of beverage and related to its global quality similarly to human impression, that is, without necessarily quantify specific substances as a selective chemical sensor, but using concept of global selectivity. Components 1 and 2 of principal component analysis explain 97.67% of information from global quality differences among samples analyzed. It can be noted that samples 1 and 7 discriminate very well from other samples, since sample 1 has the best global quality (pure arabica coffee) and sample 7 has the worst global quality (pure robusta coffee). Blends are set between these two extremes. A feasible explanation for electronic tongue selectivity (Relvas et al., 1997; Correa et al., 2004) used in this paper is the fact that it is composed of sensors manufactured from conductive polymers extremely sensitive to environment pH changes and acids presence, since its conduction process is modified by presence of H+ ions (named polymer doping process). In presence of acids, the polymer constituting the electronic tongue modifies from base condition to emeraldine salt, resulting in changes in sensor electrical answer and, therefore, in digital impression of beverage being evaluated (Firmino, 2002). Results show that electronic tongue perception has discriminated samples consistently. Sample 7 which present robusta coffee 100% and have higher pH, is put in PCA in a different place in relation to other samples, thus showing that sensor has reacted according to presence of acids in blends.

CONCLUSIONS

Robusta addition in arabica coffee increases pH, density, caffeine contents and soluble solids contents, thus decreasing moisture, acidity and beverage sweetness. Sensory analysis results

show that superior quality coffee changes to traditional when 30 to 50% of robusta are added, gradually decreasing quality.

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Consumer Preference Measured Using Hedonic Ranking R-Index Measures for Coffee (*Coffea arabica*) Cultivated at Different Altitudes in Veracruz, Mexico

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SUMMARY

The main interest in this experiment is finding out whether or not coffee grown at an altitude as low as 110 meters above sea level is unacceptable according to the consumers. Consumers (100) performed preference tests using a Rank-Rating procedure with four samples of coffee (Coffea Arabica var. Caturra) from four different altitudes (110, 360, 700, 1060 meters above sea level). The R-index values between samples were calculated and did not indicate a significant preference between samples.

INTRODUCTION

There is a tradition that coffee grown at altitudes as low far down as 110 meters above sea level is unacceptable. However, this has not been tested regarding consumer acceptance. It is possible that coffee grown at such altitudes might well be acceptable and liked by regular consumers. Avelino et al. (2005) reported a positive relation between altitude and preference. However, the coffee preferences were measured using a trained panel and the altitude tested were above 1100 meters above sea level. Traditionally, the 9-point hedonic scale is used as a measure of liking (Jones et al., 1955; Peryam and Girardot, 1952; Peryam and Pilgrim, 1957; Rosas-Nexticapa et al., 2005) although various other scaling techniques have been developed, eg., unstructured line scales (Vickers et al., 1998; Lange et al., 1999) magnitude estimation (Pearce et al., 1986, Lavenka and Kamen, 1994) a Food action Rating Scale (Schutz, 1965) 'smiley face' scales (Basker, 1989; Léon et al., 1999), and the LAM 0Scale (Cardello and Schutz, 2004; Schutz and Cardello, 2001).

Regarding the cognitive mechanisms involved in scaling, it appears that scaling is a relative process Mellers (1983 a, b). The sensation strengths of the stimuli in an experiment are compared with each other and numbers assigned accordingly from high to low. Essentially, the process is one of ranking the stimuli in order of intensity, while using the numerical estimates to describe the spacing between the ranks. This is supported by the following experiments. Lawless (Lawless, 1983; Lawless and Malone, 1986) used context effects and the fact that judges would space stimuli with even relatively small physical differences, across the whole length of a scale, to argue that scaling was relative. Context effects (Leeet al., 2001a; Parducci, 1963, 1965, 1968; Rankin and Marks, 1991; Riskey, 1986; Riskeyet al., 1979; Schifferstein, 1994, 1995, 1996; Schifferstein and Frijters, 1992; Stillman, 1993;

Vickers and Roberts, 1993) would not occur unless scaling was relative. Mellers 1983 (a) used such effects to argue for a relative cognitive process.

Laming (1984) used arguments that included the limits to transmission of information when making category judgments, which support the relative cognitive mechanism. Research into scaling errors (Jeon et al., 2004; Koo et al., 2002; Lee et al., 2001b; Parket al., 2004; Kim and O'Mahony, 1998) also supports this idea. The more the judges were able to re-taste stimuli and modify their scores, the fewer were their scaling errors. Scaling errors here were defined as giving a perceptibly stronger stimulus a score equal to or less than a perceptibly weaker stimulus. This result would not only occur if scaling were relative.

If scaling is a process of ranking the stimuli with the consumers using the scaling scores to represent the spacing between the ranks, then any other method that produces a rank order and a measure of the distances between ranks is an acceptable substitute for hedonic scaling, since it would give the same information. This idea has been exploited when measuring preferences for various foods, beverages and personal products (Lee and O'Mahony, 2005; O'Mahony et al., 2004; Pipatsattayanuwong et al., 2001). Consumers were required merely to rank the products for liking and R-Index values (Brown, 1974; O'Mahony, 1992) were used to indicate the spacing between the products. They can be used to indicate the spacing between adjacent products in the rank order or the distance from a target product. The latter ranked rating approach was used in the present study. For consumers, ranking has the advantage that it is a task with which consumers are familiar; on the street consumers are sometimes awed by rating scales.

The goal of the present research was to use a hedonic ranking procedure, with an R-index analysis, to assess the liking for black coffee derived from beans grown at 110 meters above sea level in relation to coffee derived from beans grown at higher altitudes.

MATERIALS AND METHODS

Coffee Samples

Four samples of green coffee cultivated at different altitudes - 110, 360, 700 and 1060 meters above mean sea level were presented to consumers for assessment. Samples of *Coffea arabica* var. Caturra were selected according to production in the area under investigation (Misantla, Ver. near the gulf coast of Mexico). Attention was focused on obtaining uniform wet-processed samples. In this paper samples are identified with a number referring to the altitude of origin.

All samples of green coffee were stored under dry and cool conditions at room temperature. Green coffee batches of 300 g were cured, roasted and ground according to the international standard ISO 6668:1991. The beverage was prepared following the same standard. The coffee beverage was kept in thermal containers until its temperature reached 60 °C. The samples were presented in white porcelain cups.

Judges

A total of 100 Mexican consumers (27F, 73M; age range 17-68 years) were intercepted in a popular local café (Gran Café de la Parroquia, Veracruz, Mexico), and screened to ensure that they were consumers of black coffee, drinking at least four cups per week. They were then invited to a designated location inside the café for testing.

Procedure

After establishment of rapport, four cups of coffee were presented to the judge for assessment of liking. The first cup was presented to the judge who sampled and assessed it for liking. Then, next, the second cup was presented and assessed in the same way. The judge was then asked to rank these two in order of liking. A third cup was then assessed and added to the rank order. This was followed by a fourth and final cup. The order of presentation of the samples was counterbalanced over judges. The cups were given one at a time so that the sample coffees could all be tasted at 60 °C. Judges were able to request further cups for retesting as often as desired until they had decided on a final ranking. Because the data were to be pooled over all judges, the ranking could be forced-choice; accordingly ties were not allowed. However, no consumers requested to give tied ranks.

RESULTS AND DISCUSSION

The R-index response matrix derived from the rankings by the 100 consumers is shown in Table 1. The coffee sample that was liked the most was ranked first had been given the first rank; the one liked the least was ranked fourth. The total of the rankings from all the consumers are shown for coffee samples grown at various heights above sea level (in meters). The target coffee sample in the study was grown at an altitude of 110 meters above sea level. Therefore, comparison was made in terms of the difference in acceptability between this product and those grown at higher altitudes. Hedonic R-index values with 110m sample as the 'noise' and the other altitudes as 'signal' samples, were calculated and are given in Table 2. Significance was tested using the method of Bi and O'Mahony (1995) for which the critical value was 59.66% ($p = 0.05$, two-tailed). As a check for sufficient sample size, the present sample was split into two separate samples of 50 consumers each. The results gave the same conclusions, suggesting sufficient sampling.

It can be seen from the Table that the R-Index values were small, indicating only slight preferences. Also, none of the values were significantly different from the target. From this, it can be concluded that any hedonic differentiation between coffee samples grown at an altitude of 110 m and coffee grown at higher altitudes is slight and for this sample size, no greater than chance. Inspecting the data, it can be seen that as comparisons are made with higher altitudes, the R-Indices increase. Comparison with the 1060 sample is almost significant. Yet the preference is still slight (hedonic $d' = 0.31$). It is possible that coffees grown at higher altitudes might show significant differences in preference. Avelino et al., 2005, reported positive relation between altitude and coffee preferences. However, the altitudes studied were above 1100 meters above sea level, which was the highest tested in the present study, and the preferences were done with a trained panel. More studies are needed to confirm this positive relationship between altitude and coffee preferences.

Table1. R-index response matrix derived from consumers hedonic rankings for black coffee.

Altitude (m above sea level)	1st	2nd	3rd	4th
1060	32	19	24	25
700	28	33	26	13
360	20	27	30	23
110	20	21	20	39

Table 2. Hedonic R-index values between coffee grown at 110 meters above sea level and higher elevations.

Comparisons	R-Index	Critical value
110 - 360	45.93	59.66
110 - 700	53.43	59.66
110 - 1060	58.64	59.66

CONCLUSION

It can be concluded from the present research that there would seem little justification for rejecting coffee grown at the lower elevation of 110 meters above sea level.

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A Prediction Methodology to Analyze Relationships Between Instrumental and Sensory Characteristics for Retronasal Aroma of Espresso

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SUMMARY

To develop the quantitative evaluation and designing methods for the consumer-oriented retronasal aroma of espresso, instrumental analyses by gas chromatography/olfactometry (GC/O CharmAnalysis™) and electronic nose system (E-nose, αFOX4000, Alpha M.O.S.) and sensory evaluations were carried out on the aroma compounds collected by the retronasal aroma simulator (RAS) (Roberts and Acree, 1995), and then an artificial neural network (ANN) model (JMP6, SAS institute Inc.) was applied to specify the causal relationships between analyzed properties and sensory characteristics. The odor profiles by GC/O showed the significant differences in the origin and the roasting degree ($p < 0.05$). While, the sensory factor extracted by sensory evaluation showed the significant differences in the roasting degree ($p < 0.05$). Sweet-caramel, smoke-roast, and acidic odors were predicted as the odor descriptions affecting the sensory characteristics based on the ANN model. Also, three metal oxide semiconductor (MOS) sensors (LY2/Gh, P30/1, and T40/1) of E-nose were selected for the alternative evaluation method to support the sensory evaluation.

INTRODUCTION

The retronasal aroma is caused by flavor molecules traveling from the mouth to the nasal cavity via the nasopharynx and the lungs and mainly experienced during drinking the chilled espresso beverage packed in a plastic cup with a straw. RAS has been developed to analyze retronasal aroma and gives a good approximation of the flavor composition as defined by breath-by-breath measurements and increases the concentrations of odorants and sensitivity compared to the previous methods (Deibler et al., 2001).

The design of taste and flavor has been usually based on technical findings and subjective quality evaluations by experts, although that has not been always adapted to consumer preferences. Though more than 800 volatile compounds have been identified by gas chromatography/mass spectrometry (GC/MS), the correlations with sensory characteristics have not been clarified.

The objectives of this study are to specify the causal relationships between instrumentally-analyzed properties and sensory characteristics, and then to develop the quantitative evaluation and designing methods for the consumer-oriented retronasal aroma of espresso.

MATERIALS AND METHODS

Coffee samples and Brewing espresso

Arabica coffee beans from Brazil (B, no. 2/3), Ethiopia (E, mocha Sidamo grade 2), Guatemala (G, SHB), Colombia (C, supremo), Indonesia (I, Mandheling grade 1), and Tanzania (T, Kilimanjaro AA) were roasted to three roasting degrees (L26, light roast; L23, medium roast; and L18, dark roast). The roasted coffee beans (about 7.5 g) were ground, and then espresso (average characteristic: 59 g, Brix 2.8°; and 73 °C) was brewed from ion-exchange hot water with pressure of 15 atm using an automatic espresso machine (Saeco Royal Professional, Nihon Saeco K. K.). The freshly brewed espresso was diluted to the solute concentration of Brix 1.5° and kept at 10 °C.

RAS volatile sampling

Aroma compounds were collected from 200 mL espresso solutions by RAS operated on the conditions shown in Figure 1. The RAS is composed of a 1-L stainless steel blending container, a voltage controller and high torque-speed motor to give precise rotational speed of blending impeller, a N_2 gas supply at the controlled flow rate, and a water jacket to control the temperature of container. The collection time was determined to maintain the component proportion of RAS volatiles sampled by a solid-phase microextraction (SPME) fiber (Akiyama et al., 2006). The RAS volatiles in the effluent gas were collected into a sampling bag (Flek-Sampler 3 L, Omi Odor-air Service Corp.) and air sealed with an odorless silicon stopper.

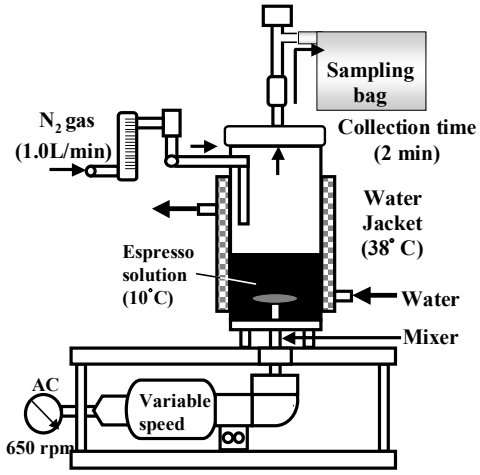

Figure 1. Schematic diagram of RAS.

GC/O

The volatile compounds in the RAS effluent gas were absorbed by 240 mg of TENAX TA (GL Sciences Inc.) and 120 mg of CARBOXEN™ 1000 (60/80 mesh, Sigma-Aldrich Co.). The isolated odor-active volatile compounds of the extracts were analyzed in triplicate by CharmAnalysis™. Sweet-caramel, sweet-fruity, nutty-roast, smoke-roast, phenolic, acidic, buttery-oily, green-earthy, green-blackcurrant, soy sauce, and others were used for the odor descriptions and the odor activities were represented as charm values.

E-nose

RAS effluent gas was manually taken using a gas-tight syringe and injected into the E-nose equipped with 18 MOS sensors under the room temperature in triplicate. The characteristics of sensors are dependent on the materials used in the manufacture of the sensors. Principal component analysis (PCA) was applied for the maximum sensor resistances to select the sensors indicating the highest factor loading for each PC as the suitable sensors for the evaluation of the retronasal aroma of espresso.

Sensory evaluation

The sensory evaluations for the RAS effluent gas were performed by five trained flavorists (ages 27 to 43, 3 males and 2 females). The flavorists sniffed each sample in a sampling bag and described the perceived quality by the hedonic rating, intensity of aroma, and 11 sensory descriptors comparing with the standard sample (Brazil L23) scored in 4-point using a 7-point sensory descriptive analysis. To select the sensory descriptors showing the significant differences among the samples ($p < 0.05$) and summarize highly-correlated descriptors ($R \geq 0.7$, $p < 0.01$), analysis of variance (ANOVA) and correlation analysis were applied to the sensory scores respectively. Also, the PC indicating the variance over 1.0 was extracted as a sensory factor showing the directions of greatest variance in the sensory scores for selected sensory descriptors by PCA.

ANN model

The total charm values analyzed by GC/O and the PC scores extracted by sensory evaluations were substituted for input and output layer in an ANN model respectively. The whole dataset was used for the calibration and cross validation and the prediction performance was evaluated by the coefficients of determination (R^2) and root mean square error (*RMSE*). The sum of products for connection weights of each odor description contributing to sensory factor were calculated to predict the odor descriptions affecting the sensory characteristics of the retronasal aroma of espresso.

RESULTS AND DISCUSSION

GC/O

The GC/O analysis detected 36 potent odorants and revealed 10 odor descriptions showing the significant differences in the origin and the roasting degree at the level of 5%. Figure 2 shows the odor profiles indicating the contributions of the production origins.

E-nose

Selected MOS sensors (LY2/Gh, P30/1, and T40/1) showed the significant differences in the origin at the level of 1% (Figure 3) and the roasting degree at the level of 5%, and were highly-correlated with the other sensors loaded by the same PC ($|R| \geq 0.7$, $p < 0.0001$).

Sensory evaluation

As shown in Table 1, an extracted sensory factor accounted for 60.4% of the sensory characteristics described by “roast”, “earthy”, “sweet”, and “soy sauce” and showed the significant differences in the roasting degree at the level of 5% except for Brazil and Colombia samples (Figure 4).

ANN model

As shown in Figure 5, sweet-caramel, smoke-roast, and acidic odors were predicted as the significant odors for the sensory characteristics of the retronasal aroma of espresso based on the sum of products for connection weights in the ANN model indicating R^2 = 0.85 and *RMSE* = 0.40.

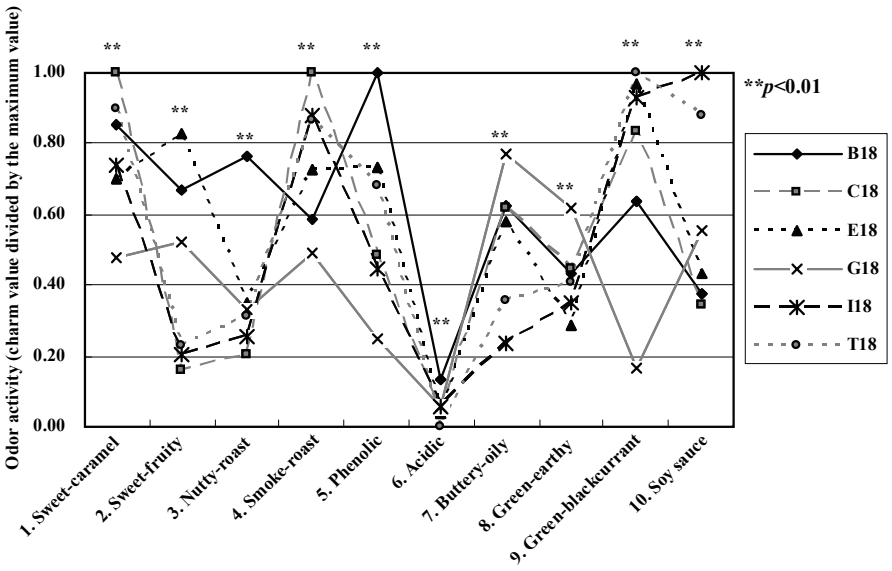

Figure 2. Odor profiles of the dark-roasted (L18) samples by the total charm values divided by the maximum values for each odor description.

Table 1. Factor loadings for sensory descriptors.

Sensory descriptor	PC1	PC2
"Roast"	0.53	0.19
"Earthy"	0.50	0.83
"Sweet"	-0.46	-0.04
"Soy sauce"	-0.51	0.53
Proportion(%)	60.4	15.8
Variance	2.4	0.6

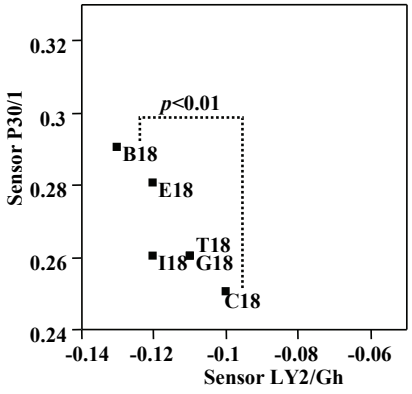

Figure 3. Plots of average sensor resistances of the dark-roasted (L18) samples.

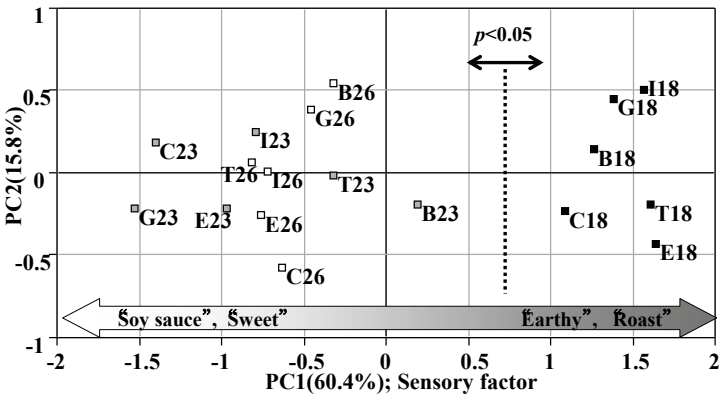

Figure 4. Plots of average PC scores of sensory factor.

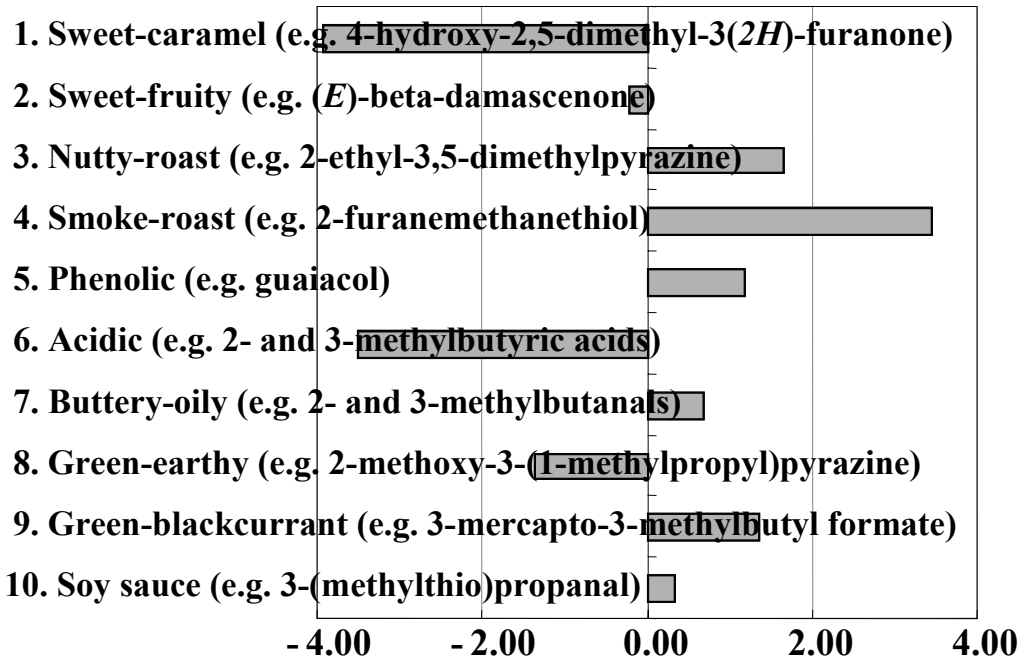

Figure 5. Sum of products for connection weights of each odor description contributing to sensory factor.

CONCLUSIONS

For the consumer-oriented designing of retronasal aroma of espresso, sweet-caramel, smoke-roast, and acidic odors should be controlled by the production origins and roasting degrees based on the odor profiles obtained by the method of the combination of RAS and GC/O. Also, three MOS sensor (LY2/Gh, P30/1, and T40/1) resistances correlated with sensory evaluation data would provide the alternative evaluation method using E-nose to support the sensory evaluation.

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Selective Hydrolysis of Chlorogenic Acid Lactones and Impact on Coffee Taste

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SUMMARY

Coffee lactones were recently isolated from coffee and suggested as key bitter impact compounds. We therefore investigated ways to remove/destroy these compounds selectively. Base and enzymatic hydrolysis were explored. The work was conducted in model systems with the major coffee lactone (3CQAL). Results were then confirmed in coffee brew. The lactones were found to be quite stable under weakly acidic conditions as found in coffee beverage. However they were quickly hydrolysed under weakly basic conditions. Enzymatic hydrolysis was also explored as a milder and more selective approach. After screening a set of lipases, esterases and tannase, the most selective and potent esterases were further studied for their substrate specificity. Hog Liver Esterase (HLE) was shown to hydrolyse selectively chlorogenic acid lactones in the presence of chlorogenic acids. On the other hand chlorogenate esterase (CE) cleaved both chlorogenic acid lactones and chlorogenic acids in a non selective way. Treated coffee samples were evaluated for bitterness by a trained sensory panel and were found significantly less bitter than the reference.

INTRODUCTION

Coffee lactones are formed during roasting by dehydration of their precursors the chlorogenic acids (Ginz, M.; Engelhardt, 2001; Frank et al., 2006). Several isomers with bitter detection thresholds in the range 30-200 µM were recently identified. In order to show that this family of compounds has indeed an impact on the sensory perceived bitterness in the beverage we investigated various approaches to neutralize these lactones either chemically or biochemically. Due to its ester and lactone functions this family of compounds is fairly labile and can easily by hydrolyzed back into the corresponding chlorogenic acids. Base and enzymatic hydrolysis were explored.

MATERIALS AND METHODS

Coffee Sample

A coffee brew model was produced from a 100% Robusta coffee (Tanzania), medium roasting level (CTN = 85), by countercurrent extraction 3 x 12 min at 120 °C. The volatile aroma was vapour stripped and the extract spray-dried without pre-concentration, (extraction yield 26.5%). Extract (powder) and aroma distillate were stored separately at -20 °C until further use.

Chlorogenic acid lactone (3CQAL)

Was synthesized as previously described (Huynh, 1994).

Base treatment

The coffee extract was dissolved at 3% t.s. in hot MQ-H2O and allowed to cool to 50 °C. After alcalinisation to pH8 by food grade NaOH10%, the coffee was stirred for 3h at 50 °C and maintained at pH8 +/-0.2 by further base addition. At the end of the hydrolysis the coffee was adjusted back to the original pH (5N, HCl, food grade), freeze dried and stored as a powder at -20 °C until use.

Enzymatic treatment

Enzymes were obtained from Sigma, Fluka or Kikkoman. For the quick screening 3% ts coffee solutions (1mL) were incubated for the given time with the enzyme (Eppendorf, thermomixer model 5437). Soluble enzymes were ultra-filtered through microcon (YM-10, Millipore) and immobilized enzymes were centrifuged (centrifuge Sigma 3-16 K, rotor 12154-H). The treated coffee samples were then analysed by HPLC as previously described to measure chlorogenic acids (Milo et al., 2001). The scale up for sensory evaluation was conducted under the best conditions found in the quick screening. With the immobilized enzyme (HLE) the sample was filtered over S&S filter (595, Φ125 mm) and the solid support washed with hot water. In the case of soluble enzymes (CE) the samples were heated at 100 °C for 5min. to deactivate the enzyme, then freeze dried and stored at -20 °C until use.

Sensory evaluation

The samples were evaluated by a trained sensory panel (12 judges) equipped with noseclips. Samples were recombined with the aroma distillate and tasted monadically and ranked on a 0-10scale. Tasting was made in duplicates. Data acquisition was achieved on a computer screen with FIZZ software (Biosystemes, Couternon, France). Panel mean score was calculated for each sample and each attribute. Duncan test was calculated to highlight differences between samples (significantly different samples are highlighted with an asterisk on charts).

RESULTS AND DISCUSSION

Quick screening of a set of lipases, esterases and tannase (Figure 1) highlighted the esterases as the most potent enzymes for the biohydrolysis of 3CQAL.

Further investigation of substrate specificity was conducted directly on a fairly bitter coffee extract from 100% Robusta beans (Figure 2). Selective hydrolysis of chlorogenic acid lactones in the presence of chlorogenic acids was achieved with Hog Liver Esterase (HLE). Chlorogenate esterase (CE) cleaved all chlorogenic acid lactones and chlorogenic acids (caffeoyl, CQA and feruloyl, FQA derivatives) in non-selective way.

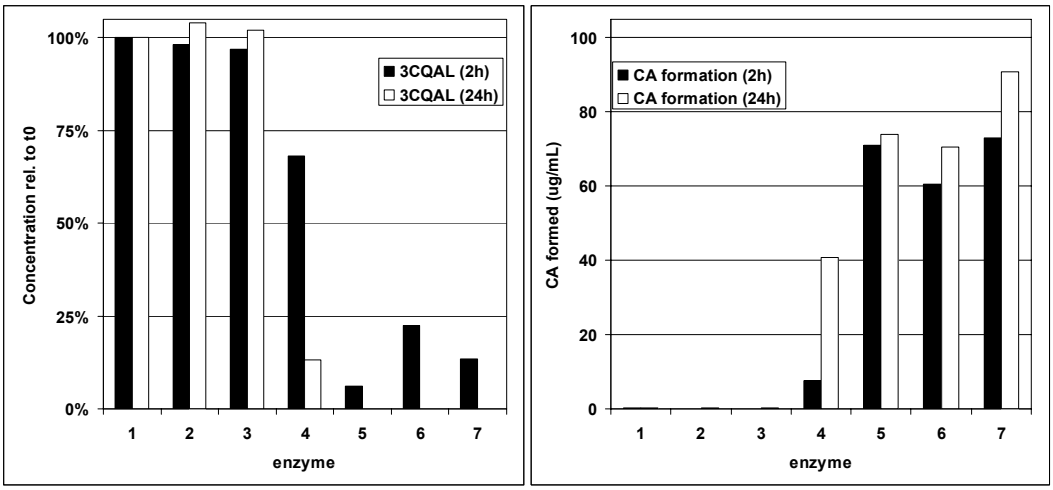

Figure 1. Enzymatic hydrolysis of 3-CQAL in model system. Conditions: 40 °C no pH control. 10 U of enzyme / 1 mg substrate. (1) Reference without enzyme. (2) *Candida rugosa* lipase. (3) *Candida cylindracea* lipase. (4) tannase from *Aspergillus oryzae*. (5) Immobilized hog liver esterase. (6) palatase. (7) porcine liver esterase. Lactone decrease (left) and caffeic acid formation (right).

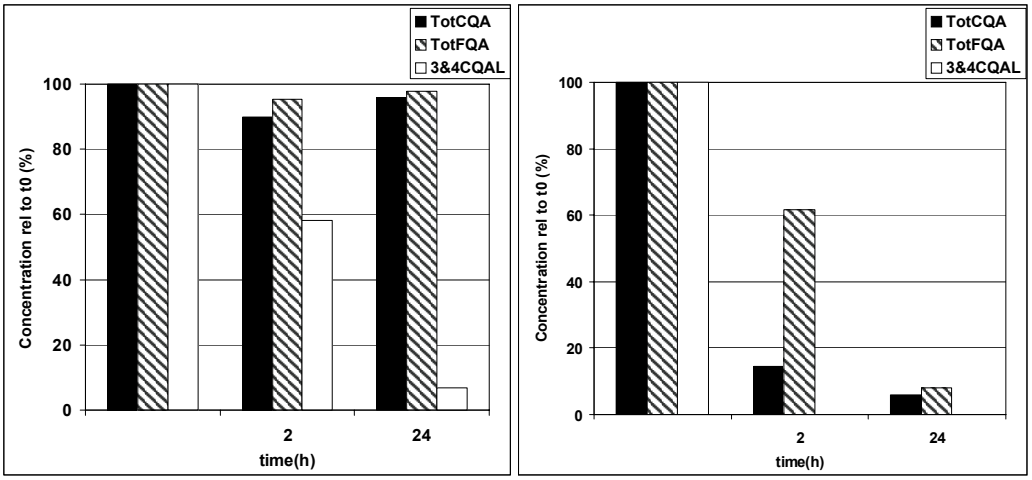

Figure 2. Enzymatic hydrolysis of lactones in coffee brew. Conditions: 40 °C no pH control. 10 U of enzyme / 1 g coffee, immobilized HLE (left), CE (right).

In both cases the hydrolysis of chlorogenic acid lactones resulted in generation of high amounts of caffeic acid (CA). Quinic acid was not detected suggesting that the enzymes cleaved the ester bond of chlorogenic acid lactones (Figure 3, Path 1A) without hydrolysis of the lactone function itself (Path 1B).

The lactones were quite stable under weakly acidic conditions as found in coffee beverage. However they were quickly hydrolyzed under weakly basic conditions such as pH8, 50 °C (Figure 4). Base treatment hydrolyzes lactones following path 1A described in Figure 3. No significant increase of caffeic and ferulic acid was observed. The hydrolysis is occurring on all the lactones including minor isomers and feruloyl equivalents.

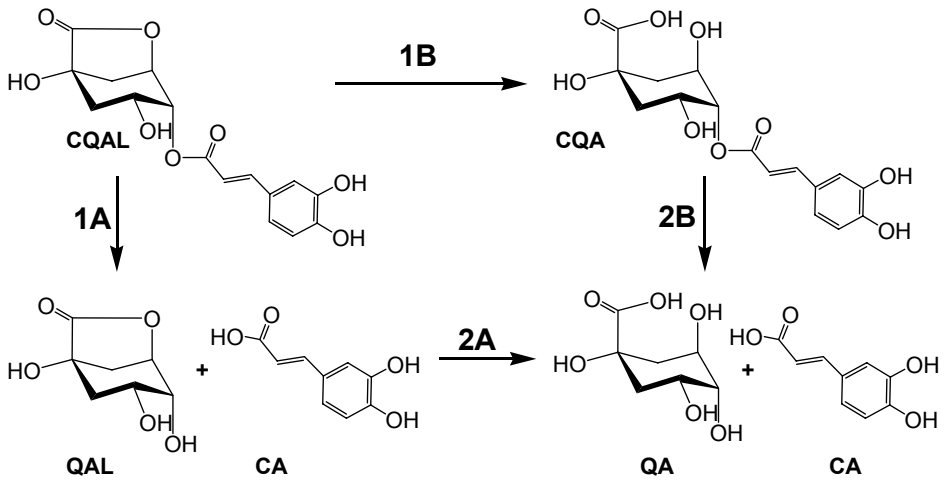

Figure 3. Possible mechanistic pathways for the biohydrolysis of chlorogenic acid lactones.

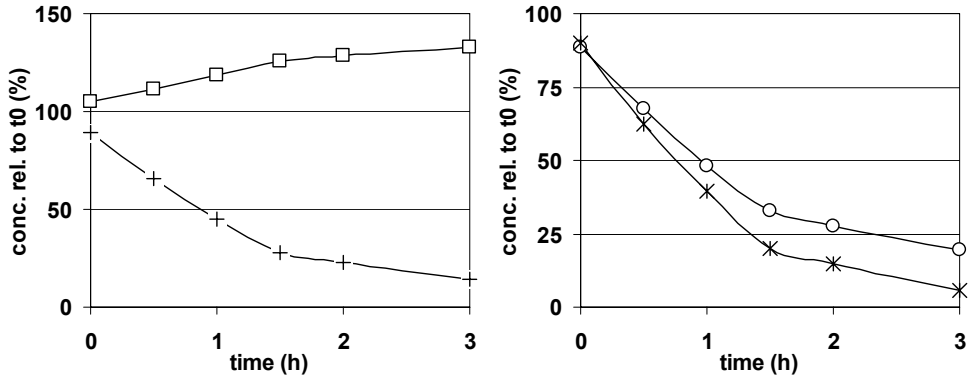

Figure 4. Chemical hydrolysis (pH8, 50°C) of coffee lactones in coffee brew. (□) total CQA, (+) total CQAL, (o) 3CQAL, (∗) 4CQAL.

For sensory evaluation the trained panel wore nose-clips in order to focus on taste alone. The enzyme and base treated coffee samples were were found significantly less bitter than the untreated reference (Figure 5). A control sample stirred without base/enzyme for the same time, at the same temperature was not different from the reference.

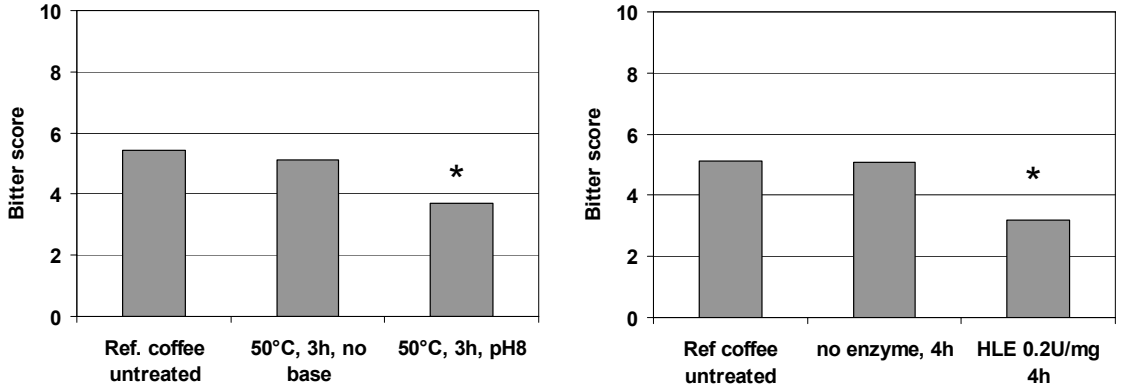

Figure 5. Sensory evaluation of hydrolyzed coffee samples: chemical hydrolysis with base pH8, 50 °C, 3 h (left), biohydrolysis with HLE (right).

Chlorogenic acid lactones are bitter impact compounds in coffee brew. The enzymatic or chemical hydrolysis transformed them into non-bitter chlorogenic or phenolic acids and had a significant impact on sensory perceived bitterness of the corresponding coffees.

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Chemical and Sensory Quality of Shaded Coffee Crop in São Paulo State, Brazil*

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SUMMARY

The sensory quality of coffee beverage results from a complex combination of chemical compounds of the beans. Experimental data have shown that trigonelline, caffeine and chlorogenic acids as well as sucrose content in green beans are related with coffee cup quality. Several papers also emphasize that the coffee quality is related with the fruit development velocity, in such way that the slower the development the higher the quality. In this sense, shading could favor coffee quality since this practice might modify the microclimate and retard the fruit cropping. In this work the effect of four coffee tree intercropping systems on the chemical composition of the green beans and on the beverage sensory profile was evaluated. Fully washed beans of *Coffea arabica* cv. Icatu Vermelho IAC-4045 grafted on Robusta IAC-Apoatã were tasted and chemically analyzed. Results revealed that the shading did not exerted significant statistical effect on the chlorogenic acid content, total titratable acidity or on the overall quality and final score of the coffee beverage comparing with the full sun grown samples. In comparison with this sample, little but statistically significant effects were observed on the trigonelline contents in grains from rubber and sucrose from coconut shaded coffee trees.

INTRODUCTION

The main purposes of the coffee tree intercropping are to favor mild climatic conditions proper to coffee growing. The intercropping system affects the local water availability, the CO_2 flux, the maximum and minimum air temperatures (Caramori et al., 1996; Pezzopane et al., 2003) and the wind's deleterious effects (Camargo & Pereira, 1994). The spacing between shade plants varies according their size and solar irradiation level of the region but the shade must allow enough solar irradiation for the coffee tree development. Therefore, it is suggested that the shade is not higher than 50% in some regions like that of the Brazilian northeast and is of around 1/3 in less irradiated coffee growing areas (http://www.ceplac.gov.br/radar/cafe.htm; Camargo, 2004)

Besides to improve the microclimate and to increase the environmental sustainability, the consortium of coffee tree with other plants represents an opportunity to improve the farmer income during times of overproduction. In this sense, rubber trees, coconut trees, avocado trees, banana trees and macadamia trees are good examples of plants used in the intercropping systems.

The productivity of the shaded coffee trees may be or may be not lower than of the unshadade plant depending on the shade level and on the agronomic technology employed (Peeters et

al., 2003). Since the plant management is appropriate the productivity of unshaded plantation is higher. (Muschler 1997, cited in Peeters et al., 2003).

The shade effects may be observed not only on the productivity but also on the chemical composition and on the grain and beverage quality, as well as on the chemical composition of the seeds as a consequence of the loading and of the fruit maturation velocity. However, the shade effects depend on the variety and on the shading level. (Vaast et al., 2003; Mushler, 2001).

The results presented herein are from a study aiming to evaluate the shade effects on the microclimate, on the beverage sensory and seed quality, on the productivity and on the chemical composition of the green bean of full sun and shaded coffee trees cultivated in Mococa, SP, Brazil.

MATERIAL AND METHODS

The green beans were from the growing cycle 2005/2006, provided by a study carried out with *Coffea arabica* cv. Icatu Vermelho IAC-4045 grafted on Robusta IAC-Apoatã planted in Mococa (latitude: 21^{o} 28' S; longitude: 47^{o} 01' W; altitude: 665 m) in full sun and in intercropped systems with grevilea trees (*Grevilea robusta*) (16x16m), rubber trees *Hevea brasiliensis* Willd. ex A. Juss.) (16x16m), banana (*Musa sp*.) "Prata Anã" (8 x 8 m) e green dwarf coconut (*Cocos nucifera* L.) (8 x 8 m). An area of 40 x 40 m with 4 m between rows and 1 m between coffee trees were used in each treatment.

The sucrose content quantification was based on the relationship between the sample and standard solutions pick areas using HPLC-PAD (Dionex) with CarboPack PA1 column and isocratic eluition with 50 mM NaOH (Salva et al. ASIC 2006). Caffeine, trigonelline and total chlorogenic acids (CGA) were simultaneously extracted in 70% methanol at 60 ^{o}C and analyzed in HPLC operating with C_{18} column and isocratic eluition with MeOH:AcOH:H_2O (50:0.5:49.5, v:v:v), and flux of 1 mL min^{-1}. The concentration calculations were based on the pick areas of the standard solutions and samples. Total titratable acidity was measured by titration with 0.1 N NaOH to pH 8.2 (method 30.1.07-AOAC, 1998).

The sensory analyses of the coffee beverage was performed by 3 tasters and assessed by overall quality and final score.

RESULTS AND DISCUSSION

Chlorogenic acid concentration in coffee green beans is close related to the fruit maturation stage and reduces as the fruit reaches the full ripening. The intercropping systems with grevilea, banana "Prata Anã", green dwarf coconut and rubber trees did not affected the CGA concentration comparing with the results of the full sun coffee samples (Table 1).

Neither total titratable acidty nor beverage overall quality and final score of the shaded coffee differed significantly of the unshaded coffees (Table 2).

Table 1. Effect of the shade coffee tree on the chemical composition of fully washed green beans of *Coffea arabica* cv. Icatu Vermelho IAC-4045 grafted on Robusta IAC-Apoatã grown in Mococa, São Paulo State, Brazil.

Shade Tree		Content (%db)			
		Sucrose	Trigonelline	CGA	Caffeine
Rubber Tree		8.6b	1.47a	5.02a	1.21a
Banana Tree		8.6b	1.39ab	4.95a	1.16ab
Grevilea Tree		8.3b	1.39ab	4.94a	1.16ab
Coconut Tree		9.1b	1.44ab	4.83a	1.14b
Full sun		8.61	1.36b	4.88a	1.18ab

Table 2. Sensory quality and total titratable acidity of the consorted *Coffea arabica* cv. Icatu Vermelho IAC-4045 grafted on Robusta IAC-Apoatã grown in Mococa, São Paulo State, Brazil.

Shade Tree		Tot. Trit. Acidity (db) mL0.1N NaOH $100g^{-1}$		Sensory Evaluation	
				Overall Quality	Final Score
Rubber Tree		96.1a		72.7	73.3a
Banana Tree		98.1a		72.7a	75.2a
Grevilea TTrTree		92.9a		79.1a	77.6a
Coconut Tree		96.2a		72.1a	71.7a
Full sun		93.1a		74.7a	74.0a

However, some experiments have showed that effect of the shading on the coffee crop may be different in different years (Vaast et al., 2005). Thus, complementary results are being collected in order to conclude the study.

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Coffee Flavour Modulation – Favouring the Formation of Key Odorants upon Roasting

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SUMMARY

The formation of 2-furfurylthiol and α-diketones upon coffee roasting were studied by biomimetic in-bean experiments and spiking of green coffee beans with potential labelled and unlabelled precursors. The target analytes in the roasted coffee samples were analysed in terms of isotope labelling pattern and abundance. The biomimetic experiments suggest that 2-furfurylthiol (FFT) is most likely not generated *via* furfural, which is in contrast to literature model studies (Tressl et al., 1993; Grosch, 1999). Incorporation of the arabinose C_5-skeleton into FFT could not be confirmed. However, our study showed that smaller arabinose fragments are integrated into the FFT molecule. The role of free amino acids and free sugars in the formation of α-diketones was clarified. In conclusion, the results of the biomimetic in-bean experiments emphasized the potential of this methodology for the verification of formation pathways in complex food systems such as coffee. Furthermore, it represents a tool for the evaluation of options to modulate the aroma profile of R&G coffee.

INTRODUCTION

The formation of important coffee aroma compounds that belong to the group of thiols and α-diketones has been extensively studied in model systems under dry heating conditions (Tressl et al., 1993; Grosch, 1999; Yaylayan and Keyhani, 1999). In arabinose/cysteine model experiments, Tressl et al. (1993) showed that 2-furfurylthiol (FFT) is formed *via* 3-deoxypentosone and furfural while maintaining the intact carbon chain. Further experiments with polysaccharides isolated from green coffee and roasted in the presence of cysteine performed by Grosch (1999) provided evidence that arabinogalactanes are key precursors of FFT. According to Yaylayan et al. (1999), α-diketones are formed from mono-, di- and oligosaccharides by Maillard-type reactions in the presence of amino acids such as glycine and alanine. Besides the direct formation from the carbohydrate skeleton, the recombination of transient intermediates has been discussed (Schieberle et al., 2003).

As shown by Limacher et al. (2007), the conclusions from model systems have to be taken with care and cannot simply be extrapolated to complex food products. Hence, to study the importance of precursors for the formation of key aroma compounds during coffee roasting under real conditions, Milo et al. (2001) developed the so-called biomimetic in-bean experiments. The authors incorporated various precursors into green coffee and quantified the key odorants generated. Using targeted omissions, they could show that 2-furfurythiol is mainly formed from water non-soluble precursors as increased FFT amounts were found in roasted coffee from water-extracted, exhausted beans. This methodology is a potential tool when it comes to studying the modulation of coffee flavour. Therefore, our study aimed at modulating coffee flavour based on the chemical understanding of formation pathways of character impact aroma compounds such as 2-furfurylthiol (FFT) and α-diketones.

EXPERIMENTAL

Preparation of bio-mimetic in-bean experiments

Hot water extraction of green coffee beans

Green coffee beans were extracted with hot water as reported in the literature (Milo et al., 2001) using some modifications. 70 kg of green Arabica coffee beans were extracted consequently four times with demineralised water at 95 °C for 2 h to obtain the water soluble substances. The extracts were collected and concentrated to a total solid content of approximately 27 %. After that, both the exhausted beans and the aqueous natural green bean extract were freeze-dried and stored at -20 °C until use.

Incorporation of bio-mimetic recombined extract (BRE)

The reconstituted green coffee extract (BRE), based on analytical results of the water soluble green coffee composition, was dissolved in demineralised water at 80 °C. 50 g of water were used for 125 g exhausted beans (EB) in order to guarantee a complete incorporation of the model solution into the coffee beans. The pH value of the compounded water soluble fraction was adjusted to pH 5.5 (corresponding to the pH of the natural extract), and water exhausted green coffee were soaked with the BRE at 50°C for at least 5 h. During soaking, the beans were gently stirred using a Rotavapor.

Omission experiments

BRE extracts were reincorporated in the exhausted coffee beans after omission of potential precursors or precursor groups. The compounded extracts did not contain any free sugars or all free amino acids. For mechanistic studies, D-[U-$^{13}C_5$]-arabinose (0.9 g/150 g beans) or [U-$^{13}C_6$-fructose]-sucrose (2 g/150 g beans; about 17 % of natural content) were added to the compounded model extract, which was omitted in all sugars, and incorporated into the water extracted, exhausted green beans. Additionally, green coffee beans were spiked with L-[3-^{13}C]-alanine (0.48 g/150 g beans).

Spiking of precursor compounds

Reference green beans were fortified with equimolar amounts of different sugars (approximately 4 g of each sugar), sucrose (about 50 % of the natural amount in coffee), cysteine (0.45 g/150 g beans) as well as alanine (0.48 g/150 g beans). Each of the precursors was dissolved in 60 g demineralized water and green coffee beans (150 g) were soaked with the prepared solution for 2 h at 50°C and for 2 h at room temperature.
All green coffee samples were roasted under the same conditions (380 s, 236°C).

Instrumental conditions

Quantification by Solid Phase Micro Extraction (SPME) combined with GC/MS

R&G coffee was suspended in hot water to obtain a slurry and after cooling spiked with defined quantities of labelled isotopes of the analytes. The prepared coffee suspensions were equilibrated (60 min, 20°C) in the sealed vials and the aroma compounds were extracted from the headspace (10 min, 40°C) using SPME (2 cm fibre coated with PDMS/DVB/Carboxen; Supelco, Bellefonte, PA, USA). Aroma compounds were thermally desorbed in the injector port of the GC at 240°C coupled to a DSQ mass spectrometer (Quadrupol, Thermo,

Brechbühler, Zürich, Switzerland). Separation of compounds was achieved on a polar silica thin-film capillary (ZB-Wax, 60 m × 0.25 mm; film thickness, 0.25 µm; Zebron, Brechbühler, Switzerland). Absolute concentrations are expressed as relative amounts compared to the reference set at 100 % (biomimetic recombined beans and green coffee).

RESULTS

2-Furfurylthiol (FFT)

The omission of sugars in the biomimetic recombined green coffee resulted in significantly increased amounts of FFT, whereas furfural content was highly suppressed to less than 40 % as compared to the reference R&G from green coffee beans reconstituted with recombined coffee extract (BRE; see Figure 1A). Spiking experiments also did not show a relationship between the formation of FFT and furfural as fortification of green beans with sucrose (50 % of natural content) increased furfural amounts up to 160 %, whereas concentrations of FFT considerably decreased (Figure 1B). Hence, it seems that the formation of FFT during coffee roasting *via* furfural as intermediate compound is of minor role. In addition, incorporation of D-[U-$^{13}C_5$]-arabinose did not yield in fully labelled FFT as it would be expected, but partially labelled FFT with $^{13}C_1$, $^{13}C_2$ and $^{13}C_3$-moieties (Figure 2A). In line with these data, spiking of green coffee with arabinose did not result in increased amounts of FFT nor furfural, which confirms the conclusion of Milo et al. (2001) that FFT is mainly formed from the non-water soluble fraction. In contrast, spiking experiment (Figure 1B) with cysteine resulted in enhanced FFT amounts, thus indicating cysteine as a suitable sulphur source.

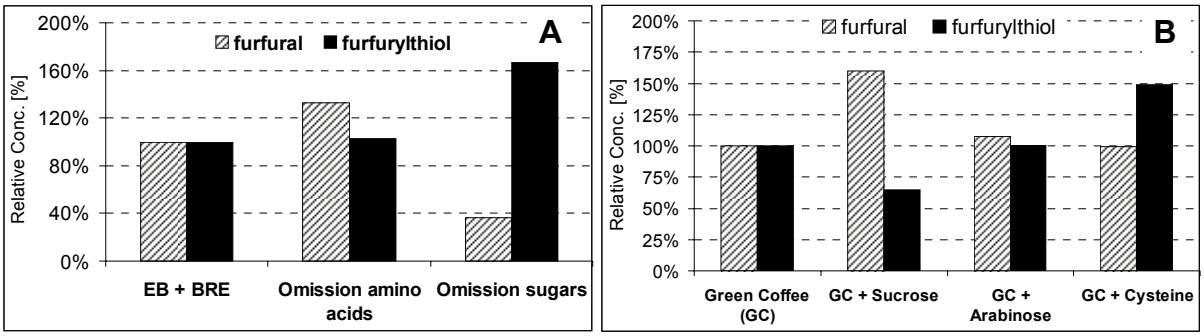

Figure 1. Omission experiments (A) and spiking of green coffee with sugars (equimolar amounts) and cysteine (B). Water exhausted beans (EB), biomimetic recombined extract (BRE).

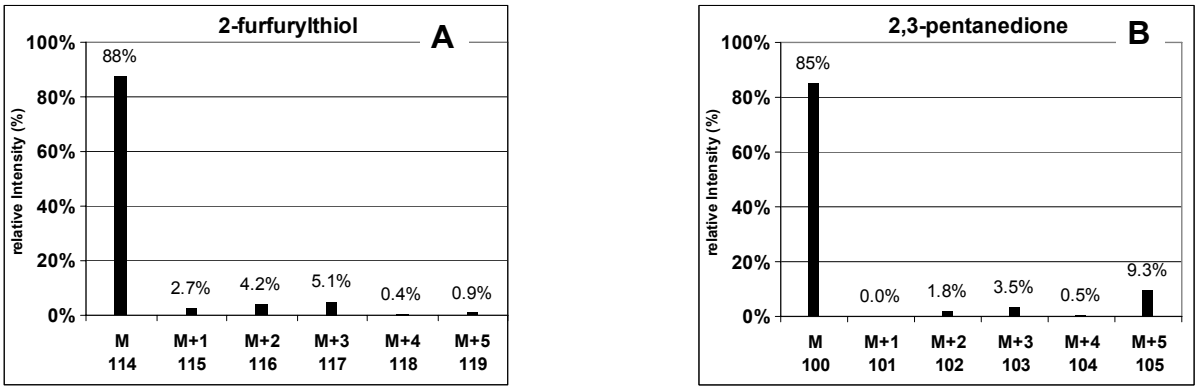

Figure 2. Incorporation of D-[U-$^{13}C_5$]-arabinose (16% of natural content) (A), and [U-$^{13}C_6$-fructose]-sucrose (17% of natural content) (B).

Diketones

Milo et al. (2001) showed in biomimetic experiments that diketones are reduced by more than 50 % when the green beans are water extracted. This result could be confirmed in our study using water extracted and roasted beans, where in particular the 2,3-pentanedione content was highly reduced (results not shown). In order to investigate the function of involved precursors, either free amino acids or free sugars were omitted in the biomimetic recombined beans. Quantitative analysis of the roasted beans revealed that the free amino acids did not influence the content of the evaluated α-diketones, as their concentrations were not affected by the omission of amino acids (Figure 3A). Furthermore, spiking of green coffee with an excess of L-alanine (Figure 3B) resulted in only small increase of said compounds. These results demonstrate that bound amino compounds like peptides and proteins can be considered as the main source of key amino acids involved in the formation of α-diketones. In contrast to the free amino acids, free sugars were found as a limiting factor in the formation of α-diketones, as their omission resulted in considerable reduction of both compounds, i.e. 2,3-butanedione decreased by more than 60% and 2,3-pentanedione by almost 90% (Figure 3A).

Incorporation of labelled precursors underlined the role of free sugars in diketone formation. While spiking of green coffee with an excess of L-[3-^{13}C]-alanine (0.48 g/150 g beans) resulted in only 5% $^{13}C_1$-labeled 2,3-pentanedione (not shown), the incorporation of [U-$^{13}C_6$fructose]-sucrose (17 % of natural content) yielded in considerable amounts of fully labelled 2,3-pentanedione (9.3 %) and to a lesser amount partially labeled M+2 (1.8 %) and M+3 (3.5 %) pattern (Figure 2B).

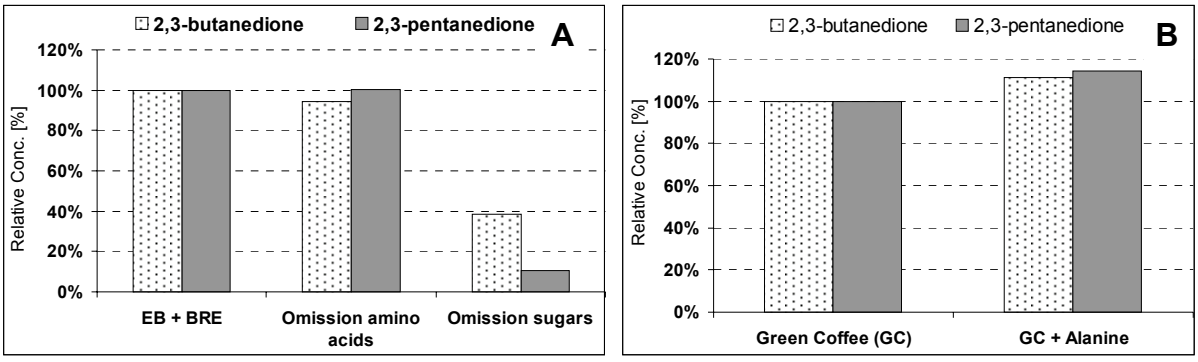

Figure 3. Omission experiments (A) and spiking of green coffee with alanine (B).

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Sensory Profiling and External Preference Mapping of Coffee Beverages with Different Levels of Defective Beans

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SUMMARY

The use of defective coffee beans in commercial blends is a common practice in Brazil. The objectives of the present study were to measure the sensory profile of coffee beverages prepared with the addition of different levels of defective beans to good quality beans, using a trained sensory panel and to investigate the consumer acceptance of the same samples, applying preference mapping analysis to address the relationship between descriptive and acceptance data. Three consumer segments were identified. Two consumer segments (67% of participants) preferred the good quality sample followed by the beverages with low level of defective coffee beans. Those samples were characterized by the trained panel as: sweet taste, characteristic aroma and flavour, and body. The third segment (33% of participants) preferred the beverages with high percentage of defects, which were described as bitter, chemical, green, burnt. Consumers in this segment did not like the good quality sample. The present results showed that although two thirds of the consumers were able to recognize and preferred good quality coffee, the remaining consumers preferred coffees containing high amounts of defective beans probably due to the acquired habit of consuming low cost/quality coffees available in the market.

INTRODUCTION

Brazil is the first world coffee producer and exporter (IBGE, 2008). Because most coffee in Brazil is harvested by the stripping method, significant amounts of immature, over-ripe and fermented seeds are observed after primary processing. These intrinsic types of "defective" beans are mechanically sorted in cooperatives, along with other types of defective and non-defective beans. For economical reasons, this low quality mixture is sold in the internal market under the name of "PVA", which stands for black (P), immature (V) and sour (A) defective beans (Pereira, 2003). Therefore, the practice of adding a small proportion of PVA to good and medium quality coffees is common in the Brazilian coffee market.

The presence of PVA in coffee blends may change sensory characteristics, yielding a more astringent and acidic beverage (França et al., 2005) and, for trained assessors, different types of defects may produce different undesirable notes (Deliza et al., 2007). Since the proportion of each individual defect in PVA mixture may vary depending on several factors, such as genetics, climate, and post harvesting methods, PVA composition may also be reflected in cup quality in different ways and intensities.

Considering that variations in taste attributes have an impact on hedonic responses (Gonçalves, 2006), this study aimed at profiling coffee beverages prepared with different levels of added defects to good quality beans, with the help of a trained sensory panel. In addition, we measured the consumer acceptance of these beverages and applied Preference

Mapping analysis to address the relationship between descriptive and acceptance data, and to identify the sensory attributes driving consumer liking.

MATERIALS AND METHODS

Good quality coffee beans (type 2) and defective beans obtained by mechanical sorting were from the Mogiana area, in São Paulo State, Brazil. Coffee beverages were prepared from six samples: 100% good quality beans (control), four blends containing different levels of defective beans (10%, 20%, 30% and 40%) added to control beans, and 100% of defective beans. The defective bean mixture or PVA, contained about 8.5% of black, 5.6% of immature and 6.3% of sour beans.

Ten selected and trained assessors evaluated the six samples using the sensory descriptors achieved from the Quantitative Descriptive Analysis (QDA) procedures, as recommended by Stone and Sidel (2004). Sixty coffee consumers (at least one cup a day) evaluated the same samples regarding acceptance using the 9-point hedonic scale varying from "disliked extremely" to "liked extremely". Both the trained panel and the consumers tasted all samples in 50mL porcelain cups coded with three digit numbers, monadically presented at 65±1 °C. The sample presentation order was balanced (MacFie et al., 1989) to prevent carry over effects and water and biscuits were provided to participants as a cleanser between samples. Data were analyzed using Preference Mapping (MacFie, 2007), Cluster Analysis and ANOVA.

RESULTS AND DISCUSSION

The means of the sensory attributes evaluated by the trained panel are presented in Table 1. The beverages with higher amount of defective beans were perceived as having higher intensity of the attributes burnt, chemical, green, astringent and bitter. On the other hand, the attribute body was perceived as less intense.

Table 1. Sensory attribute means for the analyzed coffee beverages[§].

	Sensory attributes[§§]									
	Characteristic	**Cereal**	**Burnt**	**Chemical**	**Green**	**Astringent**	**Body**	**Sweet**	**Bitter**	**Sour**
Control	6.4a	1.4	1.5b	0.9a	1.2b	1.7b	4.6ab	3.5	4.5b	3.8
10% DB	5.2ab	1.6	2.4b	1.9ab	1.8b	3.3a	4.7ab	3.3	5.1ab	3.8
20% DB	4.9ab	1.0	3.3ab	1.0ab	2.1ab	2.6ab	4.3ab	2.2	5.4ab	3.8
30% DB	4.7b	1.4	3.1ab	1.6ab	1.7b	3.1ab	4.3ab	2.9	5.8ab	3.1
40% DB	5.4ab	1.5	3.1ab	1.6ab	2.1ab	2.7ab	5.3a	2.6	5.3ab	3.5
100% DB	4.0b	2.7	4.5a	2.5b	3.3a	3.7a	4.0b	2.1	6.3a	4.3

[§]*Control= good quality beans; DB= defective beans.*
[§§]*Evaluated using non structured scales varying from 0: absent. 1: weak a 9: strong, very much.*

Figure 1 shows the results taking into account the sensory attributes and the consumer preference for the samples. The first and second dimensions accounted for by 80.6% of the variance.

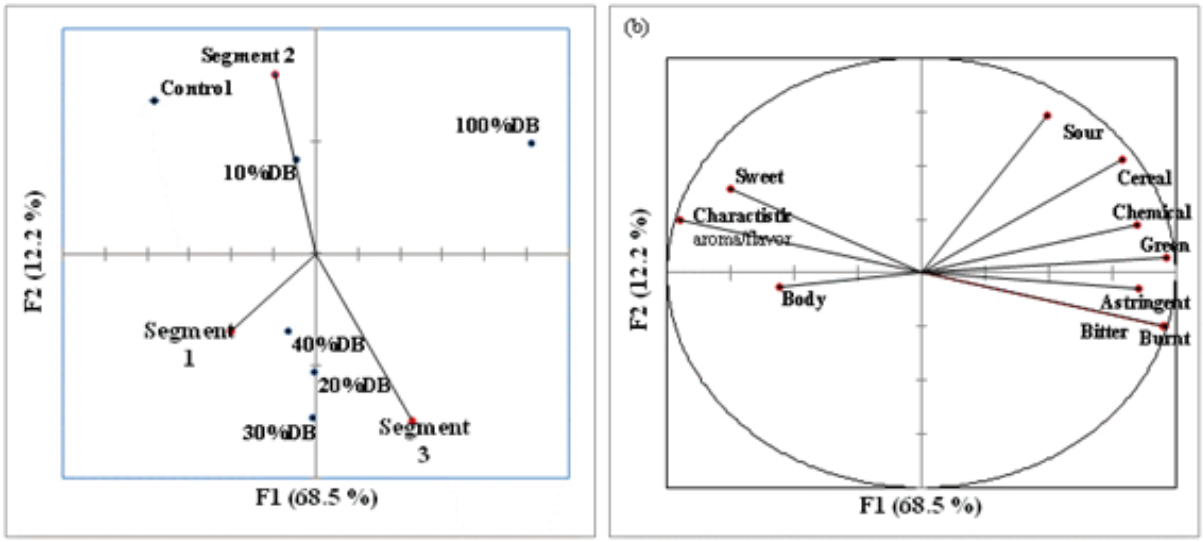

Figure 1. Preference Mapping showing (a) the position of the samples and segments of consumers and (b) the position of the sensory attributes. (DB = defective beans)

The dimension 1 separated the beverages in three groups, from the right to the left side of the map, as follows: 100% defective beans, the four blends with added defective beans, and the control sample (100% good quality beans). Probably the low amount of black (8.5%), immature (5.6%), and sour (6.3%) beans in the defective beans might have contributed to the difficulty in discriminating the blends from the other two samples (good quality and 100% DB).

This study revealed the sensory descriptions of coffee beverage which drove the acceptance of the three identified consumer segments. Two consumer segments (67% of participants) preferred the control sample followed by the beverages with low level of defective beans. Those samples were characterized by the trained panel as: sweet taste, characteristic aroma and flavour, and body. The third segment (33% of participants) preferred the beverages with high percentage of defects, which were described by the panel as bitter, chemical, green, and burnt. These consumers did not like the control sample.

CONCLUSION

The present results showed that although two thirds of the consumers were able to recognize and preferred good quality coffee, the remaining consumers preferred coffees containing high amounts of defective beans, probably due to the acquired habit of consuming low cost/quality coffees available in the market. Brazilian coffee industry should be aware of the existence of the large segment of consumers who appreciates good cup quality coffee when launching new coffee products and brands in the market.

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Analysis of Volatile Compounds of Roasted Coffee Submitted to Different Post-Harvest Processes

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SUMMARY

The objective of the present work was to identify and quantify volatile compounds of roasted coffee considering different post-harvest processes. In order to conduct the experiment, green cherry and parchment coffee were chosen. They were dried at 40, 50 and 60 °C, unpeeled, roasted, crunched and submitted to qualitative and quantitative analysis of their volatile compounds extracted by solid phase micro-extraction. The quantification of volatile compounds was obtained by CG-FID and the identification by CG-MS. Drying temperature did not influence the diversity of compounds in each sample. However, it causes quantitative differences of the same. Pyrazine was identified only in natural coffee. The content of most compounds decreased as drying temperature increased, except methilpyrazine and acetic acid.

INTRODUCTION

Generally, coffee grain presents in its chemical composition several volatile and non-volatile compounds of different intensity and concentrations. They are represented by acids, aldehydes, ketones, sugars, proteins, aminoacids, fatty acids, carbohydrates, trigolein, phenolic compounds, caffeine, and enzymes which act upon these components (Menezes, 1994). The contribution of each one of these volatile compounds for the final aroma widely varies. Synergetic and antagonic interactions among different compounds may occur. The chemical composition may be modified during post-harvest processes, that means, the modification will heavily depend on the process, drying, storage, and roasting conditions (Afonso Junior, 2001). Researches have identified and quantified volatile compounds of coffee. However, their variation as post-harvest processes vary is not well explored yet.

Due to the importance of aroma in the quality and acceptance of coffee, the objective of this work was to identify and quantify volatile compounds of natural and parchment coffee, submitted to different drying temperatures after roasting.

MATERIALS AND METHODS

Green Cherry coffee, variety Catui, moisture content approximately 0,60 d.b., harvested at Fazenda Brauna at Araponga, MG, was used in this experiment. After harvest, part of the product has its parchment removed and the rest was kept with parchment. Moreover, samples were taken to the Laboratory of Physical Properties and Quality Evaluation at CENTREINAR, Federal University of Viçosa, Viçosa, MG. Coffee samples were dried under 40,50,abd 60 °C in ventilated ovens until reaching moisture content equal to 0,11 dry basis. The reduction of moisture content was measured adopting the gravimetric method (mass loss) using an analytical weightier.

Samples were crunched using liquid nitrogen. Then, 2 g of each sample were place inside a 5 mL recipient hermetically closed. The extraction of volatile compounds from coffee was conducted by "headspace" using a 75 mm Carboxen/poly(dimethylsiloxane) (PDMS) (CAR/PDMS) fiber in a oven at 40 °C for 15 minutes (Gonzales-Rios et al., 2007).

The identification of volatile compounds was conducted by CG/MS and the quantification by CG/FID, using Gonzales-Rios et al. (2007) methodology. Volatile compounds were identified by calculating Kovats Index. The quantification, in percentual concentration, was determined by the area over each chromatogram top.

RESULTS AND DISCUSSION

Among substances identified in Table 1, pyrazine was present only in samples of parchment coffee and its concentration (in percentage) increased as drying temperature increased. Pyrazine was only identified in natural coffee, which may give these samples an oil flavor.

Pyridine, methylpyrazine, 1-hydroxypropan-2-one, 2,5-dimetylpyrazine acetic acid, 2-furancarboxyaldyde and 5-metyl-2-furancarboxyaldeyde, 2-acetylfurane, 2,3-butanediol, dihydrofuranone, furfuryl alcohol were in all samples. However, their concentration varied as the process and drying temperature varied.

Methylpyrazine and 2,4-dimethylpyrazine behaved in the same trend. Their percentual concentrations in natural coffee were higher than in parchement coffee if dried under the same temperature. In both cases, their concentration increased as temperature increased, reaching 8,43% in natural coffee dried at 60 °C.

In parchment coffee, it was not observed a variation in the proportion of acetic acid. Its variation was only observed in samples of natural coffee due to the existance of high quantity of sugars in its mucilage, favouring the syntesis of the acid. The maximum concentration of acetic acid was 19,18% in samples at 60 °C. The concentration (in percentage) of most coumpounds decreased as temperature increased, except acetic acid and methylpyrazine, which potentialize the vinegar and burnt wood flavor in those samples.

Concentrations of furancarboxyaldeyde, dihydrofuranone, furfuryl alcohol, 2-acetylfurane and 5-metyl-2-furfural decreased as temperature increased, due to the possibility of these compounds to volatilize at higher temperatures.

CONCLUSION

In conclusion, drying temperature does not alter compounds diversity. However, it directly influences its percentual concentrations. Also, the type of process distinctly characterizes the coffee, specially if high quantities of acetic acid and the presence of pyrazine in samples of natural coffee are under concern, due to the decisive influence on its aroma and flavor.

Table 1. Identification of volatile compounds in natural (C) and parchment (P) coffee, dried at 40, 50 and 60º C and their [1] Kovats Index Calculated; [2] Kovats Index tabled; [3]Concentrations (in percentage); [4] Compounds unidentified.

	C40		C50		C60		P40		P50		P60			
	$^{1}IK_1$	$^{3}C\%$	$^{*}IK_1$	$^{3}C\%$	$^{*}IK_1$	$^{3}C\%$	$^{1}IK_1$	$^{3}C\%$	$^{1}IK_1$	$^{3}C\%$	$^{1}IK_1$	$^{3}C\%$	$^{2}IK_2$	Name
1	1184,16	1,62	1182,54	2,29	1181,95	2,98	1184,34	3,70	1183,33	2,74	1183,33	2,65	1185	Pyridine
2	1264,94	0,87	1264,37	1,65	1263,83	1,81	-		-		-		1231	Pyrazine
3	1281,39	7,60	1282,44	7,92	1280,36	8,43	1280,00	6,47	1277,42	6,53	1277,21	7,88	1288	Metyl pyrazine
4	1294,31	0,23	1292,97	0,12	1294,12	0,02	-		-		-			[4]NI
5	1319,71	0,42	1319,09	4,79	1318,69	5,49	1319,31	2,57	1319,77	6,07	1319,80	3,08	1323	1-hidroxypropan-2-one
6	1326,70	6,66	1325,67	5,17	1325,50	5,05	1325,19	4,15	1326,59	4,25	1326,62	4,99	1347	2,5-dimetylpyrazine
7	1354,84	0,27			-		1358,39	0,47	-		-			[4]NI
8	1368,30	0,07			1367,91	0,73	-		-		-		1351	1-hidroxybutan-2-one
9	1439,91	6,82	1444,7	16,71	1437,51	19,18	1451,76	13,88	1452,01	13,64	1449,66	13,31	1450	Acetic acid
10	1457,13	23,08	1456,86	9,88	1456,74	8,10	1456,71	13,58	1457,8	8,98	1456,99	8,56	1449	2-furancarboxyaldeyde
11	1463,79	0,13	1463,34	3,08	1463,62	3,45	1463,67	4,34	1463,67	3,14	1463,55	3,41		1,4-diacetate 2,3-dimetylenobutane
12	1494,21	3,24	1493,85	2,89	1493,66	2,70	1494,16	3,17	1494,55	2,48	1494,65	2,46	1536	2-acetylfurane
13	1525,99	1,99	1524	2,08	1524,75	2,02	1525,30	3,09	1526,73	2,26	1526,06	2,63	1556	[4]NI
14	1533,48	7,48	1532,78	3,60	1532,70	2,94	1532,49	5,35	1533,69	3,77	1533,56	3,25		5-metyl-2-furfuraldeyde
15	1609,28	0,28	1607,93	0,18	1609,06	0,18	1608,02	0,27	1607,45	0,17	1607,88	0,28		2,3-butanodiol
16	1661,25	1,65	1660,17	1,52	1661,15	2,67	1660,04	1,62	1661,20	1,57	1660,55	1,55		Dihidrofuranone
17	1669,80	18,38	1669,80	22,62	1669,80	22,67	1669,77	25,31	1699,54	23,62	1699,70	22,08		Furametanol
18	1705,72	0,28					-		-		-			[4]NI

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Evaluation of Variety Cupping Profile of Arabica Coffee Grown at Different Altitudes and Processing Methods in Gayo Highland of Aceh (Sumatra)

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SUMMARY

A survey to evaluate cupping profile of Arabica coffee varieties grown at different altitudes was made at Gayo highland of Aceh (Sumatra). Gayo highland is the main Arabica coffee growing in Indonesia where about 70,000 ha of the area are covered by the coffee, of which about 45,000 ha are productive husbandry. Coffee from the area is mostly marketed at specialty segment using various names such as Sumatra Mandheling Coffee or Mandheling Gayo Coffee. In Gayo highland Arabica coffee is grown from about 900 m to 1,700 m. Recently several varieties are grown by the farmers without any sufficient cup profile information background. The study was aimed to describe cupping profile of each variety at different altitudes and processing methods in order to provide information to specialty coffee industry in the region. Evaluation was made for 9 varieties at three different altitude categories namely low (< 1,250 m), medium (1,250-1,400 m) and high (> 1,400 m). Coffee cherry samples were collected from farmers field then processed by two different wash methods consisted of "wet hulling" (local or traditional method) and "dry hulling" (full wash method). Cupping evaluation was done at Quality Laboratory of ICCRI and by selected exporters in addition to by selected overseas buyers involves in the specialty coffee industry. Preliminary result showed that no significant different between wet hulling and dry hulling processes on cupping profile. There was an indicator if coffee cupping profile affected by altitude. Based on cupping profile it was described that varieties of P 88, S 288 and Timtim are promising for Gayo highland.

INTRODUCTION

Recently specialty coffee market grows gradually especially in the main consuming countries. For instance, NCA (2008) reported that gourmet (specialty) coffee consumption for adults in the US increase from 14% to be 17% in 2007 and 2008 respectively. Specialty will be a good opportunity for coffee industry in the near future.

Indonesia export some 60,000 ton (1 million bags) of Arabica coffee (AEKI, 2008), of which mostly goes to specialty market segment. The main source of Arabica coffee is Sumatra mainly in North Sumatra and Aceh Provinces. In Aceh Provinces Arabica coffee is grown at Gayo highland where recently is estimated covered by some 70,000 ha.

The coffee growing area at Gayo highland shares about 50 % of the national production. Specialty Arabica coffee from Sumatra is commonly traded with several names such as Sumatra Mandheling, Lintong, Blue Batak, Retro Mandheling, Sumatra Gayo, Mandheling Gayo, Gayo Mountain Coffee, etc.

All of coffee husbandries in Gayo highland are managed by smallholding farmers with average ownership about 2 ha per household. One of the main problems arises in Gayo highland is a lot of coffee varieties used by the farmers. Several farmers made their own variety selection and grown it progenies at their farms, that several varieties have not been recognized well its cup quality profile.

This study was made in order to support specialty coffee industry in Gayo highland in obtaining unique cup quality consistently since variety provide significant effect on the quality.

MATERIALS AND METHODS

The study was carried out by making survey over Arabica coffee growing in Gayo highland. Nine varieties commonly grown grown by the farmers were identified at three different altitudes of < 1,250 m, 1250-1400 m and > 1,400 m. The varieties were Bergendal (Typica), Borbor (farmer's selection), Catimor Jaluk (farmer's selection), S 288 (Indian selection), S 795 (Indian selection), P 88 (introduction), C 50 (introduction), BP 452 A (ICCRI's selection) and Timtim (Gayo station selection).

Coffee cherries of each variety at different altitude were collected, 200 kg per picking. The cherries the processed by two ways namely fully wash (dry hulling) and local wash (wet hulling). The main different of the two processing methods are at hulling stage and drying process. On the local wash parchment removal is done when the parchment beans still wet (30-40 % moisture content) and the drying process is conducted over green beans.

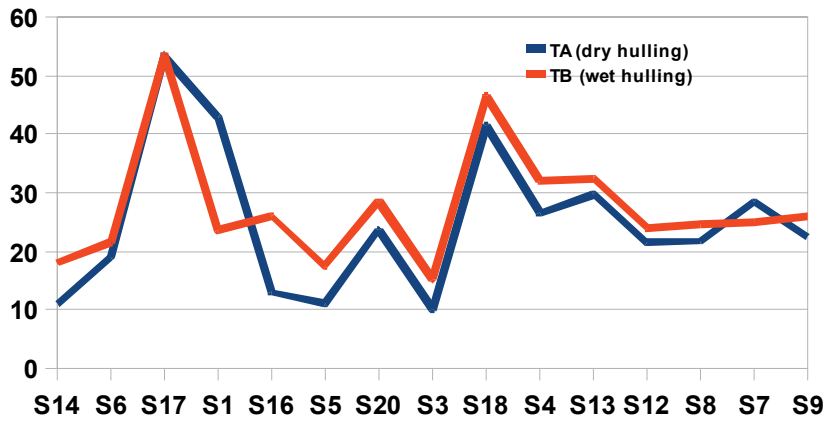
Cup quality evaluation was carried at the laboratory of ICCRI as well as specialty coffee exporters in addition to specialty coffee importers. Taste profile parameters used in this study was fragrance/aroma, flavor, body, acidity and balance. Simple statistical analysis was used to make inference of the study.

RESULT AND DISCUSSION

Effect of Processing Method

Traditionally coffee farmers in Northern Sumatra (including Gayo highland) do wet processing by applying “wet hulling”. Soon after picking mature cherries the farmers remove coffee pulp by using traditional hand pulper machine. After fermenting one night they wash their coffee and put for one to two days sun-drying, the they sell the humid parchment beans to local trader. Parchment removal of the coffee is done when the bean still moist, normally at 30-35 % moisture content. The traders dry the green coffee until 12 % moisture content (Susila, 2005).

Statistical analysis to compare total cup profile score means of all treatments showed not significantly different between “dry hulling” and “wet hulling” (Figure 1). “Wet hulling” processing provided more intent color on green beans namely grayish to blueish (Figure 2) for all varieties collected from different altitudes.



a

b

Legend:

S1 (Borbor; 900 m),	S3 (CJ; 1,050 m),	S4 (Timtim; 1,240 m),
S5 (BP 542 A; 1,300 m),	S6 (Borbor; 1,300 m),	S7 (CJ; 1,240 m);
S8 (S288; 1,240 m);	S9 (Timtim; 1,350 m),	S12 (CJ; 1,400 m),
S13 (P88; 1,400 m),	S14 (C50; 1,400 m),	S16 (Bergendal; 1,480 m),
S17 (S288; 1,350 m),	S18 (Timtim; 1,520 m),	S20 (S795; 1,350 m).

Figure 1. Effect of parchment removal stage on coffee bean. a. Comparing mean of overall cup taste profile parameters between "dry hulling" and "wet hulling". b. Color performance of green coffee.

Effect of altitude

Selection for bean and liquor qualities is highly constrained by the prevalence of large genotype-by-environment (G x E) interactions (Agwanda et al., 2003). There was an indicator that higher altitude provided better cup quality varieties on S 288, Catimor Jaluk and Borbor varieties. This indicator was in line to the report of Coffee Research Institute (2001). Exception was found on variety of Timtim where lower growing area provided better cup profile. One of the weaknesses in this survey was difficulties to obtain all varieties studied at different areas (Table 1).

Table 1. The effect of altitude on cup quality of Arabica coffee varieties grown in Gayo.

Varieties	Altitude			
	< 1,240 m	1,250 – 1,400 m	> 1,400 m	Average
P88	n.a.	n.a.	37.1	37.1
S 288	33.5	37.4	n.a.	35.5
TIM-TIM	36.0	33.1	33.1	34.1
S 795	n.a.	33.4	n.a.	33.4
C50	n.a.	n.a.	33.1	33.1
CJ	31.2	33.7	34.2	33.1
BOR-BOR	33.0	32.4	n.a.	32.7
Bergendal	n.a.	n.a.	32.0	32.0
BP 542 A	n.a.	30.9	n.a.	30.9

Promising Varieties for Gayo Highland

Result from cluster analysis based on 6 cupping parameters showed P 88, S 288 and Timtim as promising varieties for Gayo highland. The three varieties is grown at medium altitude (1,250-1,400 m). P 88 also performanced good cup quality profile with high fruity character under East Java condition (Yusianto et al., 2007).

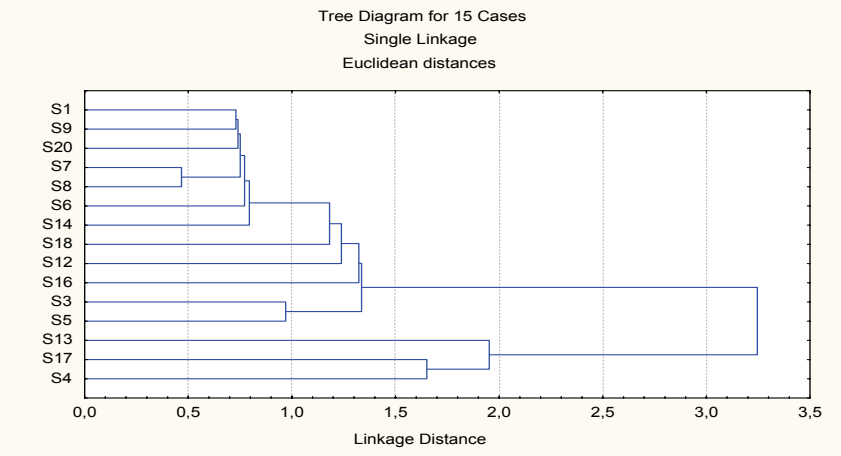


Figure 2. Cluster analysis of Arabica coffee varieties grown at different areas in Gayo highland (Legend: see Figure 1)

P 88 and S 288 showed very similar cupping profile. The two varieties provides very good fragrance and aroma, acidity, flavor, body and after taste as well as nice balance. Timtim also showed very good cup profile but bit less than the other two as well as less balance.

CONCLUSION

1. "Wet hulling" and "dry hulling" on wet processing of Arabica coffee cherries performed not significantly different effect to cup profile. "Wet hulling" method provided more intent green bean color than that of "dry hulling".
2. Promising varieties based on cupping profile for Gayo highland are P 88, S 288 and Timtim.

ACKNOWLEDGEMENT

The authors wish to acknowledge to the Provincial Government of Nanggroe Aceh Darussalaam Province through the APED Project and UNDP Indonesia in supporting budget that the study was possible. Our gratitude also goes to the Director of ICCRI for his permission and support in participating to this ASIC 2008 Conference.

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Sensory Typology: Method Used to Identify Homogeneous Groups of Coffee

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SUMMARY

The purpose of our study was to provide producing countries with an analytical approach to distinguish between coffees on a sensory basis. The aim was to establish clearly characterized groups of products capable of meeting the requirements of specific markets. The proposed method is based on studies conducted in several producing countries located in very different zones. Analyses were based on rational sampling in accordance with objectives, with the production structure in the country considered, and with prior knowledge of the variables affecting sensory quality. The latter was assessed by standardized sensory methods involving panels trained in descriptive analysis. A three-stage statistical analysis led to the establishment of coffee types. The types could be distinguished from each other significantly using major descriptors such as tastes (acid, bitter) or flavours (green, fruity). This typology provides a scientific contribution to often pre-existing commercial typologies.

RÉSUMÉ

Notre étude a pour objet de donner une démarche analytique aux pays producteurs pour différencier leurs cafés sur des bases sensorielles. Il s'agit d'établir des groupes de produits bien caractérisés pouvant répondre à la demande de marchés spécifiques. La méthode proposée repose sur des études réalisées dans plusieurs pays producteurs situés dans des zones très différentes. Les analyses sont effectuées à partir d'échantillonnages raisonnés en fonction des objectifs, de la structure de production du pays considéré et des connaissances préalables sur les variables influençant la qualité sensorielle. Celle-ci est évaluée par des méthodes sensorielles normées faisant appel à des panels descriptifs formés. Une analyse statistique en trois étapes permet de construire des types de cafés. Les types se différencient significativement sur certains descripteurs majeurs, comme les saveurs (acide, amer) ou les flaveurs (vert, fruité). Cette typologie apporte une contribution scientifique aux typologies commerciales souvent préexistantes.

OBJECTIVES

Producer countries wishing to satisfy the demand of targeted markets need to be able to describe the flavours and aromas of their coffees. The information must be clear and concise if it is to be of use to decision-makers (producer organisations or authorities). The method used to identify these coffee groups meets these objectives.

We put forward an analytic approach, whereby sensory data is collated and associated with different groups identified by statistical classification. These groups provide a way of linking sensory variables with other kinds (genetic, agricultural, environmental or socio-economic). The method put forward is based on studies carried out in several producer countries.

SAMPLING

The sampling protocol is designed for studying the influence of certain external factors on sensory characteristics. Other factors are standardised to restrict their influence on sensory characteristics (for example):

- Altitude was studied: several samples by altitude layer
- Variety was studied: several samples of the main varieties
- Year of harvest was studied: the study was repeated over several years
- Date of harvest was standardised: samples were taken only at the peak of the harvest season
- Post harvest factors were standardised: just one standardised protocol was used.

The sampling process is determined by the objectives, the production structure of the country in question and prior knowledge of variables that influence sensory quality. A description is made of the potential for coffee across a zone.

SENSORY MEASUREMENT

A descriptive sensory profile is produced for each sample of green coffee. The green coffee is sorted to eliminate defects due to postharvest handling and then roasted in a laboratory roasting appliance. Five grams of ground coffee are infused in 100 ml of water to prepare the drink. A descriptive panel (5 to 12 persons), trained in sensory analysis of coffee, note down a series of descriptors. The protocol is based on international sensory standards.

STATISTICAL DATA PROCESSING

Sensory data often displays a certain amount of unexplained variability. This variability is reduced by the use of Principal Component Analysis (PCA) (Figure 1). Only the first axes are kept and in this way part of the noise is eliminated (about 20%).

Groups are established by means of two standard typology methods (hierarchical classification and dynamic clouds), which complement each other. It is thus possible to obtain coffee types (Figure 2).

Variance analysis shows that these types differ significantly in terms of major descriptors, such as taste (acid, bitter) or flavour (green, fruity) (Figure 3). Variance analysis is used to explore links with other quantitative variables. Links with qualitative variables can be analysed using chi^2 tests (Table 1).

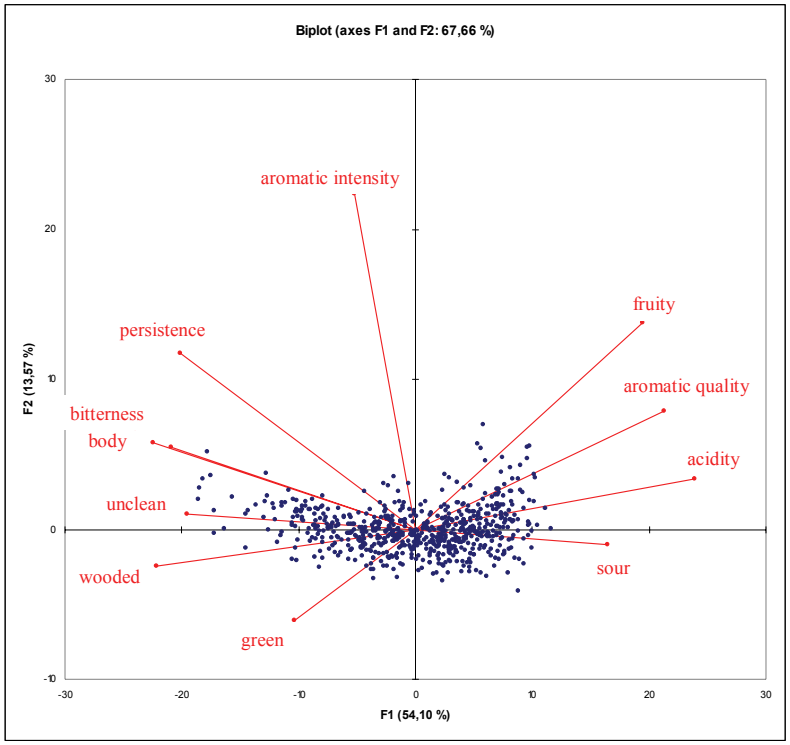

Figure 1. With PCA (Principal Component Analysis) it is possible to visualise the distribution of the sensory profiles of different coffees.

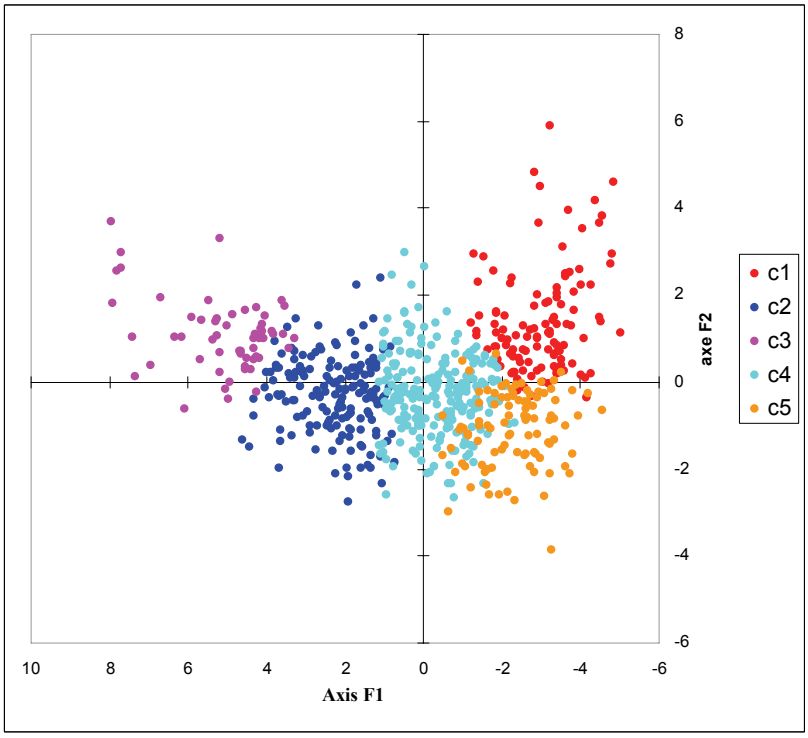

Figure 2. The classification methods are used to group together sensory profiles (in this example there are five groups designated c1 to c5). An initial structuring of the data emerges.

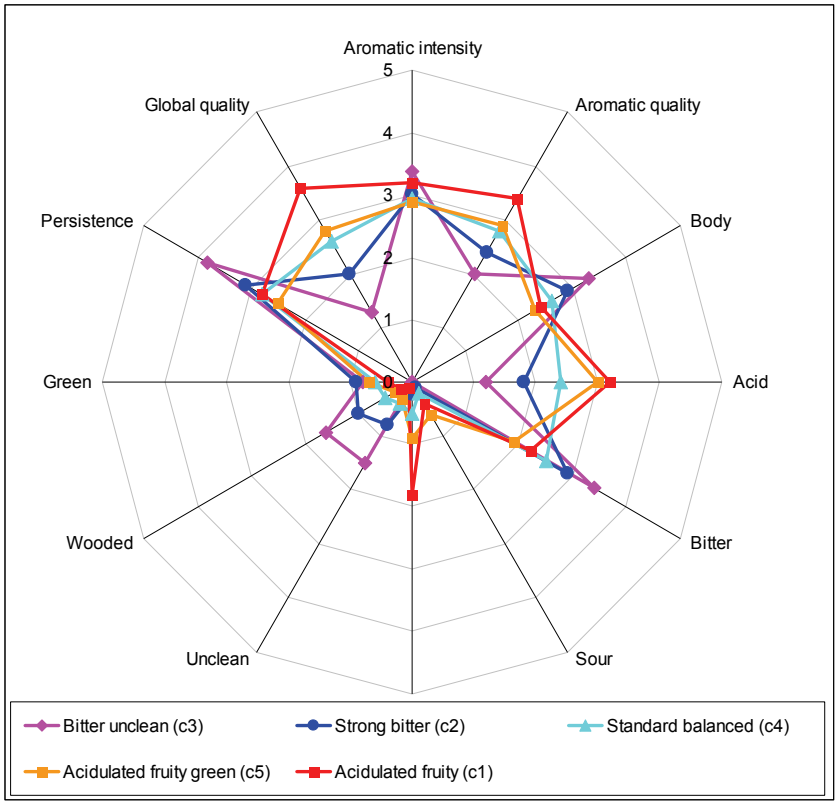

Figure 3. Average profiles of sensory groups. Using ANOVA (variance analysis), it is possible to see which sensory descriptors account for the significant differences between the groups.

Table 1. Comparison of granulometric distribution of samples by sensory group. The Chi-squared test shows that the distribution is not random.

		sensory groups				
		bitter unclean (c3)	**strong bitter (c2)**	**Standard balanced (c4)**	**Acidulated fruity green (c5)**	**Acidulated fruity (c1)**
granulometric groups	very small	3	11	7	3	0
	small	23	59	66	19	19
	big	22	52	89	26	41
	very big	8	37	66	37	45

Significantly more than the expected distribution

Significantly less than the expected distribution

CONCLUSION

By conducting sensory analysis while at the same time taking account of complementary data (environmental, geographical, socioeconomic, etc.), it is possible to use this coffee typology as a means of aiding decision-making in the producer countries. The use of this tool may help identify niche markets (for products of a certain origin or *terroir*) as well as mass markets, in which particular characteristics are sought (such as lightness or strength in coffee). This typology makes a scientific contribution to the commercial typologies that already exist in many cases.

Evaluation of Acceptability of Gourmet, Superior and Traditional Coffees

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SUMMARY

The aim of this paper was to evaluate the acceptability of three coffee samples of Gourmet, Superior and Traditional quality. This classification was confirmed by descriptive quantitative sensory analysis performed by a selected and trained team using a non structured scale from 0 to 10 cm to evaluate the fragrance of the grind and aroma, defects, acidity, bitterness, residual flavor, astringency and body of the beverage, with a final evaluation of their global quality. These samples were submitted to sensory evaluation by a group of 54 persons who consume coffee, responsible for their purchase, with no restrictions in relation to age, sex and social status. The samples were evaluated as to acceptability of color, aroma and flavor through 9 point hedonic scales. They were also evaluated using 5 point scales as ideal in relation to acidity and bitterness and through a 5 point scale related to the intention to buy. The samples were presented in a monadic manner with three number aleatory codes, according to an alignment of balanced blocks and served in 50 ml white plastic cups; sugar and sweetener were available and mineral water to be used between samples. The consumers were questioned in relation to the characteristics considered as priorities in choosing coffee and how much they agreed with the declaration: "The classification information on the coffee: Traditional, Superior and Gourmet, helps me in deciding to buy it". The test was made in individual booths lighted with fluorescent lamps and data collection was made using a *Compusense Five, version 4.8* computer software for sensory evaluation. In relation to the attributes of color and flavor, the Traditional coffee sample was considered to be better than the sample of Gourmet coffee at an error level of 5%. The Superior coffee sample with intermediate mean values did not differ from the other samples. Therefore, the consumers demonstrated better acceptance of the Traditional and Superior coffees. The information as to coffee brand, type of packaging and the Brazilian Association of the Coffee Industry – ABIC (Associação Brasileira da Indústria do Café) quality seal were considered a priority characteristic in choosing the coffee by over 50% of the consumers. The information on quality category was considered a priority by 38,9% of the consumers although 68,5% agreed with the declaration that in fact the information on coffee classification on the label helps in deciding its purchase.

INTRODUCTION

The State of São Paulo presented a Technical Standard to fix the identity and quality of roasted and ground coffee, Resolution SAA – 37 in 2001, used in purchasing coffees for public organisms. This Standard introduced descriptive quantitative sensorial analysis performed by a selected and trained team, using a non structured scale from 0 to 10 cm for evaluation of aroma, acidity, bitterness, flavor, foreign flavor, astringency and body of the beverage, with a final evaluation of the global quality and classifying by category of quality, separating the coffees in Gourmet, Superior and Traditional quality (Government Of The State of São Paulo, 2001).

This standard of coffee quality determined by the Government of the State of São Paulo, was adopted also by Municipal governments and other organisms, and even other States. The minimum acceptable level was raised from 3.5 points to 4.5 points in 2004 (Government Of The State of São Paulo, 2004). During this same year, ABIC created the Coffee Quality Program - PQC (Programa de Qualidade do Café) to inform the coffee quality which is being sold, besides allowing the consumer to identify the type of bean used by each brand and thus choose the flavor that satisfies best (ABIC, 2008). In 2007, the Government of the State of São Paulo reduced the lower limits of the global quality scale for the Superior and Gourmet coffees, equalizing them to the values instituted by ABIC in the PQC (Government Of The State of São Paulo, 2007).

The intention of both Resolution SAA-28 of June 01, 2007 in the State of São Paulo and the Program of Coffee Quality of the Brazilian Coffee Association (ABIC) in all Brazil is to avoid the appearance of unsatisfactory, inferior or adulterated quality coffees in the market, which could generate a high level of dissatisfaction among consumers of these products and hamper the growth of coffee use in the whole country.

The success of these standards reflects in an increase in domestic use of coffee. Four of the reasons that ABIC attributes to the expansion of consumption are continuous improvement of the quality of coffee offered to consumers, which increased with the Coffee Quality Program (Programa da Qualidade do Café - PQC), a consolidation of the Gourmet or Specialty coffee markets, significant improvement in the perception of coffee regarding its benefits to health, better economic conditions in Brazil, with consumption and purchasing power improvement, expansion of wage and salary mass, employability, and larger participation of consumers that migrated from classes D and E to class C (ABIC, 2008).

Before these programs consolidated in the year 2000, a study was made regarding the expectation and preferences of Brazilian consumers concerning roasted and ground coffee target product test in 10 cities with four samples, “Special” coffee, that can be considered Gourmet, “National” coffee, that can be considered Traditional and two local samples from the local market. The National product was considered better at an error level of 5% than the Special product, as to attributes of color, aroma, flavor, residual flavor and body of the beverage (Faria et al., 2000).

The aim of this paper was to evaluate the following hypotheses: did the consumer change his (her) preference as to the taste due to the presence of better quality coffees in the market and if he (she) considers the information on category of quality when purchasing, influencing his (her) choice.

MATERIALS AND METHODS

The acceptability of three coffee samples was evaluated: Gourmet, Superior and Traditional quality in this new context. The classification of the three samples used was confirmed by descriptive quantitative sensory analysis performed by a selected and trained team using a non structured scale from 0 to 10 cm to evaluate the fragrance of the grind and aroma, defects, acidity, bitterness, residual flavor, astringency and body of the beverage, with a final evaluation of their global quality (Government Of The State of São Paulo, 2007; Howell, 1998; Lingle, 1992). These three samples were submitted to sensory evaluation by a group of 54 persons who consume coffee, responsible for its purchase, with no restrictions in relation to age, sex and social status. The samples were prepared by percolation and their beverage was evaluated as to acceptability of color, aroma and flavor through hedonic scales of 9 points. They were also evaluated using 5 point scales as ideal in relation to acidity and

bitterness and through a 5 point scale related to the intention to buy. The samples were presented in a monadic manner with three number aleatory codes, according to an alignment of balanced blocks and served in 50 ml white plastic cups; sugar and sweetener were available and mineral water to be used between samples. The consumers were questioned in relation to the characteristics considered as priorities in choosing coffee and how much they agreed with the declaration: "The classification information on the coffee: Traditional, Superior and Gourmet, helps me in deciding to buy" (Meilgaard et al., 1999; Moskowitz, 1983). The test was made in individual booths lighted with fluorescent lamps and data collection was made using a computer software, *Compusense Five, version 4.8,* for sensory evaluation.

RESULTS AND DISCUSSION

A group of 54 persons who consume coffee, responsible for its purchase, 43 women and 11 men, 33% between 21 to 30 years old, 24%, 31 to 40, 26%, 41 to 50, and 17%, 51 to 60 years old, was recruited to undertake the test.

In relation to the attributes of color and flavor, the Traditional coffee sample was considered to be better than the sample of Gourmet coffee at an error level of 5%. The Superior coffee sample with intermediate mean values did not differ from the other samples. Therefore, the consumers demonstrated better acceptance of the Traditional and Superior coffees, which did not reflect in the intention to purchase (Table 1).

Table 1. Results were obtained in hedonic scale tests as to the acceptability of color, aroma and flavor and of an ideal scale of acidity and bitterness of the samples evaluated. In each line, values followed by different letters differ statistically between themselves at an error level of 5% *.

	Samples			
	Traditional	**Superior**	**Gourmet**	**D.M.S.**
Acceptability				
Color	(7,4 ± 0,9) a	(7,0 ± 1,1) ab	(6,8 ± 1,3) b	0,44
Aroma	(6,7 ± 1,2) a	(6,7 ± 1,3) a	(6,6 ± 1,6) a	0,56
Flavor	(6,7 ± 1,4) a	(6,4 ± 1,7) ab	(5,9 ± 1,9) b	0,64
Ideal				
Acidity	(3,2 ± 0,7) a	(3,4 ± 0,8) a	(3,4 ± 0,8) a	0,28
Bitterness	(3,4 ± 0,6) a	(3,1 ± 0,8) a	(3,3 ± 1,0) a	0,32
Intention to Purchase	(3,5 ± 1,1) a	(3,2 ± 1,2) a	(3,1 ± 1,3) a	0,48

**Values expressed as Mean ± Standard Deviation.*
D.M.S.: Minimum significant difference at an error level of 5% by the Tukey test.

The information as to coffee brand, type of packaging and the ABIC quality seal were considered as a priority characteristic in choosing the coffee by over 50% of the consumers (Table 2).

The information on quality category was considered a priority by 38,9% of the consumers, although 68,5% agreed with the declaration that in fact the information on coffee classification on the label helps in deciding its purchase.

Traditional coffee is even more acceptable as to flavor than Gourmet coffee and the information on classification more than the category of the coffee quality, has helped the consumer to decide for its purchase.

Table 2. Answers of the consumers in relation to the characteristics considered as priorities in choosing coffee.

Priority characteristic in choosing the coffee	% of the consumers
Coffee Brand	68,5%
ABIC quality seal	59,3%
Type of packaging	57,4%
Date of Expiration	44,4%
ABIC Purity Seal	42,6%
Price	42,6%
Quality Category (Gourmet, Superior, Traditional)	38,9%
Type of coffee (*Arábica x Conillon*)	18,5%
Others reasons	13,0%

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Evaluation of Coffee Sensory Quality Submitted to Different Degrees of Roasting: Medium and Dark

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SUMMARY

The aim of this paper was to evaluate the category of quality and sensory characteristics of two samples of raw coffee submitted to two degrees of roasting: medium and dark. The samples of raw coffee were evaluated and classified according to the Official Santos Table. The four samples of roasted coffee were evaluated as to degree of roast by reading an Agtron Coffee Roast model E10-CP spectrophotometer and classified using the Agtron System / SCAA Roast Classification Color Disk. Descriptive sensory analysis of the beverage was made by a selected and trained team of eight panelists, using a non structured scale from 0 to 10 cm to evaluate the fragrance of the grind, aroma of the beverage, defects, acidity, bitterness, flavor, residual flavor, astringency and body of the beverage, with a final evaluation of global quality. For each sample, 3 repetitions were made and the data pertaining to the sensory analysis was submitted to variance analysis Tukey test to compare the mean values. The two samples of raw coffee showed greenish color, regular aspect and good drying. Sample 1 was classified as type 6-15, one point below sample 2, classified as type 5-10. The medium roast samples represent Disk n° 55/Medium Classification and the dark roast samples represent Disk n° 25/Dark Classification. The raw coffees submitted to medium roast reached significantly better mean values for the characteristics of beverage aroma and defects at an error level of 5% than the dark roast coffee samples, which reflected on the grade for global beverage quality. The characteristics of bitterness, flavor, residual flavor and astringency of medium roast sample 2 also reached significantly better mean values at an error level of 5%, if compared to dark roast sample 2. Lighter roasts are recommended for they preserve the herb and fruit; burnt and smoke aromas are increased and acidity is reduced in darker roasts. The degree of roasting directly influences the sensory characteristics and the evaluation of beverage global quality.

INTRODUCTION

Resolution SAA-28 of June 01, 2007 and the Program for the Quality of Coffee of the Brazilian Coffee Association – ABIC (Associação Brasileira da Indústria do Café) present a classification by quality category of the coffees of Gourmet, Superior and Traditional quality and define the requisites of the physical and sensorial characteristics and global beverage quality for each one of these coffees, suggesting their composition. The 'Traditional Coffees' are those which have type 8 or better coffee beans COB (Official Brazilian Classification), with a maximum of 20% in weight of beans with black, green and burnt defects, accepting the use of past crop and light green beans with any beverage. The absence of black, green or fermented beans is recommended. 'Superior Coffees' are those which have type 2 to 6 COB

coffees, arabic or blended with robusta/conillon coffee, soft to hard beverage, with a maximum of 10% black, green and burnt defects, accepting the use of past crop and light green beans being balanced in the cup. The 'Gourmet Coffees' must have only Arabic coffee, only soft beverage, soft or strictly soft, of types 2 to 4 COB with the absence of black, green and burnt defects, black-green and fermented (Government of the State of São Paulo, 2007; 2004; ABIC, 2008).

The aim of this paper was to evaluate the category of quality and sensory characteristics of two samples of raw coffee submitted to two degrees of roasting: medium and dark. Based on the assumption that for the coffee of Traditional quality, the darker the roast, the better evaluation of global quality of its beverage, while for the Gourmet or Superior coffee, the lighter the roast the better its global beverage quality evaluation.

The quality of the coffee, as the beverage, depends on various elements: a) chemical composition of the beans, determined by genetic, environmental and cultural factors; b) methods of harvesting, processing and storage; c) roasting and preparation of the beverage; these last two, responsible for the alteration of the chemical characteristics of the beans, starting with its original composition (Silva et al., 2000).

Moura, et al. confirmed that the linear increase of time and temperature on the coffee roast presents significant negative effects for the following variables: aroma, characteristic flavor, chocolate flavor, sweetness, as well as significant positive effects on the acidity and bitterness; however, one cannot possibly foretell the global quality model in his study (Moura et al., 2007).

MATERIALS AND METHODS

The two samples of raw coffee were evaluated and classified according to the Official Santos Table (Ministry Of Agriculture, 2003). The coffee samples were roasted to reach medium and dark roasts. The four samples of roasted coffee were evaluated as to the degree of roast by reading an Agtron Coffee Roast model E10-CP spectrophotometer and classified using the Agtron System / SCAA Roast Classification Color Disk. They were ground in a Special La Cimballi mill, and the beverages were prepared by percolation using paper filter. Descriptive sensory analysis of the beverage was made by a selected and trained team of eight panelists, using a non structured scale from 0 to 10 cm to evaluate the fragrance of the grind, aroma, defects, acidity, bitterness, flavor, residual flavor, astringency and body of the beverage, with a final evaluation of global quality (Government of the State of São Paulo, 2007; 2004; Howell, 1998; Lingle, 1986). This analysis was made in red lighted booths equipped with the *Compusense Five version 4.8* computer software to collect data; the samples were presented in a monadic form according to an aleatory aligning of complete blocks with three digit codes and evaluated in relation to the reference sensory sample of known "Traditional" quality. For each sample, 3 repetitions were made and the data pertaining to the sensory analysis was submitted to variance analysis Tukey test to compare the mean values.

RESULTS AND DISCUSSION

The two samples of raw coffee showed greenish color, regular aspect and good drying. Sample 1 contained 111 defects by equivalence, consequently its classification was 6-15, one point below sample 2 with 52, which was classified as type 5-10 (Table 1).

Table 1. Quantification of the imperfect beans and equivalence in number of defects in the raw coffee samples.

Sample	Sample 1		Sample 2	
Defects	Amount of imperfect beans	Equivalence in number of defects	Amount of imperfect beans	Equivalence in number of defects
Burnt	20	10	15	7
Small husk	1	0	2	1
Bored	59	12	18	4
Conch	1	0	0	0
Light green	10	2	0	0
Dark green	5	3	0	0
Broken	399	80	199	40
Hollow / badly grained	18	4	1	0
Total	**513**	**111**	**225**	**52**

After roasting, the medium roast samples represent Disk n° 55/Medium Classification and the dark roast samples represent Disk n° 25/Dark Classification.

Table 2. Results of the descriptive sensory analysis of the 4 samples of coffee beverage made by a selected and trained team of eight panelists.

Attribute	**Sample**				**D.M.S.**
	Sample 1 Medium roast	**Sample 2 Medium roast**	**Sample 1 Dark roast**	**Sample 2 Dark roast**	
Fragrance	6,1 ± 0,9 a	6,0 ± 0,9 b	5,3 ± 0,8 c	5,7 ± 0,5 b	0,29
Aroma of the beverage	5,0 ± 0,7 a	5,0 ± 0,6 a	4,3 ± 0,7 c	4,6 ± 0,4 b	0,26
Defects	5,0 ± 0,9 b	5,1 ± 0,6 b	5,7 ± 0,7 a	5,5 ± 0,4 a	0,35
Acidity	1,8 ± 0,5 a	1,8 ± 0,4 a	1,7 ± 0,4 a	1,7 ± 0,3 a	0,17
Bitterness	5,1 ± 0,9 c	5,3 ± 0,7 bc	5,7 ± 0,7 the	5,4 ± 0,5 ab	0,31
Flavor	4,8 ± 0,7 a	4,8 ± 0,5 a	4,3 ± 0,6 b	4,6 ± 0,4 ab	0,27
Residual flavor	4,8 ± 0,7 a	4,7 ± 0,5 ab	4,3 ± 0,6 c	4,5 ± 0,4 bc	0,29
Astringency	5,0 ± 0,8 b	5,2 ± 0,5 ab	5,5 ± 0,5 a	5,3 ± 0,5 ab	0,31
Body	5,7 ± 0,5 a	5,8 ± 0,5 a	5,8 ± 0,4 a	5,8 ± 0,2 a	0,26
Global quality	4,9 ± 0,6 a	4,8 ± 0,4 a	4,3 ± 0,5 b	4,5 ± 0,3 b	0,24

Results expressed as mean value ± standard deviation.
D.M.S.: minimum significant difference at 5% error level by the Tukey test.
In each line, mean values followed by equal letters do not differ significantly among themselves at the level of 5%, by the Tukey test.

The raw coffees submitted to medium roast reached significantly better mean values for the characteristics of beverage aroma and defects at an error level of 5% than the dark roast coffee samples, which reflected on the grade for global beverage quality. The characteristics of bitterness, flavor, residual flavor and astringency of medium roast sample 2 also reached significantly better mean values at an error level of 5% if compared to dark roast sample 2 (Table 2).

Lighter roasts are recommended for they preserve the herb and fruit; burnt and smoke aromas are increased and acidity is reduced in darker roasts. The degree of roasting directly influences the sensory characteristics and the evaluation of beverage global quality.

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Study of the Thermal Generation of Aroma Compounds in Coffee Using an in-Bean Model System

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SUMMARY

In this study, water extracted (i.e. depleted) green Brazilian Arabica coffee beans are used as a model reactor to evaluate the impact of the presence of controlled amounts of various Maillard reaction precursors on the formation of aroma compounds upon roasting. The differences in the aroma compounds profiles between depleted beans and the same beans infused with sucrose, as well as with and without either L-cysteine or D-L-alanine were measured using a GC-MS system. The presence of sucrose was clearly associated with the formation of acetic acid upon roasting, whereas the addition of L-cysteine leads to increased formation of furfurylmercaptan. The presence of D-L-alanine was shown to promote the formation of pyrazines, which greatly contribute to the roasted and nutty character of the coffee aroma. This work has confirmed the suitability of using a biomimetic in-bean model system to study the generation of key aroma compounds during coffee roasting, and demonstrated its potential to gain practical insight into the role played by certain precursors in those complex reactions.

INTRODUCTION

The flavour of roasted coffee results notably from the generation of hundreds of aroma compounds during roasting. Most of the fundamental understanding of the relation between the nature of the precursors present in raw coffee and the type of aroma generated has been obtained using model systems not involving coffee beans (see extensive citations made in Milo et al., 2001). However, the conditions of the environment in which roasting reactions take place greatly influence the reaction pathways and therefore the outcomes of those reactions. In recent years, water depleted green coffee beans have been used and notably allowed to demonstrate the relative importance of extractable and non-extractable components on the generation of aroma compounds (Milo et al., 2001). Since then, in-bean infusion experiments have been used successfully to determine the possible precursors to the formation of various phenolic compounds in "natural" roasting conditions (Mueller et al., 2006). Using a similar in-bean model, the present work aims at developing a better understanding of the nature of the complex reactions taking place in coffee beans upon roasting.

MATERIALS AND METHODS

Starting material and preparation of depleted beans

All coffee used in this work was standard quality Brazil Primeiro. Water extraction of the beans was carried out on batches of 2 kg of green coffee. It consisted of three successive extraction steps carried out at 80 °C, by adding successively 8 kg, 5.2 kg and 4.8 kg of demineralised water. Extraction took place under agitation (i.e. tumbling 20 times/min.) and the beans were separated from the obtained extract by sieving between each extraction step. Three extraction batches were prepared and all the collected extracts were pooled together and

homogenised. A sample of this extract was collected, centrifuged for clarification, and freeze dried on a lab-scale freeze dryer prior to chemical analysis.

Similarly all the depleted beans were collected, dried in a fluidised bed dryer (Aeromatic, Germany) at 60 ºC for 160 minutes, then pooled together and blended homogeneously.

Spiking and roasting of depleted beans

The spiking of depleted beans with pure compounds was carried out by contacting a solution of the compounds with dry depleted beans prepared as described previously at 60 °C for 3 hours with a ratio of solution to depleted beans of 1:2 on a weight basis. The concentration of the solution was set so as to allow a concentration of sucrose of 4% (w/w) and a concentration of amino-acids (i.e. L-cysteine or D-L-alanine) of 1% (w/w) of the dry depleted beans. After infusion, the spiked beans were dried to achieve a moisture content of 10.5 ± 0.5% prior to roasting.

Roasting was carried out by batches of 150 g on a bench-top drum roaster (model BRZ2, Probat-Werke, Germany). The roasting conditions were set using the initial (i.e. non-modified) green coffee used throughout this study, so as to obtain a medium roast colour (i.e. 12 Lange units as measured using a Dr. Lange CRM Model LK-100, Dr. Lange GmbH, Dusseldorf, Germany). Those conditions were kept constant for all samples produced in the present study.

Chemical characterisation of coffee beans and water extract

The total dry matter content of the pooled water extract was determined by oven drying of a known amount at 105ºC overnight. Both the depleted beans and the dry matter of the water extract are analysed using the following methods:

- *Total and free carbohydrates:* the sample is hydrolysed with acid for total carbohydrates determination and with water for free carbohydrates determination. The sugars are then separated on an anion exchange column and detected using a DIONEX ED 40 pulsed amperometric detector.
- *Caffeine:* the caffeine is extracted in de-ionised water using an ultrasonic water bath at 40 °C and partially purified on a solid phase column prior to quantification by HPLC.
- *Trigonelline:* trigonelline is extracted in de-ionised water using an ultrasonic water bath at 40 °C and analysed by HPLC using a graphitised carbon column with UV-detection.
- *Total proteins:* the total nitrogen content of the sample is first determined using the Kjeldahl method. A corrected "protein nitrogen" content is then calculated by deducting the amount of nitrogen associated with the presence of caffeine and trigonelline (see methods provided above). The total protein content reported here is calculated by applying a factor 6.25 to the "protein nitrogen" content calculated.
- *Total Chlorogenic Acids (CGA):* total CGA content is the sum of the content of the five main isomers present in coffee (i.e. 5-caffeoylquinic acid (5- CQA), 3-CQA, 4-CQA, 3,4-di-CQA, 3,5-di-CQA and 4,5-di-CQA. The unbound CGA isomers are extracted from the sample and cleaned up using solid phase extraction. The isomers are separated by reverse phase HPLC with UV detection.
- *Key aroma compounds (KAC) analysis:* the concentration of forty volatile organic compounds selected for their particularly significant contribution to the aroma of roast and ground coffee brew, was determined by GC-MS in SIM/SCAN mode. The analysed extract was obtained by Membrane Assisted Solvent Extraction (MASE).

RESULTS AND DISCUSSION

A mass balance of the water extraction was carried out for all key components of the coffee used in order to characterise the depleted beans used (Table 1)

Table 1. Mass balance of the key components of Brazilian Arabica green coffee beans after water extraction.

Compounds	Water extract (%)	Depleted beans (%)	Loss (%)
Sucrose	72	16	12
Proteins	8	82	10
Caffeine	83	8	9
Trigonelline	79	7	14
Total GCA	68	21	11
Polysaccharides	2	84	14
Total dry matter	20	73	7

The majority of highly water soluble components of green coffee were extracted in hot water. Depleted beans were therefore constituted of the structural and water insoluble complex carbohydrates forming the beans' cell walls as well as most of the initial protein components, which are too large to diffuse through the thick cell walls. About a fifth of the initial sucrose and CGA is also retained in the depleted beans.

Taking the concentration of aroma compounds generated in depleted beans upon roasting as a reference, and expressing the relative concentration of those compounds in beans in which specific compounds have been infused, one can observe the impact of the presence of specific compounds on aroma generation (see Table 2). This study shows that the water soluble compounds present in standard Arabica coffee play a major role in the formation of many aroma compounds. However it also shows that many key aromatics of coffee are formed even in the absence of those water soluble compounds, sometimes in similar concentration to that formed in the original beans in similar roasting conditions. These results mostly confirm previously published work (Mueller et al., 2006), and provide further details regarding the nature of those compounds. When solely added the depleted beans, the presence of sucrose is shown to be mostly associated to an increased formation of acetic acid. The addition of cysteine leads mostly to an increased generation of furfurylmercaptan. The addition of D-L-alanine leads to preferential formation of 2-ethyl-3,6-dimethylpyrazine. As well as a whole range of other pyrazines, notably 2-ethyl-3,5-dimethylpyrazine and trimethylpyrazine. It is noticeable that linalool as well as pyridine form in the presence of D-L-alanine only in the absence of sucrose.

This work has confirmed the suitability of using an in-bean model system to study the generation of key aroma compounds during coffee roasting, and demonstrated its potential to gain practical insight into the role played by certain precursors in those complex reactions.

Table 2. Relative concentration of 40 key aroma compounds present in the brew of roasted Standard Brazilian Arabica as well as depleted beans (water extraction) spiked with various precursors. The reference is depleted coffee beans (water extraction) infused with pure water.

	Standard	Depleted beans (water extraction) spiked with					Aroma/flavour
Compound name	Arabica coffee (Brazil Primeiro)	sucrose (4%)	cysteine (1%)	cysteine (1%) and sucrose (4%)	alanine (1%)	alanine (1%) and sucrose (4%)	impression given by the pure compound
2-Acetylpyrazine	0.35	0.76	1.08	0.85	1.04	0.76	acrid, animal, popcorn
Methional	0.51	1.07	0.87	1.30	0.52	0.75	meaty, sulfury
1-(2Furfuryl)2formylpyrrol	0.53	1.28	0.57	1.12	0.58	0.98	minty, acid, burnt
E, E-2, 4-Decadienal	0.53	1.26	0.60	1.18	0.59	1.00	orange, citrus, fresh
Phenylacetaldehyde	0.70	1.14	0.49	0.83	0.48	0.62	green, floral
2-Ethylpyrazine	0.70	0.68	1.05	0.81	2.19	1.75	nutty, burnt praline
2, 3-Butandione	0.73	1.20	0.89	0.73	1.02	1.24	pungent, buttery, oily
Furfurylmercaptan	0.74	0.80	2.79	3.29	0.79	0.74	burnt, caramelic
5-Methyl5H6, 7dihydroCPP	0.76	0.61	0.93	0.74	2.67	1.61	peanut, earthy
2, 3-Diethyl5methylpyrazine	0.76	0.82	0.94	0.90	1.77	2.11	earthy, roasted
Trimethylpyrazine	0.94	0.79	1.08	0.97	4.42	3.76	nutty, roasted
Dimethyltrisulfide	0.95	0.85	0.39	0.34	0.97	0.96	cabbage, onion
2-Ethyl-3,5-Dimethylpyrazine	0.96	0.63	1.63	1.27	12.37	9.56	nutty, roasted, burnt
2-Acetylpyridine	1.12	0.96	0.75	0.89	1.16	1.25	pop-corn, bread
3-Methylindol (Skatol)	1.13	0.85	0.67	0.70	0.91	0.97	sweet to animal
Furfural	1.22	1.86	0.66	1.60	0.51	1.11	caramel, bread
Maltol	1.28	1.33	0.63	0.87	0.98	0.88	fruit, jam, pineapple
Furaneol	1.34	1.85	0.84	1.45	0.61	0.81	caramel. Jam,
3-Methylbutanal	1.38	1.24	0.64	0.99	0.81	1.01	toast, chocolate, malt
2-Ethyl-3,6-dimethylpyrazine	1.49	0.56	2.22	1.80	43.19	43.67	earthy, baked, potatoe
Cyclotene	1.58	1.52	0.67	1.15	0.90	1.16	caramel, spicy, burnt
Dimethyldisulphide	1.62	0.58	0.75	0.37	1.87	1.24	onion
Vinylguaiacol	1.73	0.74	0.72	0.60	0.71	0.51	spicy, smoky, tar
Furfurylmethylsulphide	1.76	0.87	0.73	0.84	1.13	1.08	garlic, burnt, coffee
2-Phenylethanol	1.80	0.96	0.66	0.76	0.56	0.53	Floral, woody, honey
Ethylguiacol	1.81	0.77	0.81	0.51	1.29	0.65	smoky, roasted, burnt
Linalool	1.90	1.05	0.79	0.95	4.68	0.86	floral, wood, citrus
2-Methylbutanal	1.99	1.03	0.90	0.98	0.92	1.00	fruity, cocoa, coffee
2-Methoxy3isopropylpyrazine	2.07	1.43	0.56	0.66	0.78	1.28	earthy, potatoe
Guaiacol	2.08	0.83	0.68	0.52	0.91	0.54	smoky, burnt
2,3-Pentanedione	2.21	1.83	0.72	1.90	0.66	1.75	buttery, pungent
MMBF	2.31	0.72	1.94	1.14	2.39	2.09	fruity, green
4-Ethylphenol	2.34	0.89	1.01	0.67	2.06	5.11	phenolic, medicinal
2-transNonenal	2.49	1.35	0.74	1.15	0.90	1.24	fatty to unpleasant
2-Methylbutanoic acid	3.18	1.23	0.79	0.87	0.71	0.68	sweaty to awful
3-Methylbutanoic acid	3.37	1.37	0.95	1.06	0.76	0.78	sweaty to awful
Acetic Acid	3.55	3.23	0.61	2.36	0.49	1.34	pungent, sour, vinegar
Pyridine	3.70	0.49	1.06	0.40	2.10	0.79	burnt, smoky
Hexanal	4.61	1.92	0.65	1.83	0.69	1.72	green, grassy
beta-Damascenone	4.69	0.54	0.78	0.74	1.98	1.37	tea, fruit, honey

Key: colour coding used for the representation of the relative content (x) compared to the reference
(the reference is depleted beans spiked with water, which has a relative content of 1)

x < 0.5	0.5 <x< 0.67	0.67 <x< 1.5	1.5 <x< 2.0	2.0 <x

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Coffee-Soy Based Beverage: Product Formulation and Consumer Acceptance

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SUMMARY

The objective of the present study was to investigate the consumer acceptance of a coffee-soy based beverage, testing different proportions of instant coffee and soymilk powder. Experiments were designed in order to model sensory responses to obtain the most preferred product using a 2^3 factorial design, with instant coffee (2 and 4%), soymilk (7 and 10%) and sugar concentration (8 and 12%) being evaluated for overall acceptability in a hedonic scale. Soymilk powder content did not influence significantly ($p > 0.05$) the consumer acceptance in the range studied. On the other hand, the sugar content had a significant and positive influence ($p \leq 0.05$) in acceptability. The opposite was observed for coffee content, which had a significant negative influence ($p \leq 0.05$). The two most preferred samples had the lowest coffee content (2%) and the highest sugar content (12%), with no statistical difference of the soymilk concentration.

INTRODUCTION

Brazil is the first coffee producer and the second largest coffee consumer market in the world (ABIC, 2008). Likewise, the country is the second largest producer of soybeans, although this legume seeds are not commonly used in the Brazilian diet (IBGE, 2008). Several epidemiological studies have shown that a regular diet including soy products or habitual coffee consumption can reduce the risk of different chronic diseases (Farah and Donangelo, 2006; Barnes et al., 2006). Considering the current consumer trend for healthier alternatives in food products, the possibility to combine in a functional beverage the health benefits of these two important Brazilian commodities, and also taking into account that there is no report of a product like this in the literature, the objective of the present study was to investigate the consumer acceptance of a coffee-soy based beverage, testing different proportions of instant coffee and soymilk powder.

MATERIALS AND METHODS

Soymilk powder and instant coffee samples were kindly provided by manufacturers. The instant coffee samples tested were chosen considering prior results (acceptability test, paired preference test and chlorogenic acid evaluation). Experiments were designed in order to model sensory responses to obtain the most preferred product by consumers. Beverage samples were prepared according to a 2^3 factorial design, with instant coffee (2 and 4%), soymilk (7 and 10%) and sugar concentration (8 and 12%), as independent variables, and four central points, resulting in 12 treatments. The dependent variable (sensory response) was the consumer acceptance score. Coffee and soymilk powder level ranges were selected following manufacturer recommendations and sugar level was based in previous experiments. Beverages were obtained by mixing the ingredients with water at room temperature on the

day before the test and kept refrigerated. Consumer acceptance tests were carried out in individual sensory booths using 84 individuals who liked and drank coffee regularly. They were chosen from staff, students and visitors at Embrapa Food Technology in Rio de Janeiro. Samples were evaluated at 12 ± 2 °C for overall acceptability using a 9-point structured hedonic scale (1= *dislike extremely*; 9 = *like extremely*) (Stone and Sidel, 1993). The order of sample presentation was balanced to prevent carry over effects and six samples were evaluated in each session by all assessors. Acceptability data were analyzed by ANOVA, using S*tatistica*™ software, v. 8.0 for windows (StatSoft®). Fisher test (LSD) was used to verify significant differences among the means, considering $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 presents the design matrix with the acceptability scores as the dependent variable. The analysis of variance of acceptance data revealed that samples were significantly different in terms of acceptability ($p \leq 0.05$) with a range of mean scores from 3.6 to 5.6 (Table 1).

Table 1. Mean acceptability scores* of soy-coffee beverages.

Samples	Coffee (%)	Soymilk (%)	Sugar (%)	Acceptability score§
3	2.00	7.00	12.00	5.6[a]
11	2.00	10.00	12.00	5.5[a]
8	3.00	8.50	10.00	5.2[ab]
2	3.00	8.50	10.00	5.1[abc]
10	3.00	8.50	10.00	4.9[bcd]
9	4.00	10.00	12.00	4.9[bcd]
4	3.00	8.50	10.00	4.8[bcd]
5	4.00	7.00	12.00	4.7[bcd]
12	2.00	10.00	8.00	4.5[cd]
7	2.00	7.00	8.00	4.3[de]
1	4.00	7.00	8.00	3.8[ef]
6	4.00	10.00	8.00	3.6[f]

**Values that are followed by different letters within rows are significantly different by Fisher´s test ($p \leq 0.05$).*

§ 1 = dislike extremely, 5= neither like nor dislike, 9 = like extremely

The Pareto chart (Figure 1), considering the independent variables showed that the sugar content had a significant and positive influence ($p \leq 0.05$), indicating that an increase in sugar content contributed to a greater acceptance of the beverage. The opposite was observed for the instant coffee content, that had a significant negative influence ($p \leq 0.05$), indicating that a decrease in coffee content contributed to a higher acceptance of the beverage. The soymilk powder content did not influence significantly ($p > 0.05$) the consumer acceptance in the range studied. The curvature was also significant ($p \leq 0.05$), so that a quadratic model should be evaluated. A central composite design needs to be considered in order to find the parameters for this quadratic model.

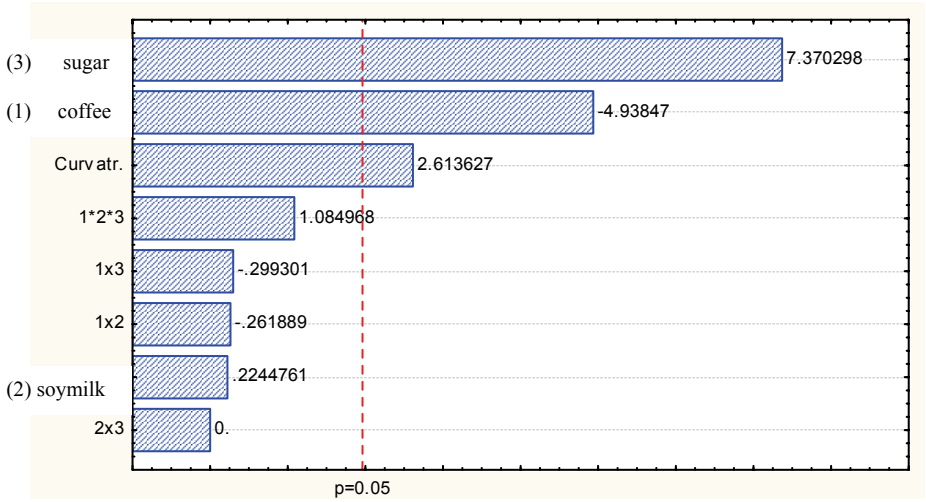

Figure 1. Pareto chart showing the statistical significance of the factors.

Figure 2 shows the means plot for the acceptability scores. It can be observed that the highest score was obtained for coffee concentration equal to 2% and sugar concentration equal to 12%. There was no statistical difference between 7% or 10% of soy milk concentration.

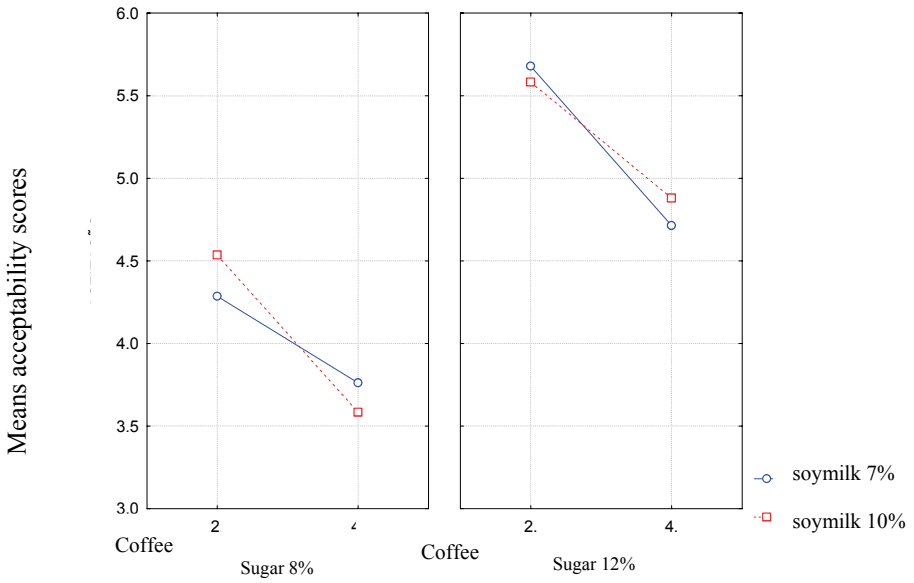

Figure 2. Means plot for the acceptability scores.

CONCLUSIONS

Samples were significantly different in terms of acceptability ($p \leq 0.05$), with a range of mean scores from 3.6 to 5.7. The two most preferred samples had the lowest instant coffee content (2%) and the highest sugar content (12%), with no statistical difference between soymilk concentrations. Further experiments will be carried out to obtain a quadratic model.

ACKNOWLEDGMENTS

The financial assistance from CBP&D Café/EMBRAPA is acknowledged. We also wish to thank Olvebra Industrial S.A and Cocam Cia de Café Solúvel e Derivados for kindly offering the samples.

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Cup Quality of New Cultivars Derived from Híbrido De Timor

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SUMMARY

Eight new cultivars and two elite lines derived from hybridizations of Catuaí and Híbrido de Timor were sampled from experimental fields in São Sebastião do Paraíso, Patrocínio, and Três Pontas, in Minas Gerais State. Sensorial analysis for cup quality were proceeded according to criteria adopted for specialty coffees, with cumulative scores for clean cup, sweetness, acidity, mouthfeel, flavor, aftertaste, balance, and overall. Cultivar Catuaí Vermelho IAC 99 was used as standard. All samples presented very good cup quality, with differentiated special attributes. The highest cumulative score for the analyzed samples of Catuaí Vermelho IAC 99 was 86 out of 100 points. Cultivars Catiguá MG2, Catiguá MG1, Araponga MG1, and Sacramento MG1, and the line H419-6-2-5-3 scored higher than 90 out of 100 points in at least one sample, which distinguished them as exceptional cup quality coffees.

INTRODUCTION

The Coffee Breeding Program in Minas Gerais, Brazil, started in 1971 at Universidade Federal de Viçosa with the introduction of a valuable germplasm of coffee, including many sources of resistance to leaf rust, specially of Híbrido de Timor. After hybridizations of these genotypes with commercial cultivars, as Catuaí, Caturra, Mundo Novo, and Bourbon, many generations of selection were made to obtain recombinants combining high yield, disease resistance, vegetative vigor, and high cup quality. To date, eight new cultivars derived from hybridizations of Catuaí and Híbrido de Timor were delivered for cultivation. Many other new cultivars are being prepared for delivery. Seven of these new cultivars and two elite lines were tested for their potential as specialty coffees.

MATERIAL AND METHODS

Seven *Coffea arabica* L. cultivars derived from hybridizations of Catuaí and Híbrido de Timor were analysed: Paraíso MG H419-1 (Catuaí Amarelo IAC 30 x Híbrido de Timor UFV 445-46); Catiguá MG1, Catiguá MG2, and Catiguá MG3 (Catuaí Amarelo IAC 86 x Híbrido de Timor UFV 440-10); Araponga MG1 (Catuaí Amarelo IAC 86 x Híbrido de Timor UFV 445-08); Pau Brasil MG1 (Catuaí Vermelho IAC 141 x Híbrido de Timor UFV 442-34); and Sacramento MG1 (Catuaí Vermelho IAC 81 x Híbrido de Timor UFV 438-52). Two elite lines were also analysed: H 419-6-2-5-3 (Catuaí Amarelo IAC 30 x Híbrido de Timor UFV 445-46) and H 514-7-10-9-3-1 (Catuaí Amarelo IAC 86 x Híbrido de Timor UFV 440-10). Cultivar Catuaí Vermelho IAC 99 was used as standard. All cultivars are registered in the Brazilian Ministry of Agriculture, where Catiguá MG3 is listed as MGS Catiguá 3. Genotypes were sampled from EPAMIG Experimental Stations located at São Sebastião do Paraíso, Três Pontas, or Patrocínio, depending on the availability. Cherry fruits were mechanically pulped.

Seeds were prepared with and without mucilage before sun dried. After hulling, seeds were kept in a coded paper bag until performance of sensorial analysis was accomplished by two professional tasters. Cup quality as analyzed according to criteria adopted for specialty coffees, with cumulative scores for clean cup, sweetness, acidity, mouthfeel, flavor, aftertaste, balance, and overall. Differentiated special attributes were also described.

RESULTS AND DISCUSSION

Table 1 shows the results of sensorial analysis of ten arabica coffee genotypes, based on criteria adopted for specialty coffees. The highest cumulative score for the analyzed samples of Catuaí Vermelho IAC 99 was 86 out of 100 points in the Três Pontas sample. Cultivars Catiguá MG2, Catiguá MG1, Araponga MG1, and Sacramento MG1, and the line H419-6-2-5-3 scored higher than 90 out of 100 points, at least in one sample, which indicated their potencial for exceptional cup quality coffees. All samples presented very good cup quality, with differentiated special attributes. Araponga MG1 was described by one of the cuppers as: "Very citric, with exotic floral nuances, live and very agreeable acidity, complex. Excellent coffee of great aroma, good when warm, lukewarm and cold". And by the second cupper as: "High acidity, high mouthfeel, intermediate sweetness. Citric flavor with a floral bit." Cataguá MG2 cultivar was described as: "Very live acidity, agreeable, very good mouthfeel, very sweet, exotic floral, very citric. Considered a complex wonderful coffee. Fantastic". The other cupper said: "Very citric, high acidity, high mouthfeel, high sweetness". Performance of sensorial analysis of new cultivars will be continued. We conclude that, new cultivars of arabica coffee derived from Híbrido de Timor present potential for very high cup quality as a result of genetic recombination and selection during breeding processes.

Table 1. Sensorial analysis scores of eight cultivars and two elite lines of arabica coffee, based on the criteria for specialty coffees. Average of two tasters. 2007.

	Três Pontas		S.S.Paraíso		Patrocínio		Average	
CULTIVAR	A	B	A	B	A	B	A	B
Paraíso MG H419-1	82.5	86.5	-	80.5	-	-	82.5	83.5
Catiguá MG1	-	-		-	94.0	86.0	94.0	86.0
Catiguá MG2	-	-	-	-	88.5	94.5	88.5	94.5
Araponga MG1	77.0	80.5	-	91.5	-	-	77.0	86.0
Sacramento MG1	79.0	79.0	-	-	91.0	88.5	85.0	83.7
Pau-Brasil MG1	85.5	83.5	-	82.5	-	-	85.5	83.0
Catuaí Vermelho IAC99	85.0	86.0	-	77.0	-	-	85.0	81.5
Catiguá MG3	-	-	-	80.5	-	-	-	80.5
H514-7-10-9-3-1	-	-	-	83.5	-	-	-	83.5
H419-6-2-5-3	-	-	-	-	93.0	87.5	93.0	87.5
A = pulped seed without mucilage; B = pulped seed with mucilage								

Potent Odorants of Roasted Brazilian Conilon Coffee

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SUMMARY

The potent odorants of roasted coffee isolated from the *Coffea Canephora* variety from Espirito Santo State, Brazil were quantified. The coffee samples were roasted at three degrees: light, medium and dark. The medium roast presented the highest essential oil content (280 mg L^{-1}). GC/MS analysis allowed the quantification of 161 compounds, 123 of which were identified. Odor activity values (OAVs) were calculated for the odorants. A group of 19 aroma impact compounds (AICs) were characterized, including methylpropanal, 3-methylbutanal, 2-furfurylthiol, furfuryl-methyldissulfide, together with several alkyl pyrazines. (*E*)-β-damascenone was identified at a higher concentration in Conillon than in Arabic coffee.

INTRODUCTION

Coffee aroma is composed by a complex mixture of countless volatile compounds that present qualities, intensities and different concentrations (Morais et al., 2003; 2007; 2006). Those compounds are generated basically during coffee-roasting by Maillard reactions, hidroxyaminoacids descarboxilations and dimerizations. Processed coffees contain more volatiles than any other food or drink (Trugo et al., 1999; 2000). During the early 80's a strong interest developed to determine the odor activity of the volatile components wich present the highest impact on the coffee aroma characterization. Studies evaluating the sensory attributes of coffee brews for worse quality robusta coffee from Ivory Coast (Nebesny and Budryn, 2006; Nebesny et al., 2007), Vietnam and Indonesia (Akiyama et al., 2005) under different conditions are known. However, no studies were found concerning the potential aroma of the compounds of the Conilon Coffee (C. canephora) produced in the Espírito Santo State, Brazil. The objective of this work is analyzing the volatile constituents and the potential aroma of the conilon brand, submitted to different roasting degrees.

MATERIALS AND METHODS

Raw material and roasting

A wet-processed *Coffea conilon* variety from Espirito Santo State, Brazil, was obtained from COOABRIEL Company. Roasting experiments were carried out with an electric microroaster (PINHALENSE®-Br) in 100 g batches of green beans (c.a. 13% humidity).

Bean roasting at 190 ± 10 °C was monitored and controlled in a visually manner. The points of light, medium and dark roasting were reached after approximately 5, 6 and 8 min, respectively.

Water content determination

Samples of roasted coffee beans were finely grounded in a household coffee grinder (CANDENCY®-USA). Sample of 5g grounded coffee beans were dried at 105 °C for 15 min using QUIMIS-Kett 600.

Isolation of the volatile fraction

The aroma components were isolated by dichloromethane countercurrent extraction (Clevenger modification apparatus) (Godefroot et al., 1981). 50 g grounded coffee beans sample was combined with 200 mL distilled water. Next, it was extracted with 50 cm^3 diclorometh ane for 2 h. After drying with anhydrous sodium sulfate, the extract was concentrated to less than 1 mL by evaporation.

Gas chromatography

The analytical conditions for GC-MS measurements are given in Table 1. Peaks in the chromatograms were characterized by the Kovats index (Adams). Compounds were identified by comparison of mass spectra data and RI using reference compounds and literature data Wiley (140, 229, 275, 330). The results express the average of three injections. 161 compounds were detected above the established detection limit (0.07% peak area, exception to β-damascenone).

Table 1. Analytical conditions for GC-MS analysis of coffee volatiles.

Gas cromatograph	GC17A – Shimadzu (JP)
Mass spectrometer	QP5000 - Shimadzu (JP)
Capillary column	DB-5, 30 m; ID 0,25 mm; film thickness 0,25 μm (Supelco, USA)
Injector temperature	240 °C
Oven temperature programming	60-240 °C (3 °C min^{-1}); 240 °C (20 min)
Carrier	He, 1cm^3. min^{-1}
Carrier flow	90 kPa column head pressure
Injection volume	1,0 μL
Ionization potential	70 eV
Interface heating	240 °C
Mass range	40 a 650 u

RESULTS AND DISCUSSION

The applied modified Clevenger technique for isolation of volatiles was very successful for the purpose of evaluation of the aroma´s profile from roasted conilon coffee. The amount of essential oil of the medium-roasted was significantly higher than the light and dark-roasting. Table 2 presents the volatile compositions identified of the essential oil for the three types of roasting. The aroma suggested for each one in diluted system (water or propileneglycol) is known (Flament, 2002; The Good Scents Company). Despite being at lower concentration, the aroma perception at half dilution approximates well of the representatives aroma of the beverage. Among the main classes of organic compounds that presented a contribution between 10 and 35% stand out ether, alcohol, ketone, furane, and pyridines (Morais et al., 2007) for the three roasting degree. However, among the dozens of volatile constituent identified in the conilon coffee only some shows strong impact in the final aroma. These constituents are called of potent odorants. From the 161 compounds detected 123 were

identified, and, among them, 19 were aroma impacts compounds (AICs). Table 2 presents the relative concentration and the odor activity relative values (OAVrelative) of these potent odorants in the essential oils. Only 11 potent odorants have odor activity above 1%. The greatest impact is provoked by: β-damascenone, 2-furfuriltiol, 3-etil-2,5-dimetilpirazina, 2-isobutil-3-metoxipirazina and furfurilmetildissulfide (this for medium and strong roasting). The aroma of the Conilon coffee in the light roasting stood out for having the largest odor activity, basically because of the β-damascenone concentration (roses odor), wich is the odorant of larger impact in the final aroma (VLPO = 0.75 ng L^{-1}) (Czerny and Grosch, 2000). With the increase of the roasting degree its concentration is reduced, in agreement with the results of Semmelroch et al. (1995). Medium and dark roasting presented similar odor activity. 2-isobutil-3-metoxipirazina together β-damascenone also has significant odor activity at medium-roasting. The strong decrease of the relative odor activity of β-damascenone and the significant increase observed for furfurylmethyldisulfide result in Conilon coffee aroma being more similar to Arabic coffee.

Table 2. Odor activity values of potent odorants in light, medium and dark-roasted Conillon Coffee.

			$OAV_{relative}$*/ROASTING			
Peak no.	R.T	Compound	Light	Medium	Dark	OPLV** (μg L^{-1})
1	1.61	Methylpropanal	21.33	3.33	2.50	0.12
2	1.76	2,3-Butanedione (Diacetyl)	0.63	0.43	0.34	4.40
3	2.50	3-Methylbutanal	4.94	3.46	7.62	0.35
4	2.48	2-Methylbutanal	1.66	1.13	1.80	1.30
5	2.80	2,3-Pentanedione	0.08	0.06	0.04	30.19
6	4.85	Hexanal	0.19	0.11	0.09	0.49
7	6.12	3- Methylbutyric acid (Isovaleric acid)	X	X	0.15	4.50
8	9.17	2-Furfurylthiol	7.00	17.00	19.00	0.01
9	14.33	Phenylacetaldehyde	0.07	0.09	0.08	4.00
10	16.00	3-Ethyl-2,5-dimethylpyrazine	8.56	9.38	8.06	0.16
11	16.40	2- Ethyl-3,5-dimethylpyrazine	1.94	2.50	2.56	0.16
12	16.51	Guaiacol	0.06	0.09	0.18	12.00
13	19.68	2,3-Diethyl-5-methylpyrazine	1.55	1.78	1.89	0.09
14	19.83	3,5-Diethyl-2-methylpyrazine	5.22	5.33	6.00	0.09
15	19.94	2-isobutyl-3-methoxymethylpirazine	20.00	38.00	34.00	34.00
16	24.02	Furfuryl-methyldisulfide	3.00	13.25	24.00	0.04
17	25.85	4-ethyl guaiacol	0.02	0.02	0.05	50.00
18	27.45	4-vinyl guaiacol	0.06	0.11	0.06	20.00
19	30.86	(E)-β-damascenone	253.33	213.33	200.00	0.00075
TOTAL ODOR ACTIVITY (Σ $OAV_{relative}$)			329.64	309.40	308.42	

** $OAV_{relative}$ = relative odor activity values, calculated by dividing the concentration by the odor threshold. The data are means of at least two assays; ** OPLV = odor perception limiar values (Czerny and Grosch, 2000).*

CONCLUSIONS

The extremely potent odorant β-damascenone (OPLV = 0.00075 μg L^{-1}) is responsible for 76.9 % of the total odor activity for light roast. This activity decreased to 64,8 % for the dark roast. The accentuated sweet-fruity, flower (roses) and tea odor of the predominant odorant β-damascenone makes the Brazilian conilon coffee very different in comparison to the Arabica brand, mainly for light roast.

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Volatile Composition of Concentrated Aroma Extracts of Soluble Coffee Beverage Obtained by Pervaporation

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SUMMARY

The present work aimed to evaluate the composition of coffee aroma concentrates obtained from pervaporation of an aqueous coffee extract, produced by a soluble coffee processing company. The pervaporation process essays were performed in bench scale, using a polydimethylsiloxane (PDMS) membrane, in which the effect of temperature on flux and permeate quality was evaluated. The volatile compounds were extracted by solid-phase microextraction (SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS). The temperature increase in the pervaporation process has enhanced the permeate flux. The comparative analysis of the chromatographic profiles pointed out relevant differences between feed and permeate, the latter presenting a greater amount of volatile compounds having differentiated intensities. Among the compounds in permeate, 11 substances were considered very important for coffee aroma: acetaldehyde, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, 2,3-pentanedione, hexanal, 2,3-diethyl-5-methylpyrazine, 3-ethyl-2,5-dimethylpyrazine, 3,5-diethyl-3-isobutylpyrazine, 4-ethylguaiacol and guaiacol.

INTRODUCTION

Brazil is the greatest coffee producer as well as exporter. Brazilian coffee is exported either as green beans, toasted coffee or soluble coffee (ABIC, 2007). The green beans represent the major exportation volume, causing financial losses to Brazil.

Soluble coffee is an alternative for daily consuming, for being quick and practical to prepare, however the drink's quality is inferior in comparison to that of the grounded and toasted coffee (Sanz et al., 2002).

To try to reduce the deleterious effect of aroma loss during soluble coffee processing, its quality is being enhanced by blending with better *robusta* coffee beans, and also by recovering the aroma to be added back to the final product. Normally, for some types of soluble coffee, natural aqueous or oily essences are reincorporated to the final product, during the agglomeration step, in order to confer better sensorial characteristics (Lucas and Cocero, 2006).

Pervaporation, a membrane separation process, is being evaluated as an alternative for recovering and concentrating volatile compounds of liquid matrices, for its separating efficiency and, mainly, for minimizing volatile losses, since it utilizes mild process temperatures. Its application is spread out through the most diverse areas of chemical industry, including food processing companies (Cassano et al., 2006).

The objective of this work was to evaluate the volatile composition of concentrated aroma extracts obtained by pervaporation of soluble coffee samples using a polydimethylsiloxane (PDMS) membrane.

MATERIALS AND METHODS

Pervaporation Process

The coffee extract (feed stream) was circulated in a plane module, measuring 28.3m^2, with a dense anisotropic membrane of PDMS (Velterop – Pervatech®). An Alternative pump kept the feed circulating constantly throughout the system. The system temperature was maintained constant with a thermostatic bath Model NT 281. The pressure difference was guaranteed by the use of a vacuum pump VARIA, model SD-90, operating totally opened. Permeate stream was collected by a condenser immersed in a liquid nitrogen bath (-196 °C). The experiments were performed in six temperatures between 20 and 45 °C, with a 5 degree interval. The PDMS membrane permeability and the volatile compounds concentration at different temperatures were investigated. All the essays were conducted in duplicate.

Solid Phase Microextraction

Volatiles were extracted by headspace solid-phase microextraction with a polydimethylsiloxane/ Carboxen/divinylbenzene fiber by keeping 1.0g of the sample in a 4mL vial for 1 hour and exposing the headspace to the fiber for 15min. The headspace extract was desorbed in a heated injector (250 °C) of a gas chromatograph. The dessorption time, inside the injector, was 3 min.

Chromatographic and Mass Spectrometry Analysis

Volatile compounds were analysed in an Agilent 6890N gas chromatograph fitted with a HP-5 fused silica capillary column (30 m X 0.32 mm X 0.25 μm). Hydrogen (1.5 mL/min) was used as carrier gas. Oven temperature was initially held at 40 °C for 3 minutes, then rised to 240 °C at 3 °C/min and kept at 240 °C for 10 min. The injector was operated in splitless mode for 2 minutes at 250ºC. The flame ionization detector (FID) was set at 300 °C. Mass Spectra were obtained in an Agilent 5973N system operating in electron impact mode (EIMS) at 70 eV, coupled to an Agilent 6890 gas chromatograph fitted with a HP-5 MS column (30 m X 0.25 mm X 0.25 μm), using the same injection procedure and oven temperature program as above. Helium was the carrier gas, at 1.0 mL/min. The identification was based on the mass spectra of the compounds compared with the data in Wiley 6th ed. library and by their calculated retention indices (RI) compared with literature data (Adams, 2007).

RESULTS AND DISCUSSION

Substances identified in pervaporate coffee are listed in Table 1. An expressive increase in the concentration of some important substances for coffee aroma, such as acetaldehyde, 2-methylpropanal, 2 and 3-methylbutanal, 2,3-pentadione, hexanal, 2,3-diethyl-5-methylpyrazine, 3-ethyl-2,5-dimethylpyrazine, guaiacol and 4-ethylguaiacol was observed in pervaporated samples. No change in the chromatographic profile with temperature of pervaporation was observed and best yields were obtained at 45 °C.

Table 1. Compounds found in soluble coffee permeate.

Peak	RI (a)	Compound
1	>400	Acetaldehyde*
2	>400	n.i.
3	610	Methyl acetate*
4	621	2-methylpropanal*
5	639	2-butanone*
6	673	3-methylbutanal*
7	679	2-methylbutanal*
8	700	n.i.
9	705	2,3-pentanedione*
10	735	n.i.
11	748	1-methyl-1H-pyrrol
12	752	n.i.
13	759	Pyridine
14	763	n.i.
15	792	2-hexanone
16	792	3-hexanone*
17	806	Hexanal
18	808	3,4-Hexanedione
19	816	tetrahydro-2-methylfuran-3-one*
20	829	4-methylthiazol
21	833	2-methylpyrazine*
22	838	furfuryl methyl ether*
23	849	furfural*
24	867	2-methyl-5-hexanone
25	871	trimethyloxazole
26	872	2-furanemethanol*
27	882	3-methyl-2-oxazolidinone
28	887	3-buten-2-one, 4-(1-aziridinil)
29	895	2,2-dimethyl-hexanone
30	897	2-heptanone
31	911	2-heptanol
32	916	furfuryl formate
33	917	2-methyl-2-cyclopenten-1-one
34	919	2,5-dimethylpyrazine
35	920	1-(2-furanyl)ethanone
36	921	1,2,5-trimethylpyrrol
37	922	2-ethylpyrazine
38	934	3-methyl-2-butenyl acetate
39	934	n.i.
40	949	n.i.
41	959	n.i.
42	962	n.i.
43	965	dihydro-3-(2H)-thiophenone
44	967	benzaldehyde
45	973	5-methyl-2-furfural*
46	989	1-octen-3-ol
47	992	dihydro-2-methyl-3(2H)-thiofenone
48	1002	furfuryl methyl sulfide
49	1002	2-ethyl-6-methylpyrazine
50	1005	2-furanmethanol acetate
51	1006	2-ethyl-5-methylpyrazine
52	1009	octanal
53	1010	2-ethyl-3-methylpyrazine
54	1012	2-formyl-1-methylpyrrol
55	1013	1,4-diclorobenzene
56	1019	1-(2-furanyl)-1-propanone
57	1024	1,2,4-trimethylbenzene
58	1029	2-vinyl-6-methylpyrazine
59	1038	2-ethyl-1-hexanol
60	1047	n.i.
61	1036	n.i.
62	1059	1-ethyl-1H-pyrrol-2-carbaldehyde
63	1066	2-butanone-1-(2-furanyl)
64	1072	2,3,4-trimethyl-2-cyclopenten-1-one
65	1079	1-phenylethanone
66	1082	2-acetyl-1-methylpyrrol
67	1084	2,6-diethylpyrazine
68	1085	2,4-dimethyl-5-ethyltiazol
69	1085	3-ethyl-2,5-dimethylpyrazine*
70	1088	2-furfurylfurane
71	1094	furfuryl propanoate
72	1095	guaiacol
73	1100	S-methyl 2-furancarbothionate
74	1105	n.i.
75	1107	nonanal
76	1110	2-methylbenzofurane
77	1115	2-acetyl-6-methylpyridine
78	1122	n.i.
79	1133	2-furanylmethyl butanoate
80	1144	n.i.
81	1145	n.i.
82	1153	1,2,3,5-tetramethylbenzene
83	1162	1,2,3,4-tetrahydronaphtalene
84	1163	2,3-diethyl-5-methylpyrazine
85	1166	3,5-diethyl-2-methylpyrazine
86	1175	n.i.
87	1185	2-methoxy-3-(2-methylpropyl)pyrazine

** Also detected in feeding. (a) Retention indices in HP-5*

CONCLUSIONS

Considering the results, it can be concluded that pervaporation is a concentration technique which can be applied for the preparation of concentrated aroma products. It is worthy to mention that the permeate is transparent, colorless and caffeine free and can be used as a flavoring agent even for decaffeinated goods.

ACKNOWLEDGMENTS

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Multiple-Approach Method for Sensory Panel Training in Producing Countries

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SUMMARY

Training stakeholders in the coffee supply chain in sensory and physical characterization is an asset for adding value to coffees and CIRAD has developed a training method for that purpose. The aim of sensory panel training method is to give in a same time a global vision of the coffee quality. Various theoretical, practical and cultural approaches are linked with sensory analysis. The participants of these training come from various mediums and their knowledge is often different. It is necessary to adapt the training to public targeted. This method is evolutionary and allows an adaptation to all situations, all publics.

RÉSUMÉ

La formation des acteurs de la filière du café à la caractérisation sensorielle et physique est un atout pour la valorisation des cafés. Pour cela, une méthode de formation a été mise au point au CIRAD.

Notre méthode de formation de jurys sensoriels a pour objet de donner une vision à la fois globale et ciblée de la qualité du café. Différentes approches théoriques, pratiques et culturelles sont regroupées autour de l'analyse sensorielle. Les participants de ces formations proviennent de divers milieux et leurs connaissances sont souvent différentes. Il faut donc adapter la formation au public ciblé. Cette méthode est évolutive et permet une adaptation à toutes les situations, tous les publics.

INTRODUCTION

Cirad experts have developed a method for training stakeholders in the coffee commodity sector on the physical and sensory characterization of coffee. This method is based on experience and on the following ISO standards: ISO 8586 (General guidance for the selection, training and monitoring of assessors), ISO 13299 (General guidance for establishing a sensory profile), ISO 6658 (Methodology – General guidance), ISO 10470 (Defect reference chart).

TRAINING GOALS

The trainees have:

- To be able to make an overall assessment of a coffee product,
- To understand how coffee preparation steps influence the coffee's ultimate sensory and physical features,
- To learn how to assess the typical character of coffee.

This method is presented in several languages on the CD-ROM (2009).

Figure 1. Training session in Kintamani Bali.

A SERIES OF TRAINING APPROACHES

The complementarity of the modules means that training can be adapted to the knowledge of each audience: participant, group, or research project.

- A theoretical approach (involving slide shows presenting the different stages in green coffee preparation) presents the different steps in market coffee preparation,
- A practical, explanatory and demonstrative approach that is related to the sensory test results,
- A cultural approach enables trainees to consider quality in terms of key evaluation criteria in different countries.

LEARNING TOOLS

Panoply of teaching tools has been developed. It includes entertaining tests that create group dynamics and encourage discussions between the participants (knowledge and experiences).

- Participative tests that promote experience exchanges between trainees,
- Blind tasting of coffee from participating countries, different origins, and the market,
- Taste bud map (Figures 1 and 2): each trainee locates his/her taste buds. Distinction between taste and flavour, and the practice of inhalation during sensory assessment,
- Slide show, theoretical courses, practical applications.

Figure 3. Tongue mapping.

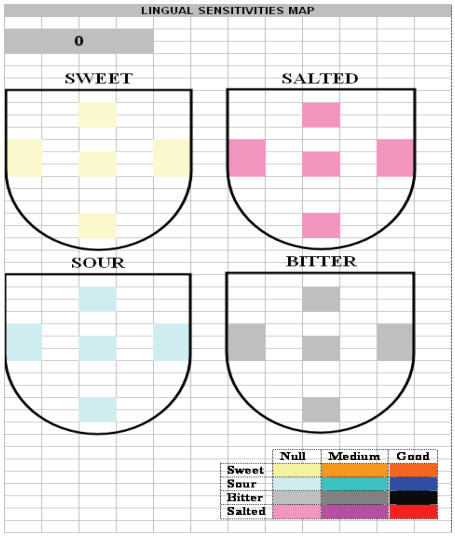

Figure 4. Tongue mapping result.

A THREE-STEPS PROCESS

Training is organized in such a way as to create progressive dynamics.

First step

- Training gain awareness on their ability to indentify basic tastes and on discussing coffee. They learn to work in a team setting.

Figure 2. Working group creation

Second step

- Trainees enhance their sensory assessment skills,
- They learn about coffee assessment terminology,
- Taste panel members are selected at the end of the second step.

Third step

- Trainees gain greater insight on the following topics: sensory analysis, harvesting and postharvest technology, green coffee sampling, coffee storage, main defects of green coffees, grain-size distribution, roasting, coffee preparation for the sensorial analysis, and tasting methods.
- The acquired knowledge is related to organoleptic coffee traits. For instance, identifying physical defects in coffee is linked with postharvest treatment, and defective coffees are tested before and after sorting, and a link is made with each sensory descriptor.

CONCLUSION

By the end of this training, participants are able to make an overall judgment of a coffee. They understand the effects of the different coffee preparation stages (from field to cup). They possess the necessary tools to assess the typicity of their coffee. Regular practice is required to maintain the knowledge acquired.

The main aspects of the method are:

- An innovative method,
- Complementary of training modules,
- Tailored for other products,
- Tailored for different target groups (researchers, local stakeholders, extension agents, funding agencies, etc…).

An educational CD-ROM has been created for that purpose and is currently being improved and translated. It should be available in 2009 and will then be proposed during training sessions.

Latitude: an Overlooked Coffee Quality Factor. Comparison in Cup Quality of Arabica Coffee from Yunnan, China

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SUMMARY

Yunnan is the only Arabica coffee producing province in China and the northernmost coffee planting area in the world (from latitude 22° N to 25.5° N). It has a unique geographic and climatic situation. In this research, 52 coffee samples were collected from three major Arabica plantation regions, namely Siamo, Dehong and Baoshan and rated by a professional cup taster group in Berlin (Germany). Surprisingly, a positive correlation between latitude and taste was found in the comparison of different Catimor 7963 samples from similar altitudes (about 1000 m.a.s.l.). It means that the coffee beans from the higher latitude regions seem to have a better taste quality than those from low latitude regions at similar altitudes. The latitude appears as a geographic component to the cup quality. Behind the latitude, there could be one or more other environment related factors. Further research is needed in collecting more data series to confirm this finding.

INTRODUCTION

Yunnan is the only Arabica coffee producing province in China. It has a unique geographic and climatic situation. It is a highland and plateau province, with the Tropic of Cancer running through its southern part. It is divided into two topography areas (Eastern part and western part). The eastern part is Yunnan highland, which has 2000-meter average altitude, spreading out with a wavy motion. It consists of low mountains and circular hills. The west has transversal mountains and deep valleys. The altitude difference is huge between high mountains and deep valleys. The average altitude of south part and north part are 1500-2200 and 3000-4000 m.a.s.l, respectively. Only in the southwest border of Yunnan, terrain is relatively flat. The coffee growing areas are on both sides of the Tropic of Cancer. There are three major Arabica plantation regions, namely Baoshan and Dehong on the subtropical side and Simao on the tropical side (See Figure 1).

Figure 1. The Yunnan province map (Courtesy: cloud-south.com).

METHODS AND MATERIALS

The 52 coffee samples belonging to 23 varieties, came from the Baoshan, Dehong and Simao coffee plantation regions in Yunnan, China. We separated all samples into three origin groups, and compared the three origins. Moreover, the samples of a single variety, cv Catimor 7963, introduced from Portugal (CIFC) were compared across the three regions.

Organoleptic analysis

The whole process of cup testing is performed under SCAA standard.

Aroma – the fragrance or odour perceived by the nose. There is a clear distinction between aroma at two different stages: aroma of the freshly ground coffee and the "in cup aroma" which is produced when water has been in contact with the ground coffee for 3-4 min (Wintgens, 2004). Generally, it means an odour with a pleasant connotation (ISO 1992). Taste refers to the tongue perception of the coffee quality. It is a very important item for the cup testing. Acidity is a sharp and pleasing taste and can range from sweet to fruity/citrus. Traditionally coffee graders consider acidity as a desirable attribute. Body is a sharp and pleasing taste (Wintgens, 2004).

Physical analysis

Total dry weight of parchment beans / Fresh coffee fruit weight (D/F): From this ratio, the amount of dry parchment beans per ton of fresh coffee fruit can be known. It is an important physical character for the quantity, but its relation to quality will be tested.

The dry weight of 17 size parchment beans/Total dry beans weight (17 size/ D): Bean size distribution is carried out by means of perforated plates commonly called screens (Wintgens, J. N., 2004). The screens with round holes are classified by the diameter of the holes, measured in 64ths of an inc (Wrigley, 1988). 17 size means a diameter of 17/64in (6,75 mm). A big bean (AA or screen size 17) is considered as high quality (Njoroge, 1998). The beans with 17 size, therefore, have a higher price.

The dry weight of 1000 parchment beans (1000 B): A high mean bean weight is considered as a high quality characteristic (Njoroge, 1998; Clifford and Willson, 1985). It is an index of the coffee beans density.

Data analysis

All the statistical analyses were performed by the statistical software of Statgraphic 5.1 and SigmaPlot 10.0. One-way Anova, coefficient variance, the simple regression and average value comparisons were calculated. The aroma and taste values are discrete numbers, namely from 1 to 7. However, the values of latitude and D/F ratio are continuous numbers. In order to solve the problem, the average values of the aroma and taste were used, as rated by three professional tasters. The average values could be considered as approximately continuously distributed. Hence, they were submitted to simple regression analysis.

RESULTS

The coffee samples from Baoshan are better than the other two sample groups in four cup testing characters as well as for total dry weight of parchment beans/fresh coffee fruit weight (D/F) under 1000 m.a.s.l.. Over 1000 m.a.s.l., the BaoShan samples were better in aroma, taste, acidity and D/F. Simao samples had better body and dry weight of 17 size parchment beans/total dry beans weight (17 size/ D). Dehong samples only had advantage in the dry weight of 1000 parchment beans (1000 B) above 1000m (Table 1). In the comparison, the BaoShan samples have better aroma at different altitudes. Regarding taste, BaoShan samples fetch the highest average score. This good performance is confirmed by another important component of overall taste, i.e. acidity.

Table 1. The best rank for cup taste related traits, among three coffee plantation regions, namely BaoShan, Simao and DeHong (all varieties confounded).

	Below 1000 m.a.s.l.	Above1000m.a.s.l.
Aroma	BaoShan	BaoShan
Taste	BaoShan	BaoShan
Body	BaoShan	Simao
Acidity	BaoShan	BaoShan
Total dry weight of Parchment beans/Fresh coffee fruit weight (D/F)	BaoShan	BaoShan
Dry weight of 17 size parchment bean/Total dry bean weight (17 size/ D)	Simao	Simao
The dry weight of 1000 parchment beans (1000 B)	Simao	DeHong

Acidity indicates the acidic balance and the presence of a sweet caramelic after taste. Traditionally coffee graders consider acidity as a desirable attribute. In terms of acidity of liquor, BaoShan samples have clearly more acidity (Table 1).

Body assesses the beverage mouth feel or the property linked with density and viscosity of the brew. Above 1000 m.a.s.l., Simao samples have higher levels. Below 1000 m.a.s.l., BaoShan samples are better (Table 1).

When ignoring the variety differences, Baoshan has better coffee on average. BaoShan is in a valley enduring dry and hot features. The coffee leaf rust disease, therefore, is not so serious. We can still find Typica and Bourbon in BaoShan, especially under 1000 m.a.s.l.. Such advantage is not noticeable in DeHong and Simao. They can only grow rust tolerant introgressed cultivars, such as those belonging to the Catimor group.

In order to know more about the local environmental implications, Catimor 7963 was used as a reference check across the three regions at a similar altitude (about 1000 m.a.s.l.) (Table 2). Again, similar results appear. BaoShan samples are better on average for all four cup testing characters and D/F. Especially in the taste item, BaoShan Samples are significantly better than others at 95% confidence. Simao samples are better in the 17 size/D parameter, meaning that coffee from Simao has more 17 size beans, namely 'AA' class. The samples from DeHong have more density, according to the 1000B parameter. Just from the cup testing point of view, it seems that the environmental conditions of BaoShan are better than those of the other two regions.

Through simple regression analysis, a positive relationship is found between latitude and taste. The correlation coefficient equals 0.74, indicating a moderately strong relationship between the variables (Figure 2).

$$\text{Taste} = -27.4 + 1.26 * \text{Latitude}$$

with a correlation coefficient (r) = 0.74.

Table 2. Comparison for cup related traits among 3 regions in China, using the standard check cv; Catimor 7963 (* = LSD significant at p = 0.05).

	BaoShan	Simao	Dehong
Aroma	X		
Taste	X*		
Body	X		
Acidity	X		
Total dry weight of Parchment beans/Fresh coffee fruit weight (D/F)	X		
The dry weight of 17 size parchment beans/Total dry beans weight (17 size/ D)		X	
The dry weight of 1000 parchment beans (1000 B)			X

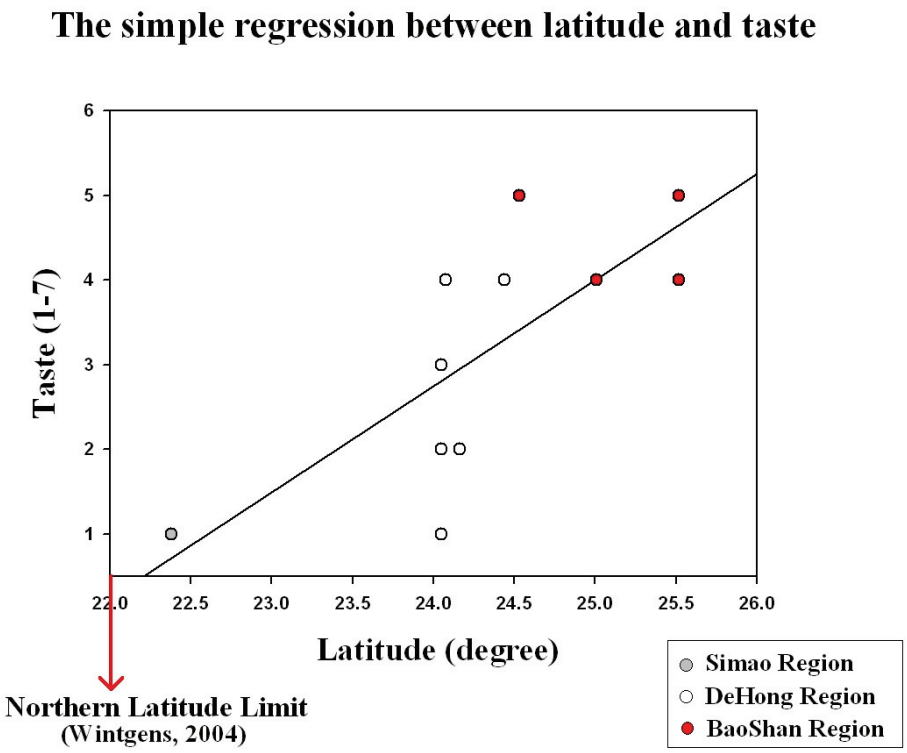

Figure 2. The simple regression between latitude and taste.

DISCUSSION AND CONCLUSIONS

From the Catimor7963 performances across three coffee planting regions, BaoShan seems to be the best place for coffee cup quality. Nevertheless, the basic data are still not sufficient, especially for the SiMao region (Figure 2). The test shows a positive correlation between latitude and taste. Hence, the coffee beans from the higher latitude regions seem to have a better taste quality than those from low latitude regions at similar altitude. However, the question is up to what latitude coffee trees could still grow. This is not only crucial for determining coffee cup quality but also for cultivation possibility through the winter period.

Coffee-growing areas are situated approximately between latitude 22° N and latitude 26° S (Descroix and Wintgens, 2004). There are a few areas of coffee outside these limits, including an area in Parana in south Brazil with low latitudes (20° to 23° S) and low altitude(between 600 and 800 m.a.s.l.) (Wrigley, 1988; Coste, 1992).

SiMao, DeHong and BaoShan regions are beyond these limits, with latitude degrees of 23°, 24°and 25°, respectively. BaoShan region has the highest latitude of the three regions. It could be one of the reasons why the coffee from BaoShan is better than others. The unique geography and climate environment of Yunnan induce such genuine coffee liquor. Generally, from May through October, the weather in China is under influence of the Chinese summer monsoon with predominantly southeast winds. In the remaining months, cold and dry air masses of the northeast branch of the monsoon circulation dominate its weather condition. However, the major part of southwest China, especially Yunnan, is not influenced by the southeast monsoon in summer but receives precipitation from the southwest monsoon (the 'Indian monsoon') (Thomas, 1997). Because of the obstruction phenomenon of the Yunnan-GuiZhou plateau (Duan et al., 2002) and the reunion with the local west air masses, the dividing line, the so-called Kunming quasi-stationary front is formed, alternating seasonally between 102 and 106° E (Thomas, 1997). During the winter, dry and warm western air

masses in the West are separated from cold and humid eastern air masses in the East (Figure 3) (Zhang, 1988). The coffee areas in BaoShan concentrate in the NuJiang Valley orientated from south to north (Figure 4). The north part is connected with Tibet and is much higher than the south part. The highest and lowest points of the valley are 4649 m.a.s.l. and 738 m.a.s.l.. The altitudinal difference is 3911 m. In the western part of the valley, there is an extension of the Himalaya Mountain, the highest one in the world. In the eastern zone, the high mountains of the Yunnan-GuiZhou plateau block off almost all the cool air mass. The whole valley is just like a corner well protected by the mountains around it.

The altitude of the coffee farms in BaoShan ranges from about 800 m.a.s.l. to 1400 m.a.s.l. It falls into subtropical climate. Especially, at the bottom of the valley, the climate is extremely hot and dry. The coffee leaf disease is restricted due to relatively low air moisture level. Therefore, in the whole Yunnan province, the Typica and Bourbon cvs. can only be planted in BaoShan.

The environmental affecting factors summarized by Descroix are Temperature, water availability, sunlight/shading, wind, soil characteristics and so on (Wintgens, 2004). The latitude appears as a geographic component to the cup quality. Behind the latitude, there could be one or more other environment related factors. Further research is needed in collecting more data series to confirm this finding, although a similar discovery was also found in Columbia before (Oberthür, 2008).

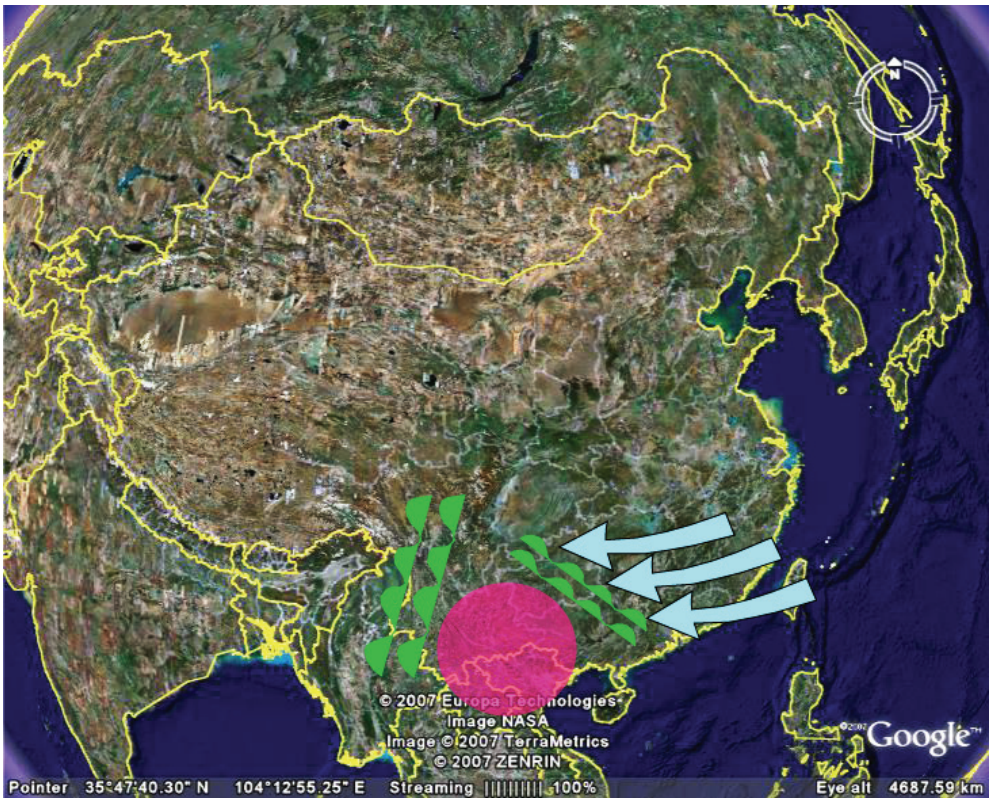

Figure 3. The general weather schema of Yunnan (Courtesy: Google Earth software).

Figure 4. The remote sensing photos of NuJiang Valley (Courtesy: Google earth software).

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Reaction Mechanisms Involved in Coffee Brew Melanoidin Formation

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SUMMARY

This aim of this paper is to introduce reaction pathways that are involved in melanoidin formation in coffee brew. To this end, all findings described so far in the literature were combined with the findings as described in the PhD thesis entitled "Coffee Brew Melanoidins – Structural and functional properties of brown-colored coffee compounds" (Bekedam et al., 2008). Based on all findings known so far, a scheme describing melanoidin-related formation pathways for coffee beans compounds was developed. The reaction pathways involved are explained in detail per coffee bean compound.

INTRODUCTION INTO NON-ENZYMATIC FOOD BROWNING

In the study described, the authors focused on the characterization of the brown-colored compounds in coffee brew. It was assumed at the start of the project that the Maillard reaction was the main contributor to brown-color formation. Indeed, many confirmations were found that indicated that the Maillard reaction was at least one of the prevalent reactions occurring during heating. However, other nonenzymatic food-browning reactions might contribute to the dark color of coffee melanoidins as well (Zamora and Hidalgo, 2005).

First, the nonenzymatic oxidation of phenols was reported to yield brown colored structures too (Zamora and Hidalgo, 2005). Green coffee beans contain high levels of phenolic compounds which is due to the chlorogenic acids. The chlorogenic acids are gradually degraded during the roasting process of coffee beans (Belitz et al., 2004). In this project, it was found that part of these chlorogenic acids were incorporated via the phenolic acid moiety through nonester bonds to coffee melanoidin structures. As a result, it seems reasonable to conclude that the dark color of coffee is also caused by oxidation of chlorogenic acids.

Second, nonenzymatic food-browning might also occur by caramelization reactions. Caramelization is the name for a cascade of heat-induced chemical reactions between sugars leading to brown-colored compounds. However, the authors expect that caramelization reactions play a limited role in food-browning of coffee beans upon roasting when compared to the Maillard reaction. This is expected since it was reported that the Maillard reaction yields much more intense colors than caramelization (Kroh, 1994). Furthermore, caramelization reactions normally involve the heating of pure monosaccharides for quite long lengths of time (Phongkanpai et al., 2006). Coffee beans contain many more reactive components, like amino groups and phenolics, which compete with monomeric sugar-sugar

caramelization reactions. Additionally, coffee beans are heated for a relatively short length of time when comparing to caramelization reaction. In the last place, the correlation that was observed between the melanoidin and proteinous material levels in the previous chapters indicates that Maillard reaction is more prevalent than caramelization reactions.

Third, lipid oxidation can lead to the formation of brown-colored compounds in food products (Zamora and Hidalgo, 2005; Adams et al., 2008). Lipid oxidation and the Maillard reaction might even be intertwined (Zamora and Hidalgo, 2005). However, it is not expected that lipid oxidation is strongly involved in coffee brew melanoidin formation because the lipid content in the beans does not decrease upon roasting (Clarke and Macrea, 1985). It is expected that substantial involvement of lipids in coffee brew melanoidin formation would automatically lead to a decrease in lipid content due to roasting, which was not the case. Furthermore, incorporation of lipids in coffee melanoidins would probably yield melanoidins that are less water-soluble due to the apolar fatty acid tail. In that case, these coffee melanoidins will likely not end up in the brew but in the residue of the brewing procedure. As a result, these water-insoluble melanoidins are not consumed in coffee brew by humans and are therefore not that interesting to investigate.

Summarizing, it is concluded that the Maillard reaction and phenol oxidation are the prevalent chemical reactions involved in the food-browning of coffee beans upon roasting. We do not dare to speculate which of these two reactions contributes most to the brown color development of the coffee beans. However, we do expect that incorporation of chlorogenic acids in coffee brew melanoidin structures, by phenolic oxidation, determines to a large extent the functional properties of coffee brew melanoidins. Some examples of these functional properties are antioxidative properties, charge properties, and extractability.

COFFEE BREW MELANOIDIN FORMATION MECHANISMS

The results presented in this study show that the structure of coffee brew melanoidins is extremely complex. The findings point towards the conclusion that “no melanoidin molecule is alike”. Therefore, it is more realistic and useful at this moment to investigate general structural properties of melanoidins than to pursue revealing the exact chemical structure of a melanoidin molecule. Our previous publications already described the identified general structural properties of coffee brew melanoidins. Now, these general structural properties, together with the information available in the literature, were used to consider degradation pathways of coffee compounds towards melanoidin-like structures.

The reaction mechanisms that were proposed to be involved in coffee melanoidin formation, were combined into one scheme as shown in Figure 1. Formulation of such a scheme was a rather complex task as different reaction pathways will have to coexist without making another pathway impossible. Even more complicated, different reaction pathways will likely require certain other reaction pathways to be able to synthesize melanoidins as those found in coffee brew. Furthermore, this proposed reaction scheme should be in agreement with findings reported in this thesis or published elsewhere in the literature. Figure 1 will be discussed in detail per green bean component in the following sections. It should be emphasized that neither the size nor the concentration of the compounds shown are identical to the precise coffee bean situation. Additionally, other reactions that are not identified so far might play a role as well. Only coffee bean compounds that played a role in melanoidin formation are discussed. The insoluble galactomannans, at least insoluble in green beans, will be discussed first as they serve as a nice introduction into the reaction scheme. Subsequently,

the water-soluble compounds will be discussed, starting with the low molecular compounds followed by the more macromolecular compounds.

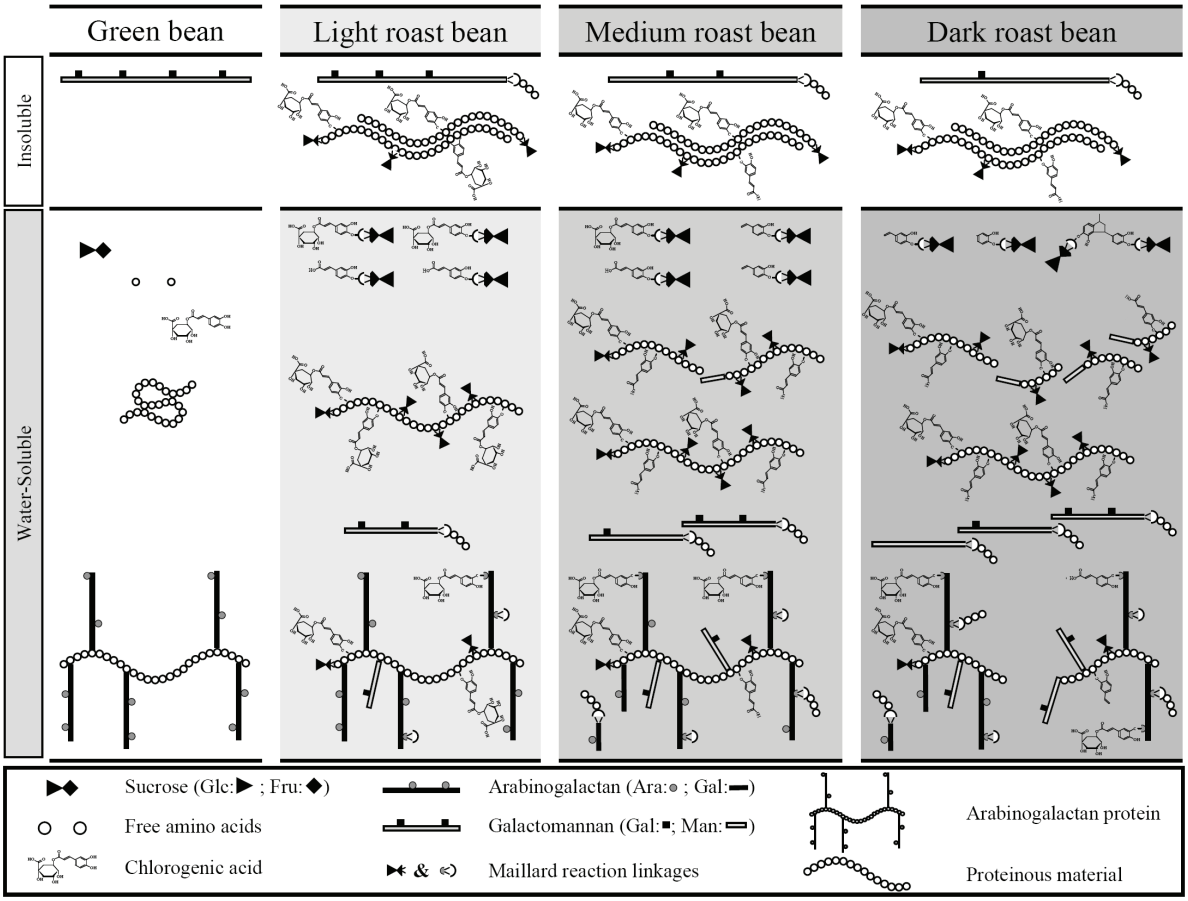

Figure 1. Proposed reaction scheme for coffee brew melanoidin formation that occurs during roasting of coffee beans (not to scale).

Galactomannans

Galactomannans are polysaccharides that consist of a mannan backbone with single substituted galactose residues. Green bean galactomannans are poorly water-soluble due to the linear character of the molecule and the architecture of the coffee bean. Therefore, green bean galactomannans are depicted in Figure 1 as "Insoluble". The degree of galactose substitution on galactomannans decreases upon roasting (Nunes and Coimbra 2001; 2002), which leads to even more linear molecules that are even less water-soluble. This debranching is visualized in Figure 1 by a gradual reduction of the amount of galactose units on the mannan backbone. Additionally, it was reported that the degree of polymerization decreases upon roasting (Nunes and Coimbra 2001; 2002). This reduction in molecular weight should lead to an improved water-solubility of galactomannan fragments. This was confirmed in our PhD research as well: the amount of galactomannans in both HMw and especially IMw coffee fractions increased drastically upon roasting (Bekedam et al., 2008). This Mw reduction, and thus improved water-solubility, is shown in Figure 1 by a gradual increase in shorter mannan chains that are present in the "Water-Soluble" part of the scheme. These shorter mannan chains can still have a quite high or intermediate Mw. This is opposed to galactomannan molecules which did not undergo sufficient weight reduction and remained "Insoluble" due to the very high Mw. It was recently reported that galactomannans can be involved in

melanoidin formation as they were found to carry brown-colored Maillard-like amino structures at their reducing end (Nunes et al., 2006). This observation made by Nunes and Coimbra (2006) is shown in Figure 1 by a linkage of a proteinous fragment which is linked through a Maillard reaction linkage to the reducing end of galactomannan. These Maillard reactions on galactomannan could occur on both water-soluble and insoluble galactomannans, as is shown in Figure 1. One should however realize that the overall contribution of these galactomannan-based melanoidins is probably rather limited as each galactomannan molecule of course only contains 1 reducing end. This is in agreement with our findings that the galactomannan-rich macromolecular coffee material, which precipitated at 20% and 40% aqueous ethanol (i.e. EP20 and EP40) was only moderately brown-colored (Bekedam et al., 2006). The last reaction pathway identified for galactomannans is their incorporation in melanoidin structures that are formed on arabinogalactan proteins (AGPs) (Bekedam et al., 2008; 2007). The galactomannan moiety increased gradually upon roasting up to 6% (w/w) of the AGP-melanoidin complexes. Whether these galactomannans are bound to the protein or the arabinogalactan moiety of AGPs and whether galactomannan is linked directly or though spacer molecules, like chlorogenic acid or an amino compound, is still unknown. The authors are of the opinion that galactomannan is most likely directly linked to the protein moiety of AGPs. However, this aspect remains to be investigated.

Sucrose

It is well known that the content sucrose decreases rapidly upon roasting (Clarke and Vitzthum, 2001). The degradation pathway for sucrose is not clear, it might be that sucrose is first hydrolyzed, followed by participation in chemical reactions. Alternatively, the part of our study that focused on low Mw melanoidin indicated that sucrose can also directly participate in chemical reactions though the fructose moiety, leaving the glucose moiety intact (Bekedam et al., 2008). This process should then first involve a caramelization-like reaction in which the fructose moiety is dehydrated. This may lead to ring-opening of the fructose by which aldehyde groups become exposed that might subsequently participate in Maillard-like reactions. Independent of the precise degradation pathways, it can be stated that sucrose degrades rapidly upon roasting and this sucrose will presumably be involved in the formation of the first melanoidin-like structures. This is depicted in Figure 1 by exclusively introducing sucrose-based Maillard reaction linkages upon initial roasting from green to light roasted beans. This sucrose is linked via fructose as it was found that intact glucose from sucrose could be released from low molecular weight melanoidins (Bekedam et al., 2008).

In the Maillard reaction, sucrose can be linked to free amino acids, to nonAGP protein, and AGP proteins (Figure 1). When linked to proteins, it should be linked to the *N*-terminal amino group or to the available ε-amino groups from e.g. lysine. Additionally, it is possible that glucose is split off from the 'sucrose' that has undergone Maillard-like reactions. Such options are not depicted though in Figure 1.

Free Amino Acids

It is well-known that free amino acids degrade rapidly upon roasting, which is due to their amino groups (Clarke and Vitzthum, 2001). Similarly to sucrose, it can be stated that free amino acids degrade rapidly and will therefore be involved in the formation of the first melanoidin-like structures. This is indicated in Figure 1 by only showing new Maillard reaction structures that involve reaction of free amino acids upon initial roasting from green to

light roasted beans. Upon prolonged roasting, no additional new Maillard reaction structures are shown that involve free amino acids.

We suggest that the majority of the free amino acids will react with degradation products from sucrose or from the arabinose moiety of AGP as these sugars are present in much larger quantities than free amino acids. This leads to the formation of low molecular weight Maillard reaction products (Bekedam et al., 2008) which are shown in the upper part of the "water-soluble" segment of the "light roasted bean" in Figure 1. Additionally, it is thought that the arabinose moiety of AGP can undergo ring-opening by a caramelization-like reaction (Bekedam et al., 2008), as suggested for the fructose moiety of sucrose as well. The aldehyde formed is subsequently available for Maillard-like polymerization reactions with an amino acid. This may lead to AGP-Maillard reaction products which are shown in the lower part of the "water-soluble" segment of the "light roasted bean" in Figure 1 (Bekedam et al., 2008).

Next to Maillard-like reactions with reducing sugars, it is also reported that chlorogenic acids may react with proteins, thus with amino compounds (Montavon et al., 2003; Rawel et al., 2005). We found strong indications that chlorogenic acids were linked to proteinous material (Bekedam et al., 2008)**.** Furthermore, results showed that low molecular weight melanoidins were formed that were rich in both amino and phenolic compounds(Bekedam et al., 2008). It is therefore that free amino acids must be capable of reaction both with sugars and with chlorogenic acids. This leads to the formation of low molecular weight Maillard reaction products containing incorporated chlorogenic acids as is shown in the upper part of the "water-soluble" segment of the "light roasted bean" in Figure 1(Bekedam et al., 2008).

Chlorogenic acids

Chlorogenic acids (CGAs) are, like sucrose and amino acids, degraded upon roasting. It was shown in our thesis that these CGAs are incorporated in coffee melanoidins (Bekedam et al., 2008). CGA degradation occurs throughout the whole roasting process (Bekedam et al., 2008; Clarke and Vitzthum, 2001). As a result, CGAs can be incorporated into melanoidin structures during the whole roasting process. This gradual incorporation of CGAs is depicted in Figure 1 by increasing the number of CGA molecules in AGP molecules from 3 CGA units in light roast, to 4 in medium roast, and finally to 5 in dark roast.

The internal ester-linkage in CGA is not degraded prior to incorporation in melanoidins (Bekedam et al., 2008). Intact CGA is predominantly incorporated via the phenolic acid moiety, being mainly caffeic acid (CA), and not via the quinic acid (QA) moiety. This newly formed linkage between the CA moiety of CGA and the melanoidin backbone occurred mostly through nonester bonds (Bekedam et al., 2008). This incorporation of CGAs into melanoidins via CA through nonester linkages is indicated in Figure 1 by linking CGA via one of the hydroxyl groups of CA to a sugar or amino moiety. The authors do not claim to know how CA is actually nonester-linked, but one of the options is via one of the hydroxyl groups.

Incorporation of intact CGAs may be followed by a release of QA upon prolonged roasting since I) almost no QA was ester-linked in low molecular weight melanoidins (Bekedam et al., 2008), and II) the phenolic groups level was higher than the ester-linked QA levels in both IMw and HMw fractions (Bekedam et al., 2008). Therefore, it can be seen in Figure 1 that incorporation of intact CGA in melanoidin structures can be followed by a release of QA.

This loss of QA from intact CGA that was incorporated in melanoidins is shown in Figure 1 as well.

In the literature, it was reported by Leloup (1995) and Frank et al. (2007) that CA can be converted into 4-vinylcatechol upon roasting. It seems reasonable to suggest that this also hold true for CA that is incorporated in melanoidins. Therefore, it can be seen in Figure 1 that incorporated CA can be converted into 4-vinylcatechol-like structures. Based on the recent paper by Frank et al. (2007), it can be suggested that 2 of these 4-vinylcatechol-containing melanoidin structures might subsequently condensate. This condensation of two 4-vinylcatechol-containing melanoidin structures can be observed for prolonged roasting from medium to dark roasted beans in Figure 1.
The last remark concerning CGAs in melanoidin formation deals with the observed anionic charge properties of melanoidins (Bekedam et al., 2007). The carboxyl group on the QA moiety from CGA contributes to the anionic properties of melanoidins under the proposed incorporation mechanism for CGAs. In the case that QA is released upon prolonged roasting, the carboxyl group from CA becomes exposed and contributes to the anionic charge properties of the melanoidins. This carboxyl group is only removed when incorporated CA is converted into 4-vinylcatechol-like structures. In that case, the melanoidins should be less negatively charged. In Figure 1, it is indicated that the amount of incorporated carboxyl groups from CGAs increases upon prolonged roasting in AGP melanoidins, being in line with the increase in negative charge in HMw material upon prolonged roasting.

Proteins

Green beans proteins are quite water-soluble (Bekedam et al., 2008). Part of these proteins is present in arabinogalactan proteins (Redgwell et al., 2002; 2005) and part is present as 'unbound' proteins, like the 11S storage protein (Rogers et al., 1999). The ratio between AGP proteins and unbound proteins in green coffee was around 1 to 50 (Bekedam et al., 2008), showing that most proteins were unbound. The unbound proteins will be discussed in this section while the AGPs are discussed in the next section. Upon roasting, unbound proteins denature and aggregate resulting in a reduction in extractability (Bekedam et al., 2008). Figure 1 suggests that the native proteins in green beans are rather "water-soluble". The denaturation and aggregation of these proteins is shown by the unfolded and aligned proteins in the "Insoluble" segment of Figure 1. Next to this denaturation and aggregation, the proteins undergo chemical reactions via their reactive groups as well. These proteins become resolubilized when the degree of chemical modification on proteins, by Maillard-like and CGA-incorporation-like reactions, is high enough. The difference between 'insoluble' and 'resolubilized' denatured proteins is shown in Figure 1 by their difference in degree of 'decoration' with sucrose or CGA molecules. Upon further roasting, it was found that more and more proteinous material ended up in the coffee brew (Bekedam et al., 2008). This could be ascribed to depolymerization of proteins into lower Mw fragments upon roasting which then automatically led to an increase in the degree of chemical modifications as well (Bekedam et al., 2008). This process of proteinous material accumulation in coffee brew caused by depolymerization of proteins into lower Mw fragments is shown in Figure 1 too. Protein fragments might also be linked to e.g. arabinogalactan or galactomannans.

Arabinogalactan protein

Arabinogalactan proteins (AGPs) are water-soluble macromolecules. However, not all AGPs were extracted into the brew prepared from green beans probably due to entanglement by the

cell wall components. AGPs were the first group of compounds of which we could decisively conclude that they are involved in melanoidin formation (Bekedam et al., 2007). There are quite some chemical reactions that may occur on AGP molecules upon roasting.

It was already mentioned that galactomannans and proteins might degrade upon roasting into smaller fragments. This degradation might just as well happen with the arabinogalactan and protein moieties in AGP, as is indicated by the presence of arabinogalactan in intermediate Mw fractions after roasting (Bekedam et al., 2008; 2007). This degradation of the protein and arabinogalactan moieties present in AGPs is shown in Figure 1 as well.

Next to degradation of AGP molecules yielding smaller molecules, AGPs can also be 'decorated' by incorporation reactions in which several coffee compounds are attached to the AGP molecules. First, after ring-opening by caramelization-like reactions yielding aldehydes, sucrose might undergo Maillard-like reactions during the initial roasting stage with the protein moiety of AGP. In a similar way, it can be reasoned that the arabinose moiety from AGP undergoes ring-opening and is transformed into an aldehyde-containing structures by caramelization-like reactions. This is in line with the findings that predominately the amount of arabinose is reduced upon roasting (Oosterveld et al., 2003; Redgwell and Fischer, 2006). This conversion reaction then yields aldehyde structures in the AGP molecules that are capable of reacting with free amino acids during the initial stage of roasting as well. Furthermore, the roasting process yields degraded protein and sugar (e.g. galactomannan) fragments that might be incorporated in the AGP structure upon prolonged roasting. Degraded protein fragments are thought to be attached via Maillard-like reaction to the reducing galactose residue that is formed by degradation of arabinogalactan as discussed above (Figure 1). Additionally, the protein fragments might be linked through arabinose molecules on AGPs, as discussed above (Figure 1). The galactomannan fragments are thought to be attached to the protein moiety of AGPs via Maillard-like linkages. The most likely possibility for these linkages is through *N*-terminal amino groups that become available by degradation of AGP's protein moiety (Figure 1). However, attachment of galactomannan to side chains of amino acids may also occur, especially during the early stage of roasting. This option is also depicted in Figure 1. It is expected that galactomannans are mainly linked to ε-amino groups during initial roasting while linkage to *N*-terminal amino groups is more abundant upon prolonged roasting.

Next to these protein and sugars, CGAs might be incorporated throughout the whole roasting process as well, as was already discussed in the CGA section (Figure 1). These incorporated CGAs might undergo a release of QA (Figure 1), and possibly transformation to 4-vinylcatechol (Figure 1). These incorporated CGAs contribute to the observed phenolic properties and the negative charge of melanoidins.

It was above already suggested that coffee bean compounds, like CGAs, are linked to the arabinose moieties from AGPs. This was suggested since a gradual availability of chemical reactive binding places is required to be able to gradually incorporate CGAs into AGP-melanoidin structures upon roasting. Linkage to the protein is not likely as the amino acid composition did not alter significantly (Bekedam et al., 2008). With respect to the arabinose, it was found that its level decreased while the level of incorporated CGAs increased (Bekedam et al., 2008). This may suggest that the arabinose moiety from AGP undergoes ring-opening, a dehydration reaction similar to caramelization reactions, which yields rather reactive aldehyde structures that might participate in subsequent reactions with e.g. CGAs. The susceptibility of arabinose towards degradation upon roasting was reported more often

(Oosterveld et al., 2003; Redgwell and Fischer, 2006). Although this suggested option for incorporation seems pretty likely, no decisive proof could be found.

The Yariv reagent used for AGP isolation is very specific for the structure of AGPs. Why the whole AGP molecule precipitates with Yariv, while the separate AGP building blocks arabinogalactan or protein alone do not precipitate with Yariv is not clear. On the basis of this specificity, it was expected and found that AGPs do not precipitate with the Yariv anymore when the structure of AGP is changed too much by degradation and/or 'decoration' reactions. Thus, precipitation of AGP structures will depend on the degree of 'decoration'/degradation. The 'decoration' and degradation of AGP structures with e.g. CGAs was already discussed above and is shown in Figure 1. Whether AGPs do or do not precipitate with Yariv, can depend solely on the degree of "decoration", i.e. in case of no or limited degradation of the AGP structure itself. In that case, the AGP macromolecular structure remains intact but is 'decorated' to such an extent that is can not precipitate with Yariv anymore. This is in line with findings that indicated the presence of macromolecular arabinogalactan material in the high Mw fractions that does not precipitate with Yariv (Bekedam et al., 2008; 2007). Degradation of the protein backbone will yield smaller sized AGP structures that do not precipitate with Yariv. These smaller sized AGP structures might then be recovered in IMw fractions (Bekedam et al., 2008; 2007) and indeed did not precipitate with Yariv (Bekedam et al., 2007). These smaller sized AGP structures, can just like the originally sized AGPs, be "decorated" with CGAs, sucrose, galactomannan fragments, and protein fragments. For clarity reasons, no distinction was made in Figure 1 between AGP structures that do react with the Yariv reagent and those that do not.

CONCLUDING REMARKS

Altogether, it can be concluded that investigation of coffee brew melanoidin formation pathways remains extremely complex. This is due to the fact that many coffee components are involved in the formation of coffee brew melanoidins. Furthermore, the size of coffee brew melanoidins might decrease due to degradation of carrier molecules or might increase due to chemical linkage of low Mw melanoidin structures as well. Additionally, the balance between soluble and insoluble coffee bean compounds is also dynamic as some compounds become extractable upon roasting while other compounds are not present anymore in brew after prolonged roasting due to conversion into roasting gasses. All these aspects together cause that reaction pathways involved in coffee brew melanoidin formation are extremely complex and poorly understood. Therefore, the proposed reaction scheme describing coffee brew melanoidin formation pathways is a major step forwards in understanding the melanoidin formation processes. This reaction scheme provides a useful tool to summarize all knowledge that is available in the literature so far. It is clear that other reactions than Maillard reactions are also involved in coffee brew melanoidin formation.

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Coffea arabica var. *laurina* Authentication Using Near Infrared Spectroscopy

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SUMMARY

On the island of Réunion, a project designed to develop a high added-value coffee brand was launched in 2002. The project focused on *Coffea Arabica* var. *Laurina* called 'Bourbon Pointu', and set out to determine growing practices and post-harvest treatments enabling the production of an inimitable coffee. The project involved the development of 106 experimental cultivation plots. The first phase of the project, from 2002 to 2007, was used to establish technical/commercial references for the production and processing of high added-value "grands crus" coffees. Soils were characterized and farms potentially suitable for the production of quality coffee were identified. Over that period more than 1126 samples were collected and analysed in diffuse reflectance from 1100 nm to 2500 nm using a NIRSystem FOSS 5000 spectrometer (Port Matilda, USA). In that way, a Near Infrared (NIR) spectral database specific to Bourbon Pointu (BP) coffees was compiled. A principal components analysis was performed on the spectral database in order to calculate Mahalanobis distances from the average spectrum for each spectrum and then fix boundaries for the population. Based on these results, samples differing from pure BP, such as "Bourbon rond", were removed from the database. The database was constructed by removing samples corresponding to abandoned trials such as certain post-harvest treatments or fertilizer tests. This led to a specific database for BP comprising 670 spectra of green coffees. From that collection, 250 random samples were analysed at the CIRAD laboratories, using reference methods, for their caffeine, chlorogenic acids, trigonelline, fat, sucrose and moisture contents. Those values were added to CIRAD's Arabica NIR database to develop specific calibration for BP coffees using Partial Least Squares Regression. This approach revealed that it was possible to authenticate BP coffee from Réunion using NIRS fingerprinting. The calibrations developed in this study made it possible to quantify the content of 6 major quality-related chemical compounds in BP green coffee.

INTRODUCTION

The island of Réunion is characterized by a wide diversity of agricultural situations. The differences are due to biophysical characteristics, such as soil, temperature, rainfall and social aspects, including types of farming and production systems. The Coffee project falls under a long-term programme to create coffee sector production as part of the Réunion region agricultural diversification programme.

This project, scheduled over a 10-year period, has focused on *Coffea Arabica* var. *Laurina,* called 'Bourbon Pointu', and set out to determine growing practices and post-harvest treatments enabling the production of an inimitable coffee The first phase of the project (2002-2007) was devoted to 1) the selection of coffee trees (within *Réunionnais* gardens) exhibiting specific agronomic, biochemical and organoleptic profiles, 2) identification of the different *terroirs*, 3) the elaboration of technical and economic references for coffee

production, 4) the study of technical-economic farming conditions suitable for the production of high quality coffee. This study was launched in 2005, the year when the 106 experimental cultivation plots produced their first yields. The aim of the study was to characterize Bourbon coffees for their biochemical properties using NIRS. The specific objectives were 1) compile a specific spectral database for Bourbon Pointu coffee (BP), 2) develop predictive equations for caffeine, moisture, trigonelline, chlorogenic acids, fat and sucrose contents. In this scheme, more than 1126 samples were collected and analysed for their NIR fingerprint, using a NIRSystem FOSS 5000 spectrometer (Port Matilda, USA). A NIR-predicted model developed for Arabica (Davrieux et al., 2003) coffees was applied to the database, samples with low or high predicted moisture contents and samples with predicted caffeine contents over 0.9% were discarded. The database was then structured using PCA analysis and Mahalanobis distances (GH) (Williams and Norris, 1990); samples with GH values over 3 were discarded. This led to a specific database for BP comprising 670 green coffee spectra. From that collection, 228 random samples were analysed for their caffeine, chlorogenic acids, trigonelline, fat, sucrose and moisture contents, at the CIRAD laboratories using a reference method. These values were added to CIRAD's Arabica NIR database to develop specific calibration for BP coffees using Partial Least Squares Regression. This approach showed that it was possible to authenticate BP coffee from Réunion using NIRS fingerprinting. The calibrations developed in this study made it possible to quantify the content of 6 major quality-related chemical compounds of BP green coffee.

MATERIAL AND METHOD

Plant material

The study was carried out over 3 production years, 2005, 2006 and 2007. A total of 1126 samples was collected over that period: 401 in 2005, 325 in 2006 and 400 in 2007. Sampling covered the 106 experimental orchards (Figure 1) and was representative of the different factors studied: *terroirs*, fertilizers, agricultural practices and post-harvest treatments. All the samples were wet processed.

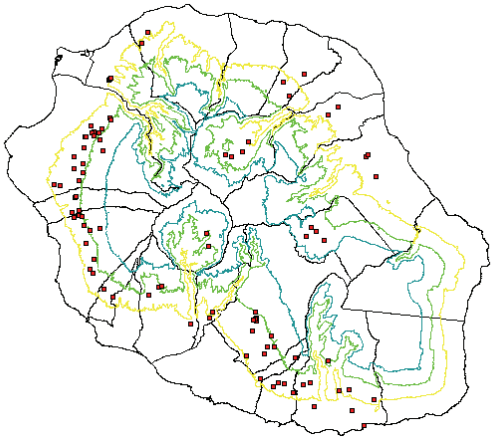

Figure 1. Réunion BP coffee experimental orchard (106) design.

After sun drying, parchment coffees were stored in a climate chamber (60% RH and 28 °C). In that way, the expected moisture content should have been around 11%-11.5%. Parchment was removed prior to analysis (Africa Hullers McKINNON) and the coffee beans were graded (vibro grader, Spectrum Industries); only grade 14 beans were analysed. The beans were cooled with liquid nitrogen and ground (< 0.5 mm) using a Rescht ZM200 grinder.

Near infrared spectroscopy

About 3 grams of homogenized powder were analysed in NIR using a FOSS 5000 spectrometer equipped with a transport module and small ring cups. Spectra were recorded as log (1/R) in diffuse reflectance from 1100 nm to 2500 nm, in 2 nm steps.

The spectra were mathematically transformed using WINISI 1.5 software (Infrasoft International, Port Matilda, USA): a second derivative of the standard normal variate and a detrend corrected spectrum (SNVD) calculated on five data points and smoothed (Savitzky and Golay smoothing) on five data points.

The spectral population was structured (Shenk and Westerhaus, 1991) using a Principal Components Analysis (PCA) and Mahalanobis distances (H) calculated on extracted PCs. The matrix expression of H calculation was: $H = X (X' X)^{-1} X'$, where H was the matrix of H distances, X the matrix of centred spectra data and $(X'X)^{-1}$ the reverse matrix of variance covariance. The Mahalanobis distance is the distance of each sample from the average sample and takes into account the total variability of the population. Generalized H distances (each individual distance divided by the average distance) were expressed as standard deviations, making it possible to define population limits and associate a probability with the H distance. A sample with an H distance over 3 had a probability of less than 1% of belonging to the population.

The parameters studied here were calibrated using Partial Least Squares Regression (mPLS). The calibration statistics used to evaluate model performances included the standard error of calibration (SEC), the coefficient of determination (R^2), and the standard error of cross-validation (SECV). The cross-validation method resulted in calculation of the SECV. For SECV, 25% of the samples were used to validate a calibration model developed with the other 75%. SECV was repeated four times and the average calculated. The Student test (t) was used to identify t-outlier samples.

The statistics, multivariate analyses and regression model were performed using WINISI 1.5 software, STATGRAPHIC Centurion XV(StatPoint, Inc., Usa) and XLSTAT version 2008 6.02 (Addinsoft, Paris, France)

Laboratory analyses

After extraction (water reflux with magnesium oxide), caffeine and trigonelline contents were determined by HPLC and UV detection at 280 nm. Polysaccharides were extracted with water (reflux) then separated and quantified using HPLC and pulsed amperometric detection. Total chlorogenic acids were extracted with methanol-water (70% w/w) then purified trough a polyamide column, eluted using alkaline methanol and quantified using a spectrophotometer at 324 nm. Fat content was determined by gravimetry using a Soxtech (FOSS) extractor and petroleum ether. Moisture content was quantified by gravimetry at 103 °C for 16 hours using a Chopin oven.

RESULTS AND DISCUSSION

The database was compiled with all the information relative to the coffee grown under the project: agronomic data, environmental data, genetic resources, post-harvest processing, qualitative data, chemical data and spectral data. The database was used to define a BP profile and understand the quality determinants. Based on this, a specific database for BP coffees was set up and used to authenticate new samples as BP. This approach required the compilation of

a database that was perfectly known (all kept samples certified BP), robust (all the possible cases foreseen, such as different cultural zones) and precisely defined (sampling, sample preparation and NIR analyses follow specific protocols).

Spectral database structuring

Based on the previously defined criteria, the following methodology was applied to set up a database representative of certified BP coffees:

1) Selection of samples based on administrative data: samples from non-retained orchards, abandoned trials, specific and/or single trials, samples unclearly tagged and off-type samples (such as "Bourbon rond" coffee) were discarded.
2) Elimination of samples with an atypical log(1/R) spectrum
3) A PCA analysis was carried out on the remaining samples and H distances calculated as a function of extracted PCs.
4) The remaining samples were predicted for their caffeine and moisture content using the NIR calibration developed at CIRAD for Arabica and BP coffees. A two-sided Grubb test with a 5% confidence level (Grubbs F., 1969), was performed on the predicted caffeine and moisture contents in order to identify and eliminate extreme values.
5) A final PCA was performed on the cleaned database in order to fix explained variability and calculated final H values for the optimum number of PCs adopted.

A total number of 1126 samples was analysed in NIR over 3 years, in accordance with the described procedure: 419 samples from abandoned trials or without clear tags, 2 samples with an atypical spectrum, 10 samples with H values over 3, 6 Arabica coffee samples from different origins, 8 samples with extreme caffeine values (Grubb's test) and 11 samples with extreme moisture values were discarded.

After 2 iterations (2 successive PCA), the calculation of H values based on the 32 CPs extracted enabled the elimination of 9 outliers corresponding to specifics *terroirs* (3), unidentified plant material (1), unsuitable soil (1) over-fermented and overripe coffees (4).

Grubb's test, performed on predicted caffeine and moisture contents, identified 10 extreme caffeine values and 11 extreme moisture values respectively.

Among the 10 extreme caffeine values (Figure 2), 8 were over 0.85%, one equal to 0.85% and one under 0.46%. In accordance with the limit of 3 times the standard deviation (0.065%) around the mean value (0.65%), the 8 samples with a caffeine content over 0.85% were discarded. Those samples were tagged as "off-type" and corresponding to "Bourbon rond" and Arabica coffees grown in Réunion.

The 11 samples with extreme moisture values, 8 over 13.5% and 3 under 8%, were also removed from the database.

The final database, structured in this way, contained 670 samples: 131 in 2005, 323 in 2006 and 216 in 2007. The descriptive statistics for predicted values using the CIRAD equation are shown in Table 1; the average moisture value was constant over the year (11.4%), and the maximum value was 13.5%.

The caffeine content was between 0.46% and 0.85% with an average value of 0.65% constant over the year. The fat content ranged from 12% to 17%. Chlorogenic acids ranged from 5.7%

to 8.4% and the average value observed in 2007 (6.6%) was smaller than in 2005 and 2006 (7.1%).

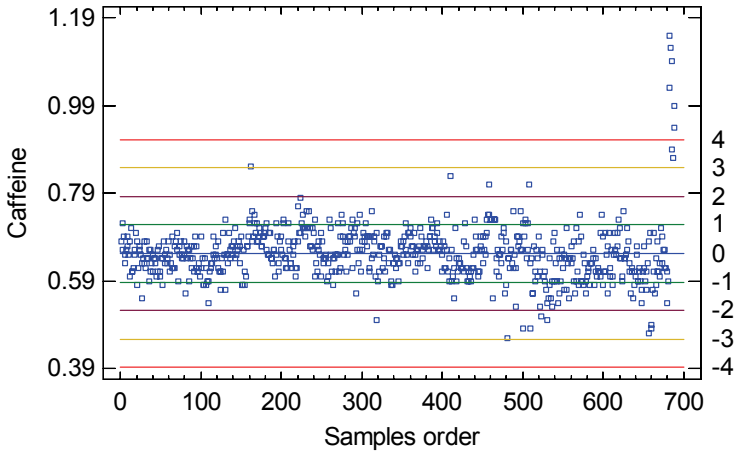

Figure 2. Grubb's test for extreme caffeine content values.

The trigonelline content was constant over the year and ranged from 0.68% to 1.14%, with an average of 0.92%. The sucrose content increased from to 2005 to 2007, from 7.6% /7.8% to 8.1% on average.

Table 1. Descriptive statistics of the 670 BP coffee samples. Predicted values*.

	Caffeine	Trigonelline	Fat	Sucrose	Chlorogenic acids	Moisture
Minimum:	0.46	0.68	12.08	6.04	5.69	8.22
Maximum:	0.85	1.14	17.33	10.30	8.39	13.55
Mean:	0.65	0.92	15.14	7.88	6.94	11.35
Std. Deviation:	0.05	0.06	0.73	0.61	0.48	0.99
Total Values:	670	670	670	670	670	670

**Expressed as a % of dry matter*

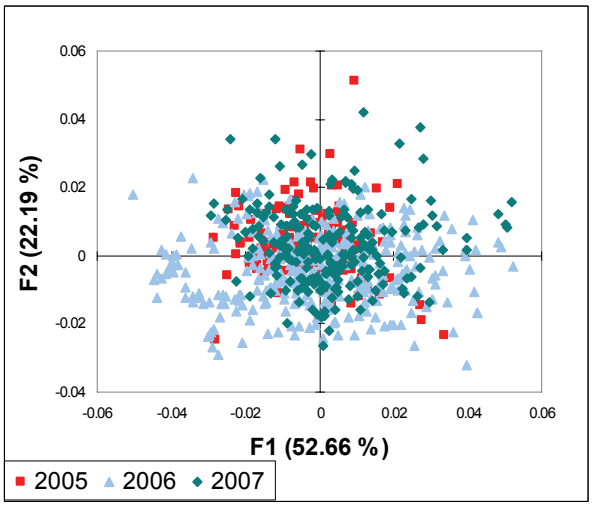

Figure 3. Scatter plot of the 670 sample scores for the first 2 PCs.

A final PCA was performed on the 670 spectra; the first 3 PCs explained 87.6% of total inertia, 52.6%, 22.2% and 12.8% respectively. The H distances were calculated using 21 PCs, the maximum H value was equal to 2.7 with no outlier. The scatter plot of the sample scores

for the first 3 PCs (Figure 3) showed a uniform distribution of the samples without any year effect.

Calibration

Of the 670 BP coffees, 250 random samples were analysed in the CIRAD laboratory for the 6 constituents and for their NIR spectra using CIRAD's FOSS 6500 spectrometer. These new data were merged with the CIRAD NIR Arabica database to create an Arabica database adapted to the specificities of BP coffees (Table 2).

Partial Least Squares Regression (PLSR) was used to establish quantitative relations between NIR spectral bands and caffeine, trigonelline, chlorogenic acid, fat, sucrose and moisture contents. The performances of the equations, in terms of R^2, SEC and SECV (Table 3) for caffeine, dry matter and fat, were highly satisfactory and the equations could be used for routine analyses. The predictive models developed for total chlorogenic acid and trigonelline contents were less efficient and those equations could be used for fine screening of samples based on these constituent predictions.

Table 2. Descriptive statistics for wet chemistry Arabica – BP coffee data base.

	Sucrose	DM	Caffeine	Trigonelline	Fat	Chlorogenic acids
Minimum:	5.26	85.10	0.45	0.67	10.46	5.10
Maximum:	10.66	93.99	1.79	1.39	17.70	11.04
Mean:	7.60	89.42	1.04	0.98	14.85	7.50
Std. Deviation:	0.94	1.49	0.32	0.11	1.52	0.90
N	551	831	606	496	356	466

The adjusted model for sucrose content was not sastifactory, probably due to a lack of fit in the wet chemistry method and the time delay between NIR and chemical analyses. This equation could be used for a rapid estimation of sucrose content and thereby the detection of extreme samples.

The scatter plot (Figure 4) for caffeine wet chemistry and NIR-predicted values highlighted the quality of the fit and showed the potential of the model for separating BP coffees with low caffeine contents from conventional Arabica coffees.

Table 3. Statistics parameters for Arabica – BP coffee NIR equations.

Constituent	N	Mean	SD	SEC	R^2	SECV
DM	751	89.49	1.47	0.13	0.99	0.14
Caffeine	577	1.03	0.32	0.05	0.97	0.06
Trigonelline	480	0.97	0.10	0.05	0.75	0.06
Fat	341	14.87	1.50	0.40	0.93	0.45
Sucrose	523	7.52	0.84	0.48	0.67	0.57
Chlorogenic Acids	449	7.48	0.84	0.42	0.75	0.47

N: Number of samples used for computation (t test); SD: standard deviation of reference values; SEC: standard error of calibration; R^2: coefficient of multiple determination; SECV: standard error of cross validation.

In the same way, PLSR models were developed for the 670 BP coffee spectra. Due to the reduced range in variation with the cleaned database specific to BP, the R^2 obtained were low (except for moisture and fat, $R^2 > 0.80$); for caffeine, R^2 was equal to 0.15 with a SD equal to 0.06%. Although the SECV and SEC error terms were of the same order of magnitude (data not shown) as those obtained on the whole database (SECV 0.05% and 0.06% for caffeine), these models were not usable for routine analysis.

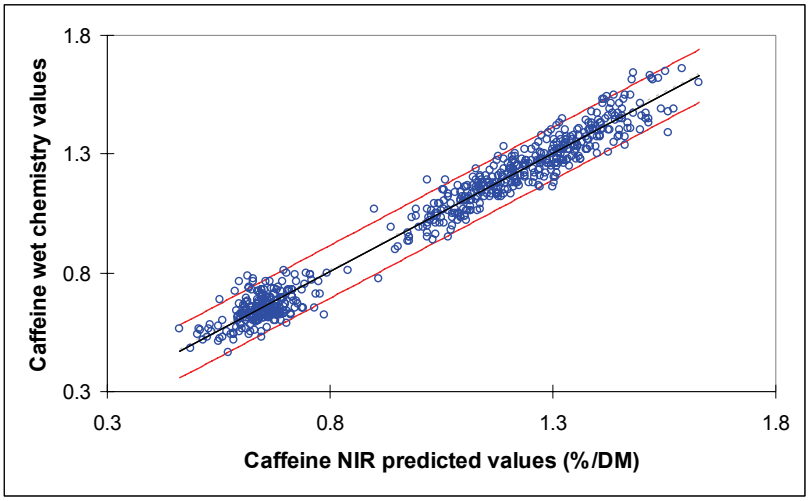

Figure 4. Scatter plot for measured caffeine vs. NIR- predicted values.

This was illustrated with the predicted caffeine content for Arabica and off-type samples removed from the BP coffee database, using the two models (Table 4).

Table 4. Caffeine content for Arabica and off-type coffees with the 2 models.

	Predicted caffeine values (% DM)			H distance vs BP database
Samples	Global equation	BP equation	Laboratory value	
Arabica1	1.18	0.584	1.27	10.5
Arabica2	1.07	0.563	1.19	15.6
Arabica3	1.15	0.646	1.25	3.9
Arabica4	1.11	0.648	1.19	3.3
Arabica5	1.15	0.659	1.24	2.1
Arabica6	1.15	0.661	1.17	2.2
Off-type1	1.15	0.694	ND	1.9
Off-type2	1.03	0.653	ND	2.5
Off-type3	1.12	0.701	ND	1.6
Off-type4	1.09	0.679	ND	0.9
Off-type5	0.89	0.623	ND	2.1
Off-type6	0.87	0.63	ND	5.0
Off-type7	0.94	0.683	1.09	2.2
Off-type8	0.99	0.693	ND	1.7

ND: non determined.

The predicted caffeine contents, using the specific BP coffee model, were underestimated for Arabica and off-type coffees, while they were correctly estimated using the global model based on numerous Arabica and BP coffee references.

At the same time, the H values calculated after projection on the final BP database scores were all higher than the outlier limit (H = 3) for Arabica 1 and 2 (Costa Rica), Arabica 3 (India), Arabica 4 (Cuba) and under 3 for Arabica 5 (Ethiopia, Moka) and Arabica 6 (Brazil). Among the off-type samples, only one had an H value over 3; off-type sample 6 was a Bourbon rond coffee from Réunion.

These results showed the need to combine a qualitative approach (H value) with a quantitative approach (PLS models) to characterize a specific coffee. In our study, the combination of the 2 strategies led to perfect identification and selection of pure BP coffee from Réunion.

CONCLUSION

The performance of the predictive models based on a broad database of Arabica coffees adapted to Bourbon pointu coffees from Réunion enabled fine characterization of these specific coffees, especially in terms of caffeine content (SECV = 0.06%, $R^2 = 0.97$). The NIR fingerprint of BP coffee can therefore be used to identify fair commercial green BP coffees (mature, fair fermented, dried) without defects by comparison with a specific BP coffee database (H value) and through its predicted biochemical profile. This study demonstrated the need to have a well-known and highly variable database to develop efficient NIR equations; calibration for BP coffees only was not possible.

Further studies will try to strengthen authentication by combining NIR fingerprints, biochemical profiles and sensory profiles. We can imagine achieving a classification by production zone using NIR and sensory analyses.

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Near Infrared Spectral Signature and their Stability across Environments

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SUMMARY

Earlier work on food plants showed that NIRS applications appeared to be effective for authenticating varieties. In our work, we confirmed that result for coffee varieties, but we also showed that inter-variety relations are not stable from one harvest to the next. We put forward the hypothesis that the spectral signature is affected by environmental factors. The purpose of this work was to find a way of reducing environmental variance in order to increase measurement reliability and enable practical application in breeding. Spectral collections were obtained on ground green coffee samples from agronomy trials. Two harvests of bean samples from 11 homozygous introgressed lines, and the cv 'Caturra' as the control, supplied from 3 different sites, were compared. For each site, squared Mahalanobis distances between the 12 varieties were estimated based on NIR spectra. Matrix correlation coefficients between matrices were assessed by the Mantel test. The higher the coefficients, the more we concluded that inter-variety relations were identical from one environment to another. We showed that very good stability was obtained for inter-variety relations across the sites when using the two harvests data. At the same time, the spectrum was regarded as a succession of phenotypic variables, each resulting from an environmental and genetic effect. Heritabilities were calculated with confidence intervals. The stability of inter-variety relations was largely increased if only the most heritable zones of the spectrum were kept. It could therefore be considered that a coffee variety can be characterized by a NIRS signature acquired over a set of harvests. We indicated how this typical signature can be used in breeding to assist in selection.

INTRODUCTION

Near Infrared Spectroscopy -NIRS is based on the absorption of electromagnetic radiation by matter (Osborne, Fearn and Hiddle, 1993). When applied to food products, this technique is of analytical use (Bertrand and Qannari, 2006) since it can extract a large amount of information concerning biochemical composition. Calibration equations are established by regression techniques taking into account a limited number of predictors which are absorbances at certain wavelengths. Another possible use of NIRS is to authenticate varieties. For instance, this technique has been used to authenticate asparagus cultivars (Perez, 2001). The chemometric discrimination of the two coffee species *C. arabica* and *C. canephora* with NIRS approaches (Esteban-Diez et al. 2007, Downey et Boussion, 1996) or Raman spectroscopy (Rubayiza et Meurens, 2005), have been tested with success. Based on NIR spectra treatment, Bertrand *et al*. 2005 investigated ways of discriminating between modern introgressed varieties and traditional ones. They demonstrated that about 90% of varieties could be successfully classified in these two categories through combined Principal Component Analysis (PCA) and Discriminant Analysis of spectral data. Here, we assumed

that a NIR spectral signature could characterize each introgressed coffee variety. As a phenotypic trait, that signature is influenced by the environment and possibly by 'variety × environment' interactions, which result from the differential performance of the variety across environments. For breeders, strong interactions are considered to be random nuisance factors. In this work, based on spectral collections acquired for ground green coffee samples from a mating design, we i) confirmed the effectiveness of the NIRS signature in discriminating between varieties and ii) we tried to demonstrate that the coffee variety NIR spectral signature was stable across environments.

We first used the total spectral information in a multivariable analysis, assuming that all the points of the whole spectrum were good predictors. We also tested a new method based on the heritability concept, to choose the best predictors among the spectra. Heritability is known to be a function of genotypic interaction and error variances. This is the degree of confidence when evaluating genotypic value through the phenotypic value and gives a measurement of genetic stability for a given trait. We considered that a NIR spectrum was a discrete sequence, each point of which was a trait used as a phenotypic predictor of the genotypic spectral signature influenced by environmental effects. By keeping only those predictors for which heritability was significant, we sought whether it was possible to improve the stability of the coffee variety NIR spectral signature. The purpose of this study was to assess the application of a Near Infrared Spectroscopy (NIRS) methodology to assist in the selection of introgressed lines

MATERIAL AND METHODS

Plant material

The experimental design employed in this study included three Colombian locations in full combination with twelve *Coffea arabica* L. genotypes (eleven advanced lines and Caturra) and two field replications (total of 72 coffee bean samples). The variety Caturra was selected as representing high-quality traditional varieties. The eleven advanced lines (A to K) were (at least generation F5) derived from crosses between Caturra and the Timor hybrid accession CIFC-1343. They were selected for their high yield, quality, and resistance to rust. The three locations studied represented the main coffee growing regions in Colombia, namely A at 1,381 m.a.s.l, B at 1,250 m.a.s.l and C at 1,635 m.a.s.l. They exhibited contrasting agro-climatic characteristics. Samples were collected during the harvest peak, using healthy ripe cherries. For each sample, 1 kg of cherries was processed by the wet method (pulping, fermentation and drying) to obtain approximately 250 g of green coffee beans. The samples of green coffee were screened through a size 17/64 inch sieve and the most defective beans were discarded.

NIRS phenotyping

NIR reflectance spectra were collected using a scanning monochromator NIRsystems spectrophotometer (model 6500) driven by NIRS2 (4.0) software (Intrasoft Intl., LLC, RD109, Sellers Lane, Port Matilda, Pa 16870). The analyses were performed on 3 gr. green coffee after grinding. For each sample, a NIR spectrum was acquired in reflectance (R) mode, where R represented reflectance energy in the 4000-9090 cm^{-1} range in 7 cm^{-1} step (Downey and Boussion 1996). The log (1/R) absorbance spectrum was obtained by the mean of those measurements and comparison with the reference. The mean quadratic error estimated from two sub-samples (two distinct samplings of the same sample) based on the raw spectrum (log 1/R) was under 300 µabs; that error was below the manufacturer's specifications and indicated satisfactory repeatability of the spectral measurement. Given these results, a single spectrum

was acquired per sample. To try and give a meaning to the bands seen in the NIR spectral range, the NIR spectra of the following main components of green coffee were also studied: caffeine, trigonelline, sucrose and chlorogenic acids (namely caffeoylquinic 5-CQA), which came from commercial standards. Total lipids were extracted from 2-g samples of dried ground green coffee using a modified Folch method (Folch, 1957).

Data analysis

As the spectrum consisted of 699 wavelengths, the same number of heritability estimations was performed. Heritability, the regression of unobservable genotypic values on observable phenotypic values, is a function of genotypic, environmental interaction and error variances. The predictor genotypic value Gi was the mean of the variety over all the test locations (Pi.. – P…). A multi-site analysis was carried out over three locations, using the following statistical model:

$$Y_{ik} = \mu + F_i + s_k + (Fs)_{ik} + E_{ik}$$

Where Y_{ik} was the phenotypic value of the i^{th} family, based on plot means at the k^{th} site, μ was the overall mean, *Fi* was the random effect due to the i^{th} family, s_k was the fixed effect due to the k^{th} station, $(Fs)_{ik}$ was the interaction between the i^{th} family and site *k*, and E_{ik} was the random error of plot means. The corresponding variances of random effects were: σ^2_F , $\sigma^2_{(FS)}$, and $2\sigma^2_e$, respectively. Estimated broad sense heritability for inbred lines (2 replications for each) evaluated at 3 locations was given by the formula:

$$H_b^2 = \sigma^2_F/(\sigma^2_F + \tfrac{1}{3}\sigma^2_{(Fs)} + \tfrac{1}{(2\times3)}\sigma^2_e) \ .$$

This estimation was performed on harvest 1 and harvest 2. The confidence intervals for heritabilities were assessed by using an "all but one" jack-knife, by removing one individual at a time from each sub-sample with a circular permutation pattern. The sampling variance of the estimated parameters was given by Tukey's formula. Trait heritability and confidence intervals were calculated using DIOGENE software, an extension of OPEP software (Baradat & Labbé, 1995).

Analysis of Mahalanobis distances between homozygous lines

In an initial analysis, all the spectral data were used. Chemometric processing consisted initially of a principal component analysis (PCA) based on the spectra on the 4,000 to 9,090 cm^{-1} segment. In an initial approach, the efficiency of near infrared spectroscopy applied to green coffees was assessed as a way of discriminating between varieties for harvest 1 and for harvest 2. Factorial scores of PCs showing an eigenvalue higher than 1 were used to calculate the discriminant function models using varieties as criteria. In a second approach, we tried to test the stability of the near infrared signature across harvests and environments. Based on the same factorial scores of PCs as previously, chemometric processing then consisted in calculating the matrices of the Mahalanobis distances between the 12 inbred lines for each site and each harvest, then for each site taking both harvests into account. The matrices were compared by a Mantel test (Mantel 1967) using GENETIX software version 4.01 (Laboratoire Génome et Populations, CNRS UPR 9060, Université de Montpellier II, Montpellier, France). That non-parametric test was used to compare two distance matrices by determining whether or not there was any correlation between the two matrices (Piepho, 2005).

In a second analysis, only those wave numbers for which absorbance heritability (log 1/R) was significant were kept. These were heritabilities whose confidence interval had a lower limit greater than zero. The sub-set of wave numbers was therefore defined by the heritability value. The same procedure as previously was applied to that sub-set. In order to compare the two approaches (i.e., with the whole spectrum or with the most heritable predictors) we compared the pseudo r of correlation obtained by comparing the two distance matrices by a Mantel test, along with a comparison of the associated probabilities. The procedure that gave the highest pseudo r associated with the greatest probability was considered to be the most efficient. Hierarchical cluster classifications were then performed on the spectral data to illustrate the stability of the relations between varieties from one site to the next.

RESULTS

Principal component analysis for harvest 1 and harvest 2

PCA was used to set up non-correlated variables which contained the maximum of the initial variance. For the two harvests, PCA provided a similar pattern for the cumulative percentage of variance explained by the first principal components. The first twelve factors that explained more than 99% of the total variance and had eigenvalues over 1 were used to calculate the discriminant function models.

Discriminant analysis for varieties

Significant classifications were obtained for both harvest 1 and harvest 2, as estimated by the P value associated with the Wilk's lambda coefficient. The percentage of correct variety classifications was perfect, since it reached 100% for both harvest 1 and harvest 2. Scatterplots presented in figure 1, based on canonical scores of the 72 samples analysed, show that the spectral signature for harvest 1 (Figure 1-A) and harvest 2 (Figure 1-B) enabled very good variety discrimination. However, the inter-variety relations are not the same in the two figures (Figure 1-A and Figure 1-B). Consequently, it is evident that it is not possible to propose selection according to relations of proximity between the variety Caturra and introgressed lines based on a single harvest.

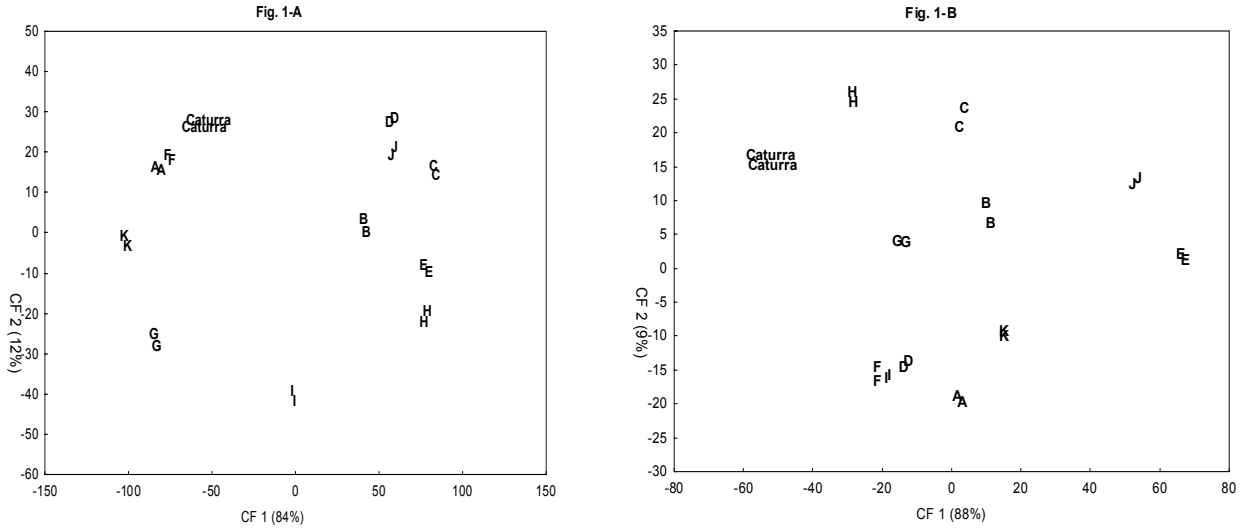

Figure 1. Scatterplot of canonical scores for the first two canonical functions resulting from the discriminant analysis of the 12 varieties studied (Caturra, A, B, C, D, E, F, G, H, I, J, K), based on NIRS acquired on ground green coffee

Heritability as a function of wave number

The near infrared spectra (log 1/R) for the ground green coffee obtained for harvest 1 were very similar to those obtained for harvest 2 (Figure 2). The heritabilities calculated for harvest 1 and for harvest 2 were also very similar, making it possible to calculate a mean heritability (h^2 mean) (Figure 3). Heritabilities were null for the spectrum interval ranging from 8,700 to 5,925 cm^{-1}. When $h^2 < 0.2$ it was found that the lower limit of the confidence interval was always less than zero, which would appear to be non-significant heritability under the experimental conditions. The heritabilities increased and became significant (confidence intervals not passing zero) between 5,800 cm^{-1} and 5,767 cm^{-1}. From 5,700 cm^{-1} to 5,235 cm^{-1}, the heritabilities were null or not significant. Lastly, in the 5,230-4000 cm^{-1} interval, the heritabilities were significant and reached high values (h^2 =0.49). It appeared that the heritabilities at adjacent wave numbers were correlated. Spectral regions where absorbance was more or less heritable thus appeared.

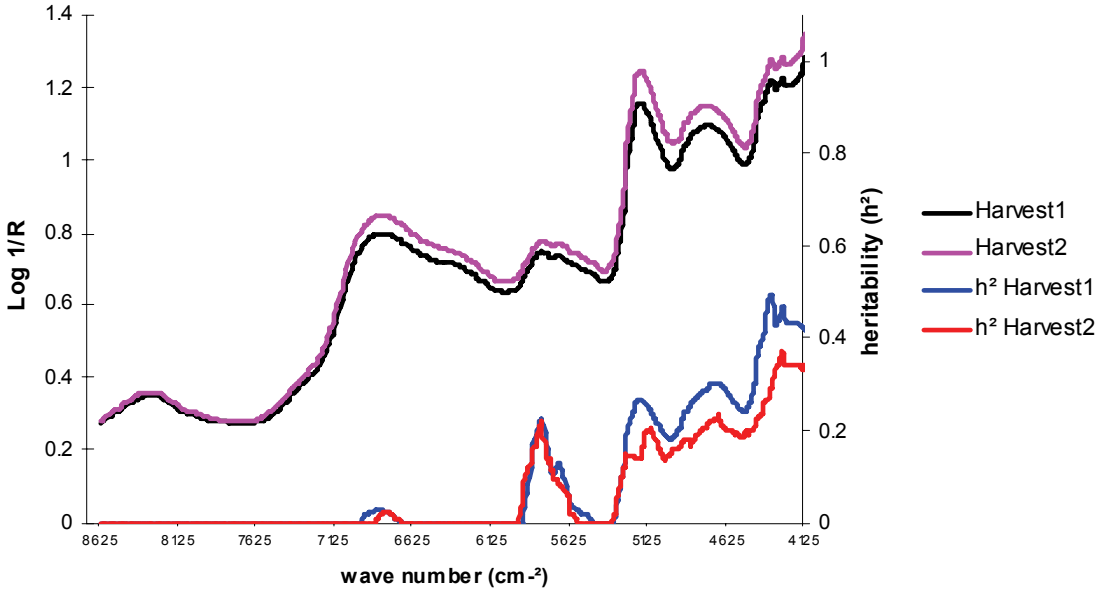

Figure 2. Near infrared diffuse reflection spectra (log 1/R) for ground green coffees and corresponding heritabilities (h^2). These are mean spectra obtained on samples from 3 sites and 12 varieties for harvest 1 and harvest 2. The heritability of log (1/R) was calculated using samples from the 3 sites and 12 varieties for harvest 1 (h^2 harvest1) and for harvest 2 (h^2 harvest2). It is represented as a function of wave number. The values of h^2 above 0.2 are significantly different from zero (confidence intervals not passing zero).

Ground green coffee is a complex solid containing a mixture of numerous molecules that absorb light simultaneously and in different ways. For this type of complex mixture, reflections at a wave number cumulate. It is therefore illusory to link log (1/R) to a wave number with a particular compound. The absorption bands of organic products observed in NIR are harmonics and combination bands whose fundamentals are located in the mid infrared. However, in infrared, spectra display few, broad reflection peaks (namely 'series of peaks'). We tried empirically to compare the spectra for the major coffee bean compounds with the heritability values obtained depending on the wave number (Figure 3).

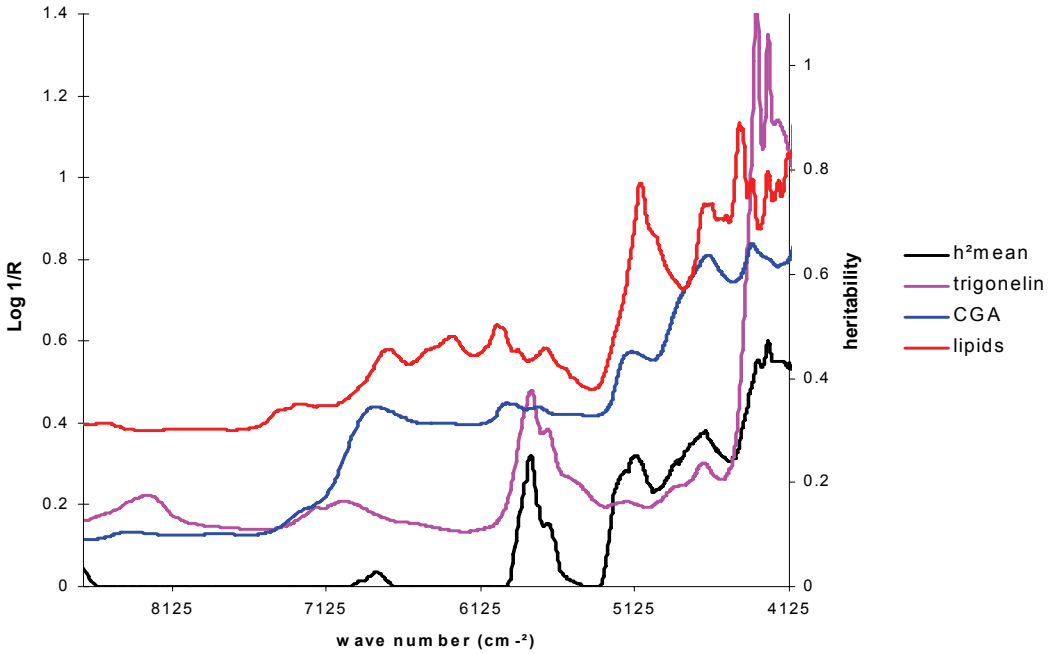

Figure 3. Near infrared diffuse reflection spectra (log 1/R) for major components of the coffee bean and absorbance heritability. These are spectra representing total lipids (lipids), chlorogenic acids (CGA), and trigonelline, accounting for 10-17%, 6-8% and 0.9-1% of dry matter respectively. The heritability (h^2 mean) of log (1/R) represented as a function of wave number was calculated using ground green coffee from the 3 sites and 12 varieties and is the mean of the two harvests (harvest 1 and 2).

The series of peaks occurring on the mean heritability curve (h^2 mean) appeared to be linked to the trigonelline series of peaks around 5,790 cm^{-1}. Starting from 5,270 cm^{-1} the trigonelline curve was very significantly correlated to those of the heritabilities ($r = 0.96$, $P < 0.00001$). A strong correlation was found between the chlorogenic acids spectrum and that of the heritabilities starting at 5,270 cm^{-1} ($r = 0.80$, $P < 0.0001$). For sucrose and caffeine (data not shown) there were no series of peaks linked to those of the heritabilities. The coefficients of correlation between the sucrose and caffeine spectra on the one hand, and that of heritability on the other hand, were highly significant and amounted to $r=0.75$ and $r=0.71$ respectively starting from 5,270 cm^{-1}. Total lipids displayed a major series of peaks around 5,080 cm^{-1} corresponding to high heritability ($h^2 > 0.25$). The second highest series of peaks for lipids around 4,625 cm^{-1} corresponded to a series of peak that was also substantial for heritability ($h^2 > 0.29$). Lastly, the series of peaks around 4,255 cm^{-1} corresponded to very high heritabilities ($h^2 > 0.46$). Starting from 5,270 cm^{-1} the total lipids spectrum was significantly correlated to heritability ($r = 0.62$).

NIR spectral signature across environments

Bean samples of 11 advanced lines and the variety Caturra supplied from 3 different sites were analysed in order to assess how the NIRS-based distances were affected by the environment. We compared distance matrices between accessions for Harvest 1, Harvest 2 and the mean of Harvest 1 and 2 (Harvest 1-2), determined at 3 different sites. For each site and each harvest, squared Mahalanobis distances between the 12 accessions were estimated based on NIR spectra. The distance matrices between varieties obtained in that way were compared by a Mantel test to determine whether there existed any correlation between two matrices (Table 1). A comparison of the three sites for harvest 1 (Table 1-A) showed that the

matrices were identical between site 1 and site 2 ($r = 0.72$ and $P < 0.001$). On the other hand, site 3 was not identical to sites 1 and 2.

Table 1. Pairwise comparisons of distance matrices between varieties for Harvest 1, Harvest 2 and the mean of Harvest 1 and 2 (Harvest 1-2), determined at 3 different sites (numbered 1, 2, 3). The tables indicate correlations between sites for harvest 1 (tables 2-A and 2-B), for harvest 2 (2-C and 2-D respectively) and for the mean of the two harvests in 2-E and 2-F. In tables 2-A, 2-C and 2-E, the correlations were calculated from the distance matrices obtained from the whole spectrum; in tables 2-B, 2-D and 2-F, the correlations were calculated from the distance matrices obtained from the heritable values, i.e. the 5,800 cm $^{-1}$- 5,767 cm $^{-1}$ and the 5,235 cm $^{-1}$- 4,000 cm^{-1} intervals. For each site, squared Mahalanobis distances between the 12 varieties were estimated based on NIR spectra. Matrix correlation coefficients between matrices are indicated and assessed according to the Mantel test. The probability of error when rejecting the null hypothesis (i.e. absence of correlation between matrices) is indicated in brackets.

2- A Harvest 1, all wave numbers 5,767], [5,235-4,000] cm^{-1} intervals

Site	1	2
3	-0.07 (0.55)	0.21 (0.11)
2	0.72 (0.00)	

2- B Harvest 1, [5,800-

Site	1	2
3	0.63 (0.00)	0.56 (0.00)
2	0.55 (0.00)	

2- C Harvest 2, all wave numbers 5,767], [5,235-4,000] cm^{-1} intervals

Site	1	2
3	0.05 (0.31)	0.03 (0.56)
2	0.04 (0.42)	

2- D Harvest 2, [5,800-

Site	1	2
3	0.02 (0.58)	0.03 (0.53)
2	0.18 (0.16)	

2-E Mean of Harvest 1-2 all wave numbers [5,800-5,767], [5,235-4,000] cm^{-1}

Site	1	2
3	0.49 (0.00)	0.21 (0.06)
2	0.42 (0.01)	

2-F Mean of Harvest 1-2,

intervals

Site	1	2
3	0.81 (0.00)	0.56 (0.00)
2	0.55 (0.00)	

By keeping only the heritable predictors, corresponding to the 5,800 cm^{-1}-5,767 cm^{-1} and the 5,700 cm^{-1} - 5,235 cm^{-1}intervals, very good identicality was found for the matrices between the three sites (Table 2-B). The distances between varieties calculated according to those intervals were significantly identical from one site to the next. For harvest 2, the relations between the distances of the varieties were different from one site to the next when all the wave numbers were considered or when only the heritable absorbance were kept. However, when considering both harvests, the analysis performed for each site showed that the distance relations were highly significantly stable between varieties (table 2-E). That stability was even greater when only considering a wave number for which absorbance was significantly heritable (Table 2-F).

Hierarchical cluster analyses were performed for each of the sites using the spectral data from the two harvests, on intervals where heritability was significant. For site 1, three major clusters, called C1 to C3, were obtained as shown on the dendrogram (Fig. 4-1). For site 2,

three major clusters, called C1' to C3' were also obtained (Fig. 4-2). The variety composition of each cluster of site 1 was strictly conserved in the clusters of site 2. For site 3, three clusters (called C1" to C3") were obtained (Fig. 4-3). Compared with sites 1 and 2, a small discrepancy was observed. Variety B was classed in cluster C3 when it was initially classed in C2 for site 1 and site 2. When considering the spectrum intervals where absorbance was heritable, the same hierarchical clusters were obtained without any discrepancy between sites (Figures 4-4, 4-5 and 4-6).

The proximity of varieties C and H with cv 'Caturra' suggested great proximity of those two varieties to the control. The distance of cluster C3 from cluster C1 (lines A, F and E) was always large. That large distance might have meant that the biochemical composition of those three lines was substantially different from that of the lines in cluster C1. All in all, the results suggested stability across environments for inter-variety relationships determined using NIRS-based Mahalanobis distances.

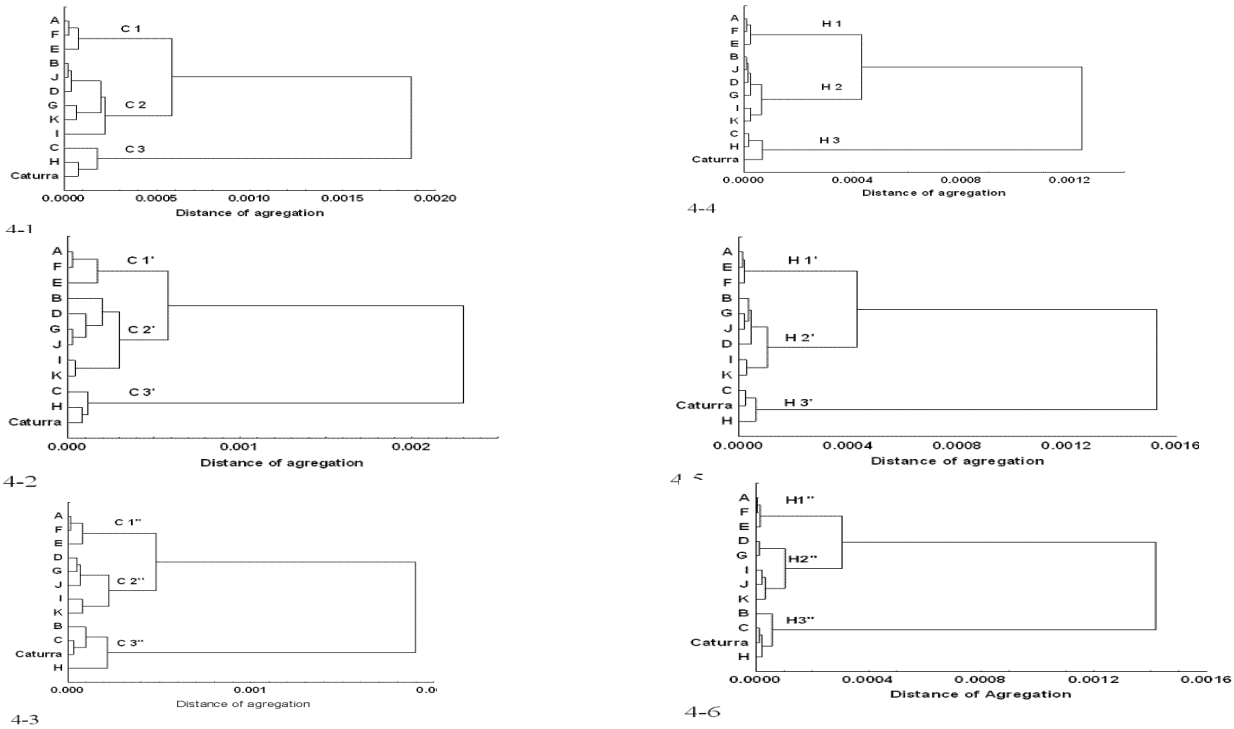

Figure 4. Hierarchical clustering analysis (Euclidian distance, Ward grouping method) performed on NIR spectra acquired on ground green coffee. Graphs 4-1, 4-2, 4-3 representing locations 1, 2 and 3 respectively were obtained from the whole spectra of the 12 varieties. Graphs 4-4 , 4-5 and 4-6 representing locations 1, 2 and 3 respectively were obtained from the heritable zones of the whole spectra of the 12 varieties. For each graph distance-based clustering enabled the definition of three main groups (i.e. C 1, C2, and C3 for graph 4-1) of varieties sharing a similar NIR signature.

CONCLUSION

We recently showed (Bertrand et al. 2008) that determining the fatty acid composition of the coffee bean is an effective tool for distinguishing between varieties. We show here on samples from different sites that the spectral signature is much more efficient in discriminating between varieties, since 100% correct classification is achieved as opposed to 79% with the fatty acid composition. Consequently, after many other authors, we show the power of this tool which proves to be much less expensive than traditional chemical analyses

for discriminating between varieties. The advanced varieties were easily distinguished from the standard arabica variety (i.e. Caturra) used as the reference variety in the trials and the recurrent parent in the backcrosses. Therefore, NIRS analyses proved to be extremely valuable for detecting in coffee beans the biochemical modifications associated with *C. canephora* DNA introgressions, as shown previously (Bertrand et al., 2005).

Unfortunately, we also show that the differences between varieties prove not to be stable from one harvest to another. Obviously, NIRS profiles are strongly affected by environmental factors. We therefore sought to minimize environmental effects. We first compared matrices obtained for a single harvest at a single site. In that case, the distances between lines were not significantly comparable. However, when calculating the distances over two harvests, the relations between lines were very stable from one site to the next. Although dynamic (i.e. environmental effects), the established relationships between lines appeared to be well conserved across locations. The NIRS-based inter-variety relationships determined at one site appeared to be stable across environments. We therefore concluded that there was stability across environments for inter-variety relationships.

The efficiency of the method was greatly increased if only certain zones of the spectrum were kept. To select those zones of the spectrum, we treated infrared spectra as a sequence of discrete variables that displayed a genetic variance and an environmental variance. The ratio of genetic variance to the sum of variances amounted to heritability. Spectral regions where absorbance displayed high heritability and spectral regions where heritabilities were not significant or null were revealed. Selecting from the spectrum only those wave numbers for which absorbance was significantly heritable amounted to choosing the best predictors, which is a conventional approach in breeding. That approach proved to be effective, since we found a significant increase in stability across environments. The study of heritable predictor distribution within the spectrum did not seem to be random. We attempted to show that some of the statistically significant heritabilities might correspond to certain major compounds of the coffee bean. It would be interesting to go into those studies in greater detail.

This stability of the NIR spectral signature is very interesting for breeders. The signature becomes a trait on which selection can be based through comparison with ideotypes. For example, in the dataset we analysed one of the possible applications was to select lines closest to cv ‘Caturra’, which is an acknowledged standard in terms of cup quality. Indeed, it can be assumed that the closer a line is to cv ‘Caturra’, the closer its biochemical composition will be and the less it will display sensory differences from the reference variety. This type of approach can prove to be particularly fruitful where introgressed lines are involved, as in our work. Indeed, it has been shown that poorly mastered introgression of major chromosome fragments of this species, with a view to introducing resistance genes, could have a substantial negative impact on beverage quality (Bertrand et al., 2003). We concluded that the NIRS methodology will be an efficient tool to assist in the selection of introgressed lines.

NIRS applications seem promising in several fields linked to breeding work, notably for genetically improving quality. In fact, in addition to cultivar authentication and traceability, NIRS can be used for rapid screening of a collection of genetic resources (pers. comm.), or as in our work, to assist in selection. Additionally, in the particular case of introgressed coffee trees, several techniques will be implemented to understand the nature of the differences observed in terms of cup quality or the biochemical composition of beans between certain introgressed genetic units and reference cultivars such as cv ‘Caturra’. NIRS will be used to choose the most different genetic units for which in–depth chemical and genomic analyses will be undertaken. Our results confirm that infrared spectroscopy is an inexpensive high through-put phenotyping tool that can be used by breeders for indirect selection based on

biochemical composition, and consequently on cup quality, in the coffee tree, but probably also in other species.

ACKNOWLEDGEMENTS

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In Search for the Geographic Origin Discrimination of the Green Coffee Bean

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SUMMARY

Nowadays, a considerably effort is being made to find reliable analytical methods for food origin discrimination and authentication. The UK Food Standards Agency (FSA) displays an interesting report on labelling research, showing that consumers strongly support Country of Origin Labelling and that consumers think it is important that labelling of food always clearly identifies the country of origin of the ingredients (Food Standards Agency, 2007). The importance of the coffee market and its globalization arise increased concern about its origin and producers respond by offering products with origin labelling to the consumer. The quality of coffee has a high variation according to its geographic origin and several attempts have been made to discriminate analytically the origin of green and also roasted coffees. Analytical methods such as gas chromatography mass spectrometry, near infrared spectroscopy, determination of organic compounds, and stable isotope analysis of specific compounds extracted from the green coffee bean have been studied extensively with promising results. The stable carbon and nitrogen isotopic ratios are related to the plants' climatic conditions during growth, mainly water and nutrient availability along with light intensity and temperature, and can be useful as indicatives of their origin, providing tools to delimit their potential cultivation areas if the conditions are significantly different. Due to this, stable isotopes are often used for establishing fingerprints in food as they integrate the isotopic signature of its provenance. The aim of this work was to apply stable isotope analysis (EA-IRMS; Elemental Analysis – Isotope Ratio Mass Spectrometry) for carbon, nitrogen and oxygen isotopic composition determination of 58 green coffee bean samples derived from Central America, Pacific, South America, Africa, Asia and Oceania. Strontium isotope abundance ratio (related with the geology and topography of each location) was also determined by MC-ICP-MS (Multicollector Inductively Coupled Plasma Mass Spectrometry). Multivariate analysis (Principal Component Analysis) of data was done in order to discriminate green coffee samples based on isotopic composition. Non-parametric measure of correlation between geographic and climatic factors and analytical data was performed to determine the relevant factors for the determination of geographical origin of green coffee beans based on isotope analysis.

INTRODUCTION

Food traceability ('from farm to fork') and food authenticity have become a major concern in antifraud and consumer protection during the past few years (Swoboda et al., 2008). Considerable efforts are currently being made to find reliable analytical methods for food origin discrimination and authentication. The importance of the coffee market and its

globalization has raised increased concern about coffee origin, and producers have responded by offering products with origin labelling. Coffee is also an important product commercialized through *fair trading* commerce which reinforces the importance of assurance of geographical origin to the final consumer.

Analytical methods such as gas chromatography mass spectrometry (Bicchi et al., 1997; Costa Freitas et al., 2001) and near infrared spectroscopy (Bertrand et al., 2005), determination of organic compounds such as chlorogenic acids (Bicchi et al., 1995), fatty acid profiles (Martín et al., 2001), tocopherols and triglycerides (González et al., 2001) and stable isotopes ratio analysis of specific compounds extracted from green coffee beans (Weckerle et al., 2002) have been studied extensively with promising results. Stable isotopes ratio, determined by EA-IRMS, is often used for fingerprinting foods because they integrate the isotopic signature of its origin. Very small differences in the chemical behaviour of different isotopes of an element can provide a very large amount of useful information about chemical (both geochemical and biochemical) processes. Isotopic fractionation frequently occurs in nature due to isotope exchange reactions and to kinetic processes, which are associated with incomplete and unidirectional processes like evaporation, dissociation reactions, biologically mediated reactions, and diffusion (Hoefs, 2004). Also, MC-ICP-MS offers high sensitivity and precision so that it is possible to explore isotopic variations of other elements. The elements of interest in radiogenic chemistry are heavy (i.e. Sr, Nd, Pb) and their isotopic fractionation is quite small and can generally be ignored. As biological processes involved in plant and/or animal metabolism do not significantly fractionate strontium isotopes, Sr isotope abundance ratio, frequently used as tracer of geological age and topography, can also be applied as a tracer of the origin of food and beverages (García-Ruiz et al., 2007). Sr isotope abundance ratio has also been used to investigate the geographical origin of different food products such as wheat (Branch et al., 2003), butter (Rossman et al., 2000), cheese (Fortunato et al., 2004) and asparagus (Swoboda et al., 2008). Isotope ratios of light (H, C, O, N, B) and heavy elements such as Sr, may supply essential information for origin discrimination of foods (García-Ruiz et al., 2007).

The aim of this work was to classify, according to geographical origin, 58 green coffee bean samples, from 20 different geographical origins based on isotopic composition of the stable isotopes carbon, nitrogen and oxygen and on strontium isotope abundance ratio.

RESULTS AND DISCUSSION

Conventional chemical methods of analysis of food allow the determination of certain components of food. In contrast, methods based on isotope analysis make use of a physical parameter, the isotope ratio, to assign the origin of food or of specific components extracted from food, allowing a differentiation of otherwise chemically identical materials (Rossman, 2001).

Vegetation takes up strontium available on the soil exchange complex and in the soil solution, which can be replenished by mineral weathering or atmosphere input. Biological processes, whether involved in plant or animal metabolism, do not significantly fractionate strontium isotopes. Therefore terrestrial vegetation will reproduce Sr isotopic composition of bio-available Sr of soil. This means that strontium isotopic composition of a plant, an animal or food product should yield information about their provenance and corresponding geologic interactions (Capo et al., 1998; Fortunato et al., 2004).

In this study, coffees from Angola, Rwanda, Malawi, Zambia, Peru, East Timor and some from Brazil showed the highest values for $^{87}Sr/^{86}Sr$ (Figure 1). All other coffees had lower levels of $^{87}Sr/^{86}Sr$.

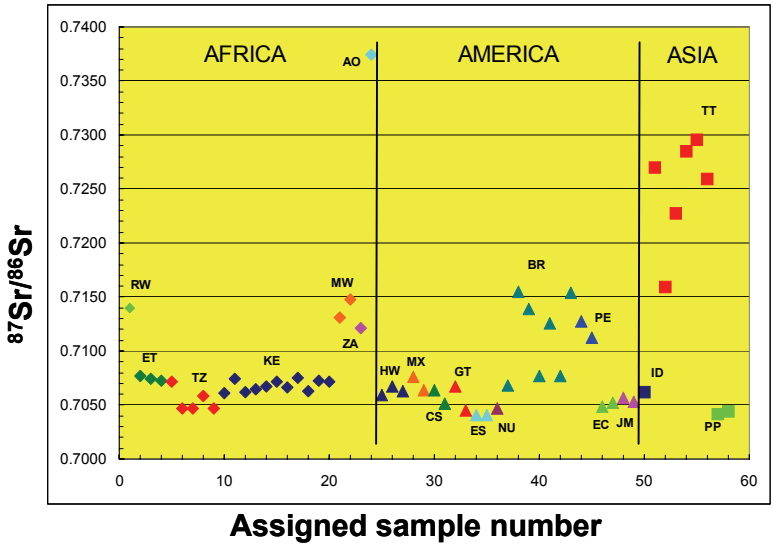

Figure 1. $^{87}Sr/^{86}Sr$ isotope abundance ratios for the 58 green coffee bean samples from different geographical origins. (Legend: RW – Rwanda; ET – Ethiopia; TZ – UR Tanzania; KE – Kenya; MW – Malawi; ZA – Zambia; AO – Angola; HW – Hawaii; MX – Mexico; CS – Costa Rica; GT – Guatemala; ES – El Salvador; NU – Nicaragua; BR – Brazil; PE – Peru; EC – Ecuador; JM – Jamaica; ID – Indonesia; TT – East Timor; PP – Papua New Guinea).

In Africa, green coffee from Rwanda, Malawi and Zambia discriminate from Ethiopia, UR Tanzania and Kenya (Figure 1). American coffees had variable $^{87}Sr/^{86}Sr$ values but in the same range. Exceptions were some green coffees from Brazil and Peru. Asian coffees from Papua New Guinea and Indonesia revealed completely different $^{87}Sr/^{86}Sr$ values from East Timor coffees. East Timor green coffees could be distinguished from all other green coffees included in this study, solely on the basis of the Sr isotope abundance ratio. Coffees from Papua New Guinea had different $^{87}Sr/^{86}Sr$ compared with the coffee obtained from Indonesia, but lower $^{87}Sr/^{86}Sr$ compared to East Timor coffee. We believe that the values found for East Timor coffees could be related to the fact that East Timor is known as a 'volcanic island'. The presence of an igneous plutonic (intrusive) rock such as granite whose $^{87}Sr/^{86}Sr$ values can be ≥ 0.710 would confirm this hypothesis as $^{87}Sr/^{86}Sr$ ratios of vegetation reflect the sources of Sr (and other nutrients) to the local environment.

Isotopic composition of the 58 green coffees revealed larger variations in $\delta^{15}N$ values than in $\delta^{18}O$ or $\delta^{13}C$ (Rodrigues et al., 2007). Principal component (PC) analysis was applied to isotopic composition of carbon, nitrogen, oxygen and strontium. Two eigenvalues with value higher than 1 were selected for PC extraction. PC1 and PC2 explain 88.18% and 7.35% of isotopic composition variation among the 58 green coffees (Figure 2). The analysis suggests a degree of discrimination between five sets of samples: Zambia, East Timor and Mexico, Peru, Costa Rica and Papua New Guinea and finally, Kenya and Ethiopia. All other coffees did not discriminate between each other. Spearman's correlation coefficient was determined in order to evaluate the correlation between values of latitude, altitude, $\delta^{18}O$ of the annual mean precipitation and of the distance to the sea, known for each geographical location included in this study (Rodrigues et al., 2007) and analytical data (Figure 3). Table 1 shows the values found for Spearman's statistic *r* for a level of confidence of 95%. Precipitation seems to be an

important factor influencing isotopic composition of oxygen of the green coffee bean. Related geographical factors such as altitude, latitude and the distance to the sea were also important and, variation in Sr isotope abundance ratio in green coffees could be related to the distance to the sea of each geographical location.

Table 1. Spearman's statistic *r* for correlations between sets of two of the variables included in this study (altitude, latitude, mean annual precipitation, $\delta^{18}O$ of mean annual precipitation, distance to the sea and isotopic composition of carbon, nitrogen, oxygen and strontium of the 58 green coffees) (only correlations with level of significance of 95% are shown).

Variables	*Spearman's R*
Altitude & Latitude	-0.68
Altitude & Distance to the sea	0.35
Altitude & $\delta^{18}O$ of Mean annual Precipitation	-0.55
Altitude & $\delta^{13}C$ of the 58 green coffees	0.27
Altitude & $\delta^{15}N$ of the 58 green coffees	0.44
Latitude & mean annual precipitation	0.34
Latitude & $\delta^{15}N$ of the 58 green coffees	-0.41
Latitude & $\delta^{18}O$ of the 58 green coffees	-0.34
Mean annual precipitation & Distance to the sea	-0.46
Mean annual precipitation & $\delta^{18}O$ of the 58 green coffees	-0.33
Distance to the sea & $\delta^{13}C$ of the 58 green coffees	0.37
Distance to the sea & $\delta^{18}O$ of the 58 green coffees	0.56
Distance to the sea & $^{87}Sr/^{86}Sr$ of the 58 green coffees	0.27
$\delta^{13}C$ & $\delta^{15}N$ of the 58 green coffees	0.42
$\delta^{13}C$ & $\delta^{18}O$ of the 58 green coffees	0.64
$\delta^{15}N$ & $\delta^{18}O$ of the 58 green coffees	0.39

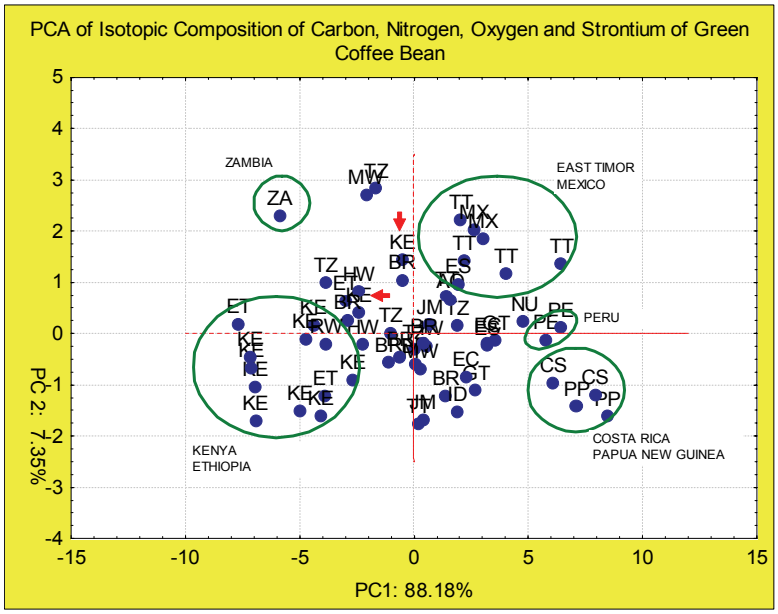

Figure 2. Principal component analysis of isotopic composition of carbon, nitrogen, oxygen and strontium of the green coffee bean from 20 different geographical origins (Legend: as in Figure 1).

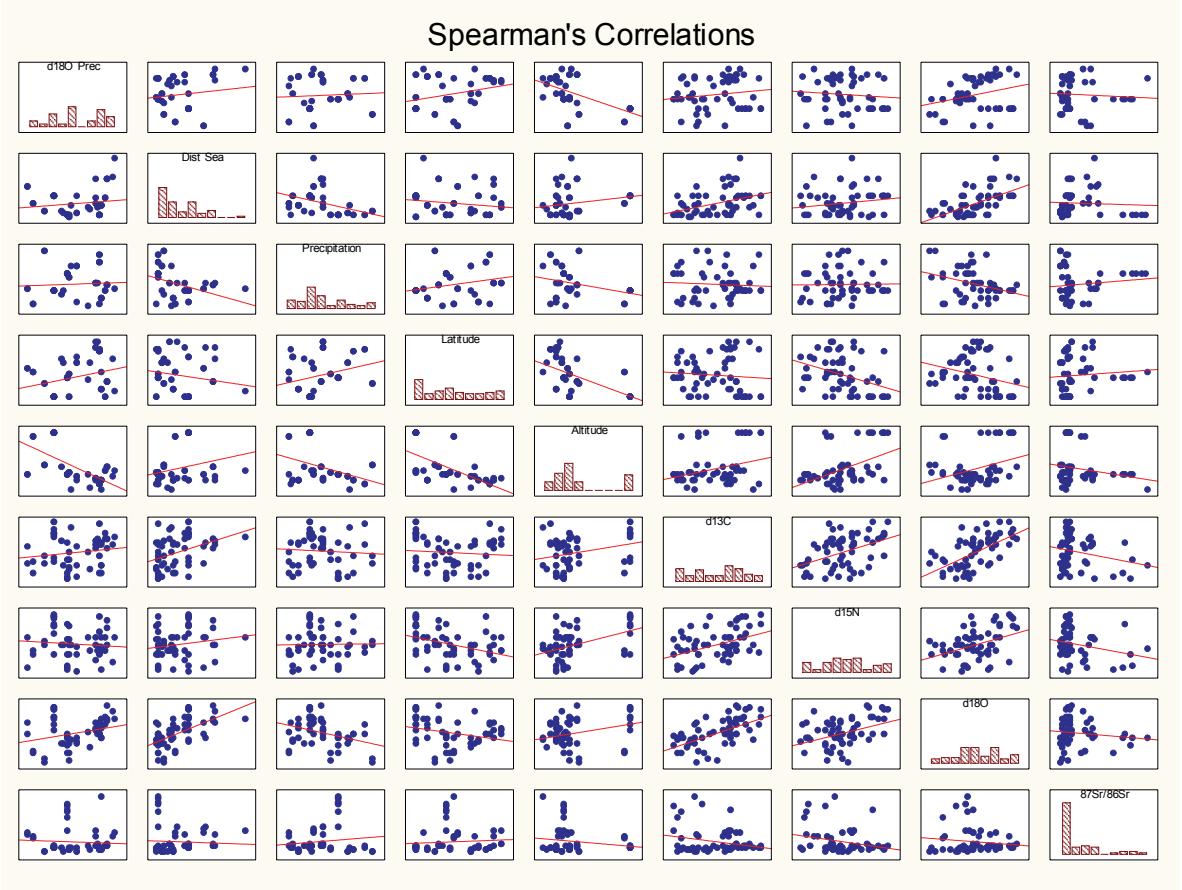

Figure 3. Spearman's correlations between variables altitude, latitude, mean annual precipitation, $\delta^{18}O$ of mean annual precipitation, distance to the sea and isotopic composition of carbon, nitrogen, oxygen and strontium of the 58 green coffees.

CONCLUSIONS

It was possible to discriminate the geographical origin of some of the coffees, especially from Africa, South America and islands. The observed differences in isotopic composition of the 58 green coffees were mainly explained by altitude, total precipitation, latitude, distance to the sea and $\delta^{18}O$ of water precipitation values associated to each geographical origin.

Despite the small number of samples, this work demonstrates the potential of the use of isotopes in sourcing the origin of green coffees. Combining C, N, O and Sr isotopic composition with climatic, geographical and geochemical information will constitute a powerful tool for origin discrimination of the green coffee bean and may contribute to certification of origin, biodiversity and sustainability projects.

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Characterization of SPME/GC-MS Headspace Profiles of Coffee Under Two Different Processing Temperatures

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SUMMARY

The objective of this study was to analyze the volatile components profile of both green and roasted coffee beans, for the evaluation of the feasibility of using such profiles for assessment of roasting degrees. Volatiles extraction and concentration were performed by solid phase micro-extraction (SPME) of the ground coffee headspace and analysis of the volatiles profile was performed by GC-MS. The characterization of headspace volatile profiles of coffee under two different processing temperatures (200 °C and 300 °C) was successfully carried out by multivariate statistical analysis of the respective SPME/GC-MS chromatograms. The analysis allowed for the discrimination between crude and roasted samples, and between samples roasted to varying degrees under different temperatures. Caffeine, although not volatile, was encountered in all headspace profiles and was one of the discriminating compounds for crude coffee. Pyridine was the main discriminating compound for coffees roasted at high temperatures and more intense degrees of roast. Although discrimination of coffee samples roasted to varying degrees was achieved by analyzing the effect of hundreds of compounds altogether, the compounds that presented the most pronounced effect on the discrimination of roasting degrees and temperatures were those generated right after the onset of pyrolysis.

INTRODUCTION

The desired aroma and flavor of the coffee beverage are developed in the roasting process, when the beans undergo reactions leading to the desired changes in chemical composition. The composition of roasted coffee depends upon the degree of roast, assessed by color or weight loss measurements (Dutra et al., 2001). Since the volatiles profile changes considerably during roasting, it should provide a more reliable measure of the roasting degree. Furthermore, previous studies have demonstrated the feasibility of assessing the degree of roast by evaluating the evolution of the composition of coffee roasting exhaust gases (Dutra et al., 2001). and by multivariate statistical analysis of coffee headspace profiles at varying roasting conditions (Liardon and Ott, 1984). Therefore, the objective of this study was to analyze the volatile components profile of both green and roasted coffee beans, for the evaluation of the feasibility of using such profiles for assessment of roasting degrees.

METHODOLOGY

Arabica green coffee samples (2004/2005 crop) were obtained from Santo Antonio Estate Coffee (Minas Gerais, Brazil). Defective coffee beans were hand picked, so that the sampling lot consisted only of good quality coffee. Samples of randomly selected 40 beans were

separated and oven roasted (Table 1). Roasting degrees were established based on visual inspection of the external color of the beans. Roasted coffee samples were stored in sealed containers at ambient temperature for a maximum period of 24h. Just before each analysis, the coffee was ground with an electric coffee grinder (C-mill 5679-01US, Bodum, USA) for 30 s. Roasting tests were conducted in duplicates.

An SPME triple phase (divinylbenzene/carboxen/polydimethylsiloxane) 50/30 μm fiber (model 57348-U, Supelco Inc., USA) was employed for the extraction of volatiles from the roasted coffee and barley headspace, according to the methodology described by Mancha Agresti et al. (2008). GC analyses were performed using a gas chromatograph (Trace Ultra) coupled to a mass spectrometer (PolarisQ) (ThermoElectron, San Jose, CA), a RTX-5MS column (5% diphenyl, 95% dimethyl polysiloxane) 30 m x 0.25 mm I.D (Restec, Ireland) and Helium as carrier gas (1 mL/min). The GC injector was operated at 250 °C in the splitless mode. The ion source (detector) and interface temperatures were 300 °C and 275 °C, respectively. Mass spectra were acquired in the electron impact mode at 70 eV, using a *m/z* range of 50 to 650 and 2 s scan time. Tentative identification of compounds was performed by comparison of the mass spectra with those of the NIST/EPA/NIH Mass Spectral Library, version 2.0 (available in the instrument software), considering a similarity level (RSI) higher than 800.

For principal component analysis (PCA), data matrices were assembled so that each row corresponded to a coffee sample and each column represented the relative peak area of a specific component. Regardless of substance tentative identification all chromatographic peaks with S/N (signal-to-noise ratio) higher than 50 were included in the final data matrix.

Table 1. Roasting conditions.

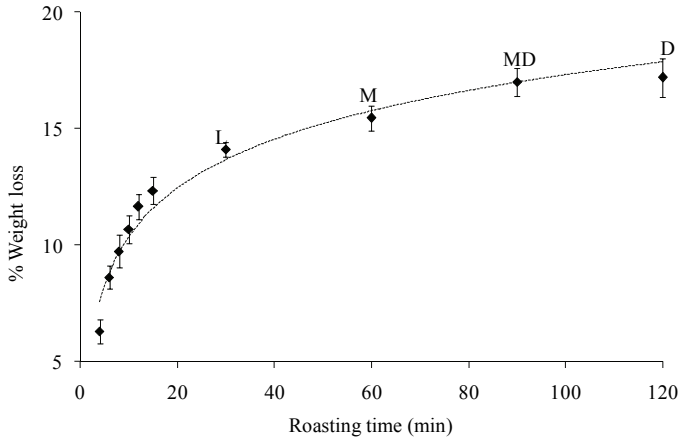
Sample ID	Roasting Degree	Oven Temperature (^{o}C)	Roasting Time (min)
L	light	200	30
M	medium	200	60
MD	medium/dark	200	90
D	dark	200	120
VL	very light	300	8
L	light	300	9
M	medium	300	10
D	dark	300	11

RESULTS AND DISCUSSION

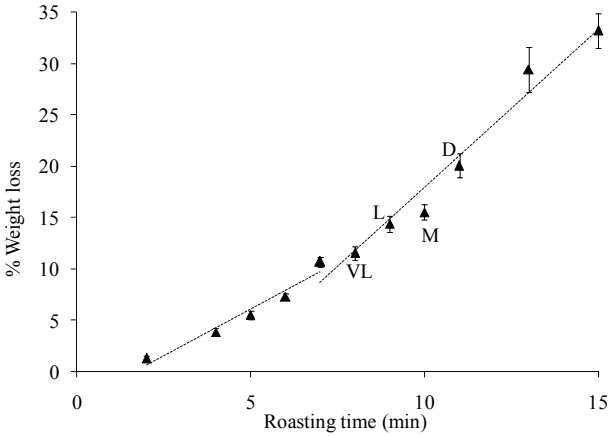
Results for weight loss during roasting are shown in Figure 1. Time scales in Figures 1a and 1b are different because coffee roasts faster at higher temperatures. Average mass loss values per roasting degree were 14%, 15% and 19%, corresponding to light, medium and dark roasts, respectively. It can be observed that roasting temperature presented a significant effect on weight loss behavior. At the lower temperature (200 °C), weight loss occurred faster during the first 20 minutes, which corresponds to the drying stage during which weight loss is mostly attributed to removal of water and crude coffee volatiles. Also, weight loss behavior was satisfactorily described by a logarithmic fit (dashed line in Figure 1a). When roasting temperature was increased (300 °C), the weight loss presented a different behavior, with the drying stage occurring at a slower rate than the pyrolysis stage. After the drying stage (0 to 7 min roasting), there was a significant increase in the weight loss rate that can be attributed to an intensive release of organic compounds, carbon dioxide and water resultant from pyrolysis and other roasting reactions. This behavior has been reported by previous studies on coffee

roasting (Dutra et al., 2001; Oliveira et al., 2005). The variation in weight loss behavior shown in the present study is an indication that the roasting temperature is affecting the volatiles profile by favoring different reactions and product removal pathways at different processing conditions.

PCA analysis of all samples is displayed in Figure 2, based on an 18 x 340 data matrix comprising two crude and sixteen roasted coffee samples, with the first two principal components (PCs) accounting for 42 and 26% of the chromatographic variance, respectively. It is clear that roasting temperature presents a significant effect on the volatiles profile, promoting a separation of the roasted coffee samples into two groups by PC1 values. Coffees roasted at 200 °C presented positive PC1 whereas coffee roasted at 300 °C presented negative PC1. PC2 values provided separation between crude and roasted coffee. Also, PC2 values decreased with an increase in roasting time, regardless of roasting temperature. The substances that presented higher influence on PC1 and PC2 are displayed in Table 2. Even though caffeine is known to be present in all coffee samples, regardless of roasting, the fact that such compound allowed for separation between crude and roasted coffees can be attributed to two factors: (i) the relative concentration of other volatile components with respect to caffeine being lower for crude than for roasted coffee; and (ii) the fact that even though caffeine is not consumed during roasting, it has been previously reported to be dragged by the water vapor present in the exhaust gas as it evolves from the coffee beans, thus presenting lower concentrations for darker roasts (Dutra et al., 2001). The unidentified compound detected at 4.25 min, characteristic of coffee roasted at 200 °C, has been pointed in a previous study (Mancha Agresti et al., 2008) as a characteristic compound of healthy and sour coffee beans. The other substances listed in Table 2 as characteristic of roasted coffees have been identified by previous studies in the headspace of roasted coffee (De Maria et al., 1996; Sanzet al., 2001; Rocha et al., 2003; Mondello et al., 2004; Zambonin et al., 2005). Among the substances affecting the principal component in the roasted coffee samples, furfural, 3-furanmethanol, 5-methyl-2-furancarboxaldehyde and 4-vinylguaiacol (2-methoxi-4-vinylphenol) were detected for both roasting temperatures. However, the amount of these substances increased with roasting temperature, as can be verified by signal-to-noise ratio values displayed in Table 2. 4-vinylguaiacol has been previously reported as a potent odorant of roasted coffee, being associated with a smoky aroma (Grosch, 2001). Pyridine was identified as the substance with the highest influence on both PC values for coffee roasted at 300 °C, being characteristic of samples submitted to a dark roast. Pyridines are formed due to trigonelline degradation, protein pyrolysis, Strecker degradation and Maillard reactions (De Maria et al., 1996). Higher concentrations of pyridines have been reported for Arabica coffee samples in comparison to Robusta ones, under the same roasting conditions. Also, the amount of pyridines depends on the degree of roast, and such compounds have been reported to be characteristic of darker roasts (Dart and Nursten, 1985).



(a)



(b)

Figure 1. Weight loss during roasting at (a) 200 °C and (b) 300 °C. VL = very light roast, L = light roast, M = medium roast, MD = medium/dark roast, D = dark roast.

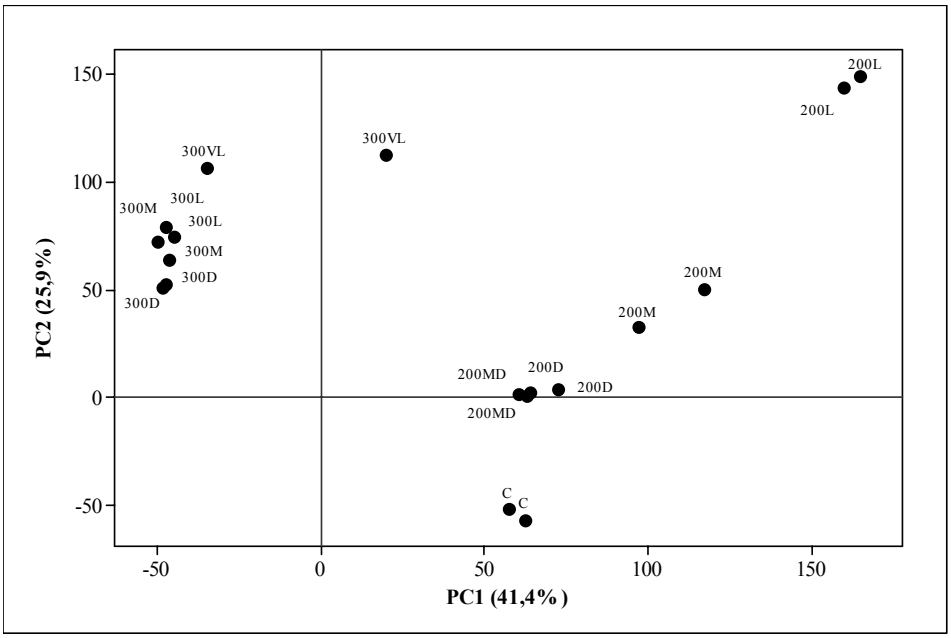


Figure 2. PCA scores scatter plots of chromatographic SPME areas of coffee volatile compounds for roasted and crude coffee (PC1 vs PC2). C = crude, VL = very light roast, L = light roast, M = medium roast, MD=medium/dark roast, D = dark roast, 200 and 300 correspond to roasting temperatures (°C).

Table 2. Tentative identification of aroma components with the most pronounced effect on PC1 and PC2 values.

Retention Time (min)	S/N[a]	Compound	*m/z* of the most intense ions (relative abundance %)
		Crude coffee	
23.24	525	unidentified	69 (100), 55(72), 67(70)
24.19	219	methyl salicylate	120 (100), 92 (90), 152 (50)
50.82	2003	caffeine	194 (100), 55 (20), 109 (10)
		Roasted Coffee – 200°C	
4.25	5410	unidentified	80(100), 79(36), 130(25)
6.69	1935	2-methyl pyrimidine	94 (100), 67 (40), 93 (15)
7.16	707	furfural	95 (100), 96 (30), 97 (10)
8.50	906	3-furanmethanol	81 (100), 98 (55), 97 (50)
13.46	1426	5-methyl-2-furancarboxaldehyde	109 (100), 110 (50), 53 (30)
15.67	3297	2-furanmethanol,acetate	98 (100), 81 (85), 140 (40)
19.14	1520	2,6-diethyl pyrazine	135 (100), 136 (50), 107 (20)
30,32	2088	4-vinylguaiacol	150 (100), 135 (65), 107 (45)
		Roasted Coffee – 300°C	
4.13	7873	pyridine	79 (100), 80 (85), 52 (50)
7.16	2970	furfural	95 (100), 96 (30), 97 (10)
8.50	2086	3-furanmethanol	81 (100), 98 (55), 97 (50)
13.46	3998	5-methyl-2-furancarboxaldehyde	109 (100), 110 (50), 53 (30)
15.12	62	2-(n-propyl)-pyrazine	94 (100), 107 (30), 58 (20)
30.32	4514	4-vinylguaiacol	150 (100), 135 (65), 107 (45)

[a]*Signal-to-noise ratio (average values including different roasting degrees).*

CONCLUSIONS

The characterization of headspace volatile profiles of coffee under two different processing temperatures was successfully carried out by multivariate statistical analysis of the respective SPME/GC-MS chromatograms. The analysis allowed for the discrimination between crude and roasted samples, and between samples roasted to varying degrees under different temperatures, regardless of compound identification. The behavior of the discriminating compounds was correlated to the weight loss curves during roasting, which in turn presented distinct behavior for different roasting temperatures. Caffeine, although not volatile, was encountered in all headspace profiles and was one of the discriminating compounds for crude coffee. Pyridine was the main discriminating compound for coffees roasted at high temperatures and more intense degrees of roast. Although discrimination of coffee samples roasted to varying degrees was achieved by analyzing the effect of hundreds of compounds altogether, the compounds that presented the most pronounced effect on the discrimination of roasting degrees and temperatures were those generated right after the onset of pyrolysis. The results presented in this study show that color and weight loss alone are not reliable as

roasting degree assessment criteria and that roasting temperature must also be taken into account.

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An Update on Ochratoxin A in Coffee After 10 Years of Research

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SUMMARY

In the nineties, the European Union programme for harmonization of mycotoxins in food, brought about a worldwide change in research inside and outside of the coffee chain to investigate the spread of OTA contamination in coffee. How it would affect human health and the economic impact on world trade were the mains aspects of concern. After more than 10 years of research carried out in Brazil, involving the coffee chain throughout from farm to cup, several conclusions and revisions should be taken into account. Until 2004, the major fungal source of OTA in coffee was *A. ochraceus* and *A. carbonarius*, with minor contribution from *A. niger*. After advances in molecular and fungal metabolite techniques, new species were described and distinguished in the section *Circumdati* and *Nigri.* Nowadays, *A. westerdijkiae* is being recognized as the main OTA producer especially in arabica coffee, while *A. carbonarius* occurs most frequently in robusta coffee. The issue of time of invasion by ochratoxigenic fungi have led to several discussions. In our studies, few cherries on the coffee trees were infected with ochratoxigenic species, indicating that infection mostly occurred after harvest, and the fungal sources were likely to be soil, equipment and drying yard surfaces. Most of the coffee samples did not contain OTA and its production was sporadic and uncommon. OTA formation during transportation was investigated; temperature inside coffee containers changed drastically during the voyage from a coffee exporting country such as Brazil to an importing country such as Italy. These changes induced condensation at the top of the containers. Therefore, the unloading of coffee in the importer countries should not be delayed. OTA intake from coffee has shown to be very low, considering the improvements adopted by several countries. The presence of this toxin in coffee in commercial roasted and ground, and soluble coffee samples has decreased considerably over this period, as a consequence of several worldwide efforts.

INTRODUCTION

Ochratoxin A (OTA) is a toxin produced by some fungal species, known to have nephrotoxic effects and carcinogenic potential in animal species. OTA consists of a chlorinated isocoumarin-derivative linked to L-phenylalanine (van der Merwe et al. 1965).

Since the occurrence of this toxin in market samples of roasted coffee was reported, surveys on the presence of ochratoxin A in coffee have been undertaken in several countries (MAFF, 1996; Patel et al., 1997; van der Stegen et al., 1997). Taniwaki (2006) revised extensively the incidence of OTA in samples of raw, roasted and soluble coffee including the geographical origin.

While the initial contamination may occur at farm level, the actual OTA formation may happen throughout the entire chain, at every stage of production. In the present paper, the work carried out is updated as the result of 10 years of investigation with the following objectives: (i) which fungi are responsible for OTA production; (ii) which stages of coffee production are susceptible to infection by fungi capable of OTA production; (iii) the influence of climatic conditions and processing practices on OTA production: (iv) which factors during coffee transportation contribute to fungal growth and OTA production; (v) what are the effects of the roasting process on OTA destruction and (vi) the evaluation of the OTA risk assessment in coffee consumption.

MATERIAL AND METHODS

Coffee samples

Samples of *Coffee arabica* were collected at different stages of maturation and processing: cherries and overripe fruit from trees, overripe fruit from the soil, from the drying yard and from barn storage on the farms.

Mycological analysis

Samples of coffee beans were surface disinfected with 0.4% chlorine solution for 1 min (Pitt & Hocking, 1997). From each sample, 50 beans were plated directly (10 particles per plate) onto Dichloran 18% Glycerol agar (DG18, Hocking and Pitt, 1980). The plates were incubated at 25 °C for 7 days. After incubation, plates were examined and representative colonies of *Aspergillus* species presumptively identified as *A. ochraceus* group and related species or *A. niger* and related species were isolated onto malt extract agar slants, and incubated to allow growth for identification.

Identification of fungi

The *Aspergillus* isolates were grown on standard media (Czapek yeast extract agar and malt extract agar; Pitt and Hocking, 1997) and identified according to Klich and Pitt (1988), Samson et al. (2004), Frisvad et al. (2004). Numbers of isolates were counted from each sample and percentage of infection by each species were calculated.

OTA production from the fungal isolates

The isolates identified as *A. ochraceus* or closely related species, *A. niger* and *A. carbonarius* were grown on yeast extract 15% sucrose agar (YES) at 25 °C for 7 days and evaluated for the production of OTA by the agar plug technique (Filtenborg *et al.,* 1983). Isolates which tested negative for OTA production by this technique, were analysed by thin layer chromatography (TLC). For this analysis, the medium and colony from a Petri dish were extracted with chloroform (50 ml) in a Stomacher for 3 min (Taniwaki, 1995). Extracts were filtered and concentrated in a water bath at 60 °C to near dryness and then dried under a stream of N_2. The residue was resuspended in chloroform, spotted on TLC plates which were developed in toluene: ethyl acetate: formic acid: chloroform (7:5:2:5) and visualised under UV light at 365 nm. An OTA standard (Sigma Chemical Co., St Louis, USA) was used for comparison.

Analysis of OTA in coffee

Coffee samples were analyzed for OTA according to Pittet et al. (1996). The samples were extracted with a solution of methanol: 3% sodium bicarbonate (50:50). The extract was filtered and diluted with phosphate buffered saline and applied to an immunoaffinity column (Ochratest, VICAM, USA) containing the monoclonal antibody specific for OTA. After washing, the OTA was eluted with high performance liquid chromatography (HPLC) grade methanol and quantified by reverse phase chromatography using a fluorescence detector. The detection limit of this method was 0.2 µg/kg. The mobile phase used was acetronitrile/4mM sodium acetate plus 0.5% acetic acid (42:58). The flow rate was 1 ml/min. The equipment used was a Shimadzu LC-10VP system (Shimadzu Corporation, Japan) set at 330 nm excitation and 470 nm emission. The HPLC was fitted with a ODS Hypersil (5 µm, 25 mm X 4.6 mm) pre column and Supelcosil^{TM} LC-18 (5 µm, 250 mm X 4.6 mm) column (Supelco Park, USA).

Conventional and Miniature Containers

A major study assessed the effects of storage in ships during transport across the tropics, a normal trade route of Brazilian coffee shipped to Europe. Three conventional containers with dimensions of 5.88 m x 2.32 m x 2.19 m and three miniature containers with dimensions of 1.86 m x 0.73 m x 0.73 m were used. In order to validate the miniature data as compared to that of the conventional containers, polystyrene was used to line three lateral walls of the miniatures. The top and one wall were not lined with polystyrene in order to simulate one segment of a real container. The miniatures were placed in a vertical position close to the end of the conventional ones. The route of this voyage from Santos, Brazil to Trieste, Italy has already been described elsewhere (Palacios-Cabrera et al., 2007).

RESULTS AND DISCUSSION

Production of coffee beans

More than 400 samples of coffee beans were examined, and from them 872 isolates of *A. niger* and *A. carbonarius* and those identified as *A. ochraceus*, but now known to be from *Aspergillus westerdijkiae,* were obtained (Table 1). The most common species found was *A. niger* (549 isolates) but only 3% produced OTA. *A. westerdijkiae* was also common (269 isolates), with 75% being able to produce OTA. *A. carbonarius* was much less common (54 isolates), with 77% being able to produce OTA (Table 2).

A. westerdijkiae was separately from *Aspergillus ochraceus* by Frisvad *et al.* (2004) These are very similar and many isolates previously identified as *A. ochraceus* are now recognized as *A. westerdijkiae*, including the original OTA-producing strain (NRRL 3174). Indeed most isolates producing OTA and previously recognized as *A. ochraceus* are now correctly placed in *Aspergillus westerdijkiae.* Amplification and sequencing of the ITS1-5.8S-ITS2 region from several Brazilian strains of both species showed specific nucleotide variations characterizing *A. westerdijkiae* and *A. ochraceus* (Fungaro et al., 2004). For ITS1, all sequences of *A. westerdijkiae* differed from the *A. ochraceus* by possessing a C instead of a T at positions 76 and 80. In addition, the strains of *A. ochraceus* showed a deletion of a T at position 89. For ITS2, specific nucleotides at position 494-495, AT, characterized the strains of *A. westerdijkiae*, instead of TC in *A. ochraceus*. Taniwaki et al. (2003) identified all species of the section Circumdati as *A. ochraceus*, since *A. westerdijikiae* had not described yet, although Fungaro *et al.* (2004) have showed that there were two distinct groups in the isolates from coffee isolated by Taniwaki et al. (2003).

The low rates of infection with potential OTA producing fungi found in Brazilian coffee cherries (Table 1) indicate few problems, but the average figures do not tell the whole story. The percentages of infection of the worst samples encountered provide a different picture (Table 3). *A. niger* was found to invade cherries on the tree, among overripe fruit sometimes reaching 100% infection. However, infection by *A. westerdijkiae*, was at most 4% in fruit from the tree, increasing to up to 16 % in fruit from the ground, and 35% in fruit during drying and storage. Such infection rates indicate little infection occurring in cherries before picking, but increasing after contact with ground, equipment or drying yard surfaces. It seems likely that damage to the skin of the fruit is needed to initiate infection. The much higher infection rates in some samples during drying and storage indicate a real potential for OTA production. Although *A. carbonarius* infection was uncommon, infection rates of fruit in samples from the drying yard in one case was 26% and from storage was up to 12%. Again these infection rates indicate a high potential for producing OTA. The high infection rates with *A. niger* indicate little problem because of the low percentages of toxigenic isolates, but do emphasise the need to distinguish between the two species of black Aspergilli.

The variability in levels of infection with species potentially capable of OTA production was reflected in the figures for ochratoxin assays. Means and ranges found in 135 samples from the various stages of harvesting and processing are given in Table 4. Samples taken from fruit on the tree contained negligible amounts of OTA, almost always below the limit of detection. Mean levels of OTA from fruit from the ground, from the drying yard and from storage were all below 4 µg/kg, again indicating overall acceptability. However, a few samples from fruit taken from the ground or during drying and storage contained much higher and often unacceptable levels. This indicates a lack of quality control during drying on some farms.

Table 1. Infection of Brazilian coffee beans with fungi potentially capable of ochratoxin A formation.

Stage	No. of Samples	*A. westerdijkiae* (%)	*A. niger* (%)	*A. carbonarius* (%)
Mature from tree	55	0.25	1.2	0
Overripe from tree	57	0.35	4.0	0
Overripe from ground	63	1.9	1.9	0
From drying yard	128	1.3	2.7	0.5
From storage on farm	105	2.0	3.2	0.5

Table 2. Percentages of isolates of three species potentially capable of OTA production*.

Species	No of isolates tested	Toxigenic (%)
Aspergillus westerdijkiae	269	75
A. niger	549	3
A. carbonarius	54	77

**Grown for one week on YES agar at 25ºC, tested by thin layer chromatography.*

Table 3. Highest infection rates observed in samples of Brazilian coffee beans (%) of fungi capable of OTA formation.

Stage	*A. westerdijkiae* (%)	*A. niger* (%)	*A. carbonarius* (%)
Mature from tree	2	6	0
Overripe from tree	4	100	0
Overripe from ground	16	10	0
From drying yard	34	30	26
From storage on farm	36	44	12

Table 4. Ochratoxin A levels in coffee beans at various stages.

Stage in production (no. of samples)	Mean (μg/kg)	Range (μg/kg)
Cherries from tree (6)	ND*	ND - 0.4
Overripe from tree (16)	ND	ND
Overripe from ground (25)	2.0	ND – 37
Drying yard (40)	2.1	ND – 48
Storage (48)	3.4	ND – 109

** Not Detected (limit of detection 0.2 μg /kg).*

Container trial

Tables 5 and 6 present the ochratoxin A (OTA) concentrations in the coffee in the conventional and miniature containers respectively, at the beginning and end of the voyage. A slight increase in OTA production was observed during transportation, especially in the top of the container located on the ship's deck, at the top of the load, close to the headspace. In this location the temperature oscillation was more drastic and consequently the humidity diffusion inside the container was more dynamic than on the other decks, mainly during the sea voyage. A similar situation occurred at the top of the prototype containers, which showed higher OTA production. From the results presented, storage below deck is the only appropriate location for container transportation, since the temperature oscillation is less drastic, and the inter-bean microenvironment is less favourable for fungal growth and toxin production.

In the light of this 10 year investigation, the following recommendations are given:

- Avoid leaving loaded containers exposed to sunshine.
- Avoid rewetting of coffee in bags by covering them during transport.
- Keep the ventilation holes in the containers unobstructed.
- Where possible avoid transshipment.
- Upon arrival, the coffee must be received and discharged as soon as possible.
- Coffee moisture should not exceed 11% anywhere, from the departure point to the roasting location.

Table 5. Initial and final ochratoxin A (OTA) concentrations (μg/kg) in coffee from the conventional containers.

Location in the vessel	Container region	OTA concentration (μg/kg)	
		Initial	Final
Deck	Bottom	ND*	0.33
	Middle	ND	0.1
	Top	ND	7.91
	Wet bags**	ND	ND
Below deck	Bottom	1.65	0.16
	Middle	0.19	<0.1
	Top	1.03	0.74
	Wet bags	ND	ND
Hold	Bottom	1.54	0.17
	Middle	0.30	0.26
	Top	ND	0.28
	Wet bags	ND	13.13

**Not Detected (limit of detection 0.2 μg /kg). **Bags were wet when the containers were opened in Italy, yet not wet initially.*

Table 6. Initial and final ochratoxin A (OTA) concentrations (μg/kg) in coffee beans transported in miniature containers.

Location in the vessel	Container region	OTA concentration (μg/kg)*	
		Initial	Final
Deck	Bottom	0.20	2.85
	Middle		1.01
	Top		2.38
Below deck	Bottom	0.20	1.22
	Middle		2.06
	Top		0.45
Hold	Bottom	0.20	0.20
	Middle		1.29
	Top		0.66

**Limit of detection 0.2 μg /kg.*

Intake assessments of OTA consumption due to coffee

A great deal of interest has been focused on the possible role of coffee in ochratoxin A consumption. JECFA has set a Provisional Tolerable Weekly Intake (PTWI) for OTA of 100 ng/kg bw/week which correspond to 14 ng/kg bw/day. From a survey of coffee drinkers, Patel et al. (1997) in the UK developed an Estimated Weekly Intake of OTA from soluble coffee. Based on a consumption of 4.5 g of soluble coffee per day, the average coffee drinker ingested 0.4 ng/kg body weight/week, while the heavy consumer (97.5% percentile) consumed nearly 20 g soluble coffee per week to give an intake of 1.9 ng/kg body weight/week. Those figures translate into 3.5 ng and 17 ng intake of ochratoxin A per day and 0.4% or 2% of the PTDI respectively (Patel et al., 1997). Comparing these data with the data obtained by Iamanaka *et al.* (2005) in Brazil, the average of OTA concentration in roasted coffee was 1.3 μg/kg. Ground and roasted coffee is the type of coffee most used by Brazilians

and many coffee producer countries for the preparation of the beverage. According to Iamanaka *et al.* (2005), considering the average Brazilian adult drinks five cups of coffee per day this would correspond to 30g of roast and ground coffee to brew five cups of the beverage (each 60 ml) prepared according to common household procedures. The probable daily intake of OTA by a 70 kg adult would be 0.56 ng/kg bw/day and this falls far below the JECFA PTDI. Examining a worst case situation of a heavy coffee drinker that may drink up to 33 cups of coffee a day (Camargo et al., 1999) this would mean an ingestion of 3.7 ng/kg bw/day which is still well below JECFA PTDI. These results indicate that coffee is not a major dietary source of ochratoxin A in the UK or Brazil and it should not be very different in most other coffee drinking countries.

CONCLUSION

As the evidence shows that ochratoxin A is formed in raw coffee beans after harvest, OTA contamination can clearly be minimized by following good agricultural practice and a subsequent post-harvest handling consisting of appropriate techniques for drying, grading, transportation and storage of raw coffee. Moreover better quality raw material, appropriate dehulling procedures and reduction of defects using colour sorting in some countries to reject defective beans can substantially reduce the concentration of OTA in raw coffee. These procedures are well established. In June 2002, the European coffee associations and bodies published the Code of Practice "Enhancement of coffee quality through prevention of mould formation" (www.ecf-coffee.org). The objective of this code of practice is to assist operators throughout the coffee chain to apply Good Agricultural Practice, Good Practices in Transport and Storage and Good Manufacturing Practices preventing OTA contamination and formation. The Codex Committee on Contaminants in Foods (CCCF) is also preparing a discussion paper on ochratoxin A in coffee and the Code of practice to prevent and reduce OTA in coffee. Preventive measures taken by all participants in the chain from tree to cup are the best way to prevent ochratoxin A contamination in coffee.

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Impact of the Time of Permanence of Coffee Fruits in the Plant and on the Ground, and of Geoclimatic Conditions on the Quality of Beverage, Presence of Defects and Incidence of Ochratoxin A

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SUMMARY

The study was conducted at the Center of Experiments of IAPAR, Londrina-PR, in coffee plantation with Sarchimor Yellow cultivar of late maturation with 4 years of age and grown space of 2.5 x 0.80 m with a plan by pit. A random design was applied, consisting of five harvest periods at intervals of 30 days (0, 30, 60, 90 and 120 days), 4 replicates and 10 plants per plot. All coffee fruits on the ground and all dried and green coffee on the plant were removed, leaving in the plant only the ripe fruits (cherry). The experiment was carried out in 2004 in the period from July to November, and in 2005 in the period from June to October. An agrimeteorological station was installed for monitoring the air temperature, relative humidity and rainfall during the experiment. In each harvest period, the coffee in the plant and fallen on the ground were collected and dried separately in boxes fitted with a screen under the full sun light down to the humidity of 12%. The green coffee beans underwent test cup (Garruti and Conagin, 1961), defect classification [black beans, burnt, black green, green, shells, shells and its pulp, malformed, insect damaged "brocados" and the total grain with defects] and ochratoxin A (OTA) analysis [AOAC 2004.10]. For the statistical analysis, data were converted into $\sqrt{Y}$. In harvest periods of 0-30 days, 31-60 days, 61-90 days and 91-120 days, the rainfall incidence were 0mm, 35mm, 252mm and 116mm, and 46mm, 39mm, 144mm and 192mm for 2004 and 2005, respectively. The mean number of defective coffee beans in 2004 was lower than that of 2005 probably due to absence of rainfall in the first 50 days of the 2004 experiment. The total amount of defective coffee beans was greater in coffee from the ground than those from the plant particularly after 60 days (150 mm of rainfall) which did not occur with the insect damaged beans that had their number increased in the plant and on the ground since the time zero of the experiment. The OTA level of contamination ranged from 0.12 to 42 μg/kg while the mean level of OTA both in the plant and on the ground remained below 5 μg/kg. In 2004, OTA was detected in the coffee from the ground from the thirty (30), while in the coffee from the plant it was detected only at harvest period of 120 days. In 2005, OTA was detected in the coffee from ground at harvest period of 60 to 120 days and in the coffee from the plant at 90 to 120 days. In 2004 and 2005, the levels of OTA in the coffee was positively correlated with the increase of black and insect damaged beans in the plant. Therefore, the permanence of coffee fruits in the plant and on the ground for more than 30 days in that particular growing area, will impact negatively the quality of beverage, increase the presence of defects and incidence of OTA.

INTRODUCTION

In Brazil predominates the harvest of coffee by stripping the coffee (derriça total) within a period of 3 to 4 months with the boom in the months of September, October and November. The maturation of the fruits is uneven with ripe and green, and dried, and fallen fruits predominating at the early and at the end of harvest, respectively. The fallen fruits are generally separated from the coffee harvested directely from the plant by cleaning the ground (varrição) previously to the harvest or by harvesting the coffee from the plant on the cloth.

The contact of the coffee with the ground increases the risk of contamination for OTA (Batista and Chalfoun, 2007; Vargas et al., 2004). The presence of defects significantly impacts the incidence and levels of OTA, and the black, insect damaged beans, malformed and broken defects are the main contributors to the occurrence of OTA (Vargas et al., 2004). High concentration for OTA in burnt beans has also been reported (Taniwaki et al., 2005). The coffee dropped on the ground loses its quality faster than the coffee on the plant (Carneiro Filho et al., 2001), with highest percentage of black beans and burnt (Carvalho et al., 1970), possibly due to the percentage of fungi inside the seed that increases with the permanence of the fruit on the ground (Krug, 1940). Some fungi have been associated with positive sensory characteristics of coffee and the other negative aspects (Taniwaki et al., 2004).

This study was carried out with the objective to assess the impact of time of permanence of the coffee in the plant and on the ground and climatic conditions in the occurrence of coffee defects, coffee drink quality and occurrence of OTA in coffee.

MATERIAL AND METHODS

The experiment was conducted at the Centre of Experimental IAPAR of Londrina-PR, (23° 23' S, 51° 11' W, altitude of 581 m), in the North of Parana State, Brazil in a coffee plantation with cultivating Sarchimor Yellow (*Coffea arabica L.*) of late maturation, 4 year age and grown spacing of 2.5 x 0.80 m with a plan by pit . An experiment entirely at random with five harvest period (0, 30, 60, 90 and 120 days), 4 replicates and 10 plants per plot was designed. All coffee fruits on the ground and all dried and green coffee on the plant were removed, leaving on the plant only the ripe fruits (cherry), as methodology proposed by Carneiro Filho et al. (2001).

The monitoring was carried out from July 27th to November 28th 2004, and in the period from June 28th to October 28th, 2005. A meteorological station was installed for monitoring the air temperature, relative humidity and rainfall during the harvest. In each harvest period, the coffee in the plant and fallen on the ground, were collected and dried separately in boxes fitted with a screen under the full sun light down to the humidity of 12%.

The coffee beans were processed and underwent test cup (Garruti and Conagin , 1961), defect classification in 300 grams [black beans, burnt, black green, green, shells, shells and its pulp, malformed, insect damaged “brocados” [Hypothenemus hampei] and the total grain with defects] and ochratoxin A (OTA) analysis [AOAC 2004.10].

The test cup was conducted by five experienced drinkers with five (05) cups of coffee per sample using the following scores: 5, 4, 3, 2, 1 and 0 for coffee drink (bebida) strictly soft, soft, just soft, hard (dura), riada and rio, respectively, as proposed by Garruti and Conagin (1961). For the statistical analysis (tukey test and Pearson correlation) the data were processed in $\sqrt{Y}$.

RESULTS AND DISCUSSION

The normal coffee harvest season in the region where the experiment was conducted occurs in the months from May to August, which coincides with the dry season most of the year. As the present study was conducted with a late maturation cultivar where the largest quantity of ripe fruits were obtained in June and July, the assessments were extended by 2 to 3 months beyond the normal harvest season in the region, covering a period of greater rainfall. Also the distribution of rainfall was different in the two periods, 2004 and 2005. These conditions enabled a better assessment of the influence of climatic factors in the quality of coffee and the levels of contamination by OTA.

In 2004, it occurred 25 days of rain accumulating a total of 403 mm within 120 days of duration of the experiment, with volume of 0.0 mm of rain, 35 mm, 252 mm and 116 mm in periods of 0-30, 31-60, 61-90 and 9 -120 days, respectively. It is important to emphasize that no rains occurred in the first 55 days of the experiment (Figure 1).

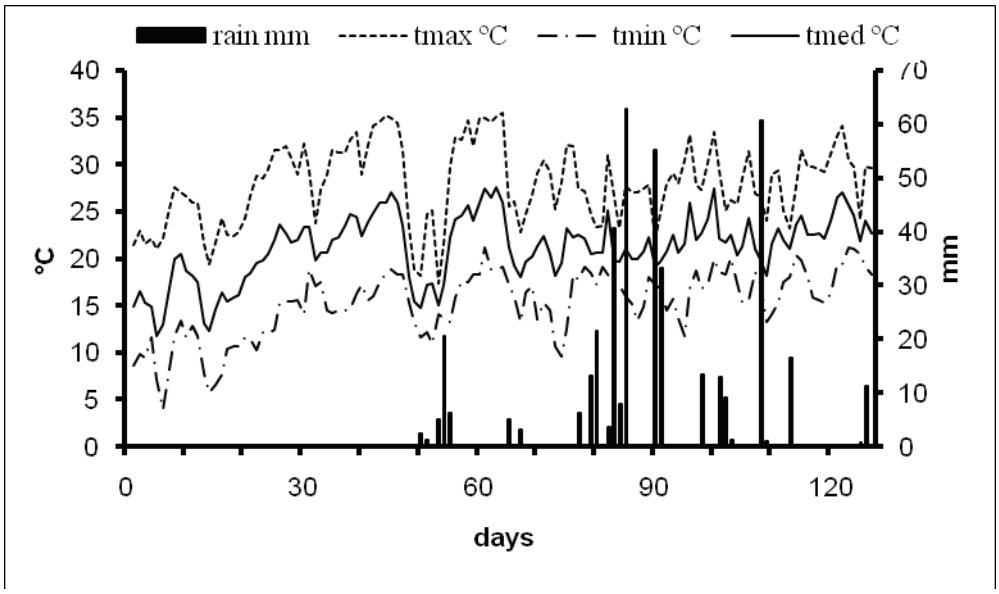

Figure 1. 2004 Climatic data during the experiment from July 27th.

In 2005, it occurred 46 days rain accumulating 421 mm distributed throughout the period with volume of rain of, 46mm, 39mm, 144mm and 192mm in periods of 0-30 days, 31-60 days, 61-90 days and 91-120 days, respectively (Figure 2).

Generally, naturally fallen coffee on the ground showed higher black, burnt, insect damaged "brocados" [Hypothenemus hampei] beans, total beans with defects and OTA contamination in the two years (2004 and 2005) assessed in relation to coffee remaining on the plant. The black beans occurred only when the coffee was collected with 90 days and significantly increased with the harvest time (120 days) in the coffee remained on the plant in 2004 and 2005. In the coffee beans dropped on the ground this defect was detected in the sample collected at 30 days and significantly increased at 90 days and 120 days (Tables 1 and 2).

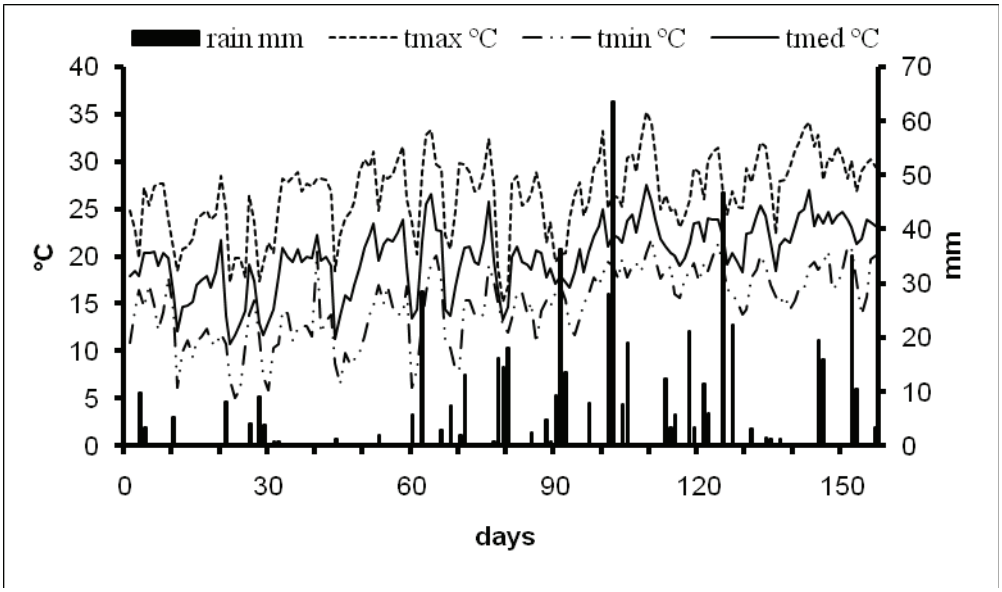

Figure 2. 2005 Climatic data during the experiment from June 27th.

The burnt beans were found on the plant since harvest at time zero (0) in 2004 and at 30 days in 2005, with significant increase from ninety days in the two years of assessment. In the ground beans, it was found burnt beans from the harvest at 30 days in 2004 and 2005 to increase from the 90 days in 2004. In 2005, the differences between the mean number of burnt beans found in different harvest times were not significant.

The number of insect damaged beans in 2004 was low, with no significant differences between the mean in both plant and ground. In 2005, possibly because the harvest has begun a month later in a year with greater incidence of rainfall the number of insect damaged beans was higher in relation to the previous year, with increasing number since the beginning. The total number of defects has increased significantly from the harvest to 90 days in the coffee on the plant and on the ground in 2004 and 2005.

There was a trend in loss of quality of drink depending on the length of stay on the coffee plant and ground. In years with less frequency of rain, it was obtained coffee drink – bebida dura − up to 60 days of residence of fruits on the plant. However, in the year with greater incidence of rain, it was detected drink riada at 60 days. After 60 days, the coffee drinks from the plant showed only rio and riada scores (0 and 1) in the two years of assessment. Coffee from the ground until 30 days of fallen returned cup dura (score 2) and occurred an increase of cups drinking rio and riada (scores 0 and 1) depending on the residence time of fruit on the ground.

The OTA in the fallen fruit occurred naturally at 30 days of permanence on the ground, depending on year and was more frequent in the coffee harvested at 60-120 days. In the coffee collected from the plant, OTA was detected at 120 days in 2004 and from 90 day in 2005, possibly due to distribution of rain in 2005. This indicates that the risk of OTA contamination is higher in coffee from the ground and is a function of local climatic conditions and of time of residence of fruit on the ground. It is important to emphasize that the mean levels of OTA observed were always below 5 μg/kg (Tables 1 and 2).

Table 1. Number of black, burnt, insect damaged beans (brocado), total defective beans in 300 grams, score of coffee drink (bebida) and ochratoxin A in green coffee per time of permanence of the fruits on the plant and on the ground in 2004. Values transformed into √ Y.

Period [day]	Number of beans in 300 grams				Drink score: 0 to 5	OTA (µg/kg)
	black	burnt	insect damaged	Total		
Plant						
0	0.00 a	1.04 a	0.00 a	4.64 a	1.46 a	0.00 a
1-30	0.00 a	1.18 a	0.00 a	4.88 a	1.56 a	0.00 a
31-60	0.00 a	1.66 a	0.00 a	5.25 a	1.41 a	0.00 a
61-90	4.40 b	5.04 b	0.61 a	8.82 b	1.18 ab	0.00 a
91-120	6.43 c	8.68 c	1.50 a	12.35 c	0.58 b	1.45 a
ground						
0*	-	-	-	-	-	-
1-30	1.22 a	3.60 a	0.75 a	10.39 b	1.41 a	0.25 a
31-60	4.36 a	4.46 a	0.00 a	8.38 a	1.31 a	1.19 a
61-90	14.50 b	11.48 b	0.43 a	19.19 c	0.80 a	2.67 a
91-120	18.53 c	13.10 b	0.75 a	23.50 d	1.14 a	1.38 a

**No fallen fruit on the ground.*
Means with same letter in the column do not differ by Tukey test with 5% probability.

Table 2. Number of black, burnt, insect damaged beans (brocado), total defective beans in 300 grams, score of coffee drink (bebida) and ochratoxin A in green coffee per time of permanence of the fruits on the plant and on the ground in 2005. Values transformed into √ Y.

Period [day]	Number of beans in 300 grams				Drink score: 0 to 5	OTA (µg/kg)
	black	burnt	insect damaged	Total		
Plant						
0	0.00 a	0.00 a	1.83 a	0.95 a	1.41 a	0.00 a
1-30	0.00 a	3.23 a	5.27 ab	2.57 a	1.41 a	0.00 a
31-60	0.00 a	1.97 a	4.84 ab	2.13 a	1.34 a	0.00 a
61-90	6.75 b	14.52 b	8.54 b	6.63 a	0.22 a	0.32 a
91-120	12.71 c	16.71 b	17.35 c	1.47 a	0.22 a	1.94 a
ground						
0*	-	-	-	-	-	-
1-30	1.73 a	4.24 a	0.87 a	7.04 a	1.41 a	0.00 a
31-60	1.15 a	3.26 a	4.24 ab	9.36 a	1.36 a	0.16 a
61-90	13.45 b	16.97 a	10.26 b	27.60 b	0.92 a	0.64 a
91-120	16.36 b	8.70 a	22.49 c	33.71 b	0.75 a	1.07 a

**No fallen fruit on the ground.*
Means with same letter in the column do not differ by Tukey test with 5% probability.

The levels of OTA in coffee on the plant showed positive and significant correlation with the number of insect damaged beans (brocado) in 2004, and with the number of black beans in the two years (Tables 3 and 4). These results are consistent with those obtained by Vargas et al. (2004). In the second year (2005), where the rains were more frequent (Figure 2), the level of

OTA in the naturally fallen coffee returned a positive correlation with the amount of rainfall (mm) and mean temperature and, negative correlation with the quality of the drink (Table 6).

The number of black, burnt beans, and total defective beans showed positive correlation with the incidence of rain, while the beverage was negatively correlated for both coffee on the plant and dropped naturally on the ground, in 2004 and 2005.

Table 3. Pearson correlation coefficient calculated in relation to incidence of rainfall, average temperature (tma), number of black beans, burnt, insect damaged beans (brocado), total defective beans in 300 grams, coffee drink (bebida) and OTA on the coffee plant in 2004.

Parameters	black	burnt	insect damaged	total defective beans	Coffee drink (bebida)	OTA (μg/kg)
rainfall	0.5643**	0.4602*	0.2988ns	0.5554*	-0.5213*	0.0844ns
Tma	0.4010ns	0.2926ns	0.1862ns	0.3867ns	-4579ns	0.0157ns
black		0.8283**	0.7521**	0.9189**	-07691**	0.5728**
burnt			0.5798**	0.9696**	-0.7154**	0.2650ns
insect damaged				0.6164**	-0.5048*	0.6152**
total defective beans					-0.7759**	0.3411ns
Coffee drink (bebida)						-0.2276ns

*, **Significant at 5% and 1% probability, respectively, t test.
nsNo significant.

Table 4. Pearson correlation coefficient calculated in relation to incidence of rainfall, average temperature (tma), number of black beans, burnt, insect damaged beans (brocado), total defective beans in 300 grams, coffee drink (bebida) and OTA in the coffee from the ground in 2004.

Parameters	black	burnt	insect damaged	total defective beans	Coffee drink (bebida)	OTA (μg/kg)
rainfall	0.6237**	0.6645**	-0.0968ns	0.6323**	-0.5418*	0.6091ns
Tma	0.5262*	0.5681*	-0.1694ns	0.4965*	-0.5190*	0.2631ns
black		0.7941**	0.1303ns	0.9743**	-0.4059ns	-0.0186ns
burnt			0.0090ns	0.8931**	-0.4039ns	-0.0009ns
insect damaged				0.1068ns	0.3023ns	-0.0923ns
total defective beans					-0.4103ns	-0.0599ns
Coffee drink (bebida)						-0.1353ns

*, **Significant at 5% and 1% probability, respectively, t test.
nsNo significant.

Table 5. Pearson correlation coefficient calculated in relation to rainfall, average temperature (tma), number of black beans, burnt, insect damaged drink (brocado), total defective beans in 300 grams, coffee drink (bebida) and OTA in the coffee from the plant in 2005.

Parameters	black	burnt	insect damaged	total defective beans	Coffee drink (bebida)	OTA (μg/kg)
rainfall	0.9029**	0.7869**	0.7527**	0.9101**	-0.9210**	0.3579ns
Tma	0.8514**	0.5937*	0.7634**	0.7989**	-0.7164**	0.3766ns
black		0.7782**	0.8793**	0.9485**	-0.7918**	0.5497*
burnt			0.5660**	0.8904**	-0.8107**	0.3847ns
insect damaged				0.8380**	-0.6670**	0.2052ns
total defective beans					-0.8490**	0.3744ns
Coffee drink (bebida)						-0.3364ns

*, **Significant at 5% and 1% probability, respectively, t test.
nsNo significant.

Table 6. Pearson correlation coefficient calculated in relation to rainfall, average temperature (tma), number of black beans, burnt, insect damaged drink (brocado), total defective beans in 300 grams, coffee drink (bebida) and OTA in the coffee from the ground in 2005.

Parameters	black	burnt	insect damaged	total defective beans	Coffee drink (bebida)	OTA (μg/kg)
rainfall	0.8478**	0.5241**	0.3703ns	0.9226**	-0.7171**	0.5929*
Tma	0.6723*	0.2097ns	0.4012ns	0.7367**	-0.6142*	0.5266*
black		0.4879ns	0.1607ns	0.9754**	-0.5387*	0.5095ns
burnt			0.3774ns	0.5574*	-0.0965ns	0.2206ns
insect damaged				0.2704ns	-0.1665ns	-0.1584ns
total defective beans					-0.5653*	0.4940ns
Coffee drink (bebida)						-0.6457*

*, **Significant at 5% and 1% probability, respectively, t test.
nsno significant.

The results indicated that the mean levels of OTA remained below the allowable limit for roasted coffee and were lower in the coffee of the plant than in the coffee from the ground. Under the conditions in which the experiment was carried out the coffee in the mature stage (cherry), can remain on the plant up to 60 days without contamination by OTA and, in years of low rainfall can remain in the plant up to 90 days without contamination by OTA. There was a positive correlation between the occurrences of black, insect damaged beans, incidence of rain and temperature, and negative correlation with quality of coffee drink (bebida. Therefore, it is important to conclude that the permanence of coffee fruits on the plant and on the ground for more than 30 days in that particular growing area will impact negatively the quality of beverage, increase the presence of defects and incidence of OTA.

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Chemical Characterization of *Coffea arabica* L. Cherries†

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†In memory of Dr. Ernesto Illy (July 18, 1925 - February 3, 2008)

SUMMARY

Coffee fruits, well known as berries or cherries, can be cross sectioned revealing four anatomical fractions: the coffee bean proper or endosperm; the hull or endocarp (parchment); a layer of mucilage or mesocarp and the pulp or esocarp. Literature data on chemical composition of anatomical fractions other than endosperm are scarce and most of them date back to the Seventhies. This is particularly true for volatile organic compounds responsible for cherry aroma being the first identification of volatile compounds released by fresh red coffee berries published in the late Ninethies. This work is aimed at characterizing the coffee cherries in terms of chemical composition by analyzing both the volatile and the non-volatile compounds (sugars, chlorogenic acids, caffeine) by means of GC-MS, HPLC and LC-MS techniques.

INTRODUCTION

The ripe fruit of coffee, generally known as coffee berry or cherry, is described as a drupe according to the botanical terminology. The drupe is a fleshy fruit surrounded by a thin skin (epicarp) with a central pulp (mesocarp + esocarp) and a inner layer (endocarp) directly surrounding one or more seeds (endosperm); typical drupes are the fruits of all members of the genus *Prunus* (apricot, peach, cherry) and of *Olea europea*. The coffee cherry contains two stones that are lying with their flat faces together, which are the future coffee beans (Figure 1).

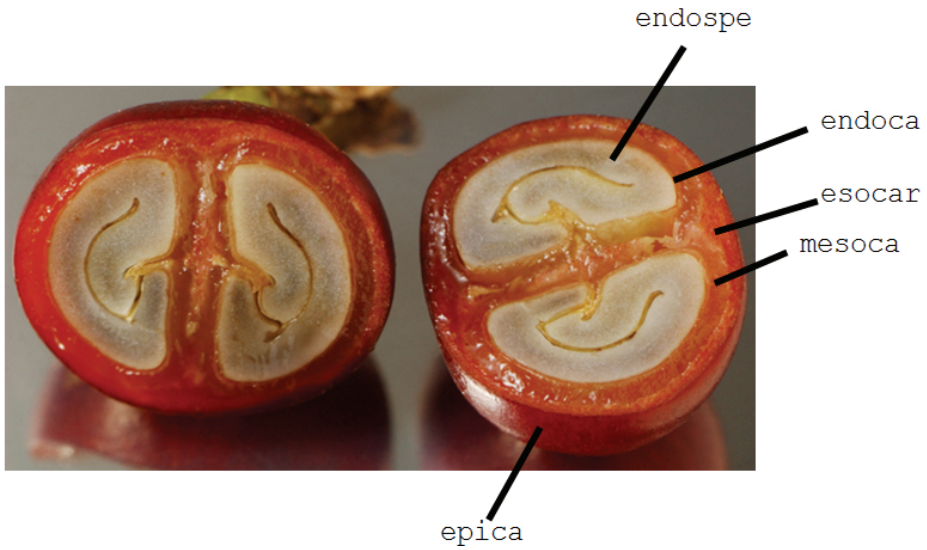

Figure 1. Section of *C. arabica* coffee cherry.

To obtain the beans, epicarp, pulp (mesocarp + esocarp) and endocarp have to be removed. In the wet processing, a mucilaginous waste product is obtained, the so called "pulp", while a solid residue is produced in the dry processing, the so-called husk (Mazzafera, 2002; Pandey, 2000).

Coffee cherries are usually considered as waste, and this is probably the main reason of the scarce interest dedicated by scientist to their chemical characterization. Chemical data, however, have been related to coffee berry disease (CBD) caused by *Colletotrichum kahawe* (Biratu, 1997) and to coffee berry borer (CBB) the major pest in coffee production. In particular, according to Mathieu et al. (1996), CBB can discriminate between the different stages of berries ripeness depending on their released effluvia.

The analysis of the volatiles released from coffee berries, revealed that *Coffea canephora* varieties had richer odorant bouquet than *Coffea arabica* ones. Volatiles compounds identified, were grouped according to chemical structures as alcohols, ketones, aldehydes, acetates, terpenes and sesquiterpenes. It has been suggested that the volatile composition of coffee berries can represent a good marker of different coffee varieties, even better than individual key compounds.

Two years later, the same group, found remarkable differences in amount and chemical nature of volatile compounds in passing from green to red berries and depending on botanical variety (Mathieu et al., 1998). Ramirez-Martines (1997) carried out the pulp chemical characterization (dry matter, condensed tannins, chlorogenic acid and caffeine contents) as affected by coffee processing: wet traditional vs. dry ecological. The comparison of the

chemical data revealed significant difference between the two different processing methods. More recently Ortiz et al (2004) analyzed the volatile emissions of *Coffea arabica* cherries at different stages of ripeness. High levels of alcohols (mainly ethanol) in all stages have been found. Overripe coffee cherries showed a volatile composition in which esters, alcohols, ketones and aldehydes dominated. In the last years 'wasteful' by-products of coffee production and their potential uses have gained interest and many studies followed this wave in the framework of sustainability and ecological topics. In this perspective, chemical information on coffee residue are restricted to compounds with specific properties and/or features which can be exploited in new applications. Recent applications of coffee husk have been proposed involving biotechnology (Pandey, 2000), agriculture (Martinez-Carrera, 2000) and cosmetic issues (Miljkovic, 2004). Fresh cherries volatile compounds, in this regards, have been neglected and previous scarce data have not yet been re-examined.

The aim of this work is to characterize unaltered fresh coffee cherries (not as by-product of coffee processing), in terms of chemical composition by analyzing both volatile and non-volatile compounds (chlorogenic acids, caffeine, trigonelline) by means of GC-MS and HPLC techniques.

MATERIAL AND METHODS

Ripe cherries from El Salvador (*Coffea arabica* L. var. Bourbon) were collected when ready to be processed, carefully packed in polyethylene bags and immediately refrigerated (4 °C) to retard, as much as possible, over-ripeness during the trip to our laboratory. Analyses were done 8 days after harvesting. During this time the cherries were kept constantly at 4 °C until sample preparation.

Analysis of the volatile part

In order to analyze pulp aroma components cherries were sectionated and the seeds were removed. Then 50g of pulp was put in 1l of distillated water together with the internal standard (dimethoxytoluene, 50 μg) and extracted for 2 hours by steam distillation (SDE) using a Lickens-Nickerson apparatus and with a mixture of pentane and diethylether (1:1, 50 ml). The organic phase was then concentrated to a final volume of 1ml by inert gas flow (nitrogen) at 40 °C. 1 μl of this concentrate was then injected to a GC-MS system by Agilent (gascromatograph 6890 equipped with a 5273 mass spectrometer by Agilent, USA).

The column was a ZB-wax (Zebron, phenomenex), 60m of length and 0,25 mm of internal diameter and 0,25 μm of film thickness; the temperature program was 35 °C for 5 minutes, raised to 210 °C at 4 °C/min, then at 20 °C/min to the final temperature of 240 °C. The injection was conducted in split mode, split ratio 4:1.

Analyses of non-volatile components

Cherries were freeze-dried using an Alpha 1-2 freeze-drier (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for a 72h period. Loss of water was of 80% for pulp and of 50% for seeds. Freeze-drying was performed on whole fruit and also on single parts (pulp and seeds plus parchment).

Caffeine, trigonelline and (3, 4 and 5-) caffeoylquinic acids quantitative determination was performed on 5g of dry matter in 100ml of boiling water; samples were centrifuged for 5 minutes at 5000rpm and filtered with a hydrophobic PTFE membrane filter (Whatman, USA).

Analyses were performed both on entire cherries and on single parts: pulp, skin and seed. Green beans were first put in liquid nitrogen and then grinded by a M20 mill (IKA® Werke GmbH & Co., Staufen, Germany).

Samples were diluted in a ratio 1:10 and then analyzed by HPLC. An 1100 HPLC system (Agilent, USA), consisting of degasser, quaternary pump, column thermostat and diode array detector (DAD) operating at 254 nm, 272 nm and 324 nm, was used. A Gemini C18 column, 5micron 250 x 4.60 mm (Phenomenex, USA) and gradient elution (methanol and 1% phosphoric acid) were used.

RESULTS AND DISCUSSION

Volatile components

Table 1 shows the volatile compounds found in cherries and their odour impressions.

Experimental results are in substantial agreement with those reported by Ortiz et al. (2004) about ripe and overripe stage of development of *C. arabica* cherry. In fact esters, alcohols and aldehydes/ketones are predominant in the volatile composition. Ortiz et al. (2004), however, did not mention the presence of linalool and linalool oxide, which have been found by Mathieu et al. (1998), as characterizing volatiles of *C. arabica* in addition to limonene. Due to the different experimental approach adopted in this study from those adopted by previous research groups (Ortiz et al., 2004; Mathieu et al., 1998), a direct comparison of the experimental data is not possible. However, the presence of linalool and linalool oxide (together with epoxylinalool) is confirmed, but not the one of limonene.

Moreover, volatile compounds found in fruits and mainly derived from three major pathway involved in the biosynthesis of aroma compounds in plants have been found (Lewinsohn et al., 2001; Dudareva et al., 2004). In particular, in addition to short chain alcohols and aldehydes (degradation of lipids), phenethyl alcohol and phenolic compounds (shikimic acid pathway) and nor-isoprenoids such as beta-ionone and geranylacetone, in addition to linalool (isoprenoid pathway) have been found.. Beta-damascenone, with a characteristic fruity aroma has been also found, as in many other fruits (Ong et al., 1998).

These results indicate that the aroma character of coffee cherry may reflect the interaction of fruity/floral notes (linalool, beta-damascenone, beta-ionone, esters) and fatty-green harsh notes (alcohols and aldehydes) with pungent and spicy notes contributing to its flavour complexity.

Table 1. Volatile compounds in coffee cherries, their odor impression and the Kovats retention indexes obtained with a DB-wax column. References: (1) Burdock (1995), (2) Flament (2001), (3) The Good Scents Company,website, (4) Kurata (2003).

N.	Compound	Odor impression	RI (Kovats Index)
1	2-Methyltetrahydrofuran	-	886
2	Acetic acid, ethyl ester	Ether-like odor reminiscent of pineapple (1)	910
3	2-Pentanone	Wine, acetone-like, characteristic odor (1)	988
4	Hexanal	Powerful, penetrating, fatty-green, grassy (2)	1075
5	3-Pentanol	-	1096
6	2-Pentanol	Mild green, fusel oil odor (1)	1107
7	n-Heptanal	Fatty, harsh, pungent odor (1)	1167
8	1-pentanol	Harsh, chemical, reminiscent of fusel oil (2)	1184
9	Furan, 2-pentyl	Earthy, mouldy, oily anisic (2)	1204
10	4-heptenal, (Z)	Fishy, sardine-like (2)	1215
11	2-heptanol	Brassy, herbaceous odor reminiscent of lemon (1)	1283
12	n-Nonanal	Fatty odor, orange and rose on dilution (1)	1360
13	2-octenal	Green-leafy; orange, honey-like (1) Nutty, tallowy (4)	1395
14	Ethyl octanoate (ethyl caprilate)	Pleasant, fruity, floral (wine-apricoat note) (1)	1398
15	Linalool oxide	Woody, floral (1)	1405
16	Acetic acid	Pungent (1)	1430
17	2,4-Heptadienal, (E,E)-	Fatty, woody, herbal flavour (2); Fishy (4)	1436
18	2,4-Heptadienal, (Z,E)-	-	1465
19	Decanal	Fatty odor, floral on dilution (1)	1468
20	3-Ethyl-2-methyl-1-heptene	-	1476
21	Benzaldehyde	Bitter almonds (1)	1498
22	Nonanoic acid, ethyl ester (ethyl nonanoate)	Odor reminiscent of cognac with a rosy-fruity note (1)	1504
23	*Unknown*	-	1500
24	Linalool	Floral (1)	1514
25	1-Octanol	Fresh, orange-rose (1)	1524
26	Decanoic acid, ethyl ester (ethyl decanoate)	Fruity reminiscent of grape (cognac) (1)	1603
27	Phenylacetaldehyde	Harsh, green odor reminiscent of hyacinth on diluition (1)	1617
28	Butanedioic acid, diethyl ester	-	1643
29	a-Terpineol	Characteristic lilac odor (1)	1664
30	*Unknown*	-	
31	2,4-nonadienal	Strong fatty, floral odor (1)	1650
32	*Unknown*	-	
33	Epoxylinalol	-	1703
34	*Unknown*	-	
35	2,4-Decadienal, (E,Z)-	Metallic tallowy (2)	1734
36	Benzoic acid, 2-hydroxy-, methyl ester (methyl salycilate)	Characteristic wintergreen-like odor (1)	1750
37	Benzeneacetic acid, ethyl ester (ethyl phenylacetate)	Pleasant, strong, sweet odor suggestive of honey (1)	1755
38	Ethyl phenyl acetate	-	1758
39	Dodecanoic acid, methyl ester (methyl laureate)	Fatty, floral odor reminiscent of wine (1)	1765
40	2,4-decadienal (E,E)	Fried, oily (2)	1779
	3-hexylFuran	-	
41	Acetic acid, 2-phenylethyl ester	-	1784
42	b-Damascenone	Tea-like, fruity (2)	1789
43	Isopropyl dodecanoate	-	1793
44	Dodecanoic acid, ethyl ester (ethyl laureate)	Floral, fruity odor (1)	1804
45	2-Ethyl-.delta.-(1(2))-bicyclo[4.3 0]nonene	-	1815
46	5,9-Undecadien-2-one, 6,10-dimethyl (geranylacetone)	-	1819
47	*Unknown*	-	1838
48	Benzenemethanol (benzyl alcohol)	Pleasant, fruity odor (1)	1846
49	ethyl-3-methylbutyl butanedioate	-	1865
50	Phenethyl alcohol	Rose-honey-like (2)	1881
51	Benzeneacetaldehyde, .alpha.-ethyl	-	1902
52	Beta-ionone	Violet (1)	1906
53	*Unknown*	-	1920
54	Cyclodecane	-	1926
55	Benzothiazole	Similar to quinoline (heavy, penetrating and nauseating, yet sweet odor) (1)	1930
56	2-Cyclohexen-1-one	-	1954
57	Tetradecanoic acid, methyl ester	-	1968
58	Tetradecanal	-	1988
59	3-ethyldibenzothiophene	-	2110
60	4-vinyl-2-methoxy-phenol	Powerful, spicy, clove-like odor (2)	2161
61	4-vinylphenol	Vanilla extract odor (1) Phenolic, smokey, medicinal (2)	2352

Non-volatile components

Quantification of caffeine, trigonelline and caffeoylquinic acids (CQA) has been performed on seed (endosperm), pulp (epicarp and esocarp) and parchment (mesocarp and endocarp) separately and on the starting material (whole cherry). Experimental data are expressed as mg/g dry matter (Table. 2).

Pulp was separated from cherry before freeze drying while parchment was separated from seed after drying (Figure 2).

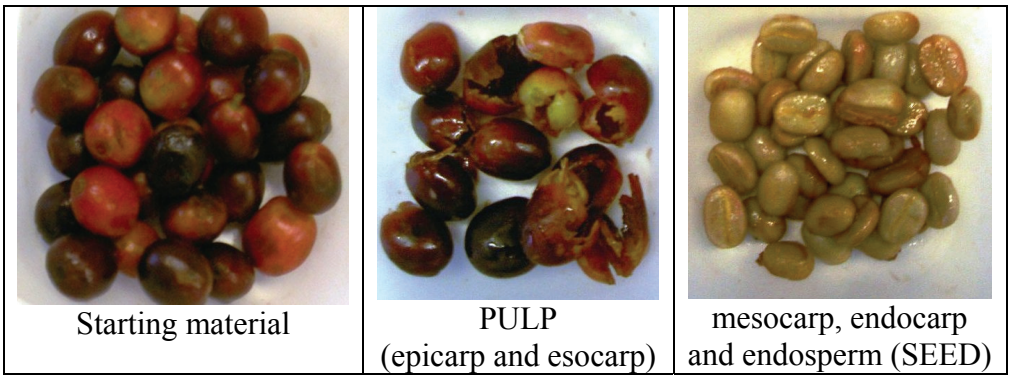

Figure2. Separation of pulp and seeds from entire fruit.

Coffee pulp dry matter content after freeze drying resulted equal to 21% in agreement with previously reported data (Molina, 1984).

Non-volatile compounds content in seed is in excellent agreement with that reported in the literature (see Table 2), whereas, in pulp, the agreement is confined to total CQA content only, being caffeine content lower than that expected. Coffee pulp caffeine content reported in literature is comprised within a wide range of values. Anyway, the caffeine content determined in the present study is remarkably lower than the lower limit of the reported range. In order to verify the possibility that sample handling and/or history might have affected pulp caffeine content, similar experimental approach and analyses have been carried out on cherries from a different origin (Brazil). A caffeine content equal to 5.5 mg/g dry matter has been found. This value is within the range reported in the literature.

The low caffeine content determined in the pulp of the coffee cherries of the present work could be due to variety, post-harvest treatments or sampling. Moreover, in spite of the care taken to preserve the sample, single cherry fermentation cannot be excluded *a priori*, in this case a lower caffeine content is expected (Roussos, 1989).

As far as chlorogenic acids is concerned, it has to be stressed that the reported data are frequently ambiguous since with the term “chlorogenic acids” it is not clear if one has to refer to the total of CQA isomers or to the predominant form (5-CQA). In all cases, the present data suggest, that the CQA isomers distribution is not different between pulp and seed being 5-CQA the predominant form. No reported data on trigonelline in coffee pulp have been found for comparison.

Table 2. Caffeine, trigonelline and caffeoylquinic acids expressed as mg/g dry matter (average n = 3 ± standard deviation). (a) Ramirez-Martinez, 2001(b) Molina, 1984, (c) Farah, 2005, (d) Illy e Viani, 1995, (e) Jaffè, 1952 (f) Bressani, 1974.

Compound	PULP (mg/g)	PULP (Literature) mg/g	PARCHMENT (endocarp and mesocarp) mg/g	SEED (endosperm) mg/g	SEED (Literature) mg/g
3-CQA	0.15 ± 0,00		0.16 ± 0.01	4.08 ± 0.24	4.83 ± 0.61 [c]
5-CQA	2.79 ± 0,05		0.26 ± 0.00	33.20 ± 2.42	31.26 ± 2.97 [c]
4-CQA	0.22 ± 0,01		0.16 ± 0.01	5.09 ± 0.34	5.44 ± 0.93 [c]
Total CQA	3.16 ± 0.06	1.1[a] ÷ 27.1[b]	0.58 ± 0.02	42.36 ± 3.00	42.0 ± 4.51 [c]
Caffeine	1.93 ± 0,01	5.1[e] ÷ 13.0[f]	0.65 ± 0.00	11.48 ± 0.43	12.0 [d]
Trigonelline	6.86 ± 0,01		3.49 ± 0.04	9.48 ± 0.37	10.0 [d]

CONCLUSIONS

The present chemical characterization of *Coffea arabica* cherries has been largely stimulated by some controversial reported data on both volatile and no-volatile compounds. As far as volatiles are concerned, in spite of different experimental approach, our findings are in substantial agreement with reported data. The presence of linalool and linalool oxide is confirmed. Several fruity/floral compounds have been also found. The very complex aroma seems to be due to the interaction of volatiles described as fruity/floral and fatty-green harsh with additional contribution of compounds characterized by pungent and spicy notes. The presence of caffeine, trigonelline and CQA in the different anatomical fractions of the coffee cherry has been ascertained. Lower caffeine content in the pulp than that expected has been found.

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Carboxylic Acid-5-Hydroxytryptamides (C-5-HT) in Coffee Brews

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SUMMARY

Carboxylic acid-5-hydroxytryptamides (C-5-HT) are known to cause stomach irritation as well as gastric lesions. However, little is known about the concentrations of C-5-HT in differently prepared coffee beverages. In this paper, the amount and the distribution of C-5-HT in filtered, boiled and manually prepared brews are presented. Whereas Scandinavian and Turkish coffee contained amounts of up to 14 mg per liter, coffees prepared with a paper filter showed concentrations of less than 1 mg per liter. Furthermore, the impact of the grind size was examined with a manually prepared coffee using a permanent gold filter. No significant difference in the C-5-HT level was observed for both the coarse and the medium grind size whereas in the brew of the finely ground coffee, the total C-5-HT content was approx. 50% higher. The proportional distribution of the different C-5-HT in the ground coffee is similar to that in the beverages prepared thereof.

INTRODUCTION

Green coffee beans contain 0.2-0.3% coffee wax, which is located in the outermost part of the beans. The main constituents of coffee wax are the so called carboxylic acid-5-hydroxytryptamides (C-5-HT).

Wurziger and Harms (1968) were the first to identify arachidic-, behenic- and lignoceric acid-5HT in coffee wax. Later on, stearic acid-5-HT as well as 20-hydroxy-arachidic- and 22-hydroxy-behenic acid-5-HT were described by Folstar et al. (1980). Kurzrock et al. (2005) introduced two carboxylic acid-5-HT with the odd-numbered fatty acids henicosanoic and tricosanoic acid. Among the unsaturated octadecadienoic and eicosenoic acid-5-HT, palmitic and eicosenoic acid-5-HT were identified by Hinkel and Speer (2005).

C-5-HT are supposed to cause stomach irritation and to have physiological effects on the liver and the bile. Furthermore, due to the ulcerogenic potential, there is a rising interest in the concentration of these substances in coffee and especially in coffee beverages. According to van der Stegen (1979), coffee brew contains up to 2.3 mg C-5-HT per liter when the beverage is prepared by boiling the coffee powder and water together while in filtered coffee no C-5-HT could be detected. However, there is no information about C-5-HT contents, neither for other preparation methods nor the C-5-HT profile in coffee brews.

In this paper, the C-5-HT contents of coffee brews prepared by using different methods and coffee makers are presented.

EXPERIMENTAL

Roasted coffee and instant coffee

The quantitative analysis of the carboxylic acid-5-hydroxytryptamides is based on published literature (Hinkel and Speer, 2006). Briefly, 5 g ground coffee were extracted with tert.-butyl methyl ether by means of accelerated solvent extraction (ASE). An aliquot was cleaned-up by automated solid phase extraction. The subsequent HPLC analysis was carried out with fluorescence detection.

The roasted coffee and the commercially purchased instant coffees were identified by our work group as pure Arabica coffee using the DIN method No. 10779.

Preparation of coffee brews

Commercial roasted Arabica coffee was ground with an industrial centrifugal mill (VTA6S, Stahwert Mühlenbau, Germany). The particle size distribution was determined using the sieve shaker (AS 200 tap, Retsch, Germany) and sieves (90-1000 µm).

To ensure comparable conditions, the coffee powder with a medium particle size and de-ionized water was used to prepare the coffee brews. The water-coffee-ratio was kept at 50 g per liter for coffee beverages and 150 g per liter for espresso coffee, respectively.

Beverages without filtration (boiled coffee) were prepared in a conventional manner. For Scandinavian brews, ground coffee was mixed with hot water and boiled gently for 10 min. After three minutes, the settled brew was decanted. Turkish coffee was brewed with cold water and boiled three times before it was decanted. A gastronomic espresso coffee maker (LaCimbali M39 DT4) was used to prepare a typical espresso.

Coffee brews with filtration were prepared in several ways: by using a commercial coffee machine with a paper filter, filtering through a permanent gold filter manually, or by using a French Press (Bodum®-Plunger).

To determine the C-5-HT content, all beverages were lyophilized. Afterwards, the dry residues were extracted and analyzed as described above. All experiments were carried out in triplicate.

RESULTS

Regarding the particle size distribution the three different charges of ground coffee was analyzed. Obviously, there were only slight differences between fine and medium ground coffee. The main particle size was between 315 and 500 µm. In contrast, coarsely ground coffee consisted primarily of particles larger than 700 µm (Figure 1).

The impact of the grind size was studied on a manually dripped coffee using a permanent gold filter (Figure 2). When comparing the brews made of coarse and medium grind size, no significant difference was observed. Regarding the finely powdered coffee, the total C-5-HT content was approx. 50% higher.

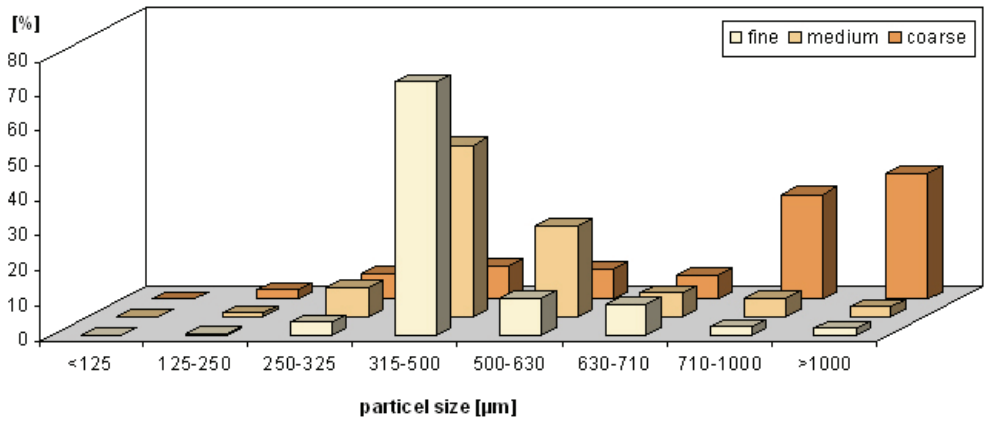

Figure 1. Particle size distribution of ground coffee.

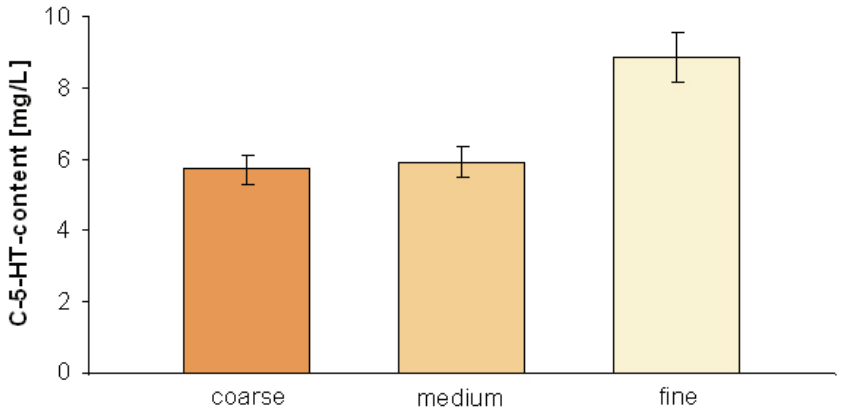

Figure 2. Influence of the grind size on the C-5-HT content (n = 3; ± SD).

Concerning the total C-5-HT content, the different coffee preparations showed strong distinctions. Whereas Scandinavian and Turkish coffee led to amounts of up to 14 mg per liter, the Espressi showed values of about 1 mg per liter. In filtered coffee, the C-5-HT content ranged between 0.2 and 6 mg per liter.

The examined instant coffees show C-5-HT amounts similar to beverages made with paper filters and the Espressi (Figure 3).

The higher C-5-HT levels in Turkish and Scandinavian coffee resulted from the water temperature and the extraction time. During preparation, the coffee powder was extracted with boiling water for five to ten minutes. Although high pressure and steam was used for espresso coffee preparation, the C-5-HT content in the brew was approx. 10 times lower.

The usage of metal-screen filters, for example in a French Press, constricted the passing of the C-5-HT into the coffee brew. In comparison with manual filtered coffee, the levels in beverages prepared with paper filters were considerably lower. Probably the C-5-HT were adsorbed to the filter paper during filtration.

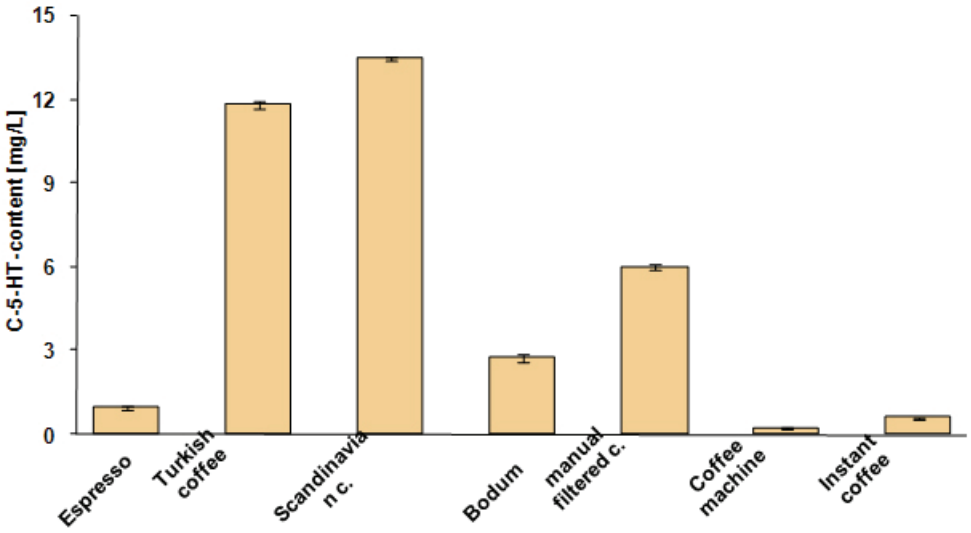

Figure 3. C-5-HT concentration in coffee brews (n = 3; ± SD)

These results are in accordance with the lipid contents in coffee brews (Sehat et al., 1993).

Moreover, the C-5-HT profiles were determined. The main constituents were amides with saturated fatty acids. Within this group, arachidic-, behenic- and lignoceric acid-5-HT represent about 90%. The minor groups consisted of 5-HT with unsaturated and hydroxy fatty acids. The C-5-HT distribution of the ground coffee was compared with the profile of the obtained coffee brew. Exemplarily the results for Turkish coffee are shown in Figure 4. No significant difference was observed, neither for the C-5-HT with saturated fatty acids nor the more polar ones with hydroxy fatty acids. The same profiles were obtained for all other coffee beverages.

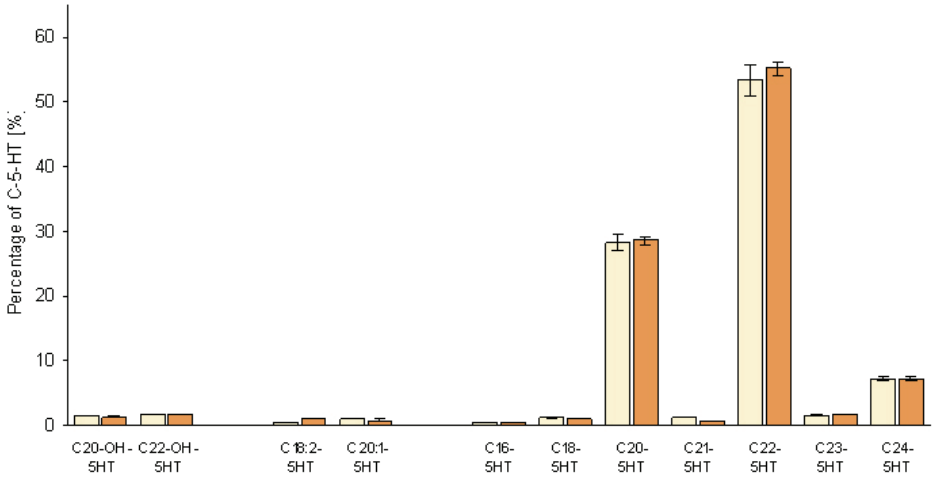

Figure 4. Percentage of C-5-HT in roasted Arabica coffee (dark) and Turkish coffee brew (light) n = 3 ± SD

CONCLUSIONS

The C-5-HT content in coffee brews is basically influenced by the preparation method. Whereas boiled coffees have amounts of up to 14 mg/L, paper filtered coffees have a total of C-5-HT contents lower than 1 mg/L. The impact of the grind size is marginal, only while

using finely powdered coffee a significantly higher C-5-HT level was observed. Ground and fresh brewed coffee show similar C-5-HT distibutions.

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Degradation Products of Carboxylic Acid-5-Hydroxytryptamides (C-5-HT)

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SUMMARY

Green coffee beans are covered by a thin waxy layer. The main constituents of the coffee wax are the so called carboxylic acid-5-hydroxytryptamides (C-5-HT). The amides of serotonin and different fatty acids were first introduced by Wurziger (Harms and Wurziger, 1968). Besides hydroxy fatty acids, several saturated, unsaturated and even odd-numbered fatty acids were identified (Folstar et al., 1980; Kurzrock et al., 2005; Hinkel and Speer, 2005). During the roasting process the C-5-HT levels are reduced to approximately 70% (van der Stegen, 1979; Hinkel and Speer, 2006). Numerous potential degradation products were suggested by Viani and Horman (1975). On the basis of heating experiments, it will be demonstrated that under roasting conditions C-5-HT are first decomposed to serotonin and then to 5-hydroxyindole, 3-methyl-5-hydroxyindole and 6-hydroxyquinoline.

INTRODUCTION

Green coffee beans are covered by a thin waxy layer. The main constituents of the coffee wax are so the called carboxylic acid-5-hydroxytryptamides (C-5-HT). The amides of serotonin and different fatty acids were first introduced by Wurziger (Harms and Wurziger, 1968).

Wurziger et al. identified arachidic-, behenic- and lignoceric acid-5HT in coffee wax. Lateron, stearic acid-5-HT as well as 20-hydroxy-arachidic- and 22-hydroxy-behenic acid-5-HT were described by Folstar et al. (1980). Kurzrock et al. (2005) introduced two carboxylic acid-5-HT with the odd-numbered fatty acids henicosanoic and tricosanoic acid. Among the unsaturated octadecadienoic and eicosenoic acid-5-HT, palmitic and eicosenoic acid-5-HT were identified by Hinkel and Speer (2005).

Roasting conditions led to a thermally induced decomposition of carboxylic acid-5-hydroxytryptamides (van der Stegen, 1979; Hinkel and Speer, 2006). Viani and Horman (1975) proposed several degradation products and pathways of formation. In order to investigate the degradation of C-5-HT during coffee roasting, heating experiments with pure palmitic acid-5-hydroxytryptamid and serotonin were performed.

EXPERIMENTAL

Roasting experiments

The roasting was carried out with palmitic acid-5-hydroxytryptamid (C_{16}-5-HT) and serotonin (5-HT) in glass vessels at different temperatures and different periods of time. After roasting, process samples were dissolved and used for GC-MS and LC-MS analysis. Serotonin and C-5-HT concentration were analysed with an HPLC-system and fluorescence detection.

RESULTS

The impact of different roasting temperatures on the decomposition of C_{16}-5-HT is shown in Figure 1. Along with the increasing thermal load, the C-5-HT level is reduced. Especially temperatures above 200 °C led to rapid degradation.

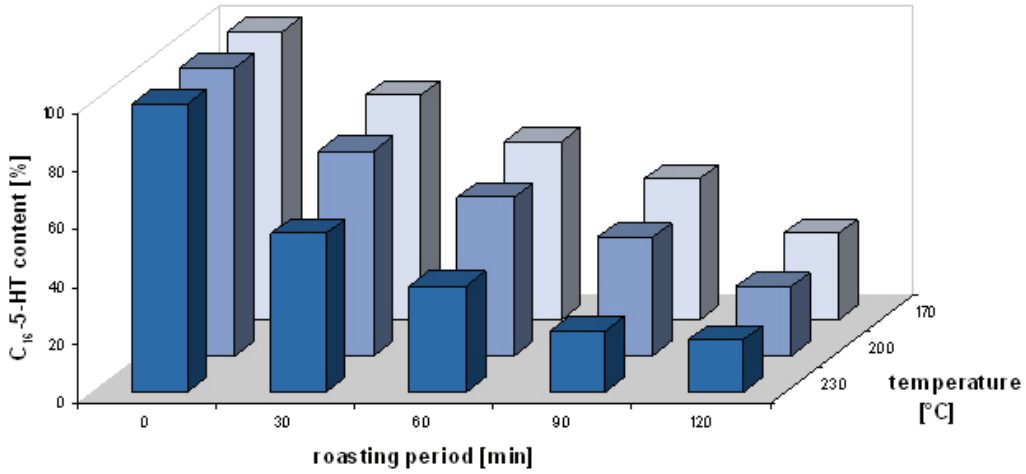

Figure 1. Impact of temperature on decomposition of C_{16}-5-HT.

At mild roasting conditions C_{16}-5-HT was decomposed to serotonin and several very small degradation products (Figure 2). This result is in line with the increase in serotonin levels during roasting (Casal et al., 2004). In contrast to Casal et al. (2004), we were able to show that the increase in serotonin levels is caused by the degradation of C-5-HT.

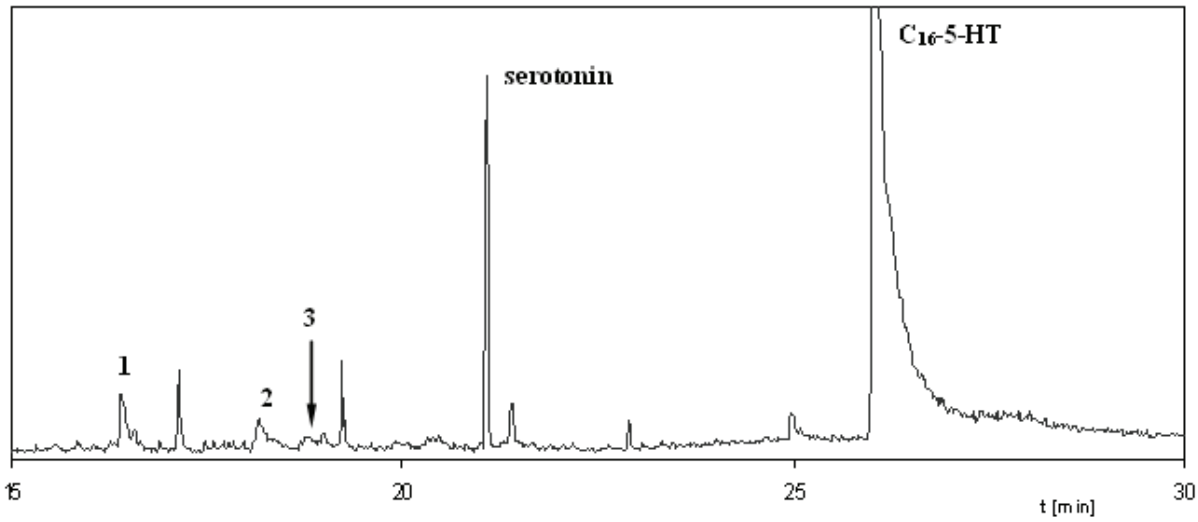

Figure 2. Degradation products of C_{16}-5-HT (1: 5-hydroxyindol, 2: 5-hydroxy-3-methylindole, 3: 6-hydroxyquinoline).

On this account serotonin was examined in further roasting experiments. After 60 min of heat treatment at 240 °C, only approx. 30% of the serotonin was degraded. In accordance to the decomposition of C_{16}-5-HT, the degradation products were identified as 5-hydroxyindole, 5-hydroxy-3-methylindole and 6-hydroxyquinoline by means of LC-MS and GC-MS (Figure 3). These results were proved with HRMS.

In comparison to the C-5-HT levels in green coffee, the serotonin concentration is approx. 200 times lower. Consequently, the degradation products are not caused by the natural serotonin but by the serotonin built up through the degradation of C-5-HT.

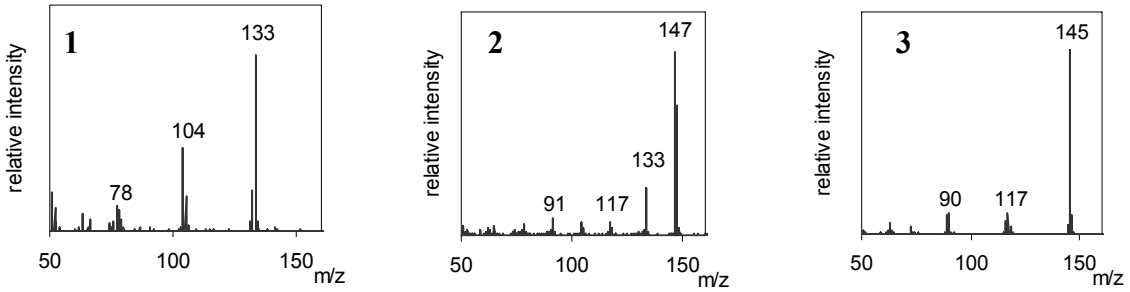

Figure 3. MS spectra 1: 5-hydroxyindol, 2: 5-hydroxy-3-methylindole, 3: 6-hydroxyquinoline.

In Figure 4, an HPLC-chromatogram of roasted Arabica coffee and the identified degradation products are shown. In addition to serotonin, only 5-hydroxy-indole and 5-hydroxy-3-methylindole could be detected. Quantification data will soon be published.

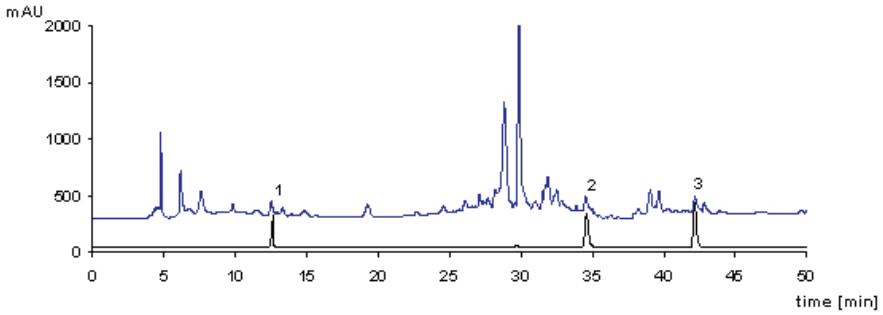

Figure 4. Degradation products in roasted Arabica coffee (1: serotonin, 2: 5-hydroxy-indole, 3: 5-hydroxy-3-methylindole).

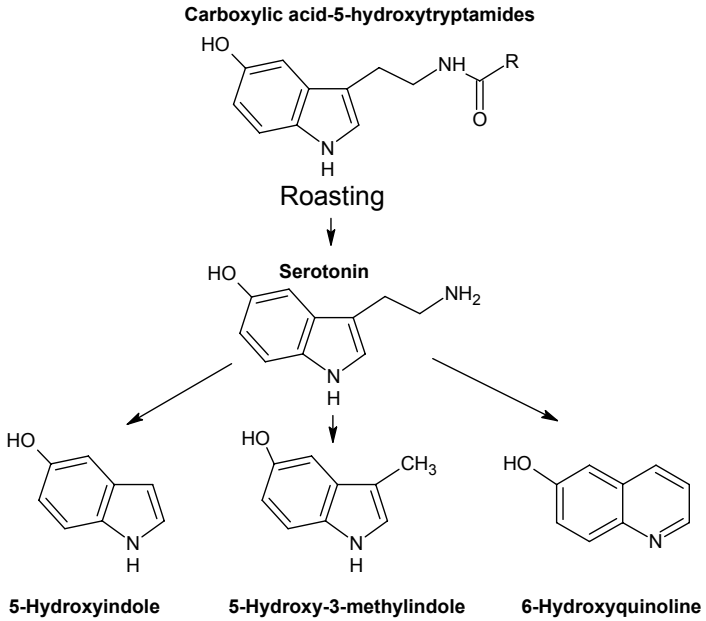

Figure 5. Thermal degradation of C-5-HT.

CONCLUSIONS

Roasting conditions led to a thermally induced decomposition of carboxylic acid-5-hydroxytryptamides. The major degradation product of C-5-HT was identified as serotonin. Model experiments approved 5-hydroxy-indole and 5-hydroxy-3-methyl-indole to be minor roasting products as suggested by Viani and Horman. In addition to the two hydroxyindoles, 6-hydroxychinolin was identified. The thermal treatment of serotonin leads to the same degradation products. Regarding the low serotonin levels in green coffee, the degradation is negligible.

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Diterpene Determination in Coffee using Fast LC-Systems of Different Manufacturers

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SUMMARY

The diterpenes are routinely analysed in our working group allowing for information about the Robusta parts in Arabica mixtures, the roasting degree etc. Due to the rather long chromatographic run-times of 70 min for the DAD-analysis and 15 min for the MS-analysis, we tested two Fast LC-Systems: the *Agilent Rapid Resolution Series 1200* and the *Waters ACQUITY UPLC* in the isocratic mode. The run-time was reduced to 2.5 min using a flow rate of 5 ml/min and 60 °C by Agilent and to 6 min. with 0.5 ml/min and 30 °C by Waters. Therefore, the experiments showed that *Fast LC* is a suitable tool for combining high resolution and highest throughput in diterpene analysis.

INTRODUCTION

The determination of the pentacyclic coffee diterpenes is important from many points of view: 16-O-methylcafestol (16-OMC) is the characteristic indicator substance for Robusta coffee in mixtures with Arabicas (Speer et al., 1991; Speer and Kölling-Speer, 2001). The cafestol/dehydrocafestol ratio allows for information about the roasting degree whereas cafestol and kahweol cause physiological effects (Speer and Kölling-Speer, 2001; 2006). The analysis is usually performed by using DIN method 10779 with the separation of the diterpenes by HPLC on a C_{18} column with an acetonitrile-water mixture (50:50) (DIN 10779, 1999).

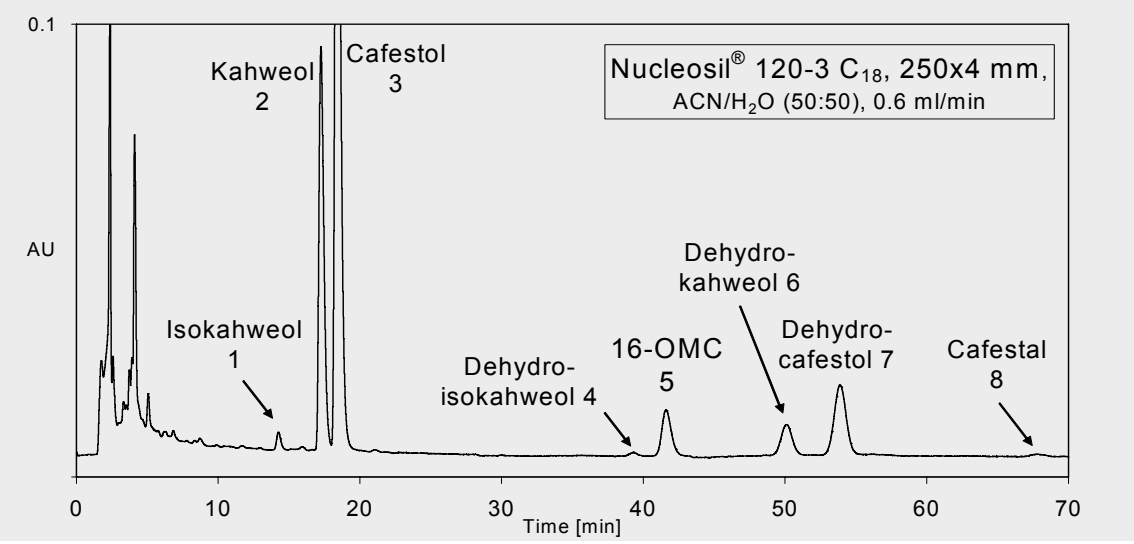

Figure 1. HPLC chromatogram of an Arabica/Robusta coffee mixture, UV 224 nm.

In our working group we generally use a Nucleosil®120-3 C_{18} column (Macherey&Nagel) (Figure 1) which separates cafestol and kahweol, solely differing in a double bond, nearly

down to base line. However, the run-time of 70 min in the isocratic mode is quite long. Using LC-MS, we separate the diterpenes with a Chromolith® column (VWR) and a flow rate of 1 ml/min within 15 min (Kölling-Speer, 2006).

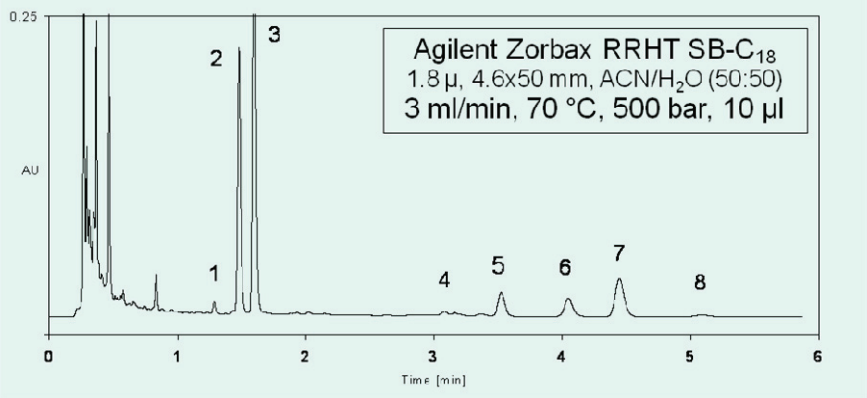

Figure 2. HPLC chromatograms of the diterpenes in an Arabica/Robusta coffee mixture.

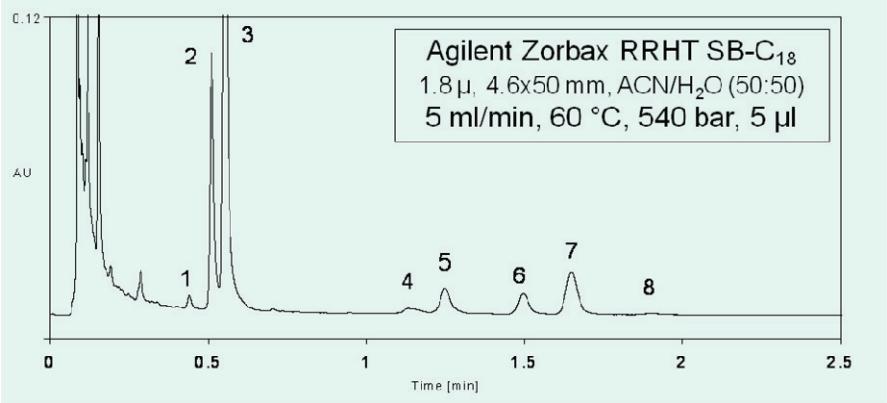

Figure 3. HPLC chromatograms of the diterpenes in an Arabica/Robusta coffee mixture.

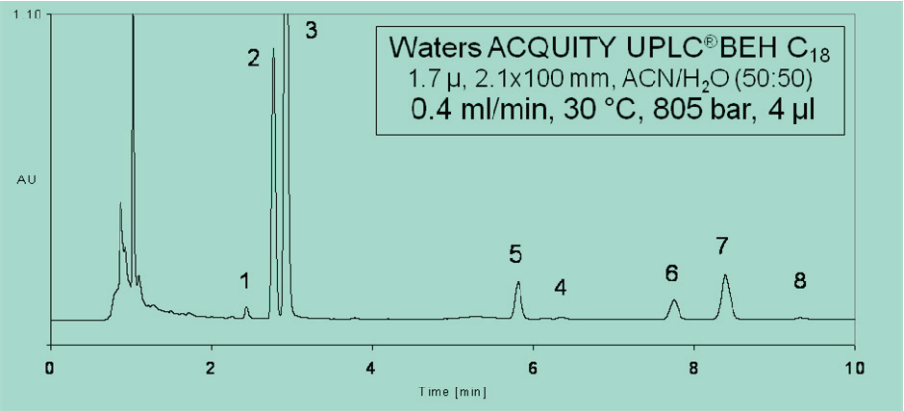

Figure 4. HPLC chromatograms of the diterpenes in an Arabica/Robusta coffee mixture.

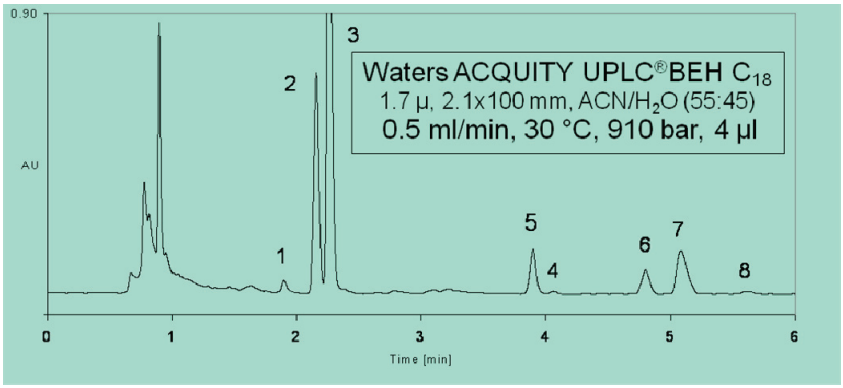

Figure 5. HPLC chromatograms of the diterpenes in an Arabica/Robusta coffee mixture.

Recently, several manufacturers have offered *Fast LC*-systems, using column material of much smaller particle sizes resulting in higher efficiency yet, also in higher backpressure. Two of these systems were tested for the diterpene analysis in the isocratic mode.

METHODS

In each case, the same sample (Arabica/Robusta coffee mixture) prepared according DIN 10779 was injected. The chromatograms are displayed at 224 nm.

RESULTS AND DISCUSSION

The **Agilent Rapid Resolution** system (equipped with an autosampler, a binary pump, a diode array detector and a column thermostat of the series 1200 SL) uses high flow rates of 3 and 5 ml/min and achieves a good separation of cafestol and kahweol even with a short column (50 mm). However, for keeping the system backpressure within limits, higher temperatures are necessary, which may possibly shorten column lifetime. The chromatographic run-times are sensationally fast at 2.5 min (Figure 3), although with a somewhat poorer resolution between the limiting peak pair than at 5.5 min. (Figure 2). Noticeably lower solvent consumption (flow rate 0.4 or 0.5 ml/min) and a noticeably lower temperature are applicable for the **Waters ACQUITY UPLC** system consisting of the same components. The run-times are a bit longer at 6 min. and 10 min. (Figuree 4 and 5); the peak resolution is nearly as good as with the Agilent experiment at 5.5 min. The particle sizes of the two manufacturers with 1.8 µ and 1.7 µ are almost the same. Practical experience will show how robust and long-living these special columns will be, provided there is careful sample filtration.

These experiments showed that *Fast LC* is a suitable tool for combining high resolution and highest throughput in diterpene analysis.

ACKNOWLEDGEMENT

We would like to thank **Agilent Technologies** and **Waters**.

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Effect of the Water Feeding Onto the Brew and the Filter Cake Using a Household Coffee Maker

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SUMMARY

The household coffee maker "Melitta Look de Luxe" allows the consumer to prepare a mild or a strong coffee brew by feeding the water through numerous small jets or through one main jet. The effects on the beverage and on the composition of the filter cake were exemplarily determined by analysing the lipophilic component cafestol and the water soluble component caffeine. Regarding the strong brew, the caffeine content was about 10 % higher than in the mild brew whereas the cafestol content with 1.8 mg/l was twice as high as in the mild brew with 0.9 mg/l. The concentrations of cafestol and caffeine in various regions of the filter cakes were also divergent depending on the regulated water feeding.

INTRODUCTION

Nowadays, the consumer has the option to select a household coffee maker from a large number of machines by different manufacturers. However, only a few of them permit feeding the water individually, such as the "Melitta Look de Luxe" with its two different settings for preparing either a mild or a strong brew or any desired brew in-between.

In our study, the effects of these two different water feedings on the beverages were investigated. In addition to the water-soluble component caffeine, cafestol as a related component of the lipid fraction with physiological properties was analysed. Furthermore, samples were taken from various parts of the moist filter cake as well as from the surface of the filter paper.

Figure 1. Water feeding by the coffee maker "Melitta Look de Luxe".

METHODS

Coffee brews were prepared by the "Melitta Look de Luxe" with the two extreme settings "mild" and "strong". All the brews were made by using 50.0 g of a commercial roasted coffee powder, 1,100 ml water (water hardness 6.5 °dH) and employing the "Melitta Original 1 x 4" paper filter. Samples were taken from various regions of the moist filter cake (Figure 3), air-dried and analysed. The determination of caffeine was carried out by applying DIN method No. 10777/2 (2000) and cafestol by DIN method No. 10779 (1999). The freeze-dried brews were saponified directly.

RESULTS

Between the mild and the strong preparations, significant effects became clear: In the case of the mild brew, the water feeding is carried out through a number of small jets (Figure 1), resulting in a filter cake with an almost plain surface (Figure 2). For the strong brew, a deep crater was produced by the water feeding through one main jet. The preparation time for the two brews was nearly the same.

As expected, the mild brew has a very gentle and light taste in contrast to the strong brew with its intensive and slightly bitter taste.

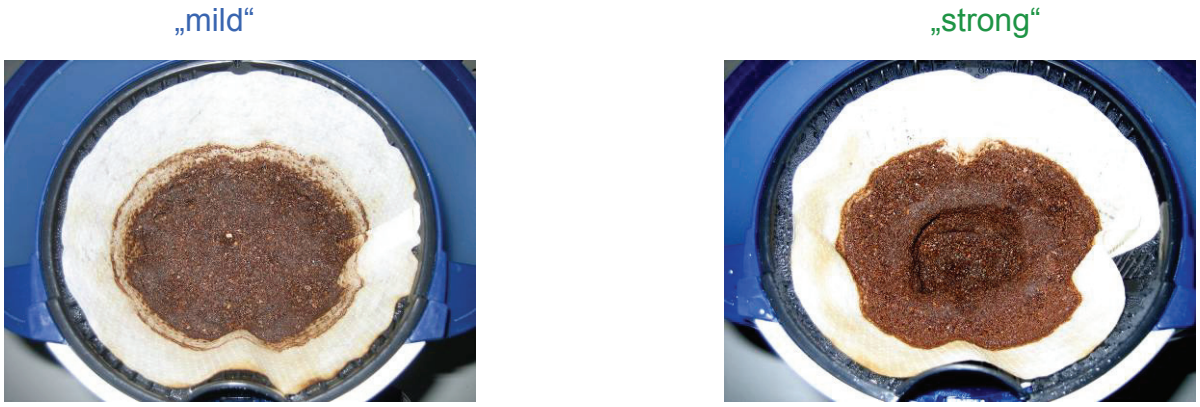

Figure 2. Filter cakes after brewing.

The effects of both preparations onto the composition of the brew and in the remaining filter cake were studied exemplarily by analysing cafestol as an essential part of the lipid fraction and caffeine as a water-soluble component of the roasted coffee powder.

Cafestol

The roasted coffee contained 4.42 g/kg cafestol. In accordance with results presented by Speer et al. (1993), Urgert et al. (1995) and Kurzrock and Speer (2001) only a small amount of cafestol was able to pass through the paper filter. However, the difference between the two water feedings was enormous. The cafestol content in the strong brew was 1.8 mg/l and therefore twice as high as in the mild brew with 0.9 mg/l.

As expected for a lipid compound, cafestol accumulated on the surface and in the lower regions of the filter cake, respectively (Figure 3).

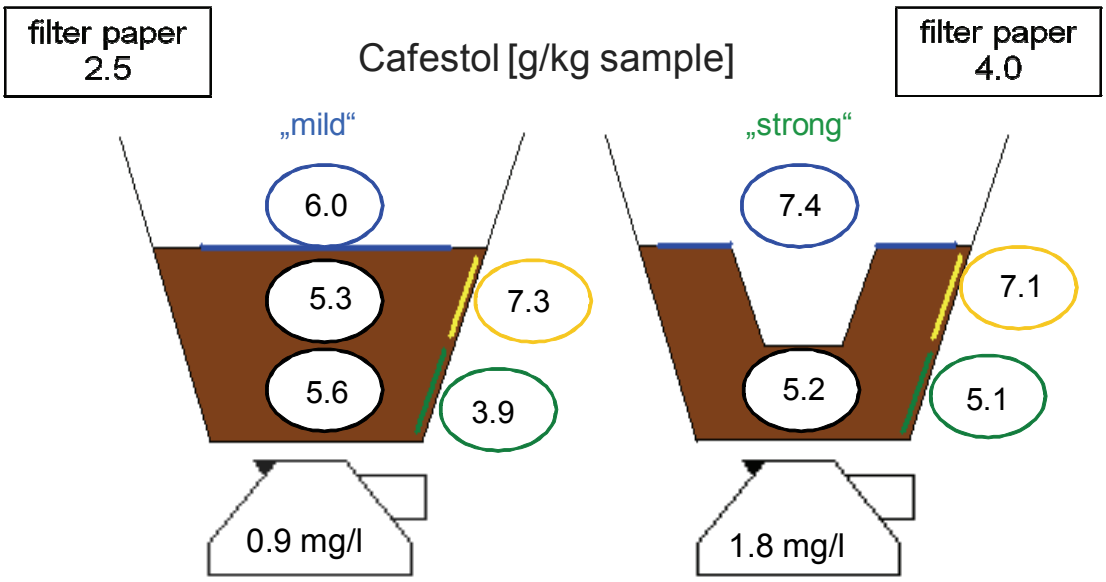

Figure 3. Contents of cafestol in the brews, filter cakes and filter papers.

Caffeine

Caffeine was determined in higher concentrations only in the lower region of the filter cakes. Almost all of the caffeine was washed out from the ground roasted coffee powder: In the strong brew, 90% were extracted whereas in the mild brew approx. 82% could be analysed (Figure 4).

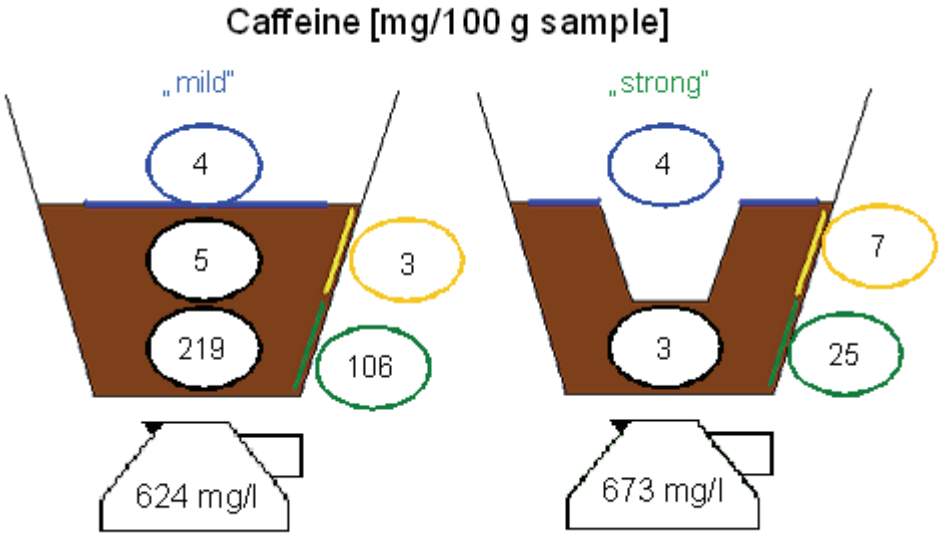

Figure 4. Contents of caffeine in the brews and filter cakes.

CONCLUSIONS

Our results revealed that the chosen way of the water feeding affects the taste and the composition of the brew and of the filter cake.

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Secokahweol – A New Diterpene Degradation Product in Roasted Coffee

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SUMMARY

During the roasting process, the diterpenes cafestol and kahweol are decomposed to several degradation products: dehydrocafestol and dehydrokahweol, cafestal and kahweal as well as isokahweol and dehydroisokahweol. With secokahweol, another peak was now elucidated in roasted Arabica coffee, intensifiying with increasing roasting degree. The molecular mass was determined to 314.1901, and the empirical formula was calculated as $C_{20}H_{26}O_3$. The IUPAC-name for this substance is 6-(hydroxymethyl)-1-[2-(5-methyl-1-benzofuran-4-yl) ethyl] bicycle [3.2.1] octan-6-ol.

INTRODUCTION

The pentacyclic diterpenes of *Coffea arabica* and *Coffea canephora var. Robusta* are well known: Arabica coffee beans contain the diterpenes cafestol and kahweol whereas Robusta coffee beans contain cafestol, small amounts of kahweol and, in addition, 16-O-methylcafestol and 16-O-methylkahweol (Speer and Kölling-Speer, 2006; Kölling-Speer and Speer, 2006).

During the roasting process, cafestol is decomposed to dehydrocafestol and cafestal whereas the degradation products of kahweol are dehydrokahweol, kahweal and, furthermore, isokahweol and dehydroisokahweol. All of these degradation products were identified by our work group for the first time a few years ago (Tewis et al., 1993; Speer et al., 2000; Kölling-Speer et al., 2005).

While analysing various commercial roasted coffees on containing Robusta parts by using DIN method No. 10779 (1999), a further additional peak was discovered in the HPLC chromatograms (Peak ? in Figure 1). It was clearly noticeable in Arabica coffees of a higher roasting degree, which was determined via the cafestol/dehydrocafestol ratio (Kölling-Speer and Speer, 1997).

METHODS

Using semi-preparative HPLC, the peak was isolated and subsequently elucidated by means of GC-MS, high-resolution mass spectrometry and different NMR and 2D-NMR experiments.

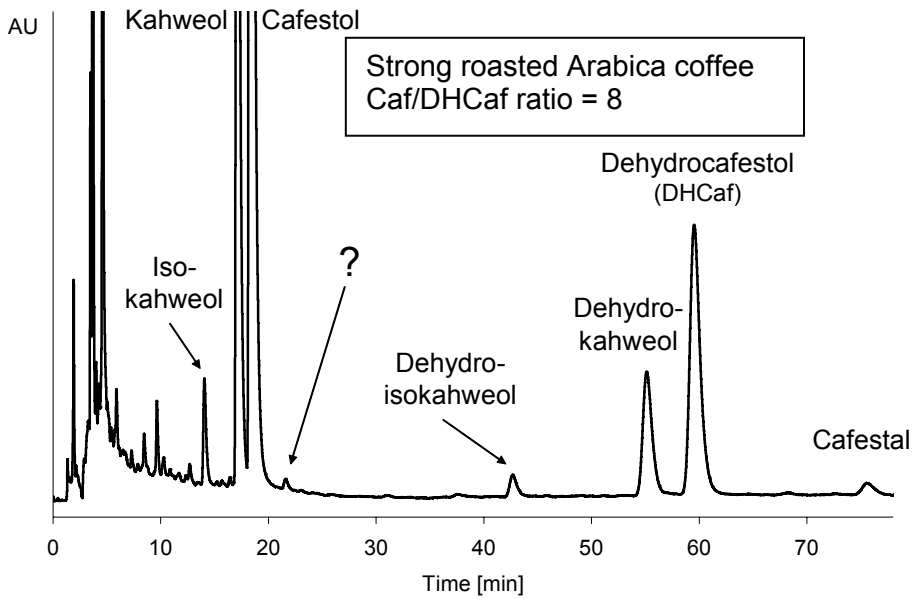

Figure 1. HPLC chromatogram of a commercial strong roasted Arabica coffee.

RESULTS

We named the new diterpene **secokahweol** (Figure 2)**,** due to its apparent derivation from kahweol and due to the cleavage of the ring. The compound had been unknown up to now.

The elucidation is presented in the following.

The molecular mass was determined to 314.1901, and the empirical formula was calculated as $C_{20}H_{26}O_3$. Therefore, secokahweol has the same molecular mass and formula as kahweol, however the mass spectra are clearly different (Figure 3).

Structural information in detail is given from the ^{1}H-NMR (Figure 4) and, in particular, from the 2D-NMR experiments below.

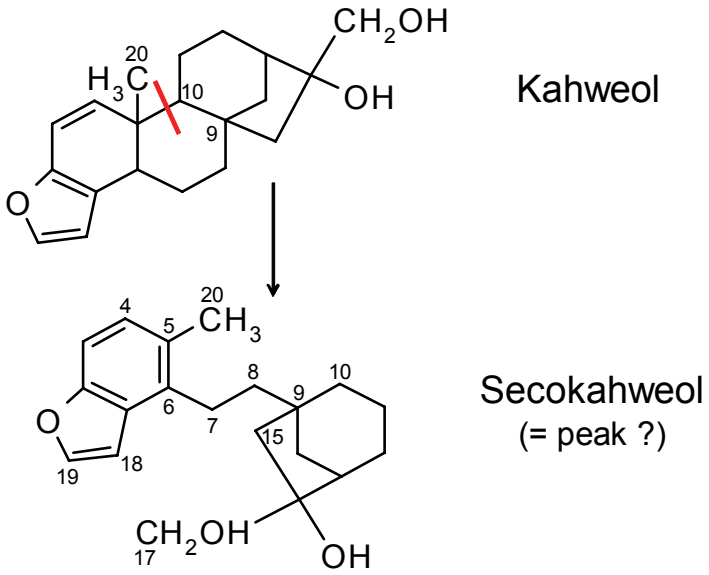

Figure 2. Structural formulae of kahweol and secokahweol.

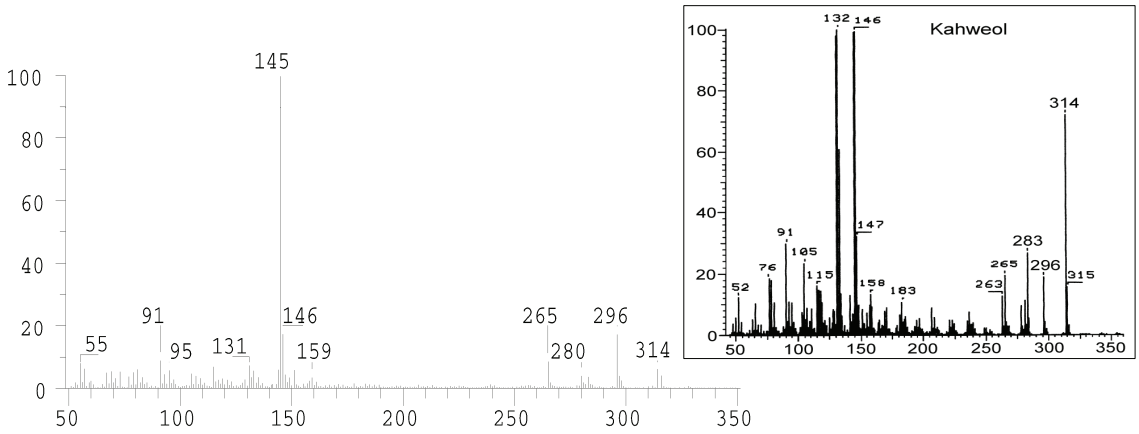

Figure 3. EI-mass spectra of secokahweol and kahweol.

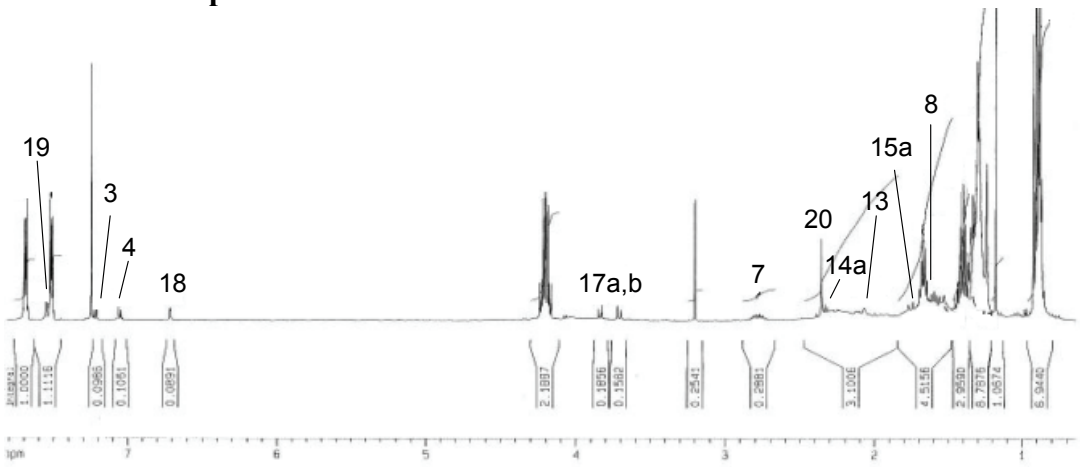

Figure 4. ^{1}H-NMR spectrum of secokahweol.

The 2D-NMR experiment NOESY (Figure 5) shows the correlations between H-7/H-20, H-7/H-18 und H-8/H-18, which prove the ring opening between C-5 and C-10.

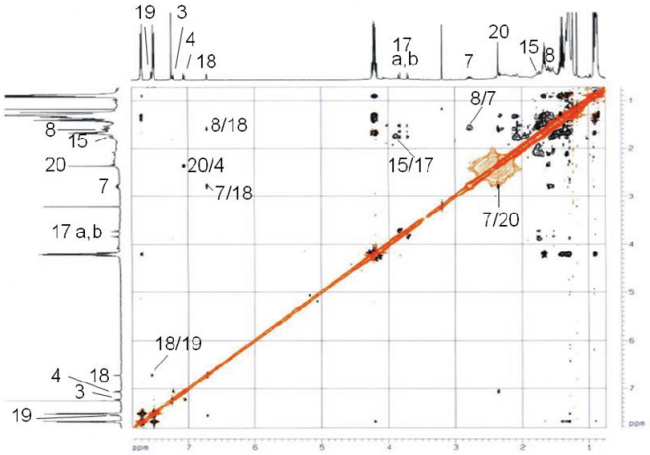

Figure 5. NOESY spectrum of secokahweol.

In addition, the cross signals in the HMBC experiment (Figure 6) between the CH_2-protons of H-7 and the C-1, C-5 und C-6-atoms as well as between the H-20 protons of the CH_3 group and the C-5 and C-6 atoms are only explicable with the ring opening.

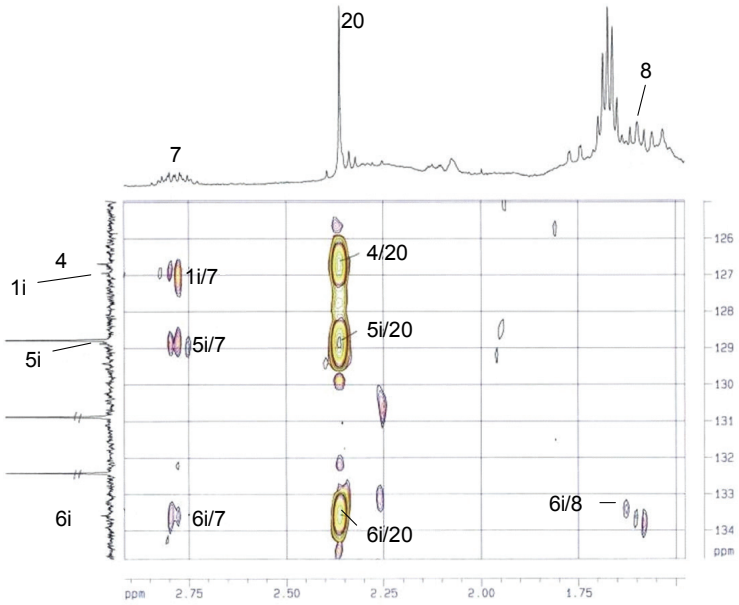

Figure 6. Cut-out of HMBC spectrum of secokahweol.

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We would like to thank Dr. Machill for acquiring the high-resolution mass spectrum.

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Effect of the Fluid Flow Speed Changes on the Chemical Composition of Coffee Samples Roasted in an Industrial Semi-Fluidized Bed Roaster

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SUMMARY

In this study, we roasted commercial blends in an industrial fluidized bed roaster at different fluid flow speeds and compared the composition of important chemical compounds responsible for coffee flavor and bioactivity. The faster the speed, the higher were the values of soluble solids and acidity in the brew and of trigonelline and chlorogenic acids in ground-roast coffee. In poor quality samples, the content of most volatile compounds was also directly correlated to speed, while in the good quality sample the inverse behavior occurred.

INTRODUCTION

Roasting is a very complex process in which many chemical reactions and physical changes in coffee seeds take place. The way roasting is performed will reflect on the quality of the beverage (Clarke and Macrae, 1985). This process involves various parameters such as chamber temperature, fluid flow speed and physical-chemical properties that will influence the temperature distribution in the beans.

There are many roasting techniques, but the basic principle of one of the most moderns roasting technologies, semi-fluidized bed or rotating cylinder, is that a forced convective flow of hot gases passes through a bed of coffee beans moved by mechanical rotation. Fluidized type bed roasters produce a significantly faster increase in bean temperature in comparison with traditional roasters. Data on the effect of the fluid flow speed changes on the chemical composition of coffee roasted in fluidized bed type roasters are scarce.

The aim of this study was to compare the composition of important chemical compounds and attributes responsible for coffee flavor and bioactivity, in seeds roasted in an industrial fluidized bed roaster, at different fluid flow speeds.

MATERIAL AND METHODS

Samples

Three sets of Brazilian commercial arabica blends with 4% (Blend 1), 50% (Blend 2) and 73% (Blend 3) of defective beans mixed with good cup quality beans were used. 480 kg of each sample were roasted separately to give medium roasting degree (Roast Color Classification System – Agtron, SCAA, USA) in an OPUS 500 Third Generation roaster (Cia. LILLA, Brazil), at three different fluid flow speeds: fast (4 min), medium (8 min), slow (15

min) and to reach a certain bean temperature which was electronically monitored. Samples were ground in a laboratory scale roaster to pass a 500 μm sieve.

Analysis

Trigonelline, chlorogenic acids (caffeoylquinic acids-CQA and feruloylquinic acids-FQA), volatile compounds, carbohydrates, lipids and water contents were determined in ground-roast coffees according to Farah et al. (2006), Toci et al. (2006), and Toci and Farah (2008); titrable acidity, pH and soluble solids were determined in the brew according to AOAC (n° 15010 and 15034, 1984).

Data elaboration

Results were considered significant when $p \leq 0,05$. The influence of variable speed flow and quality of blends were evaluated by response surface methodology (RSM) (Statistica®, version 8.0, USA).

RESULTS AND DISCUSSION

The results for non-volatile parameters are showed in Table 1. Statistical analysis revealed that fluid flow speed, quality of the blend, and the interactions between the two variables produced significant changes in the evaluated chemical parameters) of ground roast coffee, except for soluble solids.

Table 1. Non-volatile chemical composition of coffee blends roasted at different roasting speeds.

	fluid flow speed	blend 1	blend 2	blend 3		fluid flow speed	blend 1	blend 2	blend 3
Water contents g/100g	raw	9.68	9.57	9.6	Clhorogenic acid g/100g	raw	7.6	10.06	9.47
	slow roast	2.51	3.57	2.53		slow roast	0.97	0.7	1.6
	medium roast	2.58	3.87	2.85		medium roast	2.04	1.17	2.25
	fast roast	2.46	2.65	2.48		fast roast	3.00	1.99	2.94
Acidity NaOH 0,01N/g	raw	38.72	48.43	33.09	Trigonelline g/100g	raw	1.38	1.23	2.1
	slow roast	32.77	28.18	24.98		slow roast	0.28	0.14	0.25
	medium roast	40.71	28.65	28.34		medium roast	1.23	0.33	0.36
	fast roast	43.65	32.23	35.59		fast roast	0.98	0.27	0.51
pH	raw	5.71	5.81	5.87	Lipids g/100g	raw	12.91	12.72	14.75
	slow roast	5.38	6.13	5.48		slow roast	14.07	16.62	14.83
	medium roast	5.07	5.85	5.14		medium roast	15.77	15.33	15.29
	fast roast	4.93	5.63	5.09		fast roast	14.63	14.14	15.52
Soluble solids g/100g	raw	35.18	37.77	38.59	Carbohydrates g/100g	raw	86.42	86.89	83.2
	slow roast	26.95	24.72	26.45		slow roast	85.69	55.4	63.66
	medium roast	28.05	30.04	28.59		medium roast	88.62	67.53	62.42
	fast roast	29.7	33.75	31.42		fast roast	89.2	54.21	62.84

The lowest water content was found at the fast speed, which is in agreement with the literature (Baggenstoss et al., 2008). No statistical difference was found between slow and medium speed results.

The faster the speed, the higher were the values of acidity of the coffee beverage, evaluated by pH and titrable acidity.

For contents of trigonelline and chlorogenic acid (3-CQA, 4-CQA, 5-CQA, 4-FQA and 5-FQA, representing 90% of total chlorogenic acids), the faster the speed, the higher was their amount in ground roast coffee. Fast roasting is reported to produce lower 5-CQA losses (Nagaraju et al., 1997). So we can assume that the roasting speed may influence the bioactivity of the beverage.

The faster the speed, the higher were the values for soluble solids, which is in agreement with Nagaraju et al. (1997). One of the possible reasons is that since sucrose is responsible for the production of a considerable amount of soluble solids during roasting (Fito et al., 2007), it would be reasonable to assume that increasing of fluid flow speed is not sufficient to promote reactions in which sucrose participates during pyrolysis, and, therefore, minimizing losses.

No association was observed between the roasting speed, blends and the content of carbohydrates and lipids.

Thirty seven major peaks of volatile compounds were evaluated. Two different behaviors were observed upon roasting. In blend 1, which was classified as good cup quality sample, the higher the speed, the lower was the intensity of most volatile compounds. Examples were 2-methylpyrazine, 2,3,5-trimethylpyrazine, 1H-pyrrole and 2-furfurylmethanol. In blends 2 and 3, the faster the speed, the higher was the intensity of the compounds. It is known that the addition of defects (PVA) to good quality seeds leads to a decrease in the amount of crude fiber in the cell wall, composed primarily of cellulose, lignin and hemicellulose (Coelho and Pereira, 2002). In consequence, it is reasonable to assume that defective seeds will suffer a significantly faster induction of heat from outside to inside, accelerating the roasting process. Therefore, when defective seeds are added to good quality seeds, they tend to accelerate the roasting process.

CONCLUSION

The roasting speed, the quality of the blend, and the interactions between the two variables produced significant changes in the results. The faster the speed, the higher were the values of soluble solids and acidity in the brew and of trigonelline and chlorogenic acids in ground roast coffee. In poor quality samples, the content of most volatile compounds was also directly correlated to speed, while in the good quality sample the inverse behavior occurred. The implications of the chemical changes produced by different roasting speeds on the sensorial characteristics of the final beverage should be investigated

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Tryacylglycerols Changes During the Storage of Roasted Coffee

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SUMMARY

Lipids are major components of coffee, consisting of approximately 10% (w/w) of roasted coffee seeds. These components can contribute to loss of coffee quality during storage, especially through oxidation and hydrolysis. The aims of this study were to evaluate the changes in triacylglicerols (TAG) and free fatty acids (FFA) composition in roasted coffee during storage, and their dependence on storage temperature and atmosphere. The present results confirmed the occurrence of hydrolysis of the TAG fraction during storage of roasted coffee. The variables atmosphere and temperature did not influence these changes. The implications of hydrolysis and oxidation of TAG and FFA fractions on volatile compounds and sensorial characteristics of the final beverage should be investigated.

INTRODUCTION

Coffee roasting induces several chemical transformations, which result in alterations in the coffee chemical composition. After roasting, especially during storage, coffee undergoes important chemical and physical changes that greatly affect the quality and the acceptability of the brew. Many of these changes are considered almost unavoidable but may still be prevented. The rate at which these changes occur depends mostly on factors related to the environment and to technological processes, such as availability of oxygen and moisture, exposed surface area, temperature, as well as package material (Fourny et al., 1982).

Lipids are major components of coffee and correspond to approximately 10% (w/w) of roasted coffee composition. They can contribute to loss of coffee quality occurring during storage, especially through oxidation and hydrolysis. Triacylglycerols (TAG) are the main lipid class in coffee and account for approximately 75% (w/w) of total coffee lipids in freshly brewed coffee, whereas free fatty acids (FFA) account for only about 1% (Trugo, 2003). There are few studies reporting changes in the composition of FFA in roasted coffee stored under controlled conditions (Vila et al., 2005), and there are no data available concerning the TAG fraction of roasted coffee. Therefore, the aim of this study was to investigate the changes in the fatty acids contained in TAG and FFA fractions contents of these lipid fractions in roasted coffee during storage and to evaluate the influence of storage temperature and atmosphere on these components.

MATERIAL AND METHODS

Samples

Seeds of good cup quality Brazilian *Coffea arabica* classified as “strictly soft” were used. Sample was roasted in a spouted bed roaster (IRoast, Gurnee, IL, USA) at 221 °C, for 5,5

min, to give a light-medium degree according to the Roast color Classification System (AGTRON – SCAA, USA, 1995). All samples were ground to pass a 500 μm.

Storage

The storage of coffee was carried out for four months under controlled conditions of temperature (5 or 30 °C) and atmosphere (ambient air or N_2).

Sample extraction and Analysis

Total lipids in coffee samples were extracted with organic solvents (isopropanol:chloform, 1:1 v/v), by thoroughly mixing with an Ultra Turrax mixer. The lipid fractions of interest (TAG and FFA) were separated in solid phase extraction cartridges (Kaluzny et al., 1985), and methylated (Lepage and Roy, 1986). Following, resulting fatty acid methyl esters were analyzed by capillary gas chromatography (Torres et al., 2006). The quantifications of individual fatty acids and total amounts of TAG and FFA were achieved with quantitative addition of appropriate internal standards (margaric acid for FFA; trinonadecanoate for TAG; Sigma-Aldrich).

Statistical analysis

Results were considered significant when $p \leq 0,05$. The influence of variable temperature, atmosphere and time of storage were evaluated by response surface methodology (RSM), using Statistica®, version 8.0, USA.

RESULTS AND DISCUSSION

The major fatty acids considered in the coffee samples were: palmitic (16:0), stearic (18:0), oleic (18:1n-9), linoleic (18:2n-6), linolenic (18:3n-3), arachidic (20:0), gondoic (20:1n-9) and behenic (22:0) acids, in both the TAG and FFA fractions.

A continuous decrease in TAG content was observed during storage, with losses of 12% after two months and 38% after 4 months of storage. These results, indicate TAG hydrolysis (Figure 1). The content of TAG appeared to have increased during the first month of storage of coffee samples (Figure 1). It is possible that uncontrolled losses of water and/or coffee volatiles caused this apparent gain in lipids. Although the relative air humidity was high (up to 80%) during the experiment, storage atmosphere and temperature did not influence the hydrolysis of TAG and, therefore, it could be hypothesized that the water in the samples (2% w/w), which might favor hydrolysis (Akoh and Min, 2008), was the main responsible for this chemical transformation in coffee during storage.

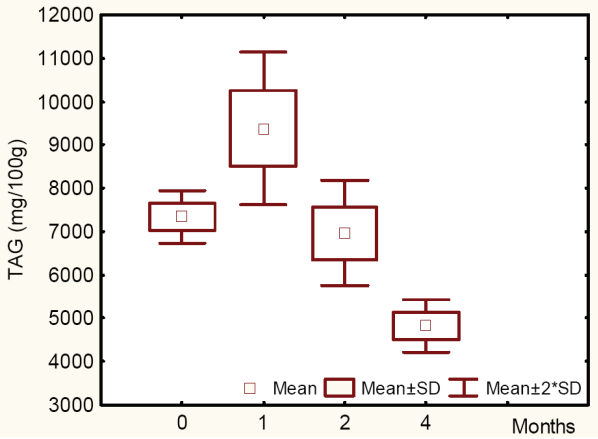

Figure 1. Box-plots analysis with TAG variations during the storage.

The content of FFA increased dramatically during the first month of storage, from very low levels in freshly roasted samples (0.4 mg/100g) to 78-109 mg/100g in the stored samples (Figure 2). Between one and two months of storage FFA contents further increased. These results of increasing FFA levels during the first two months of storage (Figure 2) are consistent with the hypothesis of TAG hydrolysis, and with the decreases observed in the TAG contents after two months of storage (Figure 1). According to the TAG levels, which continue to decrease between two and four months of storage, lipolysis seems to continue up to this period of time. However, FFA contents decreased between two and four months of storage, indicating that other chemical transformations might have affected FFA levels at this storage period. It is possible that at this storage period the rate of loss overcame the rate of FFA production through TAG hydrolysis. Oxidation of FFA could explain the apparent loss of FFA from two months of storage, since this lipid fraction is more susceptible to oxidation than esterified fatty acids in TAG molecules (Akoh and Min, 2008). It is possible that oxidation of FFA was already occurring before two months of storage, because the increase in FFA levels was lower than what was expected given the concomitant decrease in TAG. The atmosphere did not affect the contents of FFA during the whole four months of storage. In contrast, the storage temperature positively affected the amount of FFA at the first month.

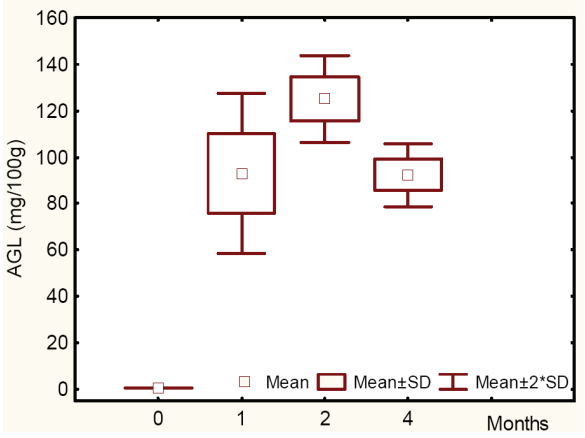

Figure 2. Box-plots analysis with AGL variations during the storage.

When individual fatty acids were considered, their loss seemed to increase with the number of double bonds, as follows. 18:1n-9, 18:2n-6 and 18:3n-3 decreased 23%, 24% and 27%, respectively, between two and four months of storage. These results are consistent to the hypothesis that FFA are at least partially degraded through oxidative reactions, since the

relative rates of oxidation of the unsaturated FA are directly associated to the number of double bonds.

CONCLUSION

The present results confirm the hypothesis of hydrolysis of the TAG fraction during storage of roasted coffee. The variables atmosphere and temperature did not influence these changes. Considering that oxidation of coffee fatty acids could lead to the formation of volatile components that would negatively affect cup quality and acceptability of the beverage, sensorial analyses should be performed in order to investigate this theme.

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Comparison of Chlorogenic Acids Contents in *Coffea arabica*, *Coffea canephora* and Hybrids Resistant to *Meloidogyne exigua*

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SUMMARY

Chlorogenic acids (CGA) are major phenolic constituents of green coffee seeds, corresponding to 5-14% of their composition. A variety of studies have attributed several biological properties to these compounds. Additionally, CGA have a marked influence in cup quality and flavor formation. *Meloidogyne exigua* is a root-knot nematode that represents a major damage to coffee plantations worldwide. Currently, the development of genetically resistant cultivars seems to be the best way of controlling root-knot nematodes. In the present study, CGA profile and contents in *C. arabica*, *C. canephora* and resistant hybrids were compared in order to evaluate the genetic influence on CGA composition.

INTRODUCTION

Chlorogenic acids (CGA) are esters between *trans*-cinnamic acids (mainly caffeic, ferulic and *p*-coumaric acids) and quinic acid. These phenolic compounds are major constituents of green coffee seeds, reaching levels up to 14% on dry matter basis (Farah and Donangelo, 2006). The major classes of CGA are caffeoylquinic (CQA), dicaffeoylquinic (diCQA) and feruloylquinic acids (FQA) (Farah and Donangelo, 2006; Clifford et al., 2006a). The total CGA content may vary according to genetics (species and cultivar), degree of maturation and agricultural practices, among other factors (Farah and Donangelo, 2006). *In vitro*, *in vivo* and epidemiological studies have attributed several biological properties to these compounds, such as antioxidant, hypoglycemic, antiviral and hepatoprotective activities (Farah et al., 2005). Additionally, CGA have a marked influence in cup quality and flavor formation (Farah et al., 2006).

Coffee is one of the most valuable primary products in world trade, being *Coffea arabica* and *Coffea canephora* the two most consumed species (www.i.c.o.org; www.abic.com.br). *C. arabica* is known to produce beverages with higher quality, which is significantly reflected in market value. However, this species is characterized by low genetic diversity, which promotes susceptibility to numerous diseases. On the other hand, *C. canephora* is a stronger plant, resistant to climate adversities, representing a valuable source of disease-resistance genes (Bertrand et al., 2003).

The root-knot nematodes are very common parasites that grow in the roots of coffee trees and represent a major damage to coffee plantations. The most important of these nematodes in Latin America, with wide dissemination in Brazilian plantations, is *Meloidogyne exigua* (Arruda and Reis, 1962). Since nematicide treatments are expensive and may be hazardous

for the environment and human health, the development of genetically resistant cultivars with *C. canephora* progenitors seems to be the best way of controlling root-knot nematodes (Hein and Gatzweiler, 2006).

In the present study, CGA profile and contents in *C. arabica*, *C. canephora* and resistant hybrids were compared in order to evaluate the genetic influence on CGA composition.

MATERIAL AND METHODS

Samples

Twenty coffee samples were analyzed: one *C. arabica* cv. Red Catuaí; one *C. arabica* cv. Rubi; one *C. arabica* cv. Yellow Bourbon; one *C. arabica* cv. Topázio; two *C. arabica* cv. Oeiras; four *C. canephora* cv. Robusta and ten different hybrids of *C. arabica* cv. Yellow Catuaí with Timor Hybrid. Arabica cultivars and the hybrids were obtained from Minas Gerais, Brazil, while Robusta samples were obtained from Turrialba, Costa Rica. All coffee seeds were harvested at the same stage of maturation.

CGA analysis

Coffee samples were extracted with aqueous methanol (40%) and clarified with Carrez reagents (Trugo and Macrae, 1984). The duplicate extractions were analyzed in a gradient HPLC system using a UV detector operating at 325 nm, according to Farah et al. (2005). The extracts were also analyzed by LC-DAD-ESI-MS, using peak mass and UV spectra according to Farah et al. (2006). To express the CGA contents per weight of dry matter, water content for each sample was determined according to the A.O.A.C. method (2000).

RESULTS AND DISCUSSION

Total CGA contents varied from 5.3 to 6.4g/100g (dry weight basis - dwb) in Arabica samples and from 6.1 to 6.7g/100 g (dwb) in Robusta samples. In coffee hybrids, total CGA contents varied from 5.3 to 6.5g/100 g (dwb) (Table 1**)**. Even though total CGA contents observed in Robusta samples are in accordance with Clifford and Ramirez-Martinez (1991), these contents are low when compared with most of the literature data for *C. canephora* (Farah and Donangelo, 2006). Such variations in CGA content may be attributed to different quality of the seeds analyzed worldwide. The seeds from Turrialba used in the present study are known for their superior quality. Total CGA contents found in *C. arabica* cultivars are in agreement with previously published data (Farah and Donangelo, 2006). In resistant hybrids, CGA contents were comparable to literature data for Timor hybrid (about 5.6 g/100 g) (Clifford and Kazi, 1987), which was expected, since samples used in this study derived from the crossing of Timor Hybrid with Arabica cultivars.

No difference was observed when total CGA contents of resistant hybrids, *C. arabica* and *C. canephora* samples were compared, although differences in CGA content of *C. arabica* and *C. canephora* samples have been widely reported (Farah and Donangelo, 2006). However, differences within CGA classes were observed. CQA was the most abundant CGA class in all samples, accounting, on average, for 67.4%, 80% and 84% of total CGA in *C. arabica*, *C. canephora* and resistant hybrid samples, respectively. The second most abundant class was diCQA, contributing with 23%, 14% and 12% of total CGA, while FQA class was responsible for 5%, 9.5% and 5% in *C. arabica*, *C. canephora* and resistant hybrid samples, respectively. Resistant hybrid and *C. canephora* samples showed similar CQA and diCQA percent distribution, whereas FQA class contribution was comparable in resistant hybrid and *C.*

arabica samples. The resistant hybrids average percent distribution of CGA classes was very similar to those observed for Timor hybrid by Cifford and Ramirez-Martinez (1991).

Table 1. Chlorogenic acids contents in *C. arabica*, *C. canephora* and resistant interespecific hybrids.

Cultivar	3-CQA	4-CQA	5-CQA	3-FQA	4 FQA+ 5-FQA	3,4-diCQA	3,5-diCQA	4,5-diCQA
Coffea Arabica								
Red Catuaí	0.44± 0.00	0.61± 0.00	3.22± 0.00	0.03± 0.01	0.25± 0.02	0.22± 0.02	0.44± 0.01	0.23± 0.01
Y.Bourbon	0.48± 0.05	0.66± 0.06	3.91± 0.45	0.03± 0.01	0.27± 0.02	0.19± 0.01	0.38± 0.03	0.22± 0.02
Rubi	0.40± 0.01	0.57± 0.02	3.31± 0.13	0.03± 0.00	0.22± 0.01	0.22± 0.04	0.46± 0.03	0.12± 0.01
Topázio	0.45± 0.03	0.60± 0.03	3.21± 0.16	0.03± 0.00	0.21± 0.02	0.24± 0.01	0.44± 0.03	0.25± 0.01
Coffea canephora								
Robusta	0.26± 0.00	0.40± 0.01	3.56± 0.01	0.03± 0.00	0.59± 0.01	0.31± 0.00	0.71± 0.01	0.28± 0.01
Robusta	0.29± 0.00	0.42± 0.06	3.74± 0.78	0.03± 0.00	0.51± 0.00	0.34± 0.00	0.96± 0.01	0.29± 0.00
Robusta	0.27± 0.03	0.40± 0.04	3.45± 0.31	0.03± 0.00	0.60± 0.06	0.34± 0.00	0.78± 0.08	0.32± 0.04
Robusta	0.25± 0.00	0.40± 0.00	3.76± 0.04	0.03± 0.00	0.58± 0.01	0.37± 0.01	0.92± 0.01	0.36± 0.00
Interespecific hybrids								
I	0.43± 0.03	0.62± 0.04	4.23± 0.25	0.02± 0.00	0.26± 0,02	0.16± 0.01	0.37± 0.02	0.18± 0.01
II	0.48± 0.00	0.67± 0.01	4.15± 0.02	0.03± 0.00	0.26± 0.00	0.20± 0.00	0.46± 0.00	0.25± 0.00
III	0.39± 0.04	0.53± 0.06	3.39± 0.42	0.03± 0.00	0.25± 0.03	0.15± 0.02	0.33± 0.04	0.20± 0.03
IV	0.46± 0.01	0.59± 0.00	3.24± 0.03	0.03± 0.00	0.31± 0.00	0.19± 0.00	0.35± 0.00	0.19± 0.00
V	0.47± 0.01	0.66± 0.01	4.35± 0.00	0.03± 0.00	0.30± 0.00	0.15± 0.00	0.34± 0.00	0.19± 0.00
VI	0.50± 0.02	0.66± 0.03	3.59± 0.14	0.03± 0.00	0.32± 0.01	0.22± 0.00	0.41± 0.01	0.20± 0.00
VII	0.44± 0.01	0.62± 0.01	4.12± 0.01	0.03± 0.00	0.29± 0.01	0.15± 0.00	0.31± 0.01	0.20± 0.01
VIII	0.47± 0.02	0.65± 0.04	3.94± 0.20	0.02± 0.00	0.26± 0.01	0.17± 0.02	0.31± 0.03	0.17± 0.02
IX	0.44± 0.01	0.62± 0.01	3.72± 0.09	0.03± 0.00	0.29± 0.00	0.19± 0.01	0.39± 0.01	0.24± 0.00
X	0.38± 0.00	0.55± 0.01	3.67± 0.04	0.02± 0.00	0.26± 0.01	0.14± 0.00	0.33± 0.00	0.21± 0.01
XI	0.54± 0.03	0.71± 0.05	3.41± 0.28	0.04± 0.00	0.26± 0.05	0.24± 0.03	0.42± 0.05	0.23± 0.05
XII	0.51± 0.01	0.67± 0.01	2.92± 0.03	0.04± 0.00	0.26± 0.01	0.25± 0.00	0.43± 0.01	0.24± 0.00

Results are shown as the means of duplicates of extraction ± standard deviation, expressed in g/100g of coffee.
CQA = caffeoylquinic acid; FQA = feruloylquinic acid; diCQA = dicaffeoylquinic acid.

Additionally, we observed in the investigated samples the presence of peaks with *m/z* and retention times compatible with less common CGA compounds by LC-DAD-ESI-MS

analysis, more specifically, *p*-coumaroylquinic acid (*p*-CoQA - *m/z* 337), diferuloylquinic acid (diFQA − *m/z* 543), caffeoylferuloylquinic acid (CFQA − *m/z* 529), di-*p*-coumaroylquinic acid (di-*p*-CoQA- *m/z* 483) and *p*-coumaroylcaffeoylquinic acid (*p*-CoCQA − *m/z* 499). Moreover, we also identified a peak consistent with caffeoyl-tryptophan (CTrp - *m/z* of 365), a hydroxycinnamic derivative, and a peak with *m/z* compatible with 1,5-γ-diferuloylquinic lactone (diFQL - *m/z* 525), which are formed by dehydration from the quinic acid moiety and formation of an intramolecular ester bond (Farah et al., 2005). Regarding these less common CGA compounds, it was possible to note some chromatographic profile differences among *C. arabica*, *C. canephora* and resistant hybrid samples (Figure 1). This result is in accordance with Clifford and Kazi (1987), who reported that these minor compounds are associated with the origin of the seed and may reflect the genotype.

In conclusion, the interespecific hybridization between Timor hybrid and *C.arabica* showed to exert influence on the major classes percent distribution and in the chromatographic profiles of minor CGA compounds, while the same was not observed among total CGA contents.

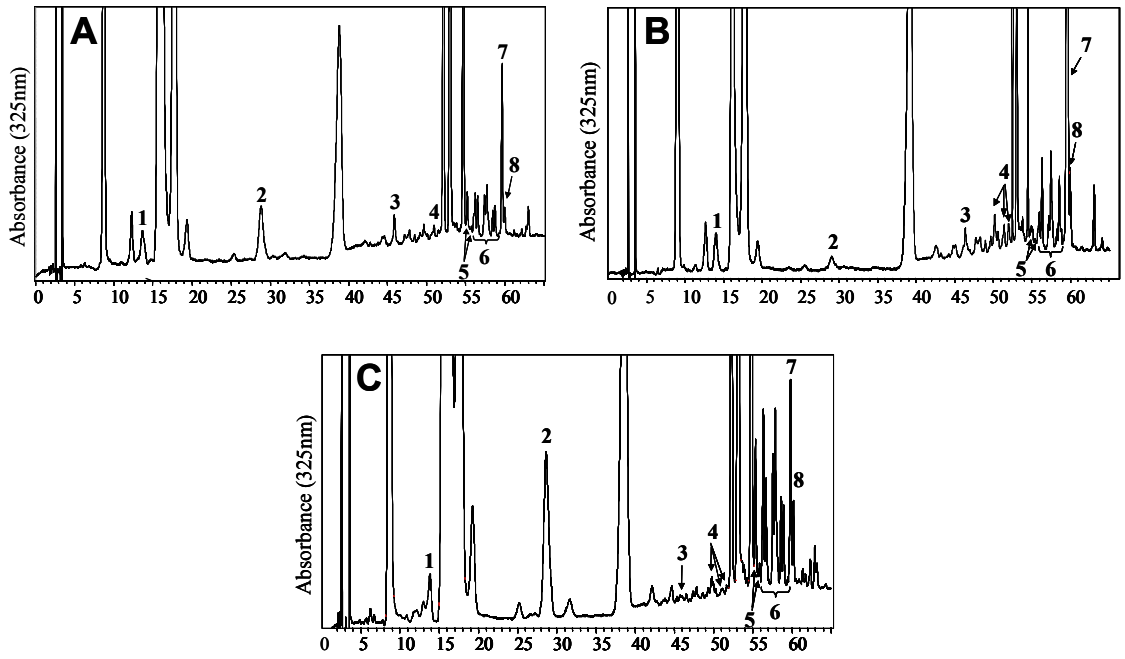

Figure 1. Minor CGA compounds in typical chromatograms from HPLC analyses of: (A) *C. arabica*; (B) *C. canephora*; (C) *hybrids resistant to M. exigua*. Peak 1 = 3-*p*-CoQA; 2 = 5-*p*-CoQA; 3 = diFQL; 4 = diFQA isomers; 5 = CoCQA isomers; 6 = CFQA isomers; 7 = CTrp; 8 = di-*p*-CoQA isomer.

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Optimization of Acid Hydrolysis for the Analysis of Conjugated Amines in Green Coffee

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SUMMARY

The objective of this study was to investigate the best parameters for the hydrolysis and extraction of conjugated amines from green coffee. Different concentrations of hydrochloric acid (1, 3, 6, 9 and 12 mol/L) and two reaction temperatures (110 °C and room temperature) were used. The free amines were separated and quantified by ion pair-HPLC and fluorimetric detection. The experiments were performed in triplicate and the means were compared by the Duncan test. The conjugated amines detected were the polyamines spermine and spermidine and the diamine putrescine. In order to hydrolyze putrescine from conjugated forms, high temperatures (110 °C) and high concentrations of HCl (9 and 12 mol/L) were needed. However, better recovery of spermidine and spermine, were obtained during hydrolysis with 6 mol/L HCl at 110 °C.

INTRODUCTION

Polyamines are ubiquitous in plant cells. They are small polycations that represent important intrinsic signals, linked to important developmental phenomena, including cell growth and division, stabilization of nucleic acids and membranes, protein synthesis and chromatin function, and biotic and abiotic stresses (Paschalidis & Roubelakis-Angelakis, 2005). Therefore, they are necessary for the successful replication of most cells and have been implicated in the regulation of senescence and morphogenesis in plants, as well as in plant responses to environmental stress (Walters, 2000).

Eukaryotic cells synthesize putrescine directly from ornitine through the activity of ornitine decarboxylase. Plants and some bacteria also synthesize putrescine indirectly from arginine, by the action of arginine decarboxylase. An aminopropyl group derived from decarboxylated *S*-adenosylmethionine is transferred to putrescine by spermidine synthase to form spermidine, and another aminopropyl group is added to spermidine by spermine synthase to form spermine.

Polyamine metabolism undergoes changes in response to infection by fungi and viruses. In fungal infection, the pattern of changes in polyamine levels depends, in part, upon whether the fungus is a biotroph or a necrotroph (Walters, 2000). Amines are present in plants free or conjugated to low molecular weight compounds (phenolic acids) or to macromolecules (proteins) (Fontaniella et al., 2001). The result of such conjugation is known as amides of hydroxycinnamic acids – HCAAs (Walters, 2003).

Conjugated amines are a widely distributed group of plant secondary metabolites which function in several growth and developmental processes including floral induction, flower formation, sexual differentiation, tuberization, cell division, and cytomorphogenesis. Although most of these physiological roles remain controversial, the biosynthesis of amides

and their subsequent polymerization in the plant cell wall are generally accepted as integral components of plant defense responses to pathogen challenge and wounding. Tyramine-derived HCAAs are commonly associated with the cell wall of tissues near pathogen-infected or wound healing regions. Moreover, feruloyltyramine and feruloyloctopamine are covalent cell wall constituents of both natural and wound potato (*Solanum tuberosum*) tubers, and are putative components of the aromatic domain of suberin. The deposition of HCAAs is thought to create a barrier against pathogens by reducing cell wall digestibility. HCAAs are formed by the condensation of hydroxycinnamoyl-CoA thioesters with phenylethylamines such as tyramine, or polyamines (Facchini et al., 2002).

Hydroxycinnamic acid conjugated amines can be prepared from acid methanol:water that has been back extracted with hexane (Panagabko et al., 2000). However, the conjugated amines are often quantified in the free form, after acid hydrolysis and extraction. There are several methods described in the literature for the hydrolysis of conjugated amines, however there is no consensus with respect to the ideal hydrolysis condition (Armas et al., 2000; Fontaniella et al., 2001; Casal et al., 2004).

The objective of this study was to investigate the best parameters for the hydrolysis (acid concentration and temperature) for optimum hydrolysis and extraction of conjugated amines from green coffee.

MATERIAL AND METHODS

Green coffee samples (*Coffea arabica*) were kindly provided by Universidade Federal de Lavras, Lavras, MG, Brazil. The standards of bioactive amines were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The reagents were of analytical grade, except those for HPLC analysis which were of chromatographic grade. Water was purified in Milli-Q (Millipore Corp., Milford, MA, USA). The mobile phases were filtered in HAWP and HVWP membranes (0.45 μm pore size, Millipore Corp.) for aqueous and organic solvents, respectively, and the extracts were filtered in HAWP membranes.

Hydrolysis of conjugated amines

Different concentrations of hydrochloric acid (1, 3, 6, 9 and 12 mol/L) and two reaction temperatures (110 °C and room temperature) were used for the hydrolysis of the conjugated amines (Armas et al., 2000; Fontaniella et al., 2001; Casal et al., 2004).

Determination of free bioactive amines

The free amines were extracted from the coffee samples (5 g) with 20 mL of 5% trichloroacetic acid and filtered through 0.45 μm pore diameter HAWP membrane. The amines were separated by ion pair-HPLC using μBondapak C18 column (300 x 3.9 mm i.d, 10 mm) (Waters, Milford, USA), and quantified after post column derivatization with *o*-phthalaldehyde and fluorimetric detection at 340 nm of excitation and 445 nm of emission (Cirilo et al., 2003).

The amines were identified by comparison of the retention time of peaks in the sample in relation to standards and confirmed by the addition of the suspect amine to the sample. The concentrations of the amines were determined by direct interpolation in individual standard curves with $R^2 \geq 0.9926$. The limits of determination were 0.02 mg/100 g for spermidine, spermine, agmatine, putrescine, cadaverine, histamine, tyramine and phenylethylamine and 0.04 mg/100 g for serotonin and tryptamine.

Statistical analysis

All of the experiments were performed in triplicate. The data were submitted to analysis of variance (ANOVA) and the means were compared by the Duncan test at 5% probability using SIGMA STAT 2.0 (Systat Software Inc, Richmond, CA, USA).

RESULTS AND DISCUSSION

The conjugated amines detected were the polyamines (spermine and spermidine) and the diamine putrescine. The levels of these amines recovered after hydrolysis of the conjugated amines are indicated on Table 1. According to the data obtained, hydrolysis of conjugates requires low pH values (acid treatment) and high temperatures. Similar results were described by Fontaniella et al. (2001).

Table 1. Levels of amines from conjugated amines in green coffee after hydrolysis under different conditions.

Amines / Conditions	Amines (mg/100 g -% CV) / HCl concentration (mol/L)				
	1	**3**	**6**	**9**	**12**
Spermidine					
room T °C/24 h	0.24 (6) b	0.26 (21) b	0.26 (18) by	0.36 (13) a	0.26 (4) b
110 °C/24 h	0.19 (15) c	0.30 (18) d	0.62 (12) bx	0.38 (6) b	0.22 (7) a
Spermine					
room T °C/24 h	0.24 (6)	0.25 (19)	0.25 (17) y	0.22 (12)	0.24 (4)
110 °C/24 h	0.31 (14) b	0.27 (25) b	0.44 (15) ax	0. 20 (3) b	0.26 (13) b
Putrescine					
room T °C/24 h	nd	nd	nd y	2.79 (12) ay	1.25 (3) by
110 °C/24 h	nd	nd	0.65 (31) cx	3.33 (5) ax	1.98 (4) bx

Mean values with different letters in the same line (a-c) or column for each amine (x-y) are significantly different (Duncan Test $p \leq 0.05$).

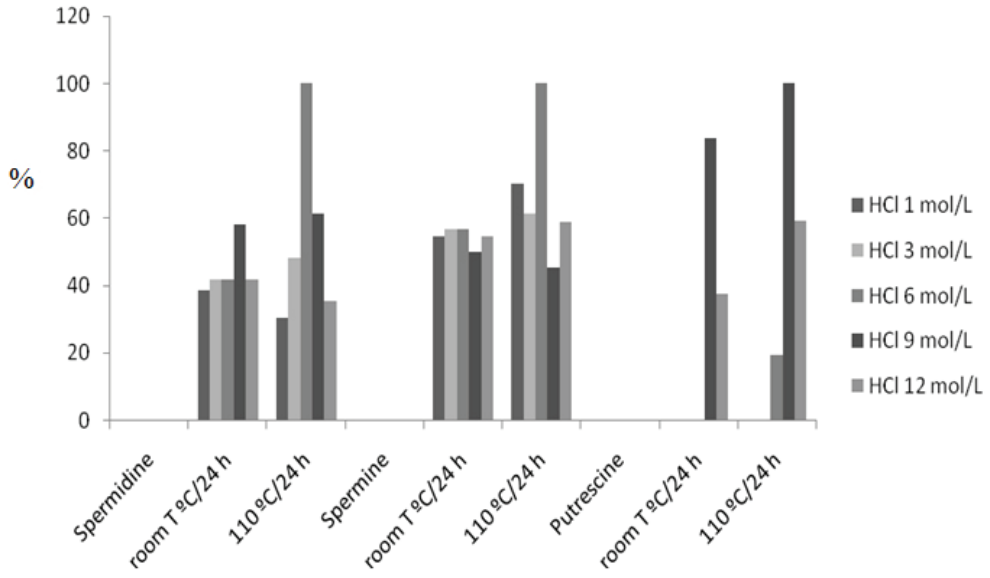

Figure 1. Recovery of bioactive amines after hydrolysis of the conjugated forms at different HCl concentrations (1 to 12 mol/L) and at room and 110 °C for 24 h.

Results can be better visualized on Figure 1. The highest amount of amine recovered was considered 100% recovery, whereas the others were calculated based on this information. Therefore, best recoveries for the polyamines spermidine and spermine were obtained with HCl concentration of 6 mol/L and at 110 °C. Under this condition, only 19% of the putrescine was recovered. Therefore, for the extraction of putrescine a higher concentration of acid was required (9 mol/L).

In order to hydrolyze putrescine from conjugated forms, high temperatures (110 °C) and high concentrations of HCl (9 and 12 mol/L) are needed. However, better recovery of spermidine and spermine, were obtained during hydrolysis with 6 mol/L HCl at 110 °C.

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The Unique Structural Features of Coffee Arabinogalactans

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SUMMARY

In order to highlight the structural features of coffee arabinogalactans, especially those loosely bound to cell walls, this work describes the purification and structural characterisation of the arabinogalactans present in the 75% ethanol fraction. These hot water soluble green coffee arabinogalactans were characterised by ^{1}H and ^{13}C NMR and, after partial acid hydrolysis, by ESI-MS/MS. Data obtained showed that these are highly branched AGPs, with a protein moiety containing 10% of 4-hydroxyproline residues. They possess a β-(1→3)-Gal*p*/β-(1→3,6)-Gal*p* ratio of 0.80, with a sugars composition of Rha:Ara:Gal of 0.25:1.0:1.5, and containing 2 mol% of glucuronic acid residues. The presence of terminally linked rhamnosyl residues in the *O*-5 position of arabinosyl residues, which was never reported for AGP's, could explain the least degradability by the human fecal microbiota of the coffee arabinogalactans.

INTRODUCTION

Arabinogalactan-proteins (AGPs) are proteoglycans widely distributed throughout the plant kingdom. They are present in the plasma membrane, within cell walls, and in the extracellular matrix, presenting different structural forms in the region adjacent to the cell walls lumen (Sutherland et al., 2004). Their role remains unclear, although having been related to many processes of plant growth, development, or adaptation, such as cell proliferation, expansion, differentiation, and somatic embryogenesis. AGPs are complex macromolecules due to their large, heterogeneous, and highly branched chains. Coffee beans are composed, approximately, by 15% of AGPs (Bradbury, 2001; Redgwell et al., 2002).

Green coffee water soluble arabinogalactans have been extracted from ground coffee with hot water followed by dialysis purification. This procedure allowed to extract 1.1% of arabinogalactans in relation to the dry and defatted green coffee weight (Nunes and Coimbra, 2001). This amount of water soluble arabinogalactans can be estimated to be approximately 7% of the total amount of green coffee bean arabinogalactans. Using graded ethanol precipitation, it was possible to obtain arabinogalactan fractions where the degree of branching and the amount of rhamnosyl residues increased with the increase of the concentration in ethanol. The major fraction (45%) of the water soluble arabinogalactans was recovered in the precipitate of a 75% ethanol solution (Nunes and Coimbra, 2001).

In the course of an ongoing project on the structural characterization of water soluble green and roasted coffee polysaccharides, we were able to isolate and purify one coffee arabinogalactan with unusual sugar composition and physico-chemical properties. These characteristics led us to structurally characterize this polysaccharide (Nunes et al., 2008).

MATERIAL AND METHODS

The extraction, purification, and characterization procedures used for extraction of coffee arabinogalactan of water soluble green coffee are summarized in Figure 1.

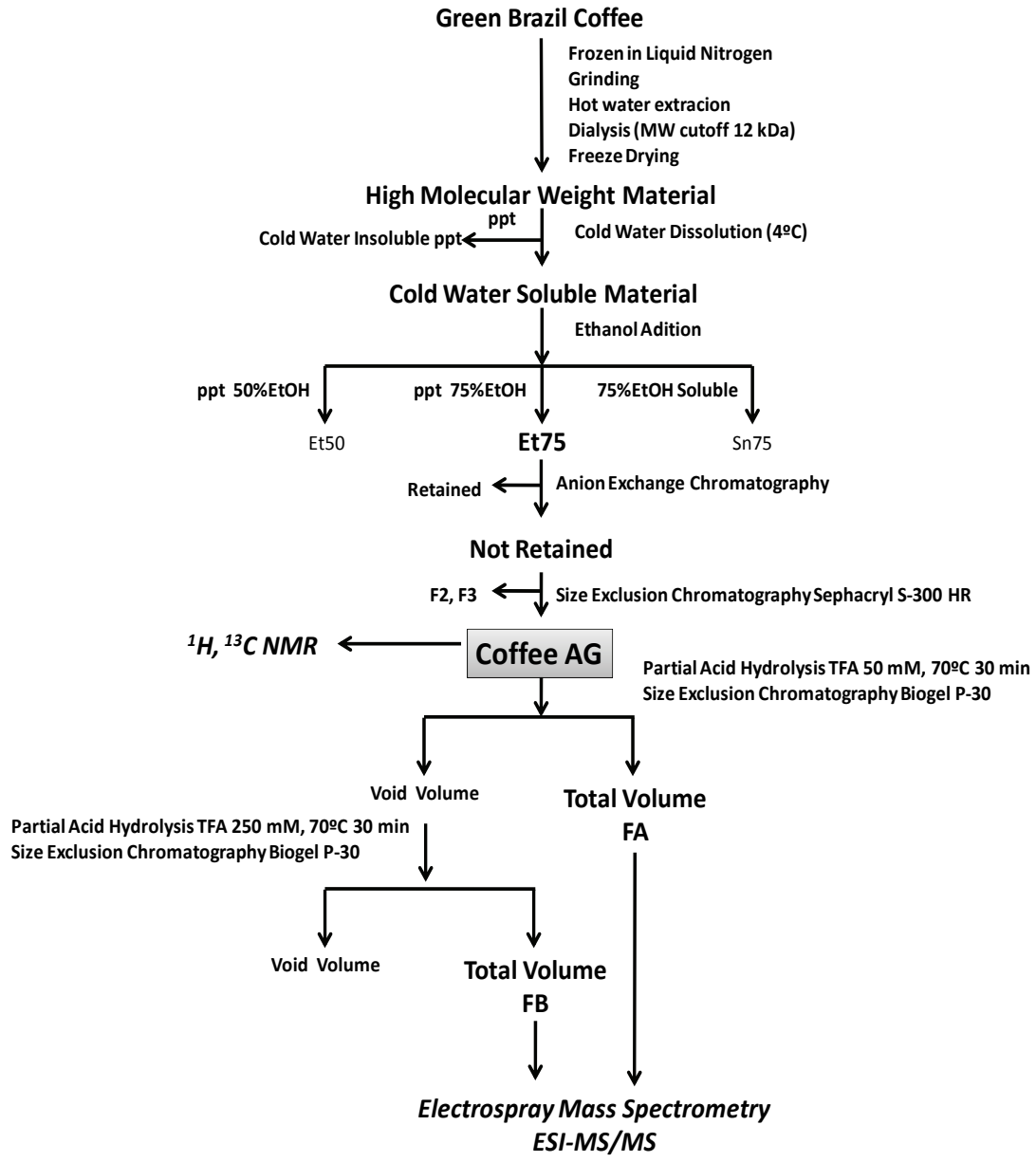


Figure 1. Extraction, purification, and characterization of coffee arabinogalactan.

RESULTS AND DISCUSSION

The AG purified from green coffee accounted for 3.2% of green coffee beans AG's. After sugar and methylation analysis it was shown to contain 9 mol% of terminally linked rhamnosyl residues, and a low amount of glucuronic acid residues (~2 mol%). Aminoacid analysis show that it contained 4.2% of protein, with a high abundance of Hyp residues (10 mol%), a typical feature of AG's. The β-Yariv precipitation assay, lead us to conclude that this coffee AG is a proteoglycan.

After partial acid hydrolysis and characterization of the oligosaccharides obtained by ESI and ESI-MS/MS, it was shown that these terminally linked rhamnosyl residues were linked to single arabinosyl and di-arabinosyl residues (Figure 2).

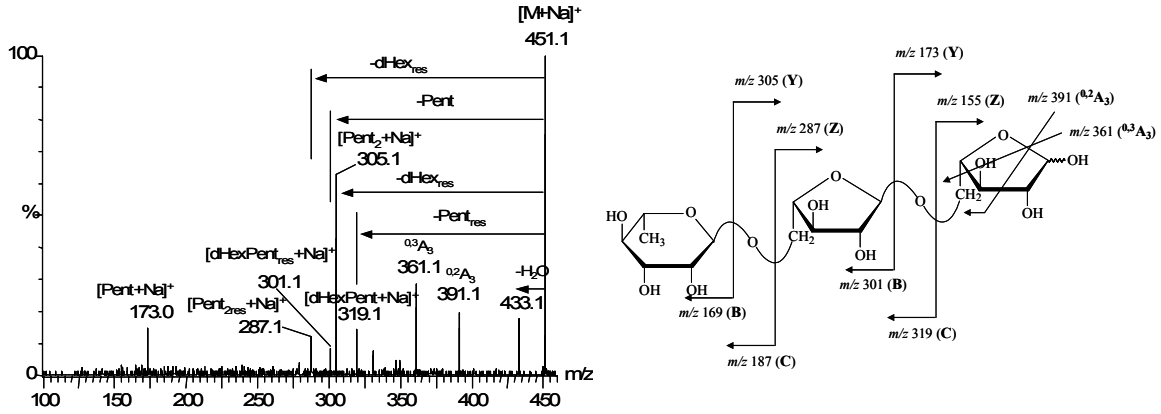


Figure 2. ESI-MS/MS of Rha-Ara$_2$ from green coffee arabinogalactan.

This arrangement of the T-Rha*p* residues was confirmed by NMR analysis of the whole polysaccharides, where it was observed a cross peak in the HMBC spectrum between the H-1 signal of T-Rha*p* and the signal of C-5 of the 5-Ara*f* residues.

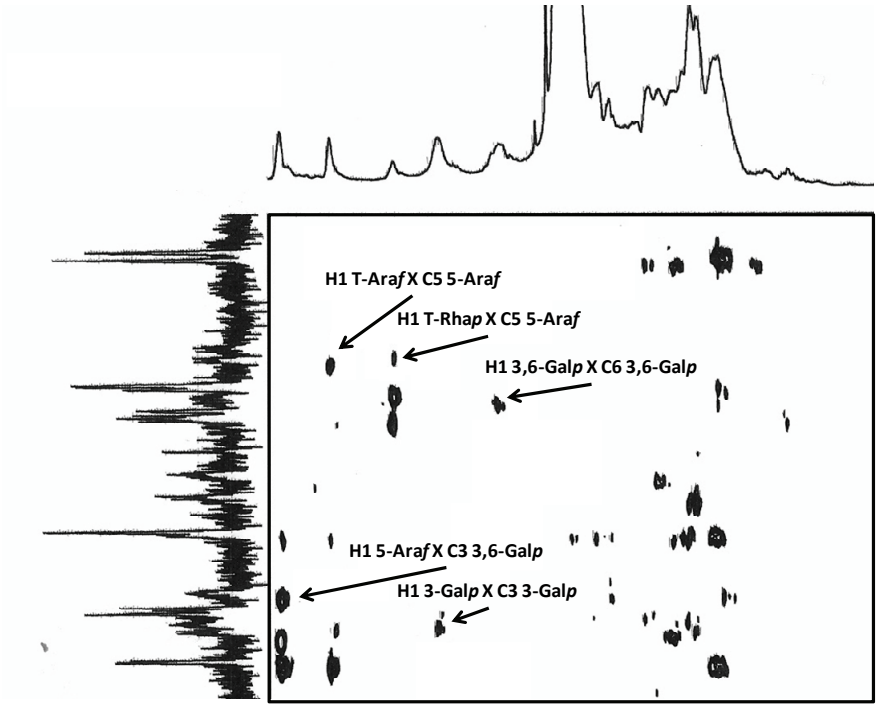
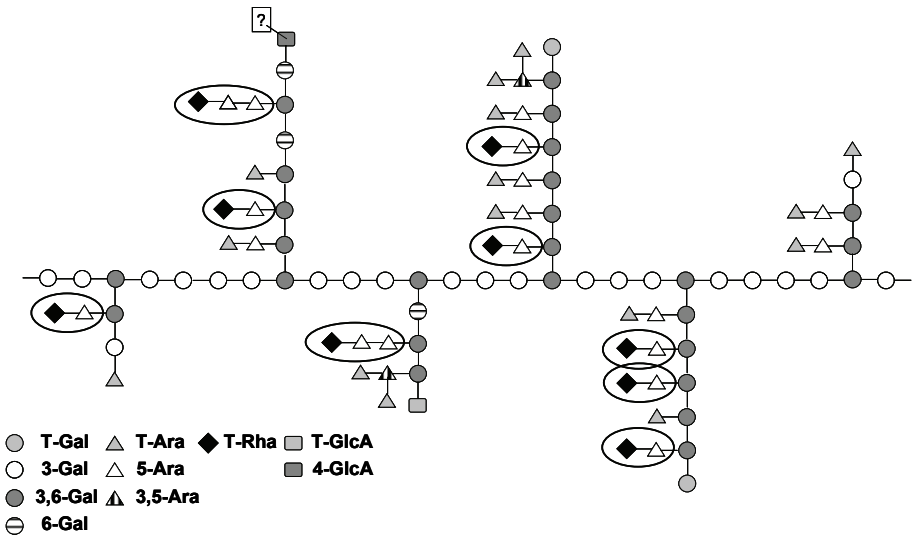


Figure 3. HMBC spectrum of green coffee arabinogalactan.

CONCLUDING REMARKS

Taking together the results from sugar and methylation analysis with the structural details given by ESI-MS/MS and NMR the following structure was deduced for the water soluble coffee rhamnoarabinogalactan:



This structure is one of all the possible combinations in terms of size of the (1→3)-linked b-D-Gal*p* backbone, (1→6)-linked b-D-Gal*p* side chains, and arrangement of the di-arabinosyl and rhamnoarabinosyl side chains. The presence of terminally linked rhamnosyl residues in the *O*-5 position of arabinosyl residues, which was never reported for AGP's, could explain the least degradability by the human fecal microbiota of the coffee arabinogalactans (Gniechwitz et al., 2007).

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Structural Analysis of Green *Coffea arabica* Beans by Consecutive Solvent Extraction

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SUMMARY

Characterization of green coffee beans is essential prior in-bean model studies to understand the role of different compounds during roasting. Whole green coffee beans were sequentially extracted at ambient temperature with several solvents. Only chlorogenic acids were not preferentially extracted in water, as nearly half the initial amount was still in the beans after extraction, probably due to their distribution or interaction with other non-extractable compounds like proteins. The depleted beans obtained after extraction were further characterised by linkage analysis of the extracts. The mannan found in the final residue had degree of polymerization of 80. The arabinogalacatan seemed to exist in two distinct forms: one having short polymer chain, highly branched and easily extractable and other firmly imbedded in the cell wall with longer and less branched backbone.

INTRODUCTION

Green coffee composition is the basis for all the reactions that are triggered during roasting. Consequently, the use of green coffee beans as a base model matrix for in-bean roasting studies is appealing but challenging. Increasing our knowledge of the composition, distribution and structure of the major compounds present in green coffee would enable a better understanding of the role played by the different families of compounds during the roasting process including their potential interactions and products derived.

The objective of our research was to characterize extracts of whole green coffee beans performed by sequential solvent extraction at room temperature using different solvents chosen by polarity. The insoluble fraction was further characterized, after grinding, by sequential extraction to extract carbohydrates.

MATERIAL AND METHODS

Coffea Arabica var. *Primero* was used for this investigation. All extracts were performed sequentially using 1:10 (w/v) of green coffee beans to solvent. Whole beans were extracted at room temperature with 3 loads of solvent over a period of 24 hours using, in order, hexane, methyl *tert*-butyl ether, methanol and water. For ground green coffee, obtained by cryo-milling (IKA, Germany), the solvents used were: 0.05 M EDTA in 0.05 M NaOAc (0 °C, 1 H), phenol-acetic acid-water (2:1:1 w/v/v) (4 °C, 3H), $NaClO_2$ (3 g/L) in 1.2mL/L AcOH (3 H, 70 °C) and 1, 2, 4 and 8 M NaOH with 0.02 M $NaBH_4$ (3H, 25 °C). All extracts were ultrafiltrated using a regenerated cellulose membrane (10 kDa) (Millipore, U.S.A.) and freeze-dried. Green coffee material (10 mg) was solubilized in *N*-methylmorpholine-*N*-oxide monohydrate (1 g) with 10 mg of *n*-propyl gallate (130 °C, 10 min.) Sucrose analysis was performed in a Dionex 50 with CarboPac PA1 column and water as eluent (1mL/min). Caffeine, chlorogenic acids and trigonelline were quantified using a HPLC with C18 phase column, phosphoric acid and acetronitrile as eluents (1 mL/min) and UV detection. Linkage

analysis was performed according to Ciucanu and Caprita (2007) and analysed using a GC-MS 6890 (Agilent, U.S.A.) installed with a HP-5 silica capillary column (J&W Scientific).

RESULTS

Sequential extraction of whole green coffee beans

The total extracted mass of whole beans was 18%. The fraction with highest yield was water (15.2%) followed by methanol (1.5%), MTBE (0.6%) and hexane (0.5%). These yields were not in accordance with the work performed by Ramalakshmi et al. (2008) that obtained the highest yield with methanol (12.1%) when using ground green coffee beans. The fact that in this work it was used whole and not ground coffee suggests water might play an important role in altering the structure of whole green coffee, maybe by swelling the beans' structure and making storage compounds more readily extractable, while methanol cannot perform this structural change. The hexane extract was solid at room temperature maybe due to high content of lipids that form a waxy in the outer shell of the beans and that account for 0.2 to 0.3% of the total bean weight, value similar to the yield of that fraction (Speer and Kölling-Speer, 2006). Nevertheless, this fraction was not characterized and further analysis must be performed to draw conclusions. Figure 1 presents the content the percentage yield of initial amount of caffeine, trigonelline, chlorogenic acids and sucrose. Extractability of all compounds increased with polarity of the solvents. The solvent showing higher extractability was water yielding 47% of caffeine, 50% of trigonelline, 24% of chlorogenic acids and 72% of the sucrose. Methanol extracted 1.4% caffeine, 1.7% of trigonelline, 0.7% chlorogenic acids and 0.7% of sucrose. MTBE was the solvent extracting the lowest amount of green coffee compounds: 0.7% of caffeine, 0.1% of trigonelline and < 0.1% of Chlorogenic acids and sucrose. In the depleted beans remained around 27% of caffeine, 30% of trigonelline, 9% of sucrose and nearly 43% of chlorogenic acids.

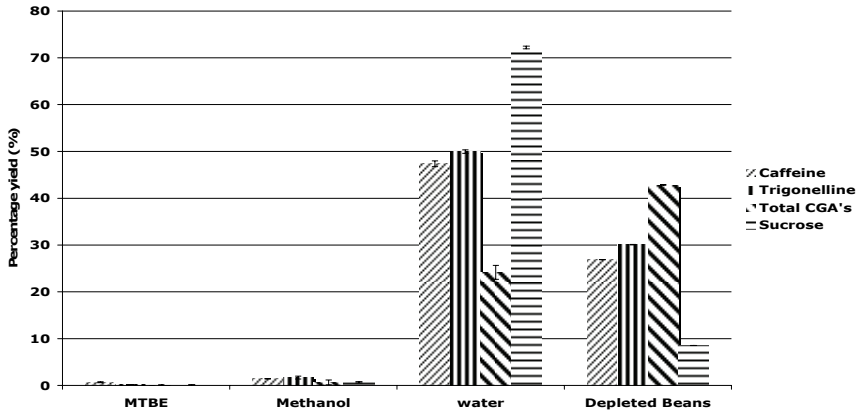

Figure 1. Yield of caffeine, trigonelline and chlorogenic acids in fractions obtained by sequential solvent extraction based on the initial content.

The high contents of chlorogenic acids, caffeine and trigonelline in the depleted beans showed extraction limitations maybe due to two main reasons. Firstly, if compounds are bound in the matrix, as for example caffeine to cell wall (Clifford, 1985) disruption of the cell wall by grinding may be necessary to release those compounds. Secondly, if these compounds exist free in the cytoplasm and if the hexane extract mainly removes the waxy layer and not membrane lipids, the compounds might be retained within the cell membrane and therefore not easily extractable in a whole coffee bean.

Carbohydrate characterization of depleted green coffee beans

The protocol followed was characterized by the low yields of extraction and significant mass losses (data not shown).

Table 1. Glycosyl-linkage analysis and characterisation of carbohydrate fractions obtained by sequential extraction of ground green *Coffea arabica* beans.

Linkage Analysis *(mol%):*	**Depleted Beans**	**EDTA**	**Phenol**	**Sodium Chlorite**	**1M**	**2M**	**4M**	**8M**	**Residue**
Galactomannan:									
***t*-Man**	1.7	7.7	4.0	1.2	5.9	5.9	2.4	2.3	0.5
4-Man	43.7	17.6	11.6	14.9	2.6	6.1	26.2	29.0	39.9
2,4-Man	2.6	0.5	2.7	0.5	0.9	1.4	1.0	1.2	2.2
4,6-Man	0.9	1.7	0.6	1.3	0.9	1.4	1.5	1.7	1.4
6- Gal	1.0	1.9	0.7	1.4	1.0	1.5	1.7	1.8	1.5
Arabinogalactan:									
***t*-Gal**	2.0	3.5	5.7	4.6	1.4	0.5	6.9	2.6	2.2
***t*-Ara**	3.8	14.3	8.5	3.7	12.6	13.7	9.7	6.7	1.3
3-Gal	16.1	6.7	7.2	61.1	12.3	3.4	21.6	28.7	24.5
5-Ara	6.5	5.6	3.2	3.0	5.8	5.7	6.3	5.8	6.6
3,6-Gal	6.9	9.7	6.2	2.4	18.4	16.6	7.0	6.1	2.2
6- Gal	0.0	0.0	17.5	0.0	0.0	0.0	0.0	0.0	5.9
Cellulose:									
***t*-Glc**	1.9	6.4	2.6	0.7	6.6	7.3	1.4	1.8	0.6
4 Glc	12.2	23.7	11.2	4.3	30.9	35.7	13.2	11.4	3.9
Relative Composition *(mol%):*									
Mannan	50.1	29.4	19.5	19.3	11.3	16.4	32.8	35.9	45.6
Arabinogalactan	35.2	39.8	48.4	74.8	50.5	39.9	51.5	49.9	42.7
Cellulose	14.1	30.1	13.9	4.9	37.5	43.0	14.6	13.2	4.4
Average Size *(end group basis):*									
Mannan	28.1	3.6	4.7	14.4	1.7	2.5	13.1	14.7	82.6
Arabinogalactan	6.1	2.2	3.4	9.0	3.6	2.8	3.1	5.4	12.1
Branching:									
4:4,6-Man	13.7	12.2	5.7	10.0	5.8	5.2	12.2	12.1	12.2
3:3,6-Gal	5.1	4.1	7.8	31.8	2.8	2.4	7.4	8.1	19.7

The depleted green beans had an average degree of polymerization (DP) for the mannan of 28 in an end group basis, similar to the value reported by Bradbury (1990). In the present work, the DP measurement was performed in whole green depleted beans and not in a mannan extract as done by Bradbury and therefore a lower DP was expected because solubilization with *N*-Methylmorpholine-*N*-oxide is known to reduce polysaccharide molecular weight (Joseleau et al., 1981). However, *n*-propyl gallate was used in the present work which is known to be a good antioxidant to stabilize cellulose during solubilization (Bok et al., 2001) and might also stabilize.

The depleted beans had a high mannan content (50% of the total sugars) and a degree of branching (DB) ara:gal 1:14. Arabinogalactan in depleted beans had a polymer average size of 6 calculated as the ratio of the sum of arabinose and galactose over the sum of terminal arabinose and galactose. The mannan DB for the depleted beans and residue was around 13 for all samples. This value is well below the reported by Bradbury (1990) of 100, but within the range 10 to 30 mannose residues per unit of galactose reported by Fischer et al. (2001). Nevertheless, the DB of mannan was homogeneous.

The final residue showed an average DP of 80 which corresponds to an average molecular weight of around 16kDa. This average DP value, never reported before for green coffee using glycosidic linkage analysis, was within the range reported by Leloup and Liardon (1993). The

difference in DP for depleted beans and residue might indicate the occurrence of two types of mannan (Oosterveld et al., 2003): a fraction loosely attached within the cell wall and other embedded in it.

During pectin extraction with EDTA, similar amounts of all carbohydrates were extracted suggesting a close interaction between pectin and main carbohydrates.

Phenol, used to solubilize proteins, showed to be a good solvent for arabinogalactans with small average size but a relative high DB. This observation can be related with the extraction of arabinogalactan-proteins (Redgwell and Fischer, 2006).

As expected, sodium chlorite had the highest arabinogalactan content of all the extracts since, besides being used to remove lignin, sodium chlorite has been shown to be a good solvent for glycoproteins and arabinogalactan (Fischer et al., 2001). The arabinogalactan present had the highest average size and DB of all extracts ara:gal 1:31. This suggests that the branching distribution of arabinogalactan within the beans is not homogeneous. Also, the DB of depleted beans is considerably higher than that found in the final residue. This difference indicates that not only the distribution is not homogeneous but that maybe two types of arabinogalactans are present in the beans; one easily accessible with high DB and low polymer size and therefore more extractable and soluble probably complexed with proteins and other large unbranched polymer (DB 20) not extractable maybe due to poor solubility and entrapment within the cell wall.

Alkali solvents extracted mainly arabinogalactans in similar ratios but with increased mannan and less cellulose. In these extracts, the arabinogalactan had a higher DB for lower concentrations of NaOH and lower DB for increased ionic strengths showing the impact of substitution degree on solubility and extractability. In addition, increasing NaOH concentration, the degree of substitution of the mannan extracted decreased probably because in similarity with arabinogalactans, the solvent ionic strength improves solubility of less soluble, due to lower branching, mannan.

CONCLUSIONS

Green coffee mannan had an average DP of 80, the highest reported to date using linkage analysis. Arabinogalactan was present in two forms being one a short-chain, highly branched and extractable polymer probably forming a complex with proteins (AGP) and other with longer average size, less branched and not extractable. *N*-Morpholine-*N*-Oxide with addition of *n*-propyl gallate showed to be good solvent for coffee carbohydrates.

Green coffee carbohydrates revealed to have a close strong interaction within the cell wall making their extraction challenging.

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Steam Pressure Coffee Extraction: Moka Coffee Maker Physics and Beverage Characterization†

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†in memory of Dr. Ernesto Illy (July 18, 1925 - February 3, 2008)

SUMMARY

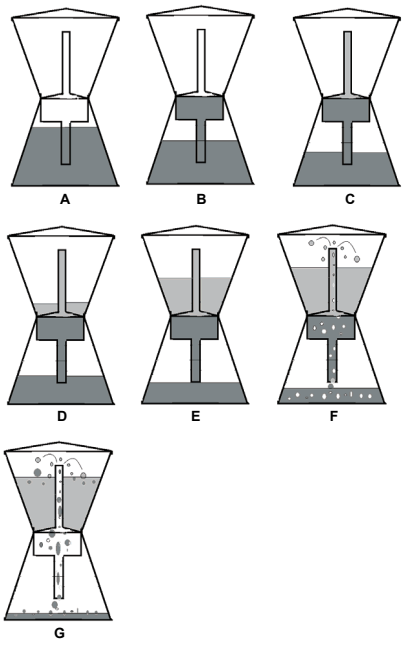
The stove-top coffee maker invented by Alfonso Bialetti in 1933, was industrially produced and commercialized from 1946 with the trademark denomination of "Moka Express" but nowadays it is well known simply as *moka*. It is an ingenious device very popular in Italy for household coffee brewing. *Moka* uses the steam pressure, produced by the water contained in an autoclave-type aluminium kettle heated by an external source (gas or electrical stove), to force upwards the same water through a roasted and ground coffee bed contained in a funnel-shaped filter. The beverage is conveyed through appropriate tubing into an upper vessel, screwed and sealed by a rubber gasket to the base kettle. The end of the brewing operation is usually announced by noisy mixture of boiling water and its vapour flowing from the upper tube, to indicate water depletion. Differently from *espresso* coffee machine, the thermal balance of *moka* is somewhat flimsy, being affected by several variables not easy to control (Petracco, 2001). The complex interplay between water pressure and temperature and physico-chemical nature of roasted and ground coffee occurring during steam pressure coffee extraction, led to a beverage characterized by harsh bitter flavour often described as "burnt", and by lack of the foam layer typical of true Italian *espresso* coffee brew. The present paper reports on *moka* thermodynamics as a function of heating power and coffee dose. *Moka* beverage has been studied by means of sequential fractionation and subsequent SBSE-GC-MS analysis.The experimental results, in addition to disclose some aspects of *moka* functioning not yet investigated in details, have been discussed in the framework of physics/beverage quality relationships.

INTRODUCTION

From its invention in 1933, the functioning of the *moka* stove-top coffee maker (see Figure 1), often misnamed *mocha*, *moca* or *stove-top espresso*, has never been the subject of detailed analysis and frequently it has been given for granted. Only very recently the *moka* coffee maker attracted the researchers' attention and its thermofluidodynamics has been investigated (Gianino, 2007; Scheri, 2007; Navarini et al., 2008).

Figure 1. Moka stove-top coffee maker (cut section).

Moka uses the steam pressure, produced by the water contained in an autoclave-type aluminium kettle (tank) heated by an external source (gas or electrical stove), to force upwards the same water through a roasted and ground coffee bed contained in a funnel-shaped filter. The beverage is conveyed through appropriate tubing into an upper vessel, screwed and sealed by a rubber gasket to the base kettle. The end of the brewing operation is usually announced by noisy mixture of boiling water and its vapour flowing from the upper tube, to indicate water depletion.



Scheme 1. Steam pressure coffee extraction in a *moka* coffee maker. A: beginning; B: imbibition (water invasion of the coffee bed); C: first drop; D: first fraction; E: middle fraction; F: initial *strombolian* phase; G: extraction tail.

The brewing process of three-cup version *moka* (150 g water and 15 g coffee powder) has been divided into two phases (Navarini et al., 2008). In the first one, up to approximately 120 g of water flowed, named *regular extraction* phase, liquid-solid extraction occurs (see Scheme 1 A-E). In this phase, depending on heating power, the initial in-tank water mean temperature during the extraction is around 68-70 °C whereas the mean final one is close to 117-120 °C. In the second phase, the final one, when water level reaches the end of the funnel-shaped filter, the short-cut between external ambient and air-vapour mixture, which no more drives in-tank water out of the tank, causes an intense evaporation (see Scheme 1 F-G). In this phase, named *strombolian phase* and announced by a well-known rattling sound, vapour-liquid-solid extraction occurs and water temperature can reach about 130 °C. The maximum relative pressure inside the water tank during coffee extraction, has been shown to range from 1.05 to 1.60 bar, but the relative pressure inside the water tank at the beginning of coffee extraction is very low and close to 0.1 bar.

The scenario emerging from this reported experimental study, is very different from that described in technical literature and believed by *moka* users (Parras et al., 2007; Varlamov and Balestrino, 2001). In particular, to describe the *moka* functioning is very common to think that standard atmosphere boiling point temperature is needed to drive the water out of the tank and moreover, the pressure rise is due to thermodynamic equilibrium between water and its vapour in saturation conditions. This description leads to think that to reach a pressure level suitable for flowing across coffee grounds, the strict thermodynamic relationship between water pressure and temperature demands a very high temperature, well above 100 °C: this process causes substances that are normally insoluble to be extracted, leaving in the cup a harsh bitter flavour often described as "burnt" (Petracco, 2001). Although it is well known in the coffee industry that high temperature extraction fluids transit in a coffee bed is noxious for the quality of the extract, according to Navarini et al. (2008) in the *moka* coffee maker this transit occurs in the *strombolian phase* only and not already at the beginning of the coffee extraction. This view suggests that substances that are responsible of the harsh bitter and "burnt" flavour of *moka* beverage should be present or more concentrated in the final phase or "extraction tail" of the *moka* brewing process.

In the present paper in addition to an experimental investigation of *moka* thermodynamics as a function of heating power, coffee dose and grinding, *moka* beverage has been studied by means of sequential fractionation and subsequent stir bar sorptive extraction (SBSE)-GC-MS analysis. SBSE is an extraction technique that utilizes glass stir bars coated with polydimethylsiloxane (PDMS) for extraction of organic compounds in aqueous samples. The device operates similar to a conventional magnetic stirring rod, with the PDMS coating enabling direct sample clean-up and analyte preconcentration while stirring. The higher extraction phase ratio of SBSE also provides better recovery and sample capacity over microextraction approaches such as solid phase microextraction (SPME). Caffeine, trigonelline and some chlorogenic acids concentrations have been also determined on the individual fractions.

MATERIALS AND METHODS

Roasted 100% *Coffea arabica* L. blend with a medium roasting degree (total weight loss: 16%) was used. Two different grind setting (sample A and B) were used and the particle size distribution were measured using a Light Scattering Particle Size Analyzer Coulter LS230. Average particle size in terms of volume mean diameter of 450-500 μm (medium grind) and 350-300 (fine grind) μm were obtained for sample A and B, respectively.

The experimental set up for thermodynamics data acquisition has been described in detail elsewhere (Navarini et al., 2008). Temperature values are affected by a global error (ΔT acquisition + ΔT thermocouple) $\Delta T = \pm 2.3$ °C whereas pressure values by a $\Delta P = \pm 0.035$ bar (Scheri, 2007).

In addition to the standard usage of the three cups version *moka* (150 g of water filling of the tank and 15 g of coffee powder filling of the funnel), two additional coffee dose were explored: 13 and 17 g. Untreated tap water (total hardness: 18-20 french degrees) was used. An electrical stove was used to heat the coffee maker for two different values of heating power: 400 W and 600 W.

Extraction yield (defined as the percentage of the brew total solids with respect to ground and roasted coffee dose) was determined according to Lopez-Galilea et al. (2007).

Beverage sequential fractionation was carried out by using a three cup version RS07 stainless steel electric *moka* coffee maker (Alessi, Italy) operating at a heating power of 365 W. This apparatus resulted to be most appropriate to perform the fractionation because it rendered particularly practical and reproducible the operation. 150 g of water filling of the tank and 15 g of coffee powder (samples A and B) filling of the funnel were used. By collecting the beverage through appropriate tubing during the coffee extraction, six 21 ± 1 mL fractions (named A1…A6 or B1…B6) were obtained. The last fraction (A6 and B6), was collected under *strombolian phase* conditions only. Three different fractionations were performed for each coffee sample. Chemical characterization was performed on fractions n° 1, 3 and 6, representative of initial, middle and final extraction phases, respectively.

For volatile compounds analysis sample extraction was performed by placing 10 mL of the desired coffee fraction in a 20 mL glass vial, adding dimethoxytoluene as internal standard and placing a PDMS coated stir bar, known as “twister”, of 10 mm x 0,5 mm (length and film thickness) supplied by Gerstel, Germany. Samples were agitated with a magnetic stirrer at 60 °C and 1000 rpm for 1 hour. After extraction the twister was removed, rinsed with water and analysed via TDS-GC-MS. Twisters were thermally desorbed using a thermal desorption unit TDS-2 equipped with auto sampler (Gerstel, Germany) and connected to an Agilent 6890 GC-5973 MS system (Agilent Technologies, Palo Alto, CA, USA). Desorption temperature goes from -120 °C to 250 °C, final temperature had been maintained for 10 min, in order to achieve a total desorption. The analysis were carried out using a HP-WAX polyethylene glycol column, 60m length x 0,15 um thickness and 250 um diameter. The flow was 1,3 mL/min and the column was kept a 35 °C for 3 min, then ramped at 4 °C/min to 215 °C, increased to 240 °C and hold for 7 min. Detection was performed using full scan mode, m/z range 41-350.

For caffeine, trigonelline and caffeoylquinic acids (3-, 4-, and 5-) quantitative determination, samples were centrifuged for 5 min at 5000 rpm and filtrated with a hydrophobic PTFE membrane filter (Whatman, USA). Diluted samples were analysed with HPLC. A 1100 HPLC system (Agilent, Germany) was used, consisting of degasser, quaternary pump, column thermostat and diode array detector (DAD) operating at 254 nm, 272 nm and 324 nm. A Gemini C18 column, 5 μm 250 x 4.60 mm (Phenomenex, USA) and gradient elution (methanol and 1% phosphoric acid) were used. An average standard deviation of 5% on analytical determination was obtained.

Statistical analysis was performed by using The Unscrambler v 9.7 (Camo Software SA, Norway).

RESULTS AND DISCUSSION

Thermodynamics data, in terms of "in tank" initial extraction temperature (Ti) (see Scheme 1 C), initial *strombolian phase* extraction temperature (Tstr) (see Scheme 1 F) and maximum extraction temperature (Tmax), together with "in tank" maximum pressure (Pmax), are reported in Table 1. In full agreement with previous data (Navarini et al., 2008) the coffee extraction in the *moka* begins at temperature significantly lower than water boiling point.

In particular, in most of the different explored experimental conditions, Ti falls in the range 56 ± 1 °C and it is not correlated to particle size, coffee dose and heating power. The resulting aqueous extract (the so called "first drop" as shown in Scheme 1 C) is characterized by a temperature within the range 50-60 °C. The other "in tank" temperatures as well as Pmax, are strongly affected by factors contributing to increase coffee bed compaction. In particular by increasing coffee dose and/or reducing the particle size, temperatures and Pmax steadily increase, as reported in Table 1.

Table 1. Extraction "in tank" temperatures and maximum pressure as function of particle size, dose and heating power.

Coffee sample	Dose (g)	Heating power (W)	Ti (°C)	Tstr (°C)	Tmax (°C)	Pmax (bar)
A	13	400	54	116	120	0.90
A	13	600	52	120	125	1.30
A	15	400	60	117	121	1.05
A	15	600	54	123	129	1.60
A	17	400	55	122	126	1.30
A	17	600	55	130	136	1.90
B	13	400	62	127	135	2.00
B	13	600	57	133	140	2.30
B	15	400	58	132	137	2.20
B	15	600	56	134	140	2.70
B	17	400	59	134	141	2.70
B	17	600	57	137	145	3.05

By fixing both coffee dose and particle size, the increase in the heating power enhances both temperatures and Pmax in addition to speed up the coffee extraction. In Figures 2 and 3, the "in tank" pressure as a function of time is reported at 400 W and 600 W heating power, respectively.

Under standard usage conditions (150 g of water filling of the tank and 15 g of coffee powder filling of the funnel) and at 600 W heating power, sample A leads to a typical extraction yield of 24.0 ± 0.1% whereas sample B to 29.4 ± 1.0%. Extraction yield ranging from 18% to 22% have been proposed as the most acceptable, as far as brew quality is concerned. The coffee brews above 24% are considered to be over-extracted (Lingle, 2001). From the present data, even if for both samples A and B the extraction yield exceeded the upper limit of 22%, sample B only leads to a substantial over-extraction.

On the basis of the thermodynamics data, the observed high extraction yield can be reasonably related to the high water temperatures reached in the tank at the beginning of the *strombolian phase*. It has been suggested (Navarini et al, 2008) that high temperature extraction fluids (vapour, water and their mixture) transit in the coffee bed is noxious for the

quality of the brew because, under these conditions, such fluids are more efficient in solubilizing less soluble compounds, generally conferring bitterness and astringency (Petracco, 2005), and/or in stripping least volatile aroma compounds which are organoleptically unpleasant and described as clove-like, smoky, burnt, medicinal/chemical (Lingle, 2001). This view is reinforced by two different reported findings. In particular, aromatics with boiling point in the range 100-250 °C, extracted by steam pressure coffee extraction are from 1.9 to 4.6 times higher than that of the most popular coffee brewing methods (Peters, 1991). Moreover, the total area of a selection of 11 volatile compounds identified in coffee brews prepared with different brewing methods by using *Coffea arabica*/*Coffea Canephora* blend, is higher for *moka* brew in comparison with coffee brews prepared with other methods (Lopez-Galilea et al., 2007) and for some volatile compounds the content in the *moka* brew is the highest.

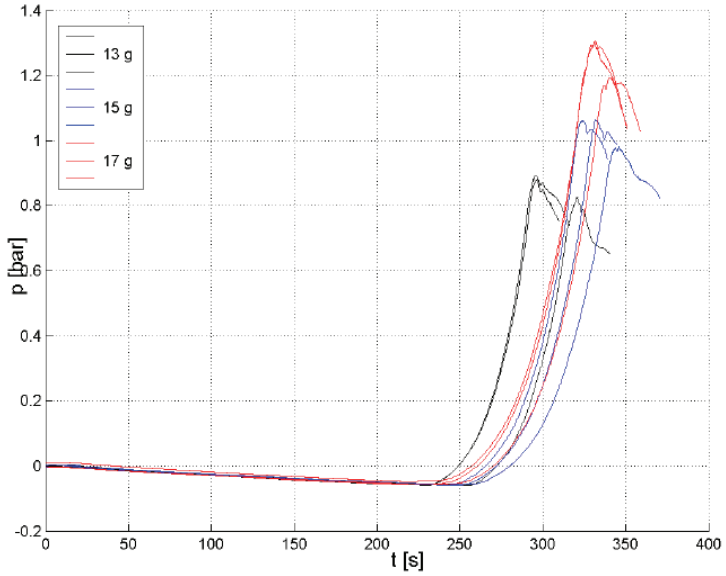


Figure 2. Sample A "in tank" pressure as a function of time. Heating power: 400 W.

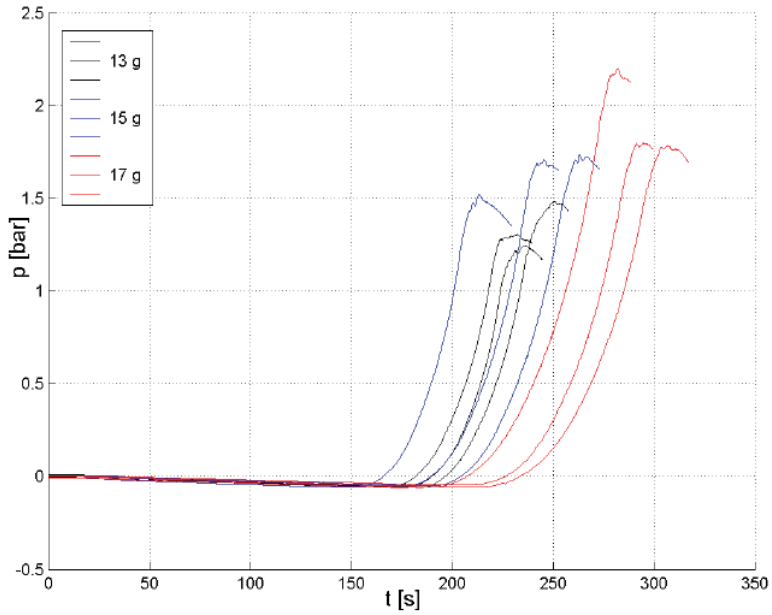


Figure 3. Sample A "in tank" pressure as a function of time. Heating power: 600 W.

In order to provide further evidence in favour to the suggested noxious role played by *strombolian* phase (or "extraction tail") on the brew quality, chemical compounds in brew fractions have been determined. For the sake of practicity and reproducibility, to perform the fractionation, a *moka* coffee maker differing in geometry, construction material, heating source and power has been used. In order to compare this apparatus with that used for thermodynamics data acquisition, unfractionated brew has been characterized in terms of extraction yield. By using samples A and B, typical extraction yields of 22.0 ± 0.4% and 26.0 ± 1.0%, have been determined, respectively. In spite of very different experimental conditions, the performances of the two different apparatuses resulted to be very close each other.

In Table 2, total solids, caffeine, trigonelline and 3-, 4- and 5-caffeoylquinic acids (3-CQA, 4-CQA and 5-CQA, respectively) concentrations in the different fractions are reported.

Table 2. Non-volatile compounds concentration in *moka* brew sequential fractions.

Fraction	Total solids (mg/mL)	Caffeine (mg/mL)	Trigonelline (mg/mL)	3-CQA (mg/mL)	4-CQA (mg/mL)	5-CQA (mg/mL)
A1	52.0 ± 1	3.18	0.48	0.81	0.93	1.65
A3	27.0 ± 1	1.70	0.21	0.35	0.41	0.74
A6	17.7 ± 1	0.70	0.09	0.15	0.17	0.31
B1	55.3 ± 1	3.08	0.46	0.72	0.85	1.52
B3	28.3 ± 1	2.15	0.28	0.44	0.52	0.93
B6	17.8 ± 1	0.56	0,05	0.12	0.14	0.26

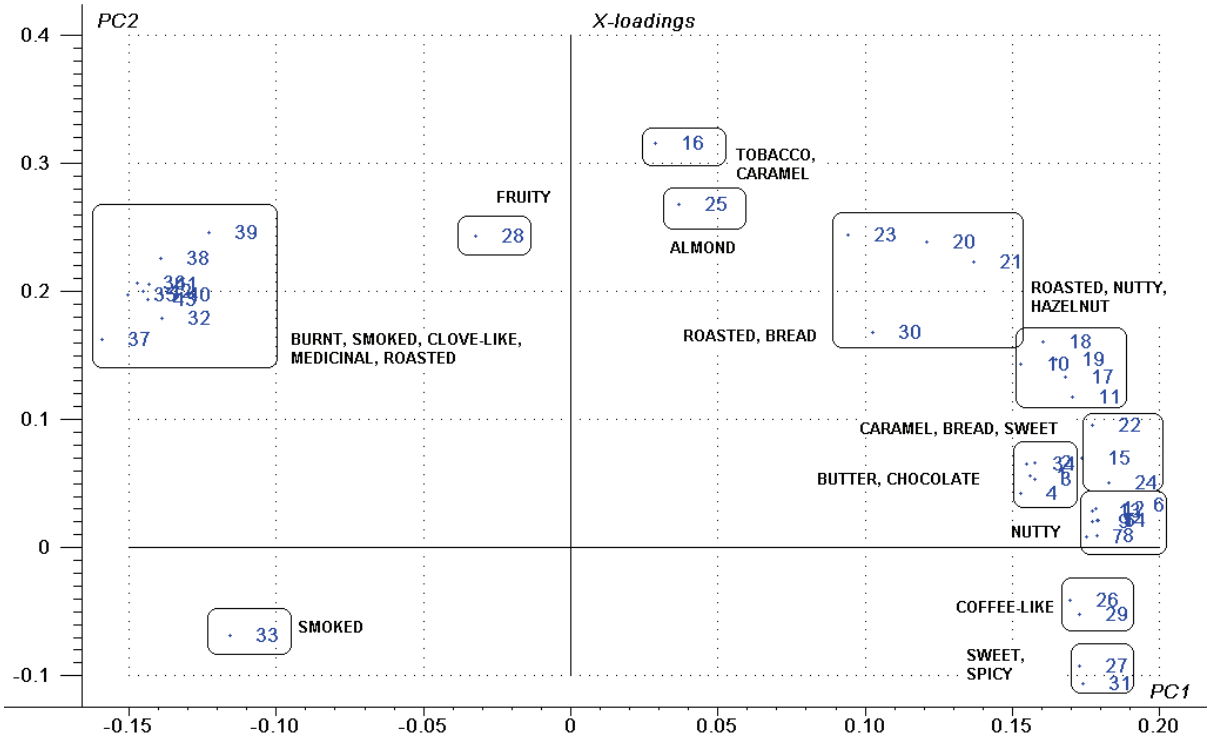


Figure 4. PCA of the *moka* brew fractions (PC1: variance explained 65%; PC2: variance explained 20%)

It is evident from Table 2, the concentration decrease in passing from the first fraction to the last one, being the latter 5-6 times less concentrated in all of the considered non-volatile

compounds. The relative distribution of the selected compounds is not altered in passing from the initial to the final extraction phases. It has to be stressed that in view of the water solubility of the considered non-volatile compounds, the figure traced by Table 2 is consistent with the expectations. In other words, the temperature gradient which developed during the *moka* extraction as well as the extraction fluids nature (vapour, water and their mixture) are not expected to influence the relative distribution of the considered compounds in the fractions. However, it is clear that from a sensory point of view, the different analytes concentrations determined in the fractions, can play a relevant role in the taste perception.

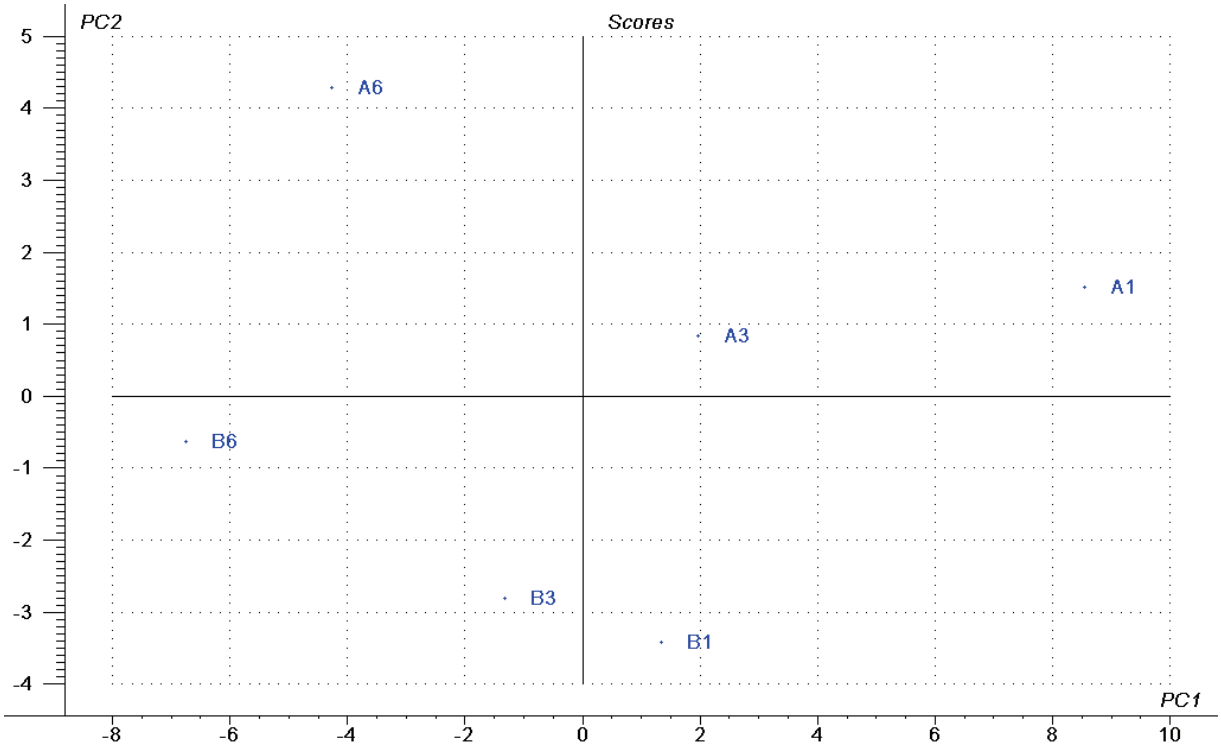


Figure 5. Principal component loadings for all the chemical compounds (PC1: variance explained 65%; PC2: variance explained 20%).

Very different the situation as far as the volatile compounds distribution in the different fractions are concerned. By statistical processing of the SBSE-GC-MS chemical data through a Principal Component Analysis (PCA), it is possible to assign the differences among the fractions to their chemical composition. As shown in Figure 4, where a bidimensional representation of PC1 and PC2 scores is reported, all the fractions collected during the steam pressure coffee extraction are separated each others. The loadings reported in Figure 5 clearly shows that fractions A1 and B1 are characterized by positive aroma, volatile compounds described, for instance, as butter, chocolate, nutty, but also by pungent notes, whereas fractions A6 and B6 are characterized by volatile compounds described as burnt, smoky, medicinal and clove-like notes. Fractions A3 and B3 are characterized by several compounds described with an overall positive aroma notes. In Table 3, the chemical compounds numbered in Figure 5 are reported together with the corresponding sensory impression taken from the literature.

Table 3. Volatile compounds identified in *moka* brew sequential fractions.

Number	Name	Sensory impression	Reference
1	2-Methylbutanal	sweet, malty, chocolate	Flament (2001), Counet et al. (2002), Schnermann & Schieberle (1997)
2	3-Methylbutanal	fruity, malty, chocolate	Flament (2001), Counet et al. (2002), Schnermann & Schieberle (1997)
3	2,3-Pentanedione	buttery, caramel, sweet, fruity, fresh	Flament (2001), Rychlik et al. (1998)
4	1-methyl pyrrole	metallic, green, beany	Flament (2001)
5	Pyridine	penetrating odour	Flament (2001)
6	Trimethyl-oxazole	burnt, nutty, hazelnut	Flament (2001), Counet et al (2002)
7	Pyrazine	pungent, corn-like	Flament (2001)
8	2-Furfuryl methyl ether	mustard, nutty, coffee	Flament (2001), US Patent 3952024
9	Methylpirazine	nutty, hazelnut, green	Flament (2001), Counet et al. (2002)
10	4-methyl-thiazole	nutty	Flament (2001)
11	Pyridine, 3-methyl-	green, earthy, hazelnut	Flament (2001), Counet (2002)
12	Pyrazine, 2,5-dimethyl-	roasted, nutty, grassy	Flament (2001)
13	Pyrazine, 2,6-dimethyl-	sweet, nutty, fried,	Flament (2001)
14	Pyrazine, ethyl-	nutty, roasted, green, buttery, rum	Flament (2001)
15	Pyrazine, 2,3-dimethyl-	green, nutty, roasted, hazelnut	Flament (2001), Counet et al. (2002)
16	Pyridine, 3-ethyl-	tobacco, green, caramel, nutty, roasted	Flament (2001), Counet et al. (2002)
17	Pyrazine, 2-ethyl-6-methyl-	earthy, musty	Maetzu et al (2001)
18	Pyrazine, 2-ethyl-5-methyl-	roasted	Flament (2001)
19	Pyrazine, 2-ethyl-3-methyl-	nutty, roasted	Flament (2001)
20	Pyrazine, 2,6-diethyl-	roasted, bread	Flament (2001), El-Saharty et al. (1998)
21	Pyrazine, 3-ethyl-2,5-dimethyl	roasted, bran	Flament (2001), El-Saharty et al. (1998)
22	2-Furancarboxaldehyde	bread, caramel, cinnamon	Flament (2001)
23	2-Furfurylmethylsulfide	roasted	Flament (2001), El-Saharty et al. (1998)
24	2-Acetylfuran	tobacco, sweet	Flament (2001)
25	Benzaldehyde	almond	Flament (2001)
26	2-Furanmethanol, acetate	floral, herbal, spicy	Flament (2001)
27	5-Methyl-2-furfural	sweet, spicy, warm	Flament (2001)
28	2-Furanmethanol, propanoate	fruity, green, pear	Flament (2001)
29	2-formyl-1-methylpyrrole	coffee	Flament (2001), Maetzu et al (2001)
30	N-methyl-2-acetylpyrrole	coffee, meaty	Flament (2001)
31	2-Furanmethanol	burnt, sweet, roasted, caramel	Flament (2001), Bredie (2006)
32	Furfurylpyrrole	roasted, chocolate, hay-like	Flament (2001), Counet et al.(2002)
33	Guaiacol	smoked, sweet, phenolic, burnt	Flament (2001), Counet et al.(2002), Maetzu et al (2001)
34	2-Acetylpyrrole	bread, walnut, cocoa, nut	Flament (2001), Schnermann & P. Schieberle (1997), Rychlik et al. (1998)
35	Difurfuryl ether	coffee, mushroom, salicilate	Winter et al. (1976)
36	3-methoxy acetophenone	smoked	Flament (2001)
37	Ethylguaiacol	smoked, clove-like, spicy, medicinal	Flament (2001), Rychlik et al. (1998)
38	2-Methyl-6-hydroxyquinoline	toasted	Flament (2001)
39	6-methoxy 2-methyl quinoline	burnt, roasted	Flament (2001)
40	Vinylguaiacol	clove-like, spicy, smoked	Flament (2001)
41	1-furfuryl-2-formyl pyrrole	burnt, pop corn	Flament (2001), Winter et al. (1976)
42	1-furfuryl-2-acetyl pyrrole	fruity	Flament (2001), Winter et al. (1976)
43	1H-Indole	stable, tarry	Flament (2001)

CONCLUSIONS

The present investigation is the first attempt to objectively correlate the steam pressure coffee extraction conditions, which develop inside a *moka* coffee maker, in terms of thermodynamics, to the beverage quality, in terms of aroma and chemical profile. Strong temperatures and pressure gradients as well as extraction fluid nature govern the *moka* coffee extraction. Differently from all the other popular preparation methods, during *moka* coffee brewing the extraction conditions vary time by time leading to an aqueous extract which results to be chemically very different from the beginning to the final phase. The latter, in which the extraction fluid is a system constituted by water, substantial vapour and their mixture at high temperature, appears to be detrimental from an aroma point of view, as suggested by the chemical data of the examined sequential fractions. In fact, the volatile compounds described as burnt, smoky, medicinal and clove-like, have been found to be responsible for the discrimination of the fractions collected under *strombolian* phase. Coffee dose, coffee powder particle size and heating power, have been found to affect the onset of *strombolian* phase and then the beverage quality from the chemical point of view.

Of course, the experimental results of the present investigation have to be validated resorting to the sensory analysis, and this will be the topic of future work.

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Coffee Sensory Quality in the Industry

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SUMMARY

This study aimed to verify the sensorial quality of commercial coffee brands supplied to Brazilian consumers according to NMQ-Program Minimum Quality Level of Coffee that is defined in the Resolution SAA-28 of June/01/2007, the 'Technical standard for defining the identity and quality of roasted coffee and ground roasted coffee'. This standard establishes criteria for assessment of the overall coffee beverage quality and classifies as Traditional, Superior or Gourmet. The research conducted with 276 samples of commercial brand roasted coffees in beans and ground showed that from 141 samples of coffee quality supposedly 'Traditional', 39.7% did not reach the sensory profile and overall quality of beverage minimum of 4.5 according to the scale of assessment established in the legislation. These coffees were classified as 'not recommended for supply'; 49.1% out of 57 'superior' coffees samples were classified as 'traditional' quality; among 49 ground and roasted coffee 'gourmet' samples, 46.9% were rated as 'superior' coffees and among 29 roasted beans 'gourmet' samples for 'expresso' coffee, 13.8% showed classification as 'superior' quality coffee. Thus, this research allowed the visualization of the importance of quality programs, particularly the NMQ Program, emphasizing the need to maintaining it in order to obtain continuous improvement of coffee quality. This program should be consolidated in the industry for the product to be much more appreciated by the consumer.

INTRODUCTION

The commoditization of the roasted and/or ground coffee market originated in the eighties, accentuated by a lack of differentiation between most of the coffee brands, implying low aggregated values, predatory competition and negative results for most of the companies. One of the consequences of this situation was the gradual loss of the coffee qualities, in a desperate search to lower production costs, as an alternative to guarantee competitiveness and the survival of the Brazilian coffee roasters. Coffee consumption was reduced by 5% between the years of 1999 and 2000, even if this period is the one, during which the product became cheaper for the consumer. To recover its position in the market and to increase sales, a proposal of classification of the roasted and/or ground coffee market was made, through the differentiation of the coffee qualities.

The Government of the State of São Paulo established technical specifications for its purchases of roasted and ground coffee, through Resolution SAA-37, starting in 2001, which considered the methodology to evaluate Traditional, Superior and Gourmet coffees (Governo do Estado de São Paulo, 2001). The classification by quality category using the technique of sensorial analysis was established for the minimum characteristics of quality which should be met by the roasted coffee, in beans or ground, with conformity within an interval of Global Quality for each category (Gourmet from 7.31 to 10; Superior from 6.51 to 7.30; Traditional from 3.5 to 6.5 and Not recommended for supply below 3.5).

In 2004, Resolution SAA-37 was revised and only the minimum acceptable global quality level was raised from 3.5 to 4.5 (Governo do Estado de São Paulo, 2001; 2004), imposing the industry to improve the quality of 'Traditional' coffees. In 2007 these Resolutions were repealed by SAA 28 of June/01/2007 (Governo do Estado de São Paulo, 2007) with the lower limit of 'Gourmet' coffee adjusted to those levels previously established in resolutions SAA 6 and SAA 7, both of May/20/2003, and the lower limit of 'Superior' coffee was also reduced, valid both for roasted bean and ground coffee (Governo do Estado de São Paulo, 2003).

Today, we live in an incessant search for 'quality' in all types of organizations, whether of products or services, as a factor of survival and competitiveness. Whatever the market requires, the companies are forced to meet. Increasing coffee consumption is the great challenge which everyone pursues in many countries, whether they are producers/consumers or only consumers of the product (ABIC, 2008).

The Brazilian Association of the Coffee Industry - ABIC, aware of the changes in these times, started the Coffee Quality Program - PQC in 2004, having understood that the alternative to solve the bottlenecks which inhibit competition with other categories of beverages was to continue to raise consumption by offering diversified and higher quality products. Therefore, Brazilian roasters are being stimulated to participate in the Coffee Quality Program - PQC, where a company is certified, receiving a symbol of quality with the sensorial characteristics of the product. One of the purposes of the Program is to inform the quality of the coffee which is being sold, it also allows the consumer to identify the type of bean used in each brand and with this information, to choose the flavor which is most liked. Currently, more than 250 brands are certified by PQC throughout Brazil (ABIC, 2008).

According to the Technical Standard to Establish the Identity and Quality of Roasted and Ground Coffee (Governo do Estado de São Paulo, 2007), 'Traditional´ Coffees are those constituted by coffee beans type 8 COB or better, with a maximum of 20% in weight of defective black, green, burnt beans, accepting the use of past year productions and light green beans in any beverage. It is recommended to avoid the presence of black, green or fermented beans. 'Superior´ Coffees are those constituted of type 2 to 6 COB coffees, Arabica or blended with Robusta/Conillon coffee, soft to hard beverage, with a maximum of 10% defective black, green and burnt beans, accepting the use of past year productions and light green coffees, balanced in the cup. The 'Gourmet´ coffees should be constituted of solely ´soft´, ´soft´ or strictly ´soft´ Arabica beverage coffees of types 2 to 4 COB with the absence of defective black, green and burnt, black-green and fermented beans.

In relation to global quality, the 'Traditional´ coffees are within the quality scale between 4.5 and 6.0, corresponding to 'Regular'. Referring to the sensorial characteristics, one perceives the presence of strange odour and flavour, low acidity, moderate bitterness and astringency and less ´body´ than the ´Superior´ and ´Gourmet´ category coffees. 'Superior' coffees fit within the quality scale between 6.0 and 7.3, corresponding to 'good' coffee and are sensorially characterized by having a characteristic aroma, low to moderate acidity, moderate bitterness, characteristic and balanced flavour, free from fermented, moldy and earthy flavour, low astringency and reasonably full-bodied. The 'Gourmet' coffees fit into the quality scale between 7.3 and 10.0, corresponding to 'very good'. In reference to the sensorial characteristics, they have marked and intense characteristic aroma, low to high acidity, typical bitterness, characteristic flavour, balanced, clean, free from foreign flavour, no astringency and full-bodied (Governo do Estado de São Paulo, 2007).

Thus, with the growing requirement of the market for 'quality' and the increase in coffee consumption, 276 samples of commercial brand roasted coffees were evaluated, in beans and

ground, to verify the behavior of the sensorial attributes and the global quality of the coffees supplied to Brazilian consumers.

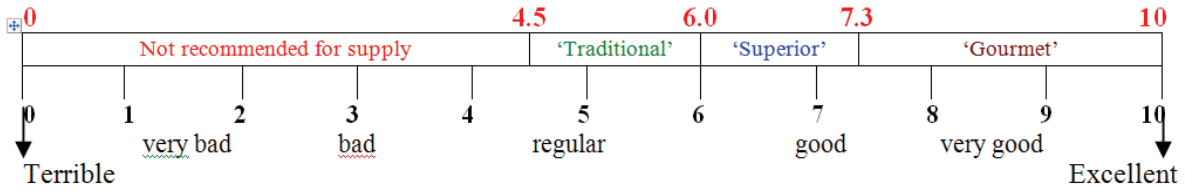
MATERIALS AND METHODS

Sensorial analyses were performed, during the period from July, 2007 to February, 2008, on 141 commercial samples of roasted and ground coffee supposedly of 'Traditional' quality, 57 'Superior', 49 'Gourmet' and 29 samples of 'Gourmet' roasted coffee in beans for ´Espresso´ coffee. The roasted and ground samples were prepared according to the methodology described in the technical standard, using a proportion of 50g of roasted and ground coffee for 500ml of mineral water at 92 °C, percolated in a paper filter and packaged in thermos bottles. The roasted bean samples were prepared in a SAECO brand automatic machine to produce an ´Espresso' coffee beverage.

A sensory quantitative descriptive analysis of the beverage was made by a selected and trained team composed of five to ten panelists using a non structured scale from 0 to 10 cm to evaluate the fragrance of the ground coffee, aroma, defects, acidity, bitterness, flavor, residual flavor, astringency and body of the beverage, with a final evaluation of the global quality of the coffee, according to terminology and evaluation sheet developed by Mori et al. (2000).

The analysis was individually performed in cabins with red lighting, equipped with the computer *Compusense Five* version 4.8 system to collect the data; the samples were presented with three digit alleatory codes and evaluated in relation to known sensorial reference samples of 'Traditional', 'Superior' and 'Gourmet' quality, respectively, comparing these to the supposed quality of the coffees evaluated.

The classification system to conclude the quality evaluation of the product was that of Technical Standard to Fix the Identity and Quality of Bean and Ground Roasted Coffee (Governo do Estado de São Paulo, 2007), with the following global quality scale:



RESULTS AND DISCUSSION

The classification of coffee by category in relation to global quality showed that from 141 samples of roasted and ground coffee of supposedly 'Traditional' quality 60.3% confirmed the 'Traditional' quality and 39.7% were classified as 'Not recommended for supply. Of the 57 samples of roasted and ground coffee supposedly of 'Superior' quality 50.9% confirmed the 'Superior' quality and 49.1% were classified as ´Traditional'. Of the 49 samples of roasted and ground coffees supposedly of 'Gourmet' quality 53.1% confirmed 'Gourmet' quality and 46.9% were classified as 'Superior'. And of the 29 samples of roasted beans of supposedly 'Gourmet' quality, 86.2% confirmed 'Gourmet' quality and 13.8% were classified as 'Superior'.

Figure 1 shows the sensorial mean profiles obtained in the sensorial descriptive analysis and Figure 2 present the configuration of the samples of roasted and ground coffees supposedly of 'Traditional', 'Superior', 'Gourmet' and 'Gourmet - Espresso' quality obtained by analysis of

the Principal Components Technique - ACP, using data of all attributes of the samples analyzed.

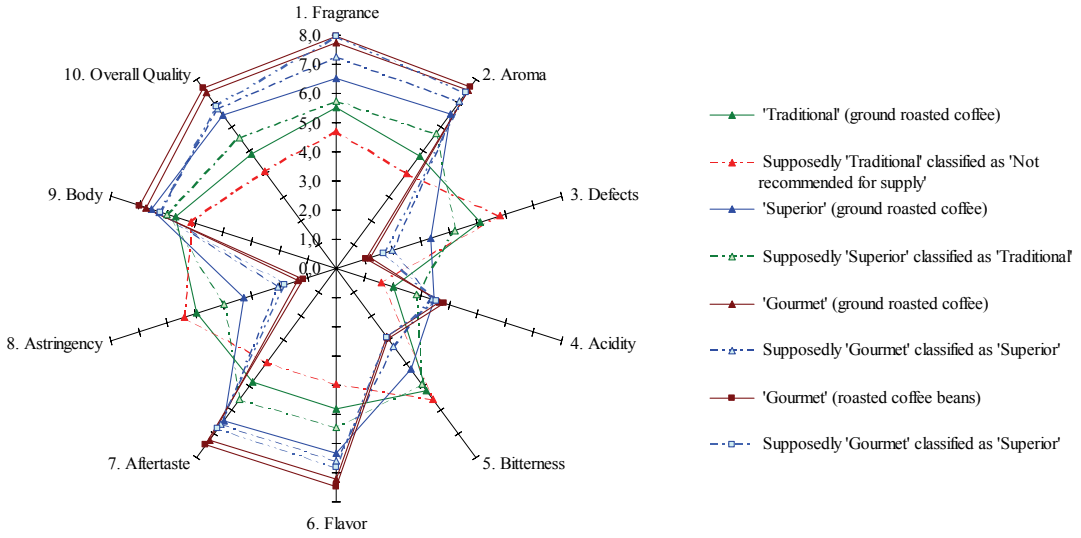


Figure 1. Mean sensorial profile of the coffee samples classified as 'Gourmet', 'Superior', 'Traditional' and 'Not recommended for supply'.

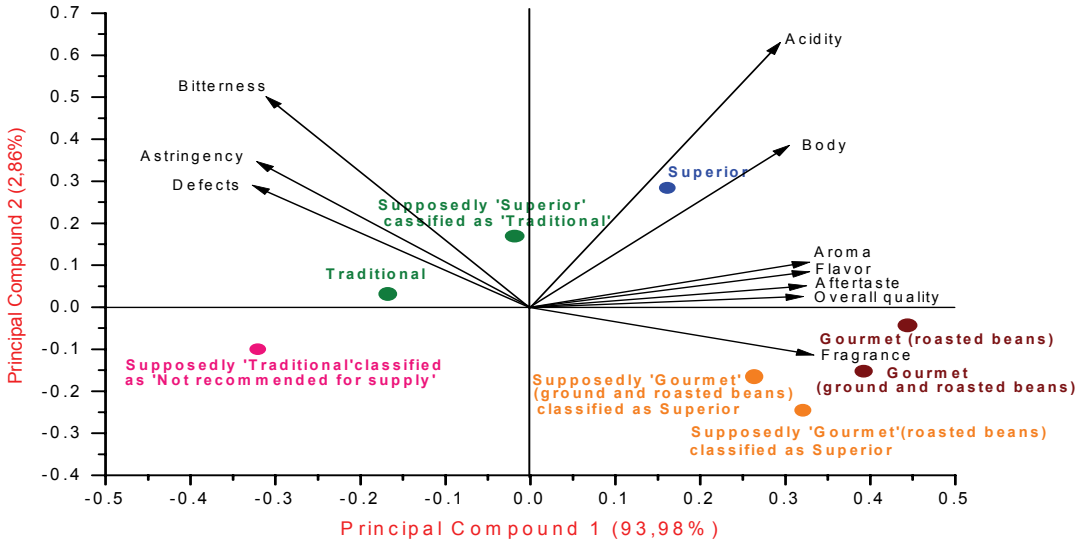


Figure 2. Configuration of the coffee samples obtained by the technique of Principal Components Analysis - ACP.

The principal components 1 and 2 explain 96.8% of the total variation, and the coffees classified as 'Gourmet' and those supposedly 'Gourmet' (roasted beans and roasted and ground) classified as 'Superior' stand out by presenting high intensity of fragrance attributes of the ground beans, residual flavour, flavour and aroma of the beverage, and consequently, high grades of global quality in the coffee beverage. The quality coffees confirmed as 'Superior' are characterized by having more body with moderate acidity and the supposedly of 'Superior' quality classified as 'Traditional' and those confirmed as 'Traditional' are characterized by presenting more defects, bitterness and astringency. The coffees classified as of 'Not recommended for supply' quality, are characterized by presenting higher intensities of bitterness, defects and astringency and low intensity in the other attributes, consequently receiving low grade in global quality of the coffee beverage.

In a general manner, it is possible to visualize the importance of maintaining and consolidating Quality Programs, specially the NMQ Program, for monitoring the quality of commercial coffees classifying them by the global quality of the beverage stimulates the industry to surpass its present position, to modernize and to go for new demands of the consumer market, which requires differentiated coffees of better quality and larger aggregated value.

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Effects of Lactic Acid Bacteria Isolated from Fermented Coffee *(Coffea arabica)* on Growth of *Aspergillus ochraceus* and Ochratoxin A Production

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SUMMARY

The main mould in coffee that produces ochratoxin A (OTA) is *A. ochraceus.* It has been shown that the native flora from fermented coffee reduces the *A. ochraceus* growth. The purpose of this study was to investigate the effects of lactic acid bacteria isolated from coffee while being fermented on growth of *A. ochraceus* and ochratoxin A (OTA) production. Three predominant lactic acid bacteria strains were isolated and identified as *Leuconostoc mesenteroides* dextranicum, *Lactobacillus brevis* y *Lactobacillus plantarum*. They were tested on growth rate and OTA production by *A. ochraceus* on MRS agar, on PDA agar and coffee meal extract agar (CMEA). The results showed that the isolated bacteria were able to inhibit growth of *A. ochraceus* and OTA production. Also, the three lactic acid bacteria and the cell-free supernatant were able to prevent spore germination of A. *ochraceus* in MRS broth. Coffee fermentations performed using a starter culture of three lactic acid bacteria and an inoculum of *A. ochraceus* confirmed a significant reduction of OTA production by *A. ochraceus*. As a way to prevent OTA production in coffee, this study offers evidence to use a starter culture of lactic acid bacteria in coffee fermentation as biological control for moulds that produce OTA in coffee.

INTRODUCTION

Coffee fermentation is the bio-chemical decomposition of the mucilage covering the pulped beans, which constitutes an obstacle to drying (Sivetz and Desrosier, 1979). Coffee fermentation is produced by natural occurring microbiota for varying lengths of time depending on climatic conditions and fruit ripeness. The microflora during the fermentation of coffee is constituted by pectolytic bacteria *Erwinia herbicola* and *Klebsiella pneumonia,* with optimal activities at pH 8.5, whereas fermentation conditions are acidic (5.3-3.5) (Avallone et al., 2001). The most frequent lactic acid bacteria isolated were *Leuconostoc mesenteroides*, *Lactobacillus plantarum* and *Lactobacillus brevis.* The yeast *Kloeckera*, *Candida* and *Cryptococcus* genuses have been isolated from coffee fermentations bacs. These microorganisms could be responsible for the alcoholic taste of the coffee beverage after overfermentation (Avallone et al., 2001). According to Avallone et al. (2002), the mucilage descomposition seems to be correlated to acidification and not to enzymatic pectolysis.

Ochratoxin A (OTA) is the main mycotoxin known in coffee. OTA occurrence in coffee beans can be due to both environmental conditions and processing conditions (Suárez-Quiroz et al., 2005). OTA was present before storage, indicating the possibility that harvesting and post-harvest handling of coffee cherries could be the critical steps leading to contamination (Suárez-Quiroz et al., 2005). Ochratoxin can be attributed to cherry contamination “in the

field" by toxigenic moulds, with toxin production occurring before or after harvesting and, in this case, during or after the process, which presupposes the persistence of OTA producing moulds. Frank (1999) observed that there was usually a reduction in the number of filamentous fungi during fermentation, to the benefit of yeasts (Frank, 2001). *A. ochraceus* was unable to grow properly in the presence of the native microbial population and was therefore susceptible to competition (Suárez-Quiroz et al., 2005). The yeast *P. anomala*, *P. kluyveri* and *H. uvarum* were found to reduce growth of *A. ochraceus* and prevent the OTA production. The previous study proposes the possibility of using yeast in biological control of OTA-producing fungi during coffee fermentations (Masoud et al., 2005). The microbial control of coffee fermentation microflora would be possible in order to limit the off-flavour development, to reduce the growth and production of OTA by toxigenic fungi and to standardize the final coffee quality. The use of an inoculum of lactic acid bacteria would be preferable in order to stay as close as possible to the natural fermentation, where the acidification is important. The LAB have shown to restrict the growth of the most important toxigenic fungi thereby reducing the formation of harmful toxins during the malting and brewing (Lowe and Arendt, 2004). Mixed inocula of LAB for use as inoculants of coffee fermentations would be to significantly reduce growth and OTA production by *A. ochraceus*. The purpose of this study was to investigate the effects of lactic acid bacteria isolated from coffee during fermentation on *A. ochraceus* growth *and* ochratoxin A (OTA) production.

MATERIALS AND METHODS

Coffee samples

Coffee cherries (*Coffea arabica*) were harvested in a plantation from the Coatepec area (Xalapa, Mexico).

Cultures

Strains of LAB were isolated from coffee fermentation tanks from Coatepec region (Veracruz, Mexico). The *A. ochraceus* strain (MULC 44640) with the potential to produce OTA was isolated from the same coffee region.

Culture media

MRS, PDA and Coffee meal agar (CMA) were utilized in this study.

Coffee samples and processing

Coffee cherries (*Coffea arabica*) were hand-picked and external mesocarp was mechanically eliminated by dry pulping (DH4 PENAGOS depulper). Strains were tested on 6-Kg batches of depulped beans contaminated with OTA producing strain of *A. ochraceus*.

OTA quantification

From coffee beans, the coffee was dried at 70 °C, frozen at –80 °C then ground. Samples were analyzed according to Nakajima. OTA was detected and quantified by HPLC (Shimadzu Corporation, Japan). The OTA content of the PDA, MRS, PDA and CMA medium was quantified using a modified version of the agar plug method (Bragulat et al., 2001; Nakajima et al., 1997).

RESULTS AND DISCUSSION

Effect of LAB predominant in coffee fermentation on growth of *A. ocharceus*

L brevis and *L. plantarun* were found to inhibit growth of *A. ochraceus* when grown together. On MRS medium *L. plantarun* was found to have a stronger effect on grown of *A. ochraceus* compared to *L. brevis.* On PDA and CMA media, the levels of growth inhibition by the three strains were low. The CMA medium may contain less specific nutrients for grown of LAB. *A. ochraceus* showed very good growth in both LAB free plates of CMA and PDA.

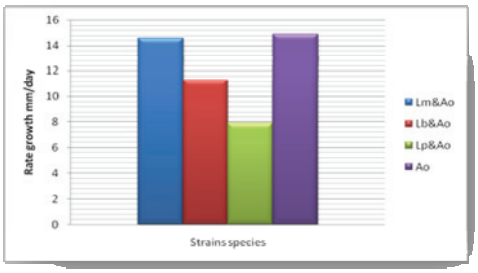

Figure 1. Growth rate of *A. ochraceus* (Ao) on MRS plates inoculated with three strains of LAB. *Ln. mesenteroides* (Lm), *L. brevis* (Lb) and *L. plantarum* (Lp) compared to the control.

Effect of LAB on production of OTA

A.ochraceus was found to produce OTA when grown on LAB-free PDA, MRS and CMA plates. When the LAB tested were co-cultured with *A. ochraceus*, OTA production was extremely reduced. Although the LAB did not inhibit growth of *A. ochraceus* completely, they were able to reduce the production of OTA.

Table 1. Ochratoxin A production by *A. ochraceus* in PDA, MRS and CMA plates inoculated with LAB strains compared to the control.

Medium	***Ln.mes. vs A. ochraceus (µg/g)[a]***	***L. brevis vs A. ochraceus (µg/g)[a]***	***L. plantarum vs A. ochraceus (µg/g)[a]***	***A. ochraceus (µg/g)[b]***
PDA	67.0	43.8	41.0	112.0
MRS	15.8	14	15.4	80.0
CMA	15.9	14.2	12.1	67.0

ANOVA (P= 0.95) α= 0.022832

Effect of LAB cells and LAB supernatant on germination of *A. ochraceus* spores.

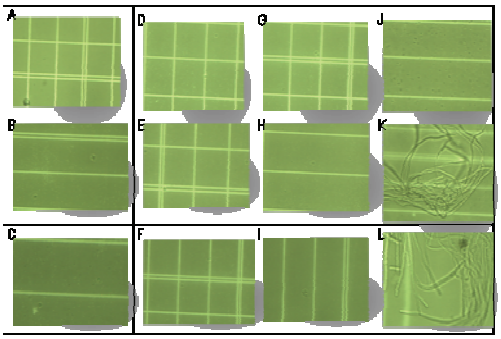

Figure 2. Germination of *A. ochraceus* spores incubated at 35 °C when co-cultured with cells of *Ln mesenteroides* in MRS both after 24, 48 and 72 h (A, B and C); with *L. brevis* (D, E and F); with *L. plantarum* (G, H and I); when inoculated without LAB (J,K and L).

Effect of LAB on OTA production by *A. ochraceus* in coffee fermentation bacs

Coffee fermentations conducted with a started culture of LAB in coffee beans contaminated with *A. ochraceus* spores were found to inhibit the OTA production compared to LAB-free coffee fermentations. Reduction OTA production might result by production of extra-cellular compounds or volatile compounds produced by LAB or the competition for nutrients, as reported for yeast (Masoud et al., 2005).

Table 2. OTA production in coffee beans inoculated with *A. ochraceus* and LAB inoculum plus *A. ochraceus* in laboratoty-scale coffee fermentations.

Harvest	***A. ochraceus (µg/kg)***	***LAB / A. ochraceus (µg/kg)***
2005-2006	2.1	0.695
2006-2007	2.5	0.260

ANOVA P= 0.95 α= 0.02529

CONCLUSION

The most important factor during coffee processing is the prevention of OTA-production by toxigenic fungi. As a way to prevent OTA production in coffee, this study offers evidence to use a starter culture of lactic acid bacteria in coffee fermentation as biological control for moulds that produce OTA. Future studies on sensory characteristics of coffee brewed and processed by this method are needed

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Analytical Method for Monitoring Freshness of Roast and Ground Coffee

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SUMMARY

An analytical method was developed for monitoring freshness deterioration of R&G coffee. The method consists of monitoring the content of a certain thiol and its oxidation product using GC-MS analysis of the sample's static headspace. It was found that the oxidation-product/thiol ratio, hereinafter the Oxidation Index (OI), clearly describes the freshness state of a given R&G product regardless of its type or origin. Results show good correlation between OI levels and operating conditions such as degassing time and exposure to oxygen. The OI advance was significantly accelerated upon oxygen exposure of the R&G coffee prior to packaging, whereas inert conditions slowed its progress. Pattern of the index for Arabica and Robusta did not differ significantly, under similar manufacture conditions. Results indicate that the OI progressively increases along the product shelf life regardless of operating conditions. Also, good correlation was found between specific index level and a point of sensory deterioration as identified by expert panel.

INTRODUCTION

Coffee freshness deterioration is known to occur via two main pathways: One is a rapid loss of highly volatile compounds, characteristic of fresh coffee; the second is aroma destruction and off-flavors formation due to oxidation processes (Barbera, 1967). Although several analytical markers were suggested to monitor freshness deterioration of R&G coffee, their use is often laborious and requires quantification and/or calibration (Clarke and Macrea, 1985; Heiss and Radke, 1977). However, the major weakness of using marker's concentration to estimate freshness relates to the fact that the amount of any single compound depends, among others, on its initial concentration. This, in turns, is affected by variables such as blend, roast degree, grinding profile etc (Geiger et al., 2005). Hence, comparing freshness state of different samples of coffee using these methods is not possible without knowledge of the samples initial conditions. In this work, we propose a rapid universal method to determine freshness state of R&G coffee regardless of its type and technological parameters.

MATERIALS AND METHODS

Turkish and Filter coffee samples were produced by Strauss-Coffee BV; Espresso products were purchased from local grocery shops.

Each coffee sample was placed in a 20 ml headspace vial for 10 min at 60 °C. 1.0 ml headspace was then collected by headspace autosampler and injected into a GC-MS. Separation was carried out on a DB-5 column. The Oxidation Index (OI) was calculated from the peak area ratio of dimethyldisulfide/methanethiol. Each data point in the figures is the mean ± SEM of 3 samples.

RESULTS AND DISCUSSION

The main contribution of this work was the ability to predict R&G coffee freshness at any point in time using the relationship between an important, sensitive aroma compound and its oxidation product. Figure 1 depicts the relevancy and sensitivity of the **Oxidation Index** (**OI**) to the presence of oxygen as measured with Turkish coffee. As can be seen 30 days after packaging, the OI of coffee packed at the presence of air increased rapidly and with a steep slope compare to its advance under inert conditions.

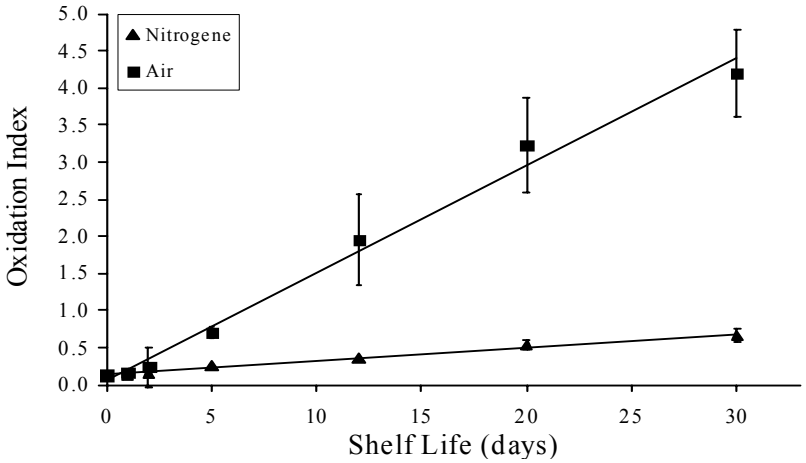

Figure 1. Oxidation of "Turkish coffee" 100gr soft packages, packed under air or nitrogen atmosphere.

A typical behavior of the OI along the shelf life of a Turkish coffee is depicted in Figure 2 where the progressive nature of coffee oxidation, despite the inert environment, is revealed. Also indicated in Figure 2 the OI level (0.7) and time (60 days) at which the Expert Sensory Panel already noted the beginning of staling. It is important to point out that OI values at which staleness is recognized by sensory methods (data not shown) vary from product to product, probably as a result of process parameters and Arabica/Robusta percentage. Several useful applications of the OI as a tool for technological assessments, process modifications as well as freshness benchmarking are shown in Figure 3-5**.**

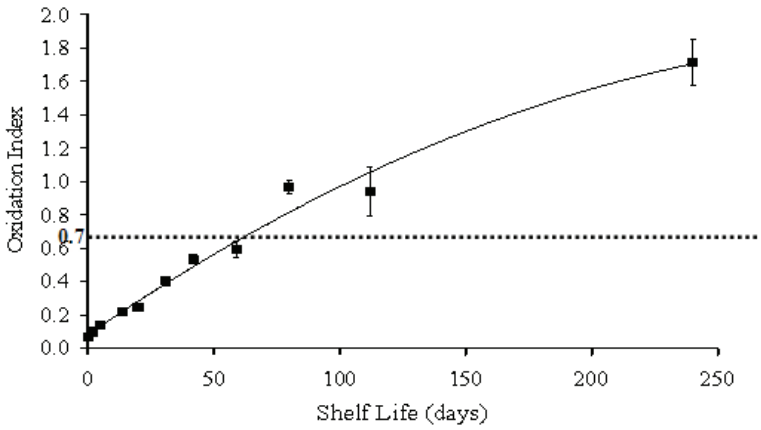

Figure 2. The oxidation progress of Turkish coffee packed under nitrogen atmosphere along 8 months shelf life.

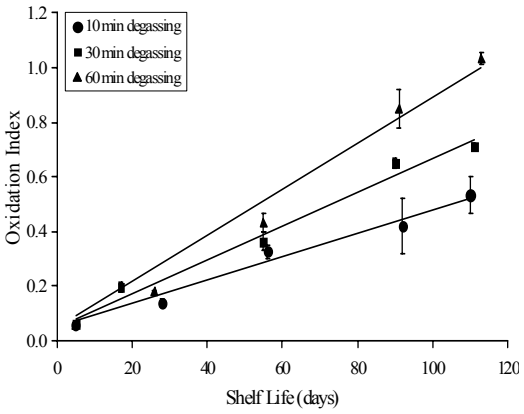

Figure 3. Effect of degassing time on the potential freshness of R&G coffee along its shelf life.

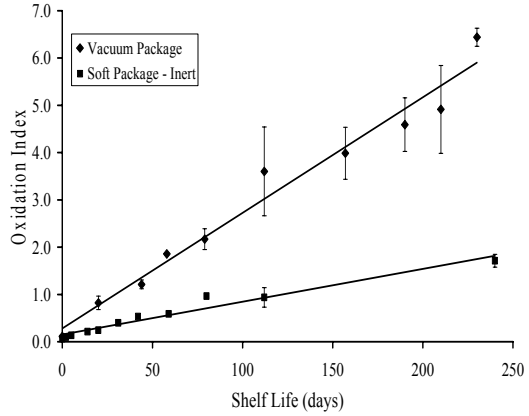

Figure 4. Inert gas-flashed soft packages Vs vacuum packages (Turkish coffee; 100g pillow and 200gr vacuum packages).

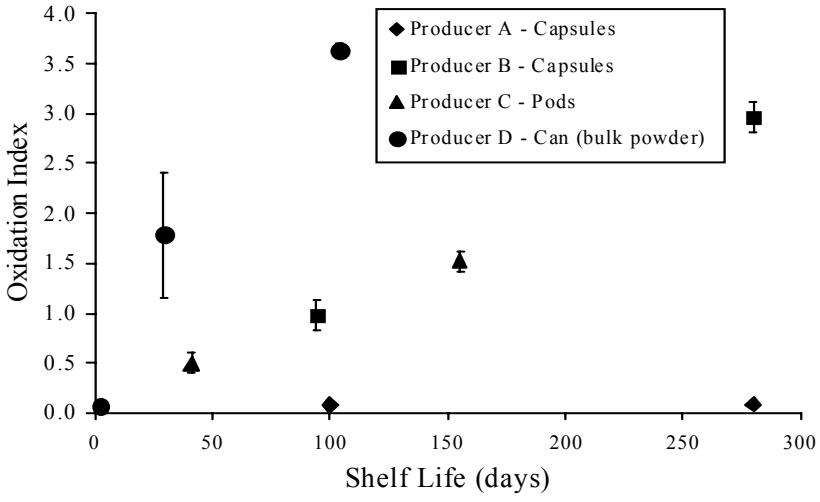

Figure 5. Espresso freshness benchmarking among various products manufactured by different producers.

Results of this study, demonstrate the sensitivity, and applicability of the OI (peak area ratio dimethyldisulfide/methanethiol) as a freshness marker of R&G coffee.

The OI revealed as a useful feedback tool for technological assessment of the production process quality. The suggested method is rapid, simple and allows to compare freshness amongst different R&G coffee products.

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Characterization of Non-Defective and Defective Roasted Arabica and Robusta Coffees by Electrospray Ionization-Mass Spectrometry (ESI-MS)

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SUMMARY

The objective of the present study was to verify if ESI-MS provides discrimination of defective and non-defective roasted coffees. Defective (black, immature, light sour and dark sour) and non-defective beans of both Arabica and Robusta species were manually separated and roasted at 200 °C for 1 h. Aqueous extracts of defatted roasted and ground coffee beans were analyzed by direct infusion electrospray ionization mass spectrometry (ESI-MS) in the both the positive (ESI(+)-MS) and negative (ESI(-)-MS) modes. Multivariate statistical analysis (PCA) was performed in order to verify the possibility of discrimination between roasted Arabica/Robusta and defective/non-defective coffees based on EI-MS profiles. Separation between Arabica and Robusta samples was observed, based on the profiles obtained in the positive mode. Grouping of fermented Arabica beans was observed for PCA analysis of both ESI(+)-MS and ESI(-)-MS spectra. Such results indicate that EI-MS analysis presents potential for the development of a analytical methodology for detection of defective beans in roasted and ground coffee.

INTRODUCTION

The presence of defective coffee beans is known to depreciate the quality of the coffee beverage consumed worldwide (Franca and Oliveira, 2008). These beans represent about 15 to 20% of the total coffee produced in Brazil and, although they are separated from the non-defective beans, they are still commercialized in the coffee trading market. Furthermore, there are no analytical methodologies that allow for detection and quantification of defective beans in roasted coffee, and, thus, an assessment of chemical attributes that could allow for differentiation between defective and non-defective beans is of relevance (Mancha Agresti et al., 2008).

A recent study (Mendonça et al., 2008) has demonstrated the feasibility of employing ESI-MS for discrimination between defective and non-defective green coffees of both Arabica and Robusta species. Aqueous extracts of green (raw) defective and non-defective coffee beans were analyzed by direct infusion electrospray ionization mass spectrometry (ESI-MS). ESI-MS profiles in the positive mode (ESI(+)-MS) provided separation between defective and non-defective coffees within a given species, whereas ESI-MS profiles in the negative mode (ESI(−)-MS) provided separation between Arabica and Robusta coffees. In view of the aforementioned, it was the aim of this work to verify if such technique also allows for discrimination of defective and non-defective beans after roasting.

METHODOLOGY

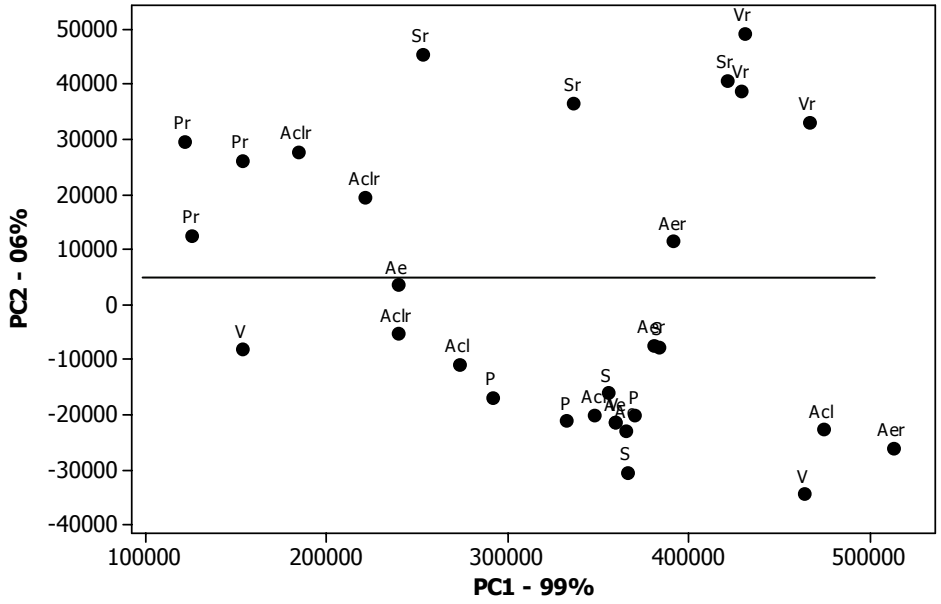
Arabica and Robusta green coffee samples (2003/2004 crop) were obtained from Samambaia Farm (Santo Antônio do Amparo, Minas Gerais, Brazil). Coffee beans were subjected to selection in an electronic sorter. The beans rejected by the sorting machine (low quality mixture) were used in this study. Black, sour (light and dark), immature and non-defective beans were manually separated from the Arabica and Robusta low quality mixtures to constitute five sampling lots (non-defective, black, immature, dark sour and light sour) for each coffee species (Arabica, Robusta), and then roasted at 200 °C for 1 h.

Ground coffee samples were defatted by ether extraction for 6 h in a Sohxlet apparatus (Tecnal, São Paulo, Brazil). Aqueous extraction of the defatted solid was performed employing an ultrasonic bath, consisting of 3 h extraction in deionized water at 25 °C (Mecozzi et al., 1999). A Q-TOF mass spectrometer (Micromass, Manchester,UK) was used for fingerprinting ESI-MS analysis. ESI-MS was performed by direct infusion of the aqueous extract, in both the negative and positive modes. The general conditions were: source temperature of 80 °C, capillary voltage of 2.5 kV and cone voltage of 40 V. Mass spectra were acquired and accumulated over 60 s and spectra were scanned in the range between 100 and 800 *m*/*z*.

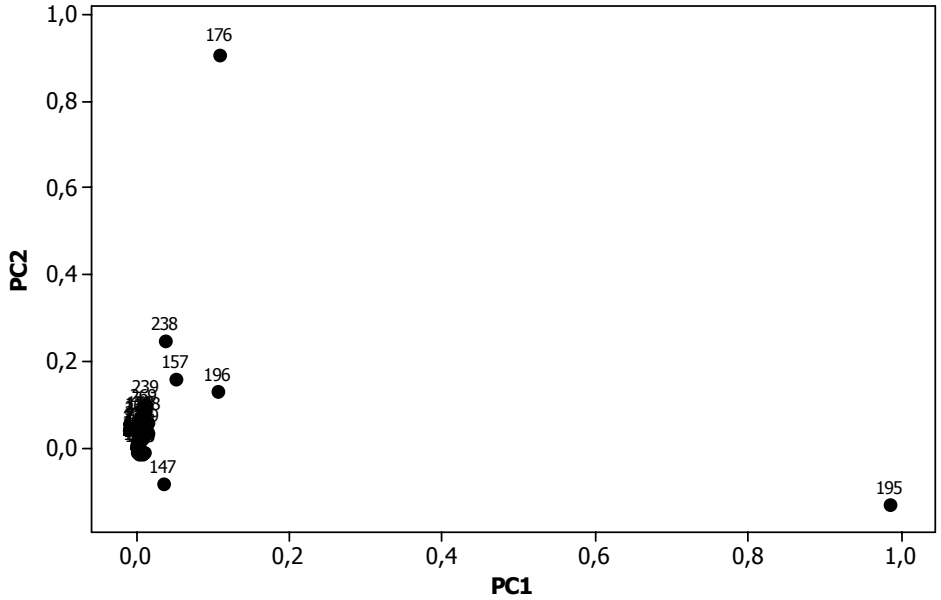
All mass spectra were handled using MassLynx 4.0 software (Waters, Manchester, UK). To discard noise signals, only the ions with a relative abundance higher than 5% were included in the final data matrix. For principal component analysis (PCA), data matrices were assembled so that each row corresponded to a coffee sample and each column represented the *m*/*z* ratios and intensities of detected ions.

RESULTS AND DISCUSSION

An analysis of the ESI(+)-MS fingerprints of non-defective and defective coffee beans of the Arabica and Robusta species, after roasting, indicated that all spectra were mainly characterized by the by the abundant ion of *m*/*z* 195 – identified as caffeine (Atoui et al., 2005) – and by a small number of low m/z cations (< 300). It was also observed that such low m/z cations seem to be associated to fermentation, in the case of Arabicas. The biplot of the Arabica and Robusta coffees ESI(+)-MS profiles is shown in Figure 1a. A clear separation between Arabica and Robusta samples can be observed, based on the second component, which presented positive and negative values for Arabica and Robusta samples, respectively. An evaluation of the loadings plot (Figure 1(b)) indicates that caffeine was the substance that presented the highest influence on PC1. The substance of m/z 176 (not identified) seems to be responsible for grouping of black beans of the Robusta species. Ionization in the negative mode provided spectra with larger number of peaks, but qualitatively similar. All spectra were mainly characterized by the by the abundant ion of *m*/*z* 191 – identified as quinic acid (Roesler et al., 2007; Santos et al., 2006), a product of thermal degradation of chlorogenic acids. PCA analysis of such spectra showed grouping of black and dark sour Arabica beans, but no separation between species was observed.



(a)



(b)

Figure 1. (a) PCA scores scatter plot and (b) loadings plot of ion abundance values obtained from the data of ESI(+)-MS fingerprints of Arabica and Robusta coffee samples. S, non-defective; V, immature; Acl, light sour; Ae, dark sour; P, black; r, Robusta.

CONCLUSIONS

A comparative evaluation of ESI-MS profiles of roasted coffees of the Arabica and Robusta species was performed, for both good quality (non-defective) and low quality (defective) coffees. ESI(+)-MS profiles allowed for separation between species due to differences in caffeine content. Grouping of fermented (black and dark sour) Arabica beans was observed in the analysis of ESI(−)-MS profiles. Results indicate that this technique presents potential for

the development of a analytical methodology for detection of defective beans in roasted and ground coffee. However, further studies are needed in order to identify specific substances that can be used as chemical markers and also the effect of roasting conditions on the methodology.

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Physical Characterization of Non-Defective and Defective Green and Roasted Arabica and Robusta Coffees

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SUMMARY

The objective of this work was to evaluate physical characteristics that would allow the discrimination of defective and non-defective coffees of both Arabica and Robusta species. It was demonstrated that separation by physical attributes can also be applied to Robusta coffees in a similar type-classification used for Arabica based on the definition of specific defects. Based on volume results for non-defective green coffee beans, it was observed that Arabica beans are larger than Robusta ones whereas no significant differences in non-defective bean densities were observed between Arabica and Robusta. A comparison between defective and non-defective beans indicates that all types of defective beans are smaller than non-defective ones, for both coffee species, prior to and also after roasting. Luminosity measurements indicated that, for both Arabica and Robusta green coffees, only black and dark sour defects can be separated from non-defective coffee based on electronic sorting by monochromatic light measurements prior to roasting. No significant differences in color parameters were observed after roasting, for both species.

INTRODUCTION

The coffee roasted in Brazil is considered to be of low quality, due to the presence of defective coffee beans, a key factor in depreciating the quality of the coffee beverage consumed worldwide. The most important types of defect are represented by black, sour or brown and immature beans (Franca and Oliveira, 2008; Mancha Agresti et al., 2008). Such defects represent about 15 to 20% of the total coffee produced in Brazil and, although they are separated from the non-defective beans, they are still commercialized in the coffee trading market. Currently there are no analytical methodologies that allow for detection and quantification of defective beans in roasted coffee, and, thus, an assessment of physical and chemical attributes that could allow for such differentiation between defective and non-defective beans is of relevance (Mancha Agresti et al., 2008). Therefore, it was the aim of this work to study physical characteristics that would allow the discrimination of defective and non-defective coffees of both Arabica and Robusta species.

METHODOLOGY

Arabica and Robusta green coffee samples (2003/2004 crop) were obtained from Samambaia Farm (Santo Antônio do Amparo, Minas Gerais, Brazil). Coffee beans were subjected to selection in an electronic sorter. The beans rejected by the sorting machine were used in this study and are designated as low quality mixture. For the Arabica samples, this mixture consisted in average of 1% black, 12% immature, 20% sour (7% dark colored and 13% light colored) and 67% non-defective beans in weight. For the Robusta samples, the low quality

mixture consisted in average of 5% black, 25% immature, 51% sour (19% dark colored and 32% light colored) and 19% non-defective beans in weight. Black, sour (light and dark), immature and non-defective beans were manually separated from the Arabica and Robusta low quality mixtures to constitute five sampling lots (non-defective, black, immature, dark sour and light sour) for each coffee species (Arabica, Robusta). Samples of randomly selected 100 beans were separated from each lot and roasted in a convective oven at 200 °C for 60 min.

Physical attributes evaluation was based on three samples of 100 beans randomly selected from each lot, before and after roasting. The average volumes of the beans were evaluated based on measurements of major, minor and intermediate diameters of individual beans. The volume was calculated based on the assumption that each bean could be approximated as half a triaxial ellipsoid (Dutra et al., 2001). Average bean density was evaluated as the ratio between the weight of the 100 beans sample and the sum of the individual bean volumes. Bulk volume was evaluated using a 50 mL graduated recipient. Color measurements were performed twenty times for whole beans and five times for ground beans for each sample, using a tristimulus colorimeter (Colortec PCM, Clinton, USA), with standard illumination D_{65}, and colorimetric normal observer angle of 10^{o}.

RESULTS AND DISCUSSION

Physical attributes of defective and non-defective Arabica and Robusta coffee beans before and after roasting are displayed in Table 1. It can be observed that non-defective Arabica beans are larger than Robusta. This also holds for defective coffees, i.e., the smallest Arabica beans (black) are still bigger than the biggest Robusta beans (non-defective). Thus, separation of Arabica and Robusta coffees by sieving can be accomplished, regardless of bean quality. A comparison between defective and non-defective beans indicates that all types of defective beans are smaller than non-defective ones. These results indicate that sieving can also be employed for separation of non-defective and defective coffee beans of a given coffee species.

An evaluation of the volumes of Arabica coffees after roasting shows that defective beans are still smaller than non-defective ones. This was not the case for Robusta coffees, with both black and non-defective beans presenting the same % increase in volume and the same weight loss. These results indicate that, after roasting, separation of defective and non-defective roasted beans by sieving will only be feasible for Arabica coffees.

No significant differences in non-defective and defective coffee bean densities were observed between Arabica and Robusta. Coffee bean density decreases during roasting, due to the simultaneous increase in volume and decrease in weight associated with the loss of water and volatile components (Franca and Oliveira, 2008; Dutra et al., 2001). After roasting, both black and dark sour Arabica beans presented higher density values in comparison to the other Arabica samples. This behavior has been previously reported for Arabica coffees of the same origin but a previous crop (Franca et al., 2005) and it is attributed to the fact that such defects present lower weight loss values and swell less than non-defective beans during roasting, thus attaining a lighter roasting degree. This was not the case for the Robusta samples, for which no significant differences between defective and non-defective beans were observed.

Table 1. Physical attributes of defective and non-defective Arabica and Robusta coffee beans[a].

	Non-defective	Immature	Light Sour	Dark Sour	Black
GREEN COFFEE					
Arabica					
Volume x 10^9 (m^3)	114.9±0.2^{a,x}	95.5±1.9^{b,x}	98.7±5.8^{b,x}	90.0±2.9^{b,x}	75.8±3.1^{c,x}
Bean density (kg m^{-3})	1293±17^{a,x}	1253±19^{ab,x}	1249±18^{ab,x}	1252±22^{ab,x}	1218±18^{b,x}
Bulk density (kg m^{-3})	625.6±2.2^{b,x}	621.4±8.1^{b,x}	648.8±1.0^{a,x}	609.0±6.8^{bc,x}	595.5±11.2^{c,x}
Robusta					
Volume x 10^9 (m^3)	73.7±2.1^{a,y}	51.7±2.0^{c,y}	70.1±1.2^{b,y}	66.0±4.4^{b,y}	42.5±2.6^{d,y}
Bean density (kg m^{-3})	1248±2^{d,x}	1275±3^{b,x}	1297±2^{a,x}	1265±4^{c,x}	1187±5^{e,x}
Bulk density (kg m^{-3})	626.6±8.0^{b,x}	642.5±1.7^{b,x}	661.6±6.9^{a,x}	641.6±6.9^{b,x}	560.5±8.0^{c,x}
ROASTED COFFEE					
Arabica					
Volume x 10^9 (m^3)	194.6 ± 7.3 ^{a,x}	160.8 ± 16.0 ^{bc,x}	170.6 ± 5.7 ^{b,x}	131.9 ± 13.1 ^{c,x}	91.0 ± 12.5^{d,x}
Bean density (kg m^{-3})	657±8^{c,y}	686±9^{c,y}	655 ± 1^{c,y}	737 ± 32^{b,x}	904 ± 12^{a,x}
Bulk density (kg m^{-3})	350 ± 2 ^{c,y}	361 ± 6 ^{c,y}	349 ± 4 ^{c,y}	396 ± 11 ^{b,y}	465 ± 8 ^{a,x}
Weight loss (%)	16,1 ± 0,4 ^{a,x}	14,5 ± 0,3 ^{b,x}	13,6 ± 0,6 ^{a,x}	12,1 ± 0,2 ^{c,y}	10,3 ± 0,5 ^{d,y}
Robusta					
Volume x 10^9 (m^3)	98.7±6,1^{a,y}	74.5±1.9^{b,y}	110.6±1.9^{a,y}	84.7±9.1^{b,y}	58.1±1.4^{c,y}
Bean density (kg m^{-3})	876±12^{a,x}	865±4^{a,x}	801±7^{b,x}	857±28^{a,x}	841±12^{a,y}
Bulk density (kg m^{-3})	467±5.0^{a,x}	468±2^{a,x}	427±4^{c,x}	446±4^{b,x}	399±4^{d,y}
Weight loss (%)	13.9±0.3^{a,y}	13.4±0.1^{a,y}	13.6±0.6^{b,x}	13.1±0.4^{ab,x}	12.6±0.4^{b,x}

[a] *Average value±Standard Deviation. Measurements of a specific parameter followed by the same letter in the same line (a,b) or in the same column (x,y) do not differ significantly by the Duncan test at 5% probability.*

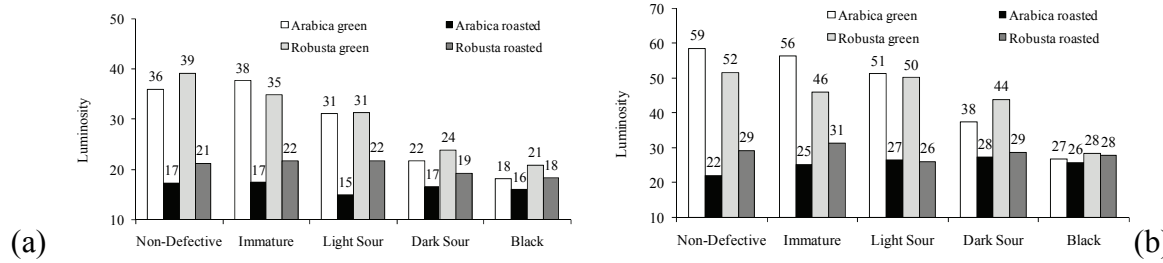

Figure 1. Average luminosity values for Arabica and Robusta coffee beans: (a) whole and (b) ground.

An analysis of luminosity values for whole beans (Figure 1a) indicates that there are no significant differences between Arabica and Robusta green coffees of the same quality. Black and dark sour beans presented lower luminosity values than non-defective, immature and light sour ones, indicating that this parameter can be successfully employed to separate black and dark sour defects from non-defective coffee prior to roasting, for both Arabica and Robusta coffees. After roasting, no significant differences in luminosity could be observed between defective and non-defective beans. Roasted Arabicas presented slightly lower luminosity values in comparison to the specific roasted Robustas, with the difference being less significant for black and dark sour beans. Luminosity values were higher for ground beans

compared to whole ones, confirming that the bean surface is darker than its core (Franca et al., 2005). No significant differences were observed for other color parameters.

CONCLUSIONS

A comparative evaluation of physical attributes of green and roasted coffees of the Arabica and Robusta species was performed, for both good quality (non-defective) and low quality (defective) coffees. Arabica coffee beans are bigger than Robusta, and defective beans are smaller than non-defective ones, regardless of coffee species. After roasting, separation of defective and non-defective roasted beans by sieving will only be feasible for Arabica coffees. Color measurements indicated that, for Arabica coffees, only black and dark sour defects can be separated from non-defective coffee based on electronic sorting by light measurements prior to roasting. Color separation between green defective and non-defective coffees will be effective for Robusta coffees.

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Discrimination Between Coffee Species (*Coffea arabica* and *Coffea canephora*) by the Content of Kahweol and Cafestol

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SUMMARY

Commercial coffee is mainly accounted for by two species: *Coffea arabica* (arabica) and *Coffea canephora* (conilon). Since these species differ in their commercial value, quality, and acceptability, adulteration and mislabeling are major concerns. High performance liquid chromatography (HPLC) has been used to characterize the composition of coffee samples and discriminate the species. The degree of roasting alters the levels of several constituents. Defective beans may also affect the final composition of the product. The aim of this work was to evaluate the possibility of discriminating arabica and conilon coffee species by their kahweol and cafestol contents. Samples of arabica, conilon, and their blends with different degrees of roasting and amounts of defective beans were investigated. After direct saponification and extraction with 3°butyl methyl ether, the samples were analyzed by reverse-phase HPLC with UV detection. A predictive model was generated and validated to estimate the proportion of conilon added to arabica coffee. The addition of conilon coffee reduced the diterpenes content. A linear model using kahweol and cafestol concentrations allowed a good fit ($R^2 = 0.93$) and the highest predictive power. Kahweol and cafestol showed potential for discrimination of coffee species.

INTRODUCTION

Commercial coffee consists almost entirely of *Coffea arabica (*arabica) and *Coffea canephora* (conilon) species. The former is considered to have superior quality and acceptability and is highly commercially valued. After roasting and milling, visual indications of the two species are eliminated and analytical techniques are required to discriminate the coffee species by their composition (Kemsley et al., 1995). HPLC has been largely used to analyze coffee compounds. The development of methods to differentiate coffee species in commercial products is necessary for the detection of either deliberate or accidental adulteration of arabica coffee with conilon. Kahweol and cafestol, two diterpenes in the unsaponifiable fraction of coffee, have been reported as potential discriminants, because their contents are different in the two species (Pettitt, 1987; Urgert et al., 1995). Their thermal stability is also higher than those of the majority of the water-soluble compounds. However, the composition of roasted coffee depends on factors other than the bean species. Roasting is responsible for the development of coffee flavor; however, it alters the original levels of several constituents. Defective beans may also affect the final composition of coffee (Farah et al., 2006; Ramalakshmi et al., 2007). The influence of roasting and defective beans must be considered when studying compounds with potential to discriminate species in commercial coffee whose amount of defective beans and degree of roasting are unknown. The aim of this work was to discriminate arabica and conilon species through measurement of their kahweol and cafestol content.

MATERIAL AND METHODS

Coffea arabica (A) and *Coffea canephora* (C) were supplied by the Instituto Agronômico do Paraná (IAPAR) and Companhia Iguaçu de Café Solúvel. In order to have a wide model, heterogeneous samples were used to allow for varieties, degree of roasting, and defective bean content variations. Light, medium, and dark roasted coffees (corresponding to around 13, 17, and 20% weight loss) were ground (0.84 mm). Three samples of each species, with different geographic origins and amounts of defective beans, were used to make the blends (A1/C1, A2/C2, A3/C3). Different contents (0, 20, 30, 40, 50, and 100%) of conilon coffees were added to arabica ones. Kahweol and cafestol levels were determined. The extraction of diterpenes was carried out by direct saponification with KOH, extraction with 3°butyl methyl ether, and clean-up with water (Dias, 2005). A reverse-phase column with isocratic elution with acetonitrile/water (55/45 v/v) was used in the detection and quantification of kahweol at 290 nm and cafestol at 230 nm.

The diterpene contents of the blends (A1/C1, A2/C2, A3/C3) were used to generate a predictive model and estimate the proportion of conilon added to arabica coffee (linear multiple regression) using Statistica™ 7.1 (Statsoft, 2006). The best model was chosen based on residual analysis, coefficient of determination, and correlation significance.

Other samples of arabica coffee cv IAPAR 59 (A4, A5) in three degrees of roasting (light, medium, and dark) and different contents of defective beans were used to verify the predictive capacity of the model generated. Sample A4 corresponded to type-8 coffee (max. of 360 defective beans/300 g of sample) (ABIC, 2008). All the defective beans were removed from a portion of A4 to obtain a defective bean-free coffee sample (A5). The percentage of conilon coffee in the samples was predicted using the previously defined model.

RESULTS AND DISCUSSION

Table 1 presents the kahweol and cafestol contents in arabica, conilon, and blends roasted to different degrees (light, medium, dark), considering the mean value for the three different blends (A1/C1, A2/C2, A3/C3). Considering the original values, the content of kahweol varied between 661 and 933 mg/100 g in arabica coffee, but it was not found in conilon. Cafestol content ranged from 275 to 478 mg in arabica and from 163 to 275 mg/100 g in conilon coffee. The literature describes levels near 730 mg of kawheol/100 g and from 100 up to 700 mg of cafestol/100 g in arabica. In *C. canephora*, 76 to 300 mg of cafestol/100 g and 0-13 mg of kahweol/100 g have been reported (Frega et al., 1994; Kurzrock and Speer, 2001; Rubayiza and Meurens, 2005; Urgert et al., 1995). The diterpenes did not degrade with increasing degree of roasting. The addition of conilon decreased the levels of diterpenes, mainly kahweol, in all degrees of roasting. The kahweol content ranged from 0 to 800 mg/100 g, while the cafestol content was reduced by half, from 400 to about 200 ng/100 g (Table 1).

The individual values found in the coffee blends generated a predictive model to estimate the proportion of conilon added to arabica. The model which used cafestol as a single variable had low predictive power. The kahweol content allowed a good fit of the model, which was improved by inclusion of the cafestol content. A linear model including both kahweol and cafestol contents (Equation 1) had the best fit ($R^2 = 0.93$, $p \leq 0.001$) and the highest predictive power (Table 2).

Table 1. Kahweol and cafestol contents in arabica and conilon coffees roasted at different degrees (light, medium, dark) and blends of conilon in arabica (0, 20, 30, 40, 50 e 100%).

	Conilon added in arabica	Roast degree		
		Light	**Medium**	**Dark**
Kahweol*	**0%**	771,7	820,0	798,0
	20%	611,0	644,3	607,0
	30%	483,5	572,5	434,5
	40%	564,0	630,0	584,0
	50%	394,5	350,5	280,5
	100%	n.d.	n.d.	n.d.
Cafestol*	**Conilon added in arabica**	**Light**	**Medium**	**Dark**
	0%	400,0	385,3	351,7
	20%	338,0	317,7	297,7
	30%	343,5	317,0	299,0
	40%	258,0	255,0	249,0
	50%	299,5	272,5	264,0
	100%	221,0	235,3	213,7

**Content of diterpene (mg/100g of sample), means values of three blends (A1/C1, A2/C2, A3/C3) ± SD.*

$$\% \text{ conilon} = 91{,}54 - 0{,}093*\text{kahweol} - 0{,}023*\text{cafestol} \quad [1]$$

The confidence limit (level of 95%) and the deviation between both values were calculated from the observed and predicted values generated by the model. The percent of conilon in arabica samples A4 and A5 was also predicted (Table 2). No dependence on the degree of roasting or the level of defects was observed. The percentage of kahweol predicted varied form 8 to -5, with a mean value of 1%, indicating that the kahweol and cafestol have discriminatory potential for the species.

Table 2. Predicted values of conilon in arabica coffee (A4 and A5).

Sample	Roast	Kahweol*	Cafestol*	Observed value	Predicted value	CL**	Deviation***
A4	Light	817 ± 5	453 ± 15	0	5	5/5	3,5
	Medium	855 ± 8	473 ± 17	0	1	1/1	0,7
	Dark	780 ± 56	472 ± 8	0	8	8/8	5,7
A5	Light	878 ± 33	427 ± 20	0	0	0/0	1,4
	Medium	923 ± 12	459 ± 3	0	-5	-5/-5	1,4
	Dark	901 ± 21	432 ± 19	0	-2	-2/-2	1,4

**Level of diterpene (mg/100g of sample), means of duplicate ± standard deviation;*
***Confidence limit (± 95%); ***Deviation between observed and predicted values.*

CONCLUSIONS

The diterpenes kahweol and cafestol showed potential to discriminate *Coffea arabica* and *Coffea canephora* in commercial blends. Kahweol provided greater discriminatory power than cafestol, since this diterpene has a wider range of variation among species. The content of diterpenes in arabica and conilon coffee with different degrees of roasting and different

fractions of defective beans allowed the generation of a model to predict the levels of conilon in blends.

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The Role of Defective Beans on the Composition of Kahweol and Cafestol in Roasted Coffee

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SUMMARY

Defective coffee beans, herein designated as PVA (black, immature and sour beans), are a crop by-product known to affect to beverage quality negatively. There are few reports regarding the lipid composition of defective beans. In particular, there have been no studies examining diterpenes, compounds present in the unsaponifiable fraction. Kahweol and cafestol, the two main diterpenes present in coffee, are known for their effects on human health. They are also considered to be potential discriminants of roasted arabica and conilon coffees, since diterpenes are present at different levels in these species. The aim of this study was to investigate the contents of kahweol and cafestol in roasted samples (*Coffea arabica* and *C. canephora* cv. Conilon medium roasted) with different proportions of defective coffee beans. The defective bean sample was characterized as presenting 37% sour, 16% black, 15% immature, 22% insect-damaged, and 10% broken beans. Reverse-phase HPLC was applied to quantify the compounds. The Cafestol content ranged from 220 (conilon) to 450 mg/100 g (arabica), while the kahweol content was around 900 mg/100 g of arabica; which was absent in conilon. There were no differences between defective and non-defective coffee samples.

INTRODUCTION

Defective beans, which represent 20% of coffee production, are the main cause for reduced roasted coffee quality. The principal defects are referred to as PVA, which stands for “preto, verde ardido”, the Brazilian denomination for black, immature and sour beans. These defects occur because of specific harvest or post-harvesting processing problems. Studies discussing defective coffee often focus on green coffee beans (Mazzafera, 1999; Ramalakshmi et al., 2007). Other studies have correlated the proportion of defective beans with the sensory characteristics of the beverages (Farah et al., 2006).

Coffee oil has been successfully used in the food and pharmaceutical industries. Coffee oil contains a relatively large amount of diterpene-rich unsaponifiable matter. Kahweol and cafestol, the main diterpenes present in coffee, have anticarcinogenic (Cavin et al., 2002) and antioxidant activity (Kurzrock and Speer, 2001), but they also raise the level of serum cholesterol in humans (Urgert and Katan, 1997). The study of these compounds is important to characterize the lipid fraction of coffee oil produced from defective beans, as lipid profile affects its potential industrial use. Kahweol and cafestol are reported as potential discriminants of roasted arabica and conilon coffees; therefore, it is also important to determine whether the presence of defective beans affects the levels of diterpenes at each species. The present study focused on the comparison of kahweol and cafestol levels in defective and non-defective roasted Brazilian coffee beans.

MATERIAL AND METHODS

Brazilian coffee, *Coffea arabica* cv. IAPAR 59 (A) and *C. canephora* cv. Conilon (C) supplied by Instituto Agronômico do Paraná were studied. Arabica and conilon coffees corresponded to type 8 (max. of 360 defective beans/300 g of sample) and type 4 (max. of 26 defective beans/300 g of sample), respectively, according to ABIC, the Brazilian coffee industry association (2008).

Beans were manually selected and all defective beans were removed from a portion of *C. arabica* sample (A) to obtain a defective bean-free coffee sample (A_0). A sample with 100% of defective beans was denominated A_{100}; the relative distribution of each kind of defect is presented in Figure 1. In addition, 10, 20, and 30% of defective beans (A_{100}) were added to sample A_0 to obtain blends A_{10}, A_{20}, and A_{30}. The letter C added to the blend name ($A_{10}C$, $A_{20}C$, $A_{30}C$) indicates the addition of 40% of *C. canephora* in *C. arabica* samples with defective beans (Table 1). Each sample was medium roasted (190-230 ºC, 5-10 min, 17% of weight loss) and ground (0,84mm particle size).

Table 1. Description of blends with different levels of defective beans and contents of *C. canephora*.

Sample*			Defects (%)
A_0			0
Blend	A_{10}	$A_{10}C$	10
	A_{20}	$A_{20}C$	20
	A_{30}	$A_{30}C$	30
A_{100}			100

** **Subscript**: level of defective beans (%)*
***C:** 40% of C. canephora in C. arabica (A_0) sample.*

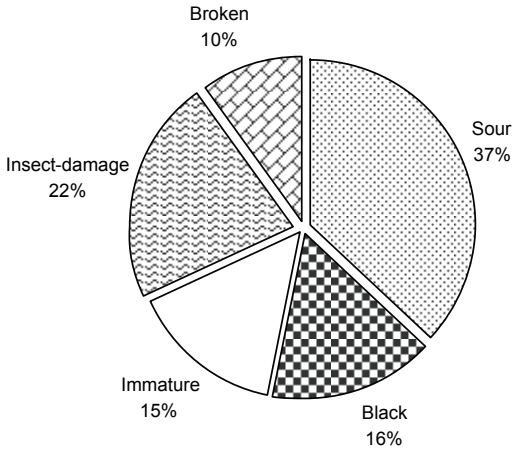

Figure 1. Relative distribution of defective beans in sample A_{100}.

The diterpenes were extracted according to DIAS (2008), using KOH (80 °C, 1 h) for saponification of the samples (0.20 g). Saponification was followed by extraction of unsaponifiable matter with 3°butyl methyl ether and cleaning-up with water. A reverse-phase HPLC column (Spherisorb ODS 1) with isocratic elution (55% of acetonitrile in water) was used for quantification. The maximum absorbance of kahweol (290 nm) and cafestol (230 nm) were assessed (Diode array UV/VIS detector). Results were expressed as mg of diterpene/100 g of roasted coffee (dry basis).

The results were analyzed by ANOVA (Tukey test, level of 5%) using Statistica™ 7.1 (Statsoft, 2006).

RESULTS AND DISCUSSION

The cafestol contents of Arabica samples agreed with the literature values, but higher amounts of kahweol (around 900 mg/100 g) than are usually cited were observed (Table 3). Levels of kahweol from 100 mg to more than 730 mg/100 g of arabica coffee have been reported, while cafestol contents between 100 and 700 mg of cafestol/100 g of arabica have been found (Kurzrock and Speer, 2001; Frega et al., 1994; Urgert et al., 1995; Lago, 2001; Rubayiza and Meurens, 2005). These variations may be due to differences between varieties or the use of different extraction methods. An aggressive extraction method that includes high temperature solvents and long processing times may also lead to the formation of artifacts and oxidation products. In addition, long high-temperature extractions can be less efficient than a method that applies mild temperature for a short time, as was done in this work. For example, while this study used 80 °C for 1 h to saponify the coffee lipids, followed by cold extraction, Frega et al. (1994) and Urgert et al. (1995) used hot extraction at 90 °C for 6 h.

The cafestol content of conilon coffee was found to be 221 mg per 100 g; however, kahweol was not found in this species (Table 3). The literature indicates that the amount of cafestol typically ranges from 76 to 300 mg/100 g of *C. canephora* (Kurzrock and Speer, 2001; Frega et al., 1994; Rubayiza and Meurens, 2005). The literature also reports that *C. canephora* contains lower amounts of kahweol than *C. arabica*, but there is no consensus about the values. Some researches indicate the absence of kahweol in *C. canephora,* while other works report amounts up to 13 mg/100 g coffee (Kurzrock and Speer, 2001; Dias, 2005; Frega et al., 1994; Rubayiza and Meurens, 2005; Nackunstz and Maier).

Table 3. Diterpenes* (mg/100g) in roasted coffee produced with defective and non-defective beans.

Arabica samples			Blends (40% of conilon in arabica)		
Sample	Kahweol*	Cafestol*	Sample	Kahweol*	Cafestol*
A_0	$923^a \pm 12$	$459^a \pm 3$	$A_{10}C$	$525^a \pm 35$	$394^a \pm 13$
A_{10}	$895^a \pm 3$	$449^a \pm 7$	$A_{20}C$	$543^a \pm 1$	$402^a \pm 10$
A_{20}	$913^a \pm 24$	$462^a \pm 10$	$A_{30}C$	$529^a \pm 23$	$408^a \pm 3$
A_{30}	$863^a \pm 17$	$430^a \pm 6$	Conilon Sample		
A_{100}	$932^a \pm 55$	$434^a \pm 15$	C	0 ± 0	221 ± 10

**Mean values with the same letter in the same column do not differ significantly (5%).*

The amount of diterpenes in *C. arabica* coffees was independent of the amount of defective beans (A_0, A_{10}, A_{20}, A_{30}, and A_{100}). The same behavior was observed for blends with conilon (Table 3). Since the diterpene content in roasted arabica and conilon coffees differs, and the occurrence of defective beans does not affect the amounts of diterpenes in these species, kahweol and cafestol show a good discriminating potential of these species in commercial blends.

Furthermore, these results are important when considering that defective beans constitute nearly 20% of the total coffee production and that they affect the sensory characteristics of the beverages negatively. The defective bean oil may be studied for alternative use in the food and pharmaceutical industries.

CONCLUSION

There are no differences in the kahweol and cafestol contents in roasted pure arabica coffee and arabica-conilon blends regardless of the presence of defective beans. Kahweol and cafestol have a good potential as discriminants of arabica and conilon species in blends.

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Residual Pesticides in Coffee

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SUMMARY

S. ISHIMITSU & CO., LTD. is a green beans importer in Japan. We have conducted residual pesticides analyses for almost all of green coffee beans we purchased since 2003. The detection rate for the samples was 3.3% after the positive list system was introduced. Residual pesticides over the maximum residue limits of Food Sanitation Law in Japan were found in some samples, but all of the pesticides disappeared after roasting. Some of these pesticides were caused by an unexplained contamination. And some of residuals were considered to be from jute bags. In some cases, the residual concentrations for jute bag samples were more than 100 times those for green coffee beans or raw materials in the bags.

INTRODUCTION

On May 29, 2006 the Ministry of Health, Labour and Welfare (MHLW) in Japan introduced the positive list system for pesticides, feed additives and veterinary drugs remaining in foods (http://www.mhlw.go.jp/english/topics/foodsafety/positivelist060228). Strict standards, less than 0.01 ppm, were applied to more than 600 pesticides for green coffee beans. If the residual levels get over the standards, the product is enjoined from domestic distribution without any risk evaluation for human health. An accidental contamination could cause violation of the law. And any trivial contamination could lead to a serious problem.

S. ISHIMITSU & CO., LTD. is a green coffee beans importer in Japan. We conduct residual pesticides analyses for almost all of green beans we purchase.

In this study, the result of analyses from June 2006 to March 2008 was summarized, and some recommendations to coffee producing countries were made.

MATERIALS AND METHODS

Green coffee beans

Green coffee beans samples are listed in Table 1. Some samples were taken from domestic stock and the others were sent from countries of origin by air.

Table 1. Details number of samples checked from June 2006 to March 2008.

Origin	Brazil	Colombia	Indonesia	Ethiopia	Vietnam	Others
Number of samples	391	368	278	255	76	498

Table 2. Target pesticides and their quantification limits (QL).

Compound	QL(ppm)	Compound	QL(ppm)	Compound	QL(ppm)
2,4,5-T	0.02	Diuron	0.002	Myclobutanil	0.02
2,4-D	0.004	Edifenphos	0.02	DDT	0.01
Acephate	0.02	Endrin	0.02	Omethoat	0.01
Acetamiprid	0.02	EPN	0.02	Oxamyl	0.02
Acrinathrin	0.02	EPTC	0.02	Parathion	0.02
Aldicarb	0.1	Ethion	0.02	Parathion-methyl	0.02
Aldicarb-Sulfone	0.3	Ethoprophos	0.02	Penconazole	0.02
Aldicarb-Sulfoxide	0.01	Etrimfos	0.02	Pendimethalin	0.02
Atrazine	0.001	Fenarimol	0.02	Permethrin	0.01
Azoxystrobin	0.001	Fenitrothion	0.02	Phenthoate	0.02
Bentazone	0.02	Fenobucarb	0.001	Phorate	0.02
BHC	0.01	Fenpropathrin	0.02	Phosalone	0.02
Bifenox	0.02	Fensulfothion	0.02	Phoxim	0.01
Bifenthrin	0.01	Fenthion	0.02	Pirimicarb	0.02
Butamifos	0.02	Fenvalerate	0.02	Pirimiphos-methyl	0.02
Cadusafos	0.02	Fluazifop	0.001	Prochloraz	0.02
Captafol	0.05	Flucythrinate	0.02	Procymidone	0.02
Carbaryl	0.001	Fludioxonil	0.02	Profenofos	0.02
Carbendazim	0.002	Fluvalinate	0.02	Propaphos	0.02
Carbofuran	0.001	Folpet	0.02	Propiconazole	0.02
Carbofuran-3-Hydroxy	0.002	Halfenprox	0.02	Propoxur	0.002
Chlorfenapyr	0.02	Hexaconazole	0.02	Prothiofos	0.02
Chlorfenvinphos	0.02	Hexythiazox	0.002	Pyraclofos	0.02
Chlorothalonil	0.02	Imidacloprid	0.01	Quinalphos	0.02
Chlorpropham	0.02	Indoxacarb	0.01	Quizalofop-ethyl	0.02
Chlorpyrifos	0.02	Iprodione	0.02	Resmethrin	0.02
Chlorpyrifos-methyl	0.02	Isofenphos	0.02	Sethoxydim	0.02
Cyanazin	0.02	Isoprocarb	0.002	Simetryn	0.02
Cyanophos	0.02	Kresoxim-methyl	0.02	Tebuconazole	0.002
Cyfluthrin	0.02	Lenacil	0.02	Tebufenpyrad	0.02
Cyhalothrin	0.02	Lufenuron	0.01	Tefluthrin	0.02
Cypermethrin	0.02	Malathion	0.02	Thenylchlor	0.02
Cyproconazole	0.02	Metalaxyl	0.002	Thiabendazole	0.001
Daminozide	0.02	Methabenz thiazuron	0.02	Thiobencarb	0.02
Deltamethrin	0.02	Methamidophos	0.02	Thiodicarb	0.002
Diazinon	0.02	Methidathion	0.02	Tolclofos-methyl	0.02
Dichlofluanid	0.02	Methiocarb	0.02	Triazophos	0.02
Dichlorvos	0.02	Methomyl	0.01	Trichlamide	0.02
Diethofencarb	0.02	Metolachlor	0.02	Trichlorfon	0.02
Dimethoate	0.001	Metribuzin	0.02	Trifluralin	0.02
Dimethomorph	0.02	Metsulfuron Methyl	0.001		
Dimethylvinphos Z-type	0.02	Monocrotophos	0.001		

Roasted beans

In case residual pesticides were detected, additional analyses were conducted after roasting. The degree of roast was about L = 25 for *arabica* coffee, and about L = 33 for *canephora* coffee.

Methods

SPT method (Satoh et al., 2001; http://www.aisti.co.jp/aisti-homepage/food.htm) was arranged and performed with GC/MS (GCMS-QP2010, SHIMADZU CORPORATION) and LC/MS/MS (Quattro Micro, Waters). The list of target pesticides is given in Table 2.

RESULTS AND DISCUSSION

The detection rate for the samples was 3.3%. Detected pesticides and their maximum residue levels (MRLs) are listed in table 3. In some cases, the concentrations of pesticides were over the MRLs. The rate of violation of Food Sanitation Law in Japan was 0.3%. All of the residual pesticides disappeared after roasting, but never allowed to be imported. With regard to green coffee beans for Japan, it would be better to take care the use of the pesticides with strict standards e.g., Fenobcarb, Isoprocarb, and Pirimiphos-methyl.

Some of the detected pesticides were not used for growing coffee trees. One of the critical sources of contamination was jute bags used for domestic distribution. In some cases, the residual concentrations for jute bag samples were more than 100 times those for green coffee beans or the raw materials (dried cherry or parchment coffee) in the bags. It is strongly recommended to keep jute bags free from pesticides. And if possible, it would be better to control jute bags including residual pesticides analyses as an acceptance check.

In case of cross-contamination from jute bags, the residual pesticides were localized on the surface of green coffee beans or the raw materials. About 60% of the pesticides could be removed by a slight polishing. Dusts or husks with high concentration pesticides generated during coffee processing could be a source of contamination too.

Table 3. List of detected pesticides with maximum concentration.

Compound	Max. Concentration (ppm)	MRL (ppm)
Carbaryl	0.002	0.01
Carbendazim	0.006	0.1
Carbofuran	0.002	1
DDT	0.03	0.01
Fenobucarb	0.007	0.01
Isoprocarb	0.006	0.01
Permethrin	0.05	0.05
Pirimiphos-methyl	0.008	0.01
Propoxur	0.002	0.01
Thiabendazole	0.01	0.01

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Method for Continuous Monitoring Rates of CO_2 Release from R&G Coffee

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SUMMARY

Monitoring and managing of CO_2 is of paramount importance in processing and maintaining the quality of R&G coffee. Current methods for estimating rates of CO_2 released from roasted beans and ground coffee involve chemical reactions, pressure measurements or infra-red spectroscopy which are, respectively, time consuming, interfere with release kinetics and complex. These apparent drawbacks, led us to the development of an easy and accurate method which is based on tracking actual weight loss due to CO_2 release from R&G sample. The system design includes analytical balance with accuracy of 1/10 of a milligram, temperature controlled chamber, coffee container equipped with water adsorbent column and data collection software. Experiments can be conducted at various temperatures and the data stored and/or plotted directly on a PC. The system was tested using Robusta coffee, ground to average size of 250 μm and the CO_2 release was monitored at temperatures of 20, 30, 40, and 50 °C. CO_2 volume, calculated from the ideal gas equation, was plotted against time over a period of 3 hrs. The results obtained are in agreement with previous results in the literature.

INTRODUCTION

Carbon dioxide is the major gas produced during coffee roasting. The content of CO_2 in roasted coffee may vary between 2-10 ml per gram (Barbera, 1967; Clarke and Macrea, 1985); large amount of it stays in the coffee even after grinding. Significant amount of this CO_2 must be removed prior to packaging to avoid technical problems and package ballooning. This so called degassing process is related to the initial CO_2 content and its duration must be controlled to prevent product deterioration due contact with O_2 at the ambient atmosphere. CO_2 release kinetics from R&G coffee was studied in earlier works. However, the methods used for measurement of CO_2 release were usually complex, laborious and non-continues (Heiss and Radke, 1977; Geiger et al., 2005; Anderson et al., 2003), making it thus, difficult to use for a large number of samples. In this work we suggest a rapid, accurate and continuous instrument enabling a computed measurement of CO_2 release form R&G coffee.

Materials and methods

Coffee samples were produced by Strauss-Coffee BV using an industrial roaster.

The CO_2 measurement apparatus was constructed from analytical balance with 0.1 mg sensitivity combined with a temperature controlled glass chamber. A Unitronics Vision 120 controller was used to control the selected parameters, collect the data and transfer to a PC. 250 ml Büchner flask was used as the coffee container. The flask is equipped with moisture trap to avoid weight loss of water (Figure 1). Roasted coffee beans were degassed f-or one hour at room temp prior to grinding. Then, 100 g were grounded to mean particle size of 212

micron and immediately placed in the weighing flask. CO_2 Release measurements were started within 1 minute from grinding.

The CO_2 volume released was calculated by converting weight loss into mole equivalents which converted to volume units using the "Ideal Gas" formula PV = nRT, where P = 1 atm, R = 0.082 $L \cdot atm \cdot K^{-1} \cdot mol^{-1}$ and T = 298 °K (STP). Microsoft® Access and Excel software were used to collect and process the data.

Figure 1. The CO_2 release, monitoring apparatus.

RESULTS AND DISCUSSION

High sensitivity and simplicity of use makes the analytical balance a very suitable tool for monitoring CO_2 loss from solid particles such as coffee. The added ability to control and monitor temperature during degassing further upgrades the system, allowing one to generate accurate and reliable data. In Figure 2 a typical plot of CO_2 release measurement over time is illustrated, showing a rapid evaporation during the first hour followed by deceleration.

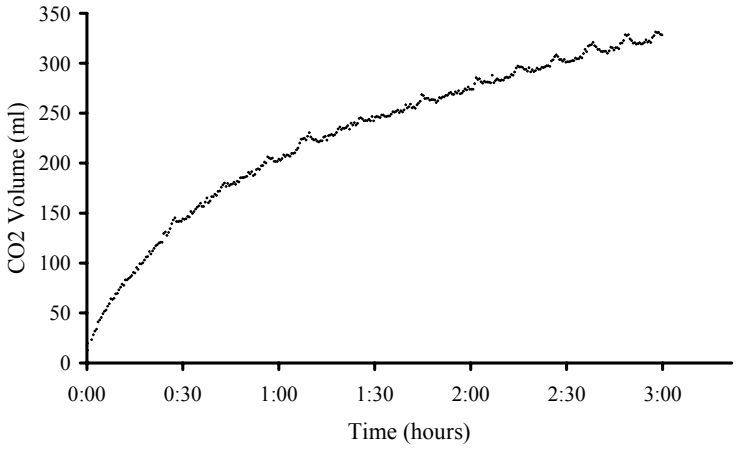

Figure 2. Typical plot of CO_2 release from R&G coffee over 3 hr measurement (100 g Vietnam G-2, 30 °C isothermal).

The strong effect of temperature on the rate of CO_2 release is demonstrated in Figure 3. which emphasizes the importance of having temperature control as an integral part of any CO_2 release measurement apparatus. Other interesting results are shown in Figure 4 where the effect of coffee origin on CO_2 release is depicted. As can be seen, there are some differences in release rates, however, the graph shape for all samples was similar.

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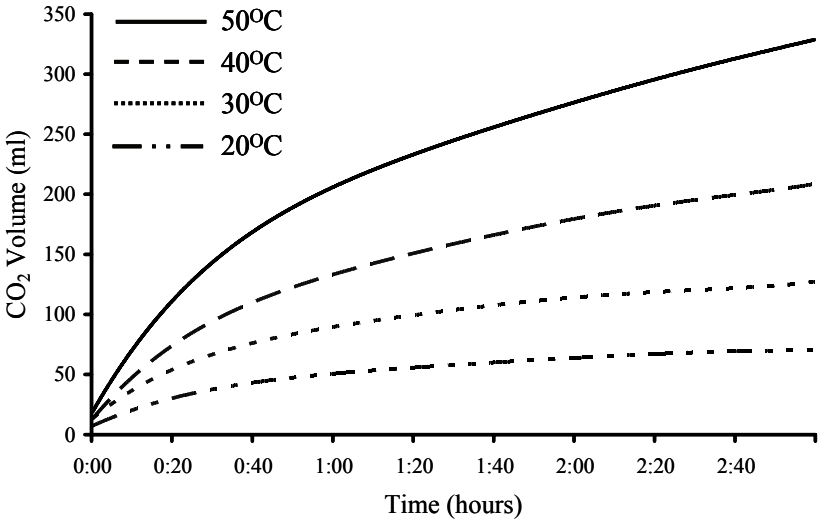

Figure 3. The prominent effect of temperature on CO_2 release rate from R&G coffee (100g Vietnam G-2,). Each graph is the mean of 3 samples.

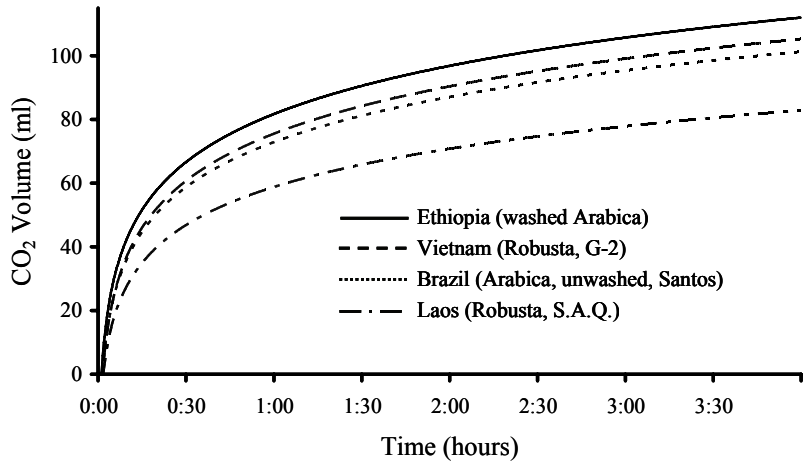

Figure 4. CO_2 release patterns of four coffee cultivars/origins (100g Vietnam G-2, 30 °C isothermal). Each graph is the mean of 3 samples.

Results obtained in this study, demonstrate the effectiveness and simplicity of the suggested apparatus as an accurate and practical tool for continuous monitoring of CO_2 release rate from coffee. In addition, the accuracy in weigh loss measurement and temperature control were shown as essential components of this apparatus.

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Quality of Shaded and Unshaded Organic Coffee: Presence of Fungi, Ochratoxin A and Caffein Content

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SUMMARY

Coffee (*Coffea arabica* L.) is a tropical crop grown in 75 countries with a total production close to 108 million t of beans. The arabica coffee plant, as the principal economic species, contributes 70% of the world's commercial coffee. The environmental conditions of coffee organic production in shaded and unshaded coffee may alter the fungi flora associated with coffee grains, as well as their population dynamics and consequently coffee quality attributes. These changes may predispose the predominance of fungal species, particularly Aspergillus section Circumdati and stimulate the synthesis of mycotoxins, such ochratoxin A (OTA). To evaluate these two organic production systems, shaded and unshaded, were collected samples from six coffee cultivars (Icatu, Oeiras, Catuaí, Obatã, Catucaí and Tupi), coffee cultivated in the Experimental Station of Embrapa Beef Cattle, Juparanã, Rio de Janeiro, Brazil. In the laboratories of Mycotoxins and Mycology and Liquid Chromatography of Embrapa Food Technology, samples were evaluated on their water activity, the percentage of OTA potentially producers fungi, OTA contamination, total nitrogen and caffeine content. The results indicated values of water activity among 0.607 to 0.645. The coffee grains from shaded production showed higher percentage of fungal contamination by *Aspergillus* sections Circumdati, Flavi and Nigri, respectively. The analysis of OTA in green coffee was performed by the High Efficiency Liquid Chromatography (HPLC). The levels of OTA in shaded coffee ranged from not detected to 0.78 μg/Kg and in unshaded coffee to not detected to 1.95 μg/Kg. It was observed on shaded organic coffee fungi contamination and OTA production levels less than the unshaded ones. Samples from unshaded coffee presented values of total nitrogen (2.04 g/100 g) and caffeine (1,11 g/100 g) levels minors than shaded, 2.14g/100 g and 1,24 g/100 g, respectively. This fact shows that shaded coffee trees could help to better nutrients up take available for plant, as well nitrogen and transforming it into caffeine, and so unshaded production provides more decaffeinated coffee than shaded. Although the organic production is also subject to natural contamination, the levels of OTA found were within the limits allowed by the European Community, parameter used nowadays, considering there is no Brazilian Regulation for this toxic metabolite. The results showed that all varieties of organic coffee in both production systems were safety to the consumers.

INTRODUCTION

Coffee (*Coffea arabica* L.) is a tropical crop grown in 75 countries with a total production close to 108 million t of beans. The arabica coffee plant, as the principal economic species, contributes 70% of the world's commercial coffee.

The organic coffee represents a small percentage in relation to traditional coffee in Brazil, but this activity has an enormous potential to promote environmental preservation with social-economic growth, although the market for organic coffee still be small (Darolt, 2002).

In 2003, Brazil was the 6th world producer of organic coffee, producing 80 thousand bags, which represents only 0.2% of its total production (Moreira, 2003) showing that despite being the world's largest producer of coffee, cultivation of organic coffee is still in ascendancy.

The cultivation of organic coffee in association with trees has been stimulated by the certification, but not a limiting condition for the conversion from conventional to organic coffee farms (Ricci, 2006), as the organic system does not propose to systematize the shaded coffee, getting this option to the producer criteria.

The environmental conditions of coffee organic production in shaded and unshaded coffee may alter the fungi flora associated with coffee grains, as well as their population dynamics and consequently coffee quality attributes. These changes may predispose the predominance of fungal species, particularly *Aspergillus* from section Circumdati that can produce ochratoxin A (OTA).

The purpose of this study was to evaluate the quality attributes of shaded and unshaded organic coffee production systems, focusing on the safety of the product, considering the eminent hazards, fungi producers of ochratoxin A (OTA) in green coffee.

MATERIAL AND METHODS

Coffee Sampling

Organic coffee (*Coffea arabica)* production area: growing at shaded and unshaded conditions at Embrapa Beef Cattle, Experimental Station (Juparanã, RJ, Brazil). The genetic material collected was from the following cultivars: Icatu, Oeiras, Catuaí, Obatã, Catucaí and Tupi.

Micobiota evaluation

Identification of filamentous fungi Filamentous fungi were initially cultured on PDA medium (Merck) and observed with an optical microscope for preliminary identification. This was done by morphotypic analysis of the colony, especially color and appearance, according Pitt and Hocking (1997). Initial fungal identification was made with microscopic slide examination of spores and mycelium (Figure 1). Further support for fungi identification was found in Christensen (1982), Nelson et al. (1983) and Pitt and Hocking (1997).

Activity of Water (A_w)

For the analysis of water activity (A_w), were separated into plastic bags around 20 g of each sample of coffee. The tests were performed at the Activity of water Analyzer from Novasina® A_w, A_w Sprint TH-500.

Ochratoxin A determination

To sample preparation 25.0 grams of ground coffee, 100 mL of NaHCO3 and 3% of solution and 100 mL of methanol to the blender and homogenize the system for 5 minutes. The samples were vacuum filtered with qualitative filter gopher Whatman 4 (kept in the dissector) and then in two other different membranes: Whatman GF / B 1 and Teflon 0.45 micrometers. 4 ml were transferred to filtrate into a 250 mL flask and added 96mL of PBS solution at 0.1%. Samples at HPLC analysis were considered as ND (not detected) when the results were below the detection limit of the method (0.5 mg/kg) (Brazil, 1999).

The results were quantified and calculated automatically by the software Empower ® (Waters Corp.).

Caffeine Determination

The caffeine content was determined by HPLC method (HPLC) using a C18 column (150 x 4.6 nm, spherical particles 5 μ), acetonitrile in 0.5% acetic acid as mobile phase, flow 1 mL / min, UV detection 280 nm, volume of injection: 20 ml and 20 minutes from time. The method was described by Grgunocich (1986) and adapted by Mello and Castro (1999). Coffee samples performed 0.1 grams; the caffeine was extracted with 10 mL of mobile phase filtering later with tissue paper. The solution obtained was centrifugated (at 14,000 rpm for 10 minutes) to ensure lower turbidity. The sample was taken to ultrasound for 10 minutes to reach room temperature. The results were comparable with 25 mg of caffeine standard.

Total Nitrogen

The determination of total nitrogen was performed by the method of Kjeldahl and the result was expressed as crude protein, after using a 5.3 conversion factor (AOAC, 2005; JONES, 1931).

Figure 1. (A) organic green coffe. (B) *Aspergillus ochraceus* (C) green coffee grains under DG18 / 7 days

RESULTS

Concerning the micobiota, it was observed higher percentage of fungal contamination by *Aspergillus* sections Circumdati, Flavi and Nigri, respectively, on the coffee from shaded production than unshaded ones.

A. niger is also potential producer of ochratoxin A, but the incidence of this fungus in coffee was very low and, according to studies made with conventional coffees, it is not capable of producing large quantities of OTA (Taniwaki, 2003). This fact may be associated to the lowest Aw found in coffee samples, which determines the optimum production of OTA. For the *A. ochraceus*, the higher OTA levels as reported in 0.85 Aw (Ribeiro et al., 2005).

Although the incidence of fungal with potential to produce OTA, the results of OTA contamination in analyzed coffee samples, in shaded coffee ranged from not detected to 0.78 µg/Kg and in unshaded coffee to not detected to 1.95 µg/Kg (Table 1, Figure 2). The results showed lowest OTA levels, below the limit set by European legislation to 5µg/kg (EC, 2005, EFSA, 2006).

Table 1. Quantification of total nitrogen, Caffeine, OTA and water activity (Aw).

Cultivar / Production System	Nitrogen total (g/100g)	Caffeine (g/100g)	OTA (µg/Kg)	Aw
Icatu / shaded	2,18	1,35	0,25	0,645
Icatu / unshaded	**1,98**	**1,18**	**1,54**	0,636
Oeiras / shaded	2,15	1,20	0,25	0,642
Oeiras / unshaded	2,01	1,08	**1,95**	0,618
Catuaí /shaded	2,25	1,24	0,25	0,632
Catuaí / unshaded	2,08	1,09	0,25	0,627
Obatã / shaded	2,07	1,30	**0,78**	**0,626**
Obatã / unshaded	2,12	1,24	0,25	0,623
Catucaí / shaded	2,15	1,27	**0,67**	**0,638**
Catucaí / unshaded	2,06	1,09	0,25	0,636
Tupi / shaded	2,02	1,10	**0,59**	**0,607**
Tupi / unshaded	1,98	0,97	0,25	0,62

Note: The detection limit of the method for the quantification of OTA is 0.5 mg / kg, so where is 0.25 µg/kg of OTA means that the values are below the limit of detection.

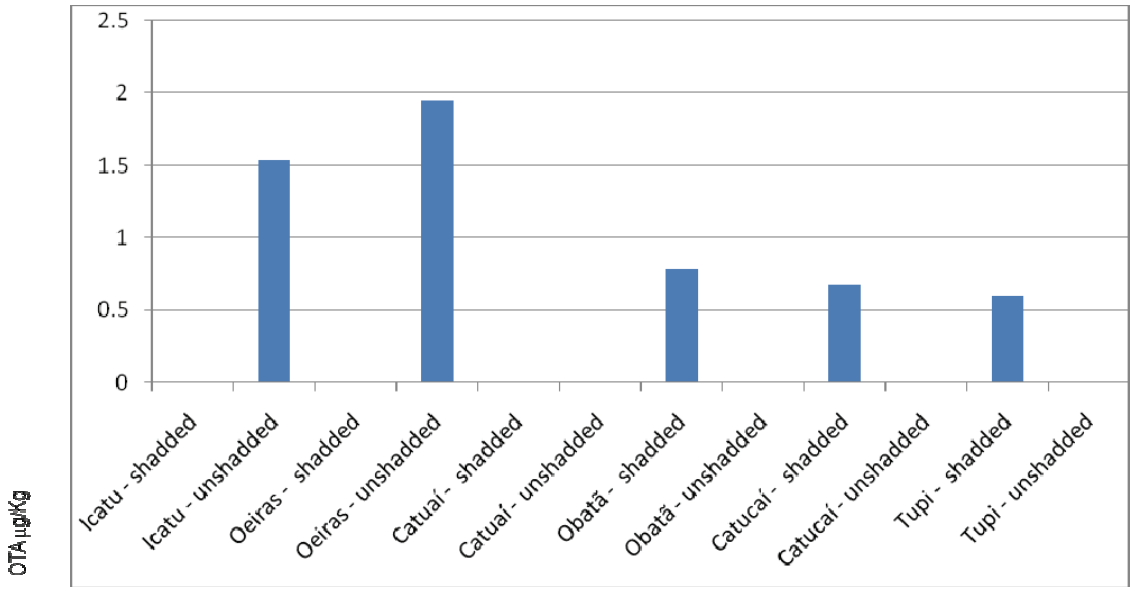

Figure 2. ochratoxin A evaluation by HPLC from shadded and unshaded organic coffee.

Coffee samples collected from unshaded system showed levels of total nitrogen and caffeine less than on shaded system (Table 1, Figure 3). This fact shows that the coffee cultivation in

shaded system could better uptake nutrients available to plant, including Nitrogen total and caffeine formation.

Samples from unshaded coffee presented values of total nitrogen (2.04 g/100 g) and caffeine (1,11 g/100g) levels minors than shaded, 2.14 g/100 g and 1,24 g/100 g, respectively (Table 1, Figure 3). This fact shows shaded coffee trees could better nutrients up take available for plant, as well nitrogen and transforming it into caffeine, and so unshaded production provides more decaffeinated coffee than shaded.

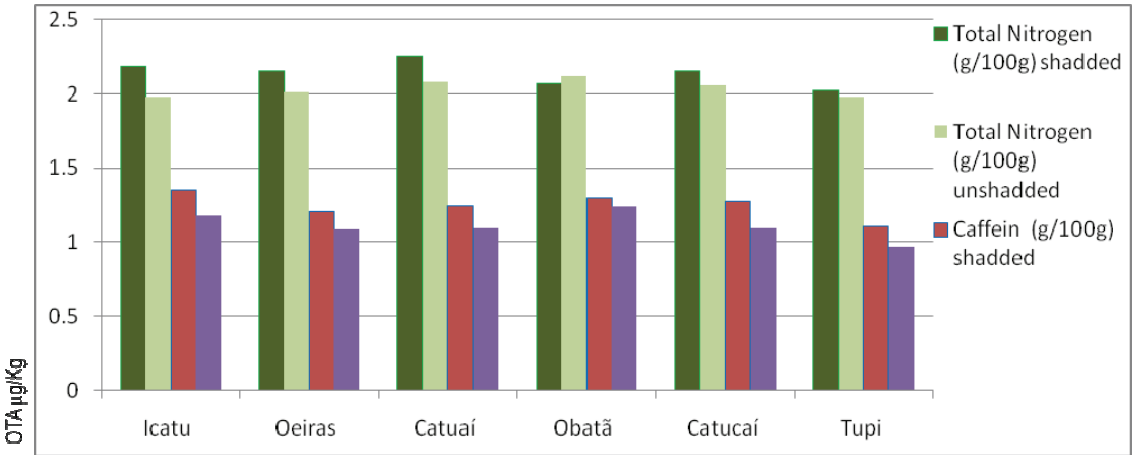

Figure 3. N total and Caffeine evaluation on shadded and unshaded organic coffee.

CONCLUSIONS

The levels of OTA found were within the limits allowed by the European Community, parameter used nowadays, considering there is no Brazilian Regulation for this toxic metabolite. The results showed that all varieties of organic coffee (**Icatu, Oeiras, Catuaí, Obatã, Catucaí e Tupi)** in both production systems were safety to the consumers.

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Amino Acids Profile in Unripe Arabica Fruits Processed by Wet and Dry Methods

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SUMMARY

The quality of natural coffee produced in Brazil is quite variable. During harvest, fruits at different stages of maturation can be found. Unripe fruits are naturally present in Brazil´s post harvest. Wet processing can effectively used. Nevertheless, an unripe portion of fruit with low quality will be produced. Mechanical depulping of immature coffee appears to be a potential way of improving its quality. The chemical, biochemical and physiological alterations that happen during post harvest result in different flavor and aroma precursor compounds that will determine coffee bean quality. Amino acids contribute to the development of typical aroma during roasting, which is considered as the major attribute related to coffee quality. These compounds act as precursor of aromatic and volatile substances while the asparagine produces acrylamide, a potentially harmful substance during the course of Maillard reaction. The amino acids were analyzed by reversed phase chromatography after derivatization with phenylisothiocyanate and ultraviolet detection. Amino acids profile of the immature coffee beans, asparagine is presented superior levels when immature coffee fruits were processed by dry processing in comparison with wet processing.

INTRODUCTION

Traditionally, green coffee is produced by two different methods, known as dry and wet processing. Both methods are aimed at reducing the water content in the raw coffee beans to about 10-12%. It is well accepted that green coffee resulting from either method of treatment, referred to as “washed” and “unwashed” Arabica, later on yields roasted beans and coffee beverages clearly differing in aroma and taste (Illy and Viani, 1995). These flavor differences are mostly attributed to differences in the thoroughness applied during either method of post harvest treatment (Maier, 1981; Sivetz and Desrosier, 1979). The wet procedure requires a strict sorting of the fruits prior to processing, because this treatment involves a mechanical depulping step, which can only be executed on fully ripe coffee cherries. There is no doubt that factor such as the grade and the homogeneity of the material affect the quality of the end product (Bytof et al., 2005).

The immature grains in Brazil’s coffee, quantitatively, one of the main problems to the offer of better quality coffee, considering the predominance of the complete crop on the harvest and on the dry processing. The processing operations can minimize this problem since correct processing techniques are applied. The wet method can be used. Nevertheless, an unripe fruits portion will be produced with low quality. The mechanical depulping of immature coffee appears as a potential way to improve its quality.

The biochemistry reactions which may occur comes post harvest of coffee grains interfere significantly in the quality and quantity of free amino acids (Selmar et al., 2002; Bytof, 2003). These alterations that happen during post harvest result in different flavor and aroma

precursor's compounds (amino acids and peptides) that will determine coffee beans quality. Qualitative analyses (Mazzafera, 1998) showed that asparagine was the main amino acid present in the immature grains coffee. Among the amino acids presented in raw grain, asparagine is the major precursor of acrylamide, substance potentially carcinogenic. During roasting of coffee, in Maillard reaction, the amino acids and reducing sugars react forming pirazines, important to development of the aroma. Although, other compounds can also be formed, including substances such as acrylamide. The presence of acrylamide in foods may represent risks to human health, including the consumption of coffee. Toxicological potential of acrylamide in foods is related not only to presence of precursors, asparagine and reducing sugars, but also the concentrations of these compounds in raw material, which may very significantly between these species, cultivation practices and processing. With this study aimed to evaluate the effects of different process performed during processing of coffee, determining the amino acids profile in unripe fruits coffee processed by wet and dry methods, especially the concentration of the amino acid asparagine, principal precursor of acrylamide.

MATERIALS AND METHODS

Coffee fruits (*Coffea arabica L.)* of Topázio cultivar, crop in the UFLA, harvest 2006/2007. After cleaning and hydraulic separation, the proportion formed by cherry and green fruits was depulped without counter-weight which regulates pressure of the drum pulper, allowing exit at the most, 10% of cherry fruits, along with green fruits, constitutes on the experiment control. Part of this fruit mixture was depulped following with the counter-weight, regulating the pressure (Borém et al., 2005), resulting in a pulped immature coffee parcel and another unripe natural immature coffee parcel. The other part of lot formed by unripe coffee, proceeding from the depulping, was left to rest in two recipients containing water and without water for a period of 12 hours. After this period, the fruits were depulped with counter-weight, regulating the pressure (Borém et al., 2005), resulting in a parcel of immature pulped coffee and another parcel of immature natural coffee. In this paper it was studied the interference of the peeling process of the green fruits and it's interaction with immersion in water for 12 hours (Borém et al., 2005). The drying of the control and other experimental parcels were realized in terraces reducing water content in the coffee beans to about 10-12% (wb).

Prior to extraction green coffee beans were ground in an analytical mill for 2 min and for obtaining a fine powder in a ball mill for further 1 min. For determination of the amino acids profile, the methodology was utilized proposed by White (1986). It was a weighed 0,5 g coffee sample completing with 80% v/v solution of methanol/HCL 0,1M. It was added 10ml of internal standard (α-aminobutyric) inside the hydrolysis tubes. The amount of 5ml were removed and filtered in a millipore filter 0.22 μm. It was measured 40 μL for each level of amino acids patterns and samples and transferred to glass tube of 8x50 mm. After the processing of drying, the tube was removed from vial of drying, adding approximately 20 μL of the solution with PITC (phenylisothiocyanate), proceeding the derivatization. After the eluent solutions preparation, the samples were put on vial and injected with liquid chromatograph previously conditioned. The column temperature should be around 50 °C and detection through waves-length of 254 nm by ultraviolet (UV). The quantification was realized by internal multilevel calibration, with assistance of the α-aminobutyric as a internal pattern.

RESULTS AND DISCUSSIONS

During the process of drying, grains modifications on its constitution could occur in response of numerous factors, such as temperature, time of exposition, variations on climate,

contributing to determine coffee´s final quality. In this sense, drying immature coffee fruits was conducted in adequate conditions of temperature and relative humidity, during this period, there wasn't climate variations which could interfere negatively on quality, obtaining grains coffee with a uniform aspect. It was observed, on this present paper, that the specific conditions of each procedure, realized during post-harvest influenced differently in the metabolism of coffee fruits, resulting in chemical and biochemical alterations which determined the differences on composition immature coffee grains. These differences on the constitution of coffee grains can be related, among other facts, to induction or inhibition process of germination, depending or not on the presence of the peel of the fruits coffee (Bytof, 2003).

CONCLUSION

The procedures used in the post harvest interfere significantly in the amino acid profile of the immature coffee beans. Asparagine is the amino acid with the highest concentration (mean = 0,1189 g/100 g) in the natural unripe coffee and significantly lower in pulped natural unripe coffee (mean = 0,092 g/100 g).

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Differentiation Between Arabica and Robusta Brazilian Roasted Coffees Using HS-SPME and SDE Gas Chromatography-Mass Spectrometry and Principal Component Analysis

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SUMMARY

The two major species of coffee, *Coffea arabica* and *Coffea caneffora* var. robusta, are markedly different in chemical composition and this reflects in the flavor fraction of each species after roasting. In this study, Brazilian roasted coffee, *arabica* and *robusta*, from several geographic regions, were evaluated. Simultaneous distillation-extraction (SDE) and headspace solid-phase microextraction (HS-SPME) were compared for their effectiveness in the extraction of volatile compounds and ability to differentiate the different species. The compounds were identified by gas chromatography-mass spectrometry (GC-MS) and principal component analysis (PCA) was applied to the major components of a flame ionization detector chromatographic response. The number of extracted compounds identified by both methods was 78 and 108 for SDE and HS-SPME, respectively. The main classes of compounds were furans, phenolics, pyrroles and pyrazines, in both extracts. When using SDE-GC-MS and FID and HS-SPME-GC-MS and FID, associated to PCA, a good separation between *arabica* and *robusta* roasted coffees were observed in both techniques. By SDE, the most representative differentiation compounds were pyridine, methyl pyrazine, furfural, 5-methyl-2-furancarboxaldehyde, 2-methoxyphenol, 3-methylbutanoic acid and 4-vinyl-2-methoxy-phenol. By SPME, the compounds were furfuryl acetate, 2-acetyl-5-methylfuran, 5-methyl-2-furancarboxaldehyde, phenol, 2-methoxyphenol, 4-ethyl-2-methoxy-phenol and 4-vinyl-2-methoxy-phenol.

INTRODUCTION

The two major species of coffee, Coffea *arabica* and Coffee *canephora* var robusta, differ considerably in chemical composition and acceptance by consumers. With respect to quality all experts and studies converges to the concept that *arabica* is the coffee related to most pleasant sensory characteristics. In this context it is clear that exists among hundreds of related chemical compounds in scientific letters some of them that influence differently the global flavor of *arabica* and *robusta* after roasting since the perception of coffee aroma is dependent upon both the concentration of individual compound and its odor threshold and the interaction between key classes of compounds (coffeeresearch.org). The application of precise physical–chemical analytical techniques in the characterization of patterns of each coffee specie or beans of same specie of different producing regions may represent important tool of classification the products targeting the consumer market. The choice of analytical strategy which allows the perception of small differences in volatiles between samples of coffee requires careful study to relation accuracy and doubtless extensive sample preparation greatly influences the reliable of results. In this study Simultaneous distillation extraction (SDE) and headspace-solid phase microextraction (HS-SPME), both associated to gas chromatography –

flame ionization detector (GC-FID were compared for their effectiveness in the extraction of volatile compounds and grouping species of brazilian coffee samples. The main disadvantage normally attributed to SDE lies in its relatively high extraction temperature and time that might result in certain chemical changes of some temperature sensitive compounds of the extracted samples (lipid oxidation and Strecker degradation of amino acids) (Garcia-Esteban et al., 2004). Since its introduction by Arthur and Pawliszyn in 1990 the technique of SPME has been widely used for the extraction and pre-concentration of an extensive range of analytes in a variety of samples. These samples commonly have components that may undergo processes of thermal decomposition, oxidation, photolysis, etc. (Garcia-Esteban et al., 2004), and the possibility of such undesirable processes during the SPME sample processing is favorably reduced due to the simplicity of sample manipulation that is characteristic of the technique. Results of the SDE-GC and HS-SPME-GC analyses were treated by principal component analysis (PCA). The compounds responsible for the differentiation between *arabica* and *robusta* were tentatively identified by Mass Spectrometry (MS) and their chromatographic linear retention index (LRI). Sampling was based on the Brazilian territorial distribution of *arabica* and *robusta* coffees.

MATERIALS AND METHODS

Coffee samples

The sampling was conducted on basis of production distribution in Brazil according to importance of each state. 24 samples were selected being 13 of Coffea *arabica* and 11 of Coffea *robusta* contemplating the various types of post-harvest treatments − Table 1 lists the regions of production chosen. The roasting was standardized as a medium roast since coffee is usually commercialized as such in the Brazilian market.

Sampling conditions for SDE

Simultaneous distillation extraction was carried out in a microscale simultaneous distillation extraction apparatus, as described in (Forehand et al., 2000). Dichloromethane was used as the solvent of capture. For each extraction, 5.00 g of grinded roasted coffee sample and 50 mL distillation water were placed into a 100 mL round bottle flask immersed in an oil bath maintained at 115 °C ; 3.0 mL dichloromethane was placed into a 5 mL round bottle flask immersed in a water bath maintained at 60 °C. The vapours were condensed by means of a cold finger maintained at -5 °C. The steam distillation process was stopped after 2.0 h. n-Decane was used as internal standard to normalize peaks areas. Each sample was analyzed in triplicate.

Sampling conditions for HS-SPME

Volatiles were collected from 1 g of grinded roasted coffee sample for 15 min on a divinylbenzene/carboxen/polydimethylsiloxane (DVB–CAR–PDMS, 50/30 μm film thickness, Supelco) fiber in a 50 mL vial after equilibration and headspace formation for 30 min at 60 °C. The concentrated analytes was desorbed for 5 min in a splitless chromatographic injection port at 250 °C. Each sample was analyzed in triplicate.

Gas chromatography and mass spectrometry

SDE or HS-SPME extracted volatiles were analyzed using an Agilent model 6890 gas chromatograph, equipped with a J&W-DB-WAX column (30 m x 0.25 mm x 0.25 μm), interfaced to a mass-selective detector (Agilent model 5973). Oven program: 40 °C (5 min) to

230 °C at 4 °C/min. Helium carrier gas was used. Detection was performed by FID. Mass spectra were recorded in the electron impact mode at 70 eV. Linear retention indices (LRI) were calculated using n-alkanes (C_9-C_{24}) series. The compounds were identified by comparing their mass spectra with computerized spectral database (NIST98.L).

Statistical analysis

The average of the three analysis made for each sample was submitted to PCA using The Unscrambler 9.1 software package.

Table 1. Origin of samples.

***arabica* samples**	**State/field processing[*1]**	***robusta* Samples**	**State/field processing[*1]**
A1-A2-A3	ES - natural coffee	R1-R2-R3-R4-R5	ES - natural
A4-A5-A6-A7	MG/*Cerrado* - natural	R6	ES – organic/natural
A8-A9	MG/*Cerrado*- semi-wet	R7	ES – semi-wet
A10	MG/south- blend/natural	R8	ES – wet coffee
A11	BA - natural	R9-R10-R11	RO - natural
A12	PE-organic/natural		
A13	SP - natural		

[*1] *The terminology used here to describe the processing is commonly found in related studies.*

RESULTS AND DISCUSSION

The Table 2 shows the number of compounds identified by class using SDE and HS-SPME, with the same chromatographic conditions. When SDE was used, 78 compounds were identified while SPME was used, 108 compounds were identified. The great majority of classes of identified compound were more extensively extracted by SPME, what can be an important factor of decision when it is necessary to differentiate samples with high degree of similarities (Nebesny et al., 2007).

As the differences in the extractive capacity between the techniques did not appear to be correlated with an discriminatory character in relation to a specific class of compounds, it could be attributed to losses related to SDE, due to the long time and the temperature of the heating that the samples were submitted, stress conditions avoided in the SPME process. The purpose of this study was to present simple and rapid methodologies of differentiation *arabica* and *robusta* roasted coffees, for this only the major peak areas were used for statistical analysis; 25 compounds obtained by SDE-GC-FID and 29 compounds obtained by HS-SPME-GC-FID, which also coincided with the highest analytical reliability.

Both methods have proved able to separate the two groups, what can be clearly shown in figure 1- where the samples were separated successfully by principal components 1 e 2 (86% of the total explained variance to SDE and 87% to HS-SPME). By SDE, the most representative differentiation compounds were pyridine; methyl pyrazine; furfural; 5-methyl-2-furancarboxaldehyde; 2-furanmethanol; 3-methylbutanoic acid and 2-methoxy-4-vinylphenol. By HS-SPME, the compounds were furfuryl acetate; 2-acetyl-5-methylfuran; 5-methyl-2-furancarboxaldehyde; phenol; 2-methoxyphenol; 4-ethyl-2-methoxy-phenol and 4-vinyl -2-methoxy-phenol. Among the two groups of 7 compounds which were able to differentiate *arabica* and *robusta* coffees, three compounds were common to both groups. This could be a signal that despite the clear distinction between analytical principles is possible in specific cases to associate the presented extraction techniques for enrichment of

the results. This work raises as a potential tool to discriminate coffees from a variety of origins as: degree of roasting; field handling; post-harvest treatment; different countries; among other possibilities (Gonzalez-Rios et al., 2007).

Table 2. Predominant Classes of compounds.

Class or function	Number of compounds	
	SDE	SPME
Acid	5	4
Alcohol	3	3
Aldehyde + ketones + Ester	4 + 11 + 6	2 + 11 + 5
Furan + pyran	14	19 + 2
Hydrocarbon + terpene	2 + 2	2 + 2
Phenolic	6	10
Pyrazine + pyridine + pyrrole	12 + 2 + 6	19 +10 + 10
Thiazole + oxazole	1	1 + 1
Others nitrogenous	3	5
Sulfur	1	2

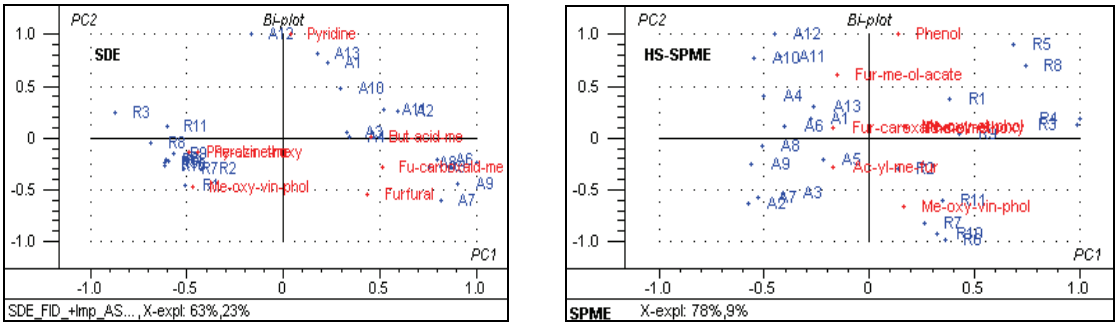

Figure 1. PCA bi-plot representation of variables (more representative compounds in discrimination) and coffee samples (A: *arabica*, R: *robusta*). Names of compounds are abbreviated.

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Colour and Quality of Green Coffee

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SUMMARY

Determinant green coffee quality parameters are correlated with grain physical appearance (uniformity, shape and size) and sensorial aspects (colour, aroma and flavour). Grain colour is an important criterion for its valorisation, acceptance or rejection. Procedures normally used for colour evaluation are quite subjective, and many authors suggest objective methods for this purpose. In the present work, evaluation of green coffee colour through visual and colorimetric methods was carried out, concomitantly with the quality analysis, in terms of contents on caffeine, chlorogenic and hydroxycinnamic acids. This study covered five cultivars from Instituto Agronómico de Campinas, Brazil. The results suggest that colour parameters *L**, *a** and *IC* are good quality indicators in terms of correspondence to chemical composition.

INTRODUCTION

Several studies have been carried out to correlate colour with quality of coffee drink (Mazzafera et al., 1988).

In this study we tried to correlate coffee colour with some chemical compounds, responsible for its quality.

MATERIALS AND METHODS

Materials

Analysis of green coffee (by humid way) were performed on dried, husked and grinded (sieve ASTM nº 35, 500 μm) seeds of 5 genotypes from Instituto Agronómico de Campinas (Brazil): *C. canephora* cv. Apoatã (IAC 2258); *C. dewevrei*; *C. arabica* cv. Catuaí (IAC 99); Icatu (hybrid of *C. canephora* x *C. arabica*) (IAC 2944); Obatã (hybrid of Villa Sarchi x Híbrido de Timor x Catuaí vermelho) (IAC 1669-20).

Methods

Colour evaluation

Dominant colour was evaluated according to NP-1795 (Norma Portuguesa, 1996) and objective evaluation was performed using a Minolta CR-300 colorimeter (Japan) with an white standard (L*=97,46; a*=–0,02; b*=1,72). The following parameters were also

calculated according to Chervin et al. (1992), McGuire (1992) and Spósito et al. (2004): ***Chroma*** (C*), ***Hue angle*** (h°), ***Total Colour difference*** (ΔE) and ***Colour index*** (IC).

Caffeine content

Extraction and quantification was carried out according to NP-1840 (Norma Portuguesa, 1986) using an HPLC Waters (USA) with UV-VIS detector 440 at 280 nm, LiChrospher 100 RP-18 column (Merck), 5 μm, 4 x 250 mm and a Rheodyne injector with 20 μl loop. Elution was carried out with 20 mM phosphate buffer and acetonitrile (9:1), 1 mL min^{-1}.

Chlorogenic acids

Extraction and determination followed Correia (1990), using an HPLC Beckman System Gold (USA), DAD 168 at 325 nm, Spherisorb S5 ODS2 column (Waters), 4,6 x 250 mm, (A) 10 mM citrate buffer, pH 2,5 and (B) methanol (gradient from 20 to 70% B, in 40 minutes), 1 mL min^{-1}, injection of 20 μl.

Hydroxycinnamic acids

Extraction and determination were performed as in Andrade et al. (1997; 1998) and Casal et al. (1999), using the same HPLC and column for chlorogenic acids, at 320 nm, (A) water and formic acid (19:1) and (B) methanol (gradient from 15 to 80% B, in 47 minutes), 1 mL min^{-1}, injection of 20 μl.

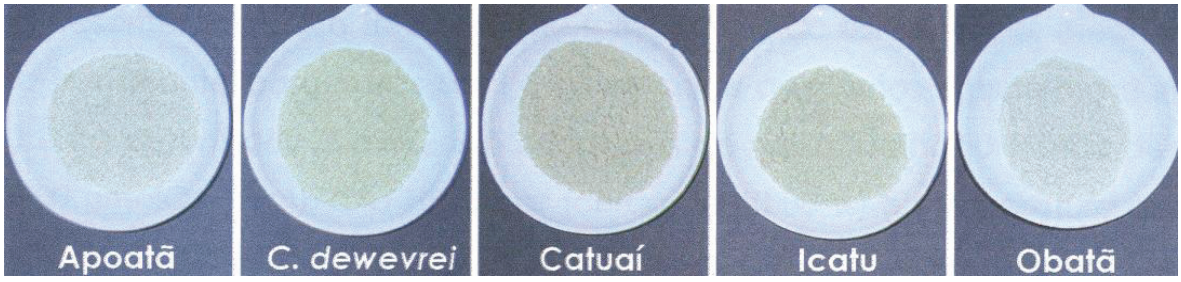
Principal Component Analysis

The association between chemical compounds and colour parameters was tested with PCA method, using STATISTICA v.6 (USA).

RESULTS AND DISCUSSION

Colour

Figures 1 and 2 show results of visual and objective colour evaluation of ground coffee.



Brownish green	Green	Greyish green	Greyish green	Whitish green

Figure 1. Colour of ground coffee grains of the 5 genotypes under analysis.

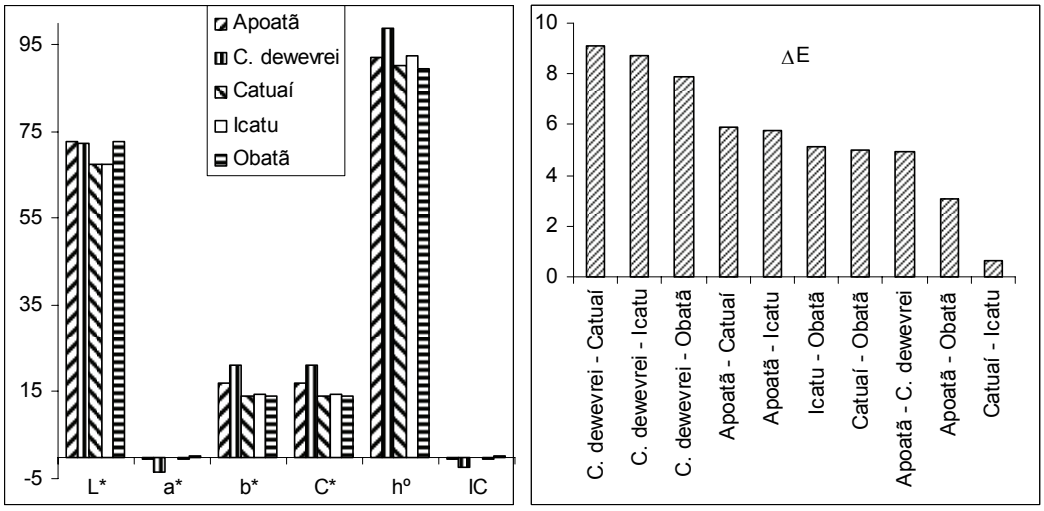


Figure 2. Analysis of colour parameters L*, a*, b*, C*, hº, IC (left) and total colour difference ΔE (right), considering the grinded samples of green coffee from the 5 *Coffea* sp. genotypes under analysis. Values represent the mean ± SE (n = 10).

Caffeine

Caffeine contents found in the 5 genotypes were (in % dry weight) were: Apoatã (2.85), *C. dewevrei* (1.12), Catuaí (1.51), Icatu (1.67), Obatã (1.10).

Chlorogenic and Hydroxycinnamic acids

Figure 3 shows results of chlorogenic and hydroxycinnamic acids content (dry weight).

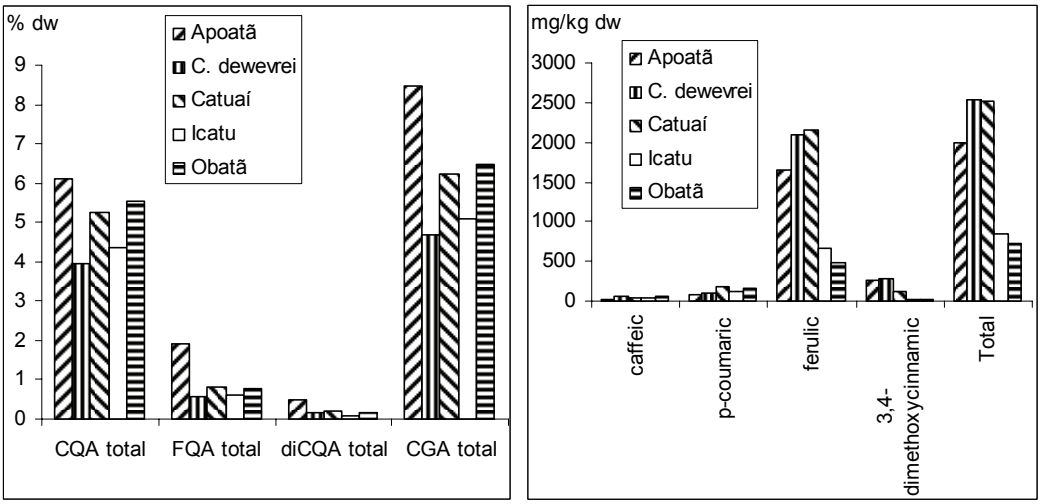


Figure 3. Chlorogenic (% dry weight) and hydroxycinnamic (mg kg^{-1} dry weight) acids. Values represent the mean ± SE (n = 3).

Principal Component Analysis

A PCA analysis will highlight the similarities and differences of the variables under study (Smith, 2002), allowing the selection of colour parameters that can be consistently associated to chemical ones in order to estimate grain quality from colour analysis. The first two PCA Factors explain 86.65% of the association between the studied parameters. Among them,

Factor 2 showed an association between chlorogenic acids, *3-CQA* and *4-CQA*, and colour parameters *a** and *IC*, which are closely correlated. In Factor 1, the association between colour parameter *L**, and ferulic acid and total hydroxycinnamic acids was also found (Figure 4).

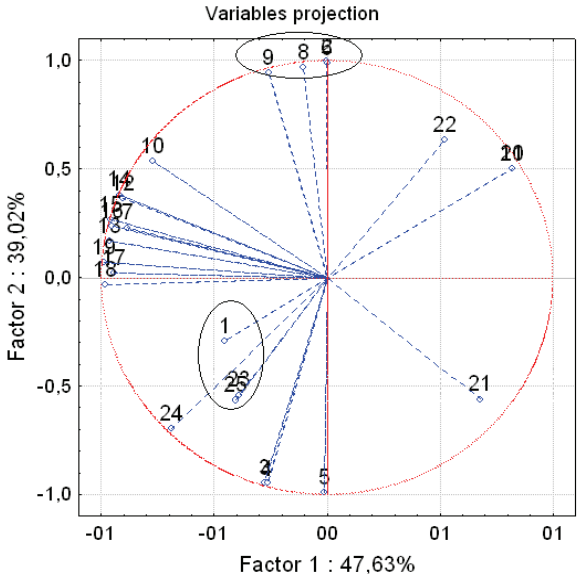


Figure 4. PCA analysis of 25 variables: 1 - L*, 2 - a*, 3 - b*, 4 - C*, 5 - h°, 6 - IC, 7 - caffeine, 8 - 3-CQA, 9 - 4-CQA, 10 - 5-CQA, 11 - total CQA, 12 - 3-FQA, 13 - 4-FQA, 14 - 5-FQA, 15 - total FQA, 16 - 3,4-diCQA, 17 - 3,5-diCQA, 18 - 4,5-diCQA, 19 - total diCQA, 20 - total CGA, 21 - caffeic acid, 22 - p-coumaric acid, 23 - ferulic acid, 24 - 3,4-dimethoxycinnamic acid, 25 - total hydroxycinnamic acids).

CONCLUSION

Results suggest that colour parameters *L**, *a** and *IC* are good indicators for the quality of these 5 genotypes of coffee, in terms of chemical composition. Further studies involving a larger number of grain origins are needed to evaluate the precision of these colour parameters as good quality probes.

ACKNOWLEDGEMENTS

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Chemical Composition of Brazilian Green Coffee Seeds Processed by Dry and Wet Post-Harvesting Methods

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SUMMARY

Coffee is the most consumed food product in the world. After harvesting, coffee fruits are processed in order to separate the seeds from skin and pulp. The way fruits are processed has a strong impact on their chemical composition, but literature data on this theme are still scarce. In this work, the levels of nine chlorogenic acids, caffeine, trigonelline and sucrose were determined by HPLC-UV and HPLC-RI systems in dry and wet post-harvested coffee seeds from seventeen Brazilian arabica cultivars and progenies.

INTRODUCTION

Coffee is the most consumed food product in the world. Brazil is the main producer and exporter of green coffee seeds (ABIC, 2008).

Traditionally, after harvesting of the fruits, green coffee seeds are obtained by one of two methods known as dry and wet processing. Although both methods aim at removing the fruit flesh of coffee cherry, they do it in different ways (Trugo and Macrae, 1984). In the dry method, the whole cherry (bean, mucilage and pulp) is dried under the sun or in a mechanical dryer, followed by mechanical removal of the dried outer parts (Rothfos, 1980). In the wet method, the pulp and skin are removed from the fresh fruit of coffee cherry through several stages such as pulping, mucilage removal (fermentation), washing and drying steps (Smith, 1985). Regarding cup quality, washed coffees are known to present better quality, less body, higher acidity and more aroma than the unwashed coffees (Mazzafera and Padilha-Purcino, 2004). It is well known that the way coffee fruits are post-harvesting processed has pronounced effects on the chemical composition of coffee seeds, but literature data on this theme are still scarce (Smith, 1985), especially regarding chlorogenic acids.

The aim of the present study was to compare the composition of chlorogenic acid compounds, caffeine, trigonelline and sucrose in Brazilian green coffee seeds produced by both dry and wet post-harvesting methods.

MATERIAL AND METHODS

Samples - Seventeen coffee samples were used in this study, being four arabica cultivars (Red Catuaí; Rubi; Yellow Bourbon and Topázio) and thirteen hybrids, eleven from the crossing of *C. arabica* cv. Yellow Catuaí with Timor Hybrid (number 1 to 11) and two from the crossing of cv. Red Caturra with Timor Hybrid (numbers 12 and 13). Coffee fruits were produced in a farm located in Paula Cândido, Minas Gerais, Brazil. After harvesting, fruits were processed by both dry and wet methods. For dry processing, the whole fruits were dried on cement layer

and on sun exposure to reach 12% of water moisture content, followed by manual de-husking. For wet processing, coffee fruits were quickly washed and mechanically pulped, and the mucilage was removed by natural fermentation for 8 h, followed by washing and de-hulling. After dry/wet processing, coffee seeds were milled to pass a 0.46mm sieve and stored in plastic bags under-20ºC until chemical analysis.

Chromatographic analyses

All analyses were performed by HPLC. A gradient system, using a U.V detector operating at 325nm was used for chlorogenic acids (CGA), according to Farah et al. (2005). Caffeine and trigonelline were determined by an isocratic system using a UV detector (Germany) employed at 272 and at 264 nm, respectively (Farah et al., 2006), while for sucrose, a R.I detector was used (Trugo et al., 1995). To express the CGA, caffeine, trigonelline and sucrose contents per weight of dry matter, water content for each sample was determined according to the A.O.A.C. method (2000).

Statistical analyses

GraphPad Prism® software, version 4.0 (San Diego, California, USA), using pared *t*-test method and considered significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

Chlorogenic acids

Nine CGA (3-, 4- and 5-caffeoylquinic acids; 3-, 4- and 5-feruloylquinic acids; 3,4-, 3,5,and 4,5-dicaffeoylquinic acids) were identified and quantified in coffees processed by dry and wet methods. The distribution profile of CGA classes of samples treated by the wet method was similar to those treated by the dry method. Coffees processed by the wet method presented significantly higher content of 4,5-dicaffeoylquinic acid ($p = 0.05$) and total CGA content ($p = 0.04$) than those processed by the dry method (Figure 1). These differences may probably result from degradation of these compounds in seeds processed by the dry method due to long periods of sun exposure (Balylaya and Clifford, 1995; Leloup et al., 2004), and from a relative CGA gain in wet processed beans caused lixiviation of other water soluble compounds (Farah et al., 2006).

Caffeine

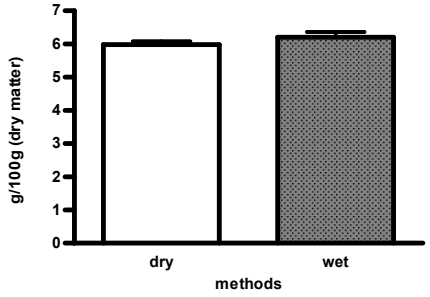
No difference was observed in caffeine content of seeds treated by dry and wet methods, probably due to the thermo-stability of this compound.

Trigonelline

Coffees processed by the wet method showed higher trigonelline content when compared to those processed by the dry method ($p < 0.0001$) (Figure 2). As with CGA, this result may derive from degradation occurred in seeds treated by the dry method. In addition, loss of some components with higher solubility in water, such as sugars, may occur in seeds processed by the wet method, promoting an increase of trigonelline content in this method.

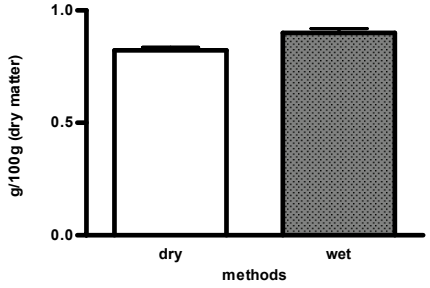
Sucrose

Sucrose content in coffee seeds processed by the dry method was significantly higher than in the wet method (p = 0.02) (Figure 3). The increase in sucrose content of seeds treated by the dry method may be a consequence of degradation of other components, such as CGA and trigonelline, as previously observed. In addition, sugars may be lost in seeds treated by the wet method due to fermentation (Wootton , 1974).



p=0.04

Figure 1. Chlorogenic acid content in green coffee seeds processed by dry and wet post-harvesting methods (n = 17).



p<0.0001

Figure 2. Trigonelline content in green coffee seeds processed by dry and wet post-harvesting methods (n = 17)

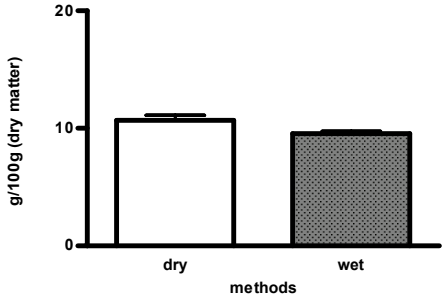


Figure 3. Sucrose content in green coffee seeds processed by dry and wet post-harvesting methods (n = 17)

CONCLUSION

Our results confirm that coffee seeds processed by the wet method are chemically different from those processed by the dry method. Sensorial analysis should be performed in order to associate the changes in sucrose, trigonelline and chlorogenic acids compounds with cup quality.

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Experimental Planning for Validation (Collaborative Study in Interlaboratorials) of Methods for Detection and Quantification of Frauds and Impurities in Roasted and Grounded Coffee

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SUMMARY

This paper describes the planning and execution of tests with the purpose of carrying out experiments for validation of methods for the detection and quantification of frauds and impurities in roasted and grounded coffee. Another purpose is to obtain estimative of parameters of some requirements for validation to be used as reference for comparisons between methods. The contamination of the samples (Figure 1) was provided by the company Toko Industry Trade Exports and Imports Ltd., member of the ABIC (Brazilian Association of Coffee Industry), using concentrations determined by previous researches in barks and sticks. Barley, the most commonly raw material used in frauds in roasted and grounded coffee, and "açaí", the raw material used nowadays as an alternative fraud, with low level of differentiation when compared to coffee, were also used as contaminants. The material was packed in sealed envelopes. Three experiments were planned for validation, one by collaborative study (experiment of accuracy) and two others by interlaboratorial study. The imaging method was used in the collaborative study while all the other methods were used in the interlaboratorial validation. The aim of this work was to compare the accuracy of the three methods and verify the existence of the following aspects: a) scientific basis of the test methods b) the presence or absence of repeated habits in all methods c) probability of false negative / positive. An interlaboratorial experiment was conducted by the Equifarma using analysis by the conventional method. The Embrapa Scientific Instrumentation was responsible for the analysis using photothermic method in another interlaboratorial experiment. Another study was done by ITAL, Embrapa/CTAA, Embrapa/Cerrados using the method of measurement by images in collaborative study. In all three experiments distributions of probability hipergeometric were used for making a system random sample with no replacement, and uniform, for coding portions of the sample to be measured, which were generated in statistical program. The experiment of accuracy followed the series of precision ISO 5725-1 to ISO 5725-6, and the interlaboratorial were carried out following the description of NATA TN 17 and EURACHEM (1998). The sample portions were large in order to avoid the necessity of replacement in case of a total loss. In the experiments the analytical capacity of the laboratories in each method were considered, and each portion sample was coded with 3-digit randomly, reducing the incidence of trends of analysts (Figure 2). For the 5 laboratories a total of 438 units were prepared and also packed in sealed envelopes. Letters with instructions, number of units to be measured, enforcement order, and way of measuring results were sent to the laboratories.

INTRODUCTION

Coffee is recognized worldwide not only by its great acceptance as beverage but also by its recognition as one of the largest commercialized product in the international market. These aspects indicate the importance of this grain to Brazil due to the agricultural aspect of its cultivation and to the industrial development of a product so well accepted. Both parameters act in social aspects because of the large number of jobs that are created and the large amount of products that are exported (De Maria et al., 1999).

The detection of impurities and mixtures in samples of roasted and grounded coffee is a constant worry, mainly because of the knowledge of occurrence of frauds in this product. The term "fraud" means the mixture, intentional or not, of foreign materials to coffee usually less than the cost of the product, which changes its quality and cause damage to consumers, especially those of economic order. In Brazil, the impurities and mixtures found more frequently in roasted and grounded coffee are shells and sticks, roasted corn, soybeans, rye, barley, "triguilho", rice, among others (Cunha et al., 2001).

The official analytical method considered for the determination of impurities in roasted and grounded coffee was developed by the Adolfo Lutz Institute. It is a subjective method, which is often time consuming and presents a great variation in the results which depends on the experience of the analyst. In order to make this determination faster, reliable and accurate a photothermic method of analysis of image was developed.

The objective of this work was to develop an experimental planning for validation of methods for detection and quantification of impurities in roasted and grounded coffee. A comparison among three methods was done: conventional (visual counting of impurities by microscopic analysis), photothermic (based on the propagation of heat generated by a light source) and analysis of image (computer analysis of microscopic images). The samples used for the analysis were roasted and grounded coffee pure and contaminated with shells and sticks, barley and "açaí".

EXPERIMENTAL

Samples of pure and contaminated coffee were obtained from the industry "Toko". In order to obtain a standard pattern of pure coffee, a sample containing a mixture of 80% arabic coffee and 20% conillon was prepared, approaching thus the commercial mixtures.

Figure 1. Pure and adulterated coffee samples received from TOKO.

Figure 2. Labels of pure and adulterated coffee.

The samples contaminated with shells and sticks, barley, and "açaí" were also obtained for evaluation by the photothermic method. This strategy permits the obtainment of an asymptotic horizontal curve, that is very close to the stage of saturation, resulting in measures comparable to those produced by image analysis (computer analysis of microscopic images) and conventional one (manual selection of impurities by microscopic analysis). To evaluate the conventional method and image one, it was accepted values near 1%, which are values determined by legislation and attend the politics pursued by ABIC.

The method of analysis of image is based on image recognition and quantification of impurities present in roasted and grounded coffee by the behavior spectrum of the sample, using software to capture, storage and process microscopic images (Figure 3).

Figure 3. Image of sample processed with 1,5% of impurity.

Figure 4. Equipment of Photothermic method.

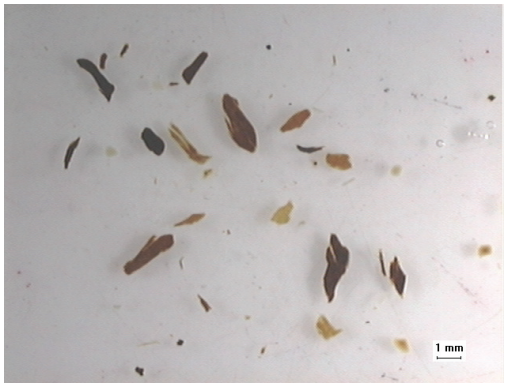

Figure 5. Conventional method – Shells.

The photothermic method is based on the propagation of heat generated by a light source on the sample, which give results that depend on the morphological structure of the sample that spreads differently. This spread is perceived by a sensor that captures the electrical signal proportional to the intensity of the heat (Figure 4). The comparison between electrical signals obtained for each sample with the standard provides the level of impurities.

The conventional process of measuring impurities is the preparation of microscopic slides, recognition of their structures, pathological and visual counting (Figure 5).

The statistical methods used were distributions of probabilities, for sampling involving distribution hipergeometric (randomly without replacement) and uniform, generated by statistical software. The trial follows a series of precision ISO 5725-1 to ISO 5725-6.

A total of 50 samples (between pure and contaminated) coded randomly was evaluated by each method, following the criteria for sampling and measuring/acceptable results in GLP and ISO 17025. The samples were prepared, packed into metal packaging standard, sealed and coded according to the planning shown in Table 1.

The result of each laboratory was sent to the coordination that has made the statistical treatment. It can be seen that the conventional method is still the most sensitive to the economic aspect and the technical approach that has been done by the quantification of samples with up to 1% of shells and sticks.

The photothermic method was not skillful to detect differences between pure coffee and fraudulent coffee, as well to quantify those fraudulences.

The conventional method has not been able to identify the materials used as fraud, and the measurement of results. It may be noticed however, that in the concentration of 1% of fraud these results showed no significant habit.

The method of image shown by the Laboratory 1, proved to be satisfactory in relation to the identification of material used as a fraud, as well as its quantification as shown in Table 1. The Laboratories 2 and 3 achieved the capture of the images and have proved skillful in identifying the material used as fraud, the proportion hits more than 90%.

Table 1. Planning trial of preparing samples for the Interlaboratorial in accordance with the method.

Shells and sticks							
Conventional (4 g)		**Image (4g)**		**Phototermic(7g)**		**Calibration(4g)**	
Concentration	**R***	**Concentration**	**R***	**Concentration**	**R***	**Concentration**	**R***
0	10	0	9	0	10	0	3
		0.25	9				
						0.4	3
		0.5	9				
						0.6	3
0.75	10	0.75	9				
						0.8	3
1	10	1	9			1	3
						1.2	3
1.25	10	1.25	9				
		1.5	9			1.5	3
1.75	10	1.75	9				
2	10	2	9				
		2.5	9			2.5	3
4	10	4	9	4	10		
5	10	5	9	5	10		
				6	10		
						8	3
				9	10		
10	10	10	9	10	10	10	3
				15	10	15	3
20	10	20	9	20	10	20	3
Barley							
10	10	10	9	10	10	10	3
20	10	20	9	20	10	20	3
Açaí							
20	10	20	9	20	10	20	3

**Numbers of repetitions.*

CONCLUSION

The conventional method is unable to identify and quantify the materials, thus it is clear that the measurement in this experiment was not good, probably due to visual deficiency or failure in the training of the analyst. Another possibility is the occurrence of visual fatigue or poor separation that reduces the counting obtained.

The photothermic method needs improvement, since the results obtained show no favorable outcome.

The method of image performed by three laboratories showed that it is possible to identify fraud using only the view of analysts trained. As the quantification can only be done using the software for processing the image, using the amount of pixels that have the same spectral response, such pixels will be part of the same category, named “impurity” and “coffee." The result of rating is the percentage occupied by each impurity in the picture observed.

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Stability of Sensorial and Biochemical Traits of Different Geographical Origin Ethiopian Arabica Coffee (*Coffea arabica* L.) Accessions Under Three Contrasting Environmental Conditions

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SUMMARY

Coffee quality is a complex trait that is influenced and determined by genetic, environment and genetic x environment interaction that finally defines both physical, organoleptic and bean biochemical composition quality profile of any ones coffee origin. Genotypic and environmental variations were the main factors that played a great role in the variation of bean biochemical composition quality profile. Organoleptic quality attributes variation was accounted for genetic, environment and genetic x environment interaction in this study. Therefore the presence of strong genotype x environment interaction for arabica coffee quality, limits development of wide adapting varieties, initiates coffee quality mapping, conservation of coffee genetic resources and establishment of core collections with respective origins.

INTRODUCTION

Coffee is the second most valuable exported commodity on earth after oil (Pendergrast, 1999) and contributes the highest to foreign currency earnings of Ethiopia (IMF, 2007). Coffee quality dictates and determines any ones coffee origin's power to penetrate the world coffee market. Jima Agricultural Research Center (JARC) coordinates national coffee research program in the country of arabica coffee origin and diversity. It is the mandate of this center to set breeding strategies giving priority for those traits that dictate world coffee market. Despite of this fact, the major focus of coffee research in Ethiopia during the early period was to develop coffee berry disease (CBD) resistance, high yielding and wide adapting varieties for release to the major coffee growing areas of Ethiopia especially for areas highly prone to CBD problem. In effect, several CBD resistant cultivars were identified, but released all over the country with out a prerequisite adaptation trial due mainly to the seriousness of CBD which was a grave threat to the Ethiopian coffee industry at that early period. However, these cultivars did not perform equally well at all environments in the country, and particularly in Hararghe it was a failure. This problem was an impetus and a good lesson for JARC to launch selection and breeding work for each locality using coffee materials from the respective location (Bayetta, 1997) and genetic x environment interaction studies in arabica coffee with due emphasis still for other desirable traits. Soon after, genetic environment interaction for yield and yield components were determined (Mesfin and Bayetta, 1987; Yonas, 2005). However, genetic x environment interaction studies for coffee beverage quality attributes was

not commenced until very recently, due to lack of coffee liquoring unit and skilled liquorers in the national coffee research center, Jima. Thus, the objective of this paper is to discuss stability performance of arabica coffee genotypes over contrasting environments.

MATERIALS AND METHOD

Known high quality selections of Harar types (Dr1, H13, H15), Sidama types (1377, 2970), Keffa types (7455, 75227, 7530), Illuababora types (74158, 74149, 74144A74139, 74304), and Wellega types (9384, 1484, 3184) were planted at Melko (JARC) and Metu in 2000, and Awada in 2001 in randomized complete block (RCB) design with three replications.

Table 1. Detail description of study areas.

Location	Altitude (m.a.s.l)	Longitude	Latitude	Rainfall	Min. T °C	Max. T °C	Soil type
Melko(Jima)	1764	036°47'00"E	07°40'00" N	1572	11.6	26.3	Eutric Nitosols
Metu	1580	035°35'57"E	08°19'14" N	1829	12.7	28.9	Umbric Nitosols
Awada	1738	038°23'16"E	06°44'57"N	1342	12.4	26.2	Nitosols and Cambisols

Source: Labouisse J-P, 2006 (Unpublished).

About 500 g of green coffee bean sample were prepared during the cropping seasons 2006/07 and 2007/08 from 10 tress of an accession per replication following the recommended wet processing procedure at JARC. Organoleptic quality attributes such as aroma (aromatic quality and intensity), acidity, bitterness, astringency, flavor and overall standard were scored using scales ranging from 0 to 5. The chemical contents of green coffee beans (caffeine, fat, trigonelline, sucrose and chlorogenic acid) were determined by Near Infrared Spectroscopy (NIRS) system (Williams and Norris, 1990).

Additive Main and Multiplicative Interaction effects (AMMI) analysis was employed to study stability. AMMMI was used to estimate stability and adaptability of genotypes by decomposing total variances in to variances due to additive main effects (genotypes and environments) and Interaction Principal Component Axes (IPCA) (Zobel et al., 1988).

Results and discussion

Combined analysis of variance over environments indicated non-significant genotype x environment interaction variance component for bean biochemical composition attributes but significant for organoleptic quality attributes except for bitterness and overall standard (data not shown). Similarly, AMMI analysis showed similar significance level of genotype x environment interaction variance components (Table 2). Significant to highly significant main effects and interaction variances were observed from AMMI analysis only for some of organoleptic quality attributes (Table 2). Significant ($P < 0.01$) variation was observed among environments for acidity, and aromatic quality whereas highly significant ($P < 0.001$) differences were observed for astringency, bitterness, caffeine, fat, flavor, overall standard and trigonelline. Highly significant difference was also observed among genotypes for all organoleptic and bean biochemical composition quality attributes except astringency, bitterness, and bean sucrose content. Genetic x environment interaction was observed

significant only for organoleptic quality attributes, but not for bean biochemical composition quality attributes.

Table 2. Mean squares of organoleptic quality attributes from AMMI analysis.

Source	Df	AC	AI	AQ	AS	FL
Environments (E)	5	1.928*	1.222	3.977*	9.227**	12.31**
Rep.with in Env.	12	0.403	0.597	0.803	1.764	0.967
Genotyope(G)	15	1.385**	1.735**	2.641**	1.056	2.316**
Genotype x Env.(GxE)	75	0.521*	0.386*	0.677**	1.818*	0.911**
IPCA1	19	0.893**	1.06**	1.803**	3.769**	1.835**
IPCA2	17	0.633*	0.181	0.419	1.814	1.195**
IPCA3	15	0.553	0.173	0.291	1.369	0.442
IPCA4	13	0.173	0.155	0.23	0.763	0.368
IPCA5	11	0.072	0.103	0.181	0.276	0.161
RESIDUAL	180	0.341	0.262	0.409	1.245	0.522

**, ** Significant at 0.05 and 0.01 probability levels, respectively, IPCA=Interaction Principal Component Axes, AC = Acidity, AI = Aromatic Intensity, AQ = Aromatic Quality, AS=Astringency, BI=Bitterness, FL=Flavor, OVS=Overall standard.*

Large percentage of variation was contributed by environment followed by genotype for most organoleptic quality attributes and bean biochemical composition characteristics. But variance contributed by genotype for aromatic intensity (51.89%), chlorogenic acid (72.95%), sucrose (45.73%), and trigonelline (46.30%) was higher as compared to environment. Only IPCA1 was significant ($P < 0.01$) for aromatic intensity, aromatic quality, and astringency, whereas both IPCA1 and IPCA2 were significant for acidity and flavor, respectively (Table 2). Highest percentage of genotype x environment interaction sum of squares was accounted by IPCA1. This axes accounted for 43.45%, 69.56%, 67.51%, 52.52%, and 51% of genotype x environment interaction mean squares of acidity, aromatic intensity, aromatic quality, astringency and flavor, respectively.

Genotypes and environments with IPCA scores of either large positive or negative value indicate high interaction whereas genotypes or environments with IPCA score of zero or nearly zero have small interaction (Crosa et al., 1990). Accordingly, stable performance of genotypes 74144A, H15 and 74158 over contrasting environments was observed for flavor, aromatic intensity, aromatic quality and acidity. Flavor is an all round organoleptic quality attribute that associated positively with other good cup quality attributes (aromatic quality, aromatic intensity and acidity). Genotypes 74144A and H15 revealed relatively low IPCA1 score of -0.1998 and 0.0028, respectively and above average flavor score (Figure 1).

Furthermore, AMMI analysis indicated that environments were profiled differently for organoleptic quality characteristics due to differences in ecological condition, genetic make up of the materials and differential response of the materials over the contrasting environments. The result indicated the need of mapping Ethiopian coffee for quality and thereby conserves coffee genetic resources within "*terrior*".

Large number of genotypes was categorized as unstable to moderately stable and very few were categorized as stable for most of the desirable organoleptic quality attributes. Thus, in view of the result of the present study, breeding strategy to develop wide adapting genotypes may not be possible.

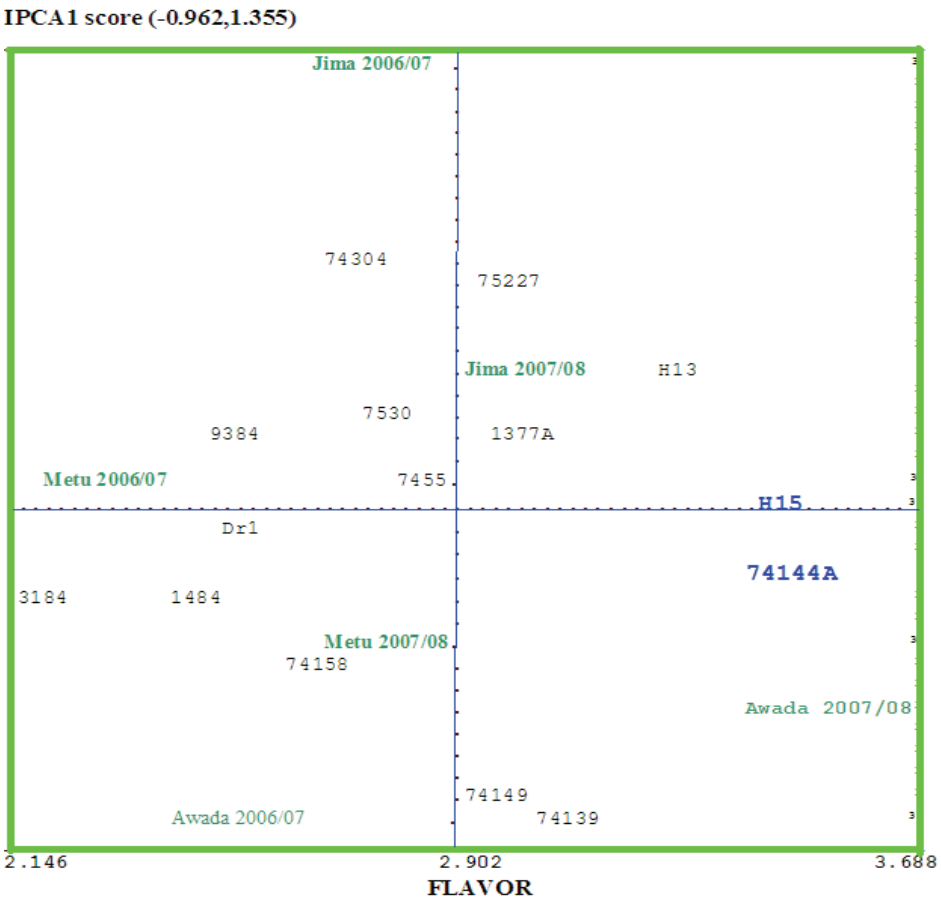

Figure 1. AMMI bi plot of flavor of 16 genotypes tested across 6 environments (3 locations and 2 years).

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A Comparison of Six Different Methodologies to Determine Raw Coffee Bean Moisture Content

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SUMMARY

The aim of this work was to compare methods to determine the moisture content on a wet weight basis (w.w.) of raw coffee beans. Six methodologies were evaluated in the moisture range from 9 to 15%: a) oven with forced ventilation (ISO 6673 method); b) oven with forced ventilation (ISO 1447 method); c) Capacitance method using the GAC 2100 equipment; d) Capacitance method using the Gehaka G 600 equipment, e) Karl Fischer Chemical method; f) oven with forced ventilation (Brazilian Agriculture Ministry method). The ISO 6673 is the official method for raw coffee and was used as a standard for comparison with the other methods. All results were analysed by the Dunnet and Tukey test at a level of 1 to probability. The results indicated that the methods tested presented higher moisture values (in the range from 0.3 to 2 percentual point) than the ISO 6673, except the capacitance method (GAC 2100). The Brazilian Agriculture Ministry method was the closest to ISO 6673 with approximately 0.3% of difference. The results from Gehaka G 600 and GAC 2100 when comparing in the range from 9 to 15% w.w showed difference in percentual point of 1.5 and 3%. The value measured with Gehaka G 600 machine was the closest value found by the Karl Fisher method. There is 1% of difference between the ISO6673 and ISO1447. All methods tested presented a statistically significant difference when compared to ISO 6673.

INTRODUCTION

The moisture content of raw coffee beans is very important on two aspects: economic, since the grain is commercialised on a mass basis and quality, since the moisture content is an indicator of storage quality. If the grains are stored at high moisture content, fungal growth may occur. For this reason, knowledge of the moisture content is useful to prevent or reduce post harvest losses, mainly during storage and transport. It is recommended that coffee beans be stored and transported at moisture contents lower than 12% (w.w.). Changes in colour, flavour and texture are observed when the moisture content is above 13% w.w. (Godinho et al., 2000; Vilela et al., 2000; Lee, 1999). There are several methods for coffee moisture content determination. The most used method in Brazil is that of the Brazilian Agriculture Ministry (1992), which uses an oven at 105 °C ± 3 °C for 24 h or up to constant weight (Godinho et al., 2000, Corrêa et al., 2000) with or without forced air circulation. Rapid electronic methods such as capacitance have been widely used at the field level and by coffee traders. This method uses electronic equipment, previously calibrated with the standard oven method, and produces the moisture content data in a few seconds. The moisture content data obtained are corrected according to the standard oven method. Currently, the Karl Fischer method is the recommended one, but the one used routinely. This method is the unique method that determines water directly. Considering the importance of the moisture content

determination in raw coffee bean, the objective of the present study was to compare six methodologies used in Brazil and overseas, using the standard ISO 6673 method (2003) as the reference method.

MATERIAL AND METHODS

Coffee sample preparation

Raw arabica coffee bean moisture content in ISO6673 of: a) 9%; b) 10%; c) 11,3%; d) 12% and e) 14.3% from 2007/2008 crop were studied.

Moisture content determination

Six methodologies were evaluated to determine the moisture content in wet weights (w.w.):

a. Oven with forced ventilation, 105ºC, 16h (ISO 6673 method);
b. Oven with forced ventilation, 105ºC, 24h (Brazilian Agriculture Ministry method);
c. Capacitance method using the GAC 2100 equipment;
d. Capacitance method using the Gehaka G 600 equipment (Brazil curve; 9 a 25%);
e. Karl Fischer Chemical adapted method, primary reference (DIS 12120);
f. Oven with forced ventilation, 130ºC, 6h, rest 15h + 4h (ISO 1447 method)

Statistical analysis

The experimental design was totally casual. ISO 6673 was used as a standard for comparison with other methods. All results were analysed with the Dunnet and Tukey test at a level of 5% of probability.

RESULTS AND DISCUSSION

The data obtained for the moisture content of coffee beans on a wet weight basis are presented in Table 1. The 5 different methodologies were compared to the ISO 6673 method and comparing by the Dunnet test.

In the Code of Practice prepared by the European Union and International Coffee Organization (ICO), the ISO 6673 (2003) method was recommended. This method uses an oven at 105 °C for 16 h, which is similar to the Brazilian Agriculture Ministry method. The only difference is the drying time, which is 24h instead of 16 h.

There is 1% of difference between the ISO6673 and ISO1447.

ISO6673 and the Brazilian Agriculture Ministry method showed significant difference in relation to Karl Fisher method, indicating that ISO6673 oven presumably doesn't take out all the water content. The water contained in the intracellular spaces of the bean should be gradually removed, because there are few exit channels. The interruption of the drying cycle, with resting time, is important so that the water in the deep layers emerges by osmosis and evaporates.

ISO1447 has a minor difference than Karl Fisher probably due to 15 hour resting period.

Gehaka G 600 has the closest values to Karl Fisher method.

According to Table 1 there were significant differences between the ISO 6673 method and the other methodologies.

Table 1. Moisture content (% w.w.) of raw coffee beans, comparing ISO 6673 with 5 other methods. Dunnet test.

Methodologies	Moisture content (% w.w.)				
Oven 105 ºC/16 h: ISO 6673	9.06 ± 0.02 a	9.90 ± 0.07 a	11.31 ± 0.02 a	11.99 ± 0.04 a	14.28 ± 0.07 a
Oven 130 ºC/6 h, rest 15 h + 4 h: ISO 1447	10.00 ± 0.08 b	10.92 ± 0.04 b	12.29 ± 0.05 b	12.88 ± 0.08 b	15.46 ± 0.12 b
Electronic capacitance: GAC 2100	8.44 ± 0.05 b	9.18 ± 0.04 b	10.62 ± 0.04 b	11.58 ± 0.08 b	13.86 ± 0.05 b
Electronic capacitance: Gehaka G-600	11.56 ± 0.05 b	12.00 ± 0.00 b	12.88 ± 0.08 b	13.42 ± 0.04 b	15.32 ± 0.04 b
Primary reference: Karl Fischer	11.92 ± 0.06 b	12.45 ± 0.19 b	13.70 ± 0.15 b	14.23 ± 0.34 b	14.74 ± 0.19 b
Oven 105 ºC/24 h: Brazilian Agriculture Ministry	9.35 ± 0.06 b	10.17 ± 0.05 b	11.57 ± 0.04 b	12.34 ± 0.03 b	14.60 ± 0.08 b
m.s.d.(5%)	0,099	0,145	0,132	0,250	0,181

m.s.d. = minimum significant difference by the Dunnet 5% of probability. Samples (average ± standard deviation) followed by the same small letters in the same column do not differ at the 5% level.

Table 2 showed the 6 different methodologies compared between them and evaluated by the Tukey test.

Table 2. Moisture content (% w.w.) of raw coffee beans, comparing ISO 6673 with 5 other methods. Tukey test.

Methodologies	Moisture content (% w.w.)				
Oven 105 °C/16 h: ISO 6673	9.06 ± 0.02 e	9.90 ± 0.07 e	11.31 ± 0.02 e	11.99 ± 0.04 e	14.28 ± 0.07 c
Oven 130 °C/6 h, rest 15 h + 4 h: ISO 1447	10.00 ± 0.08 c	10.92 ± 0.04 c	12.29 ± 0.05 c	12.88 ± 0.08 c	15.46 ± 0.12 a
Electronic capacitance: GAC 2100	8.44 ± 0.05 f	9.18 ± 0.04 f	10.62 ± 0.04 f	11.58 ± 0.08 f	13.86 ± 0.05 d
Electronic capacitance: Gehaka G-600	11.56 ± 0.05 b	12.00 ± 0.00 b	12.88 ± 0.08 b	13.42 ± 0.04 b	15.32 ± 0.04 a
Primary reference: Karl Fischer	11.92 ± 0.06 a	12.45 ± 0.19 a	13.70 ± 0.15 a	14.23 ± 0.34 a	14.74 ± 0.19 b
Oven 105 °C/24 h: Brazilian Agriculture Ministry	9.35 ± 0.06 d	10.17 ± 0.05 d	11.57 ± 0.04 d	12.34 ± 0.03 d	14.60 ± 0.08 b
m.s.d.(5%)	0,113	0,172	0,151	0,287	0,207

m.s.d. = minimum significant difference by the Tukey 5% of probability. Samples (average ± standard deviation) followed by the same small letters in the same column do not differ at the 5% level.

Table 3 presents the linear regression equations and coefficients of correlation found in this study.

Table 3. Linear regression equations and coefficients of correlation between the moisture content (Y) determined by ISO 6673 and moisture content (x) determined by the other methodologies.

	Moisture content determination (w.w.) by different methodologies*				
Statistic	A	B	C	D	E
Regression equation	y = 0,9889x - 0,1668	y = 1,3714x - 6,5674	y = 0,9486x + 1,1259	y = 0,9667x - 0,5896	y = 1,6282x - 10,521
Coefficient of correlation	0,999	0,991	0,998	0,998	0,916

**A) Oven method of the Brazilian Agriculture Ministry; B) Electronic method (capacitance GAC 2100); C) Electronic method (capacitance Gehaka G- 600; D) Oven method ISO 1447; E) Karl Fischer adapted method (DIS 12120).*

CONCLUSIONS

The results indicated that the methods tested presented higher moisture values (in the range from 0.3 to 2 percentual point) than the ISO 6673, except the capacitance method of GAC 2100.

The Brazilian Agriculture Ministry method was the closest to ISO 6673 with approximately 0.3% of difference.

The results from Gehaka G 600 and GAC 2100 when comparing in the range from 9 to 14% w/w showed difference in percentual point of 1.5 and 3%.

The results showed that there is a difference between ISO6673 and ISO1447, both with values lower than Karl Fisher method.

The value measured with Gehaka G 600 machine was the closest value found by the Karl Fisher method.

Established formula in table 3 can be used to correlate the distinct methodologies.

All methods tested presented a statistically significant difference when compared to ISO 6673.

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Comparative Evaluation of Methods to Assess Foaming Properties of Coffee

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SUMMARY

Coffee foam is a highly appreciated quality criterion for consumers. Accurate measurement of foam properties is necessary to determine molecules implied in these properties. Three methods differing in their mechanism to generate foam (i.e. whipping vs. sparging air) and in their energy input were compared on espresso and soluble coffees. Each methodology showed advantages and limitations. They provide complementary information on the mechanisms of foam formation and stability regarding the different type of energy input. The whipping methods (Vending equipment and Foam Spin) were most appropriate to discriminate coffees on the basis of their composition and/or concentration. The Foam Tube method using air sparging through a frit did not permit a strong discrimination of coffees.

INTRODUCTION

Coffee appearance and mouthfeel are key attributes driving consumer preference. Volume, texture and stability of foam are highly appreciated quality criteria for coffee consumers.

However, accurate measurement of foam properties of coffee represents a challenge. Different methodologies are possible to create foam: shaking by hand, stirring, whipping or sparging gas through a frit. Furthermore, ultrasounds can be used to control bubbles size distribution (Torres-Sanchez and Corney, 2008) and pressure to speed up foam drainage (Kruglyakov et al., 2008).

In this study, three methods differing in their mechanism to generate foam (i.e. whipping vs. sparging) were compared on espresso and soluble coffees. Method A and B generated foam by whipping the solution, with high energy (Method A) or medium energy (Method B). Method C created foam by slowly sparging air through a frit using medium/low energy input. The methodologies were tested for their ability to discriminate coffees of different composition and different coffee concentrations in terms of their foamability (foam volume) and foam stability.

MATERIAL & METHODS

Sample preparation

Soluble coffees were obtained from Nestlé. Espresso coffee was prepared from medium roasted coffee using a standard espresso machine. Coffee solutions at 0.5, 1.0 and 2.0% were prepared with ultrapure water. The solutions were incubated at 75 °C for 15 min prior to the foam experiments.

Method A / Vending equipment

The Vending equipment was developed at Nestlé. The coffee extract (84 mL) was vigorously whipped in a mixing chamber (15'000rpm during 5s). The liquid and foam parts were collected into a 100 mL glass cylinder (internal diameter: 25 mm). Pictures of the cylinder filled with foamed coffee were taken every 30s over 15min with a digital camera (Canon Powershot G9, Tokyo, Japan). Image analysis was performed with the Retrac software (N. Carter, MCRI, Oxted, UK) to determine the foam volume and liquid drainage over time.

Method B / Foam Spin

The Foam Spin was developed by Teclis (Longessaigne, France). The coffee extract (20 mL) was whipped with a 3-pals helix (5,000rpm) until a definite volume of foam (30 mL) was reached in the glass column (internal diameter: 19mm). The foam volume was measured by a grey-level scale taking pictures. The liquid volume into the foam was followed by conductivity via electrodes.

Method C / Foam Tube

The Foam Tube was developed at Nestlé. The coffee extract (40 mL) was poured into a glass column (internal diameter: 17 mm) equipped with a 2 µm frit at the bottom. Five milliliters of air were sparged at 7 mL/min through the frit into the coffee extract during 45 s. Pictures of the tube filled with foamed coffee were taken every 30 s over 15 min with a digital camera. Image analysis was performed with the Retrac software to determine the foam volume and liquid drainage over time.

RESULTS & DISCUSSION

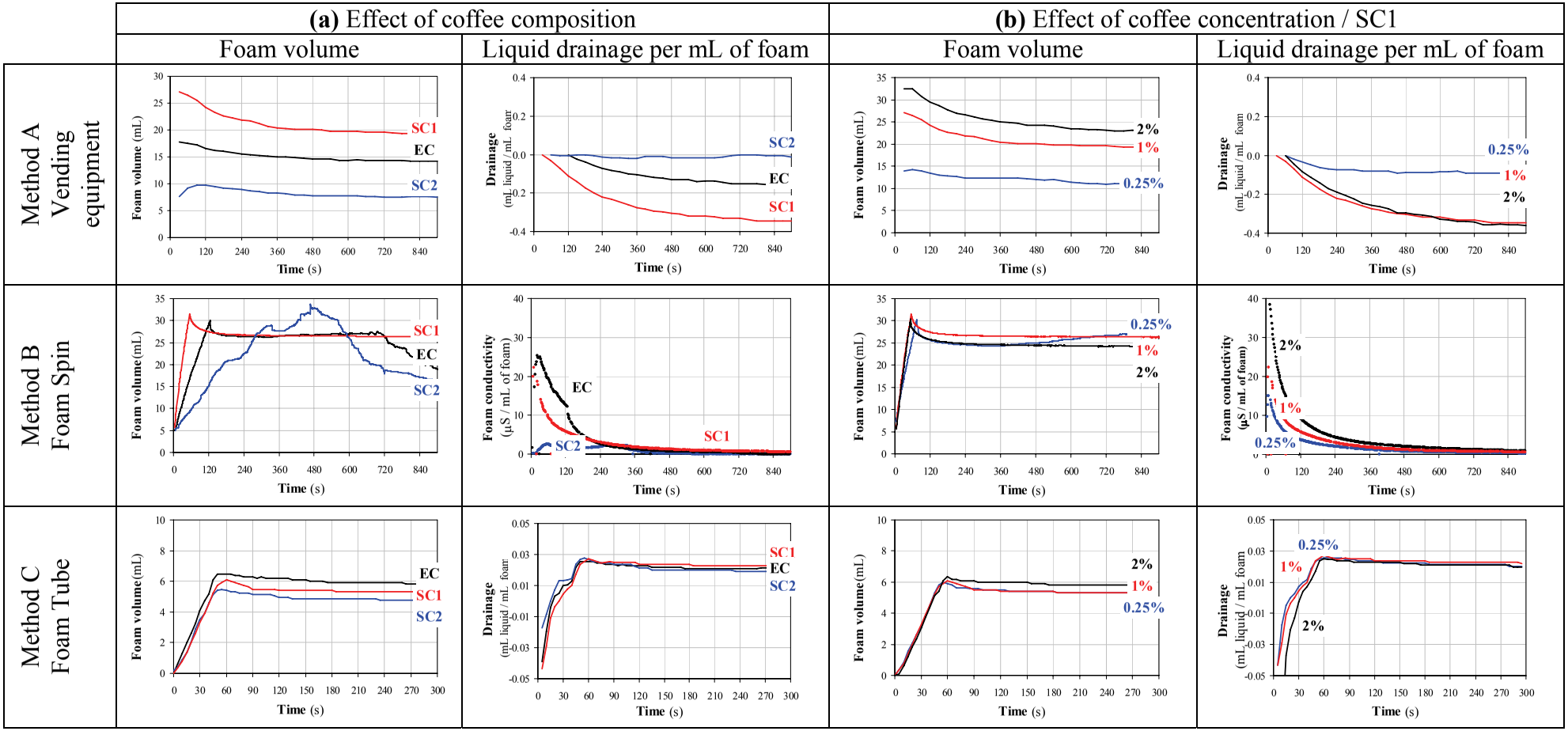
The soluble coffees (SC) were selected for their capacity to deliver foam in a cup, i.e. highly foaming (SC_1)/ poorly foaming (SC_2). An espresso coffee (EC) was also assessed to allow comparison with soluble coffee. The foaming methods were tested for their ability to discriminate samples for foam volume and stability. This was tested in two ways, i.e. using the different coffee samples (1% w/w) and using different coffee concentrations (0.25, 1 and 2% w/w).

Foaming Methods & Coffee Composition

Foam volume and stability of the three coffees were assessed at 1% w/w using the three methods (Figure 1a). Both method A and B were able to discriminate coffees of differing foaming performance with similar ranking, i.e. $SC_1 > EC > SC_2$ on the basis of foam volume. On the contrary method C was poor at discriminating foaming performance.

Method A and B are both based on the whipping technique providing high/medium energy to the extract for short (i.e. Method A 15'000rpm, 5s) or longer duration (i.e. Method B 5'000rpm, > 50 s). No redistribution of molecules at the interface is expected, thus allowing to easily differentiate for the impact of coffee composition. The impact of foam instability during foam formation is particularly obvious for SC_2 using Method B. On the contrary, Method C based on the sparging technique, allows gentle molecular rearrangement at the interface, thus limiting the direct impact of coffee composition.

Figure 1. Evaluation of foam volume and liquid drainage per milliliter of foam using the different methods (a) effect of coffee composition (b) effect of coffee concentration.



For methods A and B, the foam decay could usually be decomposed into two stages. The first stage is dominated by liquid drainage (i.e. pronounced slope in foam decrease and in liquid drainage), the second one by bubble rearrangement as described for beer foam (Sauerbrei et al., 2006). Only one stage could be distinguished by the Foam Tube methodology, probably due to fine bubbles slowing down liquid drainage. Only methods A and B permitted to clearly distinguish foam decay and these two stages.

Methods A and B clearly distinguished liquid drainage rate expressed per millilitre of foam. The measure of liquid drainage in Method B is indirect and with the assumption that the quantity of liquid at the foam/liquid interface is linearly proportional to the conductivity of the liquid. Foam decay and liquid drainage were faster with SC_1 than with EC. The high foam conductivity of EC would indicate a wet foam. Dryness of foam of SC_2, indicated by low foam conductivity, can explain its fast foam collapse.

Foaming Methods & Coffee Concentration

The foam volume and stability of the highly foaming coffee SC_1 was assessed at three concentrations 0.25, 1 and 2% w/w using the three methods (Figure 1b). Again method A was able to clearly discriminate coffees of various concentrations for their foam volume and to partly discriminate liquid drainage. On the contrary method C was poor at discriminating foaming performances. Method B poorly discriminated foam volumes and foam decay, but discriminated liquid content in foam as a function of the concentration.

CONCLUSION

Each method showed advantages and limitations. Method A was most appropriate to discriminate coffees on the basis of their composition and/or concentration. This is a simple and fairly reproducible method. However, the early stage (i.e. <100 s) of foam formation could not be monitored due to undefined interface.

Method B, was the most appropriate to evaluate the kinetics of foam formation, although image analysis would require further improvements for coffee samples.

Finally, Method C did not allow differentiating foaming behavior of coffee, neither on composition nor concentration. The gentle sparging allowed appropriate molecular re-distribution and stabilization of the interface, showing that coffee extracts even at 0.25% contain sufficient level of foaming compounds.

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Sensory and Bean Characteristics of Wild Arabica Coffee from Southeast Afromontane Rainforests in Ethiopia

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SUMMARY

A study to assess the sensory and bean characteristics of wild arabica coffee was conducted at Harenna (Bale) Afromontane rainforests in Southeast (SE) Ethiopia. Red cherries hand picked during the pick harvesting period were dry processed with better handling. And at the same time, coffee samples harvested by the farmers themselves were also bought and included in the study for comparison purpose. The major cup quality traits (fragrance, aroma, acidity, flavour body, aftertaste and overall quality of the liquor) and bean characteristics (bean size, length, width, thickness, shape index and weight) were recorded. Results showed that coffee samples bought from the farmers (less matured cherries) had lower overall cup quality as compared to those matured cherries (collected from the coffee trees in the forest). This could be due to the fact that farmers in the Harenna area pick red and green cherries together during the harvesting period. The effect of harvesting green cherries on beverage quality was more pronounced on its acidity and flavour than on its aroma and body. Maturation clearly favored the development of high-quality flavor in the coffee brew. But this had no significant effect on physical bean characteristics in Harenna forest. The sensory property of the coffee was more affected by the level of cherry maturity than bean physical characteristics. However, some of the sensory and bean characteristics were significantly correlated. The higher the proportion of the small beans in the sample, the better the quality of the coffee in SE Ethiopia, and vice versa. Comparatively, the proportion of smaller beans was lower in the SE as compared to that of the Southwest (SW), but the proportion of larger beans was higher in the SE. In both SW and SE rainforests, the proportion of beans retained on screen 16 was higher, followed by screen 17 in SE but followed by screen 15 in SW Ethiopia (data not shown). Moreover, 100 bean weight was inversely correlated with cup quality, especially in the case of matured cherries. Sensory characteristics of the coffee were positively and significantly correlated with bean width in coffee samples bought from farmers, and hence it might be an indicator of cup quality especially when green coffee cherries are harvested. Therefore, among the physical characteristics, the proportion of small beans, bean weight and bean width could be good indicators of organoleptic properties of wild Arabica coffee in SE Ethiopia.

INTRODUCTION

Coffee is one of the most consumed beverages in the world (Nebesny and Budryn, 2006), and the total annual consumption of coffee exceeds 400 billion cups (Feria-Morales, 2002). This is due to its pleasant taste and aroma (González et al., 2001). As coffee is enjoyed because of its unique properties, its quality and consumer acceptability are thus dependent on its flavor and aroma profiles (Ross et al., 2006). In coffee trading system, the quality of coffee brews

are most frequently estimated using sensory analysis (cupping). Cupping is one of the coffee tasting techniques used by cuppers to evaluate coffee aroma and the flavor profile of a coffee. And sensory attributes of coffee (fragrance, aroma, acidity, flavour, body, aftertaste, etc.) are key in determining its quality (Feria-Morales, 2002). Apart from this, coffee quality also depends on the bean physical characteristics. Physical characteristic refers to the general aspect of the product such as size, shape, length, width, density, uniformity, freedom from defects, and other external traits. In the past these were the only criteria used by merchants on the world market (Sylvain, 1958).

Coffee quality is the overall characteristics of intrinsic components of the bean, and it is influenced by different genetic and environmental factors (Brownbridge and Eyasu, 1968; Sylvain, 1958; Yadessa et al., ASIC this volume), and by processing practices (Knopp et al., 2006). The quality of green coffee mostly depends on the way in which the coffee is grown, harvested and field processed. Coffees grown properly but handled badly during harvesting will be of lower quality. Similarly, coffees harvested selectively but which fail to be field processed following the recommended optimal conditions will not be of better quality ((Feria-Morales, 2002). Quality in coffee is defined differently by the different people involved in each stage of its production (Leroy et al., 2006). Thus, to get a reliable information, sensory quality assessment should be done by trained panels to fully identify, define and understand the sensory characteristics of the coffee that determine quality. The use of trained individuals (panels) is equivalent to the use of scientific apparatus for measurement of parameters that are associated with the quality of a product. In the same way that instruments are calibrated to undertake the desired measurement, panels must be trained to ensure the systematic utilization of sensory perception throughout their senses. (Feria-Morales, 2002).

The quality of a green bean thus begins with the seed that is selected for propagation. Therefore, the chain from seed to cup is a very long one, and coffee quality depends on many different factors involved in the process across the value chain. But information on the quality aspects of wild Arabica coffee in SE Ethiopia is lacking. The objective of this study is to assess the sensory and bean characteristics of wild Arabica coffee collected from the Harenna (Bale) Afromontane rainforests in Southeast (SE) Ethiopia, and also to assess the influence of traditional coffee handling and processing practices on the quality of coffee.

MATERIALS AND METHODS

The study was carried out in the Southeastern Afromontane rainforest (Harenna forest), located in Bale Zone of Oromia Regional State, Ethiopia. The Harenna forest is a part of the Bale Mountains, one of the largest massifs in the south-eastern highlands of the country. The mountains form a sharp transition zone from high mountain vegetation to hot savannah areas within the south-eastern highlands and associated lowland physiographic regions of Ethiopia. The Harenna forest lies between 1300 and over 3000 m above sea level. Geologically, the study sites are of volcanic origin welded with volcanic ash materials (Mohr, 1971). The soil in the coffee zone is acidic to slightly acidic with a pH between 5.3 and 6.6. The rainfall pattern in the area is the bi-modal type, i.e., March to April (short rain season) and August to October (long rain season). Annual rainfall is about 1000 mm and the mean annual temperature is 18 °C (Senbeta, 2006; Kufa, 2006; Beining, 2007).

Coffee cherries were harvested at full maturity in October and November 2006. Red cherries were handpicked from the coffee trees in the forest and the samples were dry processed. And at the same time, coffee samples harvested by the farmers themselves were also bought and included in the study for comparison purpose. With the comparison of both sources (samples collected from the forest and samples bought from the farmers), the effect of traditional coffee

harvesting and handling practices was assessed as well. The dried cherries from both sources were depulped and the beans were made ready for cup tasting. Cup tasting was done at Coffee Quality Inspection and Auction Center in Addis Ababa, Ethiopia by a panel of 5 experienced cup tasters (3 from Ethiopia and 2 from Germany). All the coffees were prepared by pouring boiling water (250 ml) directly onto roasted and ground coffee (12g; medium roast; medium ground). The major coffee quality attributes (fragrance, aroma, acidity, body, flavour, aftertaste and overall quality) were assessed using the beverage quality denominations ranging from 1 to 10, corresponding to the total absence (or presence) of the criterion in the coffee, respectively.

Bean size distribution of wild coffee beans collected from Harenna Afromontane rainforest, SE Ethiopia was determined by screen analysis; perforated plate screens of different sizes (screen 19, screen 18, screen 17, screen 16, screen 15 and screen 14) were used. Weight fractions on each sieve were recorded as described in Muschler (2001), and then converted into percentage basis. Average bean density was calculated as the ratio between the weight of the 100 bean sample and the volumes of 100 beans. Bulk volume was determined by the water displacement method using a 50 ml graduated cylinder.

The obtained data were subjected to analysis of variance (ANOVA), and correlation among variables was also assessed by principal Components Analysis (PCA) based on the Pearson correlation matrix using SPSS computer program.

RESULTS AND DISCUSSION

Results showed that there was high variability in sensorial properties of wild Arabica coffee from the Harenna forests (Figure 1). Variability in cup quality of coffee samples collected from the forest coffee trees was higher as compared to those coffees bought from farmers (Table 1), suggesting good potential for using these materials in breeding programmes for improving cup quality or transferring important traits to other coffee cultivars.

Table 1. Summary of sensory characteristics of wild Arabica coffee from Harenna Afromontane rainforest in the SE Ethiopia.

Source	Statistic	Fragrance	Aroma	Acidity	Flavour	Body	Aftertaste	Overall
Collected (Forest)	Minimum	4	3.2	3	2.5	2.5	2	3.25
	Maximum	6.8	7.2	7.8	7	7.4	6.8	7.40
	Range	2.8	4	4.8	4.5	4.9	4.8	4.15
	Mean	5.61	5.63	6.07	5.53	5.91	5.13	5.87
	Std. Dev.	0.83	1.11	1.12	1.16	1.19	1.15	1.14
Bought (Farmer)	Minimum	3.8	3	4.2	3	3.8	2.6	3.13
	Maximum	6.4	6.4	6.2	6.1	6.4	5.7	6.50
	Range	2.6	3.4	2	3.1	2.6	3.1	3.38
	Mean	5.03	4.83	5.18	4.42	5.18	4.16	4.89
	Std. Dev.	1.04	1.21	0.66	1.18	0.91	1.19	1.24
P value		NS	NS	0.045	0.032	NS	0.057	0.55

Fragrance ranged from 3.8 to 6.8, aroma from 3 to 7.2, acidity from 3 to 7.8, flavour from 2.5 to 7, body from 2.5 to 7.4, aftertaste from 2 to 6.8 and overall cup quality ranged from 3.13 to 7.4 (Figure 1). Generally, coffee samples bought from the farmers had lower cup quality as compared to those collected from the coffee trees in the forest, especially beverage acidity and flavour (Figure 2). This could be due to the fact that farmers in the Harenna area pick red and

green cherries together during the harvesting period. This is because harvesting is done in a sort of campaign by family labour and/or hired labour. Most coffee farmers in Harenna area live in far away areas from the coffee forests, which is of course advantageous for the forest coffee conservation; but this scenario leads to a sacrifice in quality as farmers have to finish the coffee harvesting activity within short period of time as much as possible and return to their settlement for other activities or obligations. Moreover, the use of hired labour from the neighbouring non-coffee growing areas (Adaba, Ganale, Bidire, etc.) is also an important factor in scarification of coffee quality as payment is effected based on the quantity of coffee harvested, not on the basis of quality harvesting. The use of hired labor in the share-harvesting arrangement is also common practice Sheko area in SW Ethiopia, and hence the problem as well.

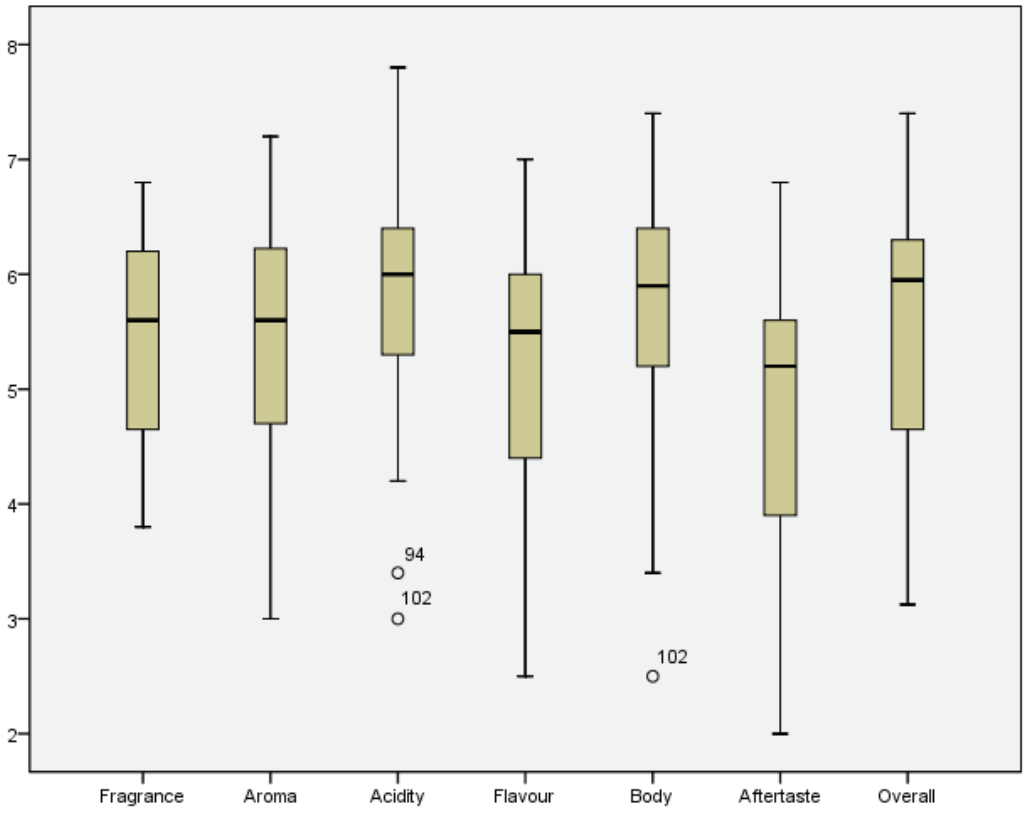

Figure 1. Sensory characteristics of wild Arabica coffee from Harenna Afromontane rainforest in the SE Ethiopia (both collected and bought samples)

Of all the cup quality traits, fragrance was little affected by the level of maturity of the cherries during harvesting; that is, variation in fragrance was relatively lower as compared to other cup quality traits, indicating that fragrance was less related to handling and processing factor (Figure 1 and 2). This study also revealed that the inherent potential or desirable cup quality of wild Arabica coffee was masked by poor harvesting and processing practices observed in the area, suggesting the need for intervention in these areas to make use of the available potential benefits of these genetic resources. Promoting action research, increasing awareness, investing more on inputs or materials required for possible use of the improved processing practices available else where or to be generated by focused research, etc. are urgently needed.

However, the level of bean maturity (sample source) had no significant effect on physical bean characteristics in Harenna forest. The sensory property of the coffee was more affected by the level of cherry maturity as compared to the bean physical characteristics. But some of

the sensory and bean characteristics were significantly correlated (Figure 4 and 5). The higher the proportion of the small beans in the sample, the better the cup quality of the coffee in SE Ethiopia (Figure 4), and vice versa.

In SE Afromontane rainforest, beans were bolder as compared to those from SW Afromontane rainforests. Stated differently, the proportion of smaller beans was lower in the SE as compared to that of the SW, and vice versa. In both SW and SE rainforests, the proportion of beans retained on screen 16 was higher, followed by screen 17 in SE (Figure 3), but followed by screen 15 in SW Ethiopia (data not shown here).

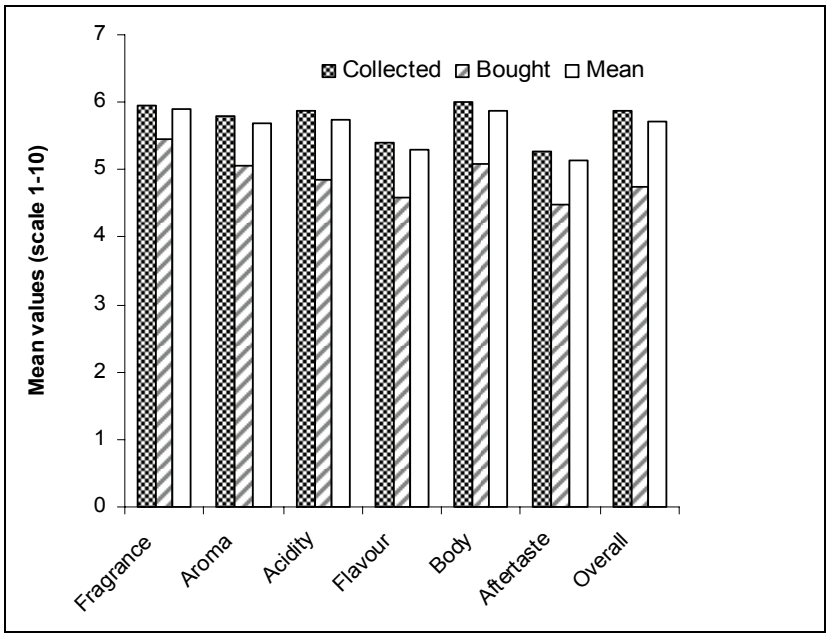

Figure 2. Mean values for cup quality traits of wild arabica coffee from Harenna Afromontane rainforest in the SE Ethiopia

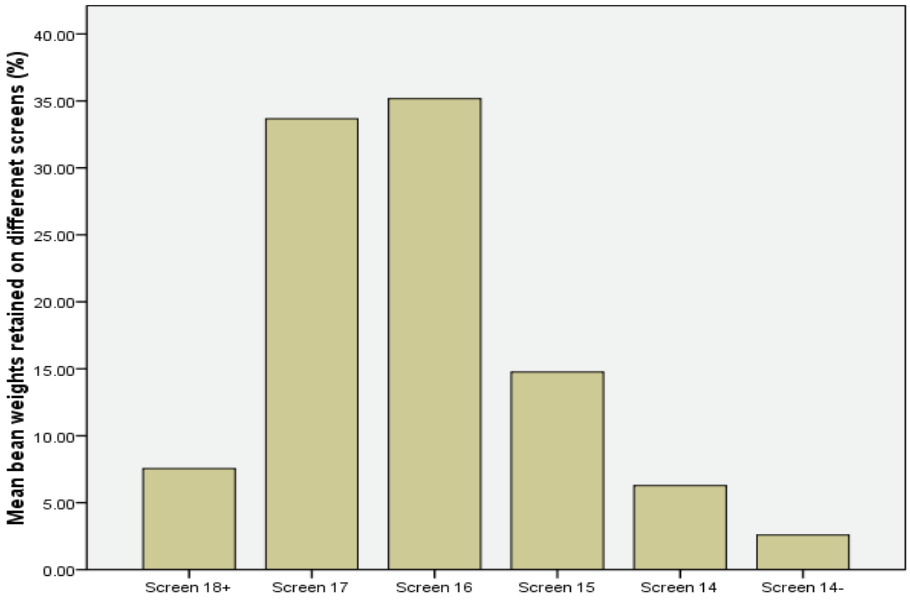

Figure 3. Bean size distribution of wild Arabica coffee from Harenna Afromontane rainforest in the SE Ethiopia

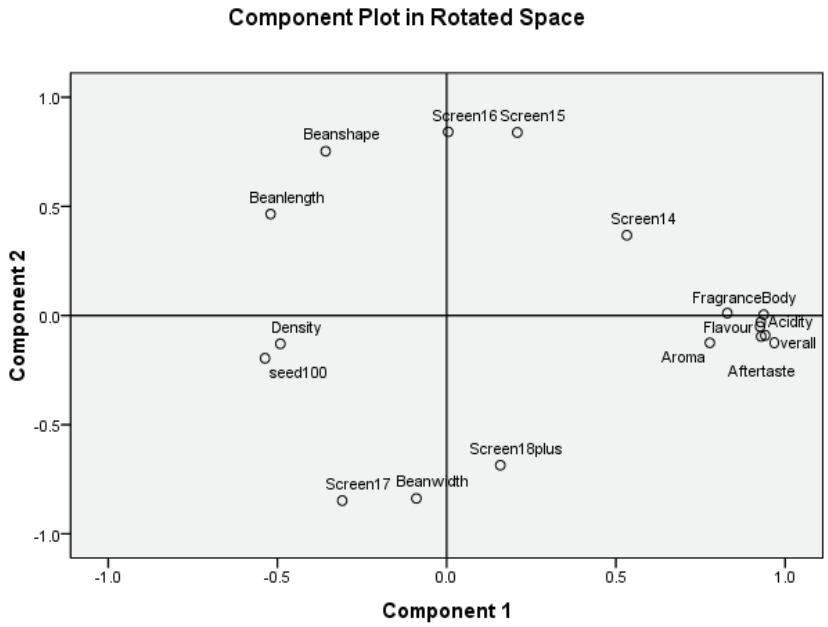

Figure 4. Principal components analysis (PCA) taking into sensory and bean characteristics of coffee samples collected from Harenna Afromontane rainforest in the SE Ethiopia (PC1 - 41.31%, PC2 - 25.36%).

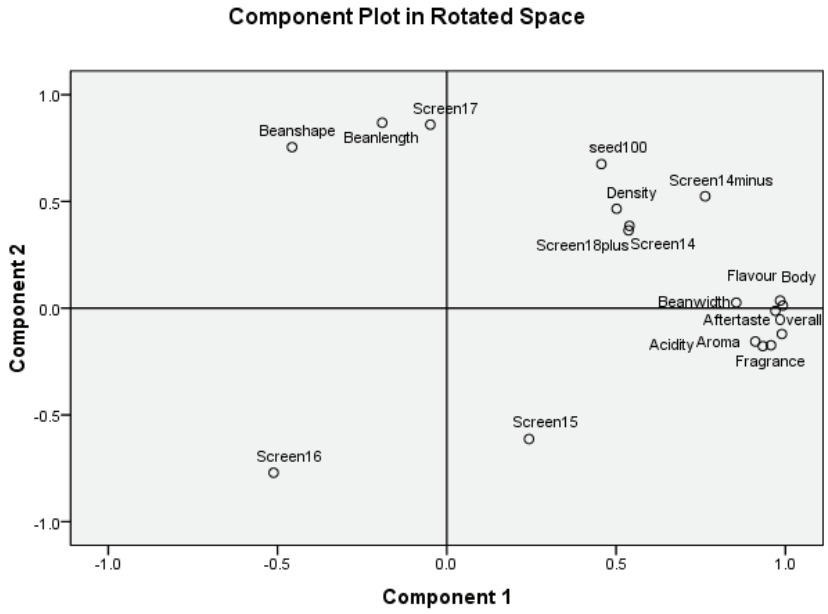

Figure 5. Principal components analysis (PCA) taking into sensory and bean characteristics of coffee samples bought from farmers (PC1-52.57%, PC2-23.97%).

As clearly indicated in Figure 4, in the case of coffee samples directly collected from the coffee trees in the forest, beverage quality was negatively correlated with bean density and 100 seed weight. But cup quality of the coffee beverage was positively and significantly correlated with bean width in the case coffee samples bought from farmers (Figure 5), and hence it can be a good indicator of cup quality especially when green coffee cherries are harvested. Therefore, among the physical characteristics, the proportion of small beans, bean weight and bean width are important indicators of organoleptic properties of wild Arabica coffee in SE Ethiopia.

CONCLUSIONS

This study showed that high variability in sensory and bean characteristics were observed in coffees samples from Harenna Afromontane rainforest in SE Ethiopia. Variation in the sensory characteristics were also noticed due to variation in the maturity of cherries, and these changes could be detected by a sensory panel. Variability was high in coffees from forests than from farmers, indicating that the inherent potential of wild Arabica coffee was masked by poor harvesting and handling practices. The major attributes of the coffee that are most affected by level of maturity were coffee acidity and flavour, with matured cherries (red cherries collected from forest trees) found to have a better coffee flavour, whereas green cherries bought from farmers found to be more bitter (less acidic). The panelists had different preferences but overall, appeared to prefer the coffee from forests (collected coffees) compared to the farmers coffee (bought coffees). The results indicate the importance of cherry maturity for cup quality of wild Arabica coffee from SE natural coffee forest.

Harvesting of green cherries more affected the sensory property of the coffee than its bean physical characteristics. But its effect on bean characteristics was also reflected on cup quality. When green coffee cherries were harvested, bean size (bean width) was a good indicator of cup quality as there was positive and significant correlation between them. But in the case of red cherries, the relationship between bean width and cup quality was much apparent. Generally, beans from SE Afromontane rainforest were bolder as compared to those from SW Afromontane rainforests of Ethiopia.

Last but not least, promoting action research to improve the processing practices and increasing awareness through training and demonstration of improved processing methods are urgently needed.

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Discrimination of Coffee Freshness and Roast Level Using SPME-Gas Chromatography and Near Infrared Spectroscopy

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SUMMARY

Coffee was harvested from Kunia, Hawaii and processed to green bean. Subsamples of the coffee were roasted to two different degrees of darkness and allowed to rest in sealed, one-way valve bags at room temperature for 8, 6, 4 or 0 weeks. The coffee was scanned as whole beans using near infrared spectroscopy (NIRS), ground, and rescanned. Subsamples of the scanned coffees then were brewed in airtight headspace vials. Aroma volatiles were captured using solid phase microextraction (SPME) and analyzed using gas chromatography (GC). The data from the NIRS and GC were then used to predict group membership using discriminate analysis. Using the NIRS data, coffee was discriminated by freshness of either ground or whole bean coffee using 8 wavelength reflectances. When the data sets were combined, 22 peaks were needed to discriminate the coffee by freshness. Roast level discrimination occurred using 2 or 1 reflectances, whole bean and ground, respectively, or 2 with a combined data set. Using the GC data, coffees were discriminated by freshness and roast level by using 9 and 1 compounds, respectively.

INTRODUCTION

Eleven regions in the state of Hawaii, USA grow coffee. Coffees from some of these regions can cost as much as $90 (USD) per roasted kilogram. Consequently, the temptation is great for some processors and farmers to misrepresent a cheaper coffee as a more expensive coffee. While laws exist to protect these coffees from such misrepresentation, it is very difficult to prove where a coffee was actually grown. Enforcement of these laws would be easier if an objective, chemical method could be used to authenticate a roasted coffee's origin.

Near infrared spectroscopy (NIRS) and solid phase microextraction-gas chromatography (SPME-GC) are rapid systems of analysis that provide large amounts of chemical data about coffee. Processing the data using the multivariate statistical technique of discriminate analysis may provide the basis of a model by which origin predictions can be made.

Because the chemical profile of coffee differs based upon the freshness and roast level, any origin authentication model must account for these changes in a roasted product. This poster reports on a preliminary study that uses NIRS and SPME-GC to discriminate roasted coffee based upon its freshness and degree of roast.

MATERIALS AND METHODS

Coffee was harvested from 10 trees in Kunia, Oahu and processed to green bean. A 3.2 kg sample of this coffee was removed, thoroughly mixed, and set aside for this experiment.

Coffee was roasted in a Probat sample roaster, using 100 g of green bean for each roast. Four replications of 2 roast levels were roasted each day of roasting. The roast levels corresponded to an approximate weight loss of 22% (wet weight basis) for the dark roast and 17% for the light roast. Coffees were roasted 8, 6, 2, or 0 weeks before analysis. After roasting, the coffees were sealed in foil bags embedded with one-way valves and stored at room temperature.

On the day of analysis, 4.5 g of coffee were removed from the bag and used for the NIRS analysis. Each individual scan was an average of 100 scans (made by the machine). Reflectance data was recorded for all wavelengths between 350-2500 nm using an ASP 350-2500 spectrophotometer (ASD, Inc., Boulder, CO, USA). Three rotations of the cuvette were made and their values averaged for use in the statistical analysis. After the whole beans were scanned, the sample was finely ground and rescanned, also with 3 rotations of the cuvette.

Once scanning was complete, a 3.3 g subsample of the ground coffee was removed and used for the SPME-GC analysis. The ground sample was placed in a 150 ml headspace vial with 60 ml of 90 °C water and sealed. After brewing for 5 minutes, a PDMS/CAR/DVB SMPE fiber was injected into the headspace and held there for 5 minutes. The fiber was inserted into an HP 5890 GC injection port (250 °C). Chromatographic conditions were: Temperature program: 0-4 min: 40 °C, 4-45 min: 3°/min increase to 163 °C, 45-51.7 min: 20°/min increase to 230 °C, 51.7-61.7 min: 230 °C; Injection: splitless; Head pressure: 10 PSI; Detector: FID; Carrier gas, helium. The column used was a Stabilwax DB, 30m length, .53mm ID. The FID detector signal was monitored using PeakSimple™ software to integrate individual peaks.

Canonical discriminate analysis was carried out using JMP 7.0.1 statistical software (SAS Institute, Inc., Cary, NC, USA). Peak reflectance values at each wavelength from the NIR spectrophotometer and peak areas from the chromatograph were used for data analysis. Forward stepwise variable selection was used to reduce the number of variables used in the discriminate analysis. Stepwise selection progressed until discrimination could be carried out with no misclassifications, less than 0.1 chance of misclassification to another group, and with the fewest number of compounds.

RESULTS AND DISCUSSION

Data from the NIRS and GC were successful in discriminating coffees by freshness and roast level with no misclassifications. The physical state of the coffee did not impede accurate classification.

Table 1 shows a summary of the discriminate analysis using the NIRS data. Within a single particle state, coffee freshness was accurately grouped using 8 wavelength reflectances.

When both whole and ground coffee were combined in a dataset, 22 wavelength reflectances were necessary for accurate discrimination.

Roast level discrimination occurred using as few as 2 wavelengths for whole bean coffee and the combined dataset. Only 1 wavelength was necessary to discriminate roast level when using ground coffee. Figure 1 shows the biplot of the discrimination of whole bean coffee based on roast level.

Table 1. Discrimination of coffee freshness and roast level using NIRS.

Discrimination	Physical state of coffee	Number of wavelengths used	Percent misclassified	p for Roy's Max Root
Freshness	Whole	8	0	<.0001
Freshness	Ground	8	0	<.0001
Freshness	Both	22	0	<.0001
Roast level	Whole	2	0	<.0001
Roast level	Ground	1	0	<.0001
Roast level	Both	2	0	<.0001

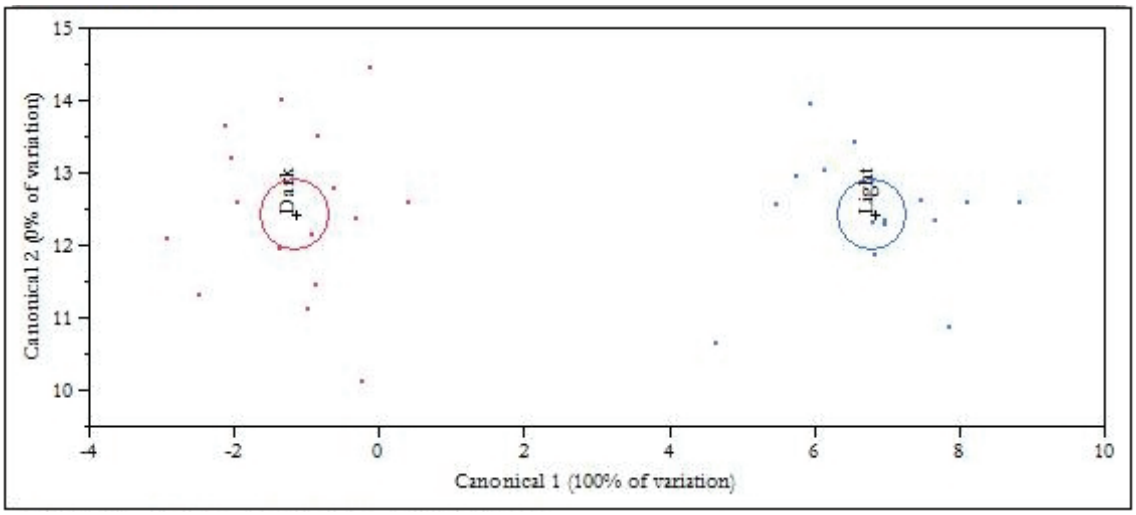

Figure 1. Biplot of roast level discrimination using whole bean coffee and NIRS data. The size of the circle corresponds to a 95% confidence limit for the mean.

Table 2 shows a summary of the discriminate analysis using the GC data. Discrimination of coffee freshness using compounds detected by the GC required 9 compounds. Roast level was discriminated using just 1 compound. Figure 2 shows a biplot of the discrimination of coffee freshness from the GC data.

Table 2. Discrimiation of coffee freshness and roast level using SPME-GC.

Discrimination	Number of compounds used	Percent misclassified	p for Roy's Max Root
Freshness	9	0	<.0001
Roast level	1	0	<.0001

Discriminate analysis of data garnered from NIRS and SPME-GC is a valuable tool for coffee analysis. Roast level and freshness were both easily predicted. Furthermore, both methods permitted discrimination of samples into one treatment independent of the other. In other words, the roast level of the coffee did not interfere with the discrimination of the coffee by freshness.

The success of the discriminations herein is encouraging for the use of these analytical methods in determining where a coffee is grown in Hawaii. When attempting to discriminate coffee origins, this data can account for differences in post-farm processing. Consequently,

true differences in the chemical profiles of the coffees grown in different places will be easier to detect.

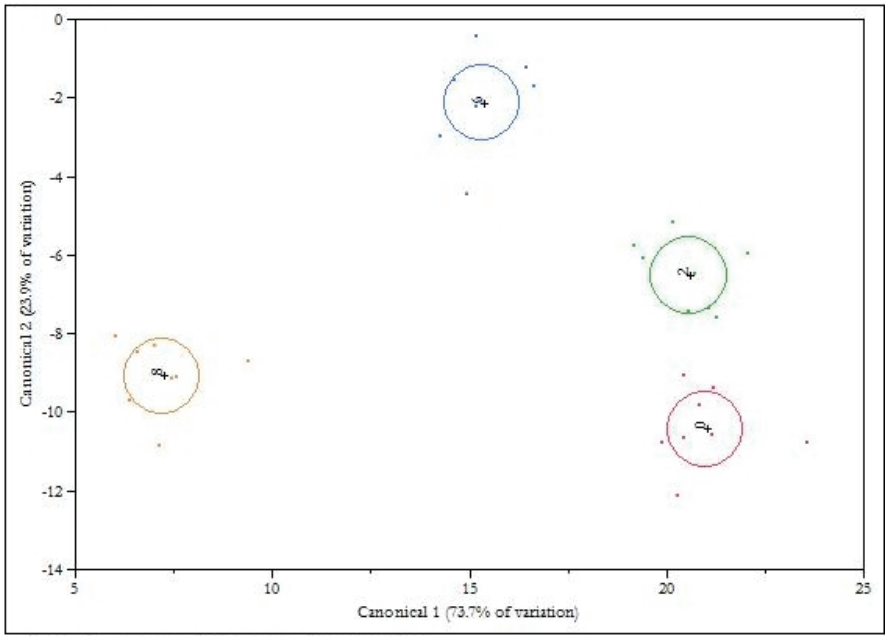

Figure 2. Biplot of coffee freshness discrimination using GC data. The size of the circle corresponds to a 95% confidence limit for the mean.

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Arabica *Versus* Robusta Green Coffee Differentiation Based on Near Infrared Spectroscopy

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SUMMARY

The interest in food authentication has been increasing in the recent years. A large number of food products have the potential to be adulterated, especially those that are expensive and have production levels which can vary as a result of climate change and harvest conditions. This is the case of coffee, particularly in recent years, due to the increasing practice of selling the coffee on the basis of its varietals and/or geographic origin. At least 66 species of the genus *Coffea* L. have been identified so far, and two of these are economically and commercially important: *C. arabica* L. (arabica coffee) and *C. canephora* Pierre (robusta coffee). The most commercially available coffee blends are produced from arabica and robusta coffees that differ not only from a botanical standpoint but also in quality. This is recognised commercially, as robusta usually sells at lower prices than arabica, more appreciated by the consumers due to its equilibrated acidity and sweetness. Although the two species show great differences in their botanical, genetic, agronomical, chemical and morphological characteristics, most of the time the analytical reference methods used to determine the chemical composition of arabica and robusta coffees in order to develop a classification model can be too elaborated and time-consuming. This is a disadvantage when trying to discriminate arabica from robusta green coffee or different mixtures of these two, during the short period of time they are kept for quality evaluation (before formal acceptance) by the company that will proceed with the roasting process. Therefore, there is a clear need for suitable analytical methods, combined with chemometrics, in order to differentiate between these two coffee types using a fast, clean and inexpensive analytical method such as near infrared spectroscopy (NIRS). In this study, near infrared spectroscopy (NIRS), combined with technical computing, has been used to discriminate between arabica and robusta types of green coffee as a means to avoid coffee adulteration. NIR spectra of 103 different coffees from 29 different geographic origins were collected. The sample set comprised of 70 arabica and 33 robusta coffees. An average spectrum was obtained for each individual sample and used separately to develop a mathematical model that allowed coffee type discrimination.

INTRODUCTION

At least 66 species of the genus *Coffea* L. have been identified so far, and two of these varieties are economically and commercially important: *C. arabica* L. (arabica coffee) and *C. canephora* Pierre (robusta coffee) (Van der Vossen, 2001). *Coffea arabica* is the species that has been known for the longest time and is also the most widespread throughout the world. It has given rise to a number of varieties (hybrids, mutants, etc.), regional types and cultivars, which reflect the influence of the environment. Today many *C. arabica* varieties are

cultivated all over the world (*C. arabica* L. var. *typica* L., *C. arabica* L. var. *maragogype* Hort., *C. arabica* L. var. *bourbon* (B. Rodr) Choussy and *C. arabica* L. var. *lamine* J. L. de Lanessan) (Coste, 1992) and coffee produced from all these varieties is commonly designated by arabica type of coffee. The species *C. canephora* takes second place in the world to *C. arabica*. The volume of business generated from this species on the international market has been increasing steadily for half a century and it may be said that one third of the coffees consumed in the world today originate from this species (Van der Vossen, 2001). The most widely cultivated variety in the world is robusta, which makes up at least 95 per cent of the *C. canephora* plantations (Coste, 1992). The hybrid of Timor should be mentioned as it is a natural hybrid between *C. arabica* and *C. canephora* used in various associations by geneticists who value it for its resistance to *Hemileia vastatrix* (coffee rust) and Coffee Berry Disease (CBD) (Coste, 1992).

Although the two species show great differences in their botanical, genetic, agronomical, chemical and morphological characteristics, most of the time the analytical reference methods used to determine the chemical composition of arabica and robusta coffees in order to develop a classification model can be too elaborated and time-consuming. Suitable analytical methods, combined with chemometrics in order to differentiate between these two coffee types using a fast, clean and inexpensive analytical method as near infrared spectroscopy (NIRS) can be very important. This technique offers many possibilities for food studies such as identification and constituent analysis of fruit, wine, meat, oil and corn (Cen and He, 2007). The combination between NIRS and PCA has been used to characterize and classify wines, fruits, cheese, roasted coffee and other foods (Huang et al., 2008) and even for sensory analysis (Andrés et al., 2007). NIRS is a fast and non-destructive technique that provides multi-constituent analysis of virtually any matrix (Reich, 2005). It covers a spectral range from 780 to 2500 nm (12,500-4000 $cm^{-}1$) and provides much more complex structural information related to the vibration behaviour of combinations of bonds (Cen and He, 2007). The analytical information of NIR spectra is multivariate in nature. To perform qualitative or quantitative NIR analysis, i.e. to relate spectral variables to properties of the different matrices, mathematical and statistical methods (i.e. chemometrics) are required to extract "relevant" information and reduce "irrelevant" information, i.e. interfering parameters (Reich, 2005).

The application of NIRS for roasted coffee authenticity is well developed by Pizarro and co-authors (Pizarro et al., 2007; Esteban-Díez et al., 2007; Pizarro et al., 2004) that have also applied the same approach for the determination of chemical components of roasted coffee like the ash content and lipids (Pizarro et al., 2004). The aim of this study was to develop a classification model for discriminating between arabica and robusta green coffee based on their NIR spectra to obtain a classification model of the two different coffee types.

RESULTS AND DISCUSSION

A visual comparison between NIR spectral profiles on the 103 green coffees can be seen on Figure 1. The average of three measurements was used for model development. MATLAB (2002) technical computing language was used to create a model, i.e. selection of spectra, average spectrum computation from the collected replicates, and principal component analysis (PCA). Factors with eigenvalues greater than 1 were selected. Discrimination between arabica and robusta green coffees is shown in Figure 2. Using the wavenumber range 12,000-4000 cm^{-1}, two principal components (PC1 and PC2) accounted for 54.48% and 15.54%, respectively, of the total variance in the spectral data set. Arabica and robusta green coffee samples were mainly separated by PC1, suggesting the presence of two different clusters associated with the two green coffee types. As can be seen in Figure 2, robusta

samples are located at the positive side of PC1 score scale and most of the arabica coffees are located at the negative side of scores scale. Although in our study we can not show which factors are influencing the measured differences in spectra, we estimate that different environments and the presence of hybrids can explain the closeness of some of the arabica coffees analysed to the robusta coffee group on PCA.

The classification model developed from the NIR spectra can be sufficient for providing a satisfactory classification of arabica and robusta coffees. In spite of the good discrimination exhibited by the selected model, which might be considered satisfactory for the classification of the coffee types considered, the relatively short distance between arabica and robusta could reveal some potential problems for classification of extreme samples within each category. Selecting wavenumber ranges where signal for each category is different can be a future approach to this problem.

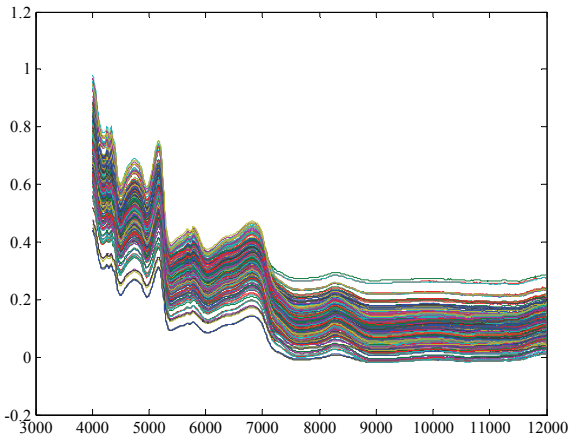

Figure 1. NIR transflectance spectra used for the green coffee type classification in the spectral region 12,000 – 4000 cm^{-1}.

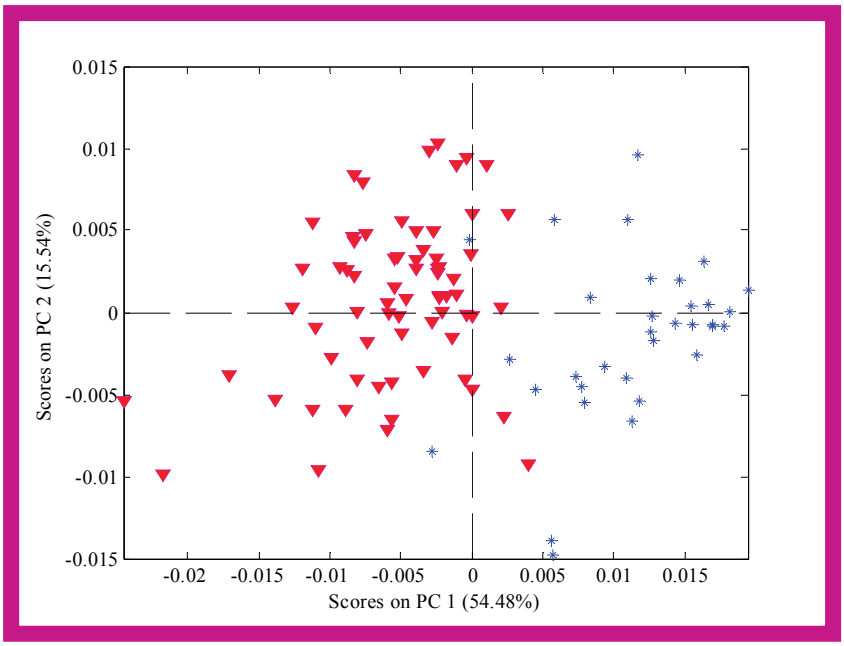

Figure 2. Scores on PC1 and PC2 for PCA analysis for the classification of green coffee varieties. Group A = arabica coffee (↖); group B = robusta coffee (∗).

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Principles and Advanced Aspects of Profile Roasting Process

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SUMMARY

The purpose of this research is to analyze the profile roasting influence on the coffee drink, on the coffee aroma and on the chemical composition of roasted coffee. In this study, the profile roasting is evaluated by the coffee bean temperature evolution along the time during the roasting process. Why do we establish a direct relation between profile roasting and bean temperature evolution? There are many parameters that seem to be the cause of the roasting process. In a roaster with heat transfer mainly based on convection the hot air flow and hot air temperature are quite important, but they do not directly cause the roast to happen. They are the main cause of the heat transfer. The heat transfer also does not roast the coffee, but it is capable to increase the coffee bean temperature. This last element is what causes the coffee beans to roast. The coffee bean temperature evolution is the determining element for all the chemical reactions that occur during the pirolises process. It is related to the necessary energy level required by each chemical reaction and many different reactions will start to happen from different bean temperatures and higher. Therefore different bean temperature evolutions will result in the formation of diverse substances along the roasting process. How the experiments were carried out in order to isolate the influence of the bean temperature evolution from other roast parameters? Each performed test generated a set of three coffee samples which were produced using three different profile recipes. These recipes resulted in roasting times of 4, 8 and 15 minutes respectively. It took the second recipe around 3 minutes to go from the first crack to the second crack. For the first recipe the elapsed time between cracks happened in less than 2 minutes and in the last recipe it happened in more than 4 minutes. These recipes were chosen to evaluate the results of a very fast roast (uneven roast), an intermediate and a baked coffee. It was always used the same Arabica coffee blends for each test to eliminate the raw material influence in the final results. With the same purpose, each set of three samples were roasted up to the same final color. In addition to it, the samples were taken with the same final moisture. This way it was possible to isolate the exclusive influence of the coffee bean temperature evolution over the roasted coffee composition. The same care was taken over other elements that could alter the cupping tests. Finally we carried out the same experiment for 4 different Arabica blends. It was possible to confirm the same trends over many cupping features and over many chemical components concentrations. The intensity of each tendency varied from blend to blend, showing the raw material influence, but the same trend showed the profile control over the process. The purpose of this research is to analyze the profile roasting influence on the coffee drink, on the coffee aroma and on the chemical composition of roasted coffee. In this study, the profile roasting is evaluated by the coffee bean temperature evolution along the time during the roasting process.

INTRODUCTION

Why do we establish a direct relation between profile roasting and bean temperature evolution

There are many parameters that seem to be the cause of the roasting process. In a roaster with heat transfer mainly based on convection the hot air flow and hot air temperature are quite important, but they do not directly cause the roast to happen. There is another element involved: the heat transfer. The heat transfer mainly caused by forced convection could be generally, and simplified, expressed by the following formula:

$$\mathbf{Q} = (K)x(Tair–Tbean)x(Vel.air)^{(k1)}x(Dg)^{(K2)}$$

The meanings of the symbols in the formula above are as following: K, K1 and K2 are constants. Tair – Tbean is the difference between the air flow temperature and the beans temperature. Vel.air is the air speed that is proportional to the air flow in the roasting chamber and Dg is proportional to the medium dimension of the beans.

This simple thermodynamic evaluation shows us that the air flow and air temperature in the roasting chamber are the main cause of the heat transfer. But the heat transfer also does not roast the coffee, but it is capable to increase the coffee bean temperature. This last element is what really causes the coffee beans to roast.

The temperature is an energy indicator related to molecular agitation. It is to say that the temperature somehow measures the molecular kinetic energy. At zero Kelvin there is no molecular movement and the higher the temperature the higher is the kinetic energy level.

All the necessary chemical components for the pyrolysis reactions are already in the green coffee beans, but no reaction occurs under ambient temperatures. There are minimum energy levels to start specific reactions of the pyrolysis and each reaction needs a different minimum energy level to start. So it implies that each chemical reaction requires different minimum temperatures to happen.

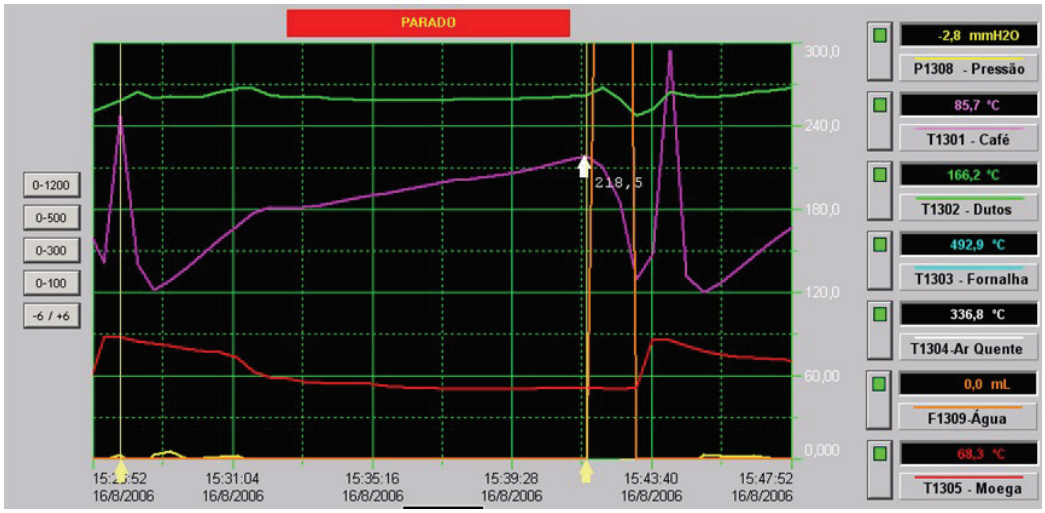
Therefore, the coffee bean temperature evolution along the time is the determining element to control the sequence and intensity of all the chemical reactions that occur during the pyrolysis process. Many different reactions will start to happen from different bean temperatures and higher. Consequently different bean temperature evolutions will result in the formation of diverse substance concentrations along the roasting process.

EXPERIMENTAL APPROACH & RESULTS

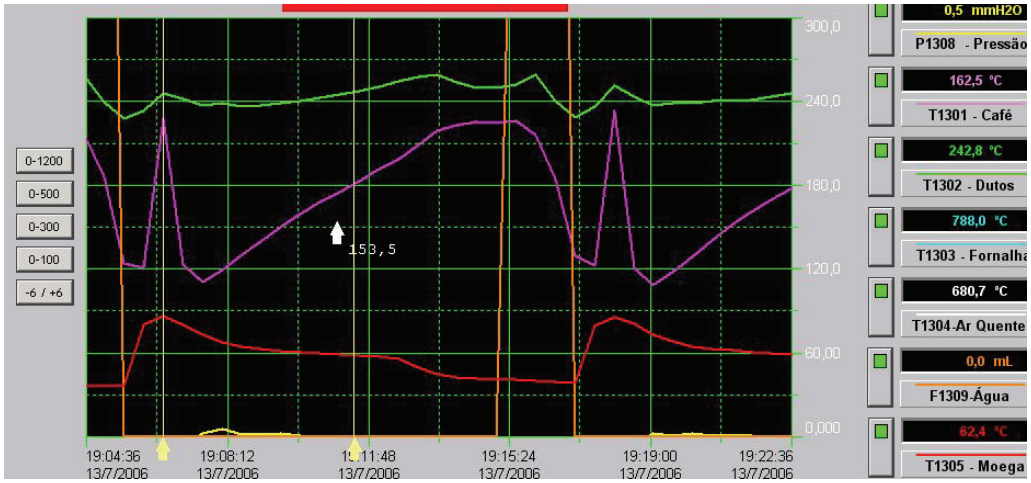
How the experiments were carried out in order to isolate the influence of the bean temperature evolution from other roast parameters

We started our experiments in 2006 and along this year and the year of 2007 we generated four sets of valid samples for our analysis. Our goal was to find a clear correlation between the roasting characteristics and three kinds of profiles: fast roast, medium roast time and slow roast time.

Recipe I:



Recipe II:



Recipe III:

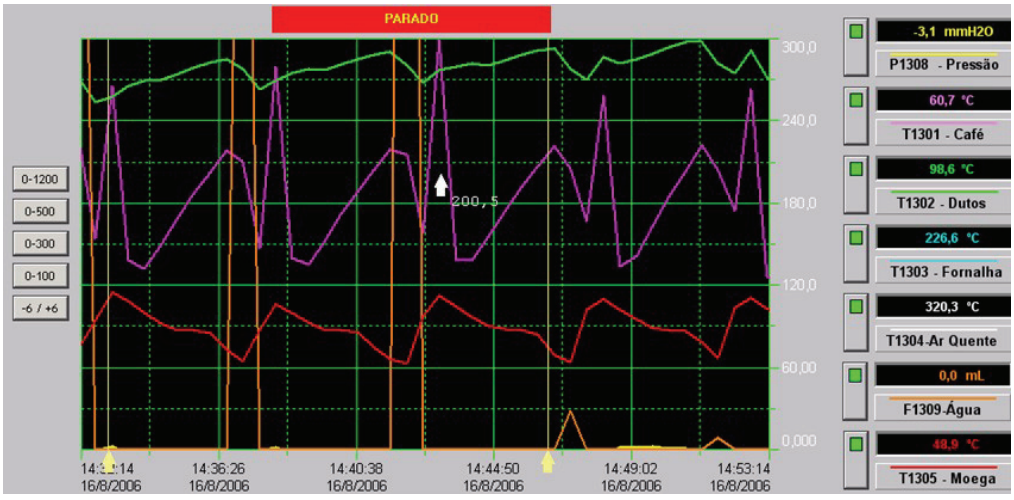


Figure 1.

As roasting characteristics we considered the cupping and the chemical analysis. In the cupping evaluation we took in account the powder aroma, the infuse aroma, the bitterness, defect perception, the acidity and the body. The defect perception was included in our evaluation because in three of our four experiments we used coffees with high percentage of black, light-green and sour beans. The defect perception indicates how intense the presence of this coffee defects could be noticed by the cuppers. This evaluation is very important for producing countries where this kind of coffee is largely used.

The chemical analysis included the titatrable acidity, the pH, the total chlorogenic acids, the concentration of extractable soluble solids, the acrylamide and the total carbohydrates.

Each performed test generated a set of three coffee samples which were produced using three different profile recipes. These recipes resulted in roasting times of 4, 8 and 15 minutes respectively. It took the second recipe around 3 minutes to go from the first crack to the second crack. For the first recipe the elapsed time between cracks happened in less than 2 minutes and in the last recipe it happened in more than 4 minutes. These recipes were chosen to evaluate the results of a very fast roast (uneven roast), an intermediate and a slow recipe that could lead us to a baked coffee. We named the Profile recipes as slow, medium and fast. The bean temperature evolution of each recipe is shown in the graphics below.

The pink line is the bean temperature evolution along the time. Looking at the graphic of recipe I we can notice that, in the beginning of the roast, there is a pick in the temperature reading. This is the moment when the roasting chamber is empty, so the system is actually reading the hot air temperature. At the moment the raw material is dumped into the roasting chamber, the temperature reading drops up to a minimum and this is the point from where the system starts to have an actual bean temperature reading.

We can notice that from the beginning of the roast and on the bean temperature starts to increase in different rhythms for different profile recipes. It gets to a point where the bean temperature reaches a maximum and then we can see a strong temperature drop. This is the moment when the roaster started the quenching procedure exactly in the instant when the coffee reached the desired final color.

As explained before, we had the target of finding a correlation between the roasting profiles and the roasting results that would neither depend on differences of raw material, nor on the final roasting color, nor on the final coffee humidity. For this purpose we establish the following experiment conditions.

Along the same experiment it was always used the same Arabica coffee blend for the thee profile recipes (the fast, the intermediate and the slow). The blends were dosed by a weighing system with a precision of 0.2% on the total blend weigh. With this procedure we eliminated any raw material interference in the final results. With the same purpose, for each experiment, the samples were roasted up to the same final color. In addition to it, the samples were taken with the same final moisture. This way it was possible to isolate the exclusive influence of the coffee bean temperature evolution along the time over the roasted coffee composition.

Finally we carried out the same experiment for five different Arabica blends, generating four sets of samples. Comparing experiment to experiment, we allowed variations in the final roasting color. One of the four blends were a blend for espresso with nether black, light-green nor sour beans. For this set of roasting samples we had a quite liter color than the other three.

It was possible to identify the same trends over many cupping features and over many chemical components concentrations. The intensity of each trend varied from blend to blend, showing the raw material influence, but we were able to establish a clear correlation between these features and the profile recipes, identifying the control the bean temperature evolution along the time has over the roasting process.

Cupping results

In the beginning of our experiments we made a pilot test and cupping analysis. We employed two professional cuppers. The first one, Mr. José Bogre, is nowadays a consultant that has worked in Brazil along more than 40 years for a large company named CIA União. Among other functions he was responsible, at that time, for the coffee final quality in a company that reached the production of 10.000 tons of roasted coffee per month. The second cupper is the Engineer Eliana Relvas. She is a food process engineer that works as consultant for Sindicafe (the coffee industry syndicate in the state of São Paulo – Brazil) and for ABIC (the Brazilian association of the coffee industry). She is well known in the Brazilian market and offers her experience for many coffee industries nationwide.

Both, José Bogre and Eliana Relvas, analyzed the same set of samples number one. The table below shows the cupping results as analyzed by Mr. José Bogre.

Table 1.

Type: 7-30-PVA 18,4%			
	Fast Roast	Slow Roast	Medium Roast
Powder aroma (0 a 10)	7,0	6,0	7,5
Infuse aroma (0 a 10)	Fruit: 2	Fruit : 2	Fruit : 2
Defect perception	Light	Strong	Light
Acidity (% de 0 a 4)	0	0	0
Bitterness (0 a 10)	5,5 Acceptable	7,00 Too strong	6,00 Acceptable
Body (0 a 10)	6,00	6,00	6,00
Cupping with sugar (0 a 10)	6,5	4,0	7,0
Beans appearance	20% uneven color	6% uneven color	11% uneven color
Beans density (g/l)	275	295	280
Color: colorete II: 88			

The table below shows the cupping results for the same set of samples as analyzed by the Engineer Eliana Relvas.

Table 2.

	Fast Roast	Slow Roast	Medium Roast
Powder aroma	5,00	5,00	5,50
Infusion aroma	5,50	4,50	5,00
Defects perception	5,00	6,50	4,50
Acidity	4,00	4,00	4,50
Bitterness	6,00	7,00	6,00
Body	6,00	6,00	6,00
After Taste	5,00	5,00	6,00
Global quality	5,00	4,50	6,00
Color: Neuhaus: 70			

The table below shows a comparison between both evaluations.

Table 3.

	Ms.Relvas			Mr.Bogre		
	Fast Roast	Slow Roast	Medium Roast	Fast Roast	Slow Roast	Medium Roast
Powder aroma	-	-	Best	-	Worst	Best
Infuse aroma	Best	Worst	-	-	-	-
Defects perception	-	Strongest	Weaker	-	Strongest	-
Acidity	-	-	-	-	-	-
Bitterness	-	Strong	-	-	Too strong	-
Body	-	-	-	-	-	-
After taste	-	-	Best	-	Worst	-
Global quality	-	Worst	Best	-	Worst	Best

After this first test we could conclude that it was worth to go further with our research. The two contracted professionals agreed about the evaluations of the bitterness, defects perception, powder aroma and the global quality. They did not totally agreed about the after taste, but adding both evaluations we could infer that the medium recipe was the best and the slow recipe the worst.

Therefore, after this pilot experiment we decided to make a Larger cupping experience that could give a much more reliable cupping evaluation, in such a way, that we would be able to eliminate the subjective aspect that may be present in some cupping trials. We prepared an event were we were able to gather 24 cuppers, employees of roasting plants from all over Brazil. For this new experiment and for all the others we mention in this paper work, we used exactly the same profile roasting recipes, so that we gather specific information about the trends of these recipes would cause to the final roasted coffee characteristics. Again, in order to reach this goal, in each experiment we changed the raw material blend and the final roasting color.

For this event the analyzed cupping elements were: powder aroma, drink aroma, bitterness, acidity, defect perception, body and global quality. We performed a blind cupping were the samples of the Slow recipe, fast recipe and medium recipe were know by the cuppers only as blue, white and green.

The table below shows the cupping final conclusion of the cuppers. When the total number of evaluations, shown in the last right column, is lower than 24 it means that some of the cuppers did not have a strait opinion about the evaluated parameter.

Table 4.

Analyzed Element	**Evaluation**	**Blue**	**White**	**Green**	**Total**
		Slow	**Fast**	**Medium**	
	Best	4	12	7	23
Powder Aroma	**Weakest**	12	3	5	20
	Best	1	11	10	22
Drink Aroma	**Worst**	14	2	6	22
	Most Intense	16	2	1	19
Bitterness	**Lest Intense**	1	10	7	18
	Strongest	4	10	6	20
Acidity	**Weakest**	8	1	6	15
	Strongest	4	5	8	17
Body	**Lightest**	9	4	5	18
	Strongest	12	3	3	18
Defects perception	**Lightest**	3	7	5	15
	Best	2	3	5	10
After Taste	**Worst**	7	3	0	11
	Best	0	4	9	13
Global Quality	**Worst**	10	1	1	12

The table below summarizes the cuppers' conclusions.

Table 5.

Evaluated element	**Evaluation type**	**Blue**	**White**	**Green**
		Slow	**Fast**	**Medium**
	Best		**X**	
Powder aroma				
	Weakest	**X**		
	Best		**X**	**X**
Infuse aroma				
	Weakest	**X**		
	Most intense	**X**		
Bitterness				
	Least intense		**X**	
	Strongest	**X**		
Defect perception				
	Weakest		**X**	
	Strongest		**X**	
Acidity				
	Weakest	**X**		
	Strongest			**X**
Body				
	Weakest	**X**		
	Most desirable			**X**
After taste				
	Least desirable	**X**		
	Best			**X**
Global quality				
	Worst	**X**		

The best powder and drink aroma were found in the coffees roasted with the fast and medium recipes. The worse evaluation came from the slow roast. The slow roast also got the worse global quality grade. The best global quality grade was obtained by the medium recipe.

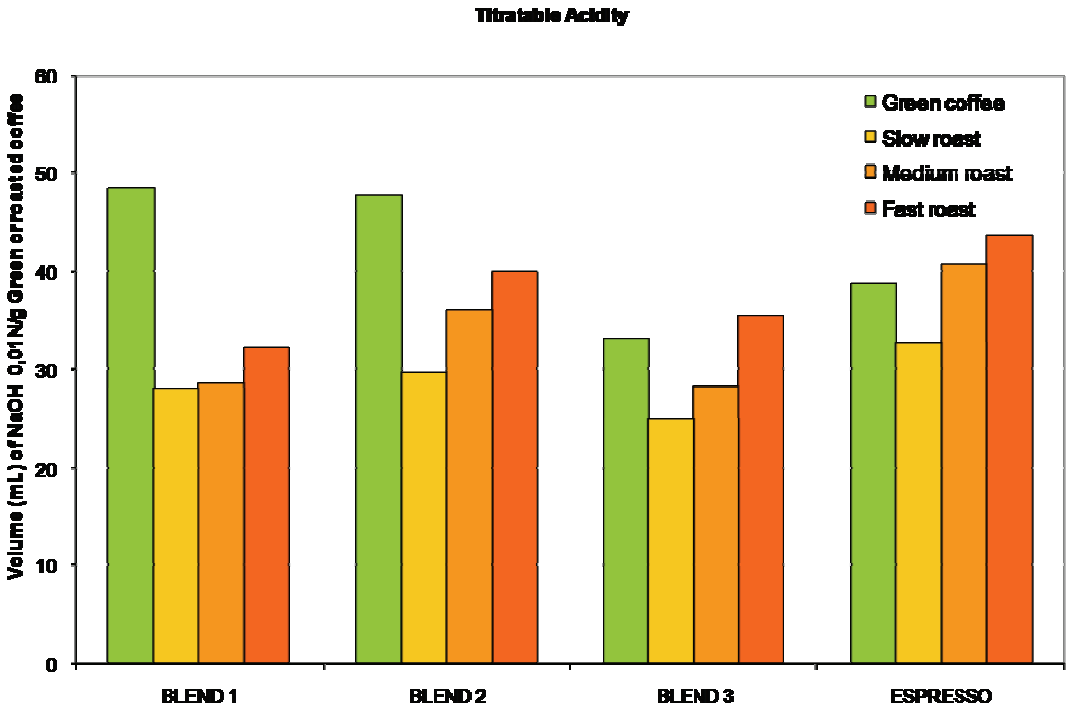
The bitterness increased along with the roasting time, but the opposite happened with the acidity. The faster the recipe was the more acid was the cupping. The same happened to the body. The faster the roast the stronger was the body.

Chemical analyses

At this moment of our research, we decided to confirm the trends we obtained in the two previous experiments with the cupping evaluations. To obtain it we executing more experiments and submitted the collected samples to chemical analysis. The first set of samples we sent to be chemically analyzed were exactly the same used in the experiment carried out with the 24 cuppers. Then, we made two more experiments using coffee with high level of defects and one more gourmet coffee with no presence of black, light-green nor sour beans. All the samples generated by these experiments were submitted to chemical analysis for comparison and to detect the expected trends.

The chemical analyses confirmed many trends observed in the cupping tests. This includes the cupping perceptions of body and acidity.

The graphics below show the titratable acidity, the PH and the chlorogenic acid for all the four experiments.



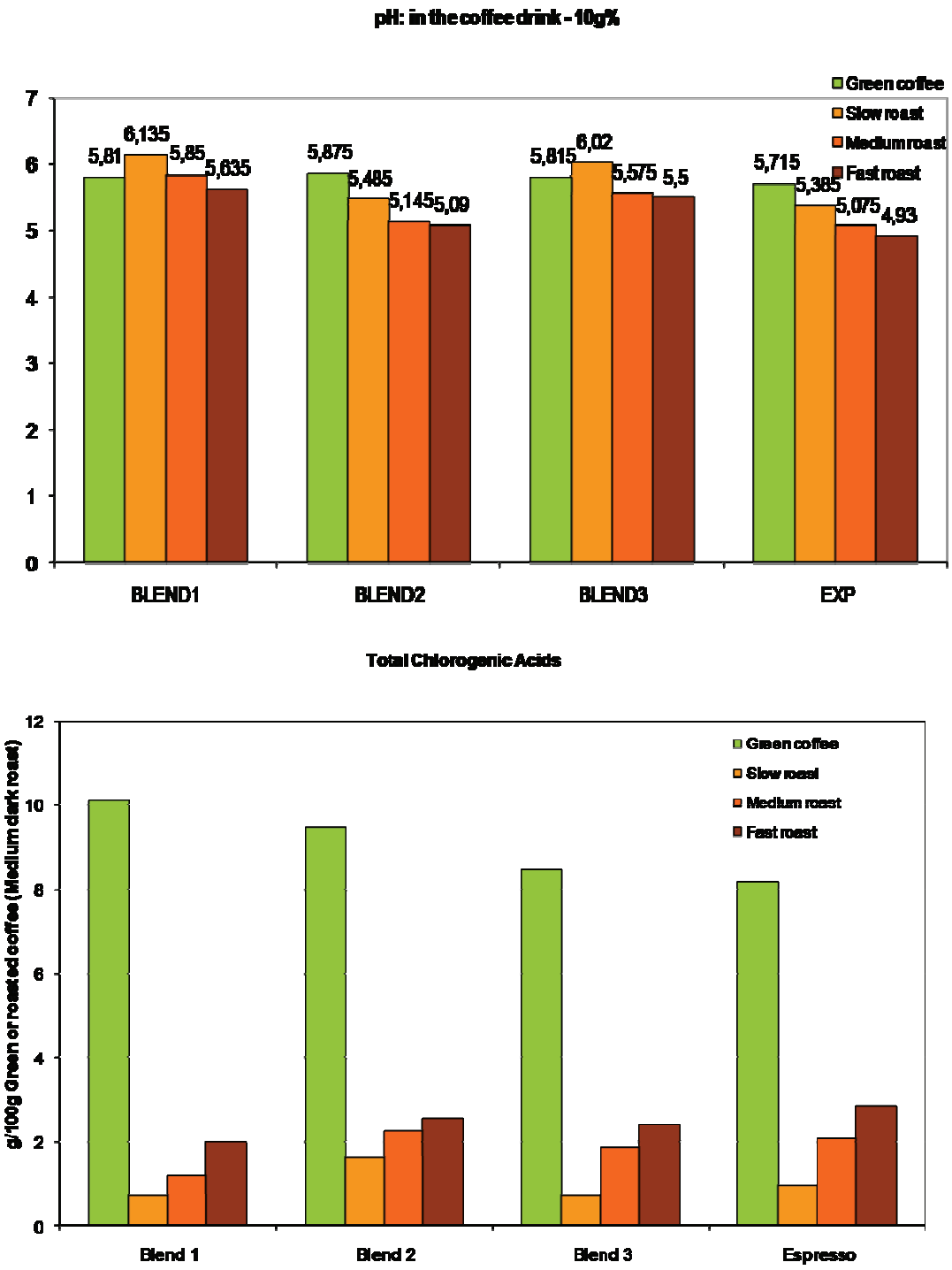


Figure 2.

Two parameter of acidity were analyzed: the concentration and the PH. The results were compatible to the cupping acidity perception: the faster the recipe the more acid was the coffee. The intensity of variation changed from blend to blend, but the trend was the same for the four of them. It is very important to mention that the chlorogenic acid followed the same trend. In some cases its concentration for the fast roast was the double when compared to its concentration on the slow recipe.

The table below shows the concentration of extractable soluble solids for the same samples.

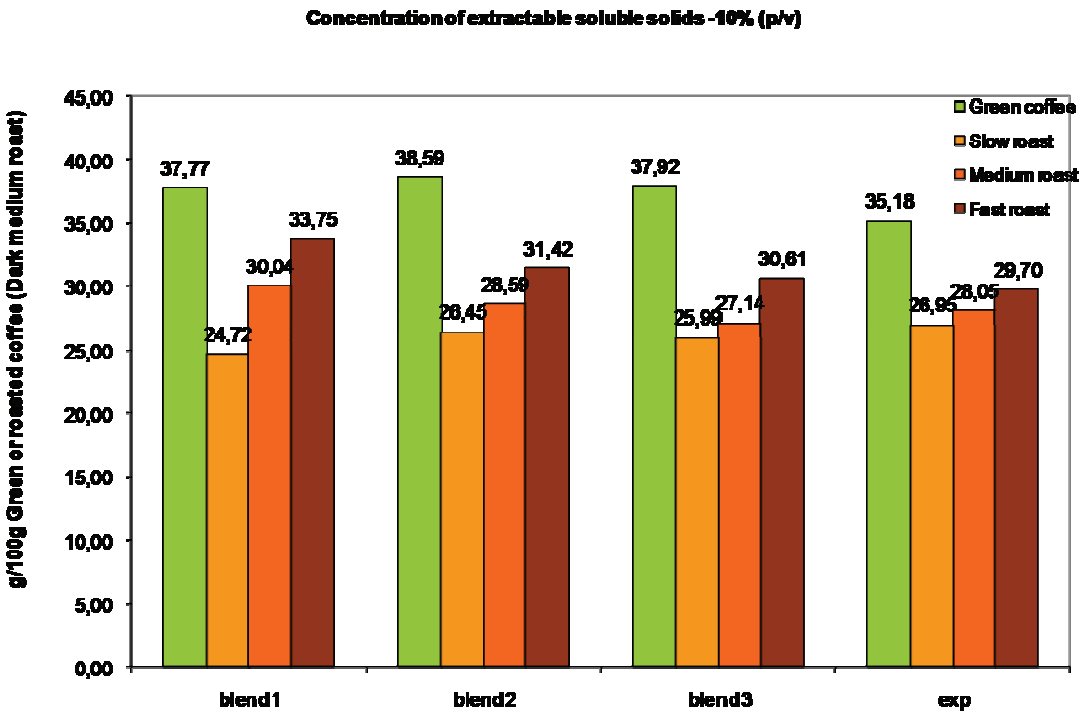
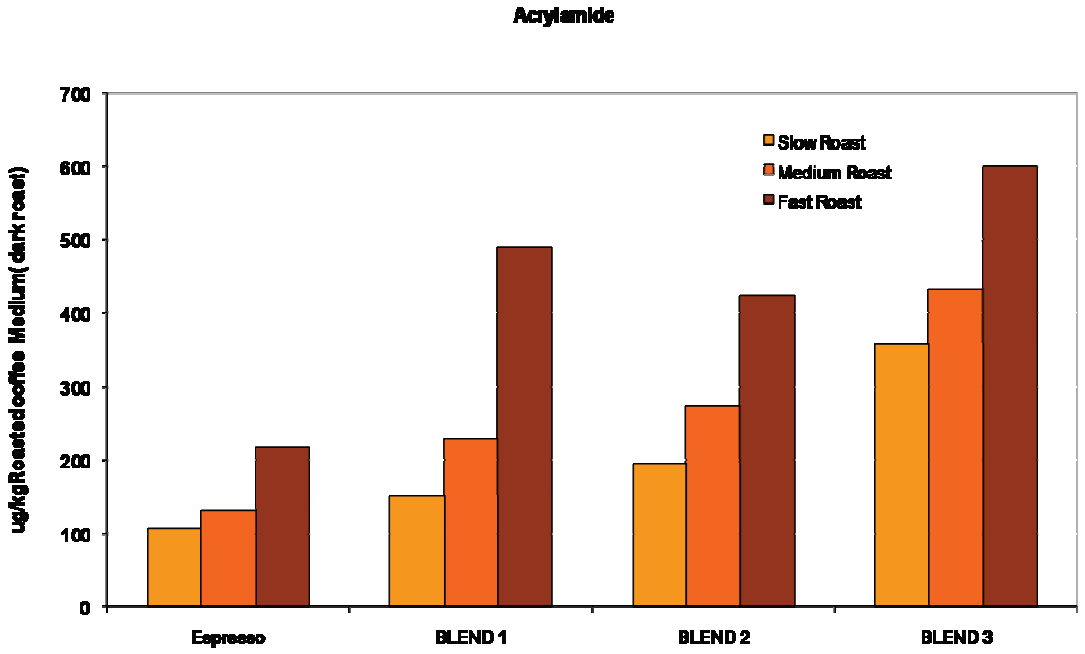


Figure 3.

The body cupping evaluations were confirmed by the analyses of soluble solids concentration. The results from the 4 sets of samples presented the highest concentrations for the fast roast and the lowest for the slow recipe. In one sample the soluble solids concentration was almost 40% higher than the one obtained in the slow recipe.

We also made some extra analysis, the acrylamide and the carbohydrate trying to also find some trend for these elements. The graphics are shown below.



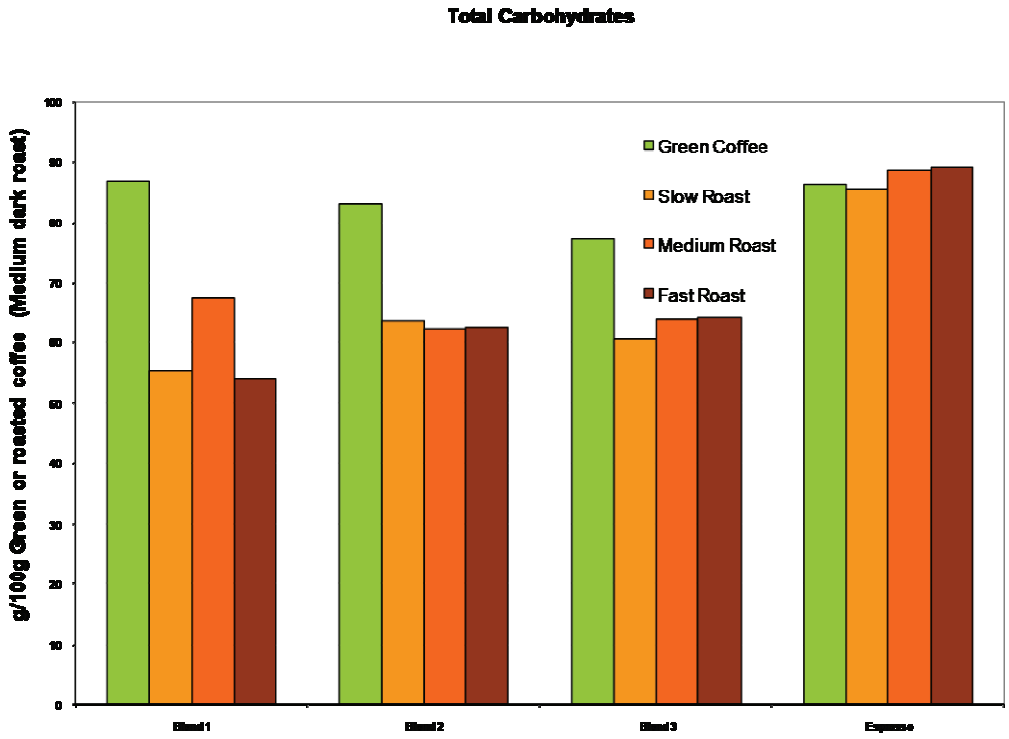


Figure 4.

The acrylamide chemical analysis showed a correlation between its concentration and the profile recipes. The faster is the roast, the higher is its concentration.

The carbohydrates studies will be abandoned for future researches using profile recipes because it was observed no correlation with the tested recipes.

The results of both, cupping and chemical analysis, showed a direct correlation between the profile recipes and the cupping and between the recipes and the concentration of many elements. The trends of acidity and body were confirmed during the cupping trials and the chemical analysis. These results show the level of control we may have over the roasting process controlling the bean temperature evolution along the time.

Coffee Roasting and Quenching Technology. Impact on Final Product Aroma Quality

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SUMMARY

The influence of different time-temperature conditions on the formation of key aroma compounds during coffee roasting was investigated. The results of this study show that, even if coffees are roasted to the same degree of roast, there are considerable differences in the concentrations of odorants depending on the time-temperature profile applied during the roasting process. Highest concentrations of Strecker aldehydes, α-diketones, and several sulfur compounds were obtained with a high temperature-short time process. However, a higher concentration of odorants does not automatically mean that the resulting beverage is of a better quality. In addition, coffees with different moisture contents were stored under protective atmosphere to elucidate the impact of the quenching process and the increased moisture contents, which may be obtained by water quenching. The coffees with an increased moisture content exhibited a considerably lower aroma stability than the low-moisture coffee. It is, however, assumed, that the faster degradation of aroma compounds is an effect of the increased moisture content only and not of the quenching method itself.

INTRODUCTION

Roasting of coffee is still an empirical standard technology and represents the key step for the development of the characteristic coffee flavor, for which coffee is so appreciated. In addition, roasting induces major physical changes within the coffee beans and determines their behavior in storage, grinding, and brewing (Illy and Viani, 1995; Schenker, 2000). In the present research project, the impact of the roasting parameters roasting time, roasting temperature, and quenching method, on aroma formation and aroma stability of roasted coffee were investigated.

MATERIALS AND METHODS

Coffee roasting trials

Batches of 100 g washed green *Coffea arabica Tip.* variety from Sumatra (Mandheling) were roasted with a laboratory scale fluidizing-bed roaster (G. W. Barth Ltd., Freiberg/Neckar, Germany). Hot air velocity was 3 m/s. Two isothermal programs, i.e., high temperature-short time (HTST) and low temperature-long time (LTLT), and one temperature profile program (Profile) were investigated. In HTST roasting, coffee was roasted for 160 s at 260 °C. In LTLT roasting, coffee was roasted for 660 s at 228 °C. For the profile roasting process, the following temperature program was applied: 180 C (180 s), from 180 °C to 232 °C in 360 s (8.7 °C/min), and 232 °C for 360 s, i.e., a total roasting time of 900 s.

Coffee quenching trials

a) For investigations on aroma stability

Batches (45 kg) of Colombian washed *Coffea arabica* with an initial water content of 10 g/ 100 g wb were roasted with a semi-fluidizing bed roaster CR-1250 from G. W. Barth Ltd. (Freiberg/Neckar, Germany). The roaster design allowed the application of water during quenching in the roasting zone and in the cooling zone. By this means, a well-controlled adjustment of the final roasted coffee moisture content was possible.

Three batches of coffee were produced with the same degree of roast, but exhibiting different moisture contents: 1.3 g/100 g wb (air-quenched), 3.2 g/100 g wb (water-quenched), and 6.5 g/100 g wb (water-quenched).

The coffees were stored under nitrogen atmosphere for a resting time of 24 hours. To prevent any contact with oxygen, the coffees were ground with a disc mill (Bühler-Miag 4000, Level 3, Bühler Ltd., Milano, Italy), which was placed in a glove box under nitrogen atmosphere (< 2% oxygen). The resulting ground coffee was packaged in valve bags under the same nitrogen atmosphere.

b) For investigations on carbon dioxide desorption

Batches of 100 g of Colombian washed *Coffea arabica* were roasted with the laboratory scale fluidizing-bed roaster as described before. A LTLT process (228 °C, 720 s was applied). The roasted coffee was cooled by air quenching, water (spray) quenching, or water (film) quenching, with resulting moisture contents of 1.9 g/100 g wb, 2.1 g/100 g wb, and 4.2 g/ 100 g wb, respectively.

Measurement of carbon dioxide desorption

Batches (80 g) of coffee beans were placed in 500 mL septum flasks immediately after roasting and quenching. Headspace pressure was measured periodically.

Aroma analysis

Coffee beans were ground with a disc mill (Bühler-Miag 4000, Level 3, Bühler Ltd., Milano, Italy). Ground coffee (5 g for the first group of compounds **1**, **2**, **5**, **6**, **10**, **16**, and **17** (see Table 1); 1 g for the second group of compounds **3**, **4**, **7-9**, and **11-15**) was weighed in a 100 mL flask and extracted with 95 g (first group), or 99 g (second group) of boiling water during 10 min under constant stirring. During extraction, the flasks were kept closed to avoid evaporation and loss of volatile compounds. After cooling, the coffee solution was spiked with definite amounts of the isotope labeled internal indicated in Table 1. The coffee solution was subsequently stirred for 10 min, and 7 mL were transferred to a 20 mL headspace vial.
Coffee aroma compounds were sampled with solid-phase microextraction at 40 °C for 10 min with a 50/30 µm StableFlex DVB/CAR/PDMS fiber (Supelco, Buchs, Switzerland). The injection was carried out at 240 °C in the splitless mode with a splitless time of 240 s. Compounds **3, 4, 7-9** and **11-15** were separated on a 60 m × 0.25 mm × 0.25 µm ZB-Wax column (Phenomenex, Aschaffenburg, Germany) with a Fisons 8000 Series gas chromatograph (Thermo Electron, Allschwil, Switzerland) with the following temperature program: 40 °C (6 min), 4 °C/min, 120 °C (0 min), 40 °C/min, 240 °C (5 min). Helium 5.6 was used as carrier gas at a constant column head pressure of 135 kPa. The gas chromatograph was coupled to a quadrupole mass spectrometer SSQ710 (Finnigan MAT, San

Jose, California), where mass spectra were recorded in the single ion monitoring (SIM) mode using electron ionization and an ionization potential of 70 eV. Compound **1** was quantified using the same set-up with the following temperature program: 40 °C (6 min), 40 °C/min, 240 °C (5 min). Compounds **2, 5, 6, 10, 16,** and **17** were separated on a 60 m × 0.25 mm × 0.25 µm ZB-1701 column (Phenomenex, Aschaffenburg, Germany) in a 2000 series TRACE GC gas chromatograph (Thermo Quest CE Instruments, Milano, Italy) with 40 °C (6 min), 4 °C/min, 120 °C (0 min), 40 °C/min, 240 °C (5 min) as temperature program**.** Helium 5.6 was used as carrier gas at a constant flow of 1.5 mL/ min. The GC was coupled to a TSQ triple quadrupole mass spectrometer (Finnigan MAT, San Jose, California) with Q1 operating in the RF-only mode. Spectra were recorded in the single ion monitoring mode. Electron ionization with an ionization potential of 70 eV was used. All SPME-GC-MS measurements were run in triplicate.

Table 1. Analytes and standards used for the quantitative analysis of aroma compounds by GC-MS.

Analyte (A)	Selected ion (*m/z*) of A	Internal standard (IS)	Selected ion (*m/z*) of IS [a]
Methanethiol (**1**)	48	[$^{2}H_3$]-**1**	51
Dimethyl sulfide (**2**)	47	[$^{2}H_6$]-**2**	50
Dimethyl disulfide (**3**)	94	[$^{2}H_6$]-**3**	100
Dimethyl trisulfide (**4**)	126	[$^{2}H_6$]-**4**	132
3-Mercapto-3-methylbutyl formate (**5**)	102	[$^{2}H_6$]-**5**	108
2-Furfurylthiol (**6**)	114	[$^{2}H_2$]-**6**	116
Methylpropanal (**7**)	72	[$^{2}H_7$]-**7**	79
2-Methylbutanal (**8**)	86	[$^{2}H_2$]-**9**	88
3-Methylbutanal (**9**)	71	[$^{2}H_2$]-**9**	73
Hexanal (**10**)	56	[$^{2}H_2$]-**10**	58
2,3-Butanedione (**11**)	43	[$^{13}C_4$]-**11**	45
2,3-Pentanedione (**12**)	100	[$^{13}C_2$]-**12**	102
N-Methylpyrrole (**13**)	81	[$^{2}H_3$]-**13**	84
Pyridine (**14**)	79	[$^{2}H_5$]-**14**	84
4-Vinylguaiacol (**15**)	150	[$^{2}H_3$]-**15**	153
2,3,5-Trimethylpyrazine (**16**)	122	[$^{2}H_{9-10}$]-**16**	131 & 132
2-Ethyl-3,5-dimethylpyrazine (**17**)	135	[$^{2}H_{9-10}$]-**16**	131 & 132

RESULTS AND DISCUSSION

Roasting conditions and formation of aroma compounds

Bulk and bean core temperatures during the applied roasting processes are displayed in Figure 1. In both the LTLT and HTST process, bean core temperature was raised quickly to above 200 °C. The temperature development in the Profile process was slower and more similar to the slow increase in temperature found in an industrial scale roaster (Baggenstoss et al., 2008).

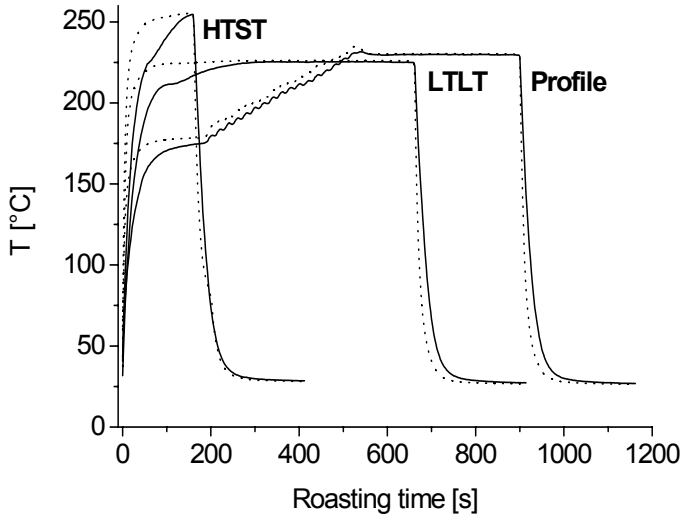

Figure 1. Evolution of bean core (—) and bulk (····) temperature during roasting with the fluidizing-bed hot-air laboratory roaster. Figure adapted from (Baggenstoss et al., 2008).

Some groups of aroma compounds followed characteristic patterns during the roasting process (Figure 2). Strecker aldehydes (methylpropanal, 2- and 3-methylbutanal), α-diketones (2,3-butanedione and 2,3-pentanedione), and 3-mercapto-3-methylbutyl formate exhibited a typical roasting kinetic, with an increase at the beginning of the roasting process, depending on their respective activation energy, a maximum at a medium degree of roast, and degradation at the end of the roasting process. Compounds such as 2-furfurylthiol, pyridine, and *N*-methylpyrrole exhibited a high activation energy, and then constantly increased during the roasting processes. Alkylpyrazines, dimethyl sulfide, methanethiol, and 4-vinylguaiacol followed a third pattern with fast formation at the first stages of the roasting process, and a flattened increase or stable concentration towards the end of roasting. Dimethyl disulfide and dimethyl trisulfide exhibited biphasic kinetics with increase at lower degrees of roast, a sharp decrease followed by further increase at the end of the process.

Those patterns together with the different kinetics followed during the applied roasting processes reveal that a targeted modulation of coffee aroma by roasting is a difficult task because of the various interdependencies between the formation mechanisms.

At the roasting end point with a lightness value L* = 21, distinct differences in the concentrations of aroma compounds were found between the high temperature-short process and the two long-time roasting processes (LTLT and Profile) (Figure 3). In particular, the concentrations of 3-methylbutanal, 2,3-butanedione, 2,3-pentanedione, methanethiol, dimethyl trisulfide, and 3-mercapto-3-methylbutyl formate were higher after HTST roasting. The differences between the two long time roasting processes were small.

The fact that in the HTST process, concentrations of Strecker aldehydes, α-diketones, and several sulfur compounds are higher, should not directly lead to the conclusion, that this coffee exhibits a better aroma quality than the other ones. Aroma quality is more a question of balance than of just the amounts of odorants. In addition, other aspects of coffee flavor, such as bitterness, acidity, and others, are also influenced by the roasting process. Until now, the question of coffee quality can only be answered by sensory tests.

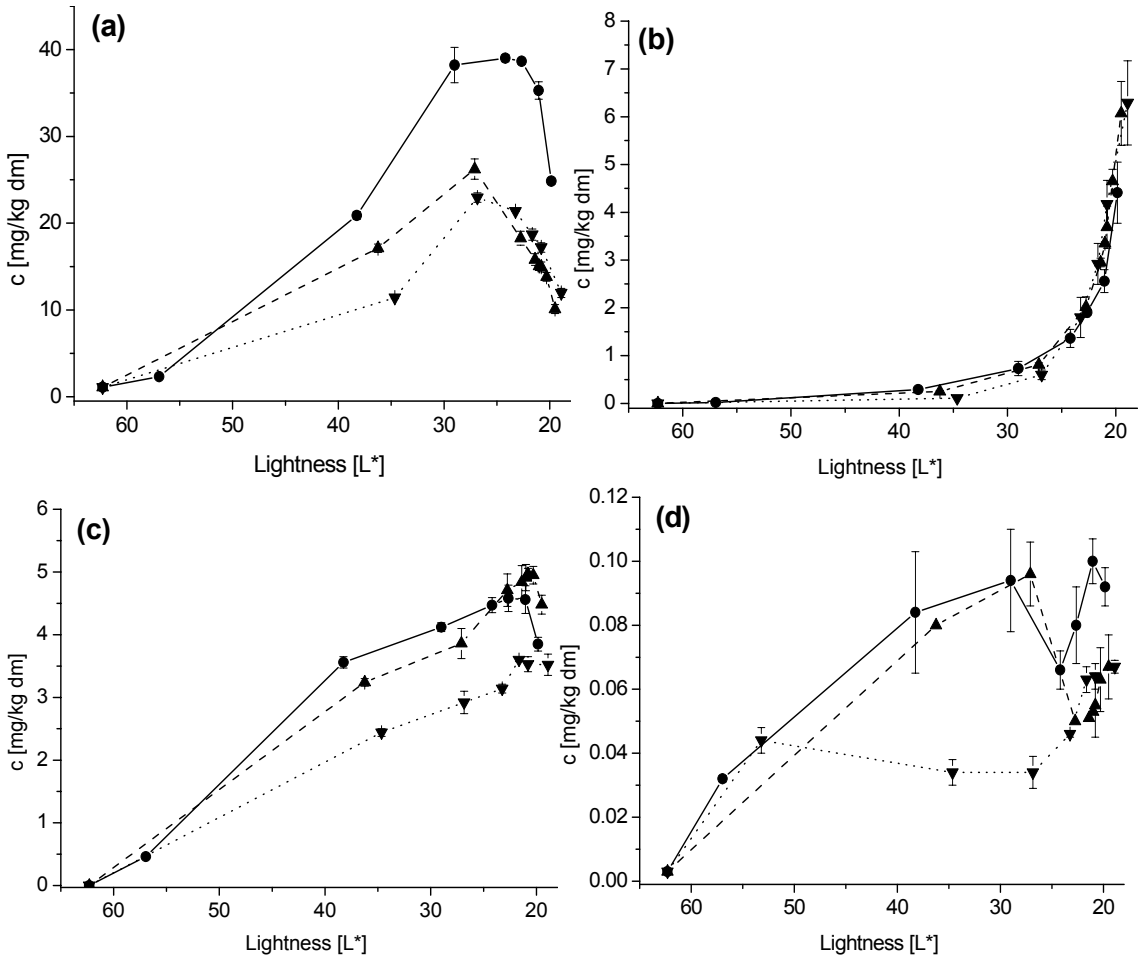

Figure 2. Different patterns of aroma formation during roasting. (a) 2,3-butanedione. (b) 2-furfurylthiol. (c) 2,3,5-trimethylpyrazine. (d) dimethyl trisulfide. Applied time-temperature conditions were: LTLT: ▲, HTST: ●, Profile: ▼).

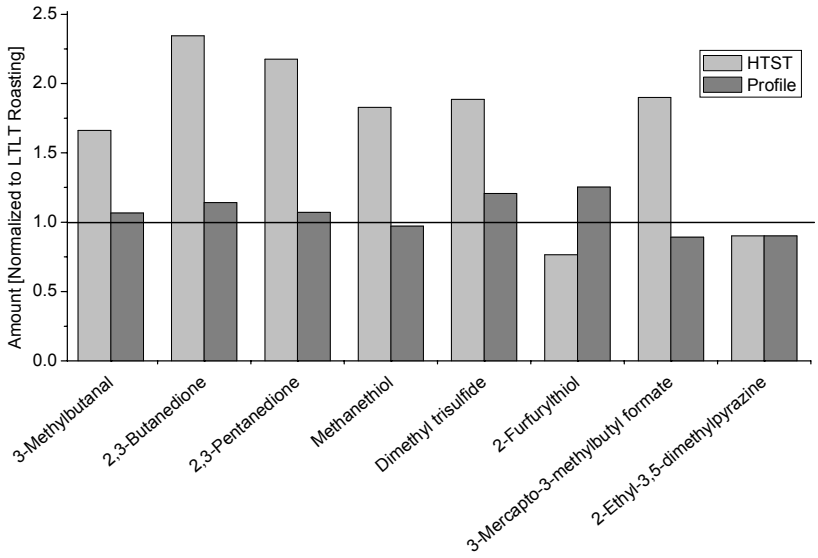

Figure 3. Relative concentrations of some odorants at the roasting end point with lightness L* = 21. The concentrations are displayed relative to those resulting from the LTLT roasting process.

Quenching of coffee and aroma stability

It was shown in earlier studies that coffee beans with increased moisture content exhibited a more important desorption of carbon dioxide during the first days of storage (Baggenstoss et al., 2007). This seemed to be independent of the specific quenching method. If an air-quenched coffee was re-moistened, a similar degassing behavior was observed as that for a water-quenched coffee with the same moisture content (results not shown). This leads to the assumption that, due to an uneven distribution of water directly after quenching, there are regions in the coffee bean with high moisture content, where a distinct relaxation of the local polysaccharide network takes place, similar to the relaxation in the cellulose network in paper as shown by Desobry and Hardy (1997).

For the present study, roasted coffees with different moisture contents after quenching were ground and packaged under nitrogen atmosphere to reduce the impact of ambient air on aroma staling. Under these optimized storage conditions, it was shown that several key odorants decreased at higher rates in roast and ground coffees with increased moisture content (Figure 4), namely dimethyl sulfide, 3-mercapto-3-methylbutyl formate, 2-furfurylthiol, 3-methylbutanal, N-methylpyrrole, 2,3-butanedione, and 2,3-pentanedione. The increase of dimethyl trisulfide, which is an end product of the oxidation of thiols, was more important with increasing moisture content. The concentration of 2,3,5-trimethylpyrazine remained stable throughout the storage trial independent of the moisture content.

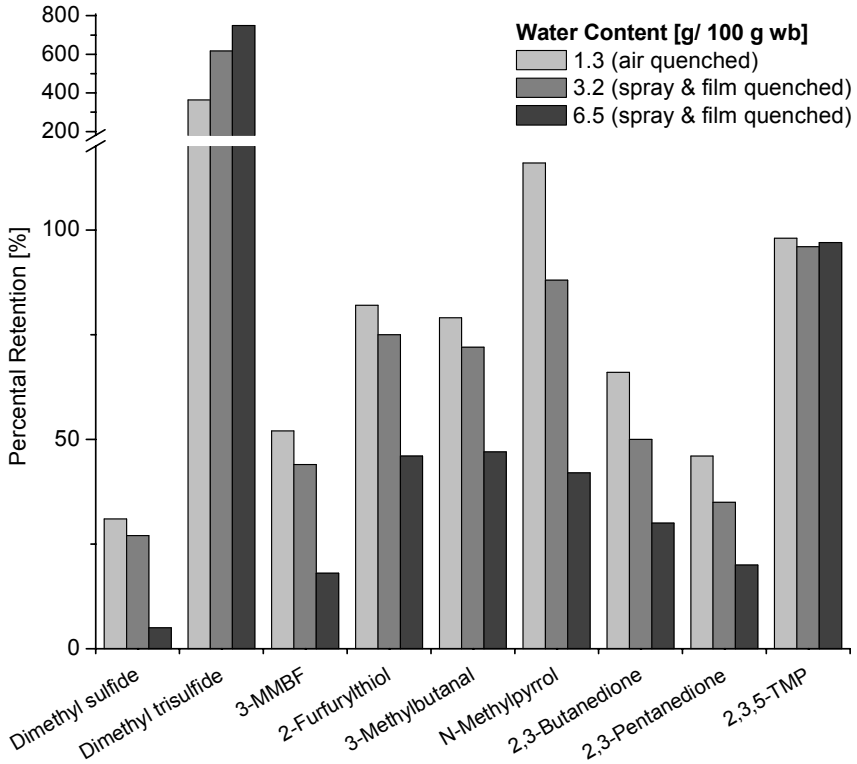

Figure 4. Percent retention (relative to t = 0 days) of some aroma compounds during storage under nitrogen atmosphere at 37 °C. 3-MMBF: 3-mercapto-3-methylbutyl formate. 2,35-TMP: 2,3,5-trimethylpyrazine.

The faster degradation rates in coffees with increased moisture content are in alignment with the theory of glass transition, which predicts that, by the plasticizing effect of water, the glass transition temperature in amorphous solid systems is reduced (Bell, 1995). This reduction

leads to a higher mobility of reactands, and hence, to higher reaction rates in amorphous systems.

Earlier studies found no changes in microstructure caused by the water quenching process (Baggenstoss et al, 2007). It is therefore assumed, that the loss in aroma stability is only due to the increased moisture content, and not to the quenching method itself.

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Quality Assessment of Roasted Coffee Blends by Hyperspectral Image Analysis

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SUMMARY

In this work, variations in the relative reflectance (%) of *arabica* and *robusta* coffee beans at different roasting times (0, 3, 6, 9 and 12 minutes) were evaluated by Hyperspectral Image analysis (HSI), with the aim to discriminate the two pure varieties. The results have made possible to identify wavelengths corresponding to the reflectance values that allow to differentiate raw and different roasted coffee varieties. In addition, the application of HSI was studied in order to identify pure samples of each variety (Arabica and Robusta) in the different ground coffee blends, where the percentage of both pure varieties changed from 0 to 100% (w/w). Hyperspectral images of coffee samples were acquired by means of a Spectralscanner in the wavelength range 400-1000 nm with a 5 nm of band pass. The data obtained were divided in two random groups: Train Data set and Test data set and the data were associated to the 17 mixtures considered. A Linear Discriminant Analysis model was calibrated in order to classify the data in the 17 classes, and the Best data set option was selected. For the train data the percentage of correct classification was 96% and for the test data set (validation) the percentage was 94%. Results demonstrated that HSI may offer a new and fast method for the detection of coffee blend adulteration.

INTRODUCTION

Food authentication is the most important issues for its quality control and safety. Assurance of the quality of roasted coffees has become increasingly important in recent years, due to the necessity to control and avoid coffee adulteration, mainly considering the great variability of the final sale price, depending on a wide range of factors, including coffee varietal and geographic origin (Pizzarro et al., 2007; Esteban-Díez et al., 2007). The two varieties of economic importance in the global coffee trade are *Coffea arabica* (*arabica* coffee) and *Coffea canephora* (*robusta* coffee) (Smith, 1985). Both varieties differ not only in relation to their botanical, chemical and sensory characteristics, but also in terms of commercial value, with *arabica* coffees achieving market prices 20-25% higher and being considered to be of better quality than *robusta* because of their superior taste and aroma. However, most commercially available coffees are produced from *arabica* and *robusta* roasted beans or blends of these two species. In the case of espresso blends, for example, it is essential to use the correct amount of high quality *arabica* coffees and the correct amount of high quality *robusta* coffees, taking care, however, to identify the correct composition and the ideal percentages of each type. *Arabica* gives qualities of aroma, delicate flavour and the right degree of acidity, while *Robusta* gives qualities of full flavour, body, chocolatiness and creaminess in the cup. So, the best blend is that which obtains a delicate and skilful balance these various characteristics. Coffees from different varieties are blended together for several reasons. One of these reasons might be to create a proprietary or signature blend that leads

consumers to equate a particular coffee profile with a particular brand image. The main goal is to make a coffee that is higher in cup quality than any of the ingredients individually (Brumen, 2005). Nevertheless high quality *arabica* coffee should be able to stand alone, so one reason for which coffees are blended in the commercial world, might be the use of lower-quality coffee or lower-economic varieties. Roasting process reduces significantly the differences between the varieties of coffee and this makes it even more difficult to tackle coffee adulteration issues. Therefore, suitable methods are required in order to discriminate between coffee varieties and to detect potential adulteration of high quality coffee beans with poorer and cheaper types, thus ensuring authenticity, quality, safety and efficacy of final product to be commercialised. After roasting, the visual criterion cannot be used and other methods must be used in order to differentiate varieties. Traditional methods of food monitoring, involving analytical techniques such as high performance liquid chromatography (HPLC) (González et al., 2001) and mass spectrometry (MS), are used (Toci and Farah, 2008), but they are time consuming, expensive and require sample destruction. Near infrared spectroscopy (NIRS) is well established as a non-destructive tool for multi-constituent quality analysis of food materials (Scotter, 1990). However, the inability of NIR spectrometers to capture internal constituent gradients within food products may lead to discrepancies between predicted and measured composition. Furthermore, spectroscopic assessments with relatively small point-source measurements do not contain spatial information, which is important to many applications for food inspection (Ariana and Guyer, 2006). Hyperspectral imaging (HSI) is an emerging platform technology that integrates conventional imaging and spectroscopy to attain both spatial and spectral information from an object. Recent advances in the application of HSI to food safety and quality assessment are reviewed, such as contaminants detection, defects identification, constituents analysis and quality evaluation (Gowen et al., 2007).

In this work was HSI was used to identify pure samples of each variety (*arabica* and *robusta*) in coffee beans at different roasting degrees and in different ground blends, where the percentage of both pure varieties changed from 0 to 100% (w/w).

MATERIALS AND METHODS

Coffee beans at different roasting degree

Samples of *arabica* (*Coffea arabica*, Santos) and *robusta* (*Coffea canephora*, var. *robusta,* India) green beans provided by Essse Caffè S.p.A. (Bologna, Italy) were used for different treatment times. Five hundred grams of green coffee beans were roasted in a laboratory roaster of 500 g capacity (mod. EXPO 500/E, STA plants s.r.l., Bologna, Italy); at different times during the process (3, 6, 9 minutes), samples of coffee beans were removed in order to submit to HSI. The roasting process was carried out in duplicate for each sample. Hyper-spectral image analysis of coffee beans (Figure 1) was carried out according to Fiore et al., 2006).

Data processing

Principal component analysis (PCA) was used to reduce the number of variables in the data matrix and to select the most discriminating parameters. The statistical package STSG Statistica for Windows, version 6.0 (Statsoft Inc., Tulsa, UK) was used.

Coffee blends

8500 g of green coffee beans of two different varieties, Arabica and Robusta, coming from 25 different production lots, were roasted for 12 minutes. For each of the 25 coffee lot, 15 different blends (w/w) of arabica and robusta coffee were obtained mixing the grains of the two pure variety. Each of 17 samples (Table 1) were splitted in two samples. Then the mixed roasted beans were grinded. 10g of each 15 blends and two pure varieties (17 samples) were placed in a Petri's disk; 50 replications for each blend were considered. For all samples were acquired hyperspectral images trough a Spectralscanner (DV Optic s.r.l., Padova- Italy).

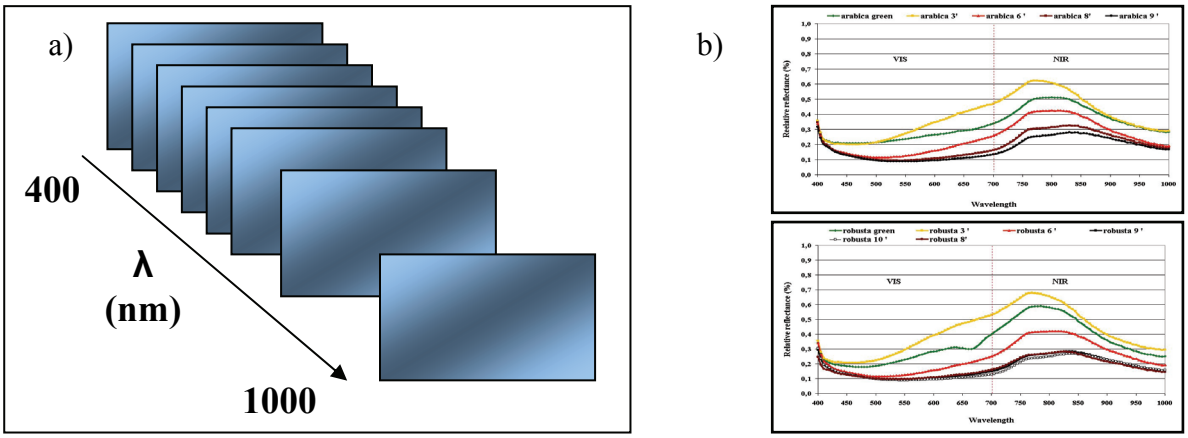

Figure 1. a) Hyperspectral image, containing 121 layers corresponding to the 121 wavelength from 400 to 1000 nm, with a 5 nm of sampling step; b) reflectance spectrums obtained by HIS.

Table 1. Percentages (w/w) of *arabica* and *robusta* pure varieties in the different ground roasted coffee blends.

Mixtures	arabica (%)	robusta (%)
1	100	0
2	95	5
3	90	10
4	85	15
5	80	20
6	75	25
7	70	30
8	65	35
9	50	50
10	35	65
11	30	70
12	25	75
13	20	80
14	15	85
15	10	90
16	5	95
17	0	100

Hyperspectral image acquisition

The environment is composed of a line spectrometer (Specim V10, Oululu, Finland) that provide a spectral dispersion from 400 to 1000nm, and a 12 bit monochrome camera (Pike F-100 B, Allied Vision Technologies gmbh, Stadtroda-Germany) with a CCD sensor having 1000 X 1000 pixels of spatial and spectral resolution respectively. The acquired lines data were transferred to a support PC through firewire *b* connection, and packed in a single file (hyperspectral image), containing 121 layers corresponding to the 121 wavelength (from 400 to 1000 nm, with a 5 nm of sampling step). The illumination system was constituted by a 21V-150W halogen lamp (EKE 21V150W, Japan) as light source and an optic fiber that transfer the radiation to a linear light diffuser (Gowen et al., 2007). A total of 850 hyperspectral images were acquired. For each of the 15 blends and for the two pure varieties 50 hyperspectral images were acquired and stored.

Analytical determinations

- Density and volume: using a suitable picnometer according to Lerici et al., 2006.
- Water activity: using an hygrometer Aqualab CX-2 (Decagon Devices Inc., Pullman, WA).

Calibration was performed using saturated solutions of known a_w.

RESULTS AND DISCUSSION

HIS applied on green and roasted coffee beans

In both coffee variety spectrums, the reflectance peaks were obtained in the near infrared region, between 700 and 1000 nm, which allowed to distinguish the different roasting degrees.

A preliminary study based on PCA has been applied for a better understanding of the discriminating efficiency of the selected descriptors and also a visualization of the samples trend. Score plot is often used to observe the structure present in the multivariate data matrix. Score of coffee bean samples obtained from covariance analysis of auto scaled data matrix are represented in the space of two principal components (PCs). Figures 2a, 2b, 2c and 2d show the plots corresponding to the sample scores (arabica and robusta green and roasted coffee beans) considering as discriminant variables the arabica and robusta coffee beans reflectance values at wavelengths between 750 and 850 nm; each (PCs) explains a great percentage of the total variance (> 90.1%). As showed in the figures (2a, 2b, 2c and 2d), the percentage of total information explained on the first PC, (PC_1) decreases from green to the more roasted samples, to indicate that increasing roasting degree, decrease the difference in arabica and robusta coffee beans.

A comparison between arabica and robusta at the same roasting degree showed that it is possible to discriminate the two varieties by means of relative reflectance values.

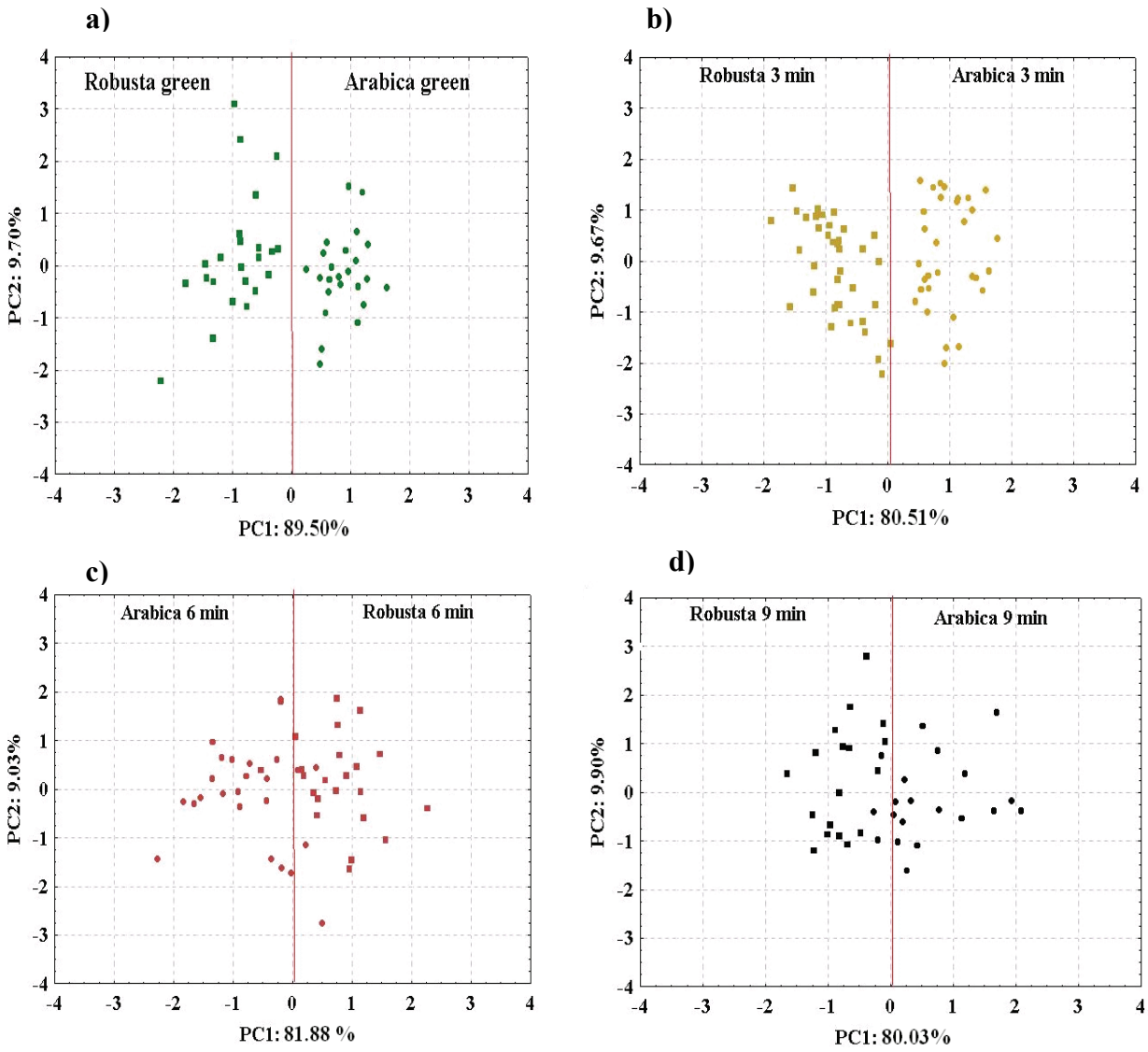

Figure 1. Score plot of arabica and robusta coffee bean samples at different roasting degrees. a) arabica and robusta green beans, b) arabica and robusta beans roasted for 3 minutes, c) arabica and robusta beans roasted for 6 minutes, d) arabica and robusta beans roasted for 9 minutes.

HIS applied roasted coffee blends

Analysis of variance (ANOVA) and the test of mean comparisons according Fischer's least significant difference (LSD) were applied on ground blend characteristic results, with a level of significance of 0.05.

The density and water activity of pure varieties and different blends of them are reported in Table 2. The ground coffee density increases significantly from pure arabica to pure robusta. In particular, the mix **1** (75%**a**-25%**r**) did not show significant differences from pure arabica, as well as the mix **3** (25%**a**-75%**r**) from pure robusta ground coffee. Also the water activity increases from pure arabica to pure robusta. As known that water content of robusta green coffee is greater in comparison of Arabica green coffee, and this difference can also be seen in their toasted. The difference of water activity values between mix **1** and mix **3** was found to be significant, contrary to the mix **2** that does not differ from other mixtures. Water activity values of pure arabica ground coffee were significantly low then pure robusta and considered mixtures.

Table 2. Average values of variables of pure and different blends ground coffee. Values in the same column followed by different letters (a-e) differ significantly at a p < 0.05 level.

Samples		Density (g/l)	a_w
	arabica 100%	0.412±0.007 ^{a,b}	0.106±0.014 ^{a}
mix 1	**75%a -25%r**	0.423±0.009 ^{b,a}	0.133±0.009 ^{b,c}
mix 2	**50%a -50%r**	0.443±0.009 ^{c}	0.149±0.003 ^{c,b,d}
mix 3	**25%a-75%r**	0.463±0.010 ^{d,e}	0.165±0.007 ^{d,c}
	robusta 100%	0.463±0.00212 ^{e,d}	0.229±0.016 ^{e}

Data analysis

The data analyses were performed using Matlab 7.0 (The Mathworks inc., USA). Each of 850 hyperspectral image was disassembled and 121 gray level image was obtained for each hyperspectral image. The 121 component image, were segmented in order to eliminate the background, using Otsu method (1979) and the mean intensity was calculated (Figure 1). Then the mean spectra from 400 to 1000 nm were calculated for all hyperspectral images (Figure 3).

Subsequently the PCA analysis was performed, considering the 17 classes, in order to individuate the component that best explain the data variance. Considering the first 6 principal components, the LDA analysis was performed, using the blend as Classification factor and the principal components as predictor factors. The data set was randomly divided in two equal groups, train set and test set. Using this approach the percentages of correct classification were calculated for the train and the test set, using progressively from 1 to all 6 principal component (Table 3).

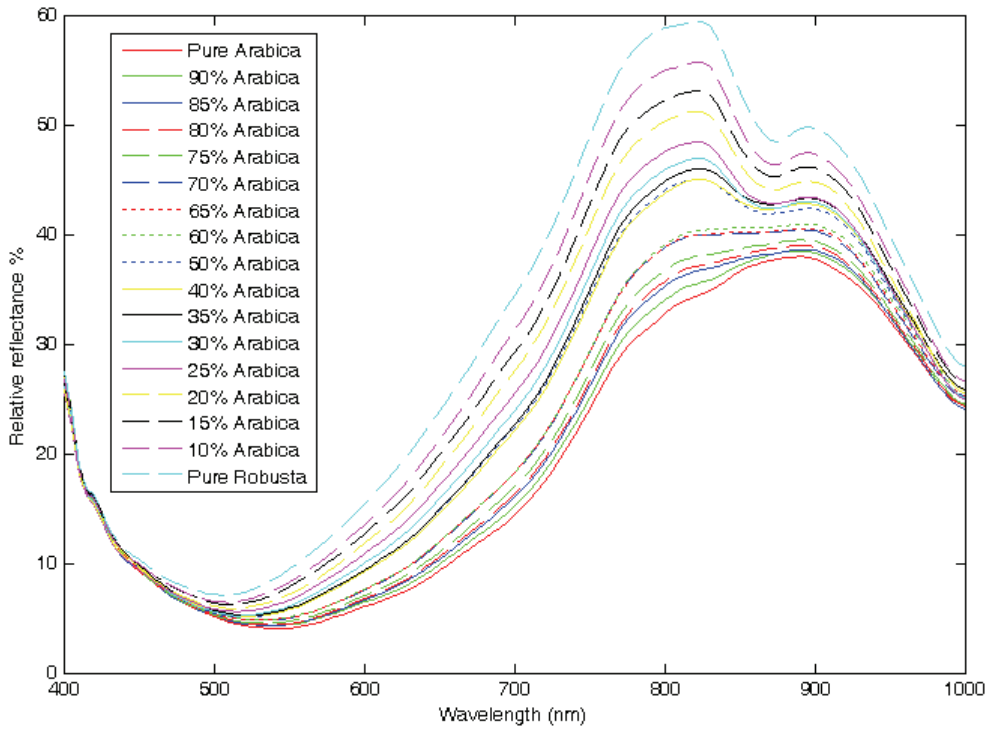

Figure 3. Mean reflectance spectra of the pure Arabica, pure Robusta and 15 blends.

Table 3. Percentage of correct classification of the 17 classes related to the Principal components number.

PC number	% of correct classification for the train set	% of correct classification for the test set
1	79.25	74.18
2	81.13	77.46
3	89.20	88.21
4	90.09	89.67
5	96.24	95.28
6	97.16	95.77

Figure 3 shows the trends of relative reflectance corresponding to arabica and robusta ground coffee blends at the same time of roasting degree (12 minutes), as a function of different wavelengths (between 400 and 1000 nm).

From an initial observation of spectra, it was possible to note that the reflectance peaks obtained in the near infrared region, between 750 and 850 nm, allowed to distinguish the different coffee blends.

In particular, the intensity of reflectance peak decreased becoming from pure robusta to pure arabica ground coffee. This reduction indicated a great absorption in this spectrum region, from arabica. A possible explanation could be due to different roasting degree reached from the two considered varieties; in fact, the high content of sugar in arabica variety, could determine a more intense pyrolysis then robusta.

Furthermore, it may be noted that the two pure varieties present reflectance peaks very different among them. In particular, pure arabica coffee show the maximum value of reflectance at around 900 nm of wavelength, while pure robusta show two peaks: the largest after 800 nm and the other one approximately to 900 nm.

It is interesting to note that, with the increase of robusta percentage in the ground blend until 40%, the reflectance peaks disappear, becoming wider and wider. From this point onwards, there is a considerable difference in both intensity and shape of the peaks. In fact, when in the ground blends robusta percentage exceeded the 40%, a clear difference in intensity of reflectance peaks was observed. Therefore, the peaks corresponding to the over 50% robusta mixtures are more spaced among them.

CONCLUSION

The results reported in this study demonstrated that the hyper-spectral image analysis may be usefully applied not only to discriminate *arabica* and *robusta* pure coffee varieties, but also to distinguish pure varieties and blends of the two species with known origin. Results demonstrated that HSI may be offer a new and fast method for the detection of coffee blend adulteration.

ACKNOWLEDGEMENTS

We acknowledge the roasting company ESSSE CAFFÈ S.p.A. (Anzola dell'Emilia – Bologna, Italy) that provided green coffee beans used in this work.

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Development of an Apparatus for Measuring the Degassing Behavior of Coffee with the Option to Examine the Influence of Protective Gases for Aroma Preservation

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SUMMARY

PROBAT developed an apparatus with which the degassing quantity and the temporal degassing behavior of ground coffee can be determined. At the same time, the influence of protective gases which are used for the ground coffee transport and storage like CO_2 and N_2 on the degassing behavior can be examined. The pressure build-up during the storage in the bin is the factor for the released gas quantity. It has been examined which effects the roasting time, grinding degree and the surrounding atmosphere in the degassing apparatus have on the degassing behavior. When using protective gases for aroma preservation, it gets obvious that nitrogen as protective gas provides in comparison to carbon dioxide rather for an accelerated degassing of the coffee. This influence is due to the different gas densities of nitrogen and carbon dioxide and thus to the partial pressure influence. Essential influencing factors for the degassing duration are partial drop of pressure and grinding degree: the finer the grain size adjustment, the quicker the degassing. As variable roasting profiles for the same coffee sort can lead to different degassing curves it is necessary to examine the particular process and the actual coffee sort to receive more precise data. The influences through process and sort can be determined and represented easily with this developed degassing apparatus.

INTRODUCTION

Within the last few years coffee roasters often asked questions about the influence of ground coffee degassing. Up to now, this influence has been regarded from different points of view (Shimoni and Labuza, 2000; Labuza et al., 2001; Matens et al., 2007; Shimoni and Labuza, 2000; Schenker et al., 2000). On the one hand, the quality requirements concerning the ground coffee, especially for the pads and pots production, are important. At the same time, the use of protective gases is discussed in connection with aroma preservation and their influence on degassing time and coffee taste. On the other hand, there are the requests of the producers for shorter pass-through times and minimized costs.

When regarding the questions of the market, it partly became clear that generally transferred data of existing examinations did not represent themselves like this in practice. Thus, based on available literature (Jansen, 2006; Meister and Puhlmann, 1989; Buchmüller and Nobis, 1997; Hinman, 1993; Barbera, 1967; Radtke and Heiss,) we thought about developing an apparatus which can easily be operated and provides the option to be used at site in order to learn about the different influences of the process.

DESIGN OF THE APPARATUS

Different demands should be kept with the apparatus. The gas quantity should be determined and recorded for a certain period of time. Representative coffee quantities should be used for the check. Certain influencing sizes like e.g. the temperature on the gas pressure should be compensable. A simple operation together with the direct presentation of the results must be given as well as the possibility of using the apparatus not only in a laboratory but also on site (Koziorowski, 2008).

A transportable rack (Figure 1) was created in which a static comparison in 6 glass bins is made possible. The cabling of the sensors was integrated in that rack and renders possible the recording of the data via a data logger with Laptop connection for direct recording. After trials with different throughflow and pressure measuring systems a Piezo pressure transmitter with a very high measuring accuracy for pressure acquisition was chosen.

Figure 1. Degassing apparatus.

Special screwings and special sealings were also used in order to guarantee a gas-tight execution. Each glass bin is equipped with a thermo-couple for temperature acquisition and a pressure relief possibility. Pressure and temperature courses are directly presented by means of the software “Easy View”. Furthermore there is the possibility of introducing selected scavenging gases like e.g. CO_2 or N_2 as well as connection additional analysis equipment e.g. for oxygen measurement. Trials with different filling quantities were made in order to have the sufficient coffee quantity in relation to the necessary free expansion volume for the different coffees and grinding degrees. It was stated that a filling quantity of 250 g of ground coffee is the optimal size.

The gas volume is determined by means of the following formula which is deposited in the software and can therefore directly be presented as serial data curve.

$$V_{Abgas}\left[\frac{Nml}{500\ g}\right] = \left[\left(\frac{(V_{Behälter} - V_{Füllung}) * (P_u + P_i)}{P_N} * \frac{T_N}{T_N + T_i}\right) - \left((V_{Behälter} - V_{Füllung}) * \frac{P_{Start}}{P_N} * \frac{T_N}{T_N + T_{Start}}\right)\right] * \frac{500}{G_{Füllung}}$$

V_{Abgas}	degassing volume	P_u	surrounding pressure	T_N	norm temperature
$V_{Behälter}$	bin volume	P_i	pressure inside bin	T_i	temperature inside bin
$V_{Füllung}$	filling volume	P_N	norm pressure	T_{Start}	temperature when measuring
		P_{Start}	inside pressure when measuring	$G_{Füllung}$	filling weight

MEASURING RESULTS

During the first trials the final alignment was compared with measuring details which could be found in literature (Shimoni and Labuza, 2000; Meister and Puhlmann, 1989; Radtke and Heiss, 1975). Clearly comparable results like e.g. for the grinding degree showed themselves (Figure 2).

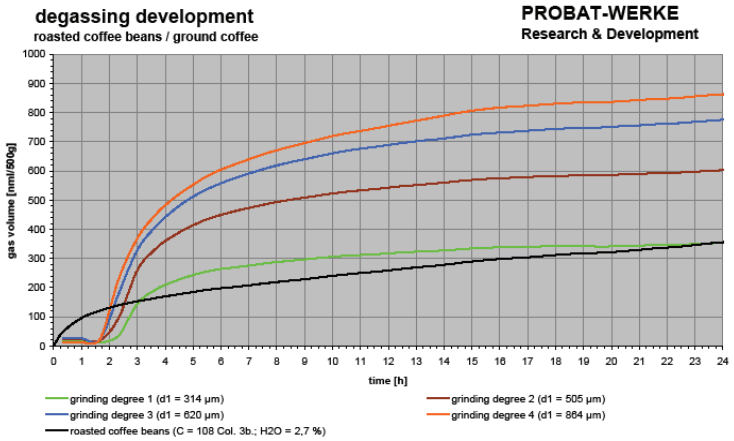


Figure 2. Measuring results – grinding degree.

It gets obvious that the finer the grinding degree the lower is the gas volume which is released. The reason for this is that more cells are destroyed during a fine grinding process by which the gas stored in the cells is already released during the grinding process.

Comparison treated/untreated green coffees

Measurements of Arabica coffee where untreated green coffees and green coffees treated as per the DCM procedure have shown that the roasting time influence on the ground coffee degassing for treated Decafs does not exist any longer. Furthermore the roasted coffee, a product of treated green coffee, degasses faster at the beginning and in comparison to untreated Arabicas they release a gas volume which is 15-20% lower (Figure 3). The reason for this is the cell structure which is changed by the decaffeinating process so that there are more and more open pores and lower cell wall thickness after the treatment and the roasting process (Figure 4 a-d).

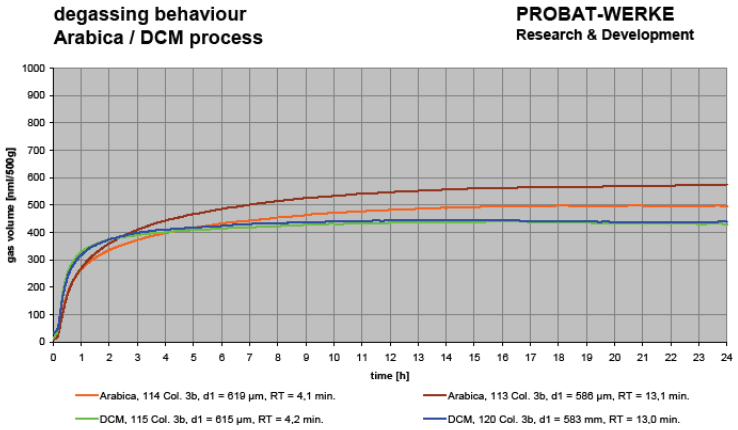


Figure 3. Measuring results – Decaf (DCM-process).

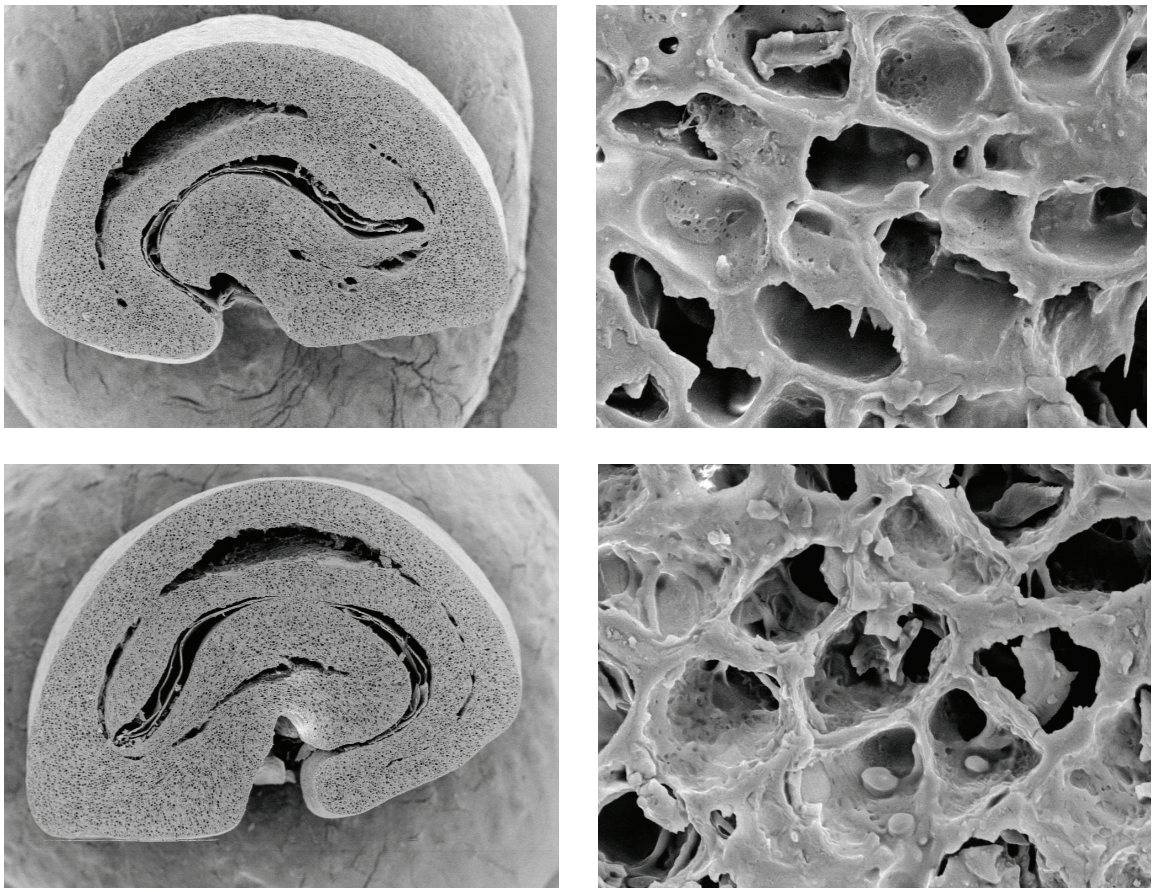


Figure 4. a/b: roasted Arabica, untreated; c/d: roasted Arabica, DCM processed.

Another Arabic coffee which was treated as per the Lendrich procedure showed a degassing quantity which is 12 % lower but the roasting time influence could not be recognised for the untreated green coffee either (Figure 5). There is no difference in the relation between the gas amounts in the comparable period of time at the beginning of the degassing process.

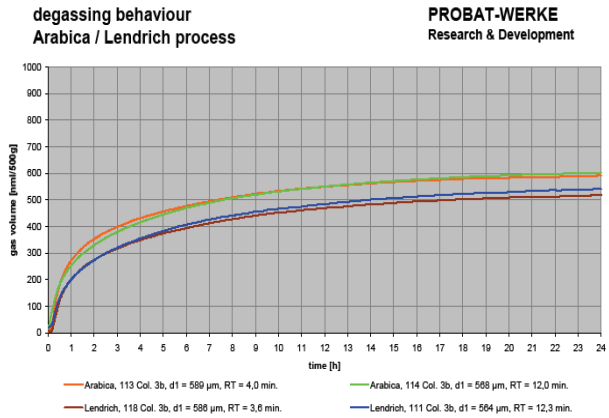


Figure 5. Measuring results – Lendrich process.

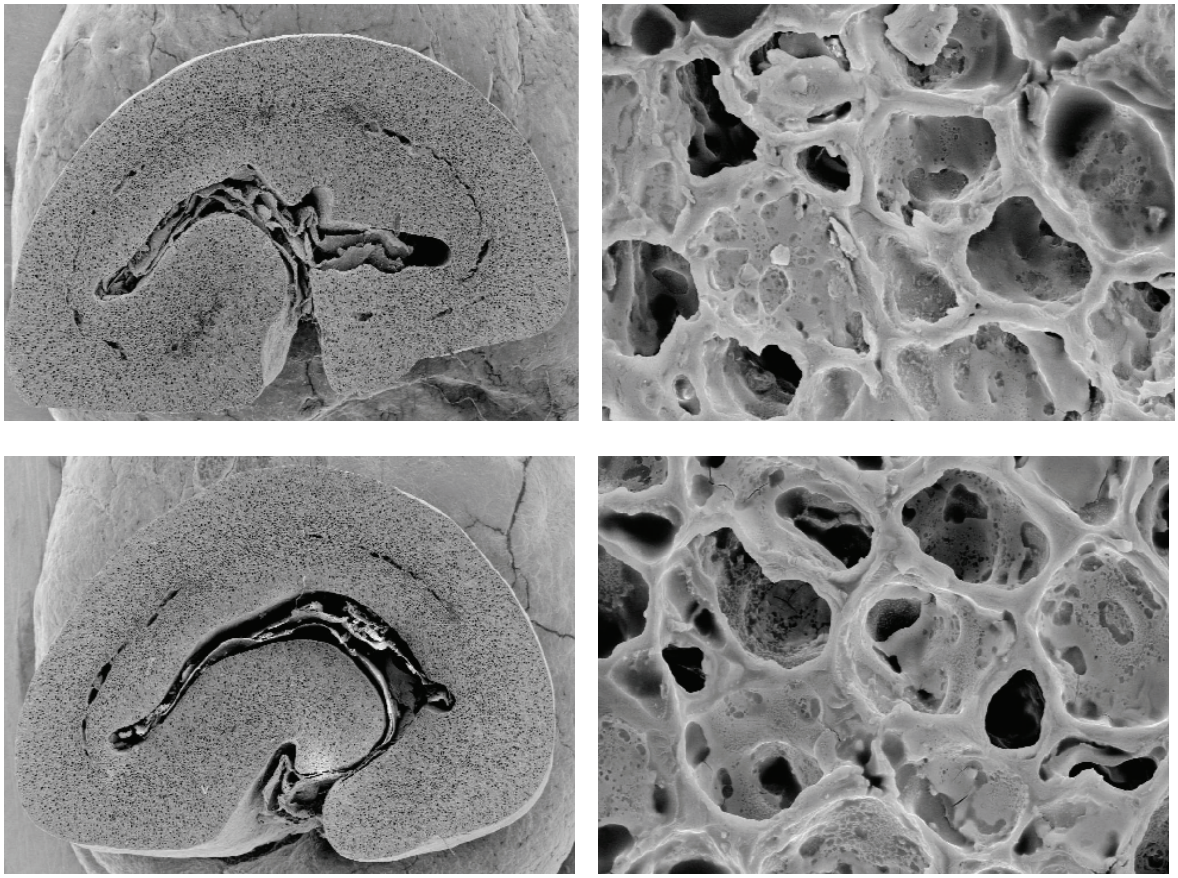


Figure 6. a/b: roasted Arabica, untreated; c/d: roasted Arabica, Lendrich process.

The lower degassing quantity then again can be explained by the modified cell structure as consequence of the pre-treatment (Figure 6 a-d).

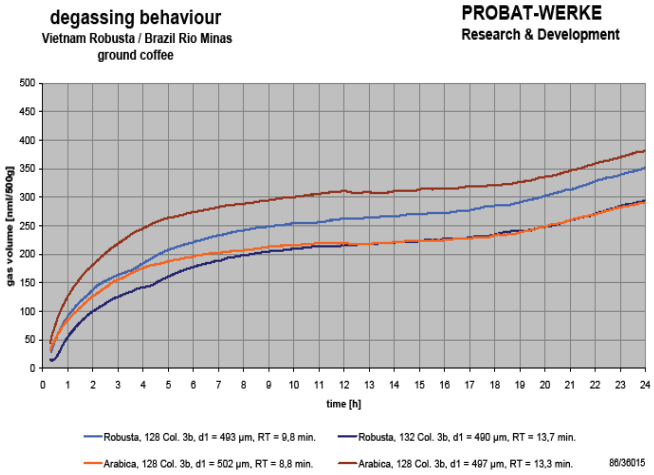
Comparison roasting time and coffee sort

An astonishing result shows the comparison of the two coffee sorts Vietnam Robusta and Brasil Rio Minas. With view to comparable grinding degrees and roasted coffee colours the Arabica with the shorter roasting time has a degassing quantity which is 25% lower compared with the coffee which was roasted longer. With regard to Robusta, the ground coffee with the

shorter roasting time has a degassing quantity which is 20% higher than the one with the longer roasting time (Figure 7). This shows that the influence of the roasting time has an opposite effect on the released gas quantity of the respective coffee sort.

We also learned about a certain effect with very dark roasted coffees which probably has to do with the "second crack". The very dark roasted coffee again has a lower degassing volume than the lighter roasted coffee sorts.

These results may be surprising but it is, however, only a further proof why general statements about the degassing behavior of the natural product are difficult. The single coffee sorts with their respective production processes must be studied individually in order to work out optimisations concerning quality and/or production.



Picture 7. Measuring results – roasting time and coffee sort.

Influence of protective gases

In order to check the influence of protective gases on ground coffee, among others, measurements on gas-tight ground coffee silos took place. 420 kg of ground coffee of equal, fresh roasted quality were filled per silo. Ground coffee with normal oxygen atmosphere of the environment was stored in silo No. 1. In order to reach oxygen values under 0.5 %, silo No. 2 was scavenged for 15.6 minutes with 3.4 m³ CO_2 and silo No. 3 for 17.3 minutes with 5.8 m³ N_2. After identical storage times samples were taken from each silo and were enclosed in the degassing apparatus in order to determine the remaining degassing quantities.

The coffee with N_2 application released a lower quantity of residual gas volume than the coffee of the other two silo compartments which refers to a higher degassing quantity in the silo (Figure 8). This degassing of coffee which tends to be accelerated under the N_2 conditions is due to the gas density, thus to the influence of the partial pressure (Figure 9).

Already during the grinding process, the coffee is protected against ageing which takes place due to the influence of air oxygen. Whether CO_2 or N_2 is chosen as protective gas is a question of cost and also depends how the taste of the coffee in the cup is perceived. There are very different opinions in the market concerning the "influence of protective gases on the taste". It is known that oxygen must be kept away from coffee in order to protect the aroma. Thereby protective gases can be of help. Test in laboratory scale can be done with the degassing apparatus with the target to find out the optimal aroma protection for the respective coffee

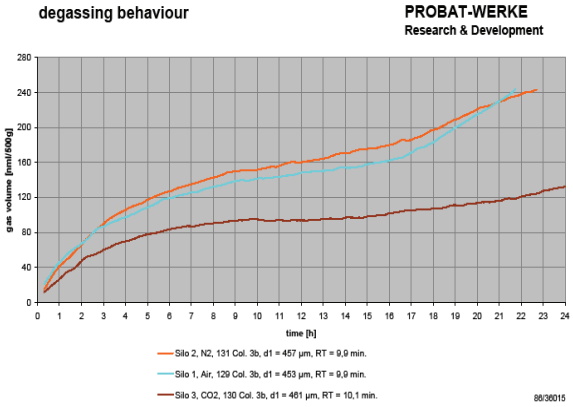


Figure 8. Degassing quantity after 2 hours at silo.

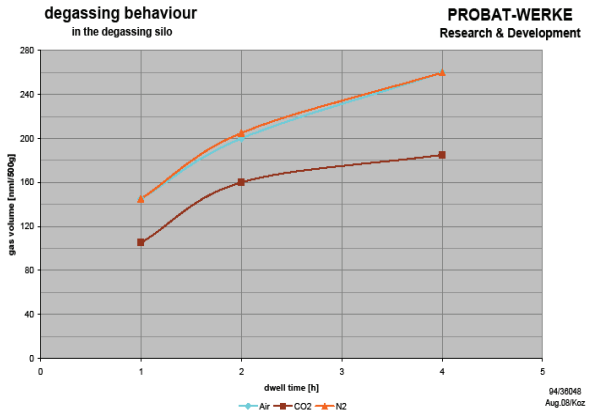


Figure 9. Degassing quantity at silo.

The PROBAT Controx System was optimised on the basis of the existing examination results and the modular construction was adapted to the demands of the market (Figure 10). This system offers the possibility of implementing an individual aroma protection.

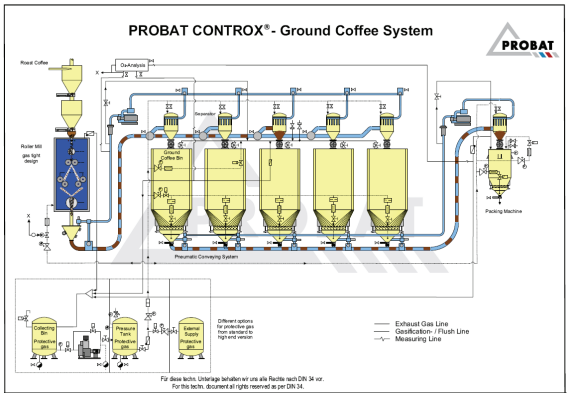


Figure 10. Optimized ground coffee system.

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Purging of Roasted Coffee Bean Containers

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SUMMARY

This study was an experimental evaluation of various purging methods used to remove residual oxygen from containers of roasted coffee beans. A pressure vessel filled with wax coated roasted coffee beans was purged with carbon dioxide gas -using pressure purging, vacuum/pressure purging, displacement purging, and vacuum/displacement purging. The residual oxygen level resulting from these purge tests was measured using an oxygen analyser. Vacuum/displacement purging was shown to be the most effective purging method – it used less purge gas than the other methods and was able to achieve a residual oxygen level of 0.0% v/v. Two cycles of vacuum/pressure purging also achieved a level of 0.0% v/v oxygen but used more purge gas. Equations previously developed for the purging of empty storage tanks were shown to be useful in roughly predicting the residual oxygen levels resulting from the purging of roasted coffee bean containers.

INTRODUCTION

Reducing the level of residual oxygen in containers of roast coffee has been shown to improve shelf life by limiting the formation of oxidized flavours. Whole bean roast coffee deteriorates at a slower rate than roast and ground coffee but still benefits from packing at low oxygen levels (Clarke 1987a). Residual oxygen levels in containers of whole bean roast coffee should be below 1% v/v and ideally should be below 0.2% v/v in order to prevent the oxidation of aromatic aldehydes – these oxidized aldehydes cause stale taste (Sivetz 2004). In one study, "just roasted taste" in whole bean roast coffee was preserved for eight weeks at 16°C by lowering the residual oxygen level to 0.3% v/v (Sivetz 2003). Unfortunately, most commercially available packaged whole bean roast coffee contains more than 1% v/v residual oxygen, usually 2% v/v or higher, immediately after packaging.

Most commercial vacuum packaging machines are not able to lower the residual oxygen level to less than 1.0% v/v when using just one application of vacuum. For example, as calculated below; a vacuum packaging machine capable of achieving a vacuum level of -950 mbar would reduce the oxygen level to only 1.3% v/v under perfect conditions – that is, if the roast coffee did not interfere with oxygen removal. From the ideal gas law, if temperature and volume remain constant, then absolute gas pressure is directly proportional to the number of moles of gas present. Therefore, the level of oxygen resulting from vacuum packaging at -950 mbar (or 63 mbar absolute pressure) would be described by Equation 1:

$$X = X_O\left(\frac{P}{P_O}\right) = 21\%v/v\left(\frac{63\ mbar}{1013\ mbar}\right) = 1.3\%v/v \qquad [1]$$

Three methods of gas purging are used for removing residual oxygen from containers of roasted coffee beans: vacuum purging, during which a combination of vacuum and inert gas is used; pressure purging; and displacement purging. Vacuum and pressure purging make use of

repeated purging cycles. These three methods can achieve residual oxygen levels of less than 1.0% v/v, thereby insuring that there is minimal flavour degradation during storage.

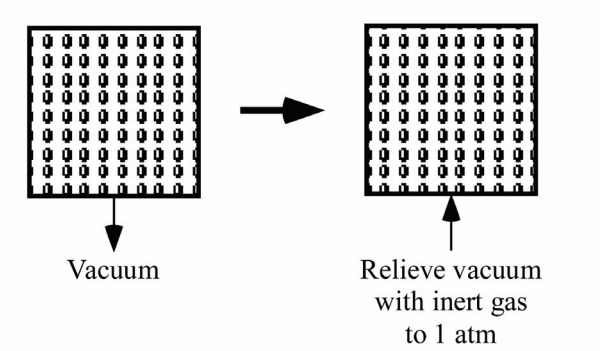

Figure 1. Typical vaccum purging sequence.

A typical sequence for vacuum purging is shown in Figure 1, at right. A vacuum is achieved, usually by means of a vacuum pump, and this vacuum is relieved with inert gas. The sequence is repeated until the desired oxygen level is reached. Equation 2, below, describes the oxygen level that results from k purging cycles, assuming X_O is the initial oxygen level, P_H and P_L are the high and low absolute pressures, and that these remain the same during each cycle. This equation, derived from the ideal gas law, is used for empty storage tanks (Kinsley 2001). Equation 3 gives the volume of inert gas used, where P_H and P_L are the high and low absolute pressures expressed in atm and V_P is the purge volume – the empty space inside the container.

$$X_k = X_O\left(\frac{P_L}{P_H}\right)^k \qquad [2]$$

$$V = k\left(P_H - P_L\right)V_P \qquad [3]$$

Vacuum purging can be used for packaging coffee in flexible pouches and metal cans. The vacuum level used during vacuum purging of rigid metal cans is limited by the strength of the can – one study determined that metal cans used for packaging soluble coffee could withstand a maximum vacuum of -836 mbar (Dantas et al 2001).

A typical sequence for pressure purging is shown in Figure 2, at right. The container or storage vessel is first pressurized with inert gas and then the container contents are vented to the atmosphere. This sequence is repeated until the desired oxygen level is reached. Equations 2 and 3 can be used to calculate the oxygen level after purging of an empty container and the volume of inert gas required, again assuming that P_H and P_L are the same for each cycle.

Pressure purging would be used prior to pressurised storage of roast coffee, either in storage vessels or metal cans - these containers are able to withstand internal pressure. Pressurised storage of roast coffee beans is reported to improve coffee flavour by retaining volatile coffee favours and by limiting oil migration to the bean surface (Illy 2007; Savonitti 2005).

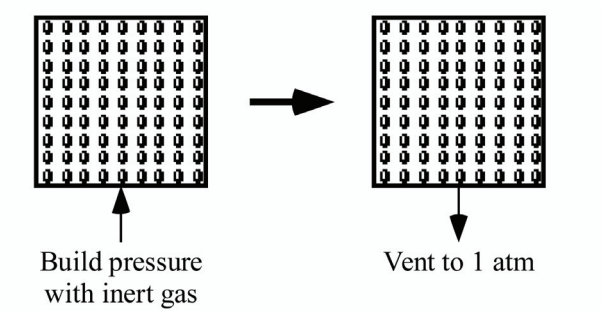

Figure 2. Typical pressure purging sequence.

Displacement purging, shown in Figure 3, at right, removes oxygen by displacement. The inert gas is injected in a steady flow into the container, usually at the container bottom, or could be deposited in the container as a liquid or solid from which gas would evaporate. Displacement purging of empty tanks works best with tall, thin tanks and when the inert gas is denser or colder than the gases in the container, resulting in minimal mixing between gases - less inert gas used as a result (Blakey et al 1984). Displacement gas purging of packed beds has been investigated for many types of materials – one study determined the time required for complete purging of oxygen from a packed bed of nylon monomer particles (Natarajan et al 2004). This study determined that a single, centrally located injection point for the inert gas was preferable to multiple injection points for the most rapid oxygen removal in a tall cylindrical tank with a conical bottom. This study also reported low residual oxygen levels could be achieved faster when a purge gas with a low diffusion coefficient was used, resulting in less mixing between the inert gas and the residual oxygen.

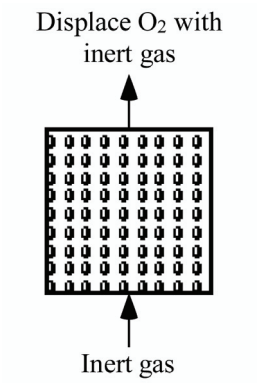

Figure 3. Displacement Purging

Figure 3. Displacement purging.

Argon, carbon dioxide, and nitrogen are the inert gases most commonly used for purging of food and beverage containers. Carbon dioxide has technical advantages when used for displacement purging or pressurised storage of roast coffee. Carbon dioxide is heavier than the other two gases – this is an advantage during displacement purging, as long as the exhaust port is positioned higher than the gas inlet. It may be made even heavier by the evaporative cooling that takes place when carbon dioxide gas is supplied from cylinders containing liquid carbon dioxide. Carbon dioxide also has a binary gas diffusion coefficient with oxygen that is 15% less than that of argon or nitrogen – this would be another advantage during displacement purging. Carbon dioxide is more effective in preventing degassing during pressurised storage of roast coffee due to the Henry's law equilibrium – more carbon dioxide

would be kept in solution in the oil and aqueous phases of roast coffee when carbon dioxide is the pressurising gas than when other gases are used.

The objective of this study was to evaluate vacuum purging, pressure purging, and displacement purging as to their effectiveness in reducing the residual oxygen level in containers of roasted coffee beans. Equation 2 was also evaluated to determine whether it would be useful in predicting the residual oxygen level in roasted coffee bean containers. Wax coated roasted coffee beans were used for this study, since roast coffee by itself rapidly absorbs oxygen.

MATERIALS AND METHODS

Wax coated roasted coffee beans were placed in a stainless steel pressure vessel and then purged with carbon dioxide gas using three different purging methods. The residual oxygen level for each method was measured using an oxygen analyser. A diagram of the experimental apparatus is shown in Figure 4, at right.

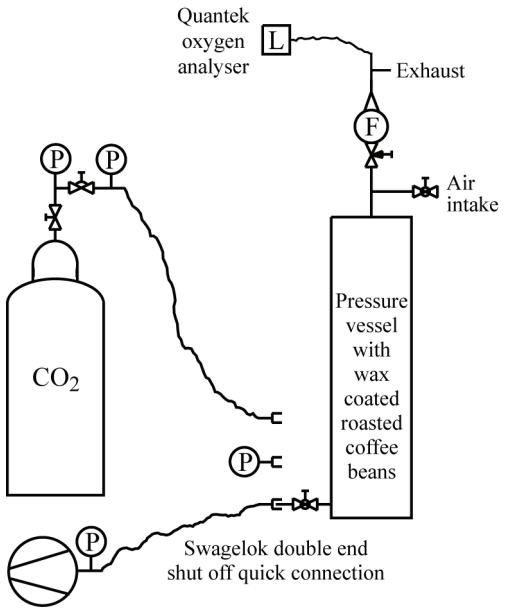

Figure 4. Experimental apparatus for gas purging of roasted coffee beans.

Carbon dioxide gas containing a maximum of 10 ppm moisture and 20 ppm oxygen was provided from a compressed gas cylinder. A vacuum of -830 mbar (183 mbar absolute pressure) was provided by the diaphragm vacuum pump. The various pressure and vacuum gauges used in this study were rated at ± 2.5% accuracy.

The pressure vessel itself had an internal volume of 608 cm³ and was a cylinder with internal dimensions of approximately 41 mm diameter and 450 mm height. The cylinder had a conical bottom with a centrally located gas injection point. The flowmeter at the top of the pressure vessel was a rotameter capable of measuring 0-20 cm³/sec and had a claimed accuracy of ±6%. A diverter plate was installed in the tee leading to the air intake valve in order to flush out this pipe section when gas flowed through the flowmeter. The pressure vessel was flushed with air between tests by operating the vacuum pump with the air intake valve open for 60 seconds – about thirty times the purge volume was pumped through the pressure vessel in this way. The Swagelok double end shut off quick connection fittings that were used at the bottom of the pressure vessel allowed 0.3 cm³ of air to enter the system when the fitting was coupled.

To determine the oxygen level in the gas leaving the pressure vessel through the exhaust port, a gas sample was pumped through the needle probe mounted at the exhaust port and then through the oygen sensor in the Quantek Model 901 oxygen analyser. This analyser uses an oxygen sensor based on an electrochemical cell with a resolution of 0.1 % v/v and a claimed accuracy of ± 0.1% v/v below 1% v/v and ±2% above 1% v/v.

Within 12 hours of packaging, most packaged roast coffee; either ground or whole bean, contains 0% oxygen because it has all reacted with the coffee (Sivetz 2003). After gas purging of roasted coffee beans, there would be a gradient in oxygen level throughout the bed of coffee beans. Gas diffusion would reduce this gradient over time. However, based on the results of a study of gas diffusion through grain bulks with porosity similar to roast coffee beans (Shunmugam et al 2005), the reaction of the residual oxygen with the roast coffee would be much faster than gas diffusion. Measuring the oxygen level at the exhaust port of the pressure vessel would provide the maximum oxygen level in the pressure vessel.

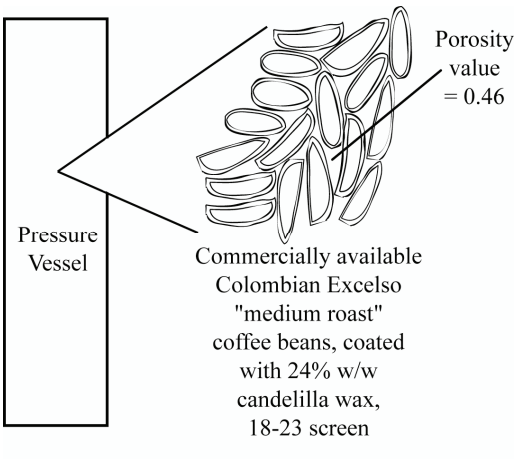

Figure 5. Wax coated roasted coffee beans used for purging stydy.

As shown in Figure 5, at right, commercially available Colombian Excelso “medium roast” coffee beans were used. These were coated with 24% w/w added candelilla wax by dipping the roasted coffee beans in molten wax. The individual width of the wax coated beans were measured – width ranged from 18/64” to 23/64”, corresponding to grading screen openings of 18 and 23. 198.5 grams of these beans were used for the purging study.

RESULTS AND DISCUSSION

The bulk density and specific gravity of the wax coated beans were determined by displacement – the bulk density was 0.41 g/cm³ and the specific gravity was 0.76 g/cm³. The porosity value calculated from these values is 0.46. This means that the interstitial space, or the space between the beans, comprised 46% of the space occupied by the beans. This value was lower than the porosity value of 0.53 identified for roasted coffee beans by a previous author (Eggers 2001). The wax coating would fill cavities in the roasted beans, resulting in a smoother surface and therefore a lower porosity. This lower porosity would result in a higher interstitial gas velocity than would be the case for uncoated roasted coffee beans.

Shown in Table 1, below, are results from pressure purging (one experiment) and a combination of vacuum and pressure purging (three experiments). In all experiments, the actual oxygen levels were close to the estimated levels calculated using Equation 2. In all but one experiment, they were practically the same or slightly lower than the estimated levels.

The second experiment, one cycle of vacuum followed by pressure to 7 bar, yielded a result slightly higher than the estimated level and with more variation than the other experiments. This may have been because there was less gas flow through the bed of coffee beans in this case – the vacuum and pressure were both applied at the bottom of the pressure cylinder. In the pressure purging experiment, venting was done through the flowmeter at the top of the cylinder. In the 2 cycle vacuum/pressure experiments, the second vacuum application was done through the air intake valve. Gas flow through the bed of coffee beans was increased as a result. The estimated volume of purge gas used was calculated using Equation 3.

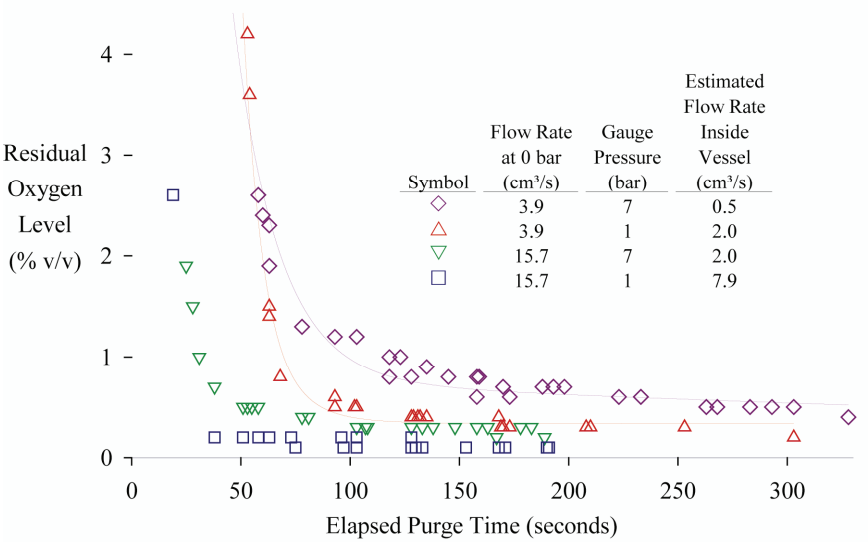

Figure 6. Residual oxygen level vs. elapsed purge time for displacement purging.

The results from displacement purging are shown in Figure 6, at right. Four or five experimental runs were conducted for each of the four combinations of flow rate and pressure listed in the table. In all cases, the residual oxygen level did not reach zero but instead tended to plateau. The residual oxygen level achieved appears to be related to the gas flow rate, specifically the gas flow rate inside the pressure cylinder, shown in the last column and calculated using the ideal gas laws from the flow rate at 0 bar and the pressure inside the pressure cylinder. The two experiments with the same internal flow rate plateaued out at about the same residual oxygen level. Of these two, the 7 bar experiment yielded a slightly lower oxygen level, possibly because the diffusion coefficient at 7 bar is one quarter of the diffusion coefficient at 1 bar and as a result there would be less mixing between gases at 7 bar.

Table 1. Actual versus estimated residual oxygen levels for various purge methods.

Sequence	P_L (atm)	P_H (atm)	Estimated O_2 Level (% v/v)	Actual O_2 Level (% v/v)	Range	*n*	Estimated Volume of Purge Gas Used (multiple of Vp)
Vac > Press 1 bar 2X	0.18	1.99	0.17	0.0 ±0.1	0.0	5	3.62
Vac > Press 7 bar 1X	0.18	7.91	0.48	0.6 ±0.1	0.3	5	7.73
Vac > Press 7 bar 2X	0.18	7.91	0.01	0.02 ±0.1	0.1	5	15.5
Atm > Press 7 bar 2X	1.00	7.91	0.34	0.1 ±0.1	0.0	5	13.8

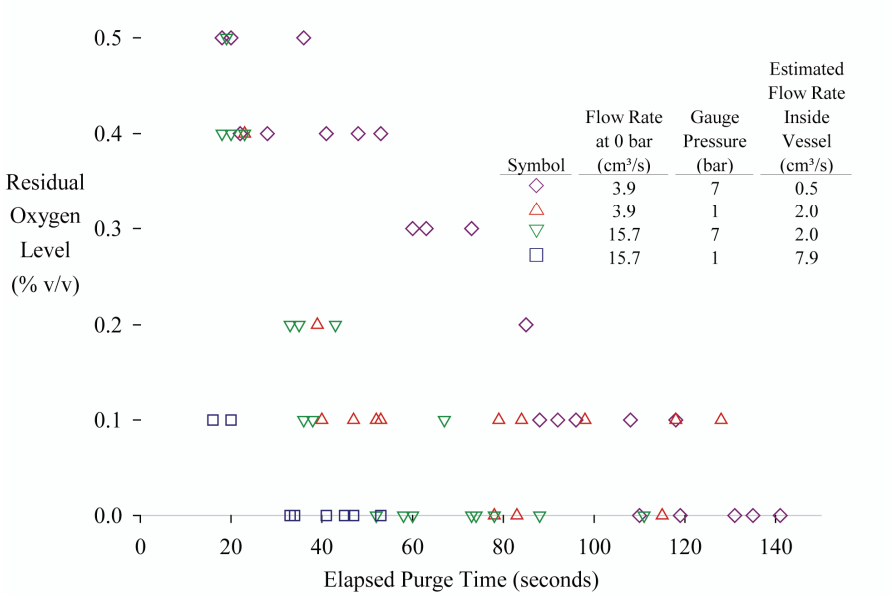

Figure 7. Residual oxygen level vs. elapsed purge time for vac > displ purging.

The results from vacuum/displacement purging experiments are shown in Figure 7, at right. In these experiments, first a vacuum of -830 mbar was applied, the pressure cylinder was pressurised to either 1 bar or 7 bar, and then displacement purging was conducted as in the previous displacement purging experiments. A residual oxygen level of 0.0% v/v was reached all cases. As before, the rate of reduction in residual oxygen level appears to be related to the gas flow rate inside the pressure cylinder, with the two experiments at 2.0 cm³/s having similar results. Of these two experiments, the experiment at 7 bar reached 0.0% v/v faster than the experiment at 1 bar, again possibly due to the lower diffusion coefficient at 7 bar.

The volume of purge gas used to reach a residual oxygen level of 0.1% v/v is shown in Figure 8, below, for displacement and vacuum/displacement methods. These values were calculated using Equation 3. As shown, the combination of vaccum and displacement purging at 1 bar used the least amount of purge gas. Of these two experiments, the higher flow rate experiment took less than half the time to achieve 0.0% v/v as the lower flow rate experiment but used slightly more gas (2.63 versus 2.26 multiples of Vp). These values were also lower than the purge gas volumes shown in Table 1 on page 5.

The purge volume, V_P, is the empty space inside the pressure vessel and equals the internal volume of the pressure vessel less the space occupied by the individual waxed coffee beans (not the bulk space). 198.5 grams of wax coated beans divided by 0.76 g/cm³, the specific gravity of the wax coated beans, yields 261 cm³ - the space occupied by the individual beans. The purge volume was then 347 cm³ (608 cm³ minus 261 cm³).

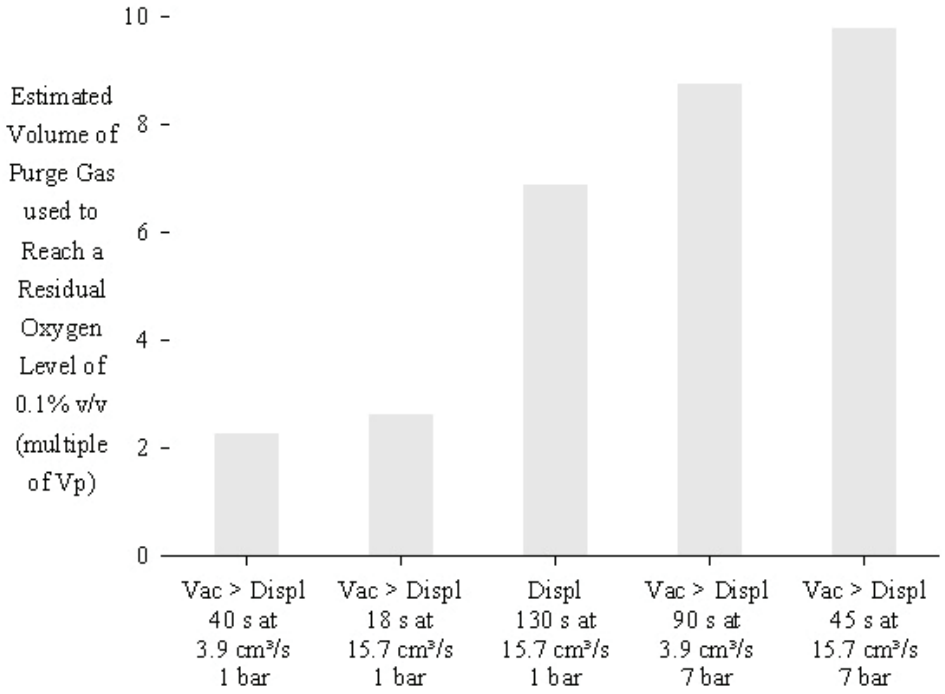

Figure 8. Estimated purge gas volume used to reach 0.1% v/v residual oxygen for purge methods.

CONCLUSIONS

A residual oxygen level of 0.0% v/v was reached by using 2 cycles of vacuum/pressure purging or by using vacuum/displacement purging.

Vacuum/displacement purging was more effective than displacement purging alone.

Vacuum/displacement purging at 1 bar used less purge gas than the other methods.

The equation developed to calculate residual oxygen levels in empty tanks was shown to be useful for rough predictions of residual oxygen levels after purging of roasted coffee bean containers.

Purging methods that involved gas flow through the bed of coffee beans were more effective than one method that used limited gas flow.

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Evaluation of Roasted Coffee Beverage Pervaporation to Aroma Concentration: Process Efficiency and Enrichment Factor

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SUMMARY

Pervaporation is a selective membrane technique in which a liquid mixture is fractionated by means of partial vaporization through a non porous permselective membrane. This process has been considered an alternative for the recovery and concentration of volatile substances from liquids mixtures, which indicates its potential for utilization in the recovery of aroma compounds. This process has as advantages over the classical processes of solvent extraction, steam extraction and supercritical extraction, such as no utilization of organic solvents and the operation under mild conditions of temperature and pressure. This work had as objective to study the pervaporation of coffee beverage in order to obtain a natural concentrated essence to be used as an additive in the food industry. Two flat sheet membranes were evaluated, polydimethylsiloxane (PDMS) and ethylene-propylene-diene terpolymer (EPDM), at three process temperatures (25 °C, 35 °C and 45 °C). Total permeate flux and the enrichment factor of some compounds were calculated for evaluating the process performance. The aromatic profile of the coffee beverage was determined by gas chromatography and mass spectrometry (GC-MS). PDMS membrane exhibited a higher permeate flux when compared to the EPDM. By increasing the temperature of the process a positive effect on the permeate flux was observed, according to the Arrhenius equation. The EPDM membrane presented higher enrichment factors for many substances, indicating that this membrane is more selective for the recovery of aroma compounds from coffee beverages. Experimental results showed that pervaporation process is a membrane technology able to recover and to concentrate coffee beverage aroma and that EPDM membrane exhibits a higher performance than PDMS membrane during such a process.

INTRODUCTION

In the food and beverage industry, the aroma of a product is of great importance for its quality and acceptance by the consumers. The aroma of a product is determined by a large number of volatile organic components, such as alcohols, esters, aldehydes, and hydrocarbons that are present in various concentrations in the beverage. During the juice processing, the temperature which the product is submitted can result in the reduction of the fresh flavour, as many of the aroma compounds are thermo sensitive.

Coffee is the second commodity more commercialized (in value) in the global trade. The annual consumption was estimated in 100 million of bags (60 kg) produced in more than 70 tropical countries. Brazil is the main growing country of coffee and has the second consuming market (ABIC, 2007). Besides its stimulatory effect, coffee is appreciated for its pleasant aroma, which is a result of roasting. Progress in instrumental analysis, particularly in gas

chromatography coupled with mass spectrometry, has shown that the volatile fraction of roasted coffee consists of a great multiplicity of compounds. More than 800 volatile compounds with a wide variety of functional groups has been identified (GROSCH, 2001). It has been reported that the volatile complex comprises by weight 38-45% furan derivatives, 25-30% pyrazines, 3-7% pyridines, 3-5% benzenoid aromatics, 1% aliphatics, 0.5% alicyclics and 1% of various sulphur compounds (which may well be particularly important for flavour/aroma) in a medium-roast Arabica coffee. Due to the complexity of components that form the volatile fraction of coffee, the identification and quantification of the aroma-active compounds are a difficult task. The temperature, in particular, is a critical point due the instability, for example, of thiols and disulphides (Guth et al., 1995).

Pervaporation is a membrane separation process that can be used to separate low quantities of specific compounds from liquid mixtures. It can be applied as an aroma separation technique, based on molecular interaction between the aroma compounds and the membrane material. It takes place under mild conditions, low temperatures and pressures, which preserve the freshness and health-promoting qualities of the food ingredients, while minimizing energy consumption. The driving force for the mass transfer is the chemical potential gradient established by applying a difference in the partial pressure across the membrane (Neel, 1991).

The sorption–diffusion mechanism has been widely used to describe the transport through pervaporation membranes. According to this mechanism the transport occurs in three steps: selective sorption of the components on the membrane surface in contact to the feed stream, diffusion of the components through the membrane and desorption of the components on the permeate side. Sorption step can be related to thermodynamic aspects as affinity between the compounds and the membrane material and diffusion to kinetic factors as mobility of the permeants in the membrane material (Karlsson, Trägardh,. 1995).

Pereira et al. (2005) emphasized that when compared to other membrane processes like ultrafiltration or reverse osmosis, pervaporation presents lower fluxes and it is still nowadays a process under development.

This work had as objective to study the pervaporation of coffee beverage in order to obtain a natural concentrated essence to be used as an additive in the food industry.

MATERIAL AND METHODS

Raw Material

The pervaporation experiments were carried out with 2 L of coffee beverage prepared with a commercial roasted and ground coffee of traditional pattern. The beverage was prepared according to packing instructions, i.e., it was used 160 g of roasted and ground coffee for 2 L of water. It was used filter paper and water at 95 °C to prepare the beverage.

Pervaporation Process

The pervaporation unity utilized was outlined in the Figure 1. It was used a plane membrane of EPDM which area was 63.6 cm^2. The prepared coffee beverage was continually circulated on the membrane surface with a centrifugal pump. The experiments were carried out at 35 °C, being controlled by a thermostatic bath. The partial pressure gradient was maintained by the use of a vacuum pump in the permeate side. The obtained permeate was collected in a

condensation apparatus submerged in liquid nitrogen (-196 °C). The total permeation rate was calculated from the permeate weight collect during a certain time. The following expression was used to calculate the total flux (*J*):

$$J = \frac{m}{A * \Delta t} \quad [1]$$

where m represents the total weight of the permeate phase, A is the membrane area, Δt is the time interval.

The samples of the beverage were collected at the initial (feed) and at the end (retentate) of the process and of the permeate in order to carried out the chromatographic analyses.

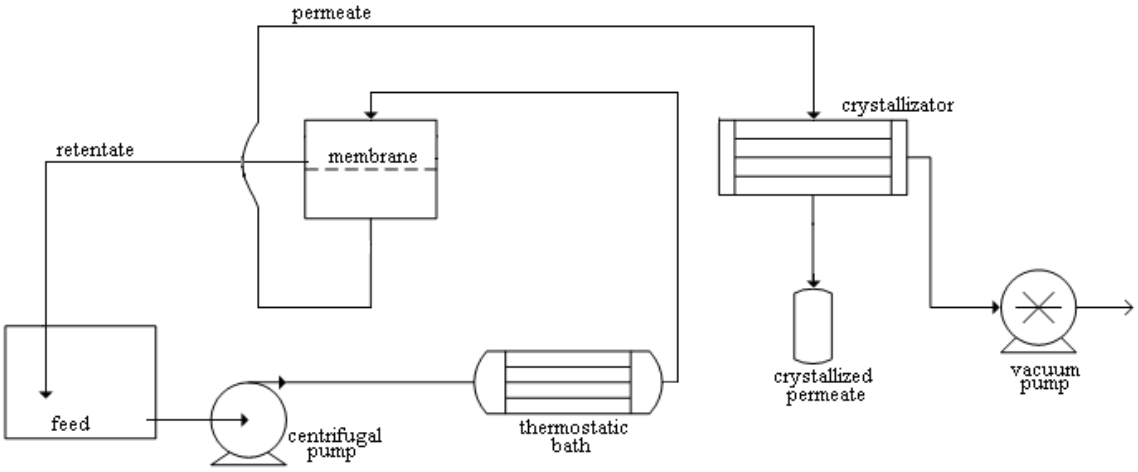

Figure 1. Pervaporation unit diagram.

The selectivity, together with the flux, determines the performance of the pervaporation process. The enrichment factor, β_i, is a measure of the selectivity and it is calculated from the concentrations of the compound *i* in the feed (F) and in the permeate (P) streams, respectively:

$$\beta_i = \frac{C_{P,i}}{C_{F,i}}$$

Analytical procedure

The qualitative analyses of the volatile compounds were made using solid-phase microextraction (SPME) and gas chromatography (GC) technique.

Solid Phase Micro Extraction –Gas Chromatography analysis

The SPME was carried with a polydimethyl siloxane, PDMS, fiber with 0.5 g of sample (feed, permeate or retentate) and 0.5 g of NaCl. The equilibrium time was 1h at room temperature and magnetic agitation. The fiber was exposure for 15 min and immediately transferred to the injector of the gas chromatograph. The dessorption time, inside the injector, was 3 min.

Solvent Extraction

Aiming at the quantification of the components the solvent extraction method was used with the addition of an internal standard before the chromatographic analysis. The identification of the components was made through the Kovats index, retention time and mass spectrometry.

Chromatographic and Mass Spectrometry Analysis

Aroma compounds were detected by using a Perkin Elmer Autosystem XL gas chromatograph fitted with a PE-5 fused silica capillary column (20 m X 0.18 mm X 0.4 μm). Hydrogen (1.0 mL/min) was used as carrier gas. Oven temperature was initially held at 40 °C for 3 minutes, then ramped at 3 °C/min to 240 °C and kept at 240 °C for 10 minutes. The injector was operated at 250 °C and in splitless mode for 2 minutes. The flame ionization detector (FID) was set at 300 °C.

Mass Spectra were obtained in an Agilent 5973N system operating in electron impact mode (EIMS) at 70 eV, coupled to an Agilent 6890 gas chromatograph fitted with a HP-5 MS column (30 m X 0.25 mm X 0.25 μm), using the same injection procedure and oven temperature program as above. Helium was the carrier gas, at 1.0 mL/min. The identification was based on the mass spectra and fragmentation profile of the compounds compared with the data in Wiley 6th ed. library and by their calculated retention indices (RI) compared with literature data.

RESULTS

Experimentally it was verified that the permeate flux increased when the process temperature increased for both evaluated membranes (Figure 2). It is known that the properties of the compounds, both in the feed solution (diffusivity and viscosity) and in the membrane (permeability) are affected by the temperature increase in the feed solution.

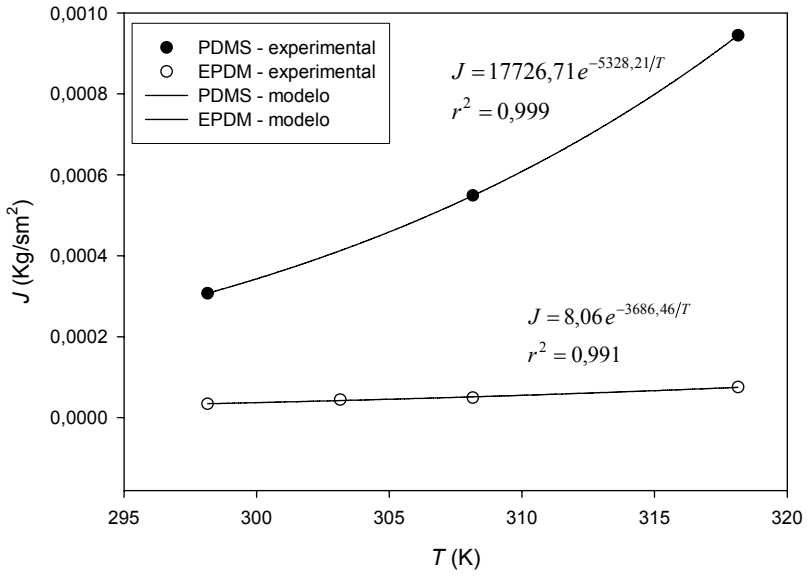

Figure 2. Effect of the process temperature on the permeate flux.

The influence of the temperature on the total permeate flux followed the Arrhenius Law as it can be observed in Figure 2. From the results the activation energies for the two systems had been determined in both the membranes (Table 1). The values of the correlations (r^2) for the two equations of total permeate flux were satisfactory confirming that the process followed the Arrhenius behavior.

Table 1. Activation energy of pervaporation process of coffee beverage.

Membrane	Activation energy (kJ/mol)	r^2
EPDM	30,65	0,991
PDMS	44,30	0,999

Table 2. Enrichment factors of some components of coffee beverage for the two membranes evaluated.

Peak	EPDM			PMDS		
	25ºC	**35ºC**	**45ºC**	**25ºC**	**35ºC**	**45ºC**
Piridira[1,2,4,5,6,7,11]	11.02	20.46	27.81	20.88	22.77	16.72
Methyl-pirazine[5,6,10]	*	*	*	18,55	19,37	*
2-furancarboxyaldehyde[2,6,7]	10.72	14.50	14.20	7.89	10.22	8.02
2-furanmethanol	*	*	*	13.15	15.58	*
2,5-dimethylpyrazine[5]	2.58	4.49	4.30	1.27	2.05	2.03
Benzaldehyde	5.85	9.02	9.39	3.55	5.11	4.20
n.i.	*	*	*	*	*	*
Phenol[1.3]	*	*	*	7.92	12.55	8.59
2-formyl-1-methylpyrrol[5.7.10]	5.36	8.22	9.28	2.58	4.18	3.72
2-acetyl pyrrol[5]	41.18	41.59	42.99	29.31	28.13	34.33
n.i.	*	*	3.54	1.25	1.60	1.57
n.i.	*	*	*	*	*	*
2-methoxy phenol	*	*	*	*	*	*
2,4-dimethylcyclopent-4-en-1,3-dione	9,89	8.38	18.33	4.25	6.52	6.88
1-(2-methylfuryl)-1-pyrrol	*	*	*	*	*	*
n.i.	*	*	0.28	*	0.42	0.33
n.i.	*	*	*	*	*	*
n.i.	*	*	*	*	*	*
n.i.	*	*	*	*	*	*
n.i.	1.02	2.77	4.42	0.69	1.50	2.29
n.i.	*	*	4.05	0.29	1.03	2.65
n.i.	*	*	*	*	*	*
n.i.	*	*	*	0.41	*	*
n.i.	*	*	*	*	*	*
Pyridine[1,2,4,5,6,7,11]	*	*	*	*	*	*
Methyl-pyrazine[5,6,10]	*	2,56	1.40	*	0.58	*
Caffeine	*	*	*	*	*	*
1,2-benzodicarboxlic acid	*	4.00	4.30	1.75	1.27	1.11

ni: not identified

By using SPME extraction before CG-MS, it was possible to identify 21 compounds in the feed sample and 52 compounds in the permeate sample. Many of these compounds are cited in the literature (De Maria & Trugo, 1998; Amstalden et. al., 2001; Nascimento et al., 2003).

Table 2 presents the enrichment factors for some compounds identified in the pervaporation with the PDMS and EPDM membranes.

It could be observed that, for the majority of the composites, the enrichment factor increased with the increase of the process temperature for both membranes demonstrating the influence of the temperature in the pervaporation of volatile compounds. However, in some cases, a small variation or even a decrease in the enrichment factor was observed.

The values of the enrichment factor of the components were higher with the EPDM membrane than with the PDMS membrane, indicating the higher selectivity of the EPDM membrane in relation to the PDMS in the pervaporation process.

The enrichment factor was higher than 1 for almost all the identified compounds, indicating that this compound was more concentrated in the permeate that it was in the feed stream.

Sensory evaluations were conducted experimentally with 23 consumers in order to evaluate coffee characteristic odor. All the permeate samples presented the aroma characteristic of coffee, indicating that the concentrated extract maintained the characteristics of the original coffee beverage.

CONCLUSIONS

It can be concluded that pervaporation is a promising technique for the recovery of coffee aroma compounds. As the feed temperature increased, flux of coffee beverage increased linearly and followed an Arrhenius type relation.

Regarding the enrichment factor, the results of this work indicate that the pervaporation is an efficient process to extract and to concentrate the volatile compounds of the coffee beverage.

ACKNOWLEDGMENTS

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Instant Coffee Aromatization Particles Containing an Encapsulated Non-Synthetic Dispersed Volatile Liquid Coffee Aroma Phase

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SUMMARY

Increasing the intensity of cup aroma released during reconstitution of instant coffee powders is one method to enhance the consumer sensory experience. At 20th ASIC, we presented a method to encapsulate synthetic coffee aromas compounded in novel buoyant water-immiscible volatile carrier liquids (VCLs) demonstrated to increase aroma release rate and peak intensity relative to use of conventional carrier liquids (Zeller et al., 2004). At 21st ASIC, we presented a method to aromatize synthetic VCLs using natural coffee aromas to further enhance the sensory quality of these high-impact controlled-release aroma-delivery systems (Zeller et al., 2006). At this meeting we present the latest chapter of our research which led to encapsulation of carrier-free volatile liquid coffee aromas having physical properties and release characteristics similar to VCLs without the need to use any non-coffee ingredients (Ceriali et al., 2005). This was enabled by first creating highly-volatile water-immiscible oil-free liquid aromas by removing the most water-soluble and least-volatile components from natural coffee aroma condensates. These liquid aromas were then encapsulated in low-density soluble coffee particles using methods previously employed to encapsulate aromatized VCLs. The resulting novel instant coffee aromatization particles contain a separate disperse phase consisting of liquid aroma droplets. These particles rapidly dissolve on the beverage surface when they are added to instant coffee powders and reconstituted in hot water. The aroma droplets released from these particles float and quickly evaporate to provide an intense authentic coffee cup aroma without producing an unsightly appearance or unpleasant residual flavor.

INTRODUCTION

Cup aroma, or preparation aroma, is the aroma released by an instant coffee product when it is reconstituted by the consumer. Preparing a hot instant coffee beverage does not provide the same aroma intensity and character as brewing roast coffee. The common practice of adding a small amount of aromatized coffee oil to agglomerated or freeze-dried coffees is effective in providing the consumer with a fresh headspace aroma when the package is opened. However, attempts to greatly increase the amount of oil needed to provide cup aroma have not been successful as a result of the formation of strong or undesirable beverage flavors and unsightly surface oil slicks. This problem was overcome by replacing coffee oil with VCLs aromatized with synthetic or natural coffee aromas and encapsulating them in floating soluble coffee capsules (Zeller et al., 2003; Zeller et al., 2003; 2004). This was demonstrated to greatly increase both aroma release rate and peak intensity, when capsules are mixed with instant coffee and reconstituted in hot water, without adversely affecting flavor or producing slicks. However, because VCL use in instant coffee products requires labeling, a further-improved

technology was developed which provides the benefits of aromatized VCLs without the need to use any non-coffee ingredients.

MATERIALS

All ground coffees, coffee aromas, coffee oil, soluble coffee extract, dried instant coffee, and milled instant coffee were derived from roasted arabica beans at Kraft Foods facilities. These materials were used to produce floating soluble coffee aromatization particles according to the methods disclosed in Ceriali et al. (2005) or Tuot (1985), as generally described below. All descriptions and data are approximate, subject to error, and nonbinding with respect to granted or pending Kraft Foods patent applications. Aromatization particles were stored in glass vials at room temperature.

METHODS

Encapsulation of Aromatized Non-Volatile Coffee Oil

Floating soluble coffee reference capsules containing an encapsulated separate disperse phase consisting of aromatized coffee oil were produced using known methods, as generally disclosed in Tuot (1985), according to the following procedure. Water was added to 500 kg ground roast coffee, having 2.3 mm particle size, to a moisture content of 50% /wt, dry-basis (db). The coffee was then contacted with saturated steam at 0.7 barg pressure for 10 min in a percolator. The steam loaded with coffee aroma constituents was removed and condensed at 5 °C to provide a weight of aqueous condensate equal to 5% of the coffee weight (db). 100 g of dried instant coffee was dissolved in 100 g of the condensate to provide an aromatized coffee solution. 10 g plain coffee oil was emulsified into the solution using a Silverson high-shear mixer at 8000 rpm for 1 min. Nitrogen gas was bubbled into the emulsion to achieve 0.75 g/mL density. 20 g of this foamed emulsion was then added drop-wise onto 400 g fine milled instant coffee powder on a vibrating bed. The mixture was equilibrated for 48 hrs in a closed container, and then sieved to recover 1-3.5 mm particles. These floating capsules, containing a core of emulsified aromatized liquid coffee oil droplets dispersed in a desiccated foamed solid soluble coffee matrix encapsulated by a dense hard coffee shell formed from the milled powder, had a volatile coffee aroma content of 100 μg/g.

Encapsulation of Volatile Coffee Essence

Floating soluble coffee capsules containing an encapsulated separate disperse phase consisting of a novel volatile water-immiscible natural coffee essence were produced using the methods generally disclosed in Ceriali (2005) according to the following procedure. Water was added to 500 kg ground roast coffee, having 2.1 mm particle size, to a moisture content of 50% /wt (db) in a percolator. A 200 mbar vacuum was then pulled on the percolator for 3 min while soluble coffee extract (53% /wt solids) was fed into the percolator. The extracted vapor loaded with coffee aroma constituents was condensed at 5 °C to remove the most water-soluble and least-volatile components. The uncondensed vapor fraction was passed into an adsorbent column loaded with Zeolite (Zeolum®, Tosoh Corp.) and then cryogenically condensed at −130 °C to obtain a frozen coffee essence in the form of a frost. 80 kg of frost was sealed in an autoclave and heated to 30 °C to remove CO_2 gas by venting to reduce pressure to 10 barg over 2 min. 40 kg of cold 53% soluble coffee extract was pumped into the autoclave, which was re-heated to 30 °C, and pressure slowly released to atmospheric over 4 hrs. 50 g of this aromatized extract was then diluted with 50 g of 53% soluble coffee extract and 20 g of this mixture blended with 80 g of 53% soluble coffee extract using a Silverson

high-shear mixer at 4000 rpm for 1 min to form a uniform dispersion. Nitrogen gas was bubbled into the dispersion to achieve 0.75 g/mL density and this foamed dispersion added drop-wise into liquid nitrogen to form 1-4 mm frozen particles. After excess liquid nitrogen was decanted, 1 part recovered particles was contacted with 20 parts fine milled instant coffee powder, equilibrated for 48 hrs in a closed container, and then sieved to recover 1-3.5 mm particles. These floating capsules, containing a core of water-immiscible liquid depleted natural coffee essence droplets dispersed in a desiccated foamed solid soluble coffee matrix encapsulated by a dense hard coffee shell formed from the milled powder, had a volatile coffee aroma content of 2120 μg/g.

Capsule Volatile Coffee Aroma Content

0.3 g of capsules was dissolved in 6 mL distilled water in a closed 22 mL glass vial and equilibrated at 60 °C for 30 min. A 0.25 mL volume of headspace was removed from the vial using an auto-sampler and injected into a gas chromatograph fitted with a capillary column and flame ionization detector. Total aroma counts were obtained, compared to appropriate calibration references to permit calculation of concentration of aroma present in the capsules, and rounded to the nearest-ten μg/g unit.

Capsule Disperse Phase and Aroma Intensity

The following three methods (A, B, and C) were used to confirm the presence of a separate liquid essence phase dispersed within the foamed solid phase of aromatization particles and, when the capsules are dissolved in water, the presence of floating liquid essence droplets that rapidly evaporate from the surface of hot beverages to provide a strong cup aroma. These methods were also used to confirm the presence of a separate liquid coffee oil phase dispersed within the foamed solid phase of the reference capsules and to demonstrate the much lower aroma intensity provided relative to capsules containing liquid coffee essence.

Method-A

3 capsules were placed in the center of a Whatman 40 filter paper disk and folded in half to cover the capsules. The capsules were crushed by applying pressure to the disk with the back of a metal spoon for 5 sec and then left for another 5 sec. A trained panel evaluated the crushed capsules in the folded disk by sniffing from 10 cm distance to assess released aroma intensity and also visibly inspected the disk to determine if it was wetted by release of a liquid coffee essence phase or liquid coffee oil phase. Results are summarized in Table 1.

Method-B

0.1 g of capsules was placed into a 200 mL cup with 1.6 g of dried instant coffee and 180 mL 75 °C water poured into the cup. A trained panel evaluated the unstirred beverage 10 sec after water addition by sniffing from 20 cm distance to assess released aroma intensity. Appearance was assessed 1 min after water addition to determine if a slick was present. The beverage was tasted 2 min after water addition to assess the effects capsules had on coffee flavor quality relative to a control beverage prepared from 1.7 g instant coffee with no added capsules. Results are summarized in Table 2; and in Table 5 using a wider range of weights.

Method-C

The capsule weight and water volume of Method-B were used, but hydration with hot (75 °C) water and cold tap water were compared. Use of cold water improved viewing of liquid aroma

droplets released from capsules containing dispersed coffee essence, because evaporation was much slower. After cold water addition, beverages were stirred for 20 revolutions and the appearance assessed 30 sec after water addition. After hot water addition, the trained panelists evaluated the aroma intensity released above the unstirred beverage after 15 sec, and the appearance after 30 sec. Results are summarized in Table 3.

Capsule Dissolution Rate

10 capsules were placed into a 200 mL cup and 180 mL 75 °C water poured into the cup. A trained panelist stirred the cup for 3 revolutions and counted undissolved capsules present at 10 sec, 30 sec, and 2 min. Results are summarized in Table 4.

RESULTS

Aromatization particles encapsulating emulsified aromatized coffee oil or dispersed coffee essence in floating capsules are referred to below as "oil capsules" or "essence capsules", respectively. Tables 1-4 provide experimental evidence essence capsules contain a separate liquid aroma phase and, relative to oil capsules, dissolve much faster and provide stronger aroma, improved appearance, and cleaner flavor. Table 5 provides experimental evidence the proportion of oil capsules used in a hot instant coffee beverage can not be suitably increased to achieve the aroma strength provided by a much lower proportion of essence capsules without adversely affecting appearance and flavor. Preferred general methods used to create frozen coffee essence frosts, aromatize soluble coffee extracts, and produce floating coffee aromatization particles are illustrated in Figures 1, 2, and 3, respectively.

Table 1. Effect of crushing capsules on perceived properties.

Method-A	**Panel Evaluation of Crushed Capsules**	
Aromatization Particles	**Relative Aroma Strength**	**Filter Paper Disk Wetting**
Oil Capsules	No Noticeable Aroma	Visible
Essence Capsules	Noticeable Aroma	Visible

Table 2. Perceived properties in hot instant coffee beverages.

Method-B	**Panel Evaluation of Hot Instant Coffee Beverages**		
Aromatization Particles	**Aroma**	**Flavor**	**Appearance**
None (Control Beverage)	Weak	Baseline	Baseline
Oil Capsules	Weak	No Difference	Visible Oil Slick
Essence Capsules	Strong	No Difference	No Difference

Table 3. Observed properties in hot and cold instant coffee beverages.

Method-C	Panel Evaluation of Hot & Cold Instant Coffee Beverages		
	Aroma	Appearance	
Aromatization Particles	Hot Water	Hot Water	Cold Water
None (Control Beverage)	Weak	Uniform Solution	Uniform Solution
Oil Capsules	Weak	- Floating Oil Droplets - Floating Particles	- Floating Oil Droplets - Floating Particles
Essence Capsules	Strong	Uniform Solution	- Pale Yellow Floating Liquid Aroma Droplets

Table 4. Observed capsule dissolution rate in hot water (for 10 capsules)

	Number of Floating Particles Visible		
Aromatization Particles	After 10 Seconds	After 30 Seconds	After 2 Minutes
Oil Capsules	10	10	10
Essence Capsules	2	0	0

Table 5. Perceived properties in hot instant coffee beverages (Method-B)

Ingredient Weights (g)	Panel Evaluation of Hot Instant Coffee Beverages		
Oil Capsules + Instant Coffee (1.7 Total)	Aroma	Flavor	Appearance
0 + 1.7 (Control Beverage)	Baseline	Baseline	Baseline
0.1 + 1.6	No Difference	No Difference	Visible Oil Slick
0.3 + 1.4	No Difference	Slight Difference	- Visible Oil Slick - Floating Particles
0.5 + 1.2	No Difference	Noticeable Difference	- Visible Oil Slick - Floating Particles
1.0 + 0.7	Slight Difference	Noticeable Difference	- Visible Oil Slick - Floating Particles
1.7 + 0.0 (No Instant)	Noticeable Aroma	Overwhelming Impact	- Visible Oil Slick - Floating Particles
Essence Capsules + Instant Coffee (1.7 Total)	Aroma	Flavor	Appearance
0.1 + 1.6	Noticeable Aroma	Similar to Baseline	Similar to Baseline

An alternative depleted coffee essence was produced without forming a frost, using the steam condensate collected for aromatizing coffee oil, and encapsulated in aromatization particles using the following procedure. The condensate was contacted with saturated steam at 0.3 barg pressure in a packed column and then condensed at 5 °C. The new condensate was again subjected to steaming, in a new packed column, and condensation under the same conditions. The final condensate had volume only 0.2 x that of the original steam condensate. The process yielded a coffee essence adequately depleted of the most water-soluble and least-volatile aroma components without the need to collect and subject the uncondensed aroma

fraction to cryogenic condensation. 1.2 kg of dried instant coffee was dissolved in 1.2 kg of the depleted essence and the resulting aromatized coffee solution was then cooled to -2 °C, foamed by bubbling with nitrogen gas to 0.75 mL/g density, poured into stainless steel trays, and frozen at -40 °C for 8 hrs. The frozen slab was ground in a cold room and sieved to obtain 1-3.5 mm frozen particles. 20 g of frozen particles were placed into bags containing 300 g fine milled instant coffee powder, equilibrated for 48 hrs, then sieved to obtain particles having 980 µg/g volatile coffee aroma content. These floating capsules provided results generally similar to those perceived for essence capsules noted in the tables. The capsules, when crushed, wetted the filter paper disk and produced noticeable aroma and, when reconstituted in hot water with dried instant coffee, provided strong aroma with no perceived difference in appearance or flavor relative to a control beverage. Similar encapsulation of the original steam condensate, not subjected to repeated steaming and condensation, provided particles having 120 µg/g volatile coffee aroma content. Those floating capsules, when crushed, provided no noticeable aroma and, when reconstituted in hot water with dried instant coffee, produced weak aroma with no perceived difference in appearance or flavor relative to a control beverage.

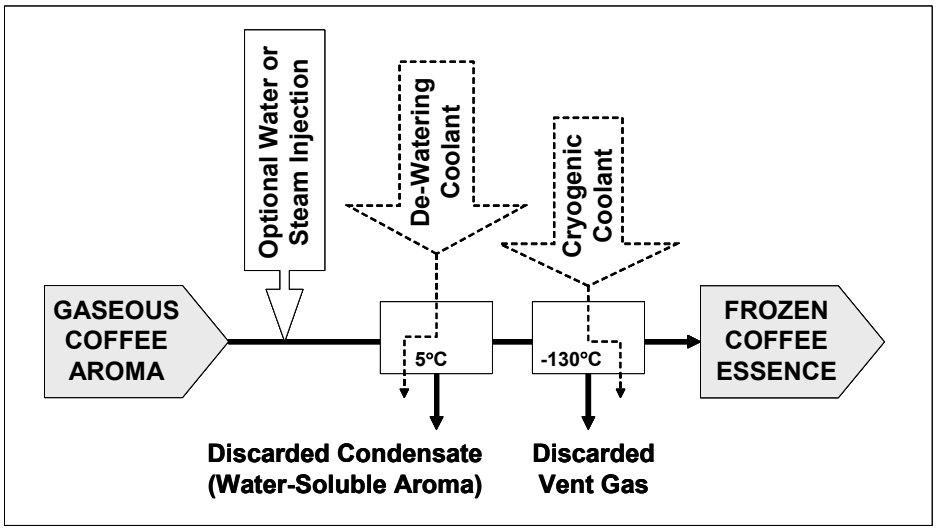

Figure 1. Method to create depleted frozen coffee essence frost.

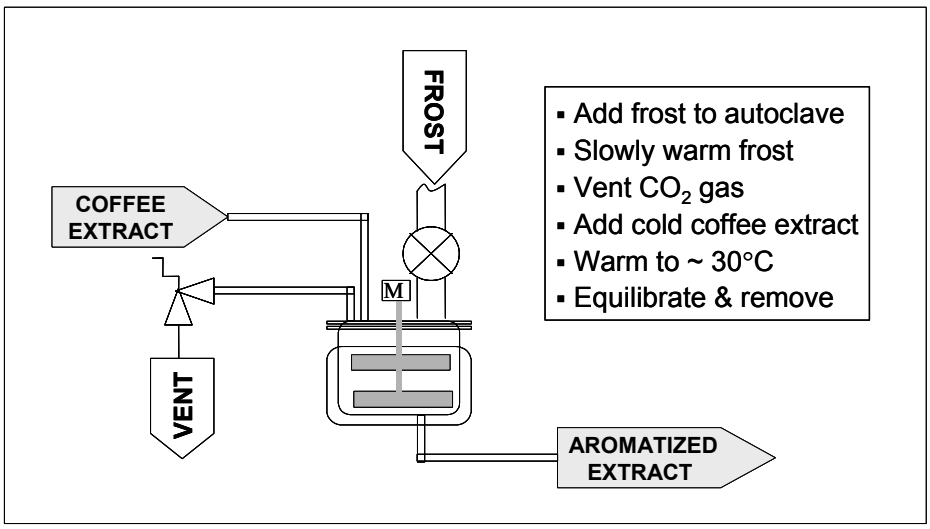

Figure 2. Method to aromatize soluble coffee extract with frost.

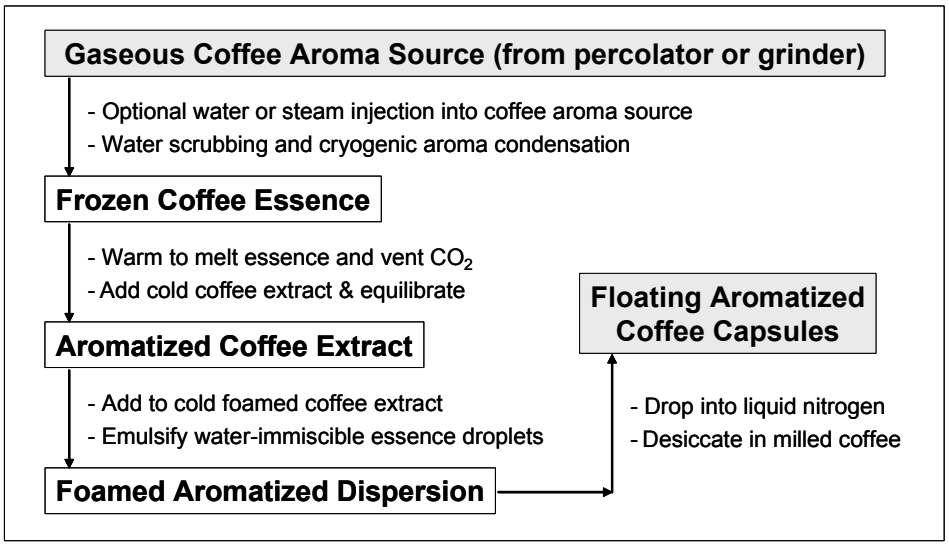

Figure 3. Method to produce floating coffee aromatization particles.

DISCUSSION

The low density of the foamed soluble coffee capsules causes them to float on the surface of hot beverages. Floating capsules containing dispersed highly-volatile coffee essences rapidly dissolve and release aroma into the air to provide a strong preparation aroma. In contrast, the relatively low volatility and high hydrophobicity of floating capsules containing coffee oil impair dissolution in water and hinder release of aroma into the air. Further, their prolonged dissolution time provides greater opportunity for aromas to partition from the coffee oil droplets into the beverage to adversely affect flavor quality. The trained panelists agreed instant coffee beverages formulated with floating soluble coffee capsules containing dispersed coffee essences provided preparation aroma intensity and quality superior to control beverages prepared without aromatization particles and to beverages formulated with floating soluble coffee capsules containing emulsified coffee oil. Instant coffee beverages formulated with floating capsules containing dispersed coffee essences were generally described as providing intense preparation aroma having fresh, rich, roast coffee character and as providing balanced instant coffee flavor having normal strength and quality attributes.

CONCLUSIONS

The intensity and quality of instant coffee preparation aroma can be greatly improved by the use of floating soluble coffee aromatization particles containing an encapsulated non-synthetic dispersed volatile liquid coffee aroma phase that has been depleted of its most water-soluble and least-volatile aroma constituents. Such depletion provides intense coffee essences having the beneficial physical properties of VCLs without the need to use any non-coffee ingredients. The hydrophobic nature of the water-immiscible essences allows them to be easily dispersed, using high-shear mixing, into foamed soluble coffee extracts to produce floating capsules. The high volatility and aroma concentration of the coffee essences encapsulated in the floating capsules provide rapid controlled-release of dispersed essence droplets onto the surface of hot instant coffee beverages and rapid evaporation to deliver a strong burst of natural coffee aroma without adversely affecting beverage flavor or creating a residual slick. Therefore, use of these novel coffee essences avoids the quality defects typically caused by attempts to use high levels of aromatized coffee oil. The production and encapsulation of the coffee essences described can be achieved using readily sourced coffee

components and equipment typically available at coffee research laboratories or instant coffee manufacturing facilities.

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Production of Activated Carbons as an Alternative Use for Defective Coffee Beans

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SUMMARY

The objective of the present study was to propose an alternative use defective coffee press cake, a solid residue from coffee oil biodiesel production, as an adsorbent for treatment of dye contaminated waters. Batch adsorption tests were performed using basic dyes (methylene blue – MB), with the adsorbent being obtained by carbonization of the solid residue at 800 °C for 1h under N_2 atmosphere. The effects of solution pH, adsorbent dosage and contact time on MB removal were investigated. The experimental adsorption equilibrium data were fitted to both Langmuir and Freundlich adsorption models, with Freundlich providing the best fit. The adsorption kinetics was determined by fitting first and second-order kinetic models to the experimental data, with the second-order model providing the best description of MB adsorption. The experimental data obtained in the present study indicated that this type of waste material is a suitable candidate for use in the production of adsorbents for removal of cationic dyes, thus contributing for the implementation of sustainable development in both the coffee and biodiesel production chains.

INTRODUCTION

Approximately 20% the Brazilian coffee production consists of defective beans, which upon roasting decrease beverage quality. These defective beans are physically separated from the non defective ones prior to commercialization in international markets. However, since to coffee producers they represent an investment in growing, harvesting, and handling, these defective beans are commercialized in the internal market in Brazil, where the roasting industry uses them in blends with non defective beans. Thus, the quality of the roasted coffee consumed in Brazil is depreciated since, after separation from the exportable portion, defective beans may be representing more than 50% of the coffee consumed in Brazil (Franca and Oliveira, 2008). In view of this situation, several studies are currently under development in order to find an alternative use for defective coffee beans. One of the alternatives being considered is biodiesel production, using the oil extracted from such defective coffee beans (Oliveira et al., 2008). This procedure has proven to be feasible, but generates a solid processing residue after removal of the oil by pressing (defective coffee press cake).

One of the effective uses of agricultural waste biomass that has gained much attention over the last decade is the production of activated carbons by thermo-chemical conversion (Crini, 2006; Oliveira and Franca, 2008). A few recent studies have shown that seed press cakes can be used for such purposes. Examples include adsorption of copper by mustard oil seed cake (Ajmal et al., 2005), methylene blue adsorption by sunflower oil cake (Karagöz et al., 2008) and removal of cadmium and chromium by Jatropha oil cake (Garg et al., 277; 2008).

Thus, the objective of the present study was to propose an alternative use for defective coffee press cake, a solid residue from coffee oil biodiesel production, as an adsorbent for treatment of dye contaminated waters.

METHODOLOGY

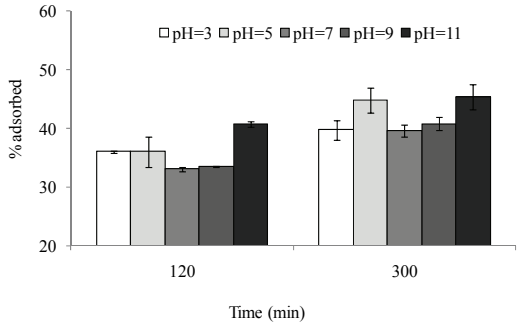
Defective coffee beans were acquired from Santo Antonio State Coffee (Santo Antônio do Amparo, MG, Brazil) and screw pressed (Ecirtec, Brazil) for oil removal. The coffee press cake was then submitted to a one-step physical activation procedure at 800 °C: the samples were put into ceramic containers and the containers placed in an electric muffle furnace at 100 °C (temperature held for 10 min), heated to 300 °C at a rate of 10 °C min^{-1} and then heated 800 °C at a rate of 30 °C min^{-1} under N_2 flow (0.5 L min^{-1} flow rate). This temperature was maintained for 60 min and the samples were then cooled, still under N_2 flow, for production of the coffee cake activated carbon (CCAC).

Batch experiments of adsorption were performed in 250 mL Erlenmeyer flasks, with the flasks being agitated on an orbital shaker at 100 rpm for pre-determined time intervals. In all sets of experiments, a pre-determined amount of adsorbent was thoroughly mixed with 100 mL MB solution. Effect of particle size (diameter = D) was evaluated in the following ranges: $D < 0.50$ mm; $0.50 \text{ mm} < D < 0.84$ mm; $D > 0.84$ mm. Effects of pH and adsorbent concentration were studied in the respective ranges of 3 to 11 and of 5 to 50 g L^{-1} at a fixed initial dye concentration (500 mg L^{-1}). Effect of contact time was evaluated at time periods ranging from 5 min to 6 hours and initial dye concentrations ranging from 100 to 750 mg L^{-1}. After the specified time periods, 2 mL aliquots were taken from the Erlenmeyer flasks and the concentration of MB was determined by a spectrophotometer (Cole Parmer 1100 RS) at 665 nm. All tests were performed in three replicates.

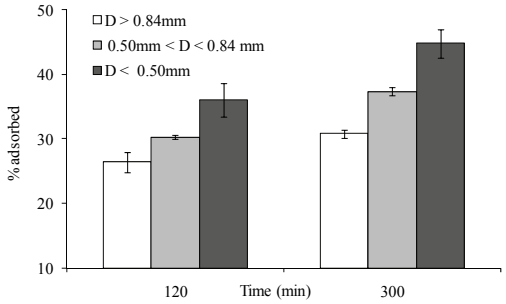
RESULTS AND DISCUSSION

Effect of adsorption parameters

In the present study, the effect of initial solution pH was investigated for values between 3 and 11 and the results are displayed in Figure 1(a). No significant differences were observed after 5 h of adsorption (based on the Tuckey test at 5% probability). Similar results were obtained by other studies, reporting similar adsorption efficiencies for pH values above and below the pH_{PZC}. In our study, even though the positively charged surface sites of the adsorbent should not favor dye adsorption due to electrostatic repulsion, dye removal was still efficient, indicating that π-π dispersion interactions between adsorbent and adsorbate are dominant in this case. This is attributed to the presence of basic groups (π electron donors) on the surface of the activated carbon given the high value of activation temperature. Given that the initial solution pH did not present a significant effect on adsorption capacity, remaining tests were conducted at pH = 5, since it is the natural pH of the aqueous MB solution. Results on the effect of particle size on the adsorption process are presented in Figure 1(b). It is clear that MB uptake increased with the decrease in particle size, due to the corresponding increase in surface area and better accessibility to pores. Similar results on particle size effect have been reported for other agricultural residues (Banat et al., 2003; Singh et al., 2003). Based on the results presented in Figure 1(b), the remaining experiments were conducted employing the smaller particle size activated carbon.



(a)



(b)

Figure 1. Effect of (a) initial solution pH and (b) particle diameter (D) on MB adsorption (initial MB concentration 500 mg L^{-1}, adsorbent dosage 10 g L^{-1}).

The influence of adsorbent dosage on the efficiency of MB removal can be viewed in Figure 2. MB removal efficiency presented a significant improvement with the increase in adsorbent dosage. After 5 h, the percent removal of MB varied from 26 to 95% with an increase in adsorbent concentration from 0.5 to 5 g L^{-1}. This can be attributed to the increase in surface area resulting from the increase in adsorbent mass, thus increasing the number of active adsorption sites. However, as expected, the amount of dye adsorbed per unit mass of adsorbent decreased with increasing adsorbent mass, given the reduction in adsorbate/adsorbent ratio. Based on the results presented in Figure 2, the remaining experiments were conducted for an adsorbent dosage of 20 g L^{-1}.

Adsorption kinetics

The adsorption data presented in Figure 3 show that a contact time of 6 hours assured attainment of equilibrium conditions regardless of the initial MB concentrations. The adsorption can be described by a two-stage kinetic behavior, for all the initial MB concentrations, with a rapid initial adsorption during the first 30 minutes, followed afterwards by a much slower rate. The faster adsorption of methylene blue during the first 30 min is an indication that methylene blue adsorption occurs mainly on the surface of the adsorbent, as reported by other studies on adsorption of methylene blue (Banat et al., 2003; Oliveira et al., 2008). Results presented in Figure 3 also show that adsorption of MB presents a strong dependency of MB initial concentration. An increase in the initial MB concentration led to an increase in the amount adsorbed, which is attributed to the increase in driving force (concentration gradient) with the increase in the initial dye concentration (Oliveira et al., 2008). The amount of MB adsorbed increased from 4.2 to 18.4 mg g^{-1} as the initial concentration was increased from 100 to 750 mg L^{-1}. No significant differences in the amount adsorbed were observed when the initial MB concentration was increased from 500 to 750 mg L^{-1}, indicating that adsorbent saturation was attained.

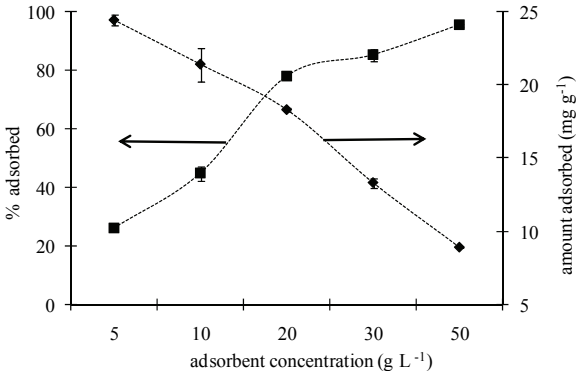


Figure 2. Effect of adsorbent dosage (■ % adsorbed; ◆ amount adsorbed per unit mass - mg g^{-1}) on MB adsorption (initial solution pH 5, initial MB concentration 500 mg L^{-1}, 5 h contact time).

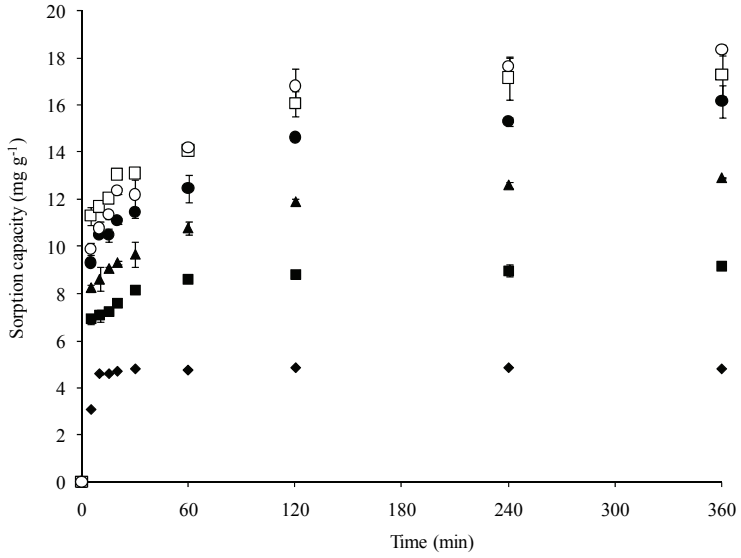


Figure 3. Effect of contact time on MB adsorption.Initial MB concentration: ◆100 mg L^{-1}, ■200 mg L^{-1}, ▲300 mg L^{-1}, ●400 mg L^{-1}, □500 mg L^{-1}, ○750 mg L^{-1}.

The controlling mechanisms of the adsorption processes were investigated by fitting first and second-order kinetic models to the experimental data. The kinetics models can be represented by the following equation:

$$\frac{dq_t}{dt} = k_n (q_e - q_t)^n \qquad [1]$$

where q_e and q_t correspond to the amount of dye adsorbed per unit mass of adsorbent (mg g^{-1}) at equilibrium and at time t, respectively; k_n corresponds to the rate constant for n^{th} order adsorption (k_n units are min^{-1} for n=1 and g $mg^{-1}min^{-1}$ for n = 2). The best-fit model selection was based on both linear regression correlation coefficient (R^2) and the difference between estimated and experimental q_e values. Results are displayed in Table 1. The first-order model did not provide a good fit, with low R^2 values and q_e values being significantly underestimated. An evaluation of both the correlation coefficients and estimated q_e values indicates that MB adsorption can be satisfactorily described by the pseudo second-order model.

Table 1. Kinetic parameters for MB adsorption.

MB initial concentration (mg L^{-1})		Pseudo first-order			Pseudo second-order		
	q_e (exp.)*	q_e	k_1	R^2	q_e	k_2	R^2
100	4.82±0.00	0.5127	0.0455	0.4972	4.849	0.226	0.9999
200	9.18±0.13	1.821	0.0104	0.8711	9.216	0.028	0.9998
300	12.92±0.01	4.605	0.0117	0.9945	13.106	0.009	0.9993
400	16.18±0.69	5.562	0.0049	0.1991	16.339	0.006	0.9947
500	17.31±0.92	6.799	0.0157	0.9897	17.637	0.007	0.9991
750	18.36±0.22	8.008	0.0108	0.9684	18.727	0.005	0.9985
*Average value± standard deviation							

Adsorption equilibrium

The adsorption isotherm is presented in Figure 4. The shape of the curve indicates favorable adsorption. Langmuir and Freundlich adsorption models were evaluated for description of the MB sorption isotherm.

Langmuir isotherm is based on a theoretical model assuming monolayer adsorption over an energetically and structurally homogeneous adsorbent surface. It can be described by the following equation:

$$q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e} \quad [2]$$

where q_e (mg g^{-1}) and C_e (mg L^{-1}) correspond to the amount adsorbed per gram of adsorbent and to the solute concentration (mg L^{-1}) in the aqueous solution, respectively, after equilibrium was reached. q_{max} and K_L are constants related to the maximum adsorption capacity (mg g^{-1}) and the adsorption energy (L mg^{-1}), respectively.

Freundlich´s equation is an empirical model based on heterogeneous adsorption over independent sites. Multilayer adsorption is considered, but the model does not account for saturation. It can be described by the following equation:

$$q_e = K_F C_e^{1/n} \quad [3]$$

where K_F is a constant that indicates the relative adsorption capacity ($mg^{1-(1/n)} L^{1/n}$ g^{-1}) and n is related to the intensity of adsorption .

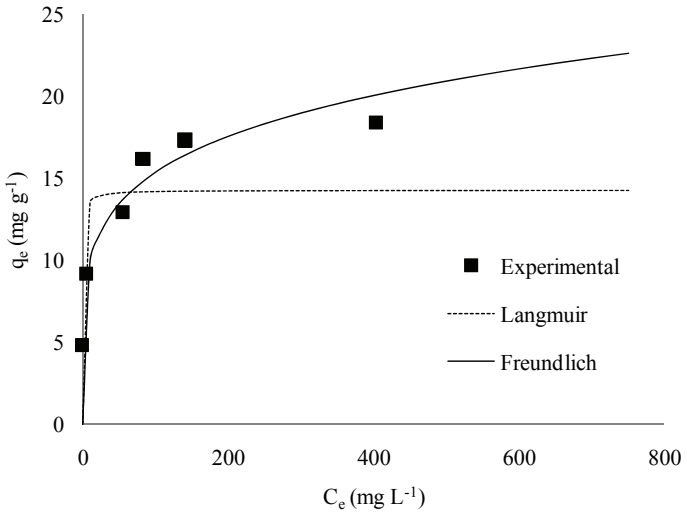


Figure 4. Adsorption isotherm.

MB adsorption was better described by the Freundlich model, indicating heterogeneous and multilayer adsorption. Maximum MB uptake capacity, based on Langmuir model, was 14.9 mg g^{-1}, a low value compared to other adsorbents employing similar types of residues, such as sunflower oil cake and date pits. However, such results do not diminish the feasibility of employing this type of adsorbent for MB removal from aqueous solutions, since it presented good adsorption capacity in comparison to other low cost residues, including date pits, apricot stones and almond shells (Banat et al., 2003; Aygun et al., 2003). Furthermore, an evaluation of the experimental curve presented in Figure 4 shows that adsorption capacity is actually higher (~19 mg g^{-1}), given that Langmuir fit was not very good. A value for 1/*n* below one indicates a normal Langmuir isotherm while 1/*n* above one is indicative of cooperative adsorption. An average value of 5.3 was obtained for 1/*n*, corroborating the heterogeneous nature of the adsorbent surface also consistent with the better Freundlich fit.

CONCLUSIONS

Experiments were conducted to investigate the potential of defective coffee press cake, a residue from coffee oil biodiesel production, as raw materials for production of adsorbents. Equilibrium data indicated favorable adsorption and were better described by the Freundlich model in comparison to Langmuir, indicating heterogeneus adsorption. The maximum value of uptake capacity obtained was 14.9 mg g^{-1}, comparable to values encountered in the literature for other similar residue based activated carbons. The results presented in this study indicate that defective coffee press cake presents great potential as an inexpensive and easily available alternative adsorbent for the removal of cationic dyes in wastewater treatments.

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The Quality Pillars: the Implementation Process of the Coffee Quality Program (CQP) in the Roasted and Ground Coffee Market in Brazil

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SUMMARY

The present study had the major objective to analyze the implementation process of the coffee quality program (CQP) and its implications on R&G coffee companies in Brazil. First, philosophy and objectives of the CQP were described by the construction of a theoretical analysis model, the 3 "Quality Pillars", with support of transactional costs economics (TCE) and concepts of quality theory. It was analyzed raw material supply of 13 coffee roasters, their productive process and marketing strategies. The research data were analyzed with support of the 3 "quality pillars" model: product quality, process quality and the quality signal, represented by CQP quality labels that communicate quality to consumers. Of 13 ABIC's associated companies researched, 7 are CQP adopters. As main results, it was possible to imply that quality was the main factor that increased vertical coordination between researched companies. Therefore, CQP implementation leads companies to adopt vertical coordination strategies with its suppliers and deliverers. On the other hand, all researched companies have difficulty in maintaining their products quality standards. Non-adopters have high difficulty to implement traceability in their production process, and it is possible to imply that traceability imposed by certification process is a competitive advantage for some adopters. Roasters researched that focus their business on product differentiation by quality attributes need as support a certification label or a strong brand to transmit credibility to consumers. It is clear that CQP has potential to become a major coordination tool in coffee agribusiness, placing ABIC as an important coordination agent.

INTRODUCTION

There are many examples of marketing segmentation by quality attributes. Such growth is due to several factors, among them increased competitiveness and consumer markets saturation, a great concern with social and environmental issues, markets deregulation, and particularly chain agents perception that their goals should be targeted to meet final consumers demands.

The Brazilian roasted and ground coffee (R&G) market is a typical example. After a long period of government regulation, domestic consumption had declined to lowest levels in history, because policy favored productivity and not quality of coffee produced. What was offered in domestic market was low quality coffee, if not tampered with impurities, such as shells and sticks, corn and barley. Consumers started to identify coffee as "all the same" and worse, as low quality coffee.

However, actions directed to quality began to change this scenario. In internal market, in the beginning of the 90's, Brazilian Coffee Roasters Association (ABIC) implemented the "Purity

seal", aiming to standardize roaster's coffee production and prevent fraud. Consumer's answer came with an increase in per capita consumption of coffee in Brazil in the 90's (Saes, 1998).

On the other hand, consumers in developed countries began to demand certified quality coffees, either by means of production processes (like organic) or by final product quality (like *gourmets*). The growing search for certified quality made coffee production expand to meet demands of different consumers' segments, with Brazil also included.

In 2004, ABIC decided to push coffee market once again by starting the implementation of the Coffee Quality Program, or CQP. In fact, the great challenge for such a quality program is to teach Brazilians different issues that distinguish a regular coffee from a high quality one.

From consumers' point of view, quality is a relative concept, it depends on their needs, interests and desires. Moreover, a product reaches quality when it meets product and/or process standards. The roaster with ABIC's CQP must attend a series of basic requirements. The three CQP pillars, in ABIC's vision are product quality, taste profile maintenance and good process practices, that involves quality in all ways.

The major objective of this study was to investigate implementation process of CQP and its implications on R&G coffee companies in Brazil. It was searched a way to describe philosophy and goals that support CQP concept through the construction of a theoretical analysis model, the "quality pillars", with Transaction Costs Economics (TCE) and quality theory support. To investigate how CQP goals were interpreted by roasters, effective actions taken were considered as well as difficulties of program implementation, using as support "quality pillars" model.

THEORETICAL FRAMEWORK

Standardization and certification within Transaction Costs Economics (TCE) perspective

TCE was systematized by Williamson (1985, 1991), but its origin dates back to 30's when Coase (1937) showed a new concept - the transaction costs. The transaction cost is the cost of making the economic system work. These are costs associated with economic activities' coordination, such as *ex ante* costs to acquire market information and to do a business deal, and *ex-post* costs, which are associated with monitoring and contracts execution enforcement (AZEVEDO, 1997; FARINA, 1997, 2000).

According to TCE, contracts are drafted under two behavioral assumptions: people have bounded rationality and can act opportunistically. As transactions differ from each other, Williamson (1985, 1991) used objective and observable elements to characterize them: the transaction specific investments, transactions frequency and uncertainty. Under associating behavioral assumptions with those three elements that characterize transactions, it is possible to identify some transactions that may be more vulnerable to opportunistic actions by one or more parties involved and their respective costs to other parts. Specific assets is the transaction attribute that weighs more in transaction costs. So, depending on transaction attributes, many costs can be incurred, which requires a different organizational structure to control them (Azevedo, 1997).

The most efficient coordination structure for each type of transaction is the one able to minimize transaction costs, ranging from market structures and vertical structures, although

hybrid forms are more common. Briefly, an important point for this work is how economic actors will deal with information asymmetry that can lead to opportunistic actions in their business transactions of buying and selling inputs and products throughout supply chain.

In this context, standards and certification appear as important coordination tools in supply chain. They communicate information to customers and consumers in a consistent and reliable way, reducing transaction costs in buyer vs. seller's relation since they eliminate and reduce quality uncertainty and create incentives for horizontal and vertical cooperation between firms (Farina, 2003; Machado, 2000; Nassar, 2003). In order to a certification to become effective, there must be cooperation and coordination between chain agents as well as incentives for staff members to integrate themselves into a certification system.

Thus, in this study it is assumed that CQP acting as a certification tool has the ability to play an important role: reduce information asymmetry between players of the coffee chain. Therefore, it could gather those agents and reduce uncertainty about certified R&G coffee quality, so that consumers have a reliable source of information to make a better buying decision.

For Machado (2000), certification is the institutionalization of standardization, because it represents the formal guarantee of systematically establish and give reputation to standards. She also points out that a key issue is the credibility of a certification process.

According to Machado (2000), to meet segmented markets, behind instruments such as labels, certificates and trademarks, firms depend on a coordination that is based not simply on price to supply raw materials or even to distribute clients' products. In agribusiness, vertical coordination occurs most commonly through intermediary organizational structures between market and hierarchy, through cooperative actions such as strategic partnerships and formal and informal contracts with few participants, all strategies in balance with competition (Machado, 2000).

Based on TCE, it is expected that the CQP certification implies on:

- Rosters should increase coordination with its suppliers, seeking to purchase raw material of superior quality, and also with a minimum quality requirement;
- Roasters can use strategies such as paying awards for quality, partnership investments or guarantees of green coffee purchase from some coffee growers. Another possible strategy is to make a backward vertical integration, i.e. roasters could acquire farms and aim to control the supply of those quality beans;
- To deliver their superior quality coffees, companies could adopt strategies in partnership with retailers to achieve consumers with higher purchasing power that could pay those coffees higher price.

Quality

Quality is a relative concept. According to Reeves & Bednar (1994), quoted by Maximiano (2000), none quality definition can be considered as the best in all situations, because each definition has both strengths and weakness, depending on the measuring criteria and generalization, management utility and importance to the consumer. Table 1 shows some quality definitions:

Table 1. Quality definitions.

EXCELLENCE	• Quality is excellence means the best that can be done, the higher performance standard in any activity field.
VALUE	• Quality means having more attributes, usage of rare materials or services more expensive. • Quality and value are relative concepts that depend on customer's purchase power.
SPECIFICATIONS	• Planned quality; product design; definition of what the product or service should be.
COMPLIANCE	• Product or service in accordance with project's specifications.
REGULARITY	• Uniformity; identical goods or services.
FITNESS FOR USE	• Quality of a design: excellent design and product/service in accordance with the project that fits a customer's defined purpose.

Source: Reeves and Bednar (1994), quoted by Maximiano (2000, p. 185).

There is a need to identify the quality vision that CQP pretends to communicate to other agents in supply-chain, i.e. what is quality in accordance with CQP parameters.

Slack et al. (1996, p. 552) proposes a definition that summarizes many quality approaches: *"Quality is consistent conformance to customers' expectations"*, the word *conformance* indicates that there is a need to meet a clear specification, ensuring that a product or service complies with specifications originally set. *Consistent* means that materials, facilities and processes have been designed and controlled to ensure that product or service meets specifications, using a set of measurable characteristics throughout time. *Customer's expectations* recognize that a product or service must satisfy customers and that they might be influenced by product's price (Slack et al., 1996).

The authors also emphasize that expectations of individual costumers differ. Thus, companies seek reconciliation between costumer's expectations and costumer's perceptions on quality. So, it is possible to introduce the "perceived quality" attribute that can be defined as the suitable degree between costumer's expectations and perceptions about a product or service.

On the other hand, Machado (2000) says that a brand reduces transaction costs helping to identify products and ensure quality pattern, regardless where the acquisition took place. Private brands and collective brands, coupled with origin specifications and production, are able to acquire a value recognized by consumers.

"Consumers perceive quality information contained in a product label. Behind these more visible elements, producers must be able to produce in accordance with a given standard and obtain a certification from third part as guarantee "(Machado, 2000, p. 106).

The "iceberg effect" is an analogy proposed by Machado (2000), in which a visible quality sign for consumer is made of several signalizing elements of the product quality. The non visible part of the iceberg, under sea level, represents costs that a company and/or chain's agents must assume responsibility with.

THE COFFEE QUALITY PROGRAM (CQP) AND ITS QUALITY PILLARS

ABIC is being an important agent in national coffee agri-system since early 90's, when it took actions to increase R&G coffee consumption in Brazil. At that time, one of its main strategies was the launching of the "Purity Seal" program, whose goal was to curb fraud and ensure

product purity, thus seeking to change Brazilian consumer's vision about coffee quality (Mário et al, 2001; Saes, 1998).

Thus, the Coffee Quality Program is an evolution of ABIC's purity program and aims to give domestic coffee consumption a continuous growth, fetched from 90's. For this reason, ABIC bets on increasing quality offered, showing consumers quality differences of R&G coffee using a label, and by doing this, creating a product segmentation and new consumption patterns (ABIC, 2006).

Through CQP one intends to ensure R&G coffee quality improvement through three major points: first is the use of coffee beans equal or better than type 8 (Brazilian Official Rank - COB), with strict limit to 20% of defective beans - PVA: black (P), green (V), soar (A) - and certified product purity. Another important point is the control of coffee characteristics maintenance along time. This information must be indicated by the roaster in the coffee "flavor profile". Product samples must be collected annually to ensure the quality standard.

In addition to ensure a minimum quality standard through a "global note" attributed to coffee, CQP raises a possibility of product segmentation through quality attributes according to cup characteristics. Thus, depending on the analysis outcome, obtained through sensory tests conducted in certified laboratories, the coffee acquires different labels, or quality seals: traditional, superior or gourmet. Finally, roasters must have a good process practices guarantee. Those roaster's qualities must be certified by third parts laboratories audits.

As CQP results, ABIC aims quality in all fronts, from certifying bodies' data collection, it intends to benchmark, so this may help rise Brazilian R&G coffee quality standard. In general, CQP aims to satisfy Brazilian coffee consumers, teaching them to demand proven quality coffee, thus enhancing their product requirements (ABIC, 2006).

METHODOLOGY

During this research period, from 2006 and early 2007, Coffee Quality Program was in its implementation stage. Therefore, the best approach to achieve this work objective was to use an exploratory qualitative research (Trivinos, 1987). The study objects are companies from the R&G coffee industry in Brazil associated with ABIC.

Relevant program data were surveyed by document analysis, *internet* and in interviews with ABIC's directors in charge of CQP development and implementation. Also, researched companies' staff and directors were interviewed. The collection method was the *focused-interview* (Alencar and Gomes, 1998). In search for a connection between these work whole theories, it is proposed a theoretical analysis model to the problem studied, as in Figure 1.

The three CQP pillars are the model basis: 1 - The first pillar is "product quality"; 2 - The second pillar is "process quality"; 3 - The third pillar is the "quality signal," which means coffee has its characteristics determined by certification maintained throughout time, represented in reality by the CQP label stamped on roasted and ground coffee packages (in brown, silver and gold) and the "taste profile" label (a clover, indicating various cup attributes).

The quality signal establishes a link between the three quality pillars and the "iceberg effect", proposed by Machado (2000). According to CQP basis, the "quality signal" and the "taste profile" goals are exactly to reach consumers and transmit product information. However,

behind these visible symbols, roasters must have the capacity of producing a R&G coffee in accordance with patterns and must obtain a certification from a third part certifier.

Giving support to the other two pillars (product quality and process quality), essential for the third's existence, we use the six quality definitions summarized in Table 1 by Maximiano (2000).

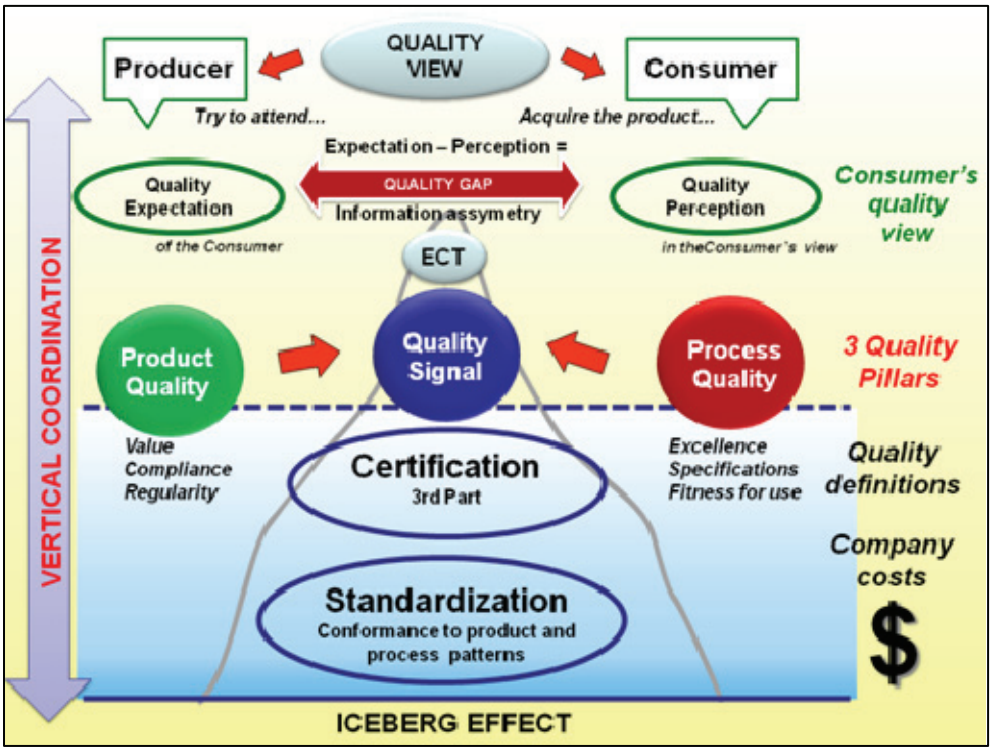

Figure 1. The three CQP quality pillars, the "iceberg effect" and the quality vision. Source: Compiled by the author, based on Machado (2000), ABIC (2006), Maximiano (2000) and Slack (1996).

In the first pillar - "product quality":

- Value: roasters use of top quality green coffee means higher acquisition costs. Compliance: R&G coffee must achieve certain specifications determined in the initial project. Regularity: products have to be uniform and identical with no quality variation.
- In the second pillar - "process quality":
- Excellence: R&G coffee quality is the best possible, resulting in the highest quality patterns throughout production process. Specifications: ensure the initially planned coffee quality pattern. Fitness for use: excellent design and product/service in accordance with the project that fits a customer's defined demand.

Vertical coordination enters in the model reaffirming that certification through quality attributes requires higher integration between supply chain actors. The last part of the model refers to the end consumer. Accordingly, a company seeks to meet consumer's quality expectations on coffee. On the other hand, when consumers buy a product and use it, they create their own product quality perception. If consumers' quality perception depends on their product expectation, the more information they have about coffee quality attributes, lower will be the gap between their product quality perception and expectation, consequently, a satisfied consumer (Slack et al., 1996).

At this point, we present the final link between our theoretical model and practice. With TCE support, the program objective is therefore to reduce information asymmetry between two players: final coffee consumers and roasters.

RESULTS

Researched roasters characterization

Thirteen coffee roasters were surveyed in the states of Minas Gerais and São Paulo. Their activities focuses were diverse, ranging from international market, gourmet coffees, institutional market, store's own-brand retail, to the traditional coffee market in Brazil. We can conclude that the sample was very representative and heterogeneous.

Table 2 below shows the sample characterization.

Table 2. Sample analysis

	Adopter							Non-adopter					
COMPANIES	**A**	**B**	**D**	**H**	**I**	**K**	**M**	**C**	**E**	**F**	**G**	**J**	**L**
Location: Minas Gerais		X	X	X				X	X		X	X	
Location: São Paulo	X				X	X	X			X			X
Size: Small (S), Medium (M), Large (L)	S	M	L	L	S	L	M	S	S	L	M	L	S
Have CQP since the program began		X	X	X		X							
Adopted CQP lately	X				X		X						
Starting CQP implementation process												X	
Prepared to adopt CQP									X	X	X	X	X
Plans to adequate it's structure in the future								X					
Presence of other certifications	X	X	X		X	X	X					X	X

Source: Research data and ABIC, 2007.

Product quality pillar

The raw material supply is crucial to achieve good quality. Roasters that match coffee processing structures, storage and green coffee sales with its roasting factory, even when they are different companies (independent legal persons), have good raw material supply. This raw material supply in quality and quantity has an explanation: they are located in Brazil's largest coffee producing and marketing centers. This also explains why there were no major changes in the coffee purchasing process by CQP adopters.

Some non-adopters companies have high quality coffees and have in common the fact that they have coffee processing units, storage and exporting companies under the same business group, that work factory independently.

The major point encountered in this quality pillar was the problem of maintaining a specific quality pattern. A CQP requirement is that companies have to maintain quality over time,

offering customers always the same quality standard established by the certification. We can conclude that this point is critical to roaster's strategies.

Quality standard maintenance is no problem for CQP adopters surveyed, mainly due to the fact that they have many supply channels to purchase raw material and are also located in major Brazil's coffee producing regions. Thus, we can conclude that the green coffee acquisition cannot be considered a specific asset according to TCE.

For non-adopters, maintain a specific quality pattern is more critical to justify not joining CQP program, especially because some of them have only a single coffee brand.

It is important to cite a sentence that summarizes the thirteen interviewed roasters vision about product quality: *"As coffee quality improves, total coffee consumed increases"* (Interview of the company F).

Process quality pillar

Traceability was identified as the critical point in this pillar, i.e. surveyed companies, both adopters and non-adopters, had or have difficulty in implementing traceability in their production process. The main reason is that they don't have an efficient system for receiving, purchasing and documenting green coffee entrance in the factory. Moreover, since CQP implementation, all adopters acquired high control in their production process.

The most interesting point refers to earnings with CQP adoption, especially with waste reduction and consequently, costs reduction. Often these gains are not aimed when a company decides to adopt the program. As example of other changes, one company modified its line and wants, time along, to improve quality of its products and intends to launch new products with CQP certification. Another company uses CQP as marketing tool against competitors:

"My product is certified, and yours?" (Company H slogan).

Quality signal pillar

Communicate credible information is one of the CQP goals. Consumers must have ways to identify their coffee's preferences and based on this data make their buying choices. There will not be a financial reward for the roaster if consumers do not recognize its efforts throughout production chain to improve quality.

All adopters have gourmet coffees in their portfolios. This reflects the need to differentiate specialty coffees from others in market through certification usage and demonstrates the importance of transmitting quality with credibility to consumers.
Analyzing adopters marketing strategies, it is possible to imply that quality is part of companies' philosophy and of its entrepreneur's behavior. One of the adopters has quality as its main objective and uses CQP successfully to transmit this philosophy to clients.

Another adopter transforms the constant search for quality in a marketing strategy to also win consumers' loyalty. Other adopters use quality as a key to achieve international market or to place their coffee in the retail shelves of *Pão de Açúcar* specialty coffee line and to be present in the best countries' stores.

Among non-adopters, the quality search is also present in some companies. One company uses quality as a strategy to conquer a large metropolitan region market. The goal is to show

consumer that coffee quality does not always need to be more expensive. Another company seeks to support its perfumer's shop, i.e., the specialty coffee and gourmet line, with institutional coffee production and own-store coffee brands for retail.

Another non-adopter focus is high cuisine, offering high quality coffee in the Brazilian market. According to the interviewee, origin certification is much more important to its marketing strategy than the quality certification offered by CQP. His distribution channel has also a special feature: direct sales to final consumers via internet. By using this strategy, the company reaches a hierarchical governance structure very difficult to be found in agribusiness: a complete vertical integration.

Companies increasingly seek market segmentation as a success key in their business. Thus, we can conclude that CQP helps adopters in defining their marketing segments and the also quality they want to offer their consumers.

CQP implementation process analysis

Given the main objective of this research, it is possible to identify which major points concerning CQP implementation process were, based on all surveyed data arranged in the quality pillars.

First, there is resistance from some companies to adopt CQP in a first moment. This fact can be considered common, since the program is complex and is in its initial implementation step. A reactive behavior also reflects some companies conduct to join the program only when the market effectively demand it, or when consumers and / or retail starts to identify CQP as a must for their purchases.

Other companies relies on strong brands to transmit credibility and quality to their customers, and don't identify CQP certification as a priority. It is a clear case of certification label versus roasters brand.

Product characteristics maintenance over time as shown in the "profile taste", avoiding quality variation has been identified as difficult to be implemented by most companies because, to compete in the low prices market, they must search for low quality / low price green coffee. This will ultimately limit competition capacity from roasters that already have CQP against others that does not have it.

Meanwhile, companies that have already joined the program have some advantages to maintain quality pattern: 1) they have a green coffee trade company combined with its roasting structure or, 2) are located in producing areas and coffee marketing centers.
However, those evidences can be applied not only for CQP adopters, the non-adopters that have high quality patterns in its products or production process also present those strategies.

There are companies that are using hybrid governance structures, with high vertical coordination; consequently, they can minimize transaction costs by reducing uncertainties about coffee quality purchased or produced by its own farms and / or partners.

In the research results it was possible to identify that some respondents recognize that CQP can become a competitive advantage in near future. Particularly considering some ABIC's strategies to boost program implementation, such as partnerships with retailers, like the chain *Pão de Açúcar*, and the requirement of certain public agencies to include CQP quality patterns in public auctions for coffee acquisitions. Since government is a great institutional client, as

this coffee acquisition criteria starts to prevail, finally cup quality will began to be a primary factor for coffee purchasing, taking off the factor price as the only acquisition prerogative.

Beyond these actions, ABIC launched in November 2006 a partnership that clear up its intention to turn CQP a coordination tool in coffee agribusiness. The "Brazil's Sustainable Coffees" is a partnership between ABIC and the Cerrado Coffee growers Associations Council (CACCER), institution in charge of "Café do Cerrado" certification. In 2008, as an evolution of those partnerships, ABIC started to gather other recognized certifications, such as UTZ Certified, Rainforest Alliance, 4C, Fairtrade, Organic, etc. It is interesting to notice that those certificates association reflects consumers' wishes to pay an additional value to products that contain all certification benefits: from social-environment protection with food safety, to products with higher sensorial attributes.

"Top-down" strategies that pull certification demand from retail to industry seem to have higher impact, and perhaps this should be the way to do it, since to reach final consumer in a strong and effective way, it would be necessary a massive marketing investment, something that is out of ABIC's plans nowadays.

CONCLUDING REMARKS

The work achieved its main objective to study CQP implementation process and its implications in R&G coffee companies in Brazil. The "quality pillars" theoretical model was developed as an attempt to gather CQP philosophy and goals with TCE basis and quality theory.

The starting point was the theoretical assumption that quality management focused on R&G coffee quality requires vertical coordination between agents in industry. Thus, to ensure CQP effective implementation, companies need strategies to create better coordination tools with their suppliers and deliverers. The key to increase coordination among economic agents is therefore to establish a quality pattern for roasters, what requires a better coffee quality acquisition, even if they have to pay an additional price for it. Those demands demonstrate the importance of "product quality" pillar for a certification program to succeed.

It was identified that traceability has been another critical point for surveyed roasters. All adopters said they encountered difficulties in implementing and documenting traceability of their production process, and it is the traceability absence that does not allow most of the non-adopters to start certification. The gains adopters identified with standardization and traceability adoption shows "process quality" pillar importance.

Surveyed companies that support their marketing strategies in quality differentiation needed a certificate or a strong brand to transmit credibility to their consumers. This explains why some companies do not want to join CQP program because they already have a strong brand to communicate with consumers and does not need a label to prove their quality to them. It is a clear certification label versus roasters brand conflict. On the other hand, roasters that link their brands with CQP label are in search for a marketing differentiation from competitors that do not have this option. Those strategies confirm the importance of the "quality signal" pillar, namely the importance of using an efficient way to transmit information, searching for an information asymmetry reduction between suppliers and customers.

This work contribution is the CQP theoretical analysis model. It provides support to better comprehend which are the certification process critical points, although we cannot widespread it, since it is a picture that shows researcher's perception about a specific study object.

Overall, there is a clear perception that CQP has potential to become an essential program for Brazilian coffee market in future, it can also acquire a status of a major coordination tool in supply chain, placing ABIC in a highlight position within coffee agribusiness, as an important coordination agent.

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International Standards on Coffee and National Ones

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SUMMARY

"ISO brews a strong cup of coffee". That was the title of an article about standardization in coffee in the ISO bulletin in 1995 (Clarke, 1995). At that time the ISO Coffee Committee had been in action for 19 years, and had issued some 20 international standards on coffee.

REASONS FOR STANDARDIZATION

The foremost aim of international standardization is to facilitate the exchange of goods and services. The International Standards Organization ISO is a legal association, based in Geneva, Switzerland. Its members are the National Standard Bodies of about 150 countries. The principal deliverable of ISO is the International Standard.

Coffee is an important, border-crossing commodity in international trade, and standards help to overcome and avoid barriers of trade, which might be generated by eventual differences in product characterization between the long distance partners of a contract. The reference to an international standard method may serve as a common language for analytical results. Standards provide simplification of contractual affairs. They are required by business, government or society, to be initiated, elaborated, published, and systematically reviewed.

STANDARDIZATION ACTORS

Standardization is carried out by independent standard bodies at national and international level. They are in general non governmental organizations, organized into technical committees and subcommittees. Within ISO's Technical Committee 34 (Food Products), Subcommittee 15(SC 15) deals with coffee. Its members are the coffee bodies of national standardization institutes who send their experts to the meetings.

The subcommittee secretariat has been run by the Brazilian Normalization Organization ABNT, since the beginning; the chair persons are experienced coffee people, including the current one, Marino Petracco from Italy.

ISO COFFEE ACTIVITIES

To develop the set of coffee standards in a consensual way, the members' delegate experts have been convening since 1976 in SC 15 plenary meetings and in working groups, and execute a broad correspondence. This is analogous to the national standardization procedures, with the important difference being an additional, intermediate level for decisions in ISO work, existing in the member bodies. They are asked to answer on enquiries sent out by the ISO subcommittee secretariat, and to vote by ballot on standards. The procedures are laid down in general ISO rules, called directives. The work is supported by the secretariat of TC Food and the ISO Central Secretariat in Geneva, where the finalized standards are issued in

print and electronic format. Internally, there are Intersecretariat meetings of TC Food and meetings of the recently established Chairman Advisory Group of ISO/TC 34.

START IN 1976

The constitutive meeting for the coffee subcommittee was held in New York in 1976, after more than a decade of activity as Working Group 2 in the subcommittee for stimulant foods SC 8 (which now is restricted to tea). The initiative came from AFNOR, the French association for normalization, and was supported by the British standards institute(BSI). Several London meetings were held in the sixties, as mentioned by R.J. Clarke, long-term chairman of ISO Coffee, in the article cited above. The last one before founding the ISO coffee subcommittee took place in Berlin 1975 at the German DIN, where the DIN coffee committee had been established 3 years previously. The mother committee, ISO/TC 34, Food Products, dates back to 1947.

THE MEETINGS

Since 1976, 17 coffee subcommittee's plenary meetings have been held in different places around the world (data from subcommittee documents circulated by the secretariat). They are listed in Table 1. At least five permanent members must attend a plenary meeting. Working group sessions are less formal.

Table 1. Places and dates of ISO coffee plenary meetings.

1. New York, USA, 7-9 June 1976	10. London, UK, 24-26 April 1991
2. Abidjan, Ivory Coast, 5-8 Dec. 1977	11. Denpasar, Indonesia, 26-28 July 1993
3. Caracas, Venezuela, 19-23 Febr. 1979	12. London, UK, 20-21 May 1996
4. London, UK, 23-25 June 1980	13. Paris, France, 14-15 Dec.1999
5. Nairobi, Kenya, December 1981	14. Rio de Janeiro, Brazil, 26-27 July 2001
6. Arusha, Tanzania, 12-15 Sept.1983	15. Habana, Cuba, 20-21 May 2003
7. Balatonfüred, Hungary, May 1985	16. Hamburg, Germany, 27-28 March 2006
8. Habana, Cuba, 16-18 Febr. 1987	17. Bogotá, Colombia, 6-8 June 2007
9. Antalya, Turky, 6-8 April 1988	18. to come

MEMBERS SINCE 1976

The member bodies are either P- or O-members, either participating actively in the work, or following the work as an observer. The first (P) group has an obligation to comment and to vote on enquiries, draft standards, and reviews presented by the secretary, and to participate in meetings. The observer (O) group receives the committee documents and has the right to submit comments and attend meetings.

Over the years, the number of subcommittee members has increased from 30 to some 55 in total. Table 2 (http://www.iso.org/iso/standards_development/technical_committees/list_of_iso_technical_committees/iso_technical_committee_participation.htm?commid=47950) indicates status changes of members since 1976. The percentage of members of producing countries is about 40%. Most mayor players in production and consumption are participating, for example Vietnam since 1995.

For ISO standards development to progress, broad participation in the subcommittee work is essential. It is realized by the member bodies and their delegate experts, who refer and discuss the items in both the national and international committees, and who need the input from their colleagues at home for the various topics covered.

The members' coffee committees, DIN coffee for example, have a balanced composition of the interested parties, with representatives of companies and academia, of official food surveillance and private analytical labs, and of consumer and coffee associations; there are about 20 coffee experts, and the secretariat is run by a specialist from DIN.

Table 2. Member bodies of ISO TC 34 subcommittee 15, Coffee, since 1976.

Member .....status	Cyprus (CYS) .....O	Jamaica (BSJ) .....O	Serbia (ISS) .....O
Algeria (INAPI) . O/-	Czechia (CNI) .....O	Japan (JISC) . O/P/O	Slovakia (SUTN) ..O
Argentina (IRAM) O	Denmark (DS)....O/-	Kenya (KEBS)	Spain (AENOR) ....O
Armenia (SARM) .O	Ecuador (INEN) P/O	..... O/P/O/P	Sri Lanka (SLSI) .. P
Australia (SAA) . O/-	Egypt (EOS) .....P	Korea, Rep. of	Switzerland (SNV) P
Belgium (NBN) P/O	Estonia (EVS) .....O	(KATS) .....O	Tanzania (TBS) P/O
Brazil (ABNT) ..... P	Ethiopia (QSAE) P/O	Libya (LNCSM) ...O	Thailand (TISI) . P/O
Bulgaria (BDS) .....O	Finland (SFS) .....O/-	Madagascar (BNM)P/-	Trinidad & Tobago
Canada (SCC) .....P/-	France (AFNOR) ...P	Mexico (DGN) .....O	(TTBS) .....O
Chile (INN) .....O	Germany (DIN) .....P	Netherlands (NEN) P	Tunisia (INNORPI)
China (SAC) .....O	Hong Kong	Norway (NSF) ... O/-	..... P/O
Colombia (ICONTEC)	(ITCHKSAR) .....O	Pakistan (PSQCA)	Turkey (TSE) .... P/O
..... P	Hungary (MSZT) .O	..... P/O	United Kingdom (BSI)
Costa Rica (INTECO)	India (BIS) .....P	Peru (INDECOPI) P.	..... P
Côte d'Ivoire	Indonesia (BSN) ....P	Philippines (BPS) . P	USA (ANSI) ..P/O/P
(CODINORM) . P/O	Iran (ISIRI) .....O	Poland (PKN) ... P/O	Venezuela
Croatia (HZN) .....O	Israel (SII) .....O	Portugal (IPQ) .. P/O	(FONDONORMA)
Cuba (NC) ..... O/P	Italy (UNI) .....O	Romania (ASRO) P/O	..... P/O
		Russia (GOST R) . P	Viet Nam (TCVN) P
		Saudi Arabia (SASO) .	Yugoslavia (SZS)
			.....P/O/-

TOPICS OF ISO COFFEE

Table 3: Topics covered by ISO TC 34 and SC 15.

Structure	Topics covered								
	Terminology Nomenclature	Sampling and sample preparation	Methods of test and analysis	Product specifications	Packaging	Storage	Transportation	Management system	Material
TC 34		**X**	**X**	**X**				**X**	
SC15	**X**	**X**	**X**	**X**		**X**	**X**		

The TC 34 business plan (Anon. http://isotc.iso.org/livelink/livelink?func=doc. Fetch&nodeid = 999731) provides the scope of activities of the TC itself and its subcommittees.

As can be seen in table 3, a product-oriented subcommittee like SC 15 has its specific agenda: - For the promotion of a basic terminology and nomenclature, broad experience and knowledge is necessary. - The topic "Sampling Procedure" also needs experience, together with statistical background. - To prepare an analytical standard - the most frequent demand - participants and contributors to ring tests have to be recruited. While the older procedures

were done via weighing, drying, sieving, and visual control, the later methods are using more complex instrumental analysis. - The “Management system” topic is dealt with on TC level. Figure 1 shows the chronological sequence of topics covered by the standards of SC 15.

Starting with the fundamentals, the first standard issued by SC 15, ISO 3509, was on basic terminology and nomenclature. This vocabulary was ready for immediate use, because it had been discussed previously. It is referenced in almost all other standards for coffee. The importance can clearly be seen on the multiple follow-up editions the standard had. Now in 2008, edition N°5 is being prepared.

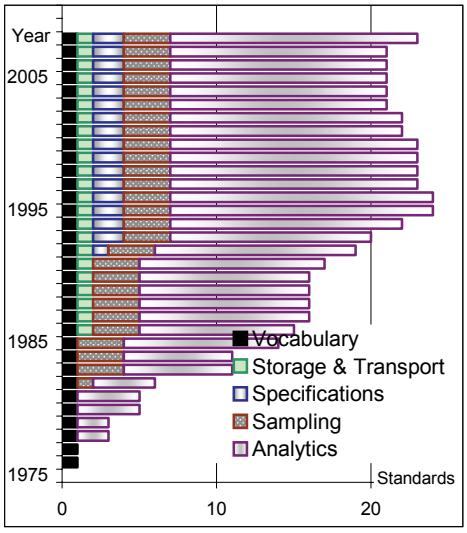

Figure 1. Chronology of the topics of ISO coffee, issue year vs. number of standards.

ISSUE OF ISO COFFEE STANDARDS SINCE 1976

The following table lists all ISO standards on coffee since 1976 in numerical order (http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=47950&published=on).

The initial vocabulary given in ISO 3509, the next standards issued were analytical ones, ISO 1446 and 1447, dealing with green coffee moisture determination. The low standard numbers reveal, that their registration was even earlier than for 3509; they were adopted from AFNOR standards in 1970 and appeared in the SC 15 list with their first review in 1978. ISO 1446 is still active, in its second edition, whereas 1447, a two stage drying procedure, was withdrawn in 2000.

In 1980, green coffee olfactory and visual examination, ISO 4149, and green coffee sieving, 4150, were issued, and followed by the first sampling procedure for green coffee in bags, 4072:1982. The respective sampling instrument was described in ISO 6666:1983, coffee triers.

Table 4. ISO standards on coffee since 1976 and actual status in September 2008.

ISO standard	Title	Status in 2008
ISO 1446:2001	Green coffee -- Determination of water content -- Basic reference method, Ed.2	International Standard confirmed 2006, NP amendment started
~~ISO 1447:1978~~	Green coffee -- Determination of moisture content (Routine method)	Withdrawn 2000
ISO 3509:2005	Coffee and coffee products – Vocabulary, Ed.4	International Standard to be revised, 2006
ISO 3726:1983	Instant coffee -- Determination of loss in mass at 70 degrees C under reduced pressure, Ed.1	International Standard confirmed 2006
ISO 4052:1983	Coffee -- Determination of caffeine content (Reference method), Ed.1	Close of review 2008
ISO 4072:1982	Green coffee in bags – Sampling, Ed.1	International Standard confirmed 2006
ISO 4149:2005	Green coffee -- Olfactory and visual examination and determination of foreign matter and defects, Ed.2	Close of review 2008
ISO 4150:1991	Green coffee -- Size analysis -- Manual sieving, Ed.2	Under revision; WD study initiated 2008
ISO 6666:1983	Coffee triers, Ed.1	Under revision; WD study initiated 2008
ISO 6667:1985	Green coffee -- Determination of proportion of insect-damaged beans, Ed.1	International Standard confirmed 2005
ISO 6668:2008	Green coffee -- Preparation of samples for use in sensory analysis, Ed. 2	International Standard revised 2008
ISO 6669:1995	Green and roasted coffee -- Determination of free-flow bulk density of whole beans (Routine method), Ed.1	International Standard confirmed 2005
ISO 6670:2002	Instant coffee -- Sampling method for bulk units with liners, Ed.2	Close of review, 2007
ISO 6673:2003	Green coffee -- Determination of loss in mass at 105 degrees C, Ed. 2	Under periodical review, 2008
~~ISO 7532:1985~~	Instant coffee -- Size analysis	Withdrawn, 2002
~~ISO 7534:1985~~	Instant coffee -- Determination of insoluble matter content	Withdrawn, 1996
ISO 8455:1986	Green coffee in bags -- Guidance on storage and transport, Ed.1	Under revision, WD study initiated 2008
ISO 8460:1987	Instant coffee -- Determination of free-flow and compacted bulk densities, Ed.1	International Standard confirmed 2003
ISO 9116:2004	Green coffee -- Guidelines on methods of specification, Ed.2	Close of review, 2007
ISO 10095:1992	Coffee -- Determination of caffeine content -- Method using high-performance liquid chromatography, Ed.1	International Standard confirmed 2005
ISO 10470:2004	Green coffee -- Defect reference chart, Ed.2	Close of review, 2007
ISO 11292:1995	Instant coffee -- Determination of free and total carbohydrate contents -- Method using high-performance anion-exchange chromatography, Ed.1	International Standard confirmed 2005
ISO 11294:1994	Roasted ground coffee -- Determination of moisture content -- Method by determination of loss in mass at 103 degrees C (Routine method), Ed.1	International Standard confirmed 2005
ISO 11817:1994	Roasted ground coffee -- Determination of moisture content -- Karl Fischer method (Reference method), Ed.1	International Standard confirmed 2005
ISO 20481:2008	Coffee and coffee products -- Determination of the caffeine content using high performance liquid chromatography (HPLC) – (Reference method), Ed.1	International Standard published 2008
ISO 20938:2008	Instant coffee -- Determination of moisture content -- Karl Fischer method (Reference method)	International Standard in print, 2008

Soluble coffee appeared 1983 with a special sampling procedure, ISO 6670, and the soluble coffee moisture determination, ISO 3726. For the latter, the lower number indicates, that it had been registered much earlier, and it took its time to be issued. The method had been presented at the 1969 ASIC Colloquium (Haevecker, 1970), with a relatively small database; when introduced to ISO, it was validated within SC 15 by its own collaborative test. The resulting ISO standard has remained unchanged since then. That same year, ISO 4052 was issued, caffeine determination for green, roast and soluble coffee. It also remained unchanged to date.

Product specification standards for green coffee are given in ISO 9116, "Green coffee - Guidelines on methods of specification", and ISO 10470, "Green coffee - Defect reference chart". The latter is very important as an internationally accepted classification of defects. In its second edition, it focused the defects' quality impacts, with a coloured picture annex, helping to identify them visually. In 1994, two standards for roast coffee were issued, followed by ISO 11292 a year later which is a challenging standard on soluble coffee's proof of authenticity.

After a period of consolidation and thorough revisions, the latest standards came in 2008. They concern a new caffeine determination for all coffee types and a moisture determination for instant coffee.

ELABORATION AND USE

Standardization starts with an identified problem, an economic or ecologic demand or a legal request, and then discussion on its relevance, followed by the preparation of science based solution plans and the discussion of their feasibility. Finally, there is an outline of a draft standard and enquiries again. A draft is to be prepared, an eventual ring test, evaluation of the test results and finalization of the draft standard. All this is done by the parties concerned and their experts, and completed in the respective standard institutions, at the national level or after introduction to ISO internationally.

Following these procedures of standardization, the resulting standard is a consensus based product; the state of the art is retained by regular reviews.

Compliance with ISO Standards is voluntary. The fact that they are developed in response to market demand, and are based on consensus among the interested parties, ensures widespread applicability and a competitive advantage for the user.

National standard collections may differ from each other, according to the national traditions and demands.

ALLOCATION OF STANDARDS TO THE TYPES OF COFFEE

The breakdown of international ISO standards on coffee according to the different coffee types over the years, is depicted in Figure 2, left side. The parallel for the national level, in this case DIN Germany, a consumer country, is shown at the right. Standards applying to different types of coffee are counted partially for each.

From the beginning, the overall distribution pattern of ISO standards shows many standards for green coffee. There are fewer for soluble, the fewest for roast coffee. This reflects the respective roles in international trade. Eventually in 2008, soluble coffee mixes became part of an ISO caffeine standard.

On the other hand, in a consumer country like Germany, the national market's demand is more oriented to the final products, roast and soluble coffee, which can be seen in the coffee national standards allocation.

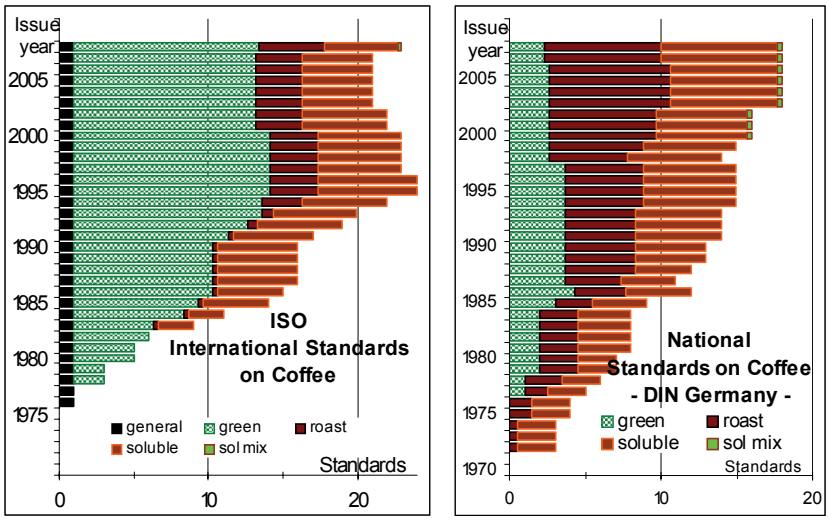

Figure 2. Allocation of standards on coffee to the coffee types at international level (ISO), and at national level in a consumer country (DIN Germany).

PROPAGATION OF STANDARDS

ISO standards are used world-wide, either as such, or officially adopted and translated by national standard organizations, irrespective of their membership in ISO Coffee.

An evaluation of the 40 separate national repertories for coffee in Europe with about 500 standards shows, that ISO standards are widely dispersed, with an individual adoption rate of the standards up to 45 %. In the ranking, the transfers are less from the green coffee side, but rather on the final products for the consumers. The most frequently adopted standards are those on mass loss of roast coffee, caffeine determination, mass loss in instant coffee and the general vocabulary. Withdrawing of a standard at ISO level is not always followed by the national one.

Conversely, national standards might also become part of the ISO repertory, when officially introduced and accepted in the ISO coffee committee. It is a matter of philosophy, whether the national standard bodies afterwards re-adopt this ISO standard, withdrawing their original one.

ISO coffee standards are cited as source in some newer methods of AOAC (1920), the well recognized American analytical collection, and vice versa. In Germany, it is the DIN standards that go down in the Official collection of analytical methods for food (1981).

Standards elaborated by the European community's normalization committee, CEN, have to be implemented as an identical national standard by CEN members. For coffee, it is the determination of Ochratoxin A in barley and roasted coffee, EN 14232 of 2003, adopted in 85% of European standard repertories, even more than CEN members.

THE GOVERNMENTAL VIEW

Food safety and consumer protection are aims of legislation, and the respective analytical standards are of concern for the official food surveillance authorities.

In 1979, the European Community, in order to harmonize national legislations, issued a directive with special Community methods for testing of soluble coffee comparable to the later ISO 3726 and 4052, and to a DIN standard for dry matter of liquid extracts. This was based on the vertical product directive on coffee extracts of 1977, in which limits were set for the dry matter content of extracts and the caffeine content of the decaffeinated coffee, the methods of analysis to be determined by the commission.

Since then, the legislative philosophy had changed: fixed official Community analysis methods were no longer maintained, but analytical performance needs to be assured. In 2001, the directive of 1979 was repealed with the explicit obligation to the member states that "methods used (should) be validated as often as possible, in particular taking account of standardization within the ISO" - an official recognition to the standardization work done at ISO.

A more global governmental activity on standardization has been carried out since 1963 by the Codex Alimentarius Commission of the UN organizations FAO and WHO, consisting of member nations' government representatives. Codex work is food oriented, in scope similar to ISO, further emphasizing consumer health and fair trade practices (homepage of Codex Alimentarius). In 1970, the question of an eventual Codex Committee for Coffee and Coffee Products for standardization came up, at about the same time when the first actions on coffee within ISO TC 34 took place. Based on a comprehensive study of the French delegation and ASIC (Alinorm 74/29), after controversial discussions, the final decision of 1991 (Anon. http://www.fao.org/docrep/meeting/005/t0490e/T0490E02.htm#ch281) was to leave the standardization on coffee to ISO "considering the existing work carried out by ISO in this area, it was not considered appropriate for the Commission to duplicate or repeat this work. The International Organization for Standardization (ISO) was invited to keep the (Codex-) Secretariat informed". That was a remarkable recognition for the ISO work on coffee. Codex kept the door open "under the new horizontal approach, (to) consider some aspects of standards applying to coffee from the broader perspective".

By referring to standards, legislation is more flexible in adapting to technical advances.

IN CONCLUSION

Standardization on coffee in ISO is a continuous process, well structured and efficient, accounting the needs and requirements of the market an the society, and run in consensus of the interested parties. The resulting international standards provide a common technical language for trade partners throughout the world, at the actual state of the art. As with national standards, the compliance to ISO standards is voluntary. ISO standards are internationally recognized and beneficial for the users.

To help for further progress, the coffee people should feel encouraged to participate in the standardization work, and for their own benefit, to use the standards.

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Homepage of Codex Alimentarius, http://www.codexalimentarius.net/web/index_en.jsp# (access Sept. 2008).

Official Methods of Analysis of AOAC INTERNATIONAL*,* Gaithersburg, MD, USA, with about 30 methods on coffee since 1920.

On-Line Roasted Coffee Quality Control Using NIR Spectroscopy

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SUMMARY

Roasting is a key stage in the production of high quality coffee and this step must be controlled and reproducible to meet consumer expectations. The coffee organoleptic and physico-chemical characteristics have to be evaluated close to the process to assure fine monitoring of the roaster conditions. In 2006, a study was done in collaboration with Kraft Foods to evaluate the feasibility of using Near Infrared Spectroscopy (NIRS) to control coffee quality at discharge from the roasters. The study focused on moisture content determination using a NirOnline Xtwo spectrometer (Nir-Online, Walldorf, Germany). For this study, 284 samples of Arabica and Robusta coffees were roasted under industrial conditions and at the laboratory, and then remoistened to obtain a range of moisture content from 1% to 6%. Coffee beans were analysed in continuous mode using the Xtwo spectrometer and in a static mode using a FOSS 6500 laboratory spectrometer (FOSS, Port Matilda, USA). Calibrations were developed using partial least squares regression to predict moisture content of the coffee batches for each instrument. The performances in terms of accuracy and robustness of each calibration for continuous mode and for static mode were compared. The standard errors of prediction (SEP) of each calibration were similar, 0.18% for the FOSS spectrometer and 0.19% for the NIR-Online spectrometer. These levels of accuracy were very close to those obtained with the FOSS instrument for ground roasted coffee and to the standard error of laboratory reference method (SEL = 0.1%). This study confirms the possibility of at-line monitoring of the roasting step using near infrared spectroscopy. The next step will be the implementation of NIRS technology in the coffee roasting chain to automate and control the roaster parameters.

INTRODUCTION

This study, carried out in 2006 in collaboration with Kraft Food (Lavérune, France) focused on continuous and rapid (about 30 ms) determination of moisture content using a NIR spectrometer (model Xtwo, Nir-Online, Walldorf, Germany) in order to monitor the roasting process. Predictive models using partial least squares regression (PLSR) were developed that gave accurate estimations (± 0.4%) of final roasted coffee moisture content.

EXPERIMENTAL PROCEDURE

For this study, 284 samples of Arabica and Robusta coffees from different geographical origins, were roasted under industrial conditions (continuously and in batches) and at the laboratory using a Probat PRE 1Z (Belgium) roaster (slow roasting) and a Neuhaus Neotec RFB (Germany) roaster (fluidized-bed fast roasting). Samples were remoistened in a controlled cabinet (FIRLABO, SP-BVEHF, Meysieu, France) at 60% RH and 28 °C, to obtain a range of moisture content from 1 % to 6 %. Coffee beans were analysed in continuous mode using the Xtwo spectrometer and in a static mode using a Foss 6500 (FOSS, Port Matilda, USA) laboratory spectrometer.

A validation set of 35 samples: 22 with final roasting moisture content and 13 remoistened samples in order to cover the total range, was analysed on both instruments. Moisture content was determined by gravimetry at 103 °C for 16 hours using a Chopin oven.

Near Infrared Spectroscopy

X-Two is a compact and rugged NIR spectrophotometer unit equipped with automatic internal referencing and a dual light source. Diode array detectors are used to provide simultaneously measured data across the complete spectral range (950 nm to 1750 nm) for one kilogram coffee bean samples. The Foss 6500 spectrometer is a grating-monochromator type and was equipped with a transport module and rectangular cell. Diffuse reflectance spectra (400 nm to 2500 nm) were recorded using about 150 g of coffee beans. The spectra were mathematically transformed using WINISI v.1.5 software (Infrasoft International, Port Matilda, USA): the second derivatives of standard normal variate and detrend corrected and smoothed spectra were computed.

Moisture calibration was performed using partial least squares regression (PLSR). Calibration statistics used to evaluate model performance included standard error of calibration (SEC), coefficient of determination (R^2), standard error of cross-validation (SECV) and standard error of prediction (SEP). The RPD ratio (RPD=SD/SEP) was also used to evaluate the performance of each equation. The Student test (t) was used to identify t-outlier samples.

RESULTS

The 284 calibration samples analyzed had an average moisture content of 3.99% with a maximum of 6.00% and a minimum of 1.95%. The average moisture content for the 35 validation samples was 4.36%, with a distribution covering the whole range of moisture content, but concentrated in the range 3.5-6% (Figure 1).

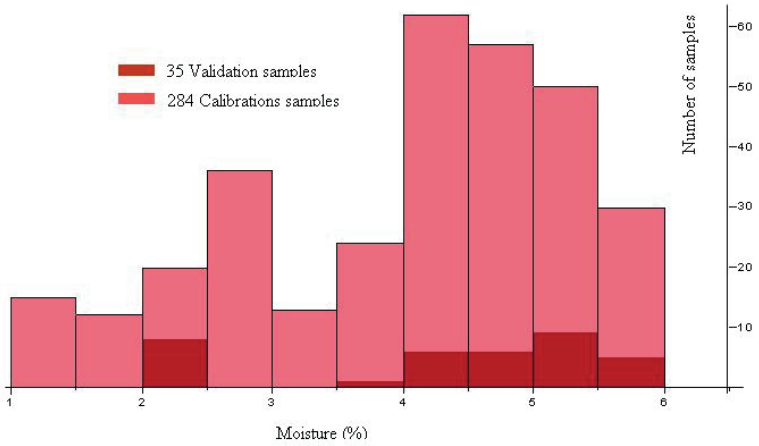

Figure 1. Moisture content distribution for calibration and validation samples.

The average NIR spectra for the NIR-online and Foss instruments (Figure 2) showed similar profiles, with a slight shift in absorbance band peaks, almost certainly due to differences in operating software wavelength codifications. These plots allowed the 3 principal absorption bands due to H-OH vibrations (1100 nm, 1340 nm and 1900 nm) to be identified. Because of its reduced spectral range (950 nm -1750 nm) the higher band (1900 nm, first overtone of H-OH) was not accessible to the NIR-Online instrument.

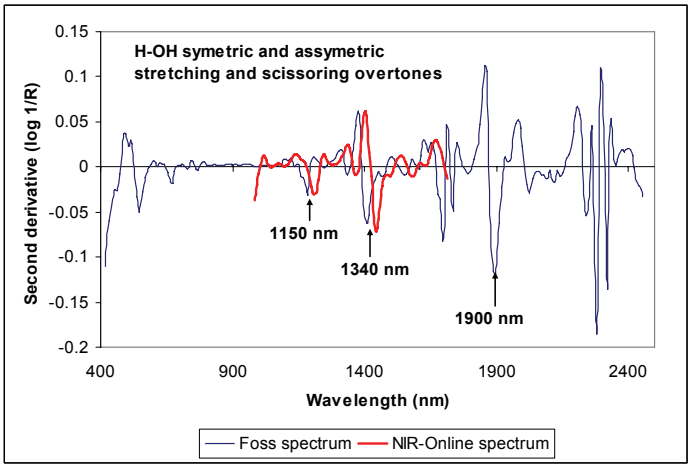

Figure 2. Average spectra of roasted beans measured on the NIR-Online and FOSS spectrometers.

The plot of the standard deviation of absorbance value calculated for each wavelength for NIR-Online spectra showed a maximum value at 1340 nm, corresponding to the first H-OH overtone absorption band (Figure 3). This high SD was caused by wide variation in the spectral fingerprint due to water.

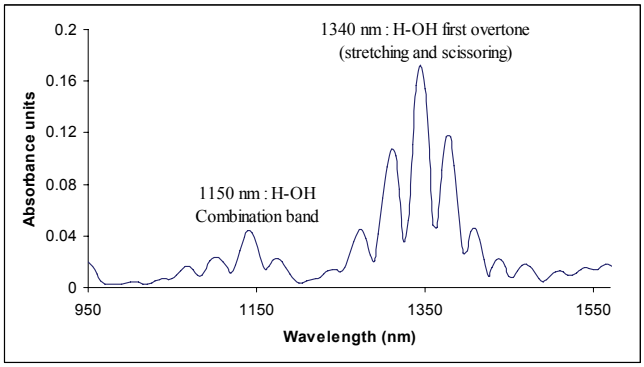

Figure 3. Standard deviations of absorbance values.

Calibration

The performances for moisture content calibration equations (Table 1) developed on each instrument were very close and highly satisfactory. Both instruments gave a RPD higher than 7, which means that predictive models can be used as routine analyses for an accurate moisture content determination.

Table 1. Performance statistics for coffee bean moisture content calibration equations.

Spectrometer	SEL (%)	SEC (%)	R^2	SECV (%)	RPD = SD/ SECV
X-Two	0.05	0,16	0,98	0,17	7,14
Foss 6500	0.05	0,12	0,99	0,17	7,28

The similar performances in terms of fitting were confirmed when predicting from the validation set of samples (Table 2 and Figure 4). For the 35 samples, the RPDs expressed as

the ratio of SD to SEP were higher than 6; 6,5 for the X-Two and 6,7 for the Foss. The SEP values were close to SECV (0,19% and 0,18% for SECV equal to 0,17%), which means that the models are robust.

Table 2. Performance statistics for prediction of coffee bean moisture content using two different calibration equations.

Instrument	SEP	R^2	Bias	SEPc	slope	RPD
X-Two	0,19	0,98	0,06	0,18	0,94	6,47
Foss 6500	0,18	0,98	-0,03	0,18	0,98	6,68

The difference between the instruments in terms of accuracy and robustness was due to the spectral range of the instruments: the wider range of the FOSS gave more information about samples (after 1700 nm) which allowed a better separation between noise and relevant information, This was illustrated by plotting the PLS loadings (Figure 5), which showed a supplementary band at 1940 nm with high coefficients for the FOSS instrument.

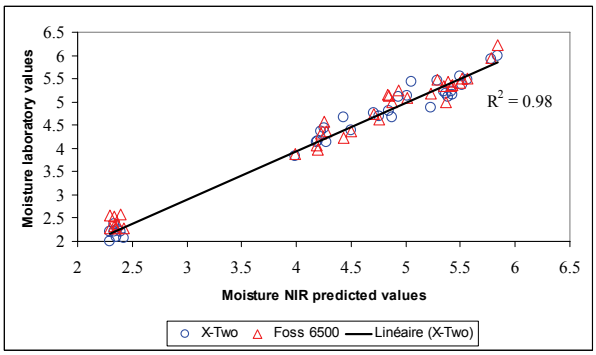

Figure 4. Scatter plot of moisture reference and NIR predicted values.

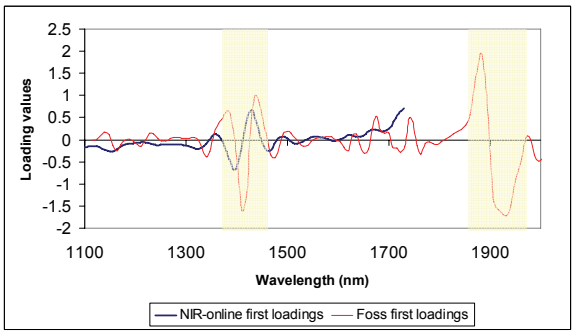

Figure 5. Loadings for the first PLS factor from the X-Two and FOSS equations.

CONCLUSION

The moisture content calibration obtained with the X-Two spectrometer could be used on-line at the end of the roasting process to control coffee bean moisture content with high accuracy. The implementation of such an instrument on-line will allow monitoring of water injection at the end of the roasting process and furthermore improve quality control over time. This approach could be expanded to include colour prediction, which would allow control of roasting degree.

Evaluation of Two Closure Systems for Glass Packages for Soluble Coffee

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SUMMARY

Two closure systems for 50g glass packages for soluble coffee were evaluated in this study: an alternative closure system, consisting of an aluminum foil-film combination (heat sealed) with plastic snap-cap and a conventional closure system (currently in use), consisting of a flexible foil (cold-bonded) and PP plasti-twist. The packages were stored at two different combinations of temperature and relative humidity (23 ± 3 ºC/70 ± 10%RH and 35 ± 3 ºC/ 80 ± 10% RH) for 360 days. Water vapour (WVTR) and oxygen (O_2TR) barrier properties of the alternative closure system showed better characteristcs than the conventional closure system. A good preservation of the initial moisture content in the product coming from the alternative package, in comparison to the conventional one, was observed, regardless of the storage condition. Headspace gas composition analyses showed that the conventional closure system maintained the composition of typical ambient air (21% O_2) inside the package, regardless of the storage condition and period. The soluble coffee in the alternative closure system, however, indicated a decrease in the amount of oxygen in the headspace and a slight increase in the carbon dioxide level, due to chemical reactions of the product (oxidation and Maillard reactions). These results are indicative of good water vapour and oxygen barrier properties of the alternative closure system, in comparison to those of the conventional system.

INTRODUCTION

The major cause of quality loss of soluble coffee is the increase in the moisture level that causes caking and clumping of the product when humidity reaches levels between 5 to 8%. Soluble coffee containing flavored coffee oils to enhance the product taste is susceptible to flavor deterioration in the presence of oxygen and moisture. Soluble coffee packed in glass containers will only maintain intact its intrinsic characteristics if the closure system used provides adequate protection against moisture uptake, oxygen absorption and loss of flavor compounds.

The aim of this study was to evaluate the stability of the soluble coffee in glass package with diferent closure systems during storage under two conditions of temperature and relative humidity.

MATERIALS AND METHODS

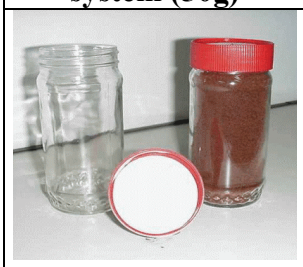
Packaging

The glass packages with two different closure systems evaluated in this study are described in Table 1.

Product

Spray-dried soluble coffee purchased from a Brazilian manufacturer**.** The initial moisture content was 2.6%d.b (2.5 to 2.7% d.b.) in the alternative package and 2.3% d.b (2.2 to 2.5% d.b.) in the conventional package.

Table 1. Main characteristics of the packages and closure systems.

Package (Net weight)	Description	Closure system	O_2TR (cm^3(STP)/ package/day)	WVTR (g water/ package/day)
 **Alternative closure system (50g)**	Glass cup with special finish (with Volan bonding agent applied), external diameter of 74 mm and 211mL of volumetry capacity	Aluminum foil-film heat-sealed with composition of Al (39 μm)/ Ionomer(33 μm) and external protective LDPE snap-cap	0.015 0.010-0.023	< 0.001
 **Conventional closure system (50g)**	Glass jar with external diameter of 50 mm and 217 mL of volumetry capacity	Cold-bonded flexible laminate with composition of metallized PET (18 g/m^2)/paper (42 g/m^2) and external protective PP plasti-twist cap	4.2 2.0-5.4	0.011 0.010-0.014

Al = aluminum foil-film; LDPE = low density polyethylene; PET – polyester; PP = polypropylene.
O_2TR - Oxygen transmission rate at 25 ℃ and 0,21atm
WVTR – Water vapor transmission rate at 38 ℃/98%RH

The soluble coffee in the alternative package was filled manually (50 g) and the aluminum foil-film was applied by heat-sealing process under previously optimized conditions in laboratory. The conventional package containing soluble coffee (50 g) had been filled and sealed under industrial conditions.

Soluble Coffee Stability Study

The stability of the soluble coffee in both glass package systems was evaluated by the moisture content of the product and the gas composition in the headspace, during 360 days at two storage conditions, 23 ± 3 °C/70 ± 10%RH and 35 ± 3 °C/80 ± 10% RH.

Moisture content of the product: was determined in accordance with the procedures and conditions described in Association of Official Analytical Chemists (1995). This method consists in drying the product in a vacuum drying chamber (10^4Pa), at 70 ± 1 °C for 16 hours.

Headspace gas composition: It consisted in taking a gas sample directly from the headspace with a gas-tight syringe introduced into the package through a septum. Next, specific gas concentrations were determined both qualitatively and quantitatively using a *Shimadzu* gas

chromatograph. The results are expressed as percentage (%) of total gas volume (Alves et al., 1998).

RESULTS AND DISCUSSION

Moisture content of the product

The moisture level of the soluble coffee in the conventional package stored at 35 °C/80% RH was significantly higher than that the soluble coffee in the same package type at 23 °C/70% RH, specially after 180 days of storage (Figure 1). The alternative closure system did not exhibit any significant increase in moisture content of the product, regardless of the storage condition and the period evaluated. However, the moisture content of the product remained below 5%d.b., as established in Brazilian rules, in both closure systems, independently of the period and conditions of storage.

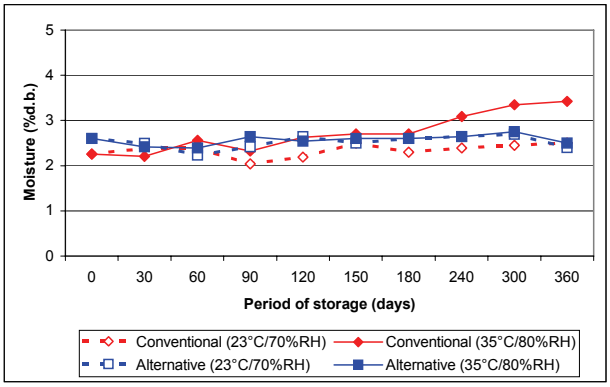


Figure 1. Moisture content of the product in the alternative and conventional packages at 23 °C/70% RH and 35 °C/80% RH.

Headspace gas composition

In both closure system, initial headspace gas composition was identical to the composition of ambient air.

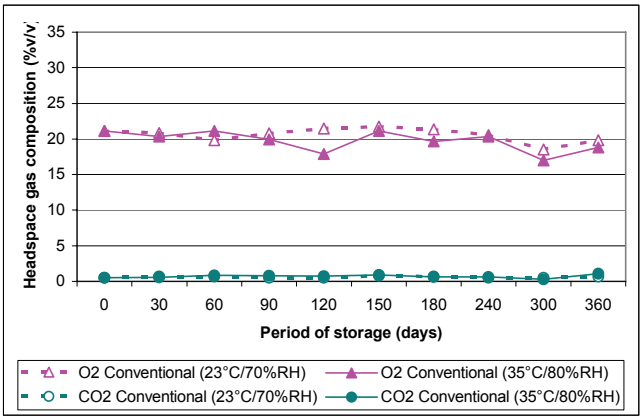


Figure 2. Headspace gas composition in the conventional closure system at 23 ºC/70% RH and 35 ºC/80% RH.

For the conventional closure system, no significative changes were detected in the gas composition of the headspace (O_2 and CO_2), it means the typical ambient air (21% O_2) inside the package was maintened, regardless of the storage condition and period (Figure 2).

However, for the alternative closure system was observed a decrease in the amount of oxygen in the headspace as well as a slight increase in the carbon dioxide level specially at 38ºC/80%RH storage condition (Figure 3). These changes were caused by the oxygen consumed by oxidation reactions in the soluble coffee. The carbon dioxide increase may originate from the carboxyl groups of the amino acids by nonenzymatic browning (Maillard reaction).

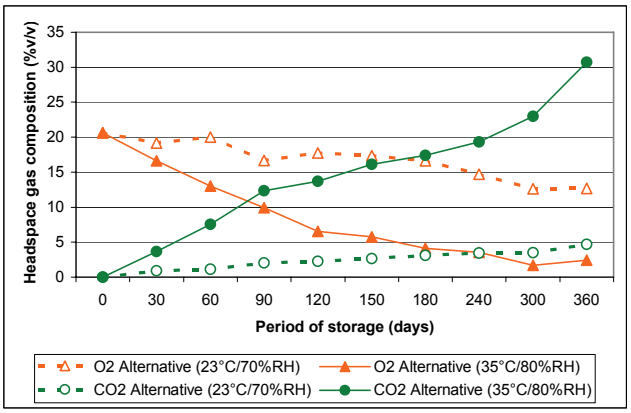


Figure 3. Headspace gas composition in the alternative closure system at 23 °C/70% RH and 35 °C/80% RH.

These results indicates that the gas barrier properties of the conventional closure system is lower and less effective than those of the alternative closure system, in that they allow constant exchange of gases between the headspace and the external enviroment. As a result, the composition of the gas mixture in the headspace of the package remains fairly constant and practical identical to the composition of ambient air, irrespective of the temperature/relative humidity at which the product is stored.

The performance of the alternative closure system, that is the modification of its headspace gas composition, results from the good barrier properties of this closure system, which did not allow diffusion of oxygen from the atmosphere into the package to compensate for the amounts of oxygen consumed by chemical reactions in the product. The build up of CO_2 in the headspace throughout storage is another finding indicative of the good gas barrier properties of the alternative closure system.

CONCLUSIONS

The alternative closure system (foil-mate) of glass package was found to be effective against water vapor and oxygen transmition rate and showed superior performance to those of the conventional closure system (currently in use). The alternative system provides higher protection for soluble coffee in terms of preserving the initial quality of the product, even when the product was stored in adverse conditions of temperature and relative humidity.

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Evaluation of Flexible Packages With Degassing Valves for Roasted and Ground Coffee

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SUMMARY

Flexible packages containing one-way degassing valves, also known as pressure relief valve, are increasing their space on the shelves of the markets. They are announced as being an economic and efficient system for the aroma conservation of roasted and ground coffee, besides to serve as a sensory attractive device. Manufacturers claim that the valves can act by holding the aromas, scavenging the residual oxygen and liberating the carbon dioxide of roasted coffee, as well as hindering the entrance of oxygen and other gases in the packaging. The objective of this research was to evaluate the performance of flexible package systems with such valve for roasted and ground coffee, by measuring the headspace gaseous composition and the integrity of commercial packages. The internal air package composition was measured with a dual headspace analyzer (MOCON PAC CHECK Model 650) in terms of oxygen concentration (O_2 detection by solid state zirconium oxide) and carbon dioxide (CO_2 detection by non-dispersive infrared). The packing integrity was tested by the bubble emission technique (ASTM E 515 – 05). Two commercial trademarks of roasted coffee and four other Brazilian brands of roasted and ground coffee with net weights ranging from 0.25 to 1.0kg, all containing degassing valves from the same manufacturer, were evaluated. According to packaging label, all the roasted and ground coffee stated 6 months of shelf life, except one roasted and ground trademark with 12 months, but this experimental analysis was carried out 3 months before packaging date. Half of tested packages presented virtually zero internal O_2 concentration (considering equipment detection limit of 0,01mg O_2/L) and above 40% of CO_2; however the other packages presented about 20% of O_2 and CO_2 levels ranging from 0,4 to 44%. By testing package integrity, it was verified that those package with high O_2 concentration also presented some kind of leak in the top seal region. Because of such leak problem the performance of the tested valves may be misjudged and more studies must be conducted to investigate such degassing valve system as well as their claimed scavenging oxygen properties.

INTRODUCTION

Coffee beans are roasted using hot combustion gases or air at temperatures above 200 °C to develop the characteristic flavors, colors, and aromas. Carbon dioxide is the major gas produced during roasting. The carbon dioxide is formed as a result of many reactions that occur including Strecker degradation, pyrolysis of sugars, and Maillard reaction (Clarke and Macrae, 1987). The carbon dioxide formed is trapped in the coffee and slowly diffuses out after roasting and grinding, thus a tempering period is required before packaging. If coffee is packaged prior to proper tempering, the eventual evolution of CO_2 may cause the package to burst. This is of concern, especially with the increased use of flexible films for packaging rather than rigid cans (Oliveira, 2006).

Flexible packages containing one-way degassing valves, also known as pressure relief valve, are announced as being an economic and efficient system for conservation of roasted and ground coffee, besides to serve as a sensory attractive device (Anjos, 2005). Manufacturers claim that the valves can act by holding the aromas, scavenging the residual oxygen and liberating the carbon dioxide of roasted coffee, as well as hindering the entrance of oxygen and other gases in the packaging (Anese et al., 2006).

The objective of this research was to evaluate the performance of flexible package systems with such valve for roasted and ground coffee.

MATERIAL AND METHODS

Two commercial brands of roasted coffee and four other Brazilian brands of ground coffee were evaluated (Table 1).

Table 1. Identification of the packaging for roasted (RC) and ground (GC) coffee.

Sample	Type of coffee	Net Weight (g)	Shelf life* (month)
A	RC	250	6
B	RC	250	6
C	RC	250	12
D	RC	500	6
E	GC	250	6
F	GC	1000	6

**Trials were carried out 3 months before expire date.*

The internal gas package composition was measured with a dual headspace analyzer (MOCON PAC CHECK Model 650) in terms of oxygen concentration (O_2 detection by solid state zirconium oxide) and carbon dioxide (CO_2 detection by non-dispersive infrared).

The packing integrity was evaluated by airleak test (Us Food And Drug Administration).

RESULTS AND DISCUSSION

From the determination of gas composition in the headspace of the packages shown in Table 2 it was found that half of tested systems presented virtually zero internal O_2 concentration (considering equipment detection limit of O_2 0,01mg/L) and above 40% of CO_2 (Samples A, C and E). The other packages presented O_2 levels ranging from 5 to 20% and CO_2 levels ranging from 0,4 to 44% (Samples B, D and F). By testing package integrity, it was verified that those package with high O_2 concentration also presented leak in the top seal region. These results indicated that the performance of the coffee brands packaging systems could not be associated with the type of coffee or their net weights.

Table 2. Gas composition in the headspace of the coffee packaging with degassing valves.

Sample	CO_2			O_2		
	Maximum (%)	Minimum (%)	Average (%)	Maximum	Minimum	Average
A	57,0	55,7	56,4 ± 0,65	< 0,01 mg/L	< 0,01 mg/L	< 0,01 mg/L
B	6,23	0,37	3,08 ± 2,93	20,4%	17,0%	18,7 ± 1,70%
C	44,5	42,4	43,4 ± 1,02	< 0,01 mg/L	< 0,01 mg/L	< 0,01 mg/L
D	33,3	6,07	17,4 ± 13,7	17,0%	4,70%	11,9 ± 6,18%
E	115	113	114 ± 0,98	< 0,01 mg/L	< 0,01 mg/L	< 0,01 mg/L
F	96,2	3,50	47,3 ± 46,4	20,0%	< 0,01 mg/L	9,99 ± 9,98%

CONCLUSIONS

The results at this phase of the studies indicated the proper performance of the desgassing valves where there were not integrity failures of the system. The packaging with failures in the heat sealing region compromise the whole system. Because of such leak problem the performance of the tested valves may be misjudged and more studies must be conducted to investigate such degassing valve system as a whole.

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Use of Aqueous Streams Produced By Bi-Polar Membrane Electrodialysis for the Modification of Coffee Flavor

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SUMMARY

Development of roast aroma in coffee is mainly attributed to Maillard reaction. It is well known that this reaction is pH dependant. Hence, shifting the pH of green coffee may be a way of shifting coffee aroma beyond what is currently available by bean selection and choice of roasting parameters. Acidification of green coffee results in bland tasting roast coffee products, quite comparable to steamed coffees. Contrary, alkalization is favorable for the formation of Maillard reaction products (e.g. pyrazines). Beverages prepared from such coffees are more intense in aroma and offer a darker cup color at identical degree of roast. The use of mineral acids or alkali for influencing the green coffee pH may be unfavorable for a number of reasons, e.g. consumer acceptance, regulatory, etc. Bi-polar membrane electro-dialysis is one elegant way of producing liquid compositions with a pH different from that of process water, i.e. an acidic and an alkaline stream. These electro-dialyzed compositions do not exhibit objectionable odor or taste and can be used to modify green coffee prior to roasting by means of soaking. Alternatively, sequential use of acidic and alkali streams is also possible. Soaking green coffee in one liquid stream and quenching roast coffee with the second offers novel coffee products without introducing acid/alkali functionality in the (semi) finished product. In summary, flavor modification in roast coffee using only green coffee and water can successfully be achieved using electro-dialyzed compositions. Limiting factors are the achievable pH range and mineral pick-up in the finished products.

INTRODUCTION

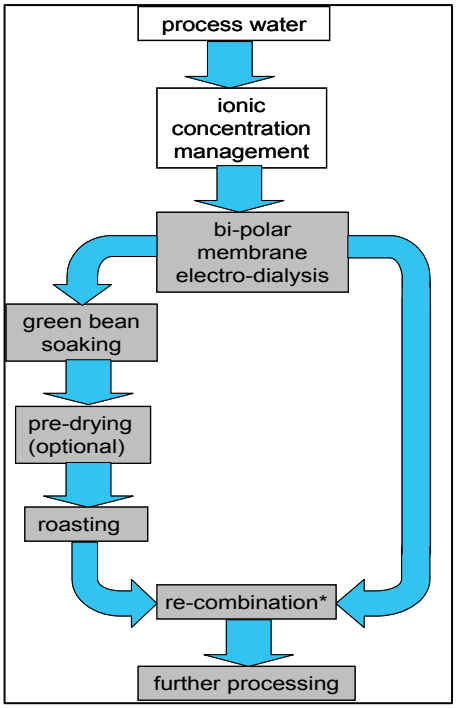
The present project aims at assessing options of modifying coffee flavor independent of bean sourcing or variation of classic roasting parameters, e.g. time and temperature. The pH dependence of the Maillard reaction (Westphal et al., 1988) has long been known. The use of bi-polar membrane electro-dialysis (BMED) in food applications has also been described (US-Patent, 2005).

Exemplary, the use of natural waters of defined mineral composition combined with a purely physical separation of ions by bi-polar membrane electro-dialysis was shown to be an elegant way of modifying the pH in green coffee without addition of commercially available chemicals.

PROCESS CONCEPT

Process water of sufficient ionic strength (salt enrichment, evaporation, etc.) is split into acidic and alkaline streams using bi-polar membrane electro-dialysis. One stream is used for soaking of green coffee, resulting in a modified green coffee pH.

After drying to the original water content (~12%) in a fluidized bed drier, all samples were roasted in a fluidized bed roaster (Neuhaus Neotech, max batch size 200g). Isothermal and target color (medium roast, 12La) roasting were carried out in 50g batches for 255s. In the examples shown, all roasted coffees were quenched with either water or the respective second stream of the BMED process using a squirt bottle.



**during quenching, soaking, extraction etc.*

Figure 1. Process concept for use of BMED streams for green and roast coffee treatment.

RESULTS

pH adjustment in green coffee has a significant impact on both coffee flavor and color. It was observed that a lower green coffee pH results in faster browning. The lower heat input required to obtain similar roast color is also reflected in better preservation of chlorogenic acids in the acidified coffee. Quenching with the respective second stream exiting the BMED unit does have little effect on the chlorogenic acid content.

pH reduction significantly reduces the yields of pyrazines, pyridines and guaiacols in roast coffee.

This is reflected in cleaner coffee aroma, comparable to steamed coffee. Contrary, pH enhancement results in darker, more intense coffees. Considerable salt pick-up is observed in neutralized samples.

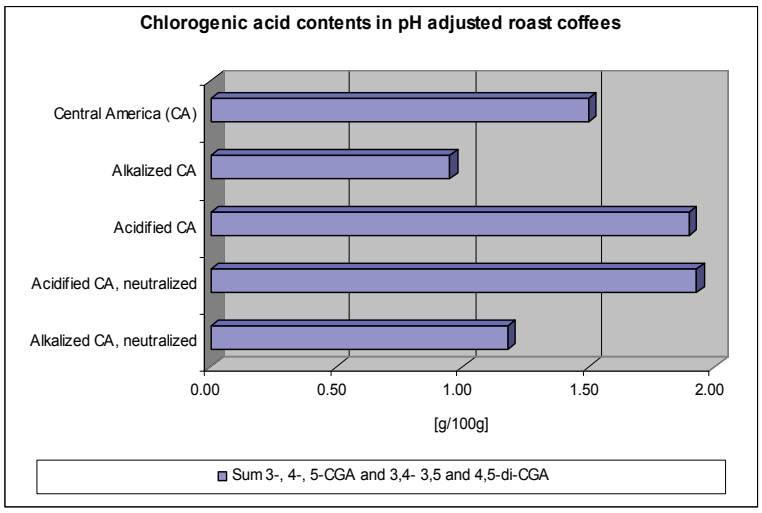


Figure 2. Chloro-genic acid contents in pH adjusted coffees (all medium roast color 12 La, 50 g/l brews.

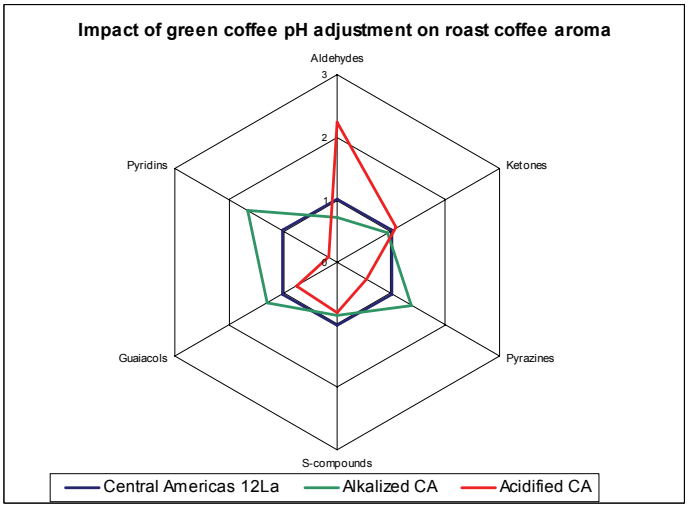


Figure 3. Impact of green coffee pH adjustment on roast coffee aroma.

Table 1. pH values and sensory impression of pH adjusted coffees (all medium roast color 12 La, 50 g/l brews).

Product	Green bean pH	Roast coffee pH	Sensory Description	Difference rating
Reference Centrals 12La	5.82	5.09	Earthy, slightly bitter, slightly fruity, medium roast, green, slightly sour	--
Process control	5.88	5.07	Similar to control, less sour, metallic, slightly salty, bitter, weak, cleaner	No-small
Alkalized Central America	7.59	5.36	Dry, more roasted, darker cup, unbalanced, sour, more bitter, intense	Moderate
Acidified Central America	5.05	4.79	Slightly citrus, dry, ligh brewt colour, sour, watery, aniseed note, clean, tea-like	Moderate
Acidified Central America, neutralized	5.05	5.14	Less aromatic, more impact, salty, cardboard, less bitter	Moderate
Alkalized Central America, neutralized	7.59	4.93	Sour, citrus, more astringent, darker appearance, smoky, intense	Moderate

CONCLUSION

As shown above, there is a variety of product benefits by manipulating coffee pH prior or after roasting. These range from production of cleaner, blander coffees from low-quality beans, without introduction of a process note as seen in steaming to production of dark but mild and highly acidic coffees.

One major technical hurdle for commercializing the process remains the generation of acidic and alkaline BMED streams of sufficient ionic strength. However, this will be associated with an mineral up-take into the coffee beyond the levels introduced by current processes, e.g. steaming and quenching. Besides the sensorial impact of different salts, finished products may be significantly different from products currently in the market.

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Coffee Oil Extraction Using Ethanol as Solvent: Kinetic Study

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SUMMARY

In the present work, it has been performed a kinetic study of coffee oil extraction with commercial ethanol from 100% *Coffea arabica* roasted beans. The beans were harvested at different maturation stages: Green, Cherry and overripe (Boia). Size and rheological analyses were used for the characterization of ground beans and coffee oil, respectively. For the solute diffusivity analysis, the oil content in raw materials, the extraction kinetic curves and the equilibrium concentrations were determined. It was concluded that oil content in the samples showed a strong correlation with the coffee beans' stage of maturation. The Green coffee presented higher values of oil content and diffusion coefficient than the other beans.

INTRODUCTION

Coffee is a truly global commodity and plays an important role in Brazil's economy. To absorb the high global supply of coffee beans, the diversification of its use in industrial processes has been encouraged. The oil extracted from roasted coffee beans is used in different food products, cosmetics and especially in the preparation of instant coffee (Silva et al., 2007). The conventional solvent used for oil extraction is n-hexane. Due to its high toxicity, the search for alternative solvents to replace n-hexane in vegetable oils extraction has been intensified. The use of ethanol as an alternative to fossil solvents shows good business prospects, since ethanol can be obtained from different natural sources, at competitive prices, in addition to its low toxicity. The ethanol production from sugar cane places Brazil in a singular position to eliminate the use of petroleum by-products in vegetable oils extraction (Lago and Freitas, 2006). Several studies on thermodynamic and kinetic behavior can be found in the literature concerning the extraction of vegetable oils with n-hexane and petroleum ether. However, operational data for vegetable oil extraction with commercial ethanol are limited. The objective of this work was to evaluate the kinetics of coffee oil extraction process using ethanol as solvent. In this study, 100% *Coffea arabica* roasted beans were used, at different maturation stages: Green, Cherry and overripe (Boia).

EXPERIMENTAL

Raw Material

The coffee samples were supplied by São Francisco farm, at São José do Vale do Rio Preto-RJ-Brazil. The coffee beans were harvested and sorted. The green and cherry are pulped and washed before the drying process. The overripe beans are sun dried immediately after harvest. Then, the beans were stored for 1 year before being toasted in the farm, according to international recommendations. The secondary processing involving roasting and grinding are the same for all dry beans. Absolute ethanol was used as solvent (VETEC).

Oil Extraction of Roasted Coffee

The samples were crushed in a bench coffee grinder (BRABENDER) and weighed in an analytical balance. Batches of 20 g of each sample were mixed with 60 g of ethanol. The mixture remained in a thermostatic bath at 65 ± 1 °C under 20 rpm. The contact time of the solvent with the coffee varied between 10 to 30 minutes with sampling at each 5 minutes to evaluate the extraction kinetics. The experimental tests were done in duplicate. The main extraction parameters, solvent/coffee ratio (w/w) and temperature were as recommended from the literature (Lago and Freitas, 2006). After the extraction, the mixture was filtered at reduced pressure, under heat, using a Büchner funnel coupled with a Kitassato for micelle and cake separation. The solvent in the micelle was recovered under vacuum at 60 °C. The extracted oil and the cake were dried by convective air at 60 °C to evaporate the residual solvent. Every fraction was weighted in analytical balance. The equilibrium concentration was determined through experimental tests as described above, increasing the contact time between the solvent and the milled coffee beans to 12 hours (Lago and Freitas, 2006). The oil content in the coffee beans was determined using a solvent/coffee ratio of 10:1 and a contact time of 28 hours (Lago and Freitas, 2006).

Size Analysis

Size analysis was performed in vibrating sieves using 14, 28, 32, 48 and 100 mesh Tyler. The Sauter mean diameter was calculated using Rosin-Rammler-Bennett model to represent the experimental data (Massarani, 1997).

Diffusion Coefficient

The diffusion coefficient was estimated considering sphere geometry for the coffee beans. Additionaly, it was considered that the coefficient is independent of the coffee concentration in the bulk phase. The mathematical model is shown in Equation [1]. The initial conditions for this equation is: $C(r,0) = C_0$ and the boundary conditions are: $(\partial C/\partial r)_{0,\forall t} = 0$ and $C(R,t) = C_{eq}$, where, $C(r,t)$, $C_{0,}$ and C_{eq} represent the oil concentration in different positions $\boldsymbol{r}$ in the bean at $\boldsymbol{t}$, the initial concentration in the sample and the equilibrium concentration, respectively. The solution of Equation [1] with the specified boundary and initial conditions is shown in Equation [2] (Brooker et al., 1992).

$$\frac{\partial C}{\partial t} = D_{ef} \cdot \left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial C}{\partial r} \right) \qquad [1]$$

$$\frac{C(t) - C_{eq}}{C_0 - C_{eq}} = \frac{6}{\pi^2} \sum_{n=1}^{3} \frac{1}{n^2} \exp\left(- \frac{n^2 \pi^2 D_{ef} t}{R^2} \right) \qquad [2]$$

Rheological Characterization

The rheological characterization of coffee oils was performed in a plate-plate rheometer (Brookfield Ultra/DVD III) at different temperatures. The obtained data of shear stress and deformation rate were adjusted by least squares method, using the Newtonian model (Fox and Mcdonald, 2001). The relation between the viscosity and the temperature was calculated using Arrhenius equation.

RESULTS AND DISCUSSION

The Green coffee presented higher content of oil in the beans (Figure 1). This result can be explained by the fact that the oil is protected by a more resistant cell wall avoiding the loss of oil during beans roasting. However, Boia coffee is overripe and has presented, after roasting, higher content oil than Cherry coffee. Probably, in this case, there was a loss of volatile compounds in beans, including water, without reducing the oil mass. Similar results have been reported in the literature (Lago and Freitas, 2006; Lago, 2001). Figure 1 compares the oil content and oil extracted at the equilibrium for the three samples analyzed. As expected, the oil mass in ethanol phase is similar for all beans due to the solvent saturation.

Figure 2 shows the particles size distribution: 99% of the particles presented diameters smaller than 1000 μm. Calculated Sauter diameter was equal to 350 μm. The beans size distribution is in the same range used for commercial extraction of vegetable oils with hexane (Rittner, 1991). The kinetic curves are shown in Figure 3. The extracted oil of each sample presented an exponential growth up to 25 minutes. From this time on, all curves presented an asymptotic trends. Lago and Freitas (2007) reported an asymptotic value to oil yield after 20 minutes.

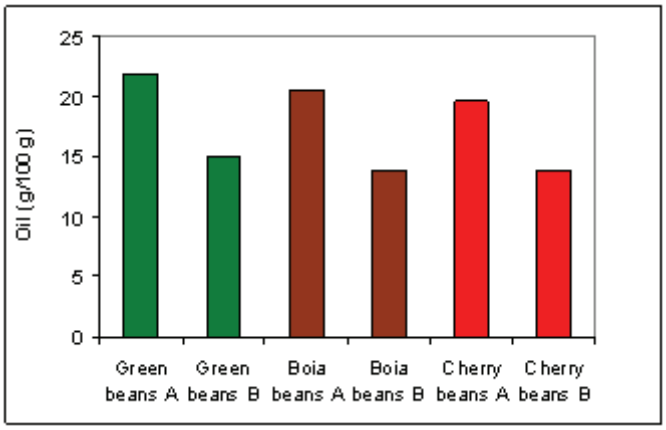

Figure 1. Coffee oil content (A) and coffee oil extracted at the equilibrium (B).

The highest diffusion coefficient was observed in the Green coffee beans, due to the higher oil contents observed in those beans (Table 1). Franco et al. (2007) obtained similar values for the diffusion coefficient of *Rosa rubiginosa* oil in commercial ethanol (1.2 E-11 to 2.2 E-10), using a solvent/coffee ratio between 15:1 and 50:1. However, the diffusivity of vegetable oils in n-hexane is about 10^2 times greater than the values obtained for extraction with ethanol (Rittner, 1991; Franco et al., 2007). Better results for extraction using ethanol can be achieved at temperatures between 70 and 75 °C (Lago and Freitas, 2006). Rittner (1991) suggests the vegetable oils extraction can be performed at 1.5 atm and 80 °C. In these conditions, the solute is completely miscible in commercial ethanol.

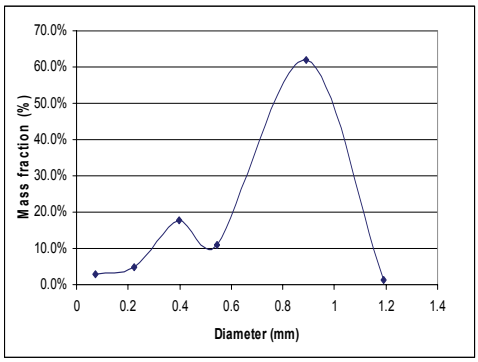

Figure 2. Size distribution.

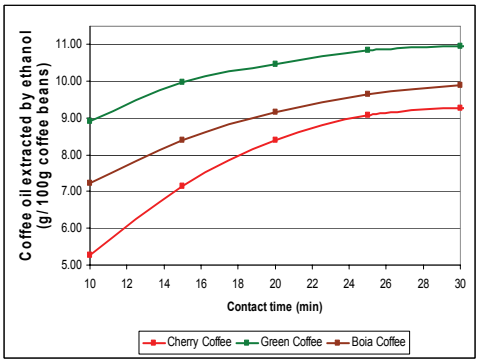

Figure 3. Kinetics curves of extraction.

In the temperature range studied, coffee oils behave like a Newtonian fluid. The viscosity decreased from 228 cP at 20 °C to 41 cP at 50 °C. The activation energy (Ea) was estimated by linear regression applied to the experimental data. The obtained value (Ea/R = 5734 K) was similar to that reported for soybean oil extracted with ethanol (5287 K) (Santos et al., 2005; Barreto et al., 2007).

Table 1. Diffusion coefficients of coffee oil from roasted beans using ethanol as solvent.

Maturation stages of coffee beans	Diffusion coefficient (m^2/s)
Green (underripe)	1.32E-11
Cherry (ripe)	7.95E-12
Bóia (overripe)	1.09E-11

CONCLUSIONS

Oils behave like Newtonian fluids for temperatures above 20 °C with viscosity strongly dependent on temperature.

The diffusion coefficients of oil in ethanol were smaller than diffusion coefficients of oil in n-hexane.

The oil yield extraction of coffee roasted beans can be improved by increasing either the solvent/coffee ratio or the extraction temperature.

ACKNOWLEDGE

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Pre-History of Instant Coffee - Early Production of Soluble Coffee

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Production of a soluble coffee is a technological challenge. Inventors were driven by a need for the product or a mass supply of the educt - both economical forces play a strong role in soluble coffee's history.

START-UP 1860 - THE MILK EXPERTISE

Coffee extract showed up first in public procurement during the North American civil war, when extract of coffee (or coffee essence) should substitute the green coffee of the field rations, which up to then had to be roasted and brewed by the soldiers, time and material consuming. The debates can be found in the Journal of the US senate of 1862 (Library of Congress).

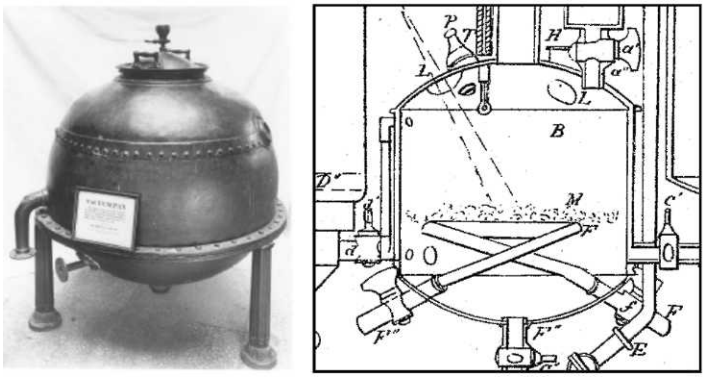

Figure 1. Borden's vacuum pan of 1853, the essential for the production of his coffee extract concentrate in 1862 (courtesy of the Smithsonian, Washington); right side patent drawing, 1856.

Three bidders were on the floor, with a stiff competition (American Desiccating Company). The winner was Gail Borden; his concentrate of coffee, milk and sugar had an acceptable taste, was the least expensive, and − the most important − Borden was able to deliver the huge amounts necessary for the troops in time. He had gained his technological expertise in concentration of milk via vacuum evaporation; he already was a mass supplier to the army with his condensed milk. His vacuum pot (Figure 1) of 1853 is shown in the Smithsonian Institution as a technological milestone (Borden Milk Company, 1932), the respective patent was issued in 1856 (Borden).

In the patents second reissue of 1863 (Borden), he expanded the description to the evaporation "when the milk is combined with sugar or extract of coffee" − exactly the product he delivered to the army. There is no description, however, how the coffee extract itself was prepared. It was supplied as a thick concentrate, in cans with one day's ration for 100 soldiers; consumer remarks were "looks like axles grease, but tastes dammed good". The product was in the market till the 20th century, and won a price at an exhibition in Buffalo in 1901 (Board of General Managers of the Exhibit).

FOCUSING ON COFFEE AROMA

Another early preparation of soluble coffee was patented to Gale in 1865. The process including extraction and drying, was focusing on protection of the aromatic volatiles. During extraction, they were removed, and condensed in fractions of varying temperatures. The one bearing the aroma, was absorbed in sugar and commingled with the aqueous coffee extract, which had been mixed with sugar before. To prevent the aroma from escaping and to serve for hardening, condensed milk might be added, gum-arabic or other additives. The resulting homogeneous mass rolled out on a table, finally formed a dense and solid cake, which could be cut into pieces to serve as single dose. The patent claims, that this can be handled and packed without being enclosed in cans, and might be used to prepare the coffee beverage or consumed as such “in the heat of a battle”. It is unknown whether it was used as intended; the civil war ended the month before Gale's patent was issued.

Another American patent of 1880 (Gue and Grant), introduced a stepwise extraction, first cold, then warm water, to separate aromatic and bitter components.

BRITISH ROOTS OF 1771 - NOT REALLY SOLUBLE COFFEE

In the United Kingdom, the earliest patent dealing with coffee preparations was of 1771 (Dring); it was called a “Compound coffee”, prepared by mixing roasted and finely ground coffee with butter - not an instant coffee as we understand it today, but it seems comparable to the eatable coffee cake of Gale, and remembers some ethnic style coffee drink with butter. The 1771 patent was cited in 1880 by v. Hof-mann from Augsburg, Germany, when he claimed to preserve the coffee aroma in tablets of ground coffee compressed with fat and bicarbonate, with patents granted all over Europe and in the US.

Figure 2. **Advertising for Camp Coffee concentrate, about 1880.**

About 1875, a thick black coffee syrup from Scotland came up, with a special trade mark and advertising (Figure 2): the liquid Camp Coffee of R.Paterson and son from Glasgow, an essence of coffee-beans, chicory and sugar, in a distinctive bottle. The origin is believed to be a request from the Gordon Highlanders for a coffee drink that could be used easily by the

army on field campaigns in India. (Camp Coffee is on the market till now; the label was slightly changed in 2006: no longer a Sikh serving coffee to the British officer, but Sikh and British sitting together with their cup of Camp Coffee.)

There might indeed have been a considerable market in the UK, whilst another British patent of 1884 (Thew) dealing with the milk to go with coffee, says: coffee extract "as is commonly sold".

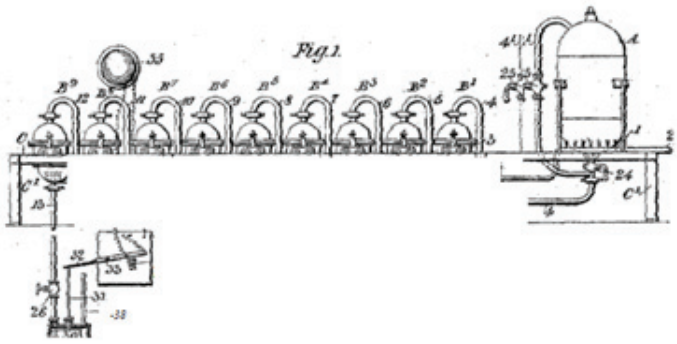

Figure 3. **Extraction battery for soluble coffee, British patent 1897.**

In 1897, the production of a pure soluble coffee concentrate, without milk or sugar, was described, an extraction of an almost modern lay-out (Figure 3), in a British patent granted to Duke from London. The process was semi continuous, counter-current, with nine extraction vessels in line, each one movable to change its place in 15 minutes intervals in the procedural series between the steam supply at the most extracted end and the roast and ground coffee feeding at the draw-off side. The vessels were hermetically sealed to avoid the deleterious air contact, the extraction was run under pressure, the draw off product was just in the right concentration to be directly cooled down and filled into bottles for consumption. Patents were also given in France, Belgium and the US.

Figure 4. The spirits of coffee - advertising tradecard from the Buffalo exposition 1901.

Nearly the same time, another multi-stage extraction was filed by Eschwege(1903) from Kent, UK, with US patents issued in 1901 and 1903: semi continuous, counter-current, but running with gravity flow and at temperatures of about 40 °C, to end up with liquid coffee extract.

With their battery type extractions, Eschwege and Duke seemed to be ahead of time. Nevertheless, a commercialization is unknown -maybe, they were overrun by the next developments.

1901: SOLUBLE COFFEE AS A PURE SOLID

Solid pure soluble coffee was presented to the public in the Buffalo Pan American exhibition 1901 by Sartori Kato from Chicago, with a patent granted in 1903. Roast coffee is defatted by pressing, then extracted in two stages; the aroma parts of the first extract were absorbed in the dried solids of the second extract, and the resulting paste dried in cold air. Thus the aromatics could be protected against rancidity. The exposition brought great publicity to this product (Eck, http://panam1901.bfn.org/visiting/food/foodbeverages.htm) and a coloured tradecard for the visitors (Figure 4) seems to reflect the spirits of coffee aromatics. Nevertheless, the real importance in the market of that time is unclear today.

INSTANT COFFEE - DEMANDS IN WORLD WAR I

The next step in soluble coffee production is connected with the name G.C.Washington of Brooklyn: a solid coffee extract, containing sugar and milk. The distribution was promoted with World War I, where again soluble coffee was in the procurement for the military. In 1918, Washington's whole production was requisitioned. The coffee was well accepted by the soldiers, and got the pet name “George” (it was labeled “George Washington's Instant Coffee”). The respective patent was issued in 1924 (Washingtin) describing the combined drying of a concentrate sugar solution, condensed milk and coffee percolate; the solid product was soluble in either hot or cold water.

A German poster for Ruwil, Der losliche Kaffee (the soluble coffee) of 1915, designer Julius Gipkens may draw the attention on the German coffee market of that time (Figure 5).

Figure 5 . Advertising for Ruwil, Soluble coffee, word trade marks registered in this field with poster by J.Gipkens 1915, courtesy of Deutsches patriotic names like “Deutscher Siegfried”, Historisches Museum, Berlin, Germany.

The trade mark Ruwil was granted to the chemist Karl Baron von Vietinghoff of Berlin, Germany, in February 1915. As declared in the register, his enterprise encompasses production of coffee extracts and merchandising, with the commodities coffee, coffee extracts, coffee substitutes and related products. For solid coffee extracts, he hold British and US patents of 1913 and 1916 respectively (v. Vietinghoff, 1916).

The poster – showing a soldier in field uniform with a box of soluble coffee and a cup- evidently should convince the families at home to send him just this soluble coffee. This intention can be seen on many other image trademarks of that time. – By then, there were also several many of them on coffee substitutes. With the beginning of the war, the importation of green coffee into Germany had been stopped by the government, and the remaining reserves were under surveillance, mostly for the troops.

After the war, there were further ascents of the soluble coffee technology towards the principles of today: Regarding the semi continuous process, in 1921, a system of four percolators and a subsequent spray drying, including the recycling of the roast aroma to the spray dryer and a dust recovery, was patented to John W.Scott, New Jersey, of the Arbuckles, a very big US coffee company. Shortly after, Arbuckles merged to the General Foods group (Pendergrast, 1999).

In a patent of 1927, a continuous extraction process was presented by Angus B.Kennedy, Cincinnati − for coal. This was also used for coffee, As pointed out by Michael Sivetz (2004) − an application not mentioned in the original patent neither in the succeeding one of 1952 (Kennedy).

THE BREAK-THROUGH ELABORATION

When the Brazilian government, suffering under a disastrous decline in coffee prices after several record harvests, approached the well reputed Swiss company Nestle to put efforts into the production of soluble coffee, a fundamental progress was initiated. The work in Switzerland started in 1930, and it took its time till 1936, to elaborate the process as a whole, with a special temperature regime in the four percolators for extraction, with a connected concentration and spray drying, and with the addition of taste neutral carbohydrates for better flavor retention, abandoned later on in the fifties; the overall inventor was M.R. Morgenthaler. The presentation to the public was in 1937, together with Swiss patents filing (no personal inventor, 1938); introduction into the market came 1938, US patents were issued in 1943 (UK trademark N°. 585860); the registered trademark Nescafe (UK trademark N°. 585860, 1938) dates back to those days.

FURTHER DEVELOPMENTS

Further developments in the various international companies were supported by applied research in the universities. Many of the early experiences of technology have found their theoretical fundaments, often with presentations at ASIC conferences.

For information on the earlier productions, main sources open to the public are national patents and national trade mark registers, giving details on the producing enterprises; even the advertising can eventually tell something on the products in the market, might it have been a poster for the street, a metal plate to be placed in the shop, or ads in the newspapers and other media.

Many of the early product forms are on the run again, competing with the classic soluble coffee, for example the soluble specials with milk and sugar, the single doses, the concentrates, the ready to drink coffees. - Today, soluble coffee has its well respected place in the food shopping cart.

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The second offer, a powder from the American Desiccating Company, was not convincing in quality, the third one, a concentrate produced by Prof. W.L.Tilden, could not be delivered in the amounts needed.

The original pot, a gift from the Borden Milk Company to the Smithsonian in 1932 -image #38448, Smithsonian Institution Archives, RU 361, box 9, f.30

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Production of Adsorbents from Coffee Husks: Effect of Thermal/Chemical Treatments on the Adsorption of Heavy Metals

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SUMMARY

Brazil is the largest coffee producer in the world, and for each coffee ton produced, the same amount of coffee husks is generated, a residue that does not present a profitable use. Therefore, the objective of the present study was to propose an alternative use for coffee husks, as adsorbents for the removal of heavy metals from aqueous solutions. Thermal and chemical treatments were evaluated in order to improve the adsorption performance of coffee husks. The activating agent was $CaCl_2$, and carbonization was carried out at 200 °C. It was observed that the percent adsorbed of Cr(VI) ions increased after the treatments. The thermal/chemical treatments did not improve adsorption performance in the case of cooper, cadmium and zinc. It was also observed that adsorption efficiency was reduced by the presence of parchment.

INTRODUCTION

The processing of coffee generates significant amounts of agricultural wastes. Coffee husks (CH), comprised of dry outer skin, pulp and parchment, are probably the major residues from the handling and processing of coffee, since for every kg of coffee beans produced, approximately the same amount of husks are generated during dry processing Saenger et al., 2001). The production of coffee in Brazil in the past 5 years ranged from 17,000 to 27,000 tons (MAPA, 2008), which represents an average of over 20,000 tons of coffee husks being produced every year. There are still no profitable uses for this type of residue and its disposal constitutes a major environmental problem in Brazil (Oliveira et al., 2008).

The use of agricultural wastes for the removal of pollutants from wastewaters is being intensively studied and seriously considered as an economically viable alternative to the conventional processes currently employed (Sud et al., 2008; Oliveira and Franca, 2008). A recent study has demonstrated that untreated coffee husks can be used as an adsorbent for removal of heavy metals from aqueous solutions, with maximum adsorption capacities in the range of 6 to 8 mg g^{-1} for Cu(II), Cd(II), Zn(II) and Cr(IV) (Oliveira et al., 2008). Hence, the objective of this work was to evaluate the feasibility of modifying the surface of coffee husks by thermal and chemical treatments in order to improve their adsorption performance.

METHODOLOGY

Dry coffee husks were acquired from Samambaia Farm, a coffee producer at Santo Antônio do Amparo, Minas Gerais State, Brazil. The husks were obtained from a dry processed coffee after de-hulling. The coffee husks were washed with distilled water (approximately 300 mL

water per g coffee husks), to remove dirt and color, and dried at 105 °C for 5 h in a convection oven. Afterwards they were treated with 2% formaldehyde solution in order to reduce organic leaching and avoid mould formation during batch adsorption (Oliveira et al., 2008). Coffee husks were then allowed to dry at room temperature for 24 h.

The adsorbent was prepared by treating the coffee husks (CH) with $CaCl_2$ at the following impregnation ratios: 50%, 100% and 150%, according to the methodology proposed by Mohanthy et al. (2005). The treated coffee husks (CaCH) were then heat treated in a muffle furnace under a stream of nitrogen at 0.3 L min^{-1} at 200 °C for 1 h. The carbonized material was cooled to room temperature under nitrogen flow.

Batch experiments of adsorption were performed using 250 mL Erlenmeyer flasks agitated on a shaker at 100 rpm for 24 h. The flasks were covered with plastic film to avoid contamination. After the specified time period, 5 mL aliquots were taken from the Erlenmeyer flask, filtered (0.45 μm) and the concentration of the metal ions was determined using an atomic absorption spectrometer (GBC AVANTA 932 PLUS, Dandenong, Australia), after treatment with a 10% HNO_3 solution. Absorption readings were taken at 324.8 nm for copper, 213.9 nm for zinc, 228.8 nm for cadmium and 357.9 nm for chromium. All determinations were performed in a total of three replicates per experiment and the average values were reported.

RESULTS AND DISCUSSION

Results for the chemical characterization of the adsorbents are displayed in Table 1. PZC values decreased as expected, given that the $CaCl_2$ treatment led to the formation of the $(-COO)_2Ca$ groups at the adsorbent surface, thus reducing the amount of protonated groups at low pH values (Khosravi et al., 2005). Activation led to an increase in carboxylic and lactonic groups and to a decrease in phenolic groups, which were in part blocked by the treatment with formaldehyde (Chen and Yang, 2005).

Table1. Chemical characterization of the adsorbents.

Adsorbent	PZC	Carboxilic groups (mmol/g)	Lactonic groups (mmol/g)	Phenolic groups (mmol/g)	Basic groups (mmol/g)
CH	6.4	0.12 ± 0.01	0.12 ± 0.02	0.97 ± 0.05	0.45 ± 0.02
CaCH 50%	5.3	0.78 ± 0.05	0.37 ± 0.01	0.84 ± 0.03	0.18 ± 0.01
CaCH 100%	5.0	0.92 ± 0.02	0.42 ± 0.03	1.01 ± 0.04	0.29 ± 0.04
CaCH 150%	4.7	1.80 ± 0.04	1.39 ± 0.03	0.15 ± 0.02	0.41 ± 0.01

Results regarding the effect of the treatments on adsorption efficiency are displayed in Fig. 1. It can be observed that CH presented reasonable values for adsorption of Zinc, Cooper and Cadmium. However, adsorption efficiency was low in comparison to an average of 90% adsorption previously reported for coffee husks (Oliveira et al., 2008). This is attributed to the presence of parchment mixed with the coffee husks used in the present study, which were not present in the previous one (Oliveira et al., 2008). The low adsorption of Chromium is attributed to the specific adsorption mechanism for such ion, which involves a reduction stage from Cr (VI) (usually present in anionic form, $HCrO_4^-$) to Cr(III). This reduction is dependent on the amount of electron donor groups (e.g, -COOH) on the adsorbent surface. The treatment with $CaCl_2$ provided a significant increase in carboxylic groups, thus favoring Cr (VI) adsorption.

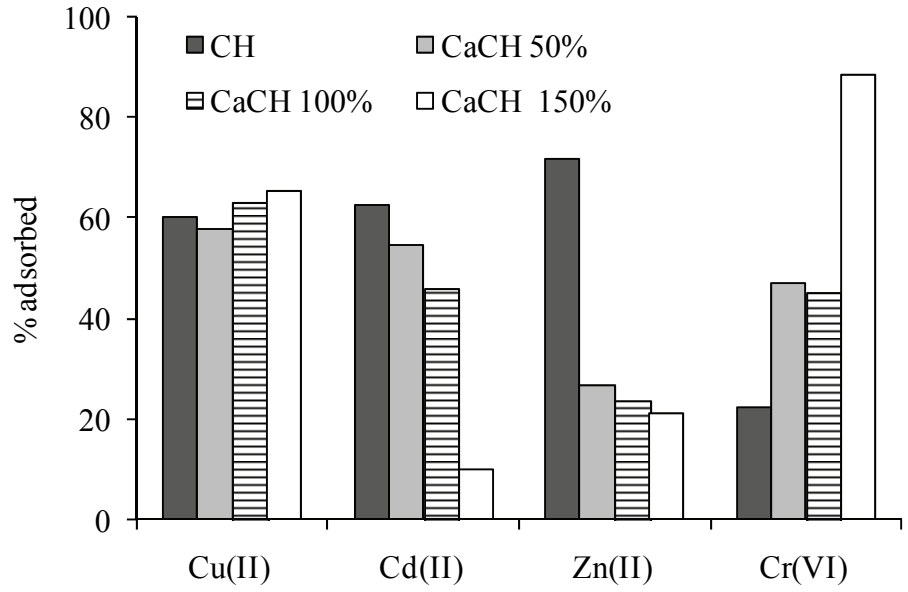

Figure 1. Adsorption capacity of untreated and treated coffee husks.

CONCLUSIONS

Experiments were conducted to evaluate the feasibility of modifying the surface of coffee husks by thermal and chemical treatments $CaCl_2$, in order to improve their adsorption performance in regard to the removal of heavy metals from aqueous solutions. Adsorbent performance was significantly improved in the case of chromium removal. However, $CaCl_2$ treatment led to a decrease in adsorption capacity for Cu(II), Cd(II) and Zn(II) removal.

ACKNOWLEDGEMENTS

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Potential Use of Coffee Compounds as Sunscreens in Cosmetic Products

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SUMMARY

Coffee oil is a sub product from decaffeination process mainly used to avoid granules fragmentation of soluble coffee. The coffee oil is rich in linoleic acid, unsaponifiable matter and antioxidants. In order to obtain another function for coffee oil and add value for this, the present research propose the use of coffee oil like sunscreen product due to the coffee oil´s capability to skin moisture, keep humidity and absorbs UVB radiation. For this, were evaluate five *Coffea arabica* cultivars, six *Coffea canephora* cultivars and one hybrid *C. arabica* x *C. canephora* from Instituto Agronomico (IAC) BAG coffee. The oil and wax yield and sun protection factor (SPF) were analysed by UV spectrophotometric method. The wax yield varies among 0.03% (IAC Apoatã 3599-1) and 0.44% (IAC Arabusta). Oil content varies of 5.08% (IAC 906 Conilon) to 21.99% (IAC 1669-20 Obatã). There is high variability and considerable SPF between the samples. For *Coffea arabica* were found SPF between 1.50 (IAC 1669-33 Tupi) and 3.65 (IAC 4045 Icatu) therefore those varieties should be able to compose a natural product that absorbs UVB radiation. The ANOVA showed high variability among plants and varieties indicating that characteristics could be select in breeding programs.

INTRODUCTION

Sunscreens are, by definition, effective in protecting against sunburn and tanning and provide protection from UV-induced DNA damage (Rosen, 2003).

The UV radiation is divided in UVA (320-400 nm), UVB (290-320 nm) and UVC (< 290 nm). The UVB and UVC radiation represents that portion of the spectrum that is capable of damaging biological organisms but UVC radiation are almost completely absorbed by ozone and very few reach the earth's surface. UVB radiation is only partially absorbed by ozone layer and can damage biological organisms while UVA is not absorbed by ozone and generally is not dangerous to biological organisms (Gibson, 2007).

There are two important coffee species: *Coffea arabica* known as Arabica and *Coffea canephora* known as Robusta both has as the main application the coffee beverage appreciated in the whole world. These species presents different chemical beans composition and could be characterized for this. Generally, *C. canephora* presents less oil than *C. arabica* however Arabica beans are smallest rich in caffeine and chlorogenic acids.

The coffee oil is a mixture of compounds like triacylglycerols, free fatty acids, vitamins and sterols. Some of these compounds present cosmetic properties like keeping humidity, skin moisture, some have antioxidant effects and UVB radiation absorption.

Linoleic acid is mainly fatty acid in the coffee oil (Folstar et al., 1975). This fatty acid is associated with curative properties: eczema relief and dermatitis cure (Beveridge et al., 1999).

About 12% of coffee oil is constituted of unsaponifiable matter. In other vegetable oils only 1 or 1.5% is unsaponifiable matter (Khan and Brown, 1953).

The coffee seeds contain also 0.2-0.3% coffee wax composed of N-alcanoil – 5 hidroxytryptamine (C – 5 – HT) (Folstar et al., 1975). This molecule has antioxidant effects (Wurziger and Harms, 1973). That antioxidants contribute for solar protection because the skin uses these for auto-protection against solar damage effects (Fuchs, 1998).

The coffee oil of roasted beans could be used as sunscreen product (Grollier and Plessis, 1988). Preliminary observations from Instituto Agronômico de Campinas (IAC) indicated a possible UVB absorption in green coffee beans too.

This research aimed to evaluate the most planted cultivars of coffee from IAC BAG Coffee of those that have potential the use like sunscreen product.

METHODS

Plant material

Coffea arabica cultivars (4 plants): Catuaí Vermelho IAC 81, Icatu Vermelho IAC 4045, Mundo Novo IAC 376-4, Obatã IAC 1669-20 e Tupi IAC 1669-33.

Coffea canephora cultivars: Apoatã (8 plants), Bukobensis (1 plant), Conilon (2 plants), Guarini (3 plants), hybrid *Coffea arabica* x *C. canephora* Arabusta (1 plant), Kouilou (8 plants), Laurenti (1 plants), Robusta (3 plants).

Methods

About 500g coffee berry fruits were harvest and dried without pulped. The fruits were processed. The coffee wax was extracted through reflux by *Butt* (Figure 1) with chloroform for 30 minutes (Folstar et al., 1975). After extraction, the seeds were dried in a forced air oven during 12 h at 60 °C and ground. Using the same *Butt*, the coffee oil was extracted with petroleum ether for 16 hours, by means of AOCS method (Firestone, 1998). After extraction, the wax and the oil were weighted and stored in freezer (-18 °C).

Figura 1. *Butt* extractor.

The SPF of samples was analyzed using UV spectrophotometry (Mansur et al., 2006). 2 µL coffee oil were dissolved in 10 ml ether originating absorbent curve like showed in Figure 2. The samples' SPF was calculated through this curve.

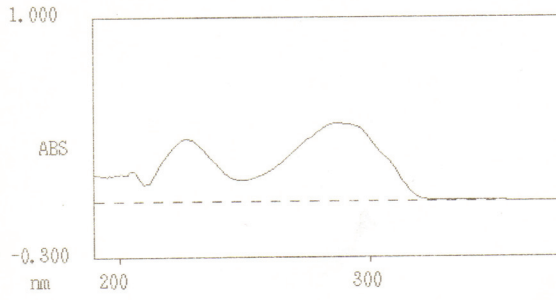

Figura 2. Absorption spectrum (190 - 400 nm) of one *C. Arabica* sample.

RESULTS AND DISCUSSION

High variability was found among Arabica and Robusta coffees inside and between cultivars. The yield of oils found for Arabica coffees are shown in Figure 3a. The better average is found for IAC 1669-20 Obatã but this cultivar is also presented in a larger variation.

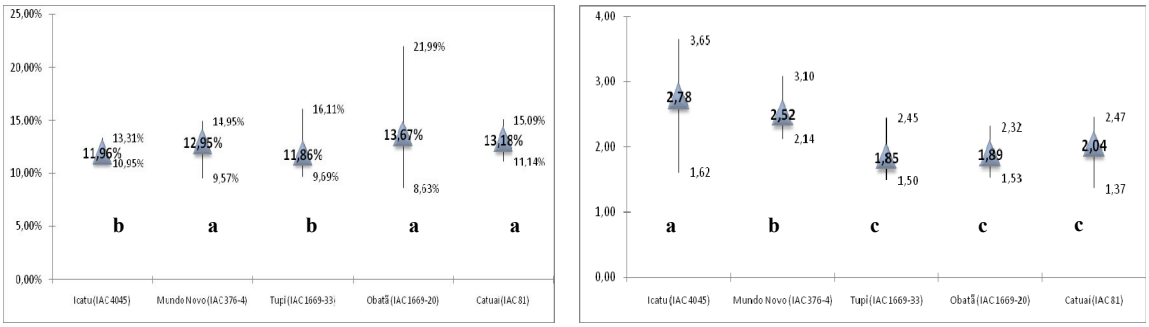

Figura 3. a) Tukey test (1%), average and variation of oil yield between Arabica cultivars. B) Tukey test (1%), average and variation of SPF between Arabica cultivars.

The average lipid content of green Arabica coffee beans is about 15%, whilst Robusta contains only 10% (Speer and Kölling-Speer, 2006). The results showed in Figure 3a agrees with this affirmation.

The best cultivar found for the SPF was IAC 4045 Icatu with SPF = 3.65. But this cultivar also presents the larger variability in its values (Figure 3b).

Robusta coffee's results are presented in Figure 4. It wasn't found any research about wax and SPF so it isn't possible to compare results. Therefore, those results can be compared with Arabica coffees and show that Arabica coffees present more SPF than Robusta coffees.

Coffea canephora presents between 6.61 (IAC Kouilou 66) and 12.27% (IAC Robusta) of lipids and has a high variability because of its origin (Aguiar et al., 2005).

In this research, we found high variability in Robusta coffees cultivars like showed in Figure 4.

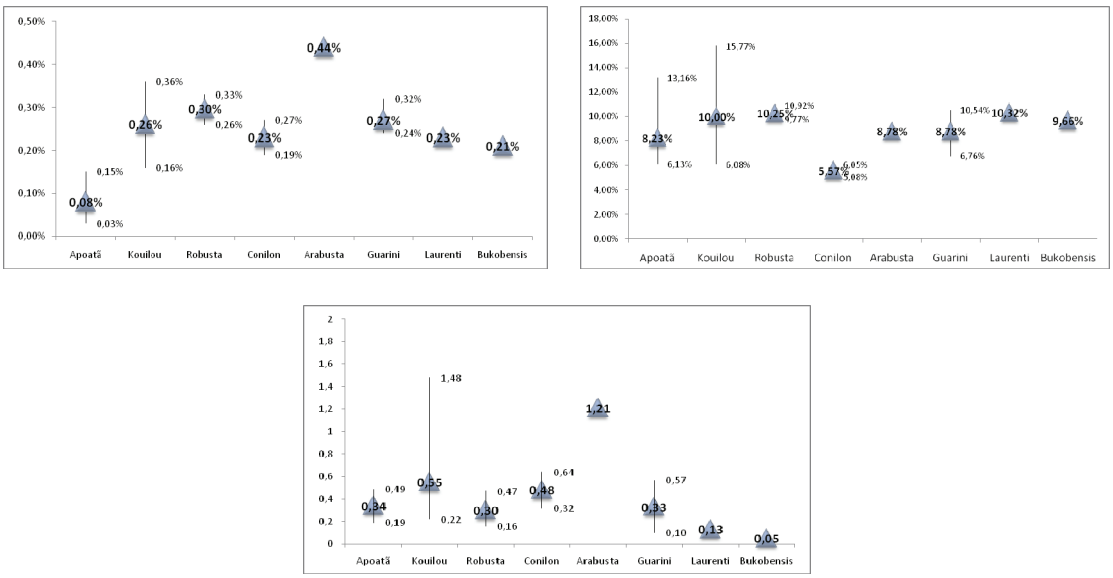

Figure 4. a) Average and variation of the amount of the wax in *Coffea canephora* cultivars. b) Average and variation in oil yield for *C. canephora* cultivars. c) Variation and average in *C. canephora* cultivars for that SPF.

Coffea canephora is an alogam specie, so ANOVA was calculated by plant and showed that there are high variability in plants.

Due to the high variability observed for all characteristics can it be conclude that the information exposed in this research can be used in breeding programs for oil and wax increases or reductions. And the SPF information can be used to produce a natural sunscreen product.

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Quality Characteristics Influencing Preference of *Coffea arabica* L. Produced in Kenya

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SUMMARY

Kenya primarily produces washed Arabica coffee (*Coffea Arabica* L.). The distinctive qualities of the coffee have earned it an important reputation on the world market. Cupping, also known as cup tasting, is the method that has been and is still used as the ultimate procedure for the assessment of coffee quality. Classification of commercial coffee traded in Kenya is done according to the quality of the beverage obtained after roasting and brewing. In the present study coffee quality assessment focused on fragrance (smell of dry ground coffee), aroma (smell of wet coffee on breaking the crust) acidity, body, presence of key flavour attributes and the preference on a six level scale. The cleanliness of the coffee in terms of off flavours was also evaluated. Completely blind tasting sessions were practiced. Analysis of variance on the regressions showed that the fragrance, aroma, acidity and flavour had a significant influence on the preference. However the body did not show any influence on the preference. The diversity of flavour profiles was demonstrated for the coffees with a variety of terms being used in their description. The presence of off flavours reduced the preference of the coffee.

INTRODUCTION

Coffee is an important foreign currency earner in Kenya and directly supports the livelihoods of many. Over 600,000 smallholders and 1300 large-scale farmers in Kenya are engaged in coffee production. Before any coffee is sold it is classified by the screen size, and cup quality. Marketing systems for coffee have determined how its quality should be defined and therefore different definitions of coffee quality can be found around the world. In Kenya liquor quality is determined on the basis of the level of acidity, body, and flavour of the brew (Devonshire, 1956; Moreno et al., 1995). The presence of defects is quite relevant in establishing coffee quality for they are associated to problems during production and processing operations. Quality of liquor determines the desirability of coffee for consumption purposes, and hence, acts as a yardstick for price determination (Walyaro, 1983; Roche, 1995). Differences in sensory characteristics of coffee samples were evaluated.

MATERIALS AND METHODS

Sampling

Dry parchment samples approximately one kilogram were collected at random from 159 factories in different coffee agro-ecological zones during the 2006/2007 main coffee season East of the Rift valley. The samples were transported to the cupping laboratory in odourless

sealed paper bags. The samples were hulled using a sample huller and graded to obtain grade AA and AB (Screen 18 and 16 respectively). One hundred grams AB grade of each coffee sample was roasted at 180- 200 °C, in an electric laboratory roaster (Probat laboratory roaster) to attain a medium roast. The roasted coffee beans were then ground to a medium grind.

Beverage quality determination

Two coffee experts evaluated the quality parameters of the samples on a scale of 0 to 5. Eleven (11) grams of the medium roast and medium ground coffees were weighed into odourless ceramic cups. Fragrance was evaluated by sniffing the dry ground coffee. Boiling water 200mls was directly poured on to the roasted coffee and brewed for four minutes. The aroma was evaluated on breaking the crust. Acidity, body, presence of key flavour attributes and the preference were evaluated on tasting the coffee. Data from the sensory evaluation was analysed using SPSS version 11.5 for windows.

RESULTS AND DISCUSSION

Analysis of variance on the regressions of the data from the sensory evaluation showed that fragrance, aroma and acidity, had an influence on the preference of the coffee at a significance level of $p \leq 0.05$. The body of the brew did not show any influence on the preference of coffee at the same level of significance. The diversity of flavour profiles was demonstrated for the coffees with a variety of terms being used in their description as shown in Table 1. Coffees which were fruity or slightly fruity were preferred and were described as good to excellent. The off flavours that were found to reduce the preference included fermented, earthy, grassy and woody. Coffees which were fermented were described as undrinkable to bad. Fermented is an off flavour in coffee that produces a highly displeasing or stinking flavour depending on the degree of over fermentation. Woody is an unpleasant wood-like character in coffee. Earthy is an odour in the coffee that produces a dust like flavour while grassy depicts the smell of grass or hay in coffee. These undesirable flavour characteristics identified can be avoided in coffee by observing the correct processing procedures.

Table 1. Diverse flavour attributes identified in wet processed coffee samples from different regions.

Identified flavour characteristics	Frequency of the Preference Levels for the Specific Flavour characteristics										Total number of samples out of 159 containing the specific flavour characteristic
	0.50	1.00	1.50	2.00	2.50	3.00	3.50	4.00	4.50	5.00	
Burnt	-	2	2	1	-	-	-	-	-	-	5
Cereal	-	-	-	-	7	2	-	-	-	-	9
Dusty (Earthy)	-	-	-	1	1	1	-	-	-	-	3
Fermented	2	2	1	2	2	-	-	-	-	-	9
Flat	-	-	-	2	2	4	1	-	-	-	9
Fruity	-	-	-	-	-	1	6	16	6	3	32
Grassy	0	1	2	1	3	2	-	-	-	-	9
Harsh	-	-	-	-	3	5	1	-	-	-	9
Ordinary	-	-	-	-	4	6	-	-	-	-	10
Peasy	-	-	-	-	-	2	-	-	-	-	2
Juicy	-	-	-	-	-	6	2	2	1	-	11
Slight fruity	-	-	-	-	-	4	11	6	-	-	21
Sour	-	-	-	-	2	1	2	-	-	-	5
Unbalanced	-	-	-	-	4	5	5	-	-	-	14
Woody	-	-	1	1	7	2	-	-	-	-	11
Total											**159**

Preference scoring scale

5. Excellent
4. Very good.
3. Good
2. Bad
1. Very bad
0. Undrinkable
- Flavour category not present at the specific preference level

CONCLUSION

All the coffees receiving a score of four and above had exceptional flavour qualities. Coffee from the specific zones where such flavour attributes were produced should be characterised and considered as benchmarks for geographical indication selection. With the market demanding differentiated coffee flavour profiles, the diversity of flavour profiles described as fruity should be pursued as a marketing tool for Kenyan coffee in the different coffee market segments.

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Brazilian Coffee: Profile of Establishments Serving this Beverage

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SUMMARY

Supply and demand for coffee are growing fast in Brazilian cities, evidencing the need for studies on the attributes to be considered when dealing with the establishment of new coffee houses. A random sample survey of 99 coffee houses was accomplished in Sao Paulo, based on the census sectors. The owners or managers were interviewed in order to obtain data on the business features and perspectives, as well as their perception on the beverage quality and the client profile. Moreover, samples of grounded coffee were taken in these coffee houses for later sensorial analysis in a laboratory. It is shown that the espresso brewing exhibits the better sensorial quality, independently of the coffee house profile. On the other hand, only in 25% of the coffee houses the filtered coffee was under recommended patterns. Finally, it is shown that entrepreneurs serving beverages with superior or gourmet quality level were more optimistic respecting to their business when compared to those who are not concerned about their product quality.

RESUMO

A oferta e a demanda por café estão crescendo rapidamente nas cidades brasileiras, evidenciando a necessidade de estudos sobre os atributos a serem considerados quando se tratar do estabelecimento de novas cafeterias. Uma amostra aleatória de 99 locais de venda de café foi realizada em São Paulo, baseada nos setores censitários. Os proprietários ou gerentes foram entrevistados para se obter dados sobre as características e as perspectivas do negócio, bem como sua percepção a respeito da qualidade da bebida e do perfil dos clientes. Além disso, amostras de café torrado foram tomadas nesses estabelecimentos para posterior análise sensorial em laboratório. Mostra-se que o preparo expresso exibe melhor qualidade sensorial, independentemente do perfil do estabelecimento. Por outro lado, em somente 25% dos estabelecimentos o café filtrado estava dentro dos padrões recomendados. Finalmente, mostra-se que os empresários que servem bebidas com qualidade em nível superior ou gourmet são mais otimistas a respeito de seu negócio quando comparados com aqueles que não se preocupam com a qualidade de seu produto.

INTRODUCTION

Currently, Sao Paulo is the principal national centre for coffee business, with a raising number of stores serving the beverage. Drinking coffee out-of-home has become an important segment of this economic sector. Vegro et al. (2007) studied the habits and preferences of coffee consumers, showing that 85% are used to drink coffee, with no statistical differences between women and men, but with more men drinking out-of-home (70% against 59%). The aim of this paper is to give a brief profile of coffee houses in Sao Paulo.

METHODOLOGY

A cluster sample survey has been used to collect data on 99 coffee houses, understood as places out-of-home where coffee beverages are served, such as coffee shops, restaurants, bakeries, fast-food shops and so on. The owner or manager responded to a questionnaire about the business situation. A sample of the ground (or ground & roasted) coffee was collected (the raw material used in preparing the beverage) for later laboratory analysis. The sensorial analysis was made according to a Brazilian legal Resolution (SAA 37, Nov. 9, 2001), and the product was classified into one of four categories, from the worst to the best: non recommendable for supply, traditional, superior and gourmet.

RESULTS AND DISCUSSION

Filtered coffee is served in 75% and espresso coffee is served only in 25% of coffee houses in Sao Paulo. The sensorial quality was considered superior or traditional for most espresso coffees samples (Figure 1). The small percentage of gourmet coffees, even when espresso is served, indicates the wide opportunities for new high quality undertakings. On the other hand, more than 50% of filtered coffee were considered non recommendable for supply. This output may be much worse, since a good raw product may result in a bad beverage when not appropriately manipulated, as stated by Pino and Vegro (2003).

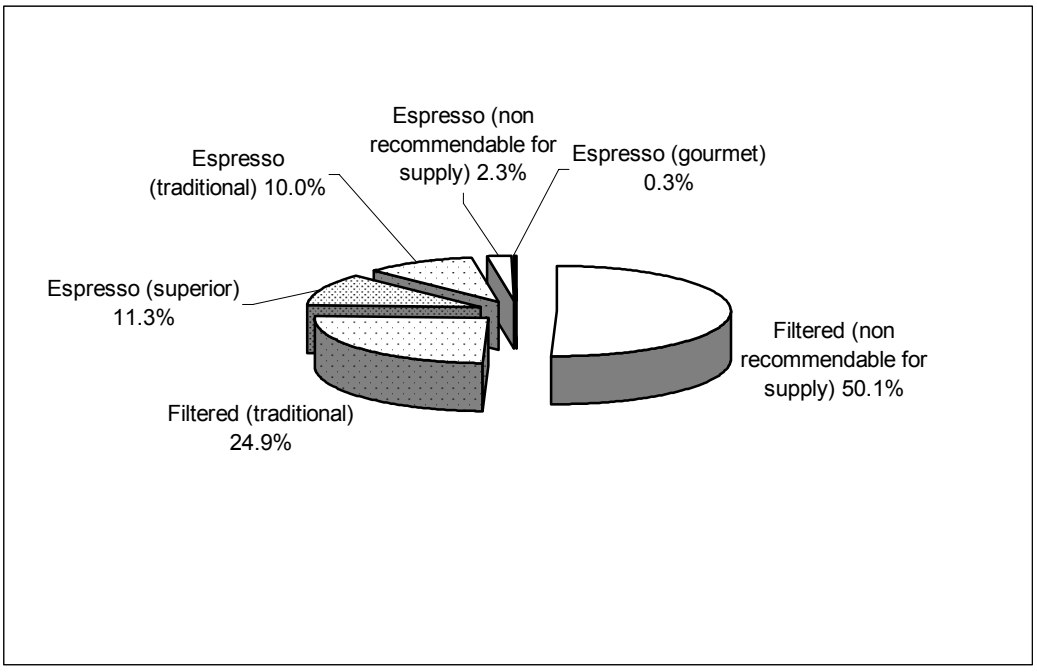

Figure 1. Sensorial quality and brewing, percentage of coffee houses, Sao Paulo, Brazil, 2006.

Filtered coffee is sold in stores serving up to 100 cups per day in any day of the week (Table 1). The large amount of non-respondents during weekends represents those working only in weekdays. However, most espresso coffee is sold in coffee houses serving up to 50 or from 101 to 200 cups per day during weekdays, and up to 50 or more than 200 cups per day during weekends. It is remarkable the trend of espresso towards the big coffee houses, mainly in the weekends, regardless of their small number. In Sao Paulo, many people use to visit malls for shopping or entertainment, and this habit influence coffee consumption during weekends.

Table 1. Business size and brewing, percentage of coffee houses, Sao Paulo, Brazil, 2006.

Period	Average number of cups sold per day	Espresso	Filtered
Weekday	Up to 50	9.1	30.9
	51 to 100	3.7	21.7
	101 to 200	8.3	16.1
	More than 200	1.7	6.3
	Non respondents	2.2	0.0
	Total	24.9	75.0
Weekend	Up to 50	7.0	23.8
	51 to 100	3.3	23.9
	101 to 200	2.9	7.7
	More than 200	5.2	4.2
	Non respondents	6.4	15.4
	Total	24.9	75.0

Source: Institute of Agricultural Economics, Sao Paulo, Brazil.

Filtered coffee rate on invoicing never exceeds 10%, but espresso rate is greater than 20% in 22% of coffee houses, mainly in those specialized in serving coffee based beverages (Table 2). Clearly filtered coffee seems to be served in non-specialized stores, contrariwise the espresso.

Table 2. Coffee rate on invoicing and brewing, percentage of coffee houses, Sao Paulo, Brazil, 2006.

Coffee rate on invoicing (%)	Espresso	Filtered
Up to 3	41.0	55.1
3.01 to 5	9.3	27.6
5.01 to 10	22.8	15.9
10.01 to 20	4.8	0.0
More than 20	22.1	0.0
Unknown	0.0	1.4
Total	100.0	100.0

Source: Institute of Agricultural Economics, Sao Paulo, Brazil.

The propensity to investment is an indicator of the potentiality or the inherent capacity for growth of each kind of coffee house (Table 3). Therefore, most places serving filtered coffee have no interest in investments, and only a few are expecting to modernize equipments. On the other hand, places serving espresso are interested not only in equipment modernization, but also in store expansion and labour qualification. The latter should be expected, as the barista, a professional who makes coffee in an espresso coffee bar, is essential to this activity.

Most owners and managers feel the sales in the day of the interview as similar to those one day, or one week, or one year, or three years ago. However, the optimistic ones see them as increasing sales (Table 4). Although there is not a perfect correlation, it seems that as better the coffee quality is, as optimistic they are.

Table 3. Propensity to investment and brewing, percentage of coffee houses, Sao Paulo, Brazil, 2006.

Propensity to investment	Espresso*	Filtered*
Store expansion	3.8	7.3
Equipment modernization	13.1	16.5
Labour qualification	8.9	1.0
Coffee drinks diversification	0.0	1.0
Other investments	0.2	0.0
No investments	9.2	52.2
Franchising store	1.9	0.0

**Multiple answers allowed.*
Source: Institute of Agricultural Economics, Sao Paulo, Brazil.

Table 4. Sensorial quality of beverage and increasing sales expected by owners and managers, percentage of coffee houses, Sao Paulo, Brazil, 2006.

Relatively to	Non recommendable for supply	Traditional	Superior and gourmet
Yesterday	27.5	34.5	30.5
One week ago	18.7	9.6	26.3
One year ago	10.0	15.5	21.1
Three years ago	22.7	37.7	19.2

Source: Institute of Agricultural Economics, Sao Paulo, Brazil.

FINAL REMARKS

Nowadays, the most dynamic segment of coffee business seems to be the out-of-home consumption in large urban centres. In Sao Paulo, filtered coffee is still prevalent, but espresso rate is increasing due to the better quality of the cups served. The process appears not to have attained a climax.

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History, Culture and Tradition: Strategies for Market Social Construction for Coffee

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SUMMARY

The search for strategies for reaching out consumers through the social construction of the market is a way of family farmers to generate income. This study assessed, from a community of farmers in the district of Alto Paraíso, State of Goiás, the insertion of local coffee production into agribusiness. In recent years, people from that region realized the existence of a coffee that can be sold in niche markets. Once the coffee is traditionally produced in agroecological systems and have characteristics of flavor, aroma and texture of the region, it can fit in the market of organic coffee and origin certification. Located in an area not traditionally producing coffee, it is believed that the best strategy for the product to reach consumers is the social construction of the market. This study is the result of a master's dissertation presented at the University of Brasilia.

INTRODUCTION

The Brazilian market is currently expanding. The domestic consumption increased 19.2% between the years 2003/2006. Also, in 2007, the demand for coffee, in Brazil, was greater than the supply. It is estimated that until the year 2010 the country will consume, per year, 21 million bags of coffee. To fill that market, the productive sector must increase production by 10%. One of the alternatives suggested by the industry to improve the supply is the opening of new areas of production (Reetz et al., 2007).

For the farmer family, the coffee market is promising. The family farming already corresponds up to 25% of Brazilian production, generating, directly and indirectly, about 8.4 million jobs. Coffee production is relatively intensive in the use of labour when compared with other plantations.

In the global market, the Brazilian coffee has been losing its share in recent years, a period when the country fell from 80% of exports, recorded at the beginning of the century to the present 25%. Commodity has been the main export product of the Brazilian coffee, but the agents of the productive chain has sought adjustment to the specialty coffee market and has increased this type of production at geometrical rates.

According to Vegro (2007), a business strategy for searching markets is to differentiate the coffee, segment the market and supply it with a product of good quality.

MATERIAL AND METHODS

The study was conducted between March 2006 and December 2007 and supported by the existing tools of theoretical knowledge of Sociology, Anthropology and Agribusiness. At first, a survey of the region's history was conducted through literature review in books, documents,

various official pages on the Internet, doctoral theses and dissertations on the subject and the region.

The research is characterized by single case study as a methodological resource. For a better understanding of the region and the activities of the district of Alto Paraíso, visits to properties of the family farmers of three production regions were carried out. Semi-structured questionnaires were applied to 28 farmers, through personal interviews, in order to raise data on the activities undertaken by families, on the living conditions and on the knowledge of traditional coffee culture of the region.

RESULTS AND DISCUSSION

The district of Alto Paraíso is located in the northeast region of the state of Goiás, about 250 kilometers from Brasília, in the Cerrado biome. This region is known for its natural beauty which combines mysticism and esoterism, attracting people from various sects and religions. At the beginning of its occupation, in the eighteenth century, the main economical activities were mining, agriculture and livestock. However, the region turned its productive vocation when people started to develop activities related to tourism instead of food production and natural resources exploitation. This milestone in its history came from the decade of 1960, with the creation of the Chapada dos Veadeiros National Park, located in the region, along with the transfer of the Brazilian Federal Capital to a nearby location. To preserve the area adjacent to the National Park, it was created in 2001, the Environmental Protection Area (APA) called Pouso Alto, which has 872,000 hectares, where a large portion of the district is inserted. Meanwhile, the trajectory of tourism exploitation was interrupted since 2000, when visitors started avoiding the region due to public health problems.

One hundred producers have been registered in the district's Rural Union, and about 70% are family farmers. In recent years, with increasing global demand for organic and origin certificated coffee, the producers realized the existence in the district of a coffee that can fit in this type of market, besides holding characteristics of flavor, aroma and texture specific to that region.

The coffee is traditionally produced in organic and agroecological systems and it is believed to be a very old variety, suitable for the production of specialty coffee. This coffee is also produced with social responsibility, especially because it is grown in properties of family farmers, being harvested and prepared with the care for the consumption of their own family.

The agricultural production in the region has the typical characteristics of the family farm established by Wanderley (1999), such as the ownership of the means of production and use of family labor. These families also present the characteristics mentioned by the Rurbano Project (SILVA, 2001) such as non-agricultural activities in urban areas and exploitation of the property with ecotourism and nature conservation.

In these properties, agricultural activity is generally carried out by only one member of the family, usually the eldest, which produces for the family consumption and has no formal education or professional qualification. With the strength of this work and support from retirement income, this people afford children studies in the city. According to the Rurbano Project, the rural property remains a refuge, an anchor, hosting the family members in times of crisis or to join for festivities.

In 67.85% of the properties visited, the agricultural production was used only for family consumption. The income of these families comes mainly from wages of one or more

members of the family that has a job in town or in the community. About 57% of these employees work for the local government and community and 43% of them work for tourism exploitation establishments such as restaurants, bars, hotels and inns. Around 28% of the households surveyed receive some king of benefit from the government, being the main income retirement pension. Only 10.78% claimed to have regular access to technical assistance and rural extension. Only one property between visited (3.57%) has more than three employees. In all others, the work of land is done only by family members, in most cases part-time. Of the people surveyed, 46.42% were over 60 years old, and that they were taking care of the activities of the property alone.

The size of the properties is not always accurate, since there is no land demarcation in the region. Of the respondents, 42.85%, claim to have ownership rights to the properties, due to residence and use of land for over 40 years. Agricultural production is diversified. They produce, mainly food, such as various types of vegetables and fruits, beans, rice, maize and cassava, in addition to the sugarcane. The cattle and small animals are used for the consumption of meat and byproducts by the family. Some medical herbs from the cerrado biome were also found in all properties visited. Few are the products marketed by farmers, usually on the weekly market in the city, where small animals, agricultural products, vegetables and fruits from the Cerrado, and prepared foods such as jellies and wines are also traded.

Coffee came to the northeastern region of Goiás in early eighteenth century, probably brought by *Bandeirantes*, the first explorers of the region. The first raids began in 1722, when the caravans came out from São Paulo towards the Brazilian interior in search for gold, and aiming to capture indians to be used as forced labour (ATTUCH, 2006). As the troops remained in the region for up to five years and the transport of food from São Paulo and Rio de Janeiro was very expensive, the products consumed by them were planted along the rivers and lagoons. The existing coffee in Alto Paraíso is probably one of the agricultural products planted in that period, since there are very old trees in the middle of the forest still preserved.
Regarding the production of coffee in the field, it is known that the region has microclimate and altitudes suitable for the regular production of good quality coffee. According to Mello (2001), the regions in Brazil that have the most favorable conditions for the production of quality coffees are the Cerrado regions. This author also cites the coffee grown in mountain regions and high humidity regions as good options for the preparation of pulped coffee. And also points out features favorable to the production of good quality coffee: a) lower temperatures, present in most regions of high altitude; b) well defined rainy season, with a dry period coincident with the time of harvest, and c) low humidity of the air, giving conditions to avoid unwanted fermentation in fruit. All these features are present in the northeastern region of Goias.

Alto Paraíso has altitude of approximately 1,300 m. Its climate is characterized by a dry and cold winter and mild and humid summer. According to Assad et al. (2000), the region presents an index of annual rainfall between 1500 and 1600 mm, with a well defined rainy season and an average annual temperature of 20.8 °C. The rainy season is characterized by the occurrence of 51% of the total annual rainfall between the months of January-April, and 42% between October-December. The dry period occurs between the months of May-September, accounting for only 7% of the total annual rainfall. Taking into account the average temperature of the air, the hottest month in the region is January (22.2 °C) and the coldest month is July (19.1 °C). With these characteristics, Alto Paraíso is located in a favorable area for the production of *Coffea arabica*, where irrigation is used to make up for the water deficit in the driest periods of the year (Assad et al., 2000).

CONCLUSION

As one of the results of the survey conducted in the region, it was observed that the northeastern region of the state of Goiás has no tradition in coffee production. Therefore producers must seek factors that can add value to their product in order to have a greater chance to compete in a niche market and with those produced in other states that traditionally produce coffee in Brazil. Once the coffee has reached the region in the eighteenth century, it can be inserted into the niche market of coffees of origin certification. That's because the region has genetic material with production in the same place for several years, and has even climate, ecosystem, soil and topography, in addition to the treatment and cultural practices of processing and storage that are local.

Moreover, the coffee produced there can be inserted in the niche market for organic or agroecological coffee. The inclusion in that market is facilitated by the fact that coffee is being grown in an area where the imposition of restriction of the use of chemicals and agricultural machinery is already institutionalized and regulated by federal and state laws.

According to Wilkinson (2004), the small volume production combined with the family farming becomes a strategic advantage, once it is associated with tradition, nature, crafts, and local values. This author emphasizes that all these factors together are rewarded by the market. It is believed, therefore, that this region, especially Alto Paraíso district and the other districts of northeastern Goiás, which have similarities in climate, topography and ecosystem, have intrinsic advantages for the establishment of a culture of coffee that can be inserted into niche markets for specialty coffees, increasing the value of the coffee produced there.

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Justifications and Motivations for the Consumption or Not of Coffee: a Mineiro Market Study

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SUMMARY

The study of coffee consumers profile and factors involved in the process of purchasing coffee is an important tool in identifying the different segments of this market and its potential. Thus it was aimed to find the motivating factors for the consumption of coffee. This way, 250 consumers were interviewed and the results were evaluated in a descriptive way. The results showed the profile of consumers and the factors involved in the consumption of coffee. In general, all groups formed associated the consumption of coffee to the habit, pleasure, family, friendship and work. It indicates the motivations for their consumption and reflects the social significance of the drink either at home, at work or in the circle of friendship and this meaning is strongly connected to the customs and habits of Brazilian society.

INTRODUCTION

The industries and institutions related to production and processing of coffee are the most attentive to the behavior and demand of consumers. The increasing of studies in this area and researches with the Brazilian consumers show such concern. The study by market segmentation is a tool that allows to analyze the particularities of each group and to serve them in a more effective way. However, the segmentation of the market for coffee must be carefully conducted because the consumer has coffee at different times and for various reasons. The consumption of coffee brings together a number of social and behavioral factors that vary individually. Thus, the act of drinking coffee may assume various connotations for the consumer and can be seen from the point of view of tangible aspect, or else the satisfaction of physiological needs, such as the satisfy hunger and thirst, and the intangible aspect, related to the achievement of desires, how to get happy (Sette, 2000; Cobra, 2006). On the other hand, understanding the reasons for what some people do not consume the drink is also important to discover the motives which influence their attitude and to investigate possible gaps that could be explored in the identification of a new market segment. This way, it was aimed to characterize the profile of consumers of Belo Horizonte / MG and study the main motivations for consumption or not consumption of coffee, and list the main substitutes of the drink.

MATERIALS AND METHODS

It was made an intentional and random sample, statistically significant, composed by 250 consumers, residing in Belo Horizonte / MG, interviewed between May the 8th up to 11th, 2007. The interviewees were asked through structured questionnaires with exploratory questions (Table 1). The questions were formulated based on a previous study (Arruda et al., 2007) and other studies with consumers of coffee (Sette, 2000).

Table 1. Summary of issues presented to consumers and not consumers of coffee.

Content of issues	
Coffee consumers	**Non coffee consumers**
Gender, age, marital status, educational level, occupation	Gender, age, marital status, educational level, occupation
What is the most consumed beverage?	Reason not to consume.
How many cups consumed a day?	Substitute of coffee.
What type of coffee consumed?	Isolated attribute of coffee appreciated.
What is the place where coffee is more consumed?	Changes suggested in the product
What time of greater consumption of coffee?	Possibility of consumption of a beverage with functionalities
What attributes observed in the purchase of coffee?	
Possibility of purchasing new product.	
Which are the elements related to the consumption of coffee?	
Disliked features in coffee	
Possibility of consumption of a beverage with functionalities	

** Issues closed with answers according to each question.*

The operating procedures of analysis were conducted with the aid of software SPSS 15.0 ®, a licensed version. The assessment was realized through exploratory descriptive analysis of data on questionnaires applied to non-consumers of coffee. For data on consumers of coffee, besides of the exploratory analysis, the test took place to verify the adequacy of the daily amount consumed and the amount recommended by the experts and the existence of difference between the amount consumed by men and women.

RESULTS AND DISCUSSIONS

The results showed that only 17% of the interviewees did not consume coffee, mainly among them those aged below 30 years, demonstrating that young consumers have shown more resistance to the consumption of coffee. This result is in agreement with the research made by Sette (2000) and Arruda et al. (2007), where a small portion of young people consumes coffee and related this consumption with overtaken habits. The interviewees pointed out as the main reason not to consume coffee the non appreciation of its taste (Figure 1). In general, negative evaluation of taste is related to the bitter taste of few coffees, usually caused by poor quality of the powder. The coffee was also rejected by medical restrictions and lack of habit of consuming it. Concerning to drinks that replace coffee, consumers showed teas, juices and milk as the main alternatives (Figure 2). Although these interviewees do not make use of coffee, its aroma is an appreciated of the product. When asked about the possibility of consumption of a product based on functional characteristics of coffee, 42.9% of respondents said they would buy it, demonstrating the possibility of exploring the market for non-consumer of coffee through innovations of the food industry. Among the consumers of coffee, it is cited by 23.3% of respondents as the most consumed beverage, and occupies a position primacy on consumer's preference. Through t-test it was observed that there is a significant difference ($p < 0.05$) between the quantity of coffee consumed daily by men and women. The consumption among men is higher, showing average of 4.2 cups, with standard deviation of 0.302, compared to women results, in which the average is 3.4 with a standard deviation of

0229 cups daily. The analysis of daily amount consumed showed no significant difference ($p > 0.05$) between the average consumption of the sample (3.75) and the amount recommended by the experts. According to LIMA (2007), the consumption of up to four cups a day is suitable for all ages, including children and adolescents. The type of coffee consumed is predominantly percolated and filtered (91%), from traditional powder (Figure 3) and the domestic environment was the main place of consumption of coffee (71%), followed by snack bars (19%) and working environment (8.6%) (Figura 4). The cafeteria is the place where coffee is least consumed, demonstrating that despite the significant growth of the sector in recent years and improvement in services and quality of products, this segment reaches a specific profile of consumers, generally those who enjoys higher quality of coffee, prepared to pay more for the product, therefore enhancing the physical space of the establishment and the services of specialized staff to serve coffee. Brand, quality, purity seal and price, were ordered as attributes observed in choosing the product. It was noticed that the purity seal is perceived as a way for validating the quality of the product and when it is present, the credibility of the mark is increased. When asked about the rejection of any attribute of coffee, 69% of respondents replied that they did not reject any feature in the drink. 31% responded affirmatively and out of those, 11% reject the variations in flavor and aroma generated from powder of poor quality, which shows that consumers are increasingly demanding regarding to the quality of coffee (COBRA, 2006), and that efforts implemented in the cultivation, harvesting and processing of coffee for a better quality, cause greater consumer satisfaction. The coffee was associated for most consumers (38.1%) to the habit, showing that they reproduce the behavior learned from their history. Pleasure was the second element most associated with the consumption of coffee, showing the degree of affection aroused by the drink, as previously noted when a comparison was made of preference for coffee and other products. It is important to highlight the association of coffee with information on social features, as friendship, social and family, revealing its role as an aggregator in different environments. The association of coffee with work and study allows to infer the relationship between these elements and its stimulant effect at the same time that permits a pause in these activities. Thus, coffee is used to meet the physiological and psychological needs of consumers.

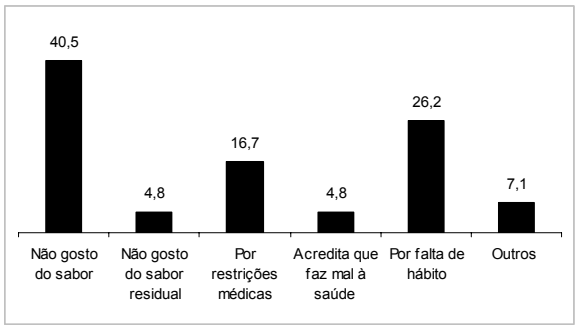

Figure 1. Major reasons for non-consumption of coffee, expressed in percentage.

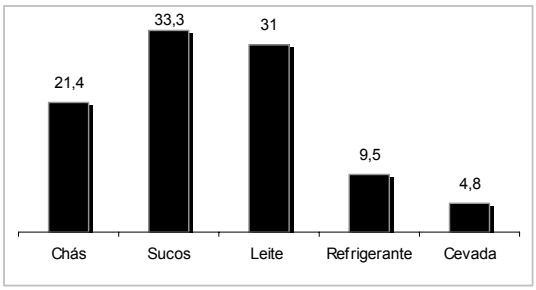

Figure 2. Products used as substitutes for coffee, expressed in percentage.

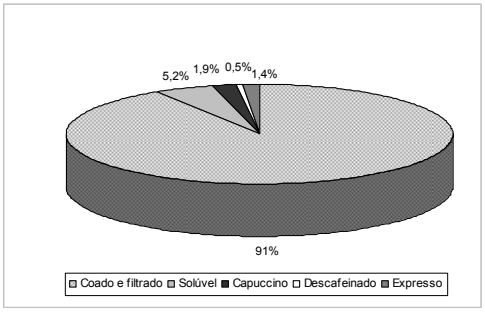

Figure 3. Type of coffee consumed by interviewees.

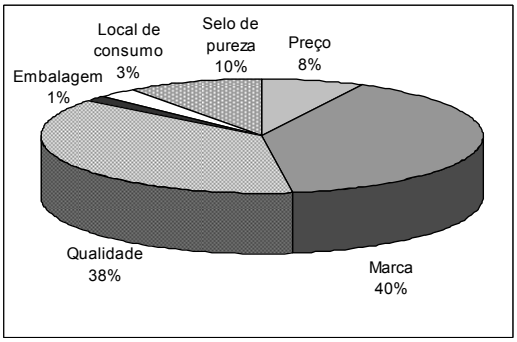

Figure 4. Attributes observed in the purchase of coffee.

CONCLUSIONS

Coffee occupies position of prominence among the drinks most consumed by Brazilians, but there is a need to position of this product among young people, as a market to be tapped. The tea, milk and juices are the substitutes for coffee and suggest the possibility of using them as a basis in the creation of a new product of coffee, adding their qualities and consumer preference of the characteristics of coffee, in the challenge of attracting new markets. The aroma is the most appreciated attribute of coffee, chosen by consumers and non consumers. In general, consumers associated the consumption of the coffee to habit, pleasure, family, friendship and work. The consumption of coffee surpasses the field of satisfaction of physiological needs, and also involves the psychological needs, such as relationships, personal and professional satisfaction.

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Application of Grouping Analysis in a Study of Coffee Consumer Market

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SUMMARY

It was used to determine groups of coffee consumers the analysis of groups based on motivating factors which influence people's behavior. This way, 250 consumers from Belo Horizonte/MG were interviewed. The results permitted us to determine the profile of consumers and the factors involved in the consumption of coffee. In general, the groups formed associated the consumption of coffee to habit, pleasure, family, friendship and work. This reflects the social significance of the beverage either at home, work or in the circle of friendship and it also means that coffee is linked to habits and customs of Brazilian society.

INTRODUCTION

The diversity of the consumer market rarely makes a unique product or service to satisfy everyone. To solve this problem, a strategy of market segmentation is used to divide the market into segments with similar characteristics. This is made through analysis of demographic differences, psychological and behavioral of consumers. This study allows the creation of targeted and profit markets, based on developing suitable products for it, which decreases the likelihood of rejection of it. Thus, there is a product or service best suited to a target audience, which makes the choice of distribution channels and communication easier. Accordingly, the cluster analysis is a useful tool in the study of groups of consumers, because it allows us to gather them together according to their similarities. The analysis of a group or cluster is the name given to a group of multivariate techniques whose primary purpose is to add objects based on the characteristics they have, forming groups with high internal consistency (in the groups) and heterogeneity taken externally (among groups). If the classification is successful, when presented graphically, the objects within the same group are close to each other and different groups are distant. This technique involves the following steps: selection of a measure of distance, choosing an agglomeration procedure; choice of the number of groups, and interpretation and description of them. Thus, it is aimed through the analysis of groups to research the consumer market of coffee in the city of Belo Horizonte / MG, in order to determine groups of consumers that allow us the study of the profile and motivations for the consumption of coffee in each group.

MATERIALS AND METHODS

It was made an intentional and random sample, statistically significant, composed by 250 consumers, residing in Belo Horizonte / MG, interviewed between May 8th up to 11^{th,} 2007. The interviewees were asked through structured questionnaires with exploratory questions (Table 1). The questions were formulated based on previous study (Arruda et al., 2007) and other studies with consumers of coffee (Interciense Informação e tecnologia: Vegro et al., 2002).

Table 1. Summary of questions posed to consumers of coffee.

Content of issues	
Gender, age, marital status, education level, occupation, income	
Consumption by habit	Consumption at the end of meals
Consumption for pleasure	Consumption to heat
Consumption to relax	Consumption in the range of work or study
Consumption at home	Consumption to interact with friends
Consumption to relax with friends	Consumption to be available for work or study
I believe that coffee brings people together	I believe that the coffee is harmful to health
Join the coffee with the act of smoking	
* Issues evaluated on a scale from 1 (strongly agree) to 5 (strongly disagree)	

Initially there was the factor analysis, using the analysis of main components and the procedure varimax. With the data of the factor analysis, the group analysis was developed using the hierarchyc group of Ward and the Euclidian distance was used as an instrument of measuring the similarities between the clusters. After evaluating data, the groups were selected, named, interpreted. For interpretation of the groups formed was carried out an analysis of factorial scores, classifying them into low, very low, medium, high and very high, where the limits represent the average (0), plus ½, plus one (1), less ½ and minus one (1) standard deviation. Finally, there was the descriptive analysis of groups formed to review the characteristics and behavior of each group.

RESULTS AND DISCUSSIONS

Depending on the analysis of the factorial loads after orthogonal rotation by VARIMAX method, it was possible to classify four factors on the motivations for consumer behavior and define them according to their homogeneous representation, as follows: psychological effect, habits, health and company effect. The analysis of group held to complement factor analysis, resulted in different groups of consumers.

Depending on the combination of factorial scores, consumers were classified into three groups based on motivation for the consumption of coffee. They were: outside the psychological effect, motivated by psychological effects, motivated by sets of factors, motivated by the society. In Figure 1 is presented the view of these groups into a plan dimensional. Since it wasn't possible to draw it in four dimensions, there are the three dimensions that represent the main factors, validating the proposal analysis.

The size of the groups must be related to the size of the circles. The summary of the characteristics of each group are presented in Table 2. In all groups formed, the coffee is a highly consumed product and it is between the more appreciated drinks by consumers.

The type used is the percolated and filtered, and the place of consumption is the domestic environment. The instant coffee, decaffeinated cappuccino and the variation cappuccino represented together a very small share of consumption. The organic and gourmet coffee were not even cited by consumers. Similarly, a search of ABIC (2005) showed that 96% of consumed coffee in Brazil in 2005, was kind of conventional roasted and ground, even with the increase in organic and decaffeinated types, compared with the previous two years. Other authors (Arruda et al., 2007; Vegro et al., 2002) also noticed this behavior.

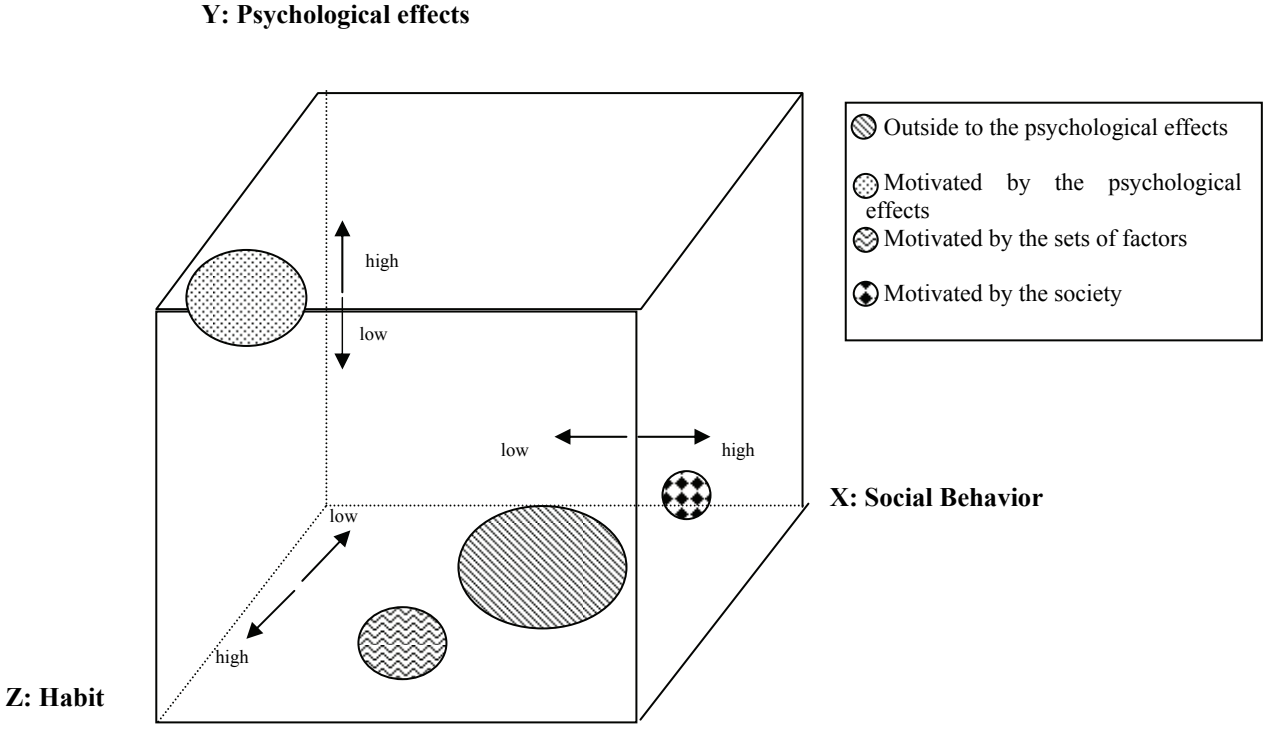

Figure 1. Groups of consumers of coffee in Belo Horizonte / MG.

Table 2. Summary of the main characteristics of formed groups.

GROUPS	PROFILE	ASPECTS OF CONSUMPTION OF COFFEE
Outside the psychological	Majority women, high schooling, various professions.	4 cups daily; type percolated and filtered, and smaller quantity soluble and expressed; quality notes; reject the bad quality of coffee and related habit, pleasure, family and friendship.
Driven by the psychological effects	Men and women, low education, professions varied.	3 cups daily; type percolated and filtered; consume at home and at work, note brand; believe that the coffee cause insomnia and related habits, family, and pleasure.
Driven by all the factors	Majority men, education and various professions.	4 cups daily; type percolated and filtered, soluble, cappuccino and expressed; consume in snack bars and cafeterias; note brand and quality; rejected the bitter taste of poor quality and coffee; related to the habit, pleasure, work and friendship.
Driven by social	Uniformity of sex, high school, various professions.	Group of largest amount consumed, over 5 cups daily; type percolated and filtered; consume at home, and snack bars, it is the type most consumed at work, they note brand, quality and price, reject coffee of poor quality and it is related to the habit, pleasure, social coexistence, friendship and work.

It was also consensus among the groups the importance of brand and quality in the process of buying the coffee, revealing the trust relationship established between the brand and the

consumer, who shows loyalty to the industry. Although there is the high degree of affection of consumers in relation to coffee, there are also features rejected by the groups: bitter taste, causing insomnia and characteristics of poor quality were raised as negative aspects related to coffee by the groups formed. The association of coffee with habits, pleasure, family, friendship and work indicates the main reasons for its consumption and reflects the social significance of the drink at home, work or the circle of friendship, and this meaning is strongly connected to habits and customs of Brazilian society. The consumers satisfy not only a physiological need, but also psychological, as relationships, personal and professional satisfaction. Coffee is seen as a great facilitator of relationships. In addition, the association of coffee to the pleasure to a quality drink has been growing for the maintenance of the product in social character (Deboçã et al., 2004; Sette, 2000). These factors are resources that can be used in the advertisements, seeking to relate pleasant moments, acts of affection and attention with family and friends, and representatives of professional success.

CONCLUSIONS

The segmentation of the market of coffee consumers through the analysis of group, based on the study of the motivations for consumption, showed that consumers in Belo Horizonte / MG follow the trend of domestic consumption, highlighting the quality of the drink, produced by the roasted and ground coffee, consumed at home or at work, in amounts close to four cups a day.

In general, all groups formed associated the consumption of coffee to the habit, pleasure, family, friendship and work. This indicates the motivations for their consumption and reflects the social significance of the beverage at home, work or in the circle of friendship and this meaning is strongly associated with the customs and habits of Brazilian society.

The coffee consumption surpasses the field of satisfaction of physiological needs, and also involves the psychological needs, such as relationships, personal and professional satisfaction.

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Enhancement of Primary Wet Coffee Processing Capacity in Kenya

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SUMMARY

Congestion in a primary coffee processing factory can have serious coffee quality implications. Therefore, due to its persistence mainly in the cooperative sector, baseline coffee processing capacity surveys have been undertaken though to a limited extent so far. These surveys have revealed the prevailing capacity status at any processing stage along the coffee processing chain. Such information can be used to: determine any additional cherry intake which can be accepted by a factory, upgrade the capacities to the correct standard design where found inadequate or establish the need for an additional new factory. Although in some cases the construction of new factories might be considered, alternative viable options which can generally reinvigorate the processing capacity are worth considering prior to making such a costly decision. Such intervention can make the coffee processing capacity to suffice without significant physical modifications along the coffee processing chain. It is deemed that by so doing, a coffee quality assurance programme shall be sustained with ease in all wet coffee factories in Kenya.

INTRODUCTION

The design of a primary wet coffee processing factory is mainly a function of the process technology characteristics at all the stages along the processing chain, pulping and pre-grading machinery capacity, method of drying (mechanical dryers or solar dependent drying tables), altitude, projected annual cherry intake capacity and the number of seasons per year (Mburu, 2004). Therefore, bottlenecks are not expected at any stage of the processing chain. However, critical congestion at the primary coffee factories during the peak season still prevails even when the annual production is below half the maximum. For that reason, a sudden drop in quality has also been observed sometimes in some consignments from coffee factories, which do commonly deliver high grade coffee. It is therefore necessary to find ways and means on how to handle such situations in an economic way without loss of quality and quantity. A strong tendency is normally to construct more factories in order to release the existing factories but bearing in mind that coffee is a seasonal crop, the factory will only be in use for only a short time of the year. However, though the construction of new factories might be considered in some cases, in general the existing processing capacity in all coffee growing areas suffices greatly. Besides, an idle factory nevertheless consumes capital in the form of depreciation among other factors. Therefore, the more factories there will be, the higher the cost of processing coffee. There being no doubt that substantial income is lost in this way, compromising the health and safety of the consumers becomes even more likely.

Besides that, there is competition between sun drying of coffee and waste disposal for the limited factory land. In most factories the need for minimizing processing water use does not feature highly in the factory management (Mburu et al., 1994). As such, processing proceeds unabated without water circulation most of the time until a crisis builds up calling for

stringent measures. The response to such a crisis is therefore too late and pollution of the rivers may not be prevented successfully. Secondly, although primary coffee factories have been encountering various problems, the causes have not been clearly identified or quantified. However, through continued research and development of the coffee processing system and technology, the various stages along the coffee processing can now be executed with higher efficiency. In view of this, coffee factory capacity surveys to establish the status of the existing processing capacities along the coffee processing chain were initiated in year 2005 and are still in progress. The provisional results of the surveys so far have exposed the specific coffee processing status at all the stages of the processing chain. The necessary interventions as the results imply will either be by simple upgrading or replacement of some facilities with the latest more advanced and economically viable technologies to improve the coffee processing capacity. This is mainly the subject of this paper.

THE CURRENT COFFEE PROCESSING CAPACITY STATUS

The results of the baseline surveys (Table 1) revealed excess fermentation capacity in the small holder processing plant (SHPP) category, the 3, 4, and 6 disc coffee factory design models to the extent of 55%, 84%, 100% and, 100% respectively while the 2 disc model had a 60% excess capacity at the storage stage. Otherwise, the rest of the processing stages in all the coffee factories were deficient in capacity.

From these results, the greatest deficit is at the drying stage mainly because of inadequate land for drying, competition between disposal of effluent and drying, frequent much longer drying times than necessary and the fact that recommendations are generally not strictly followed.

AVAILABLE PRACTICAL INTERVENTIONS

Management of the coffee factory

Training of coffee factory managers, technicians and operators to execute the process efficiently contributes greatly to the overall throughput of a primary coffee factory. This also serves to reduce drudgery normally associated with difficult coffee processing activities by for instance considering necessary breaks, shifts and other incentives. Such training should also create awareness of the critical control points and the implications of not implementing the necessary controls precisely. The technical staff should also know the utility of the machines and the equipment well in particular the aspects of troubleshooting and the corresponding repair and maintenance needs. For this purpose, capacity building in a primary coffee factory is no mean pre-requisite to the improvement of the coffee factory capacity. Managers should also know how to determine capacity as outlined by Mburu (2004) on routine basis in order to effectively control the entire coffee processing chain against the cherry intake and other variations likely to prevail at any time. Ultimately, the manager should appreciate that scheduled repair and maintenance of the entire factory machinery/equipment is vital for reliability and improved capacity.

Table 1.

Capacity Status %												
Model	Annual Cherry Intake		Fermentation		Skin drying		Final drying		Conditioning		Storage	
	Deficient	Excess	Deficient	Excess	Deficient	Excess	Deficient	Excess	Deficient	Excess	Deficient	Excess
SHPS	100	0	45.45	54.55	100	0	100	0	100	0	81.82	18.18
1	100	0	100	0	100	0	100	0	100	0	100	0
2	100	0	60	40	80	20	100	0	100	0	40	60
3	93.55	6.45	16.13	83.87	70.97	29.03	67.74	32.26	100	0	70.97	29.03
4	100	0	0	100	95.65	4.35	73.91	26.09	100	0	100	0
6	100	0	0	100	100	0	100	0	100	0	75	25

Pulping and Pre-grading

The pulping rate of a disc pulper which is in good mechanical order is 1000 kg of ripe cherry/disc/hr when the disc is rotating at 120 revolutions per minute (RPM) and in the right direction marked by an arrow on the disc surface. However, sometimes the flow of coffee ceases, or almost ceases possibly due to leaves, or twigs, having entered the pulper, causing a blockage; too high feed rate; seriously overripe coffee being pulped; undue delay between picking and pulping or insufficient water being supplied to the pulper. As a remedy to this, the machine should be stopped to clean the blockage, if necessary the breast can be removed to do so, the feed should also be cut off altogether until the machine clears itself. Overfeeding of cherry can also impose a big load to the pulper, which is likely to reduce the rotating speed. If the lowest possible feed is still too high the discs are probably worn out and ought to be sprayed to provide the required grip on the coffee. Finally the water flow should be increase accordingly. It is also worth noting that other types of pulpers need also to be attended to similarly. During pulping circulation of water recycled from the final grading of coffee contributes greatly to reduced water requirements hence easing the effluent disposal requirements. In addition to that the water input should be metered against the quantity of coffee being processed.

Ideally, the performance of the pre-grader should match that of the corresponding pulper. To achieve that, the pre-grader should be well adjusted in terms of water flow rate, screen depth and grading compartment regulation to ensure a precise cut off between the different coffee grade steams. Fortunately, the chain elevator for delivering parchment grade one to the fermentation tanks which, used to fail very frequently was ultimately replaced by a siphon system. The delivery pipe in such a system should be well inclined to the required maximum height for the pre-grader to perform optimally. Finally, in the event of an unexpected temporary breakdown of either equipment a suitable option like seeking help from a neighboring factory or overnight underwater storage of cherry should resorted to.

Mucilage removal and final grading

The fermentation process can be completed within 12 hours in the lower coffee growing areas but can take up to 4 days in the high altitude areas although, it is limited to a maximum of 48 hours. The now increasingly popular practice in all the coffee marketing sectors require 3 tanks daily to cater for all the coffee grades. However, in some cooperative factories where cherry is still sorted into 2 classes, 5 tanks are used daily. Therefore, if coffee is finally transferred out for soaking in the morning of the 3rd day following the day of pulping, then a total of 15 tanks plus 2 as a safety factor are provided. Rather than speed up the process, alternative options in the recent past have mainly considered the introduction of aqua pulpers, demucilagers, ecological units or even the adoption of chemical methods. However, such interventions perhaps accrue a substantial additional cost element, which the farmer might not be comfortable with.

In view of this, the use of concentrated enzymes made from lights coffee to complete the fermentation process within 6-8 hours presents a rather practicable solution (Calvert, 1988). By so doing, the fermentation space requirement can be reduced by 50% - 67% depending on the previous fermentation regime in practice. The benefit attributed to such an intervention is that less investment and recurrent costs will be committed towards easing congestion in a coffee factory. Besides that, the rise in the temperature of the water, concentration of sugar and enzymes due to the water circulation during pulping and pre-grading can hasten the fermentation process as well (Mburu, 1995). The fermentation process can also be enhanced by protecting the parchment from direct sunlight and the cold rain or painting the

fermentation tanks with a black paint to absorb and retain more warmth from the sun. Other suitable measures for improving fermentation include wind breaks and constructing fermentation tanks in the ground.

The final washing and grading of fully fermented parchment beans accounts for 22% of the total processing water requirements in a primary coffee factory circulating water during pulping. However, since the grading water is relatively clean, it can be circulated until the grading process is complete to reduce the processing water use. After grading parchment, recent findings (Mburu, 2007) indicate that the resultant water can also be recycled for pulping, transportation of parchment and intermediate washing during fermentation without compromising the quality of the coffee. By so doing, the processing water requirements can be lowered by almost 50%. The recycling of the final grading water speeds up the fermentation of parchment as well. Besides that, it has been proven possible to soak parchment in water (changed daily) for 7 days without affecting coffee quality (Mburu, 1997). Such a new window in technological development can make it possible to hold coffee at that stage while accepting more cherry and completing drying at the final drying tables. Besides that, pumping of wet coffee when transferring to different tanks and from the final grading channel to the skin drying tables or by gravity can help speed up the process as well.

Drying

Skin drying aims at removing the surface water and that between the parchment hull and the bean. It is one of the most critical stages in the wet processing chain since here an uncontrolled fermentation can continue when drying is done too slowly. Instead, it should be very rapid, in thin layers not exceeding 2.5 cm, involving vigorous stirring and using cold or war air without heating the beans, since otherwise high temperatures might develop inside the coffee and thus spoiling quality. It can take between one hour and a full day depending mainly on the prevailing weather, the management efficiency and the availability of space in the following stages of drying. However, prolonged skin drying times extending to overnight on the skin drying tables induces onion flavor, a general low quality and poor colours in the bean. To avoid such undesirable outcomes the operations on the tables must adhere to the stipulated recommendations.

During the skin drying stage, miscoloured and misshaped beans can be distinguished easily and therefore should be sorted out instead of at a later stage and thus making the best use of labour. Another labour saving procedure is to transport the parchment from the final washing or the soak tanks to skin drying tables by gravity through a conveyor channel. During the rain spells or when the weather is very dull and cold, skin drying might take too long a time and thus creating a bottleneck giving rise to poor qualities. A very successful way to overcome this problem is by using drying tents of plastic or PVC (Ilsley, 1973), any other cheaper but suitable alternative cover which will render useful services or appropriate mechanical driers. The temperature inside the tent will be higher than outside allowing the drying to continue. Also, during the night the parchment can remain open and thus drying will continue. Another way of performing the drying of wet parchment during rain is by stacking trays of wet parchment on racks under a corrugated iron shed. The same shed can be used for emergency bins. Otherwise, the parchment should never be left on the wet parchment tables because of lack of room on the drying tables but instead the drying area should be released.

Final drying and Conditioning drying is the most challenging link along the coffee processing chain since it is highly labour intensive and also requires a large area (Anon., 1990). However, since drying tables are too expensive to maintain and repair, they are generally not available adequately. In view of this, a reinforced PVC tent type (Ilsley, 1973)

can create conditions where in the tables remain covered with parchment and open for 24 hr a day from washing to bagging thereby shortening the drying time and hence raising the drying capacity tremendously. The heated air inside the tent also draws in a constant supply of cold air from below the tables. Further to that, the adoption of appropriate mechanical coffee driers at all the stages of the coffee processing chain except at the soft black stage can greatly improve the drying capacity.

Besides that, long rain spells in some cases can cause prolonged drying time though this is generally attributed to just poor management in most cases. This is because even in dull weather and occasional rain spells, it is easily possible to dry parchment within 14 days or even less by: opening the drying tables as early in the morning as possible and closing as late as possible in the evening during the peak time in order to make full use of each ray of sunshine; ensuring that all the tables are flat, well above the ground, constructed on proper benches if necessary, cleared from grass and bushes underneath and easily accessible; turning the coffee very frequently and; transporting the parchment to the store immediately it is fully dry. If the drying area is still insufficient, the hard parchment can be transported to the emergency bins and kept there until more tables become available again. Otherwise, the depth of coffee can be increased from 2.5 c to 5.0 cm in order to create more space on the table with effect from the medium black stage as long as stirring rate is regulated. Over drying should be avoided to ensure that parchment weight and quality is not needlessly lost and hence the financial returns accrued to it. Besides, the time wasted on over drying denies valuable space to the relatively wet coffee. Other aspects like pneumatic conveyance of dry parchment from the drying facilities to the store can save time as well.

At 10-10.5% moisture content, parchment coffee is in equilibrium with a relative humidity of about 60% at 21-22 °C of the surrounding atmosphere. However, weather conditions are quite often humid enough never to fall to this level and therefore coffee can take upto 20 days to dry satisfactory (Wooton et al 1968) and yet this coffee was at 12% after only 12 of these 20 days. It is quite likely that the 20th day was the first for more than a week during which the relative humidity fell appreciably from the invariably high night values and the final loss of moisture from 12 to 10.5% may have taken place in as little as 2 hours. During the same 8 days however, wet coffee would have lost considerable amount of water. In the field, therefore, it would be sensible practice to transfer near-dry coffee to the store in poor weather and so free drying tables for wet coffee. The unfinished parchment can be brought out again when warm and dry conditions return. These are therefore, just the sort of frustrating circumstances which justify the construction of ventilated conditioning bins. However, manually stirred conditioning bins as practiced by the cooperative coffee sector are hardly as effective as pneumatic bins.

Moisture measurement

This enables the process to proceed without unnecessary delay and important decisions to be made particularly the transfer of coffee to alternative facilities to decongest the tables or to the market. Precise measurement of the moisture content makes it certain to determine the full dry state of coffee when achieved. This avoids storage of under dry coffee and over-drying coffee leading to unnecessary losses. Therefore, in the absence of suitable moisture measurement methods, valuable time can be wasted along the coffee processing chain thereby impairing the factory processing capacity. It is imperative therefore that research in this aspect should be continued but with more vigour to provide a practicable solution since the costs of the available meters are beyond the farmer's affordability.

Storage

The coffee factory is supposed to have adequate storage capacity for the entire annual production where there is only one season, or 60% of the annual yield where 2 seasons prevail. There should also be adequate number of bags and labour for bagging and storage. That will ensure that the store does not pose any constraints to the full dry coffee from the drying tables. The store must also be well ventilated and able to sustain suitable temperature for the temporary coffee storage requirements. Finally, prompt dispatch of coffee to the miller for secondary processing and subsequent marketing is very essential.

Waste disposal

The seepage pits are recipients of raw pulping water, factory cleaning water as well as fermented wash water from intermediate washing, final washing and grading which more often than not cannot be effectively contained in the available pits (Mburu, 1998). These pits are then subject to overflow if their capacity is not adequate or if the surface rain water runoff accesses them. However, the disposal of the coffee pulp has not been a challenge in the industry as it is recycled back to the farm with eases besides being open to other practical utilization options. On the other hand minimization of the processing water requirements via its specific usage per tone of cherry offers only a partial solution. This is mainly because the effluent in the pits biodegrades into three (3) phases namely, the floating scum, the resultant relatively less loaded effluent and the sediment settled on the bottom and the sloppy sides of the pit. The scum impairs the loss of the effluent from the pit by evaporation while the sediment assisted by the water pressure makes sure that seepage ceases. The requirement of a substantial proportion of land for disposal of the effluent in competition with the drying of coffee hence still prevails.

For these reasons, it has been considered desirable to treat the effluent and separate settled solids out of the effluent prior to its release to the seepage pits (Wood *et al.,* 2000). Though this is subject to further investigation, the treated effluent would be expected to seep or evaporate out of the pit with ease thereby calling for limited but efficient pits. The pit performance can also be improved by protection from the surface water runoff from the nearby rain water catchments. Planting of suitable trees (Eucalyptus, bamboo etc) without shading is also being considered as a new option to help draw out the effluent from the pits. The improvement of the pit performance allows the release of some land to the drying process. Together with this it is important to avoid water leakages in a factory if complete processing water minimization is to be achieved. Since the processing of coffee is a chain, increased drying space will also facilitate for an increased capacity in the proceeding stages.

CONCLUSION

First, the coffee cherry delivered to a coffee factory must be suitable for the wet process because though processing cannot significantly add value to good coffee it cannot improve the quality of poor quality cherry. Secondly, a coffee factory should also be perceived as a food factory with hygienic conditions well in place particularly at the critical stages. The whole of the coffee processing system should be executed as per the existing recommendations. For this purpose, the factory managers, technicians and the operators must be well trained and very skilled in their routine duties. Since delays are undesirable particularly when the moisture content is high the resident time for coffee at any stage of the chain must be within the optimum limit. Therefore, any conditions which can easily reverse the process must not be tolerated. Rain can for instance cause severe damage to the bean quality and the nearer the coffee is dry; the more severe will be the effects of rewetting. It has also been known that

ageing of a coffee bean occurs instantly if the surface of the dry bean comes into contact with water. Fortunately, since the current coffee factory design came into effect, new and more efficient coffee processing methods have been developed or so some elements along the coffee processing chain revised using new research findings. Emerging divergent markets are also setting the stage for further reviews which will inject elasticity and the much desired safety factor with respect to processing capacity. Therefore, whenever the existing coffee processing capacity seems not to suffice these new interventions should be considered first prior to opting for an additional coffee factory. An ideal factory is on the other hand supposed to have the right coffee processing equipment at every stage of the coffee processing chain which are subjected to a strict schedule of service, repair and maintenance in order to be reliable throughout the season.

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Coffee Pulping Technology Impact over Economic Dimension*

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SUMMARY

This study is on economic impact assessment of coffee pulper technology in the São Paulo State, Brazil, from the 1990s to 2006. This innovation is an alternative procedure for post-harvest preparation of no-washed Brazilian coffees, with relevant advantages on product quality to the point of substituting the Colombian and Central American washed coffees in the blends of largest global roasters. We used indicators on transaction cost, productivity and risk, and structural changes, disposed in a relevance tree of indicators, employing *IMPACTOS* software developed by *Elabora* at the *Universidade Estadual de Campinas*. Surveys were applied *in loco*, also with deep interviews for complementary information. We used Lieckert's scale, which was converted in the interval ranging from –1 to 1, for aggregating values from the bottom to the top of the impact hierarchy structure. Values were weighted and pondered according to impact component weight given and answers cohesion. The sample was regionally stratified. The economic impacts of the technology were positive, with satisfactory data consistence. Transaction costs were lowered, investments were fostered and risks diminished.

INTRODUCTION

We intended to evaluate the economic impacts of the cherry coffee pulping technology concerning a broad range of indicators to discriminate selected impacts for different regions and agents. Creation of new technologies designed for the increase of input productivity in agriculture and, consequently, for the agricultural sector competitiveness, is a permanent guideline of the R&D policy. In the coffee production, a technology that promotes decreasing harvest and post-harvest costs and the search for quality enhancement for better drink lead the recent history of the Brazilian coffee production to be divided in two periods: before and after the emergence of the preparation of peeled cherry coffee (PC). The impacts derived from this technology for coffee preparation, had reconfigured the coffee supply pattern in the different producing belts, removing from the exclusion, the previously considered disqualified areas for the production of high quality coffees. The PC is obtained by the means of an intermediate process between the two most traditional existing modes of coffee preparation: i) natural (dry process) and; ii) the pulped ones (humid process). In the PC preparation, the grains harvested felt into the ground are washed for separating the over ripen coffee (by floatation), rocks and

pieces of soil. After that, the green, the cherry coffee and the raisins, are submitted to the pulper: a spinning framework for removing the outer skin and pulp portions from the coffee cherries and raisins. It also separates the whole green grains from the cherry and raisin coffee beans[1]. The absence of defects green and black beans) in the peeled cherry coffee lots (substantially improves the flavor of the drink. As disadvantage we can mention the fresh water needed for the PC preparation. The cumulative technological improvement of the equipment (new generations) is gradually reducing the water need for preparation. Beyond the intrinsic advantage of attainment a high quality product, obtaining a premium price comparing to the best natural coffees, the PC preparation allows the cost reduction of yard drying (up to 40% of the volume being dried during the harvesting season) and storage cost reduction (once rinds and part of the mucilage had been removed). Also, the PC can dispense the refinement as a consequence of its beans' high homogeneity. Selected electronically, the PC grains show fewer losses up to 6% of the prepared volume, comparing to the selection percentage of the lots naturally prepared. The impact assessment of adoption the coffee pulping equipment is interesting for many agents in the coffee business. From farmers, roasters to consumers, and the governmental credit policy that financed these investments, benefit from the impact information, also the equipment manufacturers can make strategic use in their business, because the impact assessment supplies elements for product improvement, market planning and sales force training. The absence of consensus on the possibility and interest of coffee producers to invest in equipments capable to improve the product quality, lead **(**Carvalho, 2000) to survey 10 farmers that had adopted the preparation of PC throughout 2 years, concluding that: a) for all the situations the production cost had increased; b) only justifies the investment in PC preparation if the market pays off; c) quality of product is more related to cost and profitability; and d) coffee producers need training to apply quality concepts and to better sell their product in the market. The inquire **(**Oliveira et al., 2005) on the economic viability of the investment on coffee pulper in Pirajú, SP, Brazil concluded that profits only reached advantageous level in the case of premium price paid for the product surpassed 50% of the regional natural coffee market price. When analyzing the determinant factors of the PC adoption (Monte and Teixeira, 2006), in the Venda Nova do Imigrante, ES, Brazil, region, the variables: yield, associative organizations, equity capital and education level, had been the most important in the decision on the adoption of the technology by coffee producers. Regarding the education level of the coffee producer, the authors concluded that the probability to find pulper machinery among those with a bachelor degree was the double comparing to the ones with the lowest education level, denoting the prevalence of capital and education for the adoption of this technology.

METHOD

The choice of the State of São Paulo for impact assessment of the adoption of pulper technology for coffee cherry and raisin peeling is strategic because in this state the coffee production presents great diversity of social and economic profiles, from small family farm to the larger entrepreneurial ones, and from micro climates less favorable to extremely vacationed location for the production of high quality coffee. Differentiated profiles allow that, in the field survey, the diversity of situations was disclosed, revealing the discriminating power of the method and the impacts intensity of the innovation at each stratum. The survey of the economic dimension was part of a complete study that dealt with other four dimensions of impact: social, environmental, managerial and quality of grains and beans. In a

[1] After separation of the rinds each coffee bean still has a parchment at the surface of it, what is dried by sun exposition and mechanical driers. The difference between the washed traditional coffee from the peeled-cherry-coffee is that the first has clean parchment while the second has mucilage residues on it.

multidimensional structure, each dimension has no relation with the other, and in the specific case of this article we deal only with the economic one. Therefore, the amount of indicators and the survey with deep interviews including closed and opened questions restricted the possibility of working with large samples, given the deep character of the study. The sample was constituted by 13 PC adopting producers. The regions chosen were: Piraju, São Manuel, Franca and Espírito Santo do Pinhal. We used the software *IMPACTOS* developed by the company *Elabora* in partnership with *UNICAMP*, for the economic dimension subdivision, weighing, elaboration of questionnaires, interviews and data reporting. The ultimate components for operating the measurement of impact, as well as their orientation (O) of scales are related below (Table 1).

Table 1. Impact components for economic dimension.

Component	Description*	O
Contractual Level	Change for transactions with written contracts.	+
Bargain Power	Capacity of appropriation of profits from buyers and with better terms of transactions.	+
Forward Processing	Adoption of an added value strategy by the means of processing, roasting and packing.	+
Geographical Asset Specificity	Dependence of the geographical location and its physical characteristics, primarily the climate conditions for taking advantage of the peeled coffee technology (PC).	-
Physical Asset Specificity	Relative amount of permanent capital (of specific use and low liquidity) in total assets.	-
Human Asset Specificity	Investment necessary for training, contracting, rewarding and to keep specialized workers in the PC facility and management.	-
Reputation	Signaling reliance to the market: long term contracting with buyers, trademarks recognition by consumers, lower cost or less bonds for loans in the market.	+
Process Vulnerability	Interruptions in the functioning and variation of the effectiveness of the equipment, damages, easiness to repair, technique assistance, electricity power breakdowns	-
Breed diversity	Number of varieties chosen in terms of harvesting period distribution for facilities optimization.	+
Economic Risk	Variations on the expected return of the enterprise, regarding gross revenue, costs and margins.	-
Fertilizer	Variation of the productivity of the fertilizer used and its marginal contribution in total product.	+
Labor	Variation of the labor productivity and its marginal contribution in total product.	+
Investment	Variation of facilities and machinery capital invested.	+
Concentration	Number and market share of buyers.	-
Security	Change in the security level of the income expected from the farm.	+
Stability	Change in the stability of the income expected from the farm.	+
Distribution	Change in the time distribution of the income along the year.	+
Amount	Change in the sum of income from the farm.	+
Income Source Diversity	Changes in the income sources portfolio.	+
Investment in facilities	Improvements in the farm facilities with new resource allocation.	+
Natural Resources Conservation	Change in the conservation of natural resources practices.	+
Legal Compliance	Changes of conformity with the legislation in the agricultural property.	+
Collective Pulper Facility	Adoption of collective processing facilities (for PC) under collective management.	+
Minimum Size Requirement	Minimum size necessary to take advantages of scale economy.	+

**After the adoption of PC (peeled cherry coffee).*

Source: components, its description, questions and impact direction orientation were submitted for key personnel representing different actors from the coffee industry.

The criteria tree was previously weighted (k) for each indicator regarding the contribution of lower components to the upper in the multi-criteria tree. For impact intensity measurement, we used questionnaires with Lieckert's scale converted into the interval (from - 1 to 1) for the purpose of aggregation, in which negative results means negative impact. The aggregation process balance the results from the bottom to the top of the criteria tree, regarding the convergence of the answers in each indicator (analyzing the frequency distributions the software calculates a measure of cohesion among interviewees). For analyzing the economic impact, we defined a limit for answers cohesion (z) in each stratum or sample, Z=0.75 as a minimum tolerance level for ambiguity, in which the best measure is given by the stratum in which $Z \geq 0.75$ and not by the aggregated result of the assessment. We asked for complementary qualitative information during the interviews, as support for the impact assessment explanation, regarding its intensity and stratification, and the interpretation and confirmation of each answer. A coefficient (α) for technology impact attribution for each component was answered in the survey, what allows isolating its contribution from the intervenient or exogenous contributions in the context of change, therefore consists the interviewee technology impact's attribution perception. Thus, we have the general context impact (IG) and its data cohesion ($Z(x)$); technology attribution for the impact (IPC) and its data cohesion ($Z(\alpha)$); and impacts from other causes (IOC). Hence, ICD + IOC = IG. Regarding cohesions (z) and weighs (k) for each component we got the resulting aggregated impact of the economic dimension (Furtado et al., 2003).

RESULTS

The impact over economic dimension, after the introduction of the coffee cherry pulper technology was analyzed focusing three main components: "Transaction costs", "Productivity and risk" and "Structural change". The results from the aggregation of these three macro-components presented IG = 0.13 (IPC = 0.07 and IOC = 0.06), showing that besides of an economic positive change at the general context, in which the technology was adopted, there is positive economic impact strictly related to the adoption of the technology. The $Z(\alpha)$ was above the tolerance limit being satisfactory ($Z\alpha = 0.79$), resulting from good convergence of answers among many interviewees. There were high "Adherence of the General Impact Structure" designed for this assessment as Z = 0.96, although with less "Adherence of the Technology"[2] impact structure and the "Components Activation" (Table 2). Partial analysis of components reveals that "Transaction Costs", IG = 0.11 (IPC = 0.08 and IOC = 0.03), were reduced after PC adoption. This component is formed by subcomponents: "Market Dependence"; "Contractual Level"; "Geographical Asset Specificity"; "Physical Asset Specificity"; "Human Asset Specificity" and "Reputation". For this group of components we observed three patterns: positive impact for the subcomponents "Market Dependence" and "Reputation"; null impact for "Contractual Level" and "Geographical Asset Specificity", and negative for "Physical Assets" and "Human Assets" Specificities. "Reputation", IPC = 0.62 and $Z\alpha = 0.75$, confirms that when a coffee producer became specialty coffee producer there is an improvement of his image at the market, primarily allowing more recognized insertion at the quality contests. Indirectly, there is an important effect over the regional reputation, which earns merit not only nationally but internationally[3]. With the process of roasted coffee trademarks segmentation, with the increased high quality and gourmet coffee supply, the roasters increased the procurement steadily for peeled cherry coffee, allowing an easier direct sell by coffee farmers. The interviewees said that it is been common to receive visiting from

[2] The "Technology Adherence" refers to the portion of components that had IPC with any kind of impact, in relation to the components that had none. It represents how much the components tree has the power for revealing the technology impact attribution in the general context.

[3] This was the case of regions as Piraju, SP; Zona da Mata, MG and Cornélio Procópio, PR.

buyers in the property searching for PC, or yet buyers requiring the future productions' reservation. It is according to the economic assumption that certainty about the product uniformity, higher frequency of transactions, and higher reputation develops routines of transaction cost reduction.

Table 2. Economic impact for peeled-cherry-coffee technology impact assessment in São Paulo, 2006.

Impact Components	k	IG	IPC	IOC	Zx	Zα
ECONOMIC IMPACT		**.13**	**.07**	**.06**	0.71	0.79
Transaction Costs	.15	**.11**	**.08**	**.03**	-	-
Market Dependence	.30	.33	.21	.12	-	-
Bargain Power	.50	.67	.42	.25	0.75	0.75
Forward Processing	.50	.00	.00	.00	0.75	0.75
Contractual Level	.10	.00	.00	.00	0.75	0.75
Geographical Asset Specificity	.10	.00	.00	.00	0.75	1.00
Physical Asset Specificity	.10	-.48	-.37	-.10	1.00	1.00
Human Asset Specificity	.10	-.28	-.21	-.07	0.25	0.75
Reputation	.10	.62	.62	.00	1.00	0.75
Productivity Variation and Risk	.70	**.13**	**.06**	**.06**	-	-
Risk	.40	.03	-.06	.06	-	-
Production Risk	.50	.20	.00	.20	-	-
Process Vulnerability	.60	-.11	-.05	-.05	0.25	0.75
Breed Diversity	.40	.67	.08	.58	0.75	0.75
Economic Risk	.50	-.15	-.11	-.04	0.25	0.75
Partial Input Productivity	.60	.19	.14	.05	-	-
Fertilizers	.33	.00	.00	.00	0.75	1.00
Labor	.33	.08	.05	.03	0.50	0.75
Investment	.33	.50	.38	.12	1.00	0.75
Structural Changes	.15	**.20**	**.10**	**.09**	-	-
Market Structure	.80	.25	.13	.12	-	-
Appropriation	.70	.26	.17	.10	-	-
Income generation	.60	.28	.17	.11	-	-
Security	.20	.17	.05	.12	0.75	1.00
Stability	.15	.17	.00	.17	1.00	0.75
Distribution	.15	.12	.07	.04	0.25	0.75
Amount	.50	.40	.30	.10	1.00	0.75
Property Value	.60	.32	.21	.11	-	-
Investment in Facilities	.50	.55	.41	.14	1.00	0.75
Nat. Resource Conserv.	.30	.14	.00	.14	1.00	0.75
Law Compliance	.20	.00	.00	.00	0.75	0.75
Income Sources Diversity	.10	.00	.00	.00	0.75	0.75
Concentration	.10	-.04	-.02	-.01	0.25	0.75
Collective Pulper Facility	.20	.33	.08	.25	0.75	0.75
Minimum Size Required	.20	.00	.00	.00	0.75	0.75
Impact Structure Adherence	0.96					
Technology Adherence	0.41					
Components Activation	0.56					
Source: research results applying software *IMPACTOS*.						

The investment in specific facilities and machinery for the preparation of CP was as expected, although, with negative contribution for the subcomponent "Physical Asset Specificity", IPC = -0.37. Depending on the producer size, the investment in machinery can reach very high money amount. Also, the "Human Asset Specificity", IPC = -0.21 was negative, due to the need for the introduction of training routines of the operations personnel with a significant increase on their wages. This specificity was higher as higher the investment and producer professionalization, revealed in Franca and Pinhal regions, therefore, there were increase in human capital in these regions, where was supposed the lesser interest or benefits for this technology regarding climate local conditions. It refutes the fundamental premise on region exclusion for technology promotion with governmental credit policy, as occurred in the FEAP/BANAGRO program in São Paulo State. The second component family "Productivity Variation and Risk", received the higher weigh in the impact tree, because it has the major importance among its peer components of the economic impact (k = 0.70). It accounted for < IG = 0.13 and IPC = 0.06, it means, the productivity gains, specially, from the input productivity increase of "Investment", IPC=0.38, which more than compensate negative results from "Economic Risk", IPC = -0.11 and from "Process Vulnerability", IPC = -0.05. The "Partial Input Productivity", IPC = 0.14, is in fact, a consequence of the pulper introduction at the coffee preparation facility. Actually, by segregating different coffee ripeness types (over ripped; green; cherry and/or raisin peeled), it optimizes operations at the yard and at the dryer. The increase in "Economic Risk" is due to the conjunction of the higher investment level and the higher cost for a product that besides receiving a premium price, follow the same volatile referential of natural coffee prices in the market and exhibits a relative concentration of buyers. Therefore, while the green coffee can find innumerous market channels, the PC is a differentiated product and the market volatility can incur losses to the producer. The exiguous marketing period of PC concurred with the end of harvest season sales, what coincides with the period of lower prices, influencing sales revenue. Situations when the producer can not wait for better terms of transaction, due to the need for generating cash flow, there were occasions that, when not finding a buyer for the PC, they were obligated to sell it as natural coffee if it starts downgrading. In the group of components "Structural Changes", its aggregation resulted IPC = 0.10. The component "Market Structure" had a positive contribution, IPC = 0.13 due to the entrance of new gourmet coffee buyers. In the general analysis, the subcomponent with the higher impact was "Investment in Facilities", IPC = 0.41, followed by the increase of "Amount" of income generated, IPC = 0.30. Certainly, the rebuild of the preparation facility for setting the pulper machinery, contributed for the increase in productivity in this step of production process. Regarding the "Amount", it was said that the regional prices were generally higher for all grades, due to the PC reputation, what increased the total revenue. In reality, the whole item "Income Generation at the Property" (composed by "Security", "Stability", "Distribution" and "Amount") was positively evaluated, IPC = 0.17. As direct result of the relevant impact derived from "Investment in Facilities", occurred positive impact in the "Property Value", IPC = 0.21.

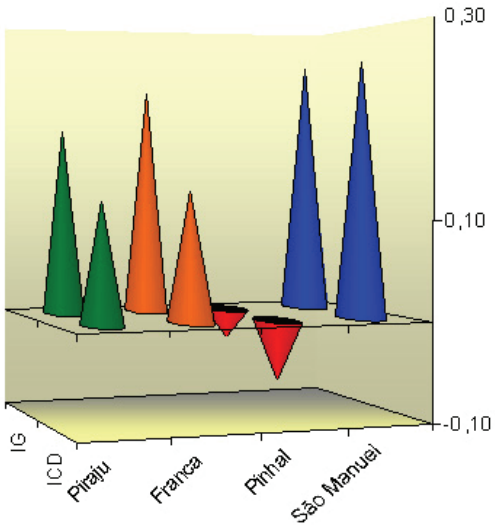

Figure 1. Economic impact of peeled cherry coffee technology, by region, in SP, 2006. Source: Research data and use of software *IMPACTOS*.

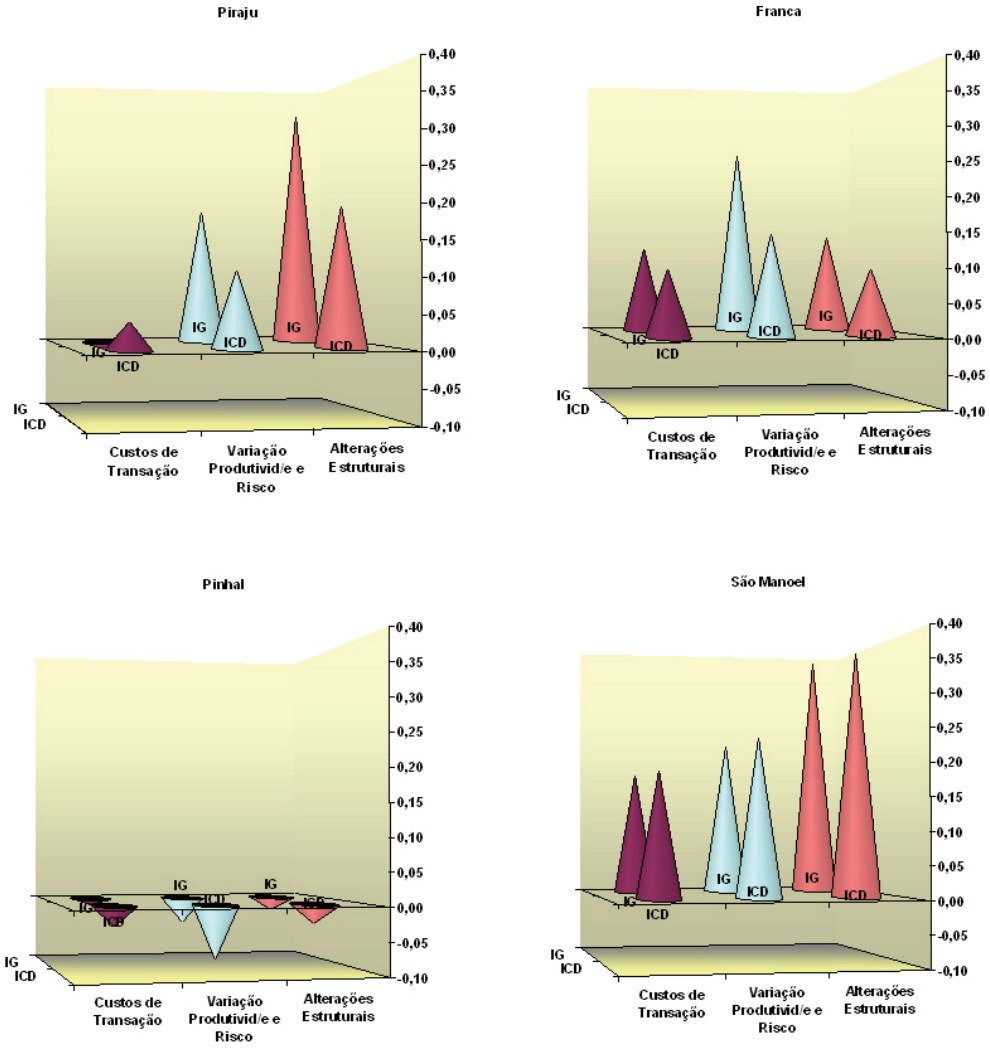

Figure 2. Macro-components for coffee pulping economic impact, selected regions of São Paulo, 2006. Source: Research results applying software *IMPACTOS*.

Concerning the regional analysis, we observed that the higher impact occurred the São Manuel region, IPC = 0.23 (Figure 1). In his region, there were coffee producers that were implementing added value strategies by the means of laughing trademarks of roasted and milled for gourmet espresso drink. Additionally, contrary to other regions, in São Manuel, the coffee producers also perceived a diminishing on the "Economic Risk", IPC = 0.67. This can explain the dismantlement of the coffee producers' local cooperative, what caused the disorganization of the marketing operations, although preserving the larger CP producers, because they weren't exclusively dependent on the cooperative for marketing their produce. The economic impact at Franca and Piraju regions was positive, IPC = 0.13 and 0.12 respectively, above the total aggregate, IPC = 0.07, that includes all four surveyed regions. Contrary to the expected, in Pinhal, the place where the innovation was early turned viable, in the beginning of 1990 years, the impact of the economic dimension was negative, IPC = -0.06. Possibly, coffee producers from Pinhal, who adopted PC, demand training and a more structured network of technology users for benefiting from the machinery (Figure 2). The regional analysis of the impact components "Transaction Cost", "Variation of Productivity and Risk" and "Structural Changes", reveals other relevant discrepancies among regions. The decrease of "Transaction Cost" was more important at regions of Franca and São Manuel. In Franca, the already recognized excellence of the naturally prepared coffee contributes to the PC quality in that location, promptly, considered by buyers as unique, increasing "Reputation".

In São Manuel, as already mentioned, the larger coffee producers who adopted PC went out of the local cooperative, because the spot market transaction costs became lower. In Pinhal, the absent of buyers that routinely demand the PC can answer for the negative evaluation of "Transaction Cost". In fact, there was PC being sold at natural coffee prices what occurred very often among these coffee producers. In this sense, the local cooperative could have a better price policy for better coffee once it already has a gourmet coffee trademark. In the subcomponent "Variation of Productivity and Risk", Piraju, Franca and São Manuel regions showed relevant and positive impacts. In the "Risk" component, the coffee producers from Piraju and São Manuel reported positive impacts, while in Franca, the impact was negative, essentially due to the "Economic Risk", IPC = -0.61. Apparently, the coffee producers that prepared CP in Franca expected difficulties to sell their product, or yet, to experience lower prices periods, enough to turn difficult to pay the mortgage of the sunken investment. In general, they are larger entrepreneurial farmers regarding other regions surveyed. This characteristic exposes them much more to the economic risk. In Pinhal, the assessment of "Productive Risk" and "Economic Risk" exhibited negative results for the IPC = -0.08 and -0.25, respectively. These results correspond to coffee producers' perception about their inability to internalize economic advantages from the technology adoption. Apparently, coffee farmers from Pinhal, inclusive the specialized in gourmet coffee production, perceived better profit opportunities in eucalyptus, comparing to the investment in the modernization of post-harvest facilities for peeled cherry coffee.

When considering "Structural Changes", for Piraju, Franca and São Manuel, it exhibits positive variations for the IPC, while in Pinhal the opposite occurs. The subcomponent "Income Generation" was positive due to "Amount", in the regions were the coffee was poorly graded, particularly, Piraju and São Manuel, ICD = 0.23 and 0.41, respectively. In Franca, as expected due to the superior natural coffee quality, the impact over the "Income Generation" was lower, ICD = 0.18. In Pinhal, contrary to other regions, the impact over the income was null for all subcomponents. The "Property Value" was benefited by the technology adoption, in Piraju, ICD = 0.24, Franca, ICD = 0.24, and São Manuel, ICD = 0.28. It happened because the "Investments' in Facilities" weigh k = 0.50 and ICD = 0.41. In Pinhal, "Property Value" was modestly positive, ICD = 0.02. Therefore, among these Pinhal's

producers, not even the possibility for mortgaging and rewarding the investment sunk at the peeled-cherry-coffee preparation facility was perceived for increasing property value.

CONCLUSIONS

Main factors as size of the farm, climate conditions and credit policy have important role in the adoption of this technology, also in its economic impacts. The study pointed positive changes in the general context for the components and agents surveyed. What strongly contributed for these changes was the peeled-cherry-coffee technology adoption. Among 24 impact components, only 5 exhibited negative impact. It increased bargain power, reduced transaction costs, increased investments in facilities but also economic risk. We refute the premise on region exclusion for technology diffusion with public credit policy, revealing qualified employment and investment in human capital impacts. It also reaffirms the role of co-operatives for small producers.

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Drying of Washed Coffee by Conventional Heat Transfer and High Frequency Techniques

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SUMMARY

In this paper the complete heat and mass transfer equations when convection heat transfer or high frequency heating are present in drying parchment coffee are introduced. Another basic and practical factors involved are also briefly discussed, such as the effects brought by choosing the right frequency of the electrical field on the heat penetration within the mass of coffee, the distribution of the electrical field within the dryer, and the maximum attainable throughput. This paper however focuses on the mathematical formulation of the drying phenomena.

INTRODUCTION

Drying is a process by which a liquid, normally water, is evaporated from a solid using thermal energy, i.e. by conduction, convection, radiation or internal heating (high frequency) techniques. Other mechanical methods are not effective if the water is contained in the interior of a capillary-porous body, since in this case it is restrained by forces which cannot be completely overcome by means of external effects such as those produced by gravity (draining, centrifuging) or compression. Most biological materials, particularly agricultural products, are such bodies according to Luikov (1966) who defined a capillary-porous body as one in which the potential created by the internal forces is much greater than the potential due to gravity. Thus thermal methods of drying are usually required, these normally involve air as the transport medium, i.e. air transfers heat to the material and also carries away moisture. In some cases however it may be convenient to use the air just as a moisture extraction agent (e.g. in conductive, radiative and internal heating methods) and in some circumstances (e.g. vacuum drying) it may not be used at all.

Industrial coffee drying methods are based on convection heat transfer, i.e. the application of hot air blown through a mass of coffee. This technique benefits from air´s ability to penetrate a given load of grain, and in addition to this allows a simple and reliable control method of the drying process, in which air temperature and flow commands coffee´s temperature level and drying rate. Convection´s major disadvantage rests on the decrease of temperature difference between drying air and product´s surface as the drying process advances, which causes a decrease in evaporation rates and drying efficiency. In order to improve the effectiveness of drying processes some authors have proposed the use of high frequency heating techniques, which does not require air as a heat transfer medium, although at present high frequency drying techniques are still much more expensive than conventional methods,. When heat is generated internally in the material by means of an electrical high frequency field the diffusivity of moisture is increased and there is also an effective negative temperature gradient, i.e. from inside to outside the coffee parchment, which increases further the evaporation rate and hence the speed of the process. Moreover it can be shown with the aid of Luikov's heat and mass transfer equations that a negative pressure gradient, i.e. higher pressures in the interior of the body, will further increase the evaporation rate.

THEORY OF DRYING: HEAT SUPPLIED FROM EXTERNAL SOURCES

When the heat is applied to the surface of the material the drying mechanism is controlled by the environmental conditions, the physical properties of the material, the temperatures of the material and heat source, and the relative position of heat source and the material. Amongst the environmental conditions we could mention the temperature, relative humidity and flow of air, and also ambient pressure. Physical properties which affect the drying dynamics when heat is supplied externally are its dimensions, density, specific heat, thermal conductivity, moisture diffusivity and optical properties, e.g. absorptivity and reflectivity. Initially, when heat is applied to the surface of the material, both the water and the dry material approach a temperature equilibrium. Provided that the surface layer of the material is wet, i.e. the internal water is constantly migrating to the outside, additional energy supplied is used to maintain this equilibrium and to evaporate the surface water. When enough heat is applied to the surface of the material then, provided internal mechanisms have forced moisture from the interior to the surface through the internal layers of the material, the molecules on the surface will acquire sufficient energy to overcome the molecular forces which bind them together. Then, due to the moisture concentration gradient between the saturated surface and the surrounding air, they will leave the surface and mix with the medium. This evaporation process depends on the temperature of the surface, which governs the amount of kinetic energy attained by the molecules and which enables them to escape, the relative humidity of the air and the air velocity. A constant temperature/evaporation phase remains until there is insufficient water migration to the surface to keep it covered by a layer of water. At this point the temperature of the wet material starts to increase since not all the energy supplied to the material can be used to evaporate water, the surplus energy is absorbed by the material itself. These two different drying phases after the initial temperature equilibrium period, are commonly referred to as the constant and falling rate drying periods.

If higher rates of moisture evaporation from the surface are required, the heat transfer resistance of the air-water/surface interface layer (where the bulk of convection occurs) should be as low as possible. An increase in the air flow rate at the surface will assist in the heat transfer across the interface layer by reducing its thickness. When processing coffee increasing the drying air temperature would not be a real alternative. When the drying process is well underway coffee's temperature is very close to that of air, and this would damage its quality, therefore increasing air's flow is the only option left for the conventional convection method. Nevertheless increasing air flow brings a dramatic increase in the electricity consumption rate. Therefore we are left with an inherently slow and temperature limited process, which initiates at considerable moisture content levels and is probably aggravated by the parchment cover, and constitutes a classic bottle-neck stage in coffee processing operations.

An alternative would be introducing other mechanisms of heat transfer to increase the total heat supplied to the surface of the material although, as will be noted later, increasing the external energy supply to the material may actually hinder the drying process and would definitely damage coffee's quality.

In order to find a relationship for the rate of evaporation of water when the material is covered by a layer of liquid, the heat transferred can be equated to the heat required to evaporate the water. This assumes that there is a continuous flow of moisture to the surface and that the material has reached a constant temperature, all heat received being used for evaporation. Considering convection alone, the rate of heat transferred is given by:

$$Q_{convection} = h_c A(T_a - T_b) = h_v \, dm'/dt \qquad [1]$$

Where A is the total area for convective heat transfer within the grain, T_a and T_b are, respectively, the temperatures of air and material, h_v is the latent heat of water vaporization, dm'/dt is the evaporation rate, and h_c is the convective heat transfer coefficient.

The moisture concentration gradient between the wet surface and air is the driving mechanism for evaporation, this potential is usually expressed in terms of the difference between the vapor pressure of coffee and that of air at the particular value of relative humidity. The vapor pressure of water is an exponential function of temperature and that of a saturated moist (or liquid) surface will correspond to the value for pure water. The vapor pressure of moist air at a relative humidity of RH is equal to that of water multiplied by the factor RH. Hence, for the evaporation potential to be a maximum, the vapor pressure of coffee grain should be high as possible relative to that of air. This can be achieved by maintaining a permanent layer of moisture on the surface of the body and using drying air whose relative humidity is as low as possible. The evaporation potential also increases with an increase in the dry bulb temperature of air (which also increases the wet bulb temperature), this is due to the resultant decrease in relative humidity of air and the increase in water vapor pressure of the layer (due to the increase in its temperature). The rate of evaporation can therefore be expressed as proportional to the difference between the vapor pressure of water on the surface of the grain, P_b, and the vapor pressure of air in the surrounding drying medium, P, i.e.

$$dm'/dt = hmA(P_b-P) \quad [2]$$

The evaporation rate, dm'/dt, can be related to a drying rate since an amount of evaporated water dm' corresponds to a reduction dm in the water contained in the material, i.e. dm' = -dm. Moreover, if the contained water is expressed in terms of moisture content dry basis (d.b.), M, we have

$$dM=dm/(\text{mass of dry solid}) \quad [3a]$$

and,

$$dM=dm/(\rho_s V) \quad [3b]$$

dM is a per unit, quantity where ρ_s is the bulk density of the dry material and V is the volume of coffee in the drying zone. Combining Equations [1]-[3] we have;

$$dM/dt = -[h_c A(T_a-T_b)]/(h_v \rho_s V) \quad [4a]$$

$$dM/dt= -h_m A[P_b-P]/(\rho_s V) \quad [4b]$$

dM/dt is the rate of variation of the moisture content of the material in a dry weight basis (kg water/h/kg dry material) for the the constant drying rate period. P_b, the water vapor pressure of the surface water, is evaluated at T_b. P is the water vapor pressure of the moist air, which is given by the product of the relative humidity of air and the vapor pressure of water evaluated at T_a. In theory the steady state characteristics of air drying (i.e. those corresponding to constant rates of evaporation) can be defined by psychrometry and conventional mass and convective heat transfer equations, and the internal mechanisms of mass and heat transfer within the coffee grain do not need to be taken into account. The use of psychrometrical estimations is based in the fact that if a material is air-dried then, as long as the surfaces in contact with air are moist, their temperature will be approximately equal to that measured by a thermometer placed in contact with the same air flow if the bulb of that thermometer is covered with a wet wick, a so called wet bulb thermometer. This is due to the fact that similar surface evaporation conditions will be

experienced by the soaked wick and the surface of the material. The practical significance of this is that, as long as the surface of the product is wet, its temperature can be read from a psychrometric chart if at least two characteristics of the drying medium, e.g. dry bulb temperature T_a (the temperature measured by an ordinary thermometer) and the relative humidity RH. In theory, knowing the temperatures of air and source and all the coefficients and dimensions involved, the drying rate and the wet bulb temperature of coffee can be found from Equation [4]. In this phase the air temperature can be maintained as high as possible so long as the temperature of the layer of water covering the grain is not greater than the temperature limits for the product. If the contribution of radiation and conduction is negligible, which is the case in most drying systems, the dry bulb temperature of air can be increased up to the point where the corresponding wet bulb temperature (read from the psychrometric chart) approaches the limits for coffee. If radiation and conduction are taken into account then it would be necessary to take into account these heat transfer methods in the energy balances shown above.

Note that, as long as the operating conditions of the system do not change, a constant rate drying will continue provided that the surface of the material is saturated with moisture. The vapor pressures of the surface and the air under these conditions are such as to result in a constant evaporation potential. The constant rate drying period ends when, at a point which is referred as the critical moisture content of the material, the internal moisture migration is reduced below that necessary to maintain an evaporative water layer on the surface of the material. This reduction in available moisture will reduce the evaporation rate from the surface and the temperature of the product will rise until it reaches that of the air, if convection only is used in the process, or a higher level if the contribution of radiation and conduction is considerable.

Although it is customary to describe the drying process in terms of different phases, or periods, it is important to note that these periods are not identical for all circumstances but are dependent on the particular material being dried, and more important, they change with the conditions under which the process is carried out. Thus there is no such thing as a constant value for the critical moisture content, it is only a transition stage which is affected by the parameters of the process. As noted above, when the falling rate period commences the constant temperature-evaporation phase ends, and the material's temperature increases, since the energy not used in evaporating water is absorbed by the product. Considering dm_h/dt as the throughput of wet coffee in weight terms, C_p as its specific heat and T, T_i and T_a as coffee's temperatures at time t, the temperature of the material when the falling rate period commences and the temperature of air, we have:

$$h_c A(T_a-T) = -h_v\, dm/dt + dm_h/dt\, (T-T_i)C_p \qquad [5a]$$

The throughput of wet coffee can be divided into two components, water and solid, i.e.

$$dm_h/dt = dm/dt + dm_d/dt \qquad [5b]$$

and the flow rate of the dry substrate can be approximated to

$$dm_d/dt = \rho_s \zeta \qquad [6]$$

where ζ is the drying throughput expressed in terms of the volume of coffee per unit time, i.e. kg/m^3. After substitution of Equation [6] in [5] we obtain:

$$h_c A(T_a- T)=-h_v\rho_s\, VdM/dt+Cp_w(T-T_i)V\rho_s dM/dt+Cp_d(T-T_i)\, \rho_s\zeta \qquad [7]$$

Where Cp_w and Cp_d are the specific heats of water and dry coffee substrate respectively.

Equation [7] relate the temperature and moisture of coffee at a given time after the (falling rate) drying period begins. However there are two unknowns in these expressions, hence even if all the coefficients, dimensions and properties referred to in Equation [7] are known, the temperature of the material, T, cannot be estimated for particular values of T_a and T_i, since the drying rate dM/dt cannot be determined from Equation [4b]. In this case the surface of the product is no longer covered by a layer of water and the vapor pressure within the material is not equal to that of a water saturated surface, it is continuously decreasing in accordance with the fall in the interior moisture content. In order to estimate the temperature of coffee in the drying process the drying rate dM/dt needs to be known, in this phase this can only be obtained by considering the heat and mass transfer mechanisms within the material.

Luikov (1966) and others have used fundamental thermodynamic relationships to derive transient equations which describe the variation of moisture, temperature and pressure within a body. The major problem in using these relationships to analyzed the internal drying phenomena is the lack of data concerning the equations' coefficients, which require several simplifications to be made. The effects in the internal drying mechanism of using intensive heating methods such as infrared or internal heating can be seen with the help of the theory developed by Luikov. With this approach it is possible to obtain the following drying equation, after some simplifications are made:

$$\partial M/\partial t = \mathrm{div}[D(M,T)\mathit{gradM}] + \mathrm{div}[\delta_t(M,T)D(M,T)\mathit{gradT}] \qquad [7]$$

The thermal gradient coefficient (δ_t) is a measure of the moisture diffusion within the grain of coffee produced by the variation of the internal thermal gradients. Both this term and the diffusivity moisture D are taken as general functions of the moisture content and temperature of the material.

Luikov gives information on the variation of D and δ_t for some capillary porous bodies. At a given temperature of the material, the diffusivity increases with moisture content to a point where it reaches a constant value. This variation is affected by the state in which the moisture is transported, i.e. vapor or liquid, and also by the physical structure of the capillary system. The thermal gradient coefficient shows a rapid increase from a particular value of moisture content, but before this point is reached the parameter is practically nil. Both parameters vary for different materials and may also vary with the drying methods used, thus it is necessary to measure them for each material and drying technique under consideration. It is possible that, if drying takes place in conditions in which δ_t is very small, the second term on the right of Equation [11] can be neglected without invalidating the result.

Note that the first term on the right of [11], i.e. div(DgradM), is negative provided that so also is the magnitude of gradM, i.e the moisture content profile within the body decreases when r increases (less water is found as the surface is approached). Thus the evaporation rate becomes greater if both the moisture gradient and the diffusivity remain high. This presents a limit, since as drying continues the diffusivity will tend to a small value as the moisture content reduces. Considering now the second term, div(δ_tDgradT), it is noted that a large temperature gradient in the direction of increasing r (i.e. higher temperatures closer to the surface) will make the drying rate more positive, i.e. it will reduce the evaporation rate. Hence if the thermal gradient coefficient, diffusivity and thermal gradients are high enough, the evaporation rate can actually be reduced. This phenomena is usually neglected in air-drying studies since it is assumed that temperature gradients are small, and therefore the effect of second term on the right in Equation [8] is neglected, leaving us with the frequently used moisture diffusion model.

Nevertheless when conductive or infrared radiation are applied on the surface of food products the high temperature gradients produced can actually be sufficient to hinder drying. Ginzburg (1969) comments on experimental observations of this effect when infrared drying methods are applied in this kind of materials. The final outcome of an intensive heating from the surface is therefore related to thermo-physical properties of the material and the resulting balance between the two terms on the right of Equation [8].

THEORY OF DRYING: THE CONTRIBUTION OF INTERNAL HEATING

When heat is generated internally in the material the diffusivity is increased and there is also an effective negative temperature gradient, i.e. from inside to outside. This increases further the evaporation rate and hence the speed of the process. Moreover it can be shown with the aid of Luikov's equations that a negative pressure gradient, i.e. higher pressures in the interior of the body, will further increase the evaporation rate. A heat transfer equation in which the internal heating effect is included can be derived by considering an energy and mass balance within a small rectangular control volume V (dxdydz) in which the body is contained, assuming that the volume so defined can gain energy from external sources (convection, radiation, conduction), ∂E_{ext}, and by internal heating, ∂E_{int}. The absorbed energy is used in heating up the material and in evaporating the water, i.e.

$$\frac{\partial E_{ext}}{\partial t} + \frac{\partial E_{int}}{\partial t} = V\rho C_p \frac{\partial T}{\partial t} + h_v \frac{\partial m'}{\partial t} \qquad [9]$$

The term $\partial E_{ext}/\partial t$ is the difference between the rate of heat flow into the infinitesimal volume V and the rate of heat flow out of it. It has been derived in several treatises in conduction heat transfer using Taylor series. It can be written as:

$$\partial E_{ext}/\partial t = \partial/\partial x(kdydz\partial T/\partial x)dx + \partial/y(kdxdz\partial T/\partial y)dy + \partial/z(kdxdy\partial T/\partial z)dz \qquad [10]$$

When heat is produced internally by means of a high frequency electromagnetic field $\partial E_{int}/\partial t$ is obtained by integrating the Poynting vector defined by

$$\boldsymbol{P} = \boldsymbol{E} \times \boldsymbol{H} \qquad [11]$$

where $\boldsymbol{E}$ and $\boldsymbol{H}$ are the electric and magnetic components of the electromagnetic field. The above involves the integration of this vector within the body along the direction of the field propagation. Thus both the direction and magnitude of the electric and magnetic components of the field define the magnitude and direction of the Poynting vector and also establish the available power which can be transferred in a given volume.

In practice the vectorial equation defined in Equation [11] is not evaluated for each grain particle. If it is assumed that the bulk material experiences a unidirectional field and similar field is experienced by all particles in the drying zone then simplified formula can be obtained Metaxas and Meredith (1983)

$$\partial E_{int}/\partial t = 1/2\ \omega\ e_o\ e''\ E_p^2\ V \qquad W \qquad [12]$$

In some cases, particularly in the microwave band of the frequency range it is difficult to obtain the above conditions. However, as will be noted, in the radiofrequency band the results are quite satisfactory, due to the much larger radiation penetration depth. They are not satisfactory, for example, if the penetration of the electromagnetic radiation is small as compared to the thickness or depth of the material to be processed.

In the case of dielectrics the mechanisms of interaction with the field responsible for the heating effect produced are explained in terms of displacement of electrons with respect to the nuclei of atoms, displacement of atoms within molecules, reorientation of molecules and an effective conduction current which might be considerable within the frequency range of interest. This component has been said to be the dominant heating mechanism in the radiofrequency band of the spectrum whereas in the microwave region the reorientation of molecules due to the existence of permanent dipoles in the molecular structure of some materials is the most significant effect (e.g. Metaxas, 1983). The cause of the heating effect produced by the reorientation of dipoles is the inability of the latter to align instantaneously with the external field, this yields a phase difference between the internal field, produced by all individual dipole contributions, and the externally applied field. Hence an effective current could be defined, which is still capacitive for the internal field, but which has a component in phase with the external field, this produces the heating effect required. This component will only occur when an oscillating field is applied whereas the conduction current is produced by movement of ions due to a potential difference at any frequency.

The heat is produced in the dielectric, therefore if the surrounding medium and the enclosure in which the product is contained are not receptive to the field the entire energy supplied is used in the load. In considering the dielectric heating characteristics of materials it is not necessary to analyze the internal structure of the product since macroscopic quantities can be measured in the laboratory. These parameters are the lumped equivalents of all the interactions between the applied field and the material under the specific conditions in which the experiment is carried out. The parameters so obtained are the dielectric constant e' and the loss tangent tgδ, the loss factor e" is the product of these two quantities. In this respect the loss factor measures the heating produced by all internal mechanisms (Senise, 1985). The dielectric constant e' represents the response of the internal polarization mechanisms to the external field, i.e. the ability of the material to store potential energy by molecular and atomic interactions. This parameter is familiar to electrical engineers, it is the ratio of the magnitude of the current produced in a dielectric under the action of an external field to that which would exist if the same field were applied to a vacuum.

Taking into account Equations [9]-[12] and [3b] we obtain:

$$\partial/\partial x\,(k dydz\,\partial T/\partial x)dx + \partial/\partial y\,(k dxdz\,\partial T/\partial y)dy + \partial/\partial z\,(k dxdy\,\partial T/\partial z)dz + 1/2\; we_o e'' E_p^{\,2}\, dxdydz = dxdydz\,\rho C_p\,\partial T/\partial t - \partial M/\partial t\; h_v \rho_s dxdydz \qquad [13]$$

hence,

$$\partial T/\partial t = h_v\,(\rho_s/\rho)\; C_p \partial M/\partial t + 1/\rho\; C_p\; div(k\,grad T) + 1/2\; we_o e'' E_p^{\,2/}\rho C_p$$

In spherical coordinates, considering that the thermal conductivity is uniform along the grain interior,

$$\partial T/\partial t = 1/2\; we_o e'' E_p^{\,2/}\rho C_p + h_v\,(\rho_s/\rho)\; C_p \partial M/\partial t + [k/(\rho Cp)]\;(\partial^2 T/\partial r^2 + 2/r\;\partial T/\partial r) \qquad [14]$$

Equations [8] and [14] form the complete mass and heat transfer model when internal heating exists within the grain. The author is not aware of any attempt to solve analytically the above heat and mass transfer problem for any grain product. For coffee drying by convective heat transfer Montoya et al, at ASIC 1989 Conference have reported on the moisture diffusivity as a function of moisture content and temperature. The same authors also reported on other important properties for coffee, such as the equivalent radius of individual parchment grains, and the bulk density and specific heat as affected by moisture content. Mathematical models

for the thermal conductivity of parchment coffee has also been made available in technical literature, as well as for the convective heat transfer coefficient, the area available for convective heat transfer on the grain surface and the latent heat of vaporization of the water contained inside the parchment grain. The dielectric constant e' and the loss factor e" have been reported by Caldas (2004). However the critical and still unavailable parameters for solving this general drying model are the moisture diffusivity and the thermal gradient coefficient when internal heating is produced within the grain. These parameters are expected to vary in a complex way and change with the heating intensity. The thermal gradient coefficient does not appear to be known for most materials of interest, although there is some information on the diffusivity. Some authors, when studying the heat and mass transfer equations in grain products such as maize and soya, have adopted the thermal gradient coefficient of pine wood since this property does not appear to have been measured by grain and seed products.

$$\partial M/\partial t = \mathrm{div}[D(M,T)\mathit{gradM}] + \mathrm{div}[\delta_t(M,T)D(M,T)\mathit{gradT}] \qquad [8]$$

$$\partial T/\partial t = 1/2\ we_o e''E_p^{2/}\rho C_p + h_v\,(\rho_s/\rho)\ C_p\partial M/\partial t + [k/(\rho Cp)]\ (\partial^2 T/\partial r^2 + 2/r\ \partial T/\partial r) \qquad [14]$$

The contribution of the internal heating effect produced by the high frequency electric field can be understood from Equation [8] and Figure 1. In this case an increase in the moisture diffusion coefficient would be expected, together with negative temperature and pressure gradients, i.e. there would be higher temperature and pressure levels in the interior of the grain than closer to its surface. When the moisture diffusion is increased and a greater flow of moisture from within the grain to its surface is produced due to greater temperature and pressure gradients, an increased thermal gradient coefficient would probably show up. These effects would cause that the two terms on the right hand side of Equation [8] add together, whereas when external heating methods are used they oppose each other as shown in figure 1 below.

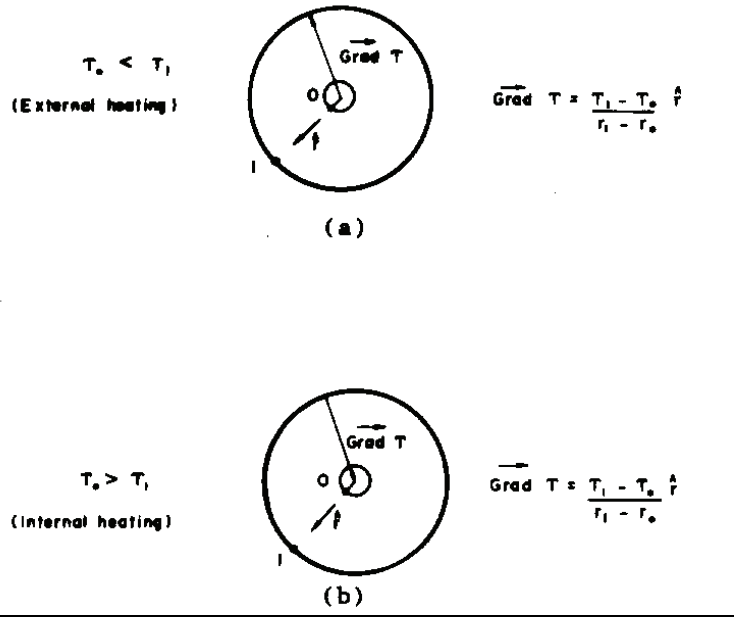

Figure 1. Temperature gradients within the grain, when heat is applied: (a) Externally, (b) Internally

If a general high frequency (HF) drying model were available and the maximum power levels which could be applied were known it would be possible to design a dryer in which a given amount of product at a particular initial moisture content would need to remain for a certain

period of time in the drying zone to reduce its moisture content to a given final value. Hence the throughput of the system could be estimated and, knowing the specifications of the equipment, it would be possible to calculate the corresponding investment and running costs. Finally these could be expressed in terms of drying costs per unit weight of the material processed. With the aid of the drying model it would be possible to vary the design parameters in order to optimize the performance and minimize the overall costs associated with the process, etc. However, as we have noted in this paper, HF drying simulation requires some information which is not available yet, basically the thermo-physical properties have not been estimated for the conditions of internal heating dissipation. Therefore up to this moment experimentation has been the only practical way of assessing the feasibility of HF drying of a number of materials. In addition to this, since in dealing with HF drying, many alternative systems can be used a method for pre-selecting the most promising techniques is also required. This is necessary for dealing with HF drying options because many options appear to be appropriate at first glance. The author carried out this analysis on a previous research work, in which the best HF drying alternatives were assessed. With these results an HF experimental drying rig was built and the drying kinetics of the most promising configurations were obtained. Some of the factors taken into account in this analysis were the following (Caldas, 1988):

Energy requirements and maximal levels of dissipated power prior to product damage or electrical breakdown (ionization).

The key parameters here are the initial and final moisture contents and the throughput required. However in HF drying there are limits to the energy dissipation within the product if physical damage, such as rupture and cracking, is to be avoided. In the case of coffee this is even more important due to the effect this could bring on the final characteristics of the beverage. In terms of energy required to drive the internal mechanisms of drying, the total needed is directly related to the density of product, the temperature rise involved, the specific heats of the dry material and water, the latent heat of water and the initial and final moisture contents. Moreover it must be taken into account that electrical discharges may develop across the air within the grain particles, or across the grain particles itself, when the electric field strength surpasses a given value. The discharges begin in high field regions either in the form of a corona discharge, in which only a limited spatial region of air is ionized and which is inherently self-extinguishing, or a sustained arc. Both cause damage to the product, and also to the equipment, and must be avoided. The value is affected by pressure but here we are usually concerned with pressures that are near atmospheric, it is also affected by frequency. However the most significant effect as far as dielectric drying is concerned is likely to be caused by non-homogeneities, such as dust and moisture droplets within the mass of grain. The author found that electrical discharges happened when the peak value of field strength in the air surrounding the grain particles where in the range of 200 KV/m, which was in accordance with technical literature. The approach used by Caldas was to estimate the field strength ($\mathbf{E_p}$) in the product for a particular value of internal heat dissipation ($\partial \mathbf{E_{int}}/\partial \mathbf{t}$) using Equation [12]. This internal field strength was then related to the "external field", i.e. that in the air surrounding the grain, where electrical discharges are likely to occur. The relationships used to relate the internal field strength in the grain to that on the surrounding air was obtained by the expressions found in Bleaney and Bleaney (1976), Smythe (1968), and Barber (1983);

$$Ep_{air} = Ep\ (e'+2)/3 \quad \text{(sphere)} \qquad (15a)$$

$$Ep_{air} = Ep(e'+1)/2 \quad \text{(cylinder)} \qquad (15b)$$

$$Ep_{air} = Epe' \quad \text{(slab)} \quad (15c)$$

Thus it is possible to express the maximum allowable applied electric field in terms of the power employed, the frequency, the volume of product in the drying zone and the dielectric properties of the material, using Equations [12] and [15]. Therefore, for a given throughput required, the power input to the dryer needed to avoid excessive electric field strength and damage to the product can be determined.

Dimensional considerations and electric field distribution

The total throughput of the system depends on the length of the drying section and the speed at which the product passes through it, these two parameters are interdependent. If the dimensions of the drying zone are limited by the space available, and if the cross section is too small, the velocity may be unacceptably high and this, associated with a given minimum drying time may result in the length of the drying zone being too great. Recirculation of the product will help but only at the cost of reducing the throughput for a given power input. Other considerations which need to be taken into account include drying uniformity which is of the utmost importance. In order to attain drying uniformity the same internal heating effect must be experienced by all grain particles. If the electric field is effectively stationary in space then the design must be such that each grain particle experiences a similar field distribution. If the field is propogating in space then added constraint is that the energy penetration must be large compared with the size of the particle or the layer of product to be heated. The concept of power penetration depth, or simply penetration depth, implies that, with a homogeneous substance, 63 % of the energy is dissipated within this depth and therefore, for uniformity, the thickness of the material should be no greater, and ideally much less, than this value.

3. Frequencies Used

Specific frequency bands are allocated for use in industrial HF heating equipment, this is done in order to avoid interference with communications and similar apparatus. The frequencies concerned are not related at all to the dielectric properties of the particular material and therefore there is no guarantee that optimum frequencies can be chosen for a particular application. In the past the frequency bands available have been centred on 6.78, 13.56, 27.12, 40.68, 434, 915 and 2,450 MHz. The HF spectrum can be split in two broad zones: microwave (MW) and radiofrequency (RF). The internal heating produced by both frequency bands are described by the same parameters, i.e. dielectric constant and loss factor. The main differences between them are the types of frequency converter and the design of the device where this radiation is applied, the so called applicator. The RF frequency converter is usually a valve oscillator whilst for microwave heating it is normally a magnetron. At RF frequencies the field can be contained within an electrode system and can therefore be described as a static field whereas at microwave frequencies the field propagates through space and must be contained within a metallic enclosure if it is to be useful in the heating context.

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Characterization of the Moment of Endosperm Cell Damage During Coffee Drying

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SUMMARY

The objective of this work was to analyze the effect of different methods of drying on the maintenance of the integrity and contraction of the cell walls and plasma membrane of natural and washed coffee. Both washed and natural coffees were submitted to three drying processes: Solar drying, 40 °C air-heated and 60 °C air-heated. During drying, coffee grains were randomly sampled and fragments of the endosperm were prepared for scanning electronic microscopy. For each sample images were generated and registered digitally. In the electromicrographs cells measurements were taken, evaluating changes in the plasma membrane as well as variations in the cellular area for different moisture content levels and periods of drying. The results allowed us to conclude that the variation in the cellular area of the coffee endosperm depends on the type of processing method and the drying conditions, albeit the phenomenon of cell contraction and expansion differed in intensity and moment of occurrence. Those changes can be related to the changes in plasma membrane integrity. The largest rate of variation of the cytoplasm was observed when the grains were dried at the temperature of 60 °C, in the intermediate phase, with moisture content between 30% and 20% (wb).

INTRODUCTION

The maintenance of the integrity of cellular membranes, among other events, is a strong indicator that the quality of the coffee was preserved after harvesting. The fruits of coffee are harvested with a high moisture content that must be reduced to 11% for its safe storage. The coffee can be processed by dry method or wet method (Bartholo and Guimarães, 1997; Borém et al., 2003). Studies of coffee quality have shown superior beverage characteristics (Brando, 1992; Malta et al., 2003; Teixeira and Gómez, 1970) for washed coffee compared to natural coffee. Both processing methods affect the content of glucose, fructose and free amino acids (Bytof et al., 2005; Leloup et al., 2004; Selmar and Bytof, 1970) however there aren't satisfactory descriptions for drying interferences. The disorganization of the membrane system is one of the initial events related to the loss of quality (Amorim et al., 1977; Prete, 1992; Ribeiro, 2003). The withdrawal of water induces contraction of the cell wall and the cytoplasm increases its viscosity (Hoekstra et al., 2001). According to the drying rate, a glass phase in the cytoplasm (Chabrillange et al., 2000; Corbineau et al., 2000; Koster and Leopold, 1988; Rogers et al., 1999) can be observed (Leprince et al., 1993) resulting in a stable system of membranes and proteins. Few studies were found linking this phenomenon with the drying conditions and coffee quality. Verification that these phenomenon occur in the endosperm during drying and how the drying parameters interfere on this process is very important.

The alterations in the ultrastructure of Arabica coffee beans have been studied. Nevertheless, no description was found for the moment when the ruptures occur.

The objective of this work was to determine, through ultrastructural analysis, the moment when microscopic ruptures occur during the drying of natural and washed Arabica coffee and to evaluate the cell contraction rate, associated to the cytoplasm and lumen area of endosperm cells.

MATERIAL AND METHODS

The harvest of the Arabica coffee, variety Topazio, was selective and manual. After harvesting, the coffee was separated according to fruit density using a mechanical washer. The unripe and overripe berries were eliminated. Part of the ripe fruits was then placed on a concrete patio for solar drying, resulting in natural coffee. The other part was peeled and immersed in water for pulping to obtain washed coffee. Both natural and washed coffees were divided into two portions, one destined for solar drying and the other for artificial drying. The portions separated for solar drying remained on the patio, under the same environment conditions, until moisture content reached 11% (wb). Sampling was done soon after harvesting and during the drying process. The samples were selected with moisture content at 40, 30, 20 and 11% (wb). The moisture content was determined by oven method at 105 ± 3 °C for 24 h.

The artificial drying was done in three 0.13 m fixed layer dryers. The air flow used was 0.33 $m^3\ s^{-1}\ m^{-2}$ and the temperatures of 40 °C and 60 °C. The ambient temperature and the relative humidity were monitored using a thermohigrograph.

To evaluate the cell contraction during drying measurements were made in the images generated by the LEO EVO 40 XVP electron microscope and registered digitally. The measurements were obtained using the Software Leo User Interface (version Leo 32.0) available in the equipment, maintaining the same working scale as the microscope. It was assumed that coffee endosperm cells have an ellipsoid shape. In each image the largest and shortest semi-axis of the cytoplasm and cell lumen were measured (Figure 1).

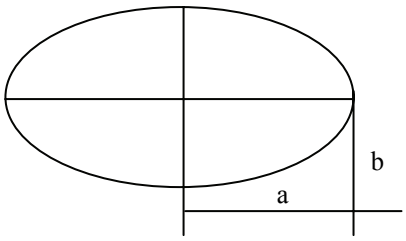

Figure 1. Schematic representation of semi-axis of an ellipse.

After these measurements the cytoplasm and cell lumen superficial area were calculated according to equation 1.

$$A = \pi\ a\ b \qquad [1]$$

where: A = area (μm²); a = largest semi-axis of the ellipsoid (μm); b = shortest semi-axis of the ellipsoid (μm)

The cell contraction during drying was studied describing the Cellular contraction rate (CCR) calculated according to equation 2. The CCR refers to the contraction of the cytoplasm superficial area in function of drying time.

$$CCR = A_0 - A_1 / t_1 - t_0 \quad [2]$$

where: CCR = cellular contraction rate ($\mu m^2\ h^{-1}$); A_0 = superficial cellular area in the earlier time of drying (μm^2); A_1 = superficial cellular area in the actual time of drying (μm^2); t_1 = actual time of drying (hours); t_0 = earlier time of drying (hours).

As shown by equation 2 the CCR values can be positive or negative, referring respectively to the contraction or expansion of the area of the cytoplasm.

RESULTS AND DISCUSSION

The Cellular contraction rate is shown in Figure 2.

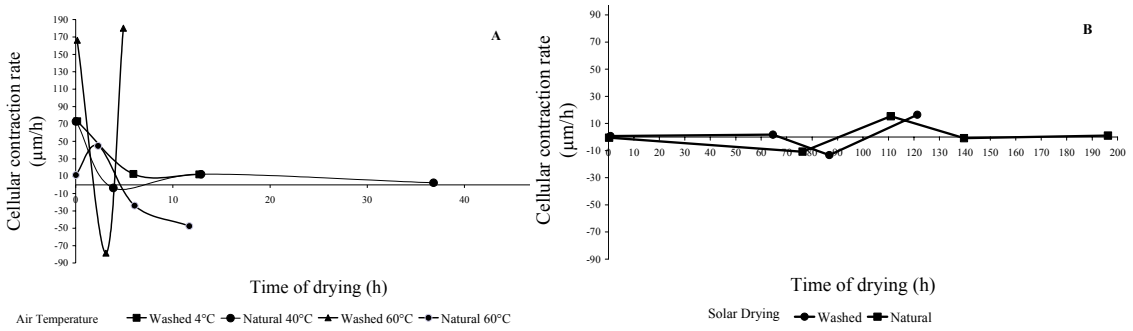

Figure 2. Cellular contraction rates during the drying for both natural and washed coffee at 40 and 60 °C (a) and solar drying (b).

The highest CCR for the washed coffee occurred in the drying at 60ºC (Figure 2a). In the first part of the curve the contraction rate varies from positive values to -78,56 µm/h, indicating contraction followed by a drastic expansion. In the final part of the curve the CCR values are again positive, reaching 180,06 µm/h, indicating a new contraction. In the drying at 40 °C the highest cell contraction rate occurred at the start of the drying process and varied from 73,00 µm/h to 11,96 µm/h. As it did not present negative values, the cell contraction decreased continuously, indicating the preservation of the cell structure.

Although the highest cell contraction rate (CCR) of natural coffee occurred in the drying at 40 °C, a gradual reduction of CCR, with a predominance of positive values, was observed. This indicates that the cytoplasm contracted continually until its final moisture content without oscillations that could signal a compromising of the cell structure (Figure 2a). In the drying at 60 °C, however, relevant oscillations of CCR occurred, varying from 44,79 µm/h to -24,00 µm/h, indicating that the area of the cytoplasm contracted until moisture content of 30% (wb), expanded until moisture content of 20% (wb) and contracted again until the end of the drying process. These variations may be related to a compromising of the integrity of the cellular membranes and, consequently, of the coffee's quality.

Relatively low cell contraction values (Figure 2b) were observed for both the washed coffee, -13,32 µm/h to 16,22 µm/h, and the natural coffee, 10,77 µm/h to 15,30 µm/h, reflecting the usual conditions in solar drying. The highest rates occurred after 80 hours of drying when moisture content reached 30% (wb). These results do not indicate any alterations that could compromise the structure of coffee endosperm cells during solar drying.

CONCLUSIONS

- The variation in the cellular area of the coffee endosperm depends on the type of processing method and the drying conditions used.
- The changes can be related to the variations in plasma membrane integrity.
- The largest rate of variation of the cytoplasm was observed when the grains were dried at 60 °C, in the intermediate phase, with moisture content of 30% and 20% (wb).
- The sun-dried coffee and the 40 °C air-heated washed coffee allow a slow and gradual cell contraction.

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The Need for Eco-Friendly Processing of Coffee in Southwestern Ethiopia: Policy Implications and Recommendations for Cleaner Production at the Birth Place of Arabica Coffee

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SUMMARY

Coffee has traditionally been the backbone of Ethiopia's economy, and will remain so for the forceable future. It is known that wet processed arabica coffee has higher demand and fetches better price in the world market. However, the conventional processing practice has apparently caused contamination of rivers in regions where wet processing is intensively practiced. Hence, the need for a more sustainable and eco-friendly approach in the sector has become so crucial. Recent reports from studies in southwestern Ethiopia have clearly indicated presence of gross pollution in most rivers that are used for wet coffee processing. Lack of regulatory standards and production protocols on wet coffee processing, and weakness to enforce existing rules and regulations at grassroots level have created a vacuum of understanding on the side of processors who often select sites for washing station establishment merely on the basis of water availability and accessibility. Hence, relevant environmental policies and laws with stringent enforcement mechanisms should be in place in order to avoid the disruptive consequences of the current production practices. In this regard, reports of the recent studies conducted in the region should serve as a wakeup call for immediate action, and not for further contemplation. Finally, summary of relevant points and recommended practices that are deemed necessary to improve sustainable production and environmentally friendly performance of coffee processing industries in Ethiopia is included in this paper.

INTRODUCTION

Coffee is produced in many parts of the country under diverse agro-ecologies and production systems. 50% - 60% of the country's foreign exchange is derived from the export of green coffee arabica beans (Mayne et al., 2002; World Bank, 2004). It is also important to notice the fact that coffee is labour intensive during harvesting and processing, and provides an important source of income from casual labour.

Despite noticeable variability in the past, the Ethiopian economy has shown remarkable and sustained growth for the last five years consecutively. Interestingly, the variability in Ethiopia's economy is mostly a result of the variability in performance of the agricultural sector (FAO 2006). Ethiopia is the largest coffee exporter in Africa, and the vast majority of coffee is exported in green bean for roasting in consuming countries. Although the total share of its coffee export in the world trade is small, Ethiopia plays an important role in the 'global value chain' because of the fine quality of its coffees.

The share of coffee in the total export value of commodities in the country has declined in recent years (Figure 1). However, the reduction is not due to the reduced importance or value

of coffee in the economy, but as a result of the rise in export value of other commodities like pulses, leather and leather products, and 'Chat'. Coffee is still the single most important commodity with tremendous effects on the country's economy.

In the year 2001/02, an estimated 32145 tons of coffee has been produced in Jimma zone alone (CSA, 2002). The volume of wet processed coffee has even increased with the remarkable rise in the total coffee production of the country in recent years. The increase in the volume of wet processed coffee is also believed to have increased the level of coffee processing waste generated along the process. Discharges from wet coffee processing plants represent a major source of river pollution where the practice is dominant for a major part of the processing season. Huge quantities of coffee processing waste in the form of pulp and effluents respectively are generated onsite and either left for natural degradation or disposed to nearby rivers causing strain on the ecological integrity of the river systems.

Recent years have witnessed important progress in the development of pollution control technology in coffee processing. Processing plants that employ improved technologies and methods substantially reduce the volume of water used in "wet" coffee processing which in turn reduces the amount of effluents discharged from the processing facilities. Additional environmentally sound measures include composting of coffee pulp with different organic residues for faster decomposition and better nutrient balance, and consequently for use as organic fertilizer.

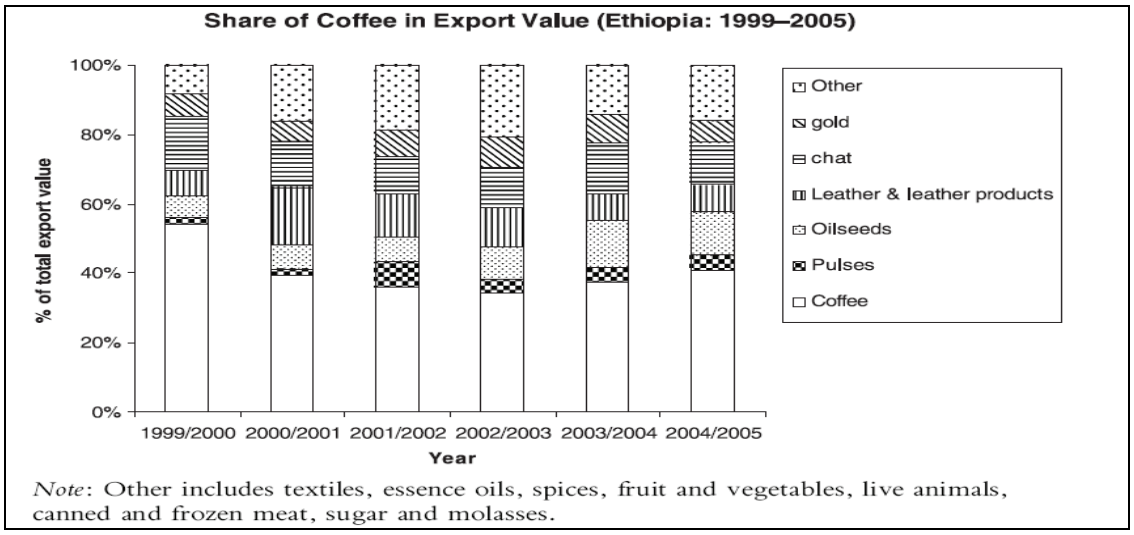

Figure 1. Trends in commodity shares of export values in Ethiopia (1999-2005) (*Petit, 2007*).

COFFEE PROCESSING AS A SOURCE OF ORGANIC POLLUTION

The conventional or traditional wet coffee processing method is associated with the use of large volumes of water resulting in huge amounts of highly polluted effluent. The disposal of this along with the pulp has become a major problem in Ethiopia like in all other producing countries. The options apparently available in almost all wet coffee processing industries are either the 'old' method in which large quantity of water is used with correspondingly huge quantity of effluents or 'ecological' methods employing technologies that minimize water usage and subsequently the effluents generated. The basic idea in the later method is to re-engineer the process, to reconvert traditional infrastructure and equipment into technology that minimizes environmental contamination (Cleeves, 2004). Technological progress has

created new techniques in pulping to minimize the amount of water needed which reduces the volumes by up to 90% when compared to traditional processing (Cleeves, 2004).

In Ethiopia, Coffee is processed using either the wet or the dry method of processing. Wet processed coffee is usually regarded as being of better quality and commands a higher price. Therefore, production of wet processed arabica coffee has sharply increased following the establishment of numerous coffee washing stations in south and southwestern Ethiopia. The rise in the number of wet processing plants has therefore resulted in the concurrent increase in processing by-products.

It is estimated that 40 m^3 of water per tone of clean coffee is the required amount for receiving the cherries, transporting them hydraulically in the pulping machine via the water current, removing the pulp, and sorting and repassing any cherries with residual pulp adhering them (Coste, 1992). Due to the high demand for water during wet processing, processing stations are often constructed near permanent water sources mainly rivers and streams, which makes the rivers more liable for contamination from the processing wastewater. The pollution resulting from 1 tonne of clean processed coffee is estimated at being equivalent to 273 m^3 of crude domestic sewage. This corresponds to daily sewage from a population of approximately 2000 persons (Calzada et al., 1989). It is therefore clear that wet coffee processing generates substantial amounts of liquid and solid waste, and the proper disposal of the effluent and pulp has become a major challenge in the coffee processing industries.

Results of studies on the effect of wet coffee processing on water quality of rivers in southwestern Ethiopia indicated high level of organic pollution, with BOD levels in most polluted rivers reaching up to 1600 mg/liter during peak processing (Table 1). Similarly, elevated levels of NH3, NO3 and PO4 were reported in the same rivers (Figure 2).

Table 1. BOD levels (mg/L) at locations above and below effluent discharge points in major rivers of Jimma Zone, Southwestern Ethiopia.

Sampling sites (rivers)	BOD (mg/L)	
	Upper course	Lower course
Dembi	2.1	42
Wurssa	4.1	366
Dengaja	2	13.3
Sunde	2	16
Kolombo	5.2	5
Torbaho	1.2	68
Bore	2.6	1200
Wanja	1	20
Temssa	3.15	1600
Urgessa	3.2	480

Source: Solomon et al. (2008)

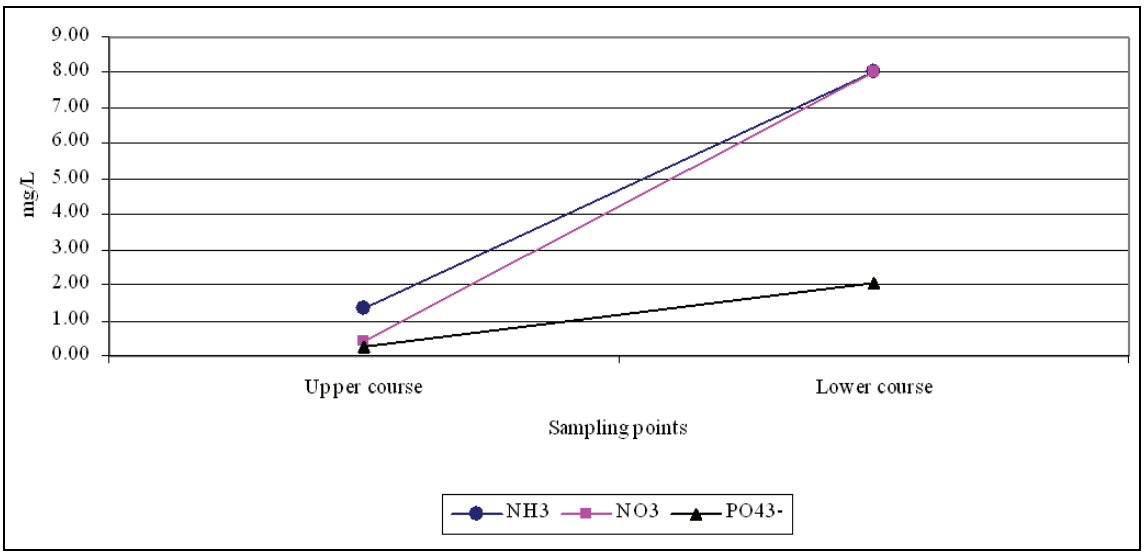

Figure 2. Average concentration of NH_3, NO_3, and PO_4 in rivers of Jimma zone (southwestern Ethiopia) above and below effluent discharge points (Solomon et al., 2008).

CONSTRAINTS AND POTENTIALS OF THE COFFEE INDUSTRY IN ETHIOPIA

The coffee industry is facing a complex set of problems that require urgent attention from policy makers, researchers and development workers alike. The problems are basically related to production, processing and marketing. It is arguably true that productivity is still constrained by shortage of improved cultivars adapted to different localities, pest and disease problems, and poor management practices. Poor post harvest practices are also responsible for losses due to reduced coffee quality. Linkage between research, extension services and producers has long been so weak, though improvements have been witnessed recently. Further strengthening of the linkage is quite important to enhance technology transfer and adoption efforts in agriculture in general and in coffee production and processing in particular.

Environmental degradation is also another area of concern, with rates of deforestation estimated at 10,000 ha/year in the coffee growing areas of the south-western parts of Ethiopia, threatening its coffee genetic resources (Gole, 2003). In addition, high level of river pollution near coffee pulping and washing stations has recently been reported from studies in south-western Ethiopia (Solomon et al., 2008).

Despite the problems and constraints facing the coffee industry in Ethiopia, there is still huge potential to boost coffee production and earnings. In line with this, the potential for product differentiation is so huge. Given the wealth of genetic resources, and large areas with exceptionally good growing conditions, the country has the potential to produce large amounts of differentiated high quality green coffee. There is also considerable potential to increase the proportion of specialty coffee export. The recent trade marking of high quality Ethiopian coffees (*Yirgachefe*, *Sidamo* and *Harar*) is good indication for the immense potential in product differentiation. While capitalizing on the virtues and tapping the potentials, serious and coordinated efforts are required from all involved in the coffee industry to reduce environmental contamination, and promote eco-friendly and sustainable production.

Policy implications and recommendations

The government's resoluteness for economic development is understandably clear. The support and incentives rendered to the private sector is also very much encouraging. As a result, expansion of investment in agriculture including coffee production and processing, is likely to boost the quantity of wet processed coffee and export earnings in general. However, without a concerted investment plan in improved technologies and strong action to enforce regulatory standards, wet coffee processing will remain to be the main source of organic pollution in rivers and streams of southwestern Ethiopia. Therefore, there should be a clear and consistent policy support and strategic plan to upgrade the nation's coffee processing systems with the objective of cutting organic pollutant discharges to surface waters in the shortest time possible.

In general, results of extensive studies in different coffee producing countries emphasize the need to effectively reduce the water volume used in wet coffee processing and washing stations. Therefore, summary of relevant points and recommended practices that are deemed necessary to improve sustainable production and environmentally friendly performance of coffee processing industries are presented below:

- Environmental audits of the coffee pulperies and the monitoring of effluent and receiving water bodies may help pollution prevention activities and regulatory efforts
- Effluent reduction through modification of operations or processes have realized a 75% reduction in water usage and correspondingly the effluent generated (Petit, 2007)
- Effluent treatment that incorporates screening settling and anaerobic decomposition, and plant-bed (introducing constructed wetlands to pulping or washing stations)
- Coffee pulp can be composted with organic residues and converted to an important organic input for soil fertility management (Solomon, 2006). Hence, other areas of coffee pulp composting like vermicomposting should get more attention from research as it may help in reducing pollution in addition to its apparent fertilizer value
- Promote and support efforts to convert coffee husk to charcoal for commercial use thereby providing a substitute to trees (helps reduce deforestation)
- Important too is 'working on regulatory measures' to ensure that effluent discharged is in compliance with the regulatory standards
- Finally, concerted efforts are needed from all actors in the coffee industry, and all actions should be coordinated to minimize contamination associated with coffee processing and sustain production of wet processed coffee

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Studies of Intermittent Drying and Tempering Period on Pulped Natural Arabica Coffee Quality

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SUMMARY

This work aimed to evaluate the effects of intermittent drying and tempering period on chemical composition and beverage quality of coffee. After two days on the ground, the coffee was carried to two experimental dryers of fixed layers with airflow of 20 m^3 min^{-1} m^{-2}. The beans mass temperature was kept at 40ºC. In order to study the effect of tempering time, were employed three periods (two, six and twelve days) and three moisture content in the beginning of the tempering (16% ± 2%, 20% ± 2% and 24% ± 2% (wb). Tempering was performed by placing the samples inside wood container and stored at ambient condition inside the laboratory room. After tempering, the coffee was carried to complementary drying until the final moisture content of 11% (wb). This experiment was carried out in a completely randomized design, factorial scheme 3x3, with three replicates. The continuous drying until the moisture content of 11% ± 1% (wb) was used as control. The results indicate a better preservation of integrity of cellular membranes when the drying process is interrupted with 24% ± 2% (wb), considering the values of electrical conductivity and potassium leaching.

INTRODUCTION

The growing demand for quality, associated to the reduction of production costs, are stimulating both producers and researchers to develop new technologies more suited to the productive system.

Drying is an important step of agricultural production and it has a huge impact on coffee quality, and several researches have evaluate the drying system, the energy consumption and de coffee quality (Cordeiro et al., 1983; Guida and Vilela, 1996).

The coffee's quality is associated to a set of factors physical-chemical which, in turn, depend on the drying conditions (Coradi et al., 2007). During the drying process, variations in the structure of the cellular membranes can occur, leading to a loss of their organization and selectivity reducing coffee's quality potential (Prete, 1992).

In studies with corn and rice, alternating drying at moderate temperatures with tempering times has led to increased quality while decreasing the overall drying time (Iguaz et al., 2006; Li et al., 1999). While many producers are adopting these methods for high-moisture content coffee, there is a lack of adequate studies and documentation to determine their effects.

The purpose of this study was to evaluate the effects of intermittent drying and tempering on the physical-chemical attributes of pulped natural coffee.

MATERIALS AND METHODS

After two days on the yard, the coffee was carried to two experimental dryers of fixed layers with airflow of 20 m³ min-1 m-2. The beans mass temperature was kept at 40 °C. In order to study the effects of tempering time, we used three periods (two, six and twelve days) and three moisture content in the beginning of the tempering (16% ± 2%, 20% ± 2% and 24% ± 2% (wb)). Tempering was performed by placing the samples inside wood container and stored at ambient condition inside the laboratory room. After tempering, the coffee was carried to complementary drying until the final moisture content of 11% (wb). This experiment was carried out in a completely randomized design, factorial scheme 3x3, with three replicates. The continuous drying until the moisture content of 11 % ± 1% (wb) was used as control. For the characterization of the coffee quality the following analyses were done: electrical conductivity, potassium leaching, total sugars, reducers and non reducers, titratable acidity, polyphenol and total soluble solids.

RESULTS AND DISCUSSION

The total content of sugars, titratable acidity, polyphenol and soluble solids did not present differences among the treatments. However, we observed significant variations for electrical conductivity (Table 1) and potassium leaching (Table 2) when the drying was interrupted with moisture content of 24% ± 2%(wb) compared to the continuous drying. These observations did not depend of tempering time.

Table 1. Medium values of electrical conductivity (µS/cm/g) of the pulped natural coffee in function of the water content in which the drying interrupted.

Moisture content (%wb)	Electrical Conductivity
11	221,71a
20	207,42ab
16	206,04ab
24	185,40b

Averages followed by the same small letter don't differ to each other, to 5% of probability for the test of Tukey.

Table 2. Medium values of potassium leaching (ppm) of the pulped natural coffee in function of the water content in which the drying interrupted.

Moisture content (%wb)	Potassium Leaching
11	52,39a
20	50,51a
16	49,03ab
24	44,11b

Averages followed by the same small letter don't differ to each other, to 5% of probability for the test of Tukey.

The coffee's deterioration and quality loss are directly related to the higher potassium leaching and electrical conductivity values that occur due to the degeneration of the cellular membranes. These results showed that the damages of the cellular membranes were lower when the drying was interrupted at 24% ± 2% (wb), independently of the tempering time, when compared to the continuous drying.

CONCLUSION

The physical-chemical analyses values had not were affect by different tempering times.

The chemical analyses had not show significant differences in function of the moisture content.

The values of electrical conductivity and potassium leaching were the lowest, when the drying process was interrupted with moisture content of 24% ± 2%(wb).

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The Effect of Different Combinations of Post-Harvest Processing on the Quality of the Cherry Coffee Beverage

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SUMMARY

The objective of the present work was to determine the effect of the unpeeling and depulping, and different ways of drying in the quality of the coffee beverage. The design of this work was in completely randomized design, split plot scheme, with four replications. The six types of coffee fruit processing were designated to the plots (natural cherry, manually unpeeled cherry, manually unpeeled and manually depulped cherry, mechanically unpeeled cherry, and mechanically unpeeled and depulped cherry), and three different drying processes were designated to the subplots (cemented ground, 35 °C constant temperature dryer, and suspended ground). All 72 samples were sent to illycaffè in Trieste, Italy, in order to perform the analysis of quality beverage characterization. Considering the conditions in which this project was performed, we may conclude that: the unpeeling and/or the unpulping of the coffee fruits did not degrade the quality of the beverage; the unpuling operation was not necessary to achieve the quality present in the coffee fruits received to perform the test; different methods of drying do not enable the obtainment of different qualities to the unpeeled and/or unpulping fruits

INTRODUCTION

To increase the competitiveness of coffee production, producers are looking for ways to improve coffee quality. The high quality coffee can get higher prices. However, to improve coffee quality more effort on research is necessary. It is important to identify which are the factors that have the higher impact on the quality of the coffee. Depending on the conditions, the coffee production may become infeasible due to competitors all over the world. Furthermore, Teixeira e Milhomem (2001) state that the competitiveness of Brazilian coffee is closely connected to the rationality of coffee production. The authors state that the demand for quality-differentiated products is an opportunity to aggregate the value of coffee. As opposed to commodities these special coffees impose with a factor that induces demand. The chemical composition of the grains is determined by genetic factors, as well as the environment and the crop. Additionally, the harvesting methods, the processing and storage are important factors that directly affect beverage quality of the coffee. Roasting and preparation methods can modify the chemical constitution of the coffee, but still the modification depends on the primary composition of the grains (Lopes, 2000). The knowledge of quality variations over the field can help the decision making process regarding the coffee production systems because of the impact that coffee quality has on the product price. Producers can identify possible causes for quality variation and use site-specific management techniques to optimize the production system. The objective of the present work was to determine the effect of the unpeeling and depulping, and different ways of drying in the quality of the coffee beverage.

MATERIAL AND METHODS

The work was developed in the Central Experimental de Beneficiamento Coletiva de Café Cereja of the Universidade Federal de Viçosa. Samples of the coffee fruit from the same field have been used, which were harvested in a coffee farm based on the town of Paula Cândido, MG. The design of this work was in completely randomized design, split plot scheme, with four replications. The six types of coffee fruit processing were designated to the plots (natural cherry, manually unpeeled cherry, manually unpeeled and manually depulped cherry, mechanically unpeeled cherry, mechanically unpeeled and manually depulped cherry, mechanically unpeeled and depulped cherry), and three different drying processes were designated to the subplots (cemented ground, 35 °C constant temperature dryer, and suspended ground). All 72 samples were dried until 11% of moisture content and stored in semi-permeable packaging. After an approximately one month period of storage, the samples were sent to illycaffè in Trieste, Italy, in order to perform the analysis of quality beverage characterization: clean beverage, sweetness, acidity, bitterness, body, flavor, balance and general concept.

RESULTS AND DISCUSSION

Except from the General Concept quality criterion (which refers to the global quality score), it was observed no significant effect from the treatment (processing and drying) on any of the quality criteria analyzed. Therefore, only the statistic analysis was detailed to this factor. In the variance Analysis to the quality factor General Concept (Table 1), the interaction between processing (plot) and drying (subplot) was significant, which implies that the effect of one factor depends on the level of the other.

Table 1. Analysis of variance and F test of Factors of variation and Fruit processing for criterion of the General Concept quality.

Factors of variation	DF	Sum of squares	Mean square	F value
Processing	5	275.60	23910.94	4782.19*
Drying	2	134.69	67.35	3.65*
Processing*Drying	10	1195.30	119.53	6.47*
Replication (Processing)	18	312.33	17.35	

**Was significative at 5% level of probability in F test.*

Thus, the unfolding of the processing/drying and drying/processing interaction effect was performed. In the first decomposition (Table 2), processing/drying, there was not interaction between these factors. Therefore, the beverage quality averages was not affected for each one of drying processing.

Table 2. Decomposition of effect of processing/drying for criterion of the general concept quality.

Drying	DF	Sum of squares	Mean square	F value
cemented ground	5	5150.83	1030.17	0,63 ^{ns}
35ºC constant temperature dryer	5	1351.50	2702.95	1,65 ^{ns}
suspended ground	5	6440.70	1288.15	0,78 ^{ns}

^{ns}There was not significant at 5% of probability in F test.

The interaction of the effect of drying on processing was determined. In this decomposition, there was interaction only between the drying and processing of the natural cherry, this is

showed in the Table 3. Next, we performed the Tukey mean test of drying processing inside the fruit processing, natural cherry.

Table 3. Decomposition of effect of drying/fruit processing for criterion of the general concept quality.

Processing	DF	Sum of squares	Mean square	F value
Natural cherry	2	1246.50	623.25	33.76*
Manually unpeeled cherry	2	32.67	16.33	0.88
Manually unpeeled and manually depulped cherry	2	11.17	5.58	0.30
Mechanically unpeeled cherry,	2	14.00	7.00	0.38
Mechanically unpeeled and manually depulped cherry	2	15.17	7.58	0.41
Mechanically unpeeled and depulped cherry	2	10.50	5.25	0.28

**Was significative at 5% level of probability in F test.*

Table 4. Tukey mean test drying/natural cherry.

35 ºC constant temperature dryer	13.00		A
suspended ground	32.50		B
cemented ground	36.25		B

**Averages followed for the same letter was not different at the level of 5% by means of Tukey test.*

The mean test showed that the drying in the dryer differed from the others, by the Tukey test, at 5% significance level. One has to take into account the fact that the coffee lot used on the tests was from the end of harvest period, the presence of fungi infestation was visually identified on the fruits that had just arrived from the country. Hence, the treatments in which the skin and pulp were removed presented superior quality scores, independent of the drying type.

CONCLUSION

Considering the conditions in which this project was performed, we may conclude that: the unpeeling and/or the unpulping of the coffee fruits did not degrade the quality of the beverage; the unpulping operation was not necessary to achieve the quality present in the coffee fruits received to perform the test; different methods of drying do not enable the obtainment of different qualities to the unpeeled and/or unpulped fruits.

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