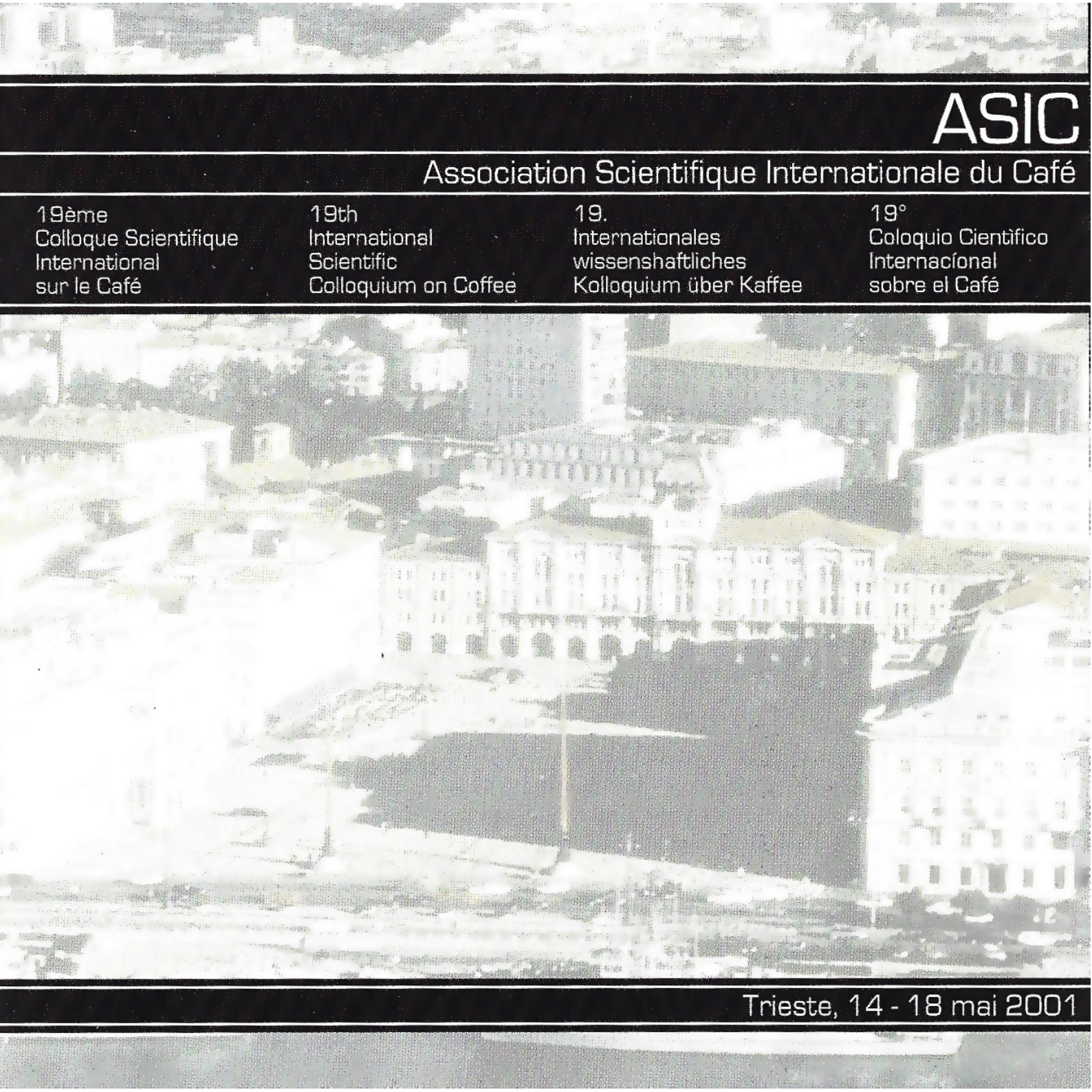
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Message from DG VIII – EU Commission

E. BARATTINI

It gives me great pleasure to represent the European Commission at the 19th session of the International Conference on Coffee Science, here in Trieste. This, in itself, is the demonstration of our interest in the ASIC activities and more in general in coffee matters.

I would like to thank the ASIC Board, especially Dr Andrea and Ernesto ILLY, for organising this today.

The European Commission, in the name of the European Community and its Member states, has been associated with coffee negotiations and international agreements since its origins. Our interest in this arises from our contractual commitments to our partners in development, large producers of coffee.

My intervention today will be focused on three main areas:

- The international Coffee Agreement 2001;
- The coffee market situation and the ways that the ICA 2001 may have an impact on it;
- The European Commission policy for development and research in the field of coffee.

At first glance, the new International Agreement of 2001, formally agreed on September 28th 2000, follows the scheme of its predecessor of 1994; however it differs on a number of additional items as:

- encouraging members to develop a sustainable coffee economy;
- fulfilling the international obligations in labour standards;
- promotion of production and consumption of coffee;
- promotion of coffee quality;
- providing a forum for the private sector, as consultative body;
- promotion training and information in order to assist transfer of technologies;
- preparation, analysis and recommendation of projects on behalf of the coffee economy.

Among these new activities and objectives, I wish to draw your attention on **two aspects** that seem **particularly important** for our debate today:

- *the private sectors participation* and the *promotion of the coffee quality.*

The 2001 coffee pact has been the first of a new generation of commodity agreements where the private sector has been called to play a key role. Its involvement is expected to generate a more intensive and effective contribution, in order to set up a strategy to meet the real needs of all the stakeholders. However, in order to ensure an efficient and coherent action, it would be essential that an adequate response would follow from all sides, producing and consuming countries.

Why this interest? Because we believe that through the globalisation process, a great opportunity is offered for a real co-operation at all levels between North and South.

Today, the coffee market is fixed at its lowest level since the beginning of the 90s. Looking at the situation historically, we observe that in constant current terms, the composite indicator price has dropped from 130 cents per pound during a period when ICO quota system was operational in the 80s, to about 51,8 cents on 10 May 2001, a drop of nearly 80 cents.

Despite many attempts to reverse the trend, the impact of these measures on the markets has been quite disappointing. I refer to the retention scheme implemented by a number of ACPC countries. One of the main difficulties was, in our opinion, the insufficient level of co-operation between the public and the private sector due to a lack of resources available, generating consequently a limited number of participants.

This is why the private sector should be ready to support public initiatives. Governments, on their side, should create adequate ground for an easy development of private initiatives and facilitate the access to the services**.** This kind of co-operation must find, of course, its natural cradle within an international and multilateral forum as ICO.

In conjunction with the private sector, I have also stressed the importance of the promotion of the quality in coffee. This is one other item that I wish to emphasise, as the new agreement identifies it as a separate objective.

To date a number of initiatives are developing, particularly in the Central American region, to produce "gourmet coffees" sold on the markets with a premium. One alternative to redress the price level.

If supported by only a limited number of producers, this action may present the risk that in the medium term, two speed coffee markets could be developed: one mass-production, at low quality and cheap, and another destined to "niche" markets, high quality and remunerative. In these terms, this strategy does not affect the total volume of coffee traded and consequently has poor chances to make a positive influence.

In order to reach an impact at global level, we should seriously think to act in concert within all parties, in order to reach a higher minimum quality standard of coffee traded, and consequently reduce the volume of product hanging over the market.

Therefore we strongly support the initiative undertaken by the International Coffee Organisation aiming at discussing during the next forthcoming Council session, possible higher quality standards for coffee. The introduction of such a scheme is under serious scrutiny and we hope that members may overcome the difficulties inevitably linked with this strategy.

The improvement of quality, of course, implies also a price increase. A legitimate fear is that consumers may not accept the "premium" for a better quality and turn themselves to alternate cheaper productions. It is in the interest of the consumer to find a good quality product, not to reject it. With low prices, consumers will be likely to suffer from deteriorating quality in mainstream coffee, as the standards inevitably decline. At medium and longer term, the risk is that the offer will become rarefied.

Therefore, I believe that a very large number of consumers will be ready to afford, if necessary, the price difference. As doctor Illy already mentioned, the wine market fits very well as an example. The differentiation of products has stimulated consumer interest rather than dampened it. This phenomenon is rejected in wide price variations that can be observed in wine. This contrasts with coffee, where the ratio between lower grades and top speciality

coffees is always quite reduced. The key to mature markets therefore is to seek added value by offering individual high quality products, both Arabica and Robusta.

However, higher quality lays down the problem of handling the low-grade coffees. In a press release of January 2001, the Speciality Coffee Association of Europe (SCAE) has estimated that 2% of exports and over 50% of coffee retained for local consumption consists of triage coffees, defined as mainly black and immature beans. It considers that the elimination of such coffee through diversion to alternative uses or even destruction would make a big contribution to a healthier coffee market.

Likewise the SCAE has proposed that countries should establish a minimum export standard for coffee, with lower grades diverted to alternative uses as: *fertiliser, animal feed mixtures, fuel.* Other possibilities could also be envisaged.

No more than a week ago, the international press reported that the ACPC is ready to destroy a consistent volume of low quality produced coffee. Once again, the International Organisation will serve as catalyst for this proposal during the forthcoming Council.

All these initiatives could be beneficial for the coffee market, at the only condition that a close co-operation should be installed among all the stakeholders, particularly between public and private sectors, co-ordinated within an international forum as the coffee Organisation.

The third item I stressed in my introductory remarks, was the information I wished to give on the European Commission policy for development and research in the coffee sector, in order to sensitise on our programs and actions.

The European Union is one of the major actors in international co-operation and development assistance. In total, the European Commission and the EU Member states provide about 50% of total international Official Development Assistance (ODA) mostly in form of grants.

However, EC Development policy is more than financial assistance alone. The European market absorbs an important share of developing countries' exports. It's the case of coffee with a share of more than 55%.

As already expressed, it should be recognised that globalisation and liberalisation reforms threaten to enhance the vulnerability of poorer small coffee, cocoa or other commodities farmers, due to extreme price fluctuations and frustrate efforts at promoting poverty alleviation.

Nevertheless, at the same time, this process provides also potential opportunities for development in trade, investment and access to know-how.

It is in this background that the European Commission guided by its Member States, responded to this challenge searching for more effective and more sustainable policy actions.

As stressed on many occasions, the main objective of Community development policy is to reduce and eventually to eradicate poverty. This objective entails support for sustainable economic, social and environmental development, promotion of the gradual integration of the developing countries into the world economy and a determination to combat inequality. These objectives correspond to the goals set in the Maastricht Treaty and have represented, of course, the general guideline of the EC action.

To be more effective, the EC's new policy aims at concentrating the activities on a more restricted number of areas. This should allow achieving a better dialogue with our partners. Six areas concerning t*rade, regional integration, macroeconomic support transport, food security, rural development and institutional capacity building,* have been identified.

Consequently, at multilateral level, the Commission is active in the following areas:

- initiatives to improve integration of developing countries in WTO;
- ongoing alliance building with developing countries and other partners in order to launch a new round of negotiations;
- use of EC commercial policy instruments. On 26 February 2001 the European Union decided to liberalise imports of all products from least-developed countries except arms and munitions "everything but arms" or EBA. In doing so, the EU extended free access to all intensive agricultural products, doing away with all remaining tariffs.

The Commission is now appealing to other developed countries to follow.

With regards to commodities, in particular, the EC is present in all agreements, in order to strengthen the dialogue with producing countries. In addition, the different preferential Agreements concluded by the EU with the African, Caribbean and Pacific countries (ACP) take into account the problem of price volatility and introduce some mechanisms aiming at stabilising the export earnings. In the former Lomé Agreement this scheme was better known as STABEX.

STABEX was a short/medium term instrument; aiming at compensating the shortfall of export earnings in commodities. In the past years, significant resources were made available for the support.

However, over the application period, the aims of Stabex changed progressively from income compensation to support for structural sector reforms. It appeared that the short-term financial compensations for export earning losses could not sufficiently contribute to introducing necessary structural reforms On the contrary, compensations turned out, in some cases, to slow down or prevent appropriate reform policies. Gradually, the emphasis of development aid from EDF shifted to support to sector policies and reforms.

With the recent Agreement of Cotonou, signed in June 2000, a new system has been introduced with the purpose to safeguard macroeconomic, local reforms and policies that are at risk, as a result of a decline in revenues. Therefore, the new Partnership Agreement stipulates that support will be given in case of short terms fluctuations in export earnings and a simultaneous worsening of the public finances.

In practice, the new system is based on an allocation of resources decided at the moment of the initial programming of EDF. Each country receives a five-year allocation for unforeseen circumstances. Thus, the volume of possible extra aid from the system will be known early and can be taken into account in a country's development plan.

The system is more targeted at supporting the overall performance of the economy, as aid can also be used in a general manner to support macroeconomic reforms or any other economic policy that is relevant to the country.

In addition to the general policy guiding the development aid that I have briefly outlined, reference must be made also to the relevant involvement of the Commission in the scientific

research and the protection of the human health.

I am referring to the **Ochratoxin** problem.

To date, no maximum limit is foreseen for OTA in coffee. However, the Commission recognises the need that all possible efforts should be made by all interested parties, in order to prevent the formation of OTA or to limit the presence in the food derived, thereof.

The European Commission is today considering the necessity of the establishment of a maximum limit for coffee by the end of 2002. A draft regulation is under preparation and it is hoped that it will be adopted quite soon. In the regulation is also foreseen that regular exchange of views between the professional organisations/interested parties and the Member States will be organised in order to discuss the efforts undertaken to reduce OTA content. Such a forum has taken place for the first time last Tuesday 8 May.

For the financial resources allocated from the Commission in scientific research, I wish to mention some initiatives undertaken in the coffee sector, where several projects are under execution These actions concern:

1. Integrated approach to prevent Ochratoxin A (OTA) in East Africa.

2. The project is expected to produce detailed information and clearer understanding of the mechanism of OTA production in coffee beans. It will lead to the development of protective cultures inhibitory to the growth of OTA producing moulds. (Kenya Tanzania, Ethiopia). Total amount: 760.000 €.

3. Coffee farm sustainability and economic viability be generating tree products, improving coffee quality and providing valuable environmental services. Region: Central America. Amount: 730,000 €.

4. Coffee wilt disease. Develop a global strategy for durable resistance in agro systems prevailing in Africa. (Uganda, Congo RDC). Amount: 720,000 €.

5. Development of bioprocess for the conservation, detoxication and valorisation of coffee pulp. The global objective is to recycle the coffee pulp and coffee husk by biotechnological processes. Region: Latin America, especially Mexico and Brazil. Amount: 390,000 €

6. Search for and creation of coffee varieties resistant to Coffee Berry Disease. East Africa, Phase I and II. Amount: 1,060,000 €

7. Nematode resistance in coffee cultivation in Central America. Phase I and II. Amount: 900,000 €.

Total of the present commitments: **4,560,000 €.**

IN CONCLUSION

Through appropriate instruments introduced by the Coffee Agreement 2001, matched with other initiatives agreed at international and multilateral level, we have seen that new ways are at our disposal to fight the present situation characterising the coffee market.

We will have to intensify our efforts to implement a comprehensive approach and more emphasis must be given to the important collaboration between the public and private sector in order to act in a coherent manner. In addition a wise policy on coffee quality, may influence positively the markets.

These efforts will have to build stronger ownership of policy initiatives to reduce poverty, not only in coffee, but more generally in all other relevant sectors, by our partner countries in Africa, Asia and Latin America.

Science, Coffee and Sustainable Development
Research focusing on quality to encourage economic growth in developing counties

A. ILLY

President of ASIC

Trieste has always been considered one of the leading cities for coffee in the world and is now confirming this role by hosting over 300 scientists (both academics and researchers) from the field of coffee worldwide – both from consumer countries, that is mainly the west, and producing countries, that is the south, involving a total of 41 countries. From 14th to 18th May Trieste will, in fact, host the 19th ASIC Conference (Association Scientifique Internationale du Café) – founded in Paris in '66 with the aim to promote, coordinate and optimize scientific research into coffee. Due to the encouragement and coordination effected by this Association, coffee is currently one of the materials undergoing the most research throughout the world.

The work performed by this scientific coffee-oriented community – involving above all physiology, chemistry, technology and agronomy – is becoming increasingly important as the results from decades of work not only demonstrate the positive impact of coffee on people's health more clearly – contrary to what was believed in the past – but also provides more concrete know-how that can be applied in producing countries to ensure improved quality at the origin. This is an important orientation that can help remedy the serious depression currently underway in the green coffee market.

Prices decreased by around 25% in the year 2000 alone, reaching the lowest ICO commodity price index since 1993 in November. As of 31/12/01, prices on the New York Stock Exchange fell from 116.50 to 56.20 cents per pound, subsequently undergoing a slow recovery.

The reason for this depression – the worst to occur over the last 30 years and which have had severe social and political effects – is excessive production, caused by the fact that new countries are now producing coffee and the other countries have increased production, although consumption has not increased. Following the boom years between '94 and '97 – generated by large deficits in production and increased speculation, when Arabic coffee prices increased to such and extent they exceeded 300 cents per pound – prices are now so low that it is impossible to even cover costs, thereby often drastically affecting already poor enemies.

There is no immediate solution to this problem. Cartels or partnerships have been implemented and disbanded a number of times, proving ineffective and extreme measures for both consumer and producing countries and have no positive effects in the long-term. Concentrating on decreasing production costs would only cause a negative effect on quality and lead to a decrease in consumption, which on one hand, would affect the entire production industry and, on the other, kill off smaller coffee growers. Due to the relative decrease in prices caused.
The Solution could be increasing the values of a product through quality, as occurred with wine – which coffee is certainly no inferior to.

The recent provision implemented by the ACPC (an Association comprising most coffee-

producing countries) regarding adoption of measures aimed at increasing and encouraging coffee consumption throughout the world (Miami – 23/4/01) is a step in this direction.

The scientific community and all those involved in coffee production must therefore undertake to concentrate their efforts in this direction – improving and directing investments into research, technology and increasing awareness.

The first means of ensuring increased consumption could be the fact that consumers begin to understand that coffee has a positive effect on health. The pleasant taste of coffee could be the second means, above all if we take into account the fact, as a rule, the higher the quality of a coffee, the lower is its caffeine content. Our desire to drink coffee is lessened when the caffeine our organism exceeds a certain level. However, consumers are also willing to pay more coffee that tastes good, thereby encouraging and funding improvement in quality to a greater extent.

Coffee must therefore no longer be considered a "commodity". Producers of primary materials must be provided the knowledge we have gained and increase the remuneration. For this purpose, scientific research takes on an ethic and economically important role in the sustainable – development of many emerging economies that currently risk collapse.

The Asic Conference in Trieste is an important and encouraging sign of this, as a large number of producers have provided contributions – both to fund research in producing countries and to ensure that scientists from poorer coffee-growing countries were able to participate. This consequently demonstrated that they understand research can help correct the distorted vision of a shortsighted market, which would sign its own death sentence by neglecting quality.

Andrea Illy
President Asic

Byline article - Il Sole 24 Ore

La scienza, il caffè lo sviluppo sostenibile
La ricerca al servizio della qualità per stimolare la crescita economica nei paesi emergenti

Andrea ILLY

Trieste, da sempre riconosciuta come una delle capitali mondiali del caffè, in questi giorni conferma questo ruolo ospitando più di 300 scienziati (accademici e ricercatori) del caffè provenienti da tutto il mondo, sia dai paesi consumatori, quindi tendenzialmente occidentali, sia dai paesi produttori; quindi dal Sud del mondo, per un totale di 41 paesi. È qui, infatti, nel capoluogo giuliano che dal 14 al 18 si sta svolgendo il 19mo convegno d'ASIC (Association Scientifique Internationale du Café) nata a Parigi nel '66 con lo scopo di promuovere, coordinare e ottimizzare la ricerca scientifica sul caffè. Grazie anche all'attività di stimolo e di coordinamento di quest'associazione, Si può dichiarare che oggi il caffè è una delle materie prime più studiate al mondo.

Il lavoro di questa comunità scientifica del caffè - che spazia soprattutto nei campi della fisiologia, chimica, tecnologia ed agronomia - assume sempre maggiore importanza perché i risultati di decenni di ricerche non solo stanno dimostrando sempre più chiaramente l'impatto favorevole del consumo di caffè sulla salute, contrariamente alle credenze del passato, ma fornisce conoscenze sempre più concrete ed applicabili ai paesi produttori per il miglioramento della qualità all'origine. È un indirizzo importante che può contribuire a sanare la profonda crisi in cui si trova il mercato del caffè verde.

Nel solo anno 2000 i prezzi hanno subito un calo di circa il 25%, raggiungendo a novembre il livello più basso dal 1993 dell'indice medio composto ICO. Alla borsa di New York, dal 3/1/2000 i prezzi sono crollati da 116.50 ct/lb (centesimi di dollaro per libbra) a 56.20 ct/lb del 17/4/2001, per poi risalire debolmente.

Le cause di questa crisi, la peggiore degli ultimi 30 anni, con il suo grave indotto socio-politico è la sovrapproduzione, dovuta all'avvento di nuovi paesi produttori e all'incremento produttivo di altri, alla quale non ha corrisposto una pari crescita dei consumi. Dopo le fiammate degli anni '94-'97, originate da un consistente deficit produttivo e amplificate dalla speculazione, in cui le quotazioni del caffè Arabica erano riuscite a superare addirittura i 300 ct/lb, i prezzi ormai sono talmente bassi che non coprono nemmeno i costi, e l'impatto di ciò ricade su economie spesso già estremamente deboli.

Non ci sono soluzioni immediate. La creazione d'accordi o di cartelli più volte intrapresi e abbandonati, sono misure estreme che si sono già dimostrate poco efficaci e che non favoriscono né i paesi consumatori né i produttori e non creano valore a lungo termine. Puntare sull'abbassamento dei costi di produzione andrebbe a scapito della qualità e porterebbe ad una contrazione de consumi che da un lato investirebbe l'intera filiera produttiva e dall'altro strangolerebbe i coltivatori più piccoli a causa dell'ulteriore abbassamento dei prezzi die ciò comporterebbe.

Come già accaduto per il vino, del quale il caffè non è meno pregiato, la soluzione potrebbe trovasi nell'incremento del valore del prodotto attraverso la qualità.

La recente risoluzione dell'ACPC (associazione che raggruppa la maggioranza dei paesi produttori) sull'adozione di misure per incrementare e promuovere il consumo di caffè nel mondo (Miami - 23/4/2001) va in questa direzione.

La comunità scientifica e tutti gli attori della filiera produttiva devono allora impegnarsi a concentrare i propri sforzi in questa direzione, rafforzando e indirizzando correttamente i propri investimenti nella ricerca, nella tecnologia e nella divulgazione della conoscenza.

La percezione da parte del consumatore dell'influenza positiva del caffè sulla- salute può costituire un primo elemento di stimolo de consumi La piacevolezza della bevanda ne può costituire un secondo, soprattutto se si considera che, in genere, più elevata è la qualità di un caffè, minore è il suo contenuto di caffeina. Quest'ultima, se supera una certa soglia all'interno del nostro organismo, provoca come reazione fisiologica la diminuzione del desiderio di consumo di caffè. Ma la piacevolezza della bevanda costituisce anche un fattore che il consumatore è disposto a pagare di più, stimolando e finanziando così l'ulteriore miglioramento della qualità.

Quindi, il caffè deve uscire dalla logica della "commodity". Ai produttori della materia prima bisogna trasferire le conoscenze acquisite e riconoscere un prezzo più elevato La ricerca scientifica assume, allora, un ruolo etico ed economico importante per lo sviluppo – sostenibile – di molte economie che rischiano il collasso.

Un segnale importante e incoraggiante in questo senso, proviene anche dal convegno di Trieste per il quale molte aziende produttrici hanno dato dei contributi sia per finanziare lavori di ricerca nei paesi produttori, sia per consentire a scienziati dei paesi più poveri di partecipare ai lavori, dimostrando di comprendere che attraverso la ricerca è possibile correggere le distorsioni di un mercato miope che trascurando la qualità del prodotto firmerebbe la propria condanna.

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Health Benefits of Coffee

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INTRODUCTION

It is widely recognised that diet is a key determinant of human health. Many epidemiological and experimental studies have implicated dietary factors in the cause and prevention of important chronic human diseases. For example, an increased risk of colon cancer has been associated with high consumption of red meat while numerous epidemiological studies have found that diets rich in fruits and vegetables correlate strongly with a reduced risk of developing cardiovascular disease and cancers. A broader range of health benefits, including prevention of osteoporosis, cataracts, diabetes and neurodegenerative diseases, is also expected to result from large intake of fruits and vegetables.

The mechanisms involved in the prevention or delay of chronic human diseases by fruits and vegetables are not yet elucidated. The presence of relatively high concentrations of certain vitamins, minerals, trace elements and dietary fibres is thought to play a significant role. In addition, it is increasingly recognised that fruits and vegetables contain a range of non-nutritional compounds possessing biological activity compatible with protective functions. Many plausible mechanisms of protection have been proposed and are currently under investigation. Improved immune function and antioxidant activity are examples of general mechanisms which may contribute to the prevention of several types of chronic diseases such as cancer and coronary heart disease. Modulation of detoxifying enzymes, control of inflammation and inhibition of tumor promotion are mechanisms important for cancer prevention. Alterations of cholesterol metabolism, control of blood pressure and decreased platelet aggregation are examples of mechanisms which may confer protection against cardiovascular disease and stroke. Food plants such as garlic or brassica, which have been identified as potentially protective against certain chronic human diseases, have been shown to contain such biologically active compounds.

In this context, coffee appears to be a very interesting food plant. It has a long history of use and in contrast to most other traditional foods, it has been the subject of extensive scientific research addressing its potential impact on human health (Debry, 1994; Schilter et al., 2001; Gray, 1998). Reviews of this information have indicated that evidence supporting a direct link between coffee intake and adverse health effects has been limited and inconsistent (Debry, 1994; Schilter et al., 2001; Gray, 1998). On the contrary, human epidemiology strongly suggests the possibility for protective health effects.

In the present paper, some emerging evidences supporting the possibility of coffee-mediated health benefits have been extracted from the large and complicated literature on coffee and health. Putative mechanisms are proposed in light of recent research conducted in experimental *in vivo* and *in vitro* models. Although scientific confirmation is clearly needed, it is argued that like for other food plants, available data on the health effects of coffee are compatible with a contribution of this common beverage to a healthy and balanced diet.

COFFEE, HEALTH AND SAFETY

Although coffee has a long history of human food use of over 1000 years, until recently most of the studies on its health effects have focused on potential adverse effects. From the large epidemiological database on coffee and health, no significant trend associating moderate coffee consumption with potential adverse effects has emerged (Debry, 1994; Schilter et al., 2001; Gray, 1998). Epidemiological studies addressing the impact of coffee intake on health outcomes of public health importance such as cancers, cardiovascular disease, osteoporosis and developmental effects have been largely inconsistent. Where present, effects were usually weak and not dose-dependent. Plausible mechanisms are often missing. Based on this literature, it appears that in general, moderate coffee consumption ranging from 3-5 cups a day is unlikely to be of any health concern (Debry, 1994; Schilter et al., 2001; Gray, 1998).

While most of the human data converge to show that moderate coffee consumption is safe, the information presently available does not allow to accurately evaluating the risk associated with higher levels of consumption (Schilter et al., 2001). It is important to note that in the situation of high intake, residual confounding factors may significantly bias the data. Many studies have indirectly seen that heavy coffee consumption was associated with lifestyles known to be important risk factors for vascular diseases, malignancies and developmental adverse effects. A recent study (Leviton et al., 1994) indicated that heavy coffee drinkers were more likely to smoke and less likely to take vitamin supplements or to consume a healthy diet (high vegetable, high vitamins, high fibres, low fat). The authors of this study proposed that heavy coffee drinkers may be at increased risk for a number of diseases not because of coffee consumption per se, but because of other aspects of their lifestyle.

HEALTH BENEFITS OF COFFEE

Coffee consumption has been associated with reduced risk of several diseases including certain cancers, Parkinson's disease, hepatic diseases, kidney stones (Curhan et al., 1998; Leitzmann et al., 1999) and suicide (Klatsky et al., 1993; Kawachi et al., 1996). In the following sections 3 examples are discussed in more details. For all of them, epidemiological evidence is briefly reviewed and plausible hypotheses for mechanisms are proposed.

Coffee and cancer

Epidemiology

The epidemiology of colorectal cancer provides the most supportive evidence of a potential coffee-dependent protection against cancer development. The relationship between coffee consumption and the incidence of colorectal cancer has been addressed in numerous studies conducted in different geographical areas implying various types of coffee brew. The data are inconsistent but many case-control studies have revealed inverse (protective) associations between coffee drinking and the risk of colorectal cancer. In its review of 1991, IARC concluded that "although it is not possible to exclude bias and confounding as the source of the apparent inverse association, the collective evidence is compatible with a protective effect" (World Health Organisation International Agency for Research on Cancer, 1991). A meta-analysis of coffee consumption and risk of colorectal cancer was published recently (Giovannucci, 1998). The results from 12 case-control studies showed an inverse association between coffee consumption and risk of colorectal cancer while five cohort studies did not support any positive or negative link. Although definitive conclusion cannot be drawn because of inconsistencies between case-control and cohort studies, this meta-analysis

suggests strongly a lower risk of colorectal cancer associated with substantial consumption of coffee (>4 cups a day).

The epidemiology of pancreatic cancer is an other interesting example in the context of coffee and health. Numerous studies have examined the potential link between coffee drinking and the risk of pancreatic cancer. Although most of the studies do not support any association, some have raised the possibility of a weak increase in pancreatic cancer for heavy coffee drinkers (World Health Organisation International Agency for Research on Cancer, 1991). Further studies and more recent analysis of the etiological factors for pancreas cancer have revealed that the weak effects of coffee, if any, are likely to be related to confounding factors such as smoking and therefore coffee consumption is not considered to represent a significant risk factor for cancer of the pancreas (Nehling and Debry, 1996; Silverman et al., 1998; Weiderpass et al., 1998). In a recent case control study, a U-shaped relationship was found between the level of coffee consumption and the risk of pancreatic cancer (Nishi et al., 1996). These authors concluded that as compared to abstinence, small to moderate amounts of coffee might prevent pancreatic cancer, whereas large amounts may increase the risk of developing this disease. This dose-response trend was confirmed through a meta-analysis involving 14 studies published between 1981 and 1993 (Nishi et al., 1996). The lowest relative risk of pancreatic cancer was found at low consumption levels ranging from 1 to 4 cups a day.

Putative mechanisms

With respect to the prevention of colon cancer, it has been postulated that coffee might inhibit the excretion of bile acids which are believed to be promoters of such disease. There is some circumstantial evidence to suggest that such a mechanism may occur. It is well documented that the coffee diterpenes cafestol and kahweol (C+K) increase serum cholesterol in man and experimental animal (Schilter et al., 2001; Ugert and Katan, 1997; Huggett and Schilter, 1995). This could be a consequence of a reduction in bile acid secretion. However, the hypercholesterolemic effects of C+K is found mainly as a result of the consumption of large amounts of boiled coffee, a brew known to contain the highest concentrations of diterpenes (Schilter et al., 2001; Ugert and Katan, 1997). In the contrary, reduced risk of colon cancer has been observed in several geographical areas including some regions where the exposure to C+K is likely to be small due to the traditional consumption of brews low in diterpenes (World Health Organisation International Agency for Research on Cancer, 1991; Giovannucci, 1998). Based on experimental model studies, alternative protective mechanisms have been proposed. Overall, these data provide support for the chemoprotective effects of coffee and coffee components.

Antioxidant activity

There is increasing evidence that oxidative damage is involved in various pathological processes such as cancer. In addition there has been a substantial body of data indicating a role for oxygen radical scavengers, such as dietary antioxidants, in reducing chronic disease including cancer. Several coffee constituents have been recently shown to possess strong antioxidant properties *in vitro* and *in vivo* (Stadler, 2001; Homma, 2001; Schilter et al., 2001). Antioxidant activity could therefore be a key mechanism supporting the chemoprotective effects of coffee on cancer development.

The antioxidant activity of coffee is attributed to the presence of compounds occurring naturally in green beans such as chlorogenic acids (caffeic, cinnamic, ferulic and isoferulic esters of quinic acid) and purine derivatives (e.g. caffeine). Roasting profoundly modifies coffee composition. With respect to antioxidants, it results into the production of chlorogenic

acid degradation products such as caffeic acid, a well documented antioxidant (Stadler, 2001; Homma, 2001). Caffeic acid has been shown to be further degraded into phenylindans which although formed at low levels (ppm) possess high antioxidant activity. In addition, melanoidins, a poorly characterised class of compounds, are formed at roasting from the reaction of free monosaccharides with chlorogenic acids or amino acids. Melanoidins have been suggested to possess radical scavenging activity. There are likely to be other uncharacterized antioxidants that are formed during the roasting process. In several experimental models, roasting has been shown to increase the antioxidant potential of coffee (Stadler, 2001). Different antioxidant mechanisms have been found with coffee, including trapping of active oxygen, radical scavenging and transition metal chelation (Stadler, 2001; Homma, 2001).

The contribution of antioxidants to the overall chemoprotective effects of coffee is still difficult to define. Although many studies have demonstrated antioxidant and antimutagenicity effects of coffee and coffee components in *in vitro* and *in vivo* test systems, others have found pro-oxidant and mutagenic activities *in vitro* (Stadler, 2001; Schilter et al., 2001). However, it is important to keep in mind that data on antioxidant activity are highly dependent upon the test system and conditions applied (Stadler, 2001). Mutagenicity of coffee has been attributed to the pro-oxidant activity of its polyphenolic constituents. At high concentrations, and in presence of large amounts of transition metals and high oxygen tension, polyphenols can reduce atmospheric oxygen to finally form hydrogen peroxide (H_2O_2), a strong reactive oxygen species (ROS). Such a pro-oxidant activity has been documented with other dietary polyphenols and antioxidants, for example from wine and black tea (Stadler, 2001). The biological significance of the pro-oxidant and mutagenic activity of coffee has therefore to be interpreted with caution since the *in vitro* assays used do not reflect adequately conditions present in physiological situations. In this context, it is important to note that in contrast to the results of the *in vitro* studies, *in vivo* experiments in rodent have not shown any evidence of mutagenicity (Nehling and Debry, 1996).

One cup of coffee has been reported to contain between 200 and 550 mg of total polyphenols (Bravo, 1998). This indicates that coffee may make a significant contribution to the overall human polyphenol intake. Further investigation addressing polyphenol absorption, distribution, metabolism and biological actions will be required to assess their actual effects *in vivo* and to evaluate their actual impact on human health.

Stimulation of cellular detoxification processes

Other potential mechanisms of chemoprotection have emerged from experimental investigations. For example, coffee has been shown to induce glutathione S-transferases (GSTs), a group of enzymes involved in detoxification processes (Abraham and Singh, 1999). The coffee specific diterpenes cafestol and kahweol (C+K) may contribute significantly to these effects. They have been reported to be anti-carcinogenic in several laboratory animals (Schilter et al., 2001). Experimental evidence has indicated that this protective activity may be related to the ability of C+K to induce detoxifying enzymes such as glutathione S-transferases (Schilter et al., 2001; Schilter et al., 1996; Cavin et al., 1998). Recently it has been suggested that besides a stimulation of detoxification processes, a reduction of carcinogen activation could also play an important role in the chemoprotective effects of C+K (Cavin et al., 1998). With respect to the hepatocarcinogen aflatoxin B1 (AFB1), C+K was shown to decrease the expression of AFB1-activating cytochrome P450s in the rat liver and to strongly induce glutathione S-transferase sub-unit Yc2 which efficiently detoxifies aflatoxin 8,9-epoxide (AFBO), the most genotoxic metabolite of aflatoxin B1 (Cavin et al., 1998). Further studies performed in human liver epithelial cell lines stably transfected to express AFB1-activating

cytochromes P450s indicated that C+K may also prevent the genotoxic effects of AFB1 in human (Cavin et al., 2001). In these test systems, 2 independent mechanisms were identified, an induction of GST-mu known to be involved in AFBO detoxification and a direct inhibition of the activity of the cytochrome P450 2B6, one of the enzyme responsible for the activation of AFB1 in human (Cavin et al., 2001). Recent investigations have suggested that the potentially beneficial effects of C+K are not restricted to xenobiotic metabolizing enzymes. Preliminary data have indicated that C+K may stimulate cellular response against oxidative stress. For example, C+K has been shown to increase the intracellular production of glutathione, one of the major antioxidant of the cells. In addition, C+K has been shown to strongly induce GST Yp, an enzyme involved in the inactivation of H_2O_2 (Schilter et al., 1996; Yin et al., 2000).

The effects of C+K are not limited to the liver. C+K have been shown to produce effects on drug metabolising enzymes in the colon resulting in an inhibition of 2-amino-1-methyl-6-phenylimidazo(4,5-*β*)pyridine (PhIP) DNA adduct formation (Huber et al., 1997). PhIP is a pyrolysis product found in cooked meat and fish. It produces colon cancer in rat and has been implicated in the etiology of human colon cancer.

The overall impact of C+K on human health is difficult to predict. At high levels, C+K has been documented to raise serum cholesterol in human (Ugert and Katan, 1997; Schilter et al., 2001). Safe levels of exposure to the cholesterol-raising diterpenes cafestol and kahweol have not been officially established. However, based on clinical human information, an exposure corresponding to 10 mg diterpenes/day is considered to have negligible hypercholesterolemic effects (Ugert and Katan, 1997; Schilter et al., 2001). Comparing this figure with occurrence data in various coffee brews reveals that except for boiled and French press coffees which contain relatively high levels of diterpenes, up to 5 cups of coffee a day are unlikely to have any appreciable effects on blood cholesterol. The level at which C+K may be expected to exert chemoprotective effects in human is not known. Animal data have suggested that effects on xenobiotic metabolizing enzymes may occur at doses which do not have any significant effects on blood cholesterol (Huggett and Schilter, 1995).

Coffee and liver diseases

Clinical and epidemiological data

Coffee consumption has been repeatedly found in clinical and epidemiological studies to reduce the levels of serum γ-glutamyltransferase, a marker of hepatobiliary pathologies (Ugert and Katan, 1997; Schilter et al., 2001), suggesting a possible protective effect on hepatic diseases. Such a hypothesis has been supported by other investigations. Several epidemiological studies have observed that coffee consumption was associated with a reduced risk of developing alcohol-induced cirrhosis (Klatsky et al., 1993; Corrao et al., 1994; Klatsky and Amstrong, 1992). In the first report, it was observed that drinking 4 or more cups of coffee a day reduced 5-times the risk of developing alcoholic cirrhosis as compared to non-coffee drinkers (Klatsky and Amstrong, 1992). In a recent study, the beneficial effect of coffee on alcoholic cirrhosis was further confirmed (Epidemiologic Group of the Italian Society of Alcohology, 2001). In addition, this study addressed the joint action of coffee consumption and hepatic viral risk factors of cirrhosis on the resulting risk of developing the disease. Coffee was found to antagonize the promoting effects of hepatitis B and C infection on cirrhosis development, suggesting a protective effect of coffee on non-alcoholic cirrhosis. Further work is required to clearly demonstrate these chemopreventive effects. For instance, it has to be clarified if the inverse association between coffee intake and cirrhosis observed in

epidemiological studies is real or if it is a consequence of coffee aversion in patients developing severe cirrhosis. Furthermore, the mechanism of action has to be established.

Putative mechanisms

The development of liver diseases induced by excessive alcohol consumption is thought to result from both nutritional deficiencies and ethanol-mediated toxic effects (Lieber, 2000; Poli, 2000). The direct toxic effects of ethanol are complex and relate partly to its pro-oxidant properties. *In vivo* and *in vitro* data have revealed that the pro-oxidant effect of ethanol depends upon its metabolism through the alcohol-inducible cytochrome P450 CYP 2E1 (CYP 2E1) (Lieber, 2000; Poli, 2000). Ingestion of large amounts of alcohol is associated with an increased CYP 2E1-dependent biotransformation of ethanol into acetaldehyde with a concomitant generation of reactive oxygen species. These free radicals will bind to macromolecules (e.g. lipids) leading to toxicity. In addition they will react with intracellular thiols, reducing cellular antioxidant defenses through a depletion of glutathione. Inhibitors of CYP 2E1 expression have been shown to reduce the production of free radicals and to prevent or treat alcoholic liver diseases.

Antioxidant activity and stimulation of defenses

Several well documented biological effects of coffee and coffee components may provide plausible hypotheses for protection mechanisms against cirrhosis. As discussed in the previous section, coffee contains high levels of antioxidants which could scavenge oxygen radicals generated by CYP 2E1-mediated ethanol metabolism (Stadler, 2001; Homma, 2001). In addition, it has been recently found that the coffee diterpenes C+K may improve cellular defense against oxidative stress through an increased in intracellular glutathione and through an induction in expression of enzymes involved in the detoxification of reactive oxygen-species (e.g. GST Yp) (Schilter et al., 2001, Schilter et al., 1996).

Effects on cytochrome P450 CYP 2E1

Other potential mechanisms refer to effects of coffee on CYP 2E1. It has been observed in liver systems that C+K alter certain cytochrome P450 activities through at least 2 different mechanisms: 1) a direct inhibition of the enzymatic activity (e.g. human P450 2B6); 2) an inhibition of enzyme expression (e.g. rat P450s CYP3A2 and CYP2C11) (20,21). The impact of coffee or coffee components on P450 2E1 activity or expression is not known. Based on the data obtained on other P450s, it is considered that effects on CYP 2E1 may constitute an alternative plausible hypotheses for a mechanism involved in the chemoprotective effects of coffee against alcoholic cirrhosis.

Coffee and Parkinson's disease

Epidemiology

Parkinson's disease (PD) is a neurodegenerative disorder leading to slowness of movement (bradykinesia), tremor and rigidity. The disease is common, affecting 1% of the population over 55 years (3% over 65). PD is characterized by the progressive degeneration of cells in a restricted part of the brain called the substantia nigra. Little is known about its etiology and much effort is devoted to the identification of risk factors which are likely to be of both genetic and environmental nature. The major risk factor for PD found up to now is age. Other determinants, such as occupational exposure to pesticides or dietary factors, are still under debate.

With respect to coffee consumption, potential links with PD are still equivocal. However, several studies have observed that coffee consumption was inversely associated with PD occurrence (Grandinetti et al., 1994; Hellenbrand et al., 1996; Fall et al., 1999; Jimenez-Jimenez et al., 1992; Ross et al., 2001). For example, In a case-control study, the past dietary habits of 342 PD patients were compared with those of controls from the same neighbourhood. Amongst differences, it was observed that patients consumed less coffee, but not tea, than controls (Hellenbrand et al., 1996). In a prospective cohort study (Ross et al., 2001) initiated 30 years ago and comprising a total of 8004 Japanese American men, coffee and caffeine intake was assessed at enrolment and 6 years later. Incident cases of PD were identified using well established criteria. Adjustments were performed for potential confounding factors including alcohol consumption and cigarette smocking which has been often found to be associated with lower risk of PD. Because of its prospective design, its rigorous assessment of dietary intake, its good follow-up and adjustment for several potential confounding factors, this study can be considered of good quality. A dose-response relationship was found: higher amounts of daily coffee intake were associated with lower relative risks of PD. This relationship was also found with caffeine. The adjusted relative risk of developing PD was 5.1 for non-coffee drinkers as compared with those who drank 28 ounces (about 900 ml) or more of coffee per day.

The epidemiological data reported above on coffee and PD have to be interpreted with caution. The observational nature of the studies does not allow concluding on causal relationship. Although intriguing, further confirmation is necessary before any definitive conclusion can be drawn. For example, it has to be assessed whether the effects observed reflect a specific action of coffee or coffee components, or if they are a consequence of preclinical PD on food consumption behaviour (Jimenez-Jimenez et al., 1992; Honig, 2001).

Putative mechanisms

Based on the available information on the biological effects of coffee, several hypotheses for mechanisms can be drawn.

Adenosine A2 receptor antagonism

Adenosine receptor agonists reduce locomotor activity in rodents, possibly through inhibition of dopamine neurotransmission. Recent evidence have indicated that adenosine A2 receptor antagonists may improve motor deficits in primates treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin known to induce a PD-like disease (Kanda et al., 1998; Ross et al., 2001). Caffeine is an adenosine A2 antagonist. Coffee/caffeine would therefore decrease clinical manifestation of PD, without having any direct biological effects on the pathogenesis of PD. Limited data in animal models support this hypothesis (Popoli et al., 1991; Ross et al., 2001).

Coffee as a source of niacin

Coffee contains relatively high levels of niacin. This vitamin is required for the synthesis of the cofactors nicotinamide-adenine dinucleotide (NADH) and nicotinamide-adenine-dinucleotide-phosphate (NADPH), which are involved in the glutathione-reductase mediated reduction of free radicals in the brain, including the substantia nigra. NADPH is also involved in the biosynthesis of dopamine. Intravenous treatment of PD patients with NADH and NADPH has been reported to lead to some clinical improvement (Birkmayer et al., 1993). Two case-control studies have indicated that niacin contained in coffee may be

neuroprotective (Hellenbrand et al., 1996; Fall et al., 1999), while this hypothesis was not supported by a propective cohort study (Ross et al., 2001).

Antioxidant effects

Dopaminergic cells are highly susceptible to oxidative stress. Stress defence mechanisms in these cells are limited and dopamine-mediated oxidative stress (through auto-oxidation and monoamine oxidase B metabolism) is significant (Schulz et al., 2000). There are increasing evidence for a role of progressive oxidative stress in the pathogenesis of PD. As discussed above, coffee may prevent oxidative stress via its antioxidant constituents. In addition, it may stimulate the production of cellular antioxidants such as glutathione and induce enzymes involved in the detoxification of reactive oxygen radicals. Data obtained with the diterpenes C+K are in agreement with such hypotheses. Data regarding both cerebral disposition and biological effects of C+K and other coffee components are necessary before drawing any conclusion regarding the relevance of antioxidant effects as a plausible mechanism explaining the possible preventive effects of coffee on PD development.

CONCLUSION

Up to recently, research on coffee and health has focused on the potential deleterious effects. Evidence to support a direct link of coffee with adverse effects has been limited and inconsistent. In fact, human epidemiology supports the safety of moderate coffee consumption and suggests the possibility for protective effects. Coffee has been associated with a reduction in the incidence of various diseases including Parkinson's disease, liver diseases and certain cancers. Plausible mechanisms can be proposed. They rely on the biological effects of coffee constituents such as polyphenolic antioxidants, diterpenes and caffeine. Similarly to other plant foods considered as potentially chemoprotective such as garlic or brassica, the data available support coffee to be a contributor of a healthy and balanced diet.

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Non-caffeine di-Cinnamoylquinide Constituents of Roasted Coffee Inhibit the Human Adenosine Transporter

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ABSTRACT

Preliminary screening of a non-xanthine constituent of roasted coffee, 3,4-diferuloyl-1,5-quinide (DIFEQ), a derivative of the chlorogenic acids, i.e. isomeric mono- and di-substituted coumaroyl-, caffeoyl-, and feruloyl- esters of quinic acid, showed inhibition of the adenosine transporter at low micromolar concentration. Displacement of [^{3}H]NBTI binding by DIFEQ in cultured U-937 cell preparations, expressing the human adenosine transporter protein, showed a K_i of 0.96 ± 0.13 μM. 3,4-Dicaffeoyl-1,5-quinide and 3,4-dicoumaroyl-1,5-quinide had similar affinities, indicating that all dicinnamoylquinides formed in the roasting process of coffee are adenosine uptake inhibitors. Inhibition of the adenosine transporter results in increased extracellular levels of adenosine. This could protect cells from various tissue insults, and possibly counteract the effects of adenosine antagonists, such as caffeine. This is supported by the fact that acute administration of DIFEQ, at 30-300 mg/kg i.p., dose dependently reduced locomotion in mice. This suggests that DIFEQ-like compounds in coffee are able to modulate the stimulant effect of caffeine, and may contribute to health-related effects of coffee.

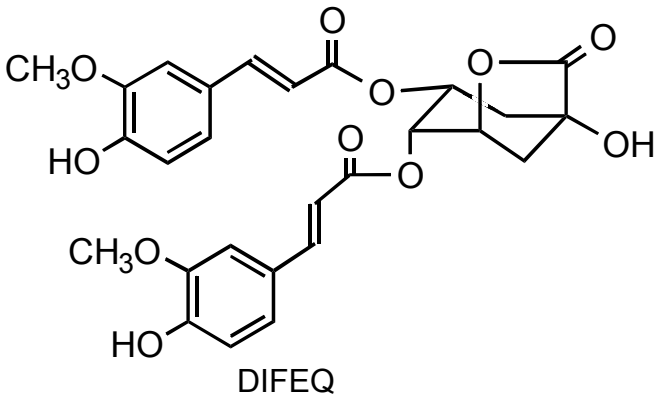
INTRODUCTION

Until now most coffee studies have focused on the effects of caffeine (Fredholm et al., 1999). However, both epidemiological and psychometric studies have revealed health-related effects of coffee consumption that cannot be explained by the effects of caffeine alone. One example is the effect of coffee on heart disease. A study of the relation between coronary heart disease and coffee intake in 20,000 individuals living in Finland found that death from coronary heart disease was higher among non-coffee consuming individuals than those who drank 5-6 cups of coffee per day (Kleemola et al., 2000). Further, coffee consumption up to 5 cups a day, but not tea, reduced coronary disease in 11,000 men and women in Scotland (Woodward et al., 1999). Another example is the subjective, immediate feeling of relaxation after a cup of hot coffee, an effect that is not evoked by the correspondent dose of caffeine. The feeling of well being and energetic arousal reported in healthy volunteers increased dose-dependently with increasing strength of freshly brewed coffee, but it did not correlate with the level of caffeine (Quinlan et al., 2000). Further, in a test of psychomotor performance in 19 healthy volunteers, 100 mg caffeine given in tea or hot water, but not when given in decaffeinated coffee, raised the threshold frequency to detect a flickering light (Hindmarch et al., 1998). Thus, there seems to be a pharmacologically active agent in coffee that is not only different from caffeine but may have the opposite effect.

We postulate that non-caffeine constituents of roasted coffee mediate some of the health effects of coffee, and that this agent may belong to the chlorogenic acid class of compounds. Chlorogenic acids are common in plants and fruits, including green coffee beans, where they can constitute over 10% of the dry weight (Clifford, 1975). Chemically, chlorogenic acids are

esters of various hydroxycinnamic acids and quinic acid (a sugar-like molecule). However, coffee is unique in that the roasting transforms some chlorogenic acids into new compounds with capacity to enter brain. The high temperature of the roasting process causes the loss of a water molecule from quinic acid, forming an intramolecular ester bond and also a large number of structural isomers (Scholz et al., 1990, 1991). These new derivatives (quinides) are neutral compounds, because they lack the carboxylic group. Dicaffeoylquinic acids constitute 1-2% of green *Robusta* and *Arabica* coffee beans (Herrmann, 1989). Assuming that all quinic acids having an unsubstituted 5-position are converted to the corresponding quinides, the total amounts of quinides present in an average fresh cup of brewed or instant coffee would equal or exceed that of caffeine.

Few pharmacological effects of quinides are known. Almost 20 years ago Boublic (1983) reported that 250 mg of instant coffee in 170 mL, approximately one fifth of that contained in a cup of coffee, displaced half the binding of the opioid receptor antagonist, naloxone. Subsequent efforts to identify the molecular entity responsible for the anti-morphine activity suggested it to be an isomer of feruloylquinide, i.e. an ester between 3-methoxy-4-hydroxycinnamic acid and one of the alcohol groups of quinic-1,5-lactone Wynne et al 1987). We have synthesized the disubstituted analog, 3,4-diferuloyl-1,5-quinide (DIFEQ), as a model compound of lipophilic quinides. Preliminary screening of DIFEQ on 64 different neurotransmitter receptors revealed that DIFEQ, in addition to blocking the binding of naloxone, also binds to the adenosine transporter protein.



DIFEQ

We now report the binding characteristics of DIFEQ on the human adenosine transporter and the acute effects of DIFEQ in open field behavior in mice. In order to ascertain the presence of DIFEQ or DIFEQ-like compounds in roasted coffee, the inhibitory activity of ethyl acetate extracts of a single cup of coffee at the human adenosine transporter binding site was determined.

METHODS

Synthetic samples of DIFEQ and its analogs were prepared in 5 steps from quinic acid and ferulic acid by the method of Wynne as described by Huynh-Ba (1995a,b). Inhibition of [^{3}H]adenosine transport was performed according to Gu et al (1993). Affinity of DIFEQ for the adenosine transporter was measured by its ability to displace (*S*)-[^{3}H](4-nitrobenzyl)-6-thioinosine ([^{3}H]-NBTI) binding in homogenates of U-937 cells, expressing the human *es* transporter (Marangos et al., 1982). Nonspecific binding was defined by co-incubation with 10 uM NBTI. Coffee extracts were prepared from 25 g regular or decaffeinated Colombian whole beans (*Coffea arabica*) or 10 g instant coffee in 200 mL hot water by extraction of an acidified solution with ethyl acetate. Extracts were dissolved in methanol (100 mg/mL), serial diluted with buffer, and assayed for their displacement of [^{3}H]NBTI binding in U-937 cell

homogenates. Eight month old mice (C57BL6/J) were tested in automated open field activity monitors measuring 27 x 27 cm (Med Assoc, Georgia VT). Locomotor activity was measured in 4 male and 4 female animals per dose and defined as the total horizontal distance traveled in a 5 min interval starting 10 min after intraperitoneal administration of DIFEQ (Florio et al., 1997). Because of low solubility in water, DIFEQ was dissolved in 10% solution of Tween 80, an agent that does not produce behavioral effects in rodents (Castro et al., 1995).

RESULTS

Inhibition of [^{3}H]adenosine transport in U-937 cell culture showed an inhibition constant K_i of 1.3 ± 0.4 uM (n = 2). Displacement of [^{3}H]-NBTI binding to the human adenosine transporter by DIFEQ showed an affinity constant K_i of 0.96 ± 0.13 uM (n = 6). The displacement curves had a Hill slope of 0.94 ± 0.02. Caffeine had no affinity for the transporter (K_i >120 uM). Affinities of DIFEQ for the adenosine A_1 and A_{2A} receptors were K_i 33 ± 5 uM and K_i >500 uM, respectively. The affinities of DIFEQ and its analogs for the adenosine transporter are shown in Table 1.

Table 1. Affinities of Mono- and Di-cinnamoylquinides for the Human Adenosine Transporter

Compound	Acronym	Structure[a] R^3	R^4	Affinity[b] (K_i uM)
3-caffeoyl-1,5-quinide	3-CAQ	OH	OH	> 100
4-caffeoyl-1,5-quinide	4-CAQ	OH	OH	52.7
3-feruloyl-1,5-quinide	3-FEQ	OMe	OH	6.6
4-feruloyl-1,5-quinide	4-FEQ	OMe	OH	21.4
3,4-dicaffeoyl-1,5-quinide	DICAQ	OH	OH	2.4 ± 0.7
3,4-diferuloyl-1,5-quinide	DIFEQ	OMe	OH	0.96 ± 0.13
3,4-dicoumaroyl-1,5-quinide	DICOQ	H	OH	0.94 ± 0.07

[a] Aromatic meta and para substituents of each of the one or two cinnamoyl ester groups
[b] Displacement of [^{3}H]NBTI binding in U-937 cell homogenates

Of the mono-cinnamoylquinides tested, only 3-FEQ showed low micromolar affinity for the human adenosine transporter. The more abundant 3-CAQ and 4-CAQ were virtually inactive. However, all the di-substituted quinides showed considerable activity with DICOQ and DIFEQ being the most potent compounds.

The ability of the extractable content in a single cup of coffee to inhibit the adenosine transporter binding is shown in Table 2. All extracts showed similar activities (12–16 mg/L), regardless of their caffeine content.

Table 2. Affinities of Ethyl Acetate Extracts for the Human Adenosine Transporter

Coffee	Amount	Extract	Quality	Affinity[a] (K_i mg/L)[b]
Arabica whole bean	25 g	0.82 g	regular	12.6
Arabica whole bean	25 g	0.76 g	decaffeinated	---
Arabica instant	10 g	0.80 g	regular	12.4
Arabica instant	10 g	0.72 g	decaffeinated	15.6

[a] Displacement of [^{3}H]NBTI binding in U-937 cell homogenates
[b] The corresponding value for DIFEQ was 0.50 mg/L

The acute behavioral effects of DIFEQ and its brain content at various times after intraperitoneal administration in mice is shown in Table 3. The actual levels of DIFEQ present in the brain after 100 mg/kg i.p. are also shown in Table 3. Analysis of the elimination rate from 10-60 min resulted in a half-life of 14 min.

A significant reduction in the spontaneous locomotor activity was seen at the highest dose that lasted for the whole 90 min test period. At the 100 mg/kg dose only the first 15 min showed a significant reduction in locomotor behavior relative to control animals.

Table 3. Effect of DIFEQ on acute locomotor activity and brain levels in mice[a]

Time	Brain level[b]	Locomotor act. 0 mg/kg[c]	100 mg/kg[c]	300 mg/kg[c]
10	32 ± 22	1436 ± 245	1080 ± 91	947 ± 145
15	14 ± 7	1193 ± 386	751 ± 84	793 ± 125
20	---	897 ± 180	838 ± 55	652 ± 133
30	7 ± 3	818 ± 136	686 ± 82	307 ± 125
60	1.1 ± 0.5	659 ± 100	551 ± 90	43 ± 22
90	---	437 ± 117	380 ± 57	44 ± 28

a) Average distance (cm) of 8 animals during 5 min in an open field
b) Amount of DIFEQ (ng) found in whole mouse brain after 100 mg/kg ip
c) Administered dose per kg body weight of DIFEQ (10 mg/mL) in 10% Tween 80

The inhibition of spontaneous open field locomotor activity after 100 mg/kg ip is clearly correlated with the experimental levels of DIFEQ found in brain. However, the behavioral effects of the 300 mg/kg dose did not show any relationship to brain levels (Figure 1).

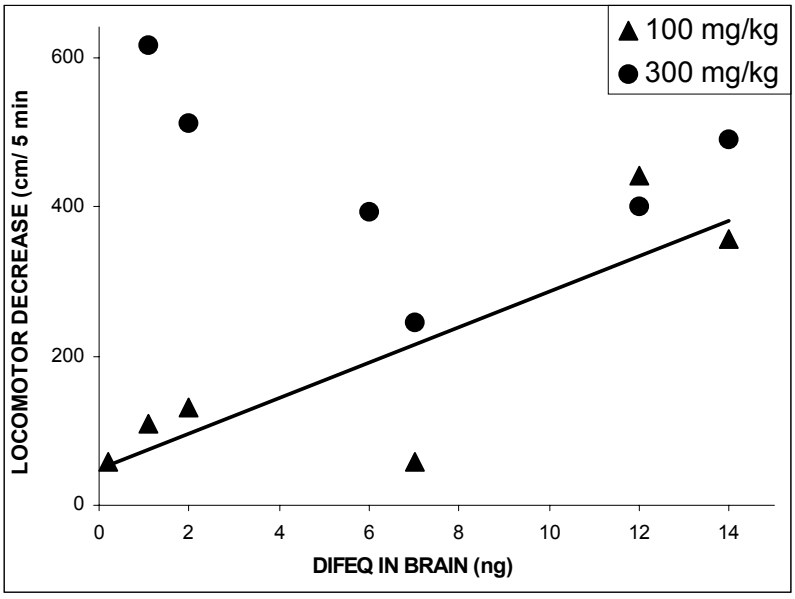


Figure 1. Correlation of Decreased Locomotor Activity and Brain Levels of DIFEQ in the Mouse

DISCUSSION

We have demonstrated that a series of neutral constituents of coffee, putatively formed in the roasting process of green coffee beans, inhibit the human adenosine transporter. The

antagonist activities of 3,4-dicinnamoyl-1,5-quinides at the adenosine transporter binding site surpasses the affinity of caffeine for the adenosine A_{2A} receptor by a factor of 3 (Fredholm et al., 1999). On the basis of weight, coffee extracts inhibited the adenosine transporter binding 25-fold less potently than DIFEQ, regardless of whether it was prepared from regular or decaffeinated whole beans or instant coffee. Since caffeine has no affinity for the adenosine transporter (Marangos, 1982), the binding activities of coffee extracts are presumably the result of DIFEQ-like constituents in roasted coffee.

Green coffee beans contain considerable quantities of 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid (Herrmann, 1989). Of these, only 3,4-dicaffeoylquinic acid can form a 1,5-quinide, due to the stereochemical requirement that the 5-position must be free of substituents for the intramolecular 1,5-lactone bond to be formed. Assuming that all available 3,4-dicaffeoylquinic acid is converted to DICAQ in the roasting process, the amount present in 25 g roasted Arabica coffee would be 60 mg DICAQ. An average cup of Robusta coffee would contain 170 mg DICAQ (Herrmann, 1989). Thus, both the amount and potency of DICAQ for the adenosine transporter equals that of caffeine for the adenosine A_{2A} receptor.

Inhibition of the adenosine transporter would have the same effects as an adenosine receptor agonist, counteracting the antagonistic activity of caffeine (El Yacoubi et al., 2000). The reduced locomotor activity seen during the first 25 min after DIFEQ administration of the 100 mg/kg dose match the brain levels of DIFEQ, in line with an increased level of adenosine in brain. The behavior after the 300 mg/kg dose, however, does not seem to be mediated by brain levels and is probably not the sole result of central effects. Both brain and plasma levels were quite low, which would explain the necessity for such a high dose to be given. It is not known whether the low bioavailability of DIFEQ is specific for the mouse or whether it holds true also for humans. DIFEQ is a lipophilic compound with a calculated log P value of 3.61, which would ensure a readily passage through the blood-brain barrier. DIFEQ is an ester and as such subject of esterases in blood, rapidly transforming DIFEQ into quinic acid and ferulic acid, which could explain the low levels of the parent compound found in mouse blood and brain.

Recent studies of adenosine transport inhibitors, as well as adenosine agonists, have demonstrated a wide range of important health related effects. Inhibition of the adenosine transporter prevents the intracellular metabolism of adenosine and prolongs the presence of high levels of adenosine (Thorn and Jarvis, 1996; Jacobson et al., 2000). This increased level of adenosine in brain causes stimulation of adenosine receptor subtypes, similar to the effects seen from unselective adenosine receptor agonists (Dunwiddie and Masino, 2001). Adenosine uptake inhibitors, such as dipyridamole, mioflazine and draflazine (Hammond et al., 2000), have been used as cardioprotective agents in heart surgery and after heart attacks (Van Belle, 1993; Rongen et al., 1995). The dual adenosine uptake inhibitor and phosphodiesterase inhibitor, propentofylline, is a promising anti-Alzheimer agent (Ringheim, 2000). Further, adenosine or adenosine agonists have shown positive results on wound healing (Grant et al., 1999), and protection against cell damage during chemotherapy (Fishman et al., 2000; Ohana et al., 2001). Thus, a compound (or a mixture of compounds such as DIFEQ or DICAQ), present in brewed or instant coffee in quantities sufficient to increase extracellular levels of adenosine in brain and periphery, have the potential to cause similar effects.

In conclusion, we report here that DIFEQ, a non-xanthine derivative of chlorogenic acid, has low micromolar affinity for the human adenosine transporter protein. The potency of DIFEQ is surpassing that of caffeine for the adenosine A_{2A} receptors. DIFEQ is a lipophilic representative of a large number of neutral, isomeric compounds formed in the roasting

process of coffee, and therefore, raises the possibility that the cardio- and liver-protective effects from coffee consumption seen in epidemiological studies, are the consequence of increased extra-cellular adenosine levels. The similar potencies for DICAQ and DICOQ suggest that the combined activities of DIFEQ-like constituents of coffee has the potential to reach pharmacologically active levels as a result of normal coffee consumption, and possibly modulate the stimulant effects of caffeine.

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Does Coffee Drinking Influence Plasma Antioxidant Capacity?

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SUMMARY

Absorption, metabolic fate and availability for antioxidant protection in humans of dietary plant constituents, such as flavonoids and related polyphenols, are still not fully explained. Moreover, the definition "plant phenols" includes thousand of compounds with different chemical structures corresponding to different antioxidant activities. As chemical structure is an important determinant in their bioavailability. the profile of phenolic compounds in plasma can be quite different from that of the original dietary source due to metabolisation and bio-transformation.

Recently, a number of beverages derived from vegetables have been tested for their in vitro and in vivo antioxidant activity (white and red wine, green and black tea, beer). A straightforward analysis of these studies confirm that (i) the capacity of a food to transfer its antioxidant activity is linked to several know and unknown chemical/biochemical/physiological characteristics, (ii) the effect of food phenols on the redox balance in vivo can not be a simple extrapolation of its activity in vitro.

Coffee contains several phenolic components, other than tocopherols, endowed with antioxidant capacity: chlorogenic acids (esters of some cinnamic acids with quinic acid), and caffeic, ferulic, p-coumaric acids in free form. Black tea contains catechins and thearubigins and theaflavines, which are oxidation products of cathechins formed during enzymatic oxidation by polyphenol oxidase in fresh tea leaves.

In this study we concentrated on the capacity of coffee to affect the plasma redox homeostasis in humans, using tea as positive control.

In two different sessions, a standard amount (200 ml) of brewed coffee or black tea was administered in fasting conditions to 10 healthy non-smoker moderate-coffee drinkers. Beverages were taken within 10 min from brewing. Plasma at time 0, and 1 and 2 hours after coffee/tea administration was analysed for: uric acid; alpha-tocopherol and glutathione (reduced and oxidised); total antioxidant capacity, by measuring the competition with the bleaching of two different target molecules (r-phycoerithryn and crocin) triggered by the peroxyl radicals generated by thermal decomposition of 2,2 azo-bis (2 aminopropane)-chlorohydrate (AAPH).

The ingestion of 200 ml of coffee in bolus produced a 5.5% increase ($P<0.05$) in the plasma antioxidant capacity (by the r-phycoerithryn test) at t=1, maintaining a 4% increase after two hours. The 4.7% increase 1 hour after the tea administration did not reach statistical significance, as a consequence of different individual responses. As for coffee, the antioxidant capacity measured by the crocin test gave a similar trend in the modulation of antioxidant activity, even if the differences were not statistically significant. No effect was seen in the case of tea drinking using this test.

The explanation of the discrepancy between the two methods employed can be found in the capacity of some molecules to affect the plasma concentration of uric acid, coupled with a different sensibility to uric acid of the two methods. In fact, only tea drinking produces a significant increase of plasma uric acid, a component of the group of molecules with antioxidant activity contributing to the AC as measured by the r-phycoerithryn test, but not by the crocin test. The other parameters of antioxidant status do not change significantly, except for a significant increase of alpha-tocopherol 2 h after tea drinking.

From all that, we can advance the hypothesis that the increase of plasma antioxidant capacity (by crocin test) induced by coffee is due to antioxidants derived from coffee, while in the case of tea the (although not statistically significant) increase is due to the increase of uric acid.

At the moment we are not able to justify why tea drinking induces an increase of uric acid and coffee drinking not. However, phenolic composition and quantitative distribution in different phenolic classes can be responsible for this phenomenon.

INTRODUCTION

Some dietary plant constituents, such as flavonoids and related phenolic, are considered powerful antioxidants *in vitro* (Sichel et al., 1991; Wang et al., 1996; Hiramoto et al., 1996; Bors et al., 1990; Ghiselli et al., 1998; Castelluccio et al., 1995; Nardini et al., 1995; Natella et al., 1999), and are supposed to b responsible for the inverse relationship between fruit & vegetable intake and risk of degenerative diseases (CHD, cancer). Polyphenols are also endowed with biological activities, such as modulation of enzymes, activation of transcription factors and in general of gene expression.

However, their absorption, metabolic fate and, availability for antioxidant protection in humans are not fully understood. Moreover, the definition “plant phenolics” includes thousand of compounds with different chemical structures corresponding to different antioxidant activities. In addition, the chemical structure (number of phenolic rings, aromatic substitution, glycosylation, conjugation with other phenolics or organic acids) is an important determinant in their bioavailability. As a consequence, the profile of phenolic compounds in plasma can be quite different from that of the original dietary source due to metabolization and biotransformation.

Recently, a number of beverages derived from vegetables have been tested for their in vitro and in vivo antioxidant activity (white and red wine, green and black tea, beer) (Duthie et al., 1998; Vinson et al., 2001; Natella et al., 2001; Hodgson and Puddey, 2000; Leenen et al., 2000; Miura et al., 2001; Gasbarrini et al., 1998; Ghiselli et al., 2000).

A straightforward analysis of these studies confirms that

- the capacity of a food to transfer its antioxidant activity is linked to several know and unknown chemical/biochemical/physiological characteristics (see the case of green and black tea with and without milk) (Serafini et al., 1996);
- the effect of food phenolics on the redox balance in vivo can not be a simple extrapolation of their activity in vitro.

The aim of this study was to assess the capacity of coffee (American style) in affecting the plasma redox homeostasis in humans in fasting conditions, using tea as positive control.

Coffee contains several phenolic components, other than tocopherols, endowed with antioxidant capacity. Among the phenolic compounds identified are chlorogenic, caffeic, ferulic, and p-coumaric acids. On the basis of 10 g coffee per cup of brew, a cup content of chlorogenic acids can range from 15 to 325 mg. An average value of 200 mg/cup has been reported for American coffee. Black tea contains catechins and thearubigins and theaflavines, which are oxidation products of catechins formed during enzymatic oxidation by polyphenol oxidase in fresh tealeaves.

METHODS

Coffee brew was prepared by using a commercial automatic brewing machine (60 g of roasted and ground coffee per liter of water). The coffee brand was Lavazza Qualità Rossa. Tea (20 g/l) was prepared by 5 min infusion in water at 100°C. The tea brand was Twining Earl Gray.

Ten healthy non-smoker moderate-coffee drinkers (2-4 cups per day) were recruited among the staff of the INRAN. A standard amount (200 ml) of brewed coffee was administered in fasting conditions. In a different session (2 weeks apart) black tea was administered as positive control. Beverages were ingested within 10 min from brewing. Subjects were asked to avoid antioxidant supplements, and to have a diet low in "coffee, wine, chocolate, tea, fruit & vegetable" in the two days preceding the experiments. Plasma was separated from blood collected just before coffee/tea administration (time 0) and 1 and 2 hours after coffee/tea administration.

Blood was withdrawn in vacutainers with EDTA (1 mg/ml) and samples were centrifuged to separate plasma.

The following tests were performed on plasma samples:

- total antioxidant capacity, AC, assessed by measuring (a) the loss of fluorescence of r-phycoerithryn when exposed to a constant flow of peroxyl radicals, generated by the thermal decomposition of AAPH (TRAP test) (Ghiselli et al., 1996); (b) the competition with the bleaching of a carotenoid, the crocin (crocin test) triggered by the peroxyl radicals generated by thermal decomposition of 2,2 azobis (2 aminopropan)-chlorohydrate (AAPH) (Tubaro et al., 1996);
- alpha-tocopherol (Lang et al., 1986),
- uric acid utilizing a kit by Boehringer Manneheim;
- SH-groups (Ellman, 1959).

RESULTS AND DISCUSSION

The ingestion of 200 ml of coffee in bolus produced a statistically significant increase (5.5%, $P<0.05$) in the plasma antioxidant capacity (TRAP) at t=1 (Table 1), maintaining a 4% increase after two hours. The 4.7% increase of TRAP 1 hour after the tea administration did not reach statistical significance, as a consequence of different individual responses.

As for coffee, the crocin test gave a similar trend in the modulation of antioxidant activity, even if the differences were not statistically significant. On the contrary, a statistically significant decrease ($P<0.005$) was seen after tea drinking using this test.

Among the three antioxidants tested in this study – alpha tocopherol, SH groups and uric acid – only this last, and only after tea drinking, increased significantly (Table 1).

The two methods employed to measure AC differ for their capacity to be affected by uric acid (plasma uric acid contribution to TRAP is about 60%, while its contribution to the crocin test is equal to zero).

In our study tea drinking produces a significant increase of plasma uric acid. The same increase is not produced by coffee. Plotting together TRAP, crocin and uric acid (Figure 1), it appears evident that the increase of TRAP after tea drinking overlaps the increase of uric acid.

In the case of coffee drinking, the increase of plasma antioxidant capacity, as measured with both methods, is clearly independent from uric acid, suggesting that other compounds (possibly polyphenols) directly participate to the AC.

Table 1. Plasma concentration of some parameters of antioxidant status (mean ± standard error of 10 subjects)

	Coffee			Tea		
	baseline	1 h	2 h	baseline	1 h	2 h
α-tocopherol, μg/ml	7.2±0.6	7.4±0.5	7.6±0.5	7.2±0.5	7.7±0.5	8.0±0.5*
SH groups, μM	398±16	412±14	409±17	432±15	437±14	413±12
Uric acid, mg/dl	5.1±0.3	5.2±0.3	5.1±0.3	5.2±0.3	5.4±0.4**	5.4±0.3*
Crocin, mM Tx eq	1.22±0.07	1.31±0.09	1.20±0.08	1.36±0.07	1.26±0.10	1.22±0.05 **
TRAP, mM ROO• eq	1.45±0.07	1.53±0.07*	1.51±0.06	1.49±0.05	1.56±0.05	1.52±0.05

** P<0.05 from baseline by paired t-test*
*** P<0.005 from baseline by paired t-test*

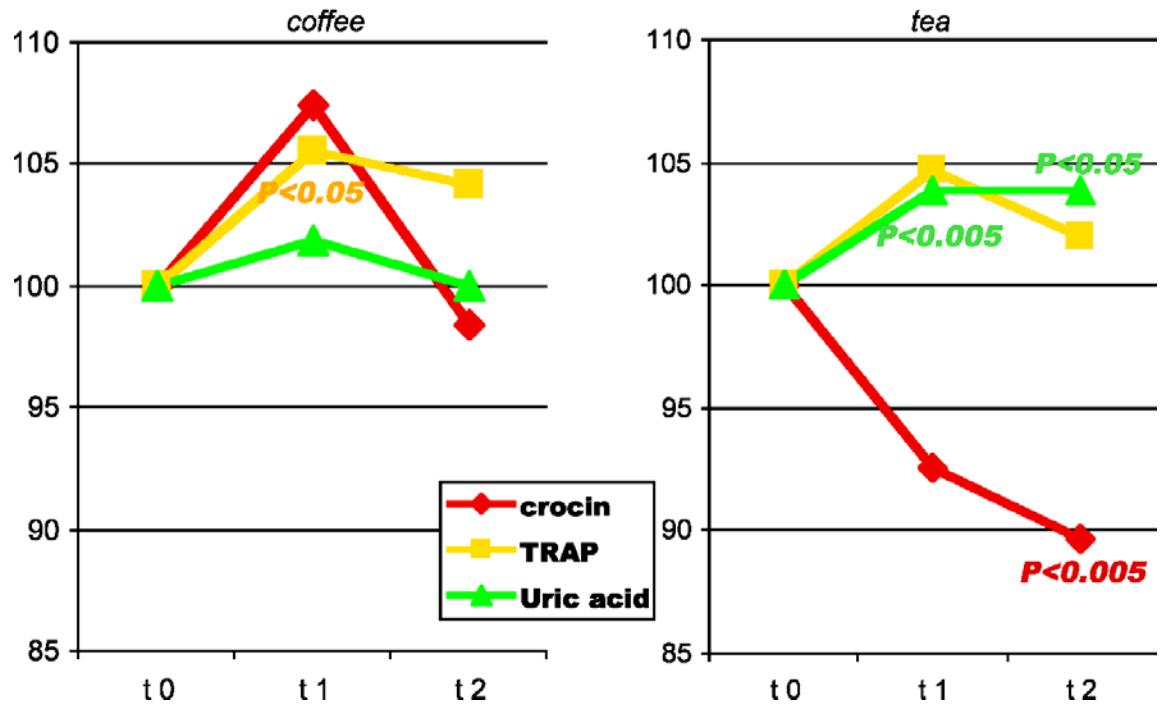

Figure 1. Plasma total antioxidant capacity and uric acid

As a conclusion we can advance the hypothesis that the increase of plasma antioxidant capacity induced by coffee is due to antioxidants derived from coffee, while in the case of tea the increase of TRAP (although not statistically significant) is due to the increase of uric acid. In previous studies we found:

- a 15% increase of plasma uric acid 1 hour after by beer drinking (whole or dealcoholized) in humans (Ghiselli et al., 2000);
- plasma uric acid 8% higher in rats drinking beer than in controls drinking water (Gasbarrini et al., 1998);
- a more pronounced increase in plasma concentration of uric acid after a meal supplemented with wine (+23%) than after a meal supplemented with an hydroalcoholic solution (+10%) (Natella et al., 2001).

Apart from the well known effect of ethanol on plasma uric acid (Yamanaka and Kamatani, 1997), its increase after administration of phenol-rich foods/beverages has been ascribed to the interference of phenols with secretion and re absorption of uric acid (Gibson et al., 1984).

At the moment we are not able to fully justify why tea drinking induces an increase of uric acid and coffee drinking not. However, our hypothesis is that the different phenolic pattern of coffee and tea is responsible for the different response of uric acid in plasma.

In conclusion, the increase of TRAP after tea administration in humans seems to be linked to uric acid increase (probably due to tea polyphenols), while the increase of TRAP after coffee administration could be directly linked to coffee polyphenols.

Moreover, our results confirm that the mere measure of "*antioxidant capacities*" can lead to confounding results in *in vivo* studies, where chemical characteristics and *in vitro* activity of an *antioxidant-rich food* can not be transposed.

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Beneficial Effects of Caffeinated Coffee and Effects of Withdrawal

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SUMMARY

There is now a large literature on the beneficial effects of caffeinated coffee on performance and mood. Recent claims have been made that the effects of caffeine do not reflect a benefit but merely the removal of negative effects of caffeine withdrawal. If such claims are true then this undermines the substantial literature on the behavioural benefits of consuming caffeinated coffee. It is essential, therefore, to provide a systematic account of this issue. Those who do not consume caffeinated products cannot have caffeine withdrawal. Comparison of non-consumers with regular consumers who have had caffeine withdrawn provides information on whether withdrawal does influence mood and performance. Similarly, if effects of caffeine can be demonstrated in this group then this casts serious doubts about the caffeine withdrawal explanation of the behavioural effects of caffeine. Three studies were conducted to examine these issues. Overall, results from these large-scale studies showed no evidence of negative effects of caffeine withdrawal on mood and performance. They also show improved mood and performance in both non-consumers, who by definition are not withdrawn, and withdrawn consumers.

INTRODUCTION

A large number of studies have examined the effects of caffeine on mental performance and many of these have demonstrated beneficial effects (see Lieberman, 1992, for a review). It is also well established that caffeine is especially beneficial when alertness is low (Lorist et al., 1994; Smith, 1994) and this has been examined by considering its effects at night (Smith et al., 1993), in the early morning (Smith et al., 1992), after lunch (Smith et al., 1990), prolonged work (Smith, 1994) and when subjects are suffering from a cold (Smith et al., 1997). Some of these studies have also shown that even low doses (<200 mg) can be beneficial when alertness is reduced. Furthermore, the practical importance of such effects has been demonstrated in a study that examined caffeine and the driving performance of sleepy drivers (Hrne and Reyner, 1996). This study clearly demonstrated that 150 mg caffeine (equivalent to 2 cups of coffee) significantly reduced driving impairments, subjective sleepiness and EEG activity indicating drowsiness.

James (James, 1994) has questioned whether the superior performance seen in caffeine conditions are due to actual enhancement or merely reflect performance being impaired in the caffeine-free conditions. A major piece of evidence against this view is that there is a vast animal literature on behavioural effects of caffeine, and these results cannot be accounted for by removal of caffeine withdrawal. Smith (1994) has suggested that the evidence for negative effects of caffeine withdrawal is not strong and this has been confirmed in recent studies of caffeine withdrawal and headaches (Smith, 1996; Rubin and Smith, 1999; Dews et al., 1999). There is a similar lack of evidence for negative effects of caffeine withdrawal on performance and Rogers et al. (1995) conclude that "... in a review of recent studies we find no unequivocal evidence of impaired psychomotor performance associated with caffeine withdrawal". Indeed, Rogers et al. (1995) have shown that the beneficial effects of caffeine on performance can be demonstrated in non-users, and users who had caffeine withdrawn for

varying periods of time (1.5 hr, 13 hr and 7 days). This confirms previous findings showing that caffeine has comparable effects when it is given after abstinence of 1 hr (Smith et al., 1994; Warburton, 1995) or 12 hrs (Smith et al., 1992).

One of the problems in comparing studies of caffeine and caffeine withdrawal is that they have used different paradigms, have different designs and vary in experimental power. Smith (1999) conducted a study to examine effects of caffeine and caffeine withdrawal in the same experiment. The first part of the study involved a normal caffeine challenge. James argued that any positive effects of caffeine actually reflect negative effects of withdrawal in the caffeine-free condition. To directly examine withdrawal effects half the subjects then to continued using caffeinated products for a week whereas the others were provided with caffeine-free drinks. If there are negative effects of caffeine withdrawal these should show up at this stage of the study. James argued that after 7 days the negative effects of caffeine withdrawal should have gone. If a caffeine challenge is then repeated with these subjects there should now be no beneficial effects of caffeine because the negative effects of caffeine withdrawal have gone. An alternative view is that caffeine will improve performance, both following short-term withdrawal and 7 days of withdrawal, and that it will be difficult to demonstrated effects of caffeine withdrawal per se. Smith's experiment tested these views and it had the following methodological features. First, two doses of caffeine were compared with placebo in a double-blind study. The fact that larger doses of caffeine produce bigger effects than the smaller doses also produces problems for the withdrawal explanation. If a person is given a dose that is equivalent to their normal intake they have not had caffeine withdrawn. Yet the literature shows that additional caffeine may lead to beneficial effects. Secondly, tests that were known to be sensitive to effects of caffeine were used. Finally, caffeine abstinence is difficult to assess unless saliva samples are taken and that was done here. The results confirmed that performance of psychomotor tasks is improved following consumption of caffeinated coffee. The improvement was generally in the form of dose response, with 3 mg/kg caffeine being associated with the best performance. These results were apparent both before and after withdrawal, which suggests that the beneficial effects of caffeine cannot be accounted for by impairments in the caffeine-free condition. The view is further supported by the absence of negative effects when caffeine is withdrawn. A direct test of James explanation of the effects of caffeine on psychomotor performance shows, therefore, that there is little support for his view.

Another method of examining whether removal of negative effects of caffeine withdrawal underlies the positive effects of caffeine on performance is to compare regular consumers and non-consumers (who by definition cannot have caffeine withdrawal). We have recently conducted three studies which have examined this topic. In the first, over 250 volunteers who have abstained from caffeine for 12 hours have been compared with over 60 volunteers who do not consume caffeinated drinks. The data from these groups show no differences in mood, performance or the reporting of headaches (Figures 1-3). Identical results have been obtained in another large-scale study looking at different performance tests.

Two studies have then examined the effects of caffeine on the performance and mood of non-consumers and withdrawn regular consumers. Both show identical significant beneficial effects of caffeine in non-consumers and withdrawn consumers (Figures 4 and 5). Overall, these large-scale studies show no evidence of negative effects of caffeine withdrawal on mood and performance. They also show improved mood and performance in both non-consumers, who by definition are not withdrawn, and withdrawn consumers.

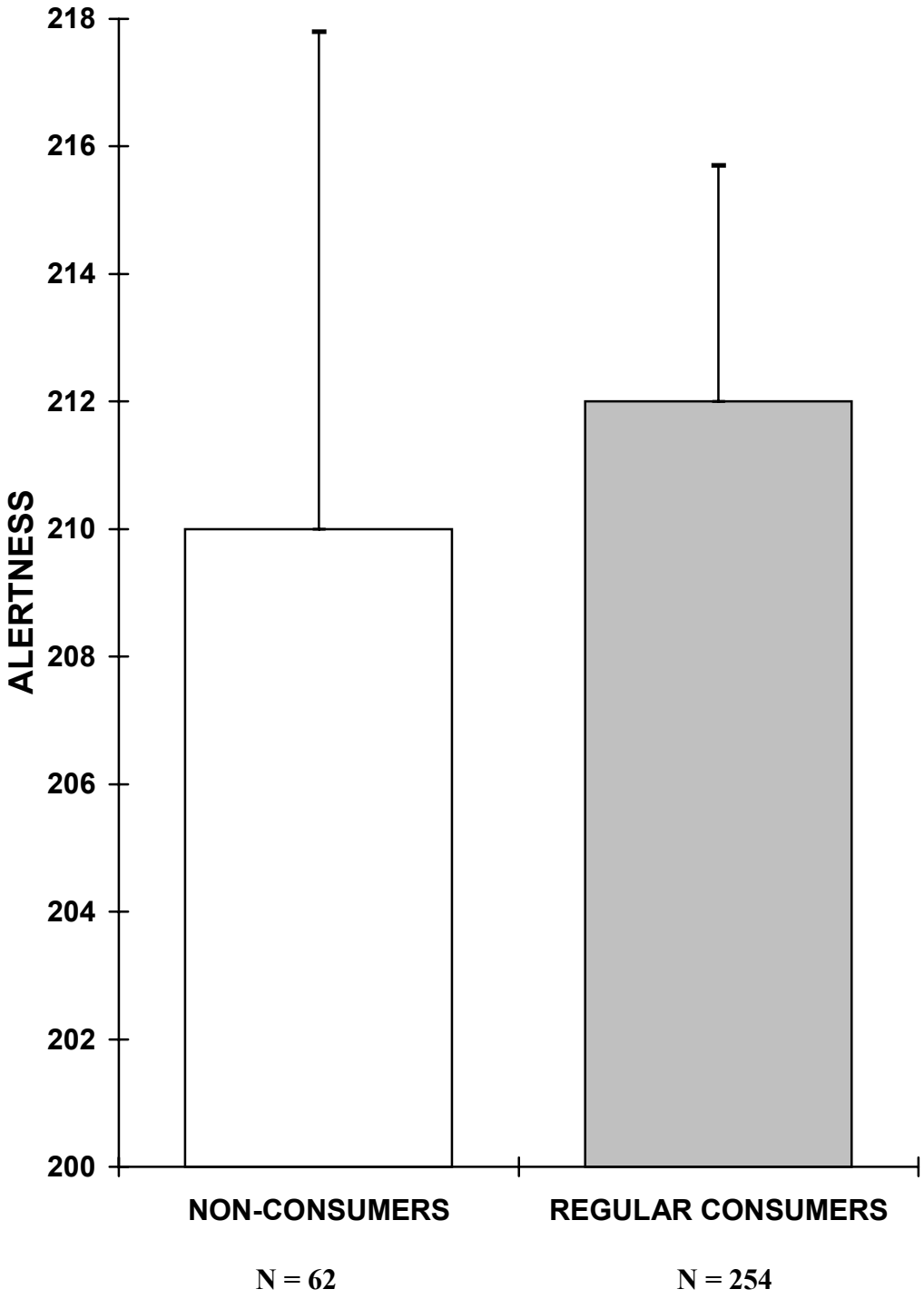

Figure 1. Non-Consumers and Withdrawn Regular Consumers: Alertness in the Morning (high scores = greater alertness; no significant difference between groups)

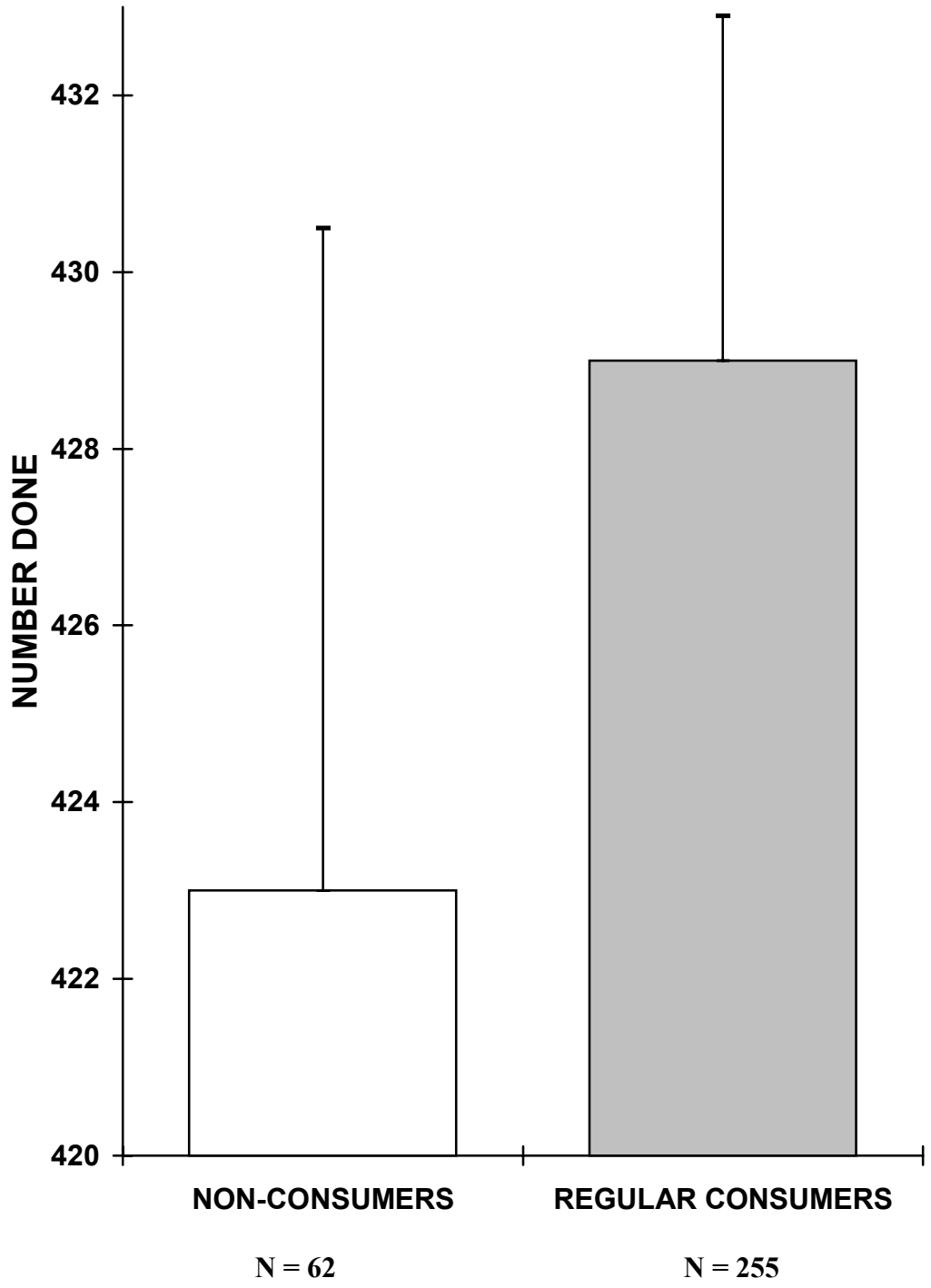

Figure 2. Non-Consumers and Withdrawn Regular Consumers: Number Done Five-Choice Serial Response Task (high scores = better performance; no significant difference between groups)

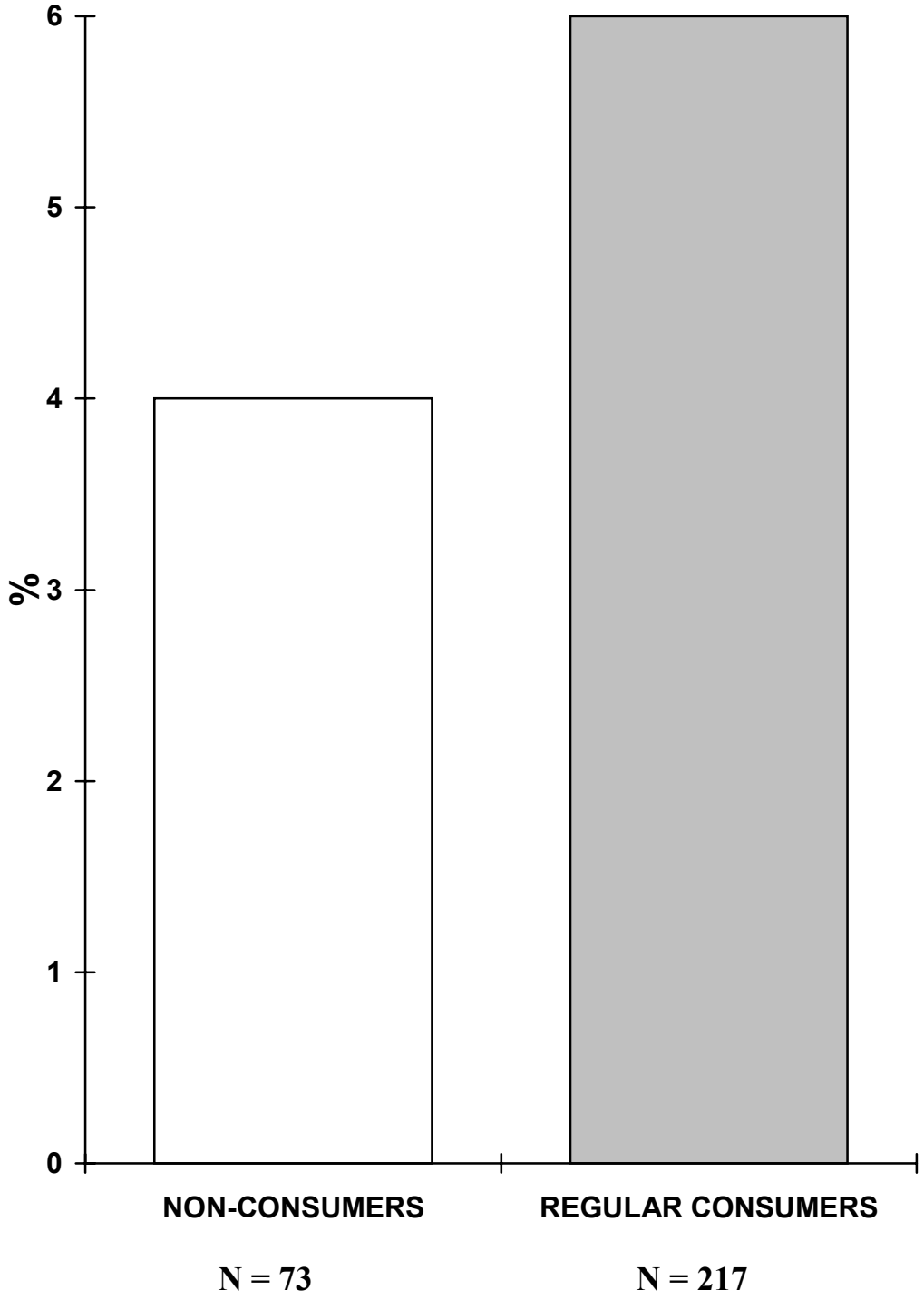

Figure 3. Non-Consumers and Withdrawn Regular Consumers: Headache (note low incidence of headache; no significant difference between groups)

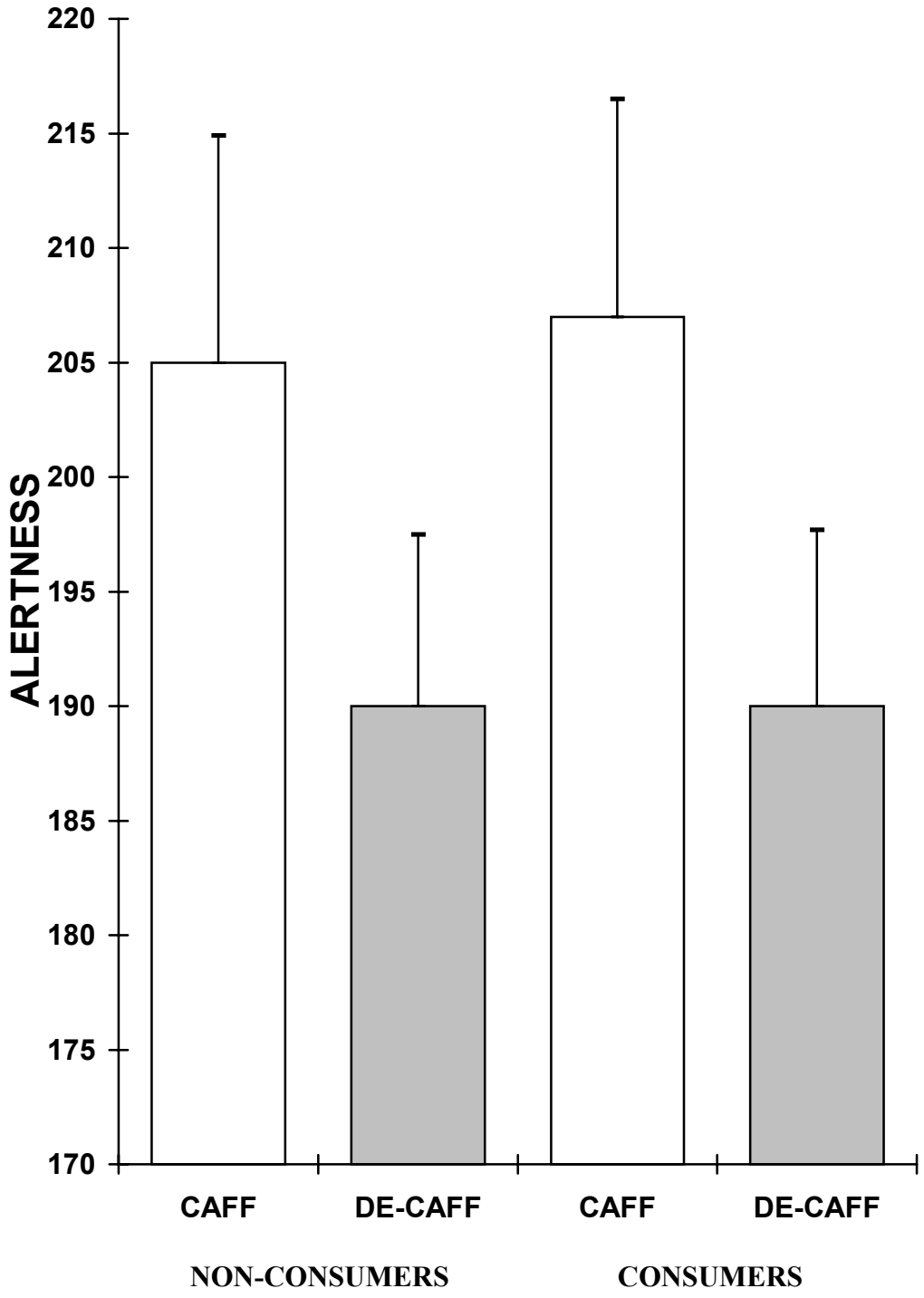

Figure 4. Non-Consumers and Withdrawn Regular Consumers: Effects of Caffeine on Alertness (high scores = greater alertness; caffeine has a significant effect in both groups)

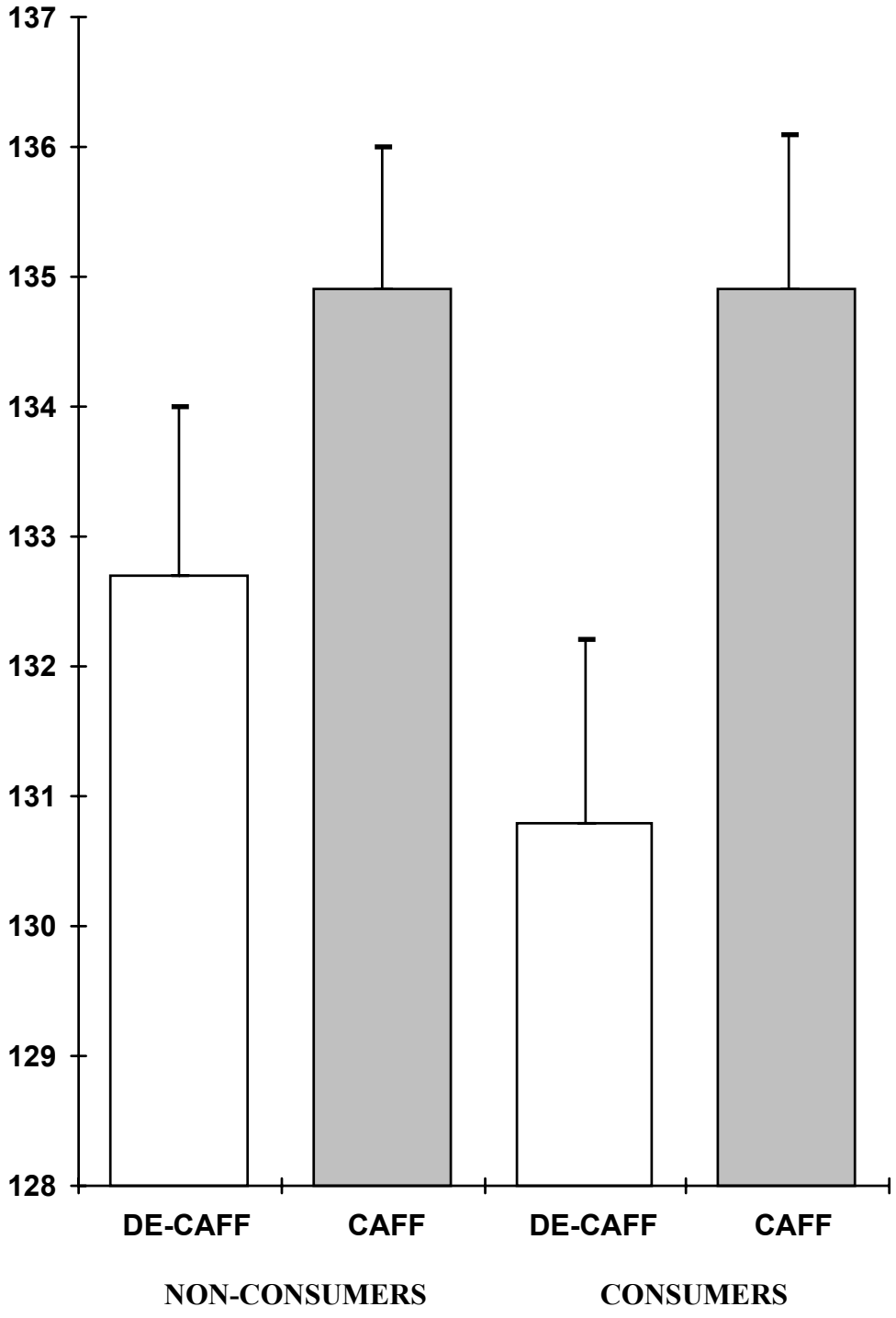

Figure 5. Non-Consumers and Withdrawn Regular Consumers: Effects of Caffeine on Semantic Memory – Number Done (high scores = better performance; caffeine has a significant effect in both groups)

Another way of investigating the effects of caffeine without the confounding effects of withdrawal is by examining the effects of caffeine in those who have recently consumed caffeine and who, by definition, cannot be withdrawn. Our earlier research has shown that effects of caffeine can be demonstrated when testing is carried out only two hours after consumption of caffeine. We have now replicated this effect and also demonstrated beneficial effects of caffeine on the encoding of new information in volunteers who have been consuming caffeinated coffee over the course of the day. This is consistent with our earlier result showing that there were few differences in the acute effects of caffeine in those who consumed caffeinated beverages for a week and those who consumed de-caffeinated drinks. Overall, results from these large-scale studies showed no evidence of negative effects of caffeine withdrawal on mood and performance. They also show improved mood and performance in non-consumers, withdrawn and non-withdrawn regular consumers.

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Caffeine and Human Memory: A Literature Review and some Data

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SUMMARY

The literature review suggested that the effects of caffeine on human memory are inconsistent and inconclusive compared with well-established caffeine effects on measures of attention and reaction time. When the literature is reviewed in relation to the dissociations in human memory identified by mainstream memory research it appears that although there are no global effects on memory, caffeine does reliably improve semantic memory (i.e. retrieval of 'general knowledge') and executive function (as measured by performance on logical reasoning tasks). The literature review also suggested that the effects of caffeine may be mediated by mediated by personality characteristics and time of day. A double blind, placebo-controlled study was able to replicate the finding that caffeine improves semantic memory and executive function performance but did not provide any evidence that caffeine interacts with baseline physiological arousal or time of day.

INTRODUCTION

There is accumulating evidence that caffeine has effects on cognitive performance and a large body of literature now describes significant short-term improvements in alertness, vigilance and reaction time after consumption of caffeine (e.g. Smith, 1994; Lieberman, 1992). In contrast however, evidence for an effect of caffeine on human memory is inconclusive and disparate and studies variously report that consumption of caffeine has no significant effect on memory, causes a decrement in memory performance or improves memory. Consequently, despite a small number of well-controlled studies that have produced positive results it is widely surmised (e.g. James, 1998) that memory is an area where caffeine has negligible effects. It is suggested however that there are a number of reasons why such a conclusion may be considered premature.

Firstly, caffeine research has generally failed to acknowledge the distinctions in memory structure and processes that have been identified in psychological research. Evidence from cognitive psychology (Baddeley, 1997) suggests that a structural level human memory is composed of short-term memory and various types of long-term memory. Caffeine research to-date has tended to draw conclusions about the effects of caffeine on memory as whole by testing one component of the human memory system, usually short-term memory.

There is also a considerable body of psychological research to suggest that there are different processes which human memory uses to lay-down and retrieve information contained in memory e.g., explicit memory (conscious use of memory), implicit memory (unconscious use of memory), recall and recognition (Eysenck and Keane, 1995). With the exception of recall, very little research to-date has attempted to investigate what effects caffeine might have on these different memory processes.

In reviewing the large body of literature that refers to caffeine and memory it is hoped to identify any memory structures or processes where caffeine has a consistent effect, any areas where the literature is inconclusive and any areas where there is, as yet, no data available. Due to the large amount of empirical evidence that supports the existence of discrete memory structures and processes (see Eysenck and Keane, 1995; Baddeley, 1997) the review will use the dissociations identified by contemporary cognitive psychology and cognitive neuropsychology as a framework for discussion. The review will not then attempt to provide an exhaustive catalogue of every study which has considered the effect of caffeine on human memory but will review only those which clearly acknowledge or are compatible with the major memory structures and processes that have been dissociated in mainstream memory research.

SENSORY MEMORY

Experimental evidence (e.g. Sperling, 1960) suggests that there are very short term, memory stores for visual and for auditory stimuli that are likely to have a role in allowing the perceptual system sufficient time to process very briefly presented stimuli (Baddeley, 1997). Sensory memory stores stimuli for approximately one third of a second before they dissipate and are referred to as iconic memory for the visual stimuli and echoic memory for the auditory stimuli. Both have been studied extensively in cognitive psychology but no published studies to-date have investigated whether sensory memory is affected by ingestion of caffeine.

SHORT-TERM MEMORY

The concept of a relatively short-term memory store was first described in detail by James (1890) who reported the existence of relatively short-term memory store characterised by limited capacity and short duration. Presently the capacity of the store, referred to as short-term memory, is thought to be approximately 7 pieces of information which can be stored for a duration of less than 5 seconds without any form of strategic rehearsal. The nature of short-term memory (STM) has been the subject of a vast amount of interest in both cognitive psychology and neuropsychology and it is perhaps not surprising that much of the literature pertaining to caffeine and memory also focuses on the potential effects caffeine might have on STM. The most commonly used method of studying STM is the free recall task where participants are simply shown a list of words and asked to recall them when the last word has been shown.

On the basis of studies that have used immediate free recall tasks to investigate the effects of caffeine on STM it is suggested that this is component of memory where caffeine has very little effect. Six studies have found no effect of caffeine (Loke et al., 1985; Loke, 1988; Mitchell and Redman, 1992; Smith et al., 1993; Barraclough and Foreman, 1994; Rogers and Dernoncourt, 1998) and only one has found a decrement (Terry and Phifer, 1986). Retrieval of information from STM can also measured using the Sternberg memory scanning paradigm (Sternberg, 1975) in which participants are asked to judge, as quickly as possible, whether a test digit or letter is contained within a series of briefly-presented multi-digit combinations. Studies using this task have also produced inconsistent results (Kerr, 1991; Hogervorst, 1998; Hindmarch et al., 1998).

WORKING MEMORY

The concept of a unitary STM has been rendered obsolete by the work of Baddeley and Hitch (1974) and Baddeley (1986) whose experimental work suggests that STM itself is composed

of three discrete sub-components. On the basis of these sub-divisions Baddeley (1986) has proposed the working memory model which is widely accepted in cognitive psychology and neuroscience as being the foremost model of short-term memory as it specifies not only the qualities of short-term memory but also the mechanism by which STM can be used in everyday tasks.

Baddeley proposes that that working memory has three discrete components; the phonological loop, the visuo-spatial sketchpad and the central executive. The components of working memory model are the phonological loop (responsible for holding speech-based information for 1.5-2.0 sec. or longer if certain strategies are used), the visuo-spatial sketchpad, which is thought to hold visuo-spatial information and the central executive which is responsible for co-ordination of the systems and for communication with long term memory. By way of example, if a person were carrying out a real-life task such as performing a numerical calculation the numbers involved would be held in the phonological loop whist the central executive directed cognitive resources to the numbers and to perform the calculation. When the calculation had been performed the central executive component would then separate out the important numbers to be retained before directing them to longer-term memory.

In practice performance of the phonological loop is measured using a digit span task similar to recalling a list of numbers whilst the visuo-spatial sketchpad is measured by tasks involving memory the physical location of stimuli. Measurement of executive function is more complex but generally involves tasks in which information has to be held and manipulated at the same time. The most famous example of this type of task has been devised by Baddeley (1968) where participants are shown a rapid sequence of sentences describing the order of letter pair (e.g. A follows B) and a letter pair (e.g. BA) and have to make a judgement as to whether the sentence is true or false. Performance in such tasks is taken to be the number of sentences a participant can answer in a set period of time and the percentage they get correct. Of the 8 studies that have investigated the short-term effects of caffeine on working memory none have been able to report any effects of caffeine on the visuo-spatial sketchpad and only one has reported a significant main effect on the phonological loop (Smith et al., 1994b). The effects of caffeine on central executive function appear to be more reliable with 3 studies describing significant increases in performance after caffeine (Smith, Kendrick and Maben, 1992; Smith et al., 1993; Smith, Maben et al. 1994). Studies which have failed to report any effects of caffeine on executive function have generally used lower doses of caffeine (e.g. Smith et al., 1997) and it is suggested that caffeine effects on central executive function may possibly be dose-dependent.

LONG-TERM MEMORY

The majority of models of memory recognise the existence of a longer-term storage facility, LTM, which is characterised by an extremely large, comparatively permanent storage capacity. Like STM however LTM is unlikely to be a unitary construct and Tulving (1972) has suggested that because of the need for the device to store a very diverse array of information it may be fractionated into episodic and semantic memory. Episodic memory refers to a system storing specific events or episodes which can be tied to a specific time such as when you went on holiday last year or what you had for dinner. Semantic memory on the other hand is a system for storing general knowledge, rules and formulas which are without any temporal associations such as the knowledge that Paris is the capital of France or how words are correctly spelt. The dissociation between episodic and semantic memory has been demonstrated in amnesics who generally have intact semantic memory as shown by unimpaired language and grammar yet who have severe deficits in episodic memory. The dissociation has been further demonstrated in PET scan studies where it is reported that

episodic memory is associated with high blood flow around the frontal cortex whilst semantic memory is associated with increased blood flow in the posterior regions of the brain (Tulving, 1989).

Episodic memory

Caffeine research appears to have paid relatively little interest to the effects of caffeine on LTM compared to STM or working memory. The three studies have attempted to investigate the effects of caffeine on LTM have all used delayed recall tasks where participants are asked to remember a series of stimuli items and then to attempt to recall them several minutes later. Unfortunately the studies have produced largely inconsistent results with Terry and Phifer (1986) demonstrating a decrease in recall after acute ingestion of caffeine but Loke et al. (1985) and Loke (1988) reporting no effects.

Semantic memory

Experimental evidence would suggest however that semantic memory is one of the few areas of memory where caffeine has a reliable effect. Five studies to date have investigated the effect of acute exposure to caffeine on semantic memory performance using the widely used Baddeley (1981) semantic test. In this test participants are shown a series of sentence (e.g. a dogs have wings or canaries have wings) and asked to make a decision as to whether the sentence was true or not. Participants are asked to complete as many trials as they can in a set period of time (usually 3-5 minutes) with performance being measured in terms of the amount of questions they attempt to answer and the percentage they get correct. In four of the five studies consumption of caffeine (40 mg to 4 mg/kg) is reported to have significantly improved memory performance (Smith et al. 1992; Smith et al., 1993; Smith et al., 1994b; Smith, et al., 1999) with only one study failing to find an effect (Smith et al., 1997).

MEMORY PROCESSES

Research into human memory also distinguishes between the processes that underlie human memory as well as the subcomponents of the human memory system. Thus far the review has discussed the potentially differential effects of caffeine on the putative distinctions within the structure of memory, an alternative strategy would be to consider the effects of caffeine on different memory processes.

Recognition memory

One of the few dissociations in memory process that has been consistently recognised in the study of the cognitive effects of caffeine is that between recall and recognition and this is probably a reflection of the long-standing interest the topic has generated within mainstream memory research. Recognition memory is measured by presenting participants with a series of word stimuli and when this is finished presenting them with another list composed of the original list interspersed with words that they have not seen before. Participants are then required to indicate which of the words in the first list were presented in the second list. Although the effects of caffeine on delayed recognition memory has been the subject of numerous studies the results obtained suggest that recognition memory is not generally affected by acute exposure to caffeine; of the 8 studies reported (Bowyer et al., 1983; Loke et al., 1985; Smith et al., 1992; Anderson and Revelle, 1994; Smith et al., 1994a; Smith et al., 1994b; Smith et al., 1997; Smith et al., 1999a) only one has reported a significant main effect (Anderson and Revelle, 1994). The significant increase in recognition memory performance described in this case was obtained using very long lists of words, up to 80, such that the

caffeine effect was probably to decrease attentional lapses during the encoding procedure which are known to mediate recognition performance (Underwood, 1978).

Implicit memory

The majority of the studies which have investigated the effects of caffeine on memory have focused on explicit memory, that is where the memory task requires conscious recollection of previously presented stimuli or experiences. There is however evidence from cognitive psychology and neuropsychology to suggest that there is a different form of memory that is revealed when '…performance on a task is facilitated in the absence of conscious recollection' (Graf and Schachter, 1985, p. 501). Implicit can be measured in a variety of ways but usually consists of an encoding task where participants inadvertently learn a list of words (e.g. by rating them in terms of how much they liked them) followed by a seemingly unconnected task, such as a series of word anagram completions. Implicit memory would be demonstrated if a higher percentage of anagrams were solved for anagrams of words encoded than for anagrams of words which had not. Although implicit memory is easily demonstrable and supported by considerable evidence no studies to-date have conducted well-controlled investigations into the effects of caffeine on implicit memory.

Levels of processing

The two basic premises of levels of processing theory are that the level or depth of processing of a stimulus mediates subsequent memorability and that deeper levels of analysis produce more elaborate and enduring memories than do shallower levels of analysis (Eysenck and Keane, 1995). One of the clearest demonstrations is provided by Craik and Tulving (1975) who manipulated encoding of lists of words to different levels before testing incidental learning via an unannounced recall test. It was found that recall was better after an encoding task involving the meaning of words (e.g. asking if the words fit into a given sentence frame) than it was after an encoding task involving superficial characteristics of the word (e.g. asking if the word was in upper or lower case).

In relation to caffeine incidental learning to different levels of processing has been used by Gupta (1991) prior to a recall task and Gupta (1993) prior to a recognition task. In both studies it was found that there were no main effects of caffeine but that there was a consistent three-way interaction between caffeine condition, level of processing and impulsivity (a index of baseline physiological arousal; Eysenck, 1967).

THE EFFECT OF CAFFEINE ON MEMORY IN CONJUNCTION WITH OTHER FACTORS

A number of studies have considered the possibility that caffeine will not have consistent effects but will have specific effects depending on an individual's baseline physiological arousal or impulsivity (Humphreys and Revelle, 1984; Anderson and Revelle, 1994). Specifically caffeine would be thought to prove of particular benefit to individuals in states of low impulsivity but to have no effect (or to cause a decrease in cognitive performance) when participants are in a high state of arousal. An individual's actual arousal levels would be dependent on trait impulsivity and also on time of day, physiological arousal being greatest in low impulsives in the morning and decreasing thereafter and being lowest in high impulsives in the morning but increasing toward evening (Blake, 1967).

Of the eight studies that have considered the possibility of an interaction between caffeine and impulsivity on memory performance four have failed to report any interaction (Bowyer et al.,

1983; Erikson et al., 1985; Arnold et al., 1987; Smith et al., 1994b). Only Anderson and Revelle (1994) have reported an interaction between impulsivity and caffeine that conforms to the prediction made by the Humphreys and Revelle's (1984) model. The other remaining studies have generally found that in high impulsives caffeine impairs performance in both recognition and free recall tasks and may interact with the type of encoding task (Smith et al., 1994a; Gupta, 1991, 1993) but that, in contrast to Humphreys and Revelle's (1984) prediction, low impulsives are unaffected by caffeine.

In conclusion the literature review suggests that there are no global effects of caffeine on human memory and that the available evidence suggests that the main effects of caffeine are limited to specific effects on semantic memory and executive function. The review has also identified a number of facets of human memory which have not yet been investigated in relation to caffeine such as sensory memory and implicit memory. There also appear to be a number of areas of research where the results, though equivocal at present, may warrant further research and foremost amongst these is arguably the potential mediation of caffeine effects by impulsivity which might explain why effects of caffeine on memory are inconsistent.

Given these conclusions from the literature review the following experiment will attempt to replicate the finding that caffeine significantly increases semantic memory and executive function performance. The study will also attempt to attempt to investigate whether these effects are mediated by impulsivity and it's associated circadian rhythmicity, specifically whether caffeine will prove to be especially beneficial when baseline arousal is low i.e. in low impulsives in the evening and high impulsives in the morning.

METHOD

Experimental design

The experiment employed a between-subjects' design with time of testing (morning vs. evening), impulsivity (high vs. low) and ingestion of caffeine (see below) comprising the between subjects factors. The 96 participants were divided into groups as shown in Table 1. Administration of caffeine was double blind.

Table 1. Experimental groups: time of testing and caffeine condition on day 1 and day 2

Group	Time of testing	Impulsivity	Caffeine condition
1 (n = 12)	Morning	Low	Caffeine
2 (n = 12)			Placebo
3 (n = 12)		High	Caffeine
4 (n = 12)			Placebo
5 (n = 12)	Evening	Low	Caffeine
6 (n = 12)			Placebo
7 (n = 12)		High	Caffeine
8 (n = 12)			Placebo

PARTICIPANTS

Ninety-six participants took part in the experiment, 48 low impulsives (EPI Impulsivity <5) and 48 high impulsives (EPI Impulsivity ≥5) with 24 males and 24 females within the low and

high impulsivity sub-groups. All were non-smokers and regular daily consumers of caffeinated coffee or tea. The demographics of the sample are shown in Table 2.

Table 2. Participant demographics and personality characteristics by high and low impulsivity (means, S.E. in parentheses)

Variable	Low impulsivity group (EPI-I < 5)	High Impulsivity group (EPI-I ≥ 5)
Age (years)	21.44 (0.32)	21.40 (0.45)
Mean caffeine consumption (mg/24h)	156.04 (17.21)	195.31 (17.58)
Impulsivity-EPI (0 low - 9 high)	2.85 (0.14)	6.21 (0.14)

PROCEDURE

Familiarisation

Participants were briefly familiarised with the test battery no more than one week prior to their first test session to ensure that they were familiar with the tests. Following demonstration of the computer tasks participants were weighed without shoes or coat so that the amount of caffeine they were to receive could be calculated.

Test procedure

The baseline test session was conducted at either 08.30 hrs or 18.30 hrs.

–08.00 Begin abstinence from self-administered alcohol and caffeine until the end of the experiment

00.1 Baseline test battery

+00.40 Administration of caffeine or placebo

+01.40 Test battery (post-caffeine/placebo)

EXPERIMENTAL BEVERAGES

All drinks were made with one rounded teaspoonful of decaffeinated coffee in 150 ml to this was added the appropriate amount of either the active or placebo solutions (each potentially containing 20 mg/ml of caffeine) such that in the active condition participants would ingest 4 mg/kg of caffeine.

MEASURES

Questionnaire

At the familiarisation session prior to the demonstration of the computer tests participants were asked to complete a detailed questionnaire that recorded demographic details, health-related behaviours, eating and sleeping habits and a profile of personality traits including the Eysenck Personality Inventory (Eysenck and Eysenck, 1968) which measures impulsvity.

Performance tasks

All tasks were presented on microcomputer.

Semantic memory

This test, following Baddeley (1981), measures speed and accuracy of retrieval of information from semantic memory. Participants were shown a sentence (e.g. dogs have wings) and asked to make a decision as to whether the sentence was true or not. Another sentence was shown automatically after a decision had been made about the first and the task continued for a total of 3 min. Indices of performance were the number of sentences judgements attempted, the number of judgements made correctly and the mean reaction time (MRT) for sentence judgements to be made.

Logical reasoning task

This task follows Baddeley (1968) and is deemed to be a test of the executive function component of the working memory model. In this task participants were shown a sentence describing the order of letter pair (e.g. A follows B) and a letter pair such as BA. Participants were then required to judge whether the statement was true or false by pressing the appropriate response key. The task went on for 3 min. and the number of statements attempted, the percentage correct and the mean reaction time (MRT) for responses was recorded.

ANALYSIS

The data was analysed using a between-subjects ANCOVA with performance at baseline used as covariate to account for individual differences in performance.

RESULTS

It was found that there were statistically significant main effects of caffeine for all indices of performance in both memory tasks with the exception of reaction time on the logical reasoning task (Table 3).

On the semantic memory test statistically significant effects of impulsivity were found for number of trials attempted where 134.19 (S.E. 1.38) trials were attempted by high impulsives and 129.60 (S.E. 1.36) by low impulsives. A main effect of impulsivity was also found for MRT for correctly answered trials with high impulsives answering correctly answered trials in a mean MRT of 1258 msec (S.E. 19) and low impulsives answering in a mean MRT of 1342 msec (S.E. 19). There were no significant interactions between caffeine and impulsivity.

CONCLUSION

It was found that caffeine significantly improved performance on all indices of performance on both tasks with the exception of MRT for correct trials for logical reasoning. This finding is in accord with the literature review and it is noted in both cases there were concurrent increases in both speed and accuracy of performance in the caffeine conditions making it unlikely that the effects of caffeine are mediated solely by speed of cognitive processing. No interactions were found between caffeine, impulsivity and time of day though for the semantic memory task high impulsives, as might be expected, attempted more trials than low impulsives and completed correctly answered trials more quickly. In summary the study has

replicated previous findings with regard to the effects of caffeine on semantic memory and executive function but does not offer any support for the theory that caffeine has any differential effects on high or low impulsives at any particular time of day.

Table 3. Memory performance: in 4 mg/kg caffeine and placebo conditions (adjusted means, S.E.s in parentheses)

Test	Index of performance	Caffeine (4mg/kg)	Placebo	Statistically significant effects
Semantic memory	Number of trials attempted	134.81 (1.39)	128.96 (1.4)	Caffeine ($p < 0.05$) Impulsivity ($p < 0.005$)
	Percentage of trials correct	95.58 (0.42)	93.20 (0.42)	Caffeine ($p < 0.05$
	MRT correct trials (msecs)	1263 (20)	1337 (20	Caffeine ($p < 0.05$) Impulsivity ($p < 0.005$)
Logical reasoning	Number of trials attempted	61.28 (1.03)	58.35 (1.01)	Caffeine ($p < 0.05$)
	Percentage of trials correct	92.94 (0.63)	91.07 (0.62)	Caffeine ($p < 0.05$)
	MRT correct trials (msecs)	3173 (92)	3301 (91)	-

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Effects of Caffeine on Dopamine and Acetylcholine Release and on Short Term Memory Function: A Brain Microdialysis and Spatial Delayed Alternation Task Study

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SUMMARY

The effects of caffeine have been studied in freely moving rats implanted with concentric microdialysis probes in the nucleus accumbens (NAc) shell and core and in the medial prefrontal cortex (PFCx). Intravenous administration of caffeine (0.25, 0.5, 1.0, 2.5 and 5.0 mg/kg) dose-dependently increased dopamine (DA) and acetylcholine (ACh) dialysate concentrations in the PFCx, while failing to affect DA in the NAc shell and core.

Such effects were duplicated by intravenous administration of the selective antagonists of adenosine A1 and A2a receptors, DPCPX (0.0625-1.0 mg/kg i.v.) and SCH 58216 (0.0625 - 1.0 mg/kg i.v.), respectively. The effect of caffeine on cortical DA and ACh transmission was also studied in rats chronically administered with caffeine (25 mg/kg/twice a day/seven days). At the end of this treatment rats become tolerant to the locomotor stimulating effects of a dose of caffeine of 1 and 2.5 mg/kg i.v.; these doses increased dialysate ACh in the PFCx but failed to affect DA release in this brain region. In non-tolerant subjects caffeine stimulated DA and ACh release in the PFCx while in tolerant subjects stimulated only ACh release.

The effect of the acute systemic administration of caffeine (1.0, 2.5 and 5.0 mg/kg i.p.) was also studied in the performance of a spatial delayed alternation task to assess working memory efficiency in rats. Administration of caffeine (2.5, 5 and 10 mg/kg i.p.) failed to affect the number of correct responses/trial in rats performing at 62±3% correct (baseline) in a spatial alternation task made up by ten couples of a forced run followed by a choice run. These observations indicate that the psychostimulant properties of caffeine are differentiated from the property of stimulating DA release in the nucleus accumbens shell, a property that caffeine, in contrast with most drugs of abuse, does not possess. This, in turn, is consistent with the lack of addictive properties of caffeine. The dissociation between tolerance to the locomotor effects of caffeine and stimulation of ACh release in the prefrontal cortex suggests that this effect might be correlated to the arousing effects of caffeine as distinct from its locomotor stimulant properties. Finally, the present results indicate that caffeine does not improve nor impair short-term memory.

INTRODUCTION

Caffeine is an ingredient of many popular beverages provided with psychostimulant properties; although this drug is currently not included in the list of drugs that fulfil DSM-IV criteria for addiction, this issue is debated. In fact, although tolerance to and withdrawal from caffeine has been demonstrated in humans and in animals after prolonged assumption (Chou et al., 1985; Nehlig, 1999; Svenningsson et al., 1999), no consistent self-administration of caffeine has been shown, in spite of various attempts, in rats and in non human primates (Nehlig, 1999; Griffiths and Mumford, 1995; Garrett and Griffiths, 1998) .

Caffeine's central effects are known to be due to blockade of adenosine A_1 and A_{2a} receptors. These receptors are widely distributed through the brain (Dixon et al., 1996; Lee and Reddington, 1986; Palmer and Stiles, 1995) , adenosine A_1 receptors being present mostly in cortical layers, hippocampus and striatum (Dixon et al., 1996; Palmer and Stiles, 1995) and A_{2a} receptors being co-localized with DA receptors in the striatum (Dixon et al., 1996; Palmer and Stiles, 1995).

Various studies have shown that a neurochemical property common to most drugs of abuse is the ability of preferentially stimulating dopamine (DA) release in the shell subdivision of the nucleus accumbens (NAc shell) (Cadoni and Di Chiara, 2000; Cadoni and Di Chiara, 1999; Di Chiara, 1999; Pontieri et al., 1996; Tanda et al.; 1996) .

In order to investigate the relationship between the psychostimulant properties of caffeine (Nehlig et al., 1992) and DA transmission we studied the effects of caffeine on DA release in the different subdivisions of the NAc (Heimer et al., 1991; Heimer and Alheid, 1991) and in the prefrontal cortex (PFCx) in caffeine-naive rats and in rats made tolerant to the locomotor stimulant effects of caffeine by caffeine pre-treatment. The effects of caffeine on dialysate acetylcholine (ACh) in the PFCx were also studied. Finally, in order to address the issue of whether caffeine may, acutely, affect short term memory function, we studied its effect on a spatial delayed alternation task (SDAT) model of working memory.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (275-300 g), housed in groups of 2-3 per cage for at least 3 days before use, were maintained on a 12:00/12:00 h light/dark cycle, with food and water available ad libitum. After surgery the rats were housed individually in Plexiglas cages where they recovered for 24-30 h prior to the microdialysis experiments.

Surgery and Microdialysis

Rats were anaesthetised with ketamine HCl (100 mg/kg i.p.) and stereotaxically implanted with a vertical concentric microdialysis probe in the left PFCx, and, in different subjects, in their left NAc shell and right NAc core. The coordinates were: **PFCx-** AP= +3.6 mm, ML= –0.7 from bregma, DV= –4.8 mm from dura; **NAc shell-** AP= +2.2 mm, ML= –1.1 from bregma, DV= –8.0 mm from dura, **NAc core-** AP= +1.6 mm, ML= +2.0 from bregma, DV= –7.8 mm, according to (Paxinos and Watson, 1998). For intravenous catheter implantation see Crane and Porrino (1989). The perfusion flow was set at 1 µl/min. The perfusion solution contained 147 mM NaCl, 4 mM KCl, 2.2 mM $CaCl_2$; in the experiments in which ACh was determined, the ACh esterase inhibitor, neostigmine bromide (0.1 µM) was added to the perfusion solution. ACh was assayed by HPLC-electrochemical detection (ECD) in conjunction with an enzyme reactor (Damsma et al., 1987). DA was separated by reverse phase liquid chromatography (150 x 4.6 mm - LC_{18}DB) and detected by HPLC-ECD.

Spatial delayed alternation task

Before testing for SDAT, rats underwent 11 days of pre-training: **days 1-5:** handling, twice a day; **days 6-7:** exposure to the T-maze (10 min twice a day); **days 8-9:** exposure to the T-maze (10 min twice a day) and food restriction (from now on 2 pellets/day, ~15 g/day); **days 10-11:** (forced alternation) each rat is allowed to explore, for 5 min, only one arm of the maze in which 5 chocolate minipellets (~0.15 g/each) were available. In the following 5 min each

rat is allowed to enter the opposite arm, for 5 min, in which 5 chocolate minipellets were available. On **day 12** the SDAT begins: each rat is put in the alley of the T-maze to perform during 10 couples of forced runs (the rat is forced to the open arm to eat the minipellet, the other arm being closed) followed by a choice run (the rat, in order to eat another minipellet, must remember the arm of the forced run and choose the opposite one). Thus, each rat might collect, at the best of his performance, up to 20 minipellets during a daily session. Rats are thus kept performing 10 trials/day sessions until a constant criterion of 7 correct performances out of 10 are reached for at least 3 days **(baseline)**; on the following day rats are given caffeine 2.5, 5 or 10 mg/kg i.p. (10 min before the beginning of the session) **(drug day)**. On the next day rats are tested (drug-free) for the last time **(post-drug day).**

Drugs

Caffeine (RBI, USA), dissolved in saline was administered i.v. (microdialysis) or intraperitoneally. SCH 58261 (Schering-Plough, Milan, Italy), and DPCPX (RBI, USA) were suspended in saline with the addition of 0.3% TWEEN 80.

Statistics

Values are expressed as changes percent with respect to baseline (100%) which was set as the average of the last six pre-treatment samples. One and two way analyses of variance (ANOVAs), with time as the repeated measure, were used to analyse the treatment effects.

Tukey's post-hoc analyses were applied for multiple comparisons, with the statistical significance set at $P<0.05$. One way ANOVAs were also applied to determine the significance of the effects of caffeine on SDAT. A statistical comparison was made between the average data of baseline, drug day and post-drug day.

RESULTS AND DISCUSSION

Effect of caffeine, DPCPX and SCH 58261 on dialysate dopamine in the prefrontal cortex, nucleus accumbens shell and core

Caffeine (0.25, 0.5, 1.0, 2.5 and 5.0 mg/kg i.v.) modified DA in the PFCx at all doses except the lowest, but not in the NAc shell and core. **PFCx:** caffeine 0.25 mg/kg: ($F_{12,36}=1.78$, $P>0.05$); 0.5 mg/kg: ($F_{12,84}=4.14$, $P<0.005$ and $P<0.05$ at Tukey's test); 1.0 mg/kg: ($F_{12,60}=4.56$, $P<0.0003$ and $P<0.05$ at Tukey's test); 2.5 mg/kg: ($F_{12,48}=3.04$, $P<0.002$ and $P<0.05$ at Tukey's test) and 5.0 mg/kg: ($F_{12,24}=4.65$, $P<0.0006$ and $P<0.05$ at Tukey's test). **NAc Shell:** caffeine 1.0 mg/kg: ($F_{6,6}=3.73$, NS); 2.5 mg/kg: ($F_{6,18}=1.38$, NS) and 5.0 mg/kg: ($F_{6,12}=0.23$, NS); **NAc Core:** 1.0 mg/kg: ($F_{6,6}=2.87$, NS); 2.5 mg/kg: ($F_{6,12}=0.3$, NS) and 5.0 mg/kg: ($F_{6,12}=0.23$, NS). Two way ANOVA showed that the effects of caffeine on PFCx DA were dose- ($F_{4,215}=10.16$, $P<0.001$) and time-dependent ($F_{9,210}=9.61$, $P<0.001$) with a significant dose x time interaction ($F_{36,170}=1.72$, $P<0.02$). The changes of DA output after caffeine 5.0 mg/kg were larger than those after caffeine 0.25, 0.5 and 1.0 mg/kg ($P<0.05$).

DPCPX significantly modified dialysate DA in the PFCx at the dose of 0.0625 mg/kg i.v., ($F_{12,24}=6.44$, $P<0.00005$, and $P<0.05$ at Tukey's test), 0.125 mg/kg i.v., ($F_{12,72}=2.30$, $P<0.015$ and $P<0.05$ at Tukey's test), 0.25 mg/kg i.v., ($F_{12,48}=1.87$, $P<0.06$ and $P<0.05$ at Tukey's test), 0.5 mg/kg i.v., ($F_{6,24}=5.56$, $P<0.0009$ and $P<0.05$ at Tukey's test) and 1.0 mg/kg i.v. ($F_{12,24}=8.91$, $P<0.00004$ and $P<0.05$ at Tukey's test). Two way ANOVA showed these effects to be dose- ($F_{4,194}=18.083$, $P<0.001$) and time-dependent ($F_{9,190}=14.96$, $P<0.001$) with a significant dose x time interaction ($F_{36,149}=4.49$, $P<0.001$). Tukey's test revealed that the

changes of DA output after DPCPX 1.0 mg/kg i.v. were significantly larger than those after DPCPX 0.0625, 0.125, 0.25 and 0.5 mg/kg i.v.. DPCPX at the dose of 0.5 mg/kg i.v., did not significantly modify dialysate DA in the NAc shell ($F_{12,12}$=0.82, NS) and core ($F_{12,12}$=0.94, NS).

Intravenous administration of SCH 58261 significantly changed dialysate DA in the PFCx at all doses tested (0.0625 mg/kg: ($F_{9,18}$=2.47, P<0.048, and P<0.05 at Tukey's analysis); 0.125 mg/kg: ($F_{12,36}$=2.18, P<0.035, and P<0.05 at Tukey's analysis); 0.25 mg/kg: ($F_{12,36}$=3.00, P<0.005, and P<0.05 at Tukey's test); 0.5 mg/kg: ($F_{12,60}$=9.61, P<0.00001, and P<0.05 at Tukey's test); and 1.0 mg/kg: ($F_{12,60}$=9.64, P<0.00001, and P<0.05 at Tukey's test)). These increases were also dose- ($F_{4,205}$=12.008, P<0.001) and time-dependent ($F_{9,200}$=9.876, P<0.001), with a significant dose x time interaction ($F_{36,160}$=2.610, P<0.001). Tukey's post hoc analysis revealed that the increases of DA output after SCH 58261 0.5 and 1.0 mg/kg were significantly larger than those after SCH 58261 0.0625, 0.125 and 0.25 mg/kg (P<0.05). At the dose of 0.5 mg/kg i.v., SCH 58261 failed to significantly modify dialysate DA in the NAc shell ($F_{10,20}$=0.95, NS) and core ($F_{10,20}$=1.21, NS).

Effects of caffeine, DPCPX and SCH 58261 on dialysate acetylcholine in the prefrontal cortex

Intravenous administration of caffeine significantly modified dialysate ACh in the PFCx at all doses tested (0.25 mg/kg: $F_{11,55}$=2.22, P<0.03 and P<0.05 at Tukey's test; 0.5 mg/kg: $F_{14,48}$=2.3, P<0.025 and P<0.05 at Tukey's test; 1.0 mg/kg: $F_{12,48}$=4.04, P<0.0001 and P<0.05 at Tukey's test, and 2.5 mg/kg: $F_{12,60}$=3.88, P<0.0001 and P<0.05 at Tukey's test). Two way ANOVA yielded a main effect of dose ($F_{3,18}$=4,83, P<0.01) and Tukey's test revealed a significant difference between the effect of 0.25 mg/kg as compared with that of 2.5 mg/kg (P<0.05).

DPCPX (0.0625, 0.125 and 0.25 mg/kg i.v.) significantly modified ACh release (one way ANOVA) at 0.0625 mg/kg ($F_{12,72}$=4.7, P<0.009, and P<0.05 at Tukey's test), 0.125 mg/kg ($F_{12,60}$=4.23, P<0.014, and P<0.05 at Tukey's test) and 0.25 mg/kg ($F_{12,36}$=7.3, P<0.0001, and P<0.05 at Tukey's test). Two-way ANOVA also showed these effects to be dose- ($F_{2,167}$=16.38; P<0.0001) and time-dependent ($F_{9,169}$=7.57, P<0.0001). Post hoc analysis showed that the increases after the dose of DPCPX 0.25 mg/kg were significantly larger than those after DPCPX 0.0625 and 0.125 mg/kg (P<0.05).

SCH 58261 significantly changed dialysate ACh at the dose of 0.0625 mg/kg ($F_{12,72}$=2.14, P<0.025, and P<0.05 at Tukey's test), 0.125 mg/kg ($F_{12,36}$=6.16, P<0.001, and P<0.05 at Tukey's test), 0.25 mg/kg ($F_{12,48}$=3.8, P<0.0001, and P<0.05 at Tukey's test), 0.5 mg/kg ($F_{12,48}$=2.17, P<0.03, and P<0.05 at Tukey's test) and 1.0 mg/kg ($F_{12,48}$=2.19, P<0.03, and P<0.05 at Tukey's test). SCH 58261 modified ACh release in the PFCx in a dose- ($F_{4,21}$=514.82, P<0.0001) and time-dependent fashion ($F_{11,231}$=5.28, P<0.00001) and with a significant dose x time interaction ($F_{44,231}$=2.04, P<0.0004). The effect of the intermediate dose of 0.25 mg/kg i.v. of SCH 58261 were significantly larger than those of the lower doses 0.0625 and 0.125 mg/kg as well as of the higher doses 0.5 and 1 mg/kg i.v. (P<0.05 at Tukey's test), indicative of a bell-shaped dose effect relationship.

Effects of chronic treatment with caffeine on caffeine-induced changes in prefrontal cortical dopamine and acetylcholine release

Caffeine (1.0 mg/kg i.v.) failed to significantly affect dialysate DA in the PFCx ($F_{12,60}$=0.80, NS) of rats chronically exposed to caffeine (seven days, twice a day, 25 mg/kg i.p.); two way

ANOVA showed a significant effect of treatment ($F_{1,122}$=21.16, P<0.001) and time ($F_{12,111}$=1.34, P<0.02) and a significant treatment x time interaction ($F_{12,98}$=2.62, P<0.005). Tukey's post hoc analysis revealed significant differences within the first 30' after caffeine administration between the effect on chronic-caffeine and that on chronic-saline treated rats. Similarly, caffeine (2.5 mg/kg i.v.) given to chronically treated rats, failed to stimulate DA release in the medial PFCx ($F_{12,72}$=1.78, NS); two way ANOVA of the effect of caffeine in chronic-saline versus chronic-caffeine treated rats, yielded a significant effect of treatment ($F_{1,150}$=20.11, P<0.001), time ($F_{12,130}$=2.34, P<0.005) and a significant treatment x time interaction ($F_{12,127}$=2.08, P<0.05). Caffeine (1.0 mg/kg i.v.) significantly modified ACh in the PFCx of caffeine pre-exposed rats ($F_{12,72}$=2.85, P<0.002, and P<0.05 at Tukey's test). Two way ANOVA showed a significant effect of time ($F_{12,108}$=3.6, P<0.0001). Similar results were obtained also when caffeine was administered at the dose of 2.5 mg/kg i.v.: caffeine significantly modified dialysate ACh in the PFCx of chronically-treated rats ($F_{12,48}$=2.95, P<0.003, and P<0.05 at Tukey's test) and two way ANOVA showed a significant effect of time ($F_{12,132}$=4.3, P<0.00001).

Effects of caffeine on spatial delayed alternation task

The administration of caffeine (2.5, 5 and 10 mg/kg i.p., 10 min beforehand) to rats performing in the SDAT failed to affect significantly the number of correct performances/session in the treatment day with respect to both baseline and post-drug day: **caffeine 2.5 mg/kg:** ($F_{2,9}$=0.12,N.S.)**; caffeine 5 mg/kg:** ($F_{2,18}$=0.34,N.S.)**; caffeine 10 mg/kg:** ($F_{2,16}$=1.17,N.S.).

The present study shows that caffeine, given intravenously at doses that elicit behavioural stimulation, increases extracellular DA in the PFCx but not in the NAc shell or core. The lack of the ability to increase DA in the NAc shell makes caffeine different from drugs provided with positive reinforcing and addictive properties that share the property of preferentially increasing DA in the NAc shell (Cadoni and Di Chiara, 1999; Pontieri et al., 1996; Tanda et al., 1997; Pontieri et al., 1995). This difference in turn is consistent with the lack of addictive properties of caffeine (Nehlig, 1999; Majchrzak et al., 1992). Therefore, the present results substantiate the hypothesis that the property of increasing DA in the NAc shell is a biochemical expression of the addictive potential of a drug (Di Chiara, 1999). However, the observation that caffeine, fails to stimulate DA in the NAc does not exclude that the NAc plays a role in the psychostimulant effects of caffeine through blockade of A_{2A} receptors that normally act antagonistically with DA D_2 receptors on striatal neurons (Fredholm and Svenningsson, 1995; Ferrè et al., 1997) .

Caffeine stimulates DA and ACh transmission in the PFCx. This effect could be related to the psychostimulant effects of caffeine but it is unclear if this change is the cause or the effect of the stimulant properties of caffeine on behaviour. Thus, it has been proposed that adenosine, by acting onto the cell bodies of pontine cholinergic projection nuclei regulates arousal (Rainnie et al., 1994; Strecker et al., 2000). These neurones, through their connection with cortically projecting cholinergic neurons (Mesulam et al., 1983; Schwaber et al., 1987) are thought to regulate cortical activity; DA transmission might be involved in this function through its projections to the magnocellular cholinergic nuclei of the basal forebrain (Zaborszky and Cullinan, 1992).

As to the mechanism of these stimulant effects of caffeine on cortical DA and ACh, the observation that they can be reproduced both by DPCPX (Bruns et al., 1987) and by SCH 58261 (Zocchi et al., 1996), coupled with the property of caffeine to act at both A_1 and A_{2A} receptors indicates that, at least in principle, both receptors can be responsible for these

effects. Thus, combined blockade of A_1 and A_2 receptors might explain the high in vivo potency of caffeine on DA and ACh transmission compared to its relatively low affinity for each receptor subtype in vitro (Fredholm and Svenningsson, 1995).

The present study also showed that chronic exposure to caffeine resulted in tolerance to its locomotor stimulant effects (Chou et al., 1985), an action that was accompanied by tolerance to stimulation of DA in the PFCx. No tolerance was obtained to the stimulant effects of caffeine on ACh release. Therefore, the stimulant properties of caffeine on motor activity (locomotion) might be related to release of DA in the PFCx: one might ask what is than the correlate of stimulation of ACh release in the cortex given the fact that, in contrast to DA, tolerance does not take place to the effect of caffeine on ACh transmission in the PFCx. We tentatively suggest that this effect is related to the arousing effects of caffeine that are likely to be resistant to tolerance.

A number of studies have attributed a critical role in the regulation of short term memory function to DA transmission in the PFCx (Sawaguchi and Goldman-Rakie, 1991; Adams and Moghaddam, 1998). Thus, given the finding that caffeine stimulates DA release in this brain region, it was intriguing to determine if caffeine-induced increases of DA release in the PFCx were paralleled by a significant improvement of short term memory function. Thus, the present study also addressed whether caffeine could affect (either ameliorate or worsen) correct performance in a rat model of spatial working memory: our results indicate that caffeine does not affect this measure of short term memory. Finally, while our results in the SDAT model, do not support the finding that caffeine improves short term memory in humans (Smith and Gallagher, 1999), they do not exclude that caffeine might positively affect some attentional aspects of performance in tasks of working memory that may escape detection in the SDAT model. In this regard, further experiments aimed to specifically address this issue are required.

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Adenosine A_{2A} Antagonists and Huntington's disease

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SUMMARY

Huntington's Disease (HD) is a neurodegenerative disorder caused by expansion of a CAG repeat in the gene encoding for Huntingtin (Htt) and resulting in progressive degeneration of the striatal GABAergic/enkephalin neurons. These neurons express both the A_{2A} and D2 receptors which stimulate and inhibit adenylyl cyclase, respectively. In this study we analyzed the possibility of an involvement of the A_{2A} receptor and its signalling components in the pathogenesis of HD. We report here that striatal cells expressing mutant Htt exhibit increased binding affinities for the selective A_{2A} receptor ligand 3H-ZM 241385. Furthermore, despite identical basal adenylyl cyclase activity in all cells, forskolin, a direct activator of this enzyme, significantly over-stimulated cAMP production in mutant Htt cells with respect to parental or wild type Htt-expressing cells. Remarkably, coupling of the A_{2A} receptor to adenylyl cyclase was also aberrantly increased. Nevertheless, in all clones, stimulation of cAMP production by 10-7 M NECA was fully counteracted by selective A_{2A} receptor antagonists as ZM 241385, SCH 58261, CGS 15943 and caffeine. Altogether, these data suggest that expression of mutant Htt induces an amplification of adenylyl cyclase-transduced signals and an aberrant coupling of the A_{2A} receptor to this transduction system. Given the involvement of adenylyl cyclase in key physiological functions including cell growth and cell survival, we speculate that these changes may alter the susceptibility of striatal neurons to cell death and may contribute to the development of HD.

INTRODUCTION

Huntington's disease is a dominantly-inherited neurodegenerative disorder featuring progressive worsening chorea, psychiatric disturbances and cognitive impairment due to brain cell loss, with basal ganglia showing the most dramatic morphological abnormalities and degeneration (Ross, 1995). The disease is caused by expansion of a polymorphic CAG trinucleotide repeat encoding a poly-glutamine tract in Huntingtin (Huntington's Disease, 1993), a 3154 aa protein with recently described antiapoptotic functions (Rigamonti et al., 2000), which may be essential for normal embryonic development (Duyao et al., 1995) and neuronal survival in adulthood (O'Kusky et al., 1999).

Adenosine is a neurotransmitter whose actions are mediated by four G-protein-coupled receptors (the A_1, A_{2A}, A_{2B} and A_3 receptors, Fredholm et al., 1994). Adenosine agonists induce potent psychomotor depression and inhibit the locomotor activity induced by dopamine agonists. This adenosine-dopamine interaction mainly depends on a post-synaptic A_{2A} receptor-D_2 receptor interaction in striatum (Ferrè et al., 1993). In this brain area, A2A receptors are co-expressed with D_2 receptors on GABAergic enkephalin neurons, which are key components of the "indirect" striatal efferent pathway involved in regulation of motor activity, and are highly affected in HD (Reiner et al., 1988). By activating A2A receptors on

these neurones, adenosine acts as a dopamine functional antagonist. Interestingly, A_{2A} receptors stimulate and D_2 receptor inhibit adenylyl cyclase, which represents a key target in mediating the opposite effects of these two transmitters (Ferrè et al., 1993).

Therefore, the possibility that changes in the adenosine-dopamine system in striatum play a role in the motor dysfunction typical of HD remains a strong one. Interestingly, adenosine has been suggested to act as a trigger for development-associated apoptosis (Jacobson et al., 1999), and, under specific conditions, activation of the A_{2A} adenosine receptor may result in cell death (Ongini and Schubert, 1998). It may hence be hypothesised that a dysregulation of adenosine-mediated cell death, likely due to an alteration of A_{2A} receptor expression or function, may contribute to neurodegeneration in HD.

Here we tested the possibility that mutant Htt may alter A_{2A} receptor signalling. In particular, we found that striatal cells expressing mutant Htt (Rigamonti et al., 2000) show an aberrant amplification of cAMP formation upon exposure to either forskolin or, more remarkably, adenosine receptor agonists, suggesting a specific interaction of mutant Htt with this transduction system. We speculate that such changes may contribute to the development of HD. Interestingly, selective A_{2A} receptor antagonists as ZM 241385, SCH 58261, CGS 15943 or caffeine retained their ability to fully counteract A_{2A} receptor-mediated cyclase stimulation also in mutant Htt-expressing cells, hence highlighting, for the first time, a potential use of these compounds in the pharmacological treatment of HD.

MATERIALS AND METHODS

Materials

3H-ZM 241385 (specific activity 17 Ci/mmol) were from Tocris Cookson Ltd (Bristol, UK). 3H-cAMP (specific activity 21 Ci/mmol) was purchased from the Radiochemical Centre (Amersham, UK). NECA (5'-N-ethylcarboxamidoadenosine), Ro 20-1724, (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone), GTPγS, cAMP and forskolin were from Sigma Chemicals Co. (St. Louis, MO). SCH 58261 were from the Schering-Plough Research Institute, Milan, Italy. Aquassure and Atomlight were from NEN Research Products (Boston, Mass., USA). All other reagents were of analytical grade and obtained from commercial sources.

Cell Culture

Parental ST14A cells and stable subclones expressing either 548 aminoacids N terminal to the protein in the wild-type (N548wt) or mutant (N548mu) versions, or the full-length wild-type (FLwt) and full length mutant (FLmu) proteins (Rigamonti et al., 2000) were grown at 33°C in the presence of Dulbecco's Modified Eagle Medium supplemented with 10% foetal bovine serum as previously described (Cattaneo and Conti, 1998). All transfected subclones have been previously demonstrated to express the exogenous proteins at comparable levels at least up to 25 passages in culture (Rigamonti et al., 2000). Expression levels are routinely checked by Western blot in early passaged cells. Cells were used when 80% confluent.

ReverseTranscriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was prepared from cells and tissue using Triazol Reagent (Gibco, BRL). Retrotranscription of RNA was performed with SuperScript II (Gibco, BRL) according to the instructions of the manufacturer and using 250 ng of random primers. 20 U of RNAseOUT (Gibco, BRL) were added to each reaction. 0.5 μg of cDNA were amplified by PCR using

DyNAzyme EXT (Finnzymes) and primers specific for the A_{2A} receptor. PCR was carried out for 40 cycles with the following parameters: denaturation at 95°C for 30 sec., annealing at 54°C for 30 sec., extension at 72°C for 7 min.

Receptor binding studies

Cells were washed with phosphate buffered saline and scraped off T75 flasks in ice cold hypotonic buffer (5 mM Tris HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized with Polytron and was centrifuged for 30 min at 48,000 g and each pool was used to perform a single saturation experiment. The membrane pellet was resuspended in 50 mM Tris HCl, 120 mM NaCl, 5 mM KCl, 10 mM MgCl2 and 2 mM CaCl2, pH 7.4, incubated with 2 I.U./ml of adenosine deaminase for 30 min at 37°C and centrifuged for 30 min at 48,000 g. The resulting pellet was resuspended at a concentration of 100-150 μg protein/100 μl and this homogenate was used for assaying binding of 3H-ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo-[2,3a][1,3,5]triazinyl-amino]ethyl) phenol) (Poucher et al., 1995). The protein concentration was determined according to a Bio Rad method (Bradford, 1976) with bovine serum albumin as a standard reference. In saturation studies, as previously described (Varani et al., 1997), cell membranes were incubated for 60 min at 4°C with 8-10 different concentrations of 3H-ZM 241385 (0.05-10 nM) in a total volume of 250 μl. Non-specific binding was defined as binding in the presence of 10 μM NECA and was about 40% of total binding. Bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/B glass-fibre filters using a Micro-Mate 196 cell harvester (Packard).

The filter bound radioactivity was counted using a microplate scintillation counter (Top Count) at an efficiency of 57% with Micro Scint 20. A weighted non linear least-squares curve fitting program LIGAND (Munson and Rodbard, 1980), was used for computer analysis of saturation experiments.

Measurement of cAMP levels

Cells were washed in phosphate buffered saline and centrifuged for 10 min at 200 g. The pellet resuspended in a buffer containing 120 mM NaCl, 5 mM KCl, 0.37 mM NaH2PO4, 10 mM MgCl2, 2 mM CaCl2, 5 g/L D-glucose, 10 mM Hepes-NaOH, pH 7.4, and centrifuged again for 10 min at 200 g. Cells (4x105 cells/tube) were then suspended in 0.5 ml incubation mixture described above containing 1.0 I.U. of adenosine deaminase/ml and 0.5 mM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone, Ro20-1724, as phosphodiesterase inhibitor) and preincubated for 10 min in a shaking bath at 37°C. Then forskolin (1 μM) or NECA (1 nM-10 μM) were added to the mixture and the incubation continued for a further 5 min. The effect of several A_{2A} receptor antagonists such as CGS 15943 (1 μM), ZM 241385 (1 μM) and SCH 58261 (1 μM) was determined by antagonism of NECA (100 nM)-induced stimulation of cyclic AMP levels. The reaction was terminated by the addition of cold 6% trichloroacetic acid (TCA). The final aqueous solution was tested for cyclic AMP levels by a competition protein binding assay carried out as previously described (Varani et al., 1998). Samples of cyclic AMP standards (0-10 pmol) were added to each test tube containing trizma base 0.1 M, aminophylline 8.0 mM, 2 mercaptoethanol 6.0 mM, pH 7.4, 3H-cAMP and binding protein, previously prepared from beef adrenals, was added and incubated at 4°C for 90 min. The samples, after the addition of charcoal, were centrifuged at 2,000 g for 10 min. The clear supernatant (0.2 ml) was mixed with 4 ml of Atomlight and counted in a LS-1800 Beckman scintillation counter.

RESULTS

Both parental striatal-derived ST14A cells and Htt-engineered subclones express the A_{2A} adenosine receptor

Reverse-transcriptase polymerase chain reaction was used to detect the presence of A_{2A} receptor mRNA levels in parental ST14A cells and in the various stable subclones expressing N548wt, N548mu, FLwt and Flmu Htt proteins. All subclones expressed the 630 bp band corresponding to the A_{2A} receptor mRNA, although to a different extent. Despite differences in mRNA levels, comparable A_{2A} receptor protein densities are found in all clones, as shown by radio-ligand binding studies performed with the highly selective A_{2A} receptor antagonist 3H-ZM 241385 (Table 1). Scatchard-plot analysis revealed the presence of a single class of specific, high affinity and saturable binding sites in both ST14A and in Htt-expressing cells, with comparable Bmax values, independently of the presence of normal or mutant Htt (Table 1).

Table 1. Binding parameters of the A_{2A} adenosine receptors antagonist [3H]-ZM 241385 in ST14A striatal membranes, in wt and muHtt in stable cell clones

	ST14A	FL wt	N548 wt	FL mu	N548 mu
Kd (nM)	2.30 ± 0.10	2.42 ± 0.08	2.48 ± 0.08	1.61 ± 0.06*	1.32 ± 0.06**
Bmax (fmol/mg protein)	92 ± 6	90 ± 8	90 ± 6	93 ± 5	90 ± 3

*Analysis was by Student's t test; * P <0.05 vs control; ** P <0.01 vs control.*

Kd values were instead significantly reduced in FLmu cells with respect to parental ST14A cells, a change which was even more significant in N548mu cells (Table 1). Comparable data were obtained with the A_{2A} receptor ligand 3H-ZM 241385. For the ST14A, FLwt, N548wt, Flmu and N548mu, Kd values were, respectively: 2.30±0.10; 2.42±0.08; 2.48±0.05; 1.61± 0.06*; 1.32±0.06** nM (* P <0.05 vs control; ** P <0.01 vs control); corresponding Bmax values were 92±6; 90±8; 90±6; 93±5; 90±3 fmol/mg protein. No changes of Kd values were detected in clones expressing either FLwt or N548wt (Table 1). These data suggest a selective increase of binding affinity in the presence of mutant Htt.

Forskolin-stimulated adenylyl cyclase response is selectively altered in cells expressing mutant Htt

Since the A_{2A} adenosine receptor is coupled to stimulation of adenylyl cyclase via Gs stimulatory proteins leading to cAMP increases, we analyzed the sensitivity of adenylyl cyclase to agents that are known to specifically activate this effector system.

Despite no changes of basal enzyme activity (Table 2), response of adenylyl cyclase to the direct activator forskolin (utilized in the presence of the cAMP-dependent phosphodiesterase inhibitor RO 201724) was notably increased in N548mu (and Flmu) cells with respect to parental or wild-type Htt cells. As shown in Table 2, an amplification of cAMP formation was already demonstrable with forskolin alone in N548mu cells.

A_{2A} receptor-stimulated adenylyl cyclase is aberrantly increased in cells expressing mutant Htt

When the adenosine analogue NECA was applied to the cells, an aberrant amplification of adenylyl cyclase response was detected selectively in cells expressing mutant Htt. In all clones NECA (0.1 nM-10 μM) significantly increased cAMP production in a concentration-dependent manner. However, the log dose-response curve for NECA in cells expressing either N548wt and FLwt or N548mu and FLmu reveals that cAMP formation was significantly higher in the latter two at almost all agonist concentrations with respect to parental ST14A cells (EC50 values: 198±15*; 93±9** nM in FLmu and N548mu, respectively, versus 270±10 in ST14A cells; *P< 0.05; **P< 0.01 vs control). No differences of NECA responses were detected in FLwt and N548wt cells with respect to ST14A cells at any of the utilized agonist concentrations (EC50 values: 253±11 and 236±13, respectively) (Table 3).

Table 2. Cyclic AMP levels in ST14A striatal cells, wt and mu Htt in stable cell clones

	ST14A	FL wt	N548 wt	FL mu	N548 mu
Basal level	18 ± 2	19 ± 2	18 ± 1	20 ± 2	20 ± 1
Forskolin 1 μM	45 ± 5	54 ± 6	47 ± 3	55 ± 5	72 ± 4*
Forskolin 1 μM + Ro 201724 0.5 mM	58 ± 4	60 ± 6	63 ± 6	78 ± 7	88 ± 5*

*The data are expressed in pmol/10^5 cells. Analysis was by Student's t test; *P <0.01 vs control*

Table 3. Stimulation of cyclic AMP levels in ST14A striatal cells, wt and muHtt in stable cell clones by NECA (1 nM-10 μM)

	ST14A	FL wt	N548 wt	FL mu	N548 mu
EC_{50} (nM)	270 ± 10	253 ± 11	236 ± 13	198 ± 15*	93 ± 9**

*Analysis was by Student's t test; *P <0.05 vs control; **P <0.01 vs control*

These results are suggestive of a selective increase of responsiveness of the adenosine A2A receptor/adenylyl cyclase system in the presence of mutant Htt. This behavior is unrelated to changes of endogenously produced adenosine since adenosine deaminase was present during the adenylyl cyclase assay.

Selective A_{2A} receptor antagonists revert A_{2A}-stimulated cAMP formation in both parental and Htt-expressing cells

To further confirm that the effect induced by NECA on cAMP formation is due to stimulation of the A_{2A} receptor subtype, we performed experiments in the presence of adenosine receptor antagonists characterised by various degree of selectivity towards the A_{2A} adenosine receptor subtype. As shown in Table 4, in ST14A cells, both the relatively-selective antagonists CGS15943 and ZM 241385, and the highly-selective antagonist SCH 58261 completely prevented the increases of cAMP induced by NECA at an agonist concentration (100 nM) which selectively stimulates adenylyl cyclase via the A_{2A} receptor. Also caffeine is able to

block completely the stimulatory effect NECA mediated. Remarkably, all the tested antagonists retained the ability to counteract NECA-induced cAMP formation also in cells expressing mutant Htt, where adenylyl cyclase activity is aberrantly increased with respect to ST14A and to wild-type Htt cells.

Table 4. Antagonism on NECA-stimulated cyclic AMP levels by typical A_{2A} adenosine antagonists in ST14A striatal cells, wt and muHtt in stable cell clones

	ST14A	FL wt	N548 wt	FL mu	N548 mu
Basal level	18 ± 2	19 ± 2	18 ± 1	20 ± 2	20 ± 1
NECA 100 nM	38 ± 5	39 ± 5	40 ± 4	48 ± 3	55 ± 6
SCH 58261 1 μM + NECA 100 nM	20 ± 4*	19 ± 3*	20 ± 2*	21 ± 2*	21 ± 3*
ZM 241385 1 μM + NECA 100 nM	18 ± 3*	19 ± 3*	18 ± 2*	19 ± 1*	19 ± 1*
CGS15943 1 μM + NECA 100 nM	19 ± 3*	20 ± 3*	18 ± 2*	19 ± 1*	21 ± 3*
Caffeine 1 μM + NECA 100 nM	20 ± 4*	22 ± 4*	22 ± 3*	21 ± 2*	21 ± 3*

*The data are expressed in pmol/10^5 cells. Analysis was by Student's t test; *P <0.01 vs cAMP stimulation NECA-mediated*

DISCUSSION

The A_{2A} adenosine receptor plays an important role in striatal function (Ferrè et al., 1993). The present study describes, for the first time, an alteration of A_{2A} adenosine receptor signalling specifically associated with the expression of mutant Htt. In particular, we have used a parental striatal cell line and the Htt-engineered derivatives express the A_{2A} adenosine receptor (Cattaneo and Conti, 1998). Both parental striatal cells and the Htt-engineered derivatives express the A_{2A} receptor, hence validating this model as suitable to study possible changes induced by mutant Htt on this receptor system. We found decrease levels of A_{2A} receptor mRNAs in mutant Htt cells. However, the density of the receptor protein was comparable in all clones as detected by binding studies. Despite no change in Bmax values, we revealed a moderate but statistically significant increase of ligand binding affinity in cells expressing mutant Htt, suggesting that mutant, but not wild-type Htt, may interfere with the kinetics of A_{2A} receptor binding by endogenous adenosine.

Our results also highlight profound changes of adenylyl cyclase response selectively in mutant Htt cells. Response to forskolin was significantly increase in these cells and more remarkably, an even higher amplification of A_{2A} receptor-mediated cAMP formation was observed. From our data it is also clear that changes of A_{2A} receptor signalling are much more evident in cells expressing truncated mutant Htt with respect to full-length mutant Htt. This finding may have intriguing functional implications, since the N548 fragment expressed in our cells mimicks one of the potential fragments suggested to be produced by proteolytic

cleavage of Htt and which may be required for the expression of mutant Htt citotoxicity and aggregates formation. Recent data demonstrate that the full length mutant protein is gradually processed over time in vivo to generate amino-terminal fragment that progressively accumulate into the nuclear and in axons and synaptic terminals. Truncated mutant Htt has been also demonstrated to carry significantly higher toxicity with respect to the full length protein. One could speculate that N548mu cells may reflect a more advanced stage of the disease where the aberrant behaviour of the A_{2A} receptor is maximally expressed.

A possible role of this receptor in the etiopathology of HD is also consistent with literature data suggesting that the A_{2A} receptor may contribute to cell death in ischemia-associated neurodegeneration, and that A_{2A} receptor antagonists may indeed prove useful in preventing such damage (Abbracchio and Cattabeni, 1999; Jones et al., 1998; Monopoli et al., 1998). In this respect, our demonstration that selective A_{2A} receptor antagonists retain their ability to fully block NECA-stimulated adenylyl cyclase may disclose new avenues in the pharmacological manipulation of Huntington's Disease.

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Carbohydrates in Coffee

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SUMMARY

Carbohydrates constitute the major fraction of both green coffee and commercial (roast and soluble) products therefrom. The principal polysaccharides are of three types: arabinogalactan, mannan and cellulose. The arabinogalactan has frequent branches whereas the mannan is lightly substituted with one-unit galactose side chains, hence the sometimes-used galactomannan terminology. Sucrose dominates the low molecular weight carbohydrate fraction in green beans.

The carbohydrates are precursors for flavor generation during roasting, whereby interactions with the proteinaceous components via Maillard reactions are particularly important. The polysaccharides are present in soluble coffee where their extractability and stability are factors that impact process and product characteristics.

INTRODUCTION

The importance of the carbohydrate fraction in coffee is evident in its high content; on a dry weight basis, it constitutes about half of the green coffee bean. Carbohydrates are also major components of roast and soluble coffee (Table 1). They are present at a lower level in R&G brews but are important as key precursors during roasting for the generation of flavor and mouthfeel-imparting compounds in the beverage. In this short review the carbohydrates in the coffee bean and the effect of processing on their chemical structures are described. The field of coffee carbohydrate chemistry has recently been more extensively covered (Bradbury, 2000).

Table 1. Approximate contents of carbohydrate in coffee

	Content, wt.%, solids basis			
Coffee	Green	Roast	R&G Brew	Soluble
Total Carbohydrate	45-60	40-50	3-6	30-45

LOW MOLECULAR WEIGHT CARBOHYDRATE

Green Coffee

The principal low molecular weight carbohydrate or sugar in green coffee is sucrose. Arabica varieties tend to contain about twice as much sucrose as Robustas with literature values ranging from 5 to 8.5% for the former and 2 to 5% for the latter (Clifford, 1985).

Use of the sensitive HPAEC-PAD method for the analysis of carbohydrates has facilitated the detection and determination of several other low molecular weight carbohydrates in coffee. Data from Rogers et al. (1999) for two varieties each of Arabica and Robusta beans are given in Table 2. The trisaccharide, raffinose and the tetrasaccharide, stachyose as well as the sugar

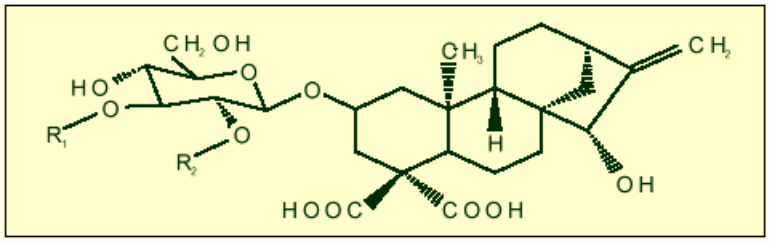
alcohol, mannitol were present in all samples. They found that the contents of the carbohydrates were affected by maturity, reflecting biochemical changes in the bean during development. The levels of glucose (8 to 12% in Arabicas, 2 to 4% in Robustas) and fructose (about half of glucose levels), were significantly higher than the sucrose contents in immature beans. Contents of these monosaccharides dropped to less than 0.1% during grain development.

Inositol hexaphosphate as well as lower levels of the pentaphosphate are present in green coffee. Total inositol phosphate levels in a series of Robusta samples (0.35 to 0.40%) were higher than in a series of Arabica samples (0.28 to 0.32%) (Franz and Maier, 1994).

Table 2. Low mol. wt. carbohydrate in mature coffee beans (from Rogers et al., 1999)

Sample	Content, % wt.								
	Arabinose	Galactose	Isomaltose	Stachyose	Raffinose	Maltose	Melibiose	Xylose	Mannitol
Robusta (ROM)	0.03	0.04	0.04	0.13	0.00	0.00	0.00	0.00	0.01
Robusta (Dormilon)	0.05	0.21	0.04	0.15	0.02	0.02	0.01	0.00	0.02
Arabica (CRM)	0.02	0.20	0.12	0.18	0.02	0.00	0.00	0.00	0.05
Arabica (Caturra)	0.03	0.06	0.04	0.10	0.02	0.02	0.00	0.00	0.03

A series of glycosides of the diterpene type is found in green coffee. The principal members of this substance class in coffee are the three related carboxyatractyl ß-D-glucosides (CKA I, II and III) depicted in Figure 1. For a series of green beans the total contents in Arabicas (up to 0.45%) were higher than in Robustas (up to 0.1%) (Bradbury and Balzer, 1999).



R_1	R_2	Compound	MW
H	H	CKA II	526
H	-C(=O)-CH$_2$-CH(CH$_3$)$_2$	CKA III	610
ß-D-Gluc$_p$(1→)	-C(=O)-CH$_2$-CH(CH$_3$)$_2$	CKA I	772

Figure 1. Carboxyatractylglycosides in green coffee (from Bradbury and Balzer, 1999)

Steaming treatments

Due to reactivity of the carbohydrates, their low mol. wt. profiles have been used to indicate the level of steam treatment in green beans. Sucrose inversion on steaming led to formation of glucose and fructose with additional rearrangement of the latter to produce the additional keto-sugar psicose. In addition, low levels of the monosaccharides: arabinose, galactose and mannose, were generated by hydrolysis of the polysaccharide fraction (Figure 2) (Luger and Steinhart, 1995).

Inositol tri- and tetra-phosphates have been found in steamed green coffees indicating the partial hydrolytic loss of phosphate groups in the inositol phosphate fraction (Franz and Maier, 1994).

Mild steaming conditions result in the quantitative removal of a carboxyl group from carboxylatractyloside molecules to yield the corresponding atractyl derivatives (Bradbury and Balzer, 1999).

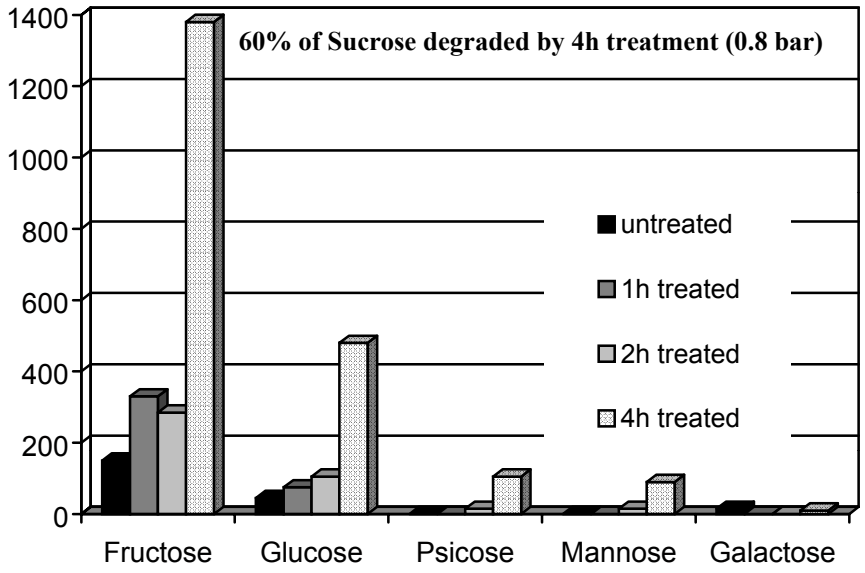


Figure 2. Effect of steaming on green coffee sugar contents (from Luger and Steinhart, 1995)

Roast coffee

The content of low mol. wt. carbohydrate in roast coffee is relatively low. Sucrose undergoes a variety of reactions which can be generally summarized into three types: fragmentation, dehydration and Maillard (Bradbury, 2000). These reactions produce a large diversity of substances that are important constituents of the commercial products of coffee. Residual contents of sucrose in most roast coffees are very low (usually <0.1%) as are those of the inversion products glucose and fructose. Low levels of other monosaccharides derived from polysaccharides are also present (Noyes and Chu, 1993).

Other low mol. wt. carbohydrates in green coffee are more stable to roasting. Although a significant degree of de-phosphorylation occurs to form tri-, tetra-, and penta-phosphate derivatives, little inositol phosphate loss occurs at ‘normal’ roast (Franz and Maier, 1994). Loss of a carboxyl group from the carboxyl derivatives is quantitative on roasting but about half of the resulting atractylglycosides survives normal roasting conditions (Bradbury and

Balzer, 1999). The sugar alcohol mannitol is also relatively stable to roasting (Noyes and Chu, 1993).

HIGH MOLECULAR WEIGHT CARBOHYDRATE

Green coffee

The coffee bean polysaccharide fraction is dominated by three polymer types: arabinogalactan (AG), mannan and cellulose. Bradbury and Halliday (1990) used linkage analysis to confirm that the AG was highly-branched and that the mannan has a linear structure. They also showed (Bradbury and Halliday, 1987) that the 'total carbohydrate' contents (the yield and type of the monosaccharides produced by acid hydrolysis) were slightly higher for Robusta compared to Arabica beans. This was attributed to higher AG contents in the former (ca. 14 versus 17%), whereas mannan and cellulose contents were similar (ca. 22 and 7%, respectively). Zapp (personal communication) found somewhat higher mannan contents in Robusta beans, whereas Fischer et al (2001) claimed that the difference in mannan content between an Arabica and a Robusta sample was of the same order as the difference in AG content and thus the total polysaccharide was similar for both types. The total polysaccharide contents found for Arabica and Robusta beans in their work were somewhat higher than those obtained in the earlier studies (Table 3).

Table 3. Total carbohydrate analysis of green coffees

	%, dry weight basis[A]					
	Arabinose	Galactose	Glucose	Mannose	Total[B]	Reference
Arabica						
El Salvador	3.6	10.7	6.7	22.5	43.5	9
Colombia I	3.4	10.4	7.0	22.2	43.0	9
Ethiopia	4.0	11.9	7.8	21.3	45.0	9
Colombia II	3.4	10.5	n.d.	21.6	n.d.	10
Brazil	3.5	10.0	n.d.	20.7	n.d.	10
El Salvador	3.4	10.7	n.d.	21.5	n.d.	10
Caturra	5.0	13.9	9.5	16.6	55.1	11
Robusta						
India	4.1	14.0	7.8	21.9	48.2	9
Ivory Coast	4.0	12.4	8.7	22.4	48.3	9
Sierra Leone	3.8	12.9	8.0	21.7	46.9	9
Cameroons	4.0	13.4	n.d.	19.2	n.d.	10
Indonesian I	3.9	11.9	n.d.	19.5	n.d.	10
Indonesian II	4.3	13.8	n.d.	19.6	n.d.	10
ROM	5.8	15.0	9.3	14.5	54.5	11

A: Determined by acid hydrolysis, values correspond to anhydro-monosaccharides
B: Does not include contribution from rhamnose (ca. 0.3%) and xylose (ca. 0.2%).
n.d.: not determined

Structures of the polysaccharides

The early work of Wolfrom and Patin (Wolfrom and Patin, 1965) showed that coffee AG had a 'Type II' structure, i.e. a ß1-3 linked backbone of galactose units with frequent single (arabinofuranosyl) and double (galactopyranosyl linked at C-3 to arabinofuranosyl) unit side chains linked at the C-6 position to galactopyranosyl units ß1-3 linked in the main chain. Bradbury and Halliday (Bradbury and Halliday, 1990) confirmed the 'Type II' classification of the AG and suggested that the polymer contained additional structural features. These included non-terminal arabinose units (linked 1-5), 1-6 linked galactose units and terminal

galactose units. This confirmed the earlier claim of galactose involvement in side chains but also indicated that galactose-galactose linkages (1-6) were also present, probably in side chains.

More recent studies by Fischer et al. (2001) on green coffee 'cell wall material' (CWM), confirmed the presence of the linkages observed above. By means of their fractionation procedure, they found evidence of highly branched AG stuctures, where almost half of the galactose backbone units were linked to a side chain. Their work suggested longer, more frequent side chains in the Robusta AG compared to the Arabica AG.

Coffee mannan has a linear ß1-4 linked mannose unit backbone with a low density of one-unit galactose side chains. Mannan polymers are apparently present as a heterogeneous mixture with a range of side chain substitutions (the average is ca. 1 in 30) and number average molecular weights of ca. 3500 (Bradbury and Halliday, 1990; Fischer et al., 2001). The presence of galactose units also leads some workers to prefer the nomenclature 'galactomannan' for this polymer in coffee.

The detection of other monosaccharides (notably rhamnose (ca. 0.3%, bean wt. basis) and xylose (0.2%)) in acid hydrolyzates of de-sugared green coffee bean preparations is indicative of low quantities of other polysaccharides (or their incorporation in the AG or mannan polysaccharides) but no definite structures have been established. Fischer et al. (2001) have published a detailed list of the glycosyl-linkages found in their green coffee CWM fractions.

Effect of roasting

The polysaccharides are the major constituents of the thick coffee cell walls and are relatively stable to roasting. Total carbohydrate analysis showed that the relative stability of the bound monosaccharide units to roasting was in the order glucose > mannose > galactose > arabinose (Figure 3) (Bradbury, 2000). The initial glucose loss (green to light roast) can be attributed to sucrose degradation. The relatively lability of arabinose can be attributed to steric (located at chain ends in the AG polymer) and linkage reactivity (bound as furanoside units) factors. Structural analysis has shown that the overall linkage pattern of the polysaccharides was basically unchanged by roasting. Microscopic examination of roasted coffees shows that much of the coffee cell wall structure is retained on roasting.

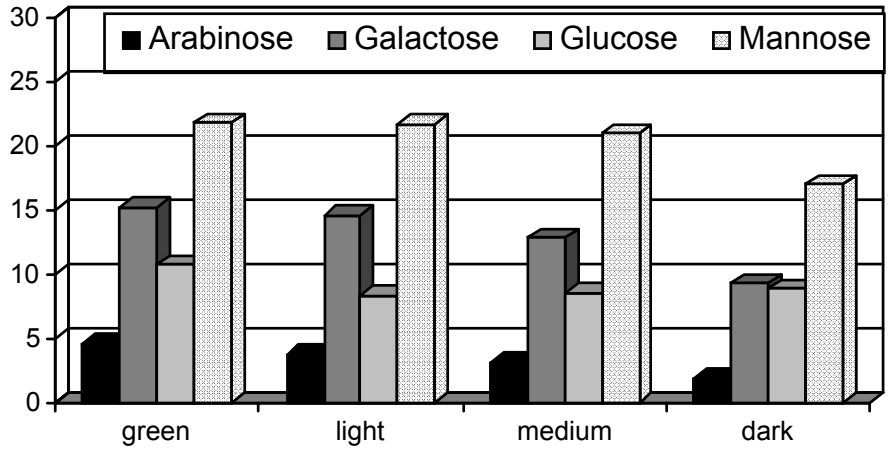


Figure 3. Total carbohydrate analysis of green and roasted Cameroon Robusta beans (from Zapp, personal communication)

Leloup and Liardon (1993) showed that the extraction yields of both AG and mannan was higher for roast than green beans at 95°C. Based on analysis of the mannan fractions from two Robusta and one Arabica 'normal' roast coffees, Bradbury (2000) suggested that molecules with higher side chain density (DB) were more readily extracted.

Undoubtedly, the polysaccharides play an important role in melanoidin (the 'unknown' non-volatile residual fraction in roast coffee) formation. As well as yielding reactive fragments they may also serve as a polymeric base on which reactive moieties from protein or phenolic compounds can be incorporated and further modified for melanoidin formation. The incorporation of polysaccharide-derived monosaccharide units in melanoidins has already been observed (Steinhart and Packert, 1993).

Implications for soluble coffee process

Although only relatively minor quantities of carbohydrate are extracted in the preparation of a R&G brew, the more rigorous extraction conditions utilized in commercial soluble coffee production leads to their incorporation at considerably higher levels.

The important role of hydrolytic scission of glycosidic linkages in polysaccharides during extraction was demonstrated in a model study by Blanc et al. (1989). They monitored the effect of autoclave extraction time and temperature (subsequent to 'atmospheric extraction' at 100°C) on the free and total carbohydrate profile for Arabica and Robusta coffees (Table 4). Arabinose, the most labile of the bound monosaccharides, was released most readily. Galactose and mannose extract contents increased with extraction time and temperature until under the most extreme conditions (190°C, 240 min) carbohydrate degradation was extensive. Glucose and fructose contents also increased, some glucose may have resulted from cellulose that had been partially depolymerized by roasting but the atractylglycosides (see above) were a likely source. Of particular interest was the formation of fructose despite the absence of a fructose containing polymer source. The sugar was probably a thermally generated rearrangement product of mannose or glucose (via the 1,2-enediol intermediate form). This intermediate would also be a source of glucose from mannose.

Table 4. Soluble free and total carbohydrate in extracts from Arabica (Santos) roasted coffee (from Blanc et al., 1989)

Conditions of 2nd stage extraction		free carbohydrate % wt.					total carbohydrate % wt.				
min	°C	Ara	Fru	Man	Glu	Gal	Xyl	Ara	Man	Glu	Gal
30	150	1.35	0.11	0.05	0.09	0.10	0.14	6.29	10.66	1.86	12.76
	160	1.74	0.10	0.08	0.10	0.19	0.16	6.00	8.95	1.40	18.40
	170	1.89	0.11	0.17	0.09	0.38	0.21	5.34	9.93	1.32	20.60
	180	1.44	0.17	0.43	0.10	0.79	0.17	3.55	13.09	1.29	18.00
	190	0.52	0.47	1.42	0.19	1.61	0.21	2.53	19.50	1.73	14.63
120	160	2.17	0.11	0.32	0.11	0.82	0.17	4.26	10.23	1.29	20.93
	170	1.19	0.24	0.82	0.14	1.55	0.20	2.65	13.40	1.45	16.40
	180	0.64	0.77	2.54	0.36	2.36	0.13	1.03	15.50	1.87	10.08
240	160	1.44	0.24	0.84	0.16	1.79	0.18	2.70	11.30	1.36	17.00
	170	0.62	0.57	2.02	0.27	2.31	0.14	2.01	13.70	1.86	11.48
	180	0.03	1.08	2.82	0.70	1.44	0.06	1.72	9.36	2.00	4.61
	190	0.00	0.03	0.43	0.01	0.01	0.08	2.05	5.91	1.66	3.19

1st stage 100°C, 30 min; 1st and 2nd stages combined before analysis

Leloup et al. (1997) monitored the effect of degree of roast on the carbohydrate composition of roast coffee extract extracted at high temperature conditions (180°C, 20 min). Although the total extracted carbohydrate was similar for light, medium and dark roasts, the carbohydrate composition varied both in terms of type and size distribution. For light and dark roasts, total yields of (arabinose plus galactose) in extract (dry wt. basis) were 22.4 and 15.4% and for (mannose) 8.7 and 16.0%, respectively. Thus AG was apparently more degraded at higher roasts, supporting earlier observations that AG was more labile than mannan on roasting. The relatively stable mannan became more accessible as the tough cell wall matrix was weakened with increasing degree of roast. HPLC showed the presence of oligomannans DP 1 to 7 in all extracts, indicative of mannan hydrolysis during extraction.

The presence of oligosaccharides up to DP 14 in a commercial sample of soluble coffee was shown by Zapp and Kuhn (1997) using MALDI-TOF mass spectrometry. The observed peaks were attributed to mannodextrins. Galactose units may also be present, either bound in the mannodextrins or in galactodextrins (sourced from AG). There was no indication of arabinose containing dextrins; these would produce molecular ions of 30 daltons less for each bound arabinose unit for a particular DP. By assuming a linear mass peak area response/molecular weight relationship based on a calibration using mannodextrins DPs 2, 3, 6 and 7, they were able to quantify the contents of DPs 2 to 14 in the sample. Yields decreased steadily with increasing molecular size.

An important aspect of the higher molecular weight carbohydrate in soluble coffee extract is solubility. AG sourced molecules remain dissolved but some of the more linear mannan-sourced molecules are less soluble and can eventually precipitate. This is an important factor in the formation of sediments in the soluble coffee process. The insolubilization is driven by association of linear regions of mannan chains to form crystalline regions (Bradbury and Atkins, 1997).

Adulteration indicators

It was shown by Blanc et al. (1989) that low molecular weight carbohydrate could be used as indicators of non-coffee bean adulterants such as husks in commercial soluble coffee products. In a major survey, the free and total carbohydrate profiles of commercial soluble coffees, determined using HPAEC-PAD chromatography, was used to indicate adulterated products. The adulterated coffees fell into two main groups: those with a high total xylose and glucose which indicated adulteration with skins or husks and those with a high total glucose content and a low xylose content which indicated adulteration with a starch based material. The limits of acceptability for the relevant low molecular weight carbohydrates have been defined by AFCASOLE (The European Soluble Coffee Manufacturer's Association) in a publication issued by the British Standards association (MAFF, 1995).

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Antioxidative Effect of Coffee Melanoidins

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SUMMARY

Melanoidins from roasted coffee show strong antioxidative effects depending on the way the coffee is treated. In this study it was possible to evaluate and describe the antioxidative potential of coffee melanoidins and to obtain an idea of the chemical source of this effect. The influence of roasting parameters was measured and quantified. Medium roasted coffee showed the strongest potential. Polymer Maillard reaction products have a remarkable antioxidative potential though it is exceeded by low molecular phenolcarbonic acids. The contribution of free chlorogenic acid isomers to the total antioxidative effect of roasted coffee equals 25% depending on the content. Despite the decomposition of chlorogenic acids in coffee during the roasting process, an increase of the antioxidative capacity is observed.

ZUSAMMENFASSUNG

Melanoidine aus Röstkaffee weisen eine starke antioxidative Wirksamkeit auf, wobei die Art der Vorbehandlung den Wirkungsgrad beeinflußt. Im Rahmen dieser Studie gelang es die antioxidative Wirksamkeit von Kaffee Melanoidinen zu überprüfen und genauer zu beschreiben sowie die chemische Herkunft dieser Eigenschaft näher einzugrenzen. Der Einfluß der Röstparameter konnte gezeigt und quantifiziert werden. Mittelstark gerösteter Kaffee zeigt die stärkste Wirksamkeit. Polymere Maillard-Produkte weisen eine deutliche antioxidative Wirksamkeit auf, welche allerdings von niedermolekularen Phenolcarbonsäureverbindungen klar übertroffen wird. Die freien Chlorogensäuren tragen je nach vorhandener Menge mit mindestens 25% zu der antioxidativen Gesamtaktivität des Kaffeegetränkes bei. Trotz des Abbaues eines Teils der im Kaffee enthaltenen Chlorogensäuren während der Röstung resultiert eine erhebliche Zunahme des antioxidativen Potentials.

INTRODUCTION

Coffee is one of the most popular beverages in the world (coffee consumed in Germany 2000: 6.7 kg per person; Deutscher Kaffeeverband, 2001). The major compounds related to the dry mass of roasted coffee are the melanoidins. They play an important role in the development of the coffee aroma during the roasting process. While the chemical structure of melanoidins in general is still mainly unknown, some interesting qualities of these substances have already been described. Different authors have postulated the antioxidative potential of melanoidins and the possible physiological effect which might occur (Franzke and Iwainsky, 1954; Prestera et al., 1993; Talalay et al., 1995). Former investigations were mainly focussed on the ability of melanoidins to improve the oxidative stability of food stuff. In our study, the radical scavenging ability was measured and described by using a modified TEAC-test (van den Berg et al., 1999).

EXPERIMENTAL

The first task in this study was to develop an adequate antioxidant assay and obtain an overview on the antioxidative potential of differently treated coffees. The coffee samples were purchased from a local market. To obtain information about the influence of the roasting temperature, specially roasted coffee samples were produced by Tchibo Frisch-Röst-Kaffee GmbH (Table 1).

Table 1. Coffee samples of different roasting degree

Coffee	Water content [%]	Roasting degree	Air temp. [°C]	Product temp. [°C]	Organic losses [%]
Columbia (arabica) raw	11.7	-	-	-	-
Columbia (arabica)	2.1	100	308	217	4.8
Columbia (arabica)	1.2	78	312	220	5.8
Columbia (arabica)	0.9	63	317	228	6.6

roasting time: 2.5min

The investigations on the beverages were completed by analysing the melanoidin fractions obtained by gel filtration chromatography. Three melanoidin fractions of different molecular size were collected. The melanoidin fractions contained different quantities of low molecular substances with antioxidative effects.

Free phenolcarbonic acids such as chlorogenic acid isomers have an important influence on the antioxidative effect of coffee. To gain information about the quantity of this influence, an HPLC method was used to separate the chlorogenic acids from the coffee. The contents of chlorogenic acids in the different roasted coffees were quantified and compared with the antioxidative effects.

Sample Preparation

The roasted coffee beans were stored at –17°C and ground in a standard coffee grinder (level 3, particle size 0.30 mm). The raw coffee was ground with a water cooled impact grinder for laboratory use. A standard method was used to prepare the beverage. 5 g of ground coffee powder were extracted with 50 g of distilled water for 5 minutes on a heated stirring device. After filtration and adequate dilution, the antioxidative capacity of the solution was measured. Sample preparation for gel filtration chromatography included an additional membrane filtration (0.45 µm).

Modified TEAC-Test

The original TEAC (Trolox equivalent antioxidant capacity) assay is based on the inhibition of the formation of 2,2´-azinobis-3-ethylbenzothiazoline-6-sulfonate) radical anions. The modification is based on the pre-generation of the ABTS radical anions with a thermo labile azo compound, 2,2´-azobis-(2-amidinopropane)HCL (ABAP). A TEAC value can be assigned to all compounds able to scavenge the $ABTS^{\bullet-}$ radical by comparing their scavenging capacity to that of Trolox, a water-soluble vitamin E analogue. The advantage of this assay is a short analysis time combined with a good reproducibility. Additionally, the test delivers two different measure values representing the fast and the slow antioxidative capacity (AC1, AC2) depending on structural properties of the sample.

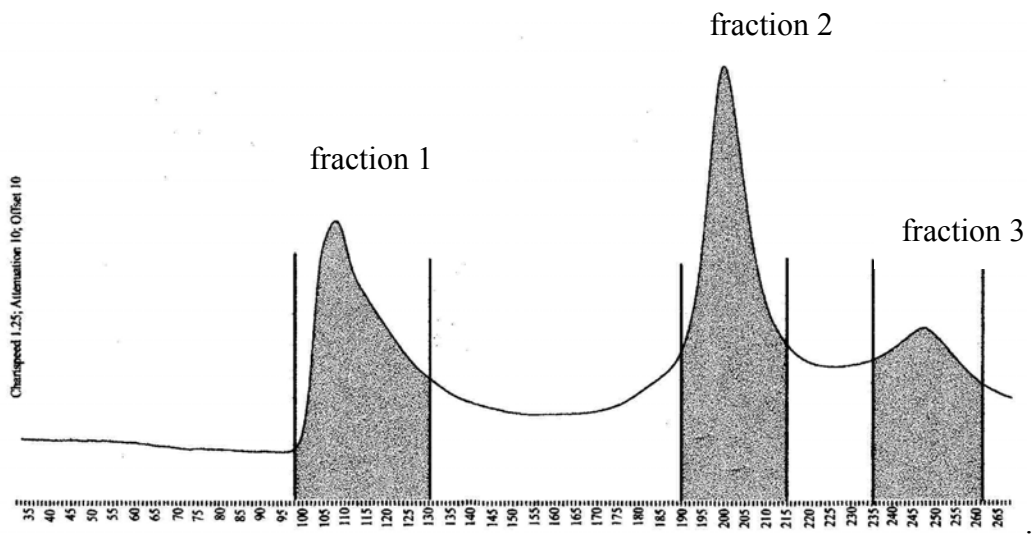

Figure 1. GFC - medium roasted coffee (405 nm, flow 4 mL/min)

Gel Filtration Chromatography

The fractionation of coffee melanoidins by gel-filtration chromatography was developed by Maier et al. 1968 (Yan et al., 1999) and is an effective method for the separation of polymers by molecular size. The coloured fractions are detected at 405nm with a UV/VIS photometer. The dextran gel Sephadex G25-fine® (Pharmacia) was used as separation medium. For preparative use, a Pharmacia XK-50 column delivered a sufficient sample capacity. The fractionation was performed in two different ways. The fractionation by peak (Figure 1) produced three main fractions which could be used for the TEAC assay. A second way of fractionation was used to detect colourless antioxidative substances which were not registered before. For this, 60 fractions of 4ml each (flow 4mL/min) were collected and analysed.

High Performance Liquid Chromatography

The three major isomers of chlorogenic acid were analysed on a Merck-Hitachi L6200 HPLC device with a programmable photodiode array detector (Waters 994). A standard RP-18 (Nucleosil 120-3C18, 3 µm) was used. The separation was started isocratic using solvent A containing 5% (v/v) acetic acid and 5% (v/v) methanol in water followed by a flushing step of 20% solvent A and 80% acetonitrile (flow 1.0 mL/min). This method (Yan et al., 1999) was transferred to a preparative HPLC-device (NovaPrep® 200, detector Merck Hitachi L-7400, column Lichrosphe® 100 RP-18, 7 µm). After removal of the chlorogenic acids, the fractions were lyophilised and the TEAC values were measured as described above.

RESULTS AND DISCUSSION

With the described TEAC assay, a variety of coffee samples was measured (Figure 3). Coffee with a strong and spicy flavour characteristic show the highest amounts of antioxidative capacity. Specially treated coffee samples like caffeine-free, mild or soluble types have a significantly lower antioxidative effect. The main reason is the loss of chlorogenic acid because of the steam treatment or other processes. The loss of antioxidative activity equals about 10-20%.

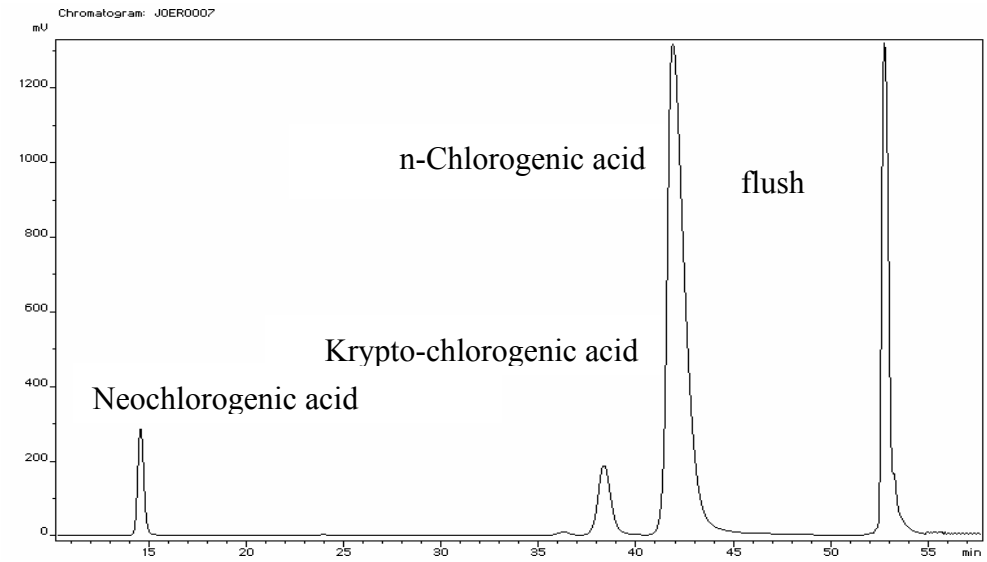

Figure 2. HPLC chromatogram of raw columbia coffee (324 nm, flow 1.0 mL/min)

The effect of the roasting degree is shown in Figure 4. The TEAC values of three differently roasted coffee samples of a Columbia coffee (Table 1) are presented in comparison with the raw coffee material. The increase of antioxidant capacity from raw to light roasted coffee equals about 25%. The medium roasted coffee shows the highest TEAC value (38.88 mmol Trolox/L). A higher roasting degree causes a decrease in antioxidative activity. An explanation for this tendency could be the formation of high molecular weight melanoidins in which the effective antioxidative components are inhibited by steric hindrance.

The separated melanoidin fractions show clear differences in molecular mass, colour and appearance (Table 2). The antioxidative capacities are also different. The results are presented in Figure 5. The fraction containing the melanoidins of the biggest size show the lowest antioxidative capacity. The highest value was measured in the middle fraction. It is also obvious that the slow antioxidative capacity (AC2) is much higher than the fast capacity (AC1). This can also be explained by steric hindrance of complex molecules. The raw coffee (Figure 4) does not show this difference between AC1 and AC2 because low molecular weight antioxidants without steric effects are predominant.

Coffee contains fair amounts of phenolcarbonic acids (e.g. chlorogenic acid, ferulic acid) which are known to have remarkable antioxidative abilities. To get an idea of the actual antioxidative effect of coffee melanoidins, the measured values for the antioxidant capacity had to be corrected by the part that is brought in by other antioxidative substances. Using an HPLC method (Figure 2) the chlorogenic isomers were separated, lyophilised and analysed. The now chlorogenic acid-free coffee was treated likewise. The results are shown in Figure 6.

To assure the comparability between normal coffee and chlorogenic acid-free coffee, the HPLC method was also applied to the normal coffee without the removal of the chlorogenic acids. Changes in the antioxidative capacity caused by organic solvent and lyophilisation were so eliminated. The loss of antioxidative capacity after the removal of the chlorogenic acids equals about 25%. This delivers a possible explanation for the differences of antioxidative capacity between normal roasted coffee and specially treated types (mild, caffeine-free).

The development of the chlorogenic acids in the course of coffee roasting leads to an important fact. The determination of the amounts of chlorogenic acids in the Columbia arabica of different roasting degrees, presented in Figure 7, indicates that a stronger roasting

results in lower amounts of chlorogenic acids. This loss of effective antioxidants should be accompanied by a decrease of the total antioxidative capacity of the sample. The antioxidative capacities measured in the coffee samples do not match with this observation (Figure 4). The roasting process causes higher values of antioxidative capacity despite the reduction of chlorogenic acids. This contradiction leads to the conclusion that substances with stronger radical scavenging effects must be formed during the roasting process. A possible explanation might also be the incorporation of fragments of decomposed chlorogenic acids in the melanoidin structure.

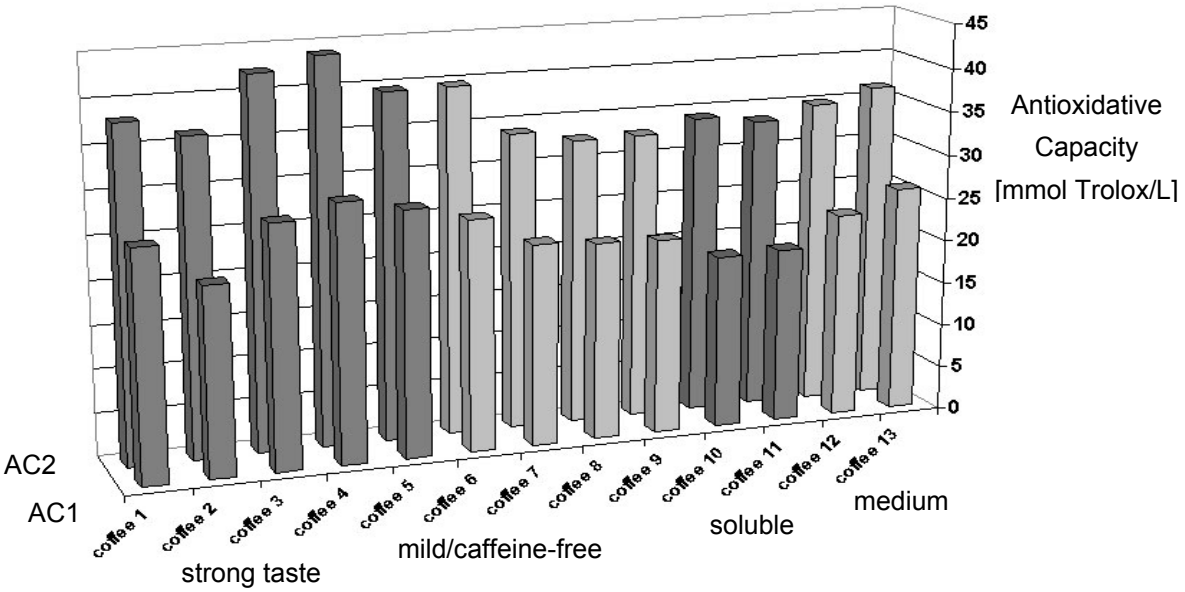

Figure 3. Antioxidative capacities of a variety of coffees measured by TEAC test

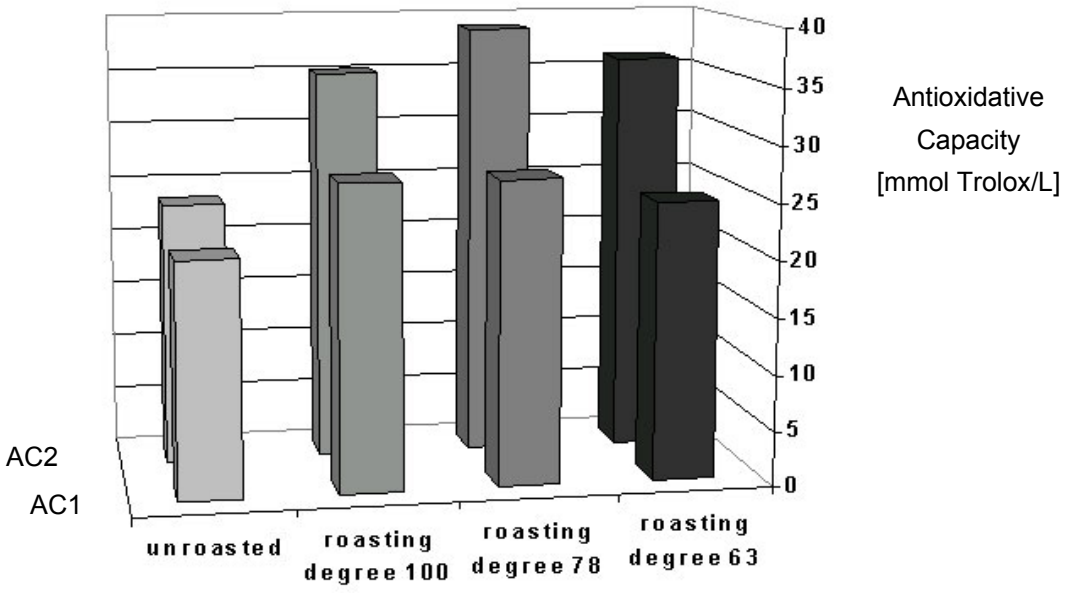

Figure 4. Antioxidative capacities of different roasting degrees of Colombian coffee measured by TEAC test

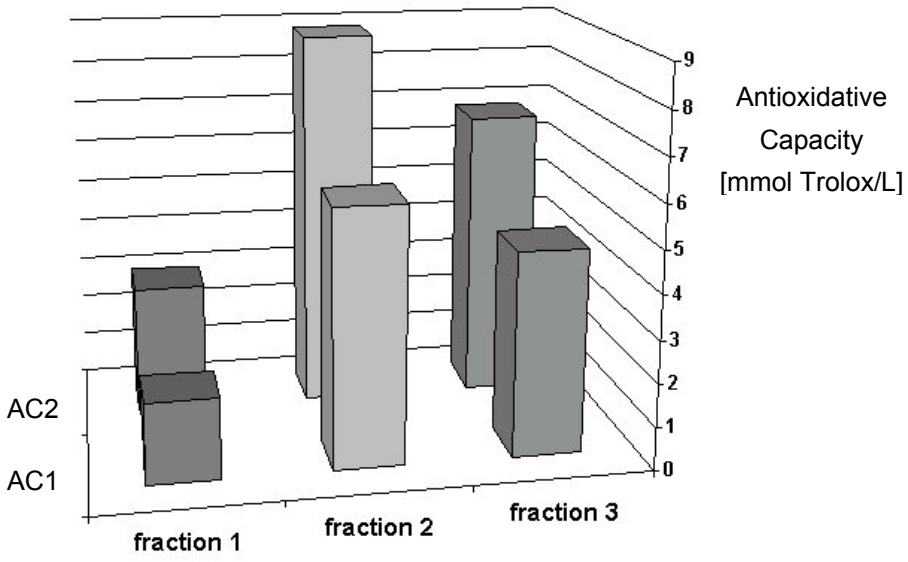

Figure 5. Antioxidative capacities of melanoidin fractions separated by gel filtration chromatography

Table 2. Sensory description of separated melanoidin fractions

Fraction	Colour of aqueous solution	Colour of lyophilised melanoidins	Structure of lyophilised melanoidins	Odour of lyophilised melanoidins
1	dark brown	light beige	voluminous, spongy	Bread, candy
2	red-orange	dark brown	powdery, crumbling	Coffee, burnt
3	yellow	beige	voluminous, spongy	Bread, candy, coffee

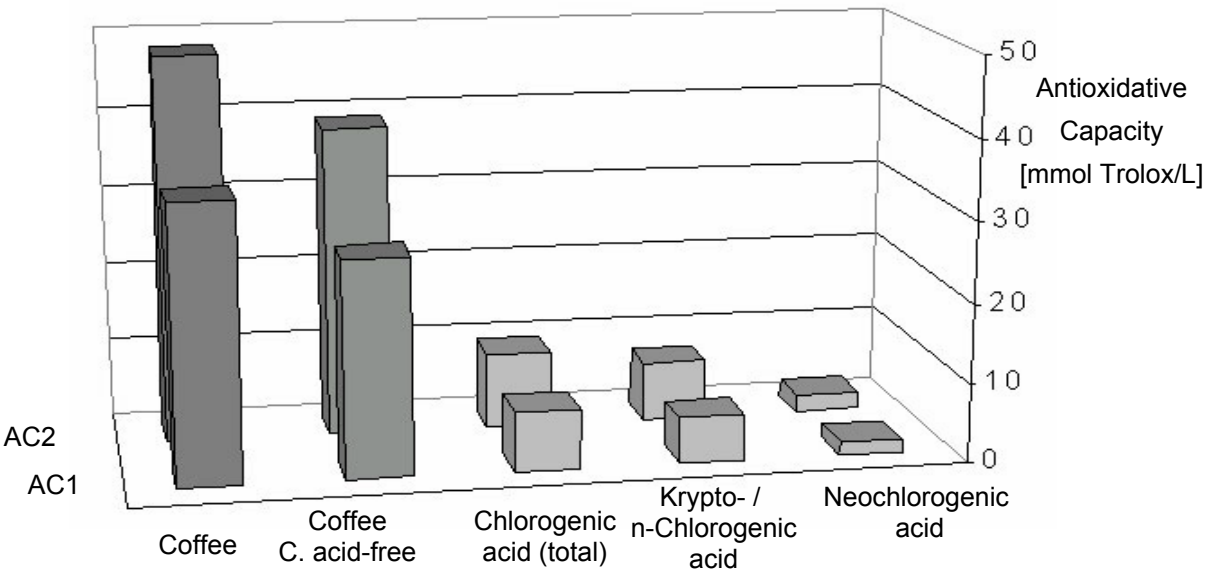

Figure 6. Antioxidative capacities of chlorogenic acid isomers and Columbia coffee after preparative HPLC separation

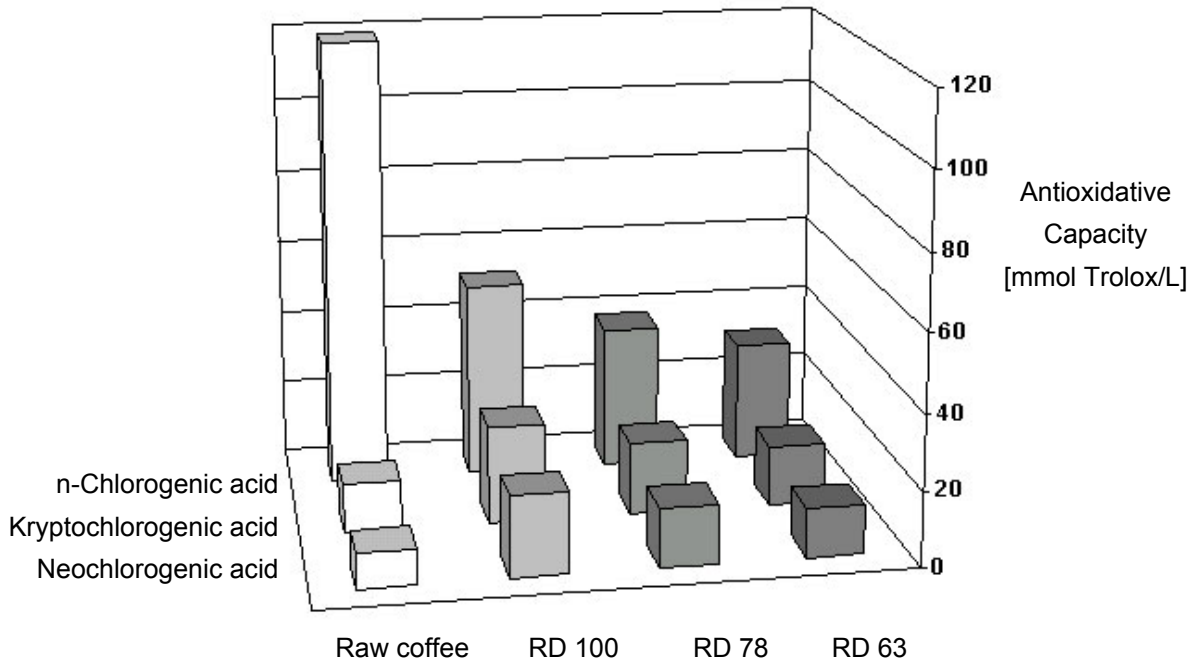

Figure 7. Chlorogenic acid contents in Columbia coffees of different roasting degrees

CONCLUSION

The modified TEAC assay is an adequate method for the description of the antioxidative potential of coffee melanoidins. The remarkable increase of the antioxidative capacity after the roasting process is astonishing compared to the decrease of chlorogenic acids in the roasted coffees. Only 15% of the total amount of antioxidative capacity result from high molecular melanoidins. The major part depends on the content of low molecular melanoidin structures and chlorogenic acids.

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Polysaccharides Composition in Arabica and Robusta Green Coffee Beans: Similar but Different?

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SUMMARY

Quantitative determination of the polysaccharide content of Arabica Caturra (*Coffea arabica* var. Caturra) and Robusta 126 (*Coffea canephora* var. Robusta 126*)* green coffee beans demonstrated that they contained identical amounts of polysaccharide. Cell wall material (CWM) was prepared from the beans and partial solubilisation of component polysaccharides was effected by sequential extraction. In Robusta more arabinogalactan was solubilised during preparation of the CWM and the water-soluble fraction of the CWM contained higher amounts of galactomannan than in Arabica. Linkage analysis indicated that the galactomannans possessed unbranched to branched mannose ratios between 14:1 and 30:1. No major differences in the structural features of the galactomannans between species was found. Compared to Arabica, Robusta appeared to contain greater amounts of arabinogalactans with more and longer side chains.

RÉSUMÉ

La détermination quantitative du contenu en polysaccharides totaux des cafés verts Arabica Caturra (*Coffea arabica* var. Caturra) and Robusta 126 (*Coffea canephora* var. Robusta 126*)* a démontré qu'il n'y avait pas de différence entre les deux espèces. Du matériel pariétal a été préparé et les polysaccharides pariétaux ont été solubilisés par extraction séquentielle. Plus d'arabinogalactane a été extrait lors de la préparation du matériel pariétal de Robusta et plus de galactomannane a été solubilisé dans l'extrait aqueux. L'analyse par méthylation a démontré que les galactomannanse possèdent des degrés de substitution entre 14:1 et 30:1. Il n'y avait pas de différences majeures entre les caractéristiques structurales des galactomannanes des deux espèces. par contre, les arabinogalactanes de Robusta semblent posséder des chaines latérales plus nombreuses et plus longues que Arabica.

INTRODUCTION

Differences in total polysaccharide content as well as structural differences between Arabica and Robusta species have been discussed in the literature. Reliable values are difficult to obtain as they generally have to be extrapolated and recalculated from a series of values obtained after various treatments and extractions which are not comparable (Bradbury and Halliday, 1990; Clifford, 1985; Clifford, 1986; Trugo, 1985). Most of these sources report a lower polysaccharide content for Robusta compared to Arabica. Clifford (1986) reported a total polysaccharide content between 38% and 48% for Robusta and 48-55% for Arabica, but experimental information detailing how the values were obtained is lacking. In contrast, Bradbury and Halliday (1990) report a total polysaccharide content of 48.1% for green Robusta coffee from Ivory Coast and "a lower polymeric carbohydrate content for Arabica beans".

Clifford (1986) also reports that Arabicas may contain more arabinogalactan (9-13%) than Robusta (6-8%) and more galactomannan (25-30% vs 19-22%). The present study compares

the total polysaccharide content of a variety of Arabica and of Robusta and report data for the structural features of arabinogalactan and galactomannan isolated from each.

MATERIAL AND METHODS

Dry, depulped and deparched beans of Arabica Caturra (*Coffea arabica* var. Caturra) and Robusta 126 (*Coffea canephora* var. Robusta 126*)* coffee which were harvested at full maturity in Ecuador were used for this investigation.

The analytical methods used have been described previously (Fischer et al., 1999; Fischer et al., 2000).

RESULTS

Total polysaccharide content

The total polysaccharide content of the beans was measured after removing the low molecular weight carbohydrates by ethanolic extraction. The ethanol-insoluble material was dialysed to remove the last traces of monosaccharide and recovered by freeze-drying. Total carbohydrate was determined by GLC analysis of the alditol acetates following Saeman hydrolysis of the total polysaccharide (Table 1). In contrast to previous reports (Bradbury and Halliday, 1990; Clifford, 1985; Clifford, 1986; Trugo, 1985; Bradbury and Halliday, 1987) we found no evidence for differences between Arabica and Robusta in their total polysaccharide content.

Table 1. Monosaccharide composition and total polysaccharide content of green Arabica and Robusta coffee. (Means values, n= 3)

Sample	Fuc	Rha	Ara	Gal	Glc	Xyl	Man	Total
				(mole %)				(%w)
Robusta	tr.	1.0	12.2	26.4	16.3	1.0	43.1	55.5
Arabica	tr.	0.4	10.6	24.5	16.7	1.1	46.7	55.8

Isolation and fractionation of CWM

Cell wall material (CWM) was prepared by a modification of an existing method used for the isolation of CWM from fruit tissue (Fischer et al., 2001). Phenol-Acetic Acid-Water (PAW) was used to inactivate endogenous enzyme activity and solubilise intra-cellular proteins which have a tendency to bind to the cell wall during its isolation. The CWM from Arabica and Robusta beans accounted for 53% and 52% of the starting material, respectively.

The PAW extract separated into a soluble and insoluble fraction during dialysis. In Robusta, the PAW-soluble fraction (Table 2) consisted of 40% polysaccharide most of which was arabinogalactan. The material that precipitated during dialysis contained very little polysaccharide and was predominantly protein (data not shown). PAW solubilised more polysaccharides from Robusta than from Arabica.

Individual polysaccharide fractions were isolated by sequential extraction of CWM with water, 1M KOH, $NaClO_2$, 4M KOH and 8M KOH and their monosaccharide composition determined (Table 3).

Table 2. Monosaccharide composition of PAW-soluble and PAW-precipitate fractions isolated during the purification of CWM from Arabica and Robusta coffee

	Monosaccharide (mole %)							**Total**
Robusta	**Rha**	**Fuc**	**Ara**	**Xyl**	**Man**	**Gal**	**Glc**	(%w)
PAW-soluble	12.4	0.4	37.9	2.3	7.0	38.7	1.3	40.2
PAW-prec.	8.7	2.4	21.7	3.1	19.2	26.1	18.8	2.7
Arabica								
PAW-soluble	6.0	0.2	33.2	3.0	11.2	40.5	5.8	12.1

Table 3. Yield and monosaccharide composition of fractions of CWM isolated from Arabica and Robusta beans (mean values; n =3).
All fractions contained traces of rhamnose and fucose

Fraction	**% CWM**	**Monosaccharide composition** (mole %)					**Total**
		Ara	**Xyl**	**Man**	**Gal**	**Glc**	(%w)
Water-sol							
Robusta	15.6	14.7	1.4	36.8	29.3	15.9	53.7
Arabica	3.5	25.9	1.3	16.7	49.3	3.4	27.3
1M KOH-sol							
Robusta	9.1	17.3	2.3	35.1	33.8	9.2	40.4
Arabica	12.8	20.5	2.7	35.1	35.3	3.2	14.9
$NaClO_2$-sol							
Robusta	4.5	32.4	0.5	5.1	57.8	1.9	36.2
Arabica	2.4	33.3	1.0	2.4	60.7	1.0	33.7
4M KOH-sol							
Robusta	4.4	14.3	4.4	33.6	34.1	12.6	48.5
Arabica	2.1	17.7	13.9	29.7	31.4	6.5	34.5
8M KOH-sol							
Robusta	5.5	23.8	1.6	17.1	53.2	3.8	31.2
Arabica	2.6	25.9	4.3	11.6	54.6	2.4	36.1
Residue							
Robusta	61.1	8.6	0.1	46.8	26.3	18.0	69.8
Arabica	76.6	7.0	3.3	51.3	19.0	18.3	70.1

The major variation in the yield of individual fractions occurred in the water-soluble fraction which had a higher yield in Robusta. The mannose content of this fraction was high. Close to 10% of the total mannose from Robusta beans was solubilised in the water fraction compared to less than 1% from Arabica.

Although there were significant differences in the comparative amounts of the $NaClO_2$-, 4 M KOH- and 8 M KOH-soluble fractions, these fractions accounted for only a small part of the total polysaccharide.

Acidified sodium chlorite is traditionally used for delignification but has also been shown to be a good solvent for glycoproteins (Selvendran and O'Neill, 1987) and it proved to be a good solvent for arabinogalactans.

The composition of the residue which accounted for 60 and 77% of the CWM of Robusta and Arabica respectively resembled that of the original CWM, demonstrating the difficulty of solubilising not only the galactomannan but also the arabinogalactan in coffee CWM.

Structural features of coffee arabinogalactan

The glycosyl linkages of the arabinogalactan extracted by chlorite (Table 4) are consistent with the documented structure of an arabinogalactan which possesses a 1-3- linked galactose backbone with a proportion of the galactose residues substituted at 0-6 by side-chains of arabinose and/or galactose residues. The degree of substitution as indicated by the ratio of 3- to 3,6-linked galactose is 0.92 Robusta and 1.45 for Arabica. This indicates that the chlorite-soluble arabinogalactan in Robusta was more highly branched than the equivalent fraction in Arabica and these differences were also apparent in the water-soluble and 8 M KOH-soluble fractions.

Table 4. Linkage analysis of $NaClO_2$-soluble arabinogalactan

Linkage	Robusta	Arabica
t-Araf	22.8	15.1
5-Araf	7.6	10.3
t-Gal	0.7	9.9
3-Gal	27.7	30.0
3,6-Gal	29.8	20.6

Structural features of coffee galactomannans

Galactomannans were recovered in most of the CWM fractions, although in Robusta coffee a higher proportion was recovered in the water-soluble fraction. After the final extraction with 8M KOH, the residue still contained close to 50% mannose. The mannans or galactomannans are composed of a 1, 4-linked mannose backbone substituted at 0-6 with single galactose residues. The water-soluble galactomannans had ratios of 4-linked to 4,6-linked mannose of 18.6 for Robusta and 28.6 for Arabica (Table 5). In other cell wall fractions, the material was more branched with ratios of unbranched to branched residues close 10, except in the 4M KOH fractions where the galactomannans were less substituted.

DISCUSSION

The results of this study showed no difference in the total polysaccharide content or any marked difference in the structural features of the galactomannans between Robusta 126 and Arabica Caturra.

Robusta 126 contained amounts of a highly soluble arabinogalactan which possessed more branch points and more extended side-chains than arabinogalactans found in Arabica Caturra. This may be one of the reasons that the arabinogalactans of Robusta were more easily

solubilised than those of Arabica. It is possible that the accommodation within the cell wall matrix of a polysaccharide with more extended sidechains may produce a cell wall with different physicochemical characteristics than one which does not possess such polymers.

Table 5. Linkage analysis of water-soluble galactomannans

Linkage	Robusta	Arabica
t-Man	2.7	3.0
4-Man	41.0	48.7
4,6-Man	2.2	1.7
t-Gal	5.3	2.1

Both varieties possessed galactomannans with ratios of unbranched to branched mannose between 10:1 and 30:1. While the overall structures did not differ significantly between the two varieties, higher amounts of the water-soluble galactomannan were recovered in Robusta 126 and it was more highly branched. The galactomannans which remained in the residue or were extracted with concentrated alkali had degrees of branching close to 20:1. Except in the water soluble fraction, no evidence was found to support the idea that Robusta contained more branched galactomannans than Arabica . The average degree of branching observed was higher than the 100:1 value reported by Bradbury and Halliday (Bradbury and Halliday, 1987; Selvendran and O'Neill, 1987) although they use harsher extraction procedures.

The main difficulty in characterising the mannans or galactomannans which are present in the coffee bean lies in the high proportion of insoluble polymers as only about 1/3 of the total CWM was solubilised by the sequential fractionation process, despite the fact that an alkali concentration of 8M was used. A certain amount of arabinogalactan remained in the residue despite its intrinsic solubility, pointing to the importance of cell-wall architecture in determining the physicochemical properties of its components. This was supported by the fact that arabinogalactans with similar structural features were recovered in all the cell wall fractions indicating that the immediate environment of the polysaccharide had more influence on its solubility than its basic structural features.

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New Aspects of Coffee Processing: The Relation Between Seed Germination and Coffee Quality

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SUMMARY

Quality of green coffee depends on the processing method. Up to now, these differences were attributed exclusively to differences in the original material used for processing. This explanation neglects the occurrence of metabolic reactions that run in the living coffee seeds and strongly depend on the physiological status of the beans. Coffee seeds are recalcitrant. This means that seed germination is already induced while they still are within the fruit. However, further development is suppressed, either by the osmotic potential of the fruit flesh or by germination inhibitors. Consequently, rather than dormant seeds, coffee beans represent germinating seeds which are blocked in their further development.

In contrast to wet processed green coffee beans, where the fruit flesh is removed directly after harvesting and thus the inhibition of further development is neutralised immediately, in dry processed coffees, the inhibition should be maintained throughout processing. Accordingly, the germination status, and thus the mobilisation of reserves influencing the composition of aroma precursors, should be quite different in wet and dry processed coffee seeds.

The processing of identical original material by both methods in parallel leads to significant differences in the cup quality of the corresponding roast coffees. These differences are based on metabolic reactions within the coffee seeds that differ markedly depending on the mode of post-harvest treatment. These reactions mainly comprise the mobilisation of reserves, liberating low-molecular substances. These, like free amino acids, are potential aroma precursors and thus influence the coffee quality. In this context the quantity and quality of free amino acids of wet and dry processed coffees was analysed. In all cases the total content of amino acids was higher in washed green coffee beans than in the corresponding dry processed ones, verifying cogently the hypothesis mentioned.

INTRODUCTION

In general, coffee processing is performed either in the dry or in the wet way. In the course of wet processing, first fully ripe coffee cherries are mechanically pulped. The resulting parchment beans are still covered by mucilaginous residues of the pulp, which are degraded during a one or two day tank fermentation. Thereby the pectins are hydrolysed and the remaining mucilage is washed off. After drying, the resulting parchment coffee is hulled, and the green coffee beans can be stored and shipped. In contrast, in the course of dry processing, the entire coffee cherries are dried directly. After husking, the green coffee beans are also ready for shipping.

It is well accepted that there are significant quality differences between the differentially processed coffees. In general, dry-processed coffees are characterised by more body, whereas wet-processed ones reveal a better aroma resulting in a higher acceptance. These undeniable

differences are widely attributed to the fact that wet processing requires a proper sorting of the fruits, because only fully ripe coffee cherries can be pulped correctly by the mechanical pulpers. Moreover, the entire wet processing requires a more thorough proceeding than the dry one. However, there are some hints in the literature, that also wet and dry processing of coffee cherries of the same quality resulted in different flavours (Chassevent et al., 1970). Thus, the question arises, if – apart from the differences of the original material used for processing – also the metabolic reactions running in the coffee beans during processing contribute to cup quality, depending on the type of the processing method used.

This work is aimed to analyse if the observed quality differences in differently processed coffees are exclusively due to differences of the original material or if they also are caused by metabolic processes occurring in the seeds during processing.

Plant physiological background

To understand the processes occurring in coffee seeds during processing, one must realise that processed green coffee represents living organisms, featuring various metabolic activities. The nature and extent of these reactions depend on the physiological status of the seeds, which among others is influenced by the water activity of the seeds. In moist seeds, i.e. during early stages of processing, the metabolic activity should be considerably high, whereas in dried ones (in the final stages of wet or dry processing) these reactions are strongly reduced due to the low water content.

Like many other tropical seeds, coffee seeds are characterised as "recalcitrant" (Roberts 1973; Ellis et al., 1990). Unlike “orthodox” seeds (e.g. wheat or peas) they do not undergo a dormancy period initiated by maturation drying. Instead, ripe coffee seeds have a water content as high as 45% (wet basis). This means that in principle they are able to germinate within the fruit (Ellis et al., 19991). However, the endogenously induced germination is obviously suppressed, either by the high osmotic potential of the fruit flesh as in the case of cocoa (Rühl et al, 1988), or by germination inhibitors like in tomato seeds (Bewley and Black, 1995), or by phytohormones like in avocados, where the germination is inhibited by absisic acid (Sembdner et al., 1988). Consequently, rather than dormant seeds, coffee beans represent germinating seeds which are blocked in their further development. Indeed, up to now it is not known, which active inhibiting principle is realised in the coffee fruits, but it is very likely that it is located - like in the other fruits - within the pulp. Thus, as soon as the seeds are taken out of the coffee cherries, i.e. when the fruits are pulped, the formerly blocked germination processes are un-locked and a typical germination metabolism can be initiated. This also applies for beans germinating out of fruits that had been shed onto the soil. Only after extensive decomposition of the pericarp – a process that can take several weeks – the germination in the coffee beans proceeds, resulting in radicule protrusion and seedling growth.

Coffee quality and seed germination

One of the characteristic features of germination metabolism is the mobilisation of reserves, i.e. fats, proteins and polymeric carbohydrates. By this, free amino acids and soluble carbohydrates should be released, which – with regard to coffee quality – represent important aroma precursors that give rise to the characteristic coffee aroma compounds during roasting. Summarising the facts so far mentioned, it can be concluded that the quality differences of technologically differently processed coffees must be influenced significantly by the metabolic processes running to different extents in the green coffees treated in the respective two ways. In the course of dry processing, the pulp (and thereby the inhibiting agent) remains

around the seed for the entire period. This means, the inhibition of further germination persists and thus the metabolic processes running in the beans should not change drastically.

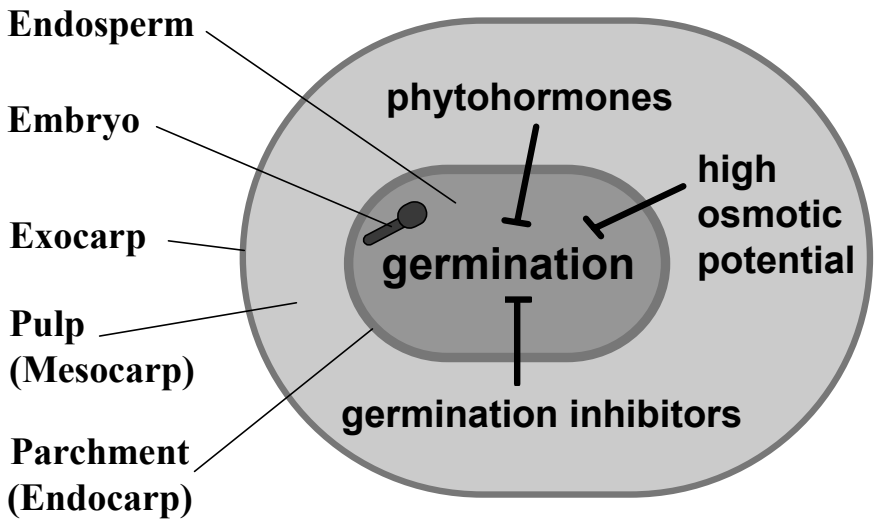

Figure 1. Inhibition of germination by active principales located in fruit flesh (pulp) of coffee seeds

Wet processing commences with the removal of the pulp, relieving the seeds from the germination inhibiting effect. The presence of sufficient water in the fermentation tanks guarantees further imbibition of the seeds. As a consequence, the germination metabolism is unlocked, leading to the mobilisation of reserves and thus to the liberation of potential aroma precursors. Hence, the question arises if the period during the wet processing, in which this metabolism takes place, is long enough to produce significant amounts of aroma precursors and to increase aroma quality. In this context we have to consider that active metabolism persists not only during the actual fermentation phase, but also in the initial period of the subsequent drying. Altogether there is a time window of 3-6 days until the water content of the seeds decreases to a point where the metabolic processes are slowed down to very low rates.

Within the next chapters we present evidence that the quality differences of washed coffees compared to dry processed ones indeed partially derive from the physiological activities within the seeds during this space of time.

MATERIALS AND METHODS

Processing

For both experimental lines – original processing and model processing in Braunschweig – exclusively ripe fruits of *Coffea arabica* L. were used. Fruits of the variety *Acaiá* were kindly provided by Ipanema Agricola Ltda., Alfenas, Minas Gerais, Brazil. Fruits of the variety *Caturra* were kindly provided by Cenicafé, Chinchiná, Colombia.

Processing in Brazil and Colombia

Wet processing

Freshly harvested fruits were mechanically pulped and submitted to tank fermentation for 22 h and 16 h (*Caturra*), respectively. Subsequently the washed parchment coffee beans were

dried on a separate plot of the plantation's sun terrace (*Acaiá*), and in mechanical dryers (*Caturra*), respectively. After 6 d (*Acaiá*) and 2 d (*Caturra*), respectively, the desired water content of 12% (wet basis) was achieved. Within 4 weeks the dry parchment coffees were sent to our lab in Braunschweig, where they were manually hulled and used for roasting. Aliquots were stored in a freezer (–70°C).

Dry processing

Mature coffee cherries from the same batches used for the wet processing were manually selected and dried as whole fruits on the sun terrace for 12 d (*Acaiá*) or 14 d (*Caturra*), respectively, until the desired water content of 12% was achieved. Within 4 weeks the dried cherries were sent to Braunschweig, where they were manually husked and used for roasting. Aliquots were stored in a freezer (–70°C).

Model processing in the laboratory

Fully ripe fruits were harvested on the coffee plantation in Brazil, subsequently transferred into "styrofoam" boxes, and sent to Germany by cargo express flight. To prevent the fruits from decomposition during the transport, some plastic bags containing ordinary ice cubes were added. Three days after harvest, the fruits arrived in Braunschweig and were subsequently submitted to model processing. During the laboratory processing periodically samples were taken and stored at -70°C for later preparation and analysis.

Wet model processing

The fruits were manually pulped and the mucilaginous parchment beans were transferred to 5 L-Erlenmeyer-flasks adding an excess of fresh water. The coffee was model-fermented under the ambient conditions of the laboratory for 36 h. During the procedure, the water was exchanged three times. The resulting parchment coffee was dried in an standard laboratory drying oven at temperatures of 35-40°C. The desired water content of 12% (wet basis) was achieved after 5 d. The beans were manually hulled. Whereas the main part was roasted, small amounts were stored at –70°C for further analysis.

Dry model processing

The mature coffee cherries were dried in a common laboratory drying oven at temperatures of 35-40°C. After 12 d drying was accomplished. The beans were manually husked and either used for roasting, or stored at –70°C for further analysis.

Extraction of free amino acids

The stored coffee seeds were transferred into liquid nitrogen and – after adding norvalin as internal standard – crushed to a fine powder. The coffee seed powder was repeatedly extracted with sulphosalicylic acid (4%).

Derivatisation and determination

The OPA-derivatisation procedure was accomplished according to Kirchhoff et al. 1989, however a Spark Holland Midas Autosampler was used for derivatisation and sample injection. The derivatives were separated on a C18 column (Nucleosil 100 5µm Macherey and Nagel 250 x 4,0 mm) using a binary gradient (MeOH, ACN, H_2O) with a flow rate of 1,3 mL/min. The derivatives were detected using a RF-551 Shimadzu fluorescence detector (334

nm ex; 425 nm em) and quantified using an external standard of a mixture of amino acids.

Roasting and sensory assessment

The roasting of the different green coffee samples was carried out by the DFA (Munich-Garching) using a Probat BRZ4 sample roaster. The sensorial assessment was accomplished by a sensory panel (11 members) of the DFA. The aroma was evaluated in triangle tests.

RESULTS AND DISCUSSION

In the experiments, identical material was used for both coffee processing methods in parallel and subsequently used for analysis. For the first time, not only green and roasted coffee beans, but also the respective "progenitors", i.e. the fresh coffee seeds and the intermediate products of green coffee preparation, have been analysed. Extensive sample preparation experiments which included original post harvest processing in the producing countries (Brazil and Colombia) and laboratory model fermentations, yielded substantial material for coffee analysis.

The sensory evaluation of the roast coffees revealed that the dry and washed coffees could be distinguished with high significance (11 of 11 panel members). In the hedonic evaluation of the overall aroma impression 9 of 11 panel members classified the "dry" roast coffee as less acceptable. These results represent an unequivocal proof that the reasons for the quality differences of technologically differently processed coffees are not exclusively due to the differences of the original material but must also be caused by the processes taking place in the beans during processing. These data confirm results from experiments of Chassevent et al. (1970) who also analysed green coffees which were obtained by applying the different processing methods on similar original material.

It is scheduled to supplement our sensory analysis by detailed analysis of the aroma impact compounds by GLC using aroma extract dilution analysis (AEDA, Grosch, 1996; Grosch et al., 1996). These studies have been initiated recently in the laboratory of Prof. Schieberle (DFA Munich).

In order to register the differences in the metabolic processes running in the differentially processed coffee beans the content of free amino acids in the raw coffee beans was analysed. Free amino acids represent typical products of reserve mobilisation, which also reveal a high significance as potential aroma precursors. Quality and quantity of free amino acids of those green coffees, which were obtained respectively from the authentic, and from the model processings, were determined. In each case the total content of amino acids was higher in washed green coffee beans than in the corresponding dry-processed coffees (Table 1).

These results were also confirmed by Casal et al. (2001) and also could be calculated from former data of Arnold and Ludwig (1996). These findings clearly demonstrate that during processing, the concentrations of potential aroma precursors change to different extents depending on the mode of processing, supporting the hypothesis mentioned above.

The time course of the liberation of amino acids points out that the content of free amino acids is determined by complex processes. Obviously, the release of amino acids – as a result of the hydrolysis of storage proteins – is overlayed by the consumption of amino acids in the course of various metabolic processes, e.g. protein biosynthesis. Whether the marked decrease of free amino acids in the first phase of wet processing is due to wash-outs in the course of fermentation, or if it reflects a general anabolic metabolism of germinating seeds remains to

be elucidated. Corresponding analysis using authentical material from original processings in Brazil and Colombia is in progress.

Table 1. Total content of free amino acids in wet and dry processed green coffees

	free amino acids [mg / kg f.w.]	
	dry processing	wet processing
plantation (Columbia)	2,760	3,070
plantation (Brazil, A)	3,570	4,310
plantation (Brazil, B)	4,030	4,360
laboratory (Germany)	5,050	5,400

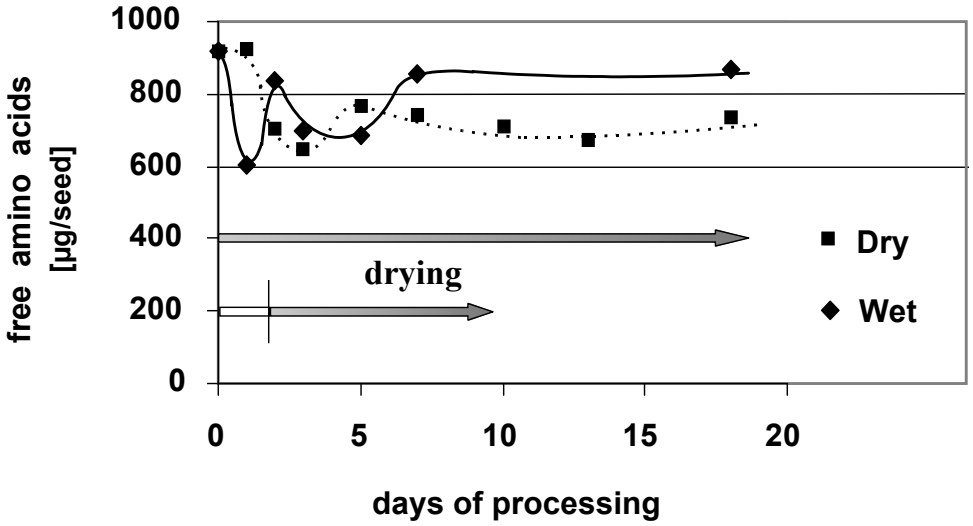

Figure 2. Changes in the content of free amino acids during model processing

Therefore, it is essential to use an additional marker for the registration of the differences in the germination status of the differentially processed coffees. One of the first metabolic reactions in germinating seeds in general is the onset of the glyoxylate cycle. It is planned to use the gene expression of the key enzyme for this metabolic pathway – that of the isocitrate lyase – as corresponding germination marker. A homologous probe of this enzyme is already synthesised and will be used for the forthcoming quantifications.

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Coffee Flavour Precursors: Contribution of Water Non-extractable Green Bean Components to Roasted Coffee Flavour

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SUMMARY

Whole green coffee beans, arabica and robusta, were extracted with hot water and roasted. Sensory evaluation of the coffee brews showed a reduction in quality: the flavour profile was unbalanced and characterized by low coffee attributes and an increase in a cereal-toasted note.

Water extraction of whole green beans resulted, after roasting, in a coffee with strongly decreased key impact odorants, like 2- and 3-methylbutanal, α-diketones and guaiacols. Guaiacols were found to be highly correlated with the amount of feruloylquinic acids in the green coffee. Among the sulfur compounds, only furfurylthiol increased significantly. This indicates that its precursors are mainly water non-extractable green bean components.

The results of this work indicate that hot water-extractable green coffee components are important for the intensity and balance of coffee flavour. However, the contribution of water non-extractable components to coffee flavour is not negligible, as key odorants are still generated upon roasting of extracted green beans.

In addition an improved and rapid method for the determination of thiols has been developed: Addition of cysteine to a roast and ground coffee slurry followed by headspace sampling using SPME and isotopically labelled internal standards is demonstrated to be a reliable and convenient technique to quantify thiols in coffee.

RÉSUMÉ

Des grains de café vert de variéte arabica et robusta ont été extraits à l'eau chaude avant d'être torréfiés. L'évaluation sensorielle de la boisson obtenue par infusion de ce café a révélé une baisse de qualité : le profil sensoriel a été trouvé plat et déséquilibré avec une perte des notes typiquement "café" et un renforcement des notes de type "céréale" et "toasté".

L'extraction préalable des grains verts provoque, après torréfaction, une importante diminution de la teneur en certains composés-clés odorants comme les 2- et 3-méthylbutanal, les α-dicétones et les guaiacols. Une excellente corrélation a été trouvée entre la quantité de guaiacols formés pendant la torréfaction et les teneurs en acides féruloylquiniques des cafés verts. Parmi les composés soufrés, seul le furfurylthiol augmente d'une manière significative dans les cafés préalablement extraits à l'eau chaude. Ceci indique que les précurseurs qui sont à l'origine de ce composé se trouvent majoritairement dans la partie non-extractible du café.

Le résultat de ce travail indique que la partie du café vert extractible à l'eau chaude est importante pour l'arôme du café, tant du point de vue quantitatif que qualitatif. Néanmoins, la partie non-extractible contribue pour une part non négligeable à la formation de certains composés-clés de l'arôme du café torréfié.

Ce travail a permis par ailleurs de développer une méthode améliiorée et rapide pour la détermination des thiols. L'additiion préalable de cystéine à une suspension de café torréfié moulu, suivie d'une analyse de l'espace de tête par SPME en présence d'un standard interne marqué s'est révélé être une technique fiable et commode pour quantifier ces composés dans le café.

INTRODUCTION

The typical aroma of coffee is generated upon roasting of green coffee belonging to the two main species, *Coffea arabica* (arabica) and *Coffea canephora* (robusta). More than 800 volatiles have been reported in the literature (Nijssen et al., 1996), but only a few are key coffee compounds characterising the aroma profile (Holscher et al., 1990; Holscher and Steinhart, 1992; Semmelroch et al., 1995; Czerny et al., 1999).

As coffee odorants belong to different chemical classes, their formation follows different pathways. This has been studied in model systems focusing on the generation of pyrazines (Reese and Baltes, 1992; Baltes and Bochmann, 1987), furfurylthiol (Parliment and Stahl, 1995) and 3-mercapto-3-methylbutylformate (Holscher et al., 1992). Although model systems give some indications on the possible reaction pathways, they do not simulate true roasting conditions, occurring within the specific environment of the coffee bean.

Recently water extracts of green coffee beans, as well as the fractions obtained by size exclusion chromatography, were evaluated for their flavour formation potential (de Maria et al., 1996; de Maria et al., 1994). The authors showed that water soluble compounds of high molecular weight are important sources of coffee aroma. However, the sensorial contribution of the compounds generated in their systems was not systematically evaluated. In this work we focus on the aroma changes generated upon roasting of green beans that were previously extracted with hot water, thus keeping the integrity of the bean structure which is essential for aroma generation during roasting.

EXPERIMENTAL

Green coffee treatment

Arabica (Caturra) and Robusta (Dormilon Ecotype) green coffee beans were obtained from two different locations in Ecuador, Pinas and Bimbe/Los Rios respectively. Ten kg of each coffee (whole green beans) were extracted at 90°C with demineralised water (3 x 50 l) in a reactor under stirring for two hours. The combined extracts (137 l) were gently concentrated at 17-20 °C under vacuum to ~15 l. Both, the concentrated extract and extracted green beans were subsequently freeze dried and stored in sealed cans at room temperature.

In another experiment, a suspension of freeze-dried extract in water (350 g in 700 ml) was re-incorporated into 1.65 kg of extracted green beans at 80°C for 4 hours. The treated, wet beans were then freeze-dried and finally roasted. Non-treated coffee was used as reference. All six coffees were roasted to the same medium roast level using a Neuhaus Neotec roaster.

Sensory analysis

The sensory profile of coffee samples were assessed by a panel of 20 subjects. Fifty g R&G coffee were used to prepare 1 l of brew, using a drip filter. Coffee brews were served hot in cups. The panellists, previously trained on individual coffee attributes, were asked to evaluate

the aroma by sniffing, and then to evaluate the flavour. No reference sample was given during the tasting.

Green coffee composition

Green beans were ground under liquid nitrogen using a Perten laboratory mill 3303 and defatted with hexane in a Soxhlet apparatus. After removing the solvent the defatted green coffee powder was used for the following analyses.

Caffeine and chlorogenic acids

Defatted green coffee powder (500 mg) was suspended in 9 ml MeOH:H_2O (7:3, v/v) and sonicated for 1 h. After centrifugation the supernatant was filtered through a 0.2 µm LC-13 disposable filter and injected in a HPLC system (HP 1090 M) for separation in a Nucleosil 100 C-18 reversed phase column (250 mm x 4 mm id, 5 µm). The compounds were eluted using a gradient of 0.1 % trifluoroacetic acid in water and acetonitrile at 1 ml /min flow rate. UV detection was carried out at 275 and 325 nm with a diode array detector.

Free sugars

Defatted green coffee powder (500 mg) was dispersed in water (70 ml) and heated to 70 °C for 30 min. After cooling the solution was diluted to 100 ml with water. Aliquots (10 ml) were passed through Sep-pak C-18 cartridges (Millipore, England) for clean-up and then filtered (0.2 µm) before HPLC analysis. The separation was performed on a CarboPac PA1 column (Dionex) using water as eluent as described in the literature (Prodolliet et al., 1995).

Total sugars

Defatted green coffee powder (100 mg) was suspended in 1.0 ml sufluric acid (72%) (w/w) and stirred for 2.5 h at 20°C. Water (11 ml) was added and hydrolysis was continued at 100°C for another 2.5 h. After cooling, the suspension was diluted to 50 ml, and an aliquot (10 ml) treated as described for free sugars.

Quantification of key aroma compounds

Determination of high volatile key impact odorants

Sample preparation: 5 g R&G coffee was suspended in 100 ml water at 90°C and vigorously shaken for 1 min. After 3 min the R&G slurry was cooled in an ice bath and a standard mixture containing $^{13}C_2$-acetaldehyde (390 µg), [$^2H_6$]-dimethylsulfide (11.2 µg), $^{13}C_4$-2,3-butanedione (55.5 µg); [$^2H_3$]-2,3-pentanedione (77.7 µg), [$^2H_7$]-methylpropanal (44 µg) and [$^2H_7$]-3-methylbutanal (57.9 µg) in dichloromethane (0.5 ml) was added. After shaking (5 min) and decantation, an aliquot (~2 ml) was placed in a headspace cell (66 ml), described by Chaintreau et al. (1995), and equilibrated at 30°C in a water bath for 30 min. The headspace was then passed through a glass tube (3½" x ¼"), containing Tenax (125 mg) at a controlled flow rate of 40 ml/min.

Volatile analysis: Volatiles were desorbed from the Tenax trap at 250°C for 10 min using an ATD 400 thermal desorber (Perkin-Elmer Corp., Norwak, CT). The volatiles were then refocussed on a second Tenax trap (–30°C) in the GC (HP 5973) before being desorbed at 260°C for 3 min onto a DBWax column (60 m x 0.53 mm x 1 µm film thickness). The

column was kept at 20°C for 5 min, then raised at 6°C/min to 180°C and without holding time at 20°C/min to 210°C where it was kept for 10 min.

Determination of thiols

Method A): R&G coffee (10 g) was suspended in dichloromethane (50 ml). A thiol standard mixture containing 15.37 µg [$^2H_2$]-furfurylthiol and 2.07 µg [$^2H_2$]-3-mercapto-3-methylbutyl formate in 0.5 ml methanol was added.

After stirring for 3 h in the dark, the mixture was filtered through a Büchner funnel, the residue rinsed with dichloromethane (2 x 10 ml) and the combined organic extracts purified by thiol selective covalent chromatography using Affi-Gel 501 (Full and Schreier, 1994). The glass columns (10 x 0.5 cm), packed with 1 ml Affi-Gel 501 suspension, were conditioned with 30 ml 2-propanol, charged with the samples (~50 ml) and then rinsed with pentane/dichloromethane (2:1, v/v, 50 ml) to remove non-bound coffee material. The bound thiols were subsequently eluted with a freshly prepared solution of dithioerythritol (1.54 g/l) in pentane/dichloromethane (2:1). The eluate was dried over Na_2SO_4 and excess dithioerythritol was precipitated in a freezer. After filtration, the samples were concentrated to 2 ml using a Vigreux column (40 cm x 1 cm), and then to 0.3 ml using a gentle stream of nitrogen.

Method B): Ground coffee (10 g) was suspended in 50 ml water (90°C) for 3 min and then cooled in an ice bath. The cold coffee slurry was spiked with the labelled thiols as described in method A. After stirring for 1 hour cysteine (300 mg) was added, and the solution stirred for further 30 min. After decantation an aliquot (0.7 ml) was transferred to a 2 ml silanised crimp-top amber vial. Headspace sampling conditions using solid phase microextraction (SPME) with a PDMS/DVB-fibre (100 µm), have been reported previously (Roberts, 2000).

Method B was also used for the determination of medium to low volatile odorants in coffee, except that no cysteine was added. For the quantification of compounds 8-14 (Table 1), a series of labelled analogue compounds were added as internal standards at a concentration of 0.2 to 5 x their corresponding analytes (Blank et al., 1999).

Volatiles were separated on a DBWAX capillary column (J&W, 30 m x 0.32 mm; 0.25 µm using a GC system (HP 5970) connected to a mass spectrometer (HP 5971). Helium was used as carrier gas at a flow rate of 0.8 ml/min. The oven temperature was initially 35°C for 3 min, then raised by 4°C/min to 220°C and kept at 220°C for 10 min. The MS was operated in the SIM mode with 70 eV.

RESULTS AND DISCUSSION

The extraction yield of whole green beans of both varieties, arabica and robusta, with hot water was approximately ~17% under the conditions chosen: 3 extraction cycles with 5-fold excess of water at 90°C under stirring, and a total extraction time of 2 hours.

In order to gain more insight into the flavour potential of the water non-extractable material of green coffee, the green bean composition was determined before and after treatment, and possible correlations with the aroma profile after roasting were studied.

Sensory evaluation

The sensory profile of a brew obtained from untreated roasted arabica coffee was compared to that originating from the corresponding extracted green coffee. As outlined in Figure 1 A), roasting extracted green beans resulted in a significantly lower overall intensity and lower coffee flavour attributes. The average sensory score of the panel for coffee flavour in the extracted arabica was only 2.5 as compared to 5 for the reference. The least significant difference (LSD) for this attribute (at 5% confidence) was 1.02 units, thus indicating the high statistical significance for the difference in coffee flavour. Only the cereal-bready note increased in R&G from extracted green coffee. Although this was not found to be statistically significant at 5%, it was clearly perceived by most subjects. The differences in the aroma profile (nasal evaluation) were similar (data not shown). Furthermore the sensory results of the robusta coffee in Figure 1 B) showed the same trend. Besides a reduction in coffee aroma and an increase in cereal character, however, typical robusta notes like earthy-mouldy and woody were also lower after the water extraction.

As also shown in Figure 1, hot water green bean extraction and reincorporation of the extract onto the beans (Arabica + Aext.) does not change the overall flavour and aroma profiles of the corresponding brews as compared to the untreated arabica and robusta samples. The only flavour attribute that distinguishes the re-incorporated sample from the references is acidity, which may have been reduced due to the loss of volatile organic acids during the various drying and concentration steps before the roasting of re-incorporated beans.

The reincorporation efficiency is also reflected in the nearly unchanged green bean composition. This is shown by the concentration of total chlorogenic acids (FQAs, CQAs, di-CQAs) and caffeine in Figure 2. About 90-95% of the initial caffeine and CQAs was found after re-incorporation of the water extract on the extracted beans.

After hot water extraction, however, only approximately 10% caffeine was left in the beans. Chlorogenic acids were reduced to 40% of the initial value, indicating that they are not as readily extractable from whole beans as caffeine.

Chlorogenic acids are well known precursors of volatile phenolic compounds in coffee. Of particular interest is the thermal decomposition of ferulic acid that leads to guaiacols and their derivatives (Fiddler et al., 1967). This chemical pathway is in agreement with the results obtained in this study. As illustrated in Figure 3, a linear correlation between the content of FQAs in green coffee and individual guaiacol derivatives in roasted coffee was found, regardless of the bean variety.

The low amounts of guaiacols in roasted coffee, obtained from extracted green beans, is also shown in Table 1 (compounds 8-10). The data indicate that the extracted green coffee still formed guaiacols upon roasting, but in a proportion corresponding to the remaining ferulic acids. In all cases a higher concentration of guaiacols was found in robusta than in arabica, which is in agreement with literature data (Tressl et al., 1978).

As determined by using stable isotope dilution assays, the concentration of other key odorants in coffee were also reduced by water extraction (Table 1). Particularly the Strecker degradation aldehydes, 3-5, were reduced by more than 50%. This result correlates well with the strong reduction of free amino acids in the extracted green beans (results not shown).

The total amino acid profile of green coffee, however, did not change due to this treatment, since most proteins are not extractable under the conditions used here.

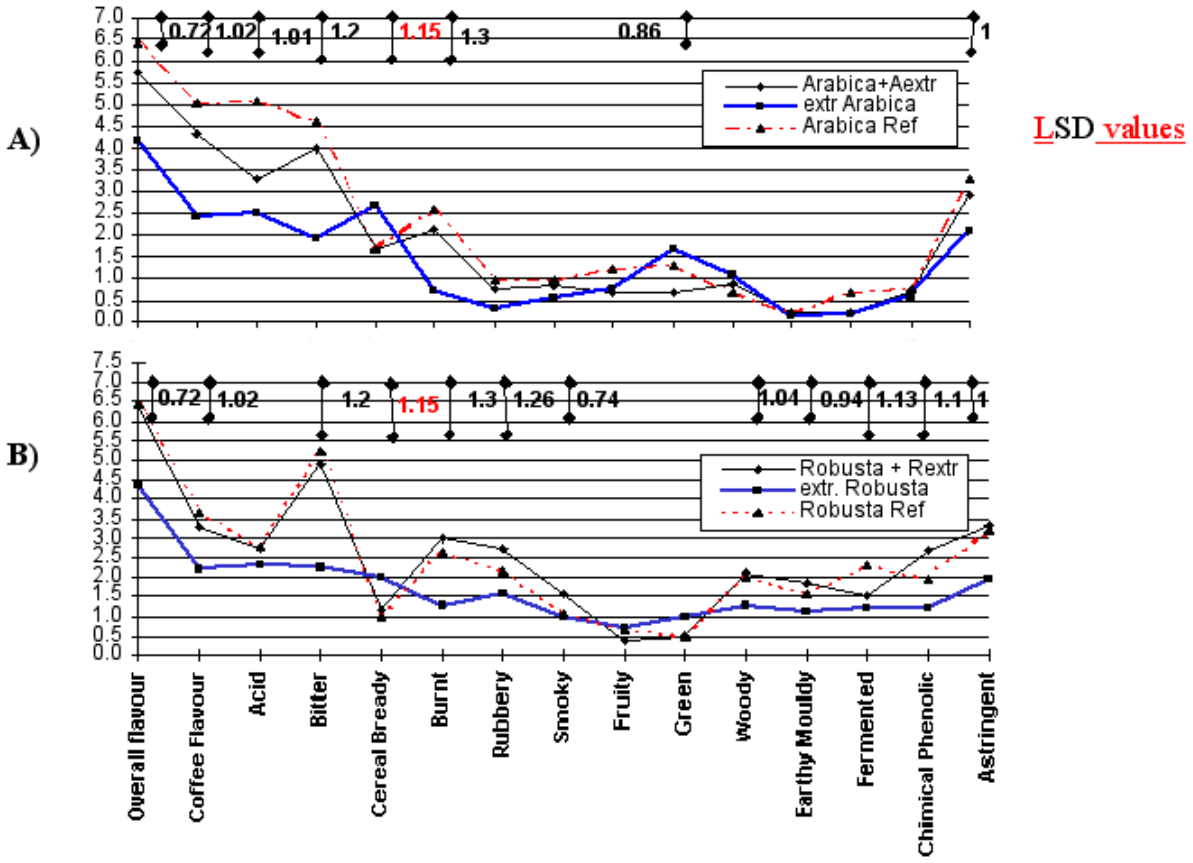

Figure 1. Sensory flavour profiles of A) arabica and B) robusta samples before and after water extraction and reincorporation. The LSD values indicate the Least Significant Difference between two intensity scores to be statistically different at 5%

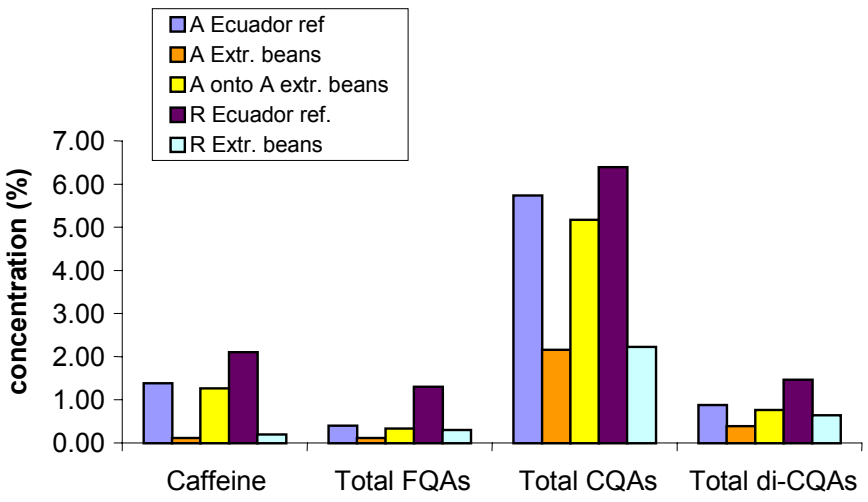

Figure 2. Profile of caffeine, total caffeoylquinic acids (CQAs), total feruloylquinic acids

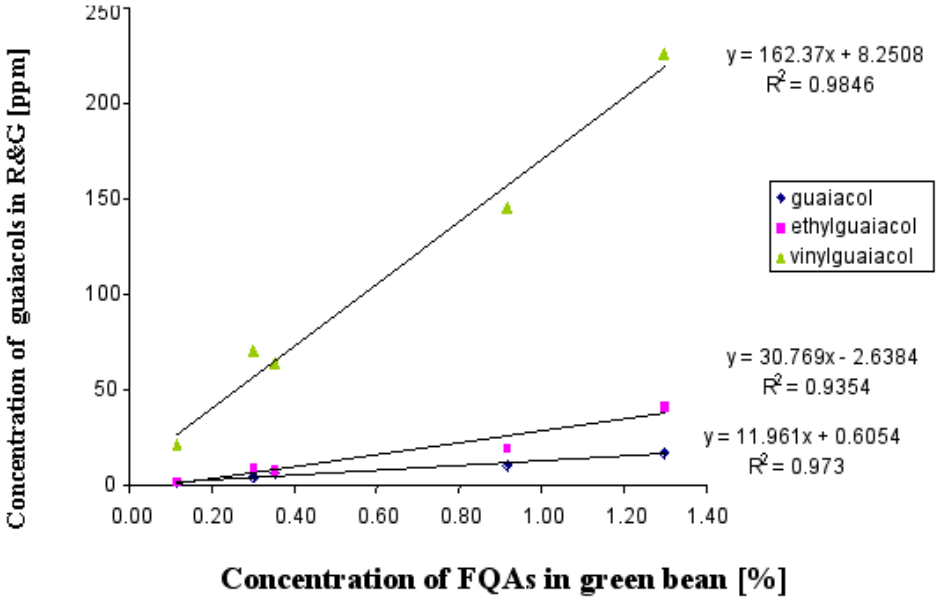

Figure 3. Correlation of feruloylquinic acid content in green bean samples with the concentration of individual guaiacols in the corresponding R&G coffees

Another group of potent odour active compounds in coffee are thiols. The precise determination of these compounds, however, is difficult due to the complexity of the coffee matrix, their low concentrations and their relative instability. In order to study the impact of green coffee extraction on the thiol formation, a simple and fast method was developed for their determination.

Under normal conditions furfurylthiol (FFT) was not detected in the headspace above a slurry of roast and ground coffee. In water containing FFT at a concentration as in these slurries, however, the compound was easily detectable. As recently shown (Hofmann et al., 2001), interactions of thiols with coffee melanoidins cause a suppression in the headspace.

We found that addition of cysteine liberates enough of these thiols in our samples to detect them in the headspace using SPME sampling. This approach was compared with that of solvent extraction combined with thiol selective enrichment, using an affinity column. As shown in Figure 4, similar FFT concentrations were found with both methods, after adding deuterated furfurylthiol as internal standard. Furthermore the amounts are within the typical range of these compounds reported in the literature (Nijssen et al., 1996) for arabica and robusta coffee. SPME sampling of a roast and ground coffee slurry after cysteine addition is, therefore, a reliable method for the quantification of thiols as long as isotopically labelled standards are used.

The quantification of two thiols, shown in Figure 5, FFT and 3-mercapto-3-methybutyl formate (3-MMBF) gave somehow surprising results. The FFT concentration doubles upon roasting of extracted beans. It is the only key compound that increases in this coffee sample. 3-MMBF, however, was generated in lower amounts. This could be explained if the main precursor for FFT is a water non-extractable green bean component while the precursor for 3-MMBF is a water soluble component. This is in agreement with the proposed precursors in the literature: based on model systems, FFT and 3-MMBF could be generated in arabinogalactane/cysteine (Parliment and Stahl, 1995) and isorprenylalkohol/cysteine (Holscher et al., 1992) systems, respectively. Cysteine as sulfur source is not the limiting

factor for the generation of volatile thiols in extracted green beans since the concentration of protein bound cysteine did not change significantly after extraction. Another possible reason for an increase in FFT in extracted beans could be the absence of reactions competing with its formation.

Table 1. Concentration of selected key odorants in R&G coffee as affected by green coffee treatment

	compound	concentration [ppm] in R&G			
		Arabica		Robusta	
		before	after extr.	before	after extr.
1	acetaldehyde	129.39	54.87	82.64	66.64
2	dimethylsulfide	1.87	1.02	2.12	1.44
3	methylpropanal	27.19	10.61	29.62	8.58
4	2-methylbutanal	36.78	17.83	47.23	15.52
5	3-methylbutanal	21.19	10.92	18.86	10.24
6	2,3-butanedione	41.20	18.33	33.01	13.89
7	2,3-pentandione	43.38	16.33	23.09	7.99
8	guaiacol	5.70	1.47	16.06	3.75
9	4-ethylguaiacol	2.39	0.59	14.21	2.83
10	4-vinylguaiacol	40.44	13.25	146.68	45.50
11	damascenon	0.24	0.07	0.38	0.06
12	2-ethyl-3,5-dimethylpyrazine	1.11	1.10	2.13	1.17
13	2,3-diethyl-5-methylpyrazine	0.36	0.18	0.77	0.22
14	furaneol	116.33	60.74	79.46	38.16

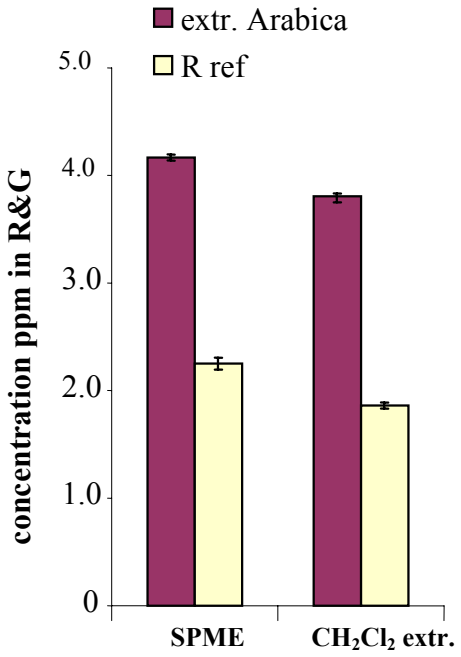

Figure 4. Comparison of FFT quantification via Headspace SPME sampling and direct solvent extraction

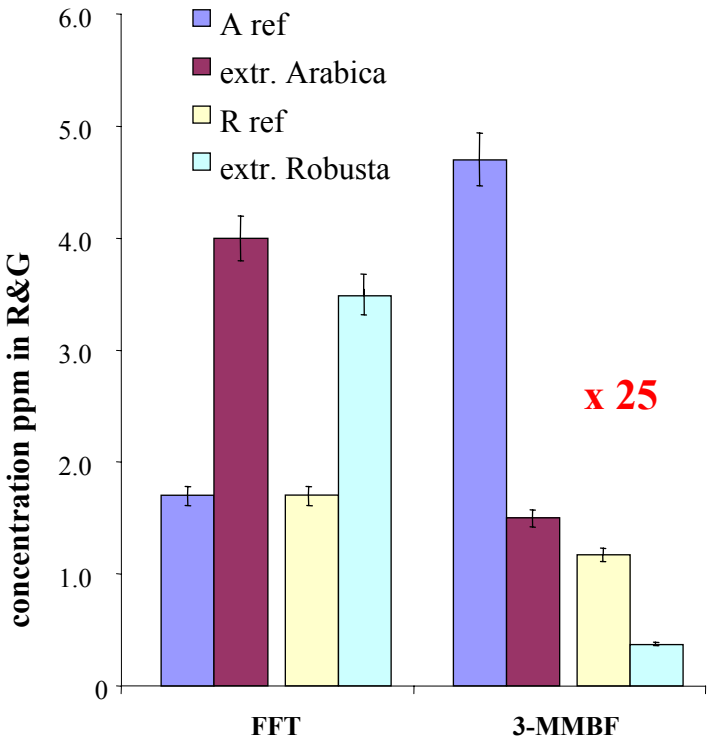

Figure 5. Concentrations of thiols in R&G as affected by the green coffee treatment. The amounts of 3-mercapto-3-methybutyl formate (3-MMBF) are multiplied by x 25

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Characterization of Free Amino Acid Enantiomers of Arabica and Robusta Coffee Varieties

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SUMMARY

This works represents an attempting to use the free amino acid enantiomers in coffee discrimination. For the purpose an extraction method was validated using extraction with sulphosalicylic acid followed by isolation by solid phase extraction on strong cation exchange columns. The amino acids were derivatised to their N-ethoxycarbonylethylesters and analysed by gas chromatography, with FID detection, on a Chirasil L-Val column.

Multivariate analysis applied to the chromatographic data show that free amino acids can be used as a tool for discrimination problems between coffee varieties. There is also some evidence that probably they can also be used for discriminating between different processing techniques, although more work has to be done in this respect.

INTRODUCTION

Efforts have been made to characterise the two most common coffee varieties using chemical data. Nevertheless, their chemical composition depends not only on the specie and variety in question but also on the degree of roasting and, to a lesser extent, on other factors such as agricultural practices, degree of maturation, storage conditions, geographical origin and green coffee processing.

The free amino acids present in green coffee are probably the single most important group of compounds in relation to the final flavour, and to a lesser extent the taste, of brewed coffee (Macrae, 1989). They represent only c.a. 5% of the nitrogen fraction and during roasting are degraded in a very high degree. Thus, only traces are found in the roasted products and subsequent brews.

Free amino acids in green coffee have been determined over the last decades by several methodologies but the results presented a wide range of values (Macrae, 1989; Clifford, 1987). In general, robusta samples contain higher levels of all the amino acids determined, with the exception of glutamic acid, which is 50% higher in the arabica samples. The presence of some particular free amino acids has been proposed as a method to differentiate coffee types (Macrae, 1989).

Few references have been reported on free amino acid levels in the bean, which depends largely on maturation (Illy and Viani, 1995). More recently, Ludwig and co-workers have studied the free amino acids in green coffees after the post-harvest treatment and storage (Arnold et al., 1994) (as in the previous works), but also in the freshly harvested beans and during a simulated post-harvest treatment (Ludwig and Arnold, 1996). Dry process promotes

appreciable changes in the amount of individual amino acids, especially glutamic acid, with a 50% increase, and aspartic acid with an irregular decrease. Fermentation during the wet process had no remarkable influence on the absolute amount as well as in the relative amounts of the free amino acids. Also storage after post-harvest treatment showed some variations but only during the first 3 weeks.

All these studies have determined the free amino acids without chiral discrimination. It is now well know that the L-amino acids (the "natural" form), either free or bounded as proteins, are subjected to racemisation to D-isomers under the influence of food processing conditions, namely heat and alkaline pH (Friedman, 1999). As the D-amino acids are also constituents of the microorganisms, their presence in fermented foods is inevitable.

In the present study the free D- and L-amino acids were analysed, in green and roasted *arabica* and *robusta* coffees, from different geographical origins, in a total of 60 samples. This work represents a contribution to the discrimination of the most representative coffee varieties and eventually to assess their green processing method.

MATERIALS AND METHODS

Chemicals

The D and L-amino acids, γ-amino-n-butyric acid (Gaba), L-pipecolic acid (PIP) and the internal standard L-p-chlorophenylalanine (IS) were all from Sigma (St. Luis, MO, USA). Ethylchloroformate (ECF), and pyridine (Py) were from Fluka (Neu-Ulm, Germany), 2,2,3,3,4,4,4-heptafluoro-1-butanol (HFB) and 5-sulphosalicylic acid dihydrate (SSA) were from Aldrich (Steinheim, Germany). All other chemicals were analytical grade from several suppliers.

The strong cation exchange columns used in the clean-up procedure were Extra-Sep-SCXD, 500 mg, 3 cc (Lida Manufacturing Corporation).

Coffee samples

Coffee samples from both *Coffea canephora* var. *robusta* and *Coffea arabica* were studied in a total of 60 samples, including green and the corresponding roasted beans. A local importer and roaster of coffee supplied all coffee samples and was able to confirm their botanical and geographical origin. A standard method was used in the roasting procedure in order to eliminate the variations due to this process.

Sample preparation

Extraction

Each powdered coffee sample was extracted with petroleum ether for 16 hour. A 1 g portion of dried defatted coffee was further extracted with 15 ml of 2% SSA solution, at room temperature, with magnetic stirring during 15 min. After centrifugation the coffee was further extracted twice with equal amounts of 2% SSA and the extracts collected, with the final volume adjusted to 50 ml.

Solid phase extraction

The packing bed was previously conditioned with 3 ml of methanol for about half an hour, and activated with methanol: 5 mM HCl (50:50) followed by 5 mM HCl, 10 ml each, at a flow rate near 2 ml/min. Then, 2.0 ml of coffee extract was loaded into the column, after being diluted to 35 ml with water, in order to reduce the ionic strength, and the pH adjusted to 2.2. The bed was washed with water and the amino acids eluted with 2 M aqueous ammonia (3 x 500 µl). The extract was dried under a N_2 stream and kept below 0ºC until derivatisation.

Derivatisation procedure

The dry residue was dissolved with 150 µl 0.1M HCl and transferred into a 1.5 ml silanised screw cap vial and the procedure followed as recently published (Casal, 2000). Briefly, 30 µl of HFB–Py mixture (2:1 v/v) was added followed by 7 µl ECF. After a brief shake, 100 µl of chloroform and NaCl were added and the vial thoroughly shaken for extraction of the derivatives into the organic layer. This phase was transferred into a 200 µl insert adjustable to the liquid sampler vials. About 1.0 µl was injected into the gas chromatographic system.

GC Analysis

Chiral discrimination of D,L-amino acids in green coffees was achieved by gas chromatography, carried out with a Chrompack CP 9001 instrument (Chrompack, Middelburg, The Netherlands) equipped with a flame ionisation detector (FID), and a automatic liquid sampler (CP-9050, Chrompack). The injector was kept at 250ºC and the detector at 280ºC. Helium as carrier gas was used at a initial inlet flow of 0.7 ml/min. Splitless injection was used with a 0.9 min purge time delay. The fused-silica capillary column (Chirasil-L-Val (25 m x 0.25 mm i.d.; Chrompack)) was used with the following temperature program: increase from 80ºC (1 min. hold) to 150ºC, at 5ºC/min.; after a 7 min. hold at 150ºC a further increase at a rate of 7ºC/min. up to 200ºC followed by a 15 min hold at 200ºC.

The GC was equipped with electronic pressure control allowing programmable gas flow during the chromatographic run. The helium was kept constant at 0.7 ml/min for the first 36 min. At this time the flow was increased to 1.7 ml/min. until the end of the chromatographic run. This increase in the carried flow rate towards the end of the chromatographic run avoided peak broadening of the last peaks, namely tryptophan, and shortened significantly the total run time.

The compounds were identified by their retention times and chromatographic comparisons with authentic standards. Quantification was based on the internal standard method using L-*p*-chlorophenylalanine.

Statistical Analysis

Free amino acid contents of green coffee beans were analysed by discriminant and cluster analysis. For discriminant analysis, according to relevant information, three groups were defined: robusta, arabica wet process and arabica dry process. Discriminant analyses were carried out following standard algorithms (Mardia et al., 1979) as implemented in Statistica for Windows package. Cluster analysis was also carried out, using Ward's method with Euclidian distances (Jacobson and Gunderson, 1986). In all cases, all data was previously standardised to zero mean and unit variance, in order to ensure equal opportunities for each variable (amino acid) to influence results.

RESULTS AND DISCUSSION

Extraction method

The extraction method was studied, based on published methodologies, in order to obtain the highest recoveries and cleaner chromatograms. Ethanol (Pereira and Pereira, 1973; Campos and Rodrigues, 1973), methanol/picric acid (Brückner and Hausch, 1989), sulphosalicylic acid (Arnold et al., 1994), and water/methanol (Palla et al., 1989; Oh et al., 1995). were tested, on green coffee, with and without previous fat extraction with petroleum ether. SSA at 2% with previous fat extraction provided the highest recoveries.

SPE extraction

The maximum SPE exchange capacity was assessed by loading several coffee extract amounts into the SCX column. It was observed that saturation was achieved with about 45 mg of defatted coffee. To avoid saturation, the loaded coffee quantity was fixed at 40 mg, corresponding to 1 g in the 50 ml total extract. The ionic strength was also tested by diluting the
2 ml-coffee extract with increasing water amounts. The best recoveries were achieved at 0.005 M corresponding to a dilution of the 2 ml extract with about 35 ml water.

Reproducibility of the method

Calibration curves were determined after subjecting standards to the same total procedure in order to compensate for the losses during protein precipitation, SPE clean up and derivatisation steps. A linear relationship was obtained with the correlation coefficients above 0.99 for all the compounds.

Precision (CV%) was calculated by five repeated extractions of the same green coffee sample. The accuracy (recovery %) was evaluated in triplicate using the same green coffee sample spiked with three known standard amounts. Overall recoveries were higher than 90%, with the exception of histidine, ornithine and lysine, probably due to the low amounts present, and also aspartic acid and pipecolic acid. These results, together with the detection and quantification limits are represented in Table 1.

The separation of proline enantiomers is not possible with this type of derivatives. Similarly, arginine, serine and threonine derivatives cannot be determined by this methodology.

Free amino acids in green and roasted coffee samples

Typical chromatograms obtained with a *Coffea arabica* sample from Brazil, green and roasted, are represented in Figures 1 and 2, respectively.

As it is easily observed in Figure 2, the roasted coffee samples presented only trace amounts of free amino acids, with no exceptions. Thus, the free amino acid determinations in roasted coffees proved to be useless, whether for discriminating varieties or assessing different roasting susceptibilities.

Beside coffee variety as a major source of variation in total and relative amounts of free amino acids, another possible source of variation is the processing type, mainly the wet and dry processes mentioned in the introduction. According to our material supplier (local

importer and roaster), samples were already labelled according to variety and processing type. Therefore, a cluster analysis was carried out in order to look for natural groupings.

Table 1. Extraction method reproducibility

	Precision CV%(n=5)	Recovery Mean %	Quantificati limit
Ala	7	94	0.4
Gly	11	92	0.4
Val	4	92	0.3
Pro	6	97	0.3
Pip	2	80	0.4
Ile	2	92	0.3
Leu	4	98	0.3
Gaba	9	90	7.9
Asp	17	75	2.1
Glu	27	106	3.1
Met	7	94	0.8
Fen	4	92	0.3
His	3	85	2.3
Orn	2	87	12.0
Lys	8	70	1.3
Tyr	4	101	0.4
Trp	6	97	0.8

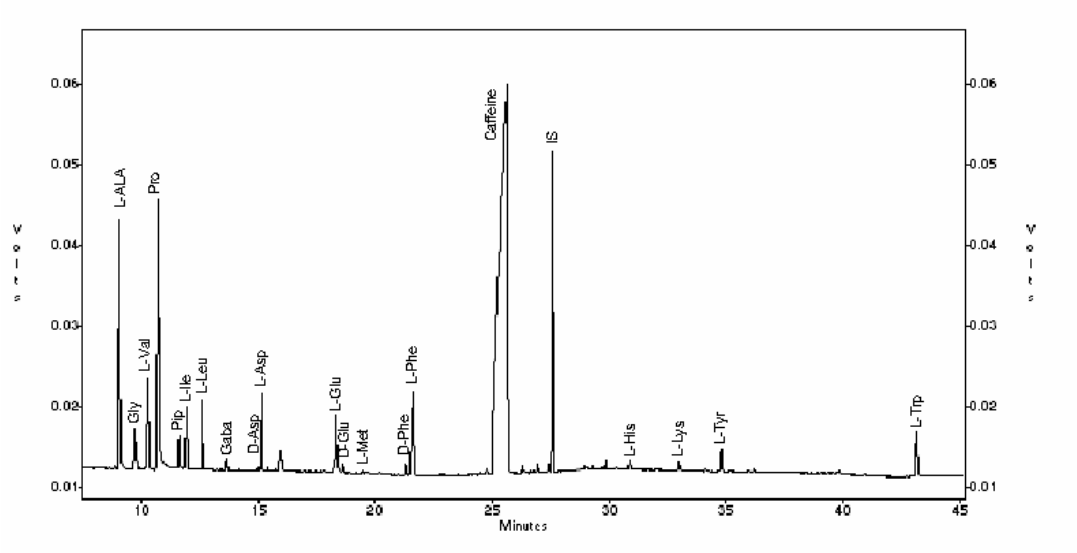

Figure 1. Free amino acids from a Brazilian arabica green coffee. (Chromatographic conditions as described in GC analysis)

The method providing the best results was Ward's method, and a dendrogram depicting the main features is shown in Figure 3. It is immediately obvious that there is a sharp distinction between robusta and arabica coffee beans, but there is no natural grouping in what concerns fermentation processes. This could mean that the process has no influence on relative amounts of amino acids, that the labelling according to process type was not reliable, or that the changes due to processing in the relative proportions of amino acids are only very secondary to other major variations.

In order to clarify this situation, several discriminant analyses were carried out, using the forward method, controlling the F to enter value, checking for the minimum number of amino acids necessary for total discrimination, i.e., with no misclassifications. Figure 4 shows the results obtained with the first four most discriminating amino acids. PIP and L-ASP exist in higher amounts in arabica beans and L-TRP in higher amounts in robusta beans. These three amino acids can therefore be used for variety discrimination. L-ALA appears as the main discriminator for process type, showing higher levels in arabicas processed by the dry method. In the following Table 2 summary results are shown for the analysis, for the significance of each discriminating dimension, and for each amino acid in the model. In Figure 4 all amino acids are affected by the respective pooled within group correlation values.

The most marked difference in the comparison between the chemical composition of washed and natural coffees lies in the content of soluble solids (Illy and Viani, 1995). Knowing that during the wet processing method a fermentation step is included, it would be somewhat natural to find higher D-amino acid contents. The results showed no differences, probably because during the dry processing method some fermentation can also occur in the drying stage. Nevertheless, further studies with more samples are recommended.

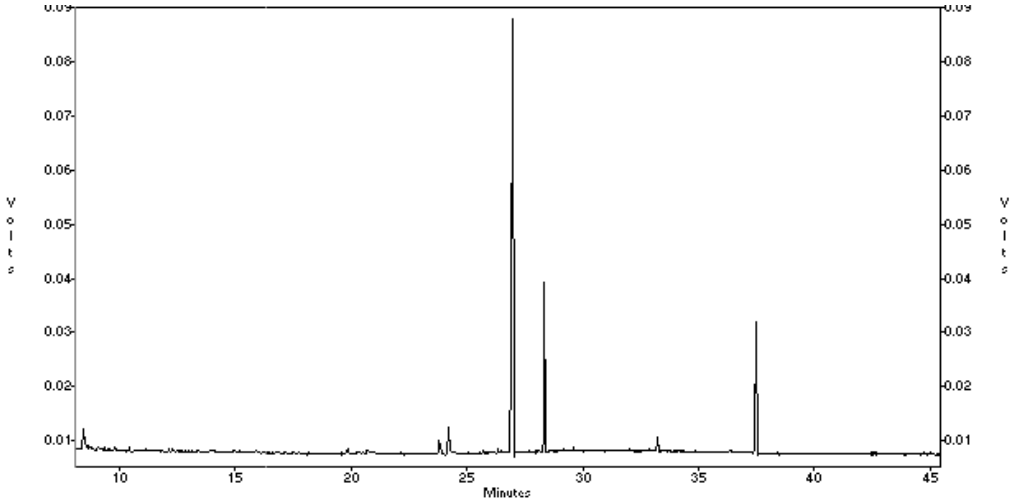

Figure 2. Free amino acids from a Brazilian arabica roasted coffee

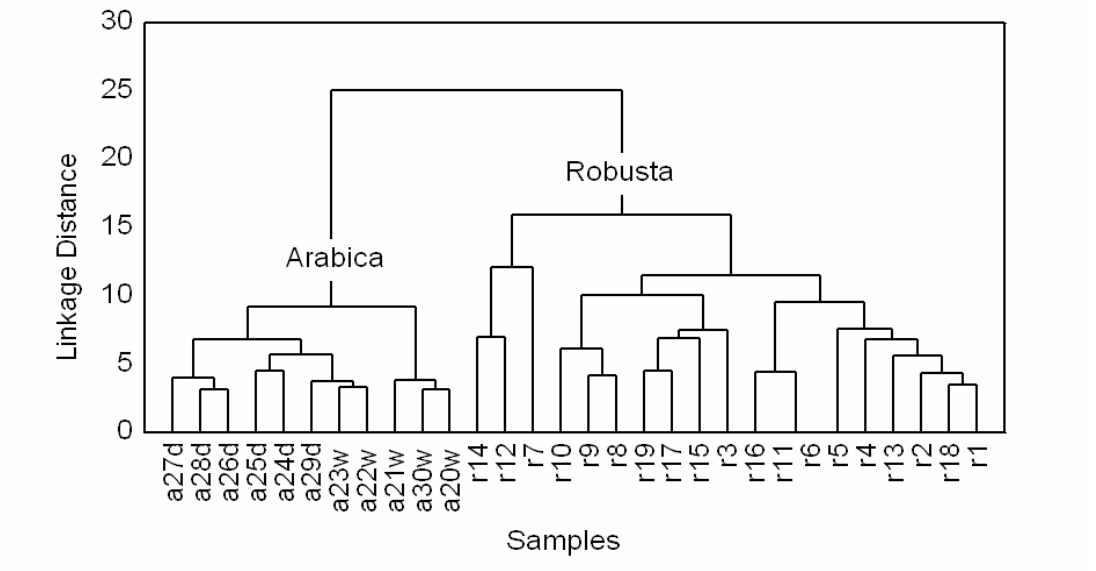

Figure 3. Cluster analysis. (a=arabica, r=robusta; d=dry process; w=wet process)

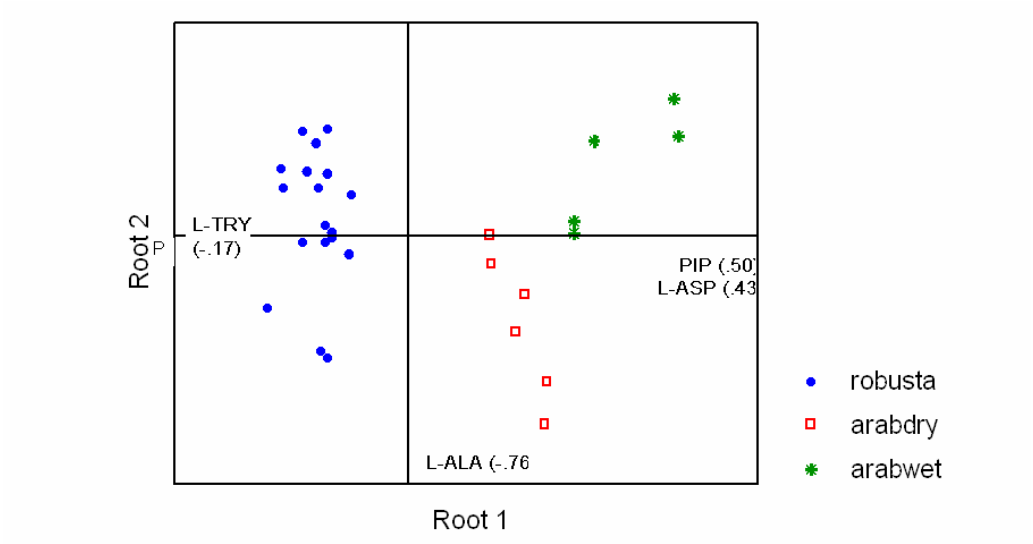

Figure 4. Discrimination of green coffee beans by the first four most discriminating amino acids

Table 2. Discriminant Function Analysis Summary
(Chi-Square Tests with Successive Roots Removed)

	Eigen- value	Canonical R	Wilks' Lambda	Chi-Sqr.	df	p-level
0	19.77889	.975640	.031671	88.03465	8	.000000
1	.51953	.584724	.658098	10.66924	3	.013666

Wilks' Lambda: .03167 approx. F (8,48)=27.715 p< .0000

	Wilks' Lambda	Partial Lambda	F-remove (2,24)	p-level	Toler.	1-Toler. (R-Sqr.)
PIP	.104620	.302729	27.63946	.000001	.611366	.388634
L-ALA	.050482	.627377	7.12726	.003718	.674584	.325416
L-ASP	.059708	.530436	10.62292	.000496	.841283	.158717
L-TRP	.053926	.587308	8.43220	.001684	.620532	.379468

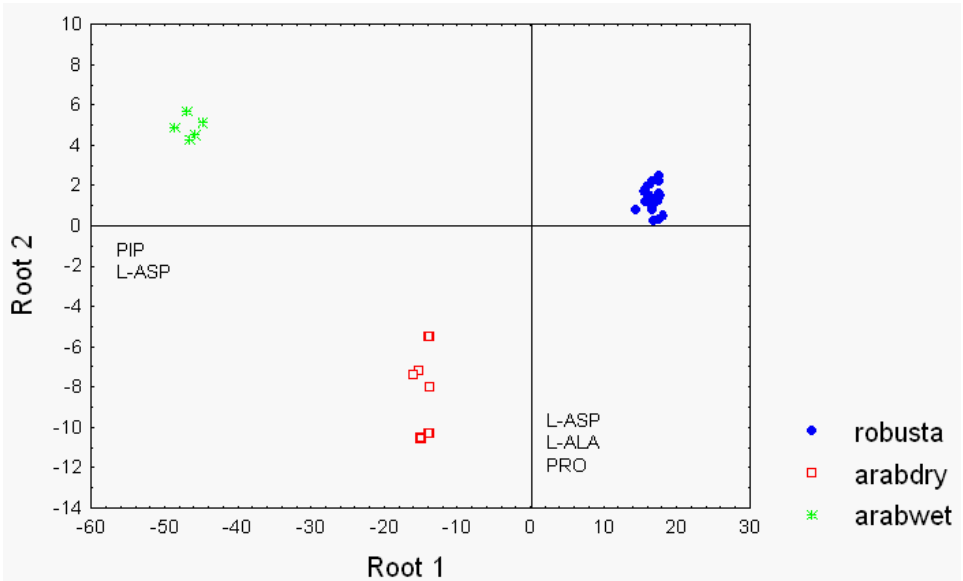

Figure 5. Discriminant analysis with all amino acids included

CONCLUSIONS

From the results obtained we can conclude that some free amino acids can be used in the discrimination of pure green arabica and robusta coffees.

Contrary to expectations, no significant variation in D-amino acid content was observed between the green processing methods. The differences in the free amino acids were very small, and all based on L-amino acids, especially L-Ala.

Chiral amino acid analyses in coffee as in other food matrices, although not yet routine procedures in food chemistry, might complement in the near future the established analytical methods used for food quality and food authentication control. In the coffee industry, however, the importance should be almost restricted to total amino acids determination, specially for roasting control.

ACKNOWLEDGMENT

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Physical Chemistry of Roasted and Ground Coffee: Shelf Life Improvement for Flexible Packaging

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SUMMARY

Traditionally coffee has been roasted, ground and then vacuum packaged in metal containers for the consumer. In the last ten years or so due to the rising cost of the whole bean and improved packaging materials, manufacturers of FRG coffee have been moving out of the can into plastic containers. This has led to a need for a better understanding of certain process steps as well as the interaction of the coffee with the environment inside a plastic container. Two key areas have been the focus of our research at the University of Minnesota.

The first has been related to the unusual nature of coffee roasting. In this process, due to both pyrolysis of cell materials and the rapid rate of one branch of the Maillard reaction, namely Strecker degradation, significant CO_2 is produced and trapped in the roasted bean. At the end of roasting and after quenching and cooling there can be up to 10 mL of CO_2 per gram in the bean. About 50% of this are lost upon grinding however the remaining CO_2 presents a problem in that with a flexible film package, the release of the gas after packaging can cause bursting of the package. Tempering the coffee for 24 to 48 hours helps to reduce CO_2 but about 10% of the shelf life are lost for each day of tempering. Our laboratory has focused on the physics of the release rate during tempering as well as after packaging. It is clear that the CO_2 is in two domains, one that represents CO_2 trapped in collapsed pockets and the other as sorbed CO_2 in equilibrium with specific sites as described by a Langmuir sorption isotherm. This work should lead to an alternative to the current practices of either vacuum packaging, use of a pressure relief valve or high CO_2 permeable films. Indeed, by flushing with the equilibrium CO_2 partial pressure and using a highly impermeable film, one can stabilize the package and prevent bursting much more economically.

Our second area of research focused on the analysis of the influence of moisture content, oxygen level and temperature on the shelf life of FRG coffee. The rate of oxidation as a function of oxygen pressure follows the typical hyperbolic function found for lipid oxidation. This correlated very well with sensory determination of end of shelf life using a new technique called Weibull Hazard Analysis. This sensory method describes the % of consumers who will be displeased as a function of storage time at given conditions. Our work showed that reducing the oxygen level to 0.5% increased shelf life by 20 fold. Water content as described by the corresponding water activity (a_w) also had a major effect, with about a 60% increase in reaction rate for ca. 1.5% increase in moisture content above the BET monolayer moisture content (about a 0.1 a_w increase). Surprisingly, temperature had the least influence, only about a 20% increase in rate for a 10ºC rise in temperature. Thus coffee at room temperature with an a_w of 0.11 has 20 weeks high quality life at 4% oxygen, while at 0.4 a_w one needs to reduce the oxygen level to <0.5% to achieve the same shelf life. In air at

0.1 a_w, the shelf life is 7 weeks while at 0.4 a_w the shelf life is 10 days, thus moisture control by quenching and choosing a low WVTR film is critical to shelf life. An Excel spreadsheet has been developed that predicts shelf life in distribution account for temperature fluctuations as well as oxygen and moisture transfer through the flexible film used for packaging.

INTRODUCTION

Coffee ranks as the second most widely traded commodity in the world in terms of dollars, only after petroleum (Pratt, 2000). The manufacturing of fresh-roasted & ground coffee (**FRG**) includes: (1) roasting for development of characteristic flavor, color and aroma; (2) quench cooling with water to stop the roasting action; (3) grinding to enable extraction of soluble solids and flavor volatiles when brewed; (4) degassing (tempering) for removal of carbon dioxide produced during roasting; and (5) packaging to prolong shelf life by preventing oxidation and loss of volatiles. Coffee beans are roasted to develop the characteristic flavors, colors, and aroma. Roasting is normally carried out at atmospheric pressure using hot air at temperatures above 200°C (Clarke and Macrae, 1987). They showed that the exit flu gas was almost 87% CO_2 and 7.3% carbon monoxide, indicating nearly complete combustion.

Carbon dioxide is the most important non - aromatic volatile found in fresh roasted ground (**FRG**) coffee. It is generated by pyrolysis of sugars and the Strecker degradation reaction (Hodge, 1953). The amount is dependent on the degree of roast, and can be up to 10 mL (NPT) of carbon dioxide per gram of coffee, although 2-5 mL (NPT) is usually reported (Barbera, 1967; Clarke and Macrae, 1987; Massini et al., 1990; Meister and Puhlmann, 1990). The amount increases with increasing roast temperature as shown in Figure 1 while the maximum amount in the coffee can be about 10 mL/g (~5 mg/g) or 0.5% (w/w) after roasting as Sivetz and Desrosier (1979) reported.

Directly after roasting the coffee bean which has now been partially pyrolyzed and is expanding in structure at the high temperature is now quench cooled with water. This can lead to both steam stripping of volatiles as well as entrapment of flavors and carbon dioxide in collapsed regions as the bean structure contracts under cooling and forms pockets that hold in the flavors. During the next step, coffee grinding, a significant amount of the carbon dioxide produced during roasting can be released. This loss is most likely due to disruption of the regions in which the gas is entrapped, as well as decreasing the path length for diffusion out of the bean. Heiss et al. (1977) reported that 45% of the carbon dioxide trapped after roasting is released during the first 5 min after grinding. Barbera (1967) demonstrated that over 70% of the carbon dioxide was released following coffee grinding into 500 μm particles (sieve size No. 35). Cartwright and Snell (1974) measured a release of 1.21 mL CO_2 and 0.002 mL O_2 (SPT) per gram of coffee during the first hour after grinding. However almost 50% of the CO_2 is retained in the coffee after grinding at the time of packaging. If the intention is to use flexible packaging, this leads to problems as the carbon dioxide can be slowly released during storage leading to loss of package integrity as the package swells and bursts.

Thus one of the major problems in the fresh roasted ground (**FRG**) coffee industry is the eventual evolution of carbon dioxide during storage after packaging, which can cause the bursting of the package especially for flexible packaging. Because of this many methods have been developed to resolve the problem including various conditioning (tempering) steps prior to packaging to allow release of the carbon dioxide as well as vacuum packaging. Such methods include gas flushing with excess carbon dioxide (over pressurization) to create an equilibrium situation in which no gas evolves from the entrapped areas (Illy, 1997), or use of films or partial film patches highly permeable to carbon dioxide to allow it to diffuse out and

packages with pressure relief valves of different designs that release the built up pressure (Radtke, 1973; Heiss et al., 1977). It should be noted that due to the extreme sensitivity of FRG coffee to oxygen, any holding time in the presence of air during tempering prior to packaging is extremely detrimental (Heiss et al., 1977; Cardelli and Labuza 2001, Cardelli, 1997). Thus there is a need to quickly desorb the CO_2 and then remove any residual oxygen from the package. In order to do so, there is a need to establish the parameters of carbon dioxide sorption equilibrium conditions after grinding (i.e. where is the CO_2 and how is it bound), as well as the desorption kinetics from the ground roasted bean so as to provide good control over carbon dioxide release and final concentration in the package.

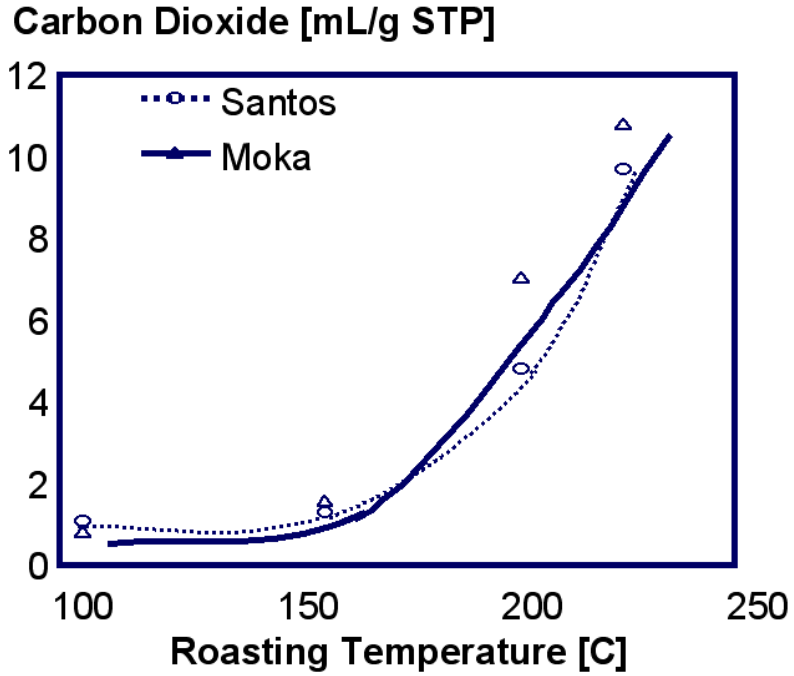

Figure 1. Effect of roasting temperature on Carbon Dioxide production mL/g coffee (Barbera, 1967)

Several physical changes take place in the coffee bean during roasting as noted. Moisture is lost and the color is darkened. Beans expand in size due to the internal build-up of gases, which along with the high temperatures allow for the formation of internal pores and pockets. Cardelli-Freire (1997) measured the glass transition temperature of coffee and found that it ranges from 130 to 170°C as a function of moisture content as seen in Figure 2. At the typical roasting temperatures, the coffee bean is raised above its glass transition temperature (T_g) and becomes rubbery and elastic. This along with the increase in gas pressure causes the structure to expand. In general when the bean goes at least 50°C above the T_g during roasting, collapse of the internal pore structure can then occur, trapping some of the gas. This is further enhanced when the bean is quickly quench-cooled with water. If the pore surface collapses enough, the structure may either prevent entirely or lead to a very slow release of carbon dioxide out of the roasted ground coffee during tempering or storage and distribution.

The pathway the bean takes during roasting, starting at 10-12% moisture while green and ending at 2-4% moisture after roasting is shown in Figure 2, known as the state diagram. After reaching maximum temperature and held to produce the desired color and flavor, then the beans are then cooled back down to room temperature in air or usually by quenching with cold water. The coffee structure then reaches a point at room temperature that is well below the glass transition temperature making the bean brittle and easy to grind. This process easily breaks up some of the pockets within which CO_2 is trapped. This accounts for the up to 50%

loss immediately during grinding. As noted before, if we cannot get rid of all the CO_2 in a reasonable time, a second approach is to pressurize the package. Illy (1997) recently reported on the use of this technique for espresso coffee based on nitrogen and carbon dioxide pressurization. This helps to maintain CO_2 inside the coffee cells, avoiding cell wall damage, package ballooning and as suggested, flavor release, but no data were presented. It is obvious that if the CO_2 pressure in the headspace is increased to that of the equilibrium value for the sorbed gas plus accounting for any CO_2 that would dissolve in the oil and water phases which becomes more significant as pressure increases, then release should be zero (i.e. the definition of equilibrium).

The weight of dry mass of the bean also decreases due to the pyrolysis reactions during roasting, which results in a decreased bean solids density and thus an increased porosity which should increase the ability of CO_2 to diffuse out during tempering. Massini, Nicoli, Cassara and Lerici (1990) reported that the structural changes of the coffee bean during roasting such as the increase in number and size of internal cavities, the dilation in size, and the decrease in weight and density, are all closely related to the amount of CO_2 released. The pore structure formed during roasting thus has a major effect on the mass transfer phenomena during tempering and during storage in the package as well as setting up the gas adsorption capacity of the roasted coffee bean (Radtke and Heiss, 1975; Saleeb, 1975). The pore networks in roasted coffee bean consist of both macropores (evacuated cells) and micropores. Different researchers have studied the macropores using light microscopy and scanning electron microscopy (SEM), showing they range from 20 to 40 μm in diameter (Schenker et al., 2000). Measuring the size of the micropores is more difficult. Saleeb (1975) measured the uptake of CO_2 at dry ice temperatures and used the Kelvin equation to determine pore size distributions. He found that the average pore size that held the majority of the CO_2 was between 17 and 33 μm and theorized that they took an inkbottle shape because of hysteresis. It should be pointed out that at normal storage conditions, even with small pores, carbon dioxide can only exist as a bound molecule or as a gas in the bean since CO_2 cannot exist as a liquid until one reaches very high pressure or the vapor pressure is decreased significantly in very small pores approaching molecular sizes. Hinman (1993) also concluded that high-energy interactions observed from the isotherm measurements could only result from the presence of pores with dimensions a small multiple of the CO_2 molecule's diameter. The results however put into question of whether there are high interaction energies or whether the gas is entrapped in collapsed pores. In either case this means that some CO_2 will be difficult to remove in tempering but it might be slowly released in packaging. Schenker et al. (2000) measured pore sizes of roasted coffee using a mercury porosimeter and found the majority of the pores ranged in diameter from 10 to 50 nm which seems to be very small but not enough to form a liquid filled capillary. The researchers also found that the pore diameters were larger for high temperature roasts than for low temperature roasts.

Coffee grinding increases the rate of CO_2 degassing due to the disruption of pore structure as mentioned above, as well as the increased surface-to-volume ratio and decreased distance that the molecules have to diffuse out of the ground bean. Typically for diffusion, if you decrease the particle size by 50%, it takes 1/4 the time to reach the same level of diffusant left in the particle (the r^2 principle). However, the reduction in particle size also allows ground coffee to be more susceptible to staling caused by oxidation as compared to whole beans, because of the larger surface-to-volume ratio and smaller diameter through which oxygen can diffuse. This is a major problem since oxygen is the main environmental factor responsible for FRG coffee deterioration as will be described later. Indeed it is the onset of sensory staleness that is used to determine the end of shelf life of coffee (Heiss et al., 1977). Although many authors reported studies on shelf life of FRG coffee and oxygen uptake (Ernst, 1979; Cros et al., 1980; Spadone and Liardon, 1989; Mori, 1985; Balasubramanyam et al., 1989; Hinman,

1991; Radtke, 1979; Radtke and Piringer, 1981; Clinton, 1980), the reported data is not sufficient to build models for shelf life prediction as a function of % O_2, water activity (a_w), and temperature.

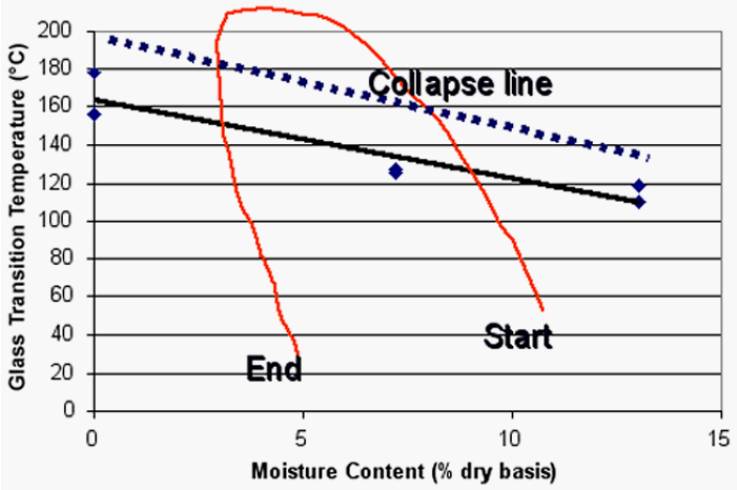

Figure 2. State diagram for coffee roasting

From this review it is obvious that the physical characteristics of FRG coffee with respect to carbon dioxide release as well as shelf life are affected by the processing conditions. We therefore conducted a series of studies to establish the physico-chemical parameters related to CO_2 sorption/desorption equilibria, CO_2 diffusion kinetics, and measured the shelf life of FRG coffee (Anderson et al., 2001; Shimoni and Labuza, 2000; Cardelli and Labuza, 2001; Cardelli, 1997). Our main goal was to apply physical chemistry principles to try to understand the parameters needed to optimize tempering as well as maximize shelf life during storage while maintaining package integrity.

CHARACTERIZATION OF CO_2 SORPTION IN FRG COFFEE

Literature evaluation

During tempering and after packaging, CO_2 reaches a meta-stable state within the coffee in the package, and it may result in an undesired pressure in the final packed product (e.g. too high – burst, too low – shrinkage). From a physical chemical standpoint, the amount of CO_2 held at equilibrium in the fresh roasted ground state will be a function of both solubility of the gas in the oil, solubility in the low moisture content (1 to 5% water), and adsorption to non-polar sites. Since carbon dioxide is linear, **O=C=O**, it is very non-polar (Dielectric constant = 0) so adsorption should be low, however it may also bind to polar sites through hydrogen bonding. These all are equilibrium processes but there can be an additional amount sorbed which is the amount entrapped in amorphous collapsed zones in the particle which is a non - equilibrium process. If for example, a particle of 1 gram size has 4 mL entrapped in it by a non-equillibrium process and its porosity was ~0.65 to 0.74, the internal pressure in the entrapped zone would be 5.5 to 6 atm if the wall were impermeable or very low permeability. If there were no adsorption or solubilization at all, then one would need an over-pressure of at least 6 atm to prevent gas release during storage and transport. This high pressure would make handling the package problematical. Thus there is a need to establish just how much CO_2 is involved in equilibrium processes of adsortion and solubility vs how much is in entrapped regions.

As to equilibrium solubility, two pieces of data exist to at least give a measure for this. For gasses dissolved in liquids such as oil or water, Henry's law applies. Henrys law (equation 1) states:

$$m = Sp \quad (1)$$

m - amount dissolved eg mg per g

S - solubiliy coefficient (amt / g atm)

p - external gas pressure

The solubility value "S" for carbon dioxide in water at 20°C is 3.45×10^{-5} mole/g×atm (Lide and Frederikse, 1995) which is 1.52 mg/g coffee atm. Using the ideal gas law, we can calculate the solubility in water at 20°C as 1.52 mg CO_2/g water atm or about 0.7 mL per g water atm. Therefore, for FRG with a water content of 4% (wb), the amount of carbon dioxide dissolved in the water will be about 2.13 x 10^{-5} mg/g coffee in air at 1 atm with a normal CO_2 concentration of 0.035% (0.00035 atm). This suggests that the amount associated with water is very small. However if all the moisture were associated with the physical structure for the entrapped gas at 5 atm pressure, this would amount to 0.3 mg/g coffee. Thus the physical distribution of the structure becomes very important.

Carbon dioxide is also soluble in the coffee oil, which is about 10 to 16% of the solids weight (Sivetz and Desrosier, 1979) after roasting. Unfortunately, no data exists for the actual solubility in coffee oil as a function of CO_2 pressure. However, coffee oil is very similar in its composition to other vegetable oils. Tomoto and Kusano (1967) determined the solubility of carbon dioxide in vegetable oils was 1.018 mL CO_2 (NTP) per mL oil (at 30°C, 1 atm CO_2). Assuming that the oil content in FRG coffee is ~15%, then the CO_2 fraction adsorbed to the oil in FRG coffee in air at the normal CO_2 pressure (0.035% CO_2) would be only 6.9 x 10-5 mL/g coffee (~15 x 10^{-5} mg/g). This fraction is also a minor portion of the total CO_2 adsorbed in FRG coffee. If all the oil were associated with the surface of the entrapped gas at 5 atm, this would amount to 0.76 mL/g FRG which is possible. Thus about 1 mL of carbon dioxide could be dissolved in the oil and water if they were totally associated with the entrapped gas whereas if not, than the amount would be minor. The oil and water, independent of where they are located would be a sink for the CO_2 if gas over-pressurization was used.

This above exercise shows that carbon dioxide can exist in the FRG entrapped in pores, dissolved in the coffee oils and moisture and possibly adsorbed in equilibrium with active sites. The goal of this first part of the study was to determine the physico-chemical parameters of this latter equilibrium carbon dioxide retention in FRG coffee as affected by roast degree, roasting time, and temperature.

Experimental procedure

Coffee (Colombian Arabica, Togo Robusta and Kenyan Arabica) were roasted using a bench-top Neuhaus fluidized-bed sample roaster RFB-L (Neuhaus-Neotec, Germany), equipped with air-flow, temperature and time control. Roast degree was determined by color measurement with a Color/Difference Meter D25-2 (Hunterlab, Virginia, US). Coffee was ground by a Super Jolly coffee grinder (Mazzer Luigi s.r.l. Venezia, Italy). A light, medium, and dark roast using both high-temperature/short-time, and a low-temperature/long-time roast was used to evaluate roasting effects.

Sorption/desorption equilibrium was determined using an apparatus designed according to Choudhary and Mayadevi (1996) with some modifications (Figure 3). The sorption unit

consisted of a sample container connected by a Swagelok double-end shut-off quick-connect system. The connection through quick connection permits the easy attachment/detachment of the sorbing sample container to the unit and the cell is automatically sealed-off when detached from the unit. The apparatus was kept in an insulated box with temperature control, and the temperature was maintained at desired values (±0.5°C). Valve M1 and M2 were opened and the entire system up to valve M3 was evacuated. When vacuum was achieved, the system was flushed with the desired gas mixture, by opening valve M3. This procedure was repeated 3 times. Then, a coffee sample was weighed into the sorption container (to 0.01 g), and connected to the apparatus. The system was flushed again by the gas mixture releasing the excess of gas flow through valve M1. Verification of the gas initial concentration in the system was done by measuring CO_2 and O_2 concentration in the excess flow using a '6600 Headspace oxygen/carbon dioxide analyzer' (Illinois Instruments Inc., 27840 Concrete Drive, Ingelside, Illinois U.S.A. 60041). Following equilibration for three weeks, carbon dioxide content in the coffee was analyzed by the Ascarite method and plotted as a function of the measured equilibrium carbon dioxide pressure in the reservoir.

Results and discussion

The Langmuir sorption isotherm (equation 2) best explains the adsorption of non-polar gases at pressures where there is no capillary condensation, which would be the case for carbon dioxide and coffee as noted earlier. The equation takes a hyperbolic form with a maximum adsorption amount called the monolayer or m_o.

$$\frac{m}{m_0} = \frac{bP}{1+bP} \qquad (2)$$

m - adsorbed CO_2 (mg/g)

m_0 - monolayer

b - the affinity of the gas to the solid surface

P - carbon dioxide pressure (atm)

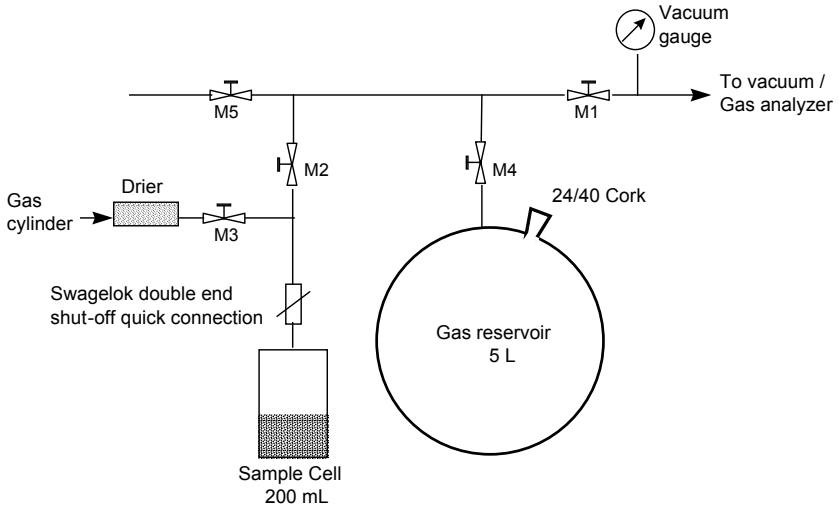

Figure 3. Schematic diagram of apparatus for sorption/desorption measurements

Figure 4 shows a typical carbon dioxide sorption isotherm with the hyperbolic shape for isotherms done at three temperatures. The plot includes the upper and lower 95% confidence

limits. As seen, above about 0.4 atm, the amount of CO_2 adsorbed flattens out at about 1.4 mg/g FRG (~0.7 mL/g), which is typical of a Langmuir isotherm. In comparison, the moisture adsorption isotherm shows a monolayer of about 35 mg water/g FRG coffee, almost 52 times greater adsorption on a molecule adsorbed per gram coffee basis. This should be expected since water is very polar (Dielectric constant ~73) and the coffee surface contains many polar binding sites. From these sorption results, one can then calculate that at 1 atm CO_2, of the 0.7 mL, there would be about 0.2 mL/g that would be solubilized in the water and oils, thus about 0.5 mL (or 0.25 mg/g) could be associated with the adsorption sites.

It should be noted that the curve is drawn through zero at a zero CO_2 level, but in fact for all experiments for different roasting times and temperatures, there was some residual amount left after the three weeks equilibrium, ranging from 0.3 to over 1 mg/g FRG coffee but there was little correlation to coffee type, roast degree or grind. This is most likely the amount entrapped in the collapsed pores, since the solubility in the low % CO_2 atmosphere should be very low. Isotherms done at 50°C showed similar flat curves. Of key here is the fact that the amount adsorbed does not seem to be a very strong function of temperature if we eliminate the effects of grind size, roast degree and moisture content. This again supports a Langmuir type adsorption process and also suggests that the CO_2 involved with this process is strongly adsorbed since raising the temperature has a small effect on amount held and in fact may increase it as new sites may be exposed.

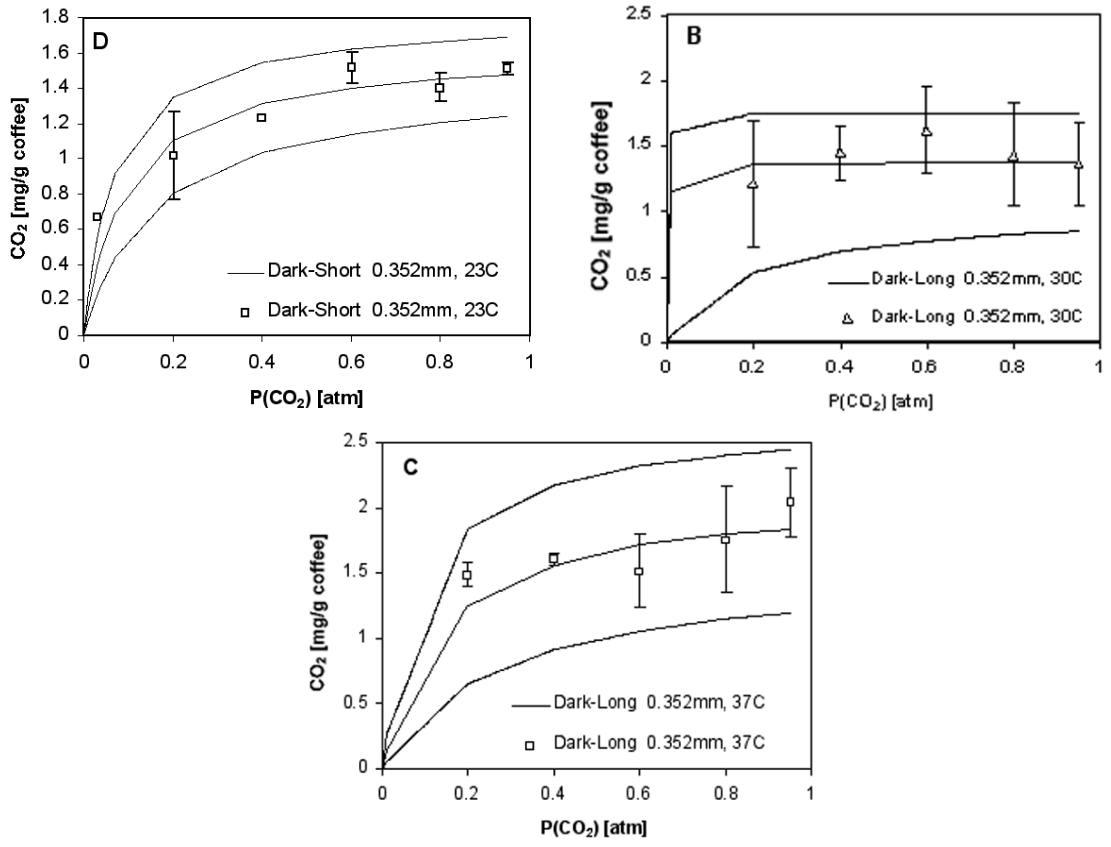

Figure 4. CO_2 Sorption isotherm for Dark- roasted coffee held at 23, 30 and 37°C

In order to verify the validity of the Langmuir isotherm it can be rearranged into a linear form to test its applicability (Eq. 3).

$$\frac{P}{m} = \frac{1}{m_0 b} + \frac{P}{m_0} \tag{3}$$

Thus the slope of the Langmuir plot (P/m vs. P) is $1/m_0$, and the intercept is $1/b\ m_0$. Figure 5 shows an example Langmuir isotherm for CO_2 adsorption. It should be noted that the fit was not good in all cases, most likely due to the complications of solubility and entrapment.

Table 1 shows the influence of some roasting conditions for a Colombian coffee. As seen, there is no general pattern except that at 23°C, the monolayer values increase when the roast is darker ($p<0.041$). The increase in m_0 values due to a darker roast could be due to the significantly different surface as demonstrated by the SEM images and supported by porosity measurements (Shimoni and Labuza, 2000). The m_o values ranged from 1.09 to 2.21 mg/g FRG coffee.

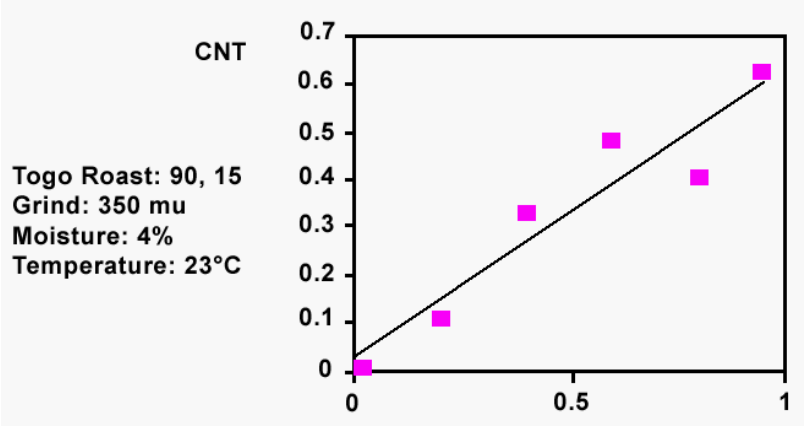

Figure 5. Langmuir plot at 23 C for Togo Roast @ 4% moisture CO_2 monolayer 1.6 mg/g FRG and $R^2 = 0.9$

As demonstrated by the results of this work, the physical sorption of carbon dioxide to FRG coffee is very likely in a sorption/desorption equilibrium to surface sites in addition to the absorption in water and oil and entrapment. Hinman (1993) reported linear CO_2 sorption isotherms for FRG coffee and extrapolated the line to 0 partial pressure which gave 0.2 mL/g absorption at room temperature. Saleeb (1975) obtained CO_2 sorption/desorption isotherms at –78.3°C and found that roughly 0.5 mL/g was retained in the coffee, however the use of this temperature complicates matters since it allows liquid condensation in capillaries. In addition, the extrapolation of particle-size/adsorbed CO_2 made by Barbera (1967) suggests that coffee ground to a particle size tending to zero still contains 8% of the whole bean original gas. An observation made by Hinman (1993) suggests that the amount of non-equilibrium CO_2 in FRG coffee decreases with an increase in temperature. Possible explanations of this phenomena may be that these collapsed structures are opened due to changes in the viscosity of the oil or to a state change of the carbohydrate polymers, although the latter is unlikely since at 4% moisture the coffee is far down in the gassy state, so typical temperatures in tempering would not support this. There is no data that can support the oil viscosity theory.

These sorption studies suggest that some amount of CO_2 is retained in the coffee regardless of the external CO_2 pressure which is critical to both tempering and storage. This CO_2 retained in the coffee is very likely the amount of carbon dioxide entrapped in the structure. This amount of carbon dioxide is very likely to be eventually desorbed from the coffee, however at a very low rate and most likely would not occur during the 24-48 hours of tempering, rather it will evolve in the package during transportation and distribution of the finished package.

There has been no definitive study to measure the rate of desorption of this CO_2 and thus the reason for the second part of this study, the kinetics of loss in tempering.

On a final note, using the data we collected, one can make an estimate of the effect of pressurization. For example, using a plastic pouch containing one pound of coffee (454 grams) which is gassed to 5 atm CO_2 pressure, this gas is initially spread in about 336 mL of pore space, assuming no package head space, only internal and interstitial pore space. Based on the Langmuir isotherms, coffee would absorb about 0.76 mL/g, which is 345 mL. This means that in a short time the pore gas pressure decreases as the carbon dioxide is adsorbed. However this is more complicated since some of the entrapped CO_2 uses up pore space and some of it is accounted for as part of the 0.76 mL/g. Thus the pressure probably is above one atmosphere after a few weeks but will continuously rise because of the pressure driving force creating a problem unless the package has good seals. The thrust then should be to maximize the amount of CO_2 that can be driven out during tempering.

Table 1. Langmuir isotherm parameters for different roast, grinding and temperatures of Colombian Arabica coffee

Roast	Particle size [D(4,3) μm]	Temp. [°C]	m_0 [mg/g]	b [1/atm]	P^a	r^2
Dark-Short	352	23	1.63	10.50	<0.0001	0.972
Dark-Short	352	30	1.05	550.43	<0.0001	0.894
Dark-Short	567	23	2.21	4.37	0.0001	0.783
Dark-Long	351	23	1.79	42.10	<0.0001	0.815
Dark-Long	351	30	1.38	504.29	0.0003	0.775
Dark-Long	351	37	2.11	7.24	0.0007	0.870
Medium-Short	360	23	1.24	37.56	<0.0038	0.861
Medium-Long	356	23	1.19	11.72	<0.0001	0.833
Light-Short	357	23	1.09	9.06	<0.0001	0.734

[a] *Significance of the Langmuir model as a model for the sorption isotherm*

KINETICS OF CO_2 DEGASSING IN FRG COFFEE

Literature evaluation

Carbon dioxide diffuses out of ground coffee faster than from whole beans. Illy and Viani (1995) reported that it took up to 1000 hours (42 days) to be released from whole beans as compared to 15 days from ground coffee. This is a smaller difference than that expected based solely on particle size (time proportional to particle size squared) indicating as before, that the gas in entrapped in small collapsed pores that have a low permeability. The greater rate is due in part to the disruption of structure during both roasting and grinding, as well as the increased surface-to-volume ratio and decreased distance that the molecules have to diffuse through. Heiss and Radke (1977) reported that 45% of the CO_2 held after roasting was released within the first five minutes after grinding. Barbera (1967) found that the amount of carbon dioxide released doubled when the particle size was reduced from 1000 to 500 μm. Unfortunately, there has been very little published on the mechanism and kinetics of carbon

dioxide release from coffee that would be useful in the design of the tempering process. Most studies (Heiss and Radke, 1977; Radke and Heiss, 1975; etc.) have measured the release of carbon dioxide in closed packages, so the gas phase CO_2 concentration increases over time reducing the driving force for release. Clarke and Macrae (1987) discussed the likelihood of two mechanisms being involved; (1) diffusion flow due to a carbon dioxide concentration differential (Fickian) and hydrodynamic flow due to pressure differentials (Poiselle). They also raise doubts that Fickian diffusion can be used to model the release since the pressure differential present is significant. Complicating matters is that tempering is a process little reported on and can be done in many ways. Degassing times are influenced by many factors including particle size distribution, degree of packing, bed depth, entrainment gas composition, temperature and flow rate of the gas as well as the binding energy of CO_2 in coffee, which as noted earlier is high based on the small changes in the isotherm with temperature. Possible methods of tempering would include:

a. silo with minimum air flow (stagnant flow)
b. silo with nitrogen gas flushing (heated or not)
c. silo using all or part of the roaster flue gas
d. fluidized bed with the above gas options
e. spiral bed drier with the above gas options
f. others

In order to understand the release of carbon dioxide, the parameters of carbon dioxide diffusion kinetics need to be determined. In the second part of this study, our goal was to determine the diffusion kinetics of carbon dioxide from freshly roasted and ground coffee and to discuss the specific transport mechanisms that could be important in this process. We looked at the effect of coffee type, roast, particle size, and degassing temperature on the kinetics of CO_2 degassing in FRG coffee and report some of this here.

Experimental procedure

Green Kenya Arabica beans and green Togo Robusta beans were used for most of the studies although early trials were done with the Colombian beans used earlier. Roasting, grinding, and CO_2 determination are described in detail by Shimoni and Labuza (2000), and Anderson et al. (2001). Briefly, green coffee beans were roasted using a bench-top Neuhaus fluidized bed sample roaster RFB-L (Neuhaus-Neotec, Germany). Two roast times were used in these experiments: a short roast of five minutes and a long roast of fifteen minutes. Roast degree was measured using a Color/Difference Meter D25-2 (Hunterlab, Virginia) on the ground coffee samples. A medium roast (Hunterlab b-value of 8.5) and a dark roast (Hunterlab b-value of 5.4) were selected for these experiments. The roasted beans were ground using a small scale Super Jolly coffee grinder (Mazzer Luigi s.r.l., Venezia, Italy). Two different grind settings were used, and the particle size distributions were measured using a Malvern Particle and Droplet Laser Beam Sizer (Malvern, Worcheser, England). Carbon dioxide content was determined by the method of Hinman (1993).

A special apparatus was built for the degassing experiments as shown in Figure 6 (Shimoni and Labuza 2000; Anderson et al., 2001). Briefly, three glass columns were used and all experiments were done simultaneously in triplicate (different columns). The columns were prepared by covering the inlet with Parafilm (American National Can, Greenwich, CT) and closing the outlet using a rubber stopper. Coffee was ground at the desired setting and then immediately added to the column, which was then assembled into the closed system described previously. Time zero was measured when the second column was filled. Once all of the columns were filled (usually within 2 to 3 minutes), they were placed in a standup incubator

set to the degassing temperature. The stoppers in the outlet were removed and replaced with carbon dioxide trapping columns. Nitrogen was brought in from the bottom of the column at a flow rate of approximately 250 ml/min. The nitrogen flowed up through the bed of ground coffee then through a 12 cm column of Drierite to remove any moisture from the gas. The carbon dioxide was then trapped using a column containing Ascarite II. A second trap containing Drierite was placed on top of the Ascarite II column. This was added to collect any moisture that was produced from the reaction of CO_2 with Ascarite II, but might not have been held for the long periods of time required for the CO_2 degassing experiments. The carbon dioxide trapping columns (Ascarite II column and the second Drierite column) were weighed to the tenth of a milligram on an analytical balance at intervals over a period of 48 hours or until the CO_2 trapping columns began losing weight. The increase in weight as a function of time was attributed to the gain of CO_2 that reacted to and was absorbed by the chemical. Measurements were taken every 10 minutes for the first half-hour, then hourly and less frequently as the rate of gain decreased.

The degassing data collected was fitted using Equation 4, the series expansion of Fick's law for unsteady state diffusion for a spherical geometry (Crank, 1975).

$$m_t = m_\infty + \frac{6(m_o - m_\infty)}{\pi^2} \sum_1^n \frac{1}{n^2} e^{-\left(\frac{D_{eff}\, tn^2 \pi^2}{r^2}\right)} \tag{4}$$

where: m_t = concentration at time = t
m_0 = initial concentration at time = 0
m_∞ = final concentration - assumed to be zero
D_{eff} = effective diffusivity - units m^2/sec
r = average radius of particle from distribution

If the final concentration is assumed to be zero, this then can be rearranged as

$$\ln m_t = \ln \frac{6(m_o)}{\pi^2} + \ln \sum_1^n \frac{1}{n^2} e^{-\left(\frac{D_{eff}\, tn^2 \pi^2}{r^2}\right)} \tag{5}$$

Thus a plot of Log_{10} m vs time should give a straight line. The first 200 terms in the series expansion were used and the equation was solved using the Excel Solver function. For the single diffusion coefficient model, the value for final CO_2 concentration was set at zero by using equation 5. The radius used in the model was from the average particle diameter, D(4,3) measured for the sample although one could have used the bed depth. Using the single particle as the diffusion length assumes that the gas flow rate is not limiting in the interstitial spaces in the bed, thus diffusion is only limited within the particle.

Desorption kinetics: results and discussion

The main objectives of the desorption study were to determine the kinetics of CO_2 degassing of FRG coffee as related to coffee variety, roast degree, grind size and temperature. Measurements of initial carbon dioxide content were taken immediately after grinding of the beans while the final content was measured after the 48 hours. As seen in Table 2, a darker roast contained more CO_2 initially than does a lighter roast as expected. There was no correlation between initial and final CO_2 because the degrees of roast and roast temperatures differ.

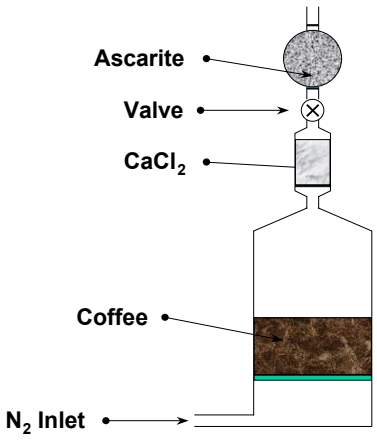

Figure 6. Apparatus for measurement of degassing kinetics

The darker roasts have an average initial CO_2 content of 6.4 mg/g, while the lighter roasts have an average content of 5.1 mg/g with an overall average of 5.7 mg/g (2.9 ml/g at STP). As seen, the Togo Robusta samples had higher initial carbon dioxide contents (average of 6.9 mg/g) than did the Kenya Arabica samples (average of 4.6 mg/g). Meanwhile the overall average final CO_2 content for Togo Robusta samples was 2.0 mg/g and for Kenya Arabica samples was 0.6 mg/g. The initial levels confirms the statement of Clarke and Macrae (1985) indicating more CO_2 production with a higher carbohydrate content coffee bean. According to Illy and Viani (1995), green Robusta coffee beans contain 54.4% carbohydrates and 8% sucrose, while green Arabica coffee beans contain 49.8% carbohydrates and 4% sucrose. The higher level at the end for Togo Robusta suggests that it traps or holds more CO_2 at equilibrium than does Kenya Arabica coffee.

Figure 7 shows an example of the diffusion loss of CO_2 using the single diffusivity model with data over the whole time period) for Colombian beans studied when the apparatus was being developed. Although giving a good fit overall, the model fails beyond the 10 hour time period. Also note that even at 48 hours, there is substantial CO_2 left in the coffee as we predicted from the sorption experiments. These differences are more obvious when plotting the Log_{10} m vs time function for the Kenyan coffee as seen in Figure 8. At about 2-3 hours (~180 minutes) the single model begins to deviate substantially as the loss of CO_2 slows significantly.

Table 2. Carbon dioxide content as a function of roast and grind size

Experiment	Coffee Type	Roast Time	Roast Degree	Grind Size	Degassing Temperature	Initial CO_2 Content (mg/g)	Final CO_2 Content (mg/g)
A	Togo Robusta	5 min	medium	570 um	40 C	6.23	1.94 ± 0.09
B	Togo Robusta	5 min	dark	350 um	40 C	7.03	0.90 ± 0.11
C	Togo Robusta	15 min	medium	350 um	20 C	5.61	2.10 ± 0.60
D	Togo Robusta	15 min	dark	570 um	20 C	8.55	3.22 ± 0.24
E	Kenya Arabica	5 min	medium	570 um	40 C	4.44	0.45 ± 0.04
F	Kenya Arabica	5 min	dark	350 um	20 C	4.97	0.61 ± 0.18
G	Kenya Arabica	15 min	medium	570 um	20 C	3.98	1.10 ± 0.16
H	Kenya Arabica	15 min	dark	570 um	40 C	5.11	0.31 ± 0.03

The degassing curves in Figure 7 and 8 indicate that there possibly are two different diffusion mechanisms occurring. Initially there should be some pressure driven flow of carbon dioxide out of the coffee particles since the pressure of CO_2 in the pores should be greater than one

atmosphere, as was noted by Clarke and Macrae (1985). In addition, there will be diffusional flow present, which, depending on the size of the pores may take place as molecular diffusion or Knudsen diffusion. There may even be a third flow regime, which is close to zero for the carbon dioxide entrapped in collapsed pores. The time at which the changeover in mechanism occurs for the first two appears to be consistent across the majority of experiments at about 120 minutes. Therefore, the data were also fitted using a dual model in which the data was broken up into segments of short times (data collected for times up to the changeover) and long times (data collected at times after the changeover). For each of these segments, the data was fit using the series expansion model of Fick's law (Equation 4) as was done for the single model.

In the example of Figure 9, using linear coordinates of CO_2 concentration versus time, both the single model and dual model appear to fit the actual data well and both have r^2 values of greater than 0.99. However, when plotted using semi-log coordinates as was shown in Figure 8 using this same data, it is apparent that slope changes and the single diffusion coefficient model does not predict as well at times greater than about 120 minutes.

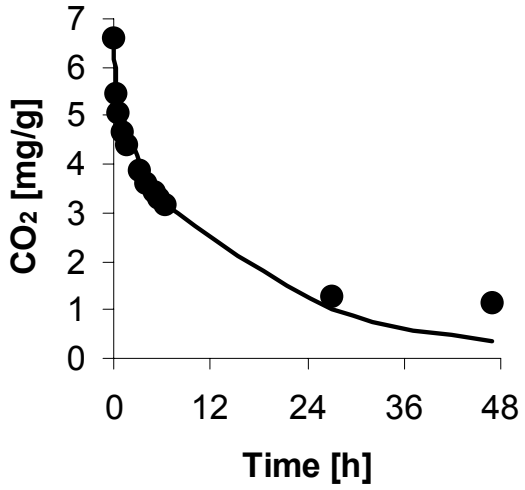

Figure 7. Degassing curve of CO_2 from FRG Colombian coffee (Dark-long roast, 92.2 μm, 25°C). Experimental data (●) and curve fit based on the calculated D_{eff}

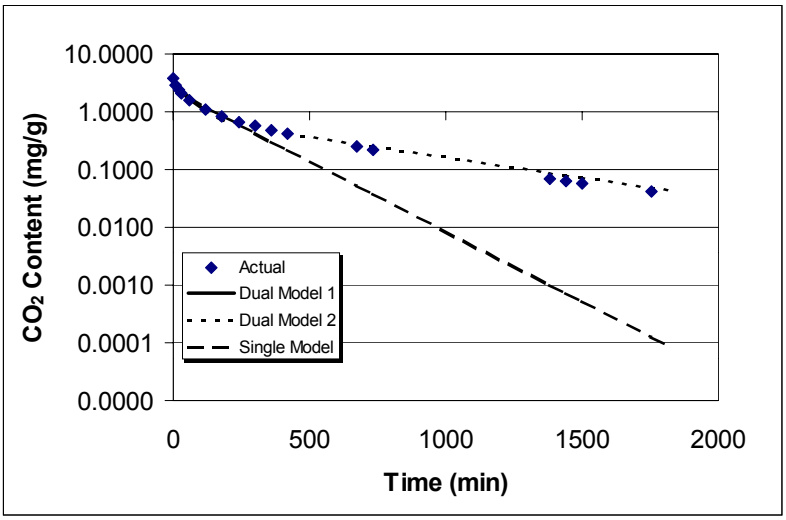

Figure 8. CO_2 degassing data using the single model and dual model in semi-log coordinates for Experiment E (Kenya Arabica, 5 min, medium roast, 570 μm, 40°C)

Table 3 summarizes the results of the calculated effective diffusivities using the two-state diffusion model as compared to the single diffusion model for the data. Each of the individual fits had a correlation coefficient (r^2) of at least 0.97, with an average over 0.98 as noted in Table 3 Using a single D_{eff} model, the overall average effective diffusivity calculated was 5.30 x10^{-13} m^2/s (range of 3.05 to 10.37 x 10^{-13} m^2/s). This is about two orders of magnitude lower than the 2 to 20 x 10^{-11} m^2/s that was determined by Bichsel (1979) and Spiro and Chong (1997) for the diffusion of caffeine in coffee during decaffeination and about one order of magnitude lower than the 15 to 27 x 10^{-12} m^2/s that was found by Xiong, Narsimhan, and Okos (1991) for diffusion of moisture from pasta. They are on the same order of magnitude as the 4 to 45 x 10^{-13} m^2/s reported by Doulia et al. (2000) for diffusion of moisture in oilseeds at 25°C. Thus, the range of effective diffusivities calculated in these experiments appears to be reasonable for a porous solid.

When the carbon dioxide diffusion data was modeled for each coffee using the dual model with two effective diffusivities, the overall average effective diffusivity was 6.18 10^{-13} m^2/s at short times and 2.38 10^{-13} m^2/s at long times. This indicates that after the first 60 to 120 minutes of tempering, the effective diffusivity on average decreases by over 50%. Given this, then what can be done to speed tempering or should one just stop at 2-4 hours when the level is below about 1 mg/g FRG coffee. Based on engineering principles several things can be done:

(1) Increase the temperature during tempering albeit this will induce oxidation;
(2) Use the smallest grind size possible. This approach might lead to handling problems such as caking or may make the overall bed less porous (note that we did not have enough data at any roast type to test this). Importantly is the amount of entrapped air in a bed. As the particle size decreases, at some point the amount of entrapped air becomes so low that diffusivity falls, as was found for moisture diffusion in cereals (Tutuncu and Labuza, 1997);
(3) Decrease the bed depth in the tempering unit, which is a physical handling problem;
(4) Increase significantly gas flow rate. This may have very little effect on the second period and could also strip away valuable flavors making the coffee stale so this is limited. There is no data available on the volume of air needed per volume of coffee to get the desired decrease in carbon dioxide and yet maintain good flavor.

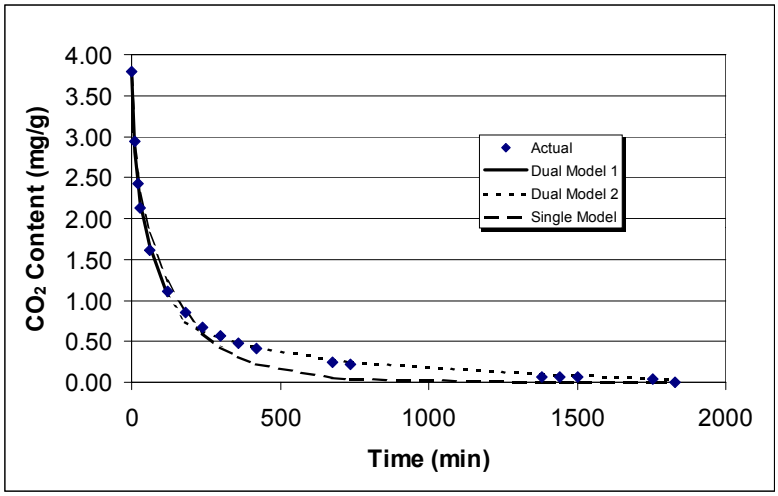

Figure 9. CO_2 degassing data modeled using single model and dual model in linear coordinates for Experiment E (Kenya Arabica, 5 min, medium roast, 570 µm, 40°C)

An analysis of our data shows that temperature has a much stronger influence on the diffusivity than expected. In the preliminary diffusion study (Shimoni and Labuza, 2000), the diffusivity was measured at 3 temperatures (25, 35, and 45°C) for Dark-long roasts from Colombian beans as seen in Figure 10.

These results suggest that the temperature effect on the diffusivity can be described by the Arrhenius equation, with activation energy E_a = 73.6 KJoule/mole which gives a temperature sensitivity of (Q_{10}) of 2.5, ie a 10°C increase in the gas temperature increased the rate by 2.5 fold.

$$D_{eff} = ke^{-\frac{E_a}{RT}} \quad (6)$$

E_a = activation energy [KJoule/mole]
T = temperature [°K]
R = gas constant [1.9872 10-3 Kcal/mole*°K]

Table 3. Carbon dioxide degassing results

Experiment	Degassing Temperature	Measured Avg. Diameter (um)	Calculated D_{eff} (m^2/s x 10^{-13}) Overall	r^2	Short time	r^2	Long time	r^2
A	40 C	587	10.4 ± 2.0	0.997	9.7 ± 1.8	0.991	9.7 ± 4.8	0.980
B	40 C	367	3.2 ± 0.4	0.991	3.7 ± 0.3	0.994	1.1 ± 0.1	0.984
C	20 C	388	5.1 ± 1.0	0.984	6.7 ± 1.4	0.998	1.1 ± 0.2	0.994
D	20 C	589	3.6 ± 0.7	0.986	5.7 ± 1.0	0.994	1.4 ± 0.2	0.987
E	40 C	486	5.6 ± 0.5	0.990	6.7 ± 0.9	0.988	1.6 ± 0.2	0.990
F	20 C	316	3.5 ± 1.2	0.975	4.4 ± 1.3	0.983	0.5 ± 0.1	0.994
G	20 C	542	3.0 ± 0.6	0.992	3.8 ± 0.9	0.994	1.0 ± 0.1	0.995
H	40 C	549	7.9 ± 0.8	0.992	8.7 ± 0.9	0.987	2.5 ± 0.4	0.985

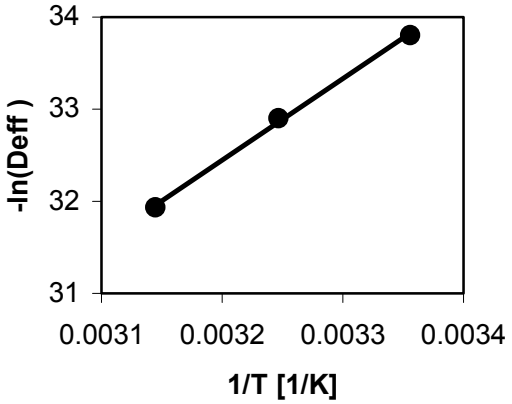

Figure 10. Arrhenius plot of ln(D_{eff}) vs 1/T for CO_2 diffusion from FRG Colombian beans

This dependence on temperature is higher that would be expected based on molecular diffusion, Knudsen diffusion, or viscous flow. Therefore surface diffusion may play an important role in the diffusion mechanism, since it has been known to have a strong dependence on temperature (Cussler, 1997).

In the second study using the beans in Table 3, when the single model effective diffusivities at 40°C are averaged and compared to the average of those at 20°C, it can be seen that a 20°C

increase in temperature raised the effective diffusivity by 70% (Q_{10} = 1.3). Similarly for the dual model, the 20°C increase caused the diffusivity on average to increase by 30% at short times (Q_{10} = 1.15). and over three times at long times (Q_{10} = 1.7). The reasons for these differences need further study but must be related to bean structure type) and roasting conditions.

The actual diffusion mechanisms taking place in FRG coffee are very complicated. There are likely influences from Knudsen diffusion, transition-region diffusion, pressure-driven viscous flow, surface diffusion, and interactions between the CO_2 gas and the coffee itself. A more rigorous diffusional model, such as those discussed by Krishna (1993), may better fit the data with the addition of other fitting terms. However, due to the variability in the data itself, it is unlikely that other models will make a significant improvement to help in tempering design. In addition, the other models would be more difficult to apply to real world applications due to the additional fitting terms that would need to be determined for each specific case. It is our conclusion that at 24 to 48 hours of degassing, coffee will still contain about 0.3 to 1.8 mg CO_2/g coffee. This most likely will not be at equilibrium and will be very hard to get rid of. Since holding at room temperature in air can cause a significant loss in shelf life, about 10% per day, it is recommended that tempering stop at 24 hours and that nitrogen gas be used. Also, since temperature increases the diffusivity, so that the time to end point decreases by ~1.3 to 2.5 fold for every 10°C rise, the gas can be heated to 40-45 C which might lead to a degassing step of less than 10 hours which would be desirable. Of course experiments in actual equipment would need to be done to verify this. In addition we would expect a benefit of decreasing the bed depth, as well as eliminating the CO_2 in the stripping gas stream, ie don't use the flu gas, as it is too high in CO_2. In no case should a stagnant flow silo be used. The optimum would be a spiral flow conveyor bed drier to minimize floor space where the flu gas is used to preheat nitrogen as the striping gas.

As a final analysis, one needs to discern the implication of tempering on the packaging system used and the loss of quality. Obviously, the reason for tempering from a physical standpoint is the need to prevent package bursting. The move from the metal can to the flexible pouch has enhanced the need for better physical chemical data so as to evaluate the conditions required to optimize flexible packaging, such as vacuum packaging, over pressurizing with carbon dioxide. The latter approach involved the use of a pressure relief valve or a high CO_2 permeable patch to allow it to diffuse out. As noted, the key in tempering is achievement of CO_2 down to levels that would not cause the flexible pouch to burst. The studies that we have done suggest that complete CO_2 loss is not required as there is some that is entrapped in collapsed regions that has a low diffusion rate out of the coffee. What has not been discovered is whether the lower diffusivity of this entrapped gas is low enough to prevent loss of package seal integrity during the 6 to 12 months needed for distribution in some areas. The low diffusion rate allows one to use an over pressure of carbon dioxide as long as the pressure level needed does not burst the package. At this point in time, although some data are available, the exact level needed for each size package is not easily calculable. In addition, the larger the package in terms of area to volume contained, the greater are the forces on the packaging seals so scale up or down is a critical issue. On top of this is the fact that during tempering, if oxygen is present in the tempering gas, there is a consequent oxidation of the coffee oils with a decrease in shelf life. The following section addresses that issue. Finally the other critical consequence of tempering is the entrainment of positive coffee volatiles in the tempering gas which leads to staling of the coffee. Once the other areas are well researched, this then is the next area to address in the continual battle to enhance coffee quality.

FRG COFFEE SHELF LIFE

Introduction: Stability Studies

Much of the research on the understanding of basics mechanisms of deterioration in dehydrated foods as influenced by moisture content was initiated by Prof. Marcus Karel in the sixties at MIT under research designed to determine the shelf life of NASA space food. At that time, Labuza et al. (1969) created a map for the rate of food deteriorating reactions as a function of water activity (a_w). This latter term describes the equilibrium thermodynamic state of the water in the product. The stability map, shown in Figure 11, allows one to identify the main reactions of deterioration likely to occur in dehydrated foods, e.g. lipid oxidation, non-enzymatic browning (NEB), nutrient loss, etc at different moisture contents. Further efforts at MIT and the University of Minnesota were concentrated on modeling the kinetics of food deterioration as a function of oxygen partial pressure, temperature and water activity (a_w).

These three parameters, O_2, a_w and T, are the major controlling factors that can vary during the storage and distribution of dehydrated foods stored in packaging which is semi-permeable to oxygen and water vapor (Karel, 1983). The approach for modeling of shelf life has been to use well characterized equations derived from basic physical-chemical principles and applying them to the study of deteriorating reactions in model systems and foods as a function of the above three parameters. The key is to know where the food is on the map so the other critical factor is the moisture sorption isotherm (moisture vs water activity) which is superimposed in the map. As seen, there is a range of moisture at the lower end for which a dry product is most stable. This range encompasses a region close to and including the moisture monolayer value, which can be calculated from the moisture vs a_w data using several techniques. Bell and Labuza (2000) have reviewed the methods to do this in detail as well as the equations needed to calculate the monolayer moisture value by the GAB equation.

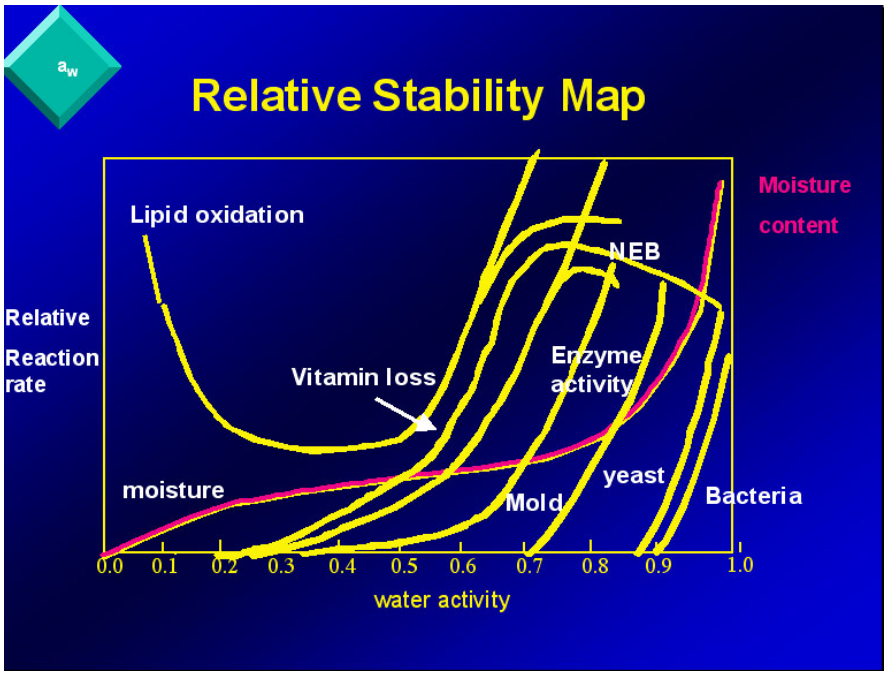

Figure 11. Relative Stability map of reactions vs. water activity

A typical moisture sorption isotherm for FRG coffee is shown in Figure 12. As seen in Table 4, using the GAB equation, the calculated moisture monolayer value is ~3.45 g water/100 g solids which is close to an a_w of 0.2. These results compare well to the published data except

for the Hayakawa value at 30 C. Since FRG coffee usually contains 2-4% water after quench cooling and probably does not change much during tempering, the moisture content during tempering and in the final package is close to the optimum for storage. Thus based on the general initial moisture range of 2 to 4%, at the industrial level, and using Figure 11, non enzymatic browning should be slow, and fat oxidation should be the most likely reaction to cause deterioration in FRG coffee with a loss in sensory qualities from a chemical reaction standpoint. It is possible that some Maillard type reactions could occur in the lipid phase but this has not been studied. The next section will review the possible kinetics of lipid oxidation in coffee.

The first such studies that incorporated the effect of oxygen partial pressure, temperature and water activity on shelf life of a dry food which deteriorates by lipid oxidation were those of Simon et al. (1971) for freeze dried shrimp and Quast et al. (1972) for potato chips. Because of the difficulty in doing this research, at different constant oxygen partial pressures, it was not until recently that similar techniques were applied to fresh roasted ground coffee (Cardelli and Labuza, 2001). The major problem is the design of a test system that will keep the oxygen level constant as the coffee reacts with the oxygen. It should be noted that no such work has been published for soluble coffee. Some results will be presented subsequently for FRG coffee.

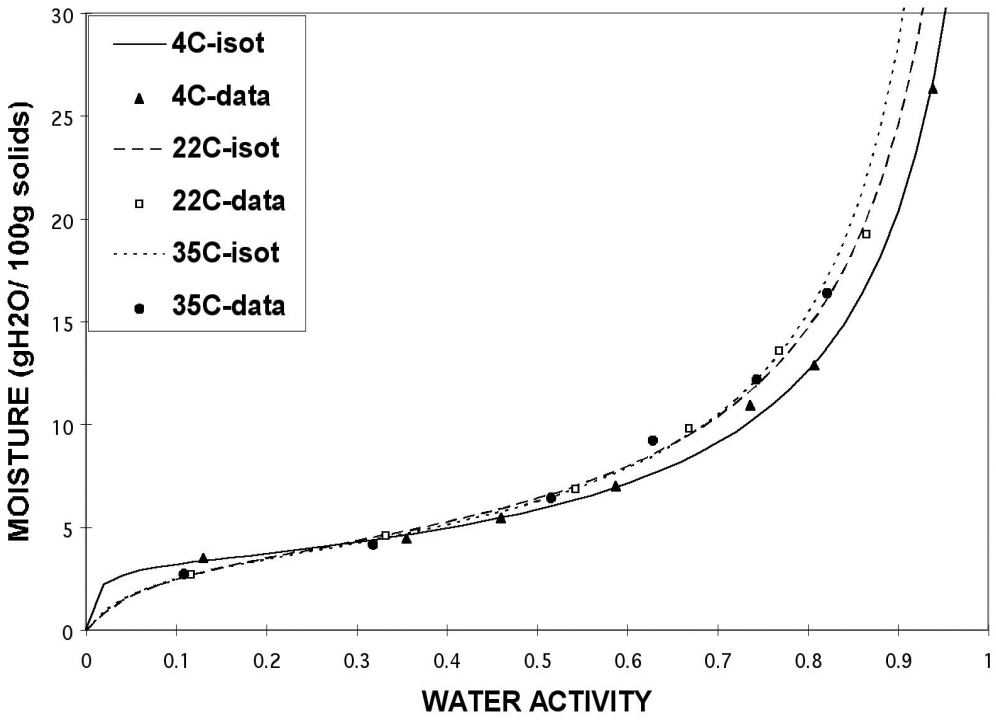

Figure 12. Moisture sorption isotherm for fresh roasted ground coffee at two temperatures. Monolayer value ~3.5 g/100 g solids

Temperature during distribution is a major factor that can affect shelf life. The Arrhenius equation (ln rate constant vs 1/T in K), previously shown above in its ability to model diffusion kinetics of CO_2 as a function of temperature, has been extensively used to model the effect of temperature on food deteriorating reactions (Labuza and Riboh, 1982). To simplify the data analysis, they showed that if you plot shelf life (time to some deterioration level) on a semi log plot vs temperature you get a straight line and from this one can calculate a temperature sensitivity or Q_{10}. This is directly related to the activation energy noted before and can be viewed as the effect of a 10 C change in temperature on shelf life, i. e. temperature

sensitivity. Figure 13 shows an example plot for vitamin C loss. Many food reactions including lipid oxidation have a Q_{10} of between 1.5 to 3, i. e. a 10°C increase in temperature gives a shelf life respectively of 66% or 33% of the original shelf life at the lower of the two temperatures. Figure 13 shows an example of the time to 20% loss of ascorbic acid in a ready to eat cereal (Kamman and Labuza, 1983) as a function of temperature and at three water activities. The plot shows excellent fit at each temperature. It should be noted that at an a_w of 0.4 the moisture content was about 4%, at 0.5 it was 8% and at 0.6 it was 12%.

As noted in Figure 13, the shelf life decreases as moisture content increases at any temperature and the temperature sensitivity is greater at the lower moisture content, i. e. 2.2 vs 1.3. This type of data has not been made available for FRG coffee until the recent studies in our lab.

The effect of moisture content (or a_w) on the rate of reactions of food deterioration has been more difficult to model as compared to temperature. An increase in moisture first dissolves more reactants and decreases local viscosity such that reaction rates increase with increasing moisture content above the monolayer. Higher moisture also plasticizes bio-polymers promoting glass to rubber transitions with a change in local free volume which also allows for increased mobility of reactants but above a certain moisture level, dilution occurs and the rates of reaction decrease again as was seen in Figure 11. The higher moistures also reduce the temperature sensitivity of the reaction as seen in Figure 13. These effects complicate the kinetics and forced scientists to use pure curve fitting techniques to predict the rate of deterioration as a function of a_w (Loncin et al., 1968) in the lower water activity range, using log shelf life vs. a_w relation. A simple plot of log shelf life vs water activity is shown for chlorophyll loss in Figure 14. As noted, going from 4% moisture to 16% (a 4-fold increase) gave a rate increase of over 120 fold, no theory explains this. This plot gives a straight line for many reactions including oxidative rancidity. In the vitamin C example of Figure 13, a doubling of the moisture at 25°C doubled the reaction rate, so the effect is very reaction specific.

Table 4. Moisture Sorption Isotherm characteristics for FRG at 22°C in comparison to other published data

SAMPLE	Initial Moisture % db	M % db	k_b	C	Q_S cal/mol e H_2O	p %
A	1.56	3.45	0.97	7.75	1200	4.5
B	3.55	3.60	0.95	15.82	1619	2.6
C	5.77	3.70	0.94	79.57	2565	1.6

Sample	T °C	m_O % db	k_b	C	Q_S cal/mol e H_2O	p %
Hayakawa et al. (1978)	20	3.22	0.92	87.2	2646	5.8
	30	1.91	1.17	31.87	2050	6.0
Quast and Teixeira (1976)	28	3.53	0.95	3.49	734	4.0
Lomauro et al. (1985)	20	3.25	0.94	61.6	2400	5.4

Because there is no unifying theory, there has been intense debate about whether there is a more appropriate model to describe the effect of moisture content on physical chemical changes in foods including reaction rates. More recently, the WLF equation has been proposed (Slade and Levine, 1985) to model the effect of both temperature and moisture on the rate of food deteriorating reactions in polymeric foods in the rubbery state. Nelson and Labuza (1994) have shown that the Arrhenius equation is a more useful model over the narrow temperature range of storage for most dehydrated foods, which would most likely include fresh roasted ground coffee. Labuza in the late 1960s introduced the concept of Q_a, similar to Q_{10}, to describe the change in shelf life for a 0.1 increase in a_w at constant temperature. This was shown to range between a factor of 2 to 3 for most deterioration reactions. Note that the chlorophyll data above has a Q_a of about 3 while the cereal data is about 2. This algorithm probably comes from an inverse linear relationship between E_a and water activity. Thus a product made to a low water activity will have the shelf life decrease as moisture is gained through the flexible package at a rate that increases with time and is greater than if no moisture were gained. Looking at the coffee sorption isotherm, one can see the importance of this in terms of quench cooling. With a monolayer at 3.5% which is at an a_w of about 0.2, quenching to 4.5% at an a_w of 0.3 essentially could reduce shelf life in half if the Q_a were two. As will be seen the Q_a for coffee is about XXXX. With respect to moisture gain during storage, Labuza et al. (1962) described the basic physical-chemical principles to model moisture gain in packaged foods as a function of % RH. Cardoso and Labuza (1983) applied these principles to the modeling of moisture gain in packaged pasta as a function of variable external % RH and temperature. Kamman and Labuza (1983) used these data to predict thiamin loss in the same pasta during storage under variable time/ temperature/ humidity conditions.

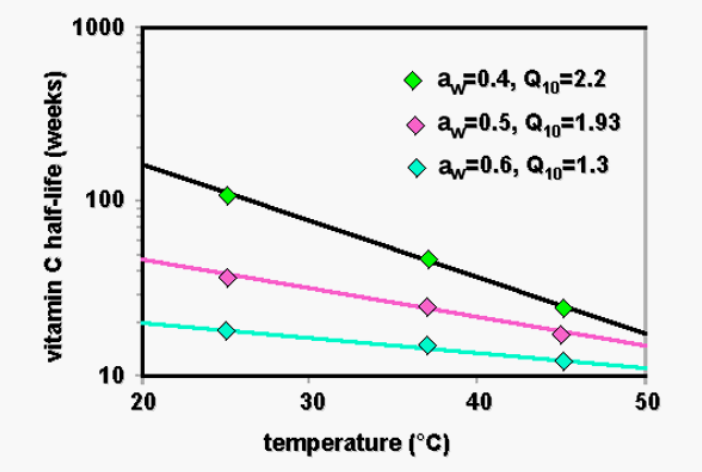

Figure 13. Time to 50% loss of vitamin C in a ready to eat cereal showing the Q10 values at the different water activities

Only a few papers have been published with algorithms for shelf life prediction of dry foods as a function of more than one environmental factor. Labuza (1971) critically reviewed the basic mechanisms of lipid oxidation and proposed a simplified model for the rate of oxygen uptake and shelf life deterioration in foods as a function of oxygen partial pressure and as affected by temperature and moisture. This model has been used to study the oxidation of freeze dried shrimp and potato chips as noted above and freeze dried beef and chicken stew (Toumy et al., 1970). Quast et al. (1972) used these basic kinetic equations and curve fitting techniques to create an algorithm for the rate of oxygen uptake of potato chips as a function of headspace O_2 concentration, % RH and the extent of oxidation. Quast and Karel (1972) combined this work with that of Simon et al. (1971) and predicted the amount of rancidity and

moisture gain in potato chips packaged in plastic pouches, as a function of headspace O_2 concentration, % RH and extent of oxidation, temperature was not varied. Villota et al. (1980) used curve fitting and physical-chemical principles to build an algorithm for the prediction of the shelf life of dehydrated vegetables as a function of temperature and moisture but not oxygen.

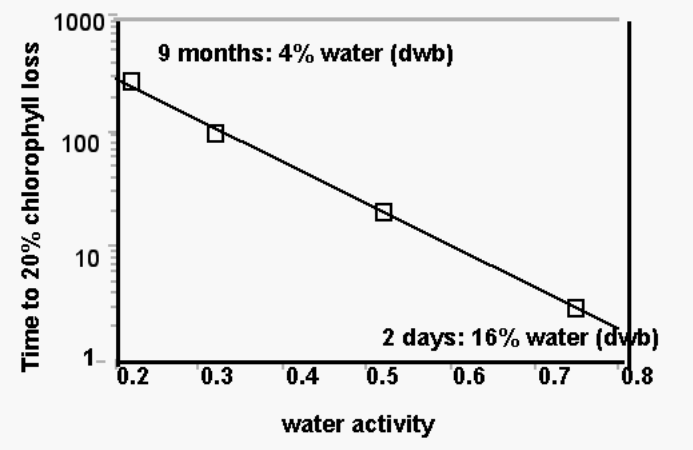

Figure 14. Exponential relationship between shelf life (time to some indices) vs. water activity

When FRG coffee (or any other dry food product) is stored in flexible plastic pouches that are permeable to both moisture and oxygen, the internal oxygen level begins to change as it reacts with the coffee and it diffuses in. Under these conditions, oxygen level in the headspace is the limiting factor controlling the rate of lipid oxidation. The uptake of oxygen (X_t) during the storage time (t) can be modeled following apparent-zero order kinetics (Eq. 7). The rate of oxygen uptake (Ro_2) is a function of the partial pressure of oxygen (pO_2) in the headspace and can be modeled by the hyperbola in Eq. (8) and shown in Figure 15. The parameters k_1 and k_2 are found from Eq. (8) by non-linear regression or by converting this hyperbola into the straight line form shown in Eq. (9) and applying linear regression.

$$X_t = R_{o_2} . t \tag{7}$$

$$R_{O_2} = k_1 \frac{p_{O_2}}{k_2 + p_{O_2}} \tag{8}$$

$$\frac{1}{R_{o_2}} = \frac{1}{k_1} + \frac{k_2}{k_1} \frac{1}{p_{O_2}} \tag{9}$$

Elder (1940) was one of the first to publish on the relationship between staling and rancidity for coffee. There is only one reference in the literature for the amount of oxygen needed to make coffee stale, i.e. Heiss et al. (1977), reported an X_o = 120 μg O_2/g for FRG coffee stored at room temperature in air (un-stated moisture). No experimental data were shown in this paper. With an unsaturated lipid content of around 8%, this amounts to about 800 μL oxygen/g lipid very close to the value Labuza (1971) gives as a guide for the end of oxidative shelf life. Thus combining the water activity effect in the activation energy we get: Eq. (10) for both T and a_w effects

$$R_{O_2} = \frac{k^*_{o1} \cdot e^{-\left(\frac{E^*_{a_1} - b_1\ a_w}{R\ T}\right)} \cdot p_{O_2}}{k^*_{o_2}\ e^{-\left(\frac{E^*_{a_2} - b_2\ a_w}{R\ T}\right)} + p_{O_2}} \quad (10)$$

Given all the above, Cardelli (1997) and Cardelli and Labuza (2001) studied the rate of lipid oxidation as a function of all three variables by measuring both oxygen uptake as well as sensory shelf life. In the oxygen uptake experiments, FRG coffee was stored under combinations of constant levels of % O_2 (0.1, 0.5, 1,3, 5, 10, 21), temperatures to simulate conditions usually found during storage and distribution (4, 22, 35) and three a_w values around the moisture monolayer (0.1, 0.28, 0.4). Headspace analysis was carried out by GC using TCD. The amount of oxygen reacted was calculated from the change in O_2 concentration in the headspace. In order to maintain constant oxygen levels the headspace had to be flushed back to the original level after each measurement. Because of the complexity of this experiment it took over one year. From this data they created some algorithms for predicting the oxygen uptake rate of coffee as a function of internal oxygen pressure, water content and temperature while under going storage in a flexible pouch. Some results will be shown below. They also introduced a third sensitivity.

In order to relate this to flexible packaging, Fickian diffusion was assumed where the rate of penetration of oxygen into a package follows Eq. (11) and is illustrated in Figure 16.

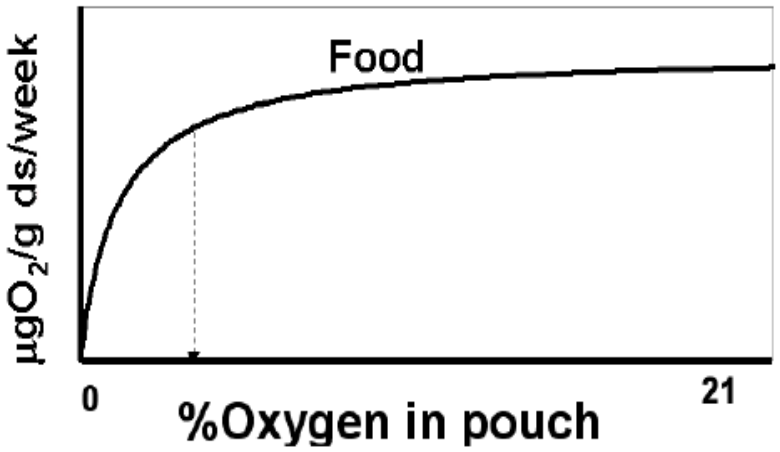

Figure 15. Rate of lipid oxidation as a function of oxygen partial pressure from Eq. (8)

Superimposing the two equations (essentially solving for them simultaneously gives rise to the conclusion that if the package is gassed to a low oxygen level but the film permeability is high, very quickly the oxygen level increases then reaches what is called a steady state. At that point the rate of reaction (consumption of the oxygen) equals the rate of permeation into the package so the rate follows Eq. (7) shown previously. This situation is best illustrated in Figure 17. Thus one can then model shelf life as a function of oxygen pressure in a flexible permeable package as a function of the O_2 level achieved.

$$\left(\frac{dV_{O_2}}{dt}\right)_{INPUT}\frac{1}{W_{coffee}}=\frac{k}{x}\cdot\frac{A}{W_{coffee}}\cdot\left(21\%-\%O_2\right) \quad (11)$$

$\frac{k}{x}$= film permeability to oxygen

W_{coffee} =weight of coffee

A= package area

$\%O_2$= oxygen level in package

Introduction: Sensory Evaluation

Sensory Shelf life can be defined as the time until a product becomes unacceptable to consumers under a given storage condition. Consumer acceptability is usually determined by means of "consumer tests" using a large number of untrained tasters in which samples with various ages are tasted together (Meilgaard et al., 1991). This is accomplished by using samples of product manufactured at different times or by freezing aged samples to stop deterioration. Both techniques introduce undesired error, i. e. batch to batch variability or the continuation of the deterioration during the frozen storage.

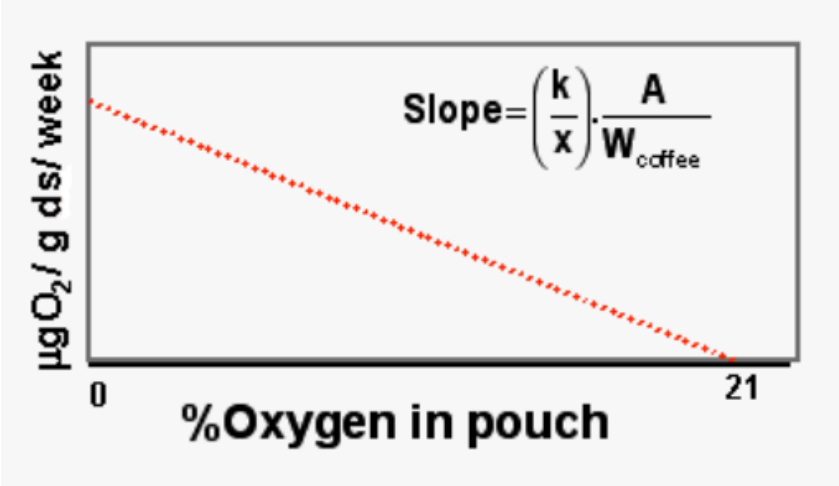

Figure 16. O_2 transfer into a flexible pouch as a function of oxygen level in package

Another technique used in industry for shelf life determination is team judgment. It is frequently applied in the early stages of product development, were prototypes are evaluated during the storage by a team familiar with the product. The main drawback is that results are limited to the tasting team making necessary the validation with consumers prior to launching.

The use of correlation between trained panel evaluation and consumer response has been reported in the literature. First, a team trained to recognize deteriorating attributes quantifies changes in their intensity during storage. Samples are frozen and then used in a second test where consumer acceptability is determined. Consumer response is correlated to trained panel data to determine the limits for the intensity of deteriorating attributes in product at the end of consumer shelf life. The shelf life of new samples is found equal to the time until intensity limits for deteriorating attributes are detected by the trained panel. The main advantage of the technique is that one set of consumer data is used multiple times when correlating to trained

panel assessment. Main disadvantages are that results depend on the type of the scale used for measuring the deterioration (Shepherd et al., 1988) and that sample storage introduces error which in turn is amplified when coupling two sets of sensory data. The technique has created intense debate in the literature. Fritsch et al. (1997) and sensory psycho-physicists support the use of trained tasters to predict consumer sensory responses (Moskowitz, 1996; 1997), where statisticians do not (Dugle, 1996).

An ideal method would be to use samples of one batch of product and evaluate them at various times during the storage with a large number of consumers. This is too costly and time consuming. One way to simplify this is to use staggered sampling designs in which reduced tasting is done in the beginning of the storage focusing on samples close to the end of shelf life. This approach has been extensively used in the mechanical and electrical industries (Johnson, 1964). Labuza and Schimdl (1988) and Fu and Labuza (1993) called the technique the "Weibull Hazard Method". More details of this method can be found in the literature (Nelson, 1969; Nelson, 1972; Gacula and Kubala, 1975; Gacula and Singh, 1984).

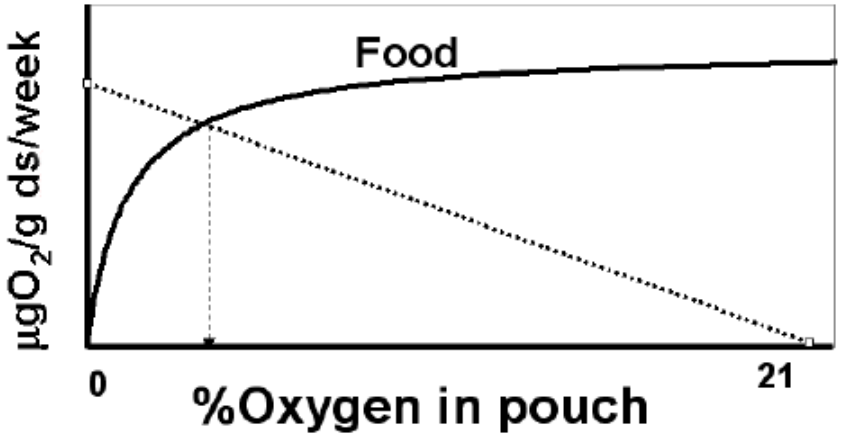

Figure 17. Steady state O_2 transfer into a flexible pouch as a function of oxygen level in package reaching a crossover value where input = reaction

Gacula (1975) was the first to use a staggered sampling design for shelf life studies in foods. The technique consists of using increasing number of samples over storage life, which are tasted to determine acceptability. It is based on the fact that the probability of failure is a bathtub shaped curve. There is a high probability of failure right after processing is good manufacturing practices were not followed, e.g. poor seals that lead to rapid oxidation. If the process is done right then this probability falls to zero. As product is stored there are more and more people who will become dissatisfied. To maximize the ability to determine end point, then one increases the number of testers over time in a special manner. End of shelf life is then the point at which a certain % of consumers becomes displeased, generally taken as 50% but this can be lowered. The main advantages over the other techniques is that all samples come from one batch and untrained consumers can be used for the evaluation. Thee result of the analysis of the sensory data is a plot such as the one in Figure 18 for an oat barn cereal that lost shelf life due to oxidation of the oat lipids.

As seen in this figure, at 38°C the oats reached a point at which 50% of consumers were displeased (Cumulative Hazard = 69.3%) at 8 weeks while at 45°C it took only about 5 weeks. This means for example, if all product were distributed at 38°C and not used by the consumer at 8 weeks, 50% of them would reject the product. This would result in loss of brand image and loss of consumers. Thus if the goal were to displease only 1% of consumers,

the distribution would need to be sure product reached consumers within 4 weeks and the product was dated with a "best to consume by" date. The other approaches would be to reformulate or use low oxygen packaging which is what is necessary for FRG coffee.

Staggered sampling methods have also been used to determine the shelf life of luncheon meats (Gacula, 1975), cassava flour (Shirose et al., 1978), ice cream (Wittinger and Smith, 1986), refrigerated meats (Andujar and Herrera, 1987), frozen foods (Tomasicchio et al., 1989), cottage cheese (Schmidt and Bouma, 1992), pasteurized milk (Duyvesteyn et al., 2001) and sausages (Thiemig et al., 1998).

The objective of this final section of the study was to develop algorithms for the prediction of the rate of oxygen uptake and loss sensory shelf life of FRG coffee as a function of oxygen partial pressure, temperature and a_w, based on basic physical-chemical principles and determining the shelf life by the staggered failure design. From this, one could then determine the extent to which the tempering process, used to reduce CO_2 levels in freshly roasted coffee, would lead to loss of high quality shelf life.

Experimental procedure

FRG Colombian Arabica coffee was stored under combinations of constant levels of $\%O_2$ (0.1, 0.5, 1, 3, 5, 10, 21), temperature (4, 22, 35) and a_w (~0.14, 0.25, 0.48). This gave three coffees with moisture contents of ~1.6, 3.55, and 5.8% (db) so they were just below, almost at and somewhat above the average monolayer of 3.5% (db). The actual water activity varies since it is a function of temperature. Sensory shelf life was determined using the Weibull hazard analysis method as described by Labuza and Schmidl (1988). Samples were brewed, and the untrained panel was asked to grade the brew as being acceptable or unacceptable. The results were transferred to a master spreadsheet for calculation and then to Weibull hazard plot as described by Gacula and Singh (1984). The criteria for the end of shelf life was set as the time for 50% probability of untrained testers, who were black coffee drinkers, to grade the sample as being unacceptable. This probability corresponds to a cumulative hazard of 69.3. The shape factor (β) was determined as 1/slope. The Weibull distribution is unskewed for $2<\beta<4$, which leads to better shelf life estimates. When the test is extended beyond end shelf life, most of samples are judged unacceptable shifting β outside the optimum range. In these situations, data were re-plotted up to cumulative hazard <100.

Shelf life: results and discussion

The results of this study indicated that the increase in $\%O_2$, a_w, and temperature, as expected, reduced the shelf life of FRG coffee. Figure 19 shows the rate of oxygen uptake for coffee stored at two levels. It is very obvious the oxidation of lipids is occurring. The study design used 7 O_2 x 3 T x 3 a_w conditions for 63 different studies which were done in triplicate. The data was modeled assuming a constant rate of oxygen uptake (Eq. 7) and the algorithm of Eq. (10) for oxygen uptake as a function of oxygen pressure. Moreover, an inverse similar function for shelf life was hypothesized, assuming that the rate of oxidation is constant at constant oxygen level and thus Eq. (7) applies. Figure 19 shows that indeed the oxygen uptake rates were linear at any given condition. Figure 20 shows plot of the algorithm for rate of uptake of oxygen. It can be seen that the plot follows the expected hyperbolic curve as a function of oxygen pressure and that a water activity increase, increases the rate of oxidation. This latter would be expected if the moistures used surround the monolayer value as depicted in Figure 11 with the highest rate being at the upper water content.

The determined parameters, for this algorithm to predict oxygen uptake rate for all conditions, are shown in Table 5. As noted for all data the fit is excellent with an R^2 of 0.988

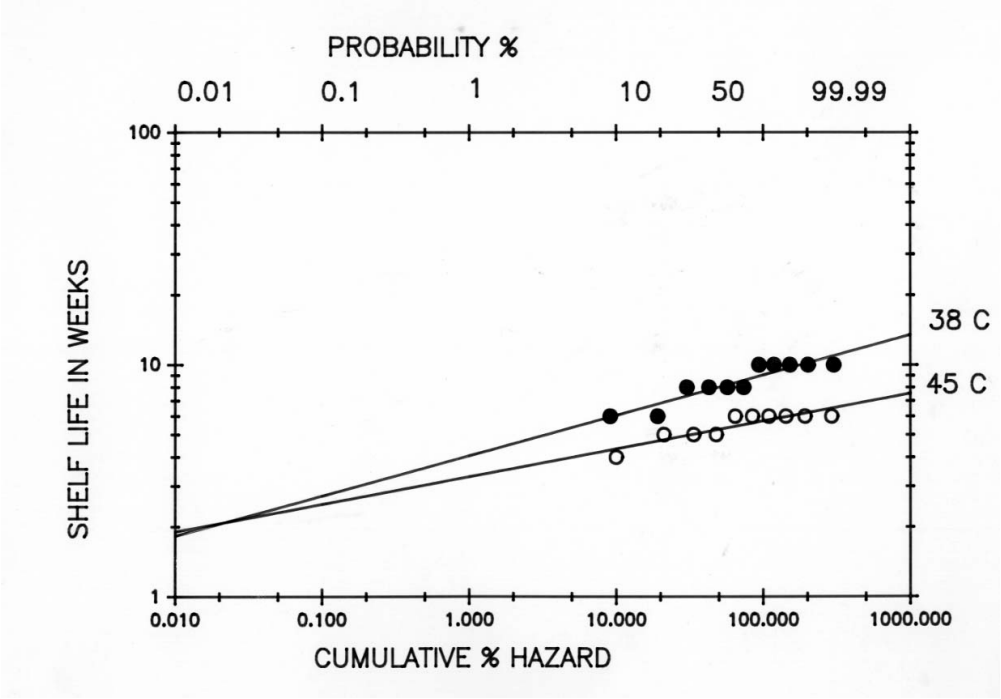

Figure 18. Weibull hazard plot of Probability of failure (top X axis) vs time in distribution for an oat bran cereal stored in air from Pickering (1984)

The second step was to then determine if loss of shelf life followed a kinetic pattern that was similar to that for oxygen uptake. The actual results of one of the sensory tests using the staggered design method are shown in Table 6.

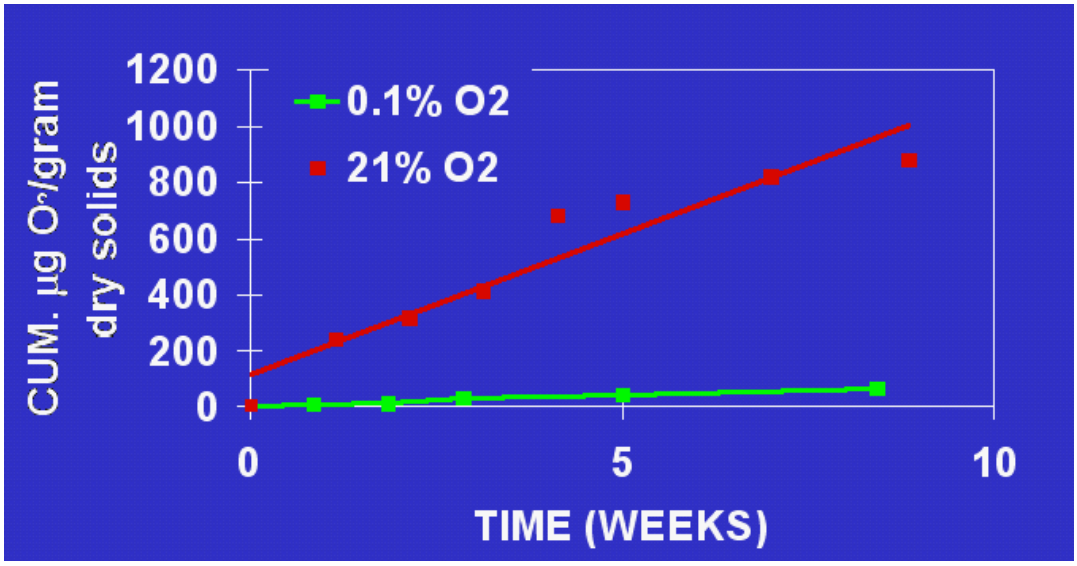

Figure 19. Typical oxygen uptake data for FRG coffee at 35°C and an a_w of 0.28

The Hazard value is then calculated from this data using the tabular approach shown in Table 7. The Hazard value is simply equal to 100 ÷ Rank #, so in Table 5, the 25th rank Hazard Score is 100 ÷ 25 = 4. Note the table is done in inverse rank order (highest rank first) and then the last column is the cumulative Hazard value. This is then use to create the Hazard

plot (log shelf life time) vs log Cumulative Hazard shown in Figure 21. As seen the time for 50% displeasure is 22.3 weeks which would be the shelf life of the product. Also note that the beta value is 3.4, this indicates that the population of the testers was a normal distribution since it fell in the range of 2 to 4. As noted earlier, this type of analysis was done for each T, a_w, O_2 condition and modeled in the same manner as for the oxygen uptake.

Figure 22 shows the results as a two-dimensional plot for the sensory shelf life FRG held at 35°C and all the other conditions. It is obvious that water activity has a large effect at all oxygen levels and that above 10% oxygen, the increase in oxygen level has only a small effect. Being below 1% oxygen has a dramatic effect on increasing shelf life.

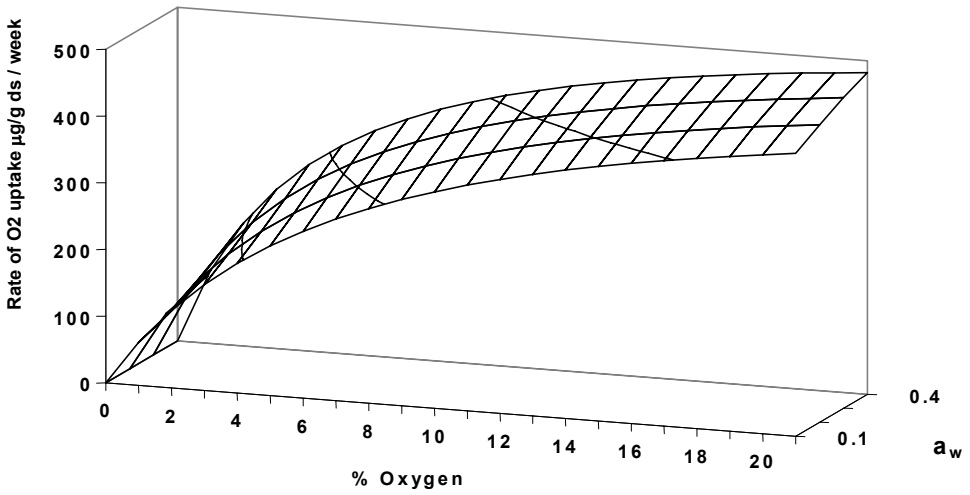

Figure 20. Rate of O_2 uptake in R/G coffee at 35°C as a function of % O_2 and a_w

Table 5. Parameters for Ro_2 uptake predicting algorithm

Parameter	Magnitude	Units
k_{o1}	370146	µg/g db/week
E_1^*	3880	cal/mole
b_1	-20.0	cal/mole
k_{o2}	398	$\%O_2$
E_2^*	2220	cal/mole
b_2	-1259	cal/mole
R^2	**0.988**	

In Figure 23, the shelf life data is plotted in a three-dimensional plot as a function of oxygen pressure and water activity, as was done for rate of oxygen uptake (Figure 20). As can been, a similar but inverted plot is obtained as would be expected if oxygen uptake were the main mode of deterioration. The algorithm used to model this was:

$$\theta_s = \frac{k_{o_4}^* . e^{\left(\frac{-E_4^* + b_4\ a_w}{R\ T}\right)} + p_{O_2}}{k_{o_3}^* . e^{\left(\frac{-E_3^* + b_3\ a_w}{R\ T}\right)} . p_{O_2}} \qquad (12)$$

As with oxygen uptake, the parameters of the algorithm were determined and are shown in Table 8. Again an excellent correlation was found with an R^2 of 0.983, very similar to that found for the oxygen uptake. This can be seen as a comparison between predicted value vs actual value in Figure 24. Thus the lipid oxidation hyperbolic model with a linear effect of a_w on the activation energy fits the shelf life data very well. It should be noted however that this was only done for one coffee variety harvested in one year. Although similar patterns should be found for other coffees, the actual parameter constants most likely would not be the same. Since it takes so long to gather this data, most likely it will not be done. Despite this the key is that these data can then be used to determine the direction and trend of the three sensitivity functions related to temperature, oxygen and moisture. This then can be used to assess factors to speed up tempering as well as improve and extend shelf life.

Table 6. Sensory results from the staggered design sensory test for FRG coffe stored at 1% oxygen, a_w = 0.28 and 35°C. (Note that negative score denoting unacceptablility is scored with a rank number beginning from the bottom and continuing right to left and upward until the last - value)

Weeks	Acceptability																		
0.0	+	+	+																
7.1	+	+	-25	+															
12.1	+	+	-24	+	+														
17.3	-20	-21	+	-22	-23														
20.1	+	-13	+	-14	+	-15	-16	+	-17	-18	-19								
23.3	-1	+	-2	-3	+	+	-4	-5	-6	-7	-8	-9	-10	-11	+	+	-12	+	+

+: acceptable sample as assessed by one untrained taster
-: unacceptable sample as assessed by one untrained taster

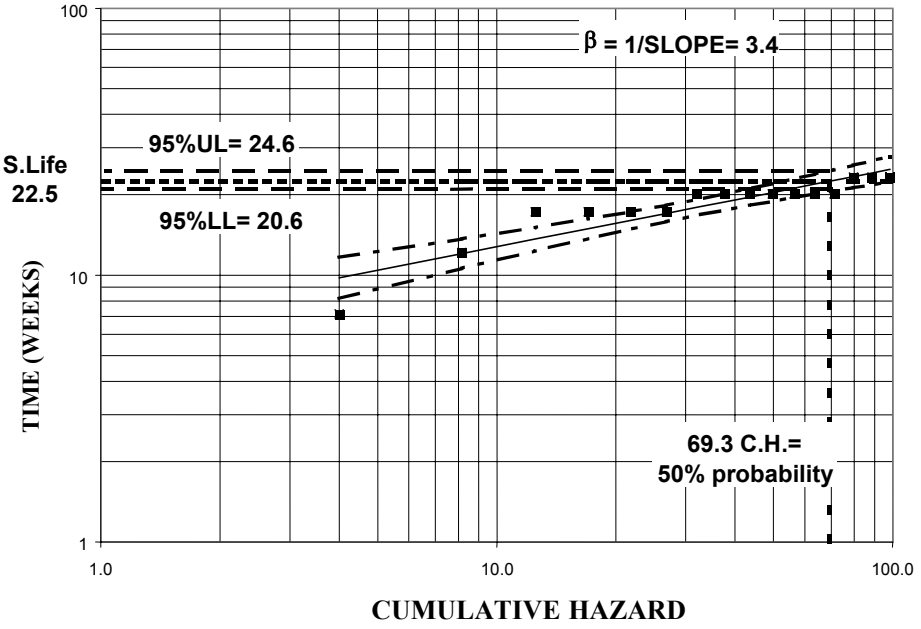

Figure 21. Weibull Hazard Plot for R/G Coffee (1% O_2, 35°C and a_w = 0.248)

Temperature is the first factor to be evaluated. Figures 24 and 25 show Arrhenius plots for both sensory score and oxygen uptake rate for one of the moisture conditions (water activity ~0.42) and for the different oxygen levels tested. From the results for both oxygen uptake rate and sensory shelf life the temperature sensitivity was calculated which is shown in Table 9 for the moisture content closest to the monolayer. As seen the temperature sensitivity effect was about the same for R_{O2} and for shelf life (Q_{10}) with an E_a of about 3 kcal/mole. This low

activation energy is indicative of a diffusion-controlled process suggesting that the deterioration is controlled by oxygen diffusion through the glassy state coffee. When diffusion is not limiting the Ea is about 15 Kcal/mole. More importantly with a low Q_{10}, this means that using a higher temperature during tempering will not do as much damage as expected. Thus if the tempering were done at 45°C, the rate increase will be only about 15%. Considering that at this moisture content the shelf life in air is 14 days at 35°C and 12 days at 45°C, the 24 hours of tempering at 45°C will be equivalent to using about 8% of the shelf life which may be acceptable.

Table 7. Weibull hazard calculation table for a FRG coffee during storage at 1% oxygen, an a_w of 0.248 and 35°C

Rank	Weeks	H value	Cum H
25	7.1	4.0	4.0
24	12.1	4.2	8.2
23	17.3	4.3	12.5
22	17.3	4.5	17.1
21	17.3	4.8	21.8
20	17.3	5.0	26.8
19	20.1	5.3	32.1
18	20.1	5,6	37.6
17	20.1	5.9	43.5
16	20.1	6.3	49.8
15	20.1	6.7	56.4
14	20.1	7.1	63.6
13	20.1	7.7	71.3
12	23.3	8.3	79.6
11	23.3	9.1	88.7
10	23.3	10.0	98.7
9	23.3	11.1	109.8
8	23.3	12.5	122.3
7	23.3	14.3	136.6
6	23.3	16.7	153.3
5	23.3	20.0	173.3
4	23.3	25.0	198.3
3	23.3	33.3	231.6
2	23.3	50.0	281.6
1	23.3	100.0	381.6

The second factor to consider is the effect of oxygen rate on rate of oxidation and loss of shelf life. The effect of an increase in rate for an increase in oxygen level indicates the sensitivity to changes in oxygen concentration as measured by Qo_2. The calculation for all data using both the sensory and oxygen up take is shown in Figure 26. As seen, between 0 and 1% O_2, the rate of deterioration accelerates about 10 fold. A much smaller effect can be seen in FRG coffee stored under conditions of 5 to 21% O_2, with only a 5% acceleration per 1% O_2 increase in this range. As seen the Qo_2c for coffee decreases from approximately 10.5 to 1 with an increase in % O_2 from 0.1 to that of air, i.e. 21%. These findings indicate how critical it is that the storage of R/G coffee be done at a low and constant % O_2 to achieve a desired shelf life. Very importantly, these results indicate that using a gas with reduced oxygen content.

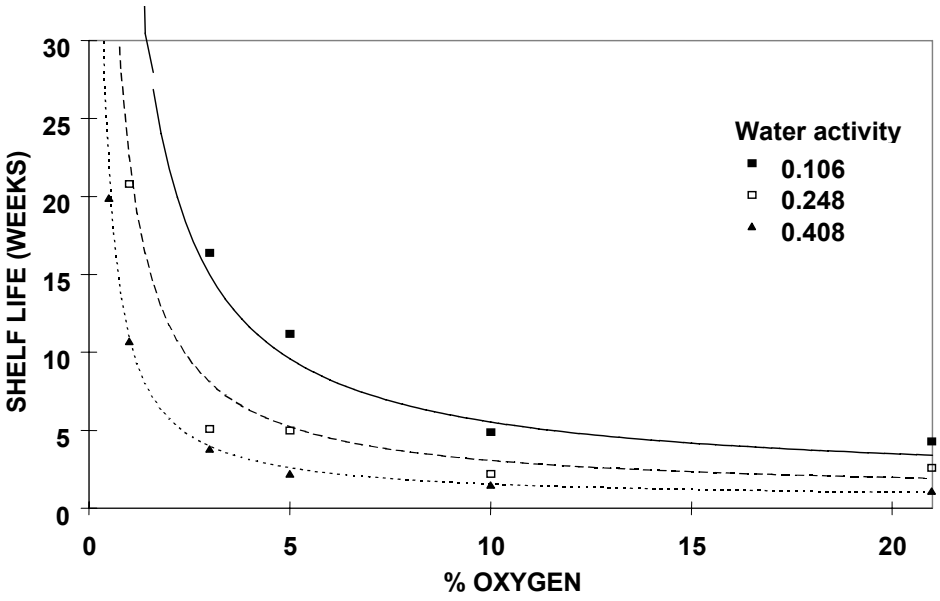

Figure 22. Sensory Shelf Life of R/G Coffee at 35°C using the Weibull method

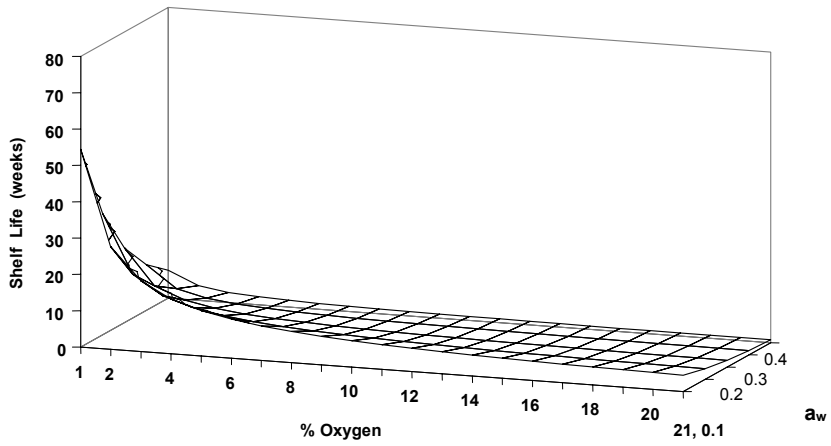

Figure 23. Shelf life of FRG coffee at 35°C as a function of oxygen partial pressure and water activity

Table 8. Parameters for shelf life predicting algorithm

Parameter	Magnitude	Units
k_{o3}	8356	$Week^{-1}$
E_3^*	6055	cal/mole
b_3	2255	cal/mole
k_{o4}	7841	% O_2
E_4^*	3352	cal/mole
b_4	-682	cal/mole
R^2	**0.983**	

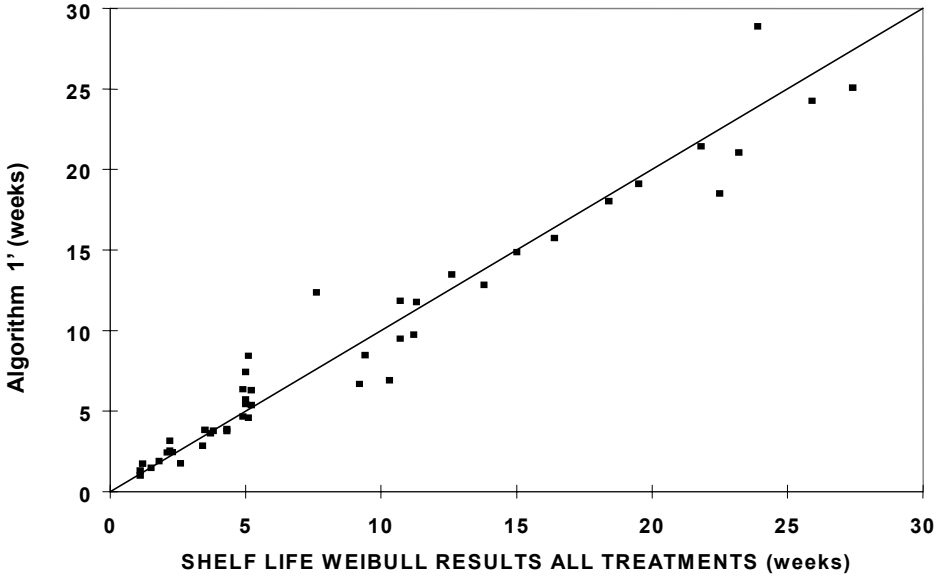

Figure 24. Comparison of predicted shelf life time vs. actual value from algorithm for all conditions tested

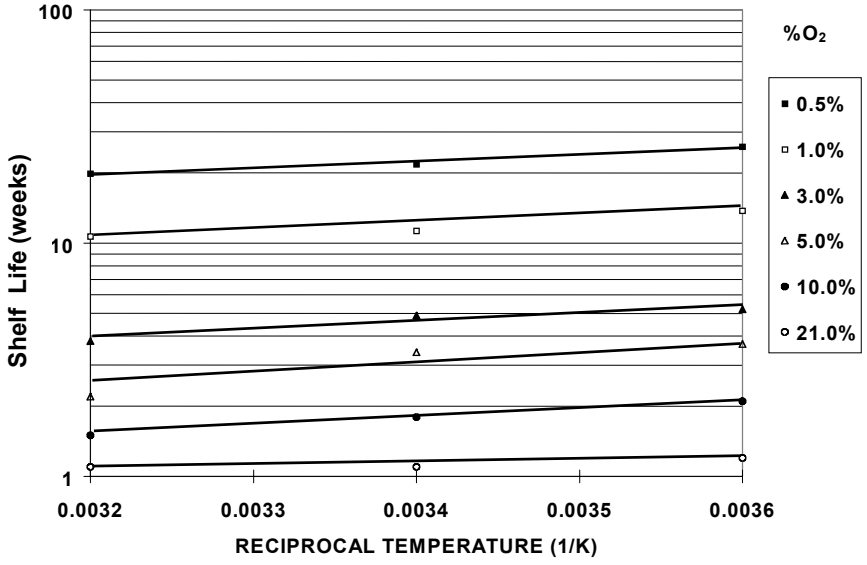

Figure 24. Arrhenius plot of sensory shelf life of FRG coffee at an a_w of ~0.42

The final factor to discuss is the effect of moisture content on shelf life, which can be assessed by evaluation of the Q_a value described previously. Figures 27 and 28 are the sensory shelf life vs water activity plots for the two higher temperatures studied. It can be seen that the lines are parallel at all oxygen levels. Table 10 shows the calculated Q_a values for FRG coffee stored at 10% O_2, 22°C and a_w = 0.14 to 0.42 for both oxidation rate and sensory testing and comparing to prior literature values. The Q_a value for sensory loss of shelf life was about 1.60 while it was less, 1.08 to 1.05, for the rate of oxygen uptake. The increase in 0.1 a_w produces about a 60% acceleration of the sensory shelf life deterioration and only 8% or less acceleration in Ro_2. This means that oxygen uptake is almost unaffected by the increase in a_w whereas shelf life is dramatically reduced with the increase in a_w. This indicates that at high a_ws, reactions other than lipid oxidation, e.g. the Maillard reaction, may participate in shelf life deterioration introducing deviations from the equations used or that the

activation energy changes for each step of the free radical reaction. As seen in Table 10, the experimental Q_a values for Ro_2 (1.05-1.08) are similar to that derived from data in Radtke (1979), i.e. 1.16. On the other hand, the Q_as for Weibull sensory shelf life (1.59-1.60) are higher than that derived from data in Clinton (1980), i.e. 1.16. With respect to tempering, this suggests that one should be sure that the tempering gas used is dehumidified since the acceleration is 60% for a 0.1 change in a_w. Since the isotherm is so flat in this region, it would take little moisture to increase the a_w by 0.1 units.

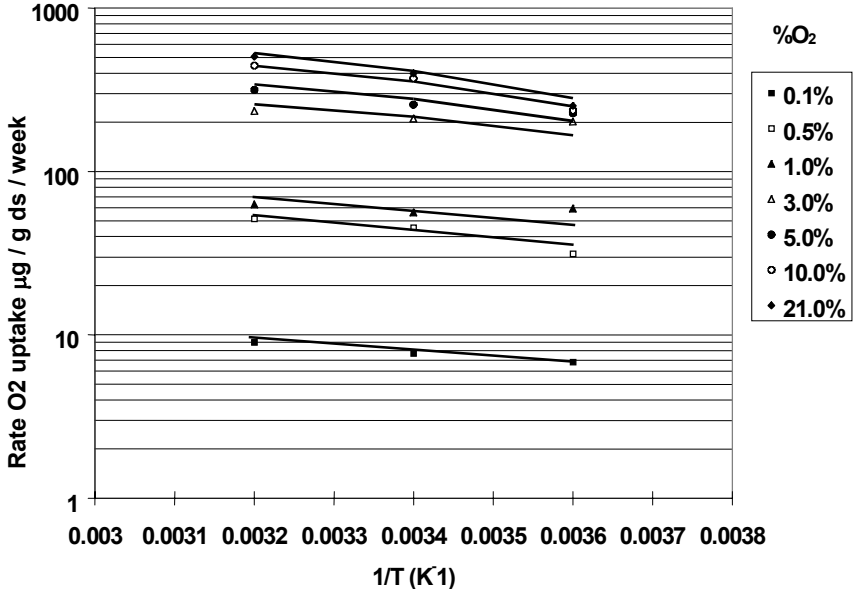

Figure 25. Arrhenius plot of rate of oxygen uptake of FRG coffee at an a_w of ~0.42

CONCLUSIONS

The shelf life kinetics study shows that temperature has a smaller effect on the sensory properties of FRG coffee than previously expected. Thus, since temperature accelerates CO_2 diffusion significantly, it would be beneficial to increase the temperature during the degassing (tempering) process.

Moisture has a significant effect on shelf life of FRG coffee, thus using the degassing period for the purpose of drying as well as CO_2 removal may increase shelf life by ~33% for every 0.1 a_w decrease.

The effect of oxygen was higher at low % O_2, thus it is recommended to use nitrogen with less than 1% oxygen during CO_2 degassing.

Table 9.Q_{10} for the sensory shelf life and rate of O_2 uptake of R/G coffee stored at a_w= 0.272

T (°C)	Q_{10} based on Sensory Shelf Life	Q_{10} based on Rate O_2 uptake	E_a cal/mole
4	1.23	1.22	3278
22	1.18	1.18	2953
35	1.15	1.16	2762

Table 10. Q_a for the shelf life and rate of O_2 uptake of R/G coffee stored at 10% O_2 and 22°C

a_w	Shelf Life		Ro_2	
	Literature*[1]	Experimental	Literature*[2]	Experimental
0.14	1.16	1.60	1.16	1.08
0.27	1.16	1.59	1.16	1.07
0.42	1.16	1.59	1.16	1.05

*[1]: *Derived from Clinton (1980)*
*[2]: *Derived from Radtke (1979)*

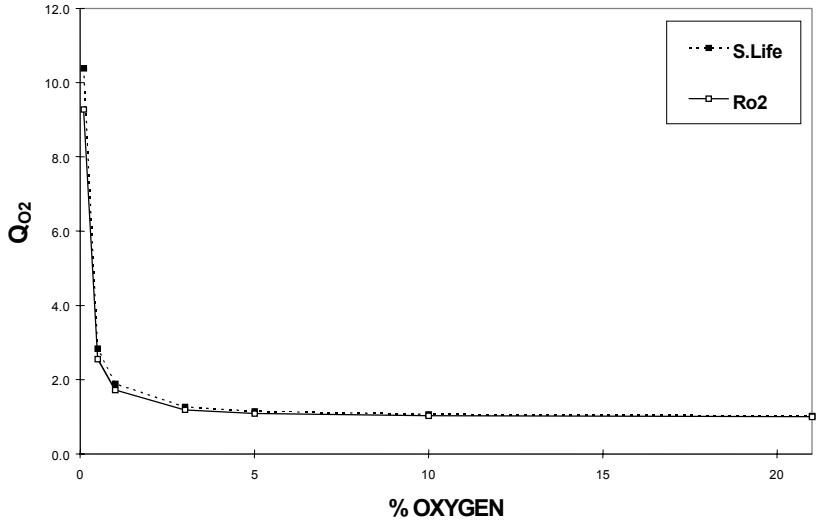

Figure 26. Qo_2 for the shelf life and rate of O_2 uptake of R/G coffee at 22°C and a_w 0.28

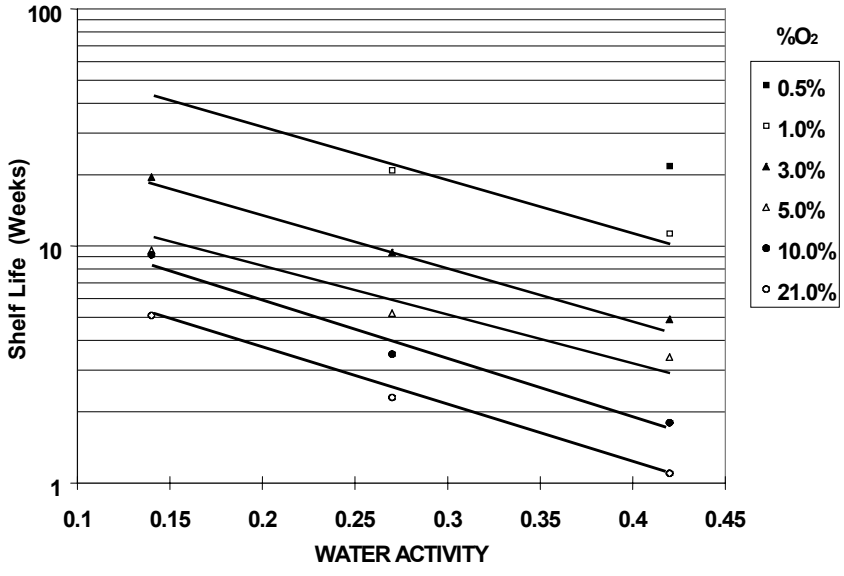

Figure 27. Sensory shelf life of R/G coffee at 22°C vs. water activity at the different oxygen levels tested

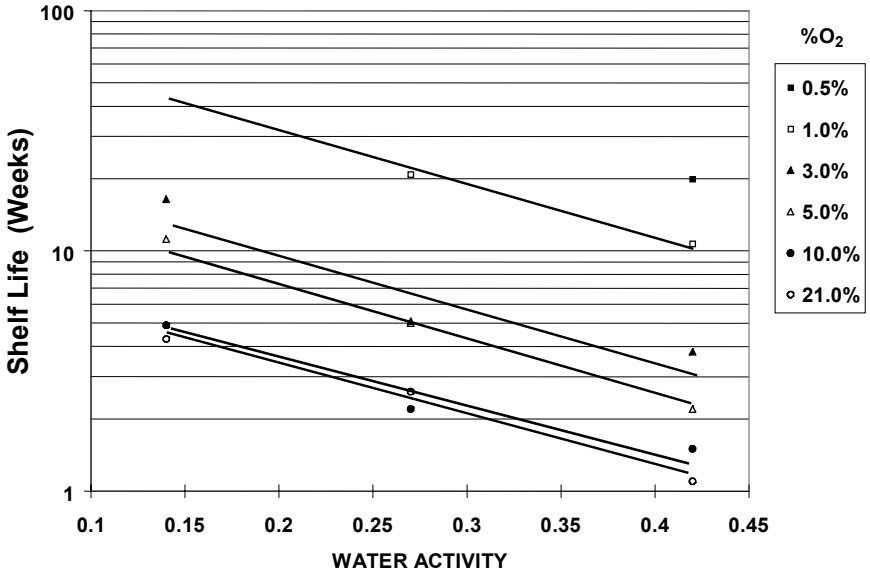

Figure 28. Sensory shelf life of R/G coffee at 35°C vs. water activity at the different oxygen levels tested

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Changes in Roasted Coffee Aroma during Storage – Influence of the Packaging

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SUMMARY

Sensory investigations revealed that after 12 weeks of storage at 20°C the aroma of a bag-stored roasted coffee powder differed significantly from the reference being stored in a can. The aroma of the bag-stored coffee exhibited a clear off-flavour which was described as musty. By application of aroma extract dilution analyses and gas chromatography/ olfactometry of headspace samples, eight odorants were identified having much lower intensities in this coffee. Quantification of the 4 odour-active aldehydes, the 2 α-dicarbonyls and the two thiols showed distinct lower concentrations in the bag-stored coffee. Model experiments, in which coffee was stored under argon or oxygen, respectively, clearly showed the stabilising effect of argon on the eight flavour compounds, whereas oxygen led to their drastic decrease.

INTRODUCTION

Besides the stimulating effect, the popularity of freshly roasted coffee is based on its unique and pleasant aroma. Therefore, many researchers have been interested since 170 years in objectifying this important quality criterion. As a result, more than 800 volatile compounds with a wide variety of functional groups are known to date (Nijssen et al., 1996). Investigations, performed in the 1990s have, however, demonstrated that only a limited number of volatiles contribute to the characteristic flavour of coffee powder. The identification of potent odorants by aroma extract dilution analyses (AEDA) (Holscher W et al., 1990; Blank et al., 1992) and gas chromatography of headspace samples (GCO-H) (Holscher et al., 1992; Semmelroch et al., 1995), quantification of odorants by stable isotope dilution assays (SIDA), a calculation of their odour activity values (Semmelroch et al., 1995; Semmelroch et al., 1996) and, finally, the reconstitution of coffee aroma (Czerny et al., 1999; Mayer et al., 2000) revealed 25 (coffee beverage) and 28 (coffee powder) compounds as the most odour-active components representing the aroma of fresh coffee products. Among these compounds, 2-furfurylthiol was confirmed as the most outstanding odorant in the flavour of the beverage as well as that of the powder.

The aroma of freshly roasted coffee is not stable and can only be maintained during a short period. Based on the knowledge of coffee odorants, it can be assumed that a decrease of aroma quality during storage is correlated with a disturbance of the odorants balance. Reasons for this effect may be the formation or the uptake of new “off-odorants” and/or a loss of odorants by evaporation or chemical reactions (e.g. by oxidation). To preserve coffee freshness over longer periods, coffee powders are stored in packaging. However, also the aroma of packed coffee is negatively affected during storage (Radtke-Granze et al., 1981; Holscher et al., 1992).

Since little is known about the influence of packaging on composition and concentration changes of coffee odorants during storage, the aim of the present study was (i) to test different types of packaging with regard to stability of coffee freshness and (ii) to identify odorants being responsible for staling.

METHODS

Freshly roasted coffee of one batch was packed under nitrogen into cans as well as into different types of bags. The samples were stored at room temperature up to 20 weeks. The aroma stability was tested by triangular tests in which the overall aroma of the bag-stored coffees was compared with can-stored coffee as the reference. The significance of the results was evaluated according to Jellinek (Jellinek, 1995). Model experiments were performed by storing freshly roasted coffee for 4 weeks either at +40°C under oxygen or at –30°C under argon in glass vessels.

Potent coffee odorants were screened by AEDA and GCO-H (Schieberle, 1995) and selected odorants were quantified by SIDA as reported recently (Mayer et al., 1999).

RESULTS AND DISCUSSION

The aroma stability of bag-stored coffees was assessed by sensory evaluation during a period of several weeks by triangular tests. Can-stored coffee (CSC) was chosen as reference because this packaging was found in preliminary experiments to have the lowest influence on coffee aroma during storage at different temperatures (data not shown). Within the first ten weeks, the aroma of the bag-stored coffees and CSC remained the same. However, from the 12^{th} week on, the aroma of the coffee stored in the packaging, consisting of an aluminium vacuum metallized polyethylene terephthalate foil laminated to a polyethylene foil (BSC), differed significantly ($\alpha < 0.01$) from the reference coffee (Figure 1). BSC was affected by an off-flavour which persisted until the end of the storage (20 weeks). The aroma of BSC coffee was then described as musty without any fresh coffee note. No significant changes in the aromas were detected of other bag-stored coffees.

To identify the compounds responsible for the off-flavor, aroma extracts of BSC and CSC coffees were investigated by AEDA. A comparison of the data showed that no off-flavor compound was generated in BSC, but the intensities of 2-furfurylthiol, 3-methyl-2-buten-1-thiol and methional were distinctly lower compared to the CSC (Table 1). The same result was obtained by GCO-H analyses of both coffees (Table 2). Additionally, methylpropanal, 2- and 3-methylbutanal, 2,3-butanedione and 2,3-pentanedione were identified as odorants having much lower FD-factors in BSC in comparison to CSC.

Quantification of the 8 odorants given in Table 2 confirmed the results. Their concentrations were much lower in BSC than in CSC (Table 3). The differences in methylpropanal, 2- and 3-methyl-butanal, 2,3-butanedione and 2,3-pentanedione suggested previously as contributors to coffee freshness (Holscher et al., 1992) amounted to a factor of 3-4. The findings of Tressl and Silwar (Tressl and Silwar, 1981) who detected a strong increase of 2-furfurylthiol during a storage of 7 weeks could not be confirmed. Contrary, the concentration of the thiol known as one of the most important coffee odorants (Czerny et al., 1999) was 5-times lower in BSC. Additionally, 3-methyl-2-buten-1-thiol and methional were also degraded in the bag-stored coffee. The results clearly demonstrate that the packaging-induced off-flavour is caused by the decrease of the eight odorants in BSC in comparison to CSC.

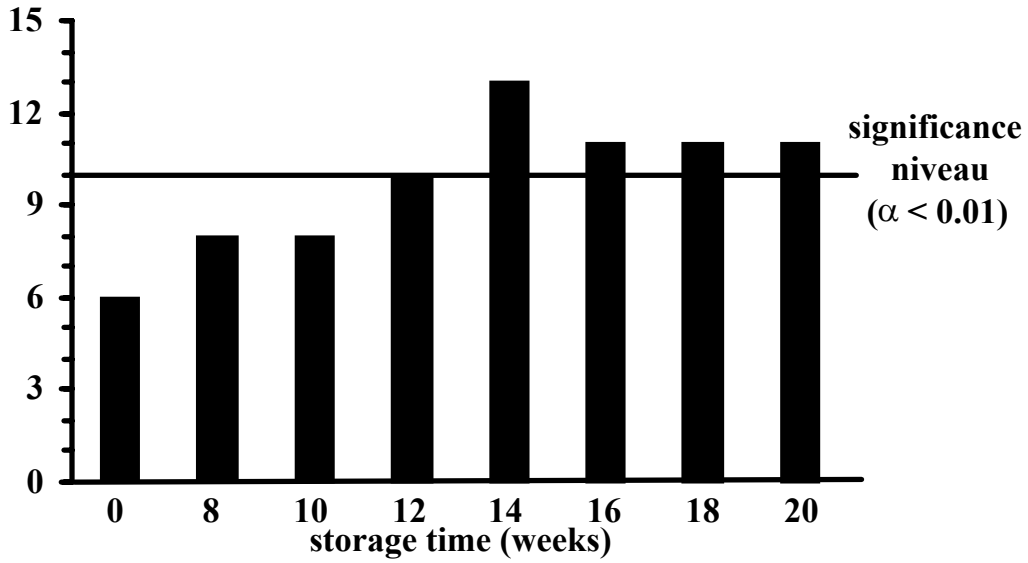

Figure 1. Results of triangular tests: number of panelists (out of 15) who recognized a difference in the overall aroma of a bag-stored coffee (BSC) in comparison to can-stored coffee (CSC)

Table 1. Potent odorants showing significant differences[a] in their odour intensities in stored coffees as evaluated by aroma extract dilution analyses

odorant	odor quality	FD-factor in	
		CSC[b]	BSC[b]
2-furfurylthiol	coffee-like	32	2
3-methyl-2-buten-1-thiol	sulphurous	8	< 1
methional	cooked potato-like	4	< 1

[a]Odorants with significant flavor intensities (differences ≥ 3 dilution steps) are listed.
[b]Can-stored coffee (CSC) and bag-stored coffee (BSC) were analysed after storage for 20 weeks at +20°C.

In the literature, oxygen has been suggested as a source for coffee staling (Holscher et al., 1992; Radtke-Granze et al., 1981; Holscher et al., 1992). To get an deeper insight into the mechanism of aroma damage by oxygen, model experiments were performed using coffee which had been stored under forced conditions at + 40°C in the presence of oxygen. After a storage period of 4 weeks, the oxygen-stored coffee (OSC) exhibited a clear stale flavor which was comparable to that of BSC. Quantification of the off-flavor compounds identified in OSC demonstrated, that the low concentrations of all odorants were nearly identical with the concentration levels in BSC (Table 4).

The influence of oxygen on coffee staling is obvious from the comparison of the OSC data and the odorant concentrations in a coffee, which had been stored under argon (ASC). In particular, 2-furfurylthiol, methional, 2,3-butanedione and 2,3-pentanedione were significantly degraded in the presence of oxygen (Table 4), but also distinct losses of Strecker-aldehydes and 3-methyl-2-buten-1-thiol were observed.

Table 2. Potent odorants showing significant differences[a] in their odour intensities of in two stored coffees as evaluated by gas chromatography/olfactometry of headspace samples

odorant	odor quality	FD-factor in	
		CSC[b]	BSC[b]
methylpropanal	malty	64	2
3-methylbutanal	malty	64	< 1
2,3-butanedione	buttery	32	2
2,3-pentanedione	buttery	16	2
2-methylbutanal	malty	8	< 1
3-methyl-2-buten-1-thiol	sulphurous	8	< 1
methional	cooked potato-like	8	< 1
2-furfurylthiol	coffee-like	4	< 1

[a]Odorants with significant odour intensities (differences ≥ 3 dilution steps) are listed. [b]Can-stored coffee (CSC) and bag-stored coffee (BSC) were analysed after storage at +20°C for 20 weeks

Table 3. Concentrations of selected potent odorants in stored coffees

odorant	concentration (µg/kg)	
	CSC[a]	BSC[a]
methylpropanal	12900	4300
3-methylbutanal	8100	2100
2,3-butanedione	19100	6500
2,3-pentanedione	23800	6500
2-methylbutanal	8800	2300
3-methyl-2-buten-1-thiol	15	4.4
methional	51	5.6
2-furfurylthiol	40	7.9

[a]Can-stored coffee (CSC) and bag-stored coffee (BSC) was analysed after storage for 20 weeks at +20°C

The losses of the odorants may be explained by investigations focussing on degradation pathways of potent aroma compounds. A new Stecker-reaction was recently proposed in which α-dicarbonyls and amino acids were shown to mainly yield the corresponding Strecker acid in the presence of oxygen (Hofmann et al., 2000). Furthermore, 2-furfurylthiol was found to be degraded in a Fenton type reaction into volatile and non-volatile dimers and oligomers. It was assumed that other odour-active thiols may react in the same way (Blank et al., 2000).

CONCLUSION

The results indicate that certain packaging may induce an off-flavour in coffee and, in consequence, can shorten the shelf-life of coffee. The staling was found to be a result of a degradation of important odorants, e.g. 2-furfurylthiol, rather than the formation of off-odorants. These compounds can, therefore, be used as indicators for objectifying coffee staling. One reason for odorant degradation is oxygen permeating into the packaging. Possibilities for this permeation seem to be a high permeability of the packaging foil and, also, a bad sealing of the bags during the packing process.

Table 4. Concentrations of odorants in coffees stored under oxygen (OSC) or argon (ASC) in comparison to the bag-stored coffee (BSC)

odorant	concentration (µg/kg)		
	BSC[a]	OSC[b]	ASC[c]
methylpropanal	4300	6900	12200
3-methylbutanal	2100	5400	8900
2,3-butanedione	6500	7300	22500
2,3-pentanedione	6500	9200	24000
2-methylbutanal	2300	5300	8700
3-methyl-2-buten-1-thiol	4.4	2.8	6.7
methional	5.6	13	102
2-furfurylthiol	7.9	8.8	217

[a]Bag-stored coffee (BSC), stored at +20°C for 20 weeks. [b]Oxygen stored coffee (OSC), stored at +40°C for 4 weeks. [c]Argon stored coffee (ASC), stored at –30°C for 4 weeks

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Staling of Roasted and Ground Coffee at Different Temperatures: Combining Sensory and GC Analysis

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SUMMARY

Temperature conditions are of utmost importance for roasted coffee degradation. An understanding of staling kinetics in ground coffee becomes fundamental for product preservation, being the effect much stronger than in coffee beans. We investigated the rate of staling of roasted and ground coffee after opening the package and storing at room-, fridge- and elevate temperature, focusing mainly on the loss of volatiles. Headspace GC analyses have been performed. Using a statistical approach, a subset of compounds able to discriminate the variable “time” has been selected, thus leading to a chemistry-related predictive model of staling. In parallel, sensory analyses have been carried out by a trained panel, concentrating on the attribute "staleness". In this case a sensory-related predictive model of staling has been studied. The models have been combined to find a correspondence between sensory perception of staleness and aroma amount. Sensory and HS GC results are coherent: they both put into evidence a decrease of the staling rate when the sample is stored in a refrigerator, thus preserving aroma for a longer time. On the contrary, storage at high temperature causes a faster deterioration of the product. The difference is due to the volatile release rate constants, which present considerable variations especially for low boiling sulphur compounds.

RÉSUMÉ

La température exerce une influence importante sur le vieillissement du café rôti. Comprendre les cinétiques du vieillissement du café moulu est fondamental pour la conservation du produit, car cet effet y est beaucoup plus marqué que dans les fèves entières. En nous concentrant sur la perte de substances volatiles, nous avons testé la vitesse de vieillissement d’un café torréfié et moulu, après l’ouverture de l’emballage et sa conservation à diverses températures (ambiante, réfrigérateur et élevée). Nous avons effectué des analyses par chromatographie à gaz sur l’espace de tête et, en utilisant une approche statistique, nous avons sélectionné un sous-ensemble de composés capables de discriminer le temps de stockage. Ceci nous a permis d’obtenir un modèle prédictif du vieillissement sur base chimique. Parallèlement, un modèle de vieillissement fondé sur l’analyse sensorielle a été établi à l’aide d’un panel de dégustateurs bien entraînés, en se concentrant sur l’attribut «vieux». Les modèles ont étés comparés pour trouver des correspondances entre la perception du «échu» et la quantité d’arôme. Les données sensorielles et analytiques sont cohérentes: les deux mettent en évidence une diminution de la vitesse de vieillissement quand l’échantillon est conservé au réfrigérateur, préservant ainsi l’arôme plus longtemps. Au contraire, la conservation de l’échantillon à une température élevée cause une détérioration plus rapide du produit. La différence est due à la perte en substances volatiles, en particulier des composés sulfurés à faible température d’ébullition.

INTRODUCTION

Coffee staling can be described as a change in the aroma profile due to degradation processes like loss of low boiling compounds and oxidation reactions. A lot of work has been carried out in the last 30 years, leading to some common and accepted conclusions on the main causes of the phenomenon. Storage temperature, penetration of oxygen in the package and loss of volatiles through diffusion are crucial for rancidity (Noomen, 1979; Vitzthum and Werkhoff, 1979; Cros et al., 1980; Kallio et al., 1990; Steinhart and Holscher, 1991; Leino et al., 1992). Moreover, moisture can accelerate the staling process (Clinton, 1980; Hinman, 1991; Nicoli et al., 1993; Cardelli and Labuza, PDF).

However most of the work that has been done so far was on coffee beans. Since the effect is much stronger in ground coffee than in beans (Vitzthum and Werkhoff, 1979; Arrackal and Lehmann, 1979), an understanding of staling kinetics in ground coffee becomes fundamental for product preservation. Hinman (1991) worked out a broad investigation on ground coffee, examining the role and impact of temperature, humidity and whole bean free-fall density on staling process through the measurement of oxygen consumption. Also Nicoli (1993) analysed beans and ground coffee in parallel, finding different constants of volatile release. From her results it is possible to infer a difference of one order of magnitude in the first order rate constants for the loss of volatiles, between beans and ground coffee.

As far as temperature is regarded Vitzthum (1979) states that - within some weeks - frozen beans do not lose aroma (1% loss) and can therefore be used as a reference, while the same sample at room temperature presents a loss of roughly 60%. Hinman (1991) extracts the kinetic constant as a function of temperature, considering three cases (31°C, 37°C, 46°C). He shows a linear trend of ln k with 1000/T (°K), with a multiplying constant -7 (i.e. the kinetic constant k increases with and exponential factor of 0.07 per °C). Nicoli (1993) worked at three different temperatures (4°C, 25°C, 40°C) showing a linear increasing trend of the kinetic constant with temperature. Many freshness indices have been proposed in order to give a semi-quantitative value to the results. Various authors (Vitzthum and Werkhoff, 1979; Arrackal and Lehmann, 1979; Vitzthum and Werkhoff, 1978) asserted that M/B ratio (2-methylfuran and 2-butanone), and M/M ratio (methanol and 2-methylfuran) are related to freshness, showing the correlation with sensory analysis to be good. Steinhart and Holscher (1991) used 13 compounds for discriminating product ageing. Kallio (1990) used a series of ratios derived from compounds that increase or decrease with storage time. All these approaches aim to use few compounds to describe the system's behaviour, and unfortunately these indices are strictly connected to the specific problem, as stressed by many authors.

Sensory analysis has been rarely used to give a quantitative response; a correlation has been usually searched to confirm a chemical trend. Sensory data have been modelled (Cros et al., 1980; Hinman, 1991; Arrackal and Lehmann, 1979; Cardelli and Labuza) to individuate an acceptability threshold as a function of air exposition and link it to product shelf-life.

In this paper we present some results of our research, where the rate of staling of roasted and ground coffee was studied. After opening, the packages were left in direct contact with air at three different temperatures, i.e. 4°C, 25°C and 40°C. We faced part of the staling problem, namely the loss of the most volatile part of aroma, on the analytical point of view, through headspace GC analyses of coffee powder. The most useful compounds were detected using discriminant analysis (Mardia et al., 1979) over storage time, in order to build a model of the volatile loss profile. In parallel, sensory analyses (i.e. sniffing tests on powder and cup tasting of espresso preparation) have been performed to establish a relationship with the analytical data.

MATERIALS AND METHODS

Coffee samples

All samples are standard production 250 g ground coffee cans of a 100% arabica coffee blend with medium roasting degree. The cans have been sampled at the end of the production line and stored at 25°C for about 3 weeks. This is necessary for the product stabilisation. Since the cans are pressurised with inert gas and remain closed, there is virtually no loss of volatiles during this period. At the beginning of the analysis campaign, the batch has been divided into three parts. The cans have been opened (time 0 in all following graphs) and stored at different thermal conditions, i.e. at 4°C (4±1°C), 25°C (T=23±2°C, rh=57±3) and 40°C (T=40.0±0.5°C), without re-closing the cans. In order to ensure proper equilibration, the products to be stored in the refrigerator or the oven have been put at the proper temperature one day before opening.

Sample preparation

Coffee powder was stored in the opened cans at different temperatures (4°C, 25°C and 40°C). Just before the analysis, 5 g of coffee powder were taken out, put into a 20 ml headspace vial and closed immediately. Opened cans were sampled at definite time steps, namely: 0 h (just opened), 2 h, 6 h, 24 h, 4 d, 10 d, 22 d, 25 d, 35 d, 45 d, 60 d, 90 d. For each temperature and time step, 5 analyses on different samples were performed in order to minimise possible procedure and analysis errors, and have sufficient statistics for data treatment.

Headspace gas chromatography

Headspace analyses of coffee powder were performed using a HP 6890 Gas chromatograph with a HP 7694 Headspace sampler. Samples were conditioned at 60°C for 30 minutes before analysis. The GC was equipped with a Gerstel cooled injection system that allowed to cryofocus (Holscher and Steinhart, 1992) the samples at –120°C during injection of 1.5 ml, so that even very volatile compounds could be analysed. Headspace Instrument parameters: Loop: 100°C, Transfer Line: 100°C, Oven: 60°C. GC-parameters: Column: DB-5, 60 m, 0.53 mm i.d., film thickness 5 μm. Gerstel Cooled injection system: Start temperature: -120°C, Initial time 2.05 min, 12°C/min to 280°C held for 8 min. GC temperature program: 35°C, 9 min isotherm then 10°C/min to 180°C and then 40°C/min to 240°C held for 10 min. Constant carrier gas (Helium) flow was set to 3 ml/min for 35 min, then to 4 ml/min for 4 min of post time at 270°C.

Parallel detection was employed using a Flame Ionisation Detector and a Pulsed Flame Photometric Detector for analysis of sulphur compounds.

Sensory analysis

Sensory analysis, which has been conducted in parallel with GC data acquisition, has been carried out by means of powder sniffing and espresso cup tasting. A trained panel of 9 assessors evaluated the samples and compiled an evaluation sheet. Espresso coffee samples have been prepared using a professional espresso coffee machine and a standard procedure, weighing 13 grams of coffee powder and brewing two cups of espresso (25±2 cc each cup) per sample. Blind cup tasting was realised using a standard reference in comparison to other samples, with different ageing. The reference was a freshly opened product belonging to the same lot of the samples. In this way the comparison could always be done with a fresh product with the same history and without any thermal stress.

The assessors were asked to evaluate stale perception and aroma quantity through powder sniffing, and sample-reference recognition as well as stale perception through espresso cup tasting. Sensory data were obtained by compiling a sheet with a 0-5 discrete scale. The evaluation sheets were then statistically treated, translating comparative sensory judgement into a quantification of sample freshness versus storage time.

Data processing

All data have been statistically treated with SYSTAT® 8.0 scripts, written by the authors, and run on a Pentium III 650 MHz 256 Mb RAM, on Windows NT 4.

RESULTS AND DISCUSSION

GC profiles

Data pre-processing

Since chromatograms have intrinsic variance due to procedure errors (sampling, injecting, instrumental error) and batch errors, a preprocessing procedure has been performed to reduce variability in the data sets. Starting with five experimental points for each timestep, we followed the procedure:

1. For each time step the overall sum of volatiles has been calculated and compared.
2. Outliers have been identified and discarded using Dixon's Q with p=0.05 (Porretta, 2000).
3. Then mean values and relative (percentage) standard deviations have been calculated and plots have been performed.
4. Compounds with too many 0 values or too high variance (according to Chauvenet criterion) have been discarded (Taylor, 1982).
5. A new check has been done, leading to Step 1.

The purged data underwent a successive statistical analysis, which led to the following results:

Freshness indice

Following the literature, several freshness indices have been evaluated. MB ratio as a function of time is plotted in Figure 1: the lower the temperature, the higher the freshness index in the first day. Afterwards experimental points are superimposed. We can therefore state that, MB ratio is not a useful index after the first hours of ageing in air, in case of ground coffee with opened and not re-closed package.

Other indices have been calculated, such as Vitzthum's M/M ratio, Kallio's But/Met (Butanedione and 2-Methylfurane), but without any significant peculiar trend.

Evolution of the amount of volatiles

A discriminant analysis has been performed on the three data sets in order to find which compounds are able to separate the evolution of volatiles with time at the different temperatures. In this way a subset of few compounds has been selected (Table 1) to model the profiles of the volatiles. The statistically based subset contains over 75% of the key compounds found by Holscher and Steinhart (1992).

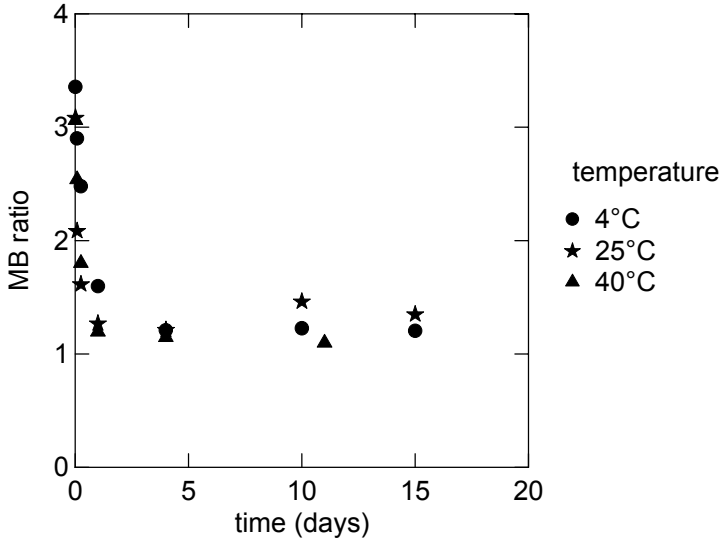

Figure 1. Changes in MB ratio of ground coffee stored at 4, 25, 40°C in presence of air

Discriminant analysis, provides linear functions which best separate cases into some predefined groups by imposing the number of classes, and trying to maximise the inter-groups distances and minimise the intra-group ones. The Mahalanobis distances between cases to be classified and the center of each predefined group are calculated. The closer a case is to the centre of one group, the more likely is to be classified to that group.

The discriminant functions have the following form:

$$f(k) = \alpha_k x_1 + \beta_k x_2 + \ldots + \psi_k x_{n-1} + \omega_k x_n$$

where f is a weighed linear composite score (Powers and Ware, 1986).

Table 1. list of the most discriminant compounds

COS, H_2S, Methanol, Acetaldehyde, Methanethiol, Methyl formiate, Ethanol, Acetone, Propanal, Furane, Isoprene, DMS, Methylacetate, CS_2, 2-Methylpropanal, Diacetyl, 2-Butanone, 2-Methylfurane, Ethylacetate, Isovaleraldehyde, 2-Methylbutanal, 3-Methylfuranthiol, 2,3-Pentanedione, Methional, Furfuryl mercaptan, FMS, MMBF, other 5 n.i. compounds

When discriminant analysis is performed on time as a grouping parameter for each data set, we find the following results, visible in Figures 2a,b,c.

At 4°C there is one cluster with experimental points taken within the first day, a second elongated one with points from 4 up to 25 days. A third cluster consists of points from 35 days on. As far as 25°C samples are regarded, we still find three clusters, but the division is different. The first one consists again of samples with less than one day; in the second one, only samples from 6 to 15 days are present, while the third one consists of samples older than 25 days (while in the case of 4°C samples 25 days old still belong to the second cluster). In case of 40°C the third cluster begins already after the 10th day (even the 4^{th} day experimental point is close to that cluster).

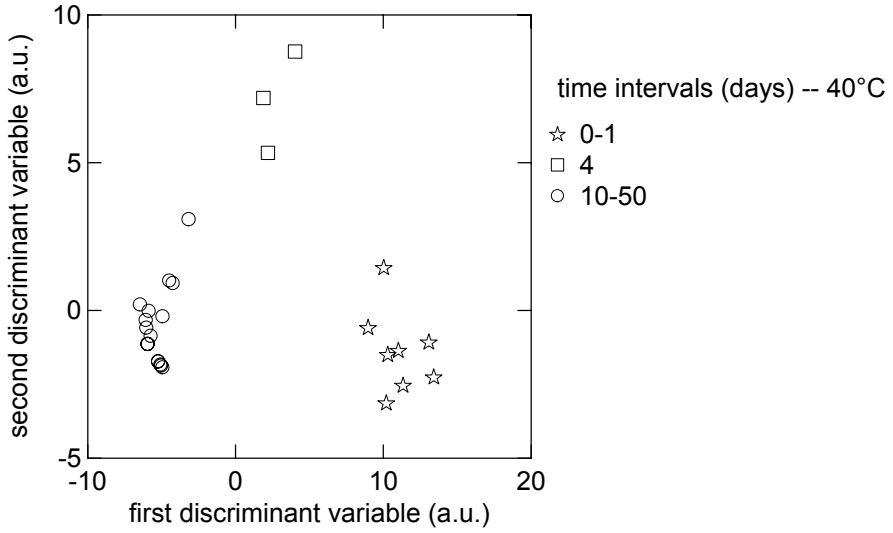

Figure 2a. Discriminant analysis on time for ground coffee at 40°C. 3 clusters can be noticed, the third one (rancid) starting already at 10 days

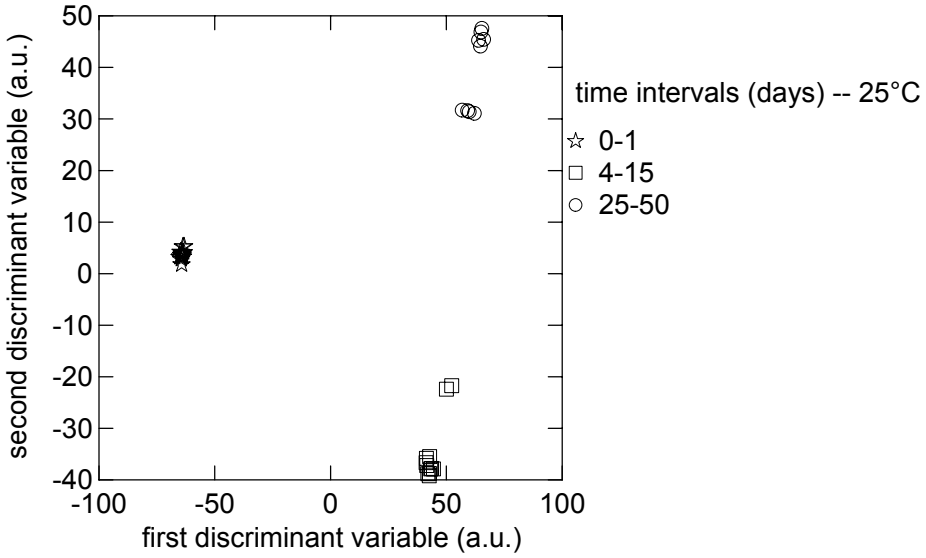

Figure 2b. Discriminant analysis on time for ground coffee at 25°C

The most discriminant compounds have been then summed, after scaling sulphur ones with a log transformation. Results are reported in Figure 3. It can be noticed that profiles show a different rate of volatile loss at different temperatures. The rate is much lower at 4°C and higher at 40°C.

There is a quick decrease of sulphur compounds at 40°C, while the slope is much smoother at 4°C, at least up to the 10th day. The trend of the most discriminative compounds is self-explaining. The 4°C profile is almost constant up to the 10th day and much higher in volatiles compared to the other two cases. The curve at 40°C already starts at a lower point and has a very steep slope, especially in the first hours.

Table 2 gives us the comparison of the relative loss of sulphur compounds. The loss of volatiles is about 10% after 1 day, 25% after 25 days and 75% after 35 days at 4°C. At 25°C the loss increases to about 40% after 1 day, over 75% after 25 days and 90% after 35 days. At

40°C the loss is more than 50% after just 6 hours and it is almost 70% after 4 days; after 25 days there is almost no low boiling sulphur compound left.

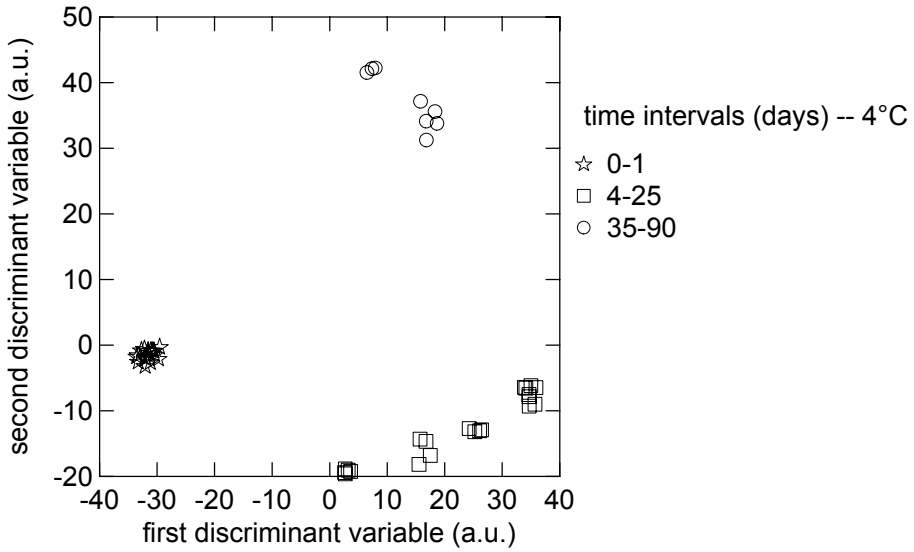

Figure 2c. Discriminant analysis on time for ground coffee at 4°C. Here the thid cluster starts after 35 days

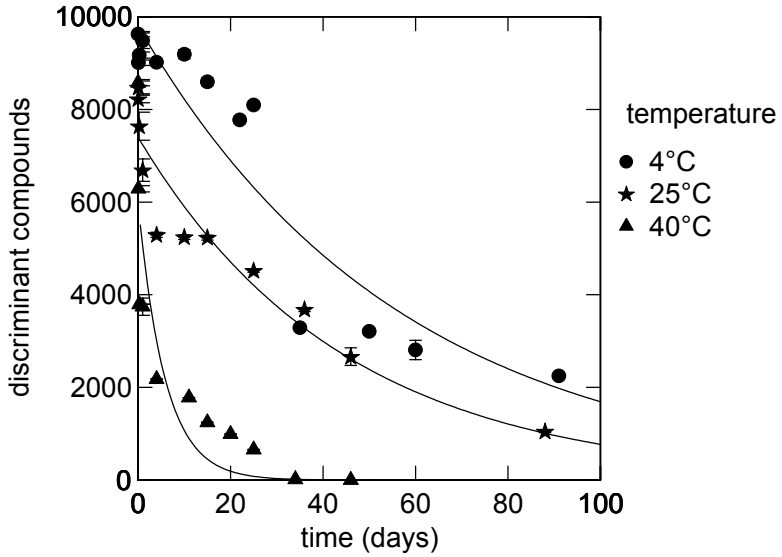

Figure 3. volatiles loss profile of ground coffee at three different temperatures: 4°, 25° and 40°C. Total amount counts discriminant compounds detected with both FID and Sulphur detectors

As far as the FID is concerned (Table 3) the loss of volatiles is about 10% after 1 day, 25% after 20 days and 75% after 35 days at 4°C. The results obtained by the sulphur detector are very similar.

Compared to the loss of volatiles at room temperature (25°C), the loss is four times higher (40%) after 1 day and more than 60% after the 4th day. After 35 days it is similar to the 4°C situation. At 40°C the loss is higher than 70% after just 6 hours and is higher than 90% after 4 days.

Table 2. Sulphur detector percentual volatile loss

Time (days)	4 °C % change	25 °C % change	40 °C % change
0.08	-5.0±0.5	-13.0±0.5	-23±2
0.25	-3.5±0.5	-25.0 ±1	-54±3
1	-13.3±0.3	-41.4±3	-56±3
4	-24.0±0.5	-52.0±2	-69±3
10	-24±1	-63.6±2	-77±2
22	-39±5	-76±6	-90±5
25	-33±3	-77±2	-94±5
35	-78±2	-93±3	-100
45	-77±3	-100	
60	-81±5		
90	-96±3		

Table 3. FID detector relative volatile loss

Time (days)	4 °C % change	25 °C % change	40 °C % change
0.08	-11±1	-10.5±0.5	-6±3
0.25	-10±2	-12±3	-70±5
1	-10±1	-39±2	-78±2
4	-14±5	-43±1	-91±3
10	-12±3	-65±3	-97±5
15	-13±4	-68±3	-97±3
22	-25±3	-77±4	-97±2
25	-26±2	-83±3	-92±3
35	-76±3	-84±4	-99±4
45	-74±5	-88±5	-100
60	-78±5	-90±4	
90	-81±4	-95±3	

Data sets have been finally fitted with an exponential model $y=A^* e^{-(t/\square)}$ which led to the following constants (Table 4).

Table 4. Parameters for the volatile loss model at three different temperatures. τ is the time constant, r^2 is the correlation coefficient

Constants	4 °C	25 °C	40 °C
τ	56.22	43.82	5.02
r^2	0.88	0.91	0.81

Results are in qualitative agreement with Nicoli (1993): the half-life of the considered low boiling volatiles is approximately 40, 30 and 4 days at 4°C, 25°C and 40°C respectively.

Sensory profiles

Data processing

Evaluations of non-recognised samples have been discarded when these were older than 1 day. Otherwise they have been set at 0 (i.e. not separable from a freshly opened sample).

In order to take into account the fact that assessors tend to increase differences when products are similar, and to minimise them when they are very different, values of samples younger

than 1 day have been corrected with a scaling factor. All data have then been fitted with the same model used for the GC data: $y=A^* e^{-(t/\tau)}$.

In the following Table 5, τ coefficients are given. It is inversely proportional to the staling rate. The value y=2.5 corresponds to "half freshness".

Table 5. Parameters for cup-tasting model at three different temperatures. τ is the time constant, r^2 is the correlation coefficient

Constants	4°C	25°C	40°C
τ	41.1	14.2	3.8
r^2	0.86	0.85	0.90
t(y=2.5)	28	10	3

As Figure 4 suggests, sensory analysis results are strongly affected by the temperature. The staling rate is actually almost 3 times higher when passing from fridge to room temperature and 10 times higher when reaching elevate temperatures. Sensory results are therefore coherent with HS GC ones: they both put into evidence a decrease of the staling rate when the sample is stored in a refrigerator, thus preserving aroma for a longer time, while storage at high temperature causes a faster deterioration of the product.

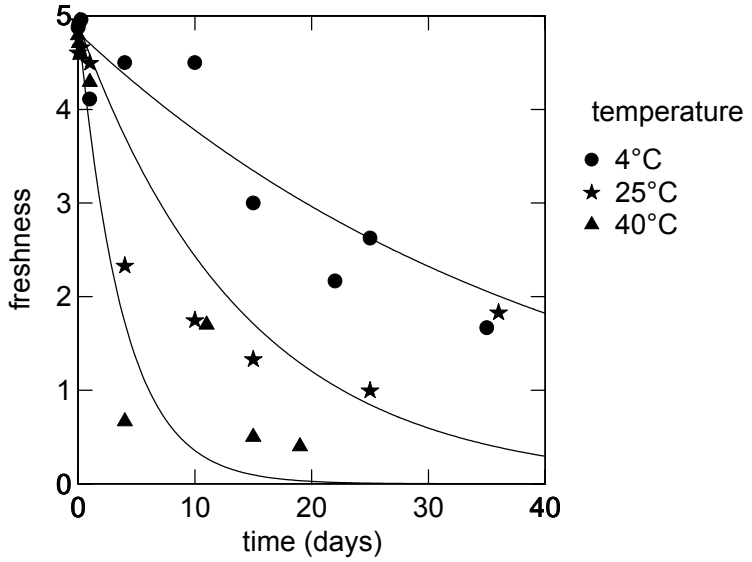

Figure 4. Ground coffee freshness profile at three different temperatures

Combining GC and sensory profiles

GC or sensory results alone are of low practical interest for product optimisation. In fact, GC results are interesting for understanding volatile loss kinetics, but are far from being directly connected to the quality in the cup. On the other hand, sensory analysis alone is time/people consuming. Moreover the panel, on the long run, can change its perception thresholds. Combining the two types of analysis becomes thus a powerful tool for getting information from both aspects, and for extending the knowledge for improving the characteristics of the product.

Comments attached to sensory evaluation sheets allowed us to set a first freshness threshold, for the perception of stale and a second one for rancidity. The thresholds have been set when 50% of the panellists found the product "old" or "unacceptable" (Cardelli and Labuza, PDF).

The values of these thresholds allowed us to calculate the corresponding day on the sensory profile. The amount of volatiles of the corresponding day has been then found on the GC graph. In such a way, a relationship between instrumental and sensory analysis has been established. This approach can be used as a tool when no panel is available. We have to remark, however, that it is quantitative reliable only with a certain blend and roasting degree. Moreover sensory analysis is performed by means of espresso tasting, i.e. the complete beverage including volatile as well as non-volatile compounds, and concentrating on the perception of stale. On the contrary, by analysing the headspace of coffee powder with our method, heavy compounds and non-volatiles substances are excluded. Therefore there cannot be a biunique correspondence between the thresholds. However, the correlation between the two kinds of analyses exists, and is a useful practical tool for passing from one level to the other, even if it is not a "physico-chemical" one, being the result of coexisting effects: loss of volatiles, and oxydation reactions.

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Instrumental Analysis and Sensory Studies on the Role of Melanoidins in the Aroma Staling of Coffee Brew

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SUMMARY

In order to investigate the chemical mechanisms involved in the rapid aroma staling during warm-keeping of coffee brews, melanoidins isolated from coffee brew were added to a model solution containing 25 aroma compounds in their "natural" concentrations mimicking the aroma of a coffee brew. Sensory analyses revealed that, in particular, the intensity of the roasty-sulfury aroma quality was reduced when melanoidins were present. Comparative aroma dilution analysis on the headspace of aqueous solutions containing the total volatile fraction isolated from coffee brew, alone, or in mixture with melanoidins visualized that the losses of the odorous thiols 2-furfurylthiol (FFT), 3-methyl-2-butenthiol (MBT), 3-mercapto-3-methylbutyl formate (MMBF), 2-methyl-3-furanthiol, and methane thiol are responsible for the aroma change. Quantification by means of stable isotope dilution assays confirmed that these thiols are rapidly degraded with increasing time during warm-keeping of coffee brews in a thermos flask. Quantitative model studies and labelling experiments with [$^2H_2$]-2-furfurylthiol, followed by ^{2}H-NMR and LC/MS spectroscopic detection gave strong evidence that these thiols are covalently bound to pyrazinium ions, the oxidation products of 1,4-bis-(5-amino-5-carboxy-1-pentyl)pyrazinium radical cations, which were recently identified in coffee as key intermediates in roasting-induced melanoidin genesis. Based on model experiments, these pyrazinium compounds were shown to rapidly react with thiols, such as, 2-furfurylthiol, thus giving rise to 2-(2-furyl)methylthio-1,4-dihydropyrazines, bis[2-(2-furyl)methylthio]-1,4-dihydropyrazines and 2-(2-furyl)methylthio-hydroxy-1,4-dihydropyrazines as the primary reaction products. The data suggest that the thiol-binding activity of pyrazinium derivatives in melanoidins undoubtedly contribute to the decrease of the sulfury-roasty odour quality shortly after preparation of the coffee brew.

INTRODUCTION

Besides its stimulatory effect, the consumer for appreciates a freshly prepared coffee brew its pleasing overall aroma. The aroma is, however, not stable, and is rapidly changing after preparation of the coffee brew. Whereas numerous studies have been performed to understand the aroma changes occurring during storage of ground coffee powder (e.g. Holscher and Steinhart, 1992; Grosch et al., 1994), the information available on the rapid aroma staling of coffee beverages are as yet very fragmentary. Heat sterilization, which is usually applied to canned coffee drinks, was found to result in a strong decrease of the roasty, sulfury aroma note of coffee beverages (Kumazawa et al., 1998). Comparative aroma extract dilution analyses before and after heating indicated a strong decrease in the odour-active thiols 2-furfurylthiol and 3-mercapto-3-methylbutyl formate (Kumazawa et al., 1998). Also the manufacturing of instant coffee from coffee brew was reported to cause a drastic decrease of thiols, such as, 2-furfurylthiol, 2-methyl-3-furanthiol and 3-mercapto-3-methylbutyl formate, whereas odour-active phenols and pyrazines did not show major changes (Semmelroch and Grosch, 1995). Based on model experiments with odour-active disulfides and egg albumin,

Mottram et al. (1996) proposed that the decrease of thiols and disulfides in foods might be the result of an interchange with sulfhydryl and disulfide groups of proteins. It is, however, as yet unclear whether the aroma changes in coffee brews are due to similar reactions involving the macromolecular melanoidins bearing various functional groups. The following investigation was aimed at studying the chemical mechanisms which might be involved in the rapid aroma staling during warm-keeping of freshly brewed coffee beverages.

MATERIALS AND METHODS

A biomimetic model of coffee brew aroma containing 25 key odorants in their "natural" concentrations was prepared as reported by Mayer et al. (2000). The 1,4-diethylpyrazinium diquaternary salt was synthesized by perethylation of pyrazine with triethyloxonium tetrafluoroborate (Hofmann et al., 1999a).

Isolation of the volatile fraction from coffee brew

Coffee powder (Coffea arabica; var. Caturra; medium roasted) was prepared by grinding the beans in liquid nitrogen. A brew was prepared by percolation of coffee powder with boiling water (50 g powder/L water). Using the SAFE device developed recently (Engel et al., 1999), an aqueous fraction of the volatiles were isolated from the brew (100 mL) by high-vacuum distillation.

Isolation of coffee melanoidins

Coffee powder (50 g) was extracted with hot tap water (1 L, 80-90°C), the aqueous solution was defatted by extraction with dichloromethane and, finally, concentrated by freeze-drying (yield: 12.5 g). Aliquots of 1.0 g of this material were dissolved in distilled water (20 mL) and either fractionated by ultrafiltration (Diaflo PM, Amicon, Witten) with an MWCO of 3000 Da to obtain melanoidins (0.35 g) after freeze-drying, or separated by gel permeation chromatography on Sephadex G-25 fine (75 x 5 cm i.d.; Pharmacia, Uppsala, Sweden) using water as the eluent (4 mL/min). Monitoring the effluent at 420 nm, the four fractions displayed in Figure 4A were collected, and freeze-dried (Hofmann et al., 2001a).

Static headspace analysis

The aqueous aroma distillate (10 mL) alone, or mixed with coffee melanoidins (125 mg), respectively, was pipetted into a septum-sealed vessel (240 mL total volume) and was equilibrated for 30 min at 45°C. Stepwise decreased headspace volumes (25 to 0.2 mL) were injected onto an HRGC column (60 m x 0.32 mm fused silica capillary; RTX-5: methyl polysiloxane-5% phenyl, film thickness: 3 µm) installed in a gas chromatograph Type CP 9001 (Chrompack, Frankfurt, Germany) and connected to a sniffing device. Separation was done by increasing the oven temperature from 30°C to 230°C at a rate of 6°C per min.

Determination of headspace concentrations of thiols

A solution of 3-methyl-2-buten-1-thiol (400 µg), 2-furfurylthiol (600 µg/L) and 3-mercapto-3-methylbutylformate (500 µg) dissolved in phosphate buffer (10 mL; 0.1 Mol/L; pH 6.0) alone, or mixed with melanoidins (125 mg), respectively, were pipetted into a septum-sealed vessel (240 mL total volume) and were equilibrated at 30°C. A comparison of the amounts of thiols present in the headspace before and after melanoidin addition was performed.

Quantification of thiols and disulfides by stable isotope dilution analysis

2-Furfurylthiol, 3-methyl-2-butenthiol, 3-mercato-3-methylbutyl formate and bis(2-furfuryl) disulfide were quantified in coffee brews either freshly prepared, or kept warm in a thermo flask, and in binary mixtures containing thiols and melanoidins, respectively, using $[^2H]_2$-2-furfurylthiol, $[^2H]_8$-3-methyl-2-butenthiol, $[^2H]_6$-3-mercato-3-methylbutyl formate and $[^2H]_4$-bis(2-furfuryl)disulfide as the internal standards (Mayer et al., 2000). Quantification was performed by mass chromatography using an ion trap detector (ITD 800, Finnigan, Bremen, Germany) running in the chemical ionisation mode with methanol as the reactant gas.

Spectroscopic measurements

GC/MS was performed using a CP 9001 gas chromatograph (Chrompack, Frankfurt, Germany) equipped with the fused silica capillary CP-WAX 52 CB (25 m × 0.32 mm, 1.2 μm film thickness, Chrompack) and coupled with the mass spectrometer Incos XL (Finnigan, Bremen, Germany). LC/MS spectroscopy was performed with a LCQ-MS (Finnigan MAT GmbH, Bremen, Germany) using electrospray ionisation (ESI). EPR and ^{2}H-NMR spectra were recorded on an ESP 300 spectrometer (Bruker, Rheinstetten, Germany) and an AMX 500 spectrometer (Bruker, Rheinstetten, Germany).

Results and Discussion

In order to investigate how the overall aroma of a coffee beverage is changing during warm-keeping, comparative sensory analyses were performed on coffee brews freshly prepared or stored in a thermos flask for 60 or 210 min, respectively. A drastic decrease of the intensity of the sulfury-roasty odour quality was observed with increasing storage time, e.g. on a scale from 0 (not detectable) to 3 (strong) the intensity dropped from 2.3 to 0.4 during storing of the brew for 210 min (Table 1). In contrast, the intensity of the sweet/caramel-like and the smoky note only increased to some extent, and the earthy aroma quality did not change significantly.

Table 1. Influence of warm-keeping on the overall aroma quality of a coffee brew

aroma quality	intensity after[a]		
	0 min	60 min	210 min
sweet/caramel	1.6	2.1	2.5
earthy	1.9	1.8	1.9
sulfury/roasty	2.3	1.2	0.4
smoky	2.0	2.3	2.3

[a]*The intensities of the given odour qualities were scored on a scale from 0 (not detectable) to 3 (strong)*

To study whether interactions between the coffee odorants and the dark brown melanoidins might be responsible for the aroma change observed, in a first set of experiments, an aqueous aroma model mimicking the overall aroma of an original fresh coffee brew was prepared using 25 coffee odorants in their "natural" concentrations. One aliquot of this model was evaluated by sensorial assessment in comparison to an original coffee brew without any further additions (Table 2), and the second aliquot was mixed with melanoidins (MW>3000 Da) which had been isolated from coffee brew by means of ultrafiltration prior to sensory analysis. The amount of melanoidins added was equivalent to their "natural" concentrations in a coffee brew.

Table 2. Influence of coffee melanoidins on the overall aroma of a biomimetic coffee aroma model

aroma quality	intensity		
	coffee brew	model w/o[a]	model w/m[a]
sweet/caramel	1.6	2.1	1.9
earthy	1.9	1.7	1.9
sulfury/roasty	2.3	2.1	1.2
smoky	2.0	1.4	1.6

[a]*The aroma profile of an aroma model (10 mL) was analysed in the absence (w/o) or presence (w/m) of coffee melanoidins (125 mg; MW>3000 Da) after storing for 30 min at 40°C*

In comparison to the aroma profile of a fresh coffee brew and the aroma model, addition of melanoidins reduced, in particular, the intensity of the sulfury-roasty odour quality (Table 2) after an equilibration time of 30 min at 40°C. These data clearly demonstrated that coffee volatiles evoking the sulfury/roasty odour quality might be either degraded, or bound by the melanoidins.

In order to elucidate those sulfury-roasty smelling volatiles which were influenced the most by the addition of melanoidins, an aqueous aroma distillate was isolated from a freshly brewed coffee beverage by means of high-vacuum distillation. An aliquot of this distillate, exhibiting the identical aroma attributes as the freshly prepared brew, was spiked with coffee melanoidins corresponding to their "natural" concentrations. After equilibration for 30 min at 40°C, aroma dilution analyses were applied comparatively onto the headspaces of both solutions. The results (Table 3) revealed 16 odorants in the fresh aroma distillate with flavour dilution (FD) factors ranking from 2 to 128. After addition of melanoidins, only 15 odorants were detected sensorially, among which the aroma impacts of 2-furfurylthiol, 3-methyl-2-butenthiol, 3-mercapto-3-methylbutyl formate, 2-methyl-3-furanthiol, and methane thiol were significantly decreased when compared to the pure aroma distillate. E.g. the FD factor of 2-furfurylthiol decreased from 32 to 2 (Table 3). Based on this observation, it can be speculated that interactions between the odorous thiols and coffee melanoidins contribute to the aroma defect developing during storage of the coffee brew.

To confirm this loss of free thiols in an original coffee brew on the basis of quantitative data, the amounts of 2-furfurylthiol (FFT) and 3-mercapto-3-methybutyl formate (MMBF) were determined in coffee brews stored in a thermos flask for 0, 30, 60, 90 and 210 min by means of stable isotope dilution assays. As given in Figure 1, the freshly brewed coffee beverage contained about 16 and 8.2 µg of FFT and MMBF, respectively. Warm-keeping of the brew led to a drastic decrease in the concentrations of both thiols, in particular of FFT, e.g. after 60 min the FFT concentration decreased by a factor of more than four. Extending the storage time to 210 min finally resulted in a complete loss of free FFT, and only small amounts of MMBF were still detectable (Figure 1).

In order to study the mechanisms of the interaction between thiols and melanoidins in more detail, aqueous solutions of 2-furfurylthiol (FFT), 3-mercapto-3-methylbutyl formate (MMBF), and 3-methyl-2-buten-1-thiol (MBT) were incubated either in the absence, or in the presence of coffee melanoidins for 30 min at 30°C. The decrease in thiol concentration was determined by means of headspace/HRGC by comparing the control (without melanoidins added) to the sample with added melanoidins (Figure 2). The results revealed that the amounts of each of the three thiols was significantly reduced in the presence of melanoidins

and showed that the decrease in concentration proceeds very rapidly (Figure 2). In particular, 50 % of FFT was "lost" after 20 min. After 30 min, the FFT was nearly absent in the headspace (Figure 2).

Table 3. Comparative aroma dilution analysis of the headspace of aroma distillates incubated in the absence or presence of coffee melanoidins[a]

odorant	aroma quality	FD factor	
		w/o	w/m
butane-2,3-dione	buttery	256	128
pentane-2,3-dione	buttery	128	128
3-methylbutanal	malty	64	64
2-methylbutanal	malty	32	64
acetaldehyde	fruity	32	32
methional	potato-like	32	16
2-furfurylthiol	roasty, sulfury	32	2
2-ethyl-3,5-dimethylpyrazine	earthy	32	32
2,3-diethyl-5-methylpyrazine	earthy	32	32
guaiacol	phenolic	16	32
dimethyl trisulfide	cabbage-like	16	32
2-isobutyl-3-methoxypyrazine	green, earthy	16	16
3-methyl-2-butenthiol	foxy, skunky	8	1
3-mercapto-3-methylbutyl formate	catty	8	2
2-methyl-3-furanthiol	meat-like	4	2
methane thiol	cabbage-like	2	<1

[a]*A high vacuum distillate (10 mL), either alone (w/o), or in the presence (w/m) of coffee melanoidins (125 mg; MW>3000 Da) were incubated for 30 min at 40°C*

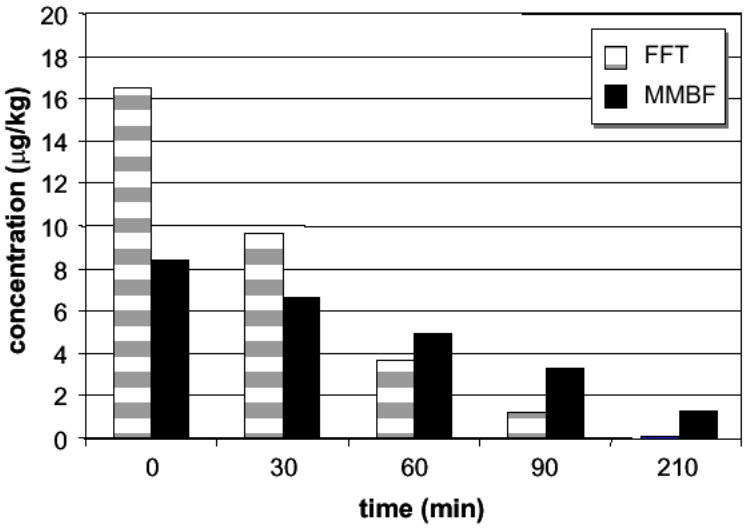

Figure 1. Influence of storage time on the concentrations of 2-furfurylthiol (FFT) and 3-mercapto-3-methylbutyl formate (MMBF) in a coffee brew maintained at 80°C in a thermos flask

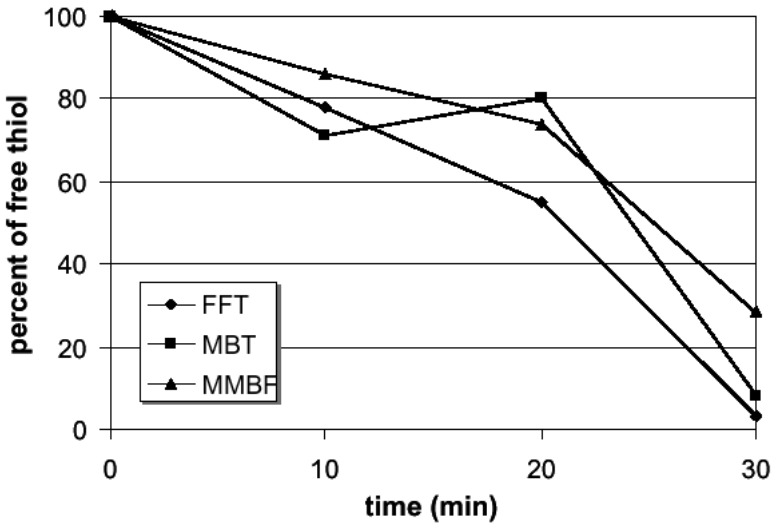

Figure 2. Influence of storage time on the headspace concentrations of thiols in aqueous solutions of 2-furfurylthiol (FFT), 3-mercapto-3-methylbutyl formate (MMBF), 3-methyl-2-buten-1-thiol (MBT), and coffee melanoidins (MW>10000 Da) maintained at 30°C

To answer the question whether these thiols are simply degraded, e.g. by oxidation, or covalently bound to the melanoidins, coffee melanoidins were incubated with [$^2H_2$]-2-furfurylthiol ([$^2H_2$]-FFT) for 90 min at 30°C, then freed again from low-molecular compounds by ultrafiltration, and, finally, analysed by ^{2}H-NMR spectroscopy. As controls, aqueous solutions of coffee melanoidins and [$^2H_2$]-FFT, respectively, were analysed. As displayed in the ^{2}H-NMR spectrum in Figure 3A, a solution of [$^2H_2$]-FFT in H_2O showed two resonance signals, one at 3.67 ppm corresponding to the deuterated methylene group in the odorant, and another at 4.70 ppm corresponding to the natural ^{2}H-abundance in tap water. ^{2}H-NMR of the coffee melanoidins did not show any signals besides the natural ^{2}H-abundance of the solvent (Figure 3B). Coffee melanoidins, however, which had been pre-incubated with [$^2H_2$]-FFT, showed additional resonance at 3.0-4.2 ppm with a strong line broadening as typically found for compounds covalently linked to macromolecules (Figure 3C). These data clearly confirmed the idea that the odour-active thiols are bound to the coffee melanoidins.

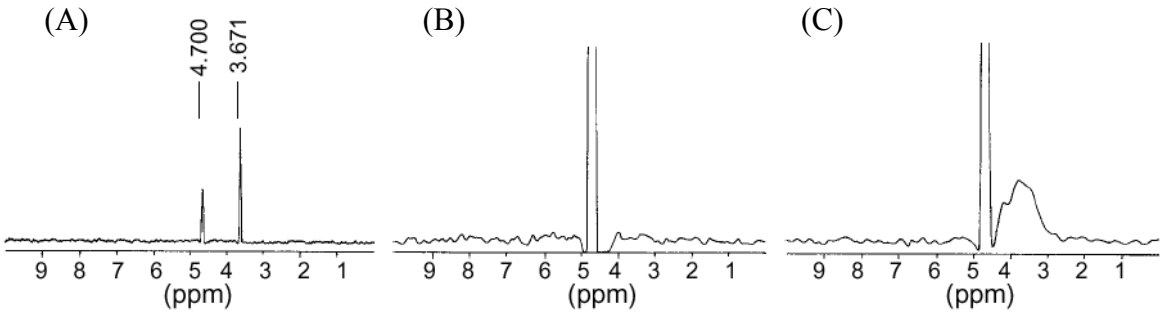

Figure 3. ^{2}H-NMR spectra (500 MHz, H_20) of (A) [$^2H_2$]-FFT (1mg/mL), (B) coffee melanoidins (100 mg/mL), (C) coffee melanoidins (100 mg/mL) after pre-incubation (90 min, 30°C) with [$^2H_2$]-FFT (2 mg) and purification

Because neither addition of the reducing agent dithioerythrytol was able to regenerate major amounts of the free thiol from a FFT/melanoidin mixture, nor the incubation of FFT in the presence of chlorogenic acids resulted in a significant decease of thiol concentration (data not

shown), it can be speculated that the thiols do neither bind via disulfide bonds, nor by nucleophilic attack to activated double bonds of chlorogenic acid moieties in the melanoidins present in coffee brews. However, the data suggest that the thiols preferably add to other reactive sites of the melanoidins.

A potential binding site might be the 1,4-bis-(5-amino-5-carboxy-1-pentyl)pyrazinium radical cations, named CROSSPY (I in Figure 4), which have recently been identified in coffee melanoidins (Hofmann et al., 1999a,b, 2001b). These radicals involved in a redox cycle of reaction intermediates were reported to be oxidized into the corresponding diquaternary pyrazinium ions (II in Figure 4), which subsequently form 2-hydroxy-1,4-dihydropyazines (III in Figure 4) upon hydratization, and regenerate the CROSSPY radicals upon redox reaction with III. The bishydroxylated dihydropyrazines (IV in Figure 4) and the hydroxydihydropyrazines III were proposed as penultimate monomers involved in melanoidin genesis by oligomerisation, e.g. via the dimeric molecule V.

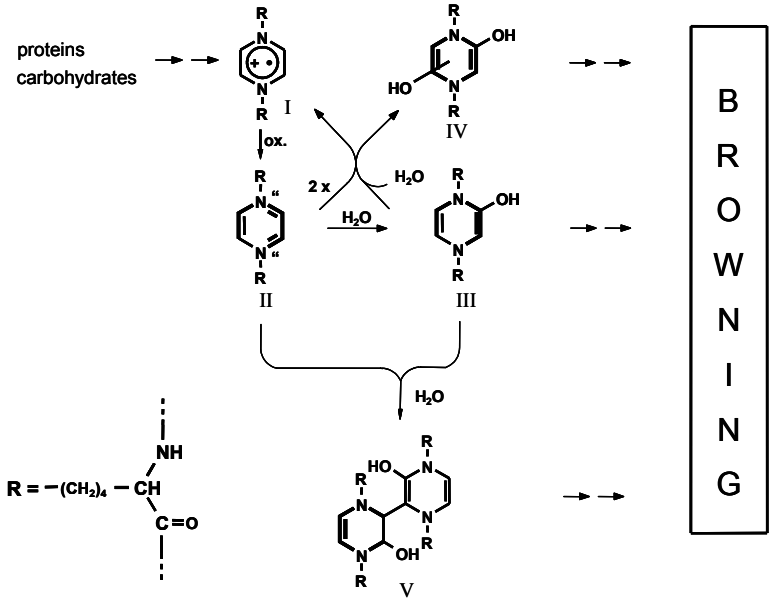

Figure 4. Melanoidin genesis via CROSSPY (I) and diquaternary pyrazinium ions (II) as the key intermediates

In order to check, whether the thiol binding activity of melanoidins is somehow correlated with the CROSSPY-associated pool of reaction intermediates, we separated the coffee melanoidins by gel permeation chromatography into four fractions (I to IV) of decreasing molecular weight (Figure 5A), and determined their radical activity by EPR spectroscopy as well as their effectiveness in thiol binding (Figure 5B). Incubation of these fractions in the presence of FFT revealed that the thiol binding activity runs parallel to the radical activity of the fractions, e.g. fraction IV showed the most pronounced effect in thiol binding and exhibited the highest radical activity, whereas fraction III was found to have the lowest potential in thiol binding and the lowest radical activity (Figure 5B). On the basis of this tight relationship, it might be speculated that the CROSSPY-associated reaction intermediates, outlined in Figure 3, are involved in thiol binding.

To study the reactions of CROSSPY-associated reaction intermediates, we recently used 1,4-diethylpyrazine diquaternary ions (Diquat) as a suitable model substance mimicking the reactions of lysine-bound pyrazinium derivatives (Hofmann et al., 1999a,b; 2001b).

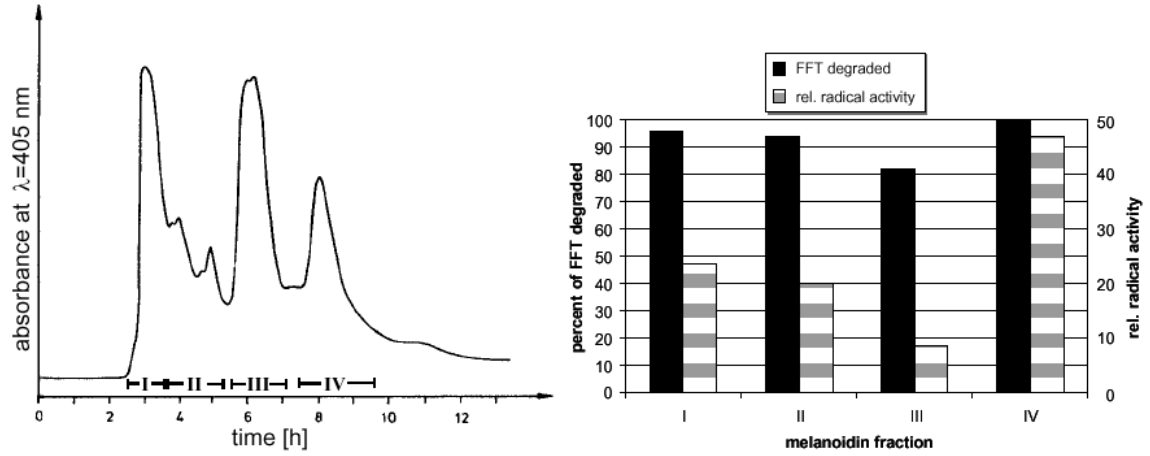

Figure 5. (A) Isolation of melanoidin fraction I, II, III, and IV from coffee brew. (B) Amount of "free" FFT present in the headspace of an aqueous solution of FFT without (w/o), or in the presence of fractions I to IV after 30 min at 30°C

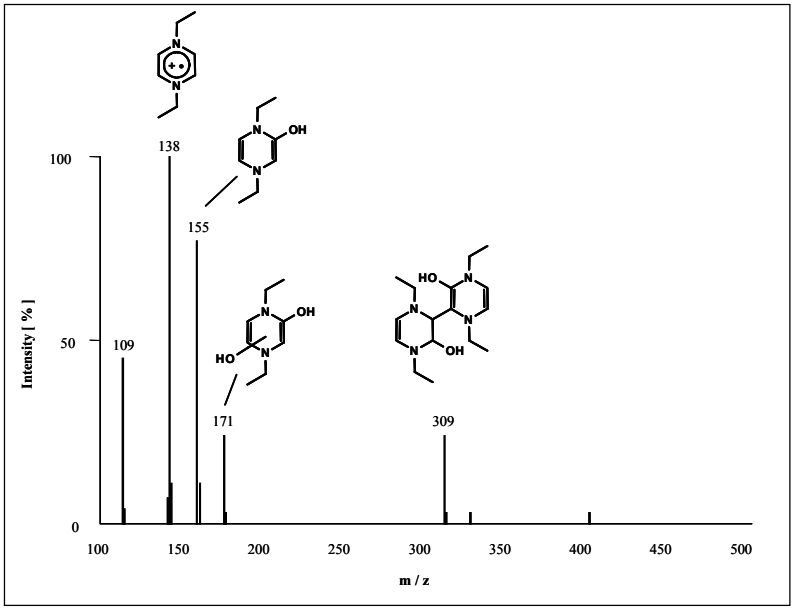

Figure 6. LC/MS spectrum of a solution of 1,4-diethylpyrazine diquaternary ions (Diquat) in water

The LC/MS spectrum (Figure 6) of a freshly prepared aqueous solution of Diquat visualized all the reaction intermediates participating at the redox pool proposed in Figure 3, namely the radical cation (m/z 138), 2-hydroxy-1,4-diethyl-1,4-dihydro-pyrazine (m/z = 155), dihydroxy-1,4-diethyl-1,4-dihydropyrazine (m/z = 171), and a dimer (m/z = 309). In order to study possible reactions between odor-active thiols and these CROSSPY-associated intermediates, an aqueous solution of Diquat was incubated in the presence of FFT at 30°C in a closed vessel. In a comparative experiment, the Diquat was substituted by coffee melanoidin fraction I. Quantification of the concentrations of "free" FFT as well as the corresponding disulfide by means of stable isotope dilutions assays revealed a rapid decrease in the amounts of the "free" thiol induced either by the addition of the Diquat solution, or the coffee melanoidin (Figure 7). Both, the Diquat-derived intermediates as well as the coffee melanoidins showed similar effects on the kinetics of FFT degradation. Although about 400 or 330 µg FFT were

bound to the coffee melanoidins or the Diquat-derived intermediates, respectively, less than 6 µg of the corresponding bis(2-furfuryl) disulfide had been generated. These data clearly demonstrate that the Diquat solution was as active in thiol binding as the coffee melanoidins, but neither the Diquat solution, nor the coffee melanoidins were able oxidize the thiol into its disulfide.

In order to gain further insights into the chemical mechanism of thiol binding, an aqueous solution of Diquat was spiked with FFT in equimolar amounts and, after 10 min at 30°C, analysed by LC/MS (Figure 8A).

The mass spectrum exhibited a molecular ion at m/z = 251 (100%), which on the basis of its LC-MS^2 spectrum (data not given) was proposed as the 2-(2-furyl)methylthio-1,4-dihydropyrazine (A in Figure 8). In addition, LC-MS^2 gave evidence that the ions at m/z=363 and 267 correspond to bis[2-(2-furyl)methylthio]-1,4-dihydropyrazine and 2-(2-furyl)methylthio-hydroxy-1,4-dihydropyrazine, respectively (A in Figure 7). For further confirmation of these structures, the experiment was repeated with $^2H_2$-labelled FFT (B in Figure 8). Comparing the LC/MS spectra (B in Figure 8) with those found in the non-labelled experiment (A in Figure 8) revealed an isotopic shift of the ions at m/z 251 to m/z 253, thus confirming the incorporation of two deuterium atoms from one molecule of FFT as given in the structure proposed for 2-(2-furyl)methylthio-1,4-dihydropyrazine. In addition, we observed an isotopic shift of two and four units for the ions at m/z 267 to 269 and m/z 363 to 367, respectively, thus verifying the structures proposed for 2-(2-furyl)methylthio-hydroxy-1,4-dihydropyrazine and bis[2-(2-furyl)methylthio]-1,4-dihydropyrazine (Figure 8).

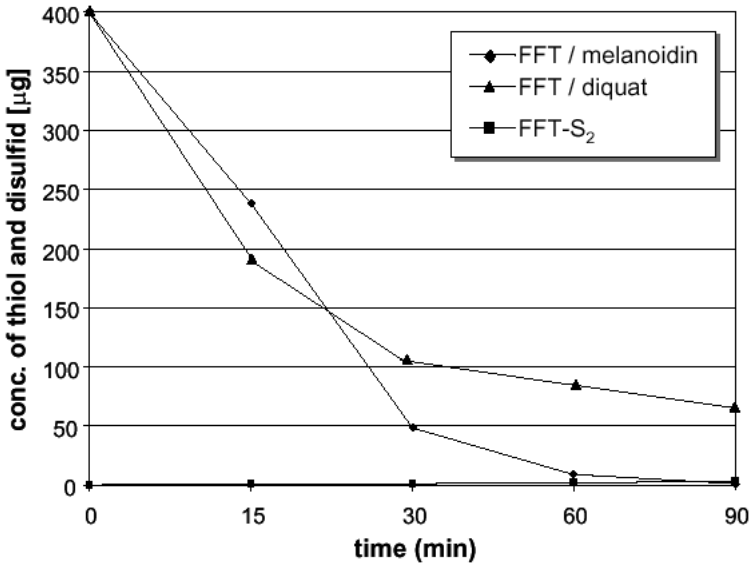

Figure 7. Influence of reaction time on the concentrations of 2-furfurylthiol (FFT) and disulfide (FFT-S_2) in the presence of melanoidins or Diquat-derived reaction intermediates, respectively

Taking all these data into account, the reaction pathways, displayed in Figure 9, were proposed for the binding of odour-active thiols to the CROSSPY-associated reaction intermediates. Oxidation of CROSSPY (I) leads to diquaternary pyrazinium ions (II), which, in the absence of thiols, react with water to form the 2-hydroxy-1,4-dihydropyrazine (III; Hofmann et al., 1999a,b, 2000a), or, in the presence of thiols such as, e.g. the 2-furfurylthiol, give rise to 2-(2-furyl)methylthio-1,4-dihydropyrazine (IV). Redox reactions of these intermediates involving the diquaternary ions then form the 2-(2-furyl)methylthio-hydroxy-

1,4-dihydropyrazine (V) and bis[2-(2-furyl)methylthio]-1,4-dihydropyrazine (VI), respectively, in the reaction with a second molecule of the thiol.

Taking all these findings into consideration, it might be concluded that the pyrazinium intermediates derived from CROSSPY, recently identified as important intermediates in melanoidin genesis in roasted coffee, are involved in the rapid covalent binding of odorous thiols to melanoidins, thus, resulting in the decrease of the sulfury-roasty odour quality shortly after preparation of the coffee brew.

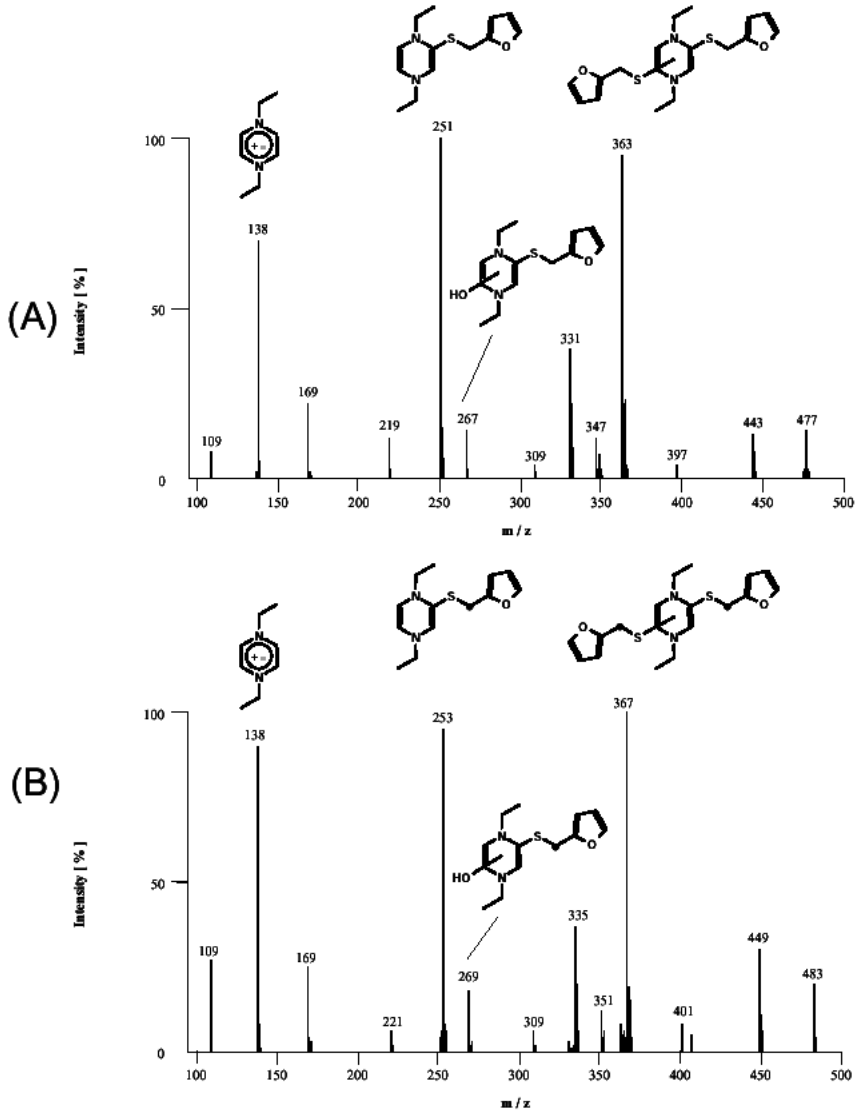

Figure 8. LC/MS spectra of an aqueous solution of Diquat and (A) FFT, and (B) $^{2}H_{2}$-FFT, respectively

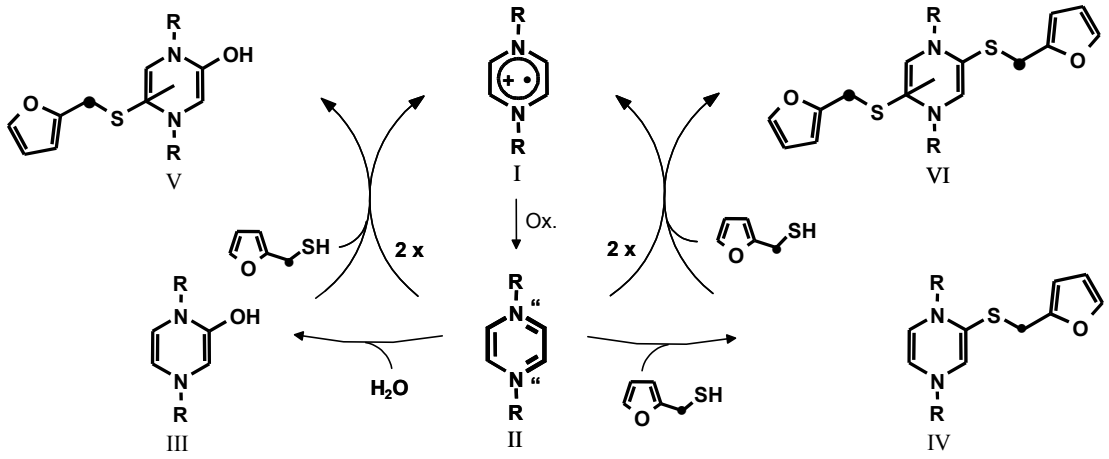

Figure 9. Reaction pathways proposed for the covalent binding of 2-furfurylthiol to CROSSPY-associated reaction intermediates

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Quantitation of Potent Aroma Compounds Above Freshly Brewed Coffee by Aroma Trapping

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SUMMARY

An analytical method is described that allows monitoring of the aroma of freshly brewed coffee as the consumer perceives it.

Coffee aroma is formed by the interaction of several potent aroma compounds (PACs), which are released from dry coffee material or coffee brew. In contrast to a traditional analytical method that would be aiming for the concentration of analytes in the matrix, an analysis of the released amount is required. Ideally this method covers all known relevant PACs, i.e. compounds of very different chemical and physical properties and concentration.

It will be demonstrated that compounds as different as Methylthiole and Furaneol together with other PACs can be monitored by means of a trapping technique and subsequent thermodesorption into a GC/MS-system. A quantitation of the released PACs is achieved by an external calibration procedure.

INTRODUCTION

In the last two decades significant progress has been made on the chemical understanding of coffee aroma by applying more and more sophisticated instrumental methods and equipment. These methods enabled coffee researchers to identify and quantify a comparatively small number of aroma relevant compounds out of a very large number of volatile but sensorially irrelevant compounds (Blank et al., 1992; Semmelroch et al., 1995)

Based on these findings, first successful recombinations were performed that verify the proper selection and quantitation of those aroma compounds (Semmelroch et al., 1996; Czerny et al., 1999). These results emphasize the necessity to better understand the balance of aroma compounds and to achieve a more detailed knowledge of the often subtle but important differences between products and preparation techniques that drive the perception and preference of consumers.

OBJECTIVE

It was the aim of this work to develop a method for an instrumental monitoring of coffee aroma as it is perceived by the consumer. This method is targeted to measure aroma released above a cup of coffee, avoiding any treatment of the coffee brew that would have an impact on its aroma balance as good as possible. For this, the assumption is made that the balanced composition of potent aroma compounds above a cup of coffee is a good measure for the aroma that reaches the olfactory system of a consumer during consumption.

ANALYTICAL APPROACH

In order to monitor the release of individual aroma relevant compounds above a cup of coffee, a special device was developed for the time-resolved aroma trapping and analysis by Thermodesorption GC/MS. Time-resolved analysis can also be achieved by non-chromatographic methods such as MS-nose instruments (Zimmermann et al., 1996; Lindinger et al., 1998). These methods are real-time methods aiming at the immediate response of a detector system. They do not include enrichment steps and are therefore less sensitive than trapping methods. They also do not involve a chromatographic separation of analytes which, in consequence, leads to a lack of selectivity. Both, lacking sensitivity and selectivity, very much limits the time-resolved quantitation of aroma relevant compounds in systems as complex as coffee aroma.

TIME-RESOLVED AROMA TRAPPING

The aroma is trapped on a series of Tenax- or Carboxen569- filled thermodesorption tubes by application of a vacuum on one side of the traps and placing the other end directly above the cup. In order to achieve identical sampling conditions for each tube and with that best possible repeatability, the tubes are positioned in equal distance to the border of the cup fixed in an aluminium ring on top of it. This set-up avoids long sampling paths enabling highly time-resolved measurements. A vacuum system as shown in Figure 1 was set up using a vacuum pump with one central dosing and stop valve, a six-port valve and six individual dosing valves for the compensation of flow rate differences across different traps. Depending on the desired time-resolution trapping times between 3 and 20 seconds were used.

The thermodesorption/GC/MS analysis was carried out using a Gerstel TDSA-TDS2 thermodesorption unit operated at a thermodesorption temperature of 250°C for 5 min and a Helium flow rate of 100 mL/min at 1bar. This unit was mounted on top of a HP6890 gas chromatograph with a Gerstel cool injection system with a Tenax-liner at –70°C during thermodesorption.

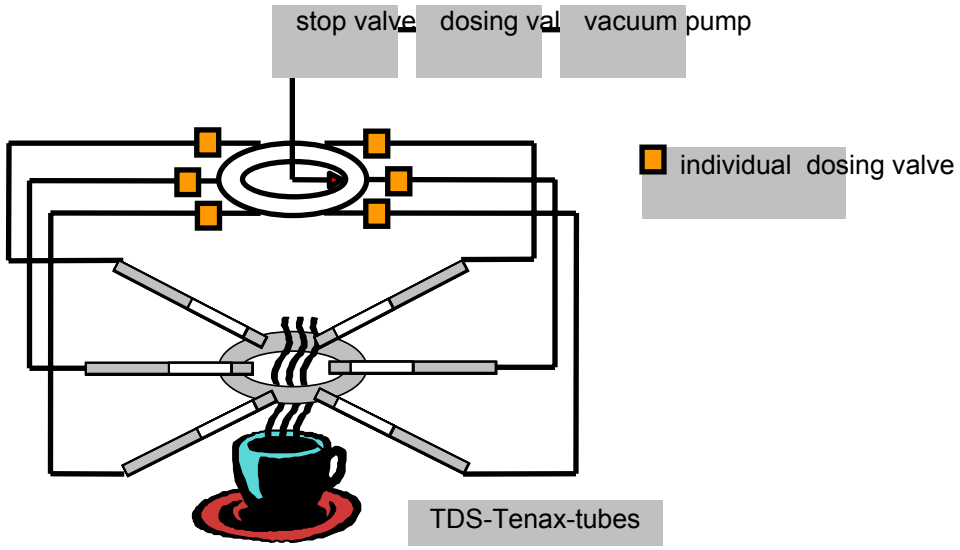

Figure 1. Sketch of sampling device for time-resolved aroma analysis

Separation was achieved with a capillary column HP1701 50 m/250 µm/1µm at a flow rate of 2 mL/min Helium under constant flow conditions and a temperature gradient of 6°C/min. A

HP5973 mass spectrometer was used as detector with an appropriate SIM-method. These conditions allow the highly selective detection of a very wide range of aroma compounds with an acceptable repeatability for most of them. The peak shape of some compounds causing often difficulties in chromatography is shown in Figure 2. Selective analysis of Methylmercaptane and Acetaldehyde is possible despite co-elution through mass selective detection, Acetic acid gives a good peak shape as a consequence of the absence of solvents, base-line separation is achieved for 2-Methylbutanal and 3-Methylbutanal and polar compounds like Maltol show an acceptable degree of tailing.

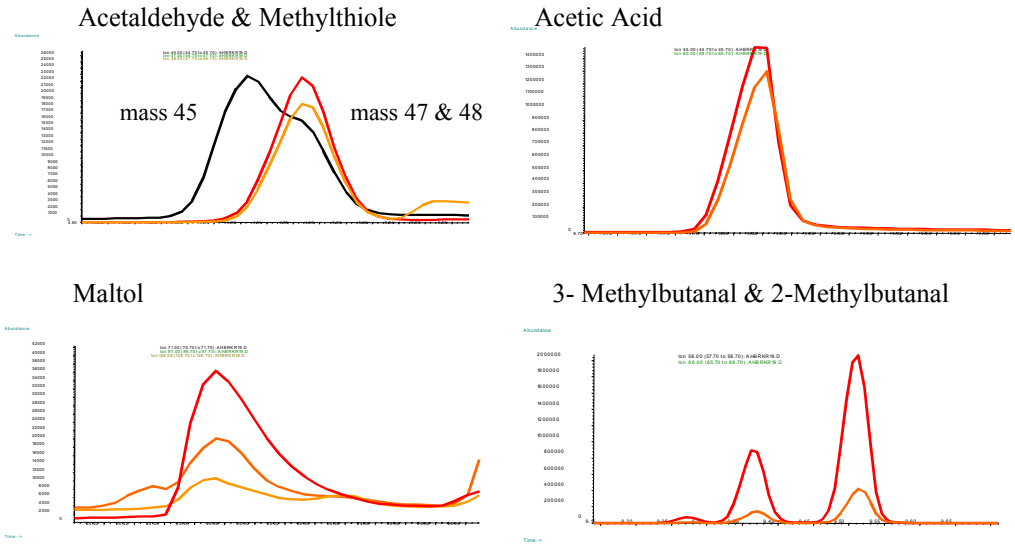

Figure 2. Peak shapes of some aroma compounds

The repeatability of Tenax-trapping and subsequent thermodesorption-GC/MS analysis was tested by repetitive analysis of the aroma in vacuum packs of roasted and ground coffee. Figure 3 shows the results for some selected compounds. Most of them are analysed at a repeatability of less than 10 percent, only very water-soluble compounds like some phenols and furanones are analysed with poor repeatability of more than 50%. However, the true analytical error is somewhat lower since variations across different coffee vacuum packs have to be considered.

Quantitation was achieved by calibration with an external standard solution of 4-Heptanone in Methanol that was spiked on cleaned Tenax-traps. Figure 4 demonstrates the good reproducibility of 5.5 percent that was achieved over a period of 4 weeks without tuning the MS. Another internal standard tested, 2,3-Dimethoxytoluene, did not perform as successfully.

RESULTS AND DISCUSSION

As an example Figure 5 shows aroma release decay curves for 18 minutes after brewing in the cup. A quick decay for most volatile compounds is observed whereas the other compounds like water-soluble and more polar compounds are released more slowly and more constantly. This is leading to a drastically modified aroma balance with time to the advantage of less volatile and water-soluble compounds. An aroma balance as is observed above the cup after several minutes after brewing is therefore similar to the balance that is perceived when consuming the coffee brew. In contrast the initial aroma balance is visualizing the balance during coffee preparation. After 2 to 3 minutes the very light compounds are decreased down to some few percent whereas the less volatile compounds decrease to values between 20 and 50 percent only, thus creating a dramatic balance shift. A not time-resolved aroma analysis

that is performed in closed systems is giving integral quantities of aroma compounds and does not reflect these important differences. The release kinetics, i.e. the variation of the aroma balance with time, are most helpful to understand the development of the aroma balance for the comparison of various types of preparation and the comparison of different types of coffee material like soluble coffee versus roasted and ground coffee. As can be seen from the decay curves the repeatability of the whole procedure is sufficient to monitor properly the development of the aroma balance with time.

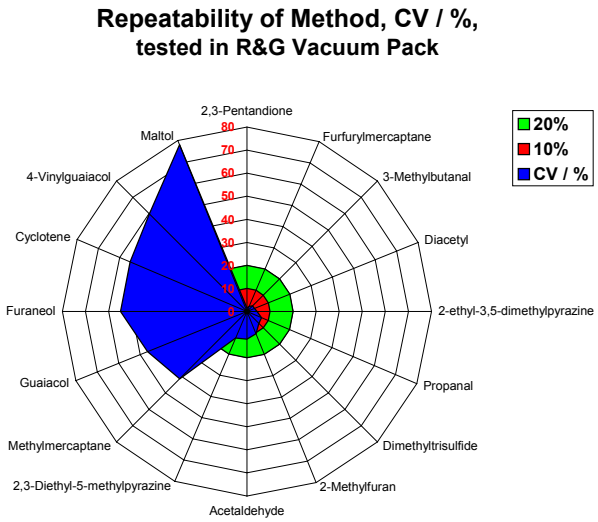

Figure 3. Repeatability of selected coffee aroma compounds sampled from five vacuum packs of roasted and ground coffee (the red and green circle help to visualize the 10 and 20 percent level)

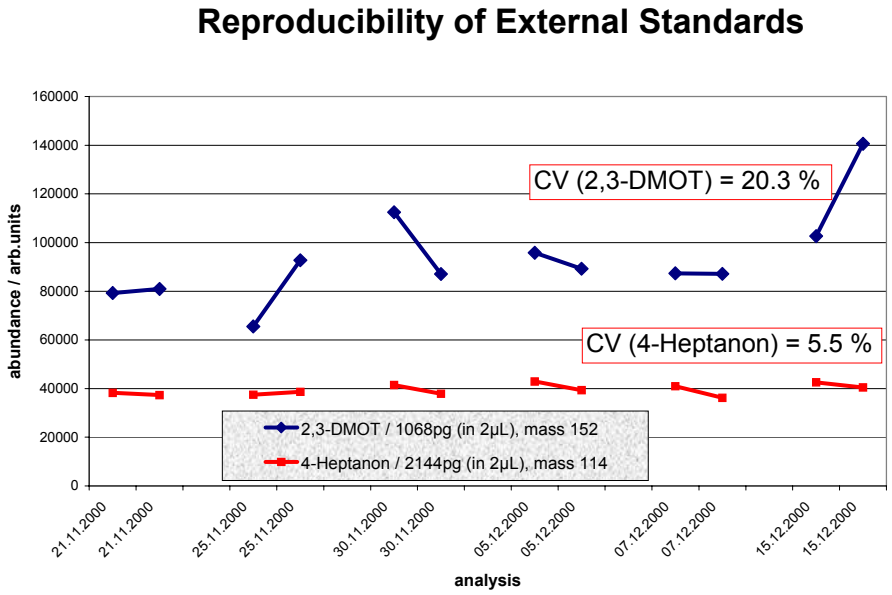

Figure 4. Repeatability of 4-heptanone in methanol after spiking on Tenax traps

In order to study initial aroma release kinetics a very high time-resolution was achieved by using the same instrumental set-up and reducing the trapping time. Figure 6 is showing the release of aroma compounds from stirred hot water after addition of some drops of highly

aromatic coffee oil. The amount of trapped aroma at a sampling time of 3 seconds was sufficient to monitor even less volatile compounds like cyclotene or phenolic compounds. A more sensitive and repeatable analysis is possible at a reduced sampling rate on the expense of time-resolution.

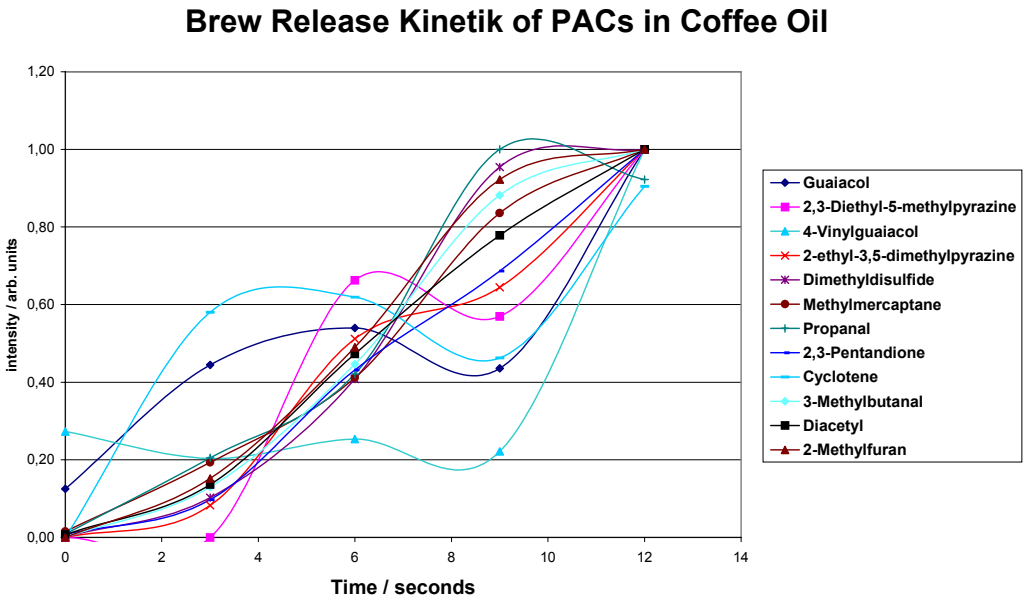

Figure 5. Aroma release curves after brewing in the cup

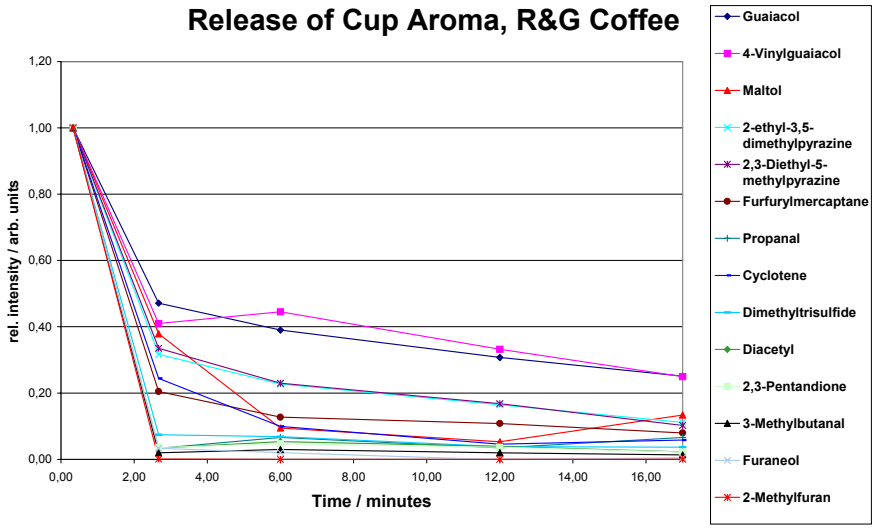

Figure 6. Highly time-resolved aroma release after addition of aromatised coffee oil to hot water (80°C)

CONCLUSION

The feasibility of a time-resolved analysis of potent coffee aroma compounds in an undisturbed release system above a cup was demonstrated. The sensitivity of the entire method allows monitoring the aroma release either at an extended time-scale or within the first seconds. A higher time-resolution than 3 seconds is not required. The method allows monitoring various parameters influencing the aroma balance as a function of time. It is applicable to roast and ground coffee as well as to soluble coffee and aromatised oil as they

are used in the production of soluble coffee or coffee beverages prepared by different brewing techniques.

In addition, a significant difference of the aroma balance was observed between the time of brewing and the time of consumption. The balance of coffee aroma compounds sampled above a cup of coffee between 4 and 15 minutes is assumed to be most representative to what a consumer perceives when consuming coffee.

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Free Radicals and other Paramagnetic Ions in Coffee Solutions

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SUMMARY

Coffee solutions contain "stable" free radicals, which are generated during roasting. EPR measurements show that the amounts and chemical nature of these free radicals change during storage in solution. They are unreactive towards the chemical spin trap, POBN, but are possibly associated with Mn(II) species during the early stages of reaction in solution. Other, unstable, free radicals are detected during incubation of coffee solutions with POBN, and dissolved oxygen has a significant impact on the levels of these free radicals generated.

INTRODUCTION

Oxidative processes are responsible in a major way for the flavour characteristics of coffee, but they also contribute to the instability of flavour during storage (Feria-Morales, 1989; Dalla-Rossa et al., 1990; Nicoli et al., 1991; Grötzbach et al., 1995a-c). The electron transfer reactions, that are characteristic of oxidative processes, often involve the generation of free radicals. Although many free radicals have very short half-lives at room temperature, especially in the fluid environment, some are stable and can be measured directly by electron paramagnetic resonance (EPR) spectroscopy, a technique which selectively detects free radicals and other paramagnetic species. Previous workers have used EPR spectroscopy to reveal a strong and stable radical signal in R&G, soluble and liquid coffee (e.g. O'Meara et al., 1957; Fritsch et al., 1974; Santanilla et al., 1981; Troup et al., 1989; Morrice et al., 1993; Goodman et al., 1994). An initial aim of the present study was to characterise fully the EPR spectral characteristics of this free radical and other paramagnetic components in soluble coffee and to investigate changes that occur with time during storage in solution.

The generation of unstable free radicals in solutions can be detected by EPR spectroscopy after reaction with chemicals known as "spin traps". These molecules react specifically with unstable free radicals to produce (more) stable radical adducts. The spectral parameters of radical adducts are dependent on the chemical nature of the trapped radical (Buettner, 1987), so it is often possible to identify the initial radical. In the present work, we have used the nitrone spin trap POBN, which generates nitroxides after reaction with free radicals, to study the generation of free radicals in coffee solutions during oxidation at 60^{o}C.

MATERIALS AND METHODS

Origin and preparation of samples

A freeze-dried soluble coffee (Nescafé Gold Blend) was purchased from a local supermarket and the spin trap α-(4-Pyridyl-1-oxide)-*N*-*t*-butylnitrone (POBN) from the Sigma Chemical Company. Deionised water was prepared with a Purite Select Analyst HP25 water purifier (Thame, Oxon, UK). The coffee was studied over periods of five days as 5% aqueous solutions in air or argon (using water degassed with argon for 5 minutes). Spin trapping measurements closely followed the procedure of Uchida and Ono (1996) for determining the

oxidative stability of beer. 466 mg of POBN were dissolved in 10 mL of 5% coffee solutions to give a concentration of 240 mM POBN. The spin trap was either added to freshly made coffee solutions, or to coffee solutions that had been aged at 60^{o}C for 18.5 hours. 1.4 mL aliquots were transferred to 4-mL amber vials (66% headspace) and incubated at 60°C on a heating block (Pierce and Warriner, Chester, UK). Vials were removed at intervals during a 4-hour period and 0.4 mL aliquots were inserted into a quartz flat cell (WG-812Q Wilmad, Buena, NJ, USA) for measurement of their EPR spectra at room temperature (20±1°C). Measurements were also made at 77 K on 0.4 mL aliquots in 4 mm o.d. quartz tubes.

EPR spectroscopic measurements

EPR spectra were all recorded on a Bruker ESP300E computer controlled spectrometer (Bruker (UK) Ltd.) operating at X-band frequencies (~9.5 GHz) and using a TM9202 cylindrical cavity. Microwave generation was by means of a klystron (ER041MR) and the frequency was measured with a built-in frequency counter. Spectra were recorded at room temperature or 77 K in 1024 points as 1st derivatives of the absorption using 100 kHz modulation frequency. Low temperature measurements were made by immersing the sample in liquid nitrogen in a quartz "finger dewar" (Wilmad WG-816B), which was inserted into the microwave cavity. The microwave power was varied between 0.01 and 32 mW to determine the saturation characteristics of coffee solutions at room temperature. The modulation amplitude was 1 mT for Mn(II) and Fe(III) spectra, and 0.5 mT for the free radicals. For solutions containing POBN, room temperature measurements were made with 10 mW microwave power and 0.1 mT modulation amplitude. Low temperature measurements used 0.1 mW microwave power and 1 mT modulation amplitude. The scan range, receiver gain, conversion time, time constant and number of scans over which spectra were accumulated, were optimised for individual spectra.

Analysis of EPR data

The intensities of single peak spectra were measured as the product of the height and width squared of the distance between inflection points of the 1st derivative recording. In order to minimise the contribution of instrumental noise, all spectra were smoothed by applying a 15-point 2nd order polynomial function (x2) after cubic background correction. Intensity measurements of the free radical signal in frozen solutions of spin trap (POBN) adducts are described in the Results and Discussion section.

RESULTS AND DISCUSSION

Free radical in a coffee solution in air at room temperature

The EPR spectrum of a 5% aqueous solution of coffee in air gave a single peak free radical signal with a peak-to-peak linewidth (Γ) of 0.58 mT (Figure 1).

With a freshly prepared sample $P_{1/2}$ was ~4 mW, but the saturation characteristics of the spectrum changed slightly with time and in the aged samples there was an increase in the microwave power at which the onset of saturation started. $P_{1/2}$ could not be measured accurately for the aged solutions, but it was in the region of 7 mW. This change in saturation characteristics indicates a change in the relaxation properties (chemical nature) of (some or all of) the free radicals responsible for the EPR signal. Reaction with oxygen would appear to be a reasonable hypothesis for this change.

Spectral intensity dropped by about 30% over a 24-hour period, but then gradually increased to exceed the original intensity after 120 hours (Figure 3a).

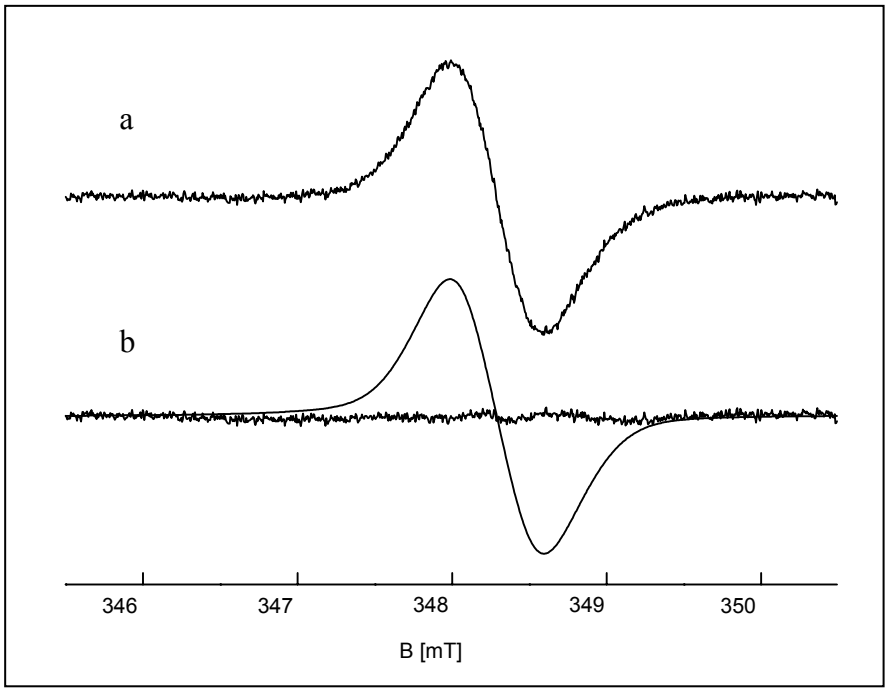

Figure 1. (a) EPR spectrum of 5% aqueous solution of soluble coffee in air at room temperature, and (b) computer fit (plus difference trace) with mixed Lorentzian/Gaussian (1:1) lineshape, g = 2.0044 and Γ = 0.58 mT

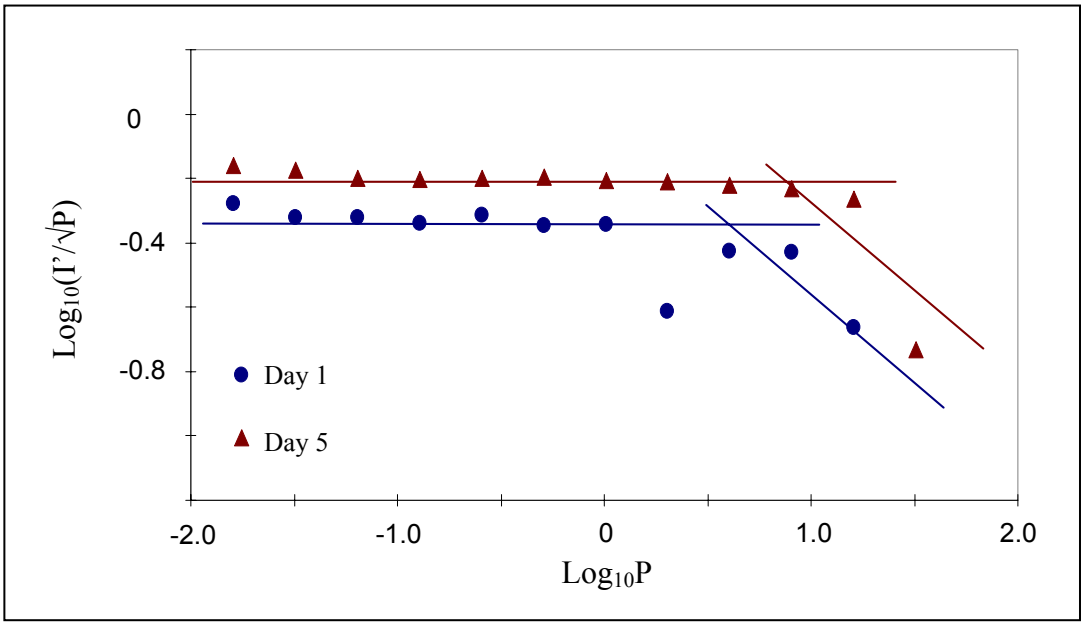

Figure 2. Relationship between free radical EPR signal of 5% soluble coffee solution and microwave power

Free radical in coffee solution under argon at room temperature

In order to investigate the possible role of oxygen in the changes in the free radical signal described in the preceding paragraph, similar measurements were made on coffee solutions stored in an inert atmosphere. The saturation characteristics of the freshly prepared sample were similar to that prepared in air with $P_{1/2}$ ~3 mW, but they did not change significantly with time over a 120 hour period. There was a decrease in spectral intensity between days 1

and 2, as in the sample stored in air, but there was no significant change in intensity over the next 4 days (Figure 3b).

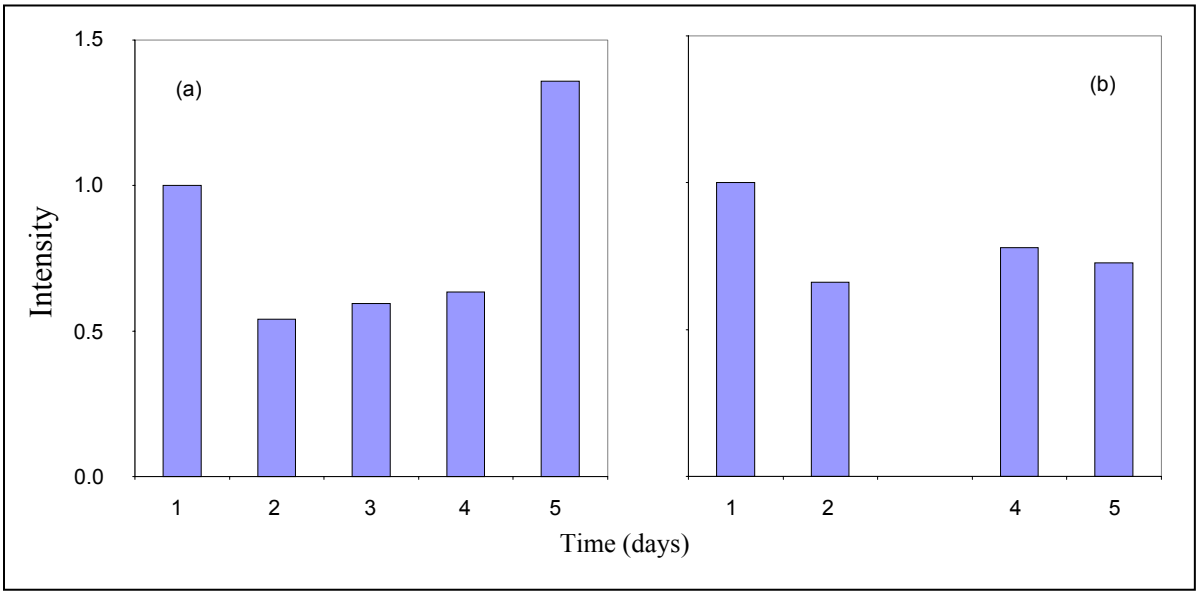

Figure 3. Variation with time of the intensity of the free radical signal in 5% aqueous solutions of soluble coffee at room temperature (a) in air, and (b) in argon atmospheres

Manganese in coffee solutions

In addition to the free radical signals, EPR spectra of coffee solutions at room temperature contain a sextet with a hyperfine coupling constant of 9.5 mT and linewidth of ~3.2 mT (Figure 4). This is consistent with the solvated Mn(II) ion, and probably originated in the unroasted coffee bean, since similar spectra are commonly seen in plant tissues (e.g. Goodman and Linehan, 1979; McPhail et al., 1982; Goodman et al., 1986; Runeckles and Vaartnou, 1992). The signal did not saturate in the microwave power range that was used for the free radical measurements described above. Its intensity decreased over the first 24 hours by an amount similar to that shown by the free radical signal, but it showed no subsequent intensity changes over a 5-day period.

Metal signals in coffee solutions at 77 K

A wide scan spectrum of 5% aqueous coffee solution at 77K (Figure 5) shows the presence of four distinct features, a low field component with g = 4.27 (at 160 mT) and three separate components with g ~2.0 (i.e. centred on 336 mT). These latter signals consist of a narrow component from the free radical(s) discussed in the previous section, a complex component from Mn(II), analogous to that seen in the room temperature spectra, and a broad single peak, which arises from high spin Fe(III) in magnetically-interacting (i.e. polynuclear) environments (e.g. Goodman, 1988). The g = 4.27 signal also arises from high spin Fe(III), but in mononuclear complexes with 'rhombic' symmetry. Other Fe(III) species may be present, but their EPR transitions are often highly anisotropic (and hence have zero height) in frozen solution measurements (Aasa, 1970). None of the Fe(III) signals showed any significant changes with ageing of the solutions over a 5-day period.

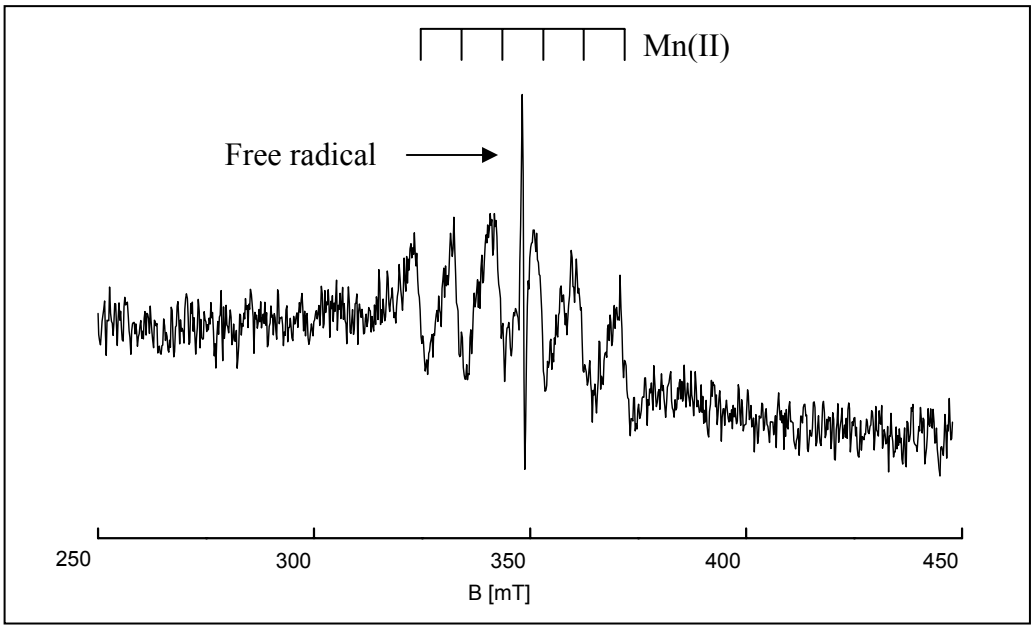

Figure 4. Wide scan EPR spectrum of 5% aqueous coffee solution at room temperature

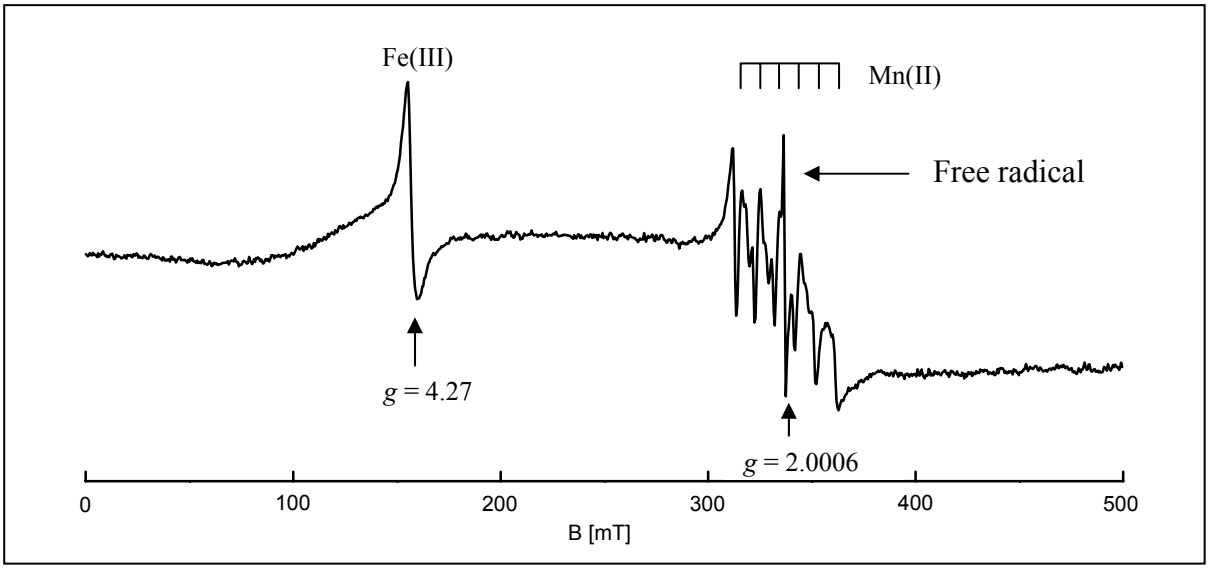

Figure 5. Wide scan EPR spectrum of 5% aqueous solution of soluble coffee at 77K

Ageing of coffee solutions at 60^{o}C in the presence of POBN

EPR spectra of POBN adducts in fluid solutions consist of 6 peaks from the ^{14}N of the nitroxide group and the ^{1}H on the α-carbon atom (i.e. that adjacent to the nitroxide) (Figure 6). The signal obtained when POBN reacted with coffee had hyperfine splittings, a (^{14}N) = 1.56 mT and a (^{1}H) = 0.26 mT, which correspond to a ·C-centred radical (Buettner, 1987). Blank et al. (2000) observed the formation of both ·OH and ·C-centred radical adducts with POBN in model coffee solutions, and the non-observation of ·OH adducts in the real coffee system may reflect the greater concentration of organic molecules, which successfully compete with the spin trap for reaction with the ·OH radicals.

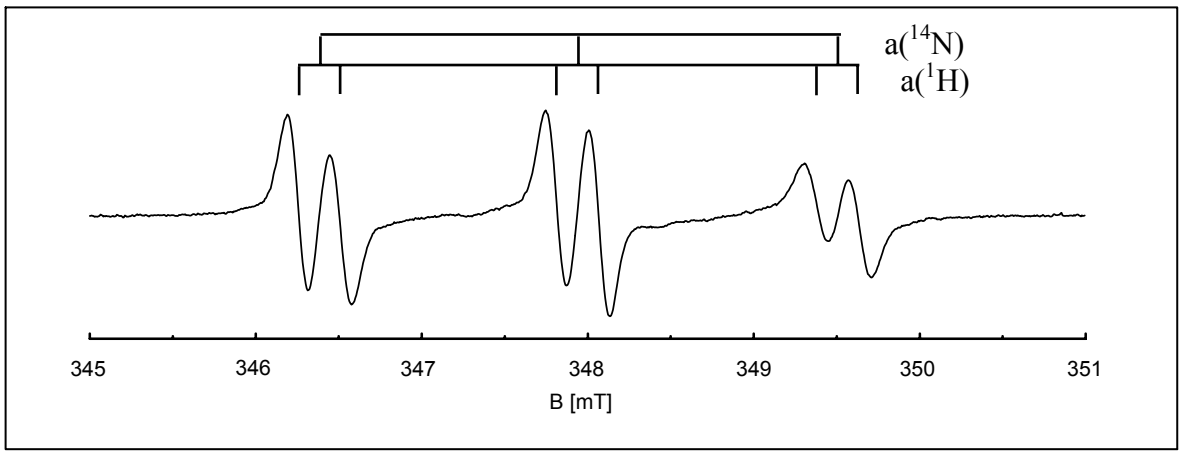

Figure 6. EPR spectrum at 20°C of the free radical adduct formed from the reaction between POBN and coffee

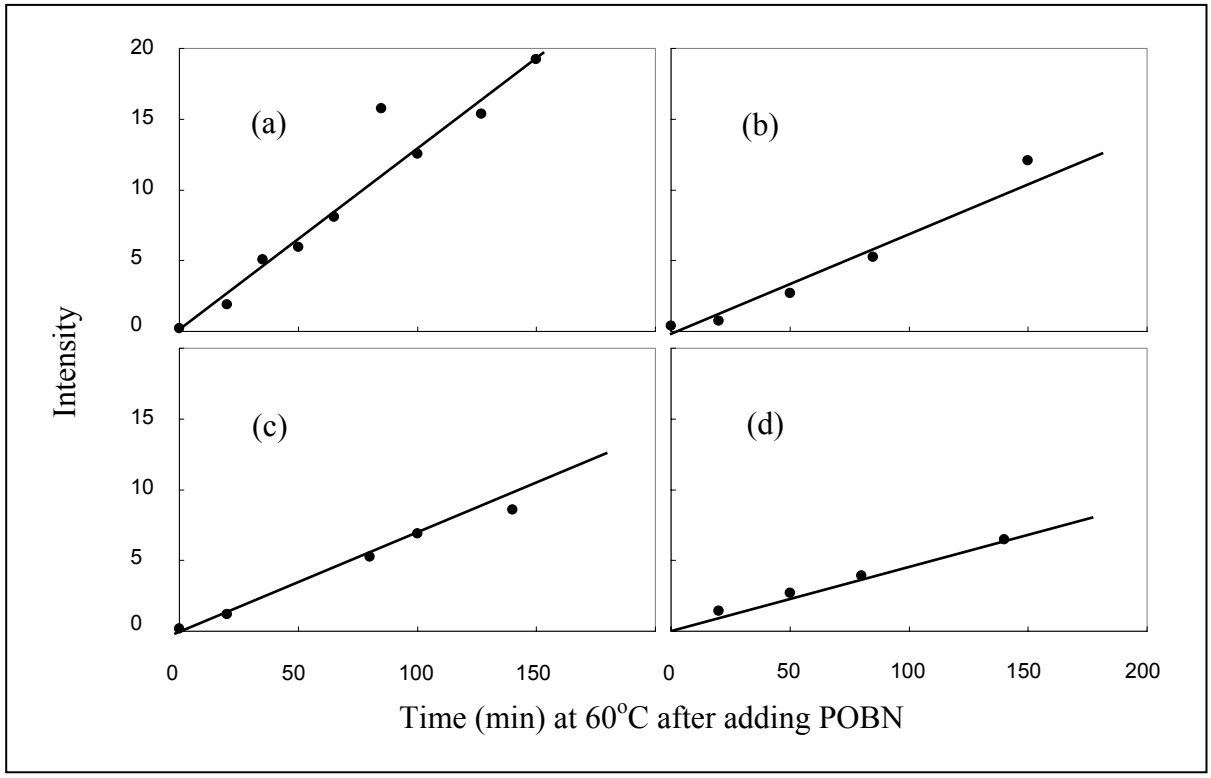

Figure 7. Variation with time of the intensity of the POBN adduct signal with fresh coffee solutions prepared with water having (a) natural oxygen content, (b) deoxygenated, and (c) and (d) after ageing for 18.5 hours at 60°C before adding POBN

For freshly made-up solutions, the rate of generation of the EPR signal during forced oxidation was appreciably slower in the deoxygenated sample than in the equivalent sample made up with water having natural oxygen content.

When similar measurements were made with coffee solutions that had been aged for 18.5 hours at 60°C before addition of the spin trap, the curves of EPR intensity against time of incubation with POBN were essentially identical and very similar that of the deoxygenated fresh coffee solution.

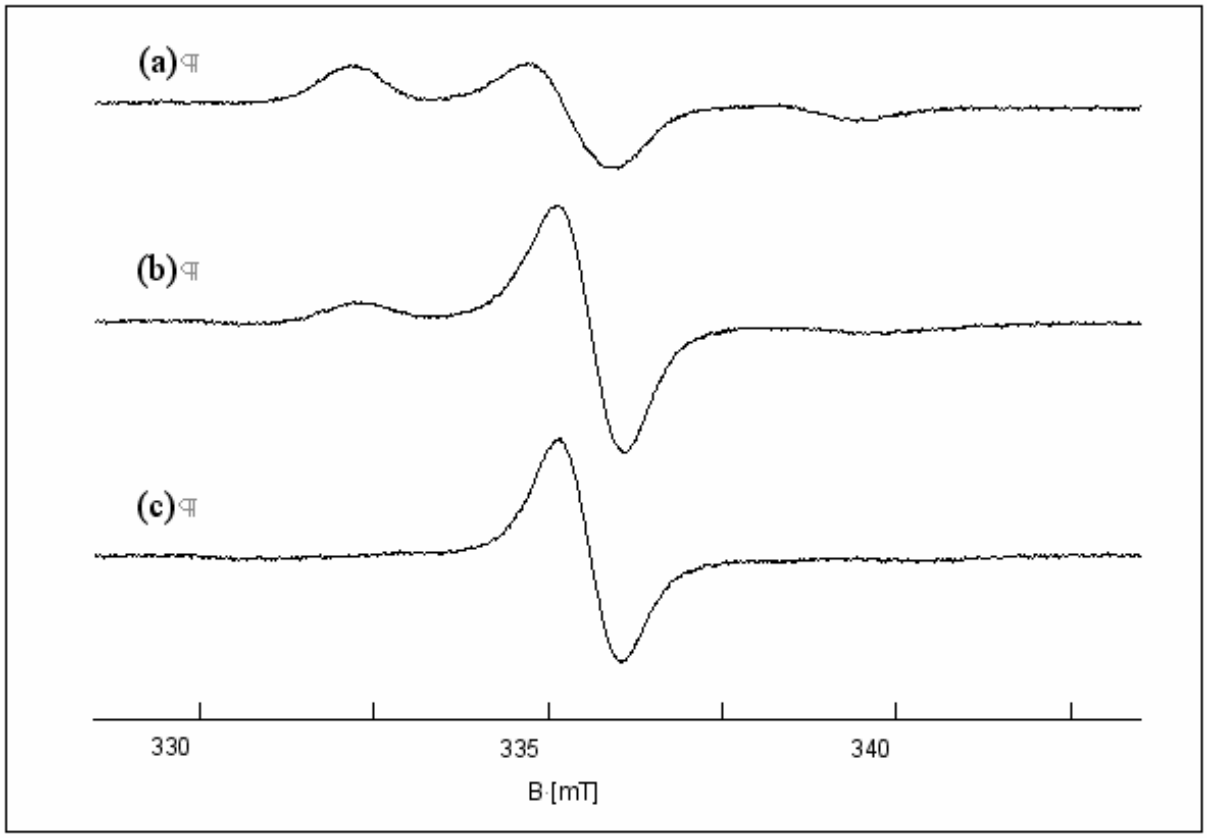

Figure 8. EPR spectra at 77 K of the products of reaction of POBN with (a) beer and (b) coffee. The free radical signal in coffee revealed by subtracting (a) from (b) is shown in (c)

Behaviour of the coffee free radical signal in the presence of POBN

The variation with time in intensity of the single peak coffee free radical signal was also studied in these spectra by deconvoluting the central peak of the 77K spectrum into its spin adduct and free radical components. This was done by subtracting from the coffee spectrum an appropriate fraction of the spectrum obtained by reaction of POBN with beer, which has no single peak free radical signal (Figure 8). A plot of the residual free radical signal as a function of time is shown in Figure 9a. This decreased by about 30% during the first 10 minutes of incubation, after which it stabilised and then maybe increased slowly. A similar result was obtained from a coffee solution incubated at 60^{o}C without the spin trap (Figure 9b), illustrating that the coffee free radical does not interact with the spin trap. The decrease in free radical signal intensity after 10 minutes incubation of the coffee solution at 60^{o}C is similar to that observed after 24 hours ageing at room temperature (see above).

DISCUSSION

The EPR spectra of soluble coffee in aqueous solutions display single line free radical signals along with signals from the paramagnetic ions Fe(III) and Mn(II). The variations with time of the free radical signals strongly suggest that two separate phenomena occur during the ageing of soluble coffee solutions. Firstly, there is an atmosphere independent (i.e. it does not require oxygen) decrease in spectral intensity over the first 24 hour period and secondly, on a longer timescale, there is an oxygen-dependent increase in signal intensity. This latter free radical component has saturation characteristics, which are slightly different from those of the initial signal and suggests that the new radical species is chemically distinct from those present in fresh coffee solutions.

The parallel behaviour of the Mn(II) and free radical components during the first 24-hour period suggests that there may be an association of these species, which results in a loss of signal from both components. This could be the explanation for the atmosphere-independent changes discussed above. Over longer periods there was no change in the Mn(II) signal intensity to match the increase in the free radical signal of the samples aged in air. In low temperature measurements, Fe(III) signals were observed from a mononuclear complex and polynuclear species, but these showed no measurable changes on ageing the samples.

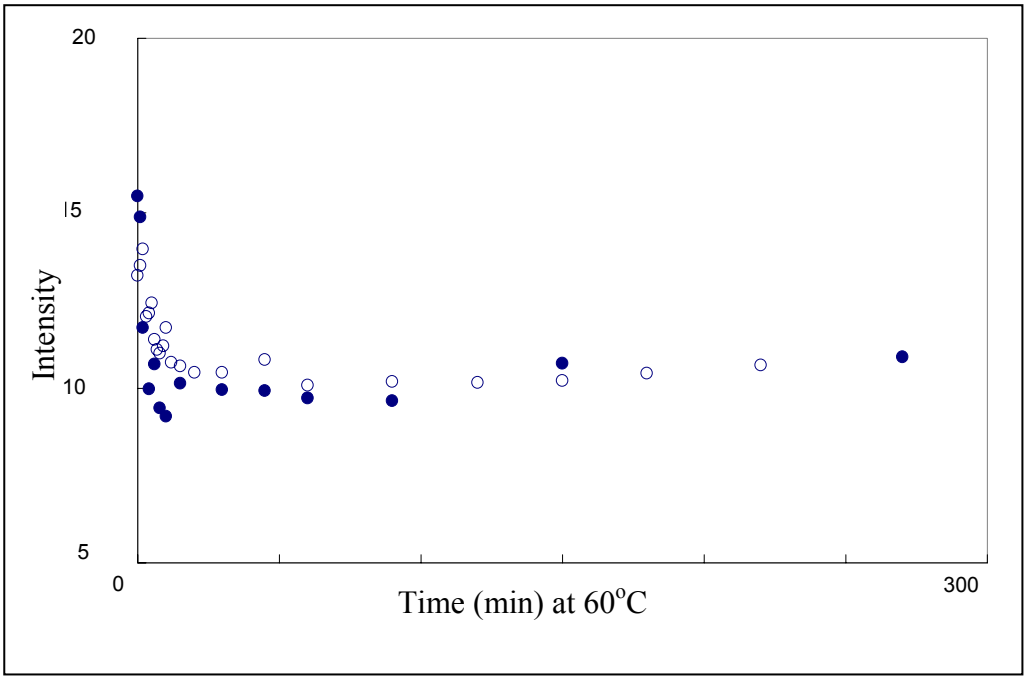

Figure 9. Variation with time of ageing in air at 60°C of the intensities of the EPR spectra at 77 K from the single peak free radical in 5% soluble coffee solutions incubated with or without POBN

In the presence of the spin trap POBN, the intensity of the adduct spectrum increased progressively with time during incubation of the coffee at 60°C. This is in contrast to the observations reported with fresh beer samples (Uchida and Ono, 1996), where there was a significant lag period prior to the detection of the adduct signals in fresh samples. One possible explanation is that the generation of ·OH radicals via a *Fenton* reaction involving H_2O_2 is a fundamental step in the spin trapping process. In beer, H_2O_2 is formed during the oxidative spoiling of the beverage and this is mediated by the presence of antioxidants. As a consequence, free radical adduct formation with a spin trap is inhibited until these antioxidants have been severely depleted. In contrast, with soluble coffee solutions H_2O_2 is present from the outset (Nagao et al., 1986) and there is no delay in the initiation of the *Fenton* reaction.

The measurements made with POBN as spin trap indicate that the trapped free radical is a C-centred entity and is not the ·OH radical. Although a fundamental role has been postulated for ·OH in the previous paragraph, and was strongly indicated by biochemical measurements in the work of Uchida and Ono (1996) with beer, its non-observation is not altogether surprising. ·OH is able to extract ·H atoms from a wide range of organic molecules, generating ·C-centred radicals in the process. These latter radicals are generally more stable, and it is likely that such reactions occur in both beer and coffee solutions, because the spin trap molecules are present at much lower levels than the organic molecules of the beverages.

ACKNOWLEDGEMENTS

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Applicability of Metal Oxide Sensors for Long Term Measurement of Aroma Concentration and Quality above Coffee Powders

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SUMMARY

Gas sensors have been widely tested since the nineties to predict the quality of unknown products after a training period with acceptable and not acceptable samples. Most of reported applications screened only a limited number of samples in a relatively short period of time. This study was aimed at screening numerous instant coffee powders characterised by in-jar aromas varying in concentration and quality during a period of 16 months (100 products). Instrumental results (gas sensors and standard gas chromatography) were compared with sensory data.

A good agreement was established between the results from the metal oxide sensors and the results measured by gas chromatography. The FOX 3000 from Alpha M.O.S. was already found to be a reliable and reproducible instrument already in a previous study. Nevertheless, important sensor drifts were observed during the study (base line and sensitivity). However, these drifts could be compensated using built-in calibration procedures. The system could recognise blind samples with a success rate higher than 72%.

Our results showed that the FOX 3000 could be used roughly to predict the concentration of total headspace aroma, and simultaneously the concentration of sulphur compounds in the headspace. This study indicated that the sensors were mainly responding to overall headspace concentration. The information delivered by the sensors on differences in aroma composition was very limited. No correlation with sensory data could be derived from such instrumental analyses up to now. This is not surprising as most key compounds of coffee aroma are present in very low concentrations in the headspace and do not contribute to the signal measured.

RÉSUMÉ

Les capteurs à gaz ou “nez électroniques” ont été souvent utilisés depuis les années 90 pour prédire la qualité d’échantillons inconnus après avoir entraîné l’instrument à distinguer des échantillons acceptables et inacceptables. Néanmoins, la plupart des applications publiées se réfèrent à un nombre limité d’échantillons analysés et, de plus, sur une courte durée. L’objectif de cette étude était d’analyser un grand nombre de cafés instantanés caractérisés par différentes concentrations, compositions et qualités d’arôme de bocal (une centaine de produits), le tout sur une période de 16 mois.

Les résultats obtenus avec le FOX 3000 se sont révélés en bon accord avec les résultats obtenus par chromatographie en phase gazeuse. Cette étude a confirmé que l’instrument développé par Alpha M.O.S. est fiable dans son fonctionnement et que la reproductibilité des

analyses est satisfaisante. Une dérive importante des capteurs a néanmoins été observée au cours de cette étude mais a pu être corrigée de façon satisfaisante avec les procédures d'étalonnage fournies avec l'instrument. Des échantillons analysés à l'aveugle après 16 mois ont été reconnus avec un taux de succès d'au moins 72%.

Nos résultats ont montré que le FOX 3000 peut être utilisé pour prédire la concentration totale de l'arôme dans l'espace de tête du bocal de façon semi-quantitative, et pour donner simultanément une idée de la concentration des composés soufrés dans l'espace de tête. Cette étude a également montré que les capteurs répondent principalement à la concentration globale de l'arôme et que les informations sur des différences de composition entre les échantillons sont limitées. Aucune corrélation n'a pu être établie à ce jour entre les descripteurs sensoriels de l'arôme et la mesure de l'arôme dans l'espace de tête, que ce soit par GC ou avec les capteurs. Ceci n'est pas surprenant au vu de la faible concentration de la majeur partie des composés odorants-clés de l'arome de café.

INTRODUCTION

Quality assurance at production floor is the only way of insuring a constant quality of the products delivered to the consumers. Beside physical and chemical controls, sensory procedures play a major role in the final liberation of coffee products, either for roast and ground coffee, or for instant powders. The overall aroma balance of coffee is very difficult to predict based on the chemical composition of the aroma, due to the low concentration of many key aroma compounds and the masking effects between potent odorants of coffee (Vitzthum et al., 1990; Grosch and Blank, 1992; Grosch, 1998; Grosch et al., 1999; Gretsch et al., 1999). Moreover, the techniques used to quantify the aroma fingerprint of a product are by far too complex and time consuming to be applied on a routine basis. However for specific applications, the knowledge of global aroma concentration together with some indications about the content of some tracers of quality would be of great interest for the manufacturer.

"In-jar" aromatisation of soluble coffee is used to deliver a fresh coffee-like aroma to the consumer at first openings of the product. The concentration and the composition of the aroma in the powder headspace are dependent on the aroma source and manufacturing conditions. Therefore, a rapid and simple method to control the global concentration of the aroma in the finished product would be useful at production floor to assure a constant quality of the product.

Gas sensors have been widely tested since the nineties to predict the quality of unknown products after a training period with acceptable and not acceptable samples. However, most of reported applications screened only a limited number of samples over relatively short period of time (Gardini et al., 2000; Bazzo et al., 1998; Schnitzler et al., 2000; Abass et al., 1999; Braggins et al., 1999; Newman et al., 1999; Luzuriaga and Balaban, 1999; Gardner et al., 2000; Ramalho, 2000). This study aimed at screening numerous instant coffee powders with in-jar aroma varying in concentration, composition and quality during a period of 16 months. Moreover, instrumental results (gas sensors and standard gas chromatography) were compared with sensory data.

Since several years, electronic sensors have been tested in our laboratory for their ability to characterise the aroma fraction released from instant coffee aromatised for in-jar aroma (first opening of the package). A first study conducted in 1996 allowed to evidence that conducting polymers were not suited for analysing coffee aroma in finished products. The reason was the high sensitivity of these sensors towards moisture and carbon dioxide, both components always present in much higher concentration than coffee aroma in the powder headspace

(Gretsch et al., 1997). More positive results were obtained with metal oxide sensors. An in depth investigation of the commercial instrument FOX 3000 supplied by Alpha M.O.S. Ltd (Toulouse, France) in 1997 revealed that the apparatus was reliable and could be used to conduct a long term study (Gretsch et al., 1998).

EXPERIMENTAL

Around 100 instant coffees conditioned in sealed glass jars were analysed for headspace aroma over a period of 16 months. Two product categories were considered (P, R) and 3 variants per category (P1-P3, R1-R3, each variant being further submitted to storage test). These products differed in headspace aroma concentrations and aroma quality already at production. The ca. 100 products were analysed in triplicate taking each time a different sealed jar with the FOX 3000 apparatus equipped with 12 sensors. The samples were also analysed in duplicate from different sealed jars using a reference method based on gas chromatography. Major features of both analytical methods are presented in the Figure 1. The samples were also described by sensory profiling using a trained panel.

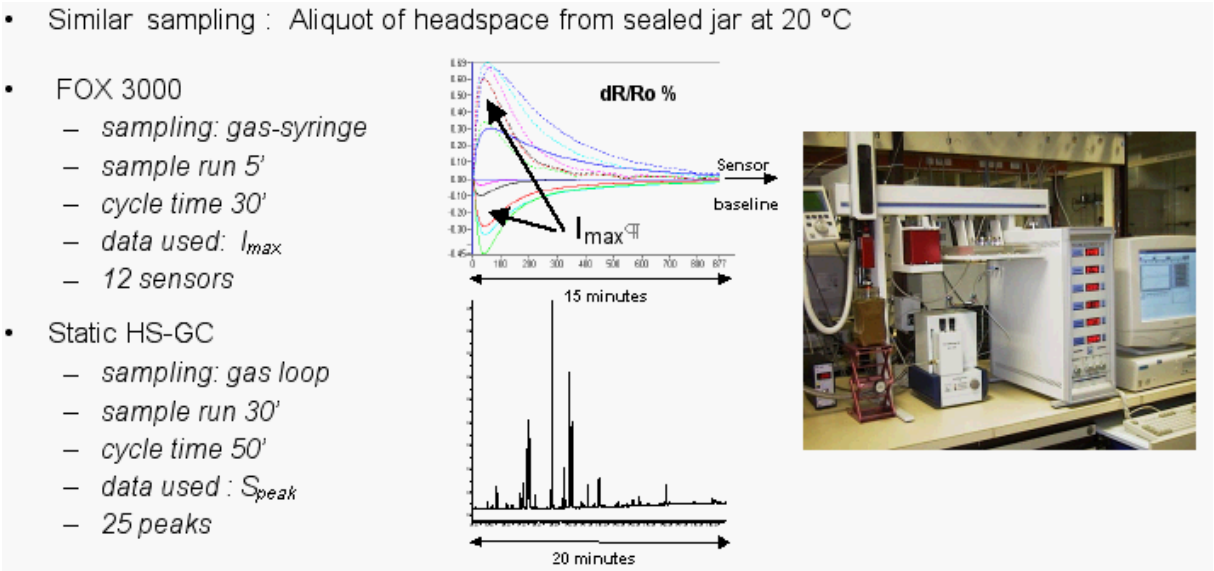

Figure 1. Comparison of aroma sampling systems, signal measured and data retained for further treatment when using the FOX 3000 or standard gas chromatography

The calibration of the response over time was performed with external gaseous standards for the analyses performed by gas chromatography. For the metal oxides, we tested two calibration procedures with the supplier. The first one proposed by default in the software used external standards. Pure chemical compounds were dissolved in MCT oil and a set of vials was prepared (one chemical per solution), stored at –20°C and analysed during the study prior to the coffee samples. The second method of calibration used the intrinsic characteristics of the sensors determined simultaneously to the analysis of coffee samples. This approach gave better results and all recognition results presented in this work were obtained with the second method. Both calibration procedures are based on protected software and the user has only access to the raw data.

RESULTS

The sensor drifts with time and calibration procedures are discussed first. Then the ability of metal oxides to measure aroma concentration is presented, following by the potential of the

system to recognise blind samples. Finally, the discrimination of the samples based on their quality is discussed.

Sensor drifts and calibration procedures

The base lines of the sensors exhibited significant drifts during the 18 months of use (Figure 2). The magnitude of the drift depended on sensor. The observed shifts were positive for most of the sensors, except for SY/AA, SY/G and SY/Gh. Sensors presenting highest drifts were SY/gCT*, SY/LG and SY/AA for chamber 1, and PA2*, T30/1* and T70/2* for chamber 2. Four of these sensors (*) were strongly influenced by the change of laboratory premises.

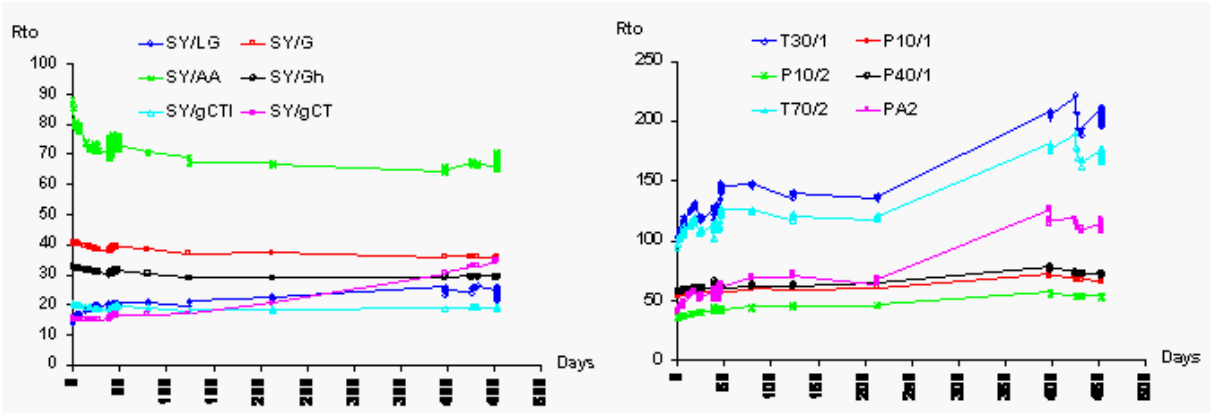

Figure 2. Evolution of sensor baseline between September 1998 and February 2000. Left: 6 sensors of chamber 1, Right: 6 sensors of chamber 2. Data points represent period of analyses with the instrument. Notice that the laboratory changed premises between days 220 and days 400

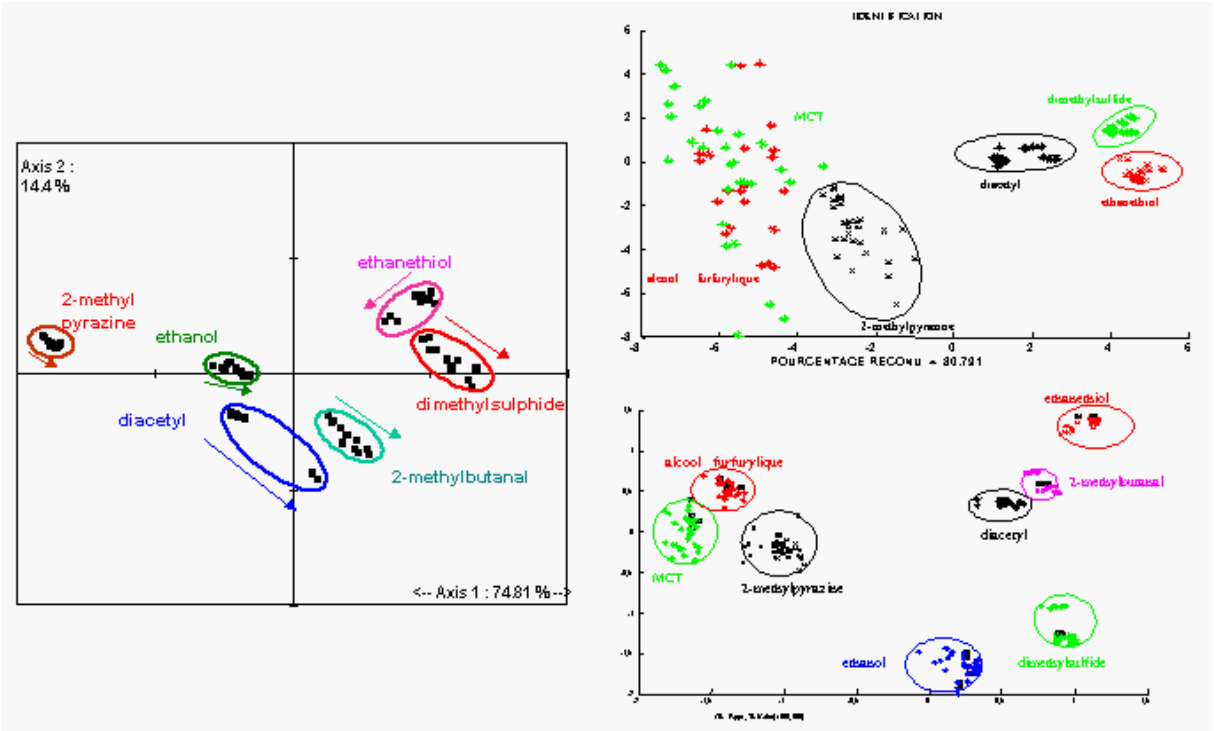

Figure 3. Comparison of raw data (left) with calibrated data (right) for standard solutions in MCT oil (top right: calibration using 2-methylbutanal and ethanol, bottom right: calibration using intrinsic parameters of sensors)

These base line drifts were accompanied by changes in sensor response during the study. Moreover the magnitudes of changes were different depending on the standard measured, as shown in Figure 3. Using the built-in calibration procedures, the first two days of analyses were taken as learning days (the system was told about the link between results and chemical compound, and a model was built using a Discriminant Factor Analysis). Samples belonging to the remaining 9 days of analyses were considered as unknown and projected in the model built with the learning period. The success rate for classifying unknown samples passed from 78% on raw data to 80-89% using the external calibration and to 100% using the internal calibration. The internal calibration was indeed the most efficient. It has not only the advantage of liberating the user from additional analyses using chemical standards, but also of providing a better correction for sensor drifts (see also Tan et al., 1998 for external calibration example).

Assessment of aroma concentration in the headspace of the coffee powders

To establish better links between global headspace intensity and the response of the sensors, a few coffee powders were coated at pilot scale with an increasing amount of aromatised oil, resulting in an increasing concentration of in-jar aroma. The sensor responses to concentration were either linear (e.g. SY/G, SY/Gh) or followed a power function of concentration (P10/2, P40/1), as shown in Figure 4 (left graph). Similar relations were searched in the long-term study, knowing that the in-jar aroma concentration of the 6 products varied between 100 ppm and 400 ppm. A discriminant analysis was performed to see which sensor would better predict the global concentration in the jar using the data from the whole study. The result evidenced that P10/2 and SY/LG responses presented the best fit with respectively, global aroma and global sulphur concentration in the jar. The relations between sensor response and ppm HS are represented in the Figure 4 (right graphs). The dispersion of the points could be explained by the jar-to-jar variation for one quality (GC and FOX data are obtained from different sealed jars, see also Table 1), and to a lesser extent by the differences in relative aroma composition between products. These results indicate that the FOX could be used to provide a rough quantification of in-jar aroma concentration.

Recognition of unknown samples

The coffees analysed were issued from two categories of products (P, R) and 3 variants per category were analysed (P1-P3, R1-R3). Using the internal calibration based on the intrinsic characteristics of the sensors measured during the analyses of the coffees, various models were built by Alpha M.O.S. depending on the level of discrimination desired. Results from the first 50% of analyses were used to build the models; the latter were then validated with remaining results (Figure 4). This study showed that the FOX could discriminate between different products, as long as the built model was restrained to the subclass considered. However, when considering the whole set of results, the discrimination was reduced somehow due to the relatively high dispersion of data, in regard to the distances between the groups.

These models were used to recognise blind samples reanalysed at the end of the evaluation period. The samples reanalysed belong to variants P1, P2 and P3 (model B in Figure 4). When considering first P or R categories (model A), the blind samples were attributed to P products with 72% success. The product variants were then calculated for both types of products categories P and R, using respectively models B or C. Although a better score was found for the P variants (Table 1), the score obtained for R variants evidenced significant overlapping. Interestingly, the product variant inside a category was more easily solved than the product category. This confirms the high correlation of sensor response with headspace global concentration as shown in the table below.

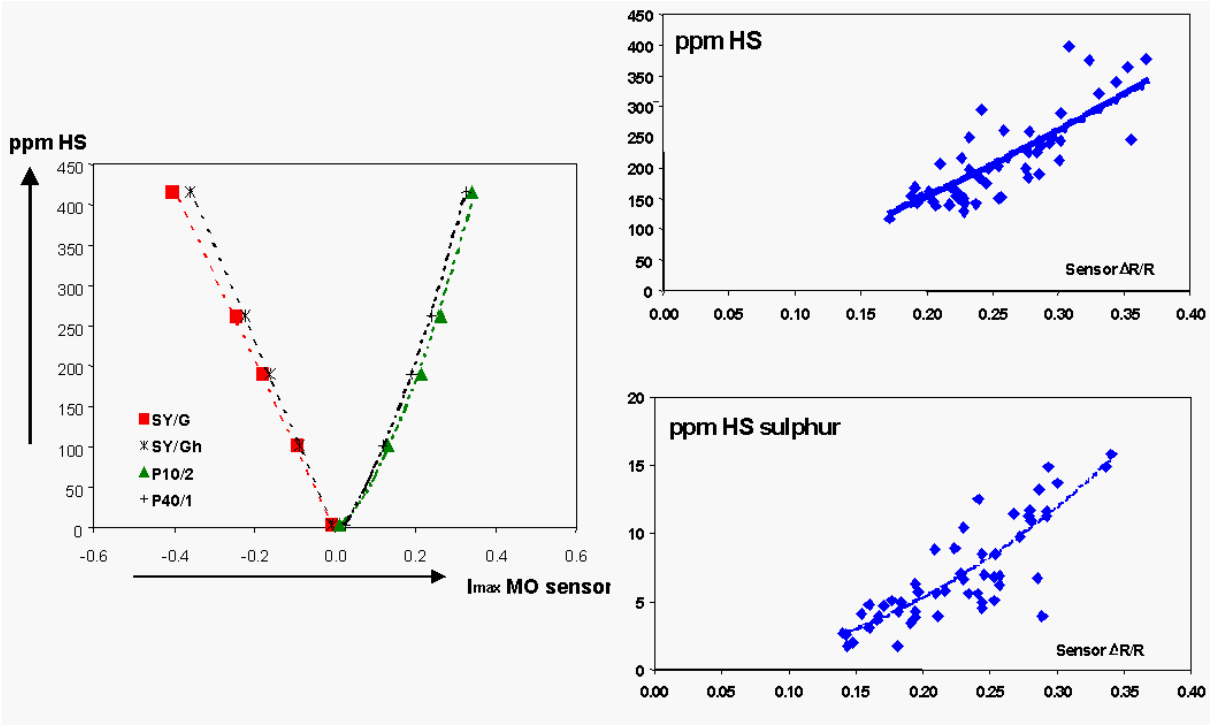

Figure 4. Response of metal oxide sensors to increasing concentration of in-jar aroma as measured in ppm by GC. Left: Samples produced at pilot scale. Correlation between in jar aroma concentration and sensor response for top right: P10/2sensor and global carbon response (FID detector). bottom right: SY/LG sensor and global sulphur response (FPD detector)

Discrimination of samples based on their quality

When looking at overall evolution of samples during storage, a good agreement was observed between GC and FOX data (Figure 5). However, this evolution was very different from the one drawn with sensory data. P3 variant was more different from the other two variants when considering instrumental data, but P1 variant was more different from the two others when looking at sensory data. Moreover based on FOX and GC data, the magnitude of change during storage was larger for P3, although sensory results exhibited a larger change for P1.

Table 1. Successful rate of unknown samples towards product categories and variants. Global headspace concentration as measured by gas chromatography, considering all analyses during 17 months

	Products P (model B)			Products R (model C)		
Product ppm aroma	**P1** 192+/-46 ppm	**P2** 234+/-15 ppm	**P3** 341+/-62 ppm	**R1** 198+/-62 ppm	**R2** 152+/-20 ppm	**R3** 205+/-59 ppm
P1 (blind)	**75%**	25%	0%	34%	**58%**	8%
P2 (blind)	6%	**94%**	0%	**79%**	21%	0%
P3 (blind)	0%	15%	**85%**	38%	8%	**54%**

When combining sensory and FOX or GC data, no correlation between quality and sensor response could be established up to now. Using a simple principal component analysis, we could see that sensory descriptors were orthogonal to the sensor or GC variables, meaning no correlation at all (Figure 6). This confirms that compounds in high concentration in the headspace above the powder are not reflecting aroma intensity and quality in the case of coffee.

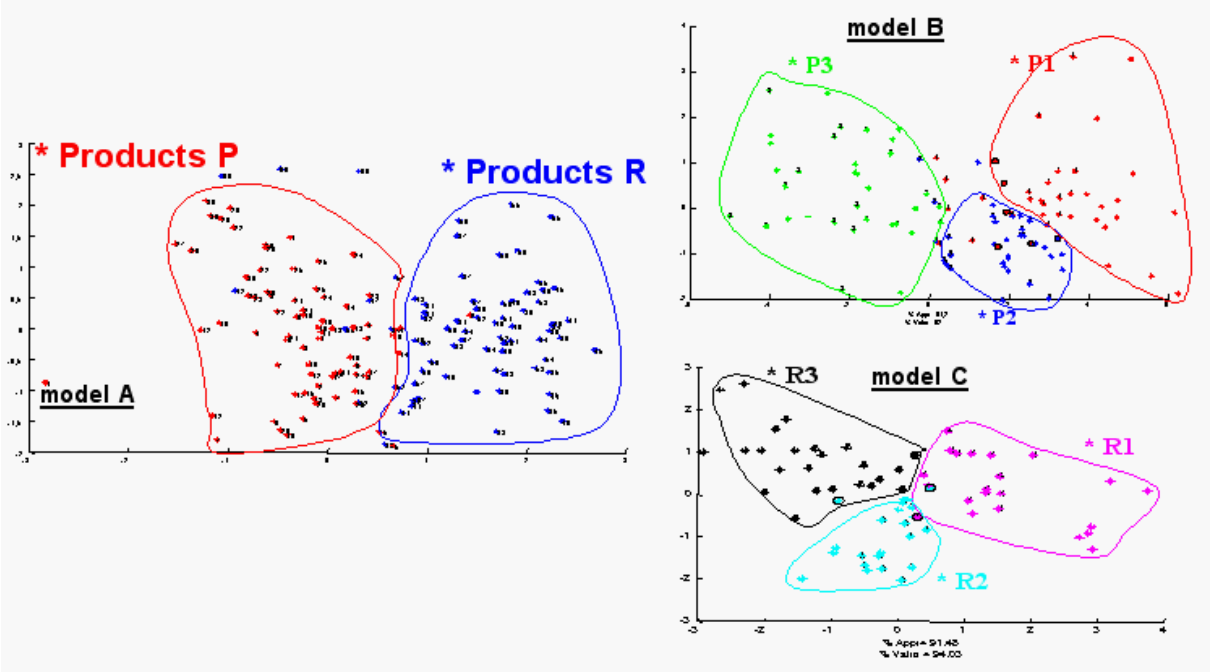

Figure 4. Models built to discriminate between samples: left: products P and R (learning score 97.4%, validation score 94.5%); top right: variants of products P (learning score 91.2%, validation score 92%); bottom right: variants of products R (learning score 91.5%, validation score 94%)

CONCLUSION

The FOX 3000 from Alpha M.O.S is a reliable instrument when operated in a controlled environment (air quality and regulation of the moisture level in the carrier gas). The reproducibility between analyses run within a day lies in the range of a few percents. A good agreement was established between the results obtained with the metal oxide sensors and the results measured by gas chromatography (overall concentration).

Significant drifts of sensor baseline and response were observed over a 16-month period of use, stressing the need for a good procedure of calibration. The recent method developed by the supplier and based on internal calibration using the physical characteristics of the sensors gave better results than the first one available, based on external chemical standards. The system could recognise blind samples with a success rate higher than 72%.

This extensive study revealed that the sensors were mainly responding to overall headspace concentration, when measuring in-jar aroma above coffee powders differing in concentration, composition and aroma quality. The information delivered by the sensors on differences in aroma composition was very limited. No correlation with sensory data could be evidenced, confirming that compounds in high concentration in the headspace above the powder are not reflecting aroma intensity and quality in the case of coffee. However our results indicate that the FOX could be used to provide a rough quantification of in-jar aroma concentration.

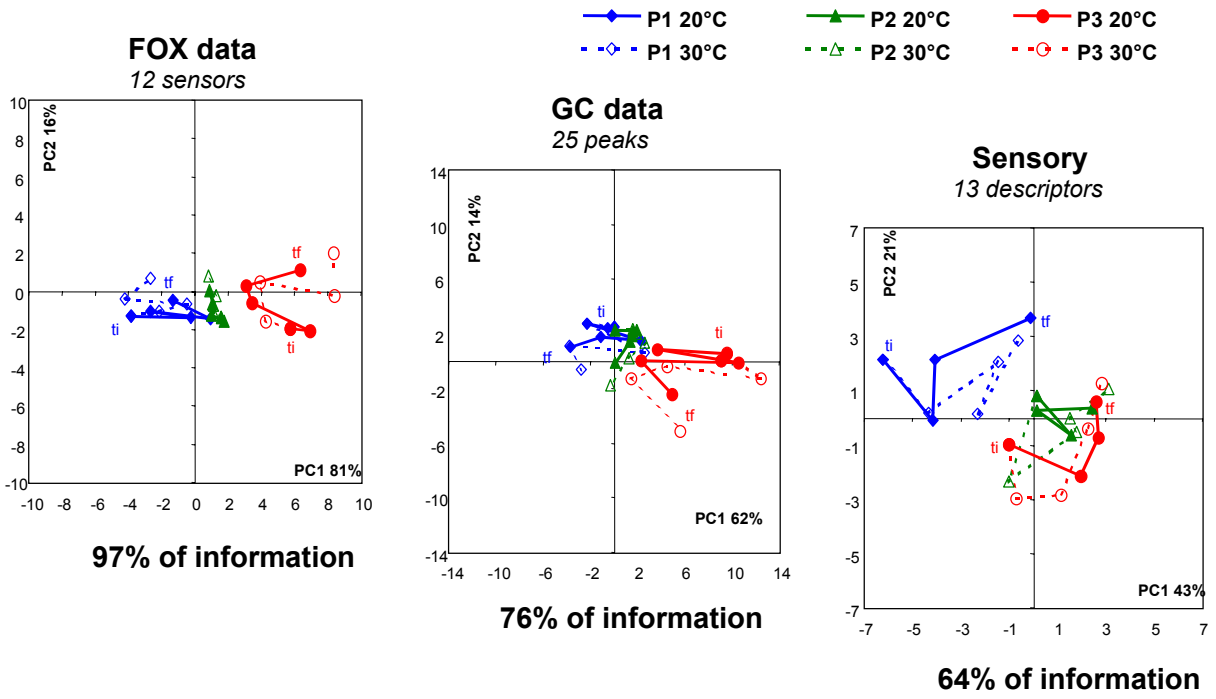

Figure 5. Evolution of P samples during storage. Comparison of Principal Component Analysis obtained from instrumental (GC, FOX) and sensory analyses

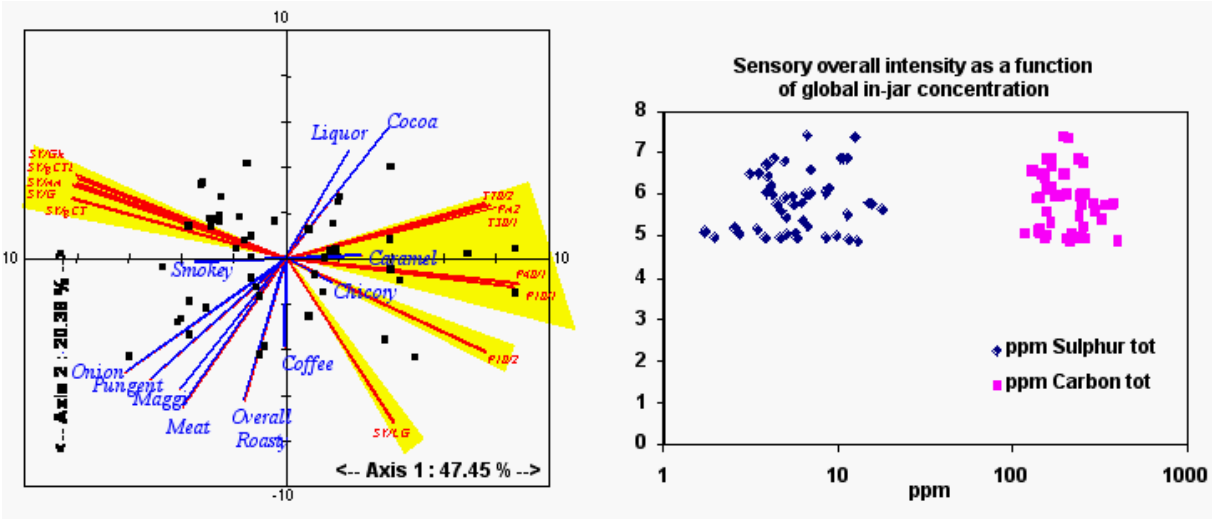

Figure 6. Principal Component Analysis obtained on FOX and sensory data (left). Plot of sensory overall intensity towards headspace concentration

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Detection of an Off-flavour in Raw Coffee: An Approach of the High Resolution Gas Chromatography/selective Odorant Measurement by Multisensor Array (HRGC/SOMSA)

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SUMMARY

Chemical sensors, e.g. metal oxides, are widely used in the so-called “electronic noses”, which have been offered commercially to be used in the control of food quality by the characterisation of the food flavour. But in practise only expensive gas chromatographic applications are used for flavour research. The HRGC/SOMSA device represents the combination of chromatographic separation and such chemical sensors. The aim of this approach in our investigation was to detect an off-flavour in steam treated coffee, described as unpleasant sweaty. The analysis of the off-flavour itself was performed by GC/MS and GC/O and 3-Methylbutanoic acid was identified as the main contributor. This result was used for the investigations with the HRGC/SOMSA device, in which the routine GC detector was replaced by a sensor array, which consists of six metal oxide sensors. After the characterisation of the metal oxides with regard to their selectivity and their sensitivity it was possible to create a sensor array with sensors sensitive to certain volatiles in the ppb range. This device was able to distinguish between "normal" and "off-flavour". The ongoing work will concentrate on the development of sensor arrays, which are able to classify food quality by selective odorant measurements with a minimum of chromatographic separation.

ZUSAMMENFASSUNG

Chemische Sensoren, z.B. Metalloxidsensoren, werden häufig in den sogenannten “Elektronischen Nasen” eingesetzt. Diese kommerziell erhältlichen Geräte werden für die Qualitätskontrolle von Lebensmitteln und die Charakterisierung von Lebensmittelaromen angeboten. In der Praxis konnten sich diese Geräte für den Bereich der Aromaforschung nicht durchsetzen, hier dominieren gaschromatographische Methoden. Das HRGC/SOMSA-System stellt eine Kombination aus Gaschromatograph und den chemischen Sensoren dar. Dieses System wurde in dieser Untersuchung für die Detektion eines off-Flavours im wasserdampfbehandelten Rohkaffee eingesetzt, welches als unangenehm schweißig beschrieben wurde. Die Identifizierung dieses off-Flavours, 3-Methylbuttersäure, wurde mittels GC/MS und GC/O durchgeführt. Dieses Ergebnis wurde genutzt um die Metalloxidsensoren des HRGC/SOMSA bezüglich ihrer Selektivität und Sensitivität auszuwählen. Der so entwickelte Sensorarray, bestehend aus sechs Sensoren, die den üblichen GC-Detektor am Ende der Trennsäule ersetzten, konnte teilweise selektiv Aromastoffe im ppb-Bereich nachweisen. Somit war es möglich bei den vorliegenden Proben zwischen normalen und off-Flavour Kaffee zu unterscheiden. Die zukünftige Arbeit soll sich mit der Miniturasierung dieses Systems, d.h. mit einem Minimum an chromatographischer Vortrennung, beschäftigen, um die Qualität eines Lebensmittels zu bestimmen.

INTRODUCTION

Flavour is the most important attribute in the evaluation of coffee quality. Odorants contributing to a given coffee flavour can be sorted out from the much larger number of none-odour molecules by using GC-techniques. In the past, new devices, so called "electronic noses", which have been offered commercially to be used in the control of food quality by the characterisation of the food flavour, have been the object of scientific presentations (Aishima, 1991; Bartlett, 1993; Fukunaga, 1995; Gretsch, 1997; Mitrovics, 1997). But still only expensive gas chromatographic applications are used for flavour research, because chemical sensors often showed deficiencies in selectivity, sensitivity and reproducibility. Furthermore sensor arrays measure the bulk of predominant food volatiles, but do not measure the major impact compounds consistently.

The HRGC/SOMSA device represents the combination of chromatographic separation and such chemical sensors (Hofmann, 1997). This device is able to classify the behaviour of different sensitive sensor layers and to elaborate knowledge on responses of sensor surfaces to certain functional groups of the aroma compounds. With these results it should be possible to develop more selective chemo-sensor arrays. Within the scope of these investigations one field of application is the perceptibility and detectability of off-flavours. Common causes for coffee off-flavours may already originate in the field, or from inaccurate conditions during harvesting or processing (Illy and Viani, 1995).

This study is on steam-treated coffee produced from one raw coffee batch. The steam-treatment of raw coffee prior to the roasting process is a widespread method for the production of less irritant coffee. After the treatment one part of the batch had an off-flavour, while the other part had a normal flavour. Therefore the aim of the study was in the first step to identify this off-flavour by conventional GC-techniques. After that experiments are to be carried out to show if the HRGC/SOMSA device, with its different metal oxide sensors, is able to detect this off-flavour.

EXPERIMENTAL

For the investigations steam treated coffee beans from one batch, one with an off-flavour, described as unpleasant sweaty and cowshed-like, were used. In the course of these experiments, both coffees were analysed three to five times by the following methods.

GC-MS and GC-Olfactometry

Coffee beans (CR3-Kaffeeveredelung M. Hermsen GmbH, Bremen, Germany) were ground in a water-cooled centrifugal mill (A10S, Janke and Kunkel, Germany). 20g of this coffee powder were filled in the external dynamic headspace device (Bücking, 1999). With a flow of 40 mL/min for 30 minutes, nitrogen was flushed above the coffee surface, and the volatiles were collected on Tenax TA and/or Carbotrap tubes. The volatile compounds were analysed on an Agilent 5890 GC equipped with a Sniffing-Port or an MS-Detector. Volatiles were desorbed by a thermal desorption device onto a cryogenic trap cooled with N_2 at –150°C. They were then injected by flash heating onto a DB-5 capillary column (30 m x 0.53mm x 1.5 µm) in the case of GC/O and onto an OV-1701 (60 x 0.25 mm x 0.5 µm) capillary column in the case of GC/MS. Compound identification was achieved by comparison of retention data on DB-5 and/or OV-1701 and mass spectral data, as well as comparison of sensory properties with those of authentic reference substances. Mass spectra were generated at 70 eV in the electron impact mode.

HRGC/SOMSA

5g of the finely ground coffee powder (see above) were filled in 20 mL vials, and were sealed and equilibrated at room temperature for 24 h. After this they were placed into the incubator oven (60°C/30 min) of the autosampler (MultiPurposeSampler MPS2, Gerstel, Germany). 7.5 mL of the headspace were injected onto a cryogenic trap cooled with CO_2 at –50°C (KAS 4, Gerstel), flash heated and injected onto a OV-1701 (60 x 0.25 mm x 0.5 µm) in an Agilent 6890 GC. The capillary column was held at 40°C for 2 min, raised at 5°C to 220°C, raised at 25°C to 270°C and held for 5 min. The effluent at the end of the column was split into an FID and the sensor array. This array was composed of a brass box (internal volume 1.8 mL) which held six metal oxide sensors (MOS) and was placed as an additional detector besides the installed FID at the heated detector base (120°C).

Table 1. Types of sensors

sensor	metal oxide (purity)	catalyst (amount)	type of conductance
WO_3	99,9%	-	n-type
WO_3	99,9%	-	n-type
ZnO/Pt	99,999%	7% (w/w)	n-type
ZnO/Pt	99,999%	7% (w/w)	n-type
UST 1330	commercial MOS, supplied by UST (Umweltsensortechnik), Geschwenda, Germany		
UST 4330			

Suspensions of semiconducting metal oxides on ceramic substrates (3x3 mm aluminium oxide platelet with platinum interdigitated electrodes) were prepared and/or commercial sensors were used (Table 1). The sensor temperature was varied between 150°C and 420°C in order to find the proper operating conditions.

RESULTS AND DISCUSSION

GC-Olfactometry and GC/MS

About 30 corresponding odorants were recognised at the sniffing port during the GC-run for both samples. There was only one difference: a potent odorant with a sweet at lower and a sweaty odour impression at higher concentration was recognised at 19.17 min in the case of the “off-flavour” coffee.

3-Methylbutanoic acid, as a reference substance, injected at the same conditions, showed the same odour impression and the same retention index.

The large amount of gas phase, which was collected on Tenax TA and/or Carbotrap tubes, resulted in a large number of peaks in the MS-chromatogram, which were not inspected in more detail. Generally this high number of volatiles, compared to the raw coffee (Czerny and Grosch, 2000), was partly generated by a “slight” Maillard reaction, which took place during the steam treatment of the raw coffee.

More important was the occurrence of a peak at 18.70 min in the “off-flavour” sample, which was identified by the retention index, the reference substance and the mass spectra as 3-Methylbutanoic acid. Figure 2 shows mass spectra of both coffees. The combination of the fragment ions at m/z 60 (100%), m/z 43 (30%) and m/z 87 (10%) is characteristic for this compound.

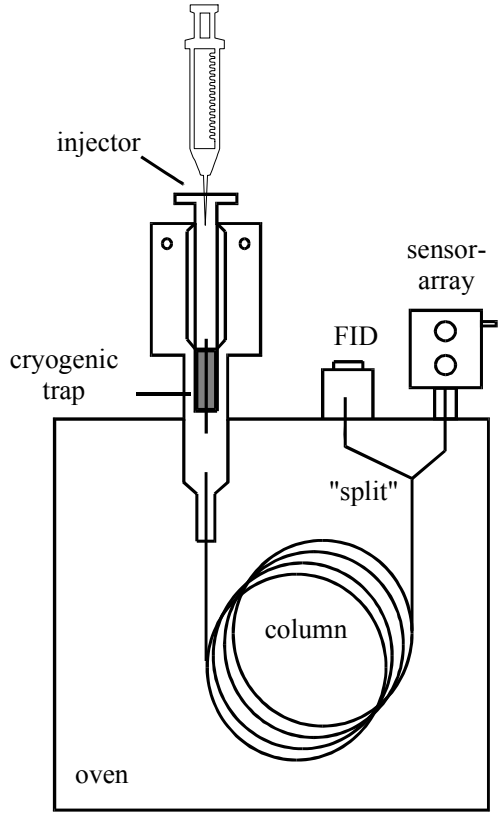

Figure 1. Scheme of the HRGC/SOMSA device. For explanation see text

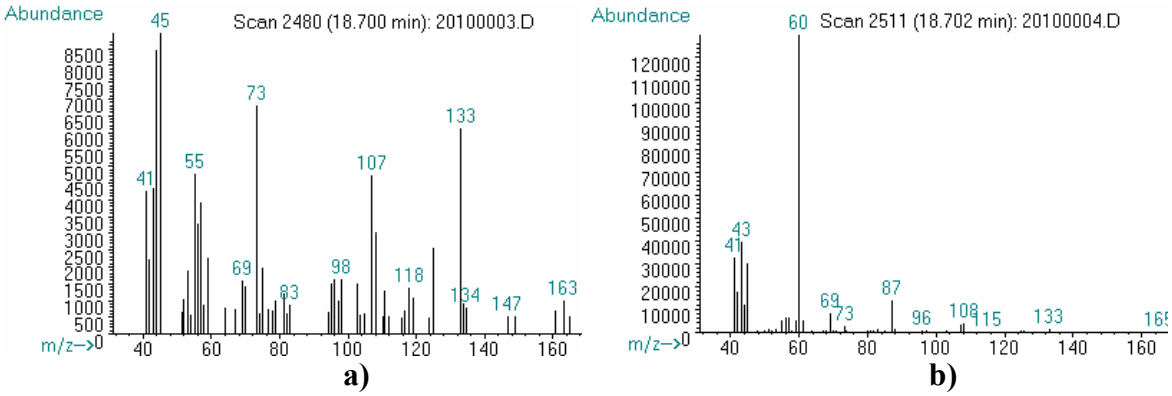

Figure 2. a) Mass spectrum (EI) at 18.70min of "normal" coffee; b) Mass spectrum (EI) at 18.70 min of "off-flavour" coffee

HRGC/SOMSA

Compared to the external dynamic headspace device, which was used for sample preparation of GC/MS and/or GC/O, the amount of trapped headspace gas was much lower (7.5 mL instead of 1200 mL). Thus the volatile concentration was much lower and the identification was more difficult. In addition a semi-polar capillary column was used for the GC separation, which is not completely optimal for short chain fatty acids. Therefore the 3-Methylbutanoic acid was below the detection limit. Furthermore metal oxide sensors have a reduced sensitivity to carboxylic acids, because their functional group, which is mainly responsible for the interaction with the sensor surface is already in the final stage of oxidation. This means that the oxidation at the surface of the sensor is reduced and consequently the change of conductance is low. Ergo the identification of the "off-flavour" coffee was not possible with its originator.

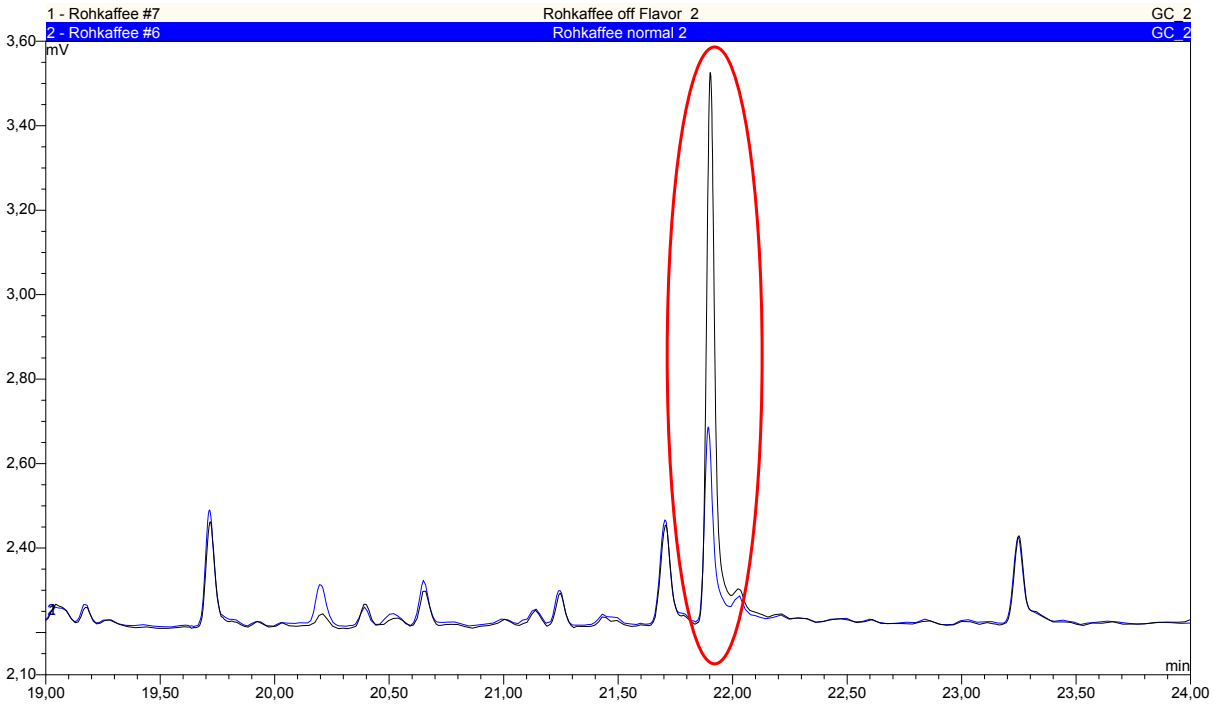

Figure 3. Detail of the GC/FID profile, labelled compound: Benzaldehyde

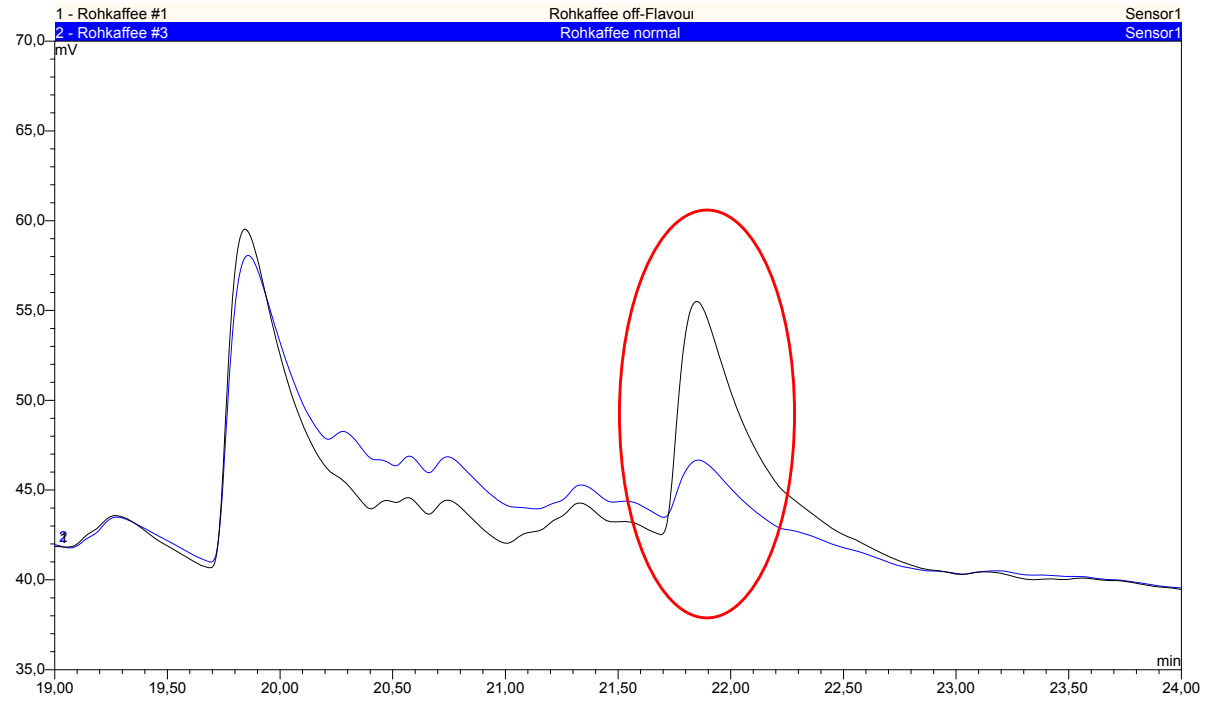

Figure 4. Detail of the HRGC/SOMSA profile, received by a WO_3-sensor at 350°C. Labelled compound: Benzaldehyde

Another compound, benzaldehyde, was selected as indicator of the off-flavour. Its concentration correlated with the concentration of 3-Methylbutanoic acid. Figures 3 and 4 show these higher amounts of benzaldehyde in the “off-flavour” coffee in comparison to the “normal coffee”.

In distinction to the other sensors (Table 1) the signal of the WO_3-sensor clearly showed the difference between the two coffee samples (Figure 4). Because of the sensor drift this result was obtained only in a reproducible way for a short time range.

CONCLUSIONS AND OUTLOOK

The HRGC/SOMSA device is a useful tool for the detection of aroma compounds. It has been shown to work in very sensitive way. The selectivity of each sensor can be rather poor, but the use of a sensor array increases the selectivity. The reproducibility is insufficient, but an update of the evaluation software, which included the calculation of the sensor drift and the use of calibration standards, would permit the use of this device during a long period.

Further, the ongoing work will concentrate on the development of sensor arrays, which are able to classify food quality by selective odorant measurements with a minimum of chromatographic separation. This would enable the dimensions of the device to be reduced to those of a hand apparatus.

ACKNOWLEDGEMENTS

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Analytical Flavour Characterisation and Classification of Arabica and Robusta Coffees from Different Origins

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SUMMARY

The aim of this study was to evaluate whether analytical methods could be used to measure variability of green and roasted coffees from different origins. Therefore, the volatile compositions of 10 Arabica and 7 Robusta coffees were studied using a combination of simultaneous steam distillation-extraction (SDE, Likens-Nickerson) and gas chromatography-mass spectrometry (GC-MS). Principal component analysis (PCA) of the semi-quantitative aroma compositions was used to visualise the relationship between the volatile patterns of the green and roasted coffees from different origins. A good classification between Arabica and Robusta species was obtained. Within the class of Arabica and Robusta species some meaningful clusters were demonstrated, allowing interesting conclusions concerning the influence of origin on the aroma pattern of coffee.

Additionally, the Arabica coffees were subjected to a descriptive sensory test for six aroma descriptors (aroma intensity, burnt/roasted, volatile acidity/fruity/flowery, 'phenolic' Robusta character, bitter taste and sour taste).

PCA was performed on the semi-quantitative aroma compositions of the Arabica coffees in combination with the sensory data. A good relationship was obtained between the aroma compositions and the sensory data, showing some interrelationships between aroma compounds and selected aroma descriptors.

RÉSUMÉ

L'objectif de cette étude était l'évaluation des méthodes analytiques pour mesurer la variabilité entre les cafés verts et torréfiés de différentes origines. Les compositions volatiles de 10 Arabicas et de 7 Robustas ont été obtenues en appliquant une combinaison de distillation à vapeur extraction simultanée (SDE, Likens-Nickerson) et de chromatographie en phase gazeuse couplée à la spectrométrie de masse (CG-SM). L'analyse en composantes principales (ACP) a été appliquée sur les compositions d'arômes semi-quantitatives et visualisait les relations entre les profils volatils des cafés verts et torréfiés de différentes origines. Une bonne classification entre les Arabicas et les Robustas a été obtenue. Par ailleurs, dans les classes mêmes des Arabicas et Robustas d'importantes 'clusters' ont été démontrées, menant à des conclusions intéressantes concernant l'influence de l'origine sur les profils aromatiques des cafés.

Additionnellement, les Arabicas ont été soumis à une analyse sensorielle descriptive évaluant six descripteurs (intensité d'arôme, brûlé/grillé, acidité volatile/fruité/fleuri, charactère 'phénolique' de Robusta, goût amer et goût acide). L'ACP a été réalisée sur les compositions aromatiques des Arabicas en combinaison avec les données sensorielles. Une relation

signifiante a été obtenue entre les profils aromatiques et les données sensorielles, montrant des relations entre les produits aromatiques et les descripteurs sélectionnés.

INTRODUCTION

As aroma is one of the most appreciated attributes of coffee, the composition of the volatile fraction of roasted coffee has been intensively studied. Several hundreds of compounds have been reported as constituents of coffee aroma (Walter et al., 1969; Grosch, 1998). At the same time different factors influencing the aroma pattern of coffee have been subjects of many investigations. For instance, it is well known that significant aroma differences exist between the 2 commercially important coffee species, *Coffea arabica* (Arabica) and *Coffea canephora var. robusta* (Robusta). Besides the botanical species, other factors (such as geographical origin, dry or wet processing of green coffee, roasting parameters, brewing methods, etc.) can affect coffee aroma.

As coffee aroma traditionally has been judged by specially trained tasters, objective methods for characterising coffee aroma become necessary in today's international coffee trade and production. Moreover, for reasons of constant aroma quality of blends, coffee processors are interested in objective analytical methods to measure variability and interchangeability of coffees from different origins. In this study simultaneous steam distillation-extraction combined with gas chromatography-mass spectrometry (SDE-GC-MS) was evaluated for objective flavour characterisation and classification of Arabica and Robusta coffees from different origins.

EXPERIMENTAL

Materials

The sampling consisted of green and medium-roasted coffee beans from 17 different origins (10 Arabica and 7 Robusta coffees) (Table 1). The coffee samples were medium roasted as single batches (3 kg) using a Neuhaus Neotec® RFB-Junior coffee roaster and medium ground in a Probat® 803C coffee grinder. After standardised roasting and grinding the coffees were packed under CO_2 in COEX OPP (oriented polypropylene) 25 µ/PET metallized 12 µ/PE 60 µ packages with an intern Danisco® valve for Arabica coffees and in PET metallized 15 µ/PE 80 µ packages with an extern, one-way Bosch® valve for Robusta coffees. All coffee samples were supplied by Fort N.V., Itegem, Belgium.

Table 1. Selection of 10 Arabica and 7 Robusta coffees

Arabica	Robusta
Brazil Santos Strictly Soft	Indonesia Flores
Guatemala Antigua	Indian Robusta (dry processing)
Colombia Supremo	Vietnam Robusta
Costa Rica Tarrazu Strictly Hard Bean	Ivory Coast Robusta
Kenya AB	Cameroon Robusta
Java Ling Tung	Uganda Robusta
India Plantation A	Indian Robusta (wet processing)
Papua New Guinea	
Haiti xxxxx	
Malawi - Burundi	

Methods

Isolation of Volatiles

A 'total volatile' analysis method based on a simultaneous steam distillation-extraction (Likens-Nickerson, SDE) was used to isolate the volatiles from the coffee samples. A sample of 25 g ground green or roasted coffee suspended in 600 ml water was extracted for 2 hours in a modified Likens-Nickerson apparatus using 60 ml dichloromethane as extraction solvent and nonane as internal standard (9.51 µg for green coffees; 170.19 µg for roasted coffees). After cooling to ambient temperature the combined dichloromethane fractions were concentrated to a final volume of 0.5 ml (green) or 5.0 ml (roasted) in a Kuderna Danish concentrator (Alltech, USA).

Gas Chromatography-Mass Spectrometry

The SDE-extracts were analysed by injection of 1 µl of aroma concentrate on a HP 5890 gas chromatograph coupled to a HP 5971A MSD mass spectrometer (Hewlett-Packard, USA). The GC was equipped with a fused silica column (HP PONA cross-linked methyl silicone, 50 m x 0.21 mm I.D., 0.5 µm film thickness). Carrier gas was helium (1 ml/min. flow rate) and the column temperature was initially maintained at 40°C for 5 min.. Subsequently it was raised from 40 to 160°C at a rate of 3°C/min., and from 160 to 220°C at a rate of 5°C/min., where it was held for 4 min. Split injection (1:5 split ratio) was used. The injector and the transfer lines were respectively maintained at 250 and 280°C. Chromatograms were recorded by monitoring (70 eV) the total ion current in the 40-260 mass range using a solvent delay of 6.8 min. Identification of the volatiles was based on comparison of the mass spectra with the mass spectra of the NBS49K library and of a self-made library. Semi-quantitative determinations of the volatile constituents were calculated by relating the peak intensities to the intensity of nonane as internal standard and were expressed as ng/g of green coffee bean or as µg/g of roasted coffee bean.

Sensory Analysis

The coffee brews (55 g/l) were prepared with Evian® water (90°C) under strictly standardised conditions using a French press (Bodum®) and served at 50-60°C in individual assessment booths of a panel room. Nine Arabica coffees, except Brazil Santos SS, were subjected to a descriptive sensory test with Brazil Santos SS as reference. The 7 assessors (2 men, 5 women) were selected from the laboratory staff and had previous experience in descriptive sensory analysis. During a training period a defined vocabulary for describing the various aroma characters of coffee was used (Dirinck et al., 2000). PCA analyses of the sensory attributes showed clusters of descriptors and 6 attributes were selected: aroma intensity, roasted/ burnt/chocolate-like/caramelly/nutty, volatile acidity/flowery/fruity, 'phenolic' (Robusta) character, bitter taste and sour taste. During a preliminary sensory training the nine Arabica coffees were assigned into three sensory classes with maximal sensory differences. At each session assessors were presented with three randomly coded coffee brews (one coffee sample from each class), and they assessed each for the intensity of the attributes listed using a continuous 100 mm-score line. All assessors attended nine sessions; each coffee sample was assessed in triplicate and the order of presentation followed a balanced experimental design. After sensory analysis the scores were converted to numerical values. For principal component analysis (PCA) mean scores for each attribute were calculated.

Statistical Analysis

To visualise the complex data matrix, principal component analysis (PCA) was performed using The Unscrambler® 6.1 (Camo, Norway) statistical software.

RESULTS AND DISCUSSION

As the aroma of coffee is mainly generated during roasting, much less volatiles were found in green coffees in comparison with roasted coffees. The green coffees from different origins had a rather similar qualitative, but a different quantitative aroma composition. An important amount of aliphatic aldehydes (pentanal, hexanal, heptanal, 2-heptenal, 2-octenal, nonanal, 2-nonenal, 2-decenal and (E,Z)- and (E,E)-2,4-decadienal), derived from autoxidation of fatty acids or esters, was found (Belitz and Grosch, 1987). Besides some typical fermentation products (e.g. 2,3-butanedione, 2,3-pentanedione, 3- and 2-methylbutanal, 3- and 2-methyl-butanoic acid), some monoterpenes (e.g. α-pinene, Δ^3-carene, limonene, terpinolene, α-terpineol) and β-damascenone, were identified in the green coffees. The typical green coffee odour compound, 2-methoxy-3-isobutylpyrazine, could also be identified (Vitzthum et al., 1976). The level of phenolic compounds in green coffees (phenol, 2-methoxyphenol (guaiacol) and 4-ethenyl-2-methoxyphenol (4-vinylguaiacol)) already demonstrated the aroma difference between Arabica and Robusta coffees. The fifty-eight aroma compounds that were identified and semi-quantified in the green varieties were classified according to their chemical structures (pyrazines, furans, pyridines, phenolic compounds, aromatic compounds, ketones, aldehydes, alcohols, terpenes, etc.). In order to visualise the volatile composition matrix, principal component analysis (PCA) was performed on the semi-quantitative data. The score plot showed a good classification between the green Arabica and Robusta coffees. Furthermore, green Arabica and Robusta coffees were clearly differentiated mutually.

The roasted coffees from different origins showed a much more intense aroma pattern and had also a rather similar qualitative, but a different quantitative composition. In comparison with the green coffee varieties some typical roasted coffee compounds, such as furans, pyrazines and pyrroles, were found. The fifty-nine identified volatile compounds were classified according to their chemical structure (pyrazines, furans, pyrroles, phenolic compounds, esters, ketones, acids, aldehydes, S-compounds, etc.) and their presence could often be related to chemical reactions during roasting and processing. Comparison of the SDE-GC-MS profiles revealed a significant difference between the roasted Arabica and Robusta varieties at the level of the phenolic compounds (phenol, guaiacol, 4-ethylguaiacol and 4-ethenylguaiacol). Robusta coffee showed 4-5-fold higher concentration of phenolic compounds compared to Arabica coffee (Figure 1).

The Robusta varieties and at a minor extent the unwashed Arabica coffees, Brazil Santos SS and Haiti xxxxx, had the highest contents of pyrazines (e.g. 2-methylpyrazine, 2,6-dimethylpyrazine) and pyrroles (e.g. 1H-pyrrole, 1-(2-furylmethyl)-1H-pyrrole). The highest amounts of furans were found for 2-furan-methanol, 2-furancarboxaldehyde and 5-methyl-2-furancarboxaldehyde. Due to wet processing the washed coffees were characterised by higher amounts of fermentation product, such as acids and ketones.

In order to visualise the volatile composition matrix, PCA was performed on the semi-quantitative data, with the duplicate analyses of the 17 coffees as objects and the 59 volatile compounds as variables (Figure 2). The score plot showed a good classification between the roasted Arabica (positive PC1) and Robusta (negative PC1) coffees, explaining 67% of the total variance (PC1 58%, PC2 9%). The Robusta varieties, especially Ivory Coast Robusta (Grain Noir), were characterised by phenolic compounds. A clear discrimination between

washed and unwashed Indian Robusta was obtained. The Arabica coffees were mainly characterised by fermentation products. PCA on the semi-quantitative SDE-GC-MS results of the Arabica and Robusta varieties separately, demonstrated some meaningful clusters within the Arabica and Robusta species, allowing interesting conclusions concerning the influence of origin on the aroma pattern of coffee.

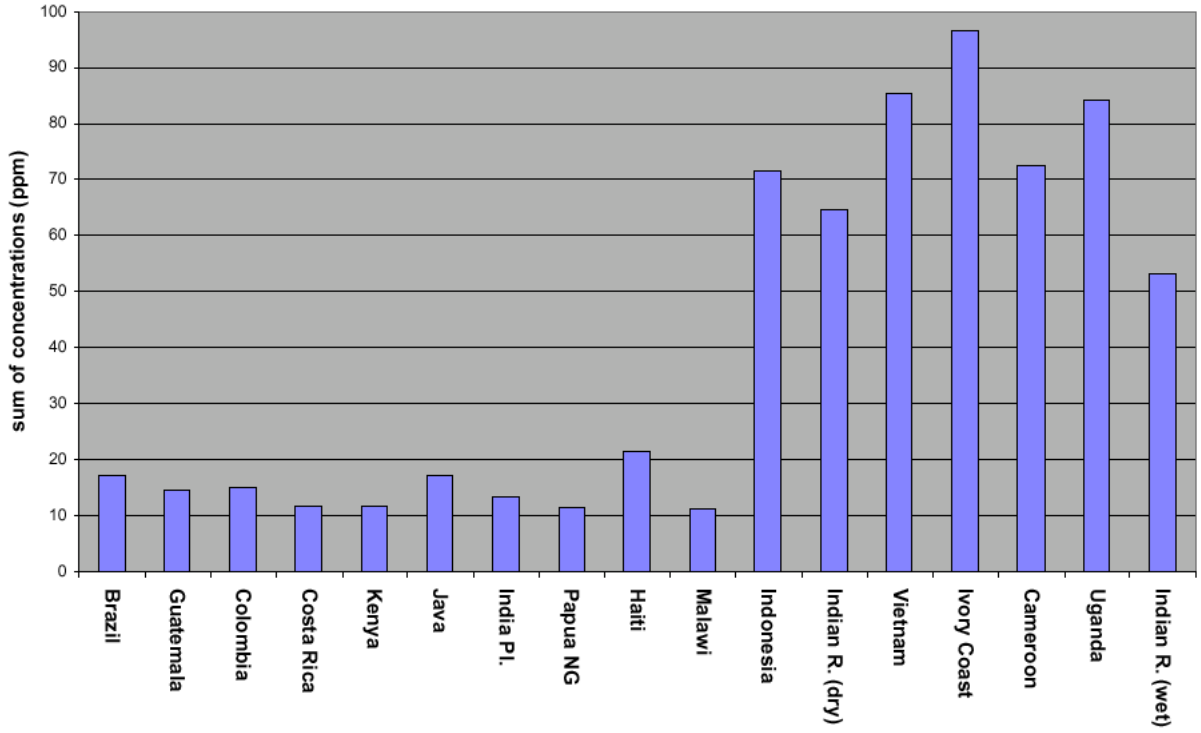

Figure 1. Histogram of concentrations of phenolic compounds in 10 roasted Arabica and 7 roasted Robusta coffees

In order to visualise some interrelationships between aroma compounds and aroma characters, PCA was performed on the semi-quantitative aroma compositions of the Arabica coffees and their sensory data from the descriptive sensory analyses. Figure 3 shows a PCA biplot of 10 Arabica coffees from different origin as objects and semi-quantitative SDE-GC-MS data of 59 volatile compounds and mean scores for the 6 selected attributes of the descriptive sensory test as variables. The PCA biplot explained 64% of the total variance (PC1 47%, PC2 17%) and the Arabica coffees were clearly differentiated, in good accordance with the sensory analyses. The unwashed Haiti had the highest 'phenolic' (Robusta) character among the Arabica varieties and was characterised by spicy phenolic compounds. At the negative side of PC1 Arabica coffees (Brazil Santos SS, Java Ling Tung and Indian Plantation A) with a high roasted/burnt/chocolate-like/caramelly/nutty character were localised. This attribute was correlated with a whole series of pyrazines, of which Brazil Santos SS had the highest level. The attributes sour taste and volatile acidity/flowery/fruity were localised together. Kenya AB and some other wet processed Arabica coffees (Guatemala Antigua, Colombia Supremo, Costa Rica Tarrazu SHB, Papua New Guinea and Malawi-Burundi) had a positive PC1 value and were characterised by a higher degree of volatile acids (propanoic acid, 2- and 3-methylbutanoic acid) and other typical fermentation products (2,3-butanedione, 2,3-pentanedione). The attribute aroma intensity was not correlated with any particular aroma character, as it was located in between the other attributes in the loading plot. A clear discrimination between unwashed (Haiti and Brazil Santos SS) and washed Arabica coffees was not obtained.

CONCLUSIONS

Simultaneous steam distillation-extraction-gas chromatography-mass spectrometry-principal component analysis (SDE-GC-MS-PCA) is a suitable method for classification and objective measurement of aroma characters in coffee. However, the disadvantage of this method is its time-consuming character. Our future research in this field will pay attention to fast methods, based on MS-based sensor technology.

ACKNOWLEDGEMENT

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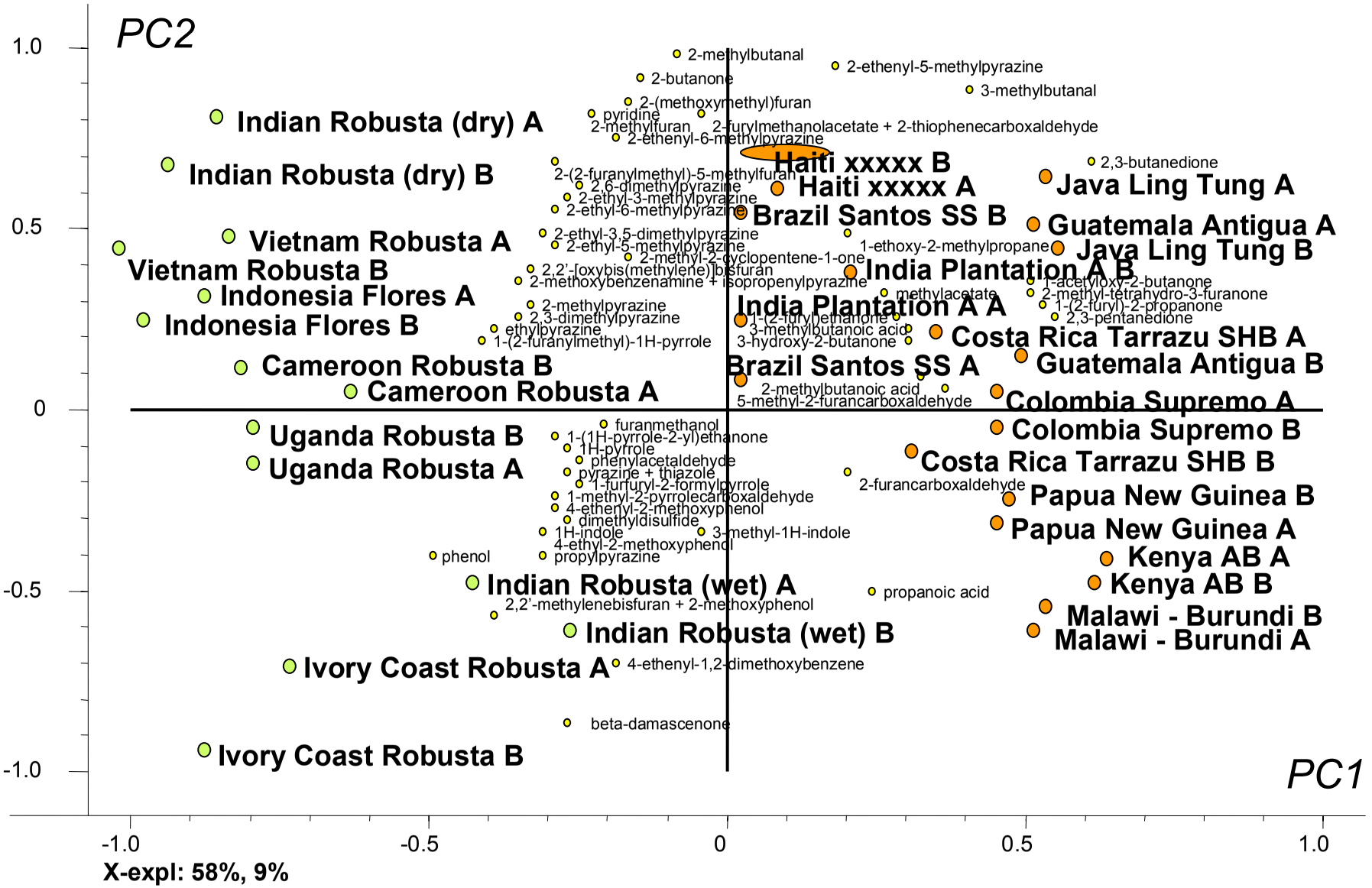

Figure 2. Principal component analysis (PCA) of volatile compositions (SDE-GC-MS) of 10 roasted Arabica and 7 roasted Robusta coffees

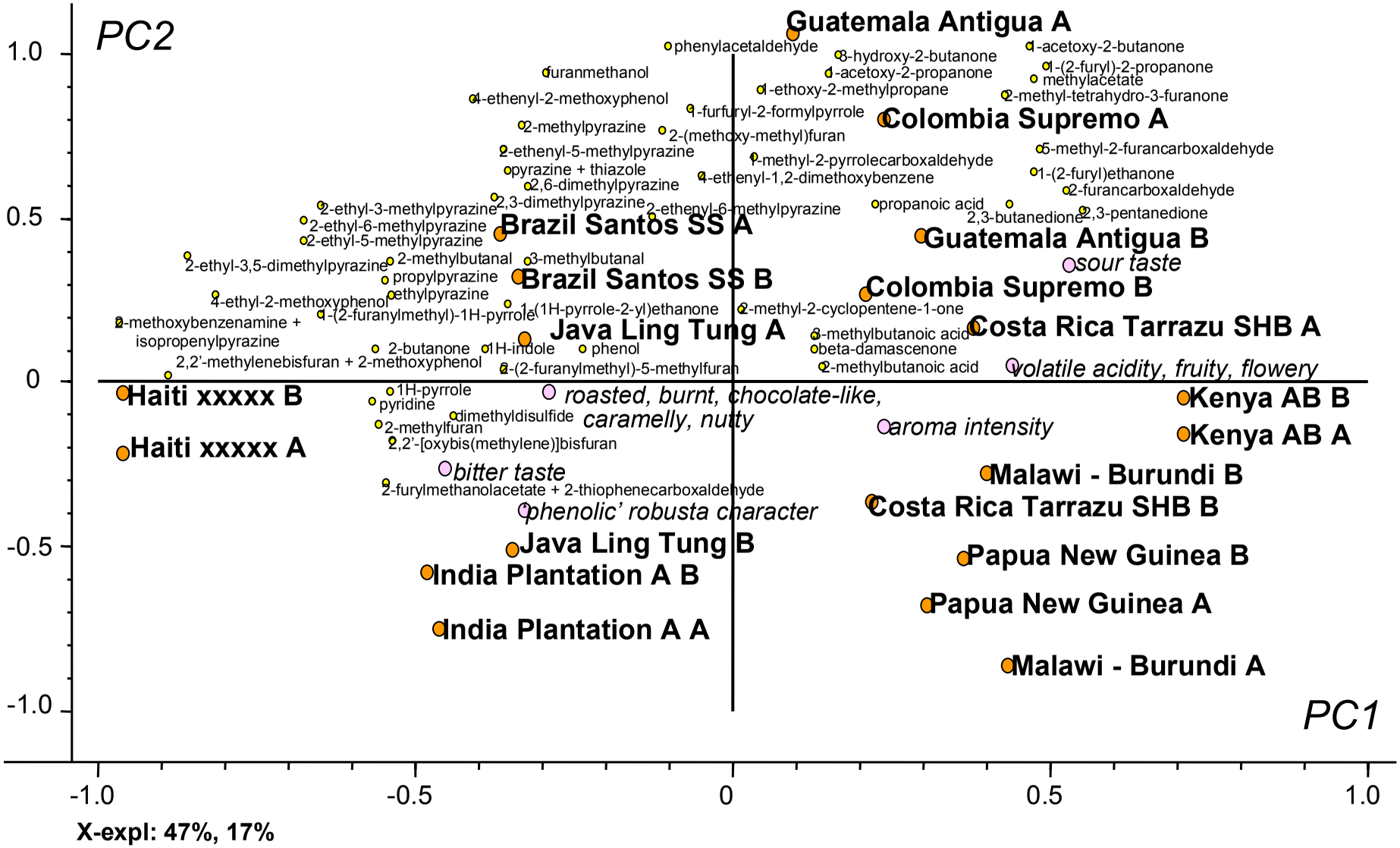

Figure 3. Principal component analysis (PCA) of volatile compositions (SDE-GC-MS) and sensory data (descriptive sensory analyses) of 10 roasted Arabica coffees

Survey of the Presence in Green Coffee of Substances Associated with Important Off-flavours, and their Correlation with Ochratoxin A Contamination

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SUMMARY

In this work an analytical method is developed for the determination of molecules responsible of important off-flavours related to fungal metabolism, such as 2,4,6-trichloroanisole and geosmin. The analysis was carried out by steam distillation of regular and decaffeinated green coffee, followed by qualitative and quantitative determination with GC-MS. In the meantime, on the same samples the concentration of ochratoxin A was determined by clean-up on immuno-affinity column followed by HPLC analysis.

The comparison of the results obtained evidences some correlation between the presence of molecules responsible of off-flavours and ochratoxin A in regular green coffee samples, which happens most evidently in coffees belonging to the Robusta species grown in some African Countries.

The analyses after decaffeination confirm the good extractive power for 2,4,6-trichloroanisole and geosmin of the solvent used in the process, methylene chloride. The final concentration of these analytes falls below the detection limit for all the samples, implying a useful effect of this extractive process over the off-flavours considered.

RÉSUMÉ

Dans ce travail on a mis au point une méthode d'analyse pour la détermination de molécules responsables d'importants arômes négatifs associées à des métabolismes mycosiques, tels que 2,4,6-trichloroanisole et géosmine. On a effectué cette analyse par distillation sous courant de vapeur sur des échantillons de café vert regular et décaféiné, suivie d'une détermination qualitative et quantitative par GC-MS. En même temps, sur les mêmes échantillons, on a déterminé la teneur en ochratoxine A par clarification sur colonne d'immunoaffinitè et par successive analyse HPLC.

A la lumière des resultats obtenus, on a mis en évidence une correlation entre la présence de molécules responsables d'arômes negatifs et la contamination par ochratoxine A dans les échantillons de café vert regular, quasi exclusivement dans les Robusta provenant des pays africains.

Les analyses effectuées après la décaféination confirment l'excellente capacité d'extraction du solvant utilisé dans le procédé, le chlorure de méthylène, par rapport à 2,4,6 trichloroanisole et géosmine. La concentration finale de ces analytes va au dessous de la limite de détection pour tous les échantillons, ce qui amène un effet positif du procédé d'extraction sur les d'arômes négatifs en question.

INTRODUCTION

Coffee flavour is one of the main reasons that attract consumers' attention and preference to this beverage. While, generally speaking, the definition of coffee quality is a complex issue, there is vast agreement that the presence of some off-flavours negatively affects the cup's appeal (Illy and Viani, 1995). Moreover, several off-flavouring taints have been ascribed – at least tentatively – to biological spoilage by micro-organisms such as bacteria or moulds (Liardon et al., 1989).

Important off-flavours in green coffee, subject of several studies, have been related to the presence of molecules such as 2,4,6-trichloroanisole (TCA) (Spadone et al., 1990), geosmin (GEO) (Gravesen et al., 1994; Johnsen and Kuan, 1987) and 2-methylisoborneol (Johnsen and Kuan, 1987; Vitzthum et al., 1990).

These substances, deriving from fungal metabolism, have an olfactory perception threshold extremely low, at the level of ppt (ng/kg) in coffee beverages, so that concentrations of ppb (μg/kg) have to be searched for in green coffee. This makes the realisation of an analytical method for their determination difficult, along with the problem that TCA and GEO are extracted together with the lipidic fraction, which covers the signal of the analytes of our interest (Tentindo, 1999).

The purpose of this work is to research the presence of TCA and GEO in green coffee, to verify if there are any differences between Arabica and Robusta samples, and to highlight the influence of the geographical area of production. In parallel, the contamination by another obnoxious fungal metabolyte, ochratoxin A (OTA), is investigated, with the purpose to find possible correlation between the presence of these off flavours and OTA.

Furthermore, the influence of solvent decaffeination process on off-flavours is investigated.

EXPERIMENTAL

The 75 lots of green coffee for the analysis, coming from 19 different countries, were selected by Demus S.p.A., Trieste and taken according to the ISO sampling method (1982). The analytical method to determine TCA and GEO was developed by G. Tentindo at the Department of Food Sciences of the University of Udine (Tentindo, 1999).

The same samples were analysed for Ochratoxin A (OTA) contamination. The study encompassed also 9 samples of decaffeinated coffee, to evaluate the effect of the treatment with methylene chloride on the molecules of our interest.

Determination of TCA and GEO

Each sample was completely ground, put in a distillation flask together with 2,3,6-trichloroanisole as internal standard and methanol, and submitted to steam distillation; then a three-step extraction with a solution of pentane/methylene chloride was performed on the distillate, and the heavy fraction dried with sodium sulphate. Then the sample was stored at –20°C until the GC-MS analysis.

For qualitative and quantitative determination was utilised a GC Varian 3400, coupled with a mass spectrometer Saturn ion trap (IDTMS). The capillary column was a DB5 (J&W) in fused silica, 30 m, i.d. 0.25 mm, with a film diameter of 0.25 μm. As transport gas was utilised helium and the injection was realised in splitless.

The detection limits were of 0.03 ppb for TCA and 0.2 ppb for GEO.

Determination of OTA

The analysis was effectuated on the same samples as above. Each sample was completely ground, homogenised and submitted to extraction, immuno-affinity clean-up and HPLC-spectrofluorimetry analysis as described by Studer-Rohr et al. (1995).

RESULTS

Correlation TCA - GEO – OTA

Table 1 shows the results of our analyses, reporting the average concentrations of TCA and GEO, together with the OTA average data, for 75 samples divided by geographical zone of production. The data highlight a higher OTA contamination in West and East African coffees.

Table 1.

SAMPLE	TCA		GEO		OTA	
	ppb	St.d.	ppb	St.d.	ppb	St.d.
West Africa 1 (R)	2,4	1,1	n.d.		1,1	1,6
West Africa 2 (R)	3,0	2,6	0,8	2,1	3,8	8,4
West Africa 3 (R)	4,8	2,8	0,8	1,1	3,0	2,1
West Africa 4 (R)	2,9	2,1	n.d.		2,5	4,8
East Africa 1 (A)	1,0	1,1	n.d.		2,2	4,2
East Africa 2 (R)	2,5	2,2	n.d.		2,7	5,3
East Africa 3 (R)	0,4	0,4	n.d.		0,6	0,4
Asia 2 (A)	n.d.		n.d.		0,1	0,3
Asia 1 (R)	0,6	0,9	n.d.		0,2	0,4
Asia 3 (R)	0,1	0,2	n.d.		0,2	0,3
Asia 4 (R)	0,1	0,2	n.d.		1,3	1,8
Central America 1 (A)	n.d.		n.d.		0.1	0.2
Central America 2 (A)	n.d.		n.d.		0,1	0,1
Central America 3 (A)	0,1	0,2	n.d.		0,4	0,8
Central America 4 (A)	0,8	0,1	n.d.			
Central America 5 (A)	0,1	0,2	0,1	0,2	0,3	0,3
Central America 6 (A)	0,3	0,4	n.d.			
South America 1 (A)	n.d.		n.d.		0,1	0,2
South America 2 (A)	n.d.		n.d.		0,1	0,3

(A) = Arabica; (R) = Robusta; n.d. = not detected; St.d. = standard deviation. For each sample several replicates has been effected.

A correspondence between the samples with a high concentration of off-flavours, (especially TCA) and those with OTA contamination (mostly in West and East African coffees) results evident, as can be better seen in Figure 1.

The presence of GEO is evident only in coffees coming from two countries of West Africa, where also the average values of TCA and OTA resulted the highest. Nevertheless, GEO content correlates pretty well (68%) with OTA contamination, even if less significantly than TCA does with OTA (86%).

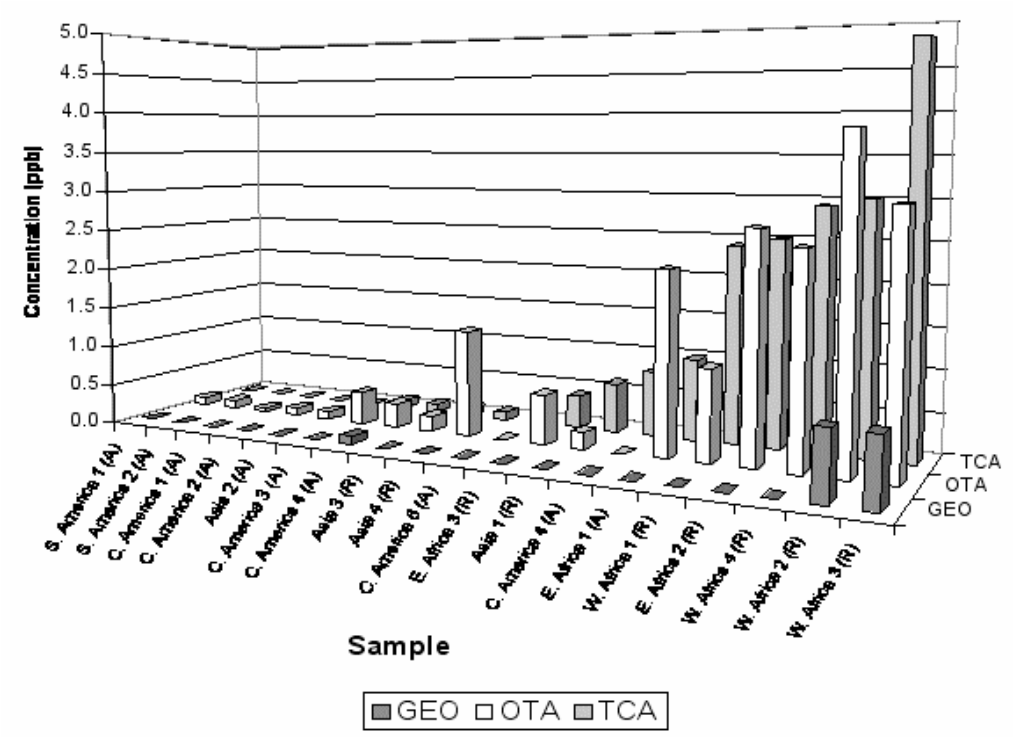

Figure 1. Correlation TCA-GEO-OTA

Effect of decaffeination

The last part of this study regards the effect of methylene chloride extraction on TCA and GEO. The results of the analyses are reported in Table 2: we can see the good extractive effect of the solvent on the off-flavours investigated – especially on TCA – leading the concentrations below the detection limit.

DISCUSSION AND CONCLUSIONS

While in Arabica coffees, especially the ones of American provenience, the concentrations of TCA, GEO and OTA resulted negligible, coffees coming from African countries, nearly all belonging to Robusta species, evidenced the highest concentrations of all contaminants. Interestingly, the comparison of the data obtained provides evidence for the existence of some correlation (up to now only of statistical nature) between the presence of molecules responsible for off-flavours and the presence of OTA, providing a prospective tool for a rough screening of regular green coffee samples against contamination.

No investigation about actual mould infection of the coffee samples was within the scope of this work. Therefore, no causal link can be here suggested between the presence of such different fungal metabolites like TCA, GEO and OTA, and possible interactions among different mycetes. Nevertheless, the possibility that agricultural or post-harvesting phenomena may favour the formation of some complex mould ecosystem should be considered by mycologists as an interesting challenge. Any elucidation of the influence of fungal metabolism on undesirable traits affecting coffee wholesomeness and quality should be more than welcome by both growers and traders.
For the moment being, a pragmatic suggestion could be put forward in order to reduce the impact of obnoxious taints on coffee quality: any measure able to make mould growth difficult, such as improved care on harvesting and thorough drying process, is likely to produce a sounder commodity.

Table 2.

SAMPLE	REGULAR GREEN COFFEE		DECAFFEINATED COFFEE	
	TCA ppb	GEO ppb	TCA ppb	GEO ppb
South America 1 (A)	n.d.	n.d.	n.d	n.d
South America 1 (A)	n.d.	n.d.	n.d	n.d
West Africa 3 (R)	4,3	n.d.	n.d	n.d
West Africa 3 (R)	5,2	n.d.	n.d	n.d
West Africa 2 (R)	4,9	n.d.	n.d	n.d
Central America 4 (A)	0,7	n.d.	n.d	n.d
Asia 1 (R)	n.d.	n.d.	n.d	n.d
Asia 1 (R)	0,7	0,2	n.d	n.d
Asia 2 (A)	n.d.	n.d.	n.d	n.d

(A) = Arabica; (R) = Robusta; n.d. = not detecte. For each sample several replicates has been effected.

The results of the analysis on green decaffeinated coffee samples allow us to verify the good extractive capability of the solvent used in the process - namely methylene chloride - for the molecules subject of this survey, especially TCA. Decaffeination shows to be a process apt to reduce undesirable off-flavours in tainted coffees.

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Volatile Composition of Arabica and Robusta Varieties for Coffee Characterisation and Classification Using a Stepwise Discriminant Approach

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SUMMARY

Two data sets involving 39 green and roasted coffee samples from different origins were considered for the differentiation and classification of Arabicas and Robustas according to the volatile fraction.

The volatile compounds were analysed by hyphenated technique consisting of a dynamic headspace directly coupled with gas chromatography-mass spectrometer system (DHS-GC-MS). Dynamic headspace sampling coupled with "cold trap" technique, enables the acquisition of an aroma profile similar to human olfactory perception. The particular technique employed in this study allowed the identification of 75 substances in the analysed green coffee samples, whereas in the roasted samples more than 80 compounds were identified.

To analyse the importance of volatile components in describing differences between the two varieties a multivariate approach, based on linear discriminant analysis, was applied. The purpose of the study was to characterise differences according to the volatile fraction before and after the roasting process. A comparison between two linear discriminant functions (LDF's), estimated with and without a stepwise variable selection, is presented here. Moreover, a cross-validation procedure, the Leave-One-Out method, was applied to test the prediction capacity of the model.

INTRODUCTION

Coffee flavour is one of the most important attribute on which depends the quality of coffe cup (Dart and Nursten, 1985). Volatile compounds are well documented in the literature (Tassan and Russel, 1974; Viani and Horman, 1974; Tressl and Silvar, 1981; De Maria et al., 1994; Grosch, 1994; Grosch, 1995). Instead, less investigated is the volatile fraction of green coffee (Pokorny et al., 1975; Amorim et al., 1977) that, on the contrary can be determinant in the selection of a coffee stock as it can define the total quality of the product.

According to the volatile fraction two data sets involving 39 green and roasted coffee samples from different geographical origins were considered for the differentiation and classification of Arabica and Robusta varieties.

The volatile compounds were analysed by hyphenated technique constituted by dynamic headspace directly coupled with gas chromatographic mass spectrometer system (DHS-GC-MS) (Barcarolo et al., 1992). Dynamic headspace sampling coupled with "cold trap" technique enables the acquisition of an aroma profile similar to human olfactory perception.

The particular employed technique allowed the identification of 75 substances in the analysed green coffee samples, whereas in the roasted samples more than 80 compounds were identified.

To analyse the importance of volatile components in describing differences between the two varieties a multivariate approach based on Linear Discriminant Analysis (LDA) was applied. Each data set was initially partitioned according to the varieties, Arabica (n_1 = 22) and Robusta (n_2 = 17). The purpose of the study was to characterise differences between the two varieties according to the volatile fraction first and after the roasting process. In a further step, in order to verify the possibility of discriminating samples according to their provenience, the geographical origin was considered as grouping variable for both data sets.

A comparison between two Linear Discriminant Functions (LDF's), one estimated with stepwise analysis and the other one with the SPSS® default method, is presented here. Moreover, the classification results, yielded by the two discriminant methods, are analysed. In particular, the model adequacy was tested by applying a cross-validation procedure, the Leave-One-Out (L-O-O) method.

THEORETICAL

Descriptive and Predictive Discriminant Analysis

As the group membership of each coffee sample was already known, LDA was applied for the two-group differentiation between Arabicas and Robustas. In general, for supervised pattern recognition Discriminant Analysis is one of the most widely used technique as quite often it enables to derive a classification rule for different applications. The estimation of a discriminant model for the characterisation of samples, whose group membership is *a priori* known, aims to determine not only the discriminating power of each variable (Descriptive Discriminant Analysis, DDA), but also a possible classification of samples of unknown origin (Predictive Discriminant Analysis, PDA) (Huberty, 1994).

The aim of the study was to characterise differences between the two varieties according to the volatile fraction. For this purpose, an approach based on discriminant analysis was employed. A classical discriminant model, given by a linear combination of discriminating variables, x_i, such as $L = c + b_1x_1 + b_2x_2 + \ldots + b_nx_n$, whose coefficients are estimated so that the "best" separation between groups is achieved, was therefore defined.

Stepwise Methodology

In discriminant analysis, the stepwise methodology provides some tools that can be used in an exploratory approach. Stepwise algorithms are used both for DDA to analyse the discriminating power of the variables under study and for PDA, where a classification rule for group membership prediction is obtained.

Stepwise methods are used for the selection of variables to be entered into the analysis. The variable selection can be based upon different criteria. Wilk's lambda (Λ), given by the ratio Within-groups Sum of Squares/Total Sum of Square (WSS/TSS), is one of the most commonly used. For this criterion the minimisation of its value is therefore involved as the smaller the Λ for a discriminating variable, the more that variable contributes to the discriminant function. The stepwise algorithm combines a forward selection and a backward elimination according to value considered for the selection criterion. In the case of Wilk's Λ stepwise method at every single step, the variable selected for entry, is the one with the

smallest Wilk's Λ value. After the first variable is entered, the criterion is re-evaluated for all variables, in order to examine both the variables not in the equation for entry (forward selection) and the variables in the equation for removal (backward elimination).

Classification and L-O-O analysis

For an assessment of discriminant model performance in terms of proportion of samples correctly classified, an external analysis based on the Leave-One-Out (L-O-O) cross-validation was performed. The aim was to validate the model estimated from the original data set, but not classifying samples using the rule based on the samples themselves as happens performing the internal analysis. In this way it is possible to obtain an empirical measure of the predictive precision of the discriminant model estimated. In fact, as its name suggests, the L-O-O procedure used here consists in testing *N* classification rules - estimated from *N*-1 samples - from time to time by using the original set after removal of one sample as test set. The *N* performance results are then averaged to obtain an L-O-O classification table containing an unbiased estimate of cases correctly classified. This procedure differs from the holdout cross-validation procedure because instead of splitting the data set into a training set used for the estimation of the model and a set used for testing its performance, the L-O-O estimator uses all the available data for training.

EXPERIMENTAL

Sampling

Thirty-nine green coffee samples (22 Arabicas and 19 Robustas) from all the world were analysed. In particular, as regards Arabica variety, 5 samples came from Africa (Kenya, Cameroon, Uganda, Ethiopia, Burundi), 8 from Central America (Nicaragua, Cuba, Guatemala, Haiti, Costa Rica, Mexico, Santo Domingo, Puerto Rico), 6 from South America (Venezuela, Peru, Ecuador, Colombia, Brazil *Santos Florada* and *Fancy*), 2 from Asia (India and Indonesia) and 1 from Oceania (Papua New Guinea).

Among the Robustas, 8 samples came from Africa (Madagascar, Congo, Cameroon, Togo, Ivory Coast, Uganda, Angola, Central African Republic), 1 from Central America (Guatemala), 1 from South America (Brazil), 6 from Asia (India *Cherry* and *Parchment*, Vietnam, Indonesia, Thailand, Java) and 1 from Oceania (Papua New Guinea).

A small amount of each sample (1 g) was milled and analysed directly using dynamic headspace equipped with "cold trap" (Huberty, 1994). Successively, the samples were roasted at 200-220°C for 10-15 min.

Analytical conditions

The sample was conditioned for a few minutes at 70°C and then stripped at the same temperature for 2 min. The compounds present in headspace were condensed in the cold trap, held at –90°C. Then the trap temperature was to 240°C in 5s so that the trapped substances were transferred into gas chromatographic column.

The green coffee samples were analysed on a GC 8000 coupled with MD 800 mass spectrometer (ThermoQuest, Milan, Italy), using fused silica column (50 m × 0.32 mm ID, 3 μm film thickness), coated with Polydimethyl Siloxane - 264 (Mega, Milan, Italia). The oven temperature was programmed as follows: 40°C for 6 min then raised to 180°C at 5°C/min and

held for 5 min; then to 200°C at 7°C/min and held for 2 min; then to 240°C at 7°C/min and held for 5 min. Helium was used as carrier gas at 04 bar pressure.

The mass spectrometer was programmed to scan ions from m/z 29 to m/z 300 at 2 cycle/s. During the analysis, the ion source was maintained at 200°C and the ionisation voltage was set at 70eV. The mass spectra were identified by matching with NIST library and published mass spectra.

Data Analysis

The identification of 75 and 85 compounds in green and roasted coffee samples, respectively, yielded two data sets which were initially partitioned according to the grouping variable defined by the coffee variety: Arabica ($n_1 = 22$) and Robusta ($n_2 = 17$).

At first, for the two data sets LDA was applied using the SPSS® default procedure for Discriminant Analysis including a sort of variable selection (Norusis, 1993). In fact, this procedure implies that variables not satisfying a minimum tolerance level - the proportion of its variance not accounted by the remaining variables – are not entered in the analysis. In fact, as they are linearly associated to others already in the model, they can be considered redundant and therefore removed from the analysis.

Successively, the Wilk's Λ stepwise procedure was carried out on the two original data sets in order to analyse if a real variable selection could contribute better to the differentiation and classification of the Arabicas and Robustas.

RESULTS AND DISCUSSION

Coupling of headspace method with cold trap technique used in this analysis permitted to obtain aromatic profiles very similar to the natural olfactory perception.

Figures 1 and 2 show typical chromatograms of green coffee and roasted coffee samples.

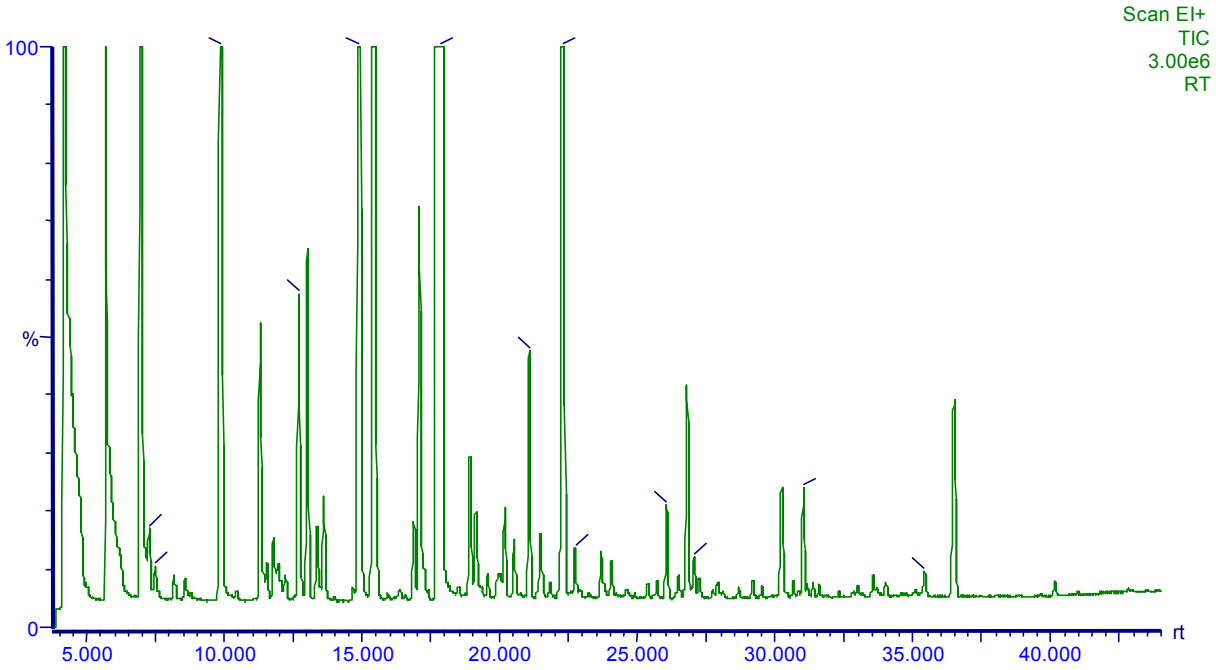

Figure 1. **Aromatic profile of green coffee sample (Cameroon)**

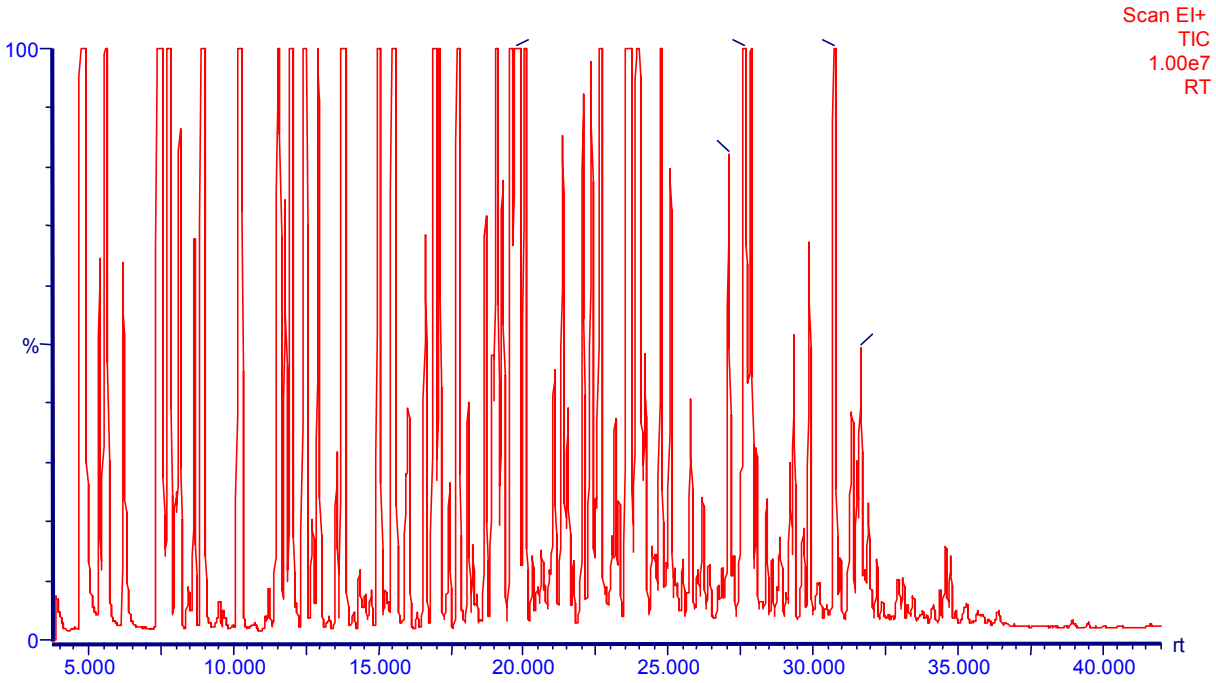

Figure 2. **Aromatic profile of roasted coffee sample (Cameroon)**

In order to obtain a discrimination and then a classification according to variety and origin, the data were processed by means of Discriminant Analysis, in particular using a stepwise method for variable selection.

Table 1 reports the summary of statistics related to the linear discriminant functions (LDF's) determined for the green and roasted coffees using the SPSS default and the Wilk's Λ stepwise procedures for Discriminant Analysis. Here are listed the eigenvalues (λ_i) associated to each LDF, the relative percent of variance, the canonical correlation measuring the association between groups and LDF, along with the *Wilks'*Λ statistics used for applying the Bartlett criterion to test the group mean equality on the discriminating variables.

It is on the basis of these statistics that the different LDF's could be compared and that the stepwise approach could be considered effective for the differentiation of Arabicas and Robustas for the green coffee as well as roasted coffee. In fact, the variance not accounted for by group differences is slightly smaller in the case of the 37-variable and 35-variable models estimated using the default procedure for LDA. As shown in the table by Wilk's Λ index, in fact, in percentage terms the variance not explained by the LDF's estimated using the default discriminant method is of 2.2% and 1.2% vs. the 5.2 % and 6.7 % for the stepwise discriminant models.

In Figures 3 and 4 are graphically plotted the histograms of the discriminant function scores for the Arabica and Robusta green and roasted coffee samples obtained using Stepwise DDA. As can be clearly seen the two coffee varieties are quite well separated in the discriminant plane and in both cases there is no overlap at all. In fact, the values of discriminant function at group means (centroids or average scores for each group) are of opposite sign both for the green coffee and the roasted coffee (Table 2) independently from the model taken into consideration.

Table 1. Summary of the MANOVA statistics for the green and roasted coffee data sets

LDF	*Eigenvalue*	*% of Variance*	*Canonical correlation*	*Wilks'Λ*	χ^2	*df*	*Sig*
Green coffee							
37-variable model	44.697	100	.989	.022	70.708	37	.001
10-variable model	18.255	100	.974	.052	94.649	10	.000
Roasted coffee							
35-variable model	81.222	100	.994	.012	85.984	35	.001
6-variable model	13.907	100	.966	.067	91.862	6	.000

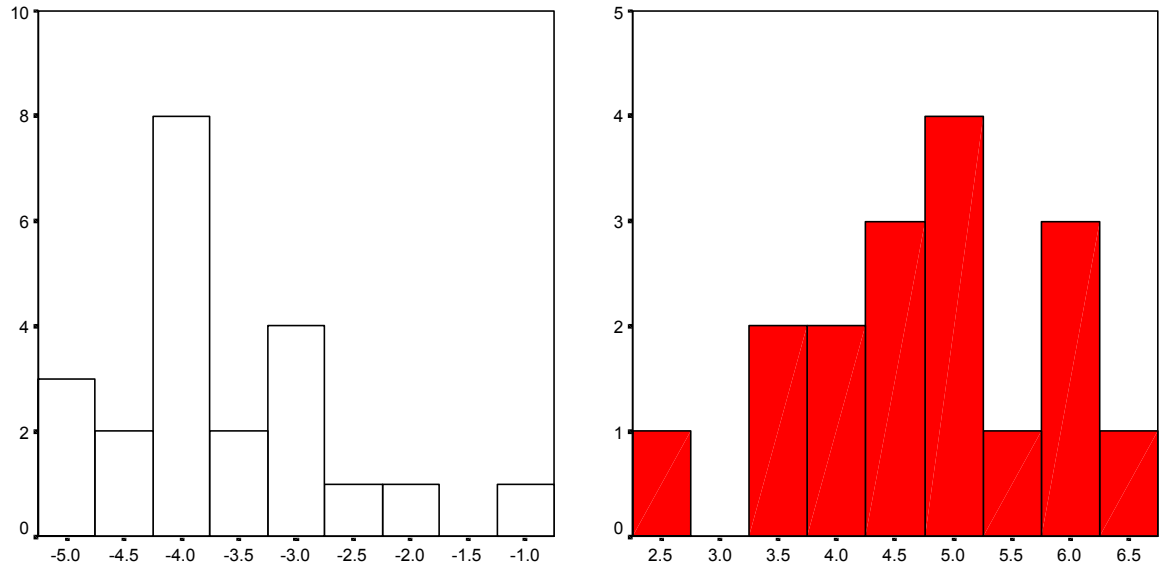

Figure 3. **Discriminant scores obtained with Stepwise DDA for Arabica (empty bars) and Robusta (filled bars) green coffee samples**

In Table 3 the classification results obtained using the original estimator in internal analysis and the L-O-O classification rule for an external analysis are listed. Here the number of samples correctly and incorrectly classified (actual group versus predicted group membership), along with the overall percentage of cases classified correctly are given. It is from this table that the prediction accuracy of the classification rules can be assessed and that validation of the discriminant functions can be carried out.

Table 2. Centroids of Arabica (G1) and Robusta (G2) groups along the LDF's for the green and roasted coffee using the default and stepwise discriminant procedures

	Green Coffee		*Roasted Coffee*	
Group	*37- variable Model*	*10- variable Model*	*35- variable Model*	*6- variable Model*
G1	5.72	- 3.66	7.72	3.19
G2	- 7.41	4.73	- 9.99	- 4.13

If, as far as descriptive capacity is concerned, the two LDF's do not show any significant difference, it is clear and evident that the validation assessment yields rather different classification results. In particular, when the stepwise approach is employed the misclassification rates are much lower than when the variable selection is not performed.

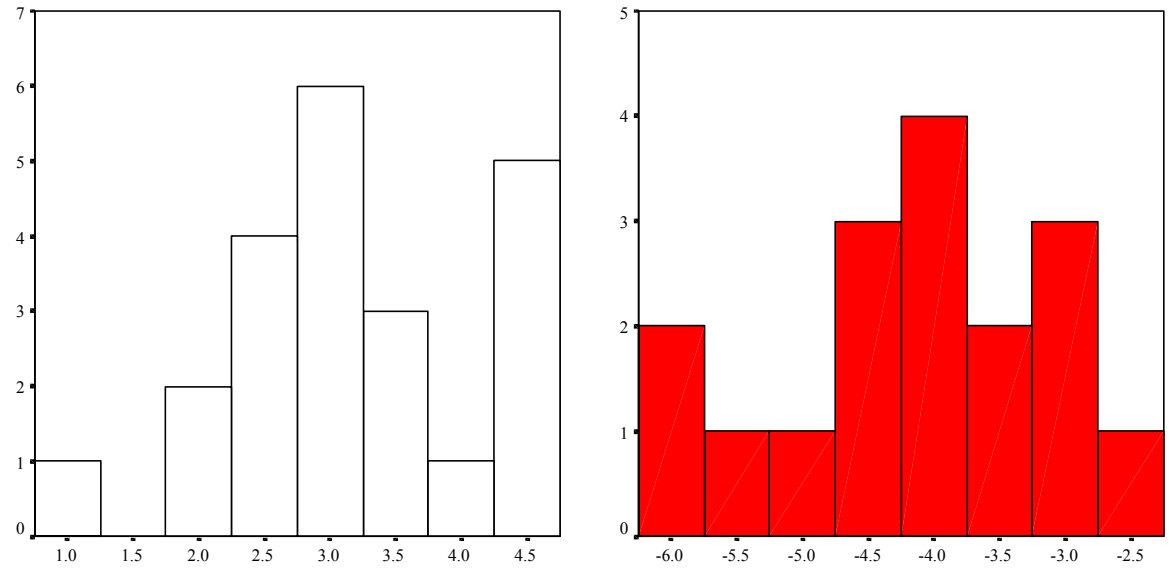

Figure 4. **Discriminant scores obtained with Stepwise DDA for Arabica (empty bars) and Robusta (filled bars) roasted coffee samples**

The variable selection using the stepwise DDA for differentiation and classification of green coffee samples identified a subset of ten variables reported in Table 4. Some discriminating compounds, such as thiophene, methylacetate and isobutanal, are really predominant in Robusta variety, whereas others, such as amylformate, 1-butanol and methyl propanoate seem to characterise Arabica variety. Other substances exhibiting clear differences are ethyl isobutyrate, typical of Brazilian Robusta, and hexanal characterizing the Arabicas from Central America.

Table 3. Summary of classification results obtained by the internal and L-O-O analysis

		Actual Group	*Predicted Group* G1	G2	*Total*	*Percentage correct*
Green coffee	37-variable model					
	Internal	*G1*	22	0	22	100 %
		G2	0	17	17	
	L-O-O	*G1*	9	13	22	43.6%
		G2	9	8	17	
	10-variable model					
	Internal	*G1*	22	0	22	100 %
		G2	0	17	17	
	L-O-O	*G1*	22	0	22	100 %
		G2	0	17	17	
Roasted coffee	35-variable model					
	Internal	*G1*	22	0	22	100 %
		G2	0	17	17	
	L-O-O	*G1*	15	7	22	71.8 %
		G2	4	13	17	
	6-variable model					
	Internal	*G1*	22	0	22	100 %
		G2	0	17	17	
	L-O-O	*G1*	22	0	22	100 %
		G2	0	17	17	

As far as the roasted samples are concerned, the six selected variables that allowed a complete differentiation of the two varieties are listed in Table 5. In particular, some of the selected

variables, namely methylacetate, thiophene, pyridine, are more prevalent in Arabicas, especially in samples from Asian countries, whereas only 2-methyl-3-buten-2-ol is mostly present in Robusta variety.

Table 4. Structure matrix for stepwise DDA of green coffee

Variable	*LDF*
Thiophene	.247
Amylformate	-.147
Isobutanal	.142
Methylacetate	.134
1-methylpyrrole	.121
Methyl propanoate	-.063
1-butanol	-.024
2-propanol	-.023
Hexanal	-.005
Ethyl isobutyrate	.003

As regards the attempt of classification according to the geographical origin, the classification results obtained here were not completely satisfactory, even if a complete separation among groups was obtained. Samples correctly classified according to the estimated rule represented only 12% of the total green coffee samples and 26% of the total roasted samples. It is very likely that the sample size for each provenience was too limited in relation to the number of variables and groups.

Table 5. Structure matrix for stepwise DDA of roasted coffee

Variable	*LDF*
2-methyl-3-buten-2-ol	.265
Methylacetate	.201
2-methyltetrahydrofuran-3-one	.186
Pyridine	.078
3-ethyl-2,5-dimethylpyrazine	-.021
Thiophene	-.006

CONCLUSION

In these later years, the volatile component analysis of a food matrix has been more and more carried out by means of the headspace technique. This method assures, in fact, a greater effectiveness in providing a real aromatic profile. Furthermore, it has been proven in this study that this technique, which does not require adsorbent polymers, is particularly appropriate to the analysis of thermolabile compounds. Indeed, the substances, once transferred from sample to the cold trap, undergo only a single heating cycle before the gas chromatographic analysis.

Satisfactory results were obtained from the analysis of samples of green and roasted coffee. Actually, thanks to the study of the volatile fraction it has been possible to completely differentiate the two coffee varieties with a success rate (in terms of cases correctly classified) of 100%.

However, these results should be considered as preliminary. It will be necessary to analyse a larger number of samples of same variety and geographical origin to identify more precisely the parameters characterising each provenience. In particular, if a classification rule for samples of unknown origin is the target of investigation.

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In-Mouth Coffee Aroma: Breath-By-Breath Analysis of Nose-Space While Drinking Coffee

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SUMMARY

Currently the vast majority of the investigations on aroma are performed under equilibrium or dynamic headspace conditions to best estimate flavour. However, eating or drinking conditions have additional important factors such as mixing, mastication and salivation, heating and others. This can lead to significant alterations of the physical and chemical state of its constituents (e.g. melting, emulsification, adsorption) and modify the release of volatiles relative to headspace profiles. Nose-space analysis is a method to sample aroma compounds directly released from humans through the nose when eating food. The main benefits of such an approach is the ability to investigate the aroma during the actual situation of consumption.

Here, we would like to present a novel approach to breath-by-breath analysis of volatiles exhaled through the nose based on Proton-Transfer-Reaction Mass-Spectrometry. First we will discuss the methods and outline the advantages of this novel method relative to current approaches. We then go on presenting breath-by-breath nose-space results while drinking coffee. Keeping the coffee for some while in the mouth, we observe a marked change in volatile distribution with time. This is indicative for a change of the perceived coffee flavour profile with time.

INTRODUCTION

The flavour of a cup of coffee is the expression of a long chain of transformations and processes, which link the seed to the cup. These include agricultural factors, storage conditions, processing steps, packaging and finally the mode of preparation. Along the long journey of the seed becoming a brew, methods have been developed to assess and predict the quality of the brew. Yet, in spite of progress in understanding the relations between agronomy, chemistry, processing and cup quality, the coffee has to be tasted to ascertain its quality.

One objective of coffee flavour research is to find chemical quality markers for the cup of coffee, which correlate with the sensory assessment of expert coffee tasters and which drive consumer preferences. Currently the majority of analytical investigations on coffee aroma are performed under equilibrium or dynamic headspace conditions. The volatile flavour compounds are either stripped by a flow of gas or extracted with solvents to be subsequently analysed by gas chromatography. While this has been extremely valuable to expedite our understanding of coffee aroma, one might wonder how well this reflects the coffee aroma profile as it is experienced during the actual situation of coffee consumption. Coffee drinking conditions have additional important factors such as mixing, mastication and salivation, heating and interactions with mouth mocusa. This can lead to significant alterations of the physical and chemical state of its constituents (e.g. melting, emulsification, adsorption)

induce chemical transformations, and modify the release of volatile flavour compounds relative to headspace (HS) studies.

Here, we discuss some new developments of "nose-space" analysis. We present a method to sample and analyse on-line volatile organic compounds (VOC) directly released from humans through the nose when consuming food (Linforth et al., 1994; Ingham et al., 1995; Linforth and Taylor, 1998; Taylor and Linforth, 2000). The main benefit of such an approach is the ability to investigate the aroma during the actual situation of consumption. We present a novel approach to breath-by-breath analysis of volatiles exhaled through the nose based on Proton-Transfer-Reaction Mass-Spectrometry (PTR-MS). First we outline the method and then go on presenting breath-by-breath nose-space results on a series of volatile coffee compounds.

NOSE SPACE ANALYSIS

The aroma (odour) of food products is related to VOCs that are released from foods and reach the olfactive epithelium in the upper part of the nose. When flavour active compounds interact with olfactive receptors, a sensory perception is triggered. VOCs can reach the olfactory epithelium from two distinct directions. Either they are sniffed directly through the nose, via the orthonasal pathway, or they reach the olfactive receptors through the oral cavity and the pharynx, via the retronasal pathway. The orthonasal aroma corresponds to an aroma, as it is perceived from a food held in front of the nose (sniffing). In contrast, the retronasal aroma corresponds to the aroma of a food, as it is perceived during food consumption (while drinking or eating). Nose-space analysis samples the air exhaled through the nose as food is being consumed and reflects the retronasal aroma composition. Hence by comparison of a headspace VOC profile with a nose-space profile, we address the question how closely the HS approach reflects the situation as it is experienced during food consumption.

The first real-time breath-by-breath analysis dates back to 1988 (Seoting and Heidema, 1988). In this work, Soeting and Heidena sampled the air flown from the nose via a membrane interface and analysed the VOC by electron impact (EI) ionisation and quadrupole mass separation. Yet a breakthrough was achieved as Taylor and Linforth from the University of Nottingham introduced API-MS (**A**tmospheric **P**ressure **I**onisation **M**ass **S**pectrometry) to achieve soft ionisation and consequently a strongly reduced fragmentation with improved sensitivity (Linforth et al., 1996; Taylor, 1996). A recent review discussed the various methods for nose-space (in-vivo) analysis (Taylor and Linforth, 2000).

More recently, a novel version of chemical ionisation (CI) for on-line dynamic measurements of VOCs has been developed, which was termed **P**roton **T**ransfer **R**eaction **M**ass **S**pectroscopy (PTR-MS) (Yeretzian et al., 2000; Lindinger et al., 1998; Lindinger et al., 1998; Hansel et al., 1995). APCI-MS and PTR-MS differ mainly in their design of the ionisation chamber. In PTR-MS the generation of the primary H_3O^+-ions is performed in an ion source chamber that is physically separated from the chemical ionisation chamber. H_3O^+-ions are generated by corona discharge in an atmosphere of water vapour and transferred to the drift tube. Within the long drift tube a controlled and stable atmosphere of water vapour and H_3O^+-ion concentrations is maintained. The HS to be analysed is introduced through a Venturi-type inlet into the drift tube, and VOCs are protonated by proton transfer from H_3O^+-ions. Separating the two processes – formation of the primary H_3O^+-ions and the chemical ionisation by proton transfer – allows optimising the design of the respective chambers individually, as well as assures that the background of ions other than H_3O^+-ions in the drift tube is maintained at the lowest possible level. Hence, the most important advantage of the two-chamber design in PTR-MS is that the optimal conditions can be adjusted independently in the two chambers. In the first chamber the generation of exclusively H_3O^+ is targeted. In

the second chamber (drift tube) the partial pressure of the VOCs and the primary ions (H_3O^+) can be individually adjusted and the reaction time in the drift tube optimised (106 ms) to assure high sensitivity, low collision energies (minimal fragmentation) and low level of cluster formation. In contrast, the design of APC-MS is such that the formation of the primary ions and the actual chemical ionisation are accomplished both in the same chamber.

From an analytical perspective, the purpose of this article is to demonstrate the potential of PTR-MS for real-time nose space analysis. From a flavour science perspective, we wish to examine possible differences between orthonasal and retronasal aroma profile and document temporal changes of the retronasal profile, while coffee is kept in the mouth.

EXPERIMENTAL

Nose-space analysis aims at sampling and analysing the air exhaled through the nose while food is being consumed. For this, nose-space air is samples via two glass-tubings fitted into the nostrils (Figure 1). The separation and diameter of the tubings have been tailor-made in order to allow the person to breathe and eat freely. The air from both tubings is combined and a small fraction of the nose-space air (14 cm^3/min) is samples at right angle and introduced into the drift-tube of the PTR-MS. The nosepiece is heated to 38°C to prevent condensation on the glass tubings.

The PTR-MS technique has been extensively discussed in a series of review papers (Yeretzian et al., 2000; Lindinger et al., 1998; Lindinger et al., 1998; Hansel et al., 1995). Briefly, it combines a soft, sensitive and efficient mode of chemical ionisation, adapted to the analysis of trace VOCs, with a mass filter (Figure 2). In this study, 14 cm^3/min gas is continuously introduced into the chemical ionisation (CI) cell (drift tube). The drift-tube contains besides buffer-gas a controlled ion density of H_3O^+. VOCs that have proton affinities larger than water (proton aff. of H_2O: 166.5 kcal/mol) are ionised by proton transfer from H_3O^+, and the protonated VOCs are mass analysed. The particularity of the chemical ionisation scheme in PTR-MS is that the generation of the primary H_3O^+-ions on the one hand, and the chemical ionisation process on the other hand, are spatially and temporally separated and individually controlled. This allows (i) maximising signal intensity by increasing the generation of primary reactant ions, H_3O^+, in the ion source, (ii) reducing fragmentation and clustering by optimising the conditions for proton transfer in the drift tube, (iii) and quantifying VOCs from measured count rates.

Consequently, the four key features of PTR-MS can be summarised as follows. First, it is fast. Time dependent variations of HS profiles can be monitored with a time-resolution of one second. Second, the VOCs are not subjected to work-up and little fragmentation is induced by the ionisation step. Hence, mass spectral profiles closely reflect genuine HS distributions. Third, mass spectral intensities can be transformed into absolute HS concentrations, without calibration or use of standards. Finally, it is not invasive. All these features make PTR-MS particularly suited to investigate fast dynamic processes, such as nose-space analysis.

RESULTS

As a starting point of the study, the HS profile of a coffee brew was measured by stripping the brew with nitrogen. The coffee brew was prepared as follows. Using a standard coffee filter machine, 25 gram of roast-and-ground coffee was extracted with 500 ml water (Vittel Bonne Source). 265 ml of the brew was put into a stripping vessel and the VOCs stripped at 36°C with a N_2-flow of 15 cm^3/min through the liquid during one hour. The VOCs in the stripped gas were measured by PTR-MS. Figure 3 shows the averaged mass-spectral intensities over

the mass range 30-165 Dalton. This spectrum represents an orthonasal reference profile to be compare with the retronasal data disussed below.

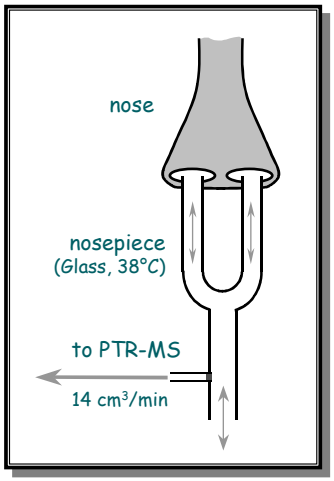

Figure 1. Nosepiece. The air exhaled during food consumption is sampled via the nosepiece and a small fraction introduced into the PTR-MS for on-line VOC analysis

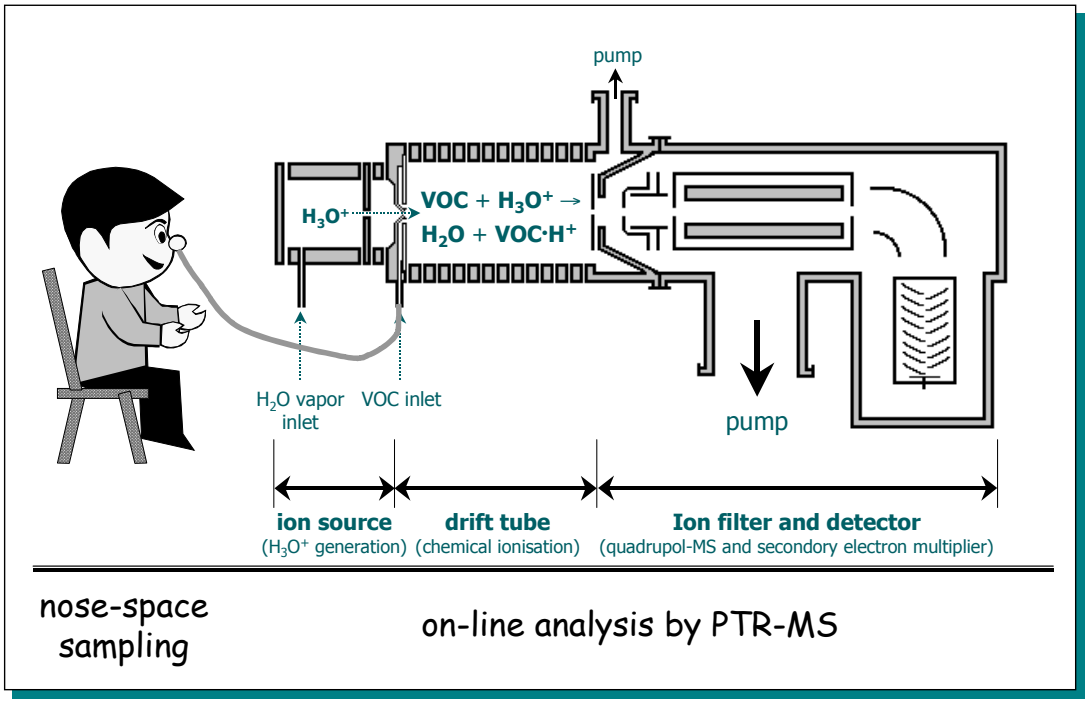

Figure 2. Experimental set-up for nose-space analysis. VOCs released through the nose when consuming food are directly analysis by PTR-MS

In Figure 3, a logarithmic intensity axis was chosen. This allows visualising mass spectral intensities over a large intensity range. As was already discussed in a previous paper (Yeretzian et al., 2000), the individual mass peaks can be assigned to VOC in the HS of coffee. E.g., the most prominent masses are assigned as follows; 45→acetaldehyde, 59→acetone, 61→acetic acid, 69→furan, 73→butanal, isobutanal, 2-butanone, 83→2-, 3-methylfuran, 87→2,3-butanedione, 2-methylbutanal, 3-methylbutanal, 97→furfural, dimethyfuran, 101→2,3-pentanedione, 111→5-methylfurfural.

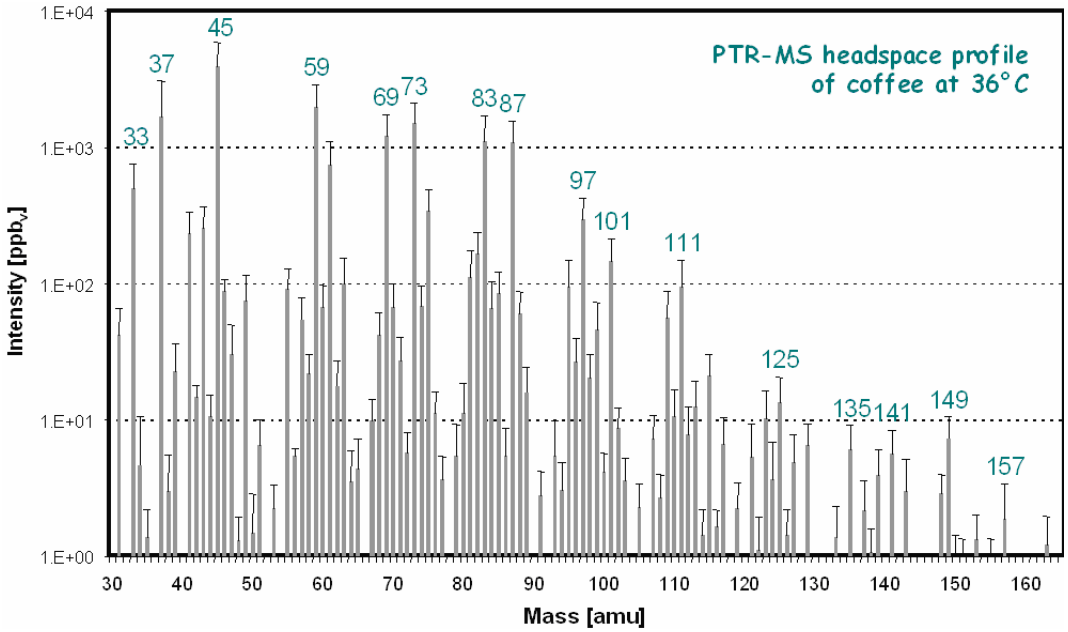

Figure 3. PTR-MS HS profile of coffee. 265 ml brew were stripped with a flow of 15 cm^3/min N_2-gas bubbling through the liquid coffee. The VOCs entrained by the N_2 gas were measured by PTR-MS. Data were averages over one hour, while the brew was kept at 36°C

The brew used for the nose-space experiments was prepared analogous to the brew for the HS experiments. In order to minimise the experimental variability, a precise protocol was develop for the preparation and drinking of the coffee. 25 gram R&G coffee was extracted with 500 ml water directly into a flask. At the end of the extraction (after exactly 4 min and 20 sec) the flask was closed with a plastic stopper and cooled at room temperature until reaching 55°C. 5 ml were filled into a small vial and brought right away to the panellist, ensuring that it is at 50°C just before the panellist consumes it. The panellist did not drink coffee during at least three hours before the experiments, and rinsed his mouth with water prior to the experiments. Nose-space sampling was done in a separate room. Figure 4 gives the time-intensity profiles for nine masses, which correspond to specific VOCs (out of the 14 masses that were actually measured). The intensity axes is adapted to each mass and plotted from the top to bottom in the order of decreasing absolute nose-space concentration.

During the first 230 sec of the nose-space experiments, air was samples through the nosepiece without having the nosepiece inserted into the nostrils. As can be seen from Figure 4, this provides a spectrum of the laboratory air (indoor-air). The intensities are most often around 1 ppb and hence negligible. Only at mass 45 and 61, 10 and 5 ppb background intensities were measured, respectively, which are still low values. Next, the nosepiece was inserted into the nostrils. Since the panellist did not have coffee in the mouth, the observed nose-space spectrum corresponds to the VOC composition of the breath-air. At the masses 59 and 69 amu, we observed strong periodic variations of the intensities, reflecting six breathing cycles (exhalation-inhalation). During exhalation we observe high intensities of acetone and isoprene, which are known to be contained in human breath. During inhalation intensities drop to essentially zero, since these compounds are absent in the laboratory-air. Besides these two compounds we also observed some acetic acid in the breath (mass 61). At approximately 410 sec, the panellist took 5 ml of coffee at 50°C into the mouth and continued to breathe at the same rate. We observed several compounds appearing abruptly in the nose-space air, and which are due to the coffee. Their intensities oscillate in accord with the breathing rhythm. These compounds are know VOC of coffee (Nijssen et al., 1996), some of which contribute to

the typical coffee aroma (Grosch, 2001). In order to investigate the impact of the mouth environment on the release of VOCs, the coffee was kept in the mouth for additional six breathing cycles. For compounds that originate from the coffee, we observe a gradual decrease of intensity with each successive breathing. After six breathing cycles, the coffee was swallowed and six additional breathing cycles were monitored. At the masses of the coffee volatiles we observe a residual intensity which is very close to the intensity prior to taking coffee into the mouth. Finally, the nosepiece is taken out of the nostrils and indoor-air is measure, as was already done at the beginning of the experiment.

DISCUSSION

Two types of background signals have to be considered when discussing nose-space data. The first relates to VOC from the laboratory air. Laboratory air was recorded in the first 230 sec and found to be negligible relative to the intensities found with the coffee in the mouths. A second type of contribution comes from VOCs contained in human breath. These were measured in a second phase of the experiment. We observed that the human breath contains several compounds at very high concentrations (such as acetone and isoprene). E.g. the intensity of acetone is more than six times as intense than the most abundant coffee VOC, acetaldehyde. Once these two types of background contributions have been evaluated, one can properly assess the VOCs originating from the coffee.

When the panellist took 5 ml coffee at 50°C into the mouth, several coffee volatiles appeared in the nose-space air. Here, we selected just 9 masses out of the 14 measured in to order to document the main observations. Prior to discussing the nose-space data, we wish to make a few comments on retronasal volatile sampling. During the first attempts to measure nose-space profiles with liquid coffee in the mouth, we were surprised not to see any signal. After several unsuccessful attempts, we came to believe that the retronasal connection of the oral cavity to the nasal cavity through the pharynx must be unconsciously closed at the back of the oral cavity, in order to avoid liquid to flow into the trachea. The panellists, therefore, trained themselves to overcome this automatic reflects. This revealed to be quit difficult in the beginning but feasible after some training. Thanks to some training we were able to record nose-space profiles as they are shown in Figure 4.

After completion of this study, we became aware of the work of Buettner, Schieberle and colleagues, who have investigated the timing and extend of the odorant transfer from the oral to the nasal cavity (Buettner et al., in press; Buettner and Schieberle, 2000). Based on real-time magnetic resonance imaging and other supporting data, they showed that the oral cavity is often isolated from the pharynx (and hence from the nasal cavity). More specifically they showed that the soft palate can close the oral cavity towards the pharynx by actively pressing onto the tongue and investigated this phenomena with solid and liquid foods.

The most striking observation of the nose-space time-intensity profiles is the strong decreases of signal intensity with time, while the coffee is kept in the mouth. In order to quantify the decrease we fitted an exponential decay curve through the maxima of the individual breathing peaks for each individual mass and determined the exponent. In Figure 5 we show one such example for mass 45. As can be seen, a good fit to a simple exponential decay function is found, with an exponent of –0.0141 and a regression coefficient (R^2) of 0.9237.

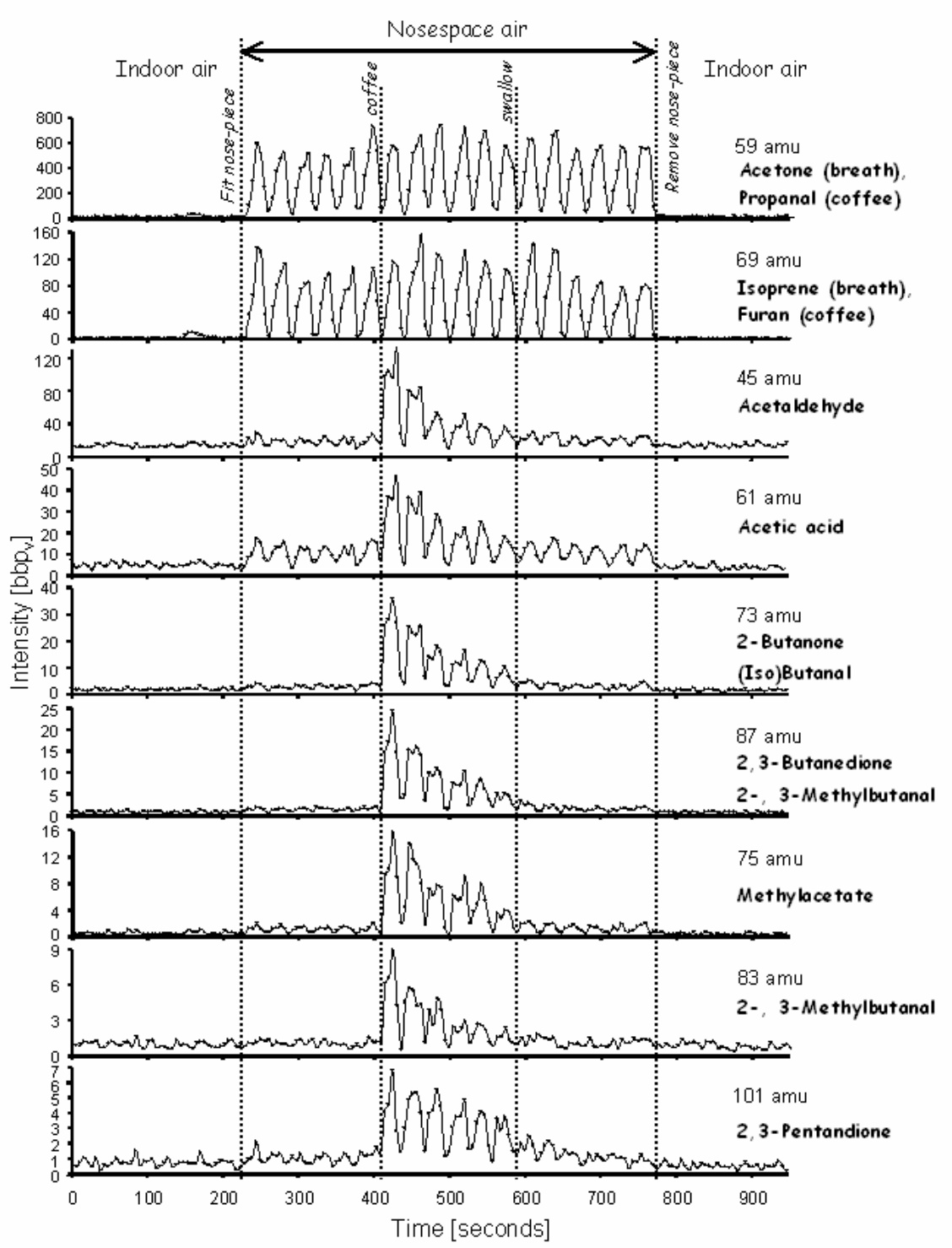

Figure 4. PTR-MS time-intensity profiles of nine VOCs during a typical nose-space experiment with coffee brew. The experiment can be divided into five consecutive phases. First indoor-air was sampled through the nosepiece in order to assess contributions from the laboratory background. We then measured the VOC composition of human breath during six consecutive breathing cycles, with the nosepiece being inserted into the nostrils, but without having coffee in the mouth. After approximately 410 sec, 5 ml coffee at 50°C was taken into the mouth and the nose-space air sampled for six additional breathing cycles, after which the coffee was swallowed and further six cycles monitored. At the end of the experiment, the nosepiece was removed from the nostrils and the indoor-air measured again. During the experiment, the nosepiece was heated to 38°C to avoid condensation

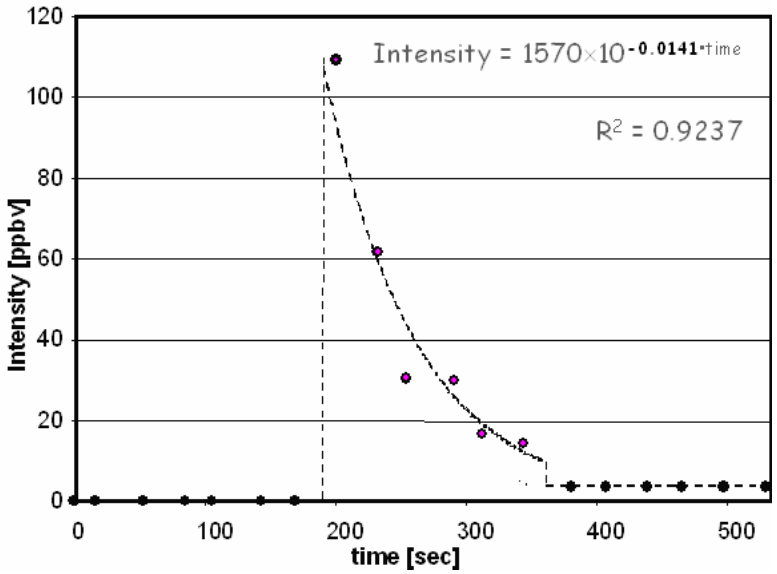

Figure 5. Exponential fit to the intensity decrease of acetaldehyde (mass 45) as a function of time. In contrast to the time-axis in Figure 4, time zero was set at the moment where the nosepiece was put into the nostrils. Furthermore, we subtracted the averaged intensity originating from the human breath and the indoor-air from the raw data. Hence the intensity prior to taking coffee in the mouth is zero. Once coffee is taken into the mouth, the intensities at masses corresponding to coffee volatiles increased initially abruptly, but then decreased gradually as the coffee was kept in the mouth. In order to assess quantitatively this decrease, we determined the maximum intensities at the individual breathing cycles and fitted an exponential function through these points. Once the coffee was swallowed, the nose-space intensities dropped to a value very close to the one prior to taking coffee into the mouth

Several processes can in principle be responsible for this decrease. One is stripping of volatiles from the liquid coffee. Based on measured partition coefficients and stripping experiments (Pollien and Yeretzian, in press), we have to conclude that the observed decrease (exponents) is at least one order of magnitude too fast than can be explained by a simple volatile stripping argument. In fact, we observe for acetaldehyde (as well as for the others) a decrease of nose-space intensity by more than 50% within 2 min. In contrast, we measured reduction by just a few percent when stripping a liquid or a coffee solution with 100 ml N_2 for about 5 min. Hence this can only account for a marginal contribution to the decrease of nose-space intensity.

Two other reasons for the fast decrease of VOCs in the nose-space can be put forward. (i) Diffusion of VOCs into the mouth mucosa will deplete the liquid coffee phase from volatiles, and hence reduce their nose-space concentrations. These compounds can be released back from the mucosa into the mouth-space once the coffee is swallowed and contribute to the lasting coffee-odour in the mouth. Currently we can hardly assess the quantitative importance of this effect, although experiments by Buettner et al. have shown that this can be quit significant (Buettner et al., in press; Buettner and Schieberle, 2000). (ii) The coffee that is taken into the mouth has a temperature of 50°C. Once in contact with the mouth tissue, the liquid starts to cool down and eventually reach 37°C. In a separate study we measured the temperature dependence of the partition coefficient for a series of coffee volatiles (Karl et al., submitted) and found that this phenomena can account for a significant if not for most of the decrease of the nose-space concentration. Quantitative studies are on the way to assess the relative contributions to the decrease of nose-space concentration with time.

Since we determined the exponents for the individual masses (Figure 5), we can calculate the volatile profile in the mouth as a function of time and follow the evolution of the mouth/nose space. This is shown in Figure 6 for three selected times. The three bottom frames show the nose-space profiles for 0, 85 and 170 sec after taking the coffee into the mouth. The frame at 0 sec corresponds to the nose-space profile as the coffee was just taken into the mouth. The spectra are normalised on the most intense peak, which is acetaldehyde (mass 45). While the coffee is kept in the mouth we can distinguish intensity changes that occur with time. This demonstrates at an analytical level, and based on just a few selected compounds, that the volatile profile is evolving rapidly in the mouth. It also suggests that a dynamic description of flavour might be more relevant to the situation of consumption than a static HS profile.

The top frame shows the intensities of the orthonasal profile (HS, Figure 1) at the same masses as for the nose-space at time zero, just below. Comparing both profiles, we see that they are distinctively different. This implies that the orthonasal HS profile is not an accurate description of the volatile profile as perceived during consumption.

CONCLUSION

The objective of this study was twofold. From an analytical perspective, we wanted to establish a nose-space method based on PTR-MS. From a flavour perspective we addressed two specific questions. First we wanted to assess the relation between the orthonasal and retronasal volatile profile. Second we were interested to see whether and how fast the retronasal volatile profile evolves with time.

Based on the results presented in this study, we believe that we have established a powerful approach that combines nose-space sampling with PTR-MS, to monitor at high time resolution the nose-space volatile profile. Considering flavour aspect, we have observed a fast exponential decrease of nose-space volatile intensities. For all compounds discussed here, the intensities decreased by more than 50% within the two minutes of keeping coffee in the mouth. While several physical phenomena can contribute to this fast decrease, the most significant is probably the cooling of the coffee from the starting 50°C to 37°C in the mouth, although absorption on the mouth mucosa might also be quit important. More quantitative studies are needed to ascertain the relative contributions. The other flavour aspect is the comparison of the orthonasal (HS) with the retronasal volatile profile. This work revealed important differences between both profiles, documenting the value of analysing coffee flavour under conditions close to the situation of consumption.

Hence these first results, based on a limited number of volatile compounds, have demonstrated significant differences between orthonasal and retronasal volatile profiles and revealed strong changes of the nose-space profiles while coffee is kept in the mouth. Further studies are on the way to extend these findings.

ACKNOWLEDGEMENTS

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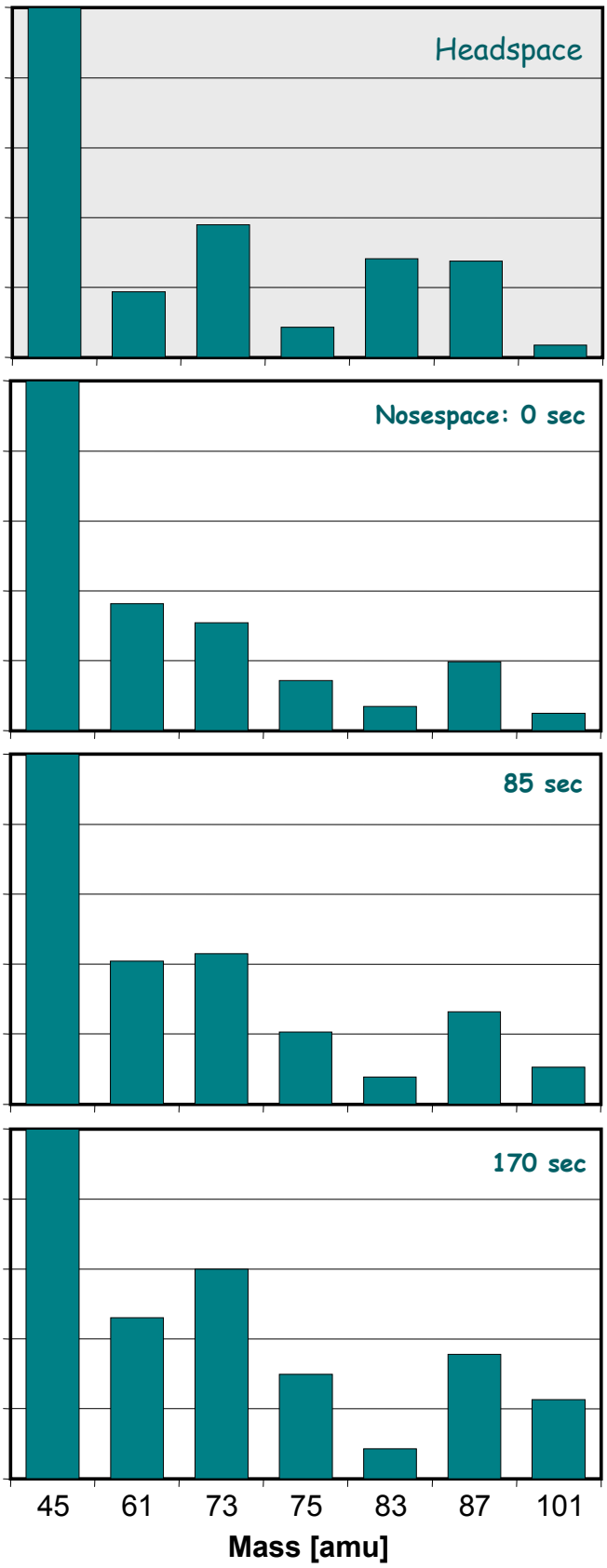

Figure 6. The top two frames allow comparing the orthonasal to the retronasal volatile profile. We see that they are quit distinctive, indicating that a traditional HS analysis might not reflect accurately the volatile profile as it is experiences by the consumer. The three bottom frames illustrate the evolution of the nose-space profile with time. A significant change with time is observed

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Effect of Water Composition and Water Treatment on Espresso Coffee Percolation

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SUMMARY

Water is an essential ingredient of drinks. However, there is little awareness of the effect of water on the quality of coffee beverages, this being particularly true for espresso coffee. In this context, water does not simply mean H_2O, but a complex solution containing many different solutes. Exception made for those that may directly contribute to taste, little is known about the role played by ionic species during the brewing of espresso coffee.

A wide range of tap and treated waters, along with solutions of selected electrolytes, were tested under strictly controlled conditions in espresso-type extraction of three different roasting grades of coffee. As a reference, ultra pure reagent-grade water was used. Among the several measured parameters, percolation time was found to be affected by water composition. Specifically, no significant differences were observed using a wide range of waters either from the tap or treated by reverse osmosis, in comparison with ultra-pure water. But then, an increase was systematically detected when testing softened waters or solutions containing bicarbonate, carbonate or hydroxide of monovalent cations.

While our experimental data confirm some previous observations, the similarity between percolation times obtained using ultra-pure water and tap waters containing quite large concentrations of bicarbonate/carbonate ions suggests that the role played by these anions in espresso extraction should be given new consideration. In particular, the effect induced by softened waters and alkaline solutions seems to be related to coffee bed particle swelling.

RÉSUMÉ

L'eau est un ingrédient essentiel à toute boisson. Cependant, on connaît peu son effet sur la qualité des boisson de café, notamment du café espresso. Là, l'eau n'est pas simplement H_2O, mais une solution complexe de nombreux solutés. Sauf pour ceux qui affectent directement le goût, le rôle de ces ions pendant la préparation de l'espresso est méconnu.

On a expérimenté l'extraction espresso sur trois cafés torréfiés plus ou moins foncés, en employant plusieurs types d'eaux (naturelles et traitées), ainsi que des solutions d'électrolytes choisis. Une eau ultra-pure a été utilisée comme référence. Le temps d'écoulement était la variable la plus affectée par la composition de l'eau: notamment, les eaux adoucies et toutes les solutions de bicarbonates, carbonates et hydroxydes de métaux monovalents ont eu pour effet de l'allonger. Au contraire, aucune différence a été observée entre eaux du robinet et eaux déionisées par osmose inverse, ou même ultra-pures.

Nos données confirment les recherches précédentes, sauf pour la similitude des temps d'écoulement obtenus avec l'eau ultra-pure et l'eau lourdement chargée en bicarbonates et

carbonates. Cela nous fait penser qu'il faudrait rediscuter le rôle de ces ions, en prenant en compte le regonflement des particules de café moulu sous l'action des eaux adoucies.

INTRODUCTION

Water is an essential ingredient of drinks. However, there is little awareness of the effect of water on the quality of coffee beverages, this being particularly true for espresso coffee. In this context, water does not simply mean H_2O, but a complex solution containing many different solutes. The most relevant ones are salts governing water hardness and alkalinity (Petracco, 2001).

The hardness of water (total hardness) depends on its alkaline-earth metals content: hard waters contain calcium and/or magnesium ions, while soft waters contain very little or none. Hard waters may be "temporarily" or "permanently" hard. Unlike the hardness of permanently hard water (deriving from Ca and Mg sulphates and chlorides), the hardness of temporarily hard water is affected by boiling because those metals are present as bicarbonates. The latter break down on heating to form carbonates, very insoluble in water especially if hot. Water alkalinity is governed by bicarbonate ions (and carbonate and hydroxyl ions if present). While pH is easily measured, it is not a very useful estimate of the total alkalinity of water, which depends on the ratio of ions representing the dissociation of carbonic acid. Bicarbonate ions plus carbonic acid (and carbonates at high pH) behave as a buffer system, causing common waters to have a pH of (say) 7.4 to 8.2.

Exception made for the ones that may directly contribute to taste (Lockhart et al., 1955), little is known about the role played by ionic species during the brewing of espresso coffee. The common observation made by coffee-machine professionals when switching from one water source to another is a change in the percolation time needed to prepare a given amount of beverage. This is even more noticeable when using espresso machines conceived for domestic utilisation, in which no direct connection to public waterworks is required, thus allowing the usage of any kind of bottled water.

Since percolation time is the main, if not the only variable at hand of the end user to obtain a given cup (Petracco, 1995), the importance of understanding the role of water in influencing it cannot be under-stressed.

As far as we know, only one study by Fond (Fond, 1995) was addressed to understand the influence of water ions on espresso type extraction. In his detailed investigation, a raw tap water having a bicarbonate content of 380 mg/l and a total hardness calculated at about 33 French degrees (°f) was treated by softening in a single ion-exchange-resin column and by demineralisation in a twin-column device. These three different types of water were used for espresso-type extraction. The percolation time was found to grow, in respect to the one measured by using the raw tap water, by about 10% with water after demineralisation and by about 53% after softening. Moreover, it was noticed that the percolation time increased significantly when the bicarbonate ion concentration (in demineralised water) was raised.

The increase in percolation time was ascribed to bicarbonate–carbonic acid equilibrium. In particular, citing Fond: "during extraction, pH of water drops from around 7.0-7.5 to 5.5-5.0, the final pH of the extract" and then "the calco–carbonic equilibrium is deeply modified with the majority of ions switching from bicarbonate HCO_3– to dissolved carbonic acid CO_2." The release of the excess CO_2 as gas, along with coffee bed particle swelling due to the wetting process of extraction water, is deemed by this author to be the main phenomenon responsible for coffee bed compaction and hence for percolation time increase.

There is a debatable point in this interpretation; namely the fact that, with the softening process described by Fond, little or no change in the carbonate/bicarbonate content of treated water is expected. Therefore a raise in percolation time of about 53% cannot be explained by a difference in bicarbonate ions content between the softened water with respect to raw tap one, which should contain essentially the same amount.

Our present investigation was stimulated by this question. Following the experimental approach proposed in (Fond, 1995), and resorting to additional characterisation techniques, a wide range of tap and treated waters, along with solutions of selected electrolytes were tested under strictly controlled conditions in espresso-type extraction of three different roasting grades of coffee (pure *Coffea arabica* blend). The experimental data were interpreted taking into consideration, beside the ionic species present in the water and in coffee, also coffee chemical components such as water-insoluble polysaccharides.

MATERIALS AND METHODS

The commercial roast and ground coffee used, pure Coffea arabica blend, was produced by illycaffè (Italy) in roasting grades C (light), N (medium) and S (dark). In order to reduce possible error sources, Easy Serving Espresso™ (ESE) coffee pods were used. The ESE serving (http://www.esesystem.com/web/english/standard/index.htm) is an industrially pre-packaged individual portion of roasted, ground, dosed out, and pressed coffee, sealed between two layers of food-use filter paper.

A Turmix MIES 515 espresso machine suitable for ESE system, properly calibrated according to the standard procedure of Quality Control Department of illycaffè Company, was used.

The following types of water were used:

- raw tap Trieste municipal water ("Trieste" for the sake of brevity):
- calcium 60 mg/l; magnesium 11 mg/l; sodium 7 mg/l; bicarbonate 200 mg/l; total hardness 19.7°f; conductivity 255 μS/cm at room temperature
- ion-exchange treated "Trieste" water ("Soft" for the sake of brevity):
- residual total hardness 3-4°f; conductivity 291 μS/cm. The treatment was performed by using a Cillit Parat 32E-96A system (Cillichemie, Italy);
- reverse osmosis treated "Trieste" water ("RO" for the sake of brevity):
- conductivity 33.5 μS/cm. The treatment was performed by using a Milli-RO system (Millipore, Italy);
- ultra-pure reagent grade water ("Milli-Q" for the sake of brevity):
- conductivity 0.1 μS/cm. Produced by a Milli-Q system (Millipore, Italy);
- several types of Italian municipal water in a wide range of total hardness (12.5-47.7°f), some of which underwent further softening treatment;
- sodium carbonate, sodium bicarbonate and sodium hydroxide solutions in Milli-Q water, within the concentration range 0.001-0.02 N.

Extraction was performed with a draw off ratio of about 5.7 (7 g R&G coffee, 40 g extract) in the following conditions: after washing the water reservoir with the selected type of water, three extractions were performed for temperature conditioning and then ten extractions were performed for percolation time and extract mass recording. Five extracts were used for pH measurements at room temperature and four extracts were used for total solids determination. For the latter, the extract (40 g) contained in a Pyrex beaker was maintained at 105°C in an oven for 48 hours and the total solids determined gravimetrically. Extraction yield [%] is defined as the ratio of total solids in the beverage and the weight of R&G coffee used to

produce it. Percolation time [s], pH and total solids [g] are given as average values (±1.0 s for percolation time and ±0.02 g for total solids). When necessary, the extract mass was normalised to 40.0 g.

Particle size distribution of coffee powder (N grade) was measured by using a Light Scattering Particle Size Analyzer Coulter LS230, equipped with a liquid module, in the range 0.1-2000 μm. Instrument set up was done following the standard procedure of Quality Control department of illycaffè. Typically, 1.0 g of coffee powder has been dispersed in 200 ml of water (or alkaline solution) at constant temperature (25, 50 or 90°C) and mechanically stirred (700 rpm) for 2 min. The resulting dispersion was introduced in the proper reservoir of the instrument and the particle size distribution was recorded. For each sample 4-8 different runs were performed to ensure reproducibility. All data were statistically treated with SYSTAT® 8.0 (SPSS Science) scripts, written by one of the Authors (RC), and run on a Pentium III 650 MHz 256 Mb RAM, on Windows NT 4.

RESULTS AND DISCUSSION

Effect of water treatment on espresso coffee percolation

In Table 1, percolation time, pH and total solids yield are reported for the three different illycaffè coffee grades. The experimental data obtained by using a sodium carbonate 0.4 g/l solution are also included in the table. Independently from the water type, both percolation time and pH increased passing from C grade to S grade. Total solid contents from C are close to those from N grade, those for S being the highest.

As clearly appearing in Table 1, percolation time is the variable that the water type affects more than others. In particular, Milli-Q, RO, and Trieste waters provided a percolation time very close each other, and for several cases practically identical within the experimental error. On the contrary, the percolation times measured by using Soft water and the sodium carbonate solution (0.4 g/l) are, independently from coffee grade, respectively about 15% and 45% higher on average. The latter findings are expectable on the basis of previous studies (Fond, 1995), but the similarity between the percolation times measured by using Milli-Q and Trieste waters is unexpected, and to some extent surprising. In fact, these findings could indicate that ultra-pure reagent grade water (virtually lacking of any solute) and raw tap Trieste municipal water (containing on the contrary a number of solutes, including a not negligible bicarbonate content) interact with the coffee bed in a similar way.

It was suggested (Fond, 1995) that carbonate/bicarbonate ions are the main responsible for the increase in percolation time observed when softened waters are used. However, since the softening treatment is not likely to significantly change the carbonate/bicarbonate content of treated water, the interpretation of the present data is not straightforward. Therefore, with the aim of providing further data, we followed two parallel approaches:

1) to study the extraction of N grade coffee by using several types of untreated and softening treated Italian municipal water

2) to study the extraction of N grade coffee as a function of the concentration of alkaline solutes.

Effect of untreated and softened municipal waters

Several Italian municipal waters characterised by total hardness either lower or higher than

that of Trieste water were tested. In Table 2, percolation time, pH and total solids yield measured by using the set of waters are reported.

Table 1. Extraction and extracts characterisation as a function of coffee roasting grade and water type

coffee grade	water	percolation time [s]	ph	extraction yield [%]
Light (C)	Milli-Q	26.5	5.3	24.5
	RO	25.5	5.3	24.5
	Trieste	27	5.3	24.7
	Soft	30	5.3	25.2
	Na_2CO_3 (0,4 g/l)	41	5.3	26.4
Medium (N)	Milli-Q	30	5.4	24.3
	RO	30	5.4	24.3
	Trieste	30.5	5.4	24.4
	Soft	35	5.4	25.4
	Na_2CO_3 (0,4 g/l)	44	5.5	26.3
Dark (S)	Milli-Q	38	5.6	25.6
	RO	35.5	5.6	25.2
	Trieste	39	5.7	25.8
	Soft	43.5	5.7	26.4
	Na_2CO_3 (0,4 g/l)	53.3	5.8	27.2

In spite of the wide range of total hardness and bicarbonate content of the examined waters, all measured parameters are very close to each other and substantially identical to those obtained with Milli-Q and Trieste waters, within the experimental error. In Table 3, percolation time, pH and total solids yield measured by using some of the previous waters after softening treatment are reported.

After softening, Trieste water extended percolation time by 13.7% with respect to untreated water, while Scandiano water did the same by 32.1% and Napoli water by 79.3%. The percolation time increase correlates with the bicarbonate content originally present in the municipal waters. These findings strongly suggest that the observed percolation time increase be not induced simply by the bicarbonate content, but more critically by the replacement of calcium (and magnesium) ions with sodium ions performed during the softening treatment.

Effect of alkaline solutions on *espresso* coffee percolation

A preliminary comparison was performed using several alkaline solutions at a fixed concentration of 0.01 N in order to check possible cation influence. In particular lithium, sodium and potassium carbonate, sodium and potassium bicarbonate, lithium, sodium, potassium and calcium hydroxide solutions were used and the corresponding percolation times compared with that of Milli-Q water.

Table 2. Effect of untreated Italian municipal waters on extraction and extracts parameters

water	total hardness [°f]	Bicarbonate content [mg/l]	percolation time [s]	ph	extraction yield [%]
Cortina	12.5	n.a.	31	5.3	24.1
Culligan	13.5	n.a.	31	5.3	24.3
Trieste	19.7	200	30.5	5.4	24.4
Forlì	27.5	n.m.	32	5.4	24.3
Napoli	45.0	520	29	5.4	24.1
Scandiano	47.7	400	28	5.3	24.1

(coffee: N grade)

Table 3. Effect of softened Italian municipal waters on extraction and extracts parameters

water	percolation time [s]	ph	extraction yield [%]
Trieste	34.5	5.4	25.4
Scandiano	37	5.4	25.3
Napoli	52	5.5	26.9

(coffee: N grade)

Independently from the monovalent cation type, hydroxide anions as well as carbonates and bicarbonates increased the percolation time. The hydroxide acted even more remarkably than the two salts, which displayed analogous percolation time increase patterns. In the case of calcium hydroxide only a modest increase of percolation time was observed.

In Figure 1, percolation time is reported as a function of the concentration of sodium carbonate, sodium bicarbonate and sodium hydroxide.

Figure 1 shows the similarity between carbonate and bicarbonate in increasing the percolation time. At a solute concentration up to 0.006 N both carbonate and bicarbonate induced a percolation time increase slightly higher than the one induced by the hydroxide, but at a solute concentration higher than 0.006 N the percolation time increase induced by the hydroxide is much more pronounced.

Phenomena other than carbon dioxide release through coffee acid neutralisation of carbonate and bicarbonate have to be taken into consideration in order to interpret the experimental data obtained with sodium hydroxide. One of these could be the coffee bed particle swelling, and in particular, the swelling of water-insoluble polysaccharides present in roasted coffee. Coffee mannan, for instance, is a linear polymer of β (1→4) linked anhydromannopyranose units and, at approximately 22% dry weight basis, is the most abundant of roasted coffee constituents (Bradbury and Halliday, 1987). Cellulose is also abundant, at approximately 8% dry weight basis (Bradbury and Halliday, 1990).

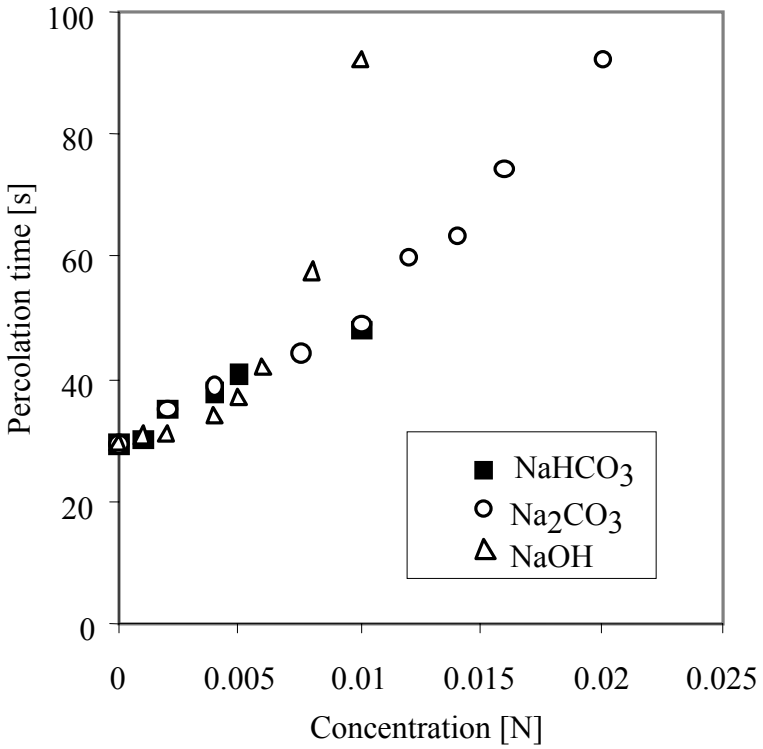

Figure 1. Percolation time as a function of solute concentration (coffee: N grade)

These polysaccharides are insoluble in water, but they swell in alkaline solutions. Mannan partially dissolves in concentrated alkali (18% aqueous sodium hydroxide) (Wolfrom et al., 1961) whereas cellulose in the same conditions swells only (Whistler, 1973). It can be hypothesised that the water-insoluble polysaccharide swelling induced by alkaline solution under conditions of pressure and temperature of the *espresso* extraction may heavily contribute to a reduced coffee bed porosity, and hence to an extended percolation time. In order to support this hypothesis, the particle size distribution of coffee powder after contact with several water types and alkaline solutions maintained at three different temperatures (25, 50 and 90°C) was measured.

The whole set of experimental data, and in particular 10 readings corresponding to the following particle diameters: 10, 27, 44, 84, 111, 177, 234, 310, 373 and 450 μm on each particle size distribution curve, were treated by discriminant analysis in order to find out whether the effect of different waters on particle swelling is recognisable or not.

Discriminant analysis is a statistical procedure to identify the variables useful for determining whether groups of data are distinguishable from each other. The aim of discriminant analysis is classification of each case into one of several mutually exclusive groups, on the basis of its measured response variables. It was introduced by Fisher in 1936, with the following basic idea: a linear combination of the measured variables can be found, through a transformation of these variables to a new scale, such that the mean distance between the classes would be maximised (Piggot, 1986).In our case, the only variables used for discriminant analysis were the granulometric ones. The discriminant functions that best separate samples have the form:

$$f(i) = \alpha_i\lambda_1 + \beta_i\lambda_2 + \ldots + \psi_i\lambda_{n-1} + \omega_i\lambda_n$$

where λ_i are the chosen diameters. No diameter smaller than 10 μm has been considered, to exclude possible errors due to an overestimation of small particles by the instrument, because of the different refractive index of colloids. Also particles bigger than 450 μm have been discarded, because of our range of interest. In Figure 2 the results of the discriminant analysis are reported.

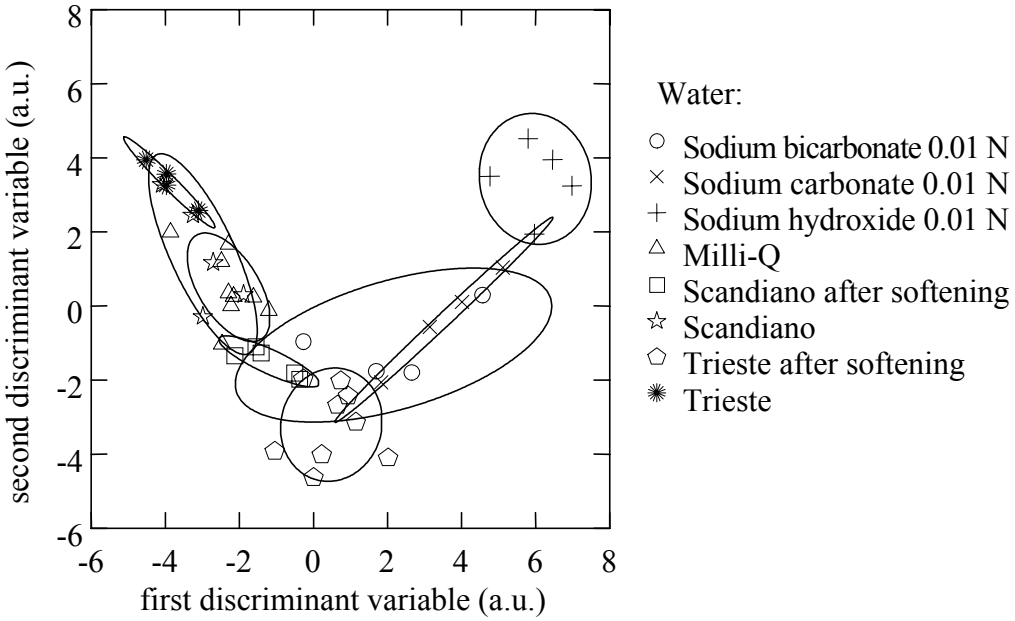

Figure 2. Discriminant analysis

It shows that the clusters relative to Milli-Q, Trieste and Scandiano overlap, forming a separate cluster. The same do the ones relative to Soft, softened Scandiano and to sodium bicarbonate and sodium carbonate (both 0.01 N) forming a second separate cluster, and the one relative to sodium hydroxide, which is well separated from the others forming a third cluster. Assuming that the observed changes in coffee particle size distribution is mainly due to the particle swelling, these findings strongly suggest that the alkalinity of the examined aqueous systems significantly influences particle swelling, whereas the latter is not perturbed changing from Milli-Q water to municipal waters with different composition.

CONCLUSIONS

While our experimental data confirm some previous observations (Fond, 1995), the similarity between percolation times obtained using ultra pure water and tap waters containing quite large concentrations of carbonate/bicarbonate ions suggests that the role played by these anions in *espresso* extraction should be given new consideration. In particular, it is known that boiling is an effective traditional way to get rid of most of water hardness and alkalinity. By heating/boiling, bicarbonates decompose according to:

$$Ca^{++} + 2\ HCO_3^{-} \rightarrow CaCO_3\downarrow + CO_2\uparrow + H_2O$$

Generally stated, bicarbonates are driven off as CO_2 (and $CO_3^{=}$) and the Ca++ hardness is precipitated as calcium carbonate, especially when the water is hot. If there is a significant content of sodium salts present as bicarbonates, there can be a rise in pH associated with boiling, because of the formation of sodium hydroxide thus:

$$Na^{+} + HCO_3^{-} \rightarrow NaOH + CO_2\uparrow$$

It is clear that the latter bicarbonate thermal decomposition may originate a particle-swelling behaviour in the coffee bed very different from the one originated by a low-sodium content water. Although the interpretation proposed in (Fond, 1995) overlooks the thermal behaviour of aqueous bicarbonate anions, in our opinion this behaviour may play a major role in the understanding of the experimental data of the present investigation. In other words, the carbon dioxide release through coffee acid mediated neutralisation of carbonates and bicarbonates (and through their thermal decomposition) may influence the dynamic process of *espresso* extraction, and this is suggested by the data reported in Figure 1, at a solute concentration up to 0.006 N.

However, the coffee bed particle swelling induced by hydroxyl anions on water-insoluble coffee polysaccharides seems to be a substantial, and under certain conditions the dominant phenomenon for interpreting the percolation time increase detected when softened waters and alkaline solutions are used. In clear agreement with Fond (1995), our investigation further confirms that coffee extraction is a complex and dynamic process, where coffee particles react with water through a multitude of phenomena. Beside carbon dioxide release and coffee particles swelling, other phenomena have yet to be introduced and understood.

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Analysis of Bitter Fractions of Roasted Coffee by LC-ESI-MS - New Chlorogenic Acid Derivatives

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SUMMARY

Chlorogenic acid lactones were identified as important compounds of the bitter methanol fraction of the roasted coffee fractionation. Seven chlorogenic acid lactones were identified in the methanol fraction, five of them for the first time in roasted coffee. Sensory tasting revealed bitterness of the lactones at 19 ppm (in aqeous solution). Schrader et al. (1996) quantified lactone contents (3-CQL + 4-CQL) at 120 ppm. Therefore it can be assumed that these compounds do have an impact on the perceivable coffee bitterness. Furthermore chlorogenic acid methylester were identified via LC-ESI-MS analysis. Structural identification of the isomers is still ongoing.

INTRODUCTION

Coffee was fractionated according to the procedure developed by Chen (1979). Fractionation is shown in Figure 1. All fractions obtained were tasted for bitterness. Sensory assessment was conducted by a panel of six panellists trained by an official method to perceive bitterness (BGVV, 1997). In the water fraction of the polyamide separation recently bitter tasting proline- and phenylalanine-based diketopiperazines were detected (Ginz and Engelhardt, 2000 and 2001), which might contribute to the perceivable bitterness of this fraction. In line with the threshold values obtained by Chen (1979), the methanol fraction was found to be much bitterer in comparison to the water fraction (for threshold values see Figure 1). Chen could not identify any constituents of this fraction. Consequently a further separation by Sephadex G10 was conducted and fractions were analysed by RP-HPLC-DAD. DAD spectra obtained in fraction VI and VII indicated the presence of compounds similar to chlorogenic acids (characteristic UV pattern between 275 and 375 nm).

For additional information all Sephadex G10 fractions were analysed by LC-ESI-MS. Signals of m/z 335 and m/z 349 (negative ESI-mode) were detected. In Figure 2 the total ion current (TIC) of fraction VII and the mass track at m/z 335 is given. Obviously a group of isomers of this compound is present. In comparison to caffeoylquinic acid (CQA) molecular weight a loss of 18 mass units leads to m/z 335, which might be explained by the loss of water. The UV- and MS/MS-spectra indicate the presence of caffeic acid in the molecule, leading to the conclusion that these compounds are chlorogenic acid lactones. The presence of quinic acid lactones, as well as chlorogenic acid lactones in coffee is well documented in the literature (Scholz-Böttcher and Maier, 1991). Bennat et al. (1994) identified 3- and 4-caffeoylquinic acid lactones (3-CQL and 4-CQL) in roasted coffee.

In a model experiment pure chlorogenic acids were thermally treated under coffee roasting conditions. Under these conditions corresponding lactones are formed as major breakdown products. The lactone fraction was isolated from an aqueous extract of the model roast by semipreparative RP-HPLC. To gain inside into the structure of the isomers, isolation via

analytical HPLC was conducted (Figure 3) and the isolated compounds were analysed by NMR. The signals at 26 min and 37 min could be identified as 3- and 4-CQL, which were already described by Bennat et al. (1994) as constituents of roasted coffee.

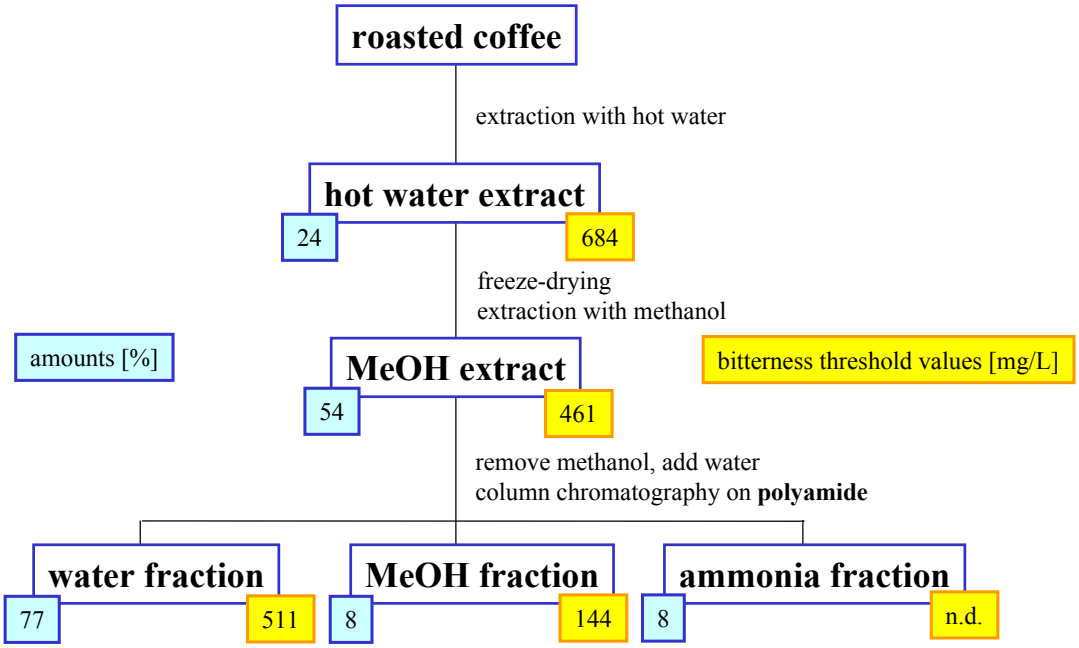

Figure 1. Fractionation of roasted coffee

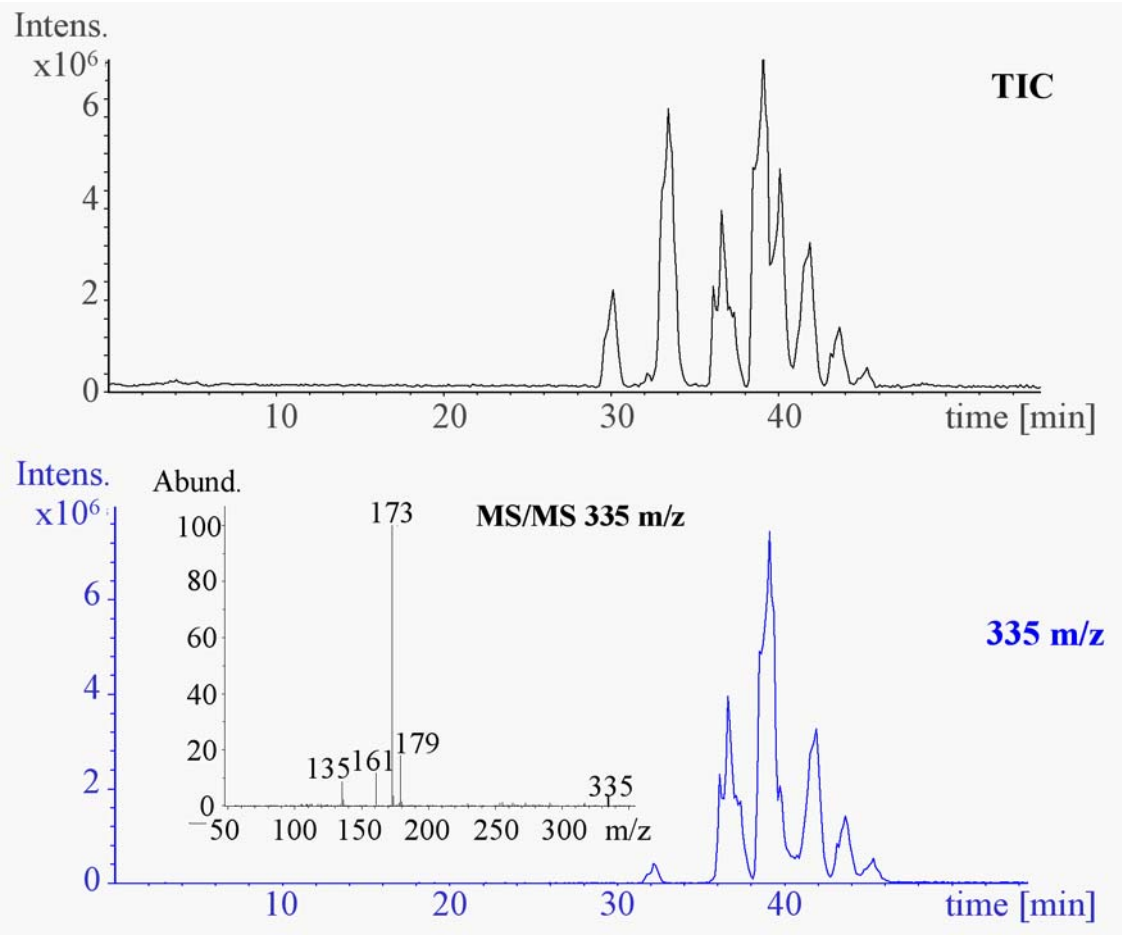

Figure 2. LC-ESI-MS analysis of fraction VII, negative ESI-Mode

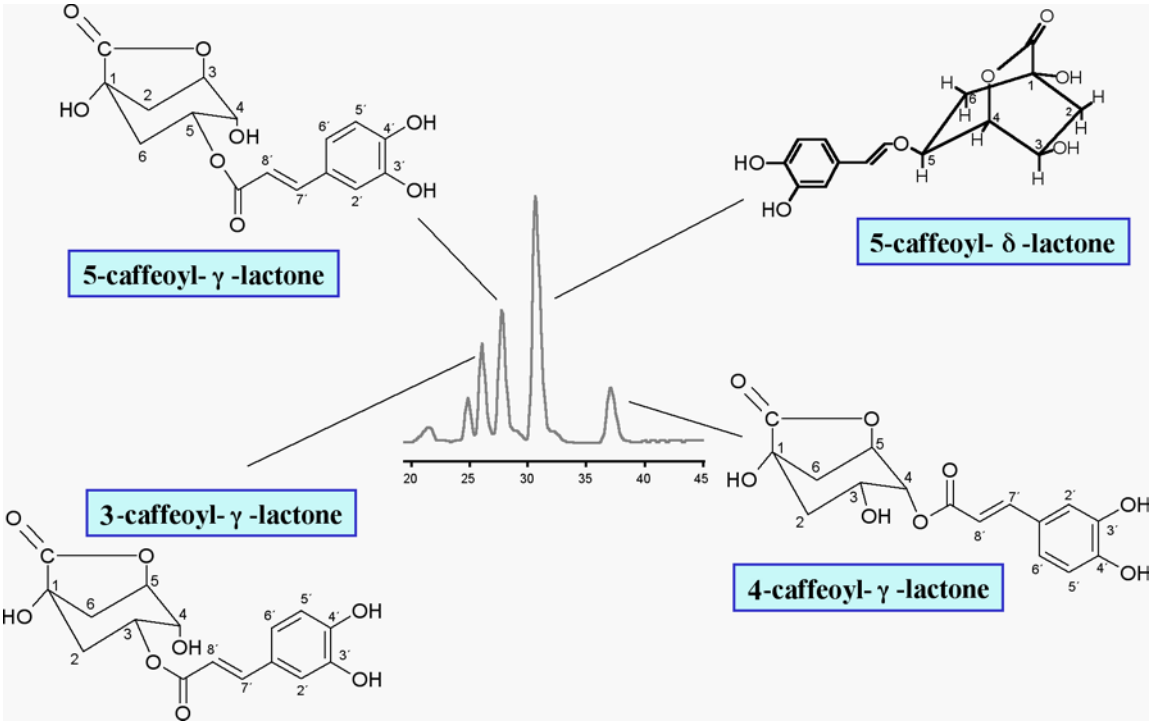

Figure 3. Separation and structures of identified lactones in model roast of 5-CQA

Comparison of the NMR-data of the isolated lactones from chlorogenic acid model roast and from roasted coffee proofed the identification of the lactones in coffee. 3-caffeoyl-γ-lactone, 4-caffeoyl-γ-lactone, 5-γ-caffeoyl-lactone and 5-caffeoyl-δ-lactone were identified as major compounds in the bitter methanol fraction of the coffee fractionation. In fraction VI of the Sephadex G10 fractionation a group of isomers of m/z 349 was detected by LC-ESI-MS analysis (negative ESI-Mode). Beside the CQL-isomers Wynnes et al. (1987) identified feruloylquinic acid lactones (FQLs) in instant coffee, but no information about their configuration is given. Furthermore it was not certain, whether or not these lactones are formed during the drying process of the instant coffee production. By comparison with synthesized reference compounds 3-feruloyl-γ-lactone and 4-γ-feruloyl-lactone were identified to be present in the methanol extract of roasted coffee.

To figure out whether these lactones are responsible for the occurring bitterness of the methanol fraction, sensory tasting of the lactone fraction isolated from the chlorogenic acid model roast was conducted. All panellists judged the lactone fraction to be bitter in a concentration of 19 ppm when tasted in aqueous solution. Schrader et al. (1996) quantified 3-caffeoyl-γ-lactone and 4-caffeoyl-γ-lactone in roasted coffee and determined concentrations of 120 ppm, an amount that is five times higher then the detected bitterness threshold value of the lactone fraction. Therefore it can be concluded that chlorogenic acid lactones do make a significant contribution to the perceivable bitterness of the roasted coffee beverage (Ginz and Engelhardt, 2001).

Beside the lactones, in the Sephadex G10 fractions VI and VII a second group of compounds was present with the typical UV spectra of chlorogenic acid like compounds. The LC-ESI-MS analysis of these fractions showed a group with mass tracks of m/z 367 and m/z 381 (negative ESI-Mode). As observed for the lactones, a number of isomers of each of these compounds are present, which again gave the same DAD- and MS/MS-spectra. The molecular weight of MW 368 and MW 382 is 15 mass units higher then the molecular weight of CQA and FQA.

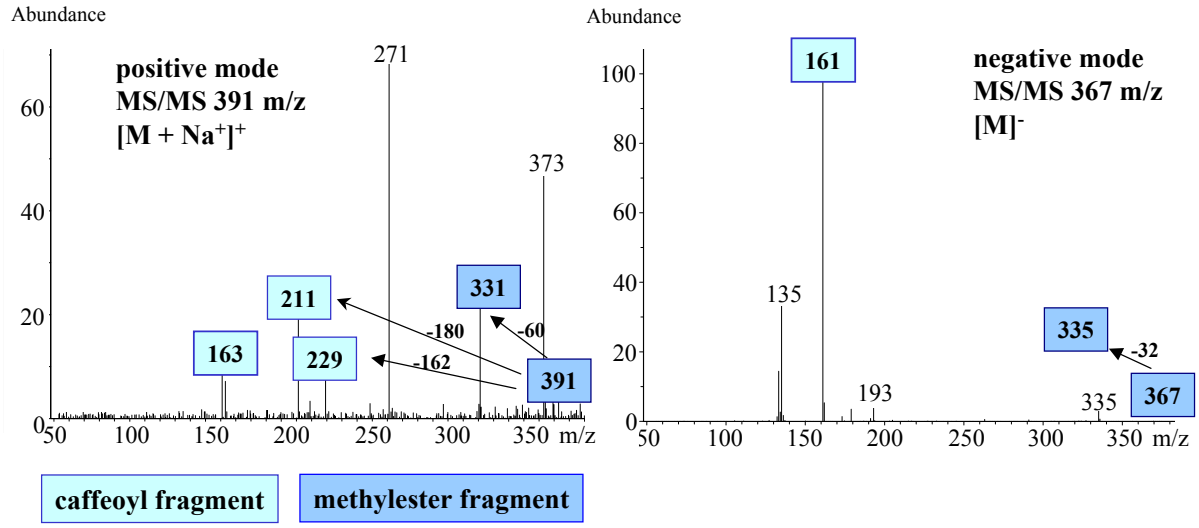

Figure 4. MS/MS-spectra of an isomer of chlorogenic acid methylesters, identified in fraction VI of the Sephadex fractionation

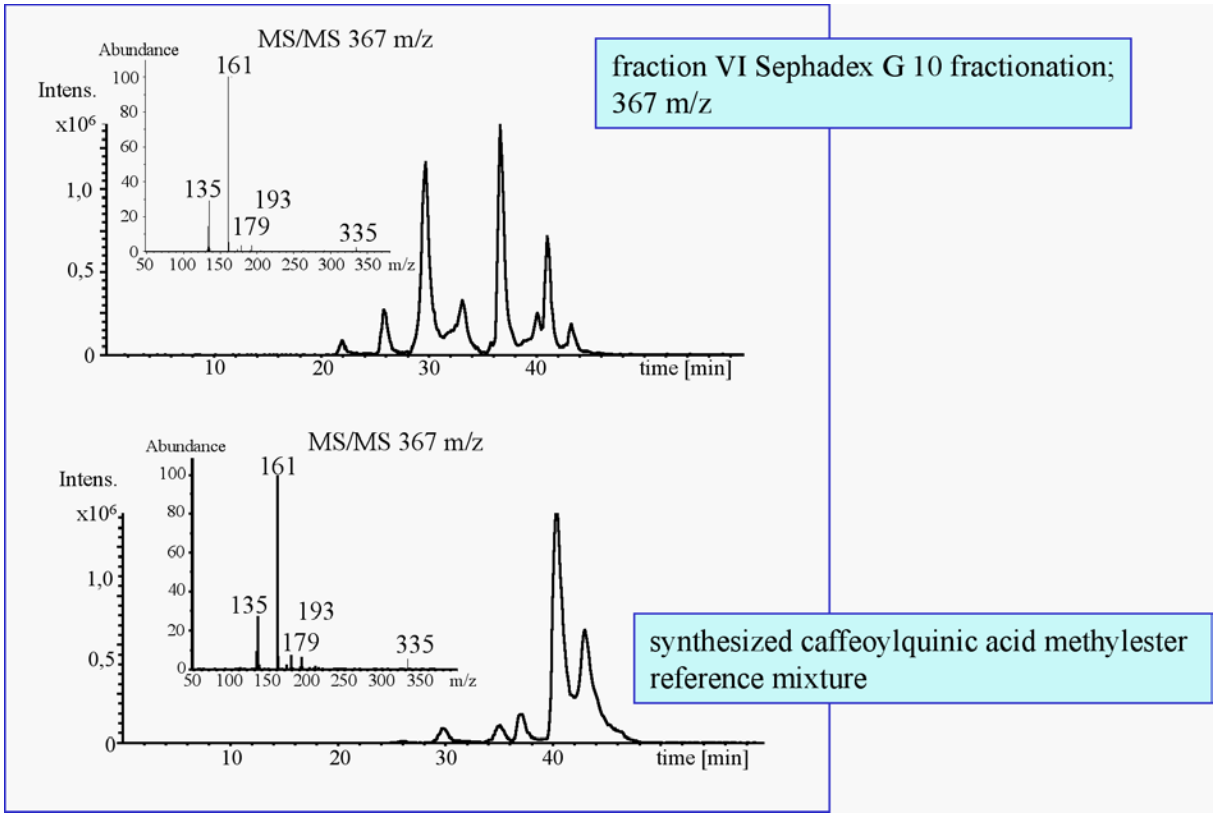

Figure 5. LC-ESI-MS analysis of fraction VI of the Sephadex G10 fractionation (a) and a mixture of synthesized caffeoylquinic acid methylesters (b); MS/MS-spectra given for comparison

The fragmentation pattern observed in positive and negative ESI-mode indicate the presence of a methylester, see Figure 4. Losses of 180 and 162 mass units (positive ESI-Mode) are characteristic breakdown pathways of caffeic acid moiety. The fragment of m/z 161 in negative ESI-Mode proofs the identification. Fragments corresponding to minus 60 (positive mode) from the molecule ion mass are typical for the presence of a methylester-group. Therefore the compounds are supposed to be isomers of caffeoylquinic acid methylester and

feruloylquinic acid methylester. Reference compounds were synthesised and a comparison was conducted via LC-ESI-MS analysis. In Figure 5 a mixture of synthesised isomeric caffeoylquinic acid methylesters is compared to fraction VI of the Sephadex fractionation. Obviously retention times and MS/MS-spectra are identical. Artefact formation during sample preparation because of the use of methanol can be neglected. Fractionation of the coffee was again conducted using ethanol instead of methanol and again chlorogenic acid methylesters were detected. However, the unambiguous verification of the structures by NMR spectroscopy is still in progress.

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Application of a Ratio Scale to Coffee Evaluation

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SUMMARY

To improve the quantitative nature of sensory tests, a ratio scale of taste, called the gust scale was used in sensory tests of coffee. By applying the gust scale to the sensory test, the intensity of acidity and bitterness in coffee could be evaluated as quantitative values with an equi-ratio property.

To estimate these gust values from physico-chemical properties, the relationship between these values and physico-chemical properties were investigated. Linear relations were observed between physico-chemical properties and the logarithm of gust values with a high correlation. Using experimental equations, gust values were estimated from titratable acidity and luminosity values.

RÉSUMÉ

Pour améliorer la nature quantitative des tests sensoriels, une échelle de rapports du goût, appelée échelle de gustation, a été utilisée dans des tests sensoriels du café. En appliquant cette échelle de gustation à un test sensoriel, les niveaux d'acidité et d'amertume du café peuvent être évalués quantitativement avec une propriété d'équi-rapports.

Pour estimer ces valeurs de gustation à partir de propriétés physico-chimiques, la relation existant entre ces valeurs et les propriétés physico-chimiques fut étudiée. Des relations linéaires furent observées entre les propriétés physico-chimiques et le logarithme des valeurs de gustation avec une corrélation élevée. En utilisant des équations expérimentales, les valeurs de gustation ont été estimées à partir de mesures d'acidité et de luminosité.

INTRODUCTION

To evaluate the perceived acidity and bitterness of coffee, sensory test has been used due to its availability, convenience and rapidness. So far, the intensities of acidity and bitterness of coffee have been assessed largely by sensory tests involving simple scoring methods or hedonic scaling methods. However, these kinds of methods have produced results consisting of a nominal or an ordinal scale (Steven, 1946), and there has been room for improvement in such quantification. Therefore, a higher quantitative scale was required to use in sensory test for coffee.

The purpose of this study was to apply a ratio scale of taste, called the gust scale (Lewis, 1948; Beebe Center and Waddel, 1948; Beebe Center, 1949), to coffee evaluation to improve the quantitative nature of sensory tests, and to investigate the relationship between the evaluated gust values and physico-chemical properties.

In this paper, the sensory test procedure to estimate gust values for the perceived acidity and bitterness of coffee is presented. In addition, the experimental formulas to estimate gust values for the intensity of acidity and bitterness of coffee from titratable acidity and luminosity at various trade varieties, different roast degrees, and several coffee brew concentrations are described.

MATERIALS AND METHODS

Coffee samples

The coffee samples used were two botanical cultivars, Arabica (Brazil No.2, Colombia Supremo, Indonesia Toraja TLA, Tanzania AA, and Zimbabwe AA+) and Robusta (India parchment AB, Indonesia AP-1 grade 2).

Five hundred grams of coffee beans were roasted with Neotec RFB-S roaster at constant temperature of 270°C. Roast degrees were produced according to the roasting time.

The L-values (luminosity in Lab chromaticity diagram) of coffee samples were shown in Table 1. The coffee beans were stored in a vacuum pack at –10°C, and ground just before use.

Table 1. L-values of roasted coffee samples

Trade variety	L-value	Trade variety	L-value
Experiment involving trade variety			
Brazil No.2	27.4	Zimbabwe AA+	27.4
Colombia Supremo	27.4	India parchment AB	27.4
Indonesia Toraja TLA	27.4	Indonesia AP-1 grade 2	27.4
Tanzania AA	27.6		
Experiment involving roast degree			
Zimbabwe AA+	29.5	Brazil No.2	29.4
	27.2		27.8
	26.2		26.3
	24.6		24.8
	22.3		22.3
	21.5		21.2
	18.0		18.0
	16.0		16.0
India parchment AB	27.3	Indonesia AP-1 grade 2	27.4
	25.1		24.4
	21.0		21.2
	18.0		18.1
	15.7		15.8
Experiment involving brew concentration			
Zimbabwe AA+	27.4	Brazil No.2	27.3
	16.0		16.0
India parchment AB	27.3	Indonesia AP-1 grade 2	27.4
	15.7		15.8

Coffee brew preparation

One hundred grams of roast and ground coffee was placed in 2liter beaker. One liter of boiling distilled water was poured into the beaker, mixed for 45 seconds, and filtered. After filtration, the brew was cooled in a cold-water bath to about 20°C.

The concentration of coffee brew was adjusted to brix 1.50% by adding distilled water for the experiments involving trade variety and roast degree. For the experiment involving coffee brew concentration, the brix of brews ranged from 1.00 to 2.50%.

Gust scale

As a scale of taste intensity based on ratio judgment, the gust scale was constructed by Beebe-Center (1948, 1949), using the data of fractionation experiments by Lewis (1948). The unit in this scale is defined as the subjective strength of a 1 percent solution of sucrose. Using the gust scale in sensory tests, the intensities of acidity and bitterness in coffees were evaluated as quantitative values with equi-ratio property.

Sensory test

The gust scale was used to evaluate the intensity of acidity and bitterness of coffee. That is, the coffee brew was served in a dark-blue glass and placed in front of 12 standard solutions arranged in decreasing order of strength from right to left. The standard solutions were labeled with the index numbers 1-12. The sensory test was performed with a panel of 5 trained testers, with 2 replications. The testers were informed that the standard solutions were ranged in decreasing order of strength from right to left, and that no particular order of sampling was required. The testers were instructed to select the solution best matching the coffee brew in terms of the intensity of acidity or bitterness.

The concentrations of standard solutions were listed in Table 2. The intervals and the ranges of standard solutions were decided based on data from unpublished preliminary experiments. The interval of the concentrations of standard solutions must be a psychological equal spacing, and since logarithmically equal spacing of psychological intensity meets this condition, the concentrations that correspond to a spacing of 0.1 log gusts were used.

Tasteless tap water was provided for oral rinsing. No swallowing of the solutions or the rinse water was permitted. All solutions and the rinse water were maintained at about 20°C.

Measurements of physico-chemical properties

Brix of coffee brew was determined by Atago RX-3000 digital refractometer. Titratable acidity was measured by neutralizing 100 ml of coffee brew with N/10-NaOH using Hiranuma auto titrator COMTITE-980. Luminosity of coffee was measured with Nippon Denshoku Kogyo color meter Model ZE-2000.

RESULTS AND DISCUSSION

Gust values for perceived acidity and bitterness

The gust values for perceived acidity and bitterness were shown in Table 3. Since the gust scale is based on ratio judgments, the equality of ratio is guaranteed. Therefore, the ratio for the intensity of taste between 2 coffee α and β is given by ψ_α/ψ_β (ψ_α: gust value for coffee α;

ψ_β: gust value for coffee β). For example, the intensity of acidity for the light roasted Tanzania (TA(27.6)) was twice that of the medium roasted Brazil (UA(24.8)). The intensity of bitterness for the light-medium roasted Brazil (UA(26.3)) was two thirds more than that of the medium roasted India parchment (WR(21.0)).

By applying the gust scale to the sensory test, the intensity of taste in coffee could be evaluated as quantitative values with an equi-ratio property.

Relationship between evaluated gust values and physico-chemical properties

Trade variety

The relationship between titratable acidity and the intensity of acidity in various trade varieties was shown in Figure 1. Titratable acidity in various trade varieties was positively related to the logarithm of gust value for perceived acidity (log ψ_α). The correlation coefficient was about 0.89. Since the acidity of India parchment AB and Indonesia AP-1 coffees were perceived as under 1 gust, these data were omitted from Figure 1.

Table 2. Concentrations of standard solutions for gust scale

log ψ^*	ψ	Tartaric acid conc. (w/v %)	log ψ	ψ	Quinine sulfate conc. (w/v %)	Index No.
0.0	1.0	0.00848	0.5	3.2	0.00084	1
0.1	1.3	0.01041	0.6	4.0	0.00110	2
0.2	1.6	0.01277	0.7	5.0	0.00143	3
0.3	2.0	0.01567	0.8	6.3	0.00188	4
0.4	2.5	0.01922	0.9	7.9	0.00245	5
0.5	3.2	0.02358	1.0	10	0.00321	6
0.6	4.0	0.02893	1.1	13	0.00420	7
0.7	5.0	0.03549	1.2	16	0.00549	8
0.8	6.3	0.04354	1.3	20	0.00719	9
0.9	7.9	0.05341	1.4	25	0.00940	10
1.0	10	0.06553	1.5	32	0.01230	11
1.1	13	0.08039	1.6	40	0.01609	12

**) ψ; Gust value (the value in ratio scale of taste)*

Figure 2 shows the relation of luminosity and the intensity of bitterness in various trade varieties. As shown in Figure 2, the intensities of perceived bitterness (log ψ_β) were almost similar in the same roast degree and the same brew concentration, regardless to trade variety. However, coffee that had weak acidity tended to have a strong bitterness value. Consequently, the perceived bitterness of robusta coffee was slightly stronger than that of arabica coffee.

Roast degree

The relationship between titratable acidity and the intensity of acidity in different roast degrees was shown in Figure 3. A linear relation was observed with a correlation coefficient 0.96. Since the acidity of dark roasted Zimbabwe AA+ and Brazil No.2 coffees and the acidity of robusta coffees could not be perceived, these data were not included in Figure 3.

Figigure 4 shows the relation of luminosity and the intensity of bitterness in different roast degrees. As shown in Figure 4, the luminosity of coffees related well to log ψ_β. The correlation coefficient was about 0.98 for arabica, 0.99 for robusta.

Table 3. Gust values for perceived acidity and bitterness

Sample (LV)	ψ_α	ψ_β	Sample (LV)	ψ_α	ψ_β	Sample (LV)	ψ_α	ψ_β	Sample (LV)	ψ_α	ψ_β
Experiment involving trade variety						*Experiment involving brew concentration*					
UA(27.4)	2.2	6.8	WA(27.4)	3.7	5.6	WA(27.4)			WA(16.0)		
CO(27.4)	2.7	6.1	WR(27.4)	<1.0	7.8	Brix=1.0%	2.1	4.6	Brix=1.0%	<1.0	11.4
IT(27.4)	2.7	6.3	UR(27.4)	<1.0	7.6	Brix=1.5%	2.9	5.5	Brix=1.5%	<1.0	15.8
TA(27.6)	2.8	5.7				Brix=2.0%	3.8	6.6	Brix=2.0%	<1.0	21.0
Experiment involving roast degree						Brix=2.5%	4.7	7.8	Brix=2.5%	<1.0	25.9
WA(29.5)	3.3	5.1	UA(29.4)	2.5	5.3	UA(27.3)			UA(16.0)		
WA(27.2)	3.8	5.9	UA(27.8)	2.2	6.8	Brix=1.0%	1.5	4.9	Brix=1.0%	<1.0	11.7
WA(26.2)	3.4	7.1	UA(26.3)	1.8	8.4	Brix=1.5%	2.1	6.2	Brix=1.5%	<1.0	16.7
WA(24.6)	2.8	8.1	UA(24.8)	1.4	8.6	Brix=2.0%	2.6	7.2	Brix=2.0%	<1.0	22.0
WA(22.3)	2.1	9.5	UA(22.3)	1.2	10.1	Brix=2.5%	3.2	8.4	Brix=2.5%	<1.0	25.6
WA(21.5)	1.5	10.7	UA(21.2)	<1.0	11.9	WR(27.3)			WR(15.7)		
WA(18.0)	<1.0	14.1	UA(18.0)	<1.0	15.1	Brix=1.0%	<1.0	6.4	Brix=1.0%	<1.0	12.6
WA(16.0)	<1.0	16.6	UA(16.0)	<1.0	17.8	Brix=1.5%	<1.0	8.9	Brix=1.5%	<1.0	17.4
WR(27.3)	<1.0	8.5	UR(27.4)	<1.0	7.6	Brix=2.0%	<1.0	11.2	Brix=2.0%	<1.0	22.6
WR(25.1)	<1.0	10.4	UR(24.4)	<1.0	9.8	Brix=2.5%	<1.0	14.8	Brix=2.5%	<1.0	28.5
WR(21.0)	<1.0	12.6	UR(21.2)	<1.0	12.3	UR(27.4)			UR(15.8)		
WR(18.0)	<1.0	15.2	UR(18.1)	<1.0	14.9	Brix=1.0%	<1.0	5.8	Brix=1.0%	<1.0	12.1
WR(15.7)	<1.0	18.8	UR(15.8)	<1.0	18.0	Brix=1.5%	<1.0	7.7	Brix=1.5%	<1.0	17.0
						Brix=2.0%	<1.0	9.8	Brix=2.0%	<1.0	22.2
						Brix=2.5%	<1.0	13.0	Brix=2.5%	<1.0	27.0

ψ_α; gust value for acidity, ψ_β; gust value for bitterness, LV; L-value, UA; Brazil No.2, CO; Colombia Supremo, IT; Indonesia Toraja, TA; Tanzania AA, WA; Zimbabwe AA+, WR; India parchment AB, UR; Indonesia AP-1

Trade variety and roast degree

The relationship between titratable acidity and the intensity of acidity when trade variety and roast degree were taken together was shown in Figure 5. Regardless of trade variety and roast degree, titratable acidity was positively and significantly related to log ψ_α with a correlation coefficient close to 0.96.

Figure 6 showed the relationship between luminosity and the intensity of bitterness. As shown in Figure 6, luminosity related well to log ψ_β with a high correlation coefficient over 0.98. Robusta coffee with weak acidity showed a more bitter tendency than arabica, especially in light roast. Therefore, the slope of equation for robusta coffee was less deep compared with arabica.

Figure 7 showed the relationship between coffee brew luminosity and the intensity of bitterness. Without distinguishing between arabica and robusta, a linear relation was observed between coffee brew luminosity and the intensity of bitterness.

According to experimental equations in Figures 5 and 6, or 7, gust values for coffee brews of brix 1.50% are estimated from the titratable acidity and luminosity.

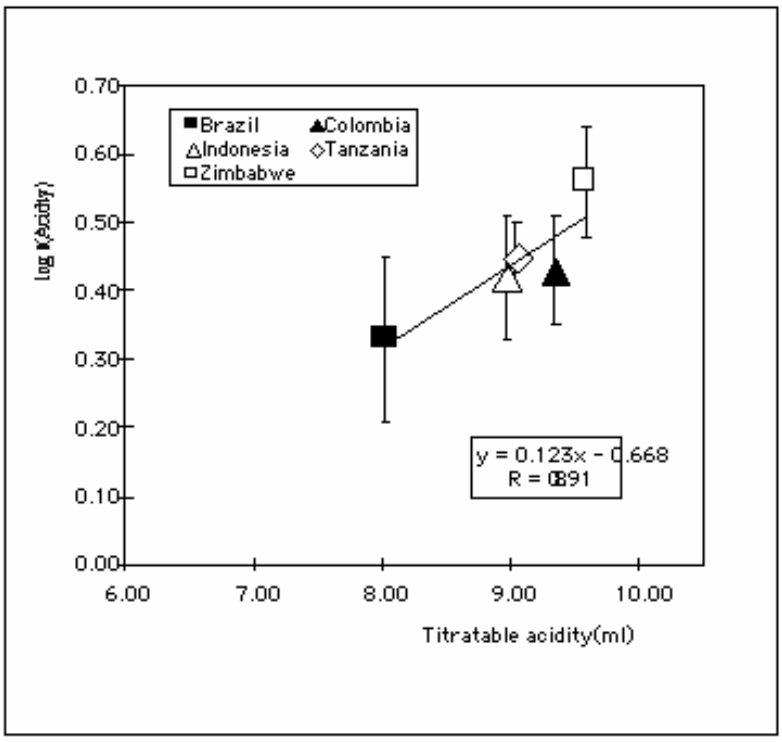

Figure 1. Relationship between titrable acidity and intensity of acidity in various trade varieties

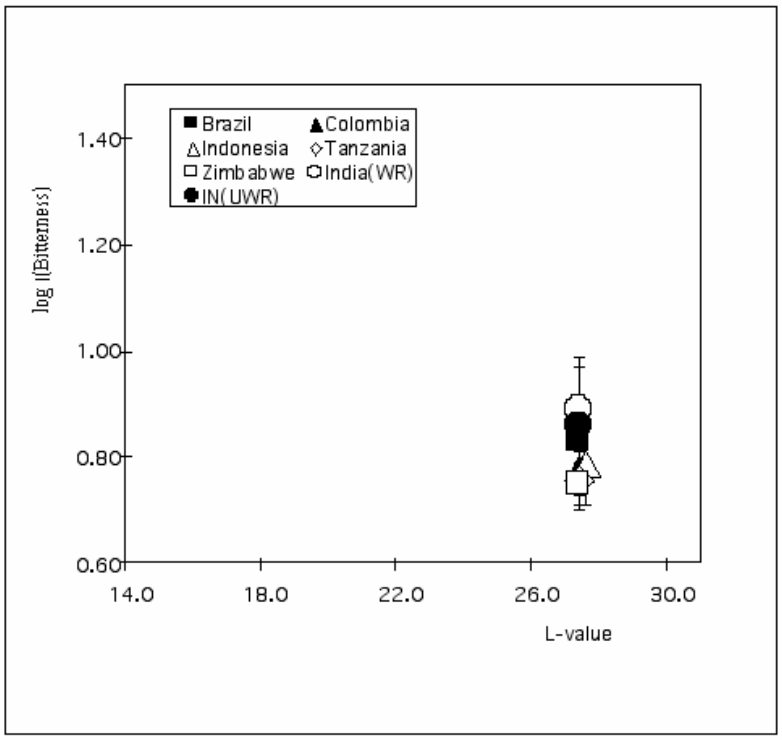

Figure 2. Relationship between luminosity and intensity of bitterness in various trade varieties

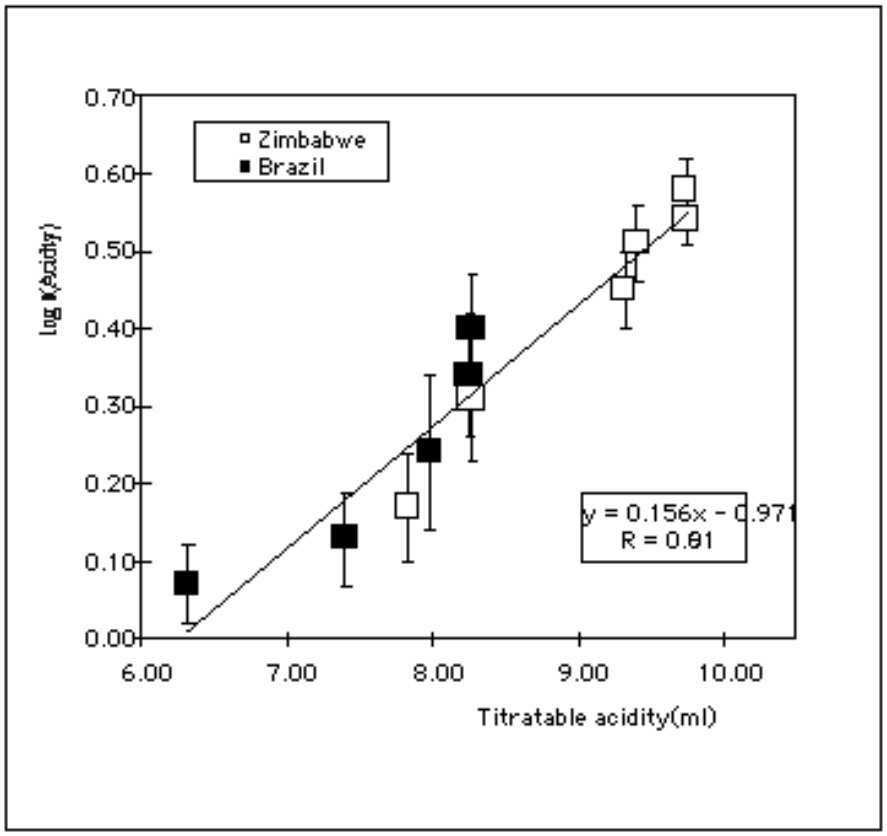

Figure 3. Relationship between titrable acidity and intensity of acidity in different roast degrees

Brew concentration

The relationship between titratable acidity and the intensity of acidity in different brew concentrations was shown in Figure 8. Since the acidity of robusta coffees and dark roasted arabica coffees could be perceived under 1 gust, these data were not plotted in Figure 8. Titratable acidity positively related log ψ_α with a high correlation. However, the equations for Zimbabwe and Brazil were different. It was considered that this might have been due to the perceived acidity being weakened by the bitterness of the coffee.

Figure 9 showed the relationship between concentration and titratable acidity for the light roasted Zimbabwe and Brazil. Titratable acidity was in proportion to concentration, which means that titratable acidity at some concentration was convertible to that in brix 1.50%.

The relationship between coffee brew luminosity and the intensity of bitterness was shown in Figure 10. The slopes of the equations for robusta and dark roasted arabica coffees were similar, while the slope of the equation for light roasted arabica coffee was gentle compared with other slopes. It was considered that this might have been due to the perceived bitterness being weakened moderately by the acidity of the coffee.

Figure 11 showed the relationship between concentration and coffee brew luminosity. Coffee brew luminosity was a linear function of concentration and slopes of 8 coffee samples were similar. Using Figure 11, coffee brew luminosity was convertible to that in brix 1.50%.

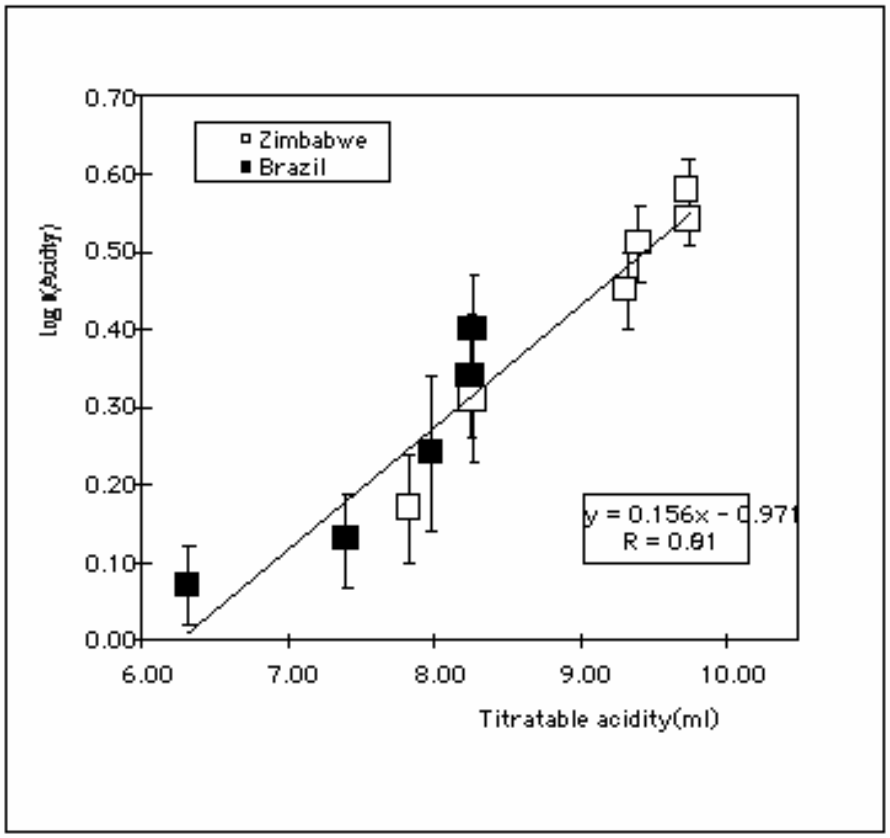

Figure 4. Relationship between luminosity and intensity of bitternes in different roast degrees

Estimation of gust values from physico-chemical properties

For roast and ground coffees, after the extraction and the adjustment of brew concentration to brix 1.50%, the gust values were estimated using these experimental equations.

For acidity: $\log \psi_\alpha = 0.154TA - 0.949$

For bitterness: $\log \psi_\beta = (-0.038LV+1.838)*Pa + (-0.031LV+1.737)*Pr$

or: $\log \psi_\beta = -0.018LV'+1.782$

ψ_α: gust value for acidity, ψ_β; gust value for bitterness,
TA: titratable acidity, LV; L-value of roast and ground coffee, LV'; L-value of coffee brew,
Pa: percentage of arabica, Pr; percentage of robusta

By converting titratable acidity and luminosity to the corresponding values at brix 1.50%, these experimental equations could be applied to coffee brews.

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Brazil Coffee Growing Regions and Quality of Natural, Pulped Natural and Washed Coffees*

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SUMMARY

Brazil is the world's largest coffee grower and second largest coffee consumer (over 25% of world production and 12,5% of world consumption). Its production in 2000 year was 31,1 million bags of 60 kg (80% Arabica and 20% Robusta). Brazil is the unique country in its ability to offer natural, pulped natural or washed coffees.

There are five Arabica coffee producing states, mostly natural processed coffee which is dried with the pulp intact in order to acquire natural sweetness. In addition, the newer natural pulped method allows the sugar of the mucilage to impart flavor and body. Washed coffees are produced in smaller quantities. By growing many varieties at different altitudes and climates and by using three different processing methods Brazil's presents the world with a coffee for every palate.

To study the quality of this diverse coffee the present investigation were conducted to generate coffee flavour descriptions and to notice those attributes that make some coffees extremely distinctive or similar to others resulting in a mapping showing a good separation of the Brazilian regions studied.

Quantitative descriptive flavour profiling of the samples was carried out following the ICO coffee sensory evaluation method. Panel members entered scores directly into a computerized sensory data collector system Compusense with statistical software for immediate analysis. The chemical composition of the green and roasted coffee beans was determined as complementary information. For the sensory evaluation of the 80 coffee samples seven trained panelists profiled in a replicate blind test and showed consistency.

The Natural coffees present strong body and aroma, mildly acidity and sweet flavor. The Natural pulped coffees present good body and aroma, low acidity and some sweetness. The washed coffees present high acidity, weak body and aroma. The mostly of natural coffees from state of São Paulo exhibit strong body and aroma, natural sweetness and lower acidity, from South Minas natural coffees exhibit a characteristic sweet flavor, heavy in body with mild acidity and from Natural Cerrado, coffee have a fine cup, strong body and excellent aroma and sweeteness. Natural coffees from the state of Bahia have strong body and pronounced sweeteness. Paraná quality coffee creates the typical flavor of Paraná natural. The state of Espírito Santo offers a wide variety of tastes: from the finest pulped naturals and naturals, which exhibit a specialty quality soft cup to the Rioy coffees.

It is possible to cluster coffee samples related to chemical constituents and sensory attributes. Vector plots of sensory, chemicals and mineral content variables can explain in a simplified way the differences between all of the samples analysed, and confirmed the unique characteristics of each producing coffee area.

INTRODUCTION

Brazil is the world's major coffee producer and the second largest consumer of this important commodity (25% of the world production and 12,5% of the global consumer market).

Brazil should better exploit both its position as the world's leading coffee producer and its natural resources to harvest a product of outstanding quality that favorably compares to the product grown by other important coffee producing countries. Its vast territory and the large variety of different soils and climatic conditions enable Brazil to produce virtually every known coffee variety and meet whatever consumer needs and preferences. Brazil is unique in its ability to offer nearly any variety and quality level of coffee that consumers from all over the world may require: Arabica or Robusta; naturals, pulped naturals or washed; single origin, varieties or blends made to be order; large volumes or specialty and also custom and niche products (such as organic coffee). There are six main coffee producing states of Brazil grow mostly natural, dry processed coffees which are dried with the pulp intact to impart natural sweetness to the product. In addition to these, pulped natural coffees, which retain most of the sugar in the mucilage and impart flavor and character to the bean, and washed coffees are grown in smaller proportions.

Arabica coffee is primarily produced by the following Brazilian states: Minas Gerais, São Paulo, Paraná, Espírito Santo and Bahia. The largest producers of Robusta coffee are: Espírito Santo, Bahia and Rondônia.

OBJECTIVE

The main objective of the study was to analyze both the sensory and chemical quality of Brazilian coffee and subsequently use these data to develop typical quality profiles for coffees produced in different Brazilian regions.

METHODS

Experiments were carried out on samples of 5 kg green Arabica coffee - either sundry and/or using an appropriate drier - and free of some defects such as black beans, unripe (immature) beans and ardidos beans. The origin of the samples was as follows:

- *samples of dry processed coffee*: 30 from São Paulo State, 19 from Minas Gerais, 2 from Paraná and 1 from Bahia;
- *samples of pulped natural coffee:* 7 from Minas Gerais, 2 from São Paulo, 1 from Espírito Santo and 3 from Paraná;
- *samples of wet processed coffee:* 4 from Bahia, 10 from Minas Gerais, 1 from Paraná and 2 from Espírito Santo.

Chemical Analysis

The physico-chemical constituents analyzed were: moisture (Instituto Adolfo Lutz, 1985), protein (A.A.C.C, 1990), raw fibers (DIEMAIR, 1963), ash, lipids, tannin, total and reducing sugars. Total acidity was determined only in samples of roasted coffee. (A.O. A. C., 1998).

Simultaneous determination of caffeine, trigonelline and chlorogenic acid was performed in coffee samples by high performance gel filtration liquid chromatography (De Maria, Trugo and Moreira, 1995)

The analysis of mineral elements and trace elements in green coffee was determined by using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES).

Roasting and Grinding

Around 5 kg of green beans were roasted until medium roasted in a conventional drum roaster at 185ºC to 210ºC for 13-15 minutes", with a weight loss of 16-17%. Upon roasting, the coffee samples were ground using a LA CIMBALI grinder.

A color measurement of the roasted and ground coffee was recorded to indicate the degree of roast at which the coffees were evaluated.

Brewing

The beverage samples were prepared by weighing and suspending 55 g of roasted and ground coffee into 1 liter of water at 95°C. The resulting beverage was then filtered through standard Melitta coffee filters and held in a vacuum flask for no longer than 30 minutes.

Sensory Evaluation

Quantitative descriptive analysis of the samples were carried out following the International Coffee Organization (ICO) coffee sensory evaluation method. The method uses a coffee vocabulary created by consumers and a trained panel to select all sensory characteristics of each individual coffee. To profile a coffee, the intensity of each aroma, flavor and mouthfeel are attributed to ICO coffee vocabulary in order to perception the intensity of selected sensory terms is assessed on a line scale. The panel members entered scores directly into a computerized sensory data collector system Compusense Five 4.0 with statistical software for immediate analysis.

A complete randomized design was selected for the assessment of the coffee samples.

Panelists received only two samples per session and each was assessed individually. The tasting temperature was between 60-70°C and an structured 10 cm scale was used to rate the intensity of each of the sensory characteristics in a blind test.

Statistical Analysis

The statistical analysis applied to the collected sensory evaluation data and chemical composition were principal component analysis (PCA) and cluster analysis.

RESULTS

Chemical composition of raw and roasted coffees

The results of the physico-chemical analysis carried out to determine the chemical composition of raw and roasted coffee samples are presented in Table 1, which shows the minimum, mean and maximum values relative to the following composition parameters:

moisture, ash, lipids, protein, raw fibers, total and reducing sugars, acidity, tannin, chlorogenic acid, trigonelline and caffeine.

The greatest variations in the chemical composition of the raw coffee samples tested were relative to the levels of lipids and crude fibers.

As to roasted coffee, the data presented in Table 1 show that the most significant degree of variation occurs in terms of lipids and total acidity levels.

Table 1. Chemical Composition of Raw and Roasted Coffees

Determination	Raw Coffee			Roasted Coffee		
	Minimum	**Mean**	**Maximum**	**Minimum**	**Mean**	**Maximum**
Moisture /100g)	8.1	**9,7**	12.7	0.6	**1,5**	3.9
Ash (g/100g)	3.0	**3,4**	3.8	3.6	**4,0**	5.0
Protein /100g)	9.8	**13,2**	15.9	11.0	**15,0**	16.5
Lipids /100g)	6.8	**12,3**	14.8	7.7	**14,0**	17.3
Total sugar (g/100g)	4.5	**8,9**	11.2	0.4	**1,7**	3.6
Reducing sugar (g/100g)	<0.1	**0,4**	1.0	0.1	**0,9**	2.2
Tannin (g/100g)	1.7	**2,9**	4.7	2.0	**2,8**	4.4
Crude Fiber (g/100g)	9.8	**12,1**	15.7	10.7	**12,7**	18.6
Acidity (ml NaOH N/100g)	n.d.	**n.d.**	n.d.	11.8	**20,4**	29.7
Chlorogenic Acid (g/100g)	4,4	**5,3**	7,0	2,0	**3,0**	3,9
Trigonelline(g/100g)	0,7	**0,9**	1,2	0,4	**0,6**	0,9
Caffeine (g/100g)	0,8	**1,0**	1,3	0,7	**1,1**	1,4

n.d.=not determined

The most significant variations were observed in terms of lipids, total sugars, tannin and protein contents.

Also the values relative to acidity varied widely between the samples analyzed.

Only moisture and protein contents were found to be relatively constant among the samples tested, while the values relative to all the other composition parameters investigated varied significantly.

Caffeine was found to be present at concentrations ranging between 0,8 and 1,3 g/100 g, whereas the level of chlorogenic acid varied from 4,3 to 7,0 g/100 g and the values relative to trigonelline from 0,6 to 1,25 g/100 g.

Caffeine was found to be present in roasted coffee at concentrations ranging between 0,7 and 1,4 g/100 g., while the content of chlorogenic acid varied from 2,0 to 3,9 g/100 g and that of trigonelline from 0,4 to 0,9 g/100 g.

According to the literature available in this field, the concentration of chlorogenic acid and trigonelline is expected to be considerably lower in roasted coffee than in raw coffee since these compounds are heat-unstable and decompose at roasting temperatures into a series of other substances that decisively contribute to the formation of the typical coffee flavor and aroma. Caffeine, however, remains stable even at the highest roasting temperatures. For that reason, any changes that occur in the concentration of caffeine are due to the loss of moisture that takes place during the roasting process.

Illy and Viani (1996) reported a mean value of 1,2% caffeine for Arabica coffee and 2,2% for Robusta. The levels of trigonelline determined in Arabica ranged from 0,6 to 1,3%, while in Robusta trigonelline was found to be present at concentrations varying between 0,3 and 0,9%. The level of chlorogenic acid varied from 6 to 7% in Arabica coffee and was found to be constant at 10% in Robusta.

Coffee Mineral Composition sorted per state of Brazil

The mean value, standard deviation and maximum and minimum levels of each mineral element are presented-sorted per state and expressed in mg/kg, in Tables 2 to 7.

The variations observed in the mean values were relative to the mineral elements calcium, magnesium and potassium.

Particularly high levels of Cu, Fe and Ni were detected in samples from 3 municipalities located in the State of São Paulo and 1 from Bahia. The levels of Al, Ba, Co, Fe and Zn varied significantly from state to state. The levels of the elements Ca, P, Mg , Mn and K were found to be similar in the samples from the states SP, MG, ES, BA and PR and these findings show that coffee may be considered to be a good source of these minerals. The variations in the mineral content of coffee samples produced in different states are probably dependent on a number of factors, such as coffee variety, soil type, the time of year the coffee is harvested, the use of specific fungicides and fertilizers.

Quantitative Descriptive Sensory Analysis

Coffee samples can be divided into groups according to their sensory attributes. Classifying the samples based on their geographical origin is not really feasible since different coffees grown in the same state or region often prove to be quite different in terms of overall and sensory quality characteristics. Therefore, it would be much more interesting and technically meaningful to classify the coffee samples according to a set of properties, as shown in Figure 1. Statistical evaluation in which the cluster technique is applied to the mean values of each individual sensory attribute classifies the coffee samples into three clusters, as shown in Figure 1.

Cluster 1 – Samples with cereal aroma : 8, 25, 26, 27, 49, 50, 53, 54, 55, 56, 57, 58, 60, 61, 62, 63, 64, 67, 68, 69, 72, 73, 74, 75, 76, 77, 78, 79, 80.

Cluster 2 **–** Samples with a burnt aroma, bitter taste, more intense body characteristics and harshness (astringency): 1, 4, 10, 12, 28, 39, 40, 41, 42, 43, 46, 59, 65, 66, 70, 71.

Cluster 3 **–** Samples with more intense typical R&G coffee fragrance (odor) and less intense cereal, caramel, chocolate and burnt aromas: 2, 3, 5, 6, 7, 9, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 44, 45, 47, 48, 51, 52.

Figure 2 shows that principal components 1 and 2 account for 44,3% of the differences between the samples of ***dry processed*** coffee. The differences observed are relative to the following attributes: typical R&G coffee fragrance, ashy-like, burnt, cereal, nutty and woody-like flavors, sweet taste, body characteristics (fullness) and astringency. In general, samples 52 and 53 possessed the most pronounced typical R&G coffee fragrance while sample 4 presented the lowest degree of intensity for this attribute. Sample 80 was identified as having the most intense ashy-like aroma, whereas samples 17, 18 and 29 presented the lowest intensity. The burnt aroma of sample 46 was much more intense than that of sample 33 and the range of variation for this attribute was particularly significant. The intensity of the cereal aroma of samples 25, 66 and 80 differed greatly from that of sample 35, presenting mean values that were much higher compared

to the mean values of cereal aroma of all the other coffee samples investigated. The intensity of the nutty aroma of sample 25 differed significantly from that of sample 34, which presented the lowest mean value for this attribute. Sample 10 presented the highest mean value for woody-like aroma and differed significantly from samples 5, 6, 16, 29, 31, 33, 34, 36, 60, 65 and 66. Sample 78 from the state of Paraná presented the highest value for sweet taste and differed significantly from all the other samples evaluated as to the intensity of this sensory attribute. Samples 2, 11, 13, 15 e 31 were identified as those with the less intense sweet taste. Sample 66 was considered to be the sample with the best body characteristics (fullness) and differed significantly from sample 36, which was the sample that presented the poorest body characteristics of all the samples analyzed. As to astringency samples 40 and 60 were found to present the highest values for astringency and differed considerably from samples, 68, 73, 74 and 77, which received the lowest ratings for this attribute. Furthermore, Figure 2 also clearly shows that the larger part of the coffees grown in the state of São Paulo - particularly those from the Pinhal region – present a markedly more intense typical R&G coffee fragrance, while sample 79 (Campinas) presented an intense nutty and sweet taste.

The same comment applies to certain samples of ground coffee from the State of Minas Gerais (samples 48 and 68). The coffee samples from the Poços de Caldas region (samples 73 and 74) and those produced in the state of Paraná (samples 77 and 78) stand out from all the other samples in terms of sweet taste, while the overall aroma of samples 10 (Espírito Santo de Pinhal-SP) and 59 (Varginha-MG) carries pronounced caramel-like notes. The following samples presented high values for bitterness, astringency and body: samples 46 (Rio Paranáíba-MG), 66 (Ubaporanga-MG), 70 (Campinas-SP), 65 (Santa Bárbara do Leste-MG), 40 (Arceburgo-MG) and 39 (Varginha-MG).

Figure 3 shows that principal components 1 and 2 account for 48,3% of the differences between the samples of ***pulped natural*** coffee. The most significant differences observed are relative to the sensory attributes sweet taste and harshness (astringency). Sample 76 (Paraná) was identified as having the most pronounced sweet taste, while samples 21 and 22 (São Paulo) received the lowest rating for this attribute. As for harshness (astringency), samples 38, 43, 47 e 61 (all from the state of Minas Gerais) were considered to be more astringent than sample 76 (Paraná). Some coffees present a markedly high degree of intensity for certain sensory attributes. This is especially the case of the following samples: sample 42, which carries a strong woody-like aroma; sample 43, with intense burnt/smoked aroma type; the coffee processed with mucilage produced in the highlands of the State of Espírito Santo presented enhanced body characteristics (fullness); sample 63 (natural pulped) presented an accentuated caramel flavour; and sample 75, which presented a pronounced cereal aroma. The main characteristic of samples 58, 61, 69 and 75 is their highly intense and typical R&G coffee fragrance (odour). As to all the other samples, none of them received any outstanding score for any of the sensory attributes investigated.

Figure 4 shows that principal components 1 and 2 account for 43,6% of the differences between the samples of ***wet processed*** coffee.

Samples 49, 50, 51, 56 and 57 presented the most pronounced typical R&G coffee fragrance. On the other hand, the main characteristics of sample 26 was its strong caramel-like aroma. Wet processed coffees tend to present a significant range of variation for sensory quality characteristics such as acidity, sweet taste and harshness (astringency). Samples 62 and 72 were found to be very similar in terms of acidity and significantly different from sample 67, which received a much lower rating for acidity. Samples 14, 49, 50, 51, 56 and 57 were found to be very similar as to the intensity of sweet taste. As for astringency, sample 56 was found to be significantly different from sample 67, which was the sample that presented the lowest rating for this attribute.

TABLE 2 - Coffee Mineral Composition of São Paulo State

Element	Mean		Standard Deviation	Min. Value	Max. Value
Aluminium	20,0	±	29,6	1,02	137,0
Barium	7,12	±	3,89	1,74	17,6
Calcium	1253	±	167	961	1586
Cobalt	0,390	±	0,330	0,000	1,61
Copper	31,3	±	71,2	8,36	369
Iron	152,0	±	328,0	23,0	1842
Phosphoru	1463	±	103	1290	1688
Magnesium	1835	±	140	1526	2084
Manganes	35,2	±	11,9	21,4	60,5
Nickel	11,53	±	26,10	0,130	141,5
Potassium	15147	±	957	12906	17003
Zinc	8,50	±	10,20	3,71	57,0

TABLE 3 - Coffee Mineral Composition of Bahia State

Element	Mean		Standard Deviation	Min. Value	Max. Value
Aluminium	17,8	±	8,4	12,4	32,2
Barium	1,64	±	0,84	0,427	2,41
Calcium	1119	±	287	795	1503
Cobalt	0,069	±	0,095	0,000	0,183
Copper	57,0	±	90,9	12,0	219
Iron	60,1	±	26,3	34,7	93,0
Phosphoru	1405	±	127	1230	1537
Magnesium	1777	±	165	1639	2050
Manganes	20,3	±	3,8	15,0	24,6
Nickel	2,08	±	2,21	0,468	5,26
Potassium	14509	±	1674	12252	16262
Zinc	11,6	±	13,0	4,77	34,8

TABLE 4 - Coffee Mineral composition of Minas Gerais State

Element	Mean		Standard Deviation	Min. Value	Max. Value
Aluminium	14,7	±	18,2	0,610	106,2
Barium	4,42	±	3,12	0,88	13,6
Calcium	1487	±	721	919	5014
Cobalt	0,150	±	0,160	0,000	0,550
Copper	16,1	±	4,6	6,21	30,5
Iron	40,3	±	17,2	23,3	99,2
Phosphoru	1658	±	457	12	2773
Magnesium	2046	±	624	1303	3813
Manganes	34,3	±	1,2	14,2	64,6
Nickel	1,01	±	1,19	0,000	6,61
Potassium	16084	±	3383	11315	25806
Zinc	6,41	±	1,96	3,46	12,4

TABLE 5 - Coffee Mineral Composition of Parana State

Element	Mean		Standard Deviation	Min. Value	Max. Value
Aluminium	6,48	±	2,9	3,57	10,82
Barium	3,52	±	1,59	1,07	5,40
Calcium	1223	±	130	1068	1428
Cobalt	0,020	±	0,030	0,000	0,070
Copper	12,3	±	0,8	11,1	13,3
Iron	29,1	±	5,8	23,2	39,7
Phoshorus	1242	±	105	1145	1374
Magnesium	1431	±	267	1136	1868
Manganes	31,1	±	7,6	17,9	38,4
Nickel	0,610	±	0,10	0,520	0,790
Potassium	12244	±	2602	9656	15365
Zinc	4,34	±	1,1	3,58	6,35

TABLE 6 - Coffee Mineral Composition of Espirito Santo State

Element	Sample 50	Standard Deviation	Min. Value	Max. Value
Aluminium	11,1	11,0	4,3	23,8
Barium	3,63	2,28	1,02	5,21
Calcium	1279	205	1091	1497
Cobalt	0,180	0,160	0,000	0,330
Copper	12,0	1,8	10,4	14,0
Iron	37,1	7,6	29,5	44,8
Phosphoru	1289	61	1220	1337
Magnesium	1581	121	1441	1651
Manganes	36,2	10,3	24,7	44,5
Nickel	1,64	0,57	1,06	2,21
Potassium	12761	952	11773	13673
Zinc	5,05	0,7	4,35	5,72

TABLE 7 - Coffee Mineral Composition of Brasil

Element	Mean		Standard Deviation	Min. Value	Max. Value
Aluminium	16,3	±	22,5	0,610	137,0
Barium	5,23	±	3,64	0,430	17,6
Calcium	1347	±	508	795	5014
Cobalt	0,230	±	0,270	0,000	1,61
Copper	24,3	±	50,5	6,21	369
Iron	85,3	±	213,0	23,2	1842
Phosphoru	1523	±	338	1145	2773
Magnesium	1885	±	462	1136	3813
Manganes	33,7	±	11,8	14,2	64,6
Nickel	5,27	±	17,17	0,000	141,5
Potassium	15219	±	2668	9656	25806
Zinc	7,36	±	7,35	3,46	57,0

CONCLUSIONS

Coffee samples can be divided into groups according to their chemical composition and sensory attributes.

The vectors that correspond to the chemical and sensory attributes explain in a simplified way the differences between the samples analyzed after dividing the coffees into 3 categories according to the method they were prepared (dry processed, pulped natural, wet processed).
In general, dry processed coffee produces a beverage with a strong aroma, moderate acidity, strong body and natural sweetness.

Cup coffee brewed from natural pulped coffee gives a beverage with good aroma and body, low acidity and reduced sweetness.

Wet processed (washed) coffee produces a markedly acid tasting beverage with less intense body characteristics and less pronounced typical coffee aroma.

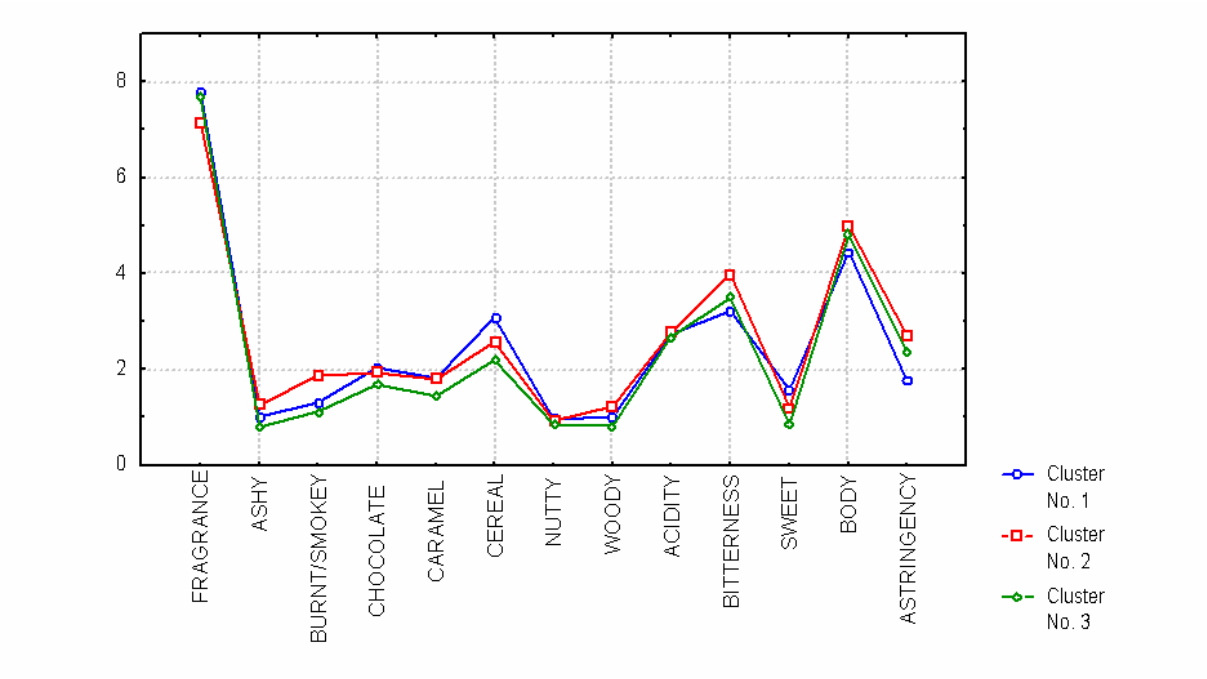

Figure 1. C*luster* analysis of sensory attribute means

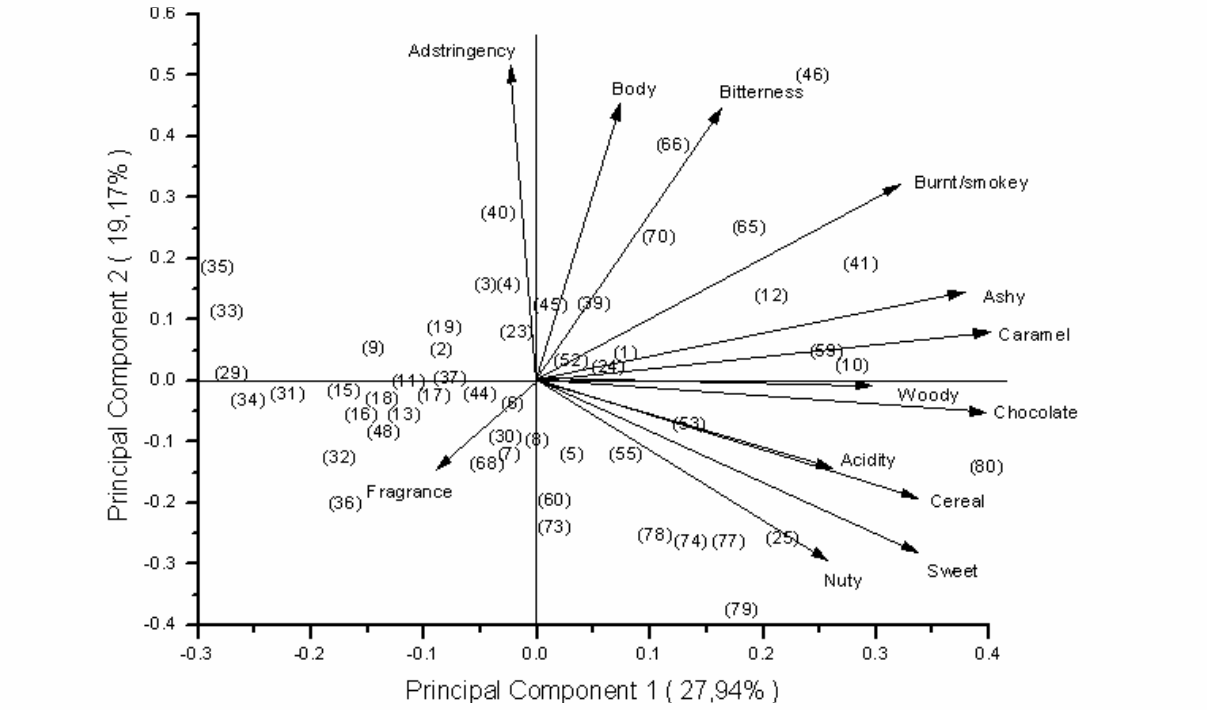

Figure 2. Principal component analysis of Natural coffees

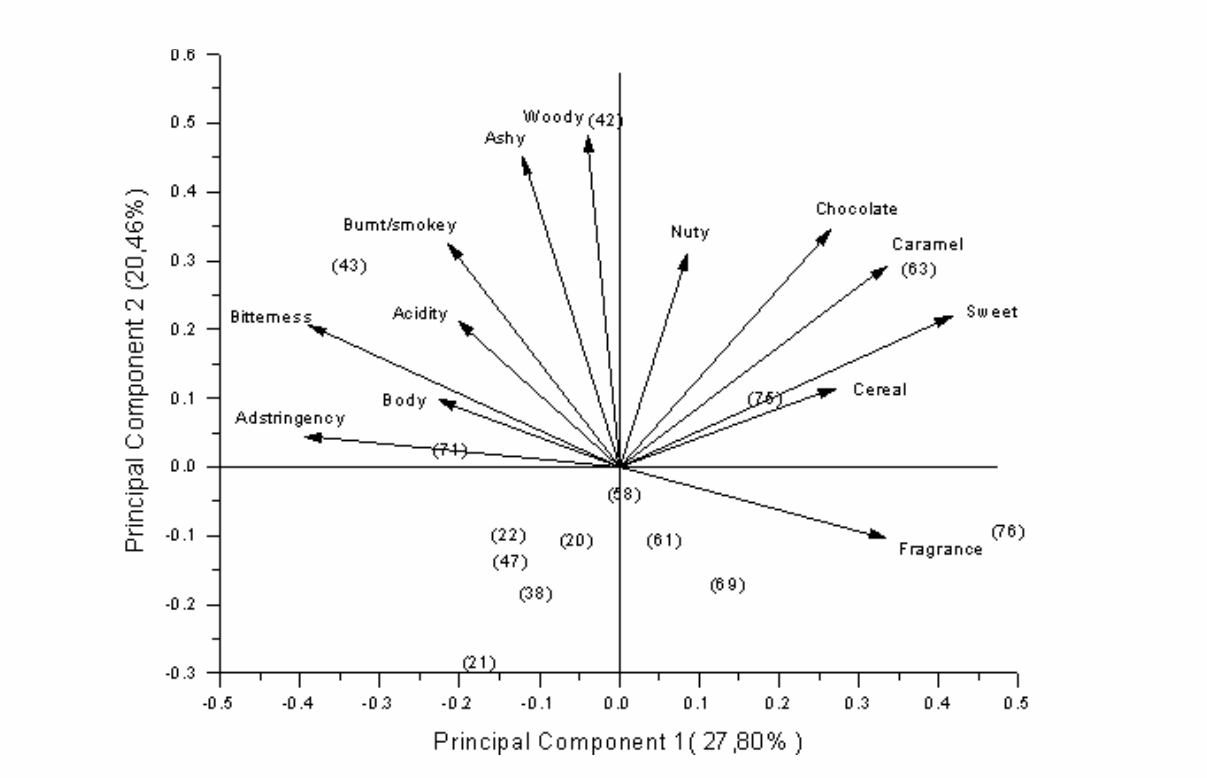

Figure 3. Principal component analysis of Natural Pulped coffees

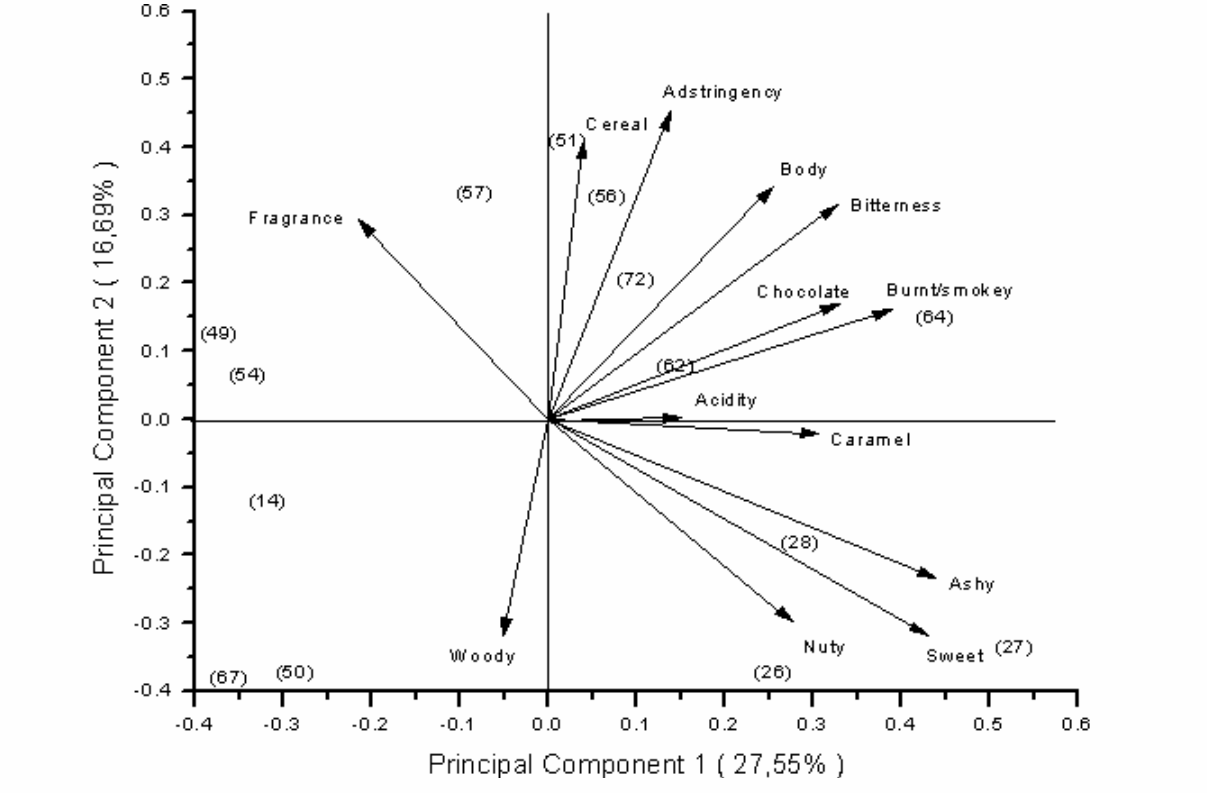

Figure 4. Principal component analysis of Washed coffees

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NMR Microscopy as a Non-destructive Tool to Probe Water and Oil in Green Coffee

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SUMMARY

Drying and rehydration of plant material such as seed is an important industrial operation and requires knowledge of the moisture diffusion throughout the material. This is especially true for wheat grains that are usually partially rehydrated by soaking in water, a process known as tempering, prior to milling. A similar process is being applied to green coffee beans especially of the Robusta type to improve the organoleptic properties of the coffee beverage.

The principal aim of this study was to evaluate changes in the physico-chemical properties of water molecules in green coffee beans after washing or steaming performed during the industrial processing at Pacorini Silocaf (Trieste). In particular, nuclear magnetic resonance (NMR) microscopy was applied to probe water distribution and mobility in Vietnam Robusta green coffee beans (*Coffea canephora*). High resolution ^{1}H spin-echo images revealed non-destructively details of the bean anatomical structure and provided maps of some of the most relevant water magnetic parameters such as longitudinal (T_1) and transverse (T_2) relaxation times and diffusion coefficients (D). Moreover, by means of chemical shift imaging methods it was possible to determine the distribution of water and lipids in dry and wet processed beans. This pioneering study demonstrates that NMR microscopy can be used to probe non-destructively water distribution and mobility in green coffee subjected to different industrial processes of washing and steaming and to reveal microstructural details that could not be obtained by using other techniques.

RÉSUMÉ

La séchage et la réhydratation des échantillons végétaux comme les semences est une opération industrielle importante et requiert la connaissance de la diffusion de l'eau à travers les tissus. Cela est vrai surtout pour les grains de blé qui sont communément partiellement réhydratés par imbibition, un procédé connu comme le *tempérage*, avant la mouture. Un procédé similaire est appliqué aux grains de café vert surtout de l'espèce Robusta pour améliorer les propriétés organoleptiques du produit final. Le but principal de cette étude était l'évaluation des changements physico-chimiques des molécules d'eau dans les grains de café vert après le lavage et la vaporisation effectués pendant le traitement industriel chez Pacorini Silocaf (Trieste). En particulier, la microscopie par résonance magnétique nucléaire (RMN) du proton a été utilisée pour évaluer la distribution spatiale et la mobilité de l'eau dans des

grains de café vert Robusta (*Coffea canephora*) cultivé au Vietnam. Des images spin-écho à haute résolution ont révélé d'une manière non destructrice les détails de la structure anatomique des grains et fourni une cartographie des principaux paramètres magnétiques comme les temps de relaxation longitudinale (T_1) et transversale (T_2) et le coefficient de diffusion (D) de l'eau. De plus, en utilisant une séquence de *déplacements chimiques* on a pu évaluer la distribution de l'eau et des lipides dans les grains avant et après le traitement industriel. Cette étude démontre que la microscopie RMN peut être utilisée pour la détermination non destructrice de la distribution et de la mobilité des molécules d'eau dans le café vert soumis à des traitements industriels de lavage et vaporisation et pour l'analyse de la microstructure que on ne pouvait pas effectuer par d'autres techniques.

INTRODUCTION

Water content is a certainly important ingredient of coffee beans and crucially affects the quality of the final product. A knowledge of the water content of green and roasted coffee products is required because it influences the water activity and stability during storage; moreover, there are often limits set by national and international legislation. However, the determination of water distribution is not simple. The difficulties, which are not peculiar to coffee, arise because different methods of water content determination give different emphasis to free water, bound water, other volatile substances, dry matter lost by pyrolysis and other components that interfere in chemical determinations (Clarke, 1985).

In recent years there has been an increasing interest in applying nuclear magnetic resonance (NMR) methods for non-invasive and non-destructive measurement of water and lipids in agricultural and food products (McCarthy, 1994; Schmidt et al., 1996). In particular, magnetic resonance imaging (MRI) has been applied for the determination of water and oil distribution and mobility and the investigation of dynamic phenomena during food processing (Ruan and Chen, 1998; Hills, 1998). The recent developments of MRI technology, such as NMR microscopy (Callaghan, 1991) which provides images with resolutions of 10-100 μm, have greatly expanded the capability of this powerful and flexible research tool.

The principal aim of this study was to evaluate changes in the physico-chemical properties of water molecules in green coffee beans after washing or steaming performed during the industrial processing at Pacorini Silocaf (Trieste). In particular, nuclear magnetic resonance (NMR) microscopy was applied to probe water distribution and mobility in Vietnam Robusta green coffee.

MATERIALS AND METHODS

Coffee samples

Ripe Robusta green coffee beans (*Coffea canephora*) collected in Vietnam during different periods of the year were supplied by B. Pacorini S.p.A. Trieste. Due to the high heterogeneity, only the most represented coffee beans were selected for NMR microscopy. Typically, the NMR measurements were performed on the following coffee samples: native coffee, coffee subjected to a standard rehydration process (washing) and coffee rehydrated by a steaming procedure.

NMR microscopy

NMR microscopy experiments were performed at 24°C on a Bruker AM300 console equipped with a standard microimaging unit and coupled to a Spectrospin vertical wide bore (WB)

superconducting magnet operating a 7.05 T (300 MHz for ^{1}H). A standard microimaging probe was used for all measurements, with radio-frequency (RF) coil diameters of 20 or 10 mm. In the case of the 20-mm RF insert, up to a maximum of six coffee beans were studied simultaneously. Typically, images were acquired with a 256×256 pixel matrix, 20 mm field of view (FOV), 600 μm slice thickness, 78 μm/pixel in plane resolution and 8 averages. Relaxation times were obtained using a modified spin echo sequence to reduce the effect of molecular diffusion (Haase et al., 1993). T_2 values were calculated from five images acquired with an echo time (TE) ranging between 6.8 and 25.4 ms. Data from the same pixel in each of these images were fitted to a single exponential decay, according to the equation:

$$M = M_0 \exp(-TE/T_2) \quad (1)$$

where M_0 represents the proton density and hence the relative distribution of water protons within the sample. T_1 values were calculated from a series of seven images with a repetition time (TR) ranging between 0·1 and 6 s. T_1 maps were obtained by fitting the data to equation:

$$M = M_0 [1- \exp(-TR/T_1)] \quad (2)$$

where M_0 is the equilibrium longitudinal magnetisation in the absence of saturation transfer.

Water diffusion experiments were performed using the 10-mm RF insert and applying the pulsed gradient spin echo (PGSE) sequence (Stejskal and Tanner, 1965). Images were acquired with a 128×128 pixel matrix, 15 mm field of view, 860 μm slice thickness, 117 μm/pixel in plane resolution and 16 averages. Diffusion maps were obtained from eight images with the diffusion sensitising gradient g up to 0.45 T/m applied along the z axis. The duration of the PGSE pulses δ was 5 ms and their separation Δ = 10 ms, thus giving the b values up to $2{\cdot}8\times10^9$ s/m^2. The diffusion coefficient D was calculated from a linear fit of lnM against the b value according to the equation:

$$\ln M = \ln M_0 - \gamma^2 g^2 \delta^2 (\Delta - \delta/3) D = \ln M_0 - bD \quad (3)$$

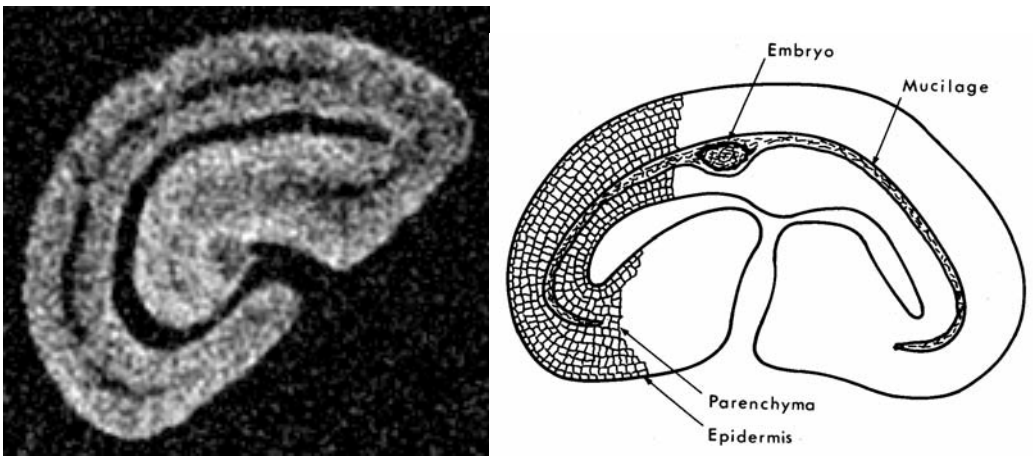
where M and M_0 represent the longitudinal magnetisation in the presence and absence of the diffusion sensitising gradient, respectively.

The saturation transfer experiment was used to measure magnetisation transfer between the restricted macromolecular protons and the free water protons (Wolff and Balaban, 1989). For obtaining the M_S values, a 1×10^{-5} T saturation pulse of 10 kHz or 3 kHz offset was applied for 2 seconds before each scan. The M_0 values were measured without irradiation, using the same pulse sequence and the same experimental parameters.

Chemical shift imaging (CSI) was performed using a standard pulse sequence. A Gaussian selective pulse of 500 Hz bandwith was employed for selective excitation of the water and oil protons. The T_1 relaxation time of water protons was also measured spectroscopically using the inversion recovery technique. T_1 values were calculated from a series of 12 experiments with recovery delays ranging from 1 ms to 15 s.

RESULTS AND DISCUSSION

The NMR microscopic images acquired in this study show different patterns of contrast depending on the sequence used to generate the images. The image contrast may depend partly on water distribution and partly on oil distribution.



a) **b)**

Figure 1. (a) High resolution ^{1}H NMR image from a transverse section of a green coffee bean (*C. canephora*). The contrast derives largely from proton density variations (TE = 6.8 ms, TR = 1.3 s). The slice thickness was 300 μm with an in-plane pixel resolution of 39 μm × 39 μm. The total acquisition time was 2 h 15 min. (b) Diagrammatic transverse section of coffee bean. The major part of the bean is formed of parenchymatous storage cells located in the outer region whereas in the middle part of the section there is a layer of mucilaginous material

Figure 1 (a) shows a high resolution transverse ^{1}H NMR spin-echo image of a ripe coffee bean in which the contrast derives largely from proton density variations. The high contrast across the image slice plane demonstrates that there is big difference in proton density between two distinct regions of the coffee bean. The outer region of the bean presents in fact a relatively strong signal whereas a much weaker signal is observed in the central region. Also shown in Figure 1 (b) is a diagrammatic transverse section of coffee bean showing its microscopic structure. It is evident that there is a close correspondence between the bright region of the image shown in Figure 1 (a) and the parenchymatous storage cells present in the outer part of the coffee bean (Figure 1 (b)). On the other hand, the dark region in the middle of the bean (Figure 1 (a)) corresponds well to the layer of mucilaginous material (Figure 1(b)).

In Table 1 are reported the average values of proton density (ρ) and relaxation times (T_1 and T_2) measured for a sample of Vietnam green coffee.

Table 1. Average magnetic parameters for different samples of Vietnam Robusta green coffee

coffee	proton density (ρ)	longitudinal relaxation time T_1 (ms)	transverse relaxation time T_2 (ms)
native	11.5	395	16.5
after washing	12.6	430	15.5
after steaming	12.8	460	14.2

From Table 1 it is clear that the two procedures of washing and steaming cause an increase of proton density in the coffee beans. The value of proton density measured for the native green coffee is certainly due to the simultaneous contribution of water and lipid hydrogen atoms whereas the increase in proton density detected for the “washed” and “steamed” coffee beans

can be ascribed to a larger quantity of water. The two procedures also cause an increase in T_1 values and a decrease in T_2 values.

Since the MRI signal intensity is not always proportional to the proton density, the total water content in coffee beans was also determined by a conventional method based on dielectric current. This rapid and non-destructive method of determination commonly used for routine quality control relies on the change of dielectric constant of coffee with its moisture content. Both methods of analysis gave similar results. Concerning the water distribution within the coffee beans, four regions which represented both the parenchymatous and the mucilaginous structures were chosen as shown in Figure 2.

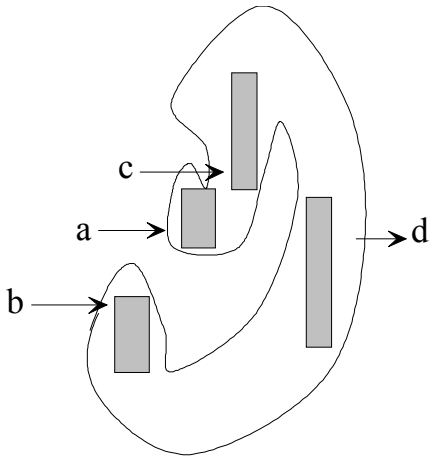


Figure 2. A schematic view of a coffee bean image with the four regions of interest wherein the average magnetic parameters were calculated

The average proton density values calculated for one coffee bean (Table 2) clearly indicated that the water content of the cellular structure is approximately 2 fold that of the mucilaginous layer. These results confirm the possibility to use quantitative NMR for the accurate determination of water content in different tissues of the bean. The great advantage of the NMR microscopic method lies in the ability to study the same coffee specimen repeatedly and non-destructively. Although the image resolution is not comparable to that of light microscopy, NMR microscopy can represent an interesting complement to the histology of coffee beans that poses a number of technical problems (Dentan, 1985). T_1 values of coffee beans also showed a longer longitudinal relaxation time in the outer region formed of parenchymatous storage cells (Table 2).

T_2 values of coffee beans were obtained from the same sets of data acquired for constructing the proton density maps. In all cases T_2 values ranged between 13.0 and 16.2 ms, and resulted to be longer in the middle part of the coffee bean that corresponds to the mucilaginous layer (Table 2). The slightly lower transverse relaxation time (T_2) observed in the cellular part (Table 2) is probably due to local magnetic field inhomogeneities caused by the cellular structure.

For a better understanding of the variations in the magnetic parameters so far studied, the diffusion coefficient (D) of water molecules was also measured. This parameter indicates the amount of spatial spreading of molecules undergoing random translational motion per unit time. In this study, diffusion maps showed a different water diffusion in the two anatomical regions of the coffee beans. In the parenchymatous region the diffusion coefficient was fairly low (Table 2) resulting an order of magnitude smaller than that measured in pure water. On the other hand, the diffusion coefficient measured in the mucilaginous region was almost by a

factor of two larger than that determined in the cellular part (Table 2). The fairly low diffusion of water molecules observed in the outer region is presumably a result of restrictions to molecular motion posed by the cellular membranes. In the central part no such obstacles are present, therefore, the water diffusion coefficient is larger although still much lower than that of water in water. An explanation to this finding could be the high viscosity of the mucilaginous material. Due to the high heterogeneity within the coffee beans, the values reported in Table 2 differed from those determined for other coffee samples. The trend of these values, however, remained unchanged.

Table 2. Average values of proton density (ρ), transverse relaxation time (T_2), longitudinal relaxation time (T_1) and diffusion coefficient (D) determined for four different regions of a green coffee bean as indicated in Figure 2

parameter	a (cellular)	b (cellular)	c (mucilaginous)	d (mucilaginous)
ρ	19.7	20.4	12.6	10.1
T_1 (ms)	540	630	460	420
T_2 (ms)	14.5	13.0	16.2	16.2
D (cm^2/s)	4.5×10^{-7}	5.8×10^{-7}	1.1×10^{-6}	1.1×10^{-6}

Finally, the absence of magnetisation transfer effect indicated that all water protons in the coffee bean are in a close contact and relax at the same rate.

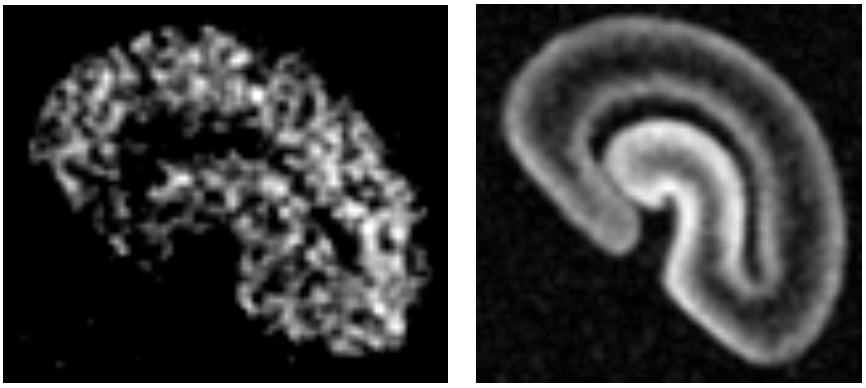
Table 3. Average values of the longitudinal relaxation time T_1 of water (w) and oil (o) protons measured for native and treated green coffee by using an inversion recovery sequence at two different echo times

	native	after washing and steaming	after steaming (3000Kg/h)[1]	after steaming (4000 Kg/h)[1]
T_{1w} (ms), TE=0 ms	334	201	218	234
T_{1o} (ms), TE=0 ms	378	288	310	316
T_{1w} (ms), TE=8 ms	450	454	465	450
T_{1o} (ms), TE=8 ms	389	406	402	408

[1] *Steaming conditions used during the industrial process at Pacorini Silocaf.*

In order to discriminate on the NMR images the water signal from that deriving from the lipids (oil) chemical shift MR imaging was used. In Figure 3 are reported the chemical shift images of a native green coffee bean. It is interesting to note that the distribution of water within the bean can not be correlated to any defined anatomical structure whereas the lipids appear to be mainly present in the parenchyma.

This pioneering study demonstrates that NMR microscopy can be used to probe non-destructively changes in the physico-chemical properties of water molecules in Robusta green coffee after industrial processes of washing and steaming. Moreover, by applying chemical shift imaging methods it is possible to determine the distribution of water and lipids in dry and wet processed coffee beans.



a) **b)**

Figure 3. Chemical shift images of a native green coffee bean: a) water distribution, b) oil distribution

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We are grateful to O. Jarh and V. Mlynárik for helpful discussions and advice. Two fellowships (to A.P. and P.S.) from B. Pacorini S.p.A.(Trieste) and from the Consortium for the International Development of the University of Trieste are gratefully acknowledged.

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Effects of Steam Treatment on Diterpenes

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SUMMARY

Steaming of green coffee beans affected the diterpene fraction of green coffees and of roasted coffees. The concentrations of total kahweol and total cafestol were only slightly influenced by steaming, whereas the concentrations of free cafestol and free kahweol decreased significantly when the degree of treatment increased. This phenomenon could be observed in green coffees as well as in roasted coffees. In addition to the diterpenes kahweol and cafestol the dehydration products dehydrokahweol and dehydrocafestol were analysed in roasted coffees. These degradation products, however, could not be detected even in the very intensively steamed green coffees (120 minutes at 2 bars). In roasted coffees the concentrations of dehydrokahweol and dehydrocafestol decreased with the time of treatment, but grew with increasing degree of roasting. In the coffees, which were steamed for 120 minutes at 2 bars the concentration of free kahweol, was below the detection limit (< 0.01 mg/g lipid).

INTRODUCTION

Coffee is the most favourite beverage of the Germans. In order to enable as many people as possible to drink coffee, the industry offers processed coffees, besides the conventional coffee (Figure 1). These include decaffeinated coffees as well as caffeine-containing but steam-treated coffees, which are supposed to be particularly stomach-friendly.

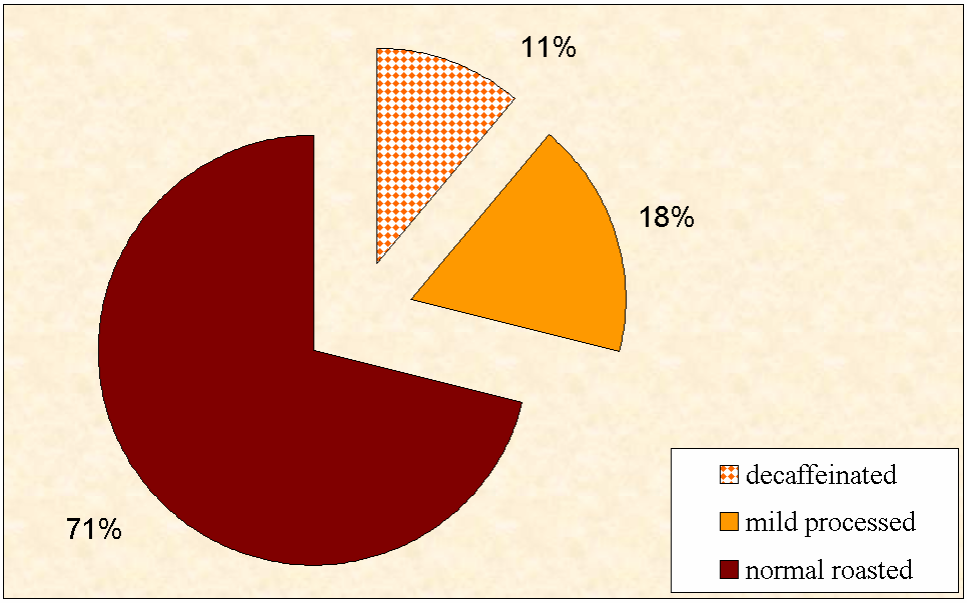

Figure 1. German Coffee Market in 2000 (data from Deutscher Kaffee-Verband e. V.)

By steaming coffee beans prior to the roasting process not only are the cellular structures changed, but also the concentration of individual substances is influenced. During the

steaming process new compounds are formed. Pronounced changes were detected in various substances, such as chlorogenic acids, melanoidins and carbohydrates, amino acids and organic acids (Sturm, 1983; Held, 1985; van der Stegen and van Duijn, 1980; Cliffort, 1997; Lücker, 1999; Kletschkus, 1990; Packert, 1993; Steinhart et al., 1990; Luger, 1996; Nehring and Maier, 1992; Steinhart and Luger, 1995; Steinhart and Luger, 1997; Schröder et al., 1997).

So far, there has been little investigation in this respect on lipids, with the exception of carbon acid-5-hydroxytryptamides (Maier et al., 1999; Wurziger and Harms, 1969). It was, therefore, the aim of our research to investigate more closely the effects of industrial steaming on the diterpenes, which occur in coffee oil in a quantitative proportion of about 18%.

RESULTS AND DISCUSSION

In our experiments we analysed the behaviour of Arabica coffees because these coffees are more important for the German coffee market than Robusta coffees. To investigate the influence of steaming a green Colombian Arabica coffee was steam-treated, at first, according to the procedure described by Lendrich et al. (1933).

The total diterpenes were analysed using the DIN-method No. 10779 (1999). The free diterpenes were determined by the procedure presented by Kölling-Speer et al. (1999).

The concentrations of total kahweol and total cafestol analysed in the unsaponifiable matter were only slightly influenced by steaming, whereas, the concentrations of free cafestol and free kahweol decreased. This applied to green coffees as well as to roasted coffees. Mainly the content of free kahweol was significantly reduced. The focus was, therefore placed on the contents of free diterpenes.

In roasted coffees, the diterpenes kahweol and cafestol were analysed and in addition their dehydration products dehydrokahweol and dehydrocafestol. These degradation products, however, could not be detected in steamed green coffees. Dehydrokahweol and dehydrocafestol were formed in higher rates when the coffee was steamed before roasting (Figure 2).

INFLUENCE OF VARIOUS STEAMING PARAMETERS (TIME AND PRESSURE)

For further investigations a green coffee from Brazil was steam-treated under various conditions to study the influence of variable parameters like time and pressure in detail. The green coffee was steamed at 1 bar for 30 and 120 minutes and at 2 bars for 30, 90 and 120 minutes. These experiments were done by CR3-Kaffeeveredelung M. Hermsen GmbH (Bremen, Germany). Lots of 100 grams-500 grams from these coffees were taken up and were roasted using a hot-air fluidised-bed roaster. The roasting was done by Tchibo Frisch-Röst-Kaffee GmbH (Hamburg, Germany). Furthermore, the colour value of the individual coffees was determined by Color Tester LFM 1 (Dr. Lange, Berlin, Germany).

As an example, the results for the medium roasted coffee steamed at 2 bars are presented in Figure 3. Dehydrokahweol and dehydrocafestol could not be detected even in the very intensively steamed green coffees (120 minutes at 2 bars). Therefore, the thermal load by steaming is not sufficient for separating a water molecule from kahweol or cafestol.

In roasted steamed coffees the concentrations of dehydrokahweol and dehydrocafestol decreased with the time of treatment, but grew with increasing degree of roasting. A similar

behaviour could be observed in the total dehydro compounds and the free dehydro compounds. Coffees, which were, steamed for 120 minutes at 2 bars (132°C), showed contents of free kahweol with concentrations lower than 0.01 mg/g lipid, which was below the limit of detection. That is, the free kahweol was completely degraded by intensive steaming.

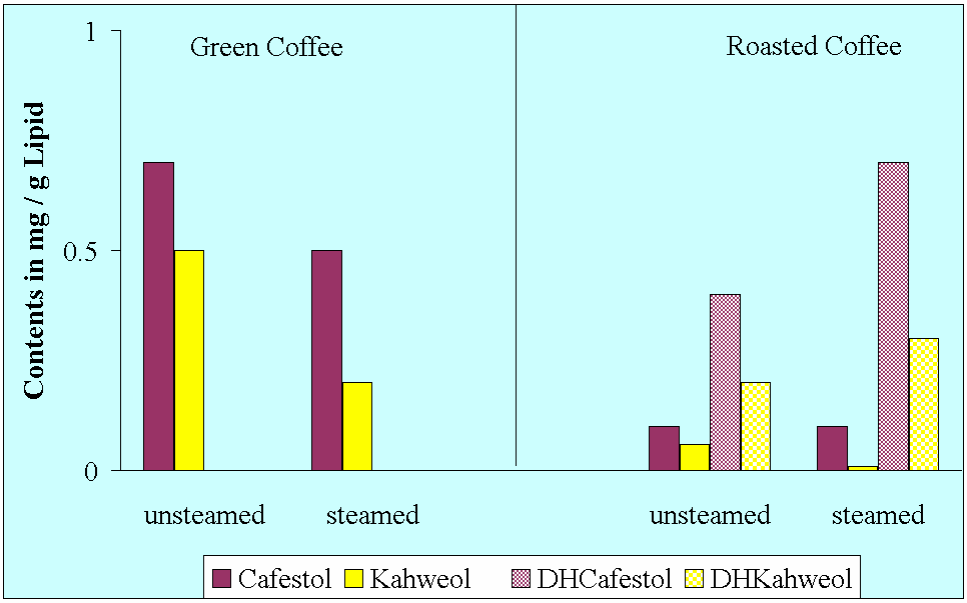

Figure 2. Contents of free diterpenes in coffee

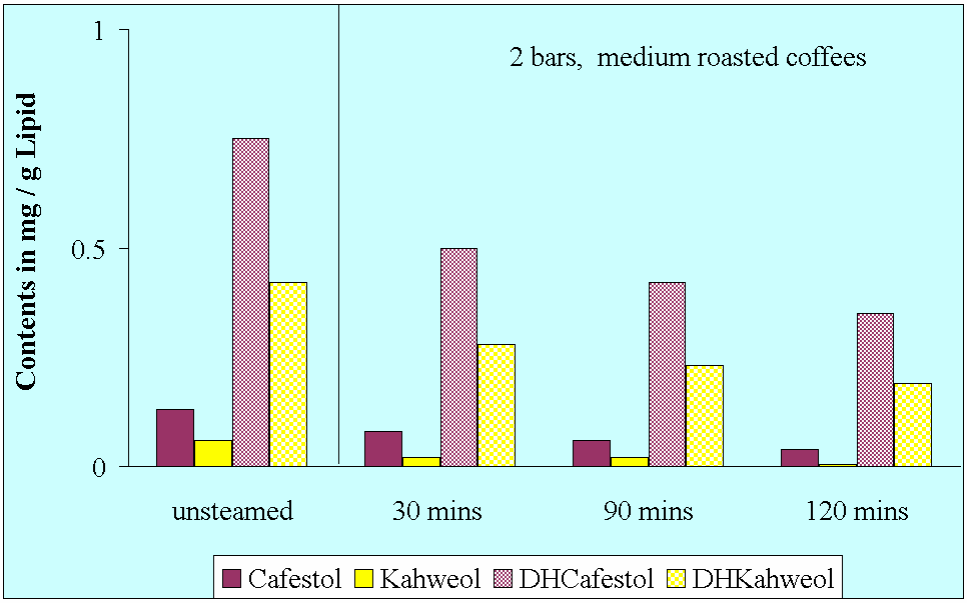

Figure 3. Contents of free diterpenes in untreated and treated roasted coffees

In Figure 3 the results of the medium roasted coffee from Brazil were presented. Furthermore, the data for light and dark roasted coffees were analysed. For a better comparison of roasted coffees with steamed and roasted coffees different diterpene contents were related to each other and evaluated on their degrees of roasting. One of these relationships is the ratio of the content of the total cafestol to the content of the total dehydrocafestol. At all stages of treatment this ratio is proportional to the degree of roasting. The degree of the change is clearly dependent on the stage of treatment, the greatest change resulted when the coffee was

steamed for 120 minutes at 2 bars. If the roasting was very strong, the ratios of total cafestol to total dehydrocafestol became quite similar at all treatment stages.

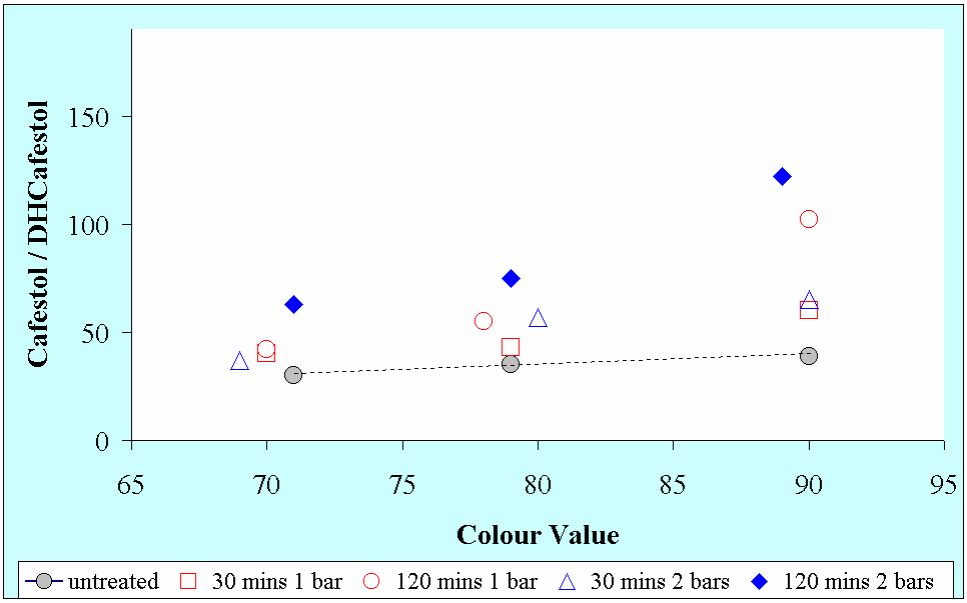

Figure 4. Ratio of total cafestol to total dehydrocafestol related to colour value

A similar result was obtained when the ratio of total free diterpenes to the colour value was investigated.

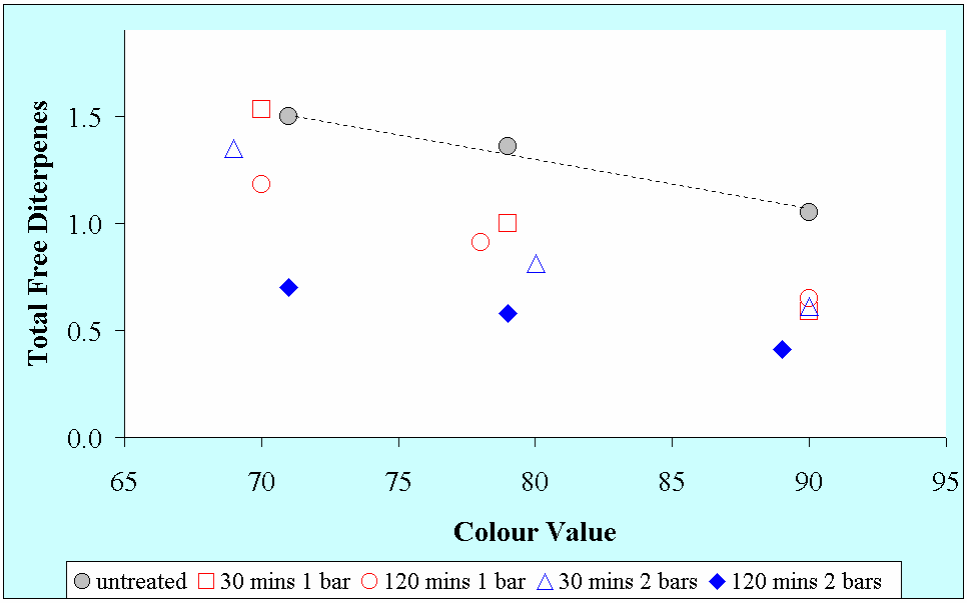

Figure 5. Ratio of total free diterpenes related to the colour value

ANALYSIS OF ARABICA COFFEES FROM DIFFERENT COUNTRIES

Besides the coffee from Brazil, other Arabica coffees from Kenya, Colombia and Costa Rica were also investigated. However, it was not our aim to obtain data for all steaming stages in each case, although we tried to summarise the results in one graph. The coffees were only differentiated by the chosen steaming process and the roasting degree (Figure 6).

The untreated coffees were marked by dark circles. Thus, with the exception of the strongly roasted brands of coffee and in spite of the differences in their diterpene concentrations due to their origin, the very intensively steamed roasted coffees could be differentiated from the unprocessed ones.

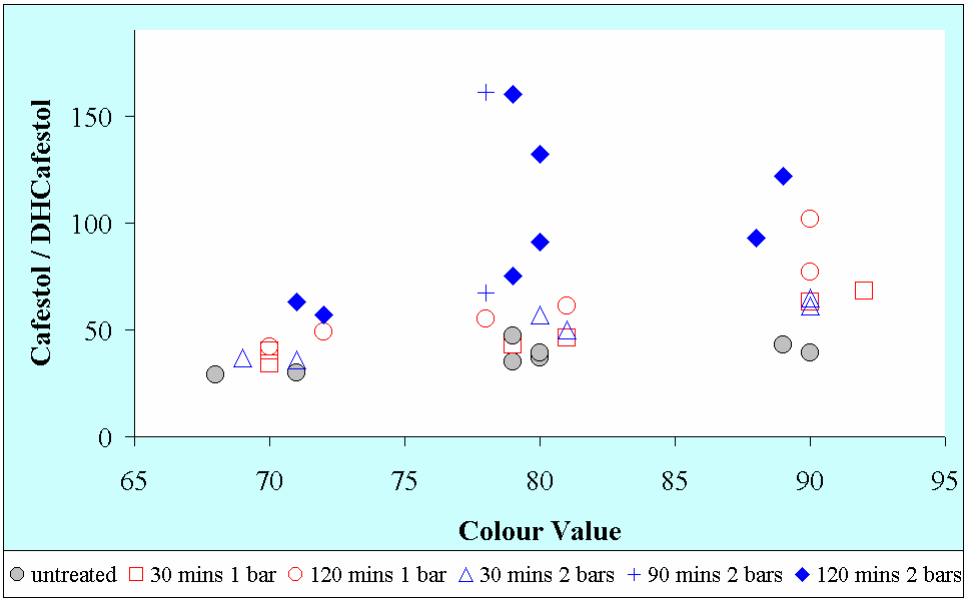

Figure 6. Influence of roast level on Cafestol/DHCafestol ratio for Arabica coffees from different countries

COMMERCIAL COFFEE SAMPLES FROM THE GERMAN MARKET

Seven pure Arabica roasted coffees were analysed, which were declared as “mild” processed. The contents for the free kahweol ranged between 0.03 and 0.06 mg/g lipid, but no coffee investigated obtained a kahweol content that was below 0.01 mg/g lipid.

It has been found difficult to assess steamed roasted coffees, particularly if the non-steamed coffee is not available for comparative analysis. However, if both steamed and non-steamed coffees are available, the content of free kahweol will be a helpful tool in the assessment of steamed coffees. Furthermore, the content of free kahweol may be an objective indicator for the optimisation of the techniques used at present.

ACKNOWLEDGEMENT

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Hydroxycinnamic Acids as a Genotype Discrimination criteria for Green Coffee Beans

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SUMMARY

High performance liquid chromatography (HPLC) profiles comparisons of hydroxycinnamic derivatives from green coffee beans of Variedad Colombia (Caturra x Híbrido de Timor), first offspring from the crossing of Caturra x Híbrido de Timor, three Híbrido de Timor accessions, two varieties of Coffea arabica and three accessions of *Coffea canephora*, were done with multivariate analysis using quantitative data. This analysis allowed to discriminate *C. canephora* from the other genotypes studied, among the *C. canephora* accessions and between Típica and Caturra varieties. From all the compounds that participated in the discrimination, three of them were isolated by semipreparative HPLC and identified for the first time. Their structures were elucidated by mass spectrometry (FAB and EI) and NMR analyses (^{1}H, ^{13}C y COSY ^{1}H-^{1}H) as hydroxycinnamic glucosides

RESUMEN

La comparación de los perfiles cromatográficos obtenidos por CLAE de los derivados hidroxicinámicos del grano de café verde, de la variedad Colombia, la primera generación del cruce de Caturra por Híbrido de Timor, tres introducciones del Híbrido de Timor, dos variedades de *Coffea arabica* y tres introducciones de *C. Canephora*, mediante análisis multivariado permitió discriminar *C. Canephora* de los otros genotipos estudiados, entre las introducciones de *C. Canephora* y entre las variedades de *C. arabica*, Caturra y Típica. De todos los compuestos que participaron en la discriminación, tres de ellos fueron separados por CLAE semipreparativa e identificados por primera vez . Sus estructuras fueron elucidadas por espectrometría de masas (IE, BAR) y por RMN (^{1}H, ^{13}C y COSY ^{1}H-^{1}H), como derivados hidroxicinámicos glucosidados.

INTRODUCTION

Among the hydroxycinnamic acids found in green coffee beans, chlorogenic acid and its isomers account for the majority. Therefore, there were found in a lesser extent in free form and/or glycosidically bound (Amorim et al., 1974). However hydroxycinnamic glycosides until now have only been definitely identified in a few cases, such as the melilotoside reported by Rakotomalala in *Coffea Stenophylla* (1992).

Besides, in different scientific investigations, authors have employed HPLC-UV and principal component analysis (PCA), of chlorogenic acids in order to characterize green or roasted coffees of different origins and qualities (Bicchi et al., 1993; Bicchi et al., 1995; Clifford and Jarvis, 1988).

The aim of this work was to perform a comparative analysis of hydroxycinnamic acids profiles of green coffee beans of genotypes of interest to Cenicafé's plant breeding program. Analytical profiles were obtained by HPLC-PDA (photodiode detector array) and the comparison was based on multivariate techniques. Coffee samples included Variedad Colombia, a composite variety resistant to rust (Hemileia vastatrix), developed in Colombia (Castillo and Moreno, 1988). He purpose was to establish the main differences in hydrocynnamic acids between among these various coffees. Another objective was to isolate and to identify through spectroscopy techniques some of the unidentified compounds, which are important for the discrimination.

MATERIAL AND METHODS

Materials

The samples consisted of seeds sun dried and grounded in the presence of liquid nitrogen of the variedad Colombia (advanced generation of Caturra X Híbrido de Timor crossing), first offspring from the crossing of Caturra x Híbrido de Timor (F1), Típica and Caturra varieties of *Coffea arabica,* of three Híbrido de Timor accessions (1343, 832 and 2252), and of three accessions of *C. canephora* (BP4, BP46 and Centro 1). These materials were supplied from the living collection of coffee resources center in Chinchiná, Caldas, Colombia.

Extraction

Hydroxycinnamic acids from each genotype were extracted from 10 g of sample using 80% ethanol following the method proposed by Rakotomalala (1992), extracting the caffeine present in the samples with chloroform from the aqueous extract before ethyl acetate extraction. Ethyl acetate extract was dried and the residue was dissolved in 50 mL of methanol for HPLC analysis. The residual aqueous fraction was passed through a conditioned XAD column, after washing with 1L of distillated water. The eluted with 500 mL of methanol was concentrated, lyophilized, weighted and redissolved in methanol for HPLC analysis.

Chromatographic separation

The high performance liquid chromatography analysis was carried out on a HPLC system consisting of two Waters Associates Model 600 pumping units, a variable wavelength UV detector (Waters 996 photodiode array detector), a valve loop injector Rheodine Model 7725 fitted with a 5 μL loop (Waters Associates), a RP-18 precolumn and a (300 mm x 3.9 mm., 4 μm) Novapack RP-18 column. The elution solvents were (A) 0.5% formic acid in 6% acetonitrile and (B) 0.5% formic acid in 34% acetonitrile. Samples and standards were analyzed at room temperature (25^{o}C) using the following elution program: 0% A to 100% A in 42 min of linear gradient and then isocratic 100% A up to 47 min. Flow rate was 0.9 mL min^{-1}. The detection was carried out at a range of wavelength of 220-340 nm.

Quantification

The quantification of each compound was performed using the external standard method, integrating the area of each of the chromatographic peaks and relating it to 5-caffeoylquinic acid standard. A calibration curve was generated using five points of 5-CQA at 0.05, 0.10, 0.25, 0.50, 0.75, mg mL^{-1}. Triplicate from each genotype were prepared. All quantified compounds showed UV-spectrum characteristics of hydroxycinnamic derivatives.

Isolation and Identification of discriminating compounds

Most compounds of the ethyl acetate fraction were identified in a previous publication (Guerrero et al., 2001). The compounds G_2 and G_5 were separated from the aqueous fraction through HPLC semipreparative using Waters Alliance system of chromatographic separation, a μ-bondapack (7.8 mm x 300 mm x10 μm) RP-18 column, a loop of 200 μL. The elution solvents were: 0.5% formic acid in 6% acetonitrile (A); 0.5% formic acid in 34% acetonitrile (B) and the flow rate was 2.0 mL min^{-1}. The following elution program was used: 70% A (5 min. isocratic) to 30% A in 35 min of linear gradient then 30% A to 0% A in 5 min and finally 0% A to 70% A in 5 min.

The C_7 compound was isolated from the ethyl acetate fraction with the same conditions above described but with the following elution program: 75% A (25 min. isocratic) to 65% A in 5 min then 65% A (5 min. isocratic) to 50% A in 3 min after 50% A to 0% A in 2 min finally 0% A (10 min. isocratic) to 75% A in 15 min.

Identification of these compounds were made through UV spectrum, mass spectrum obtained by FAB (*m*-nitrobenzyl alcohol), electronic impact (IE), ^{1}H NMR and COSY ^{1}H-^{1}H (600 Mhz). Confirmation of these structures was made through enzymatic hydrolysis using a glycosidase. The acetylated derivatives (AC_2O-Pyridine) of the all compounds and of the products of its hydrolysis were analyzed by GC-MS with a system of chromatograph Hewlett Packard 6890, with a column HP-5MS (5% phenyl-methylsiloxane) (30 m x 0.25 μm). Helium was using as the carrier gas at a flow rate of 15 mL/min. The injector temperature was 280ºC. The temperature program was 100ºC for 2 min, then raised 10ºC/min until 320ºC was reached. This final temperature was held for 3 minutes.

Statistical analysis

The tests used were principal component analysis (PCA), correspondence factorial analysis (CFA), hierarchical ascending classification (HAC) and discriminant factorial analysis (DFA). Analyses were performed using SAS and STAT-ITCF statistical software.

RESULTS AND DISCUSSION

Significant differences in the profiles of hydroxycinnamic acids compounds were found among the genotypes studied, making possible their discrimination. The HPLC chromatograms of the compounds of the EtOAc fraction (C) (Figure 1A) and the compounds of the aqueous fraction (G) (Figure 1B), illustrate the greatest differences between Típica and *C. canephora* BP4 genotypes.

Values of the quantification of each compound for all genotypes were included in one single matrix in order to perform the multivariate analysis. The PCA summarized the data of total separated peaks (22 compounds of the EtOAc fraction and 8 compounds of the aqueous fraction), in five principal components or axis accounting for 88% of the variation. Figure 2 shows genotype distributions on the 1-2 plane. The axis 1 mainly discriminated the samples on the base of contents of the 4-feruloylquinic (C_6), 3,4-dicaffeoylquinic (C_9), 4,5-caffeoylferuloylquinic (C_{14}), caffeoylferuloylquinic (C_{16}, C_{18}, C_{19}) acids and the compound G_2 (unknown), all of which are positively associated with this axis. The unknown compounds C_5 and G_4 participate with a negative relation in this same axis. The 3,5-dicaffeoylquinic (C_{10}), 4,5-dicaffeoylquinic (C_{11}), the dicaffeoylferuloylquinic (C_{17}) acids likewise the unknown compounds G_6 and C_7 have an important participation in the makeup of axis 2 with

a positive gradient. The G_7 and G_5 compounds (both unknowns), contribute with a negative gradient. The new compounds G_2, G_5 and C_7, were isolated for its identification.

Compounds G_2 and G_5

The molecular weight of G_2 and G_5 were deduced by FAB (M+1= 517 and 531 respectively). The principal fragments of IE mass spectrum of G_2 are m/z 163 (100%) and m/z 180 (80%) and of G_5 are m/z 177 (75%) and m/z 194 (80%) which give information about of the aglycones. The GC-MS analysis of acelylated derivatives and of the products of enzymatic hydrolysis indicated the presence of glucose in both compounds. The UV absorption at 290 nm and 324 nm indicated the presence of hydroxycinnamic derivatives. The ^{1}HNMR spectrums of both compounds exhibited similar signals, the principal difference was the presence of OCH_3 group in G_5, and thus it could be deduced that G_2 has a caffeoyl moiety while G_5 has a feruloyl moiety. Olefinic protons coupling constants of G_2 (δ H-7' at 7.47 ppm and H-8' at 6.2 ppm, J=16 Hz) and G_5 (δ H-7' at 7.60 and H-8' at 6.38 ppm), indicated *trans* configuration. The aromatic protons signals for caffeoyl moiety (δ H-2'at 6.94 ppm, H-5' at 6.68 ppm and H-6' at 6.85 ppm) and for feruloyl moiety (H-2' at 7.2 ppm, H-5'at 6.8 ppm and H-6'at 7.10 ppm) and signals of H-5 of quinic acid of both compounds (H-5 at 5.25 ppm to G_2 and at 5.35 ppm to G_5) agreed with signals reported by other authors for similar compounds (Chuda, Ono, Ohnishi-Kameyama, Nagata and Tsushida, 1996; Morishita, Iwahashi and Kido, 1986). Anomeric proton signals of G_2 and G_5 (5.25 ppm and 5.35 ppm respectively), indicated α-configuration for both compounds (Agrawal, 1992), then G_2 was identified as: 4'-O-(α-D-glucopyranosil)-5-*trans* -caffeoylquinic acid (Figure 3) and G_5 as 4'-O-(α-D-glucopyranosil)-5-*trans* -feruloylquinic acid (Figure 3). All protons were assigned by analysis of its ^{1}H-^{1}H COSY spectrum, this information and the other spectrum signals are in a previous study (Guerrero, 2001).

Compound C_7

The molecular weight of C_7 was determinate by FAB (M^+ 1 = 647). This compound is a similar glucoside to G_2 and G_5. ^{1}HNMR spectrum showed the presence of two *p*-coumaroyl moieties, one of which with olefinic protons (H-7'at 6.80 ppm and H-8' at 5.72 ppm, J= 13Hz) with *cis* configuration and the other one (H-7'''at 7.53 ppm and H-8''' at 6.22 ppm, J= 16Hz) with *trans* configuration. Aromatic protons signals of the *p-cis*-

CONCLUSIONS

Comparison of chromatographic profiles of the hydroxycinnamic derivatives through multivariate analysis, allowed us to discriminate the *C. arabica* genotypes from the ones of *C. canephora*, the introductions of *C. canephora* among themselves and the Típica variety from the Caturra variety. Híbrido de Timor introductions, Caturra variety and their descendants (Colombia Variety and generation F1) were classified in the same group. Furthermore, it was possible to isolate and to identify three new hydroxicinnamic glucosides, which are also discriminating compounds.

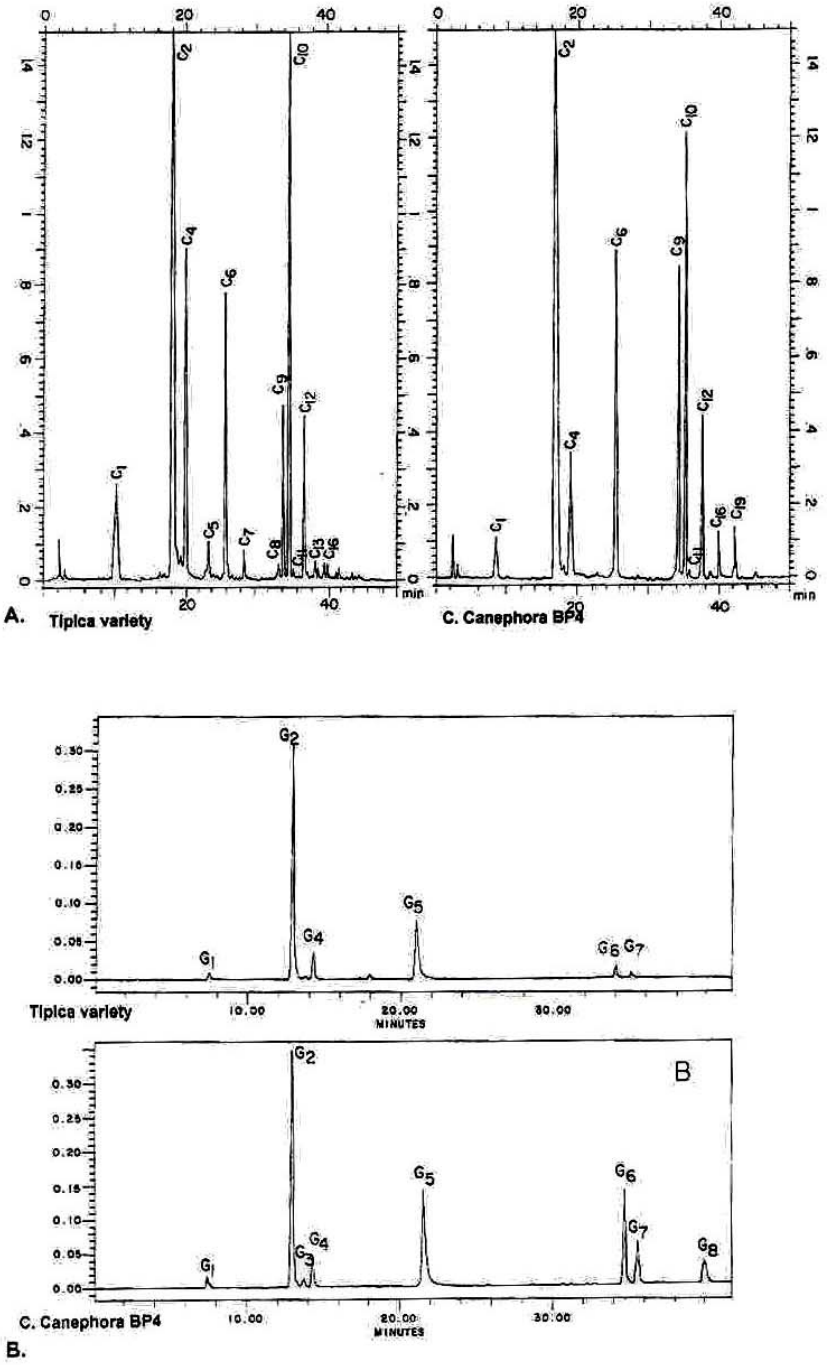

Figure 1. HPLC separation of hydroxycinnamic acids in green coffee beans (A) Hydroxycinnamic derivatives of EtOAc fraction of Típica variety of *C. arabica* and BP4 introduction of *C. canephora (*B) Hydroxycinnamic derivatives of aqueous fraction of Típica variety of *C. arabica and* BP4 introduction of *C. canephora*

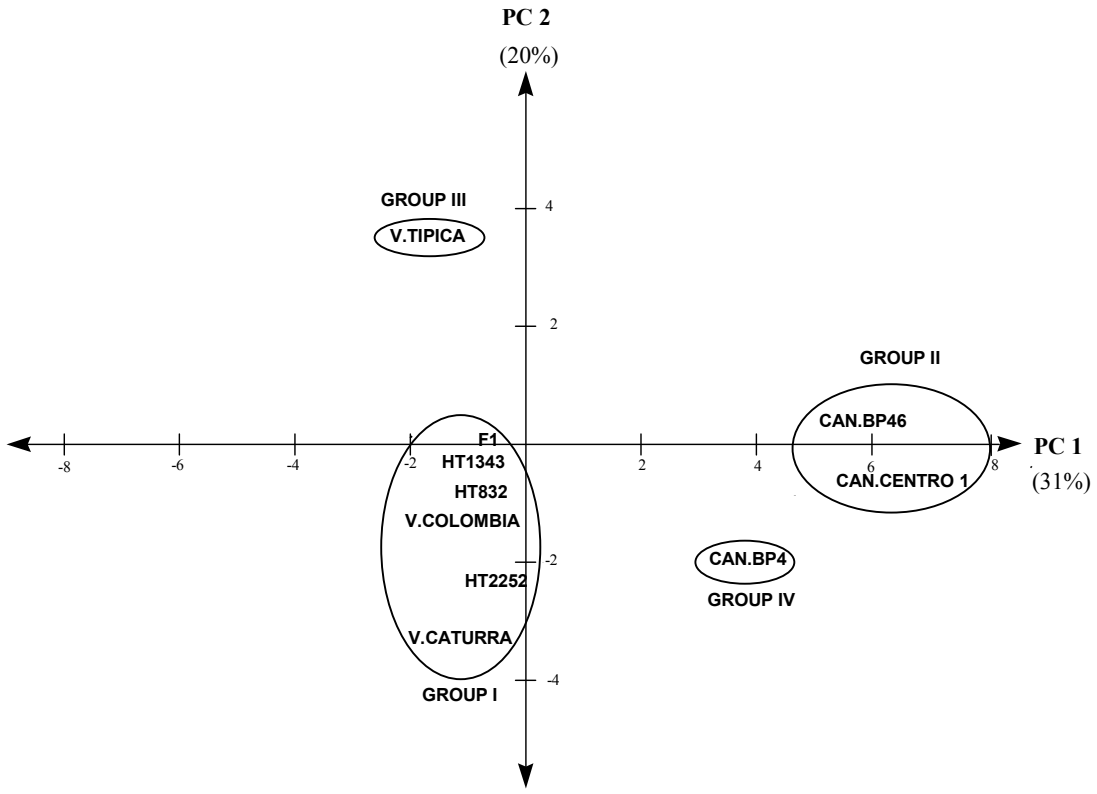

Figure 2. Representation of the genotypes on the plane 1-2 of the principal components analysis and their agroupment according to hierarchical ascending classification. Híbrido de Timor (HT); *C. canephora* (CAN); Variety (V)

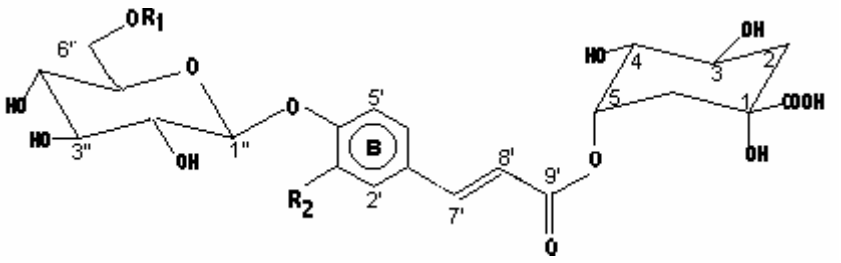

Figure 3. Structures of Compounds

	R1	R2
G_2	H	OH
G_5	H	OCH_3
C_7	*p-trans*-coumaroyl	H

ACKNOWLEDGMENTS

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Caffeine-Caffeic Acid Crystallography

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SUMMARY

This paper completes the body of research presented at the 17th and 18th ASIC conferences on use of caffeic acid (CA) to decaffeinate coffee extracts via formation of a crystalline caffeine complex. The primary objective of this paper is to document visual images of the caffeine-CA complex and its components relevant to decaffeination. Several properties of the crystalline complex were also studied, including stoichiometry, habit, melting point, and density. Light microscopy was used to distinguish complex crystals from caffeine and CA crystals grown in pure water. Scanning electron microscopy was used to more effectively detail the altered appearance of complex crystals grown in aqueous coffee solution. These images constitute the first recorded instance of a physical caffeine complex being successfully crystallized from a coffee extract.

INTRODUCTION

Use of caffeic acid (CA) to decaffeinate aqueous caffeine solutions and coffee extracts was reported at the 17th ASIC Conference in Nairobi (Zeller and Saleeb, 1997). Application to non-aqueous caffeine solvents was reported at the 18th ASIC Conference in Helsinki (Zeller and Saleeb, 1999). Decaffeination was achieved by crystallization of a 1:1 molar caffeine-CA complex from solution (Zeller et al., 1985; Saleeb and Zeller, 1985; Kaleda et al., 1988). This paper summarizes the elucidation of several physical properties of crystalline caffeine-CA, including stoichiometry, crystal system, water of crystallization, thermal transitions, and density. In addition, microscopic images are presented that contrast the appearance of pure caffeine and CA crystals from caffeine-CA crystallized from water and soluble coffee.

MATERIALS

Reagent grade CA (3,4-dihdroxycinnamic acid; MW=180.15; 97% purity; predominantly *trans*) was sourced from Aldrich and recrystallized by dissolving 8.0 g in 1 L distilled boiling water and cooling to room temperature. U.S.P grade anhydrous caffeine (MW=194.19) was sourced from Sigma. Caffeine-CA complex was prepared by dissolving 4.5 g recrystallized CA in 300 mL boiling distilled water, adding 5.1 g caffeine, and cooling to room temperature. Caffeine was used at 5% molar excess relative to caffeine to ensure caffeine-CA crystals did not contain any CA crystals. Crystals were decanted after 24 hours, unless otherwise stated, and paper filtered through a Buchner funnel under vacuum. Air was passed through the filter bed for several hours to remove all surface moisture from the crystals.

EXPERIMENTAL

Titration

0.20 g quantities of CA and caffeine-CA crystals were added to 450 mL distilled water and titrated with standardized 0.1 N NaOH solution. Inflection points were visually identified

from plotted curves to determine titration endpoints corresponding to complete neutralization of the CA carboxylic acid group.

Density

Caffeine-CA density was measured to one-decimal-place accuracy by displacing mineral oil (density=0.85 g/cc) in a 10 mL graduated cylinder at 22°C. Mineral oil density was measured by weighing 10 mL in the same cylinder. A Micromeritics helium pycnometer was used to measure CA and caffeine-CA crystal density at 22°C to two-decimal-place accuracy.

Thermal Analysis

A Perkin-Elmer DSC7 Differential Scanning Calorimeter was used to analyze hermetically sealed caffeine, CA, and caffeine-CA crystals at a heating rate of 10°C/min between 0-350°C. The same samples were then cooled at 10°C/min to determine if recrystallization occurred. A Perkin-Elmer TGA7 Thermogravimetric Analyzer was used to heat CA and caffeine-CA crystals on an open balance pan at a rate of 10°C/min between 30-600°C.

Crystal System Identification

McCrone Associates, Inc. (Westmont, IL, USA) was contracted for crystallographic analysis of CA and caffeine-CA supplied by the authors. Senior Research Microscopist, S. Stoeffler, mounted the crystals in viscous silicone oil for examination by polarized light microscopy. The morphologies of a number of crystals were examined, as well as their optical properties, including interference figures and extinction behavior between crossed polars, to identify crystal system.

Microscopy

A Leica light microscope (LM) and CamScan scanning electron microscope (SEM) were used to produce black & white Polaroid photographic images (4.5x3.5inch original image area). The images were subsequently digitized for publication using a Hewlett Packard optical scanner. Crystals were mounted in mineral oil for LM photography. Crystals were mounted and metal sputtered for SEM analysis.

RESULTS

Titration

CA and caffeine-CA titration curves are illustrated in Figure 1. CA had an endpoint of 11.1 mL 0.1 N NaOH, corresponding exactly to the 0.00111 moles CA present. Titration of an equal weight of caffeine-CA would predict an endpoint of 5.3mL for a 1:1 molar complex, since it would contain 0.00534 moles CA. However, an endpoint of only 5.1 mL was obtained. This result was sufficiently close (0.96x target) to confirm a 1:1 molar caffeine-CA complex and suggested that water of crystallization might be present since an endpoint of exactly 5.1 mL would be predicted for caffeine-CA monohydrate.

Density

Addition of 3.965 g caffeine-CA crystals to 5.045 g (5.9 mL) mineral oil gave 8.7 mL total volume with 2.8 mL displaced volume to yield 1.4 g/cc crystal density. Helium pycnometry

analyses, completed in triplicate, yielded 1.44 g/cc caffeine-CA density and 1.47 g/cc CA density.

Thermal Analysis

DSC scans of caffeine, CA, and caffeine-CA are superimposed in Figure 2. Caffeine gave a sharp melt peak at 238°C (ΔH~104 J/g) with recrystallization upon cooling (not shown), in agreement with the literature (Beilstein's Handbuch der Organische Chemie, 26^4, 2338). CA revealed endothermic melting (peak=225°C) with resultant exothermic decomposition (peak=230°C), in agreement with the literature (Beilstein's Handbuch der Organische Chemie, 10^3, 1834). Caffeine-CA revealed two relatively broad endotherms ($peak_1$=144°C; onset~141°C; ΔH~102J/g) ($peak_2$=188°C; onset ~183°C; ΔH~166J/g) with no recrystallization of caffeine-CA or caffeine upon cooling.

TGA analysis indicated the first caffeine-CA endotherm was accompanied by 4.8% weight loss, very close (1.04x target) to 4.6% weight loss that would be expected for a monohydrate. Microscopic observation of caffeine-CA crystals on a hot stage confirmed onset of melting at 183°C. TGA heating of caffeine-CA beyond its melt point revealed substantial weight loss (peak =230°C), corresponding to decomposition of CA.

Crystal System Identification

CA and caffeine-CA were found to possess monoclinic crystal structure. The following is taken from McCrone project report MA36813. Caffeine-CA crystals were typically long, fairly thick rods with slightly irregular cross-sections that were roughly square or rectangular and the larger rods often occurred as twins. Based on the observation of interference figures, the crystals were classified as biaxial. Based on their morphology and extinction behavior, the crystals were further classified as belonging to the monoclinic crystal system. CA crystals were in the shape of thin, rhomboid plates. Based on the observation of interference figures, the crystals were classified as biaxial. They were further classified as monoclinic, based on their morphology and symmetry.

Microscopy

Differences in crystal habit between caffeine, CA, and caffeine-CA crystallized from water are evident in LM images presented in Figure 3. SEM images presented in Figure 4 illustrate several different morphologies of caffeine-CA, revealing a mixture of 4-sided and 8-sided prisms having parallel opposing faces that crystallize together from water.

SEM images of caffeine-CA crystallized from soluble coffee (Zeller and Saleeb, 1997) are presented in Figures 5 and 6. The images illustrate the adverse effect of this environment on crystal growth. Essentially all caffeine-CA recovered after 24hrs was in the form of hard sediment comprised of a polycrystalline mass of prisms (Figure 5a,b) having obvious crystal defects. In addition, a much smaller amount of individual caffeine-CA crystals (Figure 6a,b) having rounded appearance with more severe defects was collected from the decanted coffee extract after two weeks. It is speculated that chlorogenic acid (CGA) liberated from the soluble caffeine-CGA complex upon addition of CA to the coffee solution may have contributed to the observed caffeine-CA defects.

DISCUSSION

The literature reports white hexagonal prisms for anhydrous caffeine (Beilstein's Handbuch der Organische Chemie, 26^4, 2338) used in this study and white monoclinic needles for caffeine monohydrate crystallized from water (Beilstein's Handbuch der Organische Chemie, 26^4, 2338.; Sutor, 1958). The literature also reports yellow CA crystals from methanol-water having monoclinic structure and 1.46 g/cc density (Garcia-Granda, 1987). Investigation of caffeine-CA structure and crystal properties was not found in the literature. Although caffeine-CA titration, DSC, and TGA analyses provide circumstantial evidence of a crystal monohydrate, supplemental chemical or spectroscopic analysis could provide tangible evidence and form the basis for another paper. Similar analysis of the physical properties of caffeine-CA crystallized from soluble coffee solution is another area for potential research.

CONCLUSIONS

Based on the evidence presented, we conclude the following. Caffeine-CA crystallizes from water as a 1:1 molar complex monohydrate in the form of yellow prisms having monoclinic structure and 1.44 g/cc density. It loses water of crystallization at 144°C, melts at 188°C, and decomposes at 230°C. Caffeine-CA crystallizes from soluble coffee predominantly as hard sediment comprised of a polycrystalline mass of moderately defective prisms.

Properties of Crystalline Compounds Relevant to this Investigation

Property	Caffeine	Caffeic Acid	Caffeine-CA
Stoichiometry	---	---	1:1
Water of Crystallization	anhydrous	anhydrous	monohydrate
Appearance	white prisms	yellow plates	yellow prisms
Crystal System	hexagonal (Beilstein's Handbuch der Organische Chemie, 26^4, 2338.)	monoclinic	monoclinic
Density (g/cc)	1.23 (Beilstein's Handbuch der Organische Chemie, 26^4, 2338.)	1.47	1.44
Dehydration Temperature (°C)	---	---	144
Melting Point (°C)	238	225(d)	188
Thermal Decomposition (°C)	---	230	230

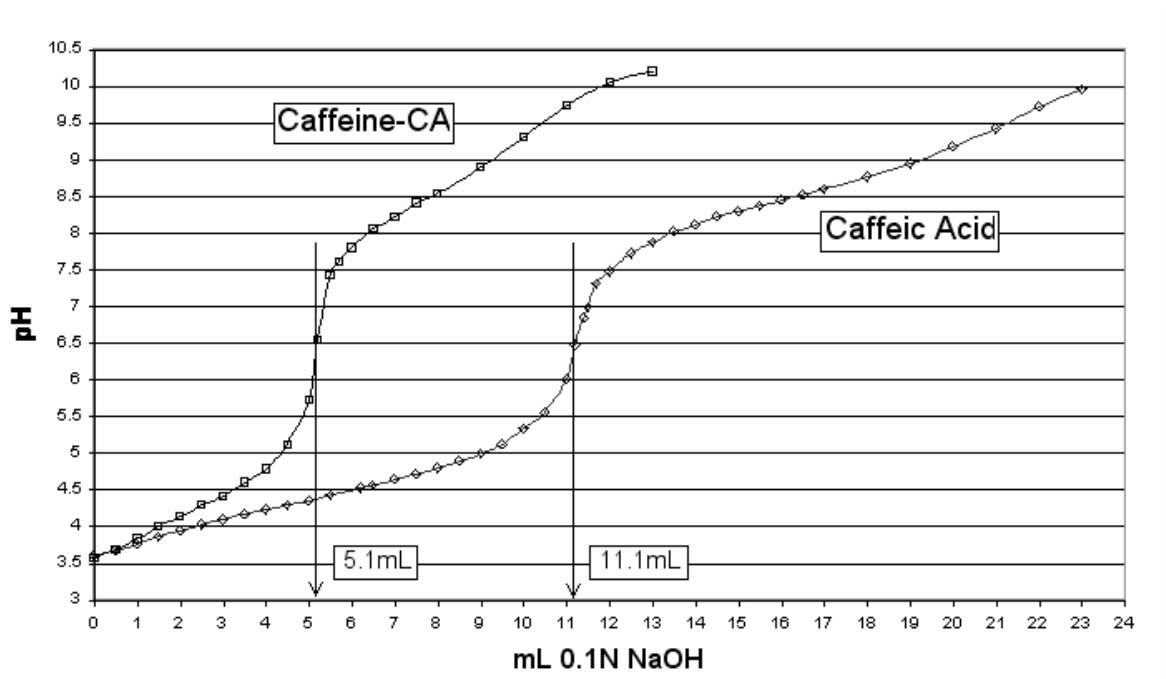


Figure 1. Tritation of Caffeine-CA versus Caffeic Aci. 0.20g crystals in 450mL water

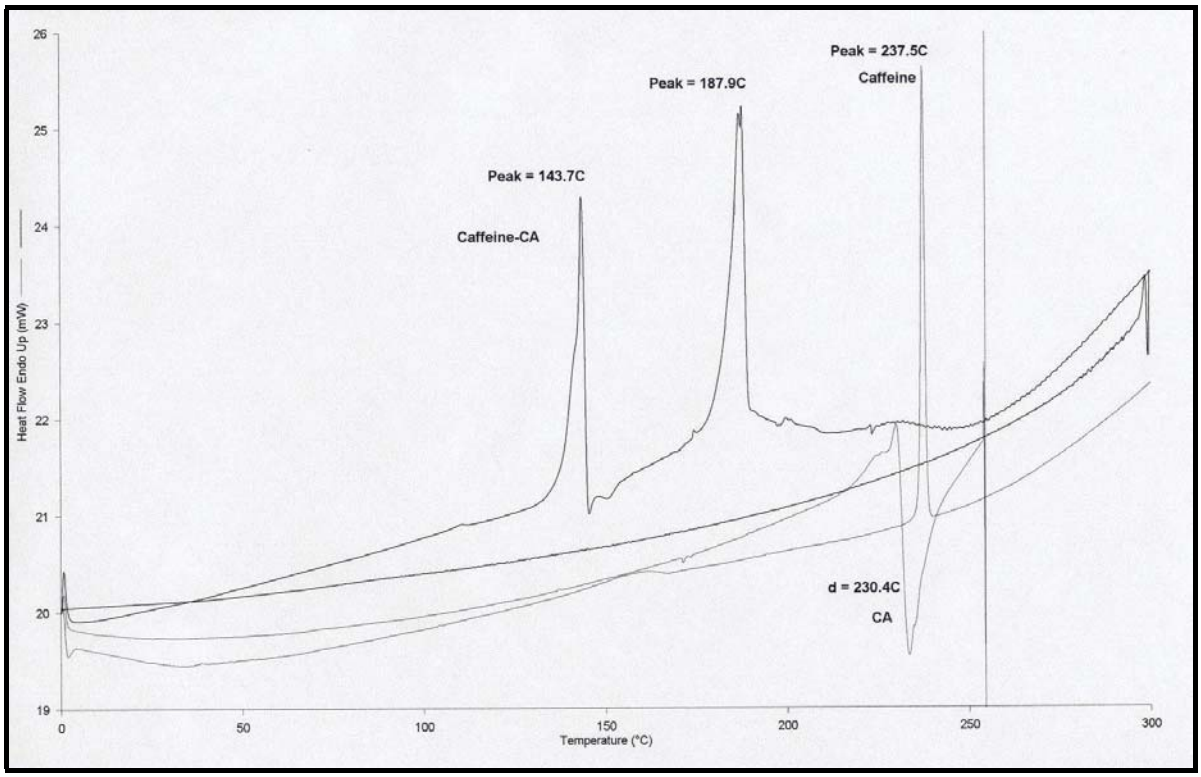
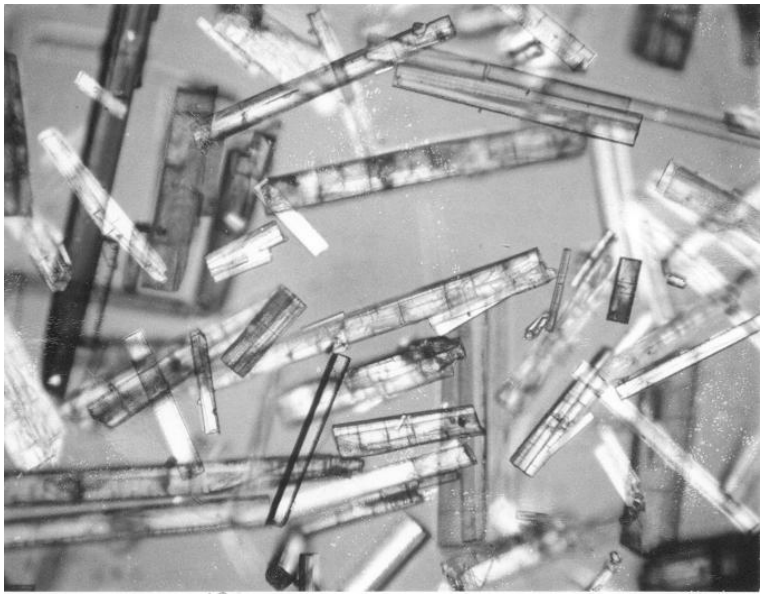


Figure 2. DSC Profile of Caffeine-CA versus Caffeine and Caffeic Acid (CA)



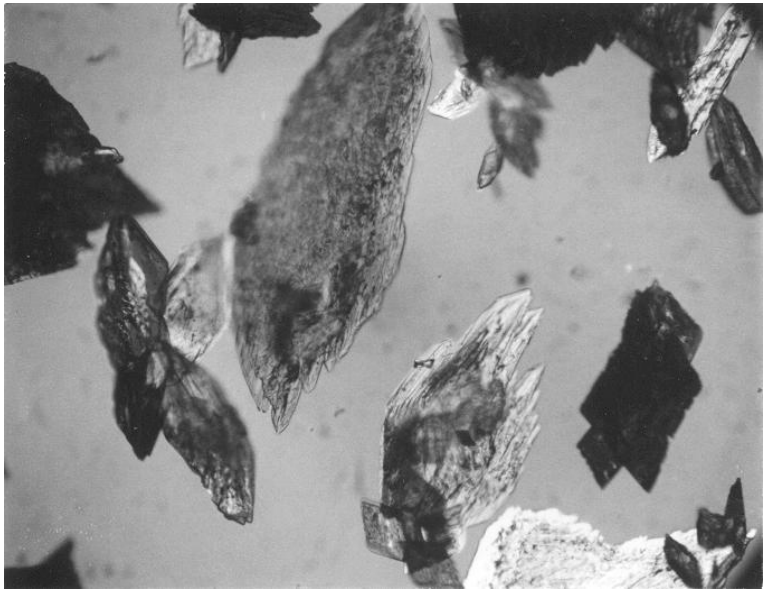
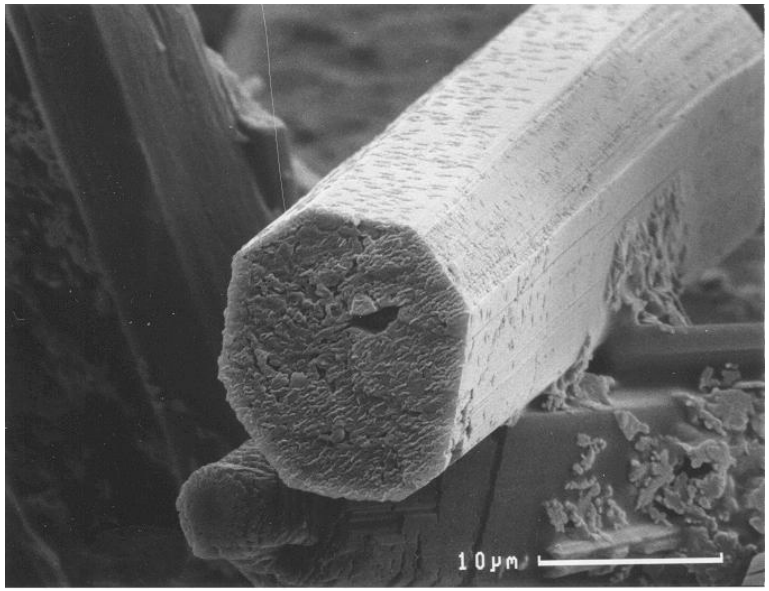
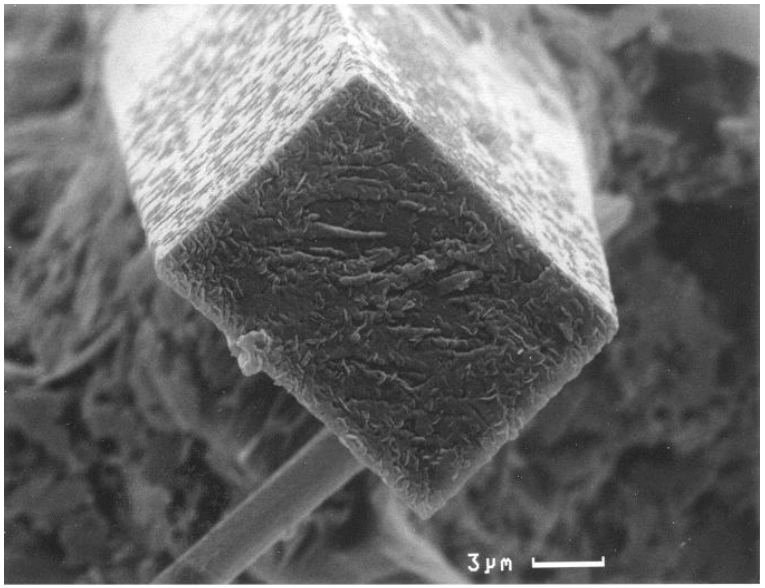


Figure 3. Comparison of Crystal Habit (LM 108x). Top-Left: Caffeine; Bottom: Caffeic Acid (CA); Top-Right: Caffeine-CA Complex





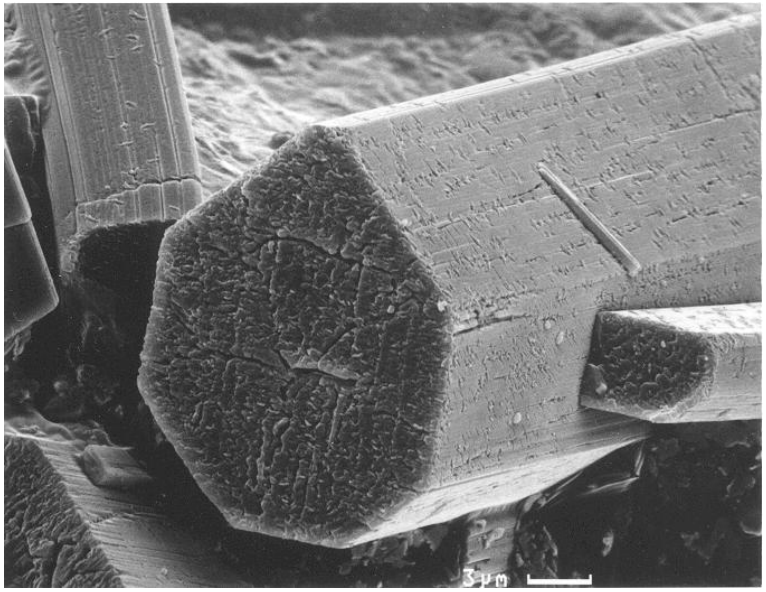


Figure 4. Caffeine-CA Morphology Variation (SEM 2750-3500x)

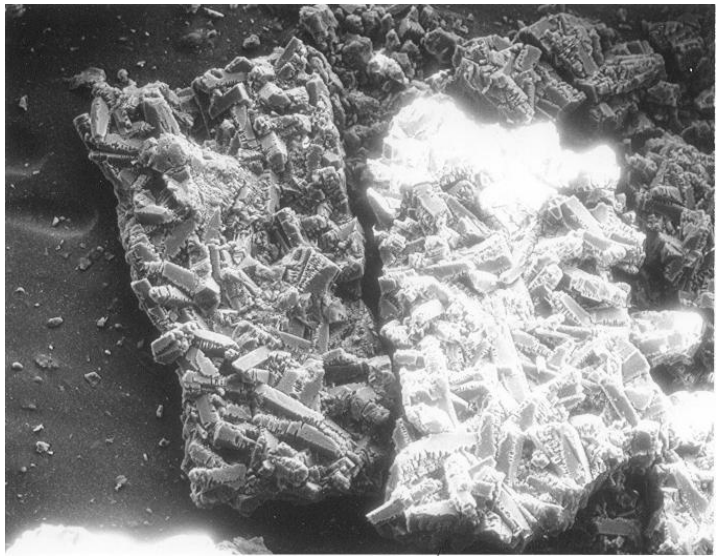


Figure 5a. Caffeine-CA from Coffee (24hrs/ SEM 100x)

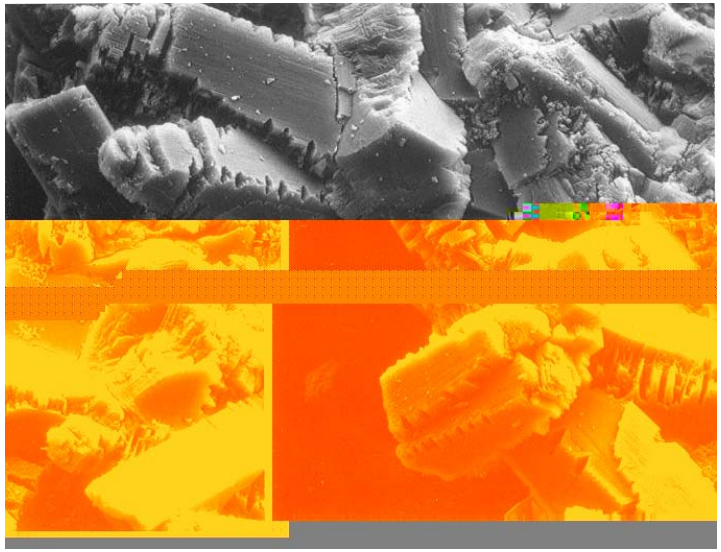


Figure 5b. Caffeine-CA from Coffee (24hrs/ SEM 500x)

Figure 6a. Caffeine-CA from Coffee (2wks/ SEM 100x)

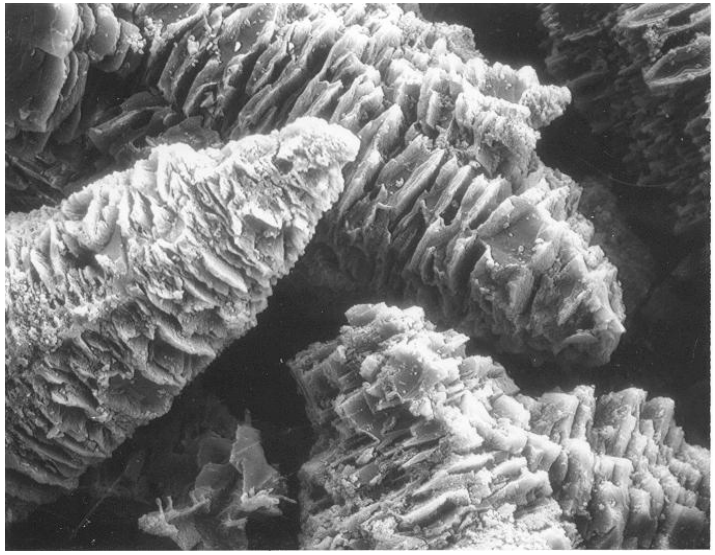


Figure 6b. Caffeine-CA from Coffee (2wks/ SEM 500x)

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Coffee Bean Carbohydrates as Relates to Quality of Kenyan Arabica Coffee (*Coffea arabica* L.)

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SUMMARY

Variation of sugars in three different quality classes of grade AB wet processed raw arabica coffee (Classes 4, 5, and 7) were investigated by HPLC method. Several sugars were separated among which three were identified. These were fructose, glucose and sucrose. The level of sucrose decreased with declining quality of the coffee. Conversely, fructose and glucose levels increased as the coffee quality declined.

INTRODUCTION

Coffee quality is a major factor in marketing Kenya coffee. Coffee payments to planters are based on quality. Assessment of coffee quality is carried out by experienced coffee liquorers after roasting a sample of the coffee already graded by size. Wet processed coffee is categorised into ten classes one being the best and ten the lowest in terms of quality. This study was carried out to investigate any variations in levels of fructose, glucose and sucrose of raw coffee beans relating them to quality classes.

MATERIALS AND METHODS

Raw commercial coffee samples of grade AB and quality classes 4, 5 and 7 were obtained from Coffee Board of Kenya sample room in Nairobi, Kenya. The samples were ground to pass through sieve number 40 (diameter 0.425 mm) (Anonymous, 1995). Sugars were extracted and analysed by High Performance Liquid Chromatography (HPLC) (Osborne and Voogt , 1978).

RESULTS

Fructose, glucose and sucrose levels of green coffee of quality classes 4, 5 and 7 are presented in Figure 1. Six peaks were observed in the HPLC chromatograms of the raw coffee. Three of the peaks were identified by use of standards as glucose, fructose and sucrose.

DISCUSSION

Among the sugars which were identified sucrose levels (relative concentrations) declined with loss in quality while fructose and glucose levels increased with loss in quality. The increase in the levels of glucose and fructose could probably be due to breakdown of more complex carbohydrates to simple sugars either during processing or during storage (Trugo, 1985). Further studies will target all the coffee grades and quality classes, storage stages and varieties.

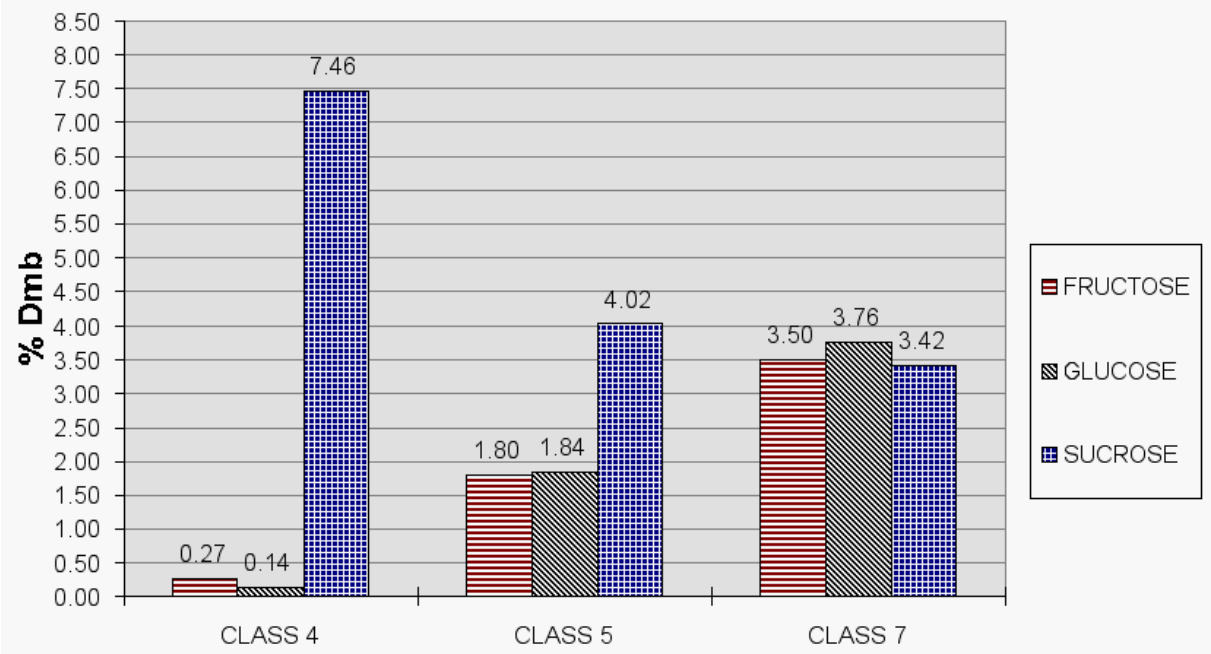

Figure 1. Fructose, glucose and sucrose contents of raw arabica coffee of different quality classes (% Dmb)

ACKNOWLEDGEMENT

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Trugo L. C. (1985). Coffee chemistry vol.1. (Eds R.J. Clarke and Macrae R.) Elsevier Applied Science, London pp. 83-114 .

16-O-Methylkahweol in Robusta Coffees

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SUMMARY

Recently a diterpene was described that was assumed to be 16-O-methylkahweol. This diterpene was now identified clearly as the 16-O-derivative of kahweol by means of GC and LC analysis with mass spectrometric detection and by its UV spectrum. 16-O-methylkahweol could be analysed both in various green and roasted Robusta coffees, and furthermore in leaves, but not in beans of different Arabica coffee species.

INTRODUCTION

The two most important commercial coffee species, *Coffea arabica* and *Coffea canephora* var. *Robusta*, differ in their pentacyclic diterpenes: 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 (16-OMC) (Speer and Kölling-Speer, 2001). Recently, a further diterpene was tentatively identified as 16-O-methylkahweol. Being found exclusively in Coffea stenophylla, it should be used for taxonomic classifications (De Roos et al., 1997).

According to our investigations 16-O-methylkahweol (16-OMK) is present in Robusta coffee beans as well (Speer et al., 2000; Kölling-Speer and Speer, 2001).

RESULTS AND DISCUSSION

In the HPLC chromatograms of various Robusta coffees, a small peak was discovered in addition to the well known diterpenes **(**Figure 1**)**. By comparing the UV spectrum of the peak with those of the known diterpenes **(**Figure 2, Figure 3**)** and by analysing the results of GC/MS and LC/MS the peak was clearly identified as 16-O-methylkahweol. Comparing the CI-mass spectrum of 16-OMK **(**Figure 4A**)** with that of 16-OMC **(**Figure 4B**)**, the typical mass fragment ions are diminished by two for 16-OMK.

Twenty-four green Robusta coffees from different cultivation areas of Africa, Asia, and South and Central America were analysed for their diterpene contents using the DIN method No. 10779. In contrast to the findings of de Roos et al. (1997), 16-OMK was clearly identified in twenty-one of the twenty-four Robustas that were tested, including coffee from Ivory Coast (about amounts see Poster Contents of Diterpenes in Green Coffees, ASIC 2001).

16-OMK was also detected in roasted commercial coffees, both in pure Robusta coffees and in mixtures with Arabica coffees. Investigations revealed that 16-OMK is mainly esterified with fatty acids as described before for the other three diterpenes (Speer, 1991; Kurzrock and Speer, 1997).

Furthermore, 16-OMK was found in leaves of various Arabica coffee species, but not in beans of Arabicas. This could be expected because the 16-O-methyl derivative of cafestol is detectable in leaves of Arabicas as well but not in the beans (Kölling-Speer and Speer, 1997).

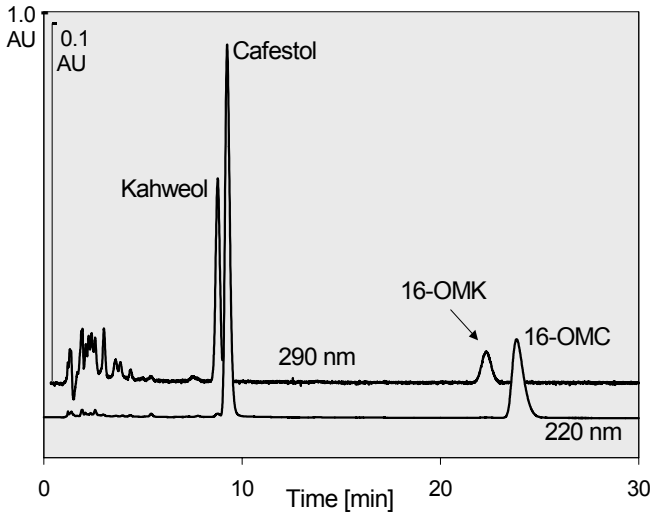

Figure 1. HPLC chromatogram of an Indonesia Robusta coffee

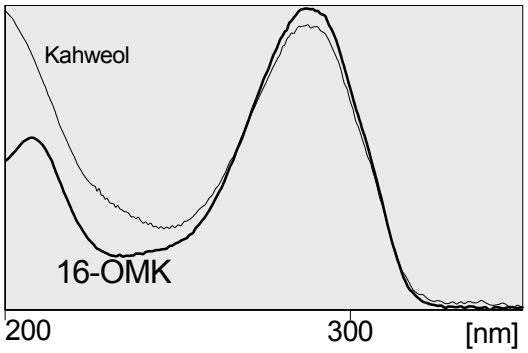

Figure 2. UV-spectra of kahweol and 16-OMK

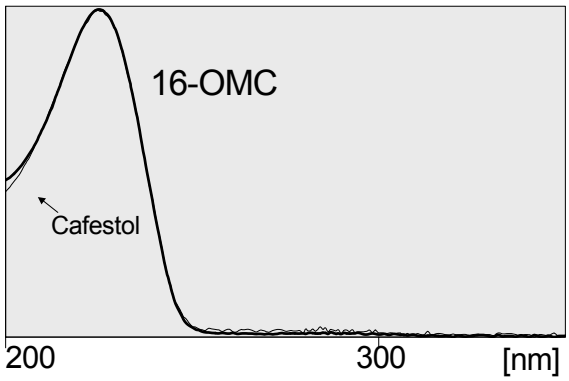

Figure 3. UV-spectra of cafestol and 16-OMC

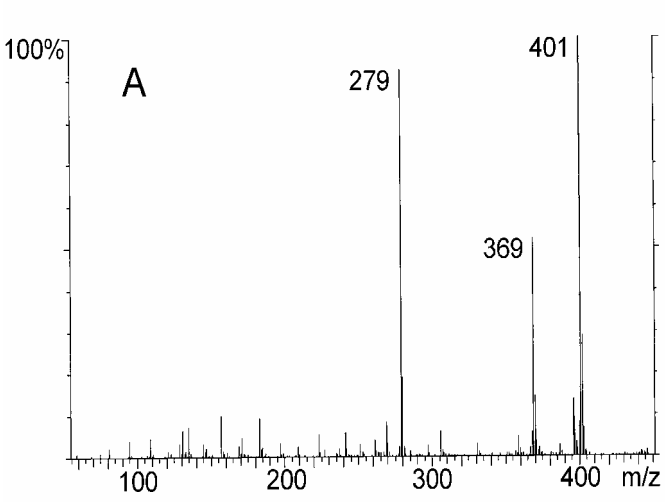

Figure 4A. CI-mass spectrum of silylated 16-OMK; mass fragment ions: m/z 401 (M+1), m/z 369 (M-OCH_3) and m/z 279 (M-OCH_3-OTMS)

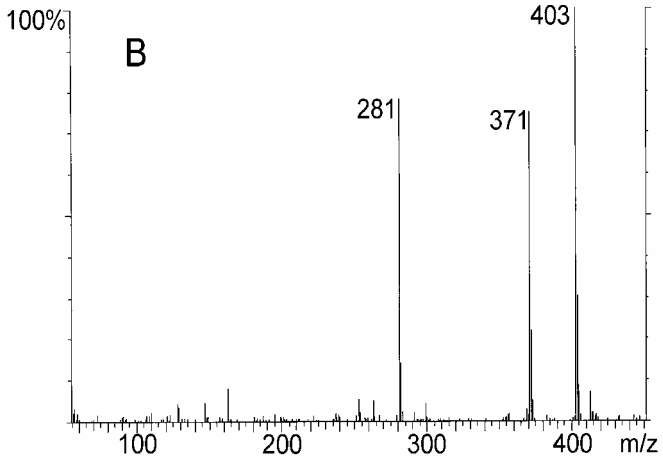

Figure 4B. CI-mass spectrum of silylated 16-OMC; mass fragment ions: m/z 403 (M+1), m/z 371 (M-OCH_3) and m/z 281 (M-OCH_3-OTMS)

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Contents of Diterpenes in Green Coffees Assigned for the European Market

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SUMMARY

In twenty Arabica and twenty-four Robusta green coffees from Africa, Asia, and South and Central America, the contents of the diterpenes cafestol, kahweol, 16-O-methylcafestol, and 16-O-methylkahweol were analysed using the DIN method No. 10779. Quantitative data of 16-O-methylkahweol in Robusta coffees are presented for the first time.

INTRODUCTION

Various diterpenes were analysed in coffee beans: cafestol and kahweol in Arabica coffees and cafestol, kahweol, and 16-O-methylcafestol (16-OMC) in Robusta coffees (Figure 1). The quantitative data published so far are comparable but only imprecisely because of differing extraction solvents and methods of determination (HPLC, GC). Furthermore, the contents were referred to lipid on the one side and to dry weight on the other side (Speer et al., 1991; Speer et al., 2000; Frega et al., 1994; Trouche et al., 1997). No quantitative data were available for the diterpene 16-O-methylkahweol (16-OMK), which was recently detected in Robusta coffees (Speer et al., 2000; Kölling-Speer and Speer 2001).

Therefore, the contents of the four diterpenes were now determined by means of a uniform method in numerous Arabica and Robusta coffees. The green coffees, which were obtained from the Deutscher Kaffee-Verband e.V. in April and October 2000, were assigned for the European market

METHOD

The determination was carried out using the DIN method No. 10779 (1999). This method includes extraction of the coffee oil with tBME, subsequent saponification and isolation of the unsaponifiable matter. Quantification of the diterpenes was obtained by RP-HPLC with UV-detection at 220 nm and 290 nm on the basis of external calibration using standard substances. Only the content of 16-OMK was calculated as kahweol. The detection limits of all four diterpenes were about 0.010 g/kg.

RESULTS

Twenty Arabica and twenty-four Robusta coffees from various cultivation areas were investigated.

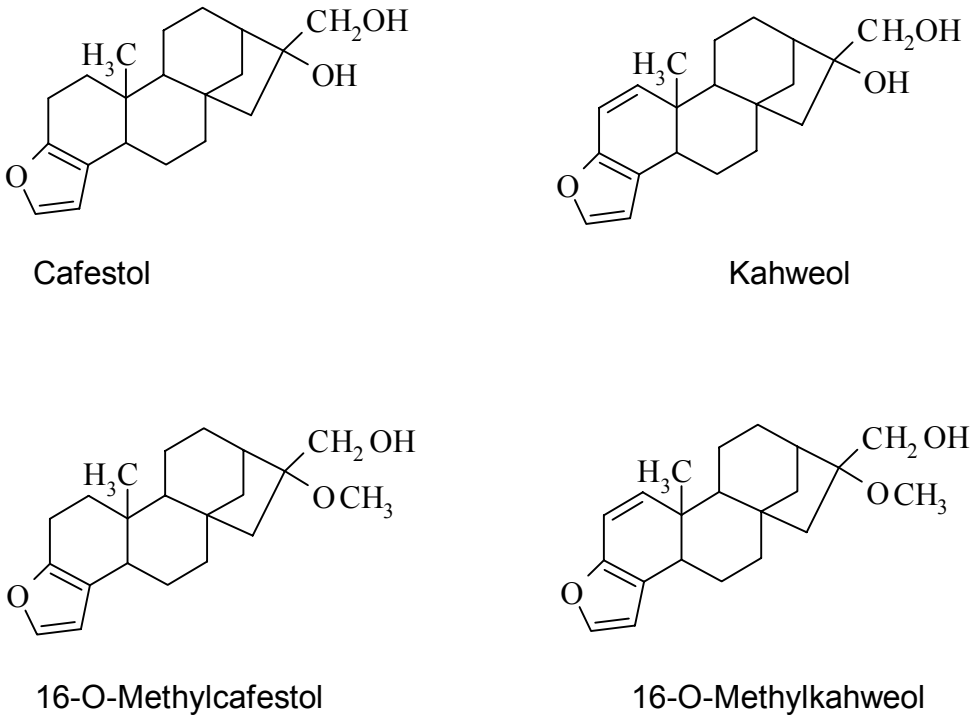

Figure 1. Structural formulae of the four diterpenes

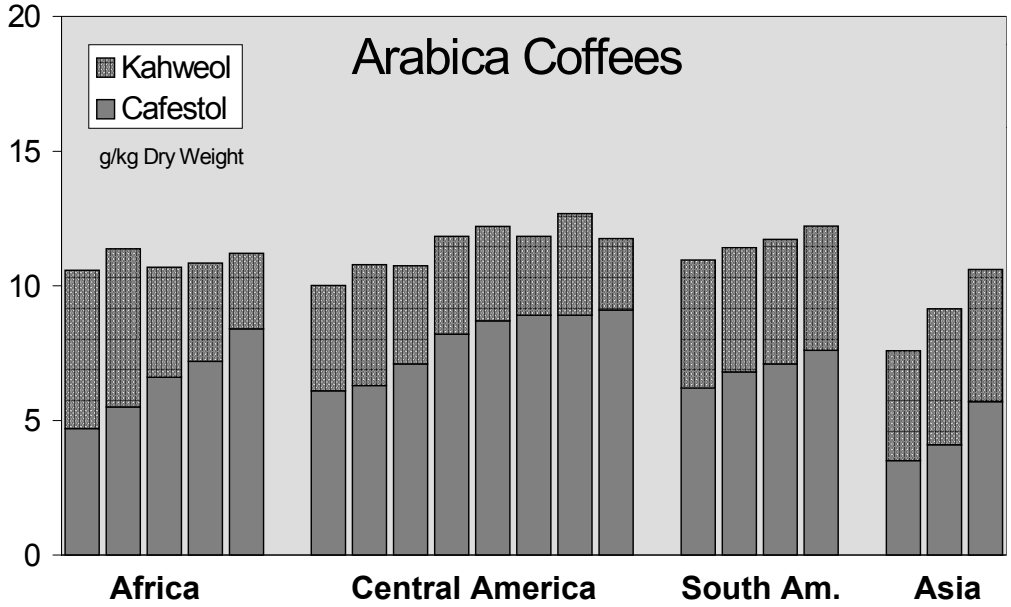

Figure 2. Contents of cafestol and kahweol in Arabica coffees

In the Arabica coffees (Figure 2), only cafestol and kahweol were found with more cafestol than kahweol in each case. 16-OMC was not detected, which was expected because 16-OMC is the reliable indicator substance for Robusta coffee. The content of cafestol ranged from 3.5 to about 9.1 g/kg, and kahweol was determined from 2.7 to about 5.8 g/kg dry weight of coffee.

In the Robusta coffees (Figure 3), in addition to cafestol and the expected 16-OMC, kahweol and 16-OMK were detected. The contents of the main diterpenes cafestol and 16-OMC ranged from 2.2 to 6.8 g/kg and from 0.9 to 2.4 g/kg dry weight, while the contents of kahweol and 16-OMK as minor components were significantly lower. The values of kahweol

were in most cases smaller than 0.1 g/kg, and the amounts of 16-OMK were below 0.03 g/kg dry weight.

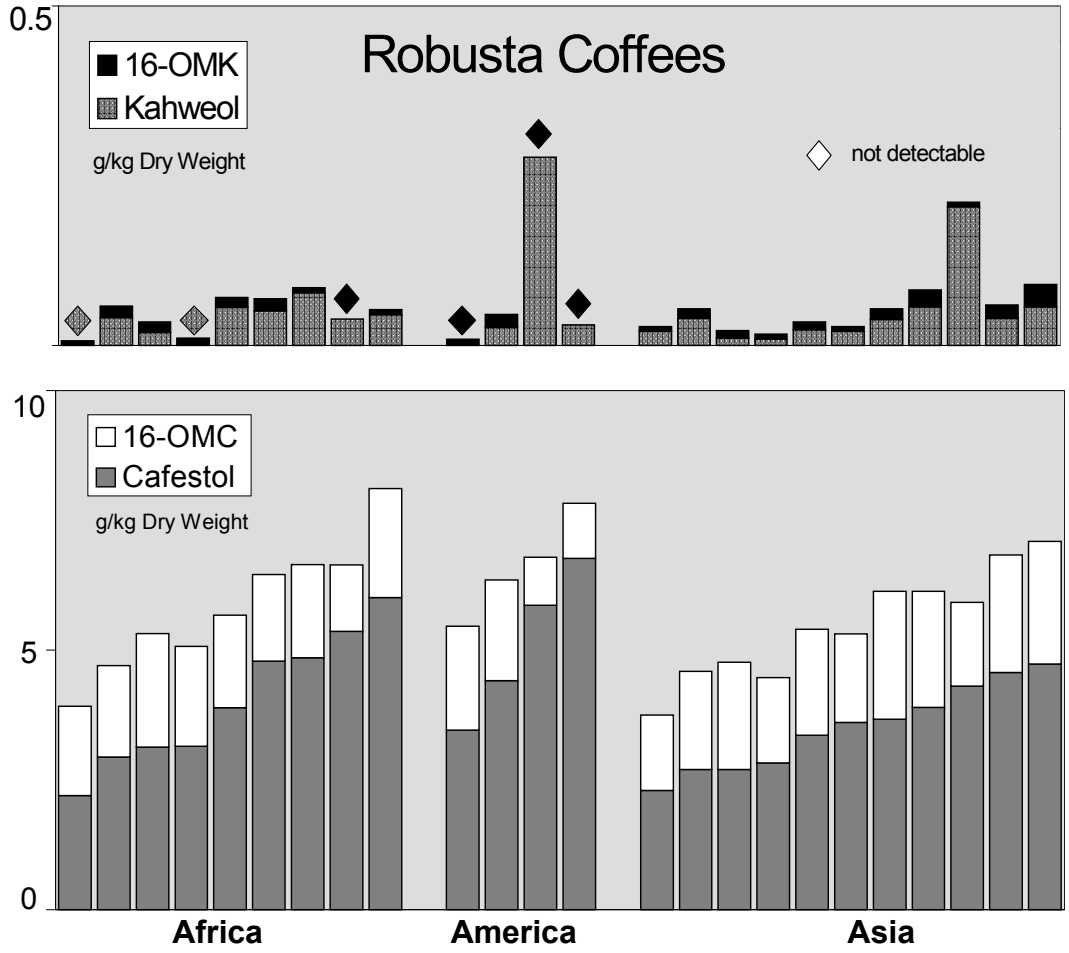

Figure 3. Contents of cafestol, 16-OMC, kahweol, and 16-OMK in Robusta coffees

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Identification of Kahweol Fatty Acid Esters in an Arabica Coffee by LC/MS

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SUMMARY

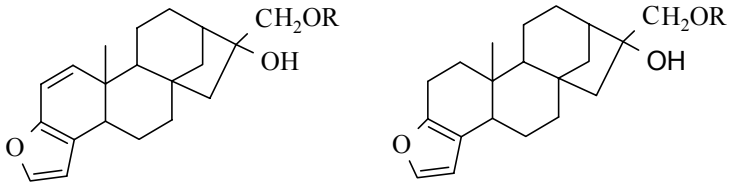
In order to identify kahweol fatty acid esters in Arabica coffees, the isolated diterpene esters were analysed by means of LC/MS/DAD. After studying the fragmentation of synthesised kahweol palmitate we identified 12 kahweol esters, 7 of which are described here for the first time.

ZUSAMMENFASSUNG

Zur Identifizierung der Kahweolfettsäureester im Arabica-Kaffee wurden die isolierte Diterpenesterfraktion mittels LC/MS/DAD analysiert. Nach Ermittlung des Fragmentierverhaltens bei der LC/MS am Beispiel von synthetisiertem Kahweolpalmitat wurden insgesamt 12 Kahweolester identifiziert, von denen 7 Verbindungen bisher noch nicht beschrieben worden sind.

INTRODUCTION

Kahweol and other diterpenes (Figure 1) of coffee oil were increasingly at the centre of interest because of their physiological effects. In human subjects, e.g., they may increase the cholesterol level and the activity of gluthathione-S-transferase (Ratnayake et al., 1993; Heckers et al., 1994; Urgert et al., 1995; Urgert and Katan 1997; Lam et al., 1982).



Kahweol Cafestol

R = H : Free Diterpene
R = Fatty acids : Diterpene Esters

Figure 1. Structural formulae of the diterpenes

Investigations have shown that only a small portion of kahweol is free (< 200 mg/kg d.m.) (Kölling-Speer et al., 1999), while the main part is esterified with fatty acids. Because only few compounds are known at present, we tried to identify further kahweol esters by means of LC/MS.

LC/MS

Instruments: HPLC Series 1100 of HP equipped with a diode-array detector and a Bruker Esquire mass spectrometer.

Chromatography: Nucleosil 120-3 C_{18}, 250 x 4 mm, acetonitrile/isopropanol (70+30, v/v), 0.6 ml/min. Mass spectrometry: positive ionisation, ion source: atmospheric pressure ion source (APCI), APCI-temperature: 350°C, gas temperature (nitrogen): 330°C, nebulizer gas pressure: 60 psi, flow rate of the dry gas: 4.0 ml/min, CID voltage: 50 V.

RESULTS

The isolated kahweol esters (Kurzrock, 1998; Kurzrock and Speer, 1997) were elucidated using LC/MS/DAD (Figure 2). For studying the fragmentation of the esters in LC/MS, we analysed first synthesised kahweol palmitate (Figure 3A) (Kurzrock, 1998; Kurzrock and Speer in press).

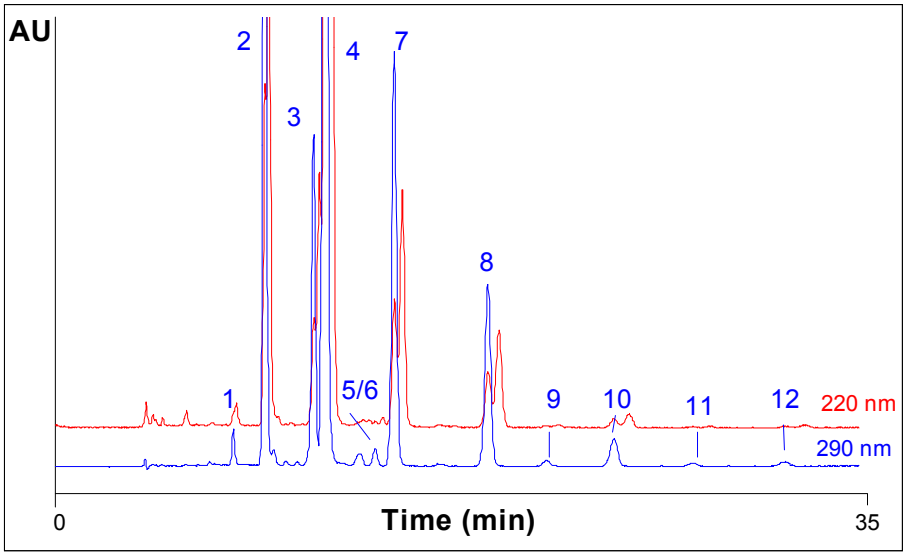


Figure 2. HPLC-chromatogram of diterpene esters (λ = 220 nm: cafestol esters and kahweol esters, λ = 290 nm: only kahweol esters, numbers: see Table 1)

Based on these results, the different kahweol esters (Figure 3B) of an Arabica coffee could be identified afterwards by means of their retention times, their UV-spectra, their ions $[M+H]^+$, and $[M+H-H_2O]^+$ as well as by means of the fragment ions m/z 279 and m/z 131 (fragment ions of kahweol).

Altogether, 12 different kahweol fatty acid esters were detected. Besides the known compounds seven new kahweol esters (*Table 1) were identified.

Table 1. Kahweol fatty acid esters identified by LC/MS/DAD

Peak no.	Kahweol Ester	$[M+H]^+$	$[M+H-H_2O]^+$
1*	kahweol linolenate	575	557
2	kahweol linoleate	577	559
3	kahweol oleate	579	561
4	kahweol palmitate	553	535
5*	kahweol heptadecanate	567	549
6*	kahweol gadoleinate	607	589
7	kahweol stearate	581	563
8	kahweol arachidate	609	591
9*	kahweol henicosanate	623	605
10*	kahweol behenate	637	619
11*	kahweol tricosanate	651	633
12*	kahweol lignocerate	665	647

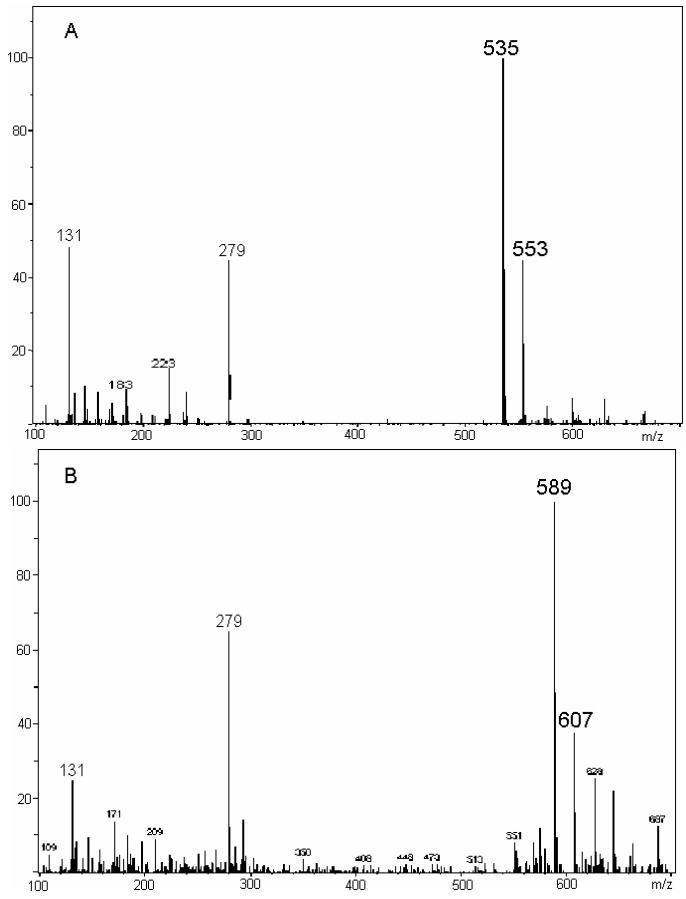


Figure 3. LC/MS mass spectra of a): kahweol palmitate (synthesized) and b): kahweol gadoleinate

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Liquid-Air Partition Coefficients of Coffee Flavour Compounds: A Novel Approach Using Proton-Transfer-Reaction Mass-Spectrometry

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INTRODUCTION

Liquid-air partition coefficients of aromatic volatile organic compounds (VOC's) are of importance to the optimisation of food processing techniques, and to the understanding of a variety of food properties. For instance, they are of significance for the design of controlled release systems, the modelling and optimisation of aroma recovery processes, and the above-the-cup aroma of beverages.

Controlled flavour release

Understanding the link between food composition, food material properties, food microstructure and the ensuing flavour release characteristics will help in the design of controlled release systems. In particular in view of the growing demand for novel, functional and low-fat products with new food textures and compositions, a more rational approach to match flavour release properties to consumer expectations will be warranted.

Aroma recovery in food processing

Recovery of specific aroma fractions from food is becoming a widespread practice in the food industry. Labile flavour fractions are extracted early on in the process and are reincorporated only towards the end of process. This allows processed foods to retain their fresh flavour characteristics. Knowledge of partition coefficients allows modelling and optimising recovery process.

These are just two examples relevant to the food industry, which would benefit from an accurate experimental determination of liquid-gas partition coefficients. In a recent publication, we introduced a novel approach to measure partition coefficients (Henry's law constants, HLC's), using PTR-MS (Karl et al., submitted). Comparing to alternative state-of-the-art techniques, this novel approach is fast and less prone to artefacts.

Here we would like to apply this approach to a series of coffee flavour compounds. First we determine the partition coefficients of 12 compounds in water and compare these to values reported in the literature. We then discuss the extension of the method to liquid coffee and report values for two impact flavour compounds of coffee (2-methylpropanal and 3-methylbutanal).

EXPERIMENTAL

Definition of partition coefficients

This paper is concerned with the partitioning of a volatile compound between a liquid and a gas phase under equilibrium conditions. Depending on the scientific field, different definitions are used to express the liquid-gas partitioning (Sander, 1999). Here, we shall use the Henry's law constant which is defined as HLC = H[M/atm] = c(aq)/p(gas). c(aq) is the molar concentration of the VOC in the liquid and p(gas) is the partial pressure in the gas-phase. HLC is the partition coefficient at standard conditions for an ideal, dilute solution. As concentrations and partial pressures increase, deviations from Henry's law become noticeable. For this reason, solutions, which obey Henry's law, are often termed ideal dilute solutions. An alternative definition used in the food and flavour science, and which expresses the partitioning in terms of volatility, is K = c(gas)/c(aq), with c(gas or liq) being the concentration (w/v) of the VOC in the liquid and in the gas respectively. Both definitions are related via the following transformation: HLC = 1/(R*T*K) 1/{[(l*atm) /(mol*K)] * [K] * [(mg/l)/(mg/l)]}.

Dynamic measurement of HLC

Recently we introduced a novel technique to measure HLCs (Karl et al., submitted). It combines a stripping cell first proposed in 1979 by D. Mackay (Mackay et al., 1979) with Proton-Transfer-Reaction Mass-Spectrometry (PTR-MS) (Lindinger et al., 1993; Yeretzian et al., 2000). Figure 1 gives a schematic overview of the experimental set-up. Small bubbles of air are introduced through a sintered glass disk into a stripping vessel. The vessel contains either water with traces of pure VOCs, or a reconstituted soluble coffee solution. On their way up through the liquid solution, the gas-phase inside the bubbles (~1 mm ∅) equilibrates with the liquid, according to Henry's law. As described in (Karl et al., submitted), checks were made in order to assure that equilibrium is reached in the air bubbles reaching the surface of the solution. Among all those we looked at, a distance of 20 cm between the sintered glass disk and the surface was sufficient to reach equilibrium, ethanol being the one with the longest equilibration time. Here we have used a 40 cm equilibration length.

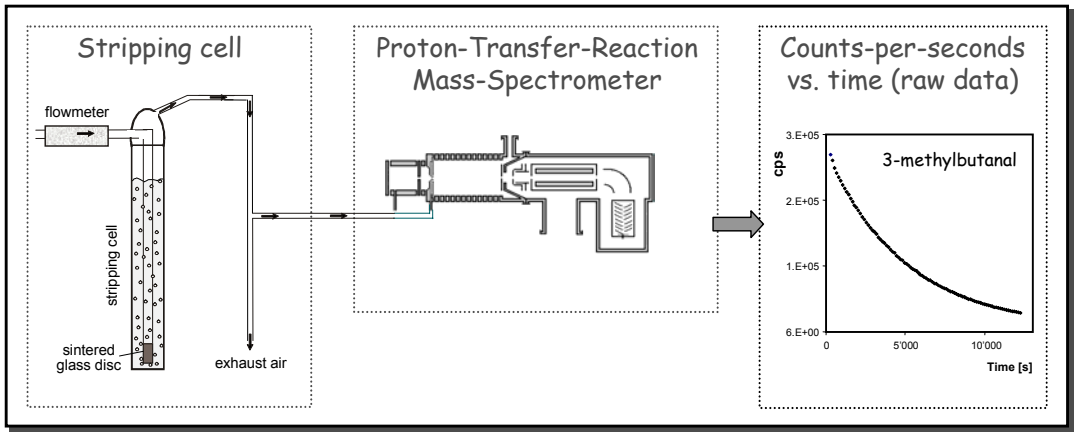

Figure 1. Experimental set-up to measure HLC's. Humidified air is introduced into the stripping cell (V=100 ml, Liquid Height = 40 cm), which then bubbles through the cell removing dissolved volatile compounds from the liquid according to Henry's Law (left frame). The concentration of the VOC's as a function of time is measured online by continuously introducing the stripped gas into the PTR-MS (middle frame). We observe an exponential decrease in the HS concentration from which we can calculate the HLC (right frame)

Due to mass transport of dissolved VOC's by means of the air bubbles, their concentration in the solution, and consequently in the HS, gradually decrease as a function of time according to:

$$\ln(cps(t)) = -\frac{F}{H \cdot V \cdot R \cdot T} \cdot t + \ln(cps_0) \quad (1)$$

where cps_o is the initial counts-per-seconds in the HS, and cps(t) the counts-per-seconds at time t. F [liter/s] is the gas flow through the vessel, H [M/atm] the Henry's law constant, V [litre] the liquid volume, R the molar gas constant (0.08205 litre atm mol^{-1} K^{-1}) and T [K] the temperature. Plotting the measured counts-per-seconds on a logarithmic scale versus time, we obtain a linear relation. The only unknown factor in the slope is the HLC. Hence, solving the equation for the HLC we obtain the following expression:

$$H\left[\frac{M}{atm}\right] = -\frac{F\left[l/s\right]}{slope\left[s^{-1}\right] \cdot V[l] \cdot R\left[\frac{l \cdot atm}{mol \cdot K}\right] \cdot T[K]} \quad (2)$$

Since only the ratio of count rates is needed to calculate the HLC, the system does not have to be calibrated and experimental errors originating from adsorption on glass surfaces and tubings are cancelled out (Buttery et al., 1969).

RESULTS AND DISCUSSION

The accuracy of the method relies on the efficacy with which the volatile compounds are stripped by the gas. More specifically, in order to determine the slope of the cps(t) vs. time plot with sufficient accuracy, one ought to reduce the concentration in the liquid by at least 5%. For highly volatile compounds, this can be easily achieved with a moderate gas flow within minutes. For less volatile compounds longer stripping times are required.

In order to quantify the impact of volatility on the time-scale of the experiment we analysed three coffee flavour compounds in water, which are known to have very different volatility: ethyl-2-methylbutyrate (high volatility), 2-methylbutanal (medium volatility), and pentanedione (low volatility). Using 100 ml solutions and a flow of 100 ml/min, Figure 2 shown the ln(cps) vs. time plot for all three compounds on an identical scale. Ethyl-2-methylbutyrate can be stripped to more than 50% within 30 minutes, which is more than enough for an accurate determination of the slope. In contrast, hardly any reduction is observed in the case of pentanedione within the same time frame. Based on these experiments (100 ml/min gas-flow; 100 ml solution) we can state that:

- for highly volatile compounds - HLC < 1 [M/atm] - the slope can be accurately determined within one hour.
- for medium volatile compounds - 1 M/atm < HLC< 10 [M/atm] - the liquid must be stripped for several hours.
- for low volatile compounds - HLC > 10 [M/atm] - time for analysis can exceed ten hours.

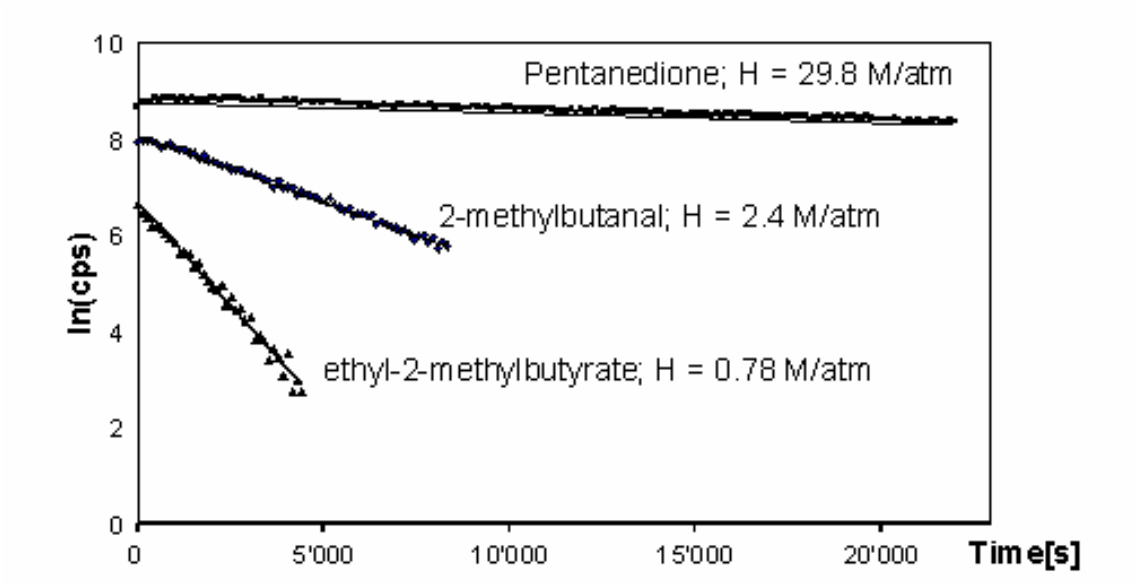

Figure 2. The volatility of compounds determines the time-scale of the experiment. 100 ml solutions of three compounds of very different volatility were stripped at gas flow of 100 ml/min. The HLC's, determined from the slopes, are also given

A way of reducing the stripping time is to increase the flow rate. But there are limits to that, since foam can form at higher flow rates. This is particularly a problem in coffee solutions, which starts to foam already at strip-rates of 100-200 ml/min. This problem can largely be resolved by a modification of the striping cell design. In short, instead of using one single stripping cell, a configuration of two stripping cells in row is used. The first stripping cell contains a solution of the VOC to be analysed, whereas the second contains initially pure water. For a discussion of the double-cell configuration please see a forthcoming publication (Karl et al., submitted).

Using the single-cell configuration (Figure 1) we have determined the HLC's at 25°C in water for a series of VOC's of coffee. As shown in Table 1, the measured values agree well with published data.

Currently only 2-methylpropanal and 3-methylbutanal were measured in coffee (reconstituted soluble coffee at 0.5% solid content). They are both of medium volatility and can be measured at a relatively low flow rate (100 ml/min). But even at this low flow rates it was necessary to add some antifoaming agents (10 ml/l of Struktol: alcoxylated fatty acid esters (GmbH&Co.)) to prevent foaming within the 2-3 hours of the experiment. Checks were made with 3-methylbutanal in water, to evaluate the impact of the antifoaming agent on the HLCs. It was found that the HLC was only affected for Struktol-concentrations above 50 ppm. Hence we believe that the antifoaming agent does not affect the values reported in Table 1. Currently, we are putting our effort in finding means to eliminate the antifoaming agents without having foam formation in coffee.

Figure 3 show the ln(cps) vs. time plots for 3-methylbutanal in water and in coffee. Irrespective of the matrix, the slope is the same in both systems, indicating that the coffee matrix does not affect the HLC. The vertical shift along the concentration axis is due to the different absolute concentrations of 3-methylbutanal in the systems. Since only the slope enters the calculation of HLC, absolute concentrations do not interfere with the determination of the HLC. Analogous to 3-methylbutanal, HLC of 2-methylpropanal in water and coffee are indistinguishable (Table 1).

Table 1. Measured HLCs in water and in coffee with standard deviations. Al values are reported at 25°C, if not otherwise noted. We also included values from the literature. To avoid foaming, we added in the experiments with coffee 10 mg/l of Struktol (antifoaming agent). Furthermore, in order to increase the concentration of the investigated compounds in coffee, we doped the coffee solution with 1 mg/l of the VOC's. * Personal communication by P. Pollien and D. Roberts (to be published)

	HLC_{water} [M/atm] Literature	K_{water} [(mg/l)/(mg/l)] Literature	ref.	HLC_{water} [M/atm]	K_{water} [(mg/l)/(mg/l)]	Std dev. if replicate	$HLC_{0.5\%\ coffee}$ [M/atm]	$K_{0.5\%\ coffee}$ [(mg/l)/(mg/l)]	Std dev. if replicate
3-methylbutanal	2.02 (28°C)	2.00E-02	(Nelson and Hoff, 1968)	2.63	1.56E-02	3.4%	2.63	1.55E-02	
2-methylbutanal	*2.51 (30°C)	1.60E-02	(Roberts and Pollien, personal communication)	2.44	1.67E-02				
2-methylpropanal	5.11	8.00E-03	(Roberts and Pollien, 1997)	3.60	1.14E-02	3.4%	3.64	1.12E-02	5.7%
methanethiol				0.92	4.44E-02				
dimethylsulfide	0.615 (20°C)	6.76E-02	(Vitenberg et al., 1975)	0.48	8.46E-02				
dimethyldisulfide	0.840 (20°C)	4.95E-02	(Vitenberg et al., 1975)	0.61	6.70E-02				
2-ethyl-3,5(6)dimethyl-pyrazine	*83.3 (30°C)	4.86E-04	(Roberts and Pollien, personal communication)	73.2	5.59E-04				
ethyl acetate	1.0 - 5.49	0.745E-2 - 4.09E-2	(Jones et al., 1988; Dewulf et al., 1999)	3.48	1.17E-02				
ethyl butyrate	3.03	1.35E-02	(Landy et al., 1996)	2.18	1.87E-02	1.4%			
ethyl-2 methylbutyrate	2.68 (30°C)	1.50E-02	(Amoore and Buttery, 1978)	0.78	5.21E-02				
2.3-butanedione	36.5 (30°C)	1.10E-03	(Amoore and Buttery, 1978)	44.2	9.25E-04				
2.3-pentanedione	*19.1(30°C)	2.10E-03	(Roberts and Pollien, personal communication)	29.8	1.37E-03				

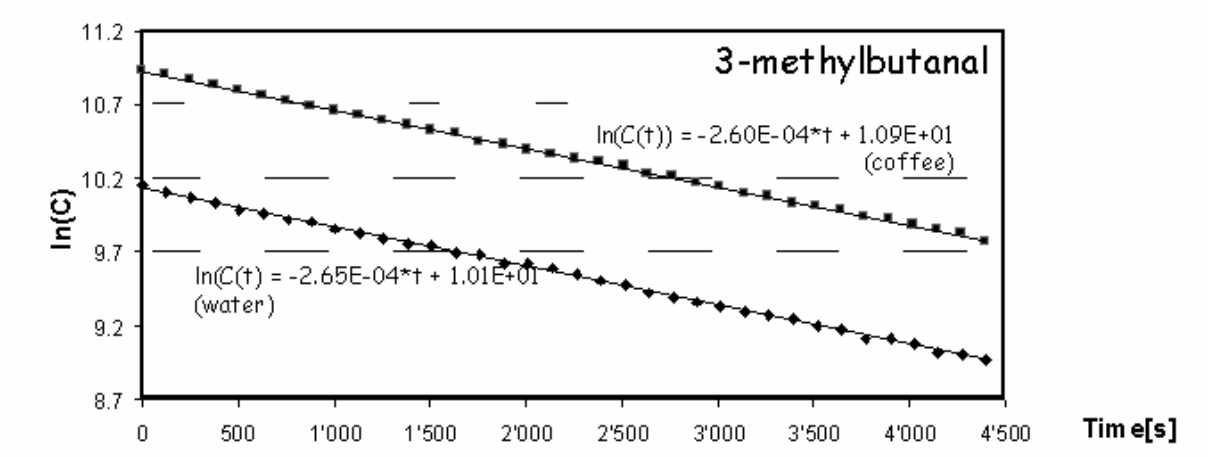

Figure 3. The HLC of 3-methylbutanal was determined in water and in coffee. The HLC was essentially indistinguishable as can be seen from the slopes. Both experiments were performed in 100 ml solutions with addition of 10 ppm Struktol (antifoam agent)

CONCLUSIONS

A novel, fast and robust method for the determination of HLC's is applied to VOC's of coffee, in water and in coffee solution. A solution of a VOC is stripped with a constant gas flow, and the HS concentration of the VOC measured on-line by PTR-MS. The HLC is determined from the slope of a ln(cps) vs. time plot. Hence it is independent of the absolute concentrations, and no calibration or internal standards are needed. For highly volatile compounds, the HLC can be determined within less than one hour with a standard deviation of better than 3%. For medium and low volatile compounds the time-scale of the experiment varies from one to several hours.

We have determined the HLC's of 12 flavour active VOC's in water. For two of them 2-methylpropanal and 3-methylbutanal we also measured HLC's in coffee solutions, and found no difference with values for water solutions. In coffee, foam formation at higher strip-rates complicates the experiments, and we are currently working on modifications of the set-up to circumvent this problem.

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Dry Matter, Condensed Tannins, Chlorogenic Acid and Caffeine Contents in Pulp Obtained by Ecological (Dry) Pulping of Ripe Coffee Berries

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SUMMARY

In some coffee producing countries, especially those concerned about the contamination of water streams, there is a trend toward replacing the traditional wet-processing of ripe coffee berries by the ecological (dry) processing method. A comparison was made of the dry matter, condensed tannins, chlorogenic acid and caffeine contents between coffee pulp produced by ecological pulping and by manual pulping. The two types of pulp were obtained from the cultivars Catimor and Yellow Catuai. The dry matter content decreased around 20% in the pulp obtained from Catimor by ecological pulping as compared with that obtained manually, whereas in Yellow Catuai there was no variation between the two pulps. Condensed tannins, chlorogenic acid and caffeine contents were also significantly (P<0.05) different in the two types of pulp obtained from Catimor, whereas in Yellow Catuai there were no significant differences between them. It is apparent that the transportation of the Catimor coffee berries to the ecological pulper was made with the aid of water and this affected the composition of the pulp obtained (lower dry matter content and loss of some constituents). Otherwise there is no difference with regard to the chemical composition of the two types of pulp as shown by the Yellow Catuai cultivar.

RESUMEN

En algunos países productores de café, especialmente aquellos preocupados con la contaminación de los cursos de agua, existe una tendencia a reemplazar el tradicional beneficio húmedo de las cerezas maduras de café con el método de procesamiento ecológico (seco). Se hizo una comparación de los contenidos de materia seca, taninos condensados, ácido clorogénico y cafeína entre pulpas de café producidas por despulpado ecológico y por despulpado manual. Los dos tipos de pulpa se obtuvieron de los cultivares Catimor y Catuai Amarillo. El contenido de materia seca en la pulpa de Catimor obtenida por despulpado ecológico disminuyó cerca de 20% comparado con el que se obtuvo por despulpado manual, mientras que en Catuai Amarillo no hubo variación alguna entre las dos pulpas. Los contenidos de taninos condensados, ácido clorógenico y cafeína fueron también significativamente (P<0.05) diferentes en los dos tipos de pulpa obtenidos de Catimor, mientras que en Catuai Amarillo no hubo diferencias significativas entre ellas. Es aparente que la transportación de las cerezas de café de Catimor hacia el despulpador ecológico se hizo con la ayuda de agua y ésto afectó la composición de la pulpa resultante (bajo contenido de materia seca y pérdida de algunos constituyentes). Por lo demás, no hay diferencia alguna con respecto a la composición química de los dos tipos de pulpa tal como se demostró con el cultivar Catuai Amarillo.

INTRODUCTION

Coffee wet-processing factories are located generally in the vicinity of water streams and part of the produced wastes are discharged into these streams. In order to reduce the risk of water streams contamination in some coffee producing countries there is a trend toward replacing the traditional coffee wet-processing with the so-called ecological processing. In the latter method of processing no water is used as transportation means and for the pulping itself, whereas for the removal of the mucilage a small amount of water is used (~1.25 l/kg of green coffee).

The pulp produced by the coffee ecological processing presumably has the same chemical composition as the one obtained by hand (manual) pulping (Ramírez-Martínez, 1998). Although there are many possible uses for the coffee pulp (production of feeds, beverages, vinegar, biogas, caffeine, pectin, pectic enzymes, protein, mushrooms, and compost), the chemical composition of the pulp obtained by ecological pulping has not been examined. There was a need to analyse the almost intact coffee pulp produced by the ecological pulping and to find new or improved uses for this kind of coffee pulp (Ramírez-Martínez and Clifford, 2000). In this paper a comparison of the contents of certain constituents is made between the pulps obtained ecologically and manually.

MATERIALS AND METHODS

Ripe coffee berries of the cultivars Yellow Catuai (*Coffea arabica* var. Catuai) and Catimor (Timor Hybrid × *C. arabica* var. Caturra) were supplied by the National Agricultural Research Institute (INIA) at Bramón, Táchira, Venezuela. Representative samples of pulp were obtained by manual pulping and by ecological pulping using PENAGOS equipment consisting of a conic vertical coffee pulper coupled to an ascending vertical mucilage remover.

Dry matter determination was made by oven drying to constant weight at 105°C. Condensed tannins were extracted by mixing 10 g of coffee pulp with 50 ml of 70% acetone in an Oster blender and centrifuging at 20000 × g for 10 min; the pellet was dispersed twice in 70% acetone and re-centrifuged. The pooled supernatants were concentrated almost to dryness in a rotavapor and the resulting slurry was dissolved in 25 ml of acetone. This final solution was subjected to Porter's autooxidative reaction (Porter et al., 1986) and the colour developed was measured at 550 nm using as standard a condensed tannin preparation from Canephora coffee pulp (González de Colmenares et al., 1994).

For the extraction of chlorogenic acid (5-caffeoylquinic acid) and caffeine a similar procedure to that for tannins was used, with 80% methanol as the extracting solvent. The methanolic extract was assayed for chlorogenic acid and caffeine using a 250 mm x 4.6 mm Hichrom Spherisorb 5µ ODS2 analytical column with a flow of 0.5 ml/min (Ramírez-Martínez, 1988; Clifford and Ramírez-Martínez, 1991). The HPLC analysis was made by linear gradient elution using methanol (A) and 0.5% phosphoric acid (B): 20% of A in B to 100% A in 60 min. Chlorogenic acid, caffeine and all other reagents were standard analytical grade items from reputable commercial sources.

RESULTS AND DISCUSSION

Figure 1 shows the chromatograms of the HPLC analytical separation of chlorogenic acid and caffeine obtained from both Yellow Catuai pulps. It can be seen that the two chromatographic

profiles of the methanolic extracts from coffee pulp obtained by ecological and manual pulping are identical.

Dry matter contents for both types of pulp prepared from Catimor and Yellow Catuai are presented in Table 1. It is evident that the pulp obtained from Catimor coffee berries by ecological pulping has a lower dry matter content (~20% less) than that obtained by manual pulping. The value of the dry matter content of the pulp obtained by ecological pulping is similar to that of the pulp obtained by the traditional wet-processing (Ramírez-Martínez et al., 1999). These results suggest that the Catimor coffee berries were fed to the vertical conic coffee pulper with the aid of water.

In Table 2 the contents of condensed tannins, chlorogenic acid and caffeine are presented for the two types of Catimor pulp. It is observed that condensed tannins and chlorogenic acid contents are significantly (P<0.05) lower in pulp obtained by ecological pulping than in pulp obtained manually, whereas caffeine content is higher. These unexpected results can be explained on the basis of the loss of some constituents (among them condensed tannins and chlorogenic acid) in the pulp obtained by ecological pulping and a seeming increase in caffeine due to the lower solids content.

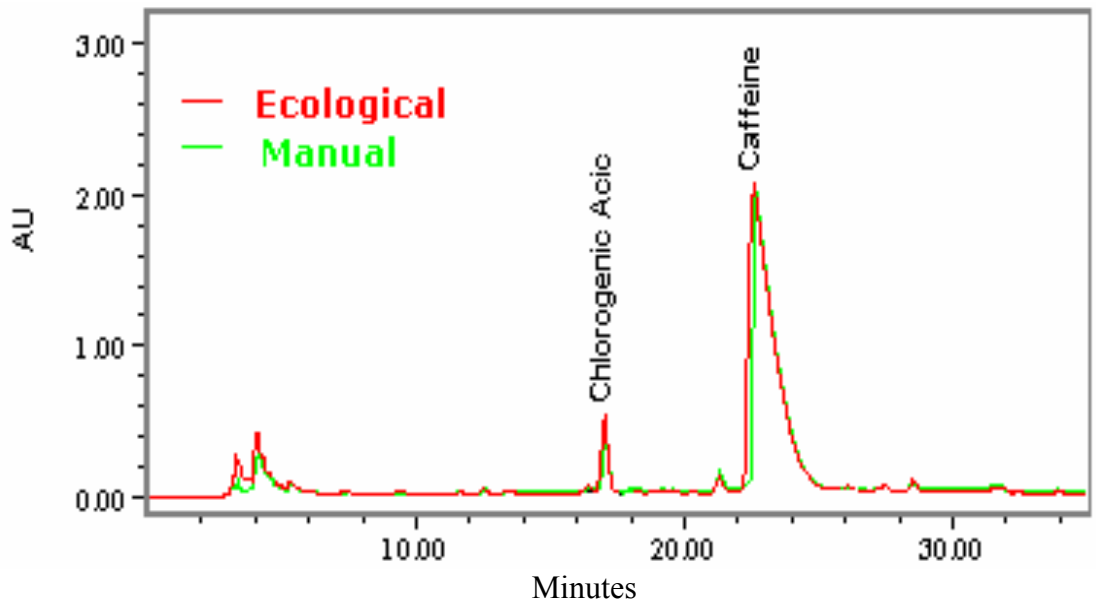

Figure 1. Chromatograms of the analytical HPLC separation of chlorogenic acid and caffeine obtained from Yellow Catuai pulps

Table 1. Coffee pulp dry matter content (% dry basis)

Pulping Type	Cultivar	
	Catimor	Yellow Catuai
Ecological	13.0	17.8
Manual	16.4	17.1

Table 3 shows the contents of condensed tannins, chlorogenic acid and caffeine in coffee pulp obtained from Yellow Catuai by ecological pulping and by manual pulping. It was found that there are no differences statistically significant (P<0.05) between the two pulping types with regard to the content of each of these constituents.

Table 2. Condensed tannins, chlorogenic acid and caffeine contents[a] (% dry basis) in Catimor pulp obtained by ecological and manual pulping

Pulping Type[b]	Condensed Tannins	Chlorogenic Acid	Caffeine
Ecological	1.43 ± 0.16	0.12 ± 0.03	1.24 ± 0.03
Manual	2.40 ± 0.48	0.21 ± 0.07	1.05 ± 0.17

[a]*Mean ± S.D., N=6*
[b]*Pulping type produced differences statistically significant (P<0.05)*

Table 3. Condensed tannins, chlorogenic acid and caffeine contents[a] (% dry basis) in Yellow Catuai pulp obtained by ecological and manual pulping

Pulping Type[b]	Condensed Tannins	Chlorogenic Acid	Caffeine
Ecological	1.60 ± 0.50	0.11 ± 0.03	1.13 ± 0.12
Manual	1.91 ± 0.59	0.11 ± 0.03	1.15 ± 0.06

[a]*Mean ± S.D., N=6*
[b]*Pulping type did not produce differences statistically significant (P<0.05)*

As previously suggested (Ramírez-Martínez, 1998), the unleached coffee pulp produced by the ecological pulping method proved to have contents of some constituents similar to the pulp obtained manually, and thus to be richer in nutrients than the pulp obtained by the wet-processing method. Sometimes water under pressure is used to hasten the loading process of the conic vertical pulper. This practice is contrary to the purpose of using ecological equipment to process ripe coffee berries in order to preserve the environment uncontaminated. Besides, if there is a genuine interest in a profitable utilisation of coffee pulp obtained by ecological pulping as a raw material for commercial purposes, no water whatsoever should be used as a means of transportation of coffee berries to the pulper.

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Differenciation of Arabica and Robusta Coffee Cup by Physico-chemical and Sensory Parameters and Multivariate Analysis

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SUMMARY

The quality of the cup of coffee depends on the parameters related to the ground roast coffee used for its preparation, and the technological conditions of extraction. With regards to the ground roast coffee used for the preparation, comparison of brew coffee from Arabica and Robusta varieties have been studied from a physico-chemical point of view (Illy, et al., 1995; Nunes et al., 1997) but no sensory comparative studies had been found.

The aim of this work was to differentiate and classify Arabica and Robusta brew coffee by the sensory and physico-chemical characteristics using Principal Component Analysis (PCA) and Discriminant Analysis (DA).

The results of the PCA showed that the PC1 separated Robusta and Arabica samples perfectly. This component (which explained 53.7% of the variance), was mainly related to the foam aspects (foam index, lipids and persistence), and with the physico-chemical and sensory parameters related to the taste of the cup (pH, acidity, caffeine, 5-CQA, bitterness, astringent, total solids, concentration, extraction, residual taste). The PC2 (that explained 16.8% of the variance) included some variables related to the body of the cup (body and solids on filtrate). Some interesting correlation among the variables were found.

The DA proposed a very simple function that explained 100% of the variance and which allowed for classification of the coffee samples into their respective group with a success rate of 100%.

INTRODUCTION

Espresso is an increasingly popular drink. Every day more than 50 million cups of Espresso coffee (EC) are consumed throughout the world (Illy and Viani, 1995; Butler, 1999). By definition, EC is a "polyphasic beverage prepared from roast and ground coffee and water alone, 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 solution of sugars, acids, protein-like material and caffeine, with dispersed gas bubbles and solids" (Illy and Viani, 1995). These characteristics of EC are responsible for its peculiar sensorial properties. A fine EC should have a great amount of persistent, consistent and hazelnut foam with "tiger-skin" effect, a bitter/acid balance taste and a strong body (Illy and Viani, 1995; Cipolla , 1999).

An important factor in the final quality of EC is the ground roasted coffee used which could be defined by the type of coffee and the roasting process. Comparison of EC from Arabica and Robusta varieties have been studied from a physico-chemical point of view (Illy and Viani, 1995; Nunes et al., 1997) but no sensory comparative studies have been found.

The aim of this paper was to differentiate and classify EC's prepared from different botanical varieties (Arabica and Robusta) according to the sensory and physico-chemical characteristics related to visual foam appearance, taste and mouthfeel, using multivariate analysis. Furthermore, correlation among different parameters have been studied.

MATERIALS AND METHODS

Arabica EC (pure *Coffea arabica* from Colombia) and **Robusta Natural blend EC** (80:20 blend of *Coffea canephora* and *Coffea arabica*) were prepared from the 7.5 g of finely ground roasted coffee for a volume of 40 ml using an experimental EC prototype. EC preparation conditions were: water pressure: 9 atm, water temperature: 96ºC (extraction temperature: 90±2ºC), extraction time: 21±3s, holder filter diameter: 38 mm. Two lots of each coffee sample were analysed by triplicate.

The EC samples were cooled at 20ºC, and the **pH** (Orion 420 A Benchtop pH meter), **density** (densitometer), **viscosity** (Ostwald viscosimeter) and **surface tension** (Traube tensiometer) were measured.

The **foam index** was defined as the ratio (in percentage) of EC foam and liquid volumes. The **persistence of foam** was defined as the time (in minutes) that the liquid phase below the cream layer took to appear during cooling at room temperature.

Total solids were determined by oven drying 40 ml of EC to a constant weight (14 h, 102±3ºC). **Extraction rate** was defined as the percentage of total extracted solids with respect to ground roast coffee dose (7.5 g). **Concentration** was defined as the percentage of total solids with respect to the EC volume (40 ml). The **total solids on filtrate** were determined by oven drying 40 ml of EC after filtering with Whatman 1 to a constant weight (14 h, 102±3ºC).

Total lipids were determined by liquid-liquid extraction using trichloromethane.

Caffeine and Trigonelline

For the extraction of caffeine and trigonelline compounds, and cleanup, C_{18} Sep-Pack cartridge 51910 (Waters Corporation Mildford, MA) was used. Pentoxiphylline was used as the internal standard. The HPLC analysis was achieved with an analytical HPLC unit (Hewlett-Packard 1100) equipped with a Rheodyne injector of 20 µl loop, a binary pump and a Diode-array detector. A reversed-phase Hypersil-ODS (5µm particle size, 250 x 4.6 mm) column was used. The mobile phase was an acetonitrile/water (15:85) in isocratic condition at a constant flow rate of 2.0 ml min^{-1} at 25ºC. Detection was accomplished with a diode-array detector, and chromatograms were recorded at 280 nm.

Chlorogenic Acids (5-CQA)

The extraction of 5-CQA and cleanup were carried out according to Bicchi et al., *(*Bicchi et al., 1995*)* in the HPLC equipment described above. Pentoxiphylline was used as the internal standard. A Hypersil-ODS column was used. The conditions of the gradient solvent system used were 100% citrate-acetic acid buffer solution (pH=3.0) for 2 minutes, 85:15 buffer/methanol for 8 minutes, both at a flow rate of 0.8 ml min^{-1}, and 85:15 buffer/methanol for 5 minutes at a flow rate of 1.2 ml min^{-1}, at 25ºC. Detection wavelength was 325 nm.

Sensory Descriptive Analysis

The sensory properties of the EC samples were measured by trained judges using a variation of the Quantitative Descriptive Analysis method (Sidel and Stone, 1993). The attributes (Table 2) were measured in 10cm line scales, typically anchored from 0 (none) to 10 (very high). Descriptive evaluation of the EC samples was then carried out in triplicate over four sessions. Three EC were analysed per session. Each EC was prepared immediately before taste, and served in a monadic presentation in a white porcelain coffee cup labelled with 3-digit codes. The order of presentation was randomised over judges and sessions. All evaluations were conducted under standardised conditions according to UNE 87-004-79 (AENOR, 1997).

Statistical analysis

t-Student analysis was applied to the physico-chemical and sensory data. Principal Component Analysis (PCA) was applied to the analytical and descriptive ratings (based on the Pearson correlation matrix) in order to determine relationships among attributes and differences among EC samples. Factors with eigenvalues greater than 1 were selected. The varimax rotation method was applied. Discriminant Analysis (DA) was performed in order to obtain an easy equation by which EC samples could be classified. Wilks'Lambda stepwise method was used. The criteria were of 0.05 for maximum significance of F to enter and 0.10 for minimum significance of F to remove. All statistical analyses were performed using the SPSS v.10.0 software package.

RESULTS AND DISCUSSION

Table 1. Physico-chemical results

Physicho-chemical parameters (n=6)	**Arabica** X□SD	**Robusta blend** X□SD	**Signif.**
pH	5.4±0.0	5.6±0.0	***
Density (g/ml)	1.010±0.000	1.010±0.000	n.s.
Viscosity (mN/m^2x s)	1.34±0.11	1.26±0.04	n.s.
Surface tension (mN/m)	49.68±1.38	50.52±0.21	n.s.
Foam index (%)	12.30±0.24	20.26±0.54	***
Persistence of foam (min)	30.27±4.49	24.50±2.81	*
Total solids (mg/ml)	41.69±0.95	39.68±1.38	*
Extraction (%)	22.24±0.51	21.17±0.74	*
Concentration (%)	4.18±0.10	3.97±0.14	*
Total solids on filtrate (mg/ml)	39.10±1.27	38.49±1.52	n.s.
Total lipids (mg/ml)	5.85±0.24	4.77±0.42	***
Caffeine (mg/ml)	2.09±0.1	2.88±0.14	***
Trigonelline (mg/ml)	1.15±0.07	1.14±0.05	n.s.
Chlorogenic acids (5-CQA) (mg/ml)	1.30±0.04	1.50±0.05	***

*Significance level: n.s. (p>0.05); * (p<0.05);** (p<0.01); *** (p<0.001)*

Table 2. Sensory results

Sensory attributes	Arabica	Robusta blend	Signif.
(n=6)	X□SD	X□SD	
Odour intensity	6.1±1.0	5.8±1.0	n.s.
Body	5.6±0.7	5.5±1.1	n.s.
Acidity	5.9±1.4	1.9±0.4	***
Bitterness	6.6±0.7	7.6±1.2	***
Astringency	6.2±0.8	6.6±1.1	*
Flavour intensity	6.4±1.2	6.2±1.1	n.s.
Aftertaste intensity	5.3±1.3	6.2±1.0	***

*Significance level: n.s. ($p>0.05$); * ($p<0.05$);** ($p<0.01$); *** ($p<0.001$)*

Principal Component Analysis (PCA)

Four principal components (PC) with eigenvalues higher than 1 were selected by PCA. PC1 and PC2 explained 70.5% of the total variance. In each PC, the correlated parameters among the variables were included. Figures 1 and 2 show bidimensional representations of PC1 and PC2 scores for all of the variables and samples, respectively.

Arabica and Robusta EC samples were perfectly separated by PC1 (Figure 2). The main foam and taste characteristics of EC were included in PC1. Highly significant ($p<0.001$) correlation between the foam index and the total lipids (–0.849) and pH (0.908) were found. Similar results were also reported by Nunes et al. (1997). PC1 included all the taste sensory parameters (acidity, bitterness, astringency, and aftertaste intensity) and some physico-chemical parameters related with taste (caffeine, 5-CQA and pH). Although the perception of acidity depends not only on pH, but also on the individual acids (Illy and Viani, 1995; Clarke and Macrae, 1985), and pH was only poorly correlated with perceived acidity in other papers (Voilley, 1981), a highly significant correlation between acidity and pH (–0.942, $p<0.001$) was found. Many substances and classes of compounds such as caffeine, 5-CQA and trigonelline have been implicated as possible contributors to the overall bitterness perception in coffee (MacCamey et al., 1990). In our study, bitterness of EC was correlated with caffeine (0.821, $p<0.01$), and 5-CQA (0.884, $p<0.001$), but not correlated with trigonelline which was included in PC 3.

PC2 included some variables such as total solids and total solids on filtrate. Total solids were correlated with body (0.617, $p<0.05$). The body perception has been associated with insoluble materials in brew coffee (Lingle, 1996), which are included in total solids.

Discriminant Analysis (DA)

One discriminant function (DF) which only included Foam Index was obtained. The DF1 explained 100% of the total variance and permitted classification of the EC samples into their respective group with a success rate of 100%. DF1 centroids values for Arabica and Robusta blend EC were, respectively, –9.527 and 9.527.

$$(DF1)\ y = 2.393 \cdot \text{Foam Index} - 38.957$$

The DA proposed a function which was very easy to apply because the Foam Index was very simple to analyse. This physico-chemical parameter was an attribute included in PC1 by PCA.

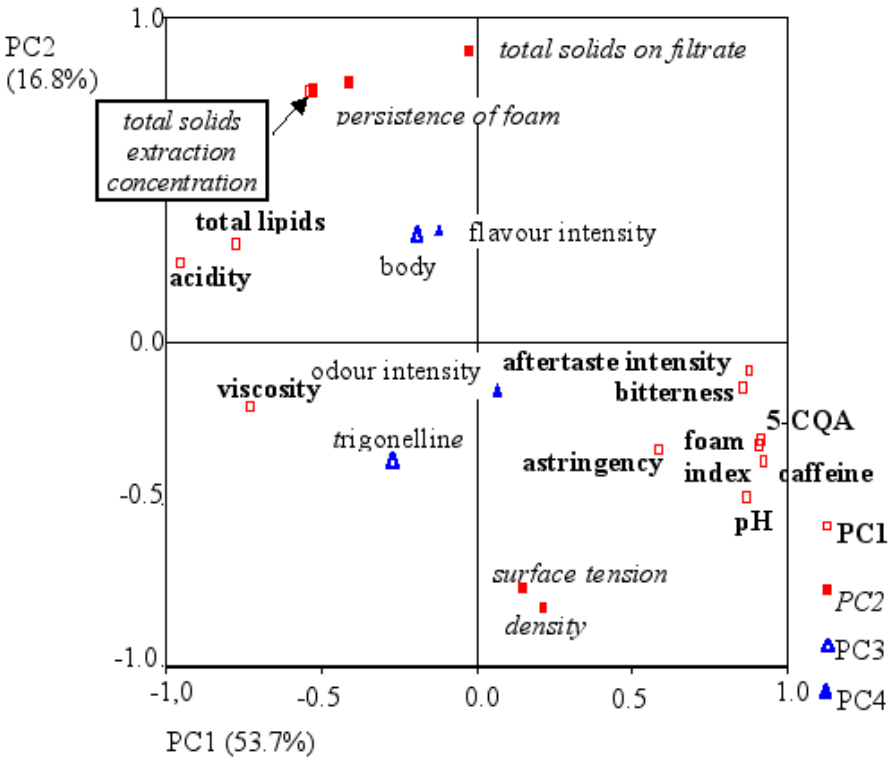

Figure 1. PC loadings for the EC variables

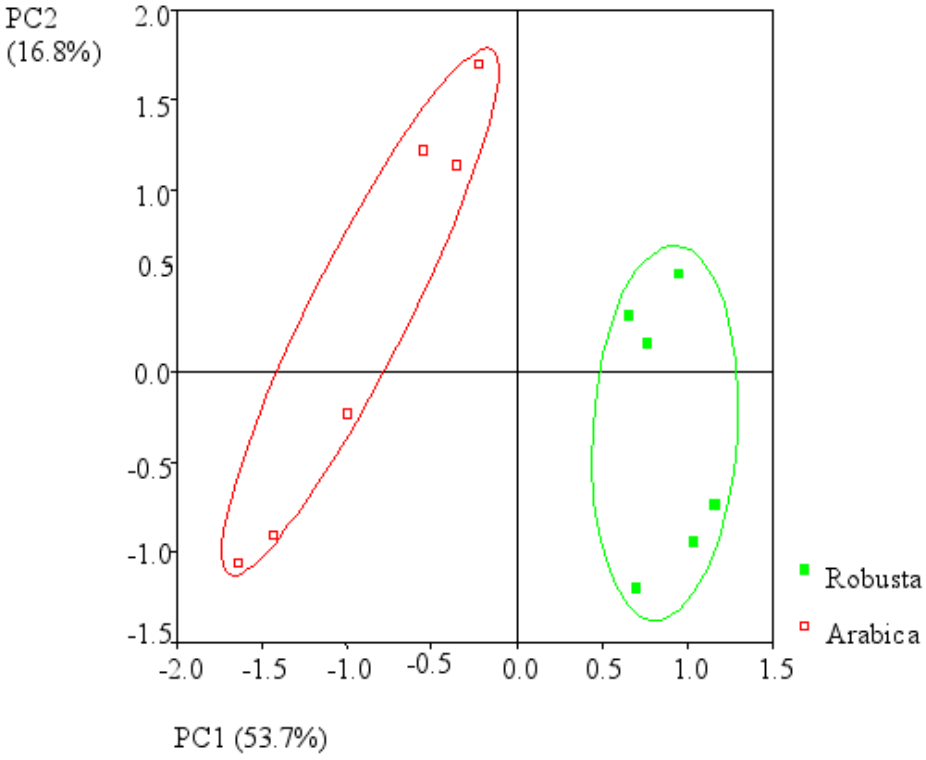

Figure 2. Normalized PCA scores of the EC samples

In conclusion, the two EC samples were separated perfectly by PCA using the PC1. The variables that differed between Arabica and Robusta EC samples were the physicho-chemical and sensory parameters related to the foam and taste of EC. But Foam Index was the main parameter which allowed the classification of each EC sample into their respective group with a success rate of 100% when it was included in a simple DF obtained by DA.

ACKNOWLEDGMENTS

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A New "Aroma Index" to Determine the Aroma Quality of a Blend of Roasted Coffee Beans

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SUMMARY

The staling of an Arabica 80:Robusta 20 blend was studied over a period of 15 months. Static Headspace-Gas Chromatography-Mass Spectrometry analysis was carried out to identify and quantify eight volatile compounds related to the ageing of coffee: Methanethiol, Thiophene, Propanone, Propanal, 2-Methylfuran, 2-Butanone, 2,3-Butanedione and Hexanal. Sensory analysis was also carried out in order to determine the loss of aroma freshness of coffee during storage. The areas of identified compounds followed the linear regression model with significance correlation coefficients in relation to aroma freshness. In order to avoid the possible effects of test conditions on results, seven new aroma indexes including methanethiol were established, obtaining high linear correlation coefficients with the aroma freshness of coffee. Five of our new aroma indexes presented excellent linear correlation coefficients ($r>|0.75|$) and one of them, Methanethiol/2-butanone, was $r>|0.9|$.

INTRODUCTION

The aroma freshness of coffee, considered to be the fine and pleasant smell arising from a freshly opened ground coffee pack or released during the grinding of freshly roasted coffee beans (Holscher and Steinhart, 1992), has long been an indicator of quality. The aroma of coffee is influenced by a large number of factors, such as specie, degree of roasting and storage (Clarke and Macrae, 1985). The changes of the coffee aroma occurring during storage are generally described as staling (Steinhart and Holscher, 1991) and are due to the rapid changes that occur to the volatiles after roasting. Although the causes of these changes are not completely understood, Holscher and Steinhart (1992) suggested two mechanisms for staling: oxidative reactions and loss of low-boiling potent-aroma components.

Vitzthum and Werkhoff (1979), Kallio et al. (1990) and Shimoda and Shibamoto (1990) had previously established that headspace techniques had been widely applied to determine the freshness of roasted coffee beans for a long time because of their simple handling and good reproducibility. Holscher and Steinhart (1992) used headspace techniques to investigate the volatiles responsible for aroma of fresh roasted coffee and concluded that these techniques permitted an objective evaluation of the aroma freshness of whole beans without exact knowledge of coffee origin and degree of roasting.

Several research groups had tried to associate coffee staling with chemical changes on coffee, giving particular importance to the search for simple chemical indicators of staling. Kallio et al., (1990) observed that the ratio of several pairs of compounds present in coffee headspace increased with storage time and that these compounds could be used as indicators of product quality. The ratio 2-methylfuran to 2-butanone concentration (M/B) was found to decrease when coffee began to become stale (Arackal and Lehmann, 1979; Vitzthum and Werkhoff, 1979). Radte and Piringer (1981) related the diminution of the S-index, the sum of the content

in several volatile compounds, to the loss of quality of coffee. According to Arackal and Lehmann (1979) “certain loss of freshness” could be detected after ten days of storage.

Methanethiol has been pointed out as a compound having a strong impact on aroma freshness showing the largest decrease already recognisable one day after roasting (Holscher and Steinhart, 1992). These results led the authors to conclude that methanethiol was the most important indicator of the loss of aroma freshness after several weeks of storage. Nevertheless, no ratio of compounds containing Methanethiol found in the literature was considered to be useful for studying the staling of coffee during storage.

The aim of this work was to follow the evolution of seven ratios of compounds including Methanethiol, over a period of 15 months of storage, using static headspace and to relate their changes to the loss of aroma freshness of coffee.

EXPERIMENTAL PROCEDURES

Materials

A commercial blend of roasted coffee beans Arabica:Robusta 80:20 (A80:R20) was obtained from a local manufacturer. Seven bags of roasted coffee beans, one for each time of analysis (0, 1, 3, 6, 9, 12 and 15 months), were stored at room temperature. Just before each analysis, the coffee was ground with a Retsch mill to a particle size of 0,75 mm.

Pure reference standards of 2-butanone, 2,3-butanedione and 2-propanone were purchased from ACROS (New Jersey, USA); 2-methylfuran, thiophene, and propionaldehyde were obtained from Aldrich (Steinheim, Germany) and Hexanal was obtained from SIGMA (Steinheim, Germany).

Static headspace-Gas Chromatography-Mass Spectrometry (SH-GC-MS)

SH-GC analysis was performed with an HP 6890 gas chromatograph (Hewlett Packard) equipped with a static headspace sampler Hewlett Packard model 7694 (Sanz et al., 2001).

Ten millilitre vials containing 2 g of roasted ground coffee were immediately sealed with silicone rubber Teflon caps and equilibrated at 90ºC for 60 minutes. After the vials had been pressurised with carrier gas for 12 s, 3 mL of the coffee headspace sample were injected into a capillary column HP-Wax (60 m x 0.25 mm x 0.5 μm film thickness; Hewlett-Packard).

The injector temperature was set at 180ºC, and helium (10 ml/min linear speed) was the carrier gas. The oven temperature was maintained at 40ºC for 6 min and programmed to 190ºC at 3ºC/min.

Mass spectrometry analysis was carried out using a Hewlett-Packard mass selective detector model 5973 coupled to the gas chromatograph. The mass spectrometer operated in the electron impact ionization mode (70 eV), with a scan range of 33 to 330 amu. The ion source temperature was set at 230ºC.

Identification of the volatile compounds

Identification of the volatile compounds was based on comparison of their mass spectra with the spectra of the Wiley library and in addition, in some cases, by comparison of their

retention time with those of standard compounds. The Kovats indexes were also calculated and compared with available literature data (Table 1).

Quantitative measurements

Areas of peaks were measured by calculation of the total area based on integration of a single ion (Table 1).

Table 1. Volatile compounds and ion for quantification

KI[a]	ID[b]	Compound	Ion for quantification
635	B	Methanethiol	47
1021	A	Thiophene	58
712	A	Propanal	58
832	A	2-Methylfuran	82
866	A	2-Butanone	43
1084	A	Hexanal	56
962	A	2,3-Butanedione	43
753	A	Propanone	43

[a]*KI, Kovats Index calculated for the HP-Wax capillary column*

[b]*ID. The reliability of the identification proposal is indicated by the following: A, mass spectrum, retention time and Kovats index agreed with standards; B, mass spectrum, compared with Wiley mass spectral data bases*

Sensory Analysis

Samples were analysed at 0, 1, 3, 6, 9, 12 and 15 months of storage in order to evaluate the loss of aroma freshness. Immediately after opening the bags, our panellists were asked to smell the coffee and to mark it according to the following scale: “fresh” (9) if a fresh roasted note was detected; “pleasant” (7) if that fresh roasted note had disappeared but it smelled good; “not fresh” (5) when neither positive nor negative notes could be detected; “rancid” (3) when rancid notes appeared and “very rancid” (1) when those negative notes were very potent. Intermediate markings were given to medium situations.

Statistical analysis

To find significant differences during storage, the data was subjected to one-way analysis of variance (ANOVA) and Tuckey’s a posteriori test using SPSS 9.0 for Windows. A significance level of $p \leq 0,05$ was used for all mean evaluations. Correlation and Regression analyses were carried out for individual compounds in relation to the loss of aroma freshness. Correlation coefficients between sensory results of aroma and values of ratios of compounds were obtained to determine possible statistical relationship between them.

RESULTS AND DISCUSSION

The storage changes may be measured using the amounts of selected volatile compounds as indicators (Kallio et al., 1990; Leino et al., 1992). Chromatographic areas of eight volatile compounds (Table 1), previously related to the ageing of coffee (Kallio et al., 1990), were found to change significantly during storage. Areas of these compounds followed the linear regression model with significance correlation coefficients in relation to the sensory results of aroma freshness (Table 2). These compounds were appropriate indicators of aroma freshness

for coffee samples according to the correlation coefficients and the significance (<0.001) of the linear regression model.

Table 2. Correlation Coefficients (r) of eight volatile compounds in relation to aroma freshness

Compound	r	Compound	r
Methanethiol	0.910***	2-Methylfuran	0.915***
2,3-Butanedione	0.918***	2-Propanone	0.913***
Thiophene	0.910***	2-Butanone	0.913***
Propanal	0.918***	Hexanal	0.910***

****Significant at p<0.001*

In order to avoid the effect of test conditions on the results, the ratios of compounds may be used as indicators of storage time (Arackal and Lehmann, 1979; Kwasny and Werkhoff, 1979; Vitzhtum and Werkhoff, 1979). Seven "Aroma Index" were calculated between methanethiol and thiophene, propanal, 2-methylfuran, 2-butanone, 2,3-butanedione, propanone and hexanal areas.

The value of each ratio at 0 months of storage was normalised at 100% and used as the reference to calculate the percentage of each ratio at the different storage times (Table 3). All aroma indexes studied showed a significant decrease during storage. Methanethiol/2-Butanone was the ratio of compounds that decreased the most when coffee was stored for one month (up to 54.90%), in comparison to Methanethiol/Hexanal which decreased up to 69.66%. The biggest differences for each ratio of compounds between the times of storage were observed between 0 and 1 month of storage; the decreases beyond 1 month were less marked. At 15-month storage, Methanethiol/Acetone was the ratio that decreased most over the whole period and Methanethiol/Propanal was the one that showed higher percentages at that time.

Table 3. Evolution of the percentages of seven "Aroma Index" at the seven times of storage

AROMA INDEX	*0*	*1*	*3*	*6*	*9*	*12*	*15*
Methanethiol/Tiophene	100^{c}	57.79^{b}	46.48^{ab}	37.09^{ab}	37.60^{ab}	26.47^{ab}	22.68^{a}
Methanethiol/Acetone	100^{f}	58.08^{e}	44.12^{d}	37.96^{d}	41.76^{c}	24.74^{b}	19.06^{a}
Methanethiol/Propanal	100^{f}	66.74^{e}	55.90^{ab}	50.13^{ab}	55.19^{ab}	47.89^{a}	40.98^{a}
Methanethiol/2-	100^{b}	56.36^{ab}	41.22^{ab}	38.52^{ab}	41.11^{ab}	31.51^{ab}	28.07^{a}
Methanethiol/2-butanone	100^{e}	54.90^{d}	40.86^{c}	35.19^{b}	30.36^{b}	25.54^{a}	23.16^{a}
Methanethiol/2,3-	100^{g}	62.18^{f}	52.05^{e}	33.96^{c}	37.03^{d}	27.73^{b}	23.33^{a}
Methanethiol/Hexanal	100^{f}	69.66^{e}	55.61^{d}	47.18^{c}	47.66^{c}	31.15^{b}	23.97^{a}

Note: Within a row different letters denote significant differences (p<0.05) between times of storage

Figure 1 represents the evolution of the coffee aroma during 15 months of storage. The biggest decrease of aroma freshness was detected between 0 and 1 months of storage (from 9 to 6), which coincided with results from Steinhart and Holscher (1991), who expressed that the biggest changes of light volatiles occur within 3 weeks. From 1 to 9 months of storage, the decrease was slower than from 0 to 1 month, and beyond 9 months, the aroma freshness reached a plateau and no differences were detected by our panellists. According to Steinhart and Holscher (1991), after 6-8 weeks of storage, staleness could be detected in their samples.

However, it was detected in our samples when coffee had been stored for 3 months since no analysis had been carried out between 1 and 3 months of storage. Finally, they defined rancidity of coffee after 4 to 5 months of storage; our panellists detected some rancidity after 6 months of storage, but clear rancidity was only perceived after 9 months.

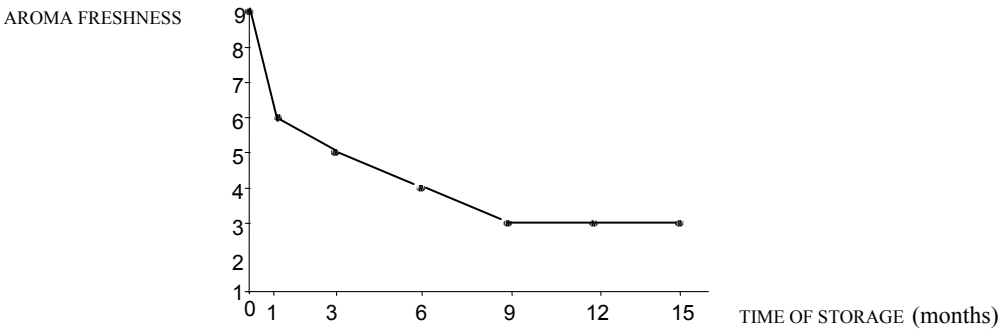

Figure 1. Evolution of the aroma of an A80:R20 coffee during 15 months of storage

Correlation between the aroma index and the sensory results of aroma of roasted coffee beans were calculated (Table 3). Good correlation coefficients ($r>0.75$) were obtained for five of the seven indexes: Methanethiol/Thiophene, Methanethiol/Acetone, Methanethiol/2-Butanone, Methanethiol/2,3-Butanedione and Methanethiol/Hexanal. Among them, the index Methanethiol/2-Butanone showed very good correlation coefficients ($r>0.9$).

These results allowed us to confirm that Aroma Indexes containing Methanethiol, the compound that decreased faster when coffee was stored, are related to aroma freshness of coffee.

Table 4. Correlation coefficients of seven aroma indexes containing Methanethiol and the aroma of coffee

AROMA INDEX	Correlation Coefficients
Methanethiol/Tiophene	0.881***
Methanethiol/Acetone	0.847***
Methanethiol/Propanal	0.683***
Methanethiol/2-Methylfuran	0.364**
Methanethiol/2-Butanone	0.926***
Methanethiol/2,3-butanedione	0.862***
Methanethiol/Hexanal	0.884***

***Significant at p<0.01*

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Determination of the Geographical Origin of Green Coffee by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

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SUMMARY

It is very important for the purposes of fair trade that an objective method is available to determine the country of origin of green coffee. Although different techniques have already been evaluated, up to now, no reliable fully validated analytical procedure has been reported. In this context, a method based on the determination of the mineral profile was investigated.

The profile of 57 elements was determined by semi-quantitative (TotalQuant) scan ICP-MS in 174 samples of Arabica green coffee originating from 12 countries (Brazil, Columbia, Costa Rica, Ecuador, Ethiopia, Guatemala, Hawaii, Indonesia, Kenya, Mexico, San Salvador, Thailand).

Different multivariate statistical analyses of the data were compared, including linear discriminant analysis, multinomial logistics discrimination, artificial neural network (ANN), k-nearest neighbours. ANN gave the best results for the discrimination of coffees from the 12 countries. Nevertheless, the average misclassification rate, estimated by cross-validation, was about 23%, and therefore an unambiguous identification of green coffee of unknown origin is not yet possible. However, Colombian and Hawaiian coffees could be well discriminated from those of other countries with average misclassification rates of 10 and 2%, respectively. This suggests that the technique has the potential, in very specific cases and given further validation, to correctly classify a green coffee of known but questionable origin.

INTRODUCTION

Coffee is grown in about 70 countries and international trade is conducted almost exclusively with green coffee. In this context, it is very important to know the country of origin of coffee for the purposes of fair international trade and to sustain claims on the geographic origin of coffee products currently on the market. No taster can reliably identify the country of origin of the coffee based on the in-cup taste and aroma, and on the aspect of the green beans due to the large number of producing countries and within-country variability. Therefore, objective methods are needed.

Thermo-gravimetric and pyrolysis mass spectrometry, chlorogenic acid profiling, infrared spectroscopy, and stable isotope techniques failed to discriminate unambiguously green coffees from different origins (Prodolliet et al., 1997). Several authors looked at minerals as potential markers of the origin of coffee (Ferreire et al., 1971; Roffi et al., 1971; Macrae et al., 1993; Krivan et al., 1993; Martin et al., 1998; Martin et al., 1999). Analysing 10 green coffees from 8 countries, Krivan et al. (1993) found manganese to be the best indicator. On the other hand, principal component and cluster analyses of the profiles of 11 elements, as determined by ICP-AES, only partially discriminated 18 coffees from 10 countries (Martin et al., 1999). The technique seems definitely promising but many more samples and minerals should be analysed to validate the approach. This study describes the analysis of 57 trace elements and

minerals by semi-quantitative (TotalQuant) scan ICP-MS in 174 Arabica green coffees originating from 12 different countries.

EXPERIMENTAL

Sampling

One hundred and seventy four Arabica green coffee samples were analysed originating from 12 different countries and 3 continents (America, Africa, Asia). All coffees were of certified origin and were obtained through the University of Hawaii (Hawaiian coffees), Nestlé representatives/buyers in some countries or the CIRAD Montpellier. The following coffees were analysed : Brazil (1990-1997 crop year, 24 samples), Colombia (1993-1997, 33), Costa Rica (1993-1997, 14), Ecuador (1994-1997, 8), Ethiopia (1994-1997, 7), Guatemala (1993-1998, 18), Hawaii (1990-1996, 24), Indonesia (1994-1997, 7), Kenya (1994-1997, 5), Mexico (1996-1997, 19), San Salvador (1997-1998, 11), Thailand (1995-1996, 4)

ICP-MS analysis

The green coffee beans were ground and digested in a high pressure asher as follows: 0.5 g product was placed into a 15 ml HPA quartz tube and 2 ml sub-distilled conc. nitric acid were added. Each tube was closed with a quartz cap and secured with a PTFE sealing tape. The tubes were placed in a 21-hole heated stainless-steel block and then pressurised at 70 bars with nitrogen. The temperature was raised stepwise from 20°C to 180°C. After cooling, the analytical solutions were diluted to 10 ml with ultrapure water. Analyses were performed on a Model ELAN 6000 ICPMS (Perkin-Elmer SCIEX). Rhodium (50 ppb) was used as internal standard. All samples were analysed in duplicates. Calibration of the TotalQuant was performed with 41 elements at the following concentrations:

- 500 ppb of Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl, Zn and Rb
- ppb of Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Sc, Tb, Th, Tm, Y and Yb

Statistical analysis

All statistical analyses were carried out using S-PLUS 2000 (Insightful Corporation, Seattle, WA, USA). Different classification methods were used (Venables and Ripley, 1999): Linear Discriminant Analysis (LDA), Multinomial Logistics Discrimination (MLD), Artificial Neural Networks (ANN) and *k*-Nearest Neighbours (kNN). Euclidean distance was used on the standardised data. Standardisation of data ensures that values are between 0 and 1 for each element “i”.

Standardisation equation : $$E_i^{scaled} = \frac{E_i - \min(E_i)}{\max(E_i) - \min(E_i)}$$

The different classification methods were validated using the following ad-hoc cross-validation: for each country, one half of the samples was randomly selected. The model was then trained on these data, and the performance was evaluated on the rest of data. This was repeated 20 times on different random subsets, in order to obtain an idea of the variability of the performance. The 20 simulations gave 20 individual error rates, which were then summarised statistically.

RESULTS AND DISCUSSION

Mineral profile

The profile of 57 trace elements and minerals was determined by TotalQuant ICP-MS in 174 Arabica green coffees originating from 12 different countries. The results are given in Table 1.

The agreement between the results obtained in this study and those reported in the literature is very good (Ferreira et al., 1971; Roffi et al., 1971; Macrae et al., 1993; Krivan et al., 1993; Martin et al., 1998; Quijano and Spettel, 1973). After K and N (1.5-3%) and Ca, Mg and P (0.1-0.2%), six elements namely Cu, Fe, Mn, Na, Rb and Si (10-100 mg/kg), were confirmed to be the major mineral constituents of green coffee. Many elements, basically all rare earth elements, but also metals like Cd, Be, Bi, Li, Pb, Pt, Sn, and Zr were detected and their concentrations are reported for the first time. Finally, green coffee appears as a safe commodity considering the low levels measured for Al, As, Cd, Cd, Cr, Hg, and Pb.

Universal classification: all countries

"What is the geographic origin of a green coffee of unknown origin?" Whatever the methodology used and the discriminant factors considered, a complete database including representative samples of each coffee-producing country should first be established. This certainly represents thousands of samples and huge efforts in sourcing the materials and in analysis. For this reason we limited the scope of the study to only 12 countries, including the main producers of Arabica coffee. Different statistical classification methods were therefore tested on the available data (3.1) to see whether the geographic origin of an unknown coffee could be predicted and assigned to one of the 12 countries used to build up the database. The data are summarised in Table 2.

The Artificial Neural Networks (ANN) model (size = 10) clearly outperformed all the other methods and resulted in the following 20 error (misclassification) rates: 15.48, 17.86, 17.86, 17.86, 19.05, 19.05, 21.43, 21.43, 22.62, 23.81, 23.81, 23.81, 23.81, 25.00, 25.00, 25.00, 27.38, 28.57, 29.76, 33.33%. On average 23.1% of the samples were incorrectly classified. In the best case, 85.5% coffees would be correctly classified (Figure 1a). In the worst case, only two thirds of the coffees would be correctly classified (Figure 1b). Increasing the number and representativeness of the samples per country would certainly improve the predictive performances of the model.

"Does the coffee under test originates from a particular country/region (to be specified) or not?" In order to see whether the proposed methodology can successfully answer this question, 3 different classifications were tested using the same green coffee database and statistical classification methods described above. However, for each classification, the data were split in 2 groups. The performances of the models are given in Table 2.

As for the all countries classification, the ANN model (size = 10) gave the best results in predicting "Colombia or not". However, error rates improved considerably. Indeed, on average only 10% of the Colombian coffees were not classified as originating from Colombia. In the best simulation the error rate even dropped down to 3.5%.

The MLD and *k*-Nearest Neighbours (k = 1) models gave the best results in predicting "Central America or not". However, the error rates were not as good as for the Colombian coffees, but still better than for the all countries classification (3.2).

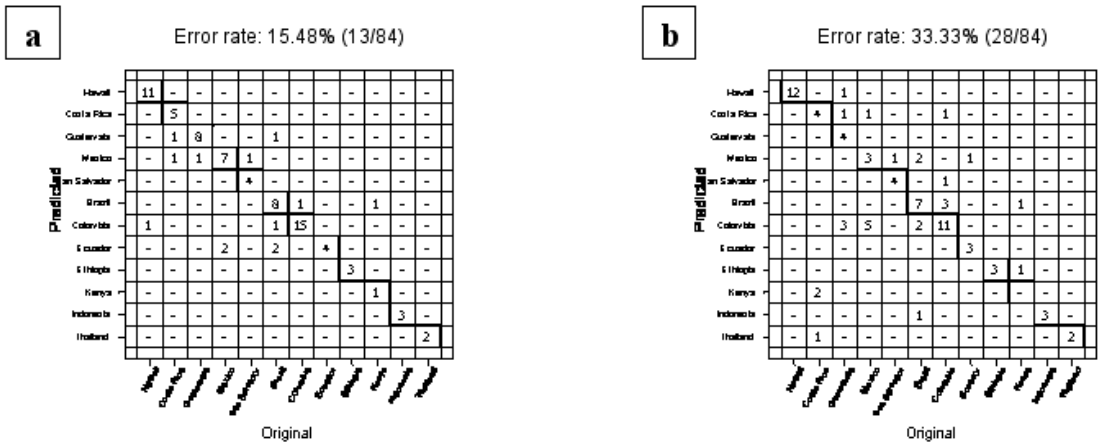

Figure 1. All-country classification - Confusion Matrix for the best (a) and worst (b) simulation using ANN

ANN (size = 5) gave again the best results in predicting "Hawaii or not" and correctly classified all samples in the best simulation (error rate = 0%). Clearly the technique seems very well suited for this application.

CONCLUSIONS

The mineral profile of coffee does not appear to be sufficiently discriminant to allow an unambiguous identification of a green coffee of an unknown geographic origin. The best and average misclassification rates, obtained applying an Artificial Neural Networks model, were 15 and 23%, respectively. Increasing the number and representativeness of the samples per country, and/or coupling this approach with another technique (e.g. NIR) would certainly improve the predictive performances of the methodology. However, the technique has the potential to correctly classify a coffee of known but questionable origin. Indeed, very low mis-classification rates were obtained when Colombian and Hawaiian coffees were tried to be identified separately.

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Table 1. Mineral profile of green coffees (µg/kg)

	Ag	Al	As	Au	Ba	Be	Bi	Cd	Ce	Co
Min.	0.00	541.50	0.00	0.00	601.96	0.00	0.00	0.00	0.33	19.52
Mean	3.25	7635.57	342.88	0.85	5504.50	2.26	0.18	12.47	24.51	157.07
Max.	18.88	61554.30	2255.92	6.57	32821.51	10.11	9.01	185.36	772.43	776.06
SD	3.47	8286.92	478.54	1.17	4081.04	2.67	0.80	18.19	78.07	138.17
RSD	106.88	108.53	139.56	138.32	74.14	118.14	448.94	145.92	318.52	87.97

	Cr	Cs	Cu	Dy	Er	Eu	Fe	Ga	Gd	Ge
Min.	118.85	1.30	8558.27	0.01	0.00	0.07	16462.25	1.19	0.04	0.00
Mean	601.96	88.69	13632.53	0.41	0.20	0.70	35986.35	19.78	0.61	1.69
Max.	6186.86	2134.22	25448.64	2.76	1.45	4.49	63233.59	44.59	3.53	14.28
SD	741.92	239.46	2322.75	0.44	0.23	0.55	6811.92	9.07	0.62	1.93
RSD	123.25	270.00	17.04	105.60	115.78	78.95	18.93	45.87	101.64	113.91

	Hf	Hg	Ho	In	Ir	La	Li	Lu	Mg	Mn
Min.	0.00	0.00	0.00	0.00	0.00	0.88	0.00	0.00	582824.90	10764.99
Mean	0.11	1.71	0.08	0.05	0.01	68.58	7.95	0.03	2155045.61	33040.22
Max.	1.84	22.08	0.64	2.24	0.14	3952.58	37.55	0.21	3917596.10	126643.65
SD	0.21	2.78	0.10	0.22	0.02	304.13	6.38	0.03	530511.17	17012.33
RSD	181.25	162.29	116.39	410.64	251.77	443.46	80.22	105.36	24.62	51.49

	Na	Nb	Nd	Ni	Os	Pb	Pr	Pt	Rb	Re
Min.	2761.26	0.00	0.19	141.93	0.00	0.00	0.07	0.00	2660.82	0.00
Mean	34480.11	0.99	2.88	819.15	0.05	43.76	0.77	0.07	30261.38	0.01
Max.	351851.48	15.72	17.10	6522.75	0.34	599.08	4.79	2.00	102708.11	0.08
SD	55344.52	1.73	3.13	823.42	0.07	64.42	0.82	0.24	19179.00	0.01
RSD	160.51	173.69	108.63	100.52	156.64	147.21	106.80	357.24	63.38	122.70

	Sb	Si	Sm	Sn	Sr	Ta	Tb	Te	Th	Tl
Min.	0.00	0.00	0.02	0.00	563.68	0.00	0.00	0.00	0.00	0.00
Mean	0.64	18558.23	0.51	24.22	7407.65	0.01	0.07	0.15	0.90	0.32
Max.	8.91	110662.13	2.93	308.94	45253.17	0.17	0.52	4.63	14.12	9.28
SD	1.00	16758.21	0.49	49.23	5652.03	0.02	0.09	0.37	1.62	0.91
RSD	156.40	90.30	95.21	203.32	76.30	190.47	119.52	243.24	180.52	281.44

	Tm	U	W	Y	Yb	Zn	Zr
Min.	0.00	0.04	0.01	0.00	0.00	4237.63	0.20
Mean	0.03	0.34	0.43	2.49	0.17	6923.82	4.56
Max.	0.22	2.95	3.96	12.88	1.23	17426.03	58.14
SD	0.03	0.37	0.58	2.17	0.20	1655.98	6.65
RSD	104.38	108.21	133.69	87.26	122.87	23.92	145.71

Specific classifications: Colombia, Central America, Hawaii vs. all other countries

Classification	**Group 1:** specific country/region	**Group 2:** other countries
Colombia vs. other countries	33 samples from Colombia	141 samples from 11 countries
Central America vs. other countries	62 samples from Mexico, Costa Rica, Guatemala and San Salvador	112 samples from 8 countries
Hawaii vs. other countries	24 samples from Hawaii	150 samples from 11 countries

Table 2. Green coffee classification - Comparison of statistical methods (errors rates in %; SD: standard deviation of the error rates)

All country classification

Model	Minimum error rate	Mean error rate	Maximum error rate	SD
LDA	31.0	39.7	50.0	5.1
MLD	22.6	27.3	32.1	2.9
ANN	15.5	23.1	33.3	4.5
kNN (k=1)	26.2	32.2	36.9	3.5
kNN (k=3)	32.1	42.1	52.4	5.0
kNN (k=5)	35.7	46.8	56.0	5.7

Colombia vs. other countries

Model	Minimum error rate	Mean error rate	Maximum error rate	SD
LDA	9.3	21.9	32.6	5.1
MLD	3.5	12.1	19.8	3.5
ANN	3.5	11.2	15.1	3.2
kNN (k=1)	5.8	12.7	17.4	3.0
kNN (k=3)	9.3	13.7	18.6	2.5
kNN (k=5)	8.1	14.3	22.1	2.8

Central America vs. other countries

Model	Minimum error rate	Mean error rate	Maximum error rate	SD
LDA	19.5	27.0	35.6	4.4
MLD	10.3	17.6	23.0	3.3
ANN	11.5	19.8	28.7	4.7
kNN (k=1)	9.2	17.2	25.3	4.8
kNN (k=3)	13.8	19.4	31.0	4.8
kNN (k=5)	11.5	24.5	32.2	5.2

Hawaii vs. other countries

Model	Minimum error rate	Mean error rate	Maximum error rate	SD
LDA	3.5	6.9	11.5	2.1
MLD	2.3	3.9	8.1	1.5
ANN	0	1.7	5.8	1.7
kNN (k=1)	1.2	4.7	6.9	1.8
kNN (k=3)	2.3	5.9	9.2	1.8
kNN (k=5)	4.6	7.4	8.1	1.9

Stachyose: A Marker of the Presence of Legume Adulterants in Soluble Coffee?

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SUMMARY

Food processors and regulatory bodies are both interested in the detection of fraudulent or accidental adulteration of food. Authenticity of soluble coffee has been and still is a major concern for the coffee manufacturer because of the strong economic interest of adulteration. Typical coffee substitutes that may be added either as (declared) ingredients or (undeclared) adulterants are extracts of chicory, malt, figs, cereals, caramel, starch or maltodextrins. A powerful tool to detect even small amounts of these potential adulterants is **H**igh **P**erformance **A**nion **E**xchange **C**hromatographic (HPAEC) profiling of sugars (free and total upon total sugars hydrolysis of polysaccharides).

Recently, some commercial soluble coffees were seized in France that showed unusual high amounts of proteins (> 20%). Although their HPAEC sugar profile proved that these products were clearly adulterated, we also analysed their HPAEC oligosaccharide profile. This appeared to be unusual since in particular one peak with the same retention time as stachyose was detected, corresponding to about 0.1 to 0.2 g/100 g of this tetrasaccharide typically found in legume plants. In the HPAEC profile of 20 authentic soluble coffees of different manufacturers and covering the most important production conditions, no corresponding peak with the same retention was observed. To identify the unknown peak in one of the suspect soluble coffees, an oligosaccharide fraction was further purified by means of classical RP-HPLC. Using methylation analysis followed by GC-MS analysis of the partly methylated alditol acetates (PMAA), it could be demonstrated that this fraction was composed of about 50% of stachyose.

We conclude that the unusual HPAEC oligosaccharide profile, together with the high protein level and the confirmed presence of stachyose in this sample point to the use of an adulterant on legume basis. Further studies will be carried out to assess the botanical source of the adulterant using PCR techniques and to improve the purification of stachyose from soluble coffee. This could lead to a new screening tool of soluble coffee authenticity and help to decide whether the undeclared addition of legume plant extracts is a more widespread issue.

INTRODUCTION

Food processors and regulatory bodies are both interested in the detection of fraudulent or accidental adulteration of food. Authenticity of soluble coffee has been and still is a major concern for the coffee manufacturer because of the strong economic interest of adulteration. Typical coffee substitutes that may be added either as (declared) ingredients or (undeclared) adulterants are extracts of chicory, malt, figs, cereals, caramel, starch or maltodextrins.

A powerful tool to detect even small amounts of these potential adulterants is **H**igh **P**erformance **A**nion **E**xchange **C**hromatographic with **P**ulsed **A**mperometric **D**etection

(HPAEC-PAD). Monitoring the HPAEC profile of soluble coffee sugars (free and total upon hydrolysis of polysaccharides) has not only become an official ISO method (11292; Prodolliet et al., 1995), but upper limits for some sugar markers such as total glucose and total xylose have also been approved by the European Soluble Coffee Industry (AFCASOLE).

We herein report that HPAEC oligosaccharide profiling, combined with protein analysis, may be used to detect the presence of protein-rich adulterants on legume base.

Table 1. Compositional data of five commercial soluble coffees as compared to those found in pure soluble coffee

product	*proteins [g/100g]*	*total glucose [g/100g]*	*total [g/100g]*	*stachyose ? [g/100g]*	*adulterated with*
pure soluble coffee	10 - 17	< 2.6	< 0.6	< 0.02	----
soluble coffee N°1	21.8	28.9	2.7	0.18	glucans, husks, leguminous
soluble coffee N°2	28.6	2.3	0.4	0.13	leguminous
soluble coffee N°3	27.6	19.3	n.d.	0.25	glucans, leguminous
soluble coffee N°4	13.3	17.2	5.0	0.17	glucans, husks, leguminous
soluble coffee N°5	24.7	1.7	0.8	0.09	husks, leguminous

OLIGOSACCHARIDE ANALYSIS

Five commercially available soluble coffees were analysed for their HPAEC sugar profiles and protein contents (Table 1). In four of them, the protein contents largely exceeded the values typically found in pure soluble coffee (21-29%, instead of 10-17%). It was hypothesised that the high protein contents could be the result of an addition of leguminous plants such as soy or chickpea. Flatulent sugars being characteristic marker compounds of such leguminous plants, we also analysed the oligosaccharide profile of these products.

As a result, a chromatographic peak with the same retention time as that of the stachyose was detected in all five products, corresponding to about 0.1 to 0.2 g/100 g of this tetrasaccharide found in high levels in legume plants. When the corresponding HPAEC profiles of 20 authentic soluble coffee samples of various industrial origins were analysed, no corresponding peak with the same retention time could be observed (Figure 1).

An extract of soluble coffee N°1 was additionally analysed using classical carbohydrate HPLC on a amino-bonded column (Figure 2). Again, a peak with the same retention time as that of stachyose was detected. From this column, fractions containing this peak were collected by repeated injection and about 20 □g of material obtained. Methylation analysis[3] (GC-MS analysis of the partially methylated alditol acetates) revealed the presence of about 50% of stachyose in the collected solid, thus confirming the identity of the chromatographic peak and the presence of trace amounts of this legume marker in soluble coffee N°1.

DISCUSSION AND CONCLUSION

We could give evidence that the HPAEC oligosaccharide profile of pure soluble coffee does normally not show the presence of a peak corresponding to the tetrasaccharide stachyose. However, the presence of such a chromatographic peak has recently been observed in five commercial soluble coffees. From one of them, a fraction eluting from a HPLC column at the same retention time as that of stachyose could be shown to be composed of about 50% of stachyose. The presence of this oligosaccharide could be explained by an addition of a legume based adulterant such as soy.

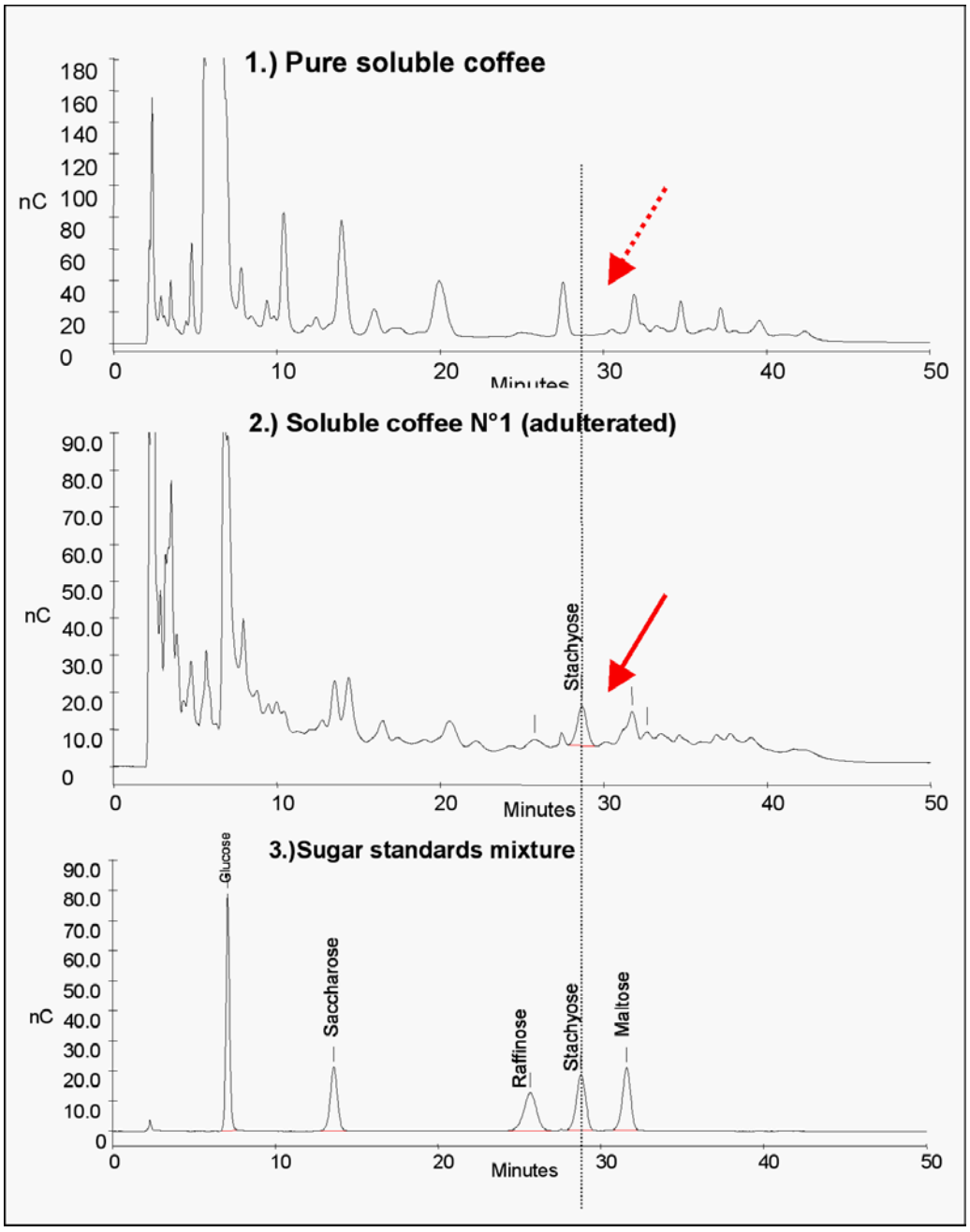

Figure 1. HPAEC oligosaccharide profile of a pure soluble coffee (ISO method 11292), of the adulterated soluble coffee sample N°1 (Prodolliet et al., 1995) and of an oligosaccharide standard mixture (Carpita and Shea, 1989)

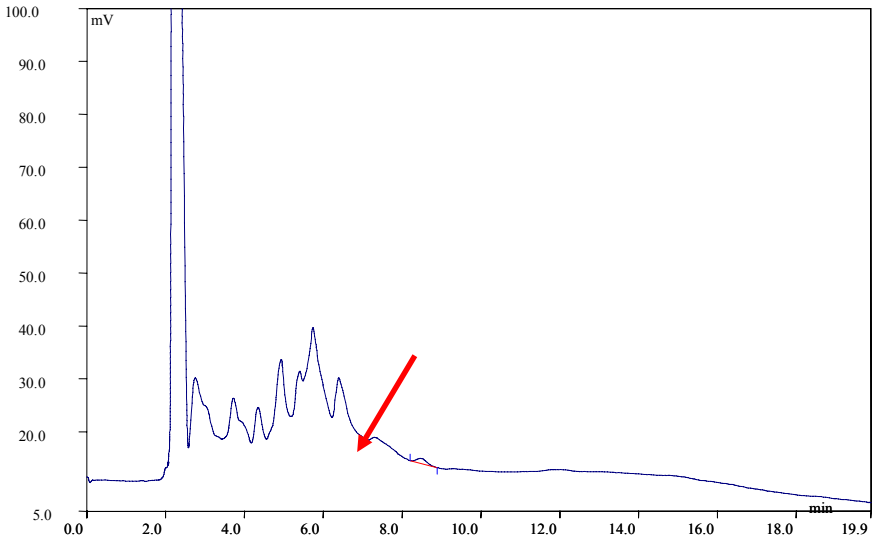

Figure 2. HPLC oligosaccharide profile of soluble coffee N°1. Stachyose elutes at about 8.5 min

This hypothesis is supported by the unusually high protein content of this sample (21.8%). However, with a view to its high total glucose content (29%), the presumed addition of soy to this product does clearly not represent the main source of adulteration. On the other hand, soy could be the main adulterant in two other products (N°2 and N°5) which based on their sugar profile appeared to be almost pure or adulterated only to a slight extent. Interestingly, the occurrence of a peak corresponding to stachyose was not correlated to a high protein content in the case of N°4. Either of the presence of stachyose or a high protein content, taken individually, could therefore be indicative of an addition of a legume based adulterant.

Because of the higher sensitivity and resolution of HPAEC-PAD with regard to classical HPLC, it would be preferable to perform the isolation of stachyose directly from the HPAEC column. This could for instance be achieved using a desalting device (carbohydrate membrane desalter). Further studies are being carried out to assess the botanical source of the adulterant in these products, in particular in coffees N°2 and N°5 which based on their sugar appeared to be only slightly adulterated by classical adulterants. Combining compositional (proteins) and classical chromatographic (HPAEC) analyses with molecular-biological methods (PCR) could thus lead to a new powerful tool of soluble coffee authenticity control and help decide whether the undeclared addition of legume based adulterants is a more wide-spread issue or not.

MATERIALS AND METHODS

The protein content was calculated according to:

$$[N_{tot} - (\text{coffeine} * 0.2885) - (\text{trigonelline} * 0.1021)] * 6.25$$

Caffeine and trigonelline contents were measured by HPLC with UV detection.

The HPAEC profiles of free and total sugars were determined according to ISO method 11292 using a Dionex DX300 instrument with PAD detector, a CarboPacPA1 column (Dionex) and water as eluent.

The HPAEC profile of oligosaccharides and flatulent sugars was run on a Dionex DX500 instrument with PAD detector, a CarboPacPA1 column (Dionex) and a 73/27 mixture of H_2O/300 mM aq.NaOH as eluent.

The HPLC profile of oligosaccharides and flatulent sugars was run on a Kontron HPLC instrument with RI detection, an amino-bonded column (Spherisorb S5 NH_2) and a 80/20 mixture of AcN/ H_2O as eluent.

Methylation analysis of the fractions collected from the HPLC column was achieved following the procedure described by Carpita and Shea (1989). The partially methylated alditol acetates (PMAA) were prepared and analysed using a Perkin Elmer Autosystem gas chromatograph with FID detector on a Supelco SP-2380 column and H_2 as carrier gas. The GC-MS analysis was performed on a Finnigan MAT 8430 instrument connected to a HP 5890 gas chromatograph (He as carrier gas). Electron impact spectra were acquired at 70 eV over the mass range 20 to 500 Da.

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Prediction of Arabica Content from Ground Roasted Coffee Blends by Near Infrared Spectroscopy

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SUMMARY

Determination of the arabica and robusta composition of roasted coffee blends is an important aim for various reasons: difference in price between robusta and arabica, particular organoleptic characteristics. Chemical analysis is long, costly and not very reliable. Near infrared spectrometry (NIRS) could be a rapid, non–destructive alternative method for determining the arabica content of ground roasted coffee blends.

The study was carried out using a spectral base comprising 352 samples of ground roasted coffee; the range of wavelengths studied was between 400 nm and 2500 nm (visible and near infrared). The samples chosen for this study came from several arabica and robusta coffee varieties of different geographical origins, for which the various post-harvest treatments and roasting parameters were representative of production conditions. This base was representative of the spectral variability seen in commercial coffees.

A principal components analysis was carried out on the matrix of spectral data. Based on the principal components extracted, the Mahalanobis distance from the mean spectrum was calculated for each spectrum. Limits of belonging to the spectral population were thus defined and the neighbourhood distances were calculated.

A mathematical model using multivariate regression methods (PLS) was developed to predict the arabica content of blends from the spectral data.

The model was validated by predicting a set of samples (23) independent from the established base. The standard error of prediction (SEP) was estimated to be 2.20% (W/W), the coefficient of determination (R^2) of the regression was equal to 0.99; these statistical criteria clearly indicate the potential of this method for use in the quality control of industrial products.

INTRODUCTION

It is important for the roasting industry to know the composition of a blend of ground roasted coffees. Studies based on the biochemical composition of arabica and robusta coffees have not succeeded in determining the arabica coffee content of blends. Near infrared spectroscopy (NIRS) could be an alternative analysis method.

We have studied here the ability of NIRS to predict arabica coffee content. Calibration was based on pure arabica and robusta blends prepared in the laboratory.

Near infrared spectroscopic method

NIRS in diffuse reflectance mode is a rapid and non-destructive technique based on the ability of organic matter to absorb light. The functional groups that respond to NIR radiation are C-H, O-H and N-H. NIRS instruments must be calibrated with the laboratory reference methods. Developing a calibration model therefore involves calculating the regression equation based on the NIRS spectra and the reference information. The set of samples must be representative of variability in the population to be predicted.

The performance of calibration in terms of data fitting and prediction accuracy is expressed by the coefficient of multiple determination (R^2), the standard error of calibration (SEC) and the standard error of prediction (SEP).

MATERIALS AND METHODS

Samples

352 ground roasted coffee blends were prepared from arabica coffees (62) and robusta coffees (45) of different geographical origins and varieties. The samples were representative of spectral and chemical variability and of the roasting methods used for commercial coffees.

Sample preparation

The blends (30 g) were prepared by weight from arabica and robusta coffees roasted and ground in the laboratory. The range of arabica contents in the blends prepared in this way varied from 0 to 100%.

Near infrared spectroscopy

NIRS: Spectrometer Nirsystem 6500. Foss-Perstorp.

Software: ISI NIRS 2 version 4.11 (InfraSoft International).

Sample presentation: 3 g of homogenized blend were analysed in diffuse reflectance from 400 nm to 2500 nm (in 2 nm steps). Analysis was duplicated.

RESULTS

Spectrum

Plotting (Figure 1) of the average spectra of arabica and robusta coffees expressed by second derivative of log (1/R) versus wavelengths enabled characterization of the major absorption bands and particularly the first overtone band and C-O (2348 nm) fat stretching and C-C (2382 nm) deformation.due to ArCH (1682 nm) and the combination bands due to C-H (2308 nm)

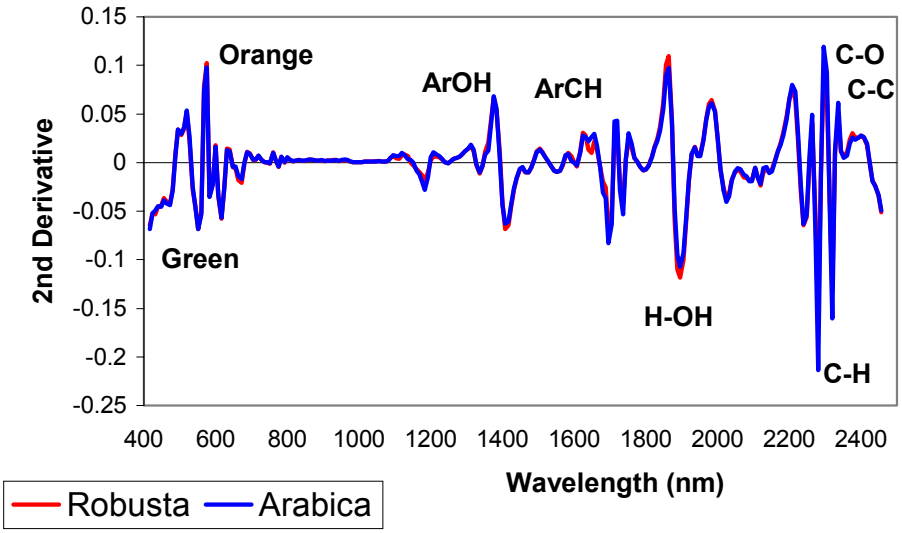

Figure 1. NIR reflectance spectra of ground roasted arabica and robusta coffees

Principal components analysis

The number of spectra was large and the related variables were strongly correlated with each other. A principal components analysis was used to extract the relevant information from the spectral matrix. The generalised Mahalanobis distance (H) is a powerful tool for :

- defining the sample boundaries, and a similarity index between spectra;
- calculating the distance of each spectrum from the average spectrum.

32 principal components (PC) explained 98.5% of the total inertia of the initial matrix. The three-dimensional scatter plot (Figure 2) of sample scores for the first three PC showed sample distribution and arabica content trends.

The first three CP explained 59.86%, 26.40% and 4.44% of initial inertia respectively.

Calibration results

Partial Least Square models (PLS) were used to establish quantitative relations between NIR spectral bands and arabica content.

The calculated model (Table 1) fitted the data, the coefficient of determination (R^2) was 0.99.

The established model was validated by predicting the arabica contents of 23 blends (5 to 80% arabica) chosen for their distribution in the main first plane (Figure 3). This procedure enabled an estimation of the SEP on an independent set of samples; the SEP estimated in this way was 2.20.

The coefficient of determination of the regression (Figure 4) between reference values and predicted values was 0.99. The linear regression slope was 1.007, the average bias was 0.53.

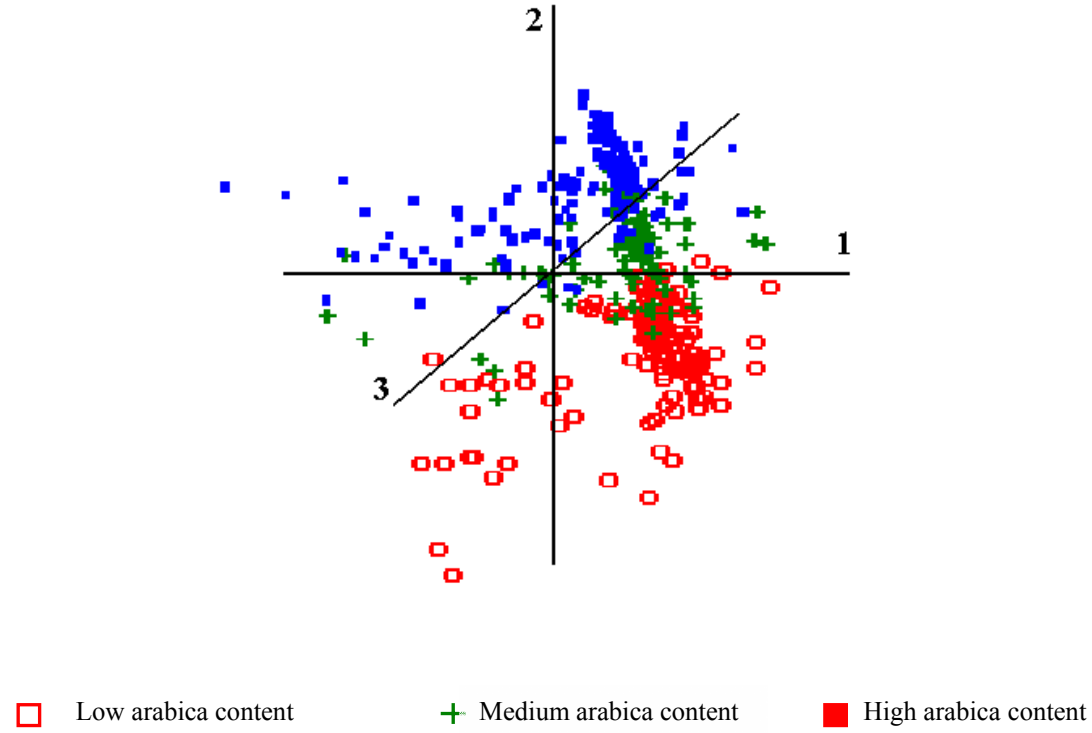

Figure 2. Scatter plot of sample scores for the first three principal components

Table 1. Calibration statistics for arabica content

Constituent	N	M	SD	SEC	R^2	SECV	SEP	Nb of PLS terms
Arabica (%)	321	51.2	34.5	1.29	0.99	1.85	2.20	15

N: total number of samples used for computation
M: mean
SD: standard deviation of the concentration values
SE : standard error of calibration
R^2: coefficient of multiple determination
SEC : standard error of cross validation
SEP: standard error of prediction

CONCLUSION

According to this study, NIRS can be considered as an effective tool for predicting the arabica content of ground roasted blends. The accuracy of the equation enables a ± 5% (w/w) estimation of the arabica content, with a confidence interval of 95%.

However, it is important to enhance the representativeness of the established base (in terms of spectral variability), by continuing to acquire new samples (of diverse varieties and geographical origins), taking into account different industrial processes.

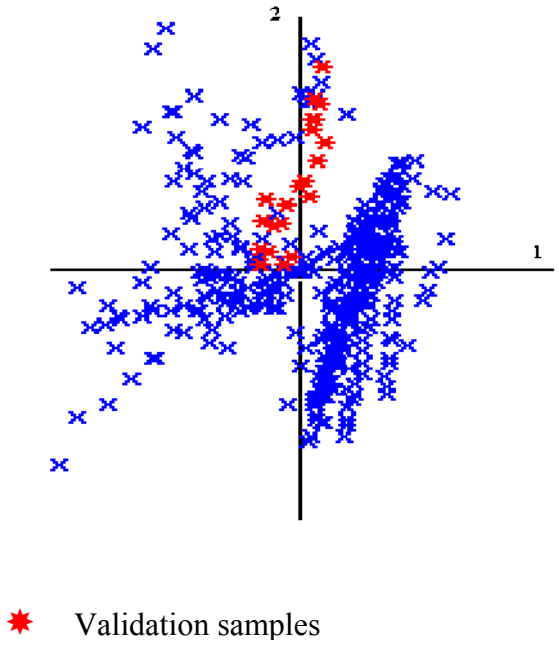

Figure 3. Scatter plot of validation sample scores for the first two principal components

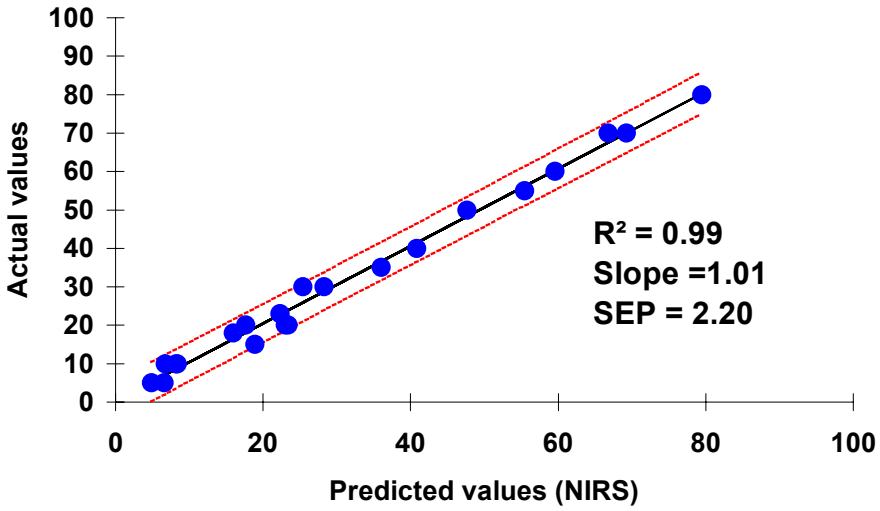

Figure 4. Correlation between laboratory arabica content and NIRS predicted values obtained on a set of 23 independent samples (confidence interval 95%)

A Study of the Formulation of Blends from Arabica and Robusta Coffees

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SUMMARY

The object of this study was to evaluate five different blends of arabica and robusta coffees by way of sensory analyses and an analysis of the behaviour of some of the chemical properties. The sensory analysis by 5 experts showed that the incorporation of 20% optimised robusta coffee did not alter the sensory characteristics of the beverage as compared to 100% arabica. Up to 50% robusta gave scores and grades above the mean, resulting in no significant difference ($p \leq 0.05$) as compared to 100% arabica. There was a linear increase in pH, % reducing sugars (w/w) and % soluble solids (w/w) with increase in the percentage of robusta. Titratable acidity and % phenolics (w/w) showed no characteristic behaviour, with no significant difference ($p \leq 0.05$) between the samples. The results showed the viability of using robusta coffee in blends with arabica coffee with respect to the sensory quality, additionally increasing the yield due to greater amounts of soluble solids, and reducing costs due to the lower cost of robusta coffee as compared to arabica coffee.

RESUMEN

El objetivo deste trabajo fue estudiar cinco formulaciones de mezclas de café arábica (bebida riada) con café robusta a través de la evaluación sensorial y del comportamiento de algunos componentes químicos. Los resultados de la evaluación sensorial, realizados por cinco experts, mostraron que en la mezcla que contiene 20% de robusta, no se detectó alteración de las características sensoriales de la bebida, comparando con la bebida de café arábica al 100% y que hasta 50% de café robusta en una mezcla, los conceptos ficaron arriba de la média, no difiriendo significativamente ($p \leq 0.05$) de las bebidas del café arábica al 100%. Los analisis químicos demonstraron crecimiento linear del pH, azúcares reductores y % de sólidos solubles con el aumento del café robusta en la mezcla, ya la acidez titulable y % de fenoles no presentaron comportamiento definido, no habiendo diferencia significativa ($p \leq 0.05$) entre las muestras. Los resultados demonstraron la viabilidad de utilizar el café robusta en mezclas con el café arábica, ya que el mismo no altera la calidad de la bebida, proporciona un mayor rendimiento, debido a la mayor cantidad de sólidos solubles que presenta y abarata los costos por ser un café mais accesible con relación al arábica.

INTRODUCTION

In agrobusiness, the variability of coffee prices creates quality problems, but also gives space for a much discussed but little studied question, that of the formulation of blends of arabica and robusta coffees for the ground roasted coffee sector. Robusta coffee, widely used in the production of soluble coffee due to its higher soluble solids content, is discriminated by the ground roasted coffee sector for not presenting the flavour and aroma considered to be characteristic of coffee. Thus this study aimed at studying five formulations of blends of

arabica (rioy beverage) with robusta coffee by way of sensory evaluations and the analysis of some chemical properties.

MATERIAL AND METHODS

Raw material

The robusta coffee (*Coffea canephora* Conillon) came from the Cooabriel Cooperative in São Gabriel da Palha/ES/Brazil, and was classified as type 5 showing 10.2% moisture content and a beverage classified as having a slight robusta taste. The arabica coffee (*Coffea arabica*) came from the Coffee Cooperative in the region of Marília/SP/Brazil, and was classified as type 5/6, showing 11.2% moisture content and a beverage classified as rioy. Both types of coffee were from the 97/98 harvest and were dry processed.

Methods

The optimum degree of roasting of the arabica coffee was determined by experts, and corresponded to a loss in weight of 20%, the mean colour co-ordinates being: L* 36.07, a* 1.97 and b* 1.74. The roasting of the robusta coffee was based on Mendes, 2001, in which the optimum range for roasting robusta coffee with the same raw material characteristics as that used in this study showed L* between 36.2 and 41.3, a* between 1.9 and 4.4 and b* between 1.7 and 6.7, with a mean weight loss of 17%. The coffee samples were roasted separately in an electric rotary drum bench roaster manufactured by PROBAT-WERKE, model PRE 1.

Table 1. Percent arabica and robusta coffees in the blends

Café Robusta	0	20	35	50	65	80	100
Café Arábica	100	80	65	50	35	20	0

Sensory Evaluation

This was carried out by five experts from the São Paulo Cereal Exchange and from the Brazilian Association of Coffee Industries (ABIC), following the evaluation norms used by the coffee sector in accordance with the Ministry of Agriculture and Supplies. The samples were presented at random with a three number code.

Chemical Analyses

- Soluble solids: A.O.A.C. method n° 15.014 (A.O.A.C., 1980).
- pH: Determined on a mixture of 2.25g ground roasted coffee in 50 ml water at 25°C, using a MICRONAL pH metre, model B-374.
- Total acidity: A.O.A.C. method n° 15.031 (A.O.A.C., 1975).
- Reducing sugars: Somoygi-Nelson method (Ashwell, 1975).
- Phenolic content: Porters test (Porter et al., 1986)

All the analyses were carried out in triplicate, followed by an analysis of variance and Tukeys test, at the 5% level.

RESULTS AND DISCUSSION

Sensory evaluation

Aroma, body, acidity and bitterness of each sample were evaluated, receiving a score on a scale from 1 to 5 (1 = bad, 5 = very good), and a grade. The average values can be seen in Table 2.

Table 2. Sensory evaluation of robusta and arabica coffee blends

Samples	*Aroma*	*Body*	*Acidity*	*Bitterness*	*Av. score*	*Grade*
0*	Reg/good	Reg/good	Normal/low	Normal	**4.0^{a}**	Good
20	Reg/good	Reg/good	Slightly low	Normal	**3.9^{a}**	Good
35	Reg/good	Regular	Slightly low	Normal	**3.3^{ab}**	Reg/goo
50	Reg/weak	Regular	Normal/low	Normal	**3.2^{ab}**	Reg/goo
65	Reg/weak	Regular	Low	Strong	**2.4^{bc}**	Regular
80	Regular	Reg/weak	Low	Strong	**2.2^{bc}**	Reg/bad
100	Reg/bad	Reg/weak	Low	Strong	**1.8^{c}**	Reg/bad

**The numbers indicate the percent robusta in each sample. Means with the same letter(s) do not differ significantly from each other ($p \leq 0.05$).*

The results indicate that samples containing 0, 20 and 35% of robusta coffee present a good intensity of the agreeable aromas typical of coffee. In the other samples, with increasing amounts of robusta, the agreeable aromas became weaker, indicating that the lack of characteristic coffee aroma shown by robusta coffee, was masking the arabica aroma. The sensation of body was felt more in the samples containing 0 and 20% robusta coffee than in the remaining samples. In this case, the robusta coffee did not intensify the sensation of body. In general, the acidity of both coffees was considered to be low, there being little difference with increase in percentage of robusta, despite arabica coffee being considered to be more acid than robusta coffee (Clifford, 1985; Clark, 1986). Bitterness was considered normal up to 50% robusta coffee, but intensified with further increases in the percent of robusta, which was to be expected, since robusta is generally considered to be more bitter than arabica coffee. The results show that the inclusion of up to 20% robusta did not alter the sensory characteristics of the beverage, as compared to 100% arabica. Using up to 50% robusta coffee in the blend, the scores and grades were above the mean of the scale used, there being no significant difference at the 5% level from the 100% arabica sample. An adequate roasting of both types of coffee impeded the perception of the medicinal flavours, characteristic of rioy beverages, both in the 100% arabica sample and in the blends.

Figure 1 shows the average evaluation scores, a linear correlation being observed between the increase in the percent robusta in the blends and the decrease in beverage score. The coefficient of determination of the straight line adjusted from the points in Figure 1 was 96%, the equation being as follows:

$$\text{Scores} = -2.407*(\%\ \text{robusta}) + 4.175$$

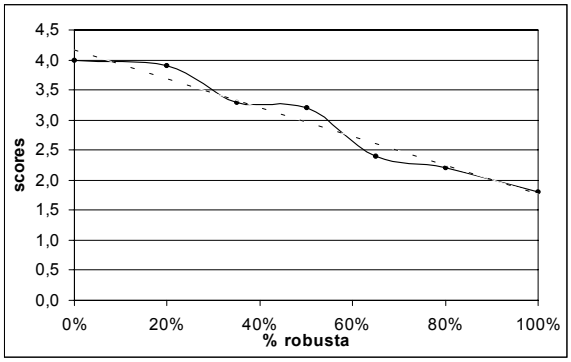

Figure 1. Sensory evaluation scores of the blends of arabica and robusta coffees

Chemical analyses

The arabica and robusta coffees showed moisture contents of respectively 1.88% and 2.01%, these values, since they are very close, not varying significantly in the blends.

Table 3. Means for the determinations of pH, titratable acidity, reducing sugars, phenolic compounds and soluble solids for the samples of arabica plus robusta coffee blends

Samples	***pH***	***Acidity****	***%red.sugars***	***% phenolics***	**% sol.**
0	6.09^{a}	123.75^{a}	0.49^{e}	1.73^{a}	21.69^{d}
20	6.10^{a}	125.00^{a}	0.58^{d}	1.66^{a}	22.34^{d}
35	6.03^{ab}	140.00^{a}	0.61^{cd}	1.62^{a}	22.71^{cd}
50	6.05^{ab}	128.75^{a}	0.68^{bc}	1.85^{a}	23.11^{bcd}
65	5.94^{abc}	137.50^{a}	0.72^{b}	1.79^{a}	23.88^{abc}
80	5.92^{cb}	132.50^{a}	0.81^{a}	1.72^{a}	24.51^{ab}
100	5.86^{c}	133.75^{a}	0.86^{a}	1.83^{a}	25.10^{a}

**Titratable acidity expressed in ml 0.1N NaOH / 100g coffee. Means in the same column with the same letter(s) do not differ significantly ($p \leq 0.05$).*

Determination of pH and titratable acidity

The results show a decrease in pH with increasing percentage of robusta in the blend. This is contrary to other reports, which state that arabica coffee is more acid than robusta coffee. One possible explanation could be the severe roasting level (20% loss in weight), since the greater the degree of roast, the lower the acidity (Clifford, 1985; Woodman, 1985; Clark, 1986).

The results did not show any well defined behaviour for titratable acidity with increase in % robusta in the mixture. The only relevant observation was that the titratable acidity of the pure arabica sample was lower than that of the pure robusta sample, in agreement with the pH determinations. Observing the grades given for sensory acidity, the lack of correlation between instrumentally measured acidity and that evaluated sensorially is clearly evident.

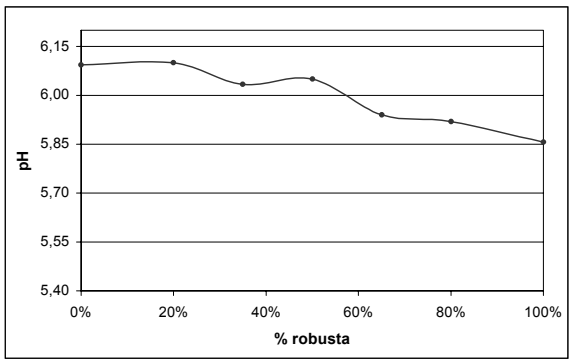

Figure 2. Means of pH measurements in blends of arabica and robusta coffees

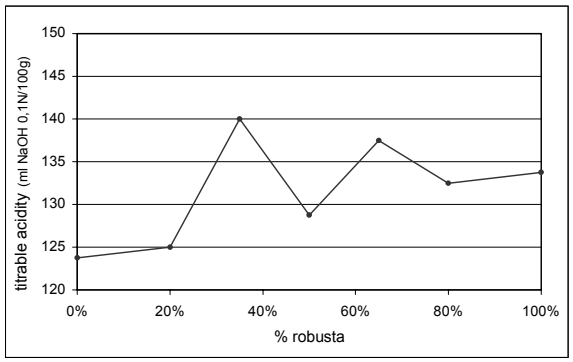

Figure 3. Means of titratable acidity in blends of arabica and robusta coffees

Determination of percent reducing sugars (dry weight basis)

The results show a direct linear relationship between percent reducing sugars and the percent of robusta in the blend. It was possible to trace the equation of the straight line, with a coefficient of determination of 99%:

% reducing sugars = 0.004*(5 robusta) + 0.490

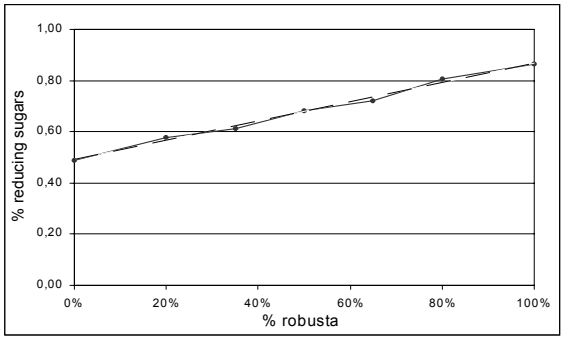

Figure 4. Percent reducing sugars in arabica plus robusta coffee blends

This behaviour was expected since robusta coffees contain a higher reducing sugar content as compared to arabica coffee.

Determination of % phenolics (dry weight basis)

The figure illustrates the lack of a relationship between the percent phenolic compounds and the percent robusta in the blends. The main group of phenolics is the chlorogenic acids, and arabica and robusta coffees show distinct compositions for these compounds. Although raw robusta coffee presents a greater chlorogenic acid composition (Clifford, 1985), these compounds undergo degradation during roasting, and in roasted coffee, arabica coffee contains more than robusta coffee (Trugo and Macrae, 1984). However this only explains the lack of a tendency to increase the % phenolics with increase in the amounts of robusta in the mixture. To explain the variation between the samples, one important fact is the amount of sample used in the analysis: 50 mg. Since the samples were mixtures, even if one grinds the two coffees together, vigorously mixing the resulting powders, it is not possible to guarantee that the 2 coffees are present in the 50 mg in the same percentage as in the original blend.

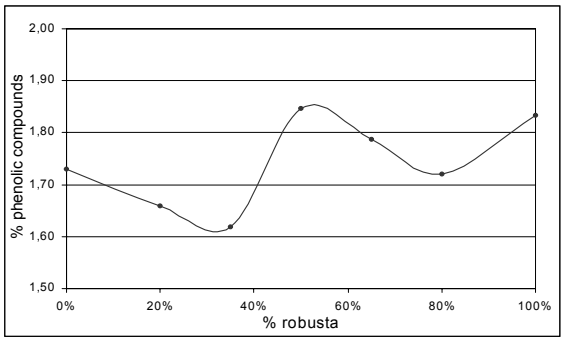

Figure 5. Mean of the % phenolic compounds (dry weight basis) in the blends of arabica and robusta coffees

Determination of the % soluble solids

As expected, the soluble solids content increased linearly with increase in percent robusta in the blends, since robusta coffee shows a greater amount of soluble solids than arabica coffee (Moraes et al., 1973/74; Vree and Yeransan, 1973). This linear relationship showed a coefficient of determination of 99%, the corresponding equation being as follows.

$$\%\text{soluble solids} = 0.035*(\%\text{ robusta}) + 21.588$$

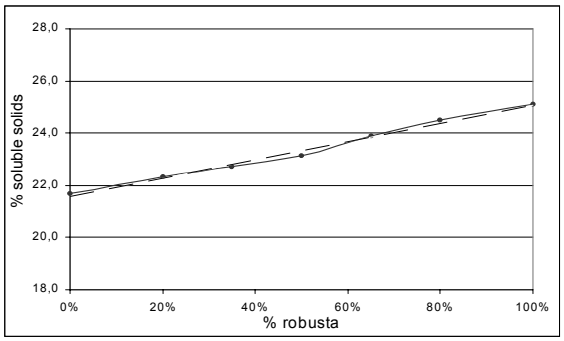

Figure 6. Means of the percent soluble solids in the blends

CONCLUSIONS

The beverage made from the blend containing 20% robusta coffee showed the same sensory characteristics as that from 100% arabica. The scores and grades of the beverages resulting from blends containing up to 50% robusta were above the average values for the scale used, there being no significant difference at the 5% level, when compared to the 100% arabica sample. The medicinal flavours characteristic of rioy coffee were not detected in any of the blends, indicating that the optimisation of the roasting process impeded the perception of this undesirable characteristic. The results showed the viability of using robusta coffee in blends with arabica coffee, since, in addition to not prejudicing the sensory characteristics of the beverages, this practice increases the yield, since robusta coffee contains more soluble solids, and also reduces costs, since robusta coffee is cheaper than arabica coffee.

The pH value, the percent (wet basis) of reducing sugars and that of soluble solids, all showed a direct linear relationship with the percent robusta in the blend, although the titratable acidity and the % phenolic compounds showed no characteristic behaviour. The method used to determine the % phenolics, may have prejudiced the results, due to the very small size of the sample used in the analysis.

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Early Lipid Oxidation in Roasted and Ground Coffee

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SUMMARY

The oxidation degree of coffee lipids in R&G upon short exposure to air at room temperature was assessed using SPME-GC/MS and POV measurements. Coffee lipids sealed under argon were heated, and hexanal was determined before and after heating by SPME HS-GC/MS. In both cases, hexanal increased depending on the exposure time of R&G, and the increase was larger after heating. The increase of hexanal after heating (reflecting lipid hydroperoxides) is in agreement with POV data.

These findings indicate that oxidation of coffee lipids readily occurred in two steps in R&G within the first 24 h exposure, first into hydroperoxides (demonstrated by hexanal data after heating) and secondly into volatiles (demonstrated by hexanal data before heating) but to a smaller extent. Also, SPME HS-GC/MS is a suitable method to accurately assess oxidation status of coffee lipids.

RÉSUMÉ

Le degré d'oxydation des lipides du café roti moulu suite à l'exposition à l'air ambiant pendant une courte période est mesuré à l'aide de SPME-GC/MS et POV. Les lipides scellées sous argon sont chauffées et l'hexanal est mesuré avant et après chauffage par SPME HS-GC/MS. Dans les deux cas, la teneur de l'hexanal augmente avec le temps d'exposition à l'air du café roti moulu, cependant à moindre mesure avant chauffage. La tendance croissante de l'hexanal déterminé après chauffage, réflectant les hydroperoxydes, est confirmée par des mesures de POV.

Ces résultats indiquent que les lipides du café s'oxydent déjà lors des premières 24 heures d'exposition du café roti moulu, et cela en deux étapes, primo en hydroperoxydes et secondo, à moindre mesure en volatiles. SPME HS-GC/GC est démontré pour être une méthode indiquée dans l'analyse de l'état d'oxydation des lipides du café.

INTRODUCTION

The staling of roasted coffee during storage is recognized to partly originate from oxygen absorption. Actually, losses in coffee flavour were shown to correlate with the exposure of roasted and ground coffee (R&G) to air (Radtke, 1979; Nicoli et al, 1993; Cardelli-Freire, 1997). Beside degradation of aroma compounds, the effects of oxygen on lipid oxidation in R&G upon storage were studied by several authors (Nicoli et al, 1993; Ortola et al, 1998; Janicek and Pokorny, 1970). As only peroxide values (POV) were monitored by these authors, the first step (i.e. formation of lipid hydroperoxides), but not the second step (i.e. decomposition of lipid hydroperoxides) of coffee lipid oxidation was observed. Accordingly, the oxidation degree of coffee lipids might not be fully assessed since it is known that catalysts (e.g. Fe ion, light, heat) accelerate the decomposition of lipid hydroperoxides and R&G contains Fe ions.

Therefore, we studied lipid oxidation in R&G during the first days of ambient storage by monitoring both lipid hydroperoxides and their volatile degradation products in coffee lipids. The aim was to study the early stage of lipid oxidation in R&G coffee, including the formation and degradation of lipid hydroperoxides.

MATERIAL AND METHODS

Extraction of coffee lipids from R&G upon exposure to air

A medium-roasted coffee blend of Arabica and Robusta (4:1) was ground using a coffee grinder (Olympia Express, set at the medium position). After 0, 1, 4, 24 and 72 h exposure to air at 20°C, R&G were sampled in a 2 l-round glass flask, evacuated to 10 mbar for 15 min. Extraction of R&G was performed under protection from air with hexane / isopropanol (v/v 3:2) according to Radin (1978). After evaporation to dryness using a Rotavapor (Büchi) at 30°C and under reduced pressure (50 mbar), coffee lipids were stored under argon at –40°C until analysis.

Hexanal in coffee lipids by SPME HS-GC/MS

Approximately 100 mg of coffee lipids were accurately weighed into a 2 ml-vial under argon. After sealing with a septum, the vial was heated at 90°C for 45 min, and cooled to room temperature. Hexanal in the vial headspace was then monitored by SPME HS-GC/MS. A polydimethylsiloxane / divinylbenzene (PDMS/DVB) 65 μm SPME fiber (Supelco) was used with 1 h-absorption at 25°C and 5 min-desorption in the GC injector at 250°C. A Trace 2000 series chromatograph equipped with an MS detector Automass Multi was used. GC conditions: DB1701 column (30 m x 0.25 mm, film thickness 0.25 μm); Oven temperature: 35°C (3 min), 4°C/min to 220°C (10 min). Hexanal in the headspace of non-heated coffee lipids was similarly measured. Analyses were performed in triplicate, and results reported as peak areas in arbitrary units.

Peroxide value (POV) measurement

Analyses were performed in duplicate. POV were obtained with coffee lipids after subtraction of the blanks, and expressed in meq O_2/kg oil.

Potentiometric method

The AOAC official method 965.33 (1995) was used except that the titration with 0.01 M-thiosulfate was performed by potentiometry according to Hara and Totani (1988) using a 718 Stat Titrino (Metrohm, Switzerland).

Ferric thiocyanate method

The ferric thiocyanate method (Hills and Thiel, 1946; Monnin and Schetty, 1964) was applied using a spectrophotometer Uvikon 930 (Bio-Tek Instruments, Basel, Switzerland) measuring the absorbance at 510 nm.

RESULTS AND DISCUSSION

Hexanal was chosen as chemical marker to study both the formation and degradation of lipid hydroperoxides from coffee lipids occurring in R&G during exposure to air at room temperature, because its main precursors, i.e. n-6 fatty acids, occur in appreciable amounts in

roasted coffee. Roasted coffee contains 10-15% lipids, of which 82-84% are triglycerides, and these triglycerides contain 40-44% linoleic acid (Ratnayake et al, 1993; Al Kanhal, 1997). SPME HS-GC/MS, which was succesfully applied to assess oxidation of vegetable oils (Recseg et al, 1999), was used to monitor hexanal in coffee oils before and after heating under argon.

Hexanal was detected prior to heating and the amounts gradually increased with exposure time of R&G to air. Similar trends were found after heating, but the amounts of hexanal were larger. During analysis, further formation of hydroperoxides from coffee lipids could be excluded thanks to the anaerobic conditions. Therefore, hexanal detected before heating was already present in coffee lipids, and that detected after heating resulted from thermal decomposition of hydroperoxides in addition to existing hexanal prior to heating. The difference reflects the amounts of hydroperoxides in coffee lipids.

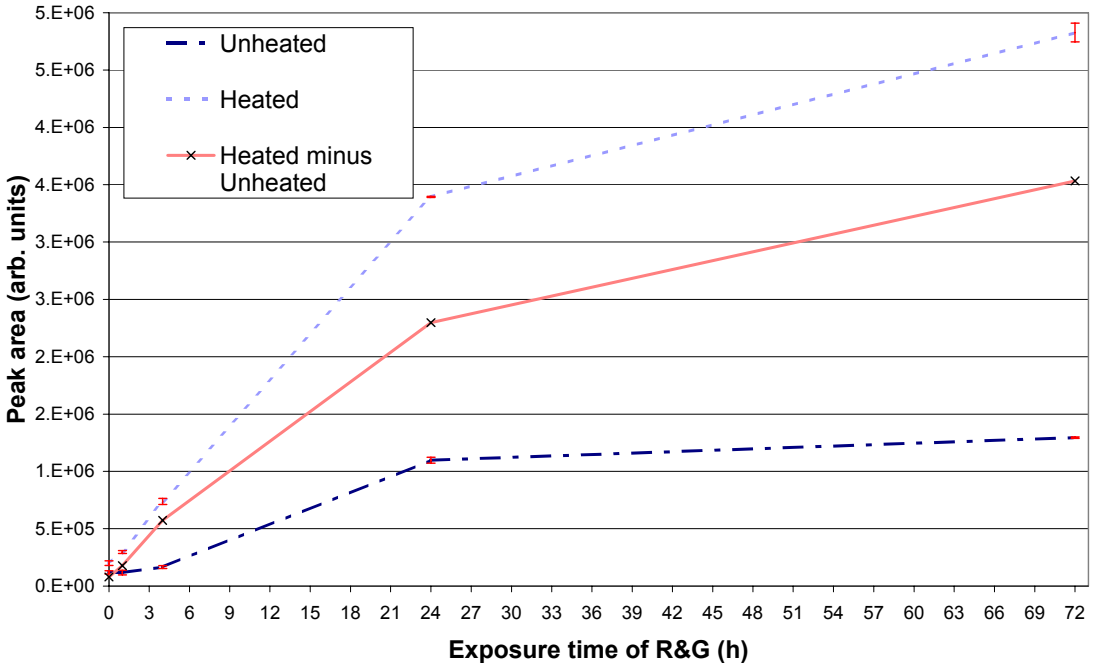

Figure 1. Hexanal detected by SPME HS-GC/MS in coffee lipid extracts before and after heating under argon

These data indicate that both the formation and decomposition of hydroperoxides from coffee lipids readily occurred in R&G when exposed to air. Such a rapid decomposition could be due to the presence of iron in R&G, which acts alone and/or together with light, as catalyst in a Fenton type reaction.

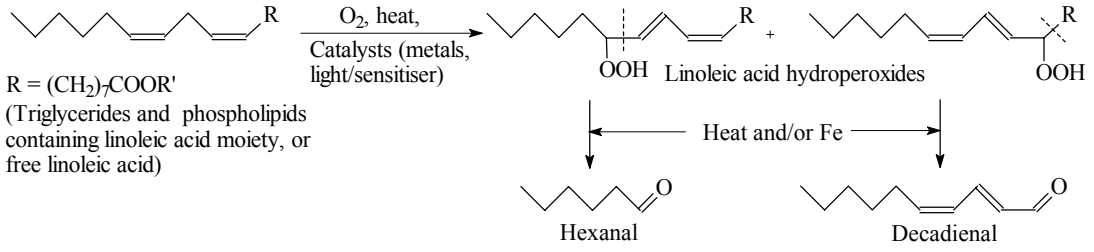

Figure 2.Oxidation of linoleic acid into hydroperoxides and their further decomposition

Although the SPME HS-GC/MS method focussing on hexanal in heated lipids only assesses thermal decomposition of hydroperoxides, results of this method are in good agreement with those of POV methods in respect to the increase of the first lipid oxidation step (i.e. generating lipid hydroperoxides) in R&G upon exposure to air

It is worth noting that negative POV values were found in coffee oils extracted from R&G exposed to air up to 4 hours. This result could tentatively be explained by a competitive reaction of reducing compounds (e.g. chlorogenic acids) which were co-extracted with coffee lipids from R&G. Accordingly, the measured amounts of thiosulfate and Fe^{2+} were lower than that necessary for the reduction of lipid hydroperoxides in coffee oil. As the consequence the measured POV values were lower than the true POV, and could be negative if the content of lipid hydroperoxides was too low to compensate the effects of reducing compounds co-extracted in coffee oils.

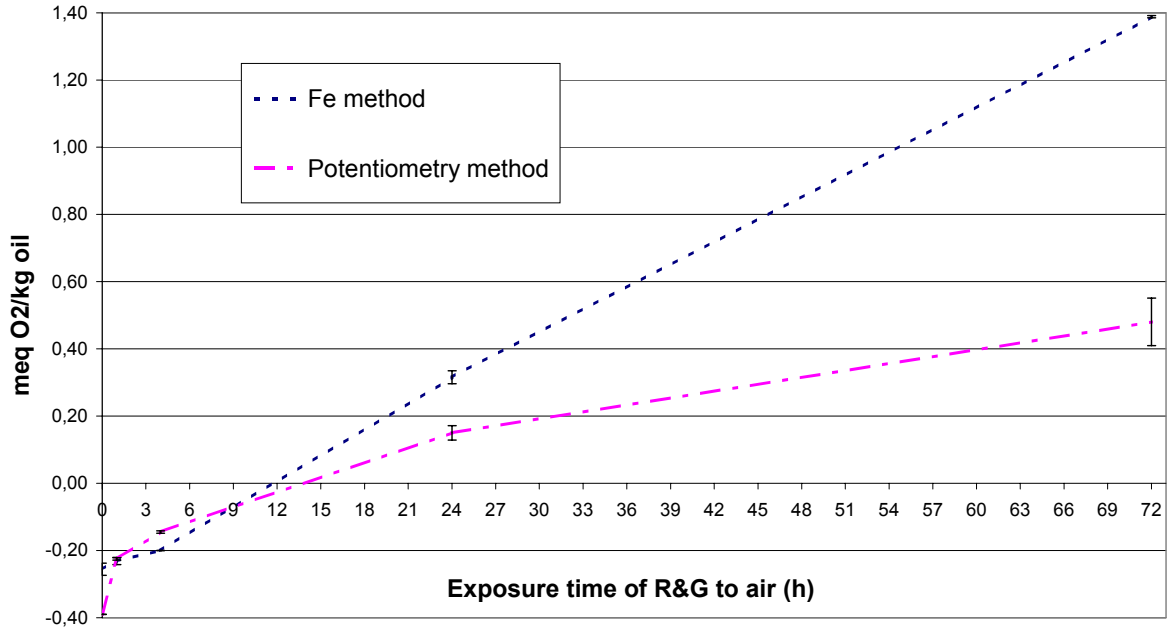

Figure 3. POV of coffee lipid extracts by Fe and potentiometric methods

Compared to SPME HS-GC/MS used in this study, other existing methods commonly used to assess lipid oxidation via hexanal, e.g. static headspace GC and SDE (simultaneous distillation-extraction)-GC, cannot provide as easily the separate information about the status of primary and secondary oxidation of the lipids. Actually, lipids are further oxidised during analysis using SDE-GC under aerobic conditions. Moreover, retroaldolisation of dodecadienal occurs during SDE to give hexanal, which overlaps with hexanal generated from lipid hydroperoxides.

CONCLUSIONS

Coffee lipids in R&G are oxidised in the early stage of R&G exposure to air. Both primary and secondary oxidation steps, i.e. formation and decomposition of lipid hydroperoxides from coffee lipids, already occurred in R&G within the first 24 h exposure to air, as evidenced by POV and hexanal measurements reported in this study. Therefore, to accurately assess oxidation status of coffee lipids in R&G, measurement of both primary and secondary lipid oxidation products is necessary. SPME HS-GC/MS as evaluated in this study can suitably achieve this task.

Although lipid oxidation products have little effect on fresh R&G (Nicoli et al, 1993), they can contribute, in particular volatile secondary oxidation products, to develop oxidised off-notes in coffee when the positive coffee flavour compounds in R&G start to decompose.

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A Preliminary Comparison of Melanoidins from Coffee and Roasted Barley Brews by Size Exclusion Chromatography (SEC)

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SUMMARY

Melanoidins are high molecular weight end products of the Maillard reaction that are responsible for the colour of foods and probably have antioxidative properties. Aims of this work were the separation and comparison of the melanoidin fractions from coffee and roasted barley brews. Defatted brews obtained from commercial soluble products were separated by membrane ultra-filtration. The obtained fractions were analysed on a Superdex Peptide column. Relevant differences were observed among coffee and barley brews.

INTRODUCTION

The Maillard reaction is responsible of most colour formation during thermal processing of foods, such as bread baking, roasting of coffee and nuts, roasting of meat, and kiln-drying of malt (Arnoldi, 2001). Nobody can deny the importance of this phenomenon in determining the quality of foods, however, many methodological difficulties have slowed down the progress in the knowledge of colour at a molecular level. The most important contribution to colour comes from polymers, which are known as melanoidins. Some attempts have been made to isolate melanoidins from foods, for example from soy sauce (Lee et al., 1987), dark beer (Kuntcheva and Obretenov, 1996), malt or roasted barley (Milic et al., 1975; Obretenov et al., 1991), and coffee (Steinhart and Packert, 1993; Homma, 1999), but their very complex, probably non-repetitive, structure has limited their characterisation.

However, studies on model systems have clearly indicated that reducing carbohydrates and compounds possessing a free amino group, such as amino acids, either in free or protein-bound form, are the basic material for their formation (Ledl and Schleicher, 1990).

Beside their obvious importance in determining the brown colour of roasted foods, in recent years great interest has developed around melanoidins for their possible role in physiology and in food stabilisation: in fact some Authors (Anese et al., 1999; Ames, 2000) have demonstrated that they possess antioxidative activity. Antioxidants are able to delay or prevent oxidation processes, typically involving lipids, which greatly affect the shelf-life of foods. In coffee the antioxidative activity (attributed to the development of Maillard reaction products) increases with roasting up to the medium-dark roasted stage, then decreases with further roasting (Nicoli et al., 1997), possibly indicating a partial decomposition of the antioxidative compounds.

However, other melanoidin physico-chemical properties may be important in foods. For example, in *espresso* coffee brew, they may have foaming properties (Petracco et al., 1999) that stabilise the foamy layer on top of the beverage, which is well known by the Italian term *crema*.

Besides amino acids and sugars, some vegetables contain high amounts of polyphenols, which probably may be incorporated in the structure of melanoidins. This phenomenon has been postulated for coffee, which contains chlorogenic acids (Heinrich and Baltes, 1987), and for barley, which contains several polyphenols and in particular bound ferulic acid (Zupfer et al., 1998), but certainly has not been sufficiently studied so far. This work aims to compare some chemical properties of the melanoidins of coffee brew and roasted barley brew, a caffeine-less coffee substitute.

EXPERIMENTAL

Materials

HPLC-grade acetonitrile and hexane were purchased from Baker, HPLC water was produced with a Milli-Q Water Purification System (Millipore), and TFA was purchased from Fluka. HPLC eluents and samples were filtered through disposable nylon 66 filters (0.45 µm, Alltech, Italy). The analysed samples were Nescafe (Nestlé, Switzerland) and Orzobimbo, a commercial lyophilised roasted barley product by STAR (Italy).

Sample preparation

Lyophilised coffee or roasted barley (0.5 g) were dissolved in 10 mL of Milli-Q water with stirring and added with 5 mL of hexane in order to extract fats. The two phases were separated by centrifugation (30 min, 10,000 rpm, 4 °C).

Separation of different molecular weight fractions

The coloured materials of the aqueous layer were separated in four fractions by ultrafiltration on Amicon membranes with molecular cut-off limits of 30,000 Da, 10,000 Da, and 3,000 Da. Each retentate was washed with MilliQ water (4 x 40 mL) and freeze-dried (total average recovery 87 %). In this way four fractions were obtained: a high molecular weight fraction HMW (> 30,000 Da); two intermediate fractions IMW1 (range 10,000-30,000 Da) and IMW2 (range 3,000-10,000 Da), and a low molecular weight fraction LMW (< 3,000 Da).

Size-Exclusion Chromatography (SEC)

SEC analyses were conducted with a Hewlett-Packard HP-1050 quaternary pump fitted with a Rheodyne injector (20 µL, loop) and equipped with a HP-1050 Diode Array Detector (HPLC-DAD). SEC separations were achieved on a Superdex® Peptide HR10/30 column (molecular weight range 7,000-300 Da, Amersham Pharmacia Biotech). The analyses were carried out in isocratic conditions, using 0.1% TFA in water/acetonitrile (80:20 v/v) as mobile phase; the flow rate was 0.4 mL/min. The chromatograms were recorded at 254, 360, and 405 nm. Complete spectral data were recorded from 220 to 550 nm for the peaks of interest. All the samples were injected after dilution at the same concentration (5 mg/mL).

Colour dilution factors (CD_{total})

In order to evaluate the browning intensity of the fractions, colour dilution factors were measured according to a recent method (Hofmann, 1998). The freeze-dried material (10 mg) was dissolved in 1 mL of water and diluted 1:1 by volume until the colour difference between the sample and two blanks of tap water could be visually detected in a triangle test.

RESULTS

Aim of this work was the comparison of some properties of the melanoidins from coffee and roasted barley. Melanoidins were extracted from brews prepared dissolving commercial samples of soluble roasted barley and coffee with standard procedures and defatted with hexane.

The coloured material was separated by ultrafiltration in 4 fractions: HMW, IMW1, IMW2, and LWM (Table 1). Major differences were observed in the relative amount of each fraction isolated from roasted barley or coffee brews. LMW is the main fraction in coffee, being HMW only 21%, whereas HMW is the main fraction in roasted barley (50%). The two intermediate fractions are in both cases not very abundant.

To get a quantitative measure of each fraction colour, it was decided to determine colour dilution factors, which have been recently proposed by Hofmann (1998) for the quantification of the colour intensity of Maillard reaction products. The same amount of each freeze-dried fraction was dissolved in 1 mL of water and diluted 1:1, until it was possible to distinguish its brown colour from tap water (higher values mean higher colour intensity). The results of these experiments are shown in Table 1. With the exception of IMW1, the colour intensity of each coffee fraction is higher than the colour intensity of the corresponding roasted barley fraction. In both cases, the ratio of the CD values of the main fractions (HMW / LMW) is 10, indicating that the major contribution to the colour of the brews comes from high molecular weight melanoidins.

Table 1. Recovery and CD of the fractions obtained by ultrafiltration from coffee and roasted barley brews

Fractions	Nominal molecular	Coffee		Barley	
	Weight (Da)	Recovery (mg)	CD_{total}	Recovery (mg)	CD_{total}
HMW	> 30,000	211	80,000	501	20,000
IMW1	30,000-10,000	18	40,000	45	40,000
IMW2	10,000-3,000	82	20,000	14	4,000
LMW	< 3,000	522	8,000	307	2,000

To have an idea of the complexity of the isolated fractions, they were diluted to the same concentration and submitted to SEC analysis on a column with molecular range 7,000-300 Da. The results shown in Figure 1 (chromatograms at 405 nm) confirm that there are important differences in the characteristics of coffee and roasted barley melanoidins. The HMW fraction of barley contains almost exclusively an unretained narrow peak with very high molecular weight, and IMW1 and IMW2 contain one very broad coloured peak at about 6,600 and 8,000 Da, respectively (the latter is considerably tailed). LMW does not contain any evident coloured peak, whereas it contains a relevant non-coloured peak at 500 Da that certainly explains the low CD value.

Considering now coffee, the HMW fraction contains only a small unretained peak, accompanied by a substantial broad peak, with maximum around 12-10,000 Da (outside the column linearity range). Both IMW1 and IMW2 contain only one major coloured peak with maxima at 5,300 and 3,900 Da, respectively, and, as in roasted barley, the LMW fraction does not show any relevant peak at 405 nm, whereas the chromatogram at 254 nm shows a relevant peak at 700 Da. Thus again, the major differences between coffee and roasted barley are related to high molecular weight melanoidins. The HMW fraction of roasted barley, besides being very abundant, contains materials with much higher molecular weight but less colour.

Perhaps that could suggest that pre-existing non-coloured polymeric materials are derivatised by chromophores deriving from the Maillard reaction. In coffee, instead, the HMW fraction has features more similar to the IMW fractions.

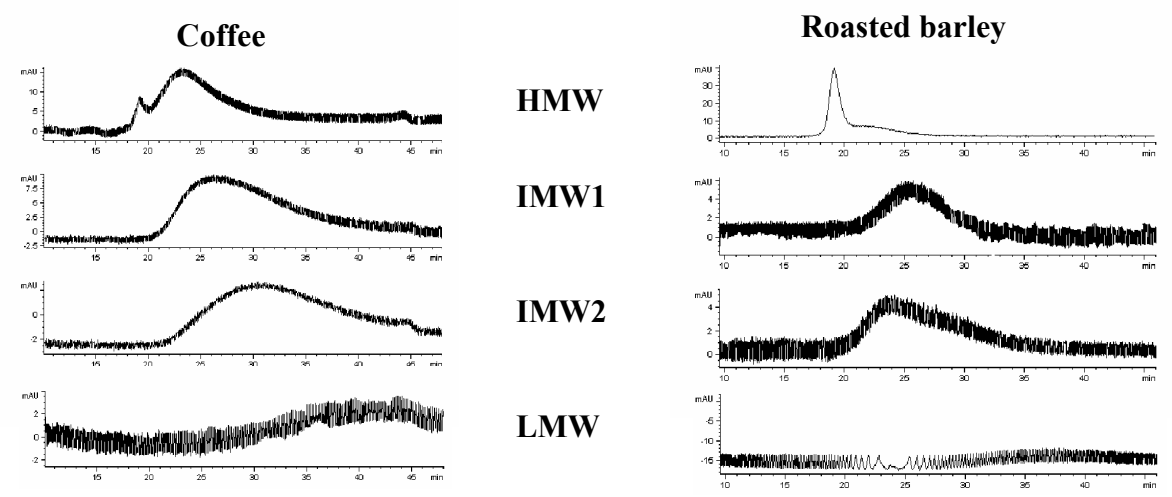

Figure 1. SEC analysis at 405 nm of coffee and roasted barley fractions obtained by ultra-filtration

Although this is only a very preliminary work that gives a limited contribution to the structural characterisation of the melanoidins of coffee and roasted barley brews, it seems to suggest that the differences could depend not only on amino acids and sugars but on other components, such as constitutive polyphenols, which could participate to the Maillard reaction. The analysis by HPLC and NMR and the determination of the antioxidative characteristics of these fractions are in progress and should furnish other relevant data for a better comprehension of the differences between coffee and roasted barley melanoidins.

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Antioxidant Properties of Ready-to-Drink Coffee Beverages

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SUMMARY

The influence of some technological variables on the evolution of the antioxidant capacity of ready-to-drink coffee brews was investigated. Results indicated that, depending on the roasting degree as well as on the packaging conditions adopted, redox reactions, which can take place during storage, are responsible for significant changes in the overall antioxidant capacity of the product. Results suggested that these reactions may be attributed to a further development of the Maillard reaction as a consequence of the extraction and solubilisation of reactants during the beverage production. Appropriate packaging and storage conditions able to preserve or enhance the intrinsic antioxidant capacity of the beverages were singled out.

INTRODUCTION

Following the disclosure of epidemiological associations between diet and health, in recent years an increasing attention have been paid towards those foods potentially exerting antioxidant capacity. In heat-treated foods, such as coffee and related beverages, this property is generally the result of the contribution of both natural and heat-induced antioxidants. In fact, during very intense heat treatments, such as the roasting of coffee, the progressive thermal degradation of naturally occurring antioxidants, mainly represented by polyphenols, has been found to be minimised by the formation of Maillard reaction products (MRPs) (Nicoli et al., 1997, 1999). These compounds, particularly those formed during the advanced stages of the reaction, represent the main antioxidant substances of coffee. Although the physiological roles of coffee MRPs have not been still clearly elucidated, MRPs are generally believed to exert a anti-mutagenic and anti-carcinogenic effects (Powrie et al., 1981; Gazzani et al., 1987; Vagnarelli et al., 1991). For this reason the possibility to maintain the intrinsic antioxidant properties during all the technological steps, which follow the roasting process, represents a challenge for the production of coffee and related products with high functional profile.

The present investigation was designed to evaluate the influence of some technological variables on the evolution of the antioxidant capacity of ready-to-drink coffee brews. These products, whose market rapidly increased in the last decade, generally undergo to a rapid decay of their original aroma and flavour. The mechanisms at the basis of this quality loss are still unknown, but they are generally accompanied by a decrease in pH, which, in turn, affects the acid-base equilibria and the partition of volatile compounds in the vapour phase (Takahashi et al., 1979; Clarke and Macrae, 1985; Engelhardt and Maier, 1985; Dalla Rosa et al., 1990; Groetzbac et al., 1995a; Skrader et al., 1996). Although there are no experimental evidences indicating that the staling of coffee brew can be attributed to redox reactions, the intrinsic antioxidant properties of the beverages are expected to be strongly affected by these undesired phenomena as well as by the storage conditions.

MATERIALS AND METHODS

Materials

Green, light, medium and dark roasted coffee beans and the correspondent soluble products obtained by the same starting materials were supplied by the Nestlè Research Center of Lausanne.

The colour of the ground coffee beans are reported in Table 1.

Sample preparation

Coffee brews were obtained by solid-liquid extraction using deionised water at 100°C. The coffee-water ratio was 10:100 (w/w). The extraction time was 10 min. After extraction, the beverages were rapidly cooled in cold running water and filtered through Whatman n. 4 filter paper. All the procedures were carried out very quickly in order to minimise the contact of the beverage with atmospheric oxygen. Coffee beverages obtained from soluble coffees were prepared by solubilising 2.5 g of powder in 100 ml of deionised boiling water. Such an amount of coffee was chosen in order to obtain brews with the same solid content of those prepared by solid-liquid extraction. After solubilisation, the samples were then immediately cooled under cold running water. Volumes of 15 ml of both series of coffee brews were introduced in 20 ml capacity vial in the presence of air or nitrogen. The vials were then hermetically closed with butyl septa and metallic caps. Dark-roasted coffee samples obtained by solid-liquid extraction and bottled in air or under nitrogen atmosphere were placed in a thermostat at 30°C for up to 17 days.

Analytical determinations

Solid content

Total solid content determination was carried out according to AOAC methods. The measurements were made in triplicate.

Colour

Colour analyses were carried out on coffee samples using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-200 measuring head. The instrument was standardised against a white tile before measurements. Colour was expressed in L*, a* and b* Hunter scale parameters (Clydesdale, 1978). Data were expressed as b*/a* ratio.

Chain breaking activity

The chain breaking activity was measured by means of the DPPH• method. According to this methodology (Brand-Williams et al., 1995), the reduction of a stable radical (2,2'-difenil-1-picrilidrazile, DPPH•; Sigma Chemical Co., St. Louis, Mo., USA) by the antioxidant or a mixture of antioxidants was followed by monitoring the decrease in absorbance at 515 nm at 25°C. A volume of 1.9 mL of 6.0 10^{-5} M methanol solutions was used. The reaction was started by the addition of 100 μL of samples, previously diluted 1: 200 (v/v) with deionised water. In all cases the DPPH• bleaching rate was proportional to the sample concentration added to the medium. Reaction rates were calculated using the equation proposed by Manzocco et al. (1998):

$$1/Abs^3 - 1/Abs_0^3 = -3kt$$

where k is the DPPH• bleaching rate, Abs_0^3 is the initial absorbance value and Abs^3 is the absorbance at increasing time t. The chain-breaking activity was expressed as the slope obtained from equation 1 ($-Abs^{-3}\ min^{-1}$) per milligram of dry matter. In the former case all the dry matter of the sample was assumed to possess antioxidant properties.

pH

The pH was measured at 25°C using a pHmeter (Crison, mod. 2002, Spain) equipped with a combination of glass electrodes and a temperature probe.

Redox potential

The redox potential measurements of the coffee samples were made with a platinum indicating electrode and a Ag/AgCL, Cl^-_{sat} reference electrode connected with a voltmeter (Crison, mod. 2002, Spain). Calibration was performed against redox standard solutions (Reagecon, Shannon, Co. Clare, Ireland) having redox potential values of 220 and 465 mV at 25°C. Electrodes were placed in a 50 mL 3-neck flask containing a volume of 20 mL of sample. Prior to analysis, oxygen was removed from the system by continuous nitrogen flushing for a period of 10 min. Millivolt values were recorded for at least 20 min at 25°C, until a stable potential was reached. A stable potential was arbitrarily defined as a change of less than 2 mV in a 3 min period. In our experimental conditions the redox potential was not significantly affected by the pH value of the coffee samples.

Statistical analysis

The results reported here are the average of at least three measurements, and the coefficients of variation, expressed as the percentage ratio between the standard deviation (SD) and the mean values, were lower than 2 for solid content and pH, 15 for color, 15 for chain breaking activity, 5 for redox potential. One-way analysis of variance was determined using the Tukey-Krammer test (JMP 3.2.5, SAS Institute Inc., Cary, NC, 1999).

RESULTS AND DISCUSSION

The antioxidant capacity was evaluated by analysing both the chain breaking activity and the redox potential values. While the assessment of the chain-breaking activity allows to estimate the quenching rate of coffee compounds toward a reference radical, the redox potential gives indication on the effective oxidation/reduction efficiency of all the antioxidants present, including the “slow” ones, which cannot be detected by the kinetic method (Nicoli and Anese, 2001). These compounds are expected to have an important role in determining and maintaining the antioxidant properties of ready-to-drink coffee beverages during storage. The influence of the roasting degree on the antioxidant capacity of coffee beverages, obtained by solid-liquid extraction, is shown in Table 2. The statistical analysis has shown that differently-roasted coffee samples had the same chain breaking activity, while the redox potential significantly decreased as the intensity of the roasting process increased, indicating a considerable gain in the overall reducing properties of the beverage. The decrease in the redox potential values can be likely attributed to the formation, during roasting, of melanoidins (i.e. products of the Maillard reaction), whose reducing properties increased with the increasing of the intensity of the heat treatment (Hayase et al., 1989; Namiki, 1990). By contrast, these heat-induced compounds had similar radical scavenging activity.

Table 1. Data on colour of green and roasted ground coffee beans

Sample	Colour		
	L*	a*	b*
Green	69.3 ± 0.24	3.3 ± 0.07	9.0 ± 0.31
Light	31.3 ± 0.79	10.8 ± 0.39	10.4 ± 0.99
Medium	25.9 ± 0.71	8.8 ± 0.18	5.8 ± 0.40
Dark	24.3 ± 0.42	6.5 ± 0.11	1.1 ± 0.20

Table 2. Chain breaking activity and redox potential values of fresh-made coffee beverages obtained by solid-liquid extraction of coffees having different roasting degree

Sample	Chain breaking activity ($-Abs^{-3}min^{-1}mg_{ss}^{-1}$)	Redox potential (mV)
Green	0.32 ± 0.13[a]	109
Light	0.273 ± 0.03 [a]	45
Medium	0.283 ± 0.13 [a]	-2
Dark	0.390 ± 0.04 [a]	-35

[a]*Chain breaking activity values are presented as the mean ± SD (n=3). Means with a common letter did not differ significantly (p<0.05)*

Table 3. Values of chain breaking activity and redox potential of beverages prepared using soluble coffee samples having different roasting degree

Sample	Chain breaking activity ($-Abs^{-3}min^{-1}mg_{ss}^{-1}$)	Redox potential (mV)
Light	5.79 ± 1.15[a]	40
Medium	6.98 ± 0.78[a]	22
Dark	2.71 ± 0.72[b]	13

[a]*Chain breaking activity values are presented as the mean ± SD (n=3). Means with a common letter did not differ significantly (p<0.05).*

Table 3 shows the chain breaking activity and redox potential values of coffee brews obtained from the soluble coffee powders with different roasting degrees. In this case, no significant differences were observed between the chain breaking activity capacity of light- and medium-roasted samples, while a statistical lower value was found for the dark-roasted one. These data, which are in agreement with previous results (Nicoli et al., 1997), show that melanoidins exhibit high chain breaking capacity, which dynamically evolves during the roasting process. By contrast, a lower redox potential value was found for the dark-roasted coffee. Also, it is interesting to observe that beverages prepared using soluble coffees exhibited higher chain breaking (10-fold increase) and redox potential values as compared to those reported in Table 2. Results suggest that extraction and spray-drying procedures applied for the production of soluble coffee powders can be responsible for a further development of

Maillard reactions. However, the slight differences in the redox potential values indicate a certain oxidative damage of the coffee brews.

It is well known that quality loss of ready-to-drink coffee beverages during storage is generally attributed to the development of a series of complex reactions whose mechanism is still unknown. The main consequences are a decrease in pH and undesirable changes in the flavour and aroma profile. Figure 1 shows the pH changes of the dark-roasted coffee beverages, obtained by solid-liquid extraction and bottled in air or under nitrogen atmosphere, vs time under accelerated storage conditions (30°C). According to previous observations the decrease in the pH of the beverage did not seem to be affected by the presence of oxygen (Nicoli et al., 1989). The comparison of the rates of the pH decrease, calculated using the zero order kinetic model by plotting the correspondent values of hydrogen molar concentration vs storage time, showed no significant differences between the two series of samples. In fact, in both cases the rate constants resulted close to 3 10^{-8} $[H^+]$ $hour^{-1}$ (r^2: 0.94 and 0.97 for ordinary and nitrogen atmospheres, respectively).

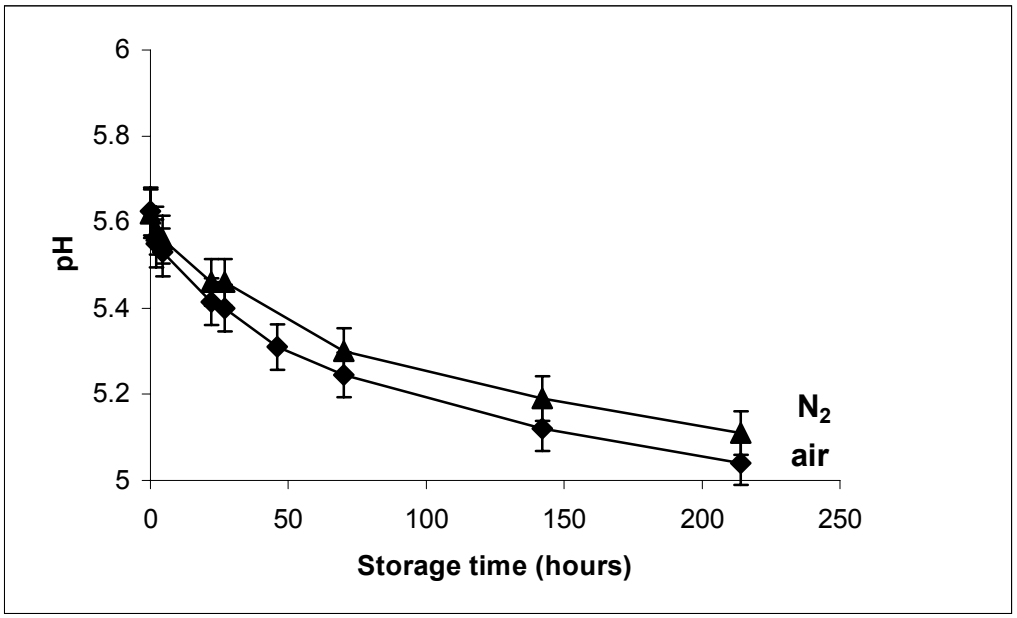

Figure 1. Changes of pH values of dark-roasted coffee brews, obtained by solid-liquid extraction and bottled in ordinary and nitrogen atmospheres, as a function of storage time at 30°C

The evolution of the chain breaking activity of dark-roasted coffee beverages, obtained by solid-liquid extraction and packed in ordinary and nitrogen atmospheres, as a function of storage time at 30°C is showed in Figure 2. For both series of samples, a gain and a further decrease in the chain breaking activity was observed. However, in our experimental conditions, the chain breaking activity values measured during storage never resulted lower than those measured for the fresh-made samples. Results clearly indicate that the radical quenching properties of coffee brews can greatly increase during storage and that this event is not related to the presence of oxygen. A similar evolution of the chain breaking activity was firstly observed in polyphenol-containing foods undergone oxidative stress (Cheigh et al., 1995; Manzocco et al., 1998; Nicoli et al., 2000). In those cases, the gain in the radical quenching properties was attributed to the partial oxidation of polyphenols to form stable macromolecular intermediates having an increased resonance delocalisation and a higher stability of the aryloxyl radicals. Since coffee polyphenols are thermally degraded during the roasting process, it is likely that other compounds may be involved in such evolution of the chain breaking activity of the coffee beverages during storage. The extraction procedures

and/or the storage conditions can probably favour a further development of the Maillard reaction with the formation of polyphenol-type intermediates, which increased the overall radical scavenging properties of the product.

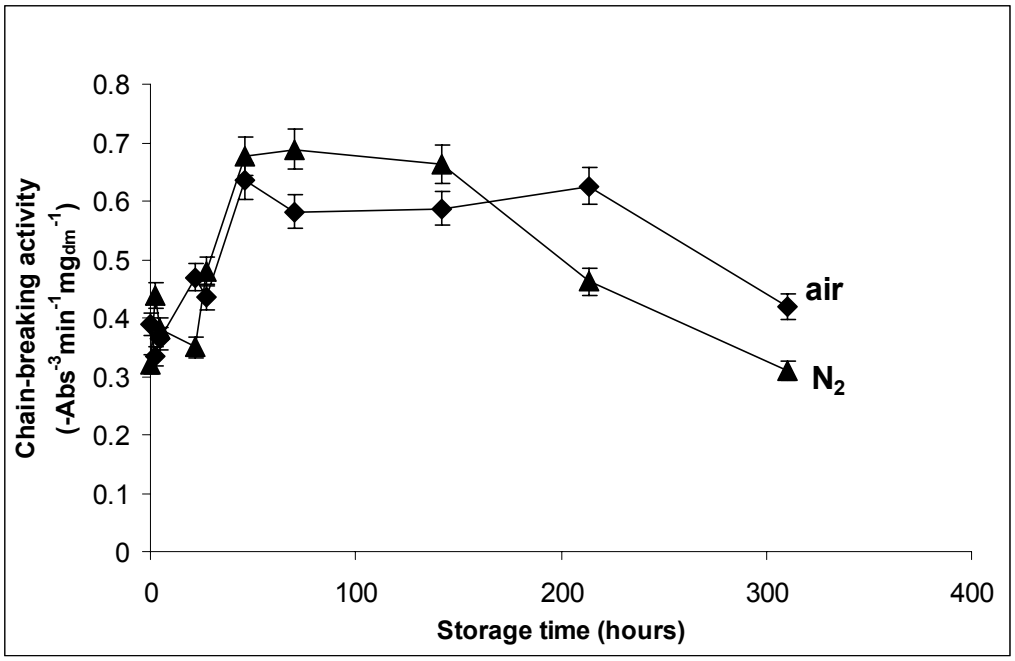

Figure 2. Changes in chain breaking activity of dark-roasted coffee brews, obtained by solid-liquid extraction and bottled in ordinary and nitrogen atmospheres, as a function of storage time at 30°C

Figure 3 shows the changes in the redox potential values of the dark-roasted coffee beverages, obtained by solid-liquid extraction and packed in ordinary and nitrogen atmospheres, as a function of storage time at 30°C. It is interesting to note that, for the samples packed in air, the gain in chain breaking activity was accompanied by an increase in the redox potential. These results indicate that, despite their increased radical quenching activity, compounds formed at intermediate storage times showed less antioxidant efficiency than those initially present. According to the Nerst's Equation, this can be attributed to the formation of compounds having higher standard redox potential and/or to the prevalence of the oxidised forms of the redox couples. The latter hypothesis seems to be confirmed by the evolution of the redox potential of the coffee brews stored under nitrogen atmosphere. In this case, no significant changes in the redox potential values were detected. It means that, in the absence of oxygen, the coffee samples maintained the same reducing properties for all the storage time, despite the chain-breaking activity changed. Whatever was the cause, the redox potential changes of air-packed coffee brews clearly indicate that the antioxidant reservoir of the product is going to be consumed.

Data on chain breaking activity and redox potential are consistent with results on the changes in colour, expressed as b*/a* ratio, of the considered coffee beverages during storage time (Figure 4). In fact, while no significant variation in the colour index was detected for coffee brews stored under nitrogen atmosphere, for the air-packed samples a significant shift from the yellow-red to the blu-red zone of the L*a*b* colour space was measured. These results can be attributed to melanoidin oxidation, in agreement with previous reports (Groetzbac et al., 1995b, 1995c).

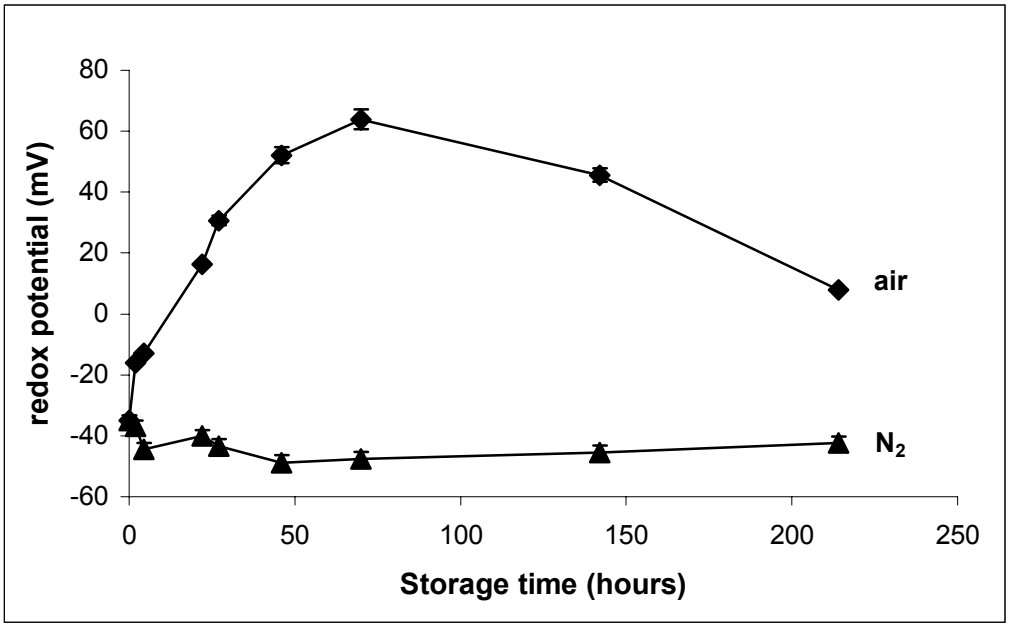

Figure 3. Changes in redox potential of dark-roasted coffee brews, obtained by solid-liquid extraction and bottled in ordinary and nitrogen atmospheres, as a function of storage time at 30°C

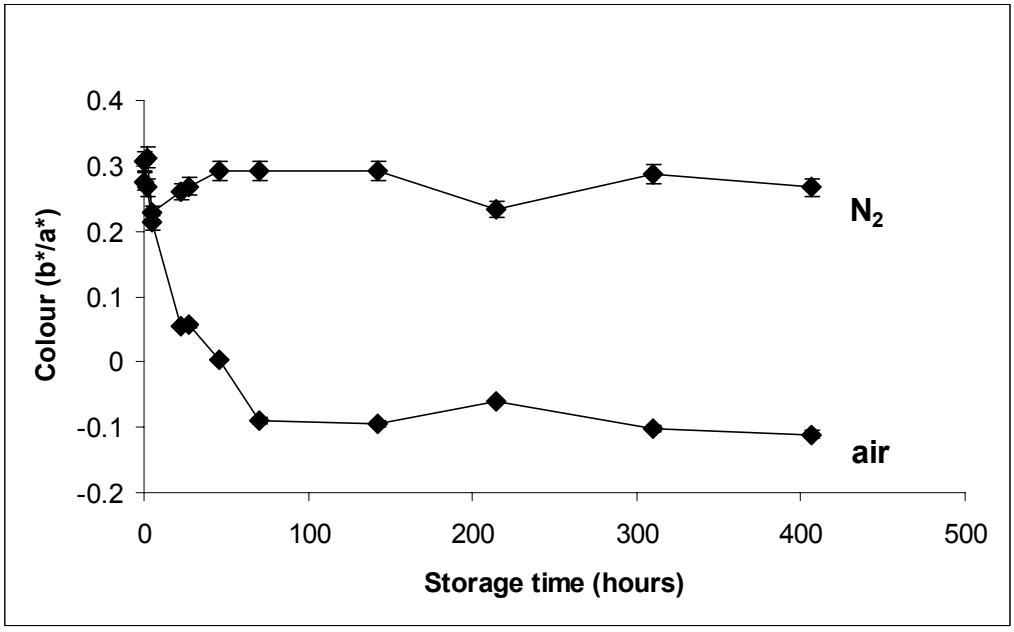

Figure 4. Changes in b*/a* ratio of dark-roasted coffee brews, obtained by solid-liquid extraction and bottled in ordinary and nitrogen atmospheres, as a function of storage time at 30°C

CONCLUSION

The overall antioxidant capacity of coffee beverages strictly depends on the roasting degree and on the intensity of possible oxidative stress related to further technological procedures. These properties were also found to dynamically change during the storage time. In particular, the radical scavenging properties of the beverage unexpectedly increased reaching a maximum at intermediate storage times. Similar behaviour of the chain breaking activity was

previously observed for polyphenol-containing foods undergone oxidative stress. Although the changes in the radical scavenging properties were not affected by the presence of oxygen, results of redox potential suggested that the exclusion of the oxygen from the head-space of the package allowed to a better retention of original colour and of the intrinsic antioxidant efficiency of the product.

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Characterisation of Arabica and Robusta Coffee Varieties According to their Trace Heavy Metals Content

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SUMMARY

Coffee is commercially available as one of the two varieties known as *Arabica* and *Robusta*. The coffee beverages are made from *Arabica* and *Robusta* roasted beans or blends of these two. The better quality coffees and usually the most expensive ones are considered to be arabicas. Therefore it is important to have analytical methods to differentiate between these two coffee varieties. The chemical composition of samples constitute a very interesting approach in order to distinguish the varieties. In this way several reports based on sterols content, analysis of the volatile components, infrared spectroscopic data, have been published.

The metal content of the coffee beans has been proved to be adequate to differentiate between the *Arabica* and *Robusta* varieties.

In this work a study of the metal content of green samples and espresso coffee has been carried out. Eight elements, Pb, Cd, Ni, Mn, Zn, Co, Cu and Cr have been analysed by graphite furnace atomic absorption spectrometry (GFAAS). These chemicals descriptors were used to characterise coffee samples from *Arabica* and *Robusta* varieties. The most important results were obtained for the elements Mn and Zn. The analysis of 37 green samples (21 *Arabica* and 16 *Robusta*) showed that Mn and Zn could be considered a very interesting elements for checking the origin of coffee. These results have been confirmed by the analysis of the corresponding espresso coffee samples; in the liquid material the levels of trace elements were generally much lower than in the green beans samples. The content of the toxic elements, Cd, Pb and Ni was low, being much less than or just about 0.5 ppm in almost all samples.

INTRODUCTION

Researches concerning the chemical composition of coffee beans have till now concentrated especially on the components that gives coffee its typical flavour and on those from which the characteristic effects of the beverage on man depend (Clarke, 1985). The contents of minerals and, peculiarly, of trace elements have been instead one of the less investigated aspects of the chemical composition of the beans. However, some recent works on this topic have shown how microelements could be used to distinguish between the two coffee varieties more common on the world market, i.e. *Arabica* and *Robusta*: especially, attempts have been made to establish the respective percentage of each variety in a possible mixture (Macrae et al., 1993; Martin et al., 1998; Maier, 1981; Krivan et al., 1993; Martin et al., 1999; Onianwa et al., 1999; Mazzotta et al., 1993). The possible determination of the right percentage of the two varieties could be very interesting, given the different commercial value attached to the two varieties, being, as everybody knows, the *Arabica* variety more precious and so more expensive than the *Robusta*.

In this work we have considered the contents of 8 microelements (Pb, Cd, Ni, Zn, Cu, Cr, Mn and Co) in 37 coffee samples of different origin, 21 of the *Arabica* variety and 16 of the *Robusta*. Both green coffee and the beverages made with the *espresso* technique were analysed. In fact we thought it would be interesting to establish also with what contribution in microelements, both biophilic and xenobiotic, coffee could supply the diet. The analysed samples are almost 80% of the world production.

EXPERIMENTAL

Before proceeding to the determination of these microelements, the coffee samples used for this work were properly prepared.

The green coffee samples were finely ground with a Retsch mill, in order to obtain particles whose diameter was minor than 1 mm. 500 mg of each sample were mineralised.

A small quantity of beans from each green coffee sample was roasted at a constant temperature of about 220°C with a Petroncini machine. After the roasting, each sample was finely ground. Espresso coffees were made with a Rossi POD System Brasilia (model Gradisca) professional espresso machine. All the espresso coffees were frozen and then lyophilised. 250 mg of each lyophilised sample were mineralised. We used the method of the dry mineralization.

The atomic absorption spectrophotometer we used is provided with a graphite furnace atomisation system and with a deuterium lamp background corrector and was equipped with the hollow cathode lamps of the microelements we searched. Each sample was mineralised thrice and then three spectrophotometric analyses were made for every element; average and standard deviation were calculated from the results.

RESULTS AND DISCUSSION

Among the biophilic microelements determined in green coffee, Mn appears to be the most interesting. In accordance with what literature reports, Mn content is in general definitely greater in the *Arabica* variety than in the *Robusta* one. Besides, often the content of this microelement appears to be comparable in samples having similar places of origin: in fact it is on average greater in the *Arabica* samples coming from Central and South America than in those coming from Africa, and there aren't significant statistic differences among the samples coming from Haiti, S. Domingo, Brazil S. Florida, Costa Rica and Ecuador, as well as among those coming from Mexico and Cuba. As regards the African products, contents are extremely similar in the samples coming from Cameroun, Kenya and Ethiopia.

The figures show also a greater presence of Zn in the *Arabica* variety; the content is peculiarly high in some samples coming from Central and South America, that is from Haiti, Mexico, Guatemala and Ecuador. These results are interesting because we can foresee the possibility of using microelements not only to discriminate between the varieties *Arabica* and *Robusta*, but even to distinguish samples of different geographic origin.

The results concerning the other biophilic microelements seem less interesting: in fact the content of Co, Cr and Cu appears to be comparable in the two varieties, with considerable fluctuations also among samples of the same variety.

As regards the investigated xenobiotic microelements, contents are generally low. The most abundant pollutant is Pb, with values sometimes higher than 0,5 ppm, but always lower than 1

ppm (and so included within the limit fixed by the Italian law for roasted coffee). The content of Cd appears to be always very low, while Ni's presence results higher than Cd's, but lower than Pb's and all the same always below 0,3 ppm (Table 1).

The results obtained from the espresso coffee samples confirm those obtained from the green products. The contents of all the investigated microelements are clearly lower in the espresso coffees than in the green ones. The most abundant biophilic microelements result always Mn and Zn: also for the espresso coffee content is higher in the *Arabica* variety than in the *Robusta* one. As regards xenobiotic elements, instead, Cd and Ni appear to be present at ppb values, while Pb's content results on average higher, but all the same always lower than 0,5 ppm (Table 2).

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Table 1. Content (μg/g) of microelements in green coffee samples

Arabica	Zn	Mn	Co	Cr	Cu	Cd	Pb	Ni
Haiti	5.71	11.84	0.035	0.113	7.01	0.014	0.018	0.085
Mexico	5.92	13.72	0.003	0.179	7.18	0.015	0.650	0.127
Cameroun	4.59	18.12	0.072	0.143	8.80	0.015	0.023	0.235
Guatemala	5.87	26.30	0.017	0.110	4.42	0.031	0.624	0.074
Cuba	3.79	13.64	0.017	0.047	5.75	0.025	0.004	0.148
Indonesia	4.16	18.29	0.012	0.177	3.99	0.019	0.712	0.126
Colombia	3.04	28.16	0.068	0.057	4.93	0.012	0.057	0.043
Nicaragua	4.37	21.18	0.104	0.073	5.38	0.009	0.023	0.078
Venezuela	3.68	46.53	0.066	0.146	4.70	0.011	0.601	0.143
S.Domingo	2.99	11.87	0.005	0.132	3.48	0.006	0.011	0.035
Puerto Rico	3.57	37.68	0.329	0.041	6.18	0.011	0.088	0.084
Brazil (Fancy)	3.15	22.62	0.035	0.045	4.82	0.008	0.028	0.026
Brazil (Florida)	2.72	11.91	0.016	0.104	13.82	0.015	0.220	0.020
Kenya	3.79	17.93	0.017	0.095	6.14	0.006	0.225	0.030
Ethiopia	3.09	17.49	0.004	0.061	4.94	0.005	0.288	0.061
India A.	2.61	23.22	0.159	0.178	4.53	0.010	0.012	0.134
Uganda	2.51	11.28	0.018	0.084	5.74	0.023	0.608	0.038
Costa Rica	2.45	12.74	0.084	0.072	4.89	0.003	0.016	0.026
Ecuador	5.39	11.94	0.003	0.080	3.92	0.010	0.270	0.013
Burundi	2.72	31.32	0.003	0.068	8.77	0.008	0.202	0.022
Peru	3.15	30.72	0.007	0.156	4.66	0.003	0.170	0.066
Robusta								
Uganda	2.99	9.88	0.064	0.071	4.47	0.035	0.087	0.090
C.d'Ivoire	2.83	7.99	0.060	0.168	8.76	0.002	0.010	0.035
India P.	3.63	11.92	0.140	0.048	7.85	0.007	0.009	0.272
India C.	2.61	11.59	0.157	0.053	7.24	0.008	0.091	0.159
Guatemala	1.33	3.44	0.007	0.074	7.92	0.028	0.270	0.020
Papua N. G.	2.83	6.18	0.014	0.073	7.72	0.016	0.719	0.225
Brazil	2.45	9.76	0.036	0.067	4.27	0.007	0.442	0.018
Indonesia	3.36	7.53	0.046	0.068	5.15	0.040	0.185	0.007
Togo	3.20	12.16	0.106	0.251	9.24	0.007	0.737	0.026
Angola	1.71	4.42	0.041	0.066	7.46	0.005	0.031	0.048
Viet Nam	2.24	18.86	0.767	0.113	3.94	0.010	0.060	0.018
Cameroun	3.63	8.90	0.065	0.100	6.92	0.003	0.030	0.020
Java	3.73	8.84	0.007	0.055	4.50	0.011	0.127	0.007
Madagascar	2.03	9.18	0.112	0.056	3.38	0.002	0.070	0.020
Thailand	2.93	15.75	0.338	0.053	3.78	0.011	0.021	0.100
C.A.R.	3.25	14.76	0.103	0.052	7.96	0.024	0.634	0.045

Table 2. Content (μg/ml) of microelements in espresso coffee samples

Arabica	Zn	Mn	Co	Cr	Cu	Cd	Pb	Ni
Haiti	0.997	0.592	0.0028	0.041	0.181	0.002	0.101	0.003
Mexico	1.187	0.514	0.0012	0.005	0.242	0.001	0.059	0.003
Cameroun	0.844	0.369	0.0006	0.005	0.370	0.002	0.116	0.002
Guatemala	2.496	1.531	0.0027	0.004	0.500	0.002	0.180	0.003
Cuba	0.836	0.672	0.0007	0.003	0.578	0.001	0.076	0.003
Indonesia	0.990	0.465	0.0004	0.004	1.413	0.001	0.076	0.003
Colombia	2.456	1.662	0.0057	0.004	0.937	0.002	0.230	0.005
Nicaragua	1.030	2.277	0.0031	0.004	0.760	0.001	0.485	0.004
Venezuela	1.871	2.286	0.0028	0.003	1.392	0.003	0.230	0.007
S.Domingo	1.252	0.651	0.0007	0.002	0.860	0.001	0.074	0.004
Puerto Rico	0.748	3.261	0.0086	0.003	0.377	0.002	0.056	0.003
Brazil (Fancy)	0.755	1.436	0.0041	0.002	0.101	0.001	0.058	0.003
Brazil (Florida)	0.582	0.650	0.0039	0.002	0.515	0.001	0.059	0.002
Kenya	0.643	0.625	0.0005	0.002	1.042	0.002	0.110	0.002
Ethiopia	0.762	0.913	0.0002	0.002	0.213	0.002	0.167	0.003
India A.	0.286	0.323	0.0004	0.002	0.169	0.002	0.029	0.002
Uganda	0.409	0.347	0.0003	0.003	0.497	0.002	0.085	0.002
Costa Rica	0.385	0.749	0.0010	0.001	0.204	0.002	0.037	0.001
Ecuador	0.782	0.934	0.0010	0.002	0.905	0.005	0.102	0.002
Burundi	1.277	1.648	0.0037	0.007	0.217	0.025	0.383	0.007
Peru	0.779	0.788	0.0006	0.003	0.742	0.005	0.078	0.002
Robusta								
Uganda	0.389	0.396	0.0011	0.002	0.096	0.001	0.045	0.002
C.d'Ivoire	0.364	0.555	0.0010	0.002	0.068	0.001	0.259	0.001
India P.	0.084	0.209	0.0012	0.001	0.110	0.002	0.011	0.001
India C.	0.274	0.226	0.0020	0.001	0.164	0.001	0.014	0.002
Guatemala	0.396	0.375	0.0005	0.002	0.195	0.002	0.027	0.001
Papua N. G.	0.121	0.100	0.0011	0.002	0.092	0.001	0.019	0.002
Brazil	0.698	0.587	0.0010	0.004	0.485	0.001	0.113	0.001
Indonesia	0.230	0.197	0.0003	0.001	0.010	0.002	0.014	0.001
Togo	0.792	0.512	0.0033	0.006	0.172	0.002	0.046	0.002
Angola	0.244	0.148	0.0010	0.003	0.291	0.002	0.033	0.002
Viet Nam	0.818	0.784	0.0134	0.006	0.150	0.005	0.286	0.007
Cameroun	0.192	0.391	0.0007	0.002	0.248	0.001	0.036	0.002
Java	1.812	0.662	0.0033	0.002	0.187	0.002	0.051	0.002
Madagascar	0.319	0.317	0.0010	0.003	0.191	0.003	0.259	0.001
Thailand	0.350	0.443	0.0039	0.002	0.600	0.001	0.152	0.003
C.A.R.	0.271	0.244	0.0010	0.001	0.061	0.001	0.033	0.001

Zinc(II)-Chelating Compounds in a Coffee Brew

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SUMMARY

The effect of roasting coffee beans (from green beans to light, medium, and dark roasts) on Zn(II)-chelating compounds formed was investigated.

In the progress from green beans to dark roasted beans, the relative Zn(II)-chelating activity reached a maximum in the medium roasted beans. Instant coffee showed a level similar to that of the green coffee beans. A chymotrypsin treatment of the green beans markedly reduced the Zn(II)-chelating activity. This suggests that proteinaceous compounds were involved with zinc-chelation in the green coffee beans, and that roasting degraded these active proteinacious compounds in the roasted beans to produce other zinc-chelating compounds.

INTRODUCTION

The effect of brewed coffee on mineral nutrition has been noticed. Brewed coffee has metal chelating-activity which results in a trace element deficiency in the areas of mal-nutrition where coffee is consumed in a large quantity. The separation and characterization of zinc(II)-chelating compounds in instant coffee were investigated. This study reports the formation process of zinc(II)-chelating compounds during the roasting of coffee beans.

MATERIALS AND METHOD

Green coffee beans of the robusta species were industrially roasted to three degrees of roasting (light, medium, and dark). The green coffee bean was analyzed as the control sample, and instant coffee was used as the reference sample whose chemical composition had previously been partially characterized.

Each sample of coffee beans were pulverized, and then extracted with boiling water for 3 min, and the resulting aqueous extract was lyophilized. The subsequent process was applied to each aqueous extract of the coffee beans and the instant coffee. The aqueous extract (10 g) was dissolved in 30 ml of a 10 mM hexamine buffer (pH 5.0) with 20 mM $ZnCl_2$ and 10 mM KCl, and the mixture was allowed to stand overnight at ambient temperature. The resulting coagulated precipitate was centrifuged, and then washed with the hexamine buffer. The zinc content in the washed solution was checked to less than 1 ppm. The washed coagulate was dissolved in 1% aqueous ammonia, and the dissolved material (Sample A) was successively passed through columns of Amberlite IRA-410(OH^-) and Amberlite IR-120B(NH_4^+). The non-adsorbed fraction (Sample A') was acidified with HCl, and the resulting precipitate (Sample Ap) was dissolved in 1% aqueous ammonia, adsorbed to Celite powder and then lyophilized. Sample Ap on Celite was chromatographed in a cellulose column developed with mixed solutions of n-propanol and 1% aqueous ammonia. The eluates were referred to as Samples Ap I and II for the 5:2 (v/v) mixed solution, Ap III for the 3:2 mixed solution, and

Ap IV for the 1:1 mixed solution. The yield and zinc concentration of a prepared sample at each separation step were determined. Green coffee beans of the robusta species were industrially roasted to three degrees of roasting (light, medium, and dark). The green coffee bean was analyzed as the control sample, and instant coffee was used as the reference sample whose chemical composition had previously been partially characterized.

The fractionated samples of the aqueous extract and Sample Ap from green coffee beans and the instant coffee were digested with bovine chymotrypsin, and then subjected to the same fractionation steps. Some of these fractions were subjected to a SDS-PAGE analysis.

RESULTS AND DISCUSSION

Change in the zinc-chelating activity during roasting

The successive separation process used for each coffee sample started from the 10 g of aqueous extract from coffee beans and from instant coffee. The zinc content in each separation step is shown as mg or μg in all the separated materials prepared from 10 g of each aqueous extract. Accordingly, the total amount of zinc determined in each separation step is regarded as the total activity of zinc-chelating compounds in the hot water extract that represents the brew for the coffee beverage.

1) Sample A. The total zinc content was 9.24 mg in the green beans, and roasting resulted in 7.73 mg for the light roast, 9.90 mg for the medium roast, and 8.80 mg for the dark roast. The zinc content in instant coffee was 1.60 mg. This large difference between instant coffee and roasted coffee beans seems to have been due to the industrial scale extraction condition. Among the green and roasted coffee beans, the amount of formed coagulants with $ZnCl_2$ was little affected by roasting.

2) Sample A'. The total zinc content was 3.83 mg for the green beans, 7.73 mg for the light roast, 6.51 mg for the medium roast, and 4.47 mg for the dark roast, suggesting that roasting reduced the total activity for zinc-chelation. Comparing Samples A and A', the amount of reduced of zinc was greatest in the dark roasted coffee beans and in the green beans.

3) Sample Ap. As shown in Figure 1, the zinc content fell from the green beans to the light roast, and then increased with increasing degree of roasting. Instant coffee showed less Zn level than that in the medium roast. Figure 2 shows the amount of zinc per gram of Sample Ap, suggesting relative Zn(II)-chelating activity of the separated compounds. In the progress from green beans to dark roasted beans, the relative Zn(II)-chelating activity reached a maximum in the medium roasted beans. Instant coffee showed a level similar to that of the green coffee beans**.**

Ap III fraction from cellulose column chromatography

The zinc from the cellulose column chromatography appeared as a single peak in the ApIII fraction for the green coffee beans, and there was almost no peak for the light roasted beans. Further roasting gave one or more peak(s) in the Ap III fraction as well as in medium and dark roasted Ap I, II and IV fractions. Their peak area increased with increasing degree of roasting.

Proteinacious compound

The appearance of the zinc peak in the Ap III fraction indicates that the zinc-chelating compound detected in the green coffee beans were degraded by roasting. Roasting the coffee beans produced new zinc-chelating compounds by reactions among the constituent sugars, amino acids, phenolic compounds, polysaccharides, and proteins. Protein was determined by Biuret reaction in the Ap III fractions from the green coffee beans and roasted beans. SDS-PAGE with Coomassie Brilliant Blue staining detected clear bands in the Ap III fraction from green coffee beans, and broad bands from the roasted beans.

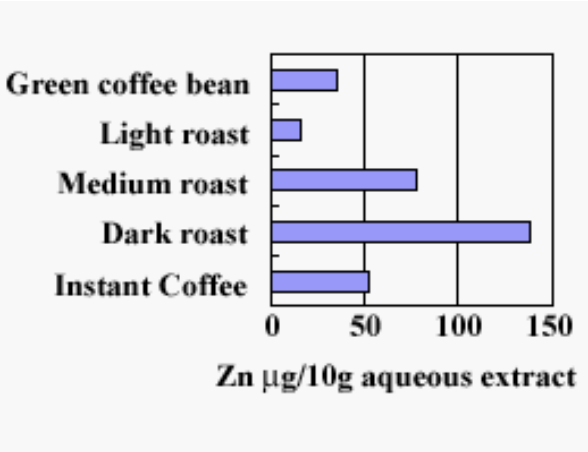

Figure 1. Total Zinc Content in Sample Ap

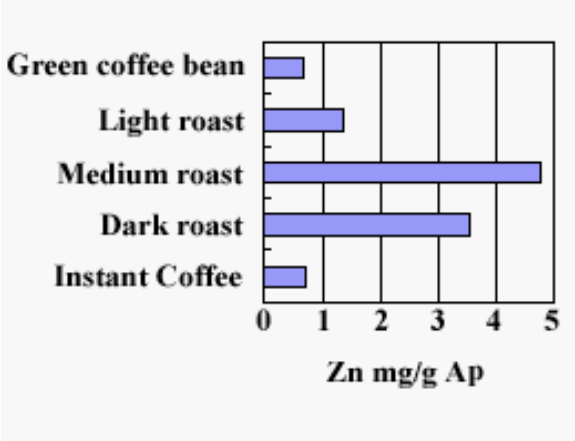

Figure 2. Zinc Content per gram of sample Ap

The aqueous extract from green coffee beans and instant coffee were digested with chymotrypsin at pH 5, before being subjected to the same separation procedure as that already mentioned. Figure 3 shows that the chymotrypsin treatment markedly reduced the zinc peak of the Ap III fraction in the green coffee beans, but not in the instant coffee in comparison with the control samples without the enzyme treatment.

It follows that the major zinc-chelating compounds in green coffee beans were proteinacious, these being degraded by reactions among the components in roasted coffee beans to form zinc-chelating compounds. The active compounds were initially increased in quantity by the degree of roasting, the relative zinc-chelating activity reaching maximum in the medium roasted beans , before dropping off in the dark roasted beans.

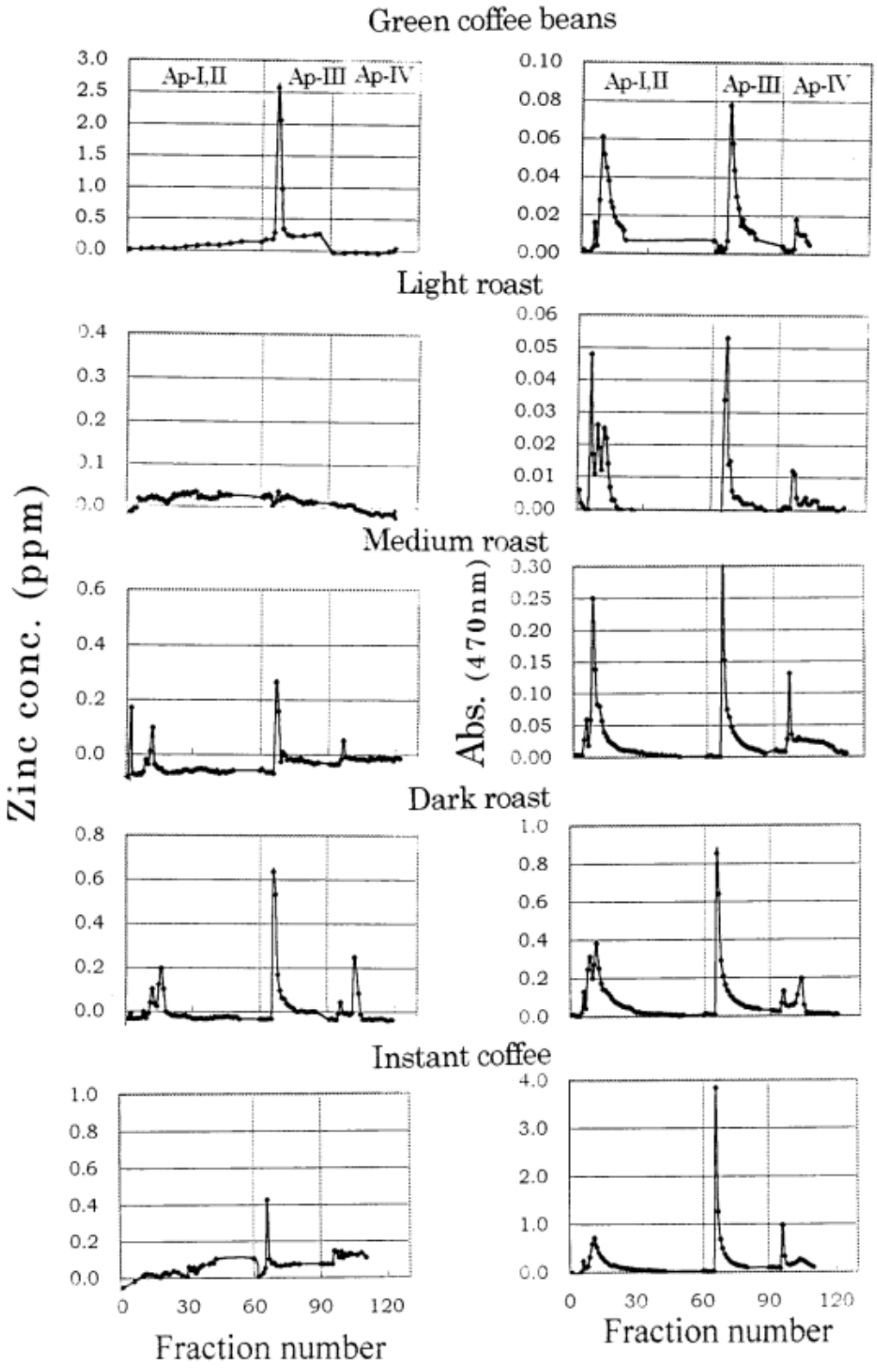

Figure 3. Effect of roasting on the Ap-III fraction of coffee beans

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Heat and Mass Transfer during Roasting – New Process Developments

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SUMMARY

Recent developments in roasting of green coffee beans are mainly based on an improvement of the heat transfer and energy recycling. The following aspects and their impacts on the roast process are introduced and discussed in detail:

- the counter-current and highly time dependent transport of heat and mass inside the bean
- the superposed heat sinks and heat sources represented by moving fronts of evaporation (endothermic enthalpy) and roast reactions (exothermic enthalpy)
- simultaneous appearance of convective, radiative and conductive transport
- variable pressure inside the bean
- variable volume of the bean in common with a non basic geometry
- variable inner structure of the bean
- variable material properties and transport coefficients
- hardly accessible measurements of local temperature inside the bean

Temperature profiles both theoretically and experimentally derived are presented considering various boundary conditions in order to conclude developments related to the roasting process. Finally some recent inventions are discussed.

ZUSAMMENFASSUNG

Neuere Entwicklungen für den Röstprozess von grünen Kaffeebohnen basieren auf der Verbesserung des Wärmetransportes und der Energierückgewinnung. Im einzelnen werden folgende Phänomene und deren Einfluss auf den Röstprozess vorgestellt und diskutiert:

- der instationäre Gegenstrom von Wärme- und Stofftransport in der Kaffeebohne
- die überlagerten, wandernden Wärmequellen (Verdampfungsfront) und Wärmesenken (Reaktionsfront)
- gleichzeitiges Auftreten von Konvektion, Wärmeleitung und Strahlung
- veränderlicher Druckverlauf in der Kaffeebohne
- veränderliches Volumen der Bohne, deren Gestalt nicht zu den Grundgeometrien gehört
- veränderliche innere Struktur der Bohne
- veränderliche Stoffeigenschaften und Transportkoeffizienten
- messtechnisch schwer zugängliche Temperaturerfassung im Inneren der Kaffeebohne

Theoretisch und experimentell bei verschiedenen Randbedingungen ermittelte Temperaturprofile werden vorgestellt und Schlussfolgerungen für die Weiterentwicklung des Röstprozesses gezogen. Anschließend werden einige industrielle Neuentwicklungen erläutert.

INTRODUCTION

A review on former ASIC Conferences demonstrates clearly the importance of Coffee Roasting as part of the coffee processing (Barbara, 1967; Sivetz, 1973; Radtke, 1975; Vincent et al., 1977; Maier, 1985. Clarke, 1985; Sivetz, 1991; Schenker et al., 1999). Although there has been a long evolution on the understanding and design of roasting processes the development has not stopped.

While a lot is understood about roasting there is still a lot to be discovered. The paper focuses on the interactions between heat transfer and the time dependent behaviour of the coffee bean.

PARAMETERS AND INFLUENCES

A central point of investigation is the bean temperature. However, it is not a uniform temperature throughout the bean volume, but it forms a three dimensional temperature field of non steady state type. So, it is very important to realise that the term 'bean temperature' has not been well defined so far.

At best the process engineer can detect the surface temperature of the bean. But the question arises, what about the temperature in the bean centre and furthermore, there is still no knowledge about the temperature gradient within the bean structure. While the principle is simple, the process is very complex. The unknown temperature field of the bean is caused by the method of heat transfer. Simultaneously the local temperatures start evaporation of the bean humidity and moreover many of chemical reactions which are combined with transfer of vapour and roast gases through the swelling bean.

In ideal case one would be able to determine the non steady temperature field during roasting in order to control the process, because it always has been stated the central idea of roasting the coffee is to have the same colour in the middle of the bean as on the outside (Bersten, 1993). This aim has not been reached so far. Experimentally one obtains something like the surface temperature by a thermal sensor actually touching only the beans or with infra red detectors, that have to be calibrated to emission coefficients depending on the surface temperature itself. Any mathematical procedure to calculate the non steady temperature field is confronted with variable material properties of the coffee bean during roasting and additionally variable geometry and inner structure of the bean. Finally a calculation of the temperature field has to consider latent energies, namely the endothermic enthalpy of vaporisation and the exothermic enthalpy of roast reactions. These latent energies appear at distinct temperatures and in consequence the problem is of non-linear character.

Figure 1 shows the interactions during roasting: the outer heat transport is in case of a drum roaster a mixture of radiation, convection and contact heat transfer. Fluidised roasting, however, is dominated by convective heat transfer. In any case the inner heat transport takes place by conduction superposed by swelling, temperature dependent material and transport properties as well as moving sinks and sources of heat energy created by vaporization and roast reactions. Furthermore the solid is a porous medium and counter current to the inner heat transport mass transport of water vapor and roast gases appears, consisting mainly of CO_2.

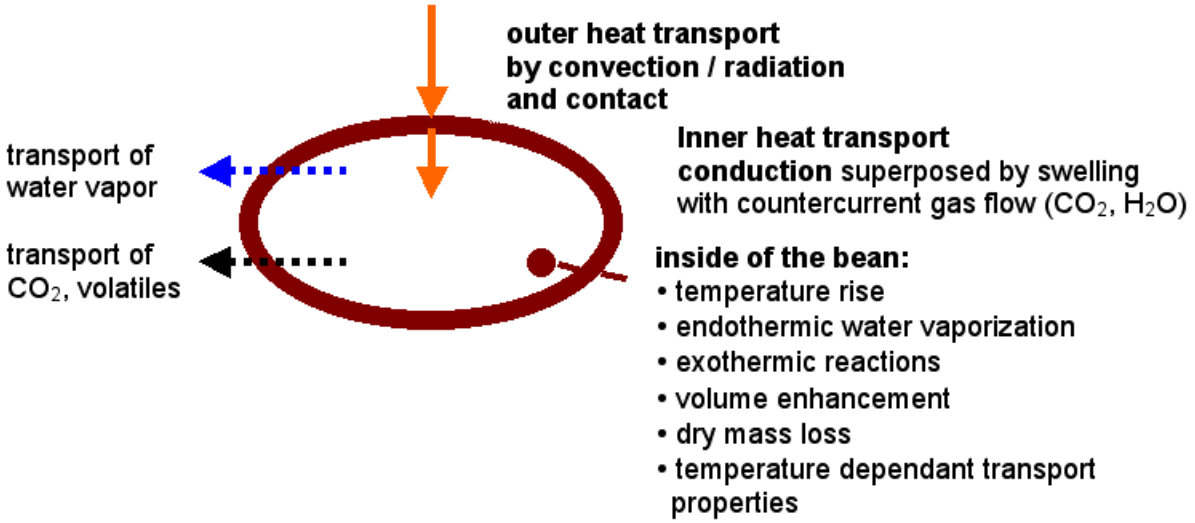

Figure 1. Roasting of coffee beans – main aspects

PHENOMENOLOGICAL PROFILES

On the basis of these fundamental interactions an idea of phenomenological profiles of temperature, water content and pressure inside the bean at various times might be of interest (Figure 2).

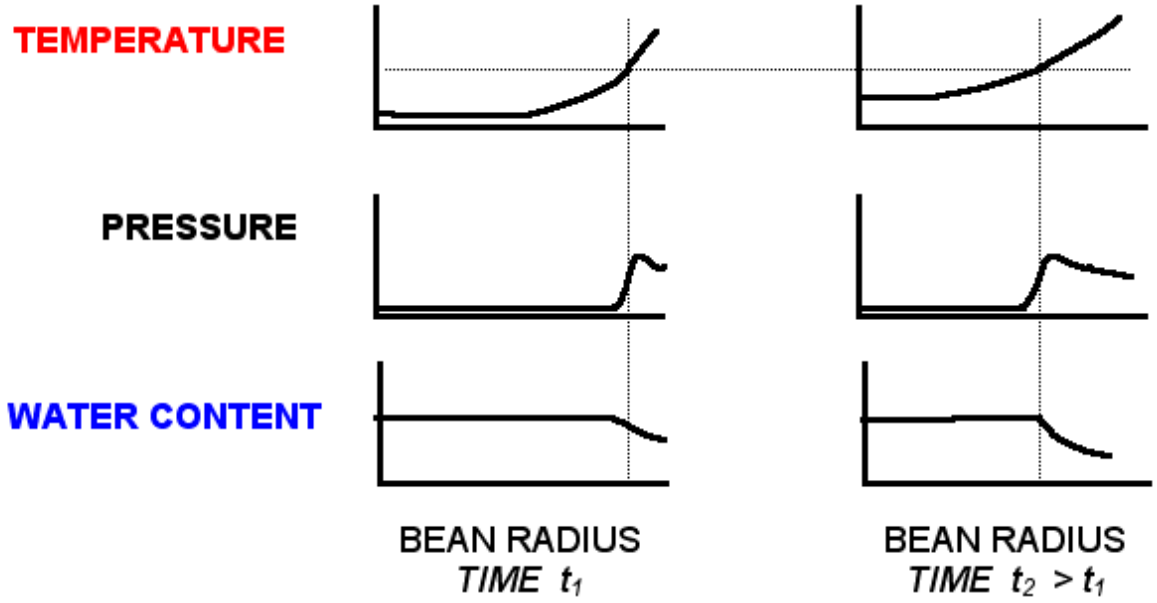

Figure 2. Assumed profiles in the coffee bean during roasting

The temperatures increase with time but at the front of evaporation moving towards the bean centre the slope of temperature becomes unsteady due to the latent energy of vaporisation. Furthermore the heat conductivity of the dried zone is smaller compared to the region in front of the moving band of vaporisation. As a consequence, the gradient of the temperature is steeper in the dried region of the coffee bean. The evaporated humidity expands and builds up a pressure peak at the moving front. Additionally thermal stresses arise due to the steep temperature gradient. So, mechanical and thermal stresses commonly lead to the well known

swelling of the bean and it is obvious that the generated water vapour is driven off to the outer surface of the bean. Furthermore it becomes understandable that sometimes beans will crack or even burst, if the moving pressure peak is too high.

Subsequently at higher temperatures the zone of roast reactions starts from the bean surface into the inner structure of the bean that has been dried before. The question arises whether both latent energies, the endothermic vaporisation zone and the exothermic reaction zone behind move simultaneously for some time or not.

The phenomenological temperature profile drops at the heat sink and is lifted at the heat source (Figure 3). Thus, due to a time section of simultaneous vaporisation and reaction, the temperature gradient between the two moving bands becomes steeper corresponding with enhanced thermal stresses of the bean material.

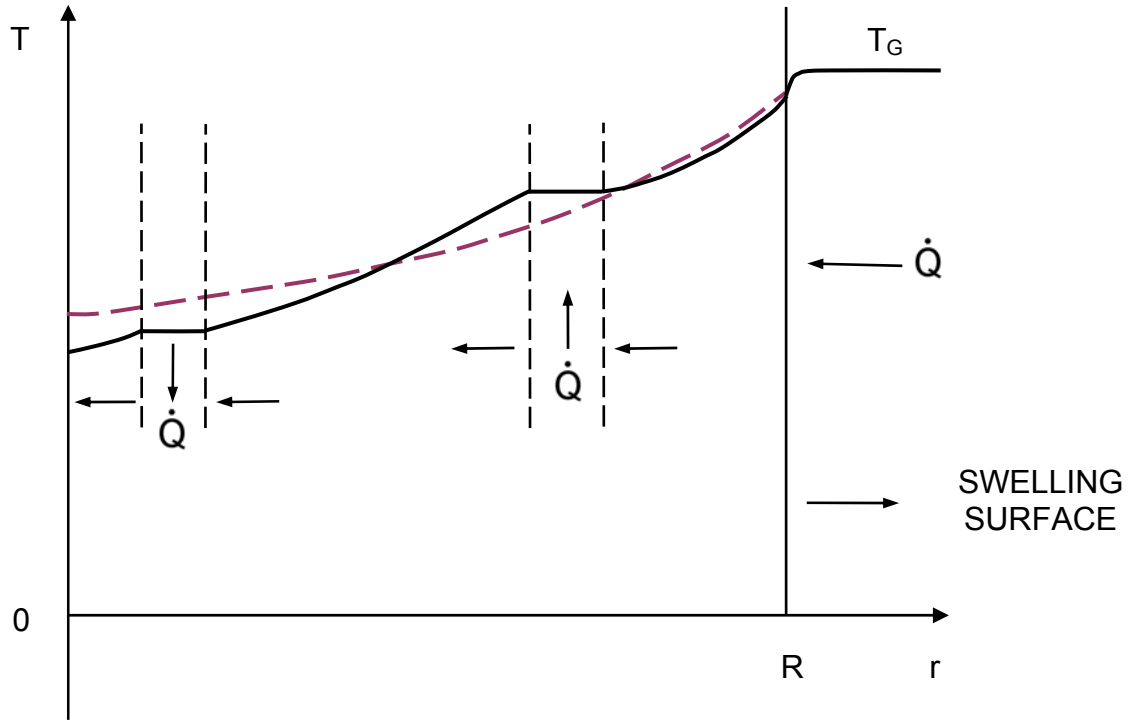

Figure 3. Temperature profile within the coffee bean

MATERIAL PROPERTIES DURING ROASTING

Considering some well known physical properties of coffee beans at green and medium roast condition, it is quite obvious, that moisture vanishes and porosity strongly increases during roasting. Both effects lead to decreasing heat conductivity, respectively to higher temperature gradients and thermal stresses. Additionally we measured some thermal data of the bean using the new ‘hot disc method’. It concerns the non steady heat wire procedure using a heat generating thin disc of only 1 mm diameter (Gustafsson, 1991). The disc is implemented onto a flat mill-cut surface of the bean. The decrease of heat conductivity is clearly to be seen, whereas the thermal diffusivity due to the volume increase during roasting remains nearly constant. The different influence of vaporisation and reaction enthalpies is roughly of the same order. Heat capacity slightly increases (Table 1).

Table 1. Thermal properties

COFFEE BEAN	THERMAL CONDUCTIVITY [W / m·K]	SPECIFIC HEAT CAPACITY [kJ / kg·K]	THERMAL DIFFUSIVITY [10^{-6} m^2 / s]	ENDOTHERMIC ENTHALPY [kJ / kg]	EXOTHERMIC ENTHALPY [kJ / kg]
GREEN	.183	1.11	0.138		
MEDIUM ROAST	.11	1.4	.11-.16	~ 200	about 160 (R. CLARKE, 1987)

In detail, we investigated the thermal data after various roasting times to get an improved knowledge of how the bean behaves along the roast process. As a consequence of the moving evaporation front the heat conductivity at the outer region of bean material, representing the area of measuring drops quickly and remains constant afterwards (Figure 4).

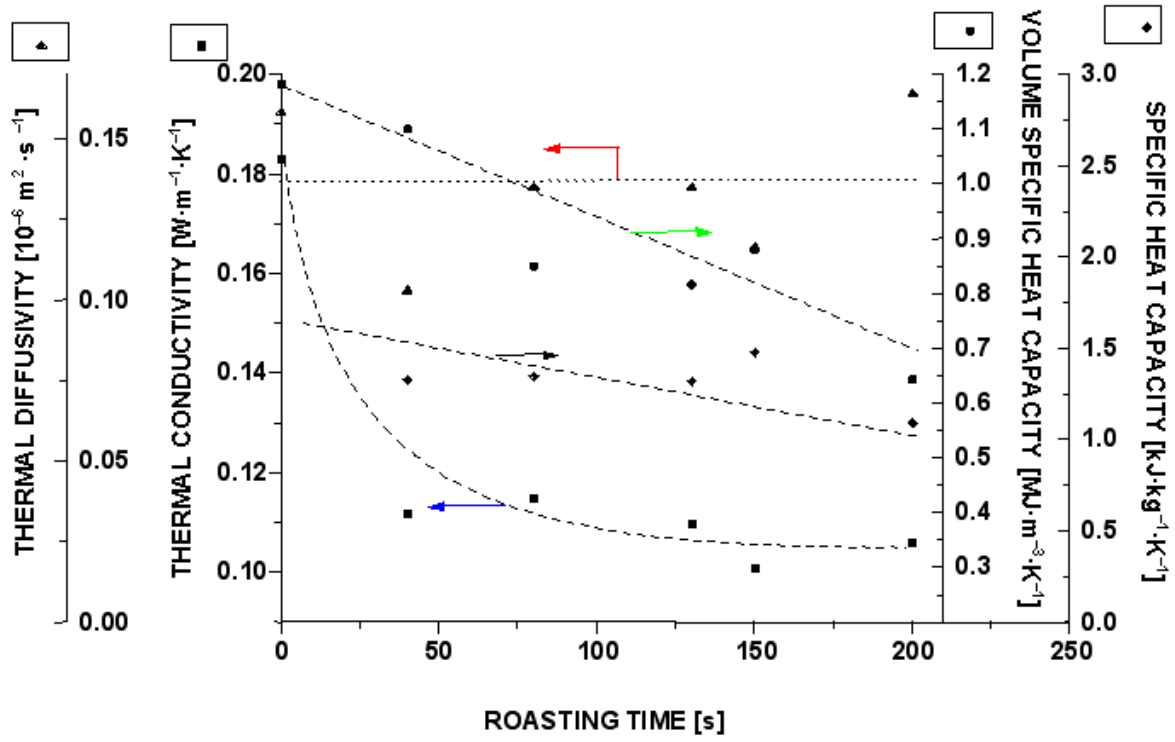

Figure 4. Thermal data of the coffee bean during roasting

Furthermore, it becomes clear that the volumetric heat capacity decreases linearly due to progressive swelling of the bean during the entire roasting process. That thermal behaviour causes the thermal diffusivity to be nearly constant.

EXPERIMENTAL WORK

Though not well defined, the bean temperature is obviously very important in roasting. Some recent results of its dependency on bean mass during roasting are shown in Figure 5.

Both bean temperature and roasting time depend strongly on the heat transfer and therefore the technology applied. The depicted results of LTLT (Low Temperature Low Time) as well as HTST (High Temperature Short Time) processes integrate into a slightly progressive function. Own measurements correspond well with the literature data. New data on the progression of bean temperature during roasting have been reported by Da Porto and Nicoli et

al. (1991) and by Schenker and Escher et al. (1999). The latter authors chose fast fluidised bed roasting of 100 g arabica beans, a hot air flow of 0.01885 m^3/s and two ways of operation: first the so called LTLT roasting with a hot gas temperature of 220°C and a roasting time between 9 and 12 minutes and second HTST roasting with a gas temperature of 260°C and a roasting time between 2.6 and 3 minutes. For the case of LTLT roasting the bean temperature and other parameters were depicted vs. roasting time: the temperature rose continuously from 20 to 190°C in 2 minutes. This increase equalled already 90% of the final increase (211°C after 14 minutes). The bean temperature data published by Da Porto et al. are valid for a laboratory roaster (HTLT) and Santos coffee. It can be seen, that in comparison with this traditional laboratory roaster the fluidised bed roaster facilitates a significantly faster increase of the bean temperature (Eggers and Pietsch)

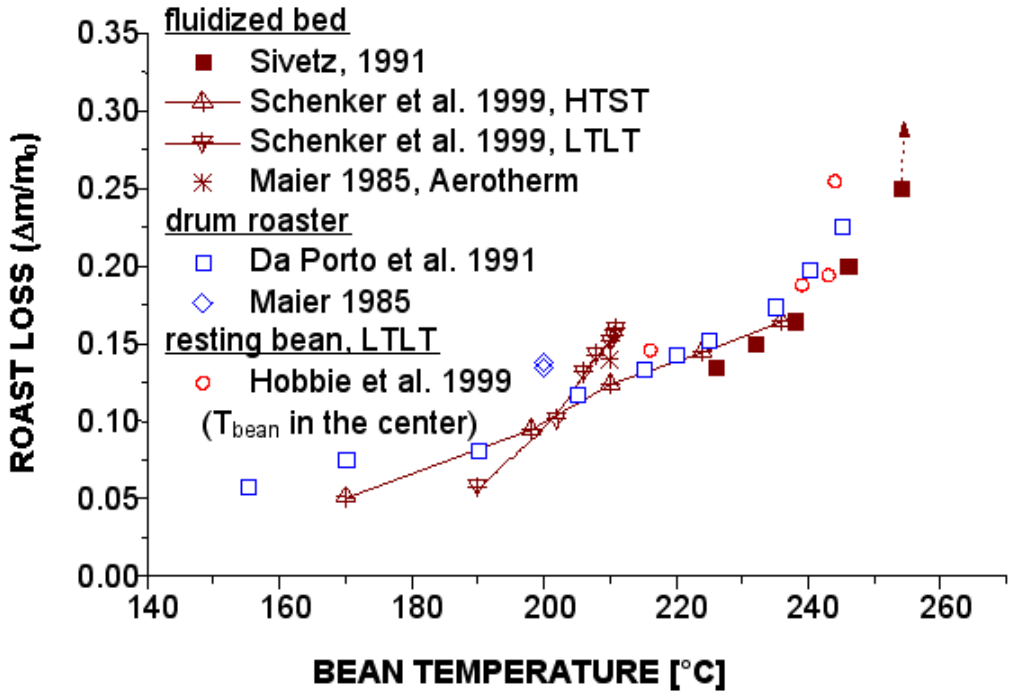

Figure 5. Roast loss vs. bean temperature – different processes

In order to obtain on-line data, namely mass and volume of the bean and additionally a profile of temperature inside the bean, we designed and built up a roasting chamber equipped with walls of glass. On the top of the roasting chamber a mass balance was installed. The aim was to measure the mass of a small sheet hanging below the balance in the roasting chamber. A special dosing facility was constructed to put at the most four green beans simultaneously onto the hanging sheet in order to start the roasting and the investigation at the same time. This way additionally enables the visualisation of the progress in roasting colour. Furthermore the beans were drilled in order to uptake very thin thermocouples with a diameter of only .25 mm. Due to the importance of the temperature investigation we checked the results via an infrared measurement which detects the surface temperature of the bean without any contact (Figure 6).

The draft shows the temperature profiles of a bean at constant roasting temperature of 237°C without forced convection.

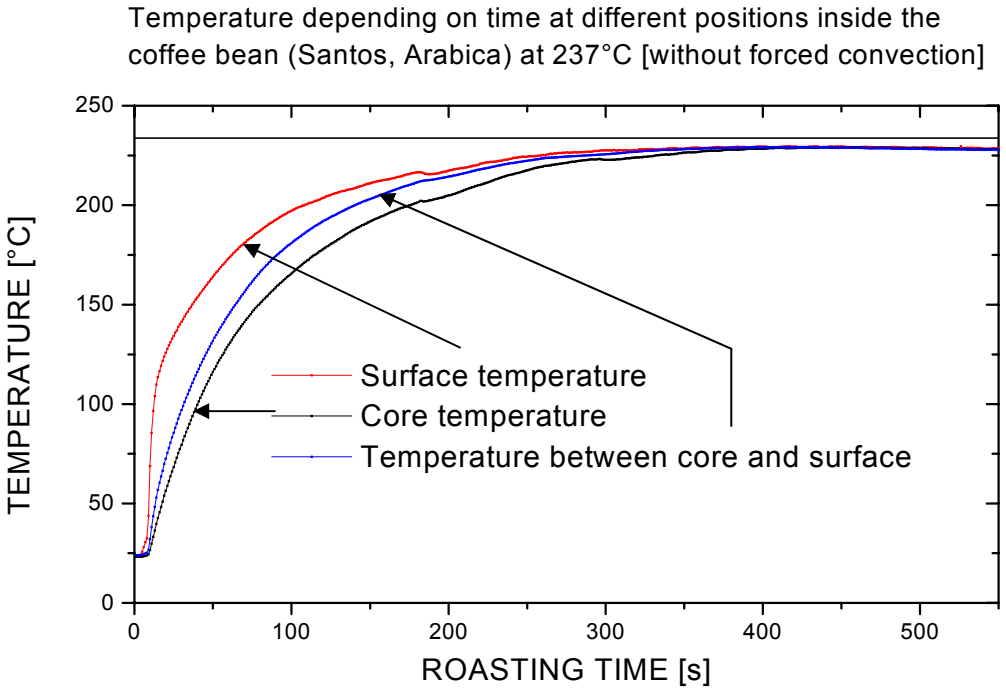

Figure 6. Temperature profiles in the bean

Starting at ambient conditions all three temperatures – at the surface, between core and surface, and core temperature itself – increase with declining slope until a nearly constant temperature has been reached. However, the temperature profiles reveal some interesting aspects of the general roasting process:

- during the first period (0-50 sec) of heating up the bean the difference between core and surface temperature passes a maximum of at least 70 degrees, whereas the temperature difference between core and half distance to the surface of the bean slowly increases up to a maximum of approximately 10 degrees remaining nearly the whole roasting time. Obviously the roasting process passes the outer sections of the bean very fast compared to the inner part of the bean. As a consequence thermal stresses may supposed to be high within the outer parts of the bean.

- a second realisation is a small temperature drop suddenly appearing at a roasting time of 200 seconds. At this time the inner temperature differences are clearly higher in comparison to the outer temperature differences. However, the temperature course drops slightly simultaneously throughout the whole bean. After this unsteady phenomenon temperatures increase more rapidly than before! As an explanation one can assume a pressure built up in the centre of the bean due to evaporating of water starting from the surface towards the central region but proceeding with decreasing velocity because the driving force of heat transfer – the temperature difference to the ambient – is diminishing all the time. So the pressure inside the bean increases until the temperatures overcome the vaporisation temperature according to the pressure. Thus the remaining water in the centre of the bean evaporates spontaneously and an endothermic flash creates the slight temperature drop. Immediately after flashing the full exothermic roast reaction is enabled to run until the core of the bean.

The explained details of the temperature profile are very interesting related to the development of new roasting processes. In order to roast the bean as homogeneous as possible a stepwise process with a slow increase of the temperature of the heating gas seems to be advantageous. On the other hand, the inner pressure of the bean is of importance to generate sufficient aroma components. Thus temperature control of the heating gas permitting not only temperature profiles with moderate differences, but also a sufficient pressure built up inside the bean has to be the objective of further development. These statements are confirmed by the results of the mass balance (Figure 7).

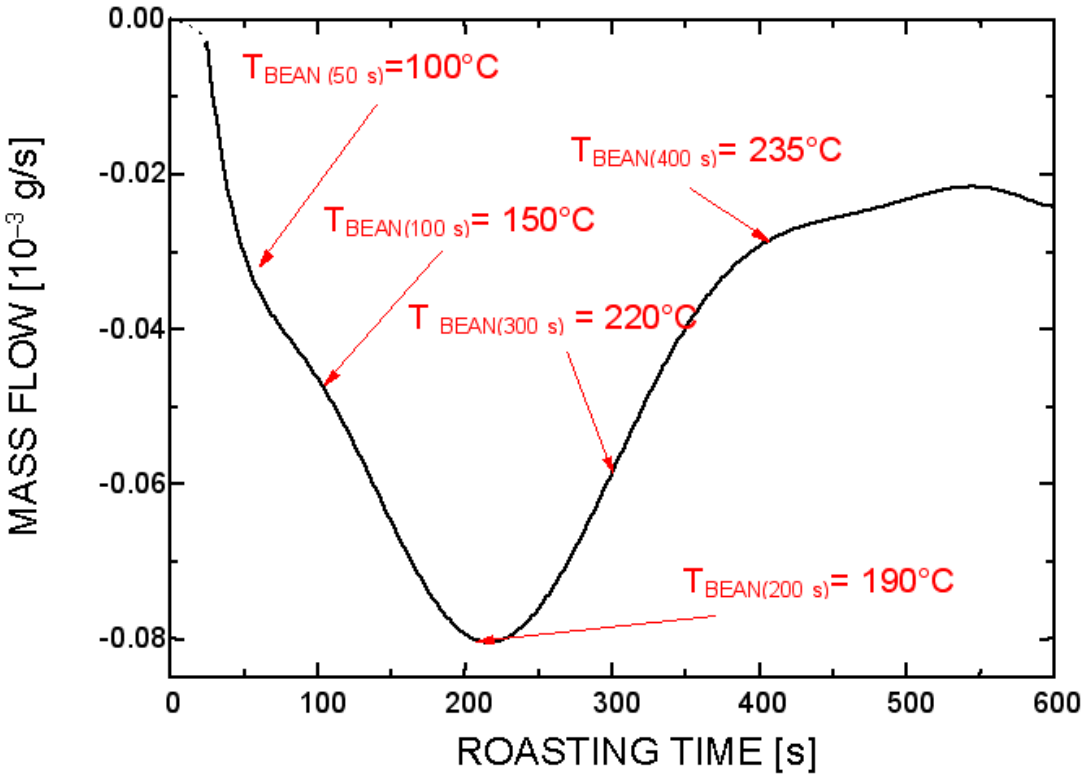

Figure 7. Mass transfer during roasting

The mass transfer during roasting increases until a peak at 200 sec. At this time the centre of the bean just reaches the temperature range while the flash of the remaining water is supposed to occur. The agreement of heat and mass transfer is clearly to be seen. Until a centre temperature of 150°C mass flow is dominated by endothermic evaporation. After that mass transport becomes more rapid indicating a superposed roast reaction with exothermic production of CO_2 until the maximum at 200°C. The remaining mass transport is caused only by exothermic reactions without any evaporation and consequently it appears as a fade-out function. Finally the experiments in our test set-up permit the investigation of the coffee bean density depending on roasting time.

Figure 8 shows the results of mass loss and swelling of the bean. The upper curve is valid for heat transfer without convection, so it corresponds to the temperature profile in Figure 6, respectively the mass profile in Figure 7. In summary the bean density falls down until half the initial value has been attained. Enhanced heat transfer via forced convection leads to a faster reduction of density but to the same final result. Running several experiments the scattering of the data becomes remarkable but it demonstrates the character of the bean as a natural material. However the spread of density during the roasting process seems to be independent of the mode of heat transfer, whether it is high or low. Thus the application of the results points out the difficulty to run a roasting process as a pure fluidised bed procedure.

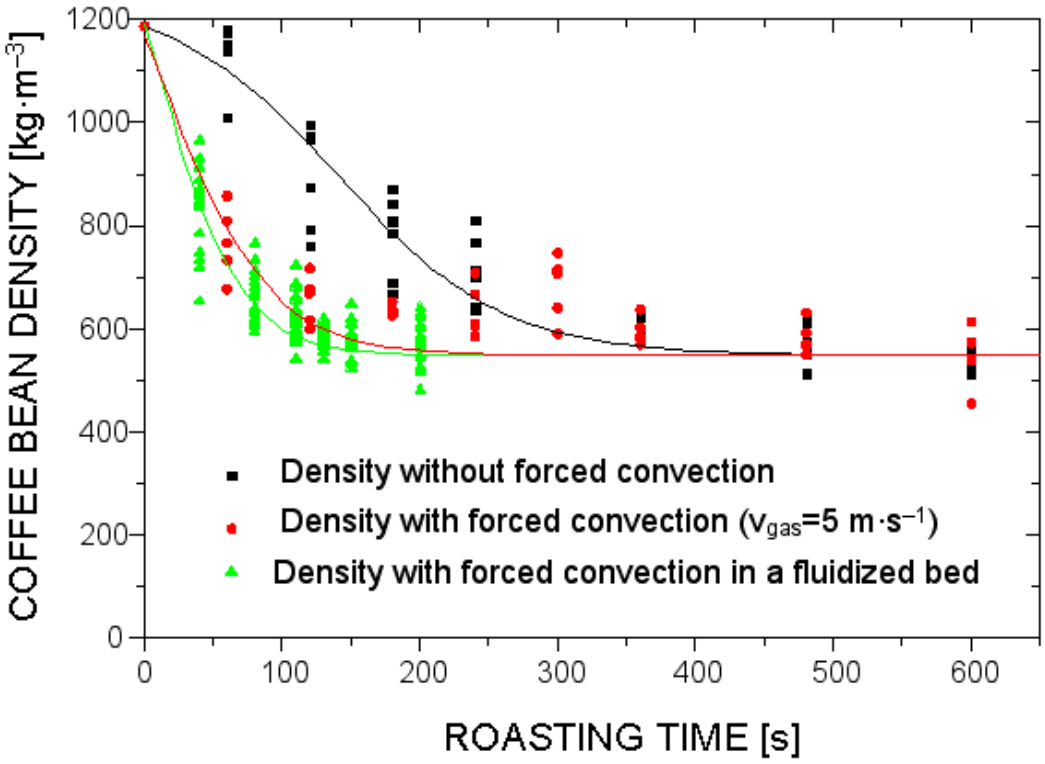

Figure 8. Density of the coffee bean during roasting

INVESTIGATION OF HEAT TRANSFER

In general the investigation of the heat transport effects from the heating gas to the surface and inside the bean is a very complex matter and only approximate calculations are possible. Nevertheless, the transfer of some knowledge from the theory of heat transfer to the roasting process offers quite interesting possibilities to further development of roasting. The heat transfer is described by dimensionless Nusselt numbers (Baehr and Stephan, 1998). The calculation provides the knowledge of the relative velocity bean – heating gas. Assuming spherical coffee beans with an average and constant diameter of 6 mm, the tendency of Nu and α can be calculated for a given gas temperature. The heat transfer coefficient α is depicted versus the Reynolds number Re in Figure 9 in a motionless system (v_{rel}=0), the heat transfer coefficient is around 14 W/m^{2}K and a minimum fluidisation velocity (e.g. Re=300) causes α-values in the range of 75 W/m^{2}K. The heat transfer to single beans is different from the heat transfer to a bed of beans. For theoretical determination of the heat transfer to a packed bed of spheres, the bed or bulk porosity φ has to be known at the same superficial velocity. Porosity values can be calculated from bean and bulk densities. Data from Vincent et al. (1997) lead to φ_{green}=0.46 and $\varphi_{roasted}$=0.53. An average value of 0.5 is close to the theoretical value for a cubic packing of true spheres (φ=0.48).

In Figure 9 the theoretical heat transfer coefficient for a packed bed with φ=0.5 is depicted. These calculated values are higher than those for single spheres at the same superficial velocity and a technical roasting process is expected to show heat transfer values of the region between both curves. Two consequences are important. On the one hand, it is possible to increase the heat transfer coefficient with higher velocities but on the other hand, the improvement is limited due to the low gradient of the Nu-functions. The inner heat transport is governed by the local heat conductivity of the bean and the moving heat sinks and sources

of evaporation and roast reactions. The mathematical solution of the corresponding partial differential equation needs a numerical finite element procedure fitted to boundary conditions of not constant type. Nevertheless, regarding the mutual effects of endothermic and exothermic enthalpy as approximately of the same magnitude (Table 1) one can estimate the dimensionless Biot number $Bi=\alpha/(\lambda/r)_{Bean}$. This data represents the measure to compare the outer heat transfer (α (W/m^2K)) with the inner, conductive heat transport $(\lambda/r)_{Bean}$. Assuming again the coffee bean as a sphere with a diameter of 6 mm and using the calculated data of Figure 9 as well as the investigated heat conductivities given in Table 1, one can conclude that neither conduction nor the transfer to the bean surface controls the process. Related to the decreasing heat conductivity of the bean during the process the Biot number increases slightly. So, the resistance to the inner heat transport will be a restricting factor only within the second part of roasting. This circumstance again confirms the statement that a stepwise increase of the roast gas temperature should be moderate.

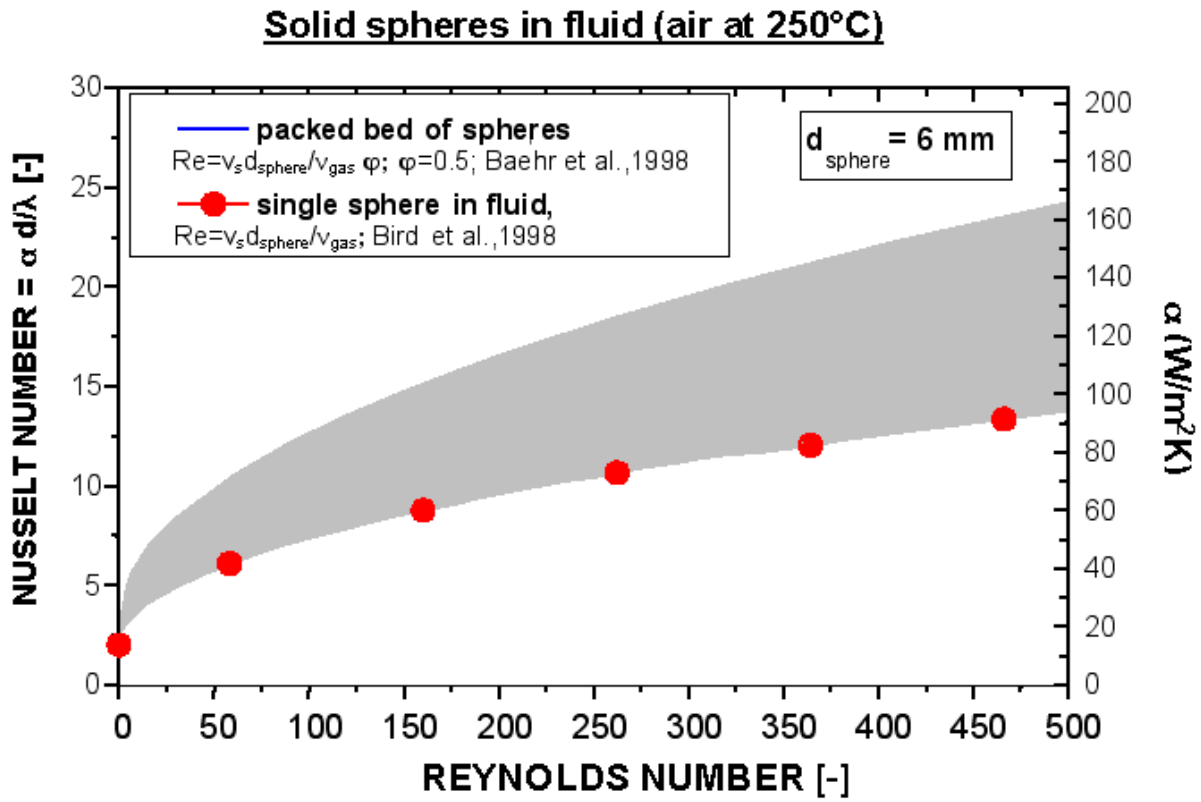

Figure 9. Heat transfer of coffee beans during roasting

The generalised investigation of heat transfer using dimensionless numbers does not explain the local heat transfer over the length of the bean. In detail, considering the boundary layer of the hot gases contacting the surface of the coffee bean offers further insight to the nature of roasting. Particularly the influence of the escaping water vapour and roast gases on the heat transfer has not been discussed so far.

Figure 10 shows the disturbance of a boundary layer. The roast gases form a cross flow and in consequence the main resistance to heat transfer will be diminished. Furthermore the thickness of the boundary layer increases in direction of the flow. Thus, the roast gases support the heat transfer in a double respect: they enhance and equalise heat transfer of forced convective nature. On the contrary, in case of free convection and radiative heat transfer the escaping gases may lead to a decreased heat conductivity within the boundary layer.

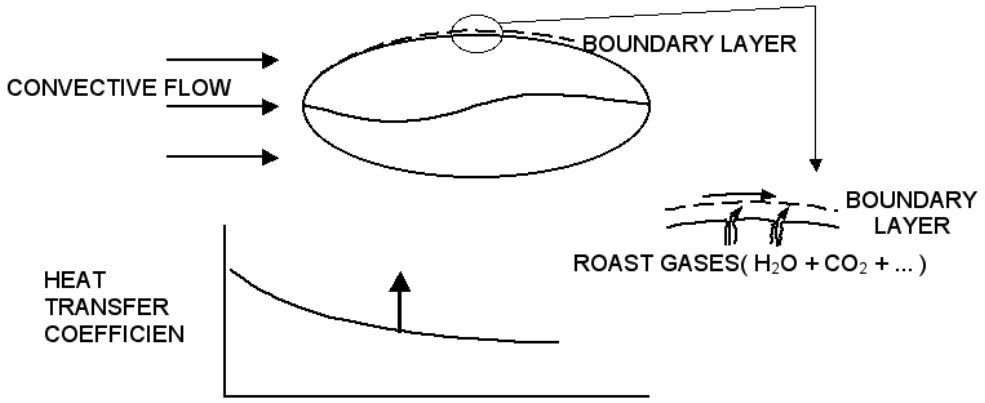

Figure 10. Influence of escaping roast gas on heat transfer

SOME ASPECTS ON RECENT AND FUTURE DEVELOPMENT

Despite of intensive research, the roasting mechanisms are not fully understood. Although many process factors have been varied and different parameters recorded, due to the enormous complexity of the subject a well-rounded model is still missing. This objective needs more detailed knowledge on

- the swelling and the time dependent inner structure of the bean
- the influence of heat radiation between the surfaces of the heated bean
- the localisation of the moving sinks and sources of vaporisation and roasting reactions

Nevertheless, in the last decade several new roaster designs have been developed by industry. Some of them are briefly described with respect to the heat and mass transport (Eggers and Pietsch).

As for traditional roasters, a tendency towards a larger coffee product variety has led to smaller uniform charges and therefore the demand for large continuous roasters is decreasing. Due to modern controls, batch roasters can make very consistent products while being able to vary the heat input over time. Roasters for operation under pressure and for steam roasting are not marketed today, although there is research activity in this field.

Recently a new rotating fluidised bed roaster has been introduced (Patent DE, 2000). The roasting and the cooling chamber are geometrically identical and their shape leads to what is called a rotating fluidised bed. The gases entering from the bottom fluidise the beans, the shape of the chamber directs them to the opposite sloped chamber wall and they move towards the bottom again. The gas flow can be well above the minimum fluidisation velocity without the risk of blowing beans out of the roasting section. So, the heat transfer is not restricted to data depicted in Figure 9. Batch operation allows different roasting processes with temperature profiles. A further new design is offered as a ‘Multiple Zone Quasi-Continuous Belt-Process’. It transports the beans on a perforated conveying belt through three heating, one quenching and one air cooling zone. Gases enter the bed from the bottom causing bean movement variable from a spouting bed to fluidisation. Independently adjustable heating or cooling zones allow temperature profile roasting in combination with a continuous processing (EU Patent, 1999).

A further industrial development is a packed bed roaster suited for conventional and high-yield low density roasting (Coffee a Cacao In.). The beans are roasted in a conical chamber

with high velocity roasting gas. The gas enters tangentially, passes through a special pattern of louvers and whirls the beans in the chamber creating a spinning packed bed. This packed bed rotates in a horizontal circle and all the beans are believed to be forced into a predestined travel path. It is possible to increase the gas velocity beyond the fluidisation point (Figure 9) of the beans since the coffee bed is kept tightly packed by centrifugal forces. These high velocities are believed to lead to high heat transfer rates so that roasting can be performed at lower temperatures (typically 275-300°C) and short times.

A further patent shows a new arrangement in which essentially all of the cooling waste air is heated and fed to the roaster (Finken et al., 1998). This leads to an increased roasting gas throughput and a lower roasting gas temperature. Stated advantages of this new roaster are more uniform roasting, reduced emissions, increased thermal efficiency and a reduced structural outlay. Finally an invention was introduced providing a method with which coffee beans are completely fluidised in order to roast and to candy the coffee beans (PCT/DE00/00205).

ACKNOWLEDGEMENTS

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When Are Coffee Beans just Right? Development of Physico-chemical Properties During Roasting

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SUMMARY

To gain a better insight into the details of the coffee roasting process, to facilitate its theoretical modelling and to improve its performance, basic physico-chemical properties of Arabica and Robusta coffee beans of different roasting degrees have been measured.

A large sample of Arabica Santos coffee beans was divided into seven equal fractions, each of which was separately roasted at 242°C for 40, 80, 110, 130, 150 and 200 seconds. A lot of Indian Robusta coffee beans was treated in a similar way. In this case, however, additional batches were prepared at 225, 250, 275 and 300 seconds roasting time. The difference between the two kinds of coffee beans as to the relative increase in size and dimensions was investigated.

Cleaved beans from different batches were inspected and photographed by Scanning Electron Microscopy (SEM); several interesting structures and features were observe to develop during roasting: bubbles/voids, deformation of inner structures and formation of Hecht filaments.

The colour of beans from each batch was quantified by their spectral remission and the data processed by computer. The development of colour during roasting and the differences between the two kinds of beans is discussed.

INTRODUCTION

To gain a better insight into the details of the coffee roasting process, to facilitate its theoretical modelling and to improve its performance, basic physico-chemical properties of Arabica and Robusta coffee beans of different roasting degrees have been measured. The parameters presented in this publication are: bean size, inner structures, colour and reflection coefficient.

So far, two different bean charges have been investigated. First an Arabica coffee, Brasil Santos. This charge was subdivided into seven fractions. Then it has been roasted at a constant temperature of 242°C for different times, ranging from 0 to 200 seconds. The India Robusta coffee was treated similarly, but four further charges with roasting times up to 300 seconds were made. The roasting machine was a fluid bed roaster.

RESULTS

Bean size

To get a reasonable simulation of the roasting process one needs to understand the development of the basic parameters according to the roasting degee. The most basic

parameter is the bean size, which is important because in further investigations the temperature profile inside the coffee bean during the roasting process shall be taken.

Figure 1. Dimensions of the coffee bean as used above

A suitable modification of this temperature profile will in the best case lead to a more uniformly roasted bean.

The size of about ninety coffee beans of nearly each roasting degree has been measured in every dimension, using a caliper. As a result one gets a distribution function that looks like gaussian and the half width increases the larger the dimension.

A comparison with the histogram of the green robusta coffee beans reveals that in fact the Robusta India coffee beans are much smaller and more spherical than the green Arabica coffee beans. The measured size relation is

2,4 : 1,9 : 1 for the green arabica beans and
1,9 : 1,5 : 1 for the green robusta beans.

To obtain results for the size increase during the roasting process the mean value of the green coffee beans was set as 100 percent for each dimension. The size increase of the partially roasted beans was compared with this reference value.

A comparison between the Brazil Santos and the Robusta India reveals an end in size increase after a roasting time of about eighty seconds. Further one can see a maximum in size increase at 110 seconds roasting time at least for the Brasil Santos.

There is one difference in increase of size between the two kinds of beans. The main axis of size increase is the Y axis for the Robusta beans ond the Z axis for the Arabica beans.

The Z axis seems more reasonable, because the resistance in Z-axis has to be less than in the other directions, according to the curvature.

Scanning Electron Microscopy

Images of green, roasted and partially roasted Robusta beans were taken. At a magnification of fifteen the green coffee bean seems to be a curled but nevertheless compact entity. After eighty seconds of roasting there is a development of cavelike structures. These structures appear on a line parallel to the surface of the coffee bean. Along this line there is a zone rich in water, where the germlayer grows into.

A look inside the cavelike structure at a magnification of 50 reveals that the walls are smooth and there are other structures which look like elastic bands. Lateron the caves disappear and

the structure of the coffee bean is degenerating. The cavelike structures will complicate the future modelling of the roasting process.

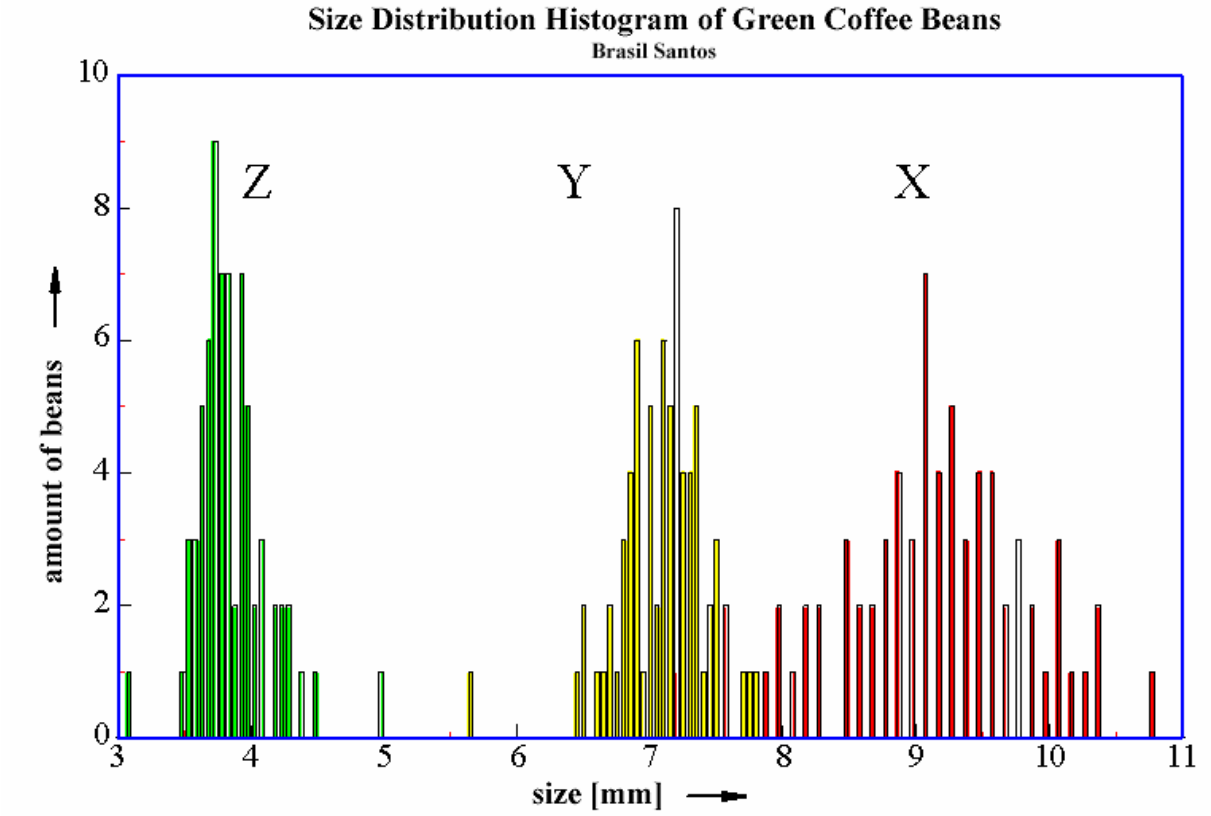

Figure 2. Size distribution histogram of green Arabica beans Brasil Santos

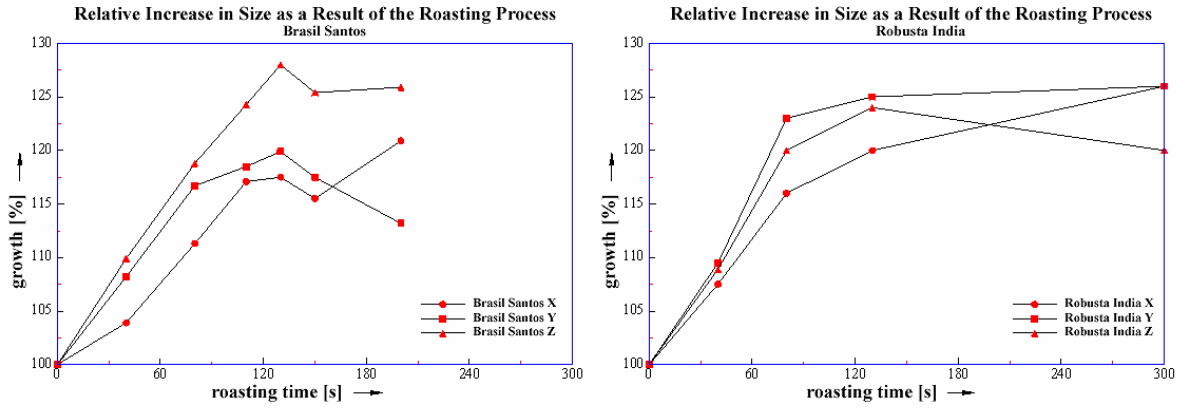

Figure 3. Robusta coffee beans cut with a scalpel at a magnification of 15

Inside the Robusta beans some needle-like structures show up after roasting times of 200 seconds. The length of the needles ranges from 10 up to 40 µm at a diameter of ca. 0,2 µm. Some of the needles grow out of the same base, the walls in the near environment of the needles seem to get thinner.

According to Maier (Maier, 1981) one might think these needles are caffeine. But the following experiment shows that they are not. We focussed the electron beam of the SEM on one of the needles, so that caffeine would have been vaporised. But the only effect was that the needles roll up like a pig's tail.

A reasonable explanation for the needles is that they might be HECHT Filaments, developing during the time the vakuole looses its cell sap.

The HECHT filaments most probably consist of galactomannan (Asante, 1974) or arabinogalactan. These needles do not show up in the Arabica beans. Perhaps the filaments will develop after longer roasting times in the Arabica beans too, or maybe they will not develop at all, because the structure of the walls is different.

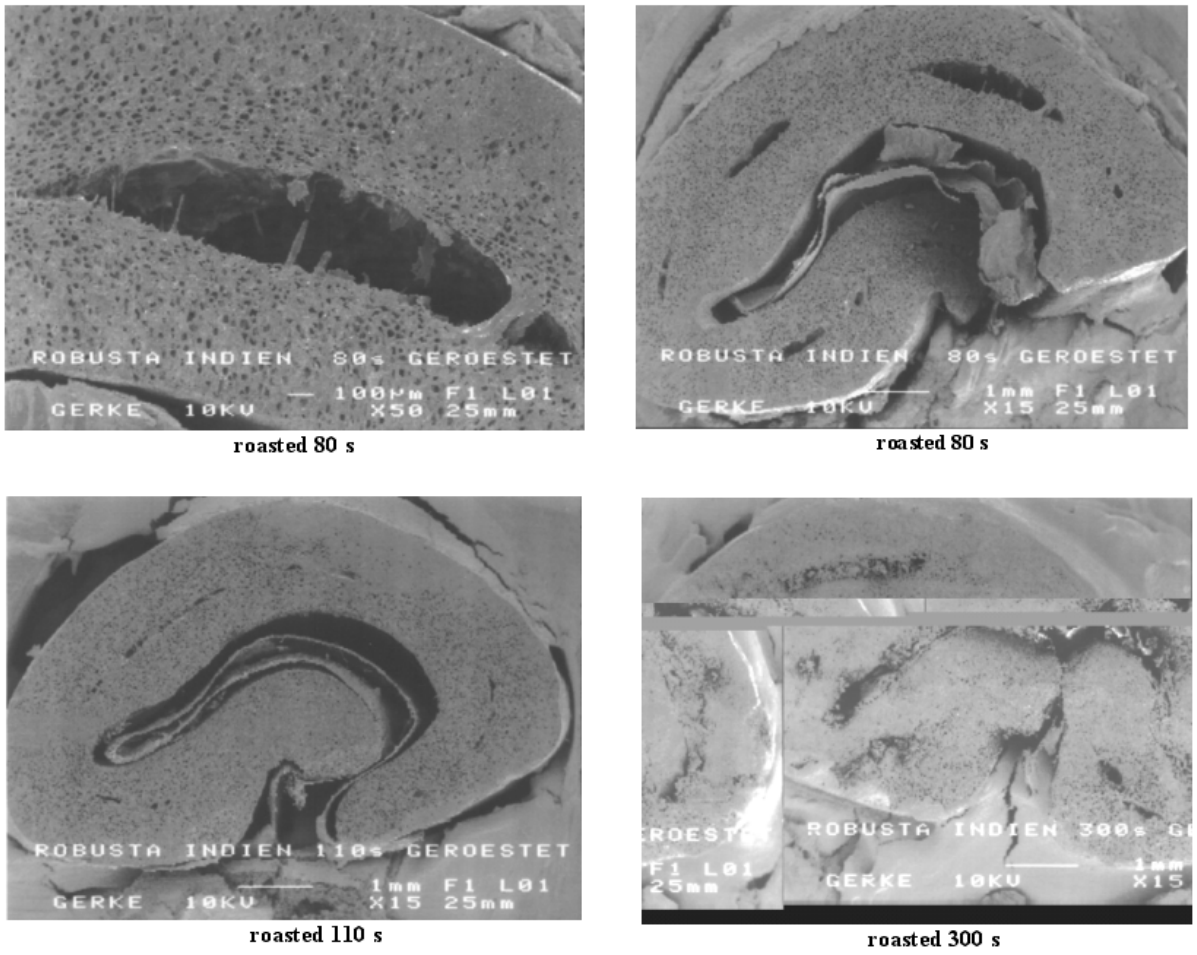

Figure 4. Robusta coffee beans cut with a scalpel at a magnification of 15

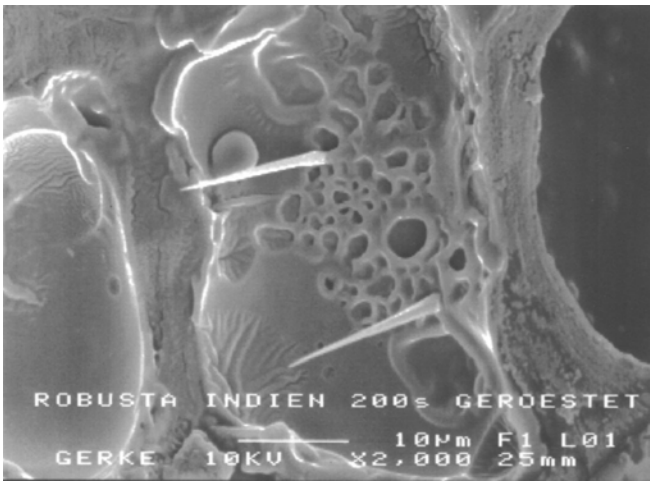

Figure 5. Robusta coffee bean, magnification of the cave-like

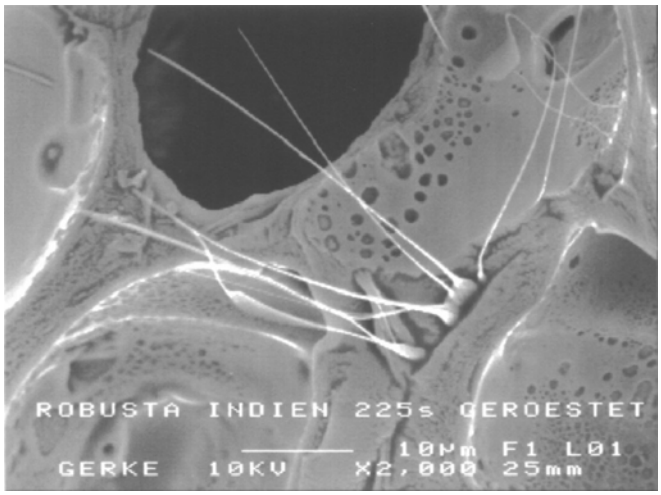

Figure 6. Robusta India, development of the needles

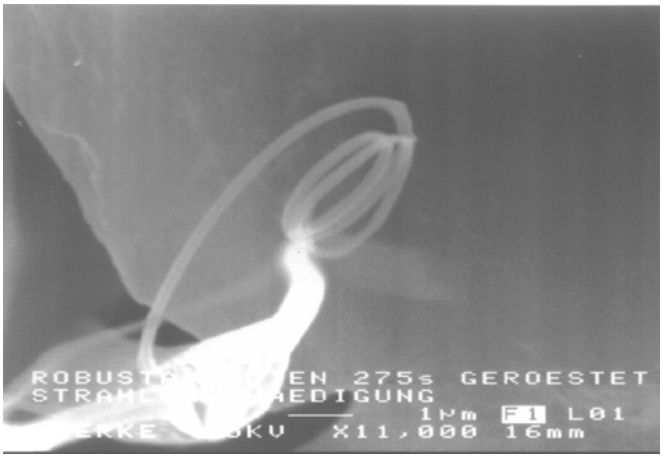

Figure 7. Deformation of the needle after focussing the electron beam on it. The pig's tail sample

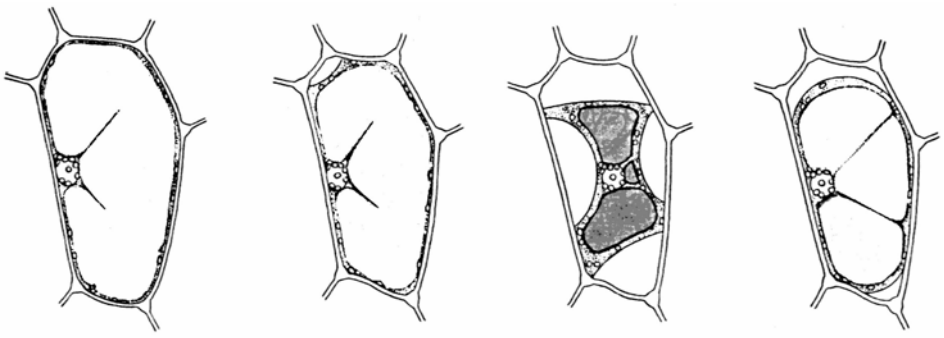

Figure 8. Development of the HECHT filaments (Schopfer, 1999)

Colour values of the coffee beans

Colour slides of coffee beans of different roasting degrees were taken and scanned. The colour values of the different roasting degrees were determined using the graphic program "Paint Shop Pro". For getting the real colour values a uniform part of the surface was

analysed. “Paint Shop Pro” delivers a histogram which gives a value for each basic colour, red, green, blue and the luminance, ranging from 0 (black) to 255 (pure colour).

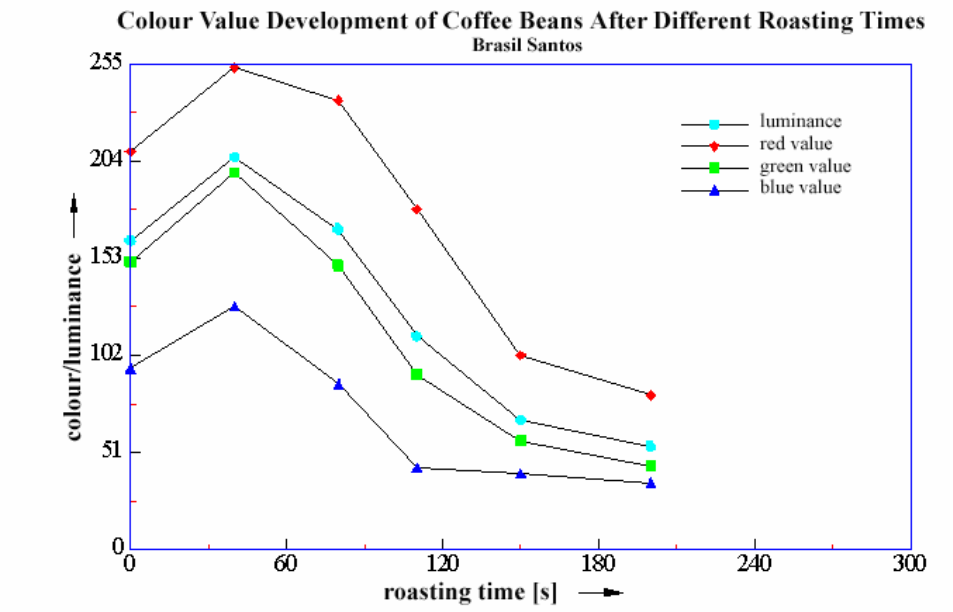

Figure 9. Colour values of the Arabica coffee beans

It is remarkable that colour values increase up to 40 seconds roasting time. This effect exists because of two physical changes of the coffee bean. As a matter of fact the silver cuticle disappears and so does the water at the surface of the coffee bean. These two effects are resulting in a brighter coffee bean. Later, the Maillard reaction leads to a brown and dark bean.

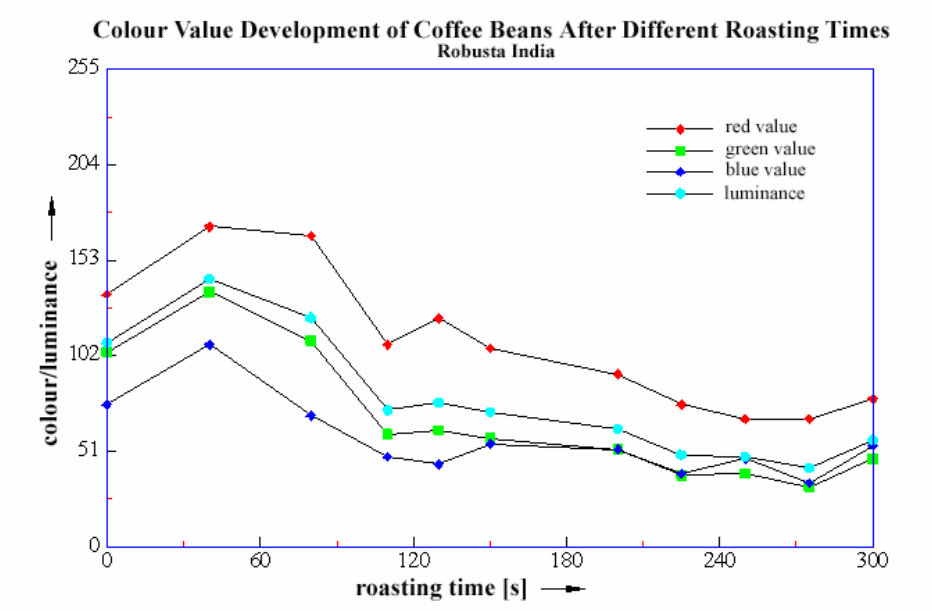

Figure 10. Color values of the regulary Robusta India coffee beans

One problem arose with the Robusta charge, because there were dark and light brown beans. Therefore both kinds of beans are investigated. One sees, that even the regularly coloured

Robusta beans are not as intensive in colour as the Arabica beans are. But they follow the same tendency.

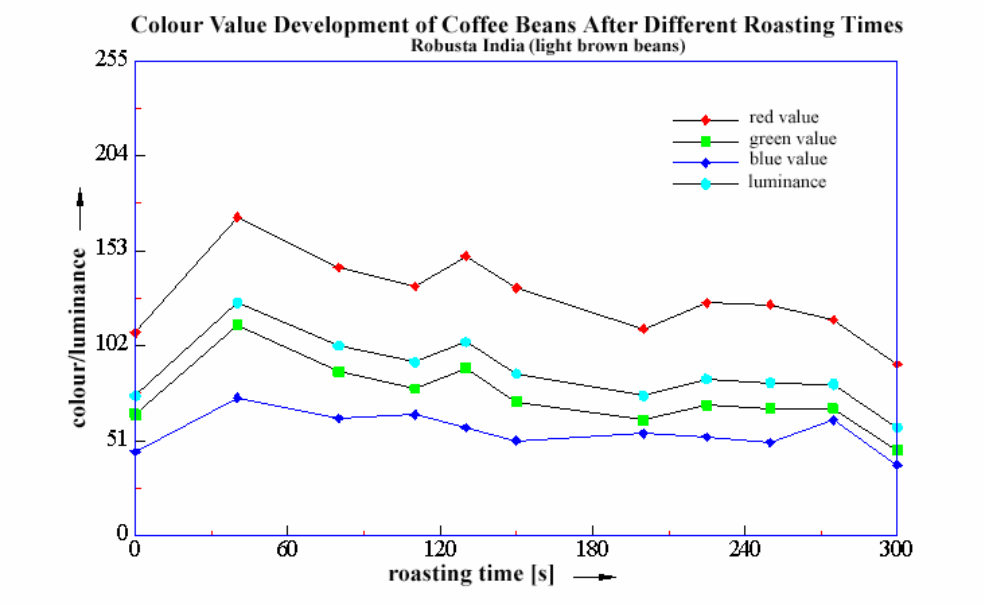

Figure 11. Color values of the light brown Robusta India coffee beans

The light brown beans do not really show a tendency, except for the increase until 40 seconds. The colour values are nearly constant.

Measurement of the reflection coefficient

The measurements of the reflection coefficient were made at PTB, the Physikalisch-Technische Bundesanstalt in Braunschweig, using an infrared spectrometer equipped with an ULBRICHT globe.

A comparison in between the data of the green coffee beans reveals no significant differences. Differences show up between the Robusta and the Arabica for identical roasting times. The peaks seem to be the same for the different curves, but the intensity is different. The reflection coefficient becomes larger the longer the roasting time lasts.

The reflection coefficient will be needed for surface temperature measurements using a pyrometer. The interesting wavelength range lies between 8 and 14 micrometers. In this range the reflection coefficient of the coffee bean is nearly constant at 0.04. Thus the coffee beans are “black” in the near infrared.

For the measurements a sample holder was painted with Nextel Velvet Coating, a special, nearly black varnish. The beans were placed on it, using black plasticine. As a measurement of the worst case, a probe holder with plasticine and a probe holder with Nextel give us the background spectra

We like to thank the FEI, Forschungskreis der Ernährungsindustrie for financial funding, Dr Jochen Wilkens from Tchibo Hamburg for coffee beans and roasting, Dr Dirk Selmar and Gerhard Bytof for discussions about the needlelike structures. As well as Henning Niemann and Dr Detlef Schiel from the Physikalisch-Technische Bundesanstalt Braunschweig for the possibility to measure the reflection coefficient.

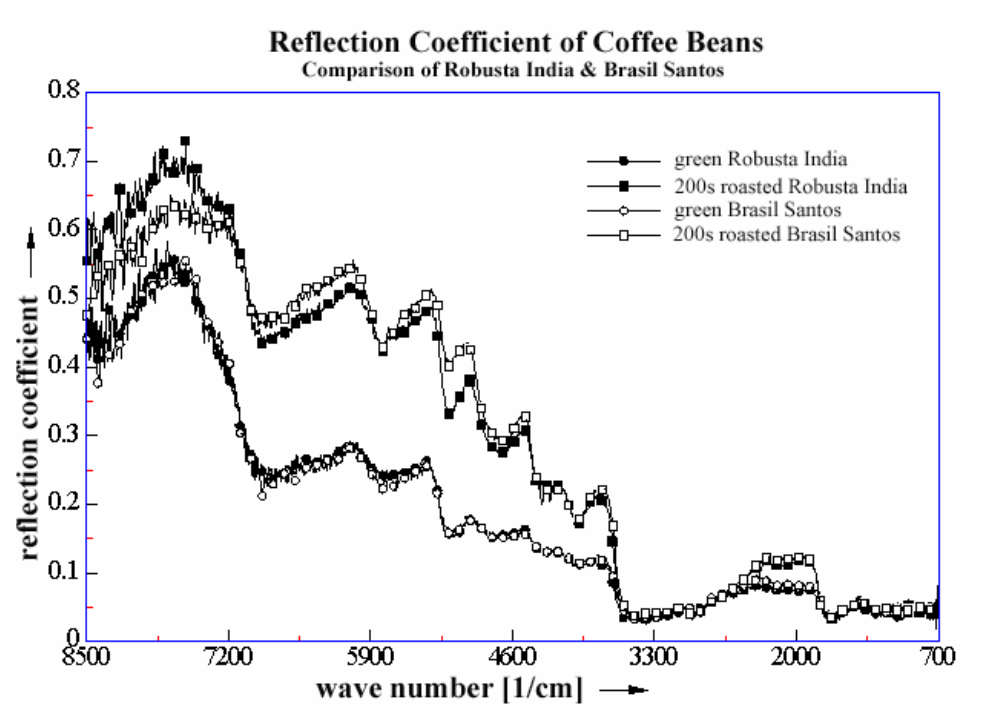

Figure 12. Measurement of the reflection coefficient, comparison between Robusta India and Brasil Santos

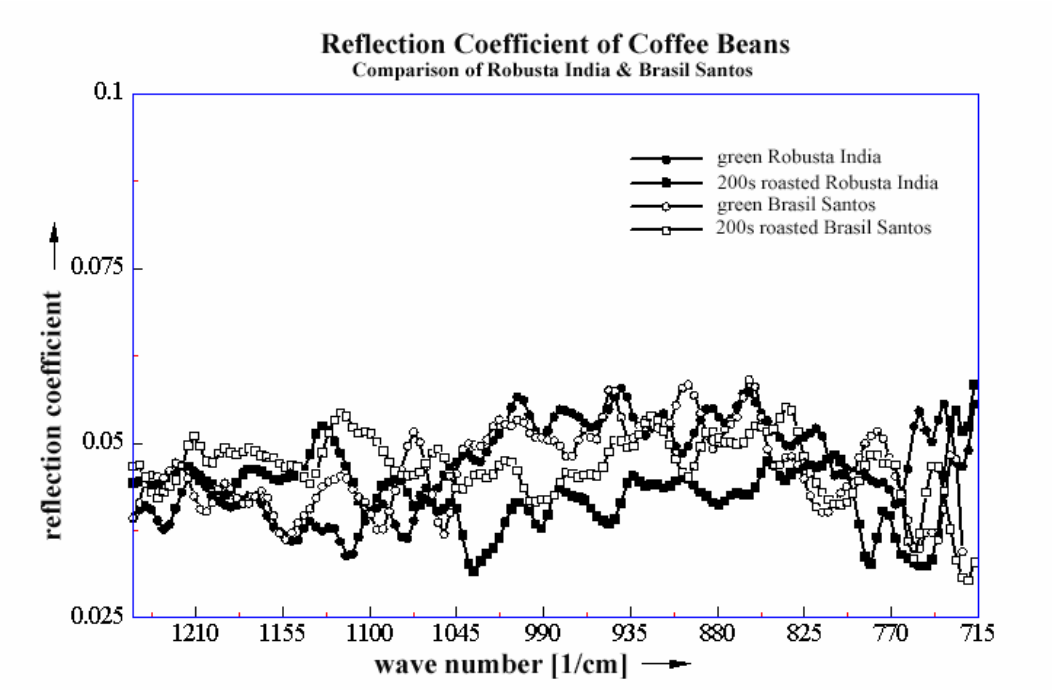

Figure 13. Reflection coefficient of coffee beans in the range between eight and 14 µm

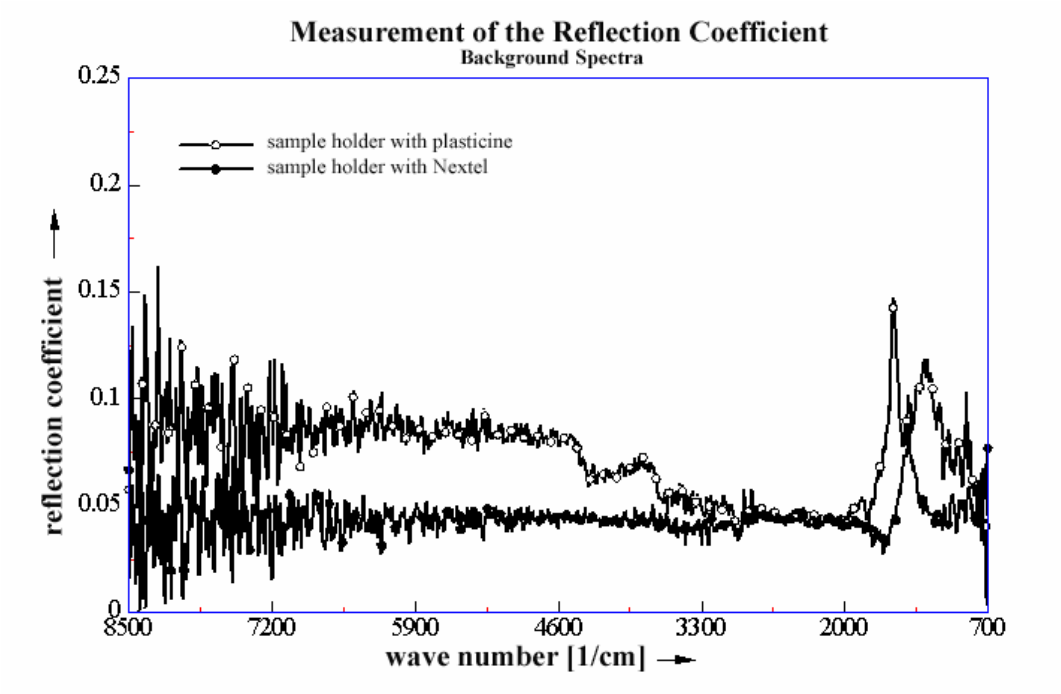

Figure 14. Reflection coefficient of the used plasticine and the sample holder

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Mechanism of Volume Expansion in Coffee Beans During Roasting

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SUMMARY

The mechanisms involved in volume expansion during roasting of coffee were investigated by means of thermal analysis. A state diagram adapted to the roasting process was determined. Roasting processes were analysed and changes of the physical state of coffee beans were set in relation to the observed volume expansion and to changes of apparent density.

The final apparent density of roasted coffee beans depends on roasting conditions, in particular on temperature, but is not related to initial moisture content. As a consequence, depending on the roasting temperature a lower initial moisture content leads to an accelerated reduction of apparent density in the first phase of the roasting process. Coffee beans with low initial moisture content roasted at a temperature above 260°C showed a pronounced tendency for oil release already at the beginning of storage.

In order to reduce mass transfer during subsequent storage, the roasting process for coffee beans is preferably carried out at product temperatures below 240°C. Furthermore, the temperature of the coffee beans should be reduced at the end of the roasting process in order to prevent temperature driven excessive reduction of apparent bean density, which would lead to an increased loss of volatiles and access of oxygen during storage.

INTRODUCTION

Coffee roasting is an empirical technology producing specific properties of roasted coffee. The most important changes during roasting are the production of specific roast flavour compounds, the development of a dark colour and the modification of texture and microstructure. Depending on raw material and roasting conditions the volume of the coffee beans increases during roasting. The observed volume increase is remarkable and influences storage stability of roasted coffee.

HYPOTHESIS

Schenker (2000) developed a model for volume expansion of coffee beans during roasting. In this model, the volume expansion is the result of driving forces namely moisture evaporation and gas formation, and of the reduction of structure resistance forces due to changes in microstructure and polymeric cell wall compounds. It was assumed that during roasting polymeric cell wall compounds change their physical state from a glassy to a rubbery or elastic state (Figure 1). Depending on the physical state of cell wall compounds during the expansion, the volume expansion may contribute more or less to the breakdown of bean

microstructure. The microstructure of roasted coffee beans is assumed to influence mass transfer properties and is responsible for storage stability with respect to flavour retention, gas evolution and oil release (Schenker, 2000). As a consequence, the optimisation of the roasting process for coffee with respect to storage stability has to aim at a maximal retention of the native bean microstructure.

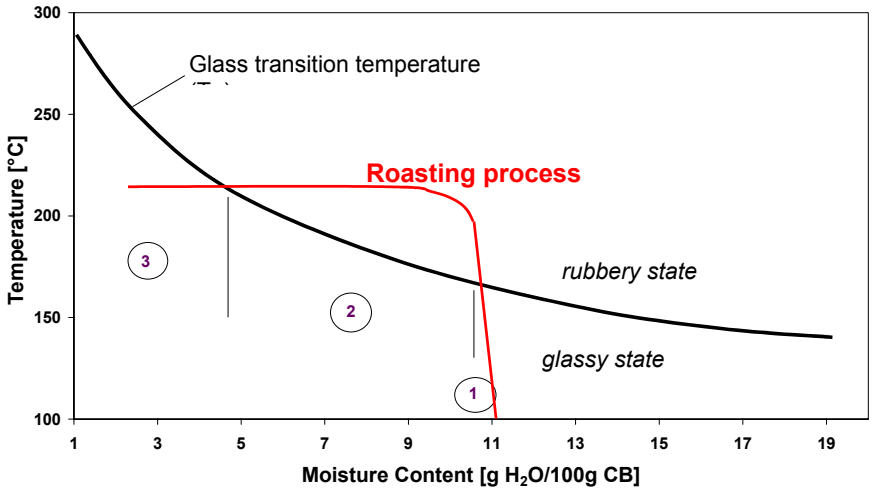

Figure 1. Modelled changes of polymeric cell wall compounds in coffee beans during roasting (Schenker, 2000)

The goal of the present project was to investigate volume expansion mechanisms during roasting of coffee beans and to discuss the volume expansion with respect of the optimisation of the roasting process. Therefore, the changes of the physical state of cell wall polymers during roasting were identified. A state diagram was developed relating of the physical state of cell wall compounds to moisture and temperature. Then, coffee beans of differing initial moisture content were roasted in isothermal roasting experiments and the observed volume expansion was assessed. Finally, based on these observations industrial roasting experiments were carried out.

IDENTIFICATION OF STATE TRANSITION PHENOMENA OF POLYMERIC CELL WALL COMPOUNDS

Dynamic mechanical thermal analysis DMTA was applied for the identification of transition phenomena of polymeric cell wall compounds. With DMTA texture modifications such as softening and hardening of materials can be identified and related to the changes of the physical state of materials.

For the analysis coffee beans of different moisture content were cross-sectioned to slices of 3mm thickness and mounted on a DMTA Solids Analyzer RSA II (Rheometrics, Piscataway USA) with plate-plate configuration. Then, the specimens were heated linearly from 30°C to 250°C with a heating rate of 5°C/min.

A DMTA thermogramme of a coffee bean slice is presented in Figure 2. Therein, the storage modulus G' is shown. The fast drop of storage modulus G' between 130 and 170°C represents a transition of polymers from the glassy to the rubbery state, the coffee bean texture is softening. The storage modulus G' increase between 200 and 230°C represents the reversion of the transition, interrupted by a melting phenomenon of a compound between 212 and 217°C. The coffee bean texture is hardening again.

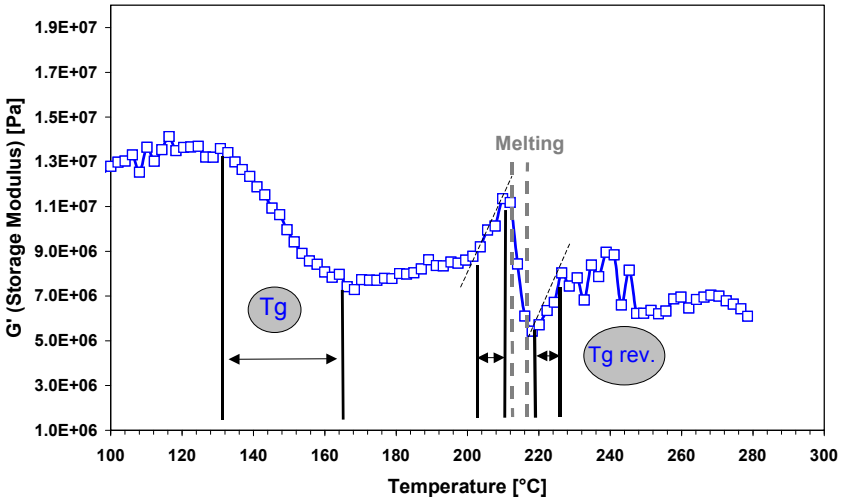

Figure 2. DMTA thermogramme of coffee beans heated with 5°C/min. *Tg* represents a glass transition phenomenon (softening) and *Tg rev* the reversion of the transition (hardening), interrupted by a melting phenomenon

We observed a strong relationship between initial moisture content and the temperature range of the glass transition. Because DMTA analysis was performed under non-moisture-controlled conditions, the moisture content as determined immediately at the beginning of the transition was taken into account.

By variation of the initial moisture content a state diagram for coffee beans was developed as shown in Figure 3. Because no sharp and pronounced transition was found in DMTA, the on- and offset of the transition was used to describe the state transition. In order to identify the compounds involved in the state modifications in green coffee beans an adapted DMTA method and a differential scanning calorimetry method (DSC) were applied to analyse the behaviour of pure amorphous or semi-crystalline polymers prevailing in coffee bean cell walls, namely cellulose, arabinogalactan, mannan and pectins. The observed state diagram of coffee beans correlates to cellulose, whereas arabinogalactan melted completely above 210°C (data not shown). Probably, the melting phenomenon observed in DMTA analysis was caused by arabinogalactan.

In conclusion a state diagram of coffee beans was developed describing the occurrence of transition phenomena (softening) of cell wall compounds during roasting (Figure 3).

PHYSICAL STATE OF COFFEE BEANS DURING ISOTHERMAL LABORATORY ROASTING

In order to analyse the influence of transition or softening phenomena on volume expansion, isothermal roasting trials were carried out on laboratory scale. The state of cell wall polymers was related to moisture content and to measured bean core temperature (Figure 4). In all roasting trials the bean core temperature passed the transition at higher moisture content within a moisture decrease of less than 1 g/100 g. At hot air temperatures of 180, 190°C and 220°C, the transition was reversed before the initial moisture was lost. In case of roasting at temperatures above 230°C the transition was reversed only during cooling. One has to bear in mind, these plots do not take the roasting time into consideration and are not related to roasting degree and colour development.

VOLUME EXPANSION DURING ISOTHERMAL LABORATORY ROASTING

Figure 5 shows the volume expansion of coffee beans as observed in isothermal roasting experiments. The volume expansion rate is highest in the first part of the roasting process at high moisture content. This refers to the first one to two minutes of the roasting process. During this stage the transition phenomena takes place. Then, after the hot air temperature is reached, the volume expansion rate is reduced probably due to the change from dynamic to isothermal heating and probably also due to reduced moisture evaporation rate.

In the second phase coffee bean cell wall compounds are in the rubbery state. The expansion is driven by the residual initial moisture, by the formation of water from non-enzymatic browning reaction and by decarboxylation reactions. On the other hand, structure resistance forces are reduced as well, and the evolution of gaseous substances is accelerated due to increased permeability of microstructure.

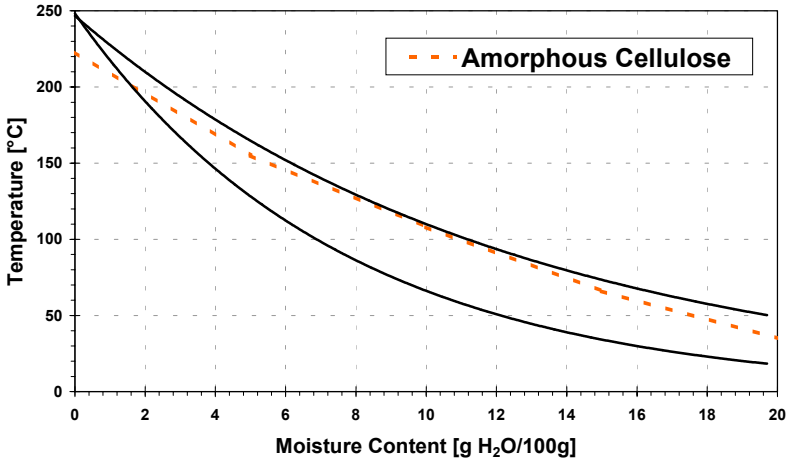

Figure 3. State diagram of amorphous cellulose and of Columbian coffee beans. Cellulose data reported by Salménn (1982)

In the third stage, the initial moisture is lost completely. In this phase the moisture content can not be reduced further because the formation of reaction water equals the evaporation. Moisture and temperature conditions are as such that cell wall polymers are in the region of glass transition (roasting trials at 220 and 230°C) or even in the glassy state (roasting trials below 220°C). Although the driving forces namely formation of reaction water and carbon dioxide are still present, the volume expansion rate is reduced depending on the state of cell wall components.

Because the relative volume is corrected for weight loss during roasting, the apparent density was used as a measure for absolute volume modification disregarding changes in the bean composition during roasting. Figure 6 sets the initial moisture content in relation to the formation of apparent density of coffee beans during isothermal roasting. The calculated change in apparent density caused by moisture loss (not accompanied by volume changes) is shown as a reference. At lower initial moisture content, the apparent density is reduced very fast at early stage of the roasting process reducing the moisture content only little. At higher initial moisture content and during progressing roasting, the density reduction is accompanied by appropriate moisture loss in the early stage of roasting. When the moisture content is becoming minimal, further density reduction proceeds depending on roasting temperature. The final apparent density is only depending on product temperature and on roasting process duration, but not on initial moisture content. In each case, the highest rate of apparent density reduction was observed after passing the transition temperature.

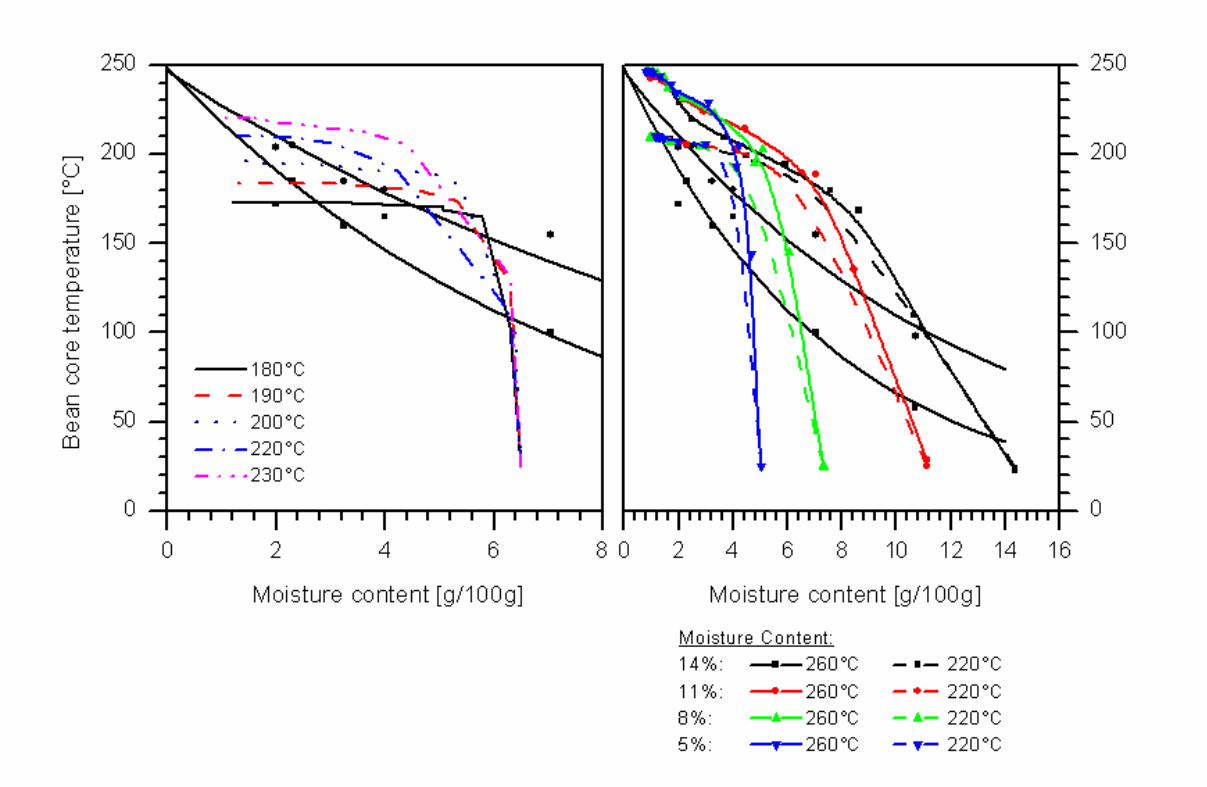

Figure 4. Changes in the state of Colombian coffee beans during isothermal roasting. Left: Hot air temperature of 180, 190, 200, 220 and 230°C, respectively Initial moisture content of coffee beans was at 6.5 g/100 g. Right: Hot air temperature of 220 and 260°C, respectively Initial moisture content between 5 to 14 g/100 g

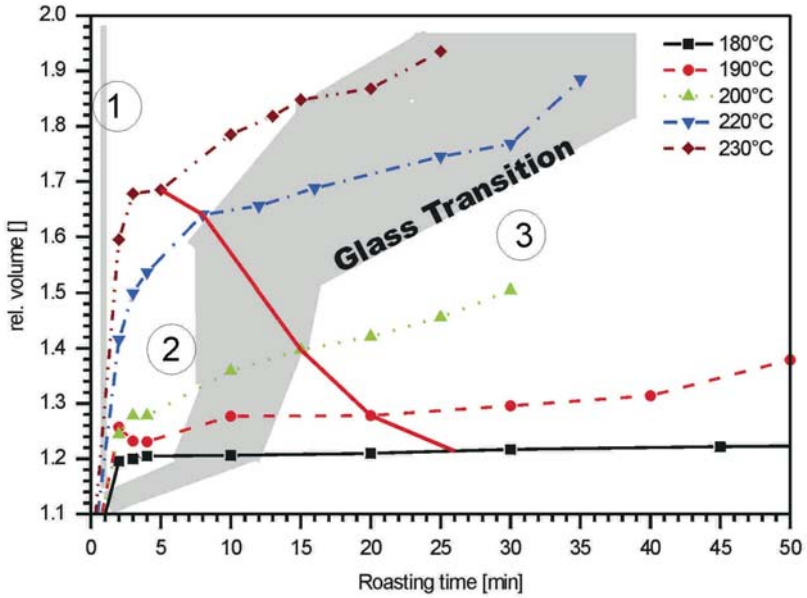

Figure 5. Formation of relative volume in coffee beans during roasting at different temperatures: Phase 1: Dynamic heating; Phase 2: Isothermal process after glass transition; Phase 3: Isothermal process, reversion of glass transition

INDUSTRIAL ROASTING TRIALS

The industrial roasting trials were performed on a partially fluidising batch roaster CR (G. W. Barth Ludwigsburg GmbH & Co, D-Freiberg/Neckar). With this roasting system, multistep roasting procedures at varying air flow were performed. Figure 7 presents a selection of industrial roasting processes in the state diagram and the corresponding reduction of apparent density in relation to moisture content. In industrial processes the elevation of product temperature is slowed down due to the inertia of the industrial system. As the actual internal product temperature could not be measured, the pile temperature was recorded as an approximation of bean temperature.

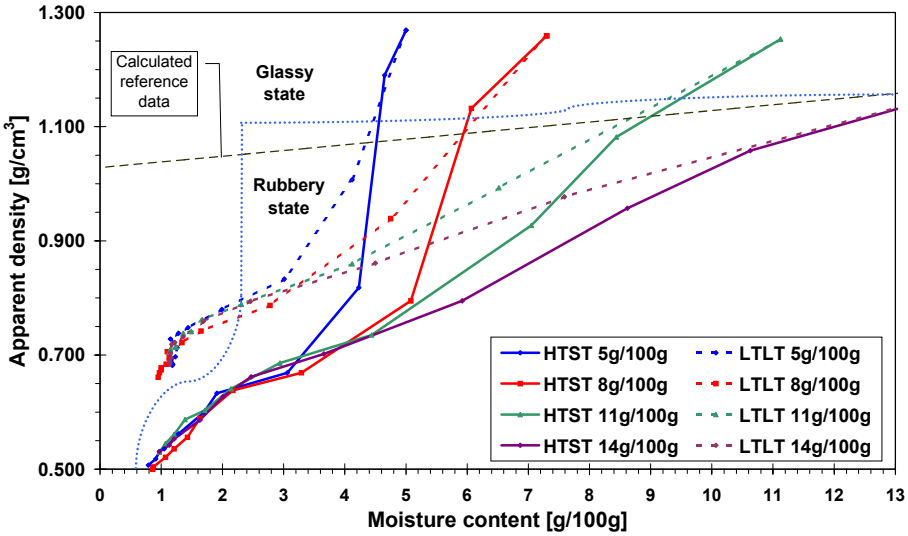

Figure 6. Changes in apparent density of coffee beans during isothermal roasting depending on initial moisture content ranging from 5 to 14 g/100 g. The calculated reference data corresponds to the change in apparent density caused by corresponding moisture loss at constant external (apparent) volume. HTST: High temperature short time process at 260°C; LTLT: Low temperature long time process at 220°C

After the initial start up phase, the coffee beans changed into the rubbery state. At the end of the roasting process or during cooling respectively, the coffee beans changed back to the glassy state. The apparent density was again reduced quickly after passing the transition temperature. Then, because the pile temperature was still rising, the reduction in apparent density was accompanied by a greater moisture loss. As soon as the pile temperature exceeded 180°C, the reduction in apparent density was more profound indicating increased volume expansion.

CONCLUSIONS

During roasting, the coffee bean volume expands due to the change of physical state of cell wall polymers to a more elastic state. Cell wall polymers in the rubbery state are less resistant towards mass transfer phenomena and allow a corresponding volume expansion of the beans. At the end of the roasting process or during cooling, the cell wall compounds are reversed to the glassy state again reducing mass transfer phenomena during subsequent storage.

The changes of the apparent density may be considered as crucial for permeability of cell wall components. The final apparent density is depending on roasting temperature, but independent from initial moisture content. As a consequence, depending on the roasting temperature a lower initial moisture content leads to an accelerated reduction of apparent density in the early

stages of the roasting process. It was found that coffee beans with low initial moisture content roasted at a temperature above 260°C showed a pronounced tendency for oil release already at the beginning of storage. Under these circumstances, a fast reduction of apparent density and a fast increase of relative volume probably lead to more permeable structures exhibiting a more pronounced tendency for mass transfer.

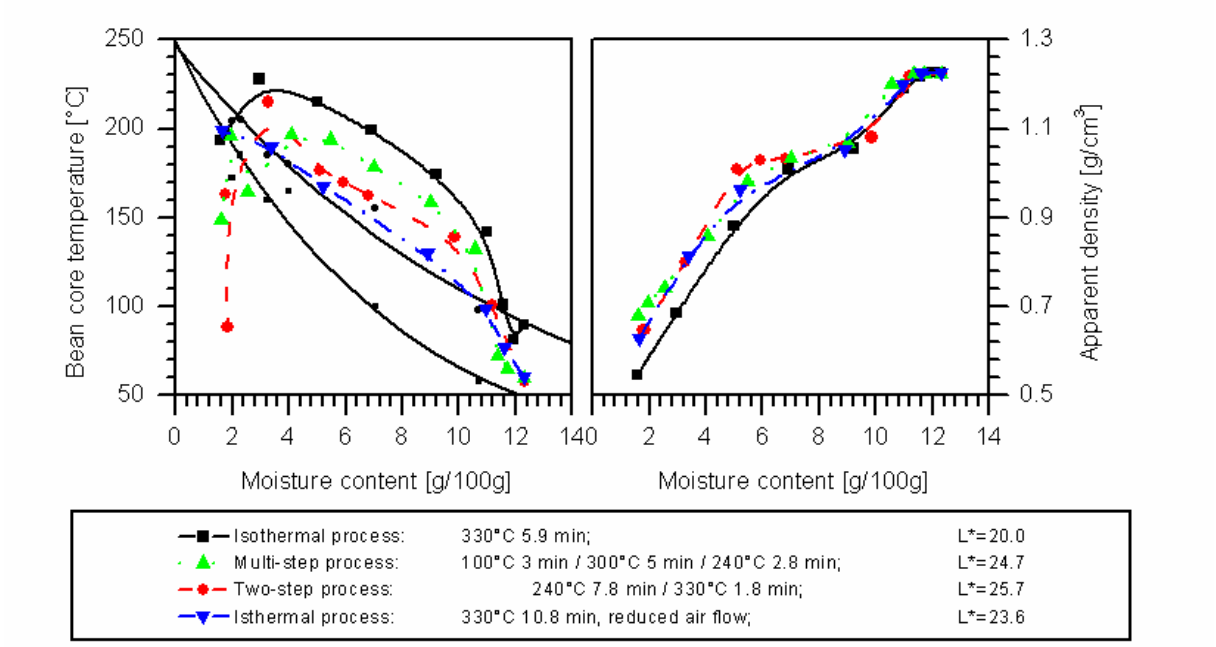

Figure 7. Left: State diagram of coffee beans roasted industrial roasting processes. Right: Reduction of apparent density of coffee beans in relation to changes in moisture content

ACKNOWLEDGMENTS

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Impact of Roasting Temperature Profiles on Chemical Reaction Conditions in Coffee Beans

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SUMMARY

Hot air roasting of coffee beans results in a large number of aroma compounds. For a given green coffee blend, the aroma profile is determined by the specific conditions for chemical reactions as controlled by the process parameters. Therefore, the influence of roasting temperatures on bean temperature, dehydration, pressure build-up, browning and the formation of key aroma compounds were investigated. Coffee was roasted in 6 different processes to equal degree of roast and the developing aroma compound profiles characterized by gas chromatography, mass spectrometry and olfactometry. A majority of aroma compounds showed the highest increase in concentration at medium stage of dehydration with bean water contents from 7 to 2% (wb). Different time-temperature histories lead to distinct aroma compound profiles. For specific flavor production, the precise control of roasting time and temperature is required.

INTRODUCTION

During roasting of coffee beans drying takes place, the microstructure is changed (Schenker et al. 2000) and complex chemical reactions are induced (Clarke and Vitzthum 2001; Illy and Viani 1995). So far, more than 800 different volatiles from a wide range of chemical classes have been identified in roasted coffee (Flament 1989; Nijssen et al. 1996). In recent years, research has been centered around the sensory relevance of these volatiles and the identification of key odorants in coffee beans and brewed coffee beverage (Blank et al., 1991-1992; Holscher et al., 1990; Semmelroch et al., 1995; Grosch et al., 1996; Semmelroch and Grosch 1996; Czerny et al., 1999).

Roasting conditions have a major impact on the physical and chemical properties of roasted coffee beans, the mode of heat transfer and the temperature conditions being the most critical process parameters (Schenker, 2000). They influence the specific conditions for chemical reactions in the beans. To date, very little literature data are available on the formation rates of aroma compounds *during* roasting and the influence of different roasting conditions on the profile of key aroma compounds in the roasted beans.

Silwar and Lüllmann (1993) roasted *Robusta* coffee samples at different temperatures for a constant duration of 5 min, resulting in products of various degrees of roast. The authors concluded from tasting cups of brewed coffee that aroma formation starts at around 170°C. At 180 to 190°C a coffee-like flavor originated, whereas the "real" flavor of roasted coffee only appeared at 220 to 230°C. Beyond this point, the flavor was judged to be over-roasted. The study also shows a continuous increase in the total number of volatiles with increasing

temperature up to 250 C, followed by decreasing numbers beyond this temperature. Furans and caramel compounds were found to be fully developed at 230 to 240°C. In contrast, 2-furfurylthiol continued to be formed up to 260°C. The formation of pyrazines generally reached a maximum at 250°C. Beyond this temperature they were assumed to be incorporated in melanoidins by those authors. Mayer et al. (1999) investigated the influence of geographical coffee bean origin and the degree of roast on concentrations of aroma compounds in *Coffea arabica*. Guaiacol and 2-furfurylthiol developed unhindered with increasing degree of roast. 2,3-Butanedione and 2,3-pentanedione developed to a maximum concentration for a medium degree of roast and exhibited lower concentrations in dark-roasted coffee beans. Gretsch et al. (1999) presented a comprehensive study on the formation of aroma compounds during roasting and their sensory impact when sniffing roast and ground coffee samples. Colombian coffee was roasted at 230°C for various periods of time to generate different degrees of roast. Sensory evaluation and instrumental analysis of the samples showed developments for concentrations of single aroma compounds and the corresponding sensory attributes during roasting. The authors found a restricted correlation between concentrations and the sensory intensities of their corresponding notes. They stated that some strong compounds, formed during the later stages of roasting, tend to mask the perception of other compounds present.

Our objective was to investigate bean dehydration, browning, and the formation of aroma impact compounds during roasting as influenced by different temperature conditions. The study aims to contribute to a better fundamental understanding of roasting and to move process optimization from art more towards science.

EXPERIMENTAL

A wet-processed *Coffea arabica* Linn. variety from Colombia was used. The water content in different lots of green beans was around 11.0 g/100 g beans. Roasting experiments were carried out with a fluidized-bed hot air laboratory roaster in batches of 100 g green beans under well-defined process conditions (Schenker, 2000; Schenker et al., 2000). Six different hot air inlet temperature conditions were applied (Table 1). Samples were removed at regular intervals and analyzed. Typical product properties have been described previously (Schenker et al., 2000).

Color was measured with a tristimulus colorimeter Chroma Meter CR-310 (Minolta, Japan). Ground coffee bean samples were placed in a petri dish and gently pressed by hand with equal force using a cylindric plastic piece to form an even surface. Results are presented in the CIE *L*a*b** color space.

For determination of water content, samples of roasted coffee beans were finely ground in a household two-disk coffee grinder. Gravimetric determination was carried out according to the Swiss Food Manual (1973). A portion of 5 g ground coffee beans was dried at 103°C for 5 h.

The volatile fraction of the roasted beans was isolated using simultaneous distillation/extraction (SDE) and vacuum distillation (Schenker, 2000). The results presented here were obtained from SDE, carried out with a Likens-Nickerson apparatus (Likens and Nickerson, 1964; reviewed by Marsili, 1997). A portion of 30 g ground coffee beans was combined with 500 mL distilled water and an internal standard of 2-butanol. The suspension was extracted with 50 mL solvent (pentane/diethyl ether mixture 1:1) for 2 h. After drying with anhydrous sodium sulfate the extract was concentrated to less than 1 mL by means of a Vigreux column (10 cm height, ∅ 1 cm).

Gaschromatography with flame ionization detector (GC-FID) was used for separation and semi-quantitative determination of aroma compounds. The analytical conditions for separation are reported by Schenker (2000). A high-resolution capillary column assured maximum separation performance, however, no pre-fractionation of the isolates was carried out. The relative amount of a compound was defined as the peak area ratio of the compound X and the internal standard (A_X/A_{IStd}).

The analytical conditions for gas chromatography mass spectrometry (GC-MS) measurements were kept close to those applied in GC-FID analysis (Schenker, 2000). Compounds were generally identified by comparison of mass spectra and retention indices (van den Dool and Kratz, 1963) with reference substances and with literature data. As an alternative to semi-quantification via GC-FID, relative amounts of a few compounds were calculated using GC-MS peak areas (Reconstructed Ion Current, RIC). In a few occasions, semi-quantitative evaluation was based on a characteristic ion of the compound in question

Table 1. Hot air temperature-time conditions in different coffee bean roasting processes to achieve a medium degree of roast (set values)

Process		Temperature	Roasting time	
			individual step	total
HTST	isothermal high temperature	260 ± 0.5°C (isothermal)	-	160 s
LTLT	isothermal low temperature	220 ± 0.5°C (isothermal)	-	600 s
HL	high temperature with a reduced final stage	240°C 220°C	150 s 210 s	360 s
LHC	continuous temperature increase from low to high	continuous increase 150→240°C 240°C	270 s 55 s	325 s
PLHC	pre-heating temperature with subsequent LHC process	150°C (pre-heating) continuous increase 150→240°C 240°C	180 s 270 s 50 s	500 s
PHL	pre-heating temperature, high temperature at medium stage and reduced temperature at final stage	150°C (pre-heating) no hot air flow (technical) 240°C 220°C	180 s 90 s 140 s 210 s	620 s

For olfactometry (GC-O), the GC-FID system was equipped with a column end split (split 1:1), leading to a sniffing port (Acree et al., 1984; reviewed by Marsili, 1997). Aroma extract dilution analysis was carried out with undiluted isolates and dilutions 1:4, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512 and 1:1024. Each sequence of GC effluents was sniffed by at least 2 persons. The Flavor Dilution factor (FD-factor) for a specific compound was defined as the greatest dilution at which this compound was still perceivable in the GC effluent. It is a measure for the possible aroma relevance of a compound. Each compound with a FD-factor of 1024 or more was specified as an "aroma impact compound" (AIC) for the respective coffee.

RESULTS AND DISCUSSION

The development of pile (hot air / bean mixture) temperatures is shown in Figure 1a. The high air to bean ratio in the laboratory roaster resulted in a rapid heat transfer. During industrial roasting with large batch sizes, the heat transfer is generally less rapid. However, one has to keep in mind that also with laboratory scale, bean core temperatures deviates from pile temperatures (Figure 1b). As the temperature increases, the water of the green beans evaporates continuously. Additionally, a substantial amount of water is generated as a result

of chemical reactions, and is also evaporated. The impact of the different temperature conditions on bean dehydration is presented in Figure 2a. With all 6 processes, the decrease of water content was closely related to the temperature conditions. A smooth and steady decrease of the bean water content was observed with isothermal conditions (HTST and LTLT).

A lot of gases, mainly CO_2 and CO, are formed during roasting. They are largely entrapped in the bean and lead to substantial pressure build-up. Gas formation and pressure are highly affected by the roasting temperature (Schenker, 2000). Pressure modelling revealed that bean pressure can easily exceed 15 bar during the final stages of high temperature roasting. Therefore, pressure may be considered as another factor that influences not only the bean microstructure, but also the conditions for chemical reactions.

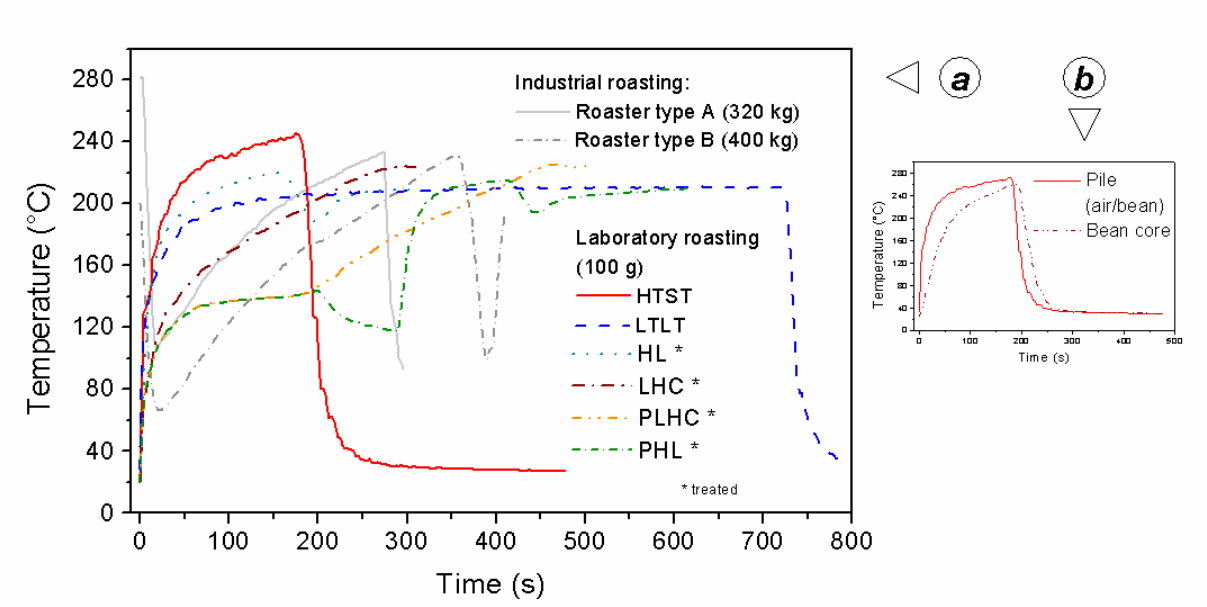

Figure 1. Temperature development in the laboratory roaster and industrial roasters. 1a: Development of pile temperatures (hot air / bean mixture). Figure 1b: Comparison of pile and bean core temperature during HTST roasting

Bean temperature, water activity and pressure are influenced by the roasting temperature. Among others, they set the conditions for chemical reactions in the bean. Figure 2b shows the impact of different time-temperature conditions during roasting on colour development of the beans. Very little browning was observed during the pre-drying stage of the PLHC and PHL processes at 150°C. In general, colour development did not set in before the water content was decreased below 10 g/100 g bean. High temperature isothermal roasting resulted in the most rapid colour development.

Table 2 gives a survey on aroma impact compounds (AICs) for high and low-temperature roasted coffee beans as identified by GC-O. Most of them are known to contribute as AICs to the sensory perception of "coffee", whereas others appear to be characteristic for the specific area of origin of the coffee. The catalogues of AICs for HTST and LTLT roasted beans were widely congruent.

Figure 3 shows the formation of selected aroma compounds during different roasting stages. The development of the 3 AICs 2-ethyl-3,5-dimethylpyrazine, propylpyrazine and 3-methylbutyric acid was similar in HTST and LTLT isothermal roasting (3c). It is characterized by low formation rates during the first third of the roasting time and a rapid

formation in the second third. During the final third of roasting, the concentrations of these compounds decreased, indicating that aroma formation is superimposed by an accelerated degradation due to the high temperature. A group of pyrazines (3a) as well as 2,3-pentanedione (3b) exhibited similar behaviour. In contrast, guaiacol, 2,3-butanedione and 2-furfurylthiol continuously increased in concentration, even during excessive roasting at high final temperatures.

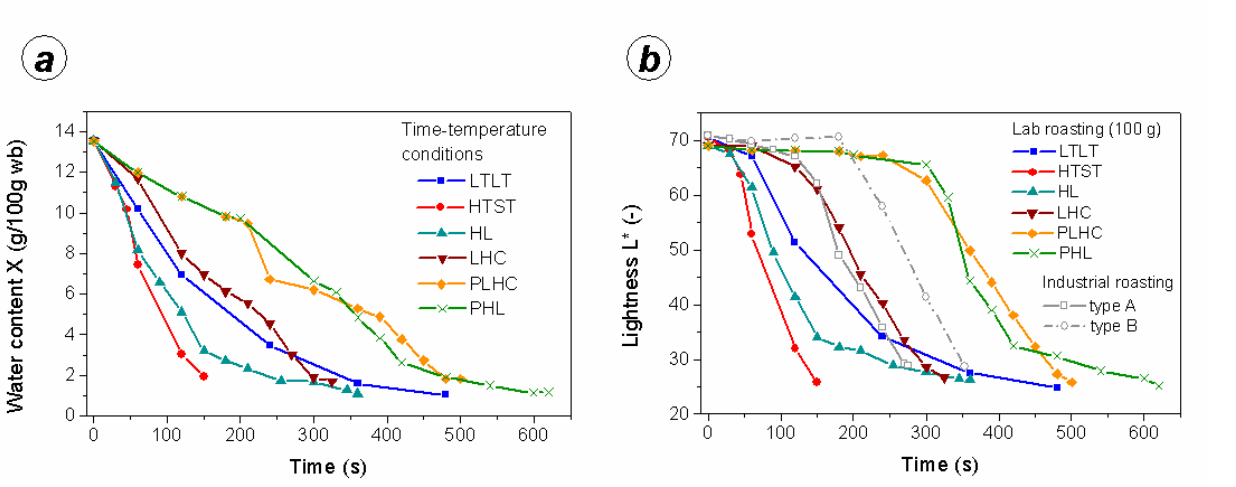

Figure 2. Impact of different time-temperature conditions during roasting on bean dehydration (2a) and on colour development (2b)

Figure 4 provides a semi-quantitative survey on the influence of the 6 different time-temperature conditions on the generation of aroma compounds. The catalogue of compounds was the same for all processes, although the quantities and the relative importance of each compound within the profile were specific for each process. Roasting temperatures below 220°C (LTLT process, reference) resulted in low aroma concentrations.

Table 2. Listing of identified aroma impact compounds from high and low temperature roasted coffee beans and their sensory quality and relevance as obtained by GC-Olfactometry

Compound	*Aroma quality*	*FD-factor*	
		HTST roasting	*LTLT roasting*
2,3-Butanedione	butter (butter)	**1024**	(256)
Beta-Damascenone	fruits, flowers, (honey, fruity, tea)	(16)	(128)
2-Ethyl-3,5-dimethyl pyrazine	(earthy, roasty, potatoes)	**1024**	**1024**
2-Furfurylthiol	bouillon, potatoes (roasty, sulfur-like, coffee-like)	**1024**	**1024**
Guaiacol	medical, glue-like, (smoky, phenolic, aromatic, spicy)	(512)	**1024**
4-Hydroxy-2,5-dimethyl-3[2H]-furanone (=Furaneol)	roasty, sweet, (caramel)	**1024**	(256)
2-Hydroxy-3-methyl-2-cyclopenten-1-one	(spices, celery-like)	**1024**	(32)
Methional	potatoes, (cooked potatoes, sweet)	**1024**	**1024**
3-Methyl-butyric-acid	sweaty, pungent, (sweaty, fermented)	**1024**	**1024**
3-Methylmercapto-	herbs	**1024**	**1024**
Propylpyrazine	potatoes, vegetables	**1024**	(64)
unknown (RI=2329)	sweet, medicine	**1024**	(4)

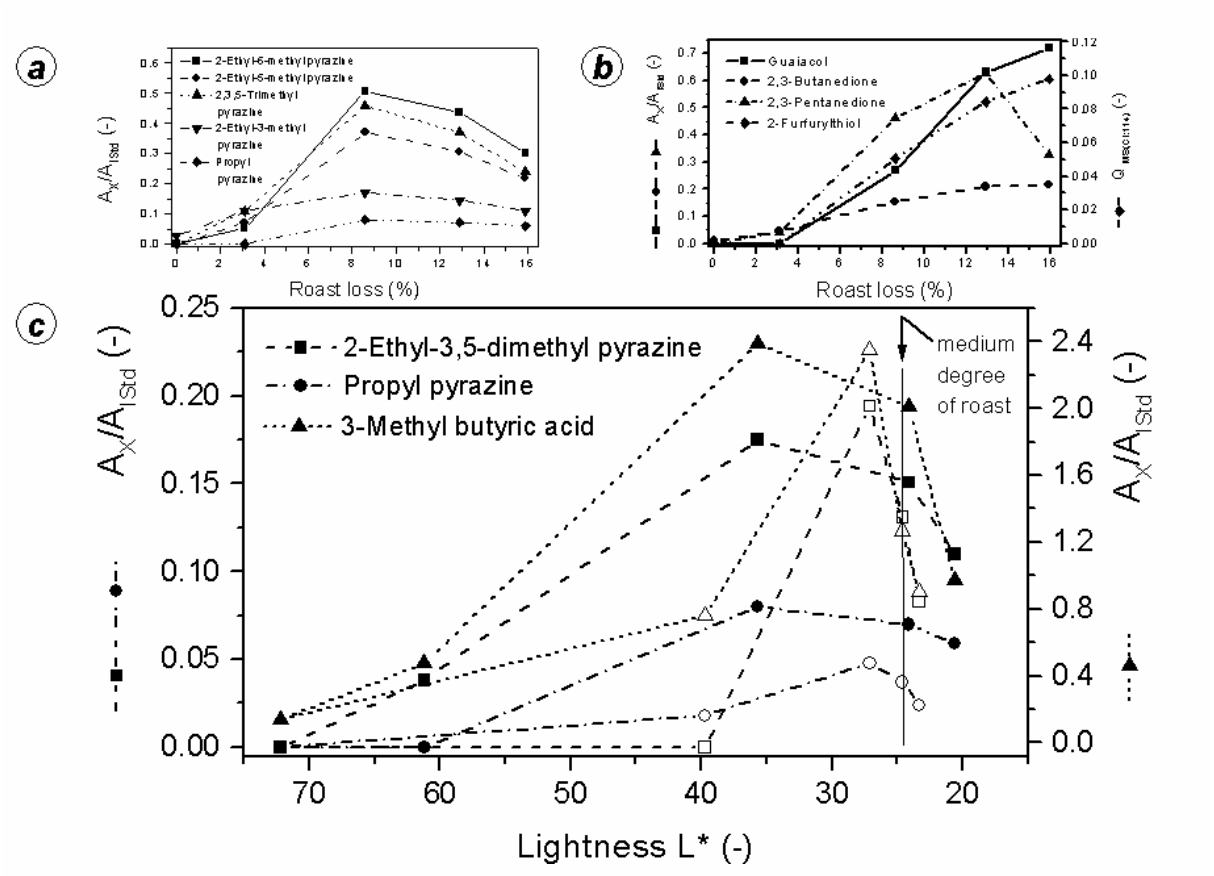

Figure 3. Quantitative development of aroma compounds during HTST roasting (solid symbols) and LTLT roasting (open symbols). 3a: Pyrazines. 3b: Guaiacol, 2,3 butanedione, 2,3-pentanedione, 2-furfurylthiol. 3c: Three selected aroma impact compounds

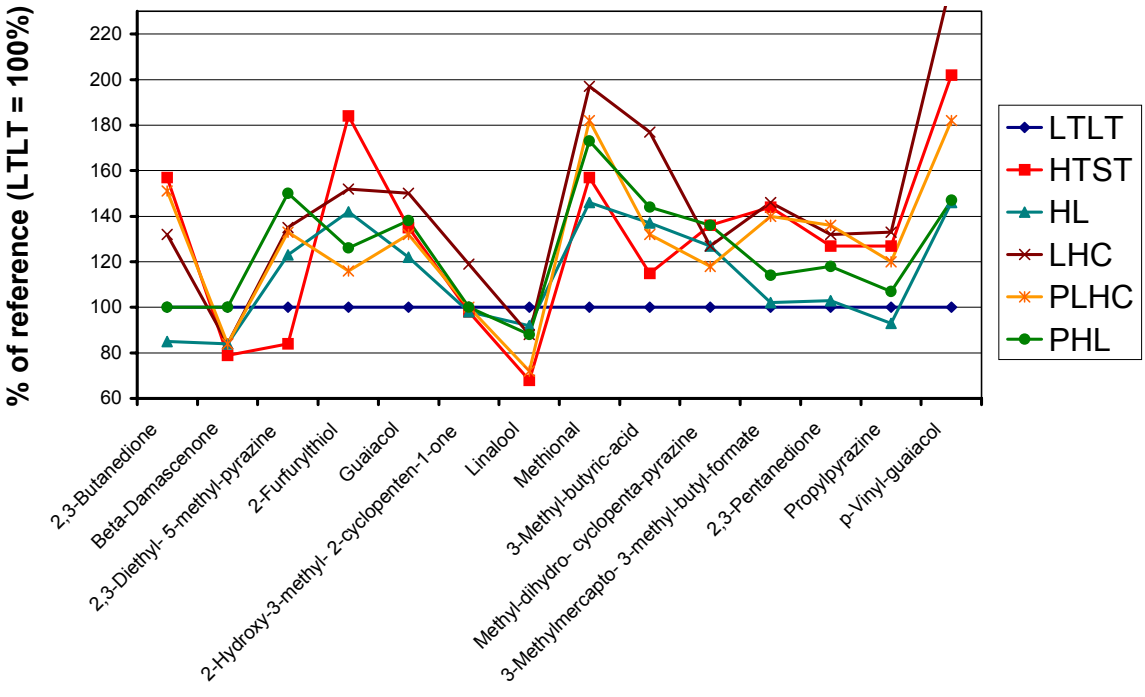

Figure 4. Profiles of aroma compounds of roasted Colombian arabica coffee beans resulting from different time-temperature conditions during roasting

CONCLUSIONS

Roasting technology cannot make up for poor quality of coffee beans. However, for a given type of a green coffee bean blend, roasting is the main flavour (odour and taste) determinant. The time-temperature conditions during roasting influence the bean core temperature, dehydration and pressure which, in turn, determine the specific conditions for aroma formation reactions. They are the major factors impinging on the potential of green coffee beans, leading to the formation of the distinct and desirable profile of aroma compounds. Regardless of the temperature conditions, the catalogue of aroma impact compounds (AIC) resulting from a given coffee blend is identical. However, the AIC *profile* is influenced by the temperature conditions. The greatest aroma formation rates during roasting were found at the medium stages of product dehydration. At least one roasting phase at medium temperature seems to be essential in generating sufficient aroma intensity, but high-temperature conditions may cause an unfavorable aroma profile. A final phase at reduced temperature causes a shift in the aroma profile. The target product colour should not be set too dark, and the process should be terminated in time because degradation of aroma compounds sets in during the final roasting stage.

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Laser / Chemical Ionisation - Mass Spectrometry as an On-line Analysis Technique for Monitoring the Coffee Roasting Process

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SUMMARY

Coffee is one of the most popular beverages all over the world. In 1999, the members of the International Coffee Organisation exported 106 118 000 bags of green coffee beans (60 kg/bag). In the old days people roasted their coffee on their own, in pans above a fire. Nowadays large scaled industrial roasting machines are used for producing several kinds of coffees, coffee powders and beverages. The roasting process is controlled mainly by roast temperature and time. One of the most recent controlling techniques measures the temperature distribution within the beans. Until now, the release of flavour active compounds during roasting has not been taken into account, to monitor and eventually control the process. The monitoring of these volatile aromatic compounds and the introduction of a feedback steering based on these results may have the potential for improving the coffee roasting process, with respect to consistency (from batch to batch) and aroma quality.

One well suited analytical method for on-line, real-time monitoring of volatile compounds in industrial processes (Grosch, 1996) as well as food processes (Maier, 1984) is the REMPI-TOFMS technique. It allows a very fast, sensitive and selective detection of selected volatile components. This is necessary, in order to analyse complex gas mixtures without the need for sample preparation, cleaning and enrichment steps.

Here we present the application of this technique to the on-line analysis of coffee roasting. Green arabica beans of a defined provenience were roasted in a small scaled laboratory roasting machine, and the temporal evolution of the volatiles released from the beans were analysis by REMPI-TOFMS @ 266 and 193 nm. A single photon ionisation process (SPI) @ 118 nm was also used in order to obtain further information about relevant coffee compounds which are undetectable with one colour REMPI.

These three wavelengths enable us to detect several flavour active and relevant compounds of coffee like phenols, furans, pyrroles, ketenes, aldehydes, and other heterocyclic, aromatic and non aromatic compounds. Due to the different time behaviour of several compounds, the ratio of certain compounds will be suitable as indicator for important process conditions like roast

level and quality of the process control (Grosch , 1990). Our newly developed device is able to switch between a REMPI @ 266 nm and a SPI @ 118 nm mode from laser pulse to pulse. The two ionisation methods, a highly selective and a medium selective photon ionisation, are combined in a small (0.6 m x 0.8 m x 1.8 m – incl. DAQ), mobile device also containing a small (19' rack) TOFMS

INTRODUCTION

In 2000 the world-wide production of raw coffee was around 100 Mio bags at 60 kg/bag. This corresponds to 14 billion US $ worth of raw material. Thus, in terms of value, green coffee is in second position - behind crude oil – among the world-wide most traded raw materials. Besides the intrinsic quality of the green beans, its value is mainly determined by the development of the flavour during roasting. The typical coffee flavour compounds are hardly present in the green beans. They are generated during roasting. In fact, roasting increases the value of green beans by a factor of 2 to 4. For instance, in 2000 the German coffee industry made a turnover of about 3.9 billion US $ using 9.2 Mio. bags of green coffee.

The normal course of events during the roasting process is the following: The green beans are dried at the beginning of the roast process. After several minutes, pyrolysis reactions start during which the beans develop their typical flavour (Grosch, 1996), their brown colour and become brittle. All of this is the result of complex chemical reactions and physical processes that still waits to be elucidated. Yet it is well known, that Maillard-Reaction including Strecker degradations and Amadori-reactions (Maier, 1984) are involved. Once the beans are roasted, they are cooled down, in order to stop the roasting progress. Any improvement of the process, be it from the perspective of flavour quality or flavour intensity, will positively affect the added value due to roasting.

Currently the roasting process is controlled by roast temperature, roast-time, weight loss and the experience of the person operating the roaster. One of the more recent parameters used to control the roast process is the temperature distribution within the beans using IR-cameras, or the surface colour distribution on the beans.

Up to now, the flavour active compounds, released during roasting, have not been taken into account, to monitor and eventually control the commercial roasters. Yet, in contract to colour, they are much more directly related to quality and flavour. In this work, we would like to explore the prospect of using the gases, released during roasting, for an on-line control of the process. The monitoring of these volatile aromatic compounds (VOC's) and the introduction of a feedback control may have the potential of improving the roasting process with respect to consistency (from batch to batch) and aroma quality. One important innovation, that we believe will affect the future of the roasting industry as a whole, is intelligent in-line process control. To come to a practical implementation of this concept, one will most probably need **fast on-line** and **real-time** techniques. Due to the fact, that many flavour active compounds of interest are in the ppmv or sub-ppmv range (in the headspace), the applied method has furthermore to be very **sensitive**. Up to now, hundreds of different compounds were found in the coffee roasting off-gas (Grosch, 1990). Hence, to monitor a single compound out of this complex gas mixture without the need of preceding cleaning steps, an appropriate analytical method has also to be extremely **selective**. All of this puts very severe demands on the method.

One potential analytical approach for on-line, real-time monitoring of volatile compounds in industrial processes (Heger et al., 1999a; Zimmermann et al., 1999a; Zimmermann et al., 1999b; Zimmermann et al., 1997a; Zimmermann et al., 1997b; Zimmermann et al., 1998a)

and in particular in food processes (Dorfner et al., 2000; Zimmermann et al., 1999c) is resonance enhanced multi photon ionisation combined with a time-of-flight mass filter (REMPI-TOFMS). Laser based mass spectrometry allows a fast, sensitive as well as selective detection of volatile compounds. Alternative on-line analysis methods are the single photon ionisation mass spectrometry (SPI-TOFMS) and the proton transfer reaction mass spectrometry (PTR-MS). While both are fast and sensitive, they have only a limited selectivity. REMPI-TOFMS used a resonant and selective laser ionisation scheme. In contrast, PTR-MS relays on a less selective chemical ionisation mode. Here we will apply laser based ionisation techniques as well as PTR-MS to the coffee roasting process and discuss the respective results.

METHODS

Laser Mass Spectrometry is a two dimensional analytical method. It includes optical selectivity in the ionisation process as well as mass selectivity by using a time-of-flight mass spectrometer.

Two different schemes of laser ionisation have been implemented: the first one is ***r****esonance* ***e****nhanced* ***m****ultiphoton* ***i****onisation* (REMPI); the second one is ***s****ingle* ***p****hoton* ***i****onisation* (SPI). REMPI enables one to increase the selectivity up to the point that one can distinguish between individual isomers of one compound (Zimmermann, 1995). For REMPI the selectivity also depends on the inlet technique (Anderson et al., 1966; Fricke, 1973; Levy, 1981; Tembreull and Lubman, 1984; Hayes, 1987; Heger et al., 1999b). In contrast to REMPI, SPI excludes only certain classes of substances, since it is a threshold ionisation method which ionises only compounds having a ionisation potential equal or below the ionising laser energy.

Using resonance enhanced multiphoton ionisation (Boesl et al., 1978; Boesl et al., 1981; Dietz et al., 1982; Lubman, 1987; Letokhov, 1987), the molecules absorb a photon and thereby reach an excited state. The molecules are ionised out of the excited state by absorbing a second photon (Figure 1). This is only possible if the following 3 requirements are fulfilled:

1) The molecule has an excited state, which corresponds exactly to the energy of the laser photon.
2) The life-time of the excited state is long enough to absorb a second photon for ionisation.
3) The energy of the second photon is sufficient to increase the internal energy of the molecules above the ionisation potential.

By tuning the wavelength of the laser light, one can selectively detect specific VOCs. Since the photon energy is adjusted to the compounds to be detected (resonant absorption), the molecules do not get a large amount of surplus energy. Therefore fragmentation is nearly completely avoidable.

The selectivity of the single photon ionisation (Van Bramer and Johnston, 1990; Schühle et al., 1988) is exclusively based on the ionisation potential of the compound to be detected. If the energy of the photons is sufficient for increasing the internal energy of the molecule above the ionisation energy, the compound will be ionised (Figure 2). Otherwise the compounds are not detectable with the adjusted wavelength. Thus SPI and PTR have quit a comparable selectivity, but both are less selective than REMPI. The degree of fragmentation can be minimised by optimising the laser wavelength to the ionisation potential of the compounds of interest (minimum excess energy above threshold).

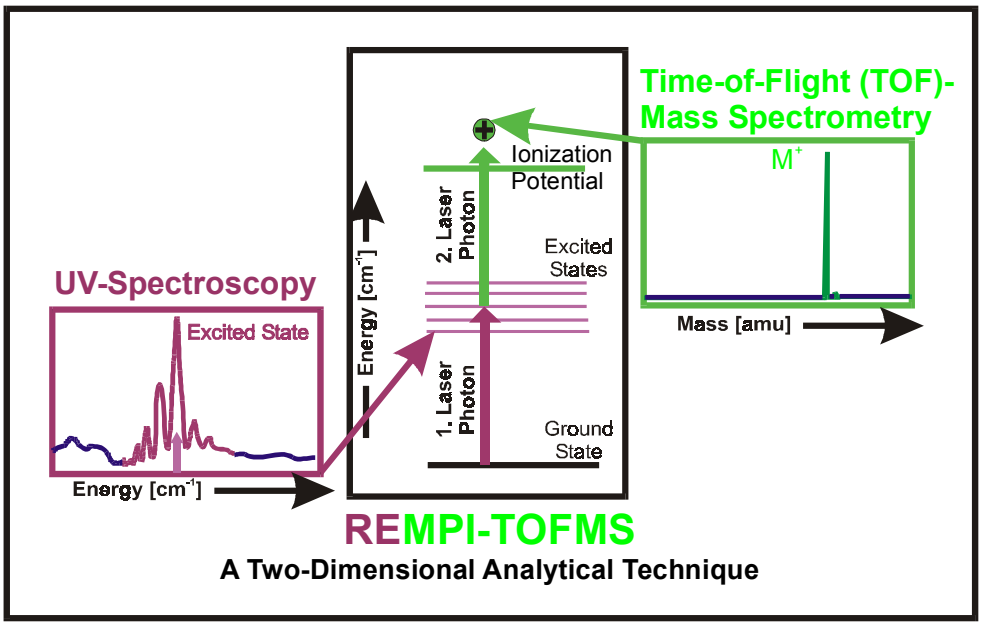

Figure 1. REMPI-TOFMS-scheme : By ionising via an excited electronic state, the UV-spectroscopic properties of the compound of interest are involved in the ionisation process. Thereby the very high selectivity – up to isomer selectivity – is implementable. Besides the selectivity of the ionisation process, ions are further characterised in the time-of-flight mass spectrometer by their molecular mass

Using a **t**ime-**o**f-**f**light **m**ass **s**pectrometer (TOFMS) (Boesl et al., 1981; Wiley and McLaren, 1955; Boesl et al., 1992; Cotter, 1994) the ionised molecules will be distinguished by their molecular mass. The time of flight is proportional to the square-root of the mass. The heavier the mass of the compound, the longer the time of flight to the detector will be. Taking an external gas standard, consisting of a series of diffusion and permeation tubes, which are containing known compounds of different mass, a mass calibration as well as an absolute quantification can be accomplished (Heger et al., 1999a; Namienik, 1984). In contrast to a sector-field mass spectrometer or a quadrupole mass spectrometer, all ionised molecules are detected in a TOFMS at once within a fraction of a second. Thus, with every laser pulse a complete mass spectrum is produced in a TOFMS.

Proton-**t**ransfer-**r**eaction **m**ass **s**pectrometry (PTR-MS) is based on a chemical ionisation step coupled with ion detection by quadrupole mass spectrometry. The chemical ionisation process runs as follows: the primary reactant ion is H_3O^+. H_3O^+ is the most appropriate primary reactant ion, when air samples contain a wide range of trace gases or volatile organic compounds. H_3O^+ ions do not react with any of the natural components of air. The primary reactant ion is produced by electron impact ionisation of H_2O vapour. The resulting H_3O^+ is transferred by an electric field into the drift tube, which simultaneously serves as a reaction chamber, containing the carrier gas molecules and the analyte gas molecules. The analyte molecules (A) react with H_3O^+: $H_3O^+ + A \rightarrow AH^+ + H_2O$. The resulting protonated analyte gas ions AH^+ and the residual H_3O^+ ions are mass-analysed in the mass spectrometer. For more details see (Lindinger et al., 1998). The proton affinity of the compound of interest is the most important property for this chemical ionisation technique. Every compound with a higher proton affinity than the reactant H_2O vapour (691 kJ/mol) will be ionised.

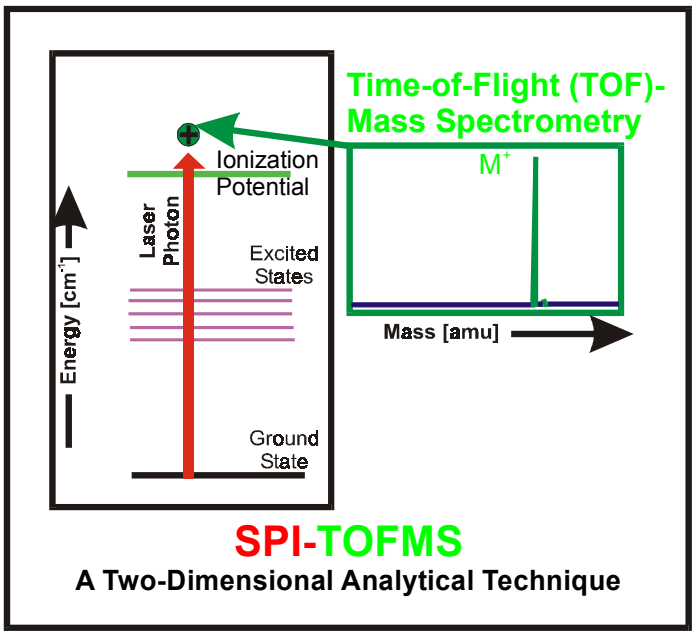

Figure 2. Single-photon-ionisation(SPI)-scheme: The ionisation of the molecule only takes place, if the energy of the photon is bigger than the difference of energy between the ground state and the ionisation threshold. The ions are distinguished in the time-of-flight mass spectrometer by their molecular mass

In PTR-MS, the headspace concentration of the detected compound depends linearly on the ion-signal intensity, whereas in REMPI-TOFMS several factors like cross section, life-time of the excited state, wavelength, power density of the laser irradiation affect the efficiency of the ionisation process.

EXPERIMENTAL

Different fixed frequency lasers, like Nd:YAG@266nm (Zimmermann et al., 1999c; Zimmermann et al., 1996), KrF-Excimer@248nm (Dorfner et al., 2000; Dorfner et al., 1998), ArF-Excimer@193nm or a Nd:YAG@355nm coupled with a third-harmonic-generation-cell (Ar/Xe) to generate 118 nm laser pulses, have been used to investigate coffee headspace gases. Here we will concentrate on investigations using a REMPI@266nm-TOFMS, and SPI@118nm-TOFMS device on the one hand and a PTR-MS device on the other hand.

In order to have a mobile and compact version, both laser based ionisation methods – REMPI and SPI – have been combined to a single instrument of moderate size, which is capable to switch between REMPI@266nm- and SPI@118nm-mode from laser pulse to laser pulse. The small (0.6 m x 0.8 m x 1.8 m – including laser source, DAQ, power supply, vacuum, …) and mobile device also contains a linear/reflectron-TOFMS in a 19’ rack (Mühlberger et al., 2000). For the investigations discussed here, we have been using, besides the compact device, also an other experimental set-up with one device for each ionisation technique, to achieve a higher time resolution. Both high resolution devices are working with a medium selective effusive molecular beam (EMB) gas inlet (Anderson et al., 1966; Fricke, 1973; Levy, 1981; Tembreull and Lubman, 1984; Hayes, 1987; Heger et al., 1999b). The REMPI@266nm-TOFMS was operated in the reflectron TOFMS mode, which increases the mass resolution at the expenses of some sensitivity. In contrast, the SPI@118nm-TOFMS was operated in the linear TOFMS mode, to compensate for loss of sensitivity due to the lower laser energy density of the 118 nm-laser system.

The PTR-MS device, we used, is a commercial device (IONICON GmbH, Innsbruck) with a modified heatable transfer line and inlet system, consisting of a quartz glass capillary with inert surfaces, in order to avoid memory effects and catalytical reactions (Hauler et al., 2001). Due to the high concentrations (saturation of signal), we had to limit the total quantity of organic compounds sampled for PTR-MS. Consequently, a home-made dilution system was necessary to adjust the signal intensity, when sampling the roast gas for PTR-MS.

The roaster, used in this study, is a laboratory Probat® BRZ 2 roaster. It has two electrically heated horizontal drums with rotating blades. Each drum has a capacity of 80 g to 100 g green beans. The temperature is adjustable from 50°C to 350°C by limiting the current through the heating rods and adjusting the flow of the fan. The fan is also used to cool the roasted beans in order to stop the roasting process once the beans were tipped out of the drum. No water quenching was possible in this type of roaster.

The roasting temperature was adjusted to 200°C, 225°C or 250°C, while the fan was adjusted to its minimum level. The temperature was not corrected during roasting, despite a short cool-down effect when filling the beans into the roaster. (Clarke and Macrae, 1987). The drum was preheated to the desired temperature. Then 80 g of green arabica beans of a defined provenience (Columbia) were filled in the drum. Afterwards the on-line probing system, which is preheated by heating cuffs, was inserted 5 cm deep into the roasting drum. Headspace gas from the roasting process was continuously extracted with a flow rate of 1.5 l/min. 10 ml/min of the overall flow is directly injected in parallel into both laser mass spectrometer devices. The length of the roasting cycles was 950 sec at 200°C, 60 sec at 225°C and 450 sec at 250°C. These roast cycles times correspond to strongly over-roasted beans (Dorfner et al., 2000). This was chosen in order to follow the process over the entire roasting process up to the point, where the coffee beans starts to become inedible.

RESULTS AND DISCUSSION

To demonstrate the different selectivity of the three measurement techniques, we have first recorded the headspace gas composition, sampled from a warmed up dry soluble coffee powder. Figure 3 shows three different mass spectra, recorded by PTR-MS (top), SPI@118nm-TOFMS (middle) and REMPI@266nm-TOFMS (bottom). Analysing the spectra, one notices that the PTR- and the SPI@118nm- mass spectra contain a large number of ketenes, aldehydes, furan derivates and only a few oxygen containing heterocyclics and aromatics, whereas the REMPI@266nm spectrum nearly exclusively consists of substituted aromatics and oxygen or nitrogen containing heterocyclics. Especially in the PTR- and SPI-mass spectra, many of the peaks correspond to a superposition of more than one compound, due to the medium selectivity of the ionisation processes. But usually one compounds dominates the signal intensity. An assignment of the most prominent mass peaks is given in Table 1. It is based on spectroscopic properties, proton affinities, literature data and on on-going investigations in our laboratory (Dorfner et al., 2001), respectively. Comparing the PTR mass spectrum and the SPI@118nm mass spectrum, one notices, that they are similar. Both contain strong signals at masses like 44 m/z (acetaldehyde), 58 m/z (acetone), 72 m/z (butyraldehyde), 74 m/z (Propanoic acid), 80 m/z (pyrazine), 82 m/z (methylfuran), 86 m/z (biacetyl), 94 m/z (phenol), 96 m/z (furfural), 100 m/z (acetylaceton / pentanedione), 108 m/z (cresol) and 110 m/z (dihydroxybenzene). But there are also some distinctive differences. In this particular sample, mass 60 m/z (acetic acid), 62 m/z (ethanthiol), 68 m/z (furan), 90 m/z, 104 m/z (styrene) and 122 m/z (benzoic acid) are only detectable using the PTR-MS technique. In contrast, the masses 79 m/z (pyridine), 98 (furfuryl alcohol), 112 (methyl furfuryl alcohol), 126 m/z (benzenetriol / maltol / HMF or enol) and 194 (caffeine) are only recorded by the SPI@118nm-TOFMS technique. The differences in signal intensities

demonstrate, that – for SPI – not only the ionisation potential is crucial but also the cross section of the ionisation step.

Looking on the REMPI@266nm mass spectrum, we can state that the range of compounds, detected by this technique, is markedly different from those seen by the two other techniques. Most compounds are aromatics (especially phenol derivates) and other are oxygen or nitrogen containing heterocyclics or their derivates, like phenol (94 m/z), cresol (108 m/z), methylstyrene (118 m/z), 4-vinylphenol (120 m/z), benzoic acid (122 m/z), dimethylstyrene (132 m/z) 4-vinylguaiacol (150 m/z) and caffeine (194 m/z). Note, that in the present setup (effusive inlet – Anderson et al., 1966; Fricke, 1973; Levy, 1981; Tembreull and Lubman, 1984; Hayes, 1987; Heger et al., 1999b) isomers can not be distinguished. The assigned isomers represent the most abundant ones (Grosch, 1996; Grosch, 1990; Tressl, 1989; Silwar et al., 1987; Vitzthum, 1975; Tressl et al., 1978; Tressl et al., 1975; Semmelroch, 1995; Grosch, 1994; Gianturco, 1967; Vitzthum and Werkhoff, 1976; Vitzthum and Werkhoff, 1975). A more comprehensive discussion of REMPI@266nm-TOFMS and its application to the analysis of coffee roast gases can be found in (Dorfner et al., 2000; Zimmermann et al., 1999c; Zimmermann et al., 1996; Dorfner et al., 1998; Dorfner et al., 2001; Dorfner et al., 1999a; Dorfner et al., 1999b; Zimmermann et al., 1997c; Zimmermann et al., 1998b; Zimmermann ey al., 1997-1998).

Some of the aromatics, shown in the REMPI@266nm mass spectrum, are detectable with SPI@118nm or PTR as well. It concerns compounds like phenol (94 m/z), cresols (108 m/z) or dihyrdoxybenzene (110 m/z). But the different ionisation techniques have different sensitivities for these compounds, due to different cross sections for the ionisation processes or different energy densities of the applied laser sources. Of course, for SPI or PTR the detection signal might be a superposition of several isobaric compounds as well.

As mentioned above, the three methods are well suited for fast, real-time, online monitoring of complex gas mixtures. All three techniques were used to monitor the coffee roasting process. All the roasting experiments were performed with the same batch of Colombian green coffee beans. The actual time resolution for the laser based methods is 0.1 sec. Since five spectra are averaged to improve the signal-to-noise ratio, the resulting resolution of the measured time-intensity profiles is 0.5 sec. The time resolution for the PTR-MS measurements is 10 sec. Since it was necessary to dilute the sampled roaster-gas for the PTR-MS applications, the results were recorded in a separate roasting experiment, where coffee was roasted to a medium-to-dark roast. The results of REMPI@266nm-TOFMS and SPI@118nm-TOFMS come from one and the same roasting cycle, in which the beans were overroasted to inedibility. Figure 4 shows some selected time-intensity-profiles of several important compounds. The approximate time limits for commercially roasted coffee are indicated in Figure 4.

The masses 60 and 80 are monitored with PTR-MS technique and correspond to acetic acid and pyrazine. Pyrazine and its derivates are reported to be potential indicators for the roast degree (Hashim and Chaveron, 1996). Acetic acid starts to increase after about 250 sec and seems to approach a plateau before the roasting process is stopped at about 460 sec roasting time, whereas pyrazine appears after 300 sec roasting time and does not reach a maximum within the roasting cycle. In 1967, Gianturco (1967) also determined time-intensity-profiles using GC-MS-technique, at a much lower time resolution than here. Besides acetic acid and pyrazine, he also looked at pyridine (79 m/z) and furfuryl alcohol (98 m/z). Here we have used SPI@118nm-TOFMS for the later two compounds (pyridine and furfuryl alcohol). The profiles from Gianturco's work correlate very well with our results. Pyridine is a degradation product of trigonellin, which itself is thermally quit stable and degrades only slowly. Thus

pyridine, whose concentration starts rising after about 450 sec, is one of the compounds, formed latest in the commercial roasting process. Pyridine has a maximum in its time-intensity-profile. This is consistent with the fact, that trigonellin is the only possible precursor and is nearly completely degenerated during the roasting process. Furfuryl alcohol also goes through a maximum, yet only in the overroasting region.

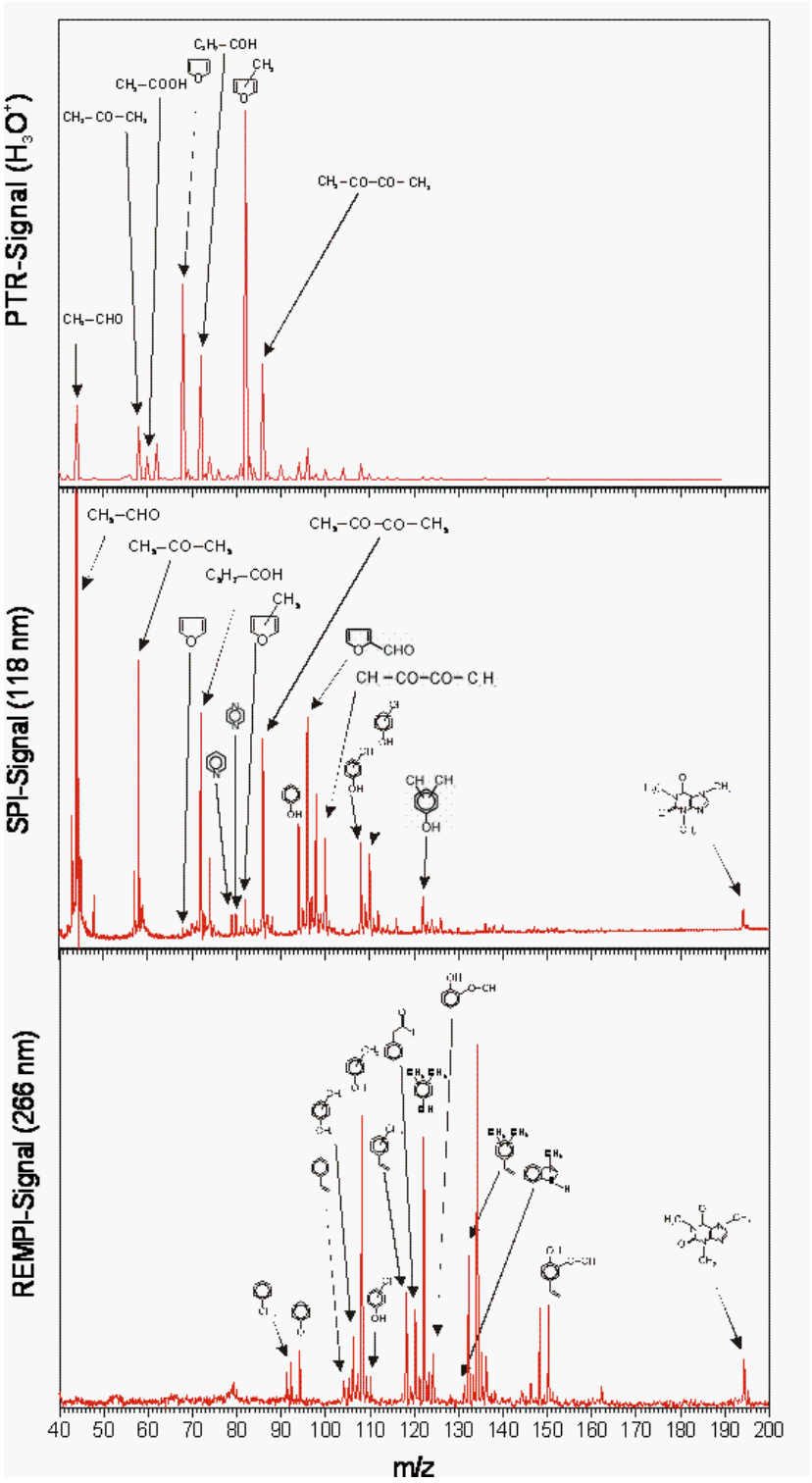

Figure 3. Typical mass spectra of the headspace gas of warmed up dry soluble coffee powder measured with PTR-MS (TOP), SPI@118nm-TOFMS (MIDDLE) and REMPI@266nm-TOFMS (BOTTOM). See text for further details

Table 1. Table of detected masses, using various ionisation techniques. PTR: proton-transfer-reaction using H_3O^+ as primary reactant; SPI: single-photon-ionisation using 118 nm laser irradiation; REMPI: resonance-enhanced-multi-photon-ionisation using 266 nm laser irradiation. Compounds that appear at the respective masses are given in the second column. For each mass, either the most prominent compound is given, as for SPI and PTR, or it represents the actually detected compound in the case of REMPI

Mass	Ionisation technique	Compound (exemplary)
44	SPI, PTR	Acetaldehyde
58	SPI, PTR	Acetone
60	PTR	Acetic acid
62	PTR	Dimethyl sulphide
68	PTR	Furan
72	SPI, PTR	Butyraldehyde
74	SPI, PTR	Propanoic acid
79	SPI	Pyridine
80	SPI, PTR	Pyrazine
82	SPI, PTR	Methyl furan
86	SPI, PTR	Biacetyl
90	PTR	Acetaldehyde dimethyl acetat
94	SPI, PTR, REMPI	Phenol / Methyl pyrazine
96	SPI, PTR, REMI	Furfural
98	SPI, REMPI	Furfuryl alcohol
100	SPI, REMPI	Pentanedione
104	PTR, REMPI	Styrene / Methional
106	REMPI	Xylene
108	SPI, PTR, REMPI	Cresols
110	SPI, PTR, REMPI	Dehydroxybenzene
112	SPI	Methyl furfuryl alcohol
116	SPI	Oxopropyl acetate
117	REMPI	Indole
118	REMPI	Methyl styrene
120	REMPI	4-vinyl phenol
122	SPI, PTR, REMPI	Dimethyl phenol / Benzoic acid
124	REMPI	Guaiacol
126	SPI	Benzenetriol, Maltol, HMF, Enol
131	REMPI	Methyl indole
132	REMPI	Dimethyl styrene
134	REMPI	Methoxy vinyl benzene
136	REMPI	4-vinyl benzenediol
146	REMPI	Trimethyl-styrene
148	REMPI	Hydroxy methyl styrene
150	PTR, REMPI	4-vinyl guaiacol
152	REMPI	Ethyl guaiacol
162	REMPI	Dimethyl hydroxy styrene
194	SPI, REMPI	Caffeine

Indole (117 m/z) and guaiacol (124 m/z) are monitored using the highly selective REMPI method. Especially indole has a unusual time-intensity behaviour. Its signal rises just after 50 sec roasting time, forms a plateau after about 270 sec and starts to increase again after 370 sec. Currently it is not clear how one has to interpret this rather unique behaviour. Indole is an early degradation product in the course of the Maillard reactions and is detectable already after 50 sec. Thus, together with 4-vinyl guaiacol, it is one of first detectable compounds during the roasting process. The guaiacol signal rises at 150 sec and ends in a broad maximum or plateau in the overroasting area. Guaiacol, a product formed from decarboxylation of trans-ferulic acid, has its largest gradient of signal intensity (changes in concentration) when the roasting process goes from medium to dark roast. Thus guaiacol

represents an important indicator compound for determining the progress of the roasting progress.

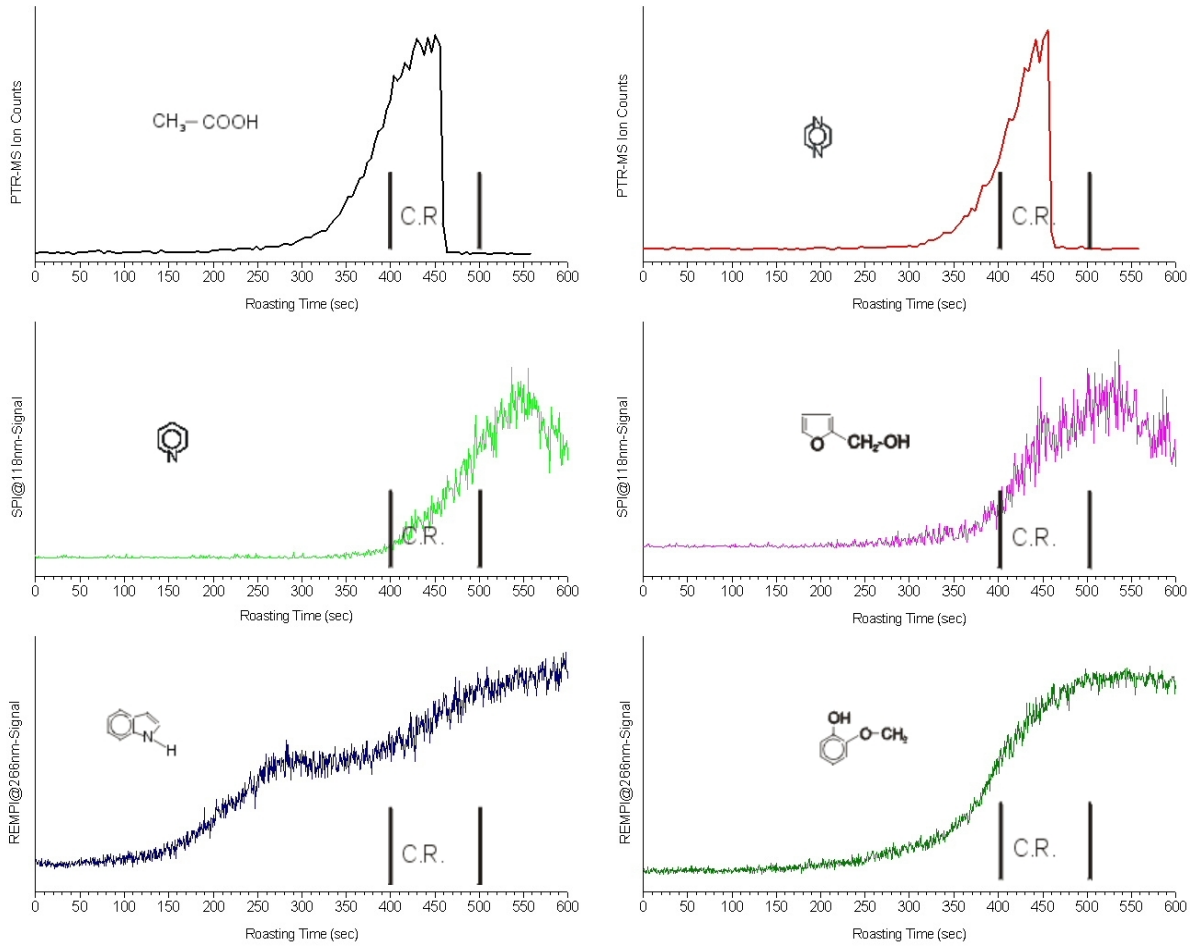

Figure 4. Time-Intensity-Profiles of several compounds recorded with PTR-MS (TOP), SPI@118nm-TOFMS (MIDDLE) and REMPI@266nm-TOFMS (BOTTOM). C.R. is the time range for a commercial roasting. For detail see text

CONCLUSIONS

Laser based ionisation and chemical ionisation techniques combined with mass spectrometers are well suited for real-time online analytical monitoring of coffee headspace. The techniques differ in their selectivity. But they all allow a soft ionisation nearly without fragmentation. The discussed approaches have different time resolutions due to characteristics of the two mass filtering techniques used (TOF ↔ quadrupol). Depending on the selectivity and sensitivity of the ionisation mode, different compounds are detectable (ketenes, aldehydes, aromatics, oxygen or nitrogen containing heterocyclics). Many compounds of interest to coffee aroma can be monitored, using easy to use fixed frequency laser sources or chemical ionisation techniques (PTR with H_2O vapour). Other compounds are detectable, using tuneable laser systems or PTR-MS in combinations with other primary reactant ions (i.e. NH_4^+).

Time-intensity-profiles of different compounds show different behaviours. Some of them confirm known Maillard-reaction pathways. But time resolved monitoring of key compounds would also help to better understand unknown reaction pathways in the roasting process. These techniques open the possibility of feedback-control of the roasting process, with the

prospect of achieving an improved and steady quality of the roasted beans. The effect of different types of roasting machines and roasting techniques, different roasting temperatures or temperature programs, different proveniences, post-harvest treatments and storage conditions as well as the influence of green coffees moisture content can be investigated and optimised. Overall, we believe that an intelligent in-line process control will improve the coffee roasting process with respect to consistency and aroma quality of the roasted product.

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The Functional Design Specifications in Coffee Processing

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SUMMARY

The basic functions of various equipment and operations are not clearly understood leading to ineffective use as well. In general the processing practice is not to the desired standard and affects coffee quality. This is because the equipment in the coffee industry are generally seen to work and not understood why they are designed to work that way. Therefore, the paper puts together available but scattered information and borrows heavily from the engineering theory to explain and thereby stimulate perception and appreciation of the users. By so doing the status of the practise and hence the end result quality can be improved.

INTRODUCTION

The Coffee processing procedure can only achieve its functional role effectively when all the components are correctly designed, manufactured, installed, operated and maintained. As such, the operators need to know the importance of the various design parameters to avoid undertaking the activities outside the limits. Currently, operations are undertaken as has been seen to occur instead of out of understanding of the importance of the procedure to coffee quality. It is important then to provide the rationale behind every operation for the operators to appreciate their perception, appreciation and attitude towards the whole process. For this purpose, the most important design parameters in the coffee industry need to be singled out and emphasised for effective use of every equipment. In view of this, the technical fundamental specifications of the processing equipment are critically reviewed. These aspects are highlighted to clearly elucidated the trio man, machine and Coffee Cherry relationship in order to improve efficiency in a coffee factory. Hence the enhancement of coffee quality.

HARVESTING

There are two seasons per year of about 3 months each. About 60% of the annual production is harvested in the main season. About 20% of coffee per season is harvested within 2 weeks or 15% within 1 week of the peak.

SORTING

This removes foreign matter, coffee not suited to the wet process and classifies cherry to facilitate pulping and to discourage mixed fermentation. Raised tables in a roofed shed are recommended to avoid drudgery. The facility must be sanitary sound and equipped with suitable mats spread on the ground or cemented surface. Adequate area must be provided for the various workers and their coffee lots.

WEIGHING SCALES

They are top loading with a range of 0-500 kg. The scale has to be visible within a distance of about 3 m. Weighing balance requires a firm level ground and located away from interference by people. Alternatively, a 20 litre tin that can accommodate about 12 kg of cherry is used.

CHERRY HOPPER

This distributes cherry evenly to a pulper. It may be dry or wet feed with water which assists the conveyance of coffee cherry to the pulper. Floors slope in 3 directions at 45° meeting with a vertical end section. Surfaces are painted with an acid resistant black paint and shaded from direct sunlight with a permanent roof.

COFFEE PULPER

Pulpers are either disc or drum type. Pulping has to completely remove and separate the skins from the berry with no bean damage. It takes place with the assistance of water.

A disc has bulbs which have a definite form, size and distribution over its surface. Bulbs have no cutting edge but are rough enough to draw the cherry past the adjacent plough fitted to the pulping bar. There are different types of bulbs for the various arabica coffee varieties.

The pulping rate is 1,000 kg cherry per hour/disc. A hand pulper can pulp 300 kg cherry per hour and 600 kg cherry per hour when powered. The power rating are 0.30 kW; 0.7 kw; 1 kW, and 1.5 kW motors for 1, 2, 3, and 4 discs respectively. The disc rotates at 120 rpm; The direction of rotation is important and is stumped on the disc. The diameter of the disc is 46 cm for accurate casting and adjustment. A disc pulper has a plough chop, Nylon fingers and Knives per disc on either side.

The **plough** is adjustable and has an initial tapering section followed by a separation section which is parallel to the disc. The plough clearance from the disc is 9 mm. **Nylon Fingers** are pressure set by the compression of a rubber block. Each finger has an independent movement to accommodate cherries of different sizes and ripeness. They are subject to readjustment within the harvesting season if the size and maturity of the cherry varies. **Knives** are provided to rapture the cherry skin and separate parchment and the skin with the aid of the discs. The clearance between the disc and the knife is 1.7 mm. This Clearance should be parallel throughout the length of the knife. The rupturing edge must always be right angled to avoid nipping the bean.

As for the Drum Pulper, the diameter of the drum is 20 cm and a length dependent on capacity. A Pulper with a capacity of 3-4 tonne per hour is driven by a 0.5-1 kW motor. It has a revolving Drum with a close fitting stainless steel cover, punched over its whole surface with bulbs. The cylinder rotates inside a fixed breast plate with pulping channels with ribs set at an angle of 45°C to the axis.

RECIRCULATION SYSTEM

The system is designed to use very small volumes of water but recirculated over 3.05 to 6.1 m only in order to save on water requirement. Pumping rate of the recirculation pump should be matched with the incoming fluid from the pulper to avoid cavitation;

PRE-GRADER

Types of pre-grader include siphon types which use water, mechanical types with chain elevators and rotary sieve which produces 2 grades. The output of an Aagaard pre-grader comprises; lights, seconds and firsts grades of coffee. Its capacity varies with the Number of discs. The screen is 34 cm wide and 160 cm long. Section lengths include: Non grading 30 cm: Parchment one (P1) 70 cm; and P2 60 cm. Grading is effected by oscillations of 6.5 cm up and 2.1 cm down. The screen depth limits are shallow 11 cm; and deep 19 cm in the water. The provided metal divide has 3 positions regulating parchment 1 and 2 proportion at optional angles of 35°C.

FERMENTATION AND SOAKING TANKS

Depth of fermenting parchment coffee should not exceed 1.0 m. Fermentation tanks need to be of masonry works whose surfaces should be made with a rich plaster mix of 1:2 (Cement:sand) applied to a much greater thickness (5 cm) than normal; Smooth, without cracks; Painted with the recommended black paints. Other protective methods include chemical resistant cements, tilling, and fibre glass lining. The tanks are: built in the ground to sustain warm temperatures; protected from cold rain water and direct sun light by simple water proof frame work or a permanent roof. Where used for soaking, the tanks should be water tight.

CONVEYANCE CHANNELS

These are employed to convey the coffee from one stage to another. Their maximum length is not critical but the gradient is 1:100 with a cross section of 30 by 30 cm. This need even be narrower for efficient conveyance and reduced water use.

FINAL WASHING AND GRADING CHANNEL

This completes washing of the degraded mucilage and mainly fine grades the fermented parchment into Parchment 1, 2, 3 and lights grades. The Dimensions of the channel are 25-30 m long; Not more than 1 m wide; 0.8 m deep and; a slope 1-1.5: 100;

DRYING TABLES

The skin drying table can be used 5 times and 2 times per day under normal and adverse conditions respectively. Skin drying tables are supposed to be 1.0 m, 1.8 m and 23 high, wide and long respectively.

A final drying table is 1.0 m high, 1.8 m wide and 23 m long. The height of the table is designed to discourage the workers from sitting on them while attending the coffee. The drying mats may be either Shade net, or Hessian. Water proofing is satisfied by use of Nylex yellow pre-film 500 gauge or yellow polythene film 0.91 m wide. The inter row spacing between the tables is 1.0 m. A ground cover with grass mowed low is a requirement.

MECHANICAL DRIERS

A mechanical dryer must: suck downward the air through the bed to aid the removal of surface moisture by gravity during skin drying; Dry coffee from an initial moisture content of 55% (wb) to 33% (wb), and also from 23% (wb) to 10% (wb); The dryer must be indirect

fired; Limiting drying temperature are not to exceed 35°C for coffee beans at any time during the drying cycle and 38°C for the air;

STORAGE AND CONDITIONING

Stacking of bags has to be on wooden platform raised 15 cm from any surface to avoid "re-wetting". One bag of parchment is about 50 kg and needs 0.1 m^2 store space, if stacked 8 bags in a row. Storage Ventilation with provision for conditioning and bagging is essential. Manual conditioning limits the distance from any surface to 1.0 m to the centre. The optimum storage conditions are a Temperature of 21oC and Relative humidity of 50% or less. The storage capacity should be provided for half the annual crop.

WASTE DISPOSAL SYSTEM

The waste disposal system must handle all the processing effluent effectively. The pulp should be conveniently discharged at sites accessible to potential user and above the factory way up from the natural water ways.

ACKNOWLEDGEMENT

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The Environmental Considerations Featuring in the Processing of Coffee in Kenya

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SUMMARY

The wastes from coffee processing in Kenya tended to pose threat to the environmental flora and fauna. To preserve the environment, appropriate technical measures are in existence. Despite their rather positive environmental impact some key factors inherent in the industry, have caused the challenge to persist and is still on. For this reason, further studies on the current environmental impact of these wastes and a thorough search for possible options for overcoming the problem are still in progress. Results of this work mainly indicate that environmental pollution especially by the effluent can only be controlled by a combination of waste minimization and treatment. Considerations in the previous work and the resultant measures so far implemented are discussed with respect to their success and constraints still prevailing. The future goals aimed at in the current research work are also highlighted in an attempt to brainstorm views from world researchers in the same field for a common approach towards overcoming environmental problems from these wastes.

INTRODUCTION

The coffee processing wastes include wet pulp, effluent, and dry husks. The annual pollution load that can arise from untreated waste waters during peak coffee processing in Kenya is equivalent to a population of 120 million people. Such pollution can affect the use of water for domestic needs, livestock watering, irrigation, fish culture and coffee processing (Anon., 1979). In the past extensive studies have been undertaken to solve these problems. Results of this endeavour are reviewed for general enlightenment and to attract inputs towards better pollution control technologies. In this regard, this presentation discusses the following existing and newly identified Environmental Related Features in the Kenya coffee Industry.

OPTIONS FOR ALLEVIATION OF POLLUTION FROM PROCESSING WASTES

The development of solutions for pollution reduction is best effected by the concept of waste minimisation followed by treatment, rather than by alleviation of the present problems by treatment alone (Mburu et al., 1994). The utilisation of the processing wastes is another potential option for pollution control.

WASTE MINIMIZATION

This can be effected by full recirculation of the processing water. During the final grading recycling the grading water can start after 5 minutes of washing. Once grading has been completed the resultant water can be reserved for pulping later, on the same day (Table 1). Recirculation of pulping water conveys wastes further away from the surface water and to easy points of utilisation. Besides effluent reduction recirculation of pulping water speeds up fermentation and makes the process effectively completed such that there is usage of less

water for intermediate and final washing (Mburu, 1994). Next to the skin lamp is a pit for collecting any discharge from the pulp.

DEWATERING *SEE PULP UTILIZATION*

It is possible to improve the management of heaps of coffee pulp, by pressing them in simple piston or screw presses. Lime addition at 0.5% will remove slimy texture of the pulp but will not improve the capacity for de-watering. The application of pressure at about 123 t/m^2 will result in the conversion of 1 tonne of wet pulp into 500 kg of liquor and 500 kg of press cake.

Table 1. Processing water use in a factory practicing full recirculation

Operation	Water (l/t of cherry)
Pulping and pre - grading	534[a]
Intermediate washing	401
Transport between tanks	801[b]
Transport to final washing channel	534
Final washing and grading	801[a]
Soaking heavy coffee	312
Cleaning factory	223
Total	3072

[a]*Since the grading water will be used for pulping, water requirement for the latter (534 l/t cherry) is excluded in the total.*
[b]*This amount can be reduced by recirculation of the water*
Source: Mburu (1996) quoted from Kamau (Unpublished)

WATER USE CONTROL

All water leaks in the factory must be fixed promptly. Every stage of processing should use just the right amount of water.

WASTE TREATMENT

The COD concentration of the effluent is 5808 mg/l while the SS concentration is 612 mg/l. The pH is 3.8 and has very low levels of nutrients implying that chemical addition is necessary for easy biological treatment in effluent ponds.

APPROACHES USED TO TREAT THE WASTE WATER

Four technologies considered appropriate within the context of Kenyan and other similar countries have been evaluated. These are: existing Kenyan seepage pit technology, with modified and improved operation; The “leeds” seepage trench correctly engineered and operated; anaerobic stabilisation ponds; and upward flow anaerobic sludge blanket (UASB) reactors.

Of the approaches tested, anaerobic ponds were least suitable despite the high COD and SS removal rate. This was due to difficulties in maintaining the pH level and mosquito infestation on the higher Hydraulic Retention Time (HRT). The UASB was more difficult than the ponds

to operate due to mechanical and electrical problems. Feed pipe and outlet pipe blockages due to small bore pipe. Problems also in pH balancing.

SEEPAGE PITS

Provision of adequate area is important to avoid overloading the existing seepage pits. But against this, the main problem is competition with the drying process for the factory area for which, priority easily goes to drying requirements. Much of the treatment capacity of a pit is by seepage of effluent through the soil before it reaches the river. Maximising the distance between the pit and the river is therefore important. Containing walls should be planted with appropriate vegetation which can strengthen the soil through an extensive root system. To operate well the side wall of a pit should not be compacted soil as compaction reduces the ability of the effluent to percolate through the wall into the surrounding soil. Pits must be of an appropriate size and number to accommodate the effluent. The correct operation and maintenance of a pit will greatly improve its performance. Operational factors such as solids separation and waste steams separation will affect the pit, as will maintenance factors such as end of season sludge removal and sidewall scrapping. Greater understanding of how pits operate, how they are designed and constructed and why they are necessary will instil a greater concern for their correct and continued use.

SEEPAGE TRENCH

This was found to be the most appropriate for the Kenyan situation. The treatment capacity of a trench is higher than that of a pit because of the larger infiltration area to volume ratio. The excavation required and hence the costs are reduced. The trench should be as deep and as narrow as possible to maximize the infiltration area whilst minimising the land use. Trenches are more suited to a wider range of site conditions than pits. For instance trenches can be built on sites with a greater gradient than which suit pits.

UTILIZATION OF THE PROCESSING WASTES

Coffee Pulp

The highest amount expected is 340 metric tonnes annually. The various utilization options of the pulp would contribute to their effective disposal and hence a clean up to boost environmental conservation. Recycling of the pulp back to the farm as manure is practised. It is possible to improve the management of the pulp, by pressing them in simple piston or screw presses. Pulp press cake is easily transportable from the site of coffee processing.

Effluent

The effluent can be used legally for irrigation by passing it through methane gas producing chambers first to render it less 'raw' and to produce gas.

Dry parchment husks

The expected amount is 32.5 metric tonnes in 4 mills. The husks are useful source of heat energy for industrial boilers, Mbuni and Kahawa coal dryers and domestic appliances. They are also used as mulch,

Coffee husks

These are obtained from hulling dry processed coffee cherry (Mbuni) and lights from the wet process. They are mainly used for making charcoal, a bio vegetal fertiliser (cofuna) and as mulch. Some are also exported for industrial use. The coffee husks produced from estate curing plants are recycled back to the farm.

GOOD HOUSE KEEPING

Factory grounds are kept clean by burning refuse or effective burying of the toxic wastes. Walk ways are paved while the rest of the ground remains grassed.

ELECTRIFICATION

Replacement of diesel powered units for coffee processing ensured no further discharge of waste diesel into the surface or subsurface environment or exhaust fumes previously emitted from diesel generators. Limited noise level now prevails.

LEGISLATION ENFORCEMENT

The existing legislation includes important provisions for environmental protection in the coffee industry, in particular limiting water use to 22.5 m^3/tonne of coffee processed and nil returnable, requiring careful disposal of coffee berry residues and maintenance of seepage pits.

The strategy for sustainable development in Kenya, developed in response to Agenda 21 will be achieved through the implementation of Kenya's National Environmental Action Plan (NEAP). This NEAP will provide a framework for integrating environmental considerations into the country's five year development plans. It will build on earlier sectoral strategies for water and environment which include the water Master plan. The National Environmental Secretariat (NES) within the ministry of Environment and Natural Resources to implement the NEAP. NEAP will also bring together existing environmental policies in Kenya, including those related to soil conservation, water pollution and water supply, under the **"Environmental Management and Co-ordination Bill"** which when enacted will include provision to set up a Standards and Enforcement Review Committee whose remit is to establish, monitor and enforce water quality and effluent standards. It will also require coffee factories to hold a discharge licence, monitor charges and submitted monitoring information to the authorities. All water quality information will be open to public scrutiny.

TRAINING

The training offered to Factory managers at the Kenya Coffee College (KCC-CRF) includes pollution alleviation from coffee processing wastes. The CRF provides liaison and advisory services to the coffee industry mainly through publications, field visits and Agricultural Shows on the need to conserve the environment.

ACKNOWLEDGEMENT

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The Optimum Conditions for Composting Coffee Pulp

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SUMMARY

The coffee pulp is one of the by-products of processing by the wet method. It has the highest potential for environmental pollution, compared with the others, if not well managed. The most attractive options to ensure that it does not contribute to environmental pollution lies in its economic utilization. In Kenya, the pulp is recycled back to the farm though not in a fully composted form. Composting studies have therefore been conducted to monitor the factors affecting the natural degradation of the pulp without any additives. The factors considered so far include, pile size and shape, ambient weather, pH and pile temperature. It has been found that, the decrease of the pile size to a minimum, rising of the pH to a maximum and a similar rise of temperature to a peak before dropping to the ambient temperature all coincide and possibly signify the end of the composting process. The processing activity has also been found to depend on the weather particularly due to the loss or gain in moisture.

INTRODUCTION

The wet coffee processing in Kenya can produce about 340,000 metric tonnes annually of fresh coffee pulp scattered all over the coffee growing regions. From the most common factory with a 3 disc pulper, about 350 metric tonnes of pulp can arise. This by-product is rather difficult to dispose and hence likely to cause environmental pollution, if allowed accumulating at the factory. All the same, the easiest way to manage it is to create suitable options for its utilization. Coffee pulp compost can supplement farmyard and inorganic fertilizers in maintaining organic matter and supplying nutrients (Adam and Dougan, 1981; Alwar et al., 1986). The coffee pulp compost can easily be handled, stored, applied to farms and the minerals are more readily available. However, optimum local composting conditions have apparently not been studied. For this reason, a local evaluation of the important parameters of the process without additives was embarked on first to pave way for further studies.

MATERIALS AND METHOD

Fresh coffee pulp was collected from a factory at the Coffee Research Station Ruiru. Doom shaped piles were formed which were treated to different levels of aeration by manual turning at different rates; Protection or exposure to the ambient weather; Moisture regulation via addition of water; different pile sizes; and Compost accelerator.

All the trials were in a completely randomised design replicated 3 times. The volume, temperature and pH of the pile were determined and recorded on daily basis during composting. The date from the beginning was also recorded in order to establish the composting duration.

RESULTS AND DISCUSSIONS

Without aeration, the piles compacted and composting was very slow inside the pile. The two weeks frequency of aeration also caused the pile to compact making it rather difficult to turn and

produced rather large clods, which were not suitable for even application in the farm. Due to these results, the turning frequency was limited to at least once a week.

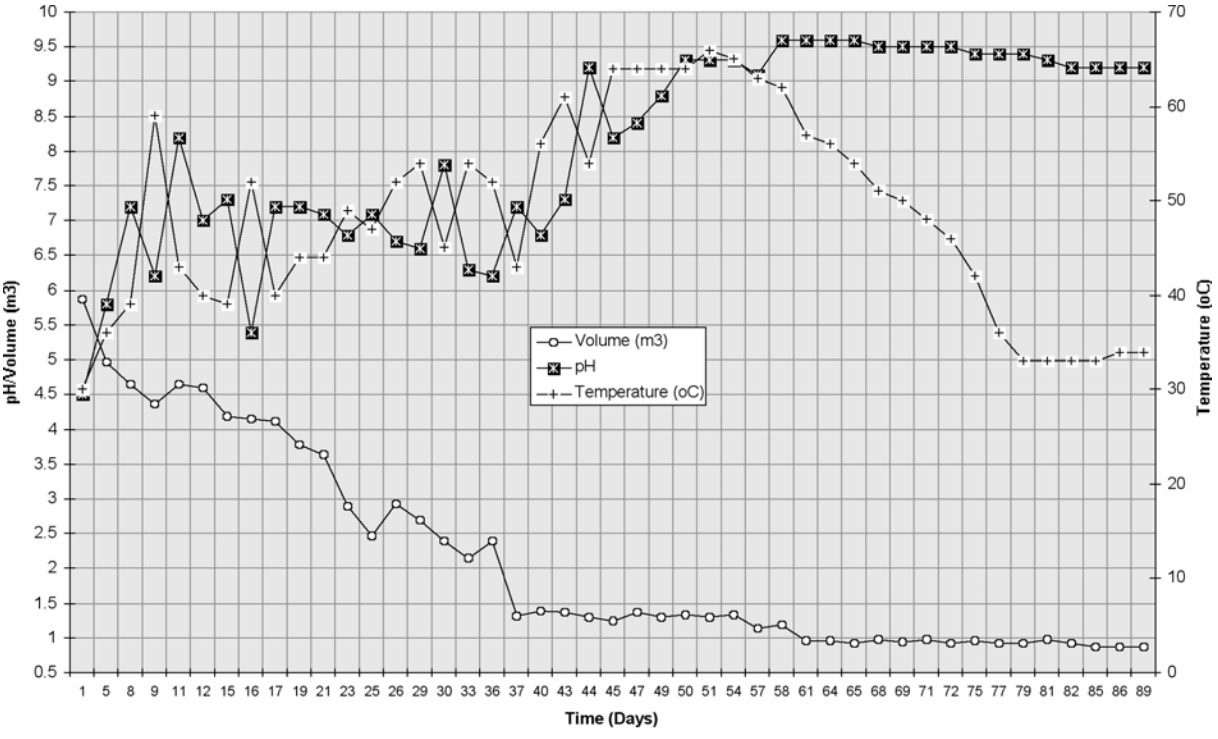

Figure 1. Composting trends

According to Figure 1, the temperature increased to a peak of 55-70^{o} before dropping to between 30-40^{o}. The pH also rose asymptotically from an initial range of 4.0 and 4.3 to a maximum of 9.6-10.1. The initial pH of the heaps was not conducive to the soils within the coffee growing zones. However, with changes in volume and the initiated rate of aeration, the pH changed to 7 after only 17 days. The volume of the pile also fell gradually to a minimum depending on the initial volume. The final volume was 0.18 of the original size. The maximum pH and temperature and the minimum volume more or less coincided.

As the frequency was increased from weekly through twice a week to daily turning the finer the final compost became. Thus reducing the material in size, making it less bulky, easy to handle and apply in the farm. Aeration of twice a week contributed to shorter composting duration than either daily or once a week (Table 1). Composting was weather dependent (Figures 2 and 3). Dry weather dried the pulp causing cessation of the process; Moderate rain reactivated the process. If it became too wet, the pH and temperature depressed. This could cease the process. Composting piles under protection from rain and sunshine had a drying tendency and moisture regulation was therefore necessary.

Table 1. Effect of aeration on composting duration

Aeration Frequency Days/week	Composting Duration Days
7	68
2	61
1	70

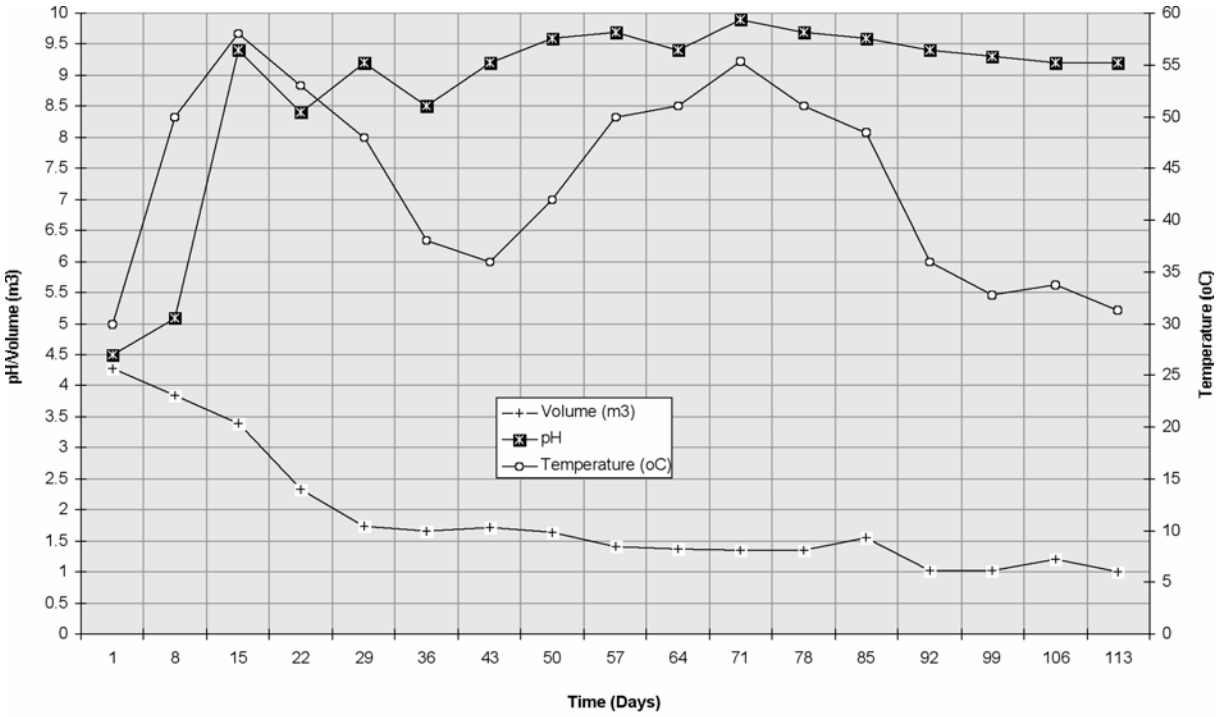

Figure 2. Effects of weather on composting

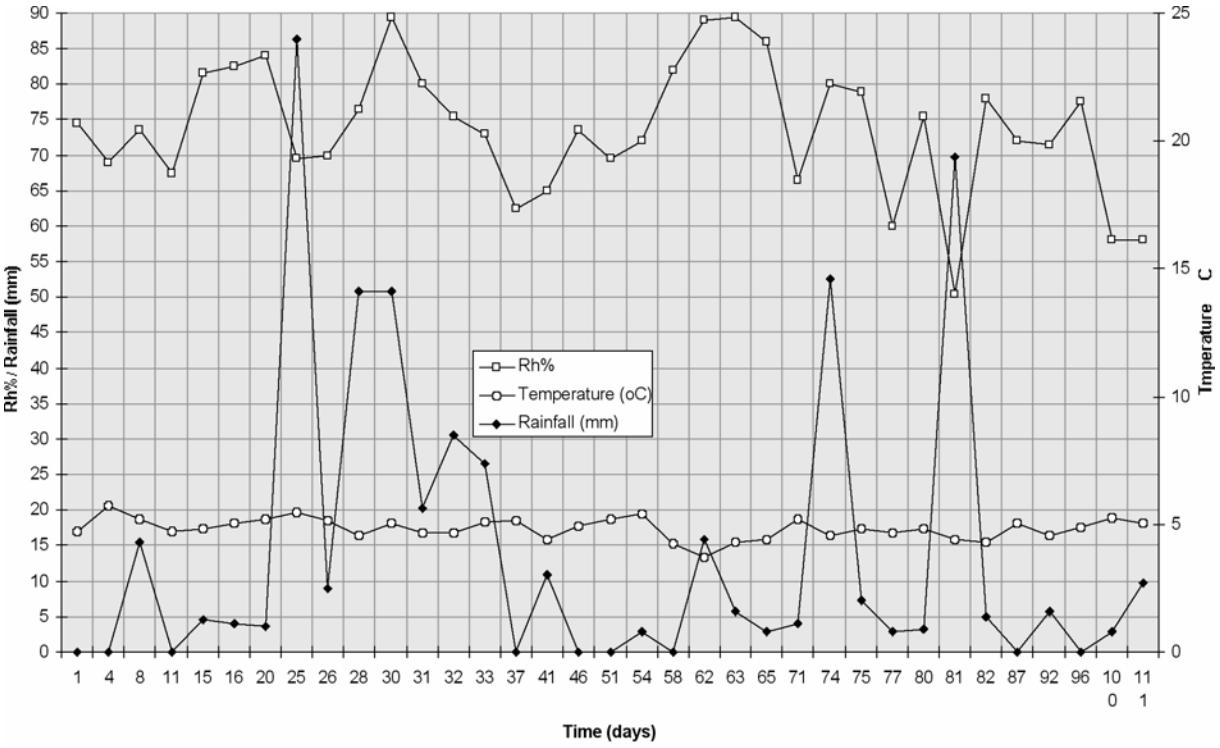

Figure 3. Prevailing weather conditions during composting

Without moisture regulation but protected from external environment composting took longer than for exposed piles (Table 2).

Table 2. Composting under cover and in the open

Aeration No of days/week	Composting Duration Days	
	Covered	Not covered
1	55	51
2	56	42
7	43	27

A Compost accelerator performed worse than natural composting (Table 3).

Table 3. Performance of a composting accelerator on coffee pulp

Treatments		
Level of AMD (ml)	Turning Frequency	Duration (Days)
200	1	76
0	1	63
200	2	75
0	2	66

For pile size of ratio 1:5:15 the composting duration were 14, 19 and 29 days respectively.

CONCLUSION

It can be concluded that the most important composting factors were pile shape and size; moisture content of the composting pulp; excess supply of oxygen and the prevailing weather conditions. The process was completed when there was no further temperature rise, and a pH of 9 or more. This verifies results previously reported by Adams and Dougan (1982), Alwar and Ramaiah (1986); and Mbole et al. (1987). For proper composting the right amount of shade was also required. Aeration at a rate of 2 days per week realised the shortest composting duration. It also gave an ideal product, which could efficiently be applied, in the farm with no difficulties.

ACKNOWLEDGEMENT

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Optical Sorting for the Coffee Industry

S. BEE

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SUMMARY

Sortex' optical sorting machines are designed to remove foreign material and add value to coffee, by removal of blemished product. Sortex have been designing and manufacturing optical sorting machines for bulk inspection and grading of foodstuffs for over 50 years.

Removal of foreign material (sticks, stones, insects etc) is now a basic prerequisite prior to processing. Simultaneously, consumers are also demanding increased quality through cosmetic enhancement of fresh pack coffee by removal of blemished and discoloured beans. Coffee taste is safeguarded by selective removal of beans that can affect flavour, such as "stinkers," over-roast or over-fermented beans. An optical sorting machine can maintain a far greater level of consistency than hand sorting and also reduces labour costs (Anon, 1987). In recent years, tighter EU and American Food and Drug Administration standards on food quality have been implemented. Optical sorting of coffee beans allows users the opportunity to strengthen their competitive position by providing a premium quality product at an increased margin.

BULK COFFEE SORTING

In a bulk sorting system, coffee beans are fed from a vibrating hopper onto a flat channelled, gravity chute. The aim is to separate the coffee beans into a uniform "curtain", or monolayer. The product then passes into an optical inspection area, where a decision on whether to accept or reject each bean is made. Defective beans are removed from the product stream with a jet of air from a pneumatic ejector.

Early optical-sorting machines used low resolution (of the order of 5 mm) photodiode technology to take views of the product from only one side. As a consequence, only surface defects facing the optical system could be detected. Nowadays, two or three high resolution Charged Coupled Device (CCD), high resolution (sub mm) line scan cameras are used to view the product from different angles as it leaves the end of the chute. Obviously, this increases the efficiency at which the system can identify defects.

DEFECTS SORTEX CAN REMOVE FROM COFFEE

Sortex' sorting machines can remove the colour defects that are typically encountered by the coffee industry, such as amber, brown, reddish (foxy), or black blemishes and even brocca (insect bites). Foreign material such as sticks or stones are also removed from coffee by optical sorting. Sortex' machines have been developed to suit both the large and small coffee processor.

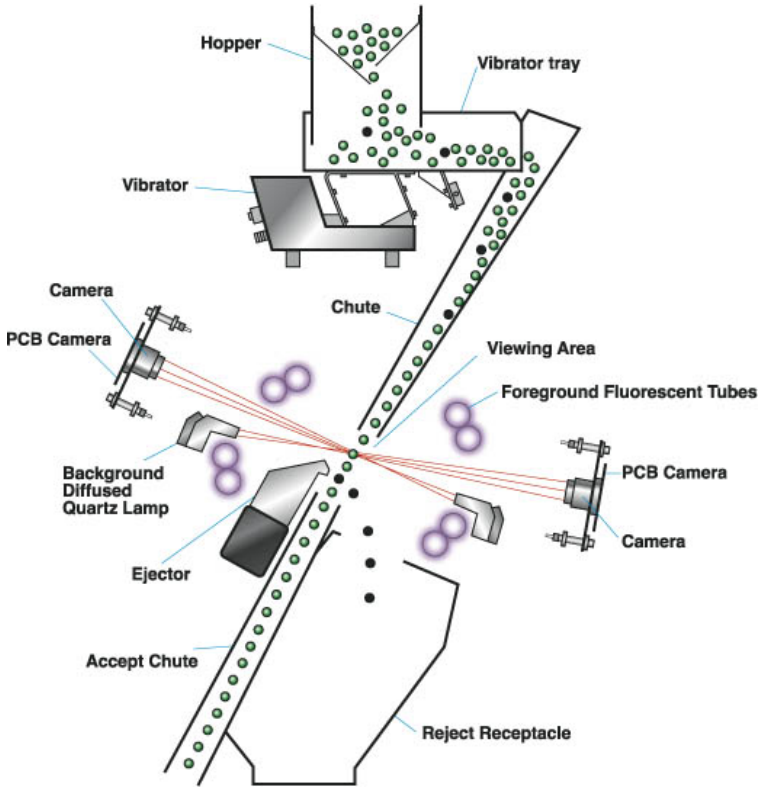

Figure 1. Schematic layout of a typical optical sorting machine

Figure 2. Amber (yellow) defects – immature berry

Figure 3. Brown defects – Over ripe or fermented

Figure 4. Reddish (“foxy”) – Damage to cherry, or due to staining from liquor

Figure 5. Black defects (whole or partial) – Usually over-ripe

Figure 6. Brocca / insect bites – antesia bug or coffee bean borer

Figure 7. Stones / Sticks / Foreign Material

Figure 8. Some coffee sort examples

MONOCHROMATIC SORTING

Defects and foreign material can be identified by selectively comparing the magnitude of reflected light at certain band-pass wavelengths. Since monochromatic sorting is typically carried out in the visible wavelengths, fluorescent tubes are used to illuminate the product at the required intensity and uniformity. Optical filters placed in front of the CCD camera allow the magnitude of detected signal at selected wavelengths to be compared with user defined thresholds. For example, the coffee beans in Figure 9 reflect over 50% of near infrared light, while a stick or a stone reflects less than half that amount, allowing differentiation between the two by setting of a threshold for rejection, at a specific band of wavelengths.

TRUE BICHROMATIC SORTING

Monochromatic sorting is effective for many applications. However, a more sophisticated approach is needed to detect the very subtle colour variations in high-value coffee. For these applications, we need to sort the product “bichromatically”, i.e. use two different band-pass wavelength filters.

Bichromatic sorting is a different technique to “dual monochromatic” sorting. Both techniques use two band-pass filters. The two band-pass filters can be either visible-visible, or visible infra-red, depending on the optical reflection properties of product to be sorted

(Figure 10). However, true bichromatic sorting allows much better subtle colour differentiation, compared to the less powerful dual monochromatic sorting technique. The true bichromatic approach calculates the ratio of the signal from band-pass filter A to B (Figure 10). This ratiometric comparison of the two band-pass signals, significantly boosts the differentiation of the system and presents a serious advantage over the dual monochromatic approach.

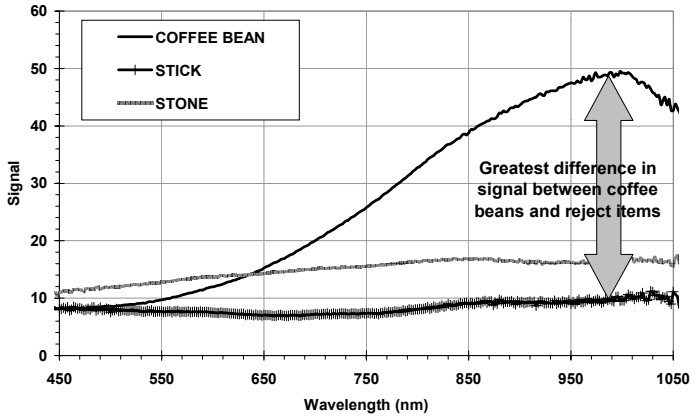

Figure 9. A typical visible reflectance spectra for Robusta coffee. In this case a near infra-red filter would be selected for monochromatic removal of foreign material

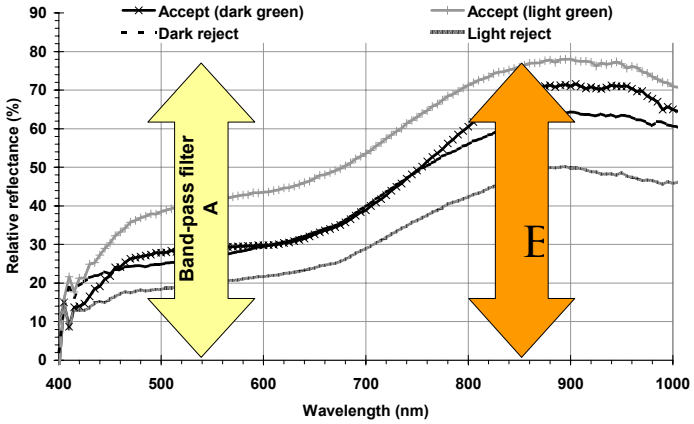

Figure 10. Spectral curves for green Arabica coffee. Bichromatic sorting is necessary, since there is no one region of the spectrum where reject material can be successfully separated from the accept material. Colour Mapping Techniques

A bichromatic sorting machine using two band-pass filters, say green and red, makes a decision based on the ratio of the two signals in conjunction with the intensity of the individual signals. The situation can be represented as a two-dimensional “colour” map by plotting the reflectivity of colour 1 versus that of colour 2 (Figure 11).

The bottom left hand corner of this map represents the reflectivity from a black particle (0% reflectivity) and the top right hand corner represents the reflectivity from a white particle (100% reflectivity). The boundary curve in is the reflectivity map contour, outlining the acceptable product, as seen by the sorting machine, for a typical product. The contour line represents the chosen accept / reject threshold. The "+" within the map contour is the background "balance point", which represents the average colour of the product.

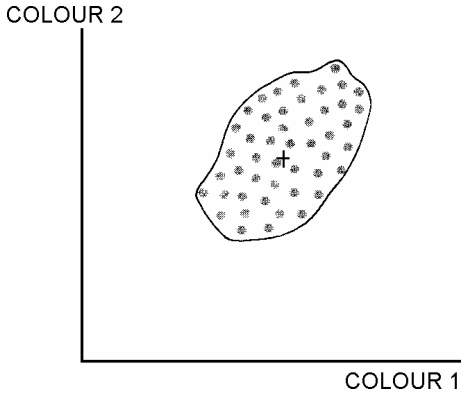

Figure 11. A bichromatic colour map, representing the distribution of colour 1 versus colour 2

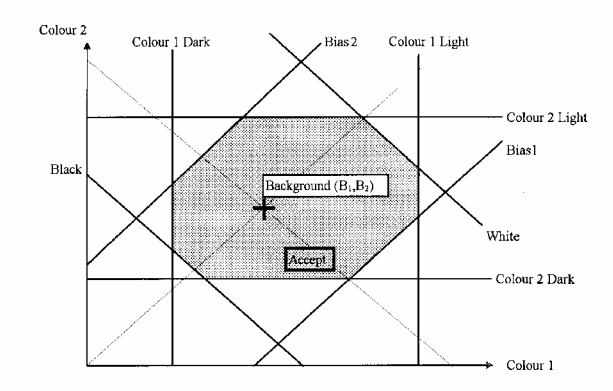

Figure 12. Bichromatic sensitivity thresholds

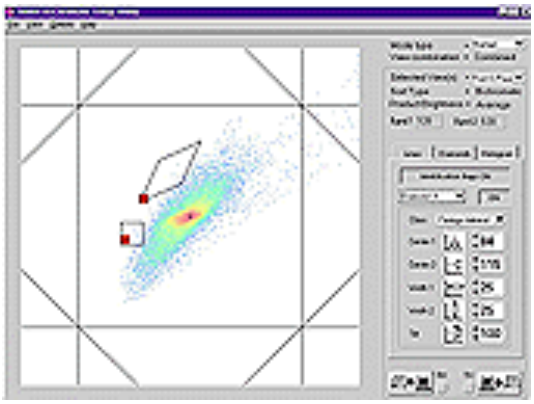

Figure 13. A bichromatic colour map, as displayed on the Sortex TrueChromatic user interface

A major part of setting up an optical sorting machine is to achieve the best overall accept / reject ratio for the product being sorted. The operator can do this by using the user interface to adjust the shape and size of the map contour, (Figure 12) to match as accurately as possible the map contour of the product batch. The sorting sensitivity increases as the machine map contour is decreased in area, as it approaches the area of the map contour of the product batch. Product within the area bounded by the threshold levels is accepted and product outside is rejected.

These techniques allow an optical sorting machine to remove a far greater range of defects, with greater accuracy and without the penalty of removing large amounts of accept product. Obviously, these techniques can be extended into three dimensions for trichromatic colour sorting.

ABOUT SORTEX

Sortex developed the first optical sorting machines for the agricultural industry. The company was established in London in 1947. Since then Sortex has grown to become the world's leading manufacturer of colour sorting machines, with clients operating across the full spectrum of food and agricultural products in more than 100 countries around the world.

The company is customer focussed and employs R&D expertise in optical design, applied physics, electronic hardware, software design and mechanical engineering.

Part of the Swiss engineering group Bühler, specialists in the design and construction of plant and equipment for human nutrition, Sortex has won the Queen's Award for Enterprise in 1968, 1972, 1987 and again in this year, 2001.

Table 1. Illustrates typical throughputs in Tonnes per hour, for different Sortex machines with standard chutes. Special high capacity chutes are also available for the 90000 range. The 90000 offers market leading high throughputs with excellent sort quality for the large coffee processor. The compact 3400 offers a lower capacity, but high quality sort for the smaller processor

Machine	Channels	Arabica	Robusta	Roasted
90000 Focus	32	-	4.0	4.0
90000 Focus	48	-	6.0	6.0
90000 Focus	64	-	8.0	8.0
90000 TrueChromatic	16	1.5	2.0	2.0
90000 TrueChromatic	32	3.0	4.0	4.0
90000 TrueChromatic	48	4.5	6.0	6.0
3400	2	0.3	0.3	0.3

THE FUTURE

Sortex is a world leader in innovation and delivery of sorting solutions to the global coffee industry. Sortex are pioneering optical sorting technology, via an active research programme. By harnessing new technologies, Sortex is committed to providing integrated solutions that fulfil the demands of the coffee processing industry.

FURTHER READING

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The Influence of Endothermic and Exothermic Energies on the Temperature Field of Coffee Beans during the Roasting process

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SUMMARY

The model demonstrates the shifting of temperature profiles by the latent energies. The precision is not sufficient so far, because the roast kinetic is still missing. For a more accurate simulation of the whole complex roasting process the model has to be extended by kinetic data and, if possible, combined with a mass balance describing the steam and the CO_2, leaving the coffee bean.

The model is very useful to simulate the heating of the coffee beans, particularly to determine the thermal property data (thermal conductivity, heat capacity) and also calorimetric data (reaction enthalpy, vaporization enthalpy) by comparison with experimental data presupposing that in each case all other data are known. Especially the thermal property data can be determined by fitting the experimental temperature curves in a heating procedure up to 100°C to calculated temperature curves, assuming that during those experiments the thermal property data are constant caused by a constant water content.

FULL REPORT

During the roasting process of coffee beans carried out at 240°C, there are different phases where endothermic and exothermic energies have a strong influence on the temperature field of the coffee bean. The heat, which is being transferred from the gas phase through a boundary layer to the surface of the coffee bean by outer heat transfer and from the surface by heat conduction to the inner regions of the coffee bean, leads to an increase of the temperature corresponding to the specific heat capacity. At temperatures above the boiling point of water, heat is being consumed by the evaporation of water that is physically bounded in the coffee bean (Örsi, 1993). The evaporation enthalpy of water at atmospheric pressure amounts about 2260 kJ per kg pure water. Assuming that the coffee bean contains 9% water (w/w) the required energy per kg coffee to evaporate the contained water amounts about 200 kJ. This value does not include the required energy to evaporate the water that's being formed during the roasting process by chemical reaction of the organic compounds. The evaporation enthalpy leads to a high energy demand of the roaster. About 30% of the energy is being used to evaporate the contained water (Garloff et al., 1996). At higher temperatures above 175°C additional heat is being generated by exothermic chemical reactions which start if the temperature is high enough to reach the activation energy of the roasting reactions (Raemy and Lambelet, 1982). The exothermic energies lead to a further increase of the bean temperature without additional heat applied from the roast gas. The coffee bean ist required to be cooled down by air or even water to avoid carbonization (Clarke and Macrae, 1987). In general the non steady temperature field of the coffee bean including the latent energies have a strong influence on the quality of the coffee product. Even though the knowledge of this three dimensional non steady temperature field would improve the specific control of the roasting process and optimize the energy demand of the roasting process respectively, it is

still unknown. To be able to simulate the non steady temperature field, the following partial differential equation based on the Fourier equation has to be solved.

$$: c_p \cdot \rho \cdot \frac{\partial \vartheta}{\partial t} = \frac{\partial}{\partial x}\left(\lambda \cdot \frac{\partial \vartheta}{\partial x}\right) + \frac{\partial}{\partial y}\left(\lambda \cdot \frac{\partial \vartheta}{\partial y}\right) + \frac{\partial}{\partial z}\left(\lambda \cdot \frac{\partial \vartheta}{\partial z}\right) + \sum \dot{W} \qquad 1)$$

The last term in equation 1) describes the flow of the different latent energies. In the case of exothermic energies it depends on the location, on the temperature, on the time a certain amount of organic compounds is being processed (reaction rate) and on the reaction enthalpy. Except the overall enthalpy, where the order of magnitude is known, the other required variables are unknown as well as the thermal conductivity and the heat capacity, which depend particularly on the humidity (Small and Horrell, 1993). High temperature gradients in the coffee bean are assumed to have a strong inluence on the thermal properties since the contained water increases the thermal conductivity and the specific heat capacity as well compared to the 'dry bean'. Since it can be assumed that there are also high humidity gradients caused by evaporating water that is moving into the roast gas, the thermal properties between the core and the surface of the bean are reasonably different. In this simulation the thermal conductivity was determined experimentally and set to a constant value of .12 [$W \cdot m^{-1} \cdot K^{-1}$]. This assumption leads to the following equation:

$$c_p \cdot \rho \cdot \frac{\partial \vartheta}{\partial t} = \lambda \cdot \left(\frac{\partial^2 \vartheta}{\partial x^2} + \frac{\partial^2 \vartheta}{\partial y^2} + \frac{\partial^2 \vartheta}{\partial z^2}\right) + \sum \dot{W} \qquad 2)$$

Additionally the latent energies have been included within an effective specific heat capacity:

$$\sum \dot{W} = c_{p,latent} \cdot \rho \cdot \frac{\partial \vartheta}{\partial t} \qquad 3)$$

Using this simplifications, equation 1) has been changed to:

$$c_{p,eff} \cdot \rho \cdot \frac{\partial \vartheta}{\partial t} = \lambda \cdot \left(\frac{\partial^2 \vartheta}{\partial x^2} + \frac{\partial^2 \vartheta}{\partial y^2} + \frac{\partial^2 \vartheta}{\partial z^2}\right) \qquad 4)$$

In this simulation the non effective specific heat was assumed to amount 2200 [$J \cdot kg^{-1} \cdot K^{-1}$] (Clarke and Macrae, 1987). The energy to evaporate the contained water, assuming a water content of about 9% (w/w), was calculated to amount 200 [$kJ \cdot kg^{-1}$] evenly spread over the temperature range between 100°C and 140°C, and the overall exothermic energy 140 [$kJ \cdot kg^{-1}$] evenly spread over the temperature range between 175°C and 240°C. From own measurements, the dependence of the density of the bean on the temperature were taken to be able to get a volume specific heat capacity (Figure 1).

The simulation has been carried out using the finite element method (FEM). As a geometrical model a half of an ellipsoid has been chosen, where the different sizes were taken from experimental size distributions as average values and the whole volume was filled with small elements. (Figure 2)

In this method the energy balance had to be solved for each element, where all equations are non linear. As solver an implicit solution method was chosen, where the solution method (frontal solution, sparse direct solution, jacobi conjugate gradient solution, incomplete cholesky conjugate gradient solution, preconditioned conjugate gradient solution) (Ames, 1992) can be chosen by the program itself automatically. A boundary condition considering heat flow from the gas to the surface has been taken, where the heat transfer coefficient h was determined by Nusselt functions (Raemy and Lambelet, 1982), assuming the coffee bean as a sphere with a diameter of 6 mm and a gas velocity of 5 $m \cdot s^{-1}$. According to those assumptions an heat transfer coefficient of 130 $W \cdot m^{-2} \cdot K^{-1}$ was calculated. The gas temperature was assumed to amount 240°C. The simulation results animated over the time are shown in Figure 3.

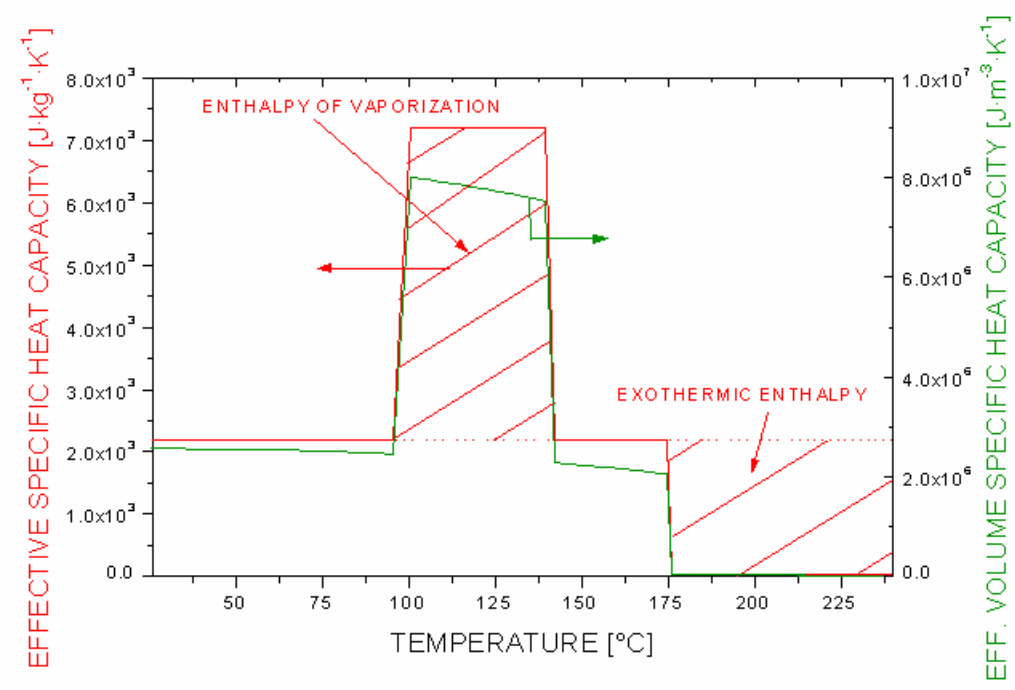

Figure 1. Effective specific heat capacity of the coffee bean (including latent energies)

In Figure 3 the transient temperature field at different roasting time is depicted. High gradients of more than 100°C from the center to the surface can be seen. The temperature profiles depending on time and depending on the location inside the bean are depicted in Figure 4.

In Figure 4 it can be seen that there are significant differences between the compared curves: The time dependent temperature curve that does not include any latent energy increase with declining slope. The temperature curve that does include the latent energies also increases, but with a more declining slope caused by the evaporation of water. After a certain point there is an inflection point in this curve where the slope is increasing caused by the influence of the exothermic energies. The temperature curves depending on the bean height (the bean height is the length of 4 mm in z-direction that can be seen in the time dependent diagram) show that without latent energy the process temperature can be obtained sooner than with latent energies included. The difference between both curves is increasing with the time until the exothermic energy is released. Then, the longer the roasting process lasts the shorter is the difference between those curves since the exothermic energy leads to an increased heating of the bean.

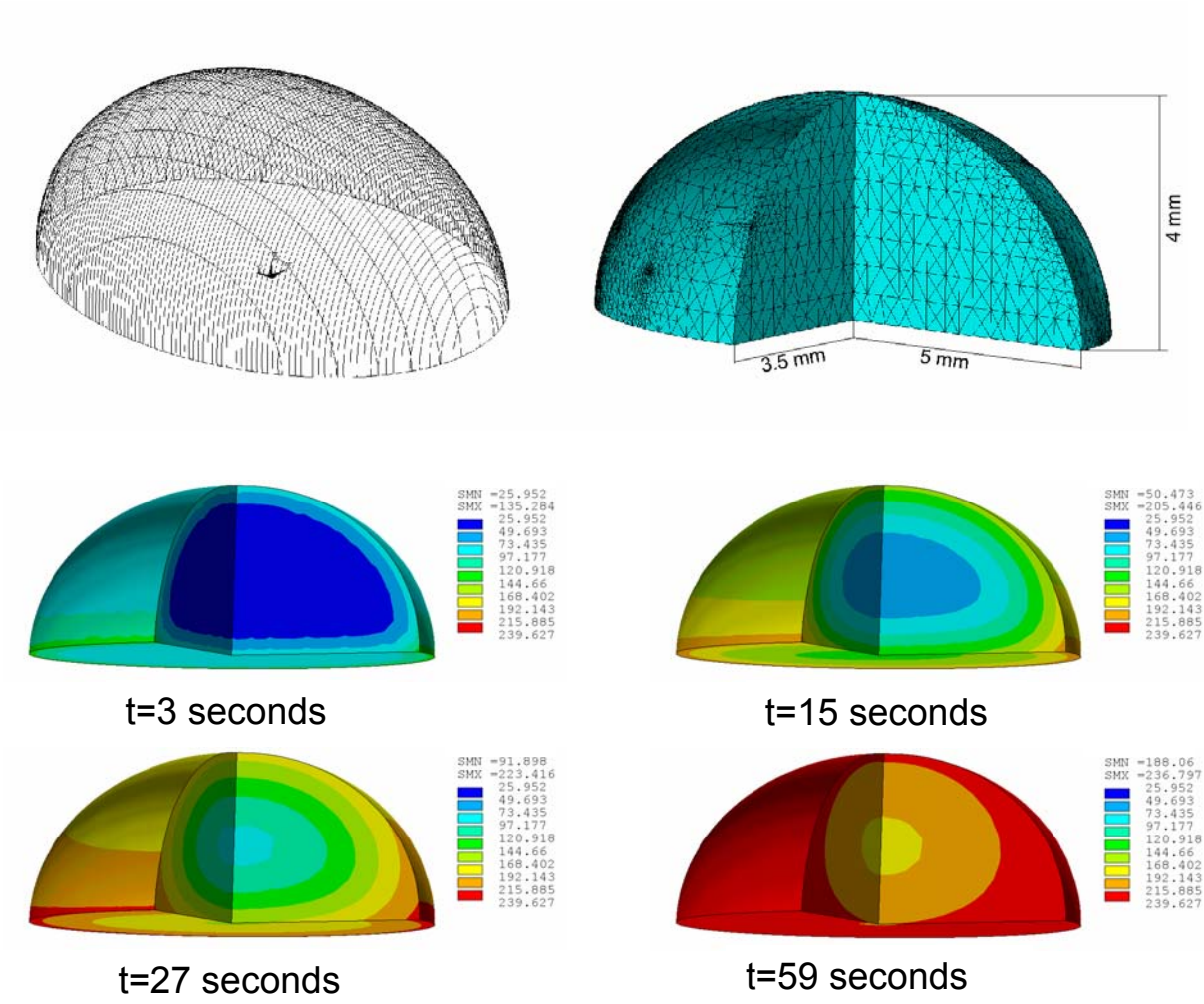

Figure 2. Geometrical model of the bean

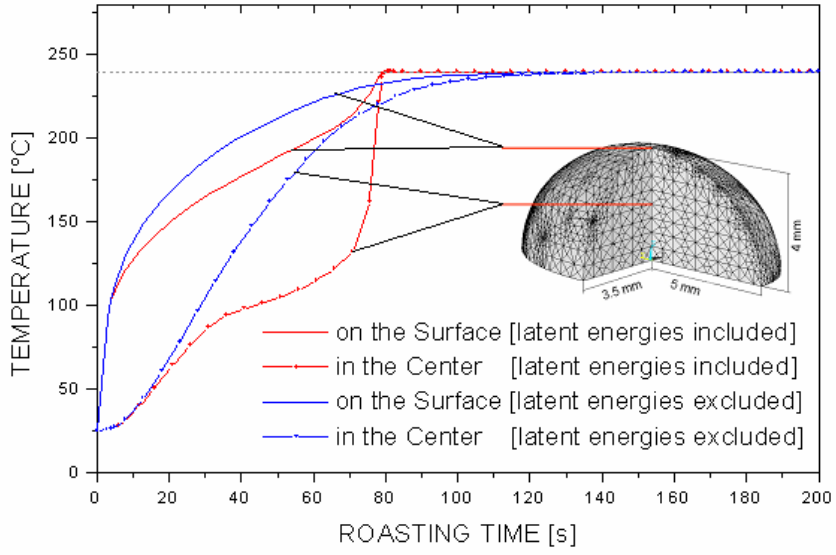

Figure 3. Simulation results, animated over the time

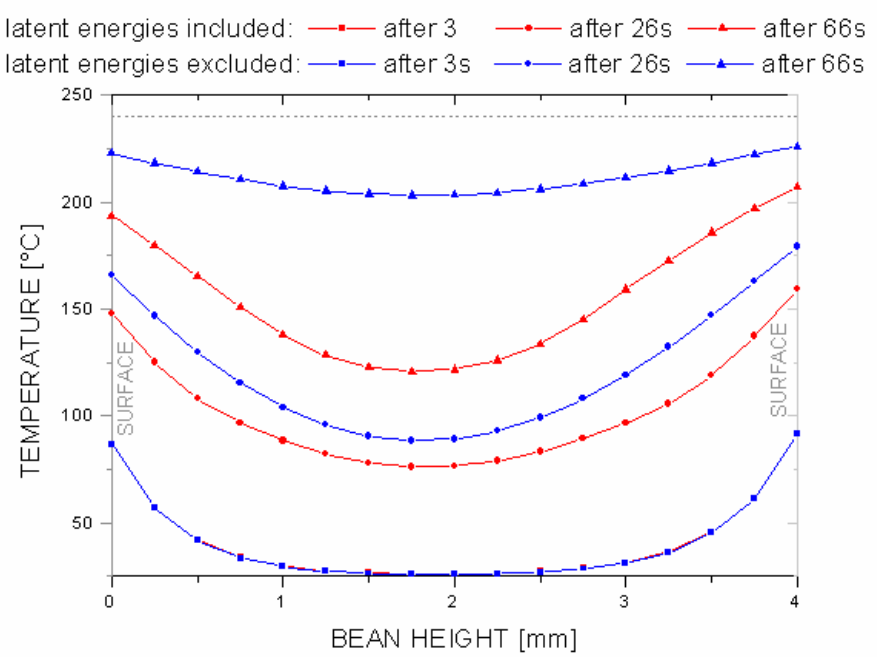

Figure 4. Temperature profile depending on time and location in the coffee

ACKNOWLEDGEMENTS

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Thermophysical Properties of Coffee as Affected by Processing

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SUMMARY

It is well known the complex and dramatic changes, which take place in coffee beans during the roasting process, are associated to a wide number of heat-induced endothermic and exothermic reactions.Although differential scanning calorimetry (DSC) is frequently used for studying the thermophysical properties of food materials, until now few studies have been carried out on heat-treated foods such as coffee.

The aim of the present investigation was to study the thermal profile of coffee beans undergone the roasting process. The influence of preliminary technological operations such as decafeinisation and/or defatting on the coffee thermal profile was also considered.

Different endothermic and exothermic phenomena were observed on coffee samples scanned in –120°-400°C range in pans no hermetically sealed. These phenomena were attributed to lipid melting, water evaporation, and to the development of non enzymatic browning reactions and carbonisation. The relevant changes in specific heat (C_p) were also considered.

Results suggest that DSC technique can represent an interesting analytical tool for the characterization and analysis of different coffee varieties. In addition on the basis of the coffee thermophysical profile suitable indications for a better optimization of the roasting process could be obtained.

INTRODUCTION

The sensorial properties, which are imparted to coffee through roasting, strongly depend on the development of a number of heat-induced phenomena. Water evaporation, glass transition, Maillard browning and reactions involving the polysaccharide fraction are only some examples of the complex pools of endothermal and exothermal phenomena taking place during roasting (Clarke and Mcrae, 1985; Lerici et al., 1980).

Although any comprehensive approach to heat transfer optimisation during coffee roasting requires the identification of the thermal phenomena occurring during the process, as well as of the relevant temperature ranges and latent heats, information regarding these properties is scarcely available, probably due to the lack of appropriate analytical techniques.

Differential scanning calorimetry (DSC) is a widely accepted tool of investigation of the thermal behaviour of foods. However, only few researchers have attempt, by means of this technique, to detect the thermal changes associated with the reactions peculiar to coffee roasting. In particular, intensive exothermic peaks occurring at *circa* 200°C and purported to be related to coffee self-ignition, have been observed by calorimetric analysis of coffee powder in sealed pans in the presence of oxygen (Raemy and Lambelet, 1982). State modifications relevant to some pure coffee compounds and the specific heat of coffee at roasting temperatures were also evaluated (Singh et al., 1997). It has been observed that the

difficulties associated with the application of this technique to complex materials, such as foods, are mainly due to the simultaneous appearance of different thermal phenomena and also to the strong dependence of the thermograms on the environmental conditions during the analysis (Raemy et al., 1983).

The aim of this work was to study the thermal phenomena occurring during coffee roasting by means of DSC analysis. In order to provide a possible identification of the state transitions and chemical reactions associated with the phenomena observed by DSC, the thermal profiles of coffee having different roasting degree were investigated. Analyses were carried out avoiding both the presence of oxygen and the development of high pressures in the pans.

MATERIALS AND METHODS

Sample preparation

Coffea arabica cv Santos (from Brasil) was used. Green coffee beans were roasted in a circulating air oven (ULE 400, Memmert, Schwabach, Germany) at 190°C for different times for up to 20 min. Prior to analyses, coffee beans were finely grounded in a coffee-grinder (Moulinex, Ireland). Additional samples were also prepared by freeze-drying the grounded green coffee (Edwards Alto Vuoto, Minifast 1700, Milano, Italy).

Analytical determination

Moisture was evaluated according to AOAC methods (1995).

Colour analyses were performed using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Japan) equipped with a CR-200 measuring head. Standard C.I.E. conditions with illuminant "C" (6774K) were used.

Density was determined according to Lerici et al. (1980).

Calorimetric measurements were made using a TA4000 differential scanning calorimeter (Mettler-Toledo, Greifensee, Swiss) equipped with a DSC 30 low-temperature measuring cell and connected to a graphWare software (TA72.2/.5, Greifensee, Swiss). Heat flow calibration was achieved using indium (heat of fusion 28.45 J/g). Temperature calibration was carried out using water (m.p. 0°C), indium (m.p. 156.6°C) and lead (m.p. 327.4°C). Aliquots of 10 mg of sample were placed into 150 μL aluminium DSC pans closed without pressure sealing. An empty aluminium pan was used as reference. Samples were heated from 0°C to 400°C at 10°C min^{-1} under nitrogen flow (10 mL min^{-1}).

Statistical analysis

Data reported in this paper are the average of at least three determinations. The coefficient of variation, expressed as the percentage ratio between the standard deviation and the mean value were lower than 2% for moisture, 3% for density and colour and 8% for enthalpy. Regression analysis was performed using Statistica (Statistica for Windows, Statsoft Inc., 1993)

RESULTS AND DISCUSSION

Table 1 shows moisture, density and colour of green and differently heat treated coffee samples. As expected, at increasing roasting time, coffee presented progressive changes in these properties, allowing samples having a light, medium and dark roasting degree to be

obtained. Figure 1 shows the thermograms from 0°C to 400°C of the green and differently roasted coffee samples. Analyses were carried out avoiding both the presence of oxygen and the development of high pressures in the pans. In fact, whilst the former could favour oxidation processes, combustion and self-ignition, the latter prevents any endothermal phenomena having activation volume >0 (i.e. caramelisation, carbonyl condensation and formation of melanoidins) (Manzocco et al., 1999).

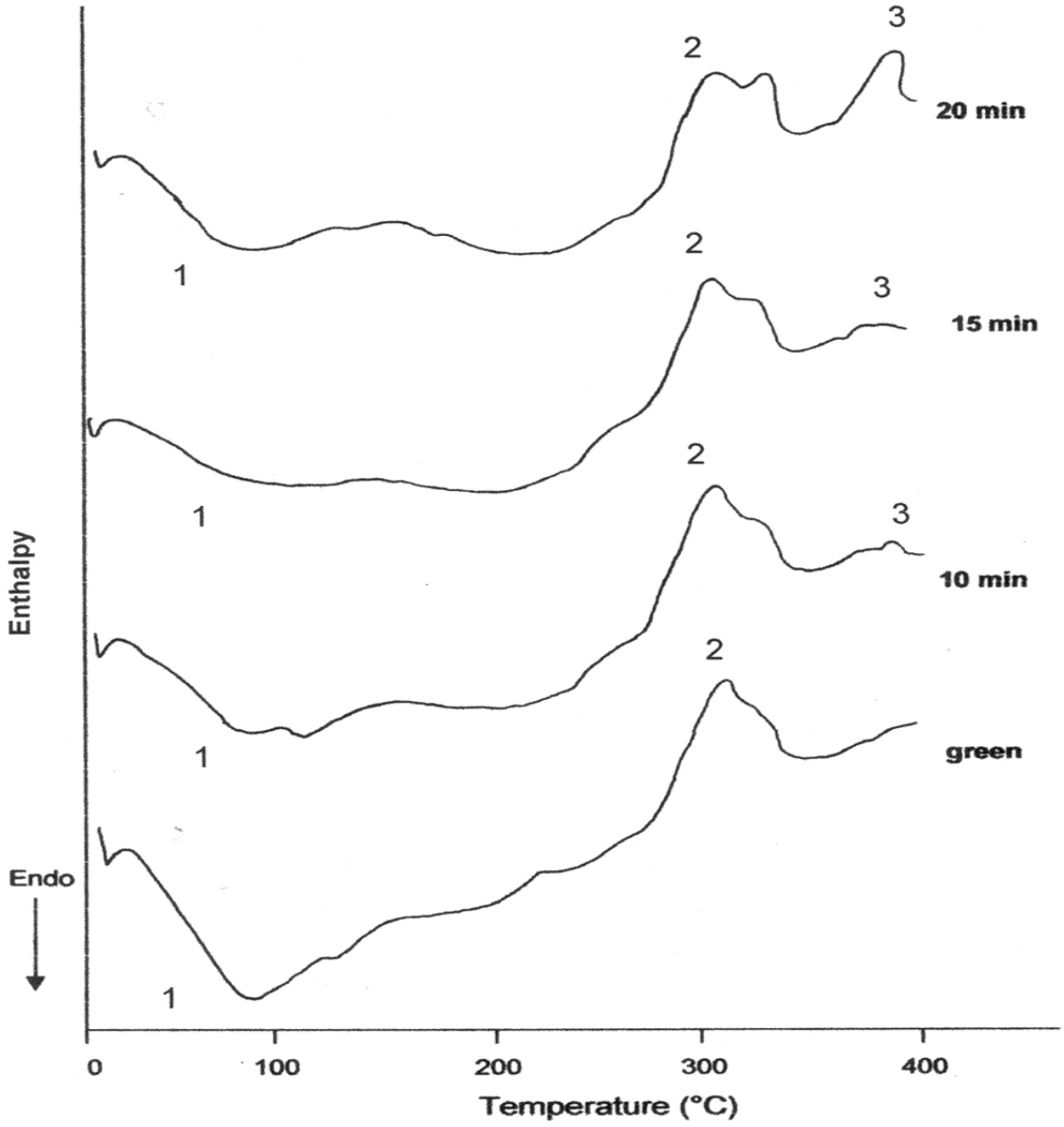

Figure 1. Thermograms of coffee roasted at 190°C for up to 20 min

It can be noticed that coffee thermograms are characterised by a significant endothermic phenomena (peak 1), followed by a sequence of endothermic and exothermic peaks above 150°C (peak 2 and 3). Peak 1 in the temperature range between 20 and 130°C was associated to the liquid-vapour transition of water. This hypothesis was confirmed by the fact that freeze-dried coffees, having moisture content lower than 0.98% (w/w) showed an almost complete disappearance of this peak (data not shown). In addition, the decrease in moisture content of the differently roasted coffee samples was associated to a progressive decrease in the enthalpy of peak 1 (Table 2) and a significant correlation between moisture content and enthalpy of this peak was found ($R = 0.96$, $p < 0.05$).

Above 150°C, the thermal profile of coffee is characterised by a complex series of endo- and exothermal peaks which can be attributed to the development of non-enzymatic browning reactions and carbonisation of the product. These results apparently contradict with the fact that only endothermal phenomena were observed upon Maillard reaction in simple model systems (Manzocco et al., 1999). In the case of coffee, it is likely that Maillard browning occurs in concomitance with the development of parallel and consecutive reactions involving not only lipid oxidation but also thermal decomposition of polysaccharides and of other compounds. The latter, being strongly exothermic (Raemy and Lambelet, 1982), hampers the detection of the endothermal peaks relevant to Maillard browning.

At very high temperatures (above 290°C), the thermal profile is characterised by a well-resolved exothermal peak (peak 2) which was associated to the carbonisation of the complex carbohydrates contained in coffee and probably contributing, under certain conditions, to its self-ignition (Raemy and Lambelet, 1982). At increasing roasting degree, a decrease in the enthalpy of peak 2 was observed (Table 2) due to the progressively lower content of these polysaccharides in the heat treated samples. In addition, the novel compounds formed as a consequence of roasting can further undergo thermal degradation leading to the appearance, above 350°C, of a new exothermic peak (peak 3), which was particularly evident in the 20 min-roasted sample.

The results acquired in this preliminary research, performed on coffee powder, suggest that DSC can represent an interesting analytical tool for qualitative and quantitative evaluation of the thermal phenomena occurring during roasting, thus allowing parameters critical to heat transfer optimisation to be achieved.

Table 1. Moisture, density and colour of coffee roasted at 190°C for up to 20 min

Roasting time (min)	Moisture (%)	Density (g/mL)	Hunter parameters		
			L*	a*	b*
0	11.24	1.15	68.1	+0.2	+17.3
10	2.52	0.90	36.7	+8.5	+15.4
15	2.29	0.72	32.7	+8.2	+12.8
20	1.68	0.60	32.1	+7.9	+10.7

Table 2. Enthalpy of DSC peaks of coffee roasted at 190°C for up to 20 min

Roasting time (min)	Enthalpy (J/g)	
	Peak 1 (20-130°C)	Peak 2 (290-350°C)
0	39.1	44.6 ± 3.1
10	24.8	41.4 ± 1.9
15	19.6	33.9 ± 2.4
20	10.9	31.5 ± 2.0

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Weight Loss in Coffee Processing

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SUMMARY

The secondary weight loss is within proportionately low, hardly perceptible bounds. One is struggling for every tenth of a per cent. If one thinks about the quantity of green coffee processed per day, it becomes clear that the product yield contributes a lot to the economic benefit.

INTRODUCTION

The reproducible output of roasted and ground coffee based on special characteristics in relation to a certain brand and within a small tolerance of quality is of high importance for all roasteries. Production needs to be realised in the most efficient manner. The operating costs and the weight loss are playing a major part in the respective calculation.

One has to keep in mind that coffee is a natural product. Depending on the coffee cultivation, growing site, soil parameters, climate, ripeness, harvest time, green bean processing and storage we can find coffees with all different kinds of quality. They are also influenced depending on age, humidity, defects and green bean size. The above mentioned factors – in combination with hardware – have a very high impact on the weight loss within coffee processing.

The presentation highlights and summarises the bandwidth of primary and secondary key factors.

The following aspects are among others important for a weight loss during the processing of coffee:

- Humidity
- Foreign substances
- Abrasion/dust
- Breakage
- Silver chaff
- Roasting reactions
- Exhaust gases

HUMIDITY

The initial humidity of the green coffee has a crucial influence on the whole weight loss. Different harvests have proven fluctuations of green coffee humidity of up to 3% for the same sort. The existing water is vaporized and evacuated during the roasting process up to a residual content of approx. 1 to 2%. A higher initial humidity inevitably entails a higher degree of shrinkage.

FOREIGN SUBSTANCES

In the interest of a dustfree, troublefree and profitable processing as far as possible, it is absolutely necessary to clean the roasted coffee prior to storage. Any dust, coffee breakage,

coffee shells, wooden parts and other coarse impurities are separated from the green coffee. The loss resulting from the separation of foreign substances depends a lot on the coffee sort and its quality. For certain sorts of Robusta, as much as 0.8% have been separated from the coffee.

Impurities, particularly small stones the size and the weight of which hardly differs from that of the green coffee beans, are separated after the roasting and cooling process. The larger and lighter roasted coffee beans provide an excellent possibility of separating the remaining stones from the coffee. Again, the weight percentage depends on the coffee sort and the coffee quality. For the Robustas, up to 0.1% of stones and similar substances have been detected.

ABRASION/DUST

The abrasion originating from the coffee is usually caused by mechanical influence and consists of dust. During processing, this dust is removed by means of exhaust devices. The weight loss hence caused surely is proportionately very low.

BREAKAGE

The breakage contained in the green coffee has already been mentioned under “Foreign Substances”. By the way, the breakage cannot be described as foreign substance, but it is inevitably separated from the green coffee beans along with the foreign substances during green coffee cleaning. The breakage which might be caused after cleaning entails an additional weight loss. Any plant parts or machines involving the use of air, e.g. roasters, coolers and destoners, hold the danger that small breakage is sucked off.

SILVER CHAFF

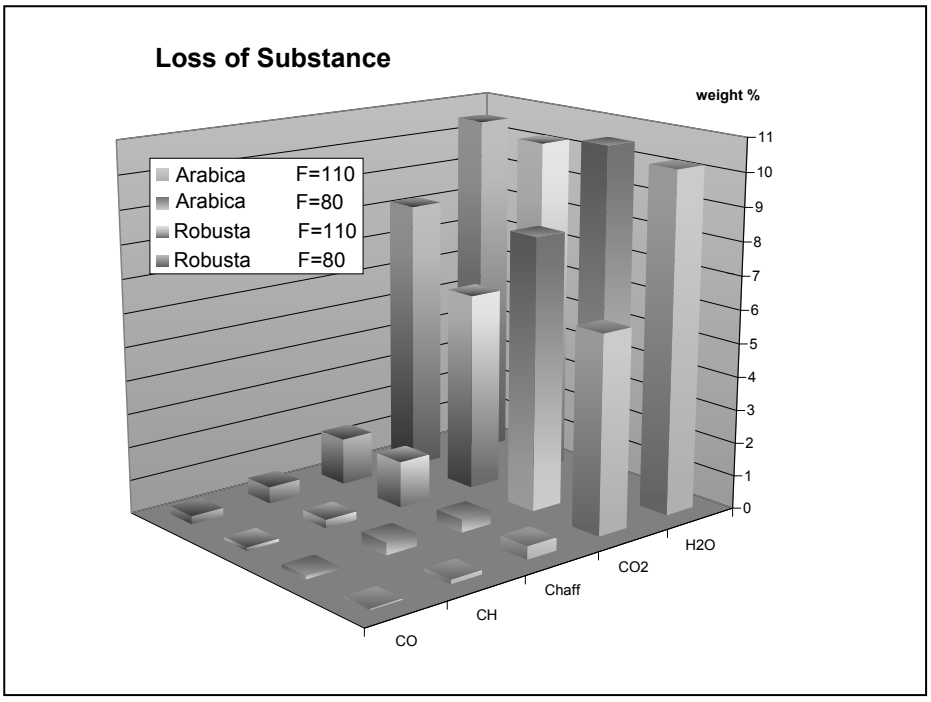
The silver chaff consists among others of the chaff situated on the bean surface. The amount of silver chaff depends on the green coffee sort and the green coffee quality. Washed Arabica coffees, for example, have a lower chaff percentage than unwashed coffees. Chaff amounts ranging from 0.3 to 0.9% have been measured for Arabica coffees and percentages of up to 1.8% have been measured for Robusta coffees.

ROASTING REACTION

The by far highest weight loss within the whole coffee processing is caused during roasting. The weight loss and/or the shrinkage caused during roasting is inseparable from the water content of the coffee, the physical and chemical changes within the coffee and the exhaust gases.

Compared with all other compounds, the percentage of carbon dioxide related to the dry substance loss is by far the highest. A certain Arabica coffee with a roasting degree of 110 scale graduation for example, shows a total loss of approx. 16.8% during roasting. The bar chart shown outlines the loss during roasting for different green coffee sorts and two different roasting degrees.

Upon reaching of a certain coffee temperature or coffee color, the roasting process is mostly instantly stopped by quenching water. The side effect that the water quenching increases the moisture content of the roasted coffee is often used for getting a certain roasted coffee humidity. The roasted coffee weight is thus increased thereby improving the yield.



DEGASSING

The pyrolytic separation of the coffee substance during roasting not only produces the organic compounds, but also the side product carbon dioxide the quantity of which, as mentioned above, by far exceeds that of all other volatile substances. The production of gas results in a strong overpressure within the bean. The cell constitution is thus loosened the volume of the coffee bean is increased. The strong pressure within the bean makes a considerable percentage of gas escape through the drying shakes and the porous structure of the cell wall. Nevertheless, a certain percentage of carbon dioxide remains within the bean with a considerable total pressure. Depending on the coffee sort and the roasting degree, this portion of carbon dioxide is approx. 1%.

In the course of time, the gas enclosed within the coffee cells is released by diffusion. The degassing speed of roasted coffee beans is extremely low. After some 24 hours, a CO_2 portion of 15%, i.e. approx. 0.15% of the coffee weight, is diffused.

A part of the gas is instantly released during grinding, due to the disintegration damaging more or less cells depending on the grinding fineness. With a central European roasting degree and filterfine grinding for example, the spontaneous degassing during grinding entails a weight loss of approx. 0.45 %.

Taking into consideration the above mentioned roasting and grinding degrees, a weight percentage of approx. 0.3 % is degassed within a dwell time of 2 hours after grinding.

The degassing of the coffee and the resulting influence on the weight reflects that an objective and exact determination of the weight loss is extremely difficult and a distinct comparison is only possible - if at all - with indication of exact data such as the dwell time.

The packaging focuses on producing packets with as little overweight as possible, i.e. with slight weight deviations.

The degassing of the coffee, therefore, is of importance for the yield. During vacuum packaging, the gas surrounding the coffee particles and consisting of CO_2 or a mixture of CO_2 and air is sucked off. Compared with the soft packet an additional weight volume of approx. 0.1% is getting lost as a result of this. A total weight loss of approx. 1.0% caused by degassing must be accepted in the field of ground coffee (filter grinding).

INFLUENCE POSSIBILITIES BY THE PRODUCER

The description of the individual aspects resulting in weight loss during the processing of coffee proves that the highest losses are caused by the roasting process itself, the green coffee sort and the green coffee quality. We differentiate between primary and secondary weight loss.

The producer has no influence on the physical and chemical processes during the roasting of coffee nor on the natural impurities in the green coffee and of the chaff volume. The related primary weight loss represents the major part but cannot be avoided during the processing of coffee.

A proper and smooth handling of the product during the whole processing, however, can quite reasonably entail a higher yield. The related loss is called secondary weight loss. The saving of weight represents measures for avoidance of this secondary weight loss.

Upon reception of the green coffee, the basis for the further process steps are already determined. If the green coffee is cracked in this section for example, which not necessarily needs to be visible, this can mean a higher loss rate for the further process. The smooth handling of the coffee right from the beginning thus is a precondition for an economic process in view of the product yield:

- Transport
- Emptying of sacks
- Cleaning
- Weighing
- Storage in silos
- Mixing

INFLUENCE BY THE ROASTING PROCESS

It has so far not yet been possible to clearly determine whether or not the roasting process itself can exert an influence on the weight loss. If at all one can talk about a saving of weight loss during the roasting process, then this is generally related to the higher final content of water in the roasted coffee bean.

Uniform, reproducible roasting results within a tight tolerance regarding the roasting degree and the roasted coffee moisture provide the possibility of exerting a favorable influence on the weight loss. A difference of the roasting degree of 2 scale graduation (Colorette 3) for example corresponds to a weight difference of approx. 0.15%.

The secondary weight loss is influenced during roasting and cooling by:

- Increase of breakage
- Product extraction
- Uniform roasting and cooling
- Reproducible roasting results
- Fluctuations of the roasting degree
- Fluctuations of the roasted coffee moisture.

The following calculation is only meant to provide a rough indication:

One bean more or less, what does it matter anyway?

Assumption:
1,000 t of roasted coffee per year, packed in 250 g-packets with each one bean too much

Sales receipt per roster and packet: DM 5.00

1000 t = 4,000,000 packets à 250 g
= 4,000,000 beans / 6,000 beans / kg = 666.7 kg

666.7 kg / 250 g-packet = 2,666.7 packets * DM 5.00 = DM 13,333.00

The result indicates the tendency that only one bean too much per packet results in a missed sales receipt of DM 13,333.00.

Influences
Weight loss (tendencies)

Primary

Humidity	humid		dry
Foreign substances	many		little
Abrasion /dust	much		little
Breakage	much		little
Silver chaff	many		little
Exhaust gas	much		little

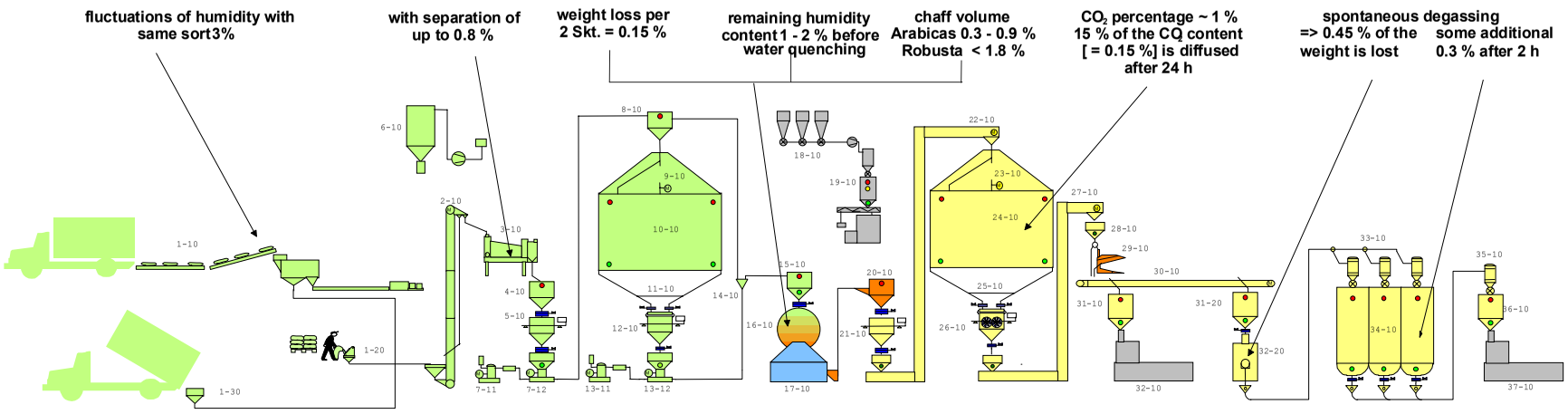
Secondary	possible increase of breakage	danger of product *
extraction	x	x
Transport	x	
Emptying of sacks	x	x
Cleaning	x	x
Weighing	x	x
Storage in silos	x	x
Mixture	x	x

Possible measures for reduction of the weight loss caused by secondary factors:

The one who exactly studies the primary factors and knows how to evaluate them for their daily work and who has his plant examined in view of secondary influences from time to time can be sure to avoid unnecessary losses caused by weight losses.

* In this context, product is defined as weight percentage within the sold coffee.

Primary Weight Loss



Secondary Weight Loss

Possible measures for: Green coffee plant

Avoidance of mistakes relating to the storage of bags (drying-out by environmental influences).

Optimum discharge of the product delivered in bags.

Favorable storage conditions.

Quality assurance by control of moisture, weight and quality, metal separator.

Right choice of screen perforation for cleaning machines.

By-pass of cleaning machines for pre-treated coffees in order to avoid breakage.

Minimization of abrasion and breakage by using suitable transport means. Mechanical transport preferred, especially in case of high performance thereby also reducing energy costs.

Roasting loss

Use of the latest roasting technique

Roasting of single components, e. g. Robusta (quality improvement and reduction of shrinkage).

Recovery of chaff and breakage from the roasting cyclone and admixture to ground coffee of minor quality.

Recovery of breakage from the cooling aggregates, e.g. outlet for very fine breakage of RZ type roasters or cooling screen with double bottom in case of drum roasters.

Selection of right screen perforation for cooling screen, e.g. slotted perforation for the roasting of small-bean or breakage coffee.

Chaff and breakage in the cooler destoner cyclone

Recovery from the cooling and destoner centriclone and use as described under item 2.

Roasted coffee transport

Selection of suitable transport means in order to avoid breakage.

Recovery of normal and very fine breakage after pneumatic and mechanic transport.

Ground coffee grinding, degassing, transport, packaging

Use closed systems in general.
Replace screw transport by suction impulse transport.
Specially formed bins with discharge device instead of geometrically unfavorable bin dimensions with discharge screws.
Minimization of returned product, e. g. Procter & Gamble plant New Orleans.
Suitable optimization measures by Probat entailed provable savings of up to US $ 1 million per year.

Study of the Roasting Parameters and Beverage Quality of Dry Processed and Wet Unfermented Processed Robusta Coffees

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SUMMARY

The objective of this study was to determine the optimum roasting degree for dry processed and wet unfermented processed robusta coffees, using a central composite design, varying roasting time and temperature. The responses were the sensory evaluation of the degree of roast, the loss of weight during roasting and the colour parameters of the roasted bean (L* C*). The results, analysed by ANOVA, RSM and Tukey's test, showed that the optimum roasting ranges for robusta coffees from the two types of processing were similar, being found in the temperature range from 12 to 20 minutes at from 205 to 215°C, the beans being characterised by an L* value between 37.5 and 39.7 and C* value between 4.6 and 6.6, with a loss of weight of from 17 to 20%. With respect to beverage quality, the more severely roasted samples showed a greater intensity of the aroma and flavour considered characteristic of robusta coffee, there being no significant difference ($p \leq 0.05$) between the two types of processing nor between the different treatments.

RESUMEN

El objetivo del trabajo fue determinar el grado óptimo de tostado para los cafés robustas procesados por vía seca y cereza descascarada, a través del método de planificación estrella, utilizando como variables el tiempo y la temperatura de tostado. Las respuestas fueran: el grado óptimo de tostado, obtenido de la evaluación sensorial; la pérdida de peso durante el mismo y parametros de color del grano (L* C*). Los resultados se analisaran por ANOVA, RSM y test de Tukey, los mismos demostraron que el rango óptimo de tostado para los cafés provenientes de los dos procesos fueron semejantes, situandose en los siguientes rangos de tiempo y temperatura:- 12 a 20 min, 205 a 215ºC; caracterizados por L* entre 37,5 a 39,7 y C* 4,6 a 6,6 y la pérdida de peso entre 17 a 20%. Con respecto a la calidad de la bebida, las muestras mas tostadas presentaron mayor intensidad de sabor y aroma característico del café robusta, no habiendo diferencia significativa ($p \leq 0.05$) entre las las dos vías de procesamiento y entre los tratamientos.

INTRODUCTION

The increasing demand for better quality coffee, together with environmental and economic concerns have led to the development of a new post-harvest technology for coffee: wet unfermented processing. This is the wet processing method without the fermentation stage, not requiring large tanks full of water followed by a discharge of organic matter, and leading to drying and storage stages which are more economical than by dry processing.

At the same time, the increasing demand by the ground roasted coffee sector for robusta coffee has created the need for technological studies to increase the quality of this coffee for its subsequent use in blends with arabica coffee.

Thus the objective of this study was to compare robusta coffee prepared by dry processing with that prepared by wet unfermented processing, optimising the roasting of these coffees by sensory methods and comparing them with respect to beverage quality.

METHODOLOGY

Raw material

Dry processed robusta coffee (RS) – type 6, and wet unfermented processed robusta coffee (RCD) – type 5. Both types of coffee were acquired from the Cooabriel Cooperative in São Gabriel da Palha/ES, Brazil.

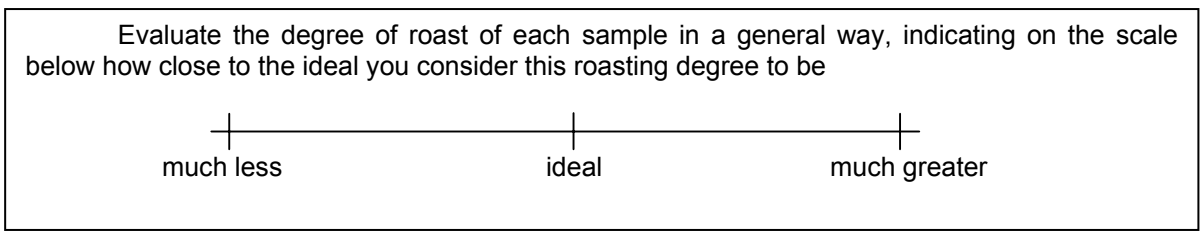
Experimental design to determine the optimum roasting ranges for the robusta coffees (RS & RCD)

A central composite design was used with two independent variables (initial roasting time and temperature), resulting in 11 treatments (Barros Neto, 1996 Khuri and Cornell, 1987) (Table 1).

Table 1. Codified and decodified variables for the experimental design (central composite design) for the optimisation of the roasting process of robusta coffees

	Codified		**Decodified**	
Assays	Time	Temperature	Time (min)	Temperature ^{o}C
1	-1.00	-1.00	8	196
2	1.00	-1.00	18	196
3	-1.00	1.00	8	224
4	1.00	1.00	18	224
5	0.00	0.00	13	210
6	0.00	0.00	13	210
7	0.00	0.00	13	210
8	-1.41	0.00	6	210
9	0.00	1.41	13	230
10	1.41	0.00	20	210
11	0.00	-1.41	13	190

The dependant variables were: the instrumental colour parameters of the coffee beans (L*, C*) measured using a ColorQuest ll/Hunter Lab spectrophotometer; the loss of weight during roasting and the mean of the scores given by 6 expert coffee tasters from the Brazilian Association of Coffee Industries (ABIC), using a 9 cm non-structured just right scale:



Evaluation of the attributes

In an attempt to characterise the degree of roast, it was suggested that 5 experts analyse the intensity of the following attributes on a non-structured 9 cm scale (Stone, 1985; Meilgaard, 1987): aromas and flavours which are burnt, roasted and characteristic of robusta coffee.

The references for the extremes were:

- strong burnt aroma & flavour: colour parameters L* = 33.88; a* = - 0.17 & b* = 0.01
- strong roast aroma and flavour: colour parameters L* = 34.96; a* = 1.49 & b* = 1.52
- characteristic robusta flavour and aroma:
- Weak: species: Conilon, prepared by dry processing, type 6, regular aroma, regular body, low acidity of the citric class, , beverage with a medium robusta taste and grade 3; colour parameters L* = 36.22; a* = 1.61 & b* = 1.76;
- Strong: species: Conilon, dry processed, type 6/7, regular aroma, regular body, low acidity of the citric type, strong robusta taste and grade 2; colour parameters L* = 35.31; a* = 1.20 & b* = 1.40.

For all the sensory analyses, the samples were presented at random in triplicate, codified with 3 digits, according to the traditional "cup test".

The results were analysed by ANOVA (Tukey) and Response Surface Methodology (RSM).

RESULTS AND DISCUSSION

Roasting optimisation

Table 2. Average scores for roasting degree of the robusta coffee samples, RCD & RS, using a non-structured 9 cm just right scale (0-much below the ideal; 4.5=ideal; 9=much above the ideal)

Assays	**4**	**9**	**10**	**5**	**7**	**6**	**3**	**2**	**11**	**1**	**8**
RCD	7.4^{a*}	7.3^{a}	4.5^{b}	3.6^{bc}	3.2^{bc}	2.9^{bc}	2.7^{bcd}	2.3^{cde}	0.9^{def}	0.4^{ef}	0.3^{f}
RS	7.7^{a}	7.4^{a}	4.1^{b}	3.2^{bc}	3.1^{bc}	2.6^{bcd}	1.6^{cde}	1.2^{cde}	0.6^{de}	0.5^{de}	0.2^{e}

** Means with the same letter in the same line no not differ significantly, $p \leq 0,05$*

For both coffees, the results indicated treatment 10 as being ideal, not presenting significant difference at the 5% level with the central point treatments (5, 6 and 7) or, in the case of RCD, with treatment 3.

These results were analysed by RSM. The adjusted models for RCD and RS only considered the parameters with significant effects at the 5% level, presenting a coefficient of determination (R^2) of 94%, the regression being statistically significant (F calculated ≈ 0.3*F tabled). In this manner the response surfaces presented in Figures 1 and 2 were constructed.

To determine the optimum roasting degree, it was decided to base this on the luminosity (L*) and chromaticity ($C^* = \sqrt{a^{*2} + b^{*2}}$) of the bean, since these data are more reproducible than time/temperature ranges, which vary according to the roaster and processing scale (Mendes, 2001). Thus the chromatic co-ordinates of all the treatments were measured and the results analysed by RSM. In this case also, the adjusted models only considered the effects which were significant at the 5% level, all the coefficients of determination being above 97%, the regression significant at the 5% level and the residues uniformly distributed. In this way the

outline surfaces shown in Figures 3 and 4 were constructed, which were superimposed on those of Figures 1 and 2 respectively.

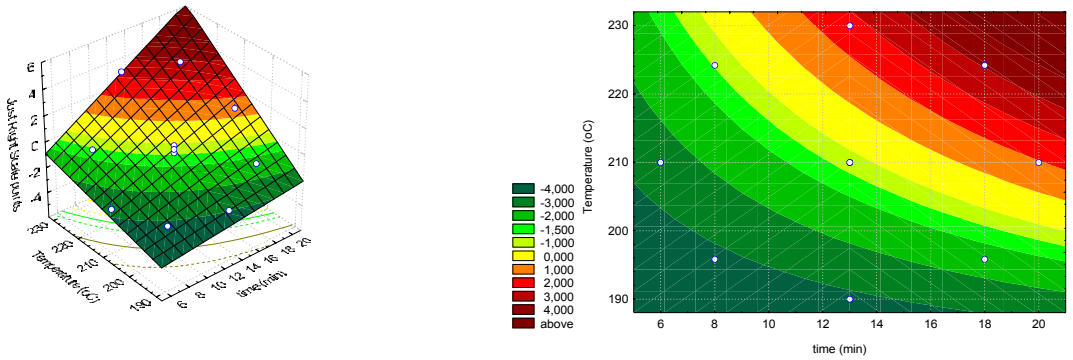


Figure 1. Response and Outline Surfaces for the average scores using the just right scale (-4.5=much below the ideal; 0=ideal; 4.5=much above the ideal) in the evaluation of the ideal roasting degree for RCD robusta coffee

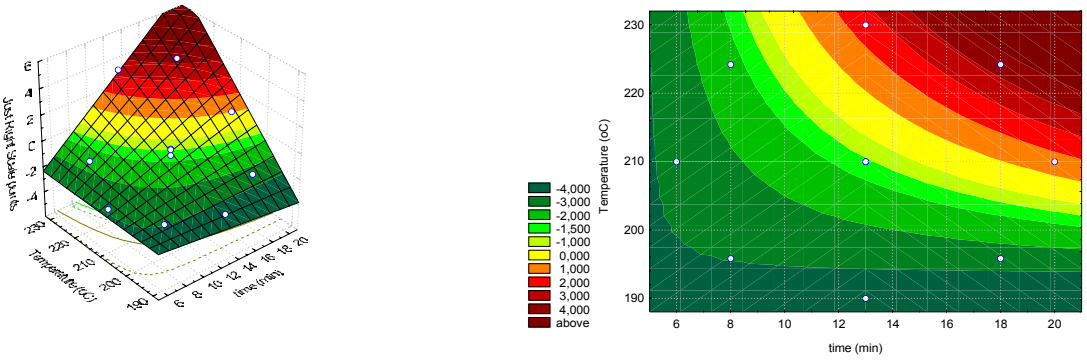


Figure 2. Response and Outline Surfaces for the average scores using the just right scale (-4.5=much below the ideal; 0=ideal; 4.5=much above the ideal) in the evaluation of the ideal roasting degree for RS robusta coffee

From the superimposed surfaces, it can be seen which ranges of L* and C* correspond to the region closest to the ideal roasting degree:

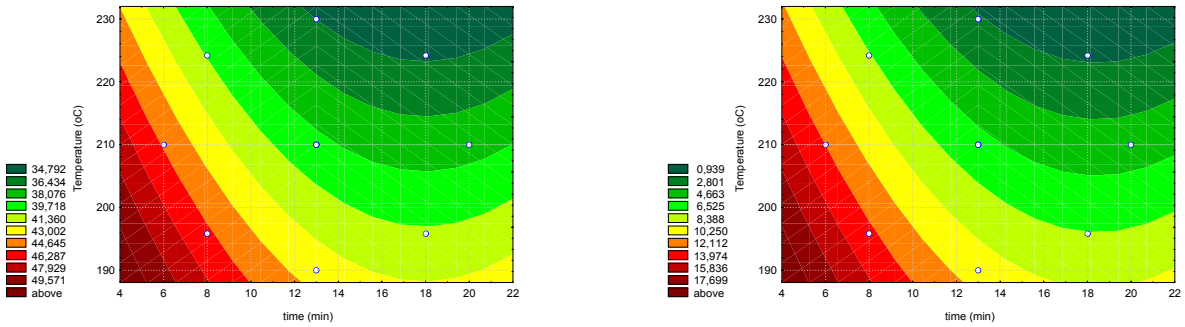
For RCD: L*: 37.4 to 39.0 & C*: 4.55 to 6.52
For RS: L*: 38.1 to 39.7 & C*: 4.60 to 6.50

Another way of characterising roasting degree is from the loss in weight during roasting, if one knows the initial moisture content. In this study the moisture contents for RCD and RS robusta coffees were 11.0% and 11.3% respectively. The loss in weight was calculated for each batch and the means analysed by RSM. As previously shown, the range of loss of weight shown to correspond to the ideal degree of roast was between 17 and 20% for both coffees.

Evaluation of the Beverage Sensory Attributes

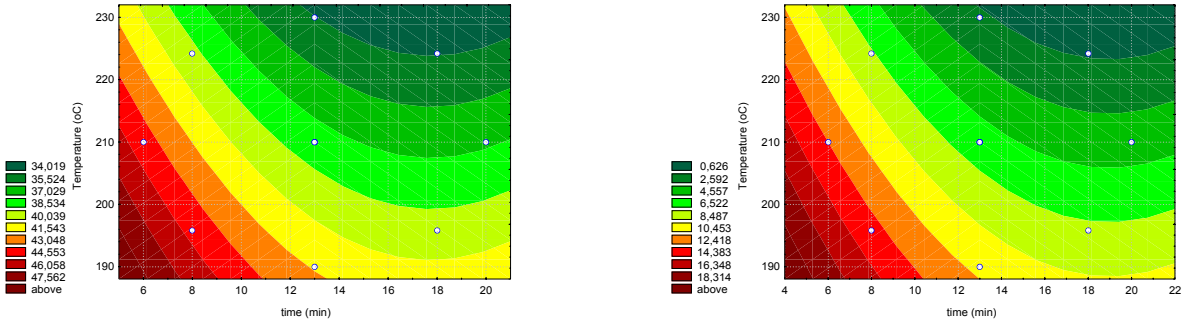
In general, the means for the attributes evaluated were not effective in statistically differentiating the samples. This was due to the high values of the minimum significant difference in all cases: the lowest, for burnt aroma of RCD, was 2.28, and the highest, for characteristic robusta flavour of RS, was 4.22. The minimum significant difference (MSD) for

Tukey's test, is calculated using the following formula: MSD = q √ (MBFresidue/n); where: q is the tabled number; n the number of judgements and MBFresidue, the quadratic mean of the residue.



a) **b)**

Figure 3. Outline surfaces for a) luminosity (L*) e b) chromaticity of RCD robusta coffee at different roasting degrees



a) **b)**

Figure 4. Outline surfaces for a) luminosity (L*) e b) chromaticity of RS robusta coffee at different roasting degrees

Thus, the larger the residue, that is, the greater the dispersion between the results, the greater the minimum significant difference. This is what occurred in this sensory analysis. The scores attributed by the judges varied a lot, leading to highly dispersed results and consequently high values for the residues. This fact confirmed what was observed during the analyses: that there is a great lack of familiarity on the part of the expert coffee tasters with respect to the suggested methodology, using a non-structured scale. It is highly relevant that these experts are highly trained to evaluate arabica coffee using the "cup test" methodology, so greater training is required for them to become familiar with the methodology suggested in this study.

Although some official organs in the coffee sector are working at establishing adequate methodology to classify robusta coffee, it was apparent that there is still a lack of criteria to evaluate this coffee, as shown by the discrepancy in the results for characteristic robusta aroma and flavour.

CONCLUSIONS

From the data presented in this paper, it appears there is no significant difference ($p \leq 0.05$) between dry processed and wet unfermented processed robusta coffees. The optimisation of the roasting degree of these coffees produced practically the same ranges for the colour

parameters L* (luminosity) and C* (chromaticity). The evaluation of sensory attributes also presented a lack of difference, even between the different roasting degrees, explained by the lack of familiarity of the judges with the methodology used and with these types of coffee.

Table 3. Means for the attributes: burnt flavour and aroma and roasted flavour and aroma for RCD and RS robusta coffees

RCD							
Assays	**BA**	assays	**BF**	assays	**RA**	assays	**RF**
9	6.6^{a}	9	6.0^{a}	9	6.2^{a}	9	5.9^{a}
4	5.0^{ab}	4	5.6^{a}	4	5.5^{ab}	4	5.6^{a}
10	2.5^{bc}	10	1.8^{b}	5	3.8^{abc}	10	3.7^{ab}
5	2.0^{bc}	7	1.1^{b}	3	3.5^{abcd}	6	3.3^{b}
6	1.8^{bc}	5	0.9^{b}	6	3.3^{abcd}	3	3.3^{b}
7	1.1^{c}	6	0.7^{b}	7	3.0^{bcde}	7	3.1^{b}
3	1.0^{c}	3	0.5^{b}	10	2.7^{bcde}	5	3.0^{b}
11	0.2^{c}	2	0.1^{b}	2	2.0^{cde}	2	1.7^{bc}
8	0.0^{c}	11	0.1^{b}	11	0.9^{cde}	11	0.5^{c}
2	0.0^{c}	8	0.0^{b}	1	0.6^{de}	8	0.3^{c}
1	0.0^{c}	1	0.0^{b}	8	0.2^{e}	1	0.1^{c}
RS							
assays	**BA**	assays	**BF**	assays	**RA**	assays	**RF**
9	6.0^{a}	9	5.8^{a}	4	6.2^{a}	4	6.1^{a}
4	5.0^{a}	4	5.1^{a}	9	5.8^{a}	9	5.6^{ab}
3	1.4^{b}	10	1.2^{b}	5	3.7^{b}	10	3.7^{abc}
6	1.3^{b}	5	1.1^{b}	6	3.6^{b}	7	3.6^{abc}
5	1.1^{b}	6	1.0^{b}	3	3.5^{b}	3	3.6^{abc}
7	1.0^{b}	3	0.9^{b}	10	3.1^{b}	5	3.3^{bc}
10	0.5^{b}	7	0.9^{b}	7	2.8^{b}	6	2.7^{cd}
2	0.4^{b}	2	0.6^{b}	2	2.4^{bc}	2	1.8^{cde}
11	0.1^{b}	11	0.4^{b}	1	0.5^{cd}	11	0.4^{de}
8	0.0^{b}	8	0.0^{b}	11	0.5^{cd}	1	0.1^{e}
1	0.0^{b}	1	0.0^{b}	8	0.1^{d}	8	0.1^{e}

Means in the same column with the same letter(s) do not differ significantly ($p \leq 0.05$)

Table 4. Means for the attribute: characteristic robusta aroma and flavour for RCD and RS robusta coffees

RCD				*RS*			
assays	**CRA**	assays	**CRF**	assays	**CRA**	assays	**CRF**
4	5.0[a]	4	5.3[a]	4	4.9[a]	4	5.7[a]
9	4.9[a]	9	4.8[ab]	9	4.7[a]	9	5.1[ab]
10	4.1[ab]	10	3.7[abc]	10	3.4[a]	10	4.9[ab]
6	3.2[ab]	6	3.5[abc]	3	3.4[a]	3	4.0[ab]
5	3.1[ab]	3	3.4[abc]	5	3.3[a]	5	3.4[ab]
7	3.0[ab]	7	3.1[abc]	7	3.0[a]	7	3.4[ab]
3	2.5[ab]	5	3.1[abc]	2	2.7[a]	6	3.2[ab]
1	1.8[ab]	1	1.7[bc]	6	2.6[a]	2	2.8[ab]
8	1.7[ab]	2	1.7[bc]	8	1.7[a]	11	2.1[ab]
2	1.6[ab]	8	1.0[c]	1	1.7[a]	8	1.6[ab]
11	0.9[b]	11	0.9[c]	11	1.3[a]	1	1.4[b]

Means in the same column with the same letter(s) do not differ significantly ($p \leq 0.05$)

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Contribution to the Study of Brazilian Coffees – Physical Characteristics

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SUMMARY

Eighty samples of green coffee were evaluated. The coffee samples came from five Brazilian states: Paraná, Minas Gerais, São Paulo e Espírito Santo and Bahia. The coffees were classified according to type, size, apparent and absolute density, a visual evaluation of the aspect/colour and drying efficiency and an instrumental evaluation of color. After roasting for 13 to 15 minutes in the temperature range from 180°C to 210°C, the coffees were ground and evaluated instrumentally by the CIELab color.

The degree of roast was determined using the Agtron disk. The results were evaluated by stastistical inference method according to the mean and standard deviation.

RÉSUMÉ

En ces études ont été évalués quatre-vingts échantillons du café cru, que ont été soumettrés au procès de torréfaction et mouture. Les échantillons de café étaient provenu de cinque états du Brésil de la région sud (Paraná); sudest (Minas Gerais et São Paulo) et nordest (Bahia). Les cafés ont été classifiqués quant à type, grandeur, densité, apparent et real, évaluation visuel quant à l'aspecte/couleur, sèche et couleur instrumental quant à lumière, couleur rouge, jaune et percentage de reflectânce. Le café aprés la torréfaction pendant treize ou quinze minutes parmi 180 C et 210 C fut sumetré a mouture et évalué quant à couleur instrumental par systema CIELab dans un colorimétre Color Eye 2020, Macbeth. Agtron par la determination de degré de torréfaction.

This study was developed through of the "Consóricio Brasileiro de Pesquisa e Desenvolvimento do café PNP&D/Café".

INTRODUCTION

The quality of coffee is directly related to climatic conditions, preparation, hulling and storage, amongst other factors which reflect on the physical, chemical and microbiological characteristics and the sensory quality of the beverage. The classification of coffee through physical analysis is a good tool for quality control. The increase in the value of the coffee and the demand for special coffee brands, encourages the improvement of this product. The classification of coffee is one of the most important items, since this is what defines its price, influencing the decision making about its commercialisation, also being used to monitor the production, evaluating its problems. On the Brazilian market there are several types of coffee called "Cafés do Brasil", that come from different regions with their greatly diversified climatic and geographical characteristics. The crop is well adapted to these characteristics and through the use of good agricultural practices, it is possible to assure a large scale production with good quality. Due to these favourable points, Brazil is the largest coffee producer of

Coffea arabica, and Coffea canephora (Robusta), with a production of 30 million bags in 2000. The internal consumption was about 13 million bags for ground roasted coffee and about 3 million bags for soluble coffee, exportation and the internal stock representing about 20 million bags (www.cafesdobrasil.com.br).In this study, 80 samples of green coffee from 05 Brazilian states with different climatic and geographical characteristics were evaluated, prepared by dry or wet processing and non-fermented wet (pulped) processing. The regions were as follows: MINAS GERAIS with 4 regions: "*Sul de Minas, Cerrado mineiro, Matas de minas e Chapada de Minas*". SÃO PAULO state with 2 regions: the north east, called "*Mogiana"* and the midwestern region. ESPIRITO SANTO state, produces coffee of the species Coffea arabica and Coffea canefora. BAHIA grows Arabica and Robusta produced in the central highlands and in the semi-arid regions in the east. PARANÁ in the south of Brazil, produces coffee in the north of the state

Considering this situation, and the different types and sources of coffee evaluated, the main goal of this study was to know more about Brazilian coffee, looking for typical characteristics of the different origins. This study showed that although the coffees came from different regions, with varying conditions of climate, varieties and processing method, the samples behaved as if belonging to a single batch, following a normal sampling distribution, slightly asymmetric for some parameters. This makes it possible to estimate the ranges and intervals of confidence of the physical parameters evaluated using a statistical inference method.

MATERIAL AND METHODS

Samples

80 green coffees from Paraná, Minas Gerais, São Paulo, Esprírito Santo and Bahia .The coffee samples were received in a random way in the period from February 1999 to December 2000.

Classification

The samples were classified according to type (Resolution 12/78 n°48 C.N.N.P.A, 1989); Teixeira, A.A, 1999); ISO 10470,1993 and size, using alternated Sieves # 19, 18, 17, 16, 15, 14, 13; for flat grains and the mokka grain sieves # 13, 12, 11, 10, 9 and 8 (Teixeira, 1999), ISO/CD 4150, 2000 and the Official Santos Table.

Density

Apparent: Mass (weight) per unit volume (Kramer and Twigg, 1970).

Real: Ratio of mass of coffee from the displaced volume of water (Machado, Prates and Fratini, 1977).

Colour CIELab

Determination using the spectrophotometer Color Eye 2020, software COMCOR 1500 PLUS-V.7,0, illuminant D65, 10°of observation.

Statistical analysis

The results were standardized following this mathematical form:

$$\text{Probability} = \frac{1}{\sqrt{2\pi\sigma}} \exp\left[\frac{(X-\mu)^2}{2\sigma^2}\right]$$

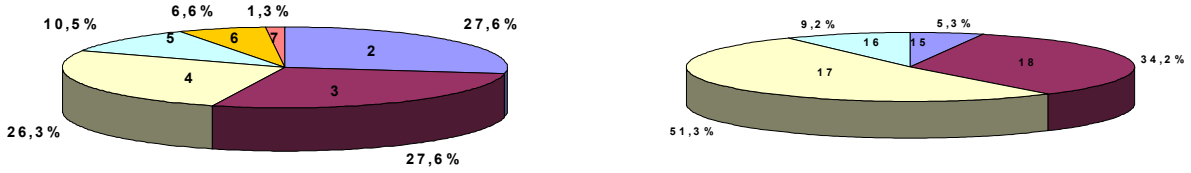
X =random variable; σ^2 *= variance and* μ*= mean* (Gacula, 1984).> In the intervals of confidence (μ - σ *to* $\mu + \sigma$, *(*μ -2σ to μ +2σ) and (μ - 3σ to μ + 3σ, are 68,27 %, 95,45% and 99,73% of the samples.

RESULTS

The coffees presented a good appearance, good drying efficiency and colour varying from a light green hue, typical of coffees prepared by the dry process (natural) to a blue-green hue, typical of coffees prepared by the wet process.

Classification

Type*:*The majority of the coffee samples evaluated were Type 2 (4 defects) or Type 3 (12 defects) with 27.6% of each, the rest being 26.3% Type 4 (26 defects), 10.5% Type 5 (46 defects) 6.6% type 6 (86 defects) and 1.3% Type 7 (160 defects). The most commonly occurring defects were insect damage and malformed and broken beans, with a low incidence of black, green and stinker beans. According to ISO 10470, 1993 and Illy and Viani (1996), the defects we found are inherent to crop, storage and hulling. Figure 1 shows the percentage distribution of classification according to type (a) and size (b) of the green coffee received. With respect to size, the majority were mesh 17 (51.3%), followed by mesh 18 (34.2%), predominating flat beans, then mesh 16 (9.2%) and a lower incidence of mesh 15 (9.2%).



(a) Size grain (b) Type of green coffee

Figure 1. Percentage distribution of classification for type (a) and size (b)

Density

The values found for apparent and real density were within the ranges cited by the Brazilian legislation, according to C.N.N.P.A. (1989), which presents a value of 0.7 g/ml for density and 1.2 g/ml for real (Sivetz, 1963). The interval for apparent density was minimum: 0.63±0.03 g/ml and maximum: 0.69±0.00 g/ml and for real density we found from minimum: 1.14±0.00 g/ml to maximum: 1.26±0.00 g/ml. Figure 2 shows the histogram and the normal distribution.

Colour CIELab

The value of the parameters for CIELab colour were distributed in five intervals of equal amplitude, thus obtaining a frequency histogram, and the values normalised according to the mean and standard deviation, to check if samples of coffee coming from different regions of Brazil could be treated as a single batch. By presenting the colour of the green, roast and ground roasted coffees in a normal distribution by construction of a Gaus curve for the

parameters luminosity, red, yellow and reflectance percentage at 740 nm. Table 1 presents the results of CIElab colour for green, roasted grain and ground roasted coffee.

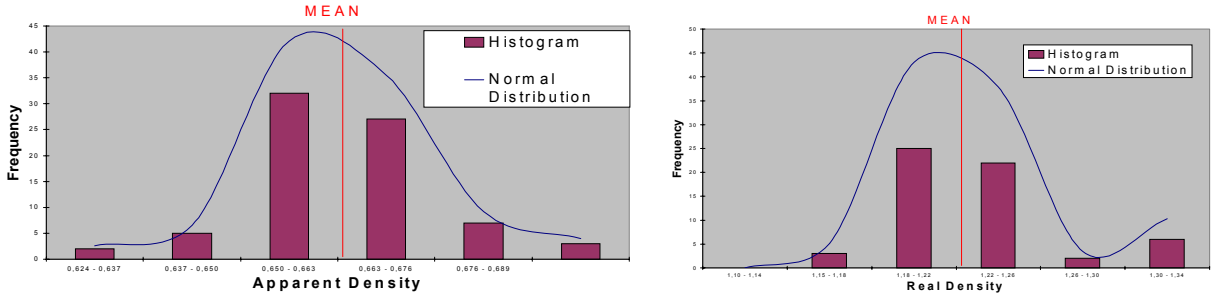


Figure 2. Histogram and normal distribution of apparent and real density

Figure 3 presents the results of the statistical analysis for CIELab colour of the green coffee.

CIELab colour of the roasted grain and ground roasted coffee

Table 2 presents the limits and intervals of confidence for the CIELab parameter for the roasted grain and ground roasted coffee, which was obtained from the normal distribution.

Table 1. CIELab colour of green, roasted grain and ground roasted coffee

Coffee	Luminosity	red	yellow	% Reflectance (740nm)
Green	47.71 ± 3.00	1.86 ± 0.62	18,95 ± 2,30	34,77 ± 3,87
Roasted grain	24.04 ± 2.23	7.36 ± 0.69	10,58 ± 1,62	14,53 ± 2,27
Ground roasted	24.18 ± 2.10	9.02 ± 1.08	19,52 ± 2,35	16,87 ± 2,99

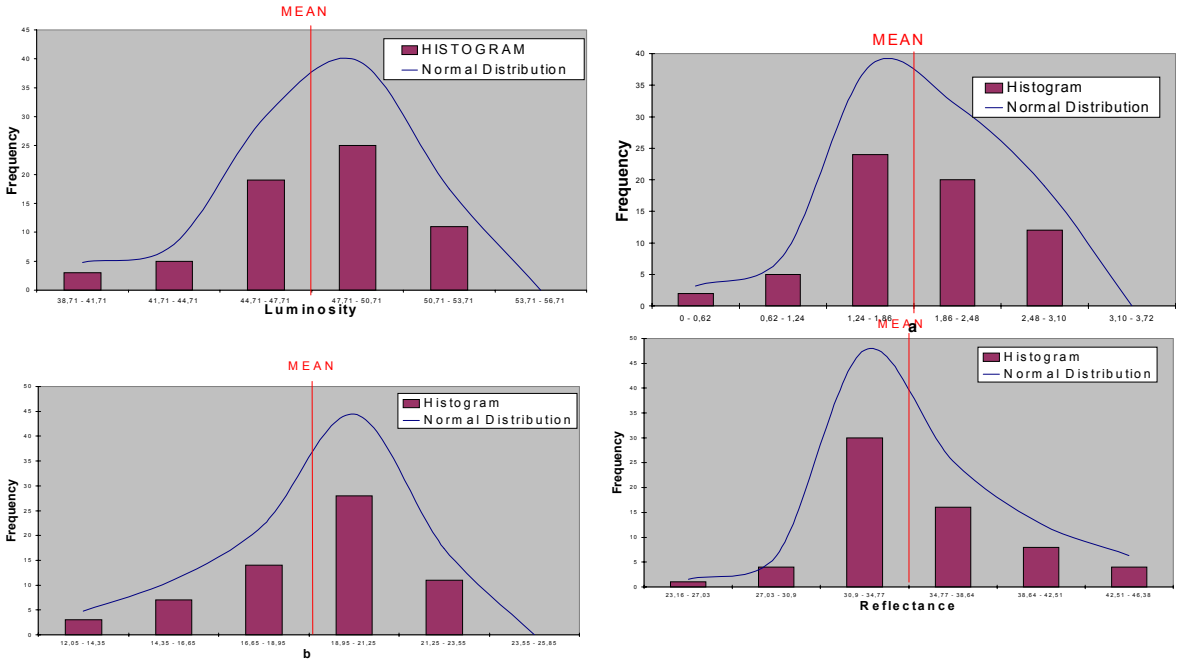


Figure 3. Histogram and normal Distribution Curve for CIELab Colour of *Green Coffee*

CONCLUSION

This study showed that although the coffees came from different regions of Brazil, with varying conditions of climate, varieties and processing methods, the samples behaved as if belonging to a single batch, following a normal sample distribution, slightly asymmetric for some parameters. This makes it possible to estimate the ranges and intervals of confidence for the physical parameters evaluated using a statistical inference method.

Table 2. Limits and intervals of confidence for the CIELab parameter for the roasted grain and the ground roasted coffee

CIELab colour	Confidence interval (%)					
	68,3 %		95,5 %		99,7%	
	Roast grain coffee	Roast and ground coffee	Roast grain coffee	Roast and ground coffee	Roast grain coffee	Roast and ground coffee
Luminosity	21,78- 26,27	22,08 -26,28	19,56 – 28,44	19,98 – 28,38	17,34 – 30,66	17,88 – 30,48
Red	6,62- 7,98	7,94-10,10	5,94 – 8,66	6,86 – 11,18	5,26 – 9,34	5,78 – 12,26
Yellow	8,98 – 12,22	8,17-12,87	7,36 – 13,84	5,82 – 15,22	5,74 –15,46	3,47-17,57
%Reflectance	12,20 – 16,80	13,88-19,86	9,99 – 19,07	10,89 – 22,85	7,60 – 21.4	7,90 – 25,84

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Influence of Extraction Temperature on the Final Quality of Colombian Coffee Cups

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SUMMARY

The final quality in a cup of coffee is influenced by the variety of coffee and the different technological variables which are implicated in the extraction of coffee. Temperature of water has one of the greatest influence on the brew attributes (Lingle, 1996).

The aim of this work was to study the effect of the temperature of the water in the final quality of the coffee cup. The pressure, dose, holder filter diameter and extraction time were fixed using an experimental brew coffee prototype. The different water temperatures studied were: 88º, 92º, 96º, and 98ºC. Colombian Arabica coffee was used. Physico-chemical (total solids, pH, index of foam, density etc), volatile headspace profile and sensory analysis of brew coffee were carried out.

The highest positive key odorants percentage (acetaldehyde, propanal, methylpropanal, 2-methylbutanal, 3-methylbutanal, 2,3-butanedione, 2,3 pentanedione, methanethiol) and the lowest negative key odorants percentage (hexanal, ethylpyrazine, 2-ethyl-6-methylpyrazine, 3,5 dimethyl-2-ethylpirazine, guaiacol) were obtained at 92ºC.

Furthermore, at 92ºC, a lower percentage of panel judges observed negative flavour notes (burnt, acrid) in the coffee cups. The panel perceived the best positive flavour attributes (fruity, cereal, fresh, straw) at 92ºC and 96ºC.

Physico-chemical, taste and mouthfeel parameters were not useful for selecting the optimal temperature of extraction because the results were in normal brew coffee values.

In conclusion, the optimal temperature of extraction in Colombian coffees was 92ºC such as showed the best positive flavour attributes as well as the best key odorant profile.

INTRODUCTION

A fine Espresso Coffee (EC) should have a great amount of persistent and consistent hazelnut foam with “tiger-skin” effect. It should also have a bitter/acid balance taste, a strong body, and an intense and good aroma and flavour (Illy and Viani, 1995; Cipolla, 1999). The final quality in a cup of EC is influenced by the variety of coffee and technological variables related to ground coffee, water and EC machine parameters which are implicated in the extraction of coffee.

The temperature of the water has been reported as the main factor in percolation (Illy and Viani, 1995) and usually a range of 92-96ºC has always been applied (Lingle, 1996). Higher temperatures are matched by increased percentage of extracted material. Extreme high

temperatures could reach an over-extraction of bitter, astringent and negative flavour compounds. By contrast, low temperatures could produce a cold EC with low aroma, taste and body. Therefore, the water temperature control is essential to quality EC.

The aim of this work was to study the effect of the water temperature on the final quality of the Colombia EC cup so as to determine the ideal water temperature.

MATERIALS AND METHODS

Arabica EC (pure *Coffea arabica* from Colombia) was prepared from the 7.5 g of finely ground roasted coffee for a volume of 40 ml, using an experimental EC prototype. EC preparation conditions were fixed: water pressure: 9 atm, extraction time: 21±3 s, holder filter diameter: 38 mm. The water temperatures applied were 88, 92, 96 and 98ºC, which was corresponded to 82±2, 86±2, 90±2 and 92±2ºC extraction temperature, respectively. Two lots of coffee sample were analysed by triplicate.

The EC samples were cooled at 20ºC, and the pH (Orion 420 A Benchtop pH meter), density (densimeter), viscosity (Ostwald viscosimeter) and surface tension (Traube estalagmometer) were measured. The foam index was defined as the ratio (in percentage) of EC foam and liquid volumes. The persistence of foam was defined as the time (in minutes) that the liquid phase below the cream layer took to appear during cooling at room temperature. The total solids were determined by oven drying 40 ml of EC to a constant weight (14 h, 102±3ºC). The extraction was defined as the percentage of total solids with respect to ground roast coffee dose (7.5 g). The concentration was defined as the percentage of total solids with respect to the EC volume (40 ml). The total solids on filtrate were determined by oven drying 40 ml of EC after filtering with Whatman 1 to a constant weight (14 h, 102±3ºC).Total lipids were determined by liquid-liquid extraction using trichloromethane.

Caffeine and Trigonelline

Extract preparation and cleanup for HPLC Analysis. For extraction of caffeine and trigonelline compounds, a C_{18} Sep-Pack cartridge 51910 (Waters Corporation Mildford, MA) was used. Pentoxiphylline was used as the internal standard. The HPLC analysis was achieved with an analytical HPLC unit (Hewlett-Packard 1100) equipped with a Rheodyne injector of 20µl loop, a binary pump and a Diode-array detector. A reversed-phase Hypersil-ODS (5µm particle size, 250 x 4.6 mm) column was used. The mobile phase was an acetonitrile/water (15:85) in isocratic conditions at a constant flow rate of 2.0 ml min^{-1} at 25ºC. Detection was accomplished with a diode-array detector, and chromatograms were recorded at 280 nm.

Chlorogenic acids (5-CQA)

The extraction of 5-CQA and cleanup were carried out according to Bicchi et al. (1997), in the HPLC described above. Pentoxiphylline was used as the internal standard. A Hypersil-ODS column was used. The conditions of the gradient solvent system used were 100% citrate-acetic acid buffer solution (pH=3.0) for 2 minutes, 85:15 buffer/methanol for 8 minutes, both at a flow rate of 0.8 ml min^{-1}, and 85:15 buffer/methanol for 5 minutes at a flow rate of 1.2 ml min^{-1}, at 25ºC. The wavelength of detection was at 325 nm.

Volatile Profile

6m L of EC sample were introduced in a 10 mL vial which was immediately sealed with silicone rubber Teflon caps. Vial was equilibrated at 60ºC for 20 min in the static headspace sampler. Volatile compounds profiles were obtained with the method described by Sanz et al (2001). The relative percentage area (compound area/total area) was calculated for thirteen compounds which had been reported as the most important odorants (Holscher et al., 1990; Blank et al., 1991; Blank et al., 1992; Semmelroch and Grosch, 1995). These compounds were separated in positive and negative key odorants.

Sensory analysis was carried out using a selected and trained panel of judges. During training, a scorecard with two parts (Sensory Descriptive Analysis and Sensory Flavour Profile) was developed. In Sensory Descriptive Analysis, the attributes (Table 2) were measured in 10cm line scales, typically anchored from 0 (none) to 10 (very high). In Sensory Flavour Profile the most frequently odour/flavour attributes described by the judges during the training process were included in the scorecard. The results were expressed as the percentage of judges that perceived each flavour attribute in each EC sample.

Overall quality of EC samples were measured by consumers using a scale from 0 (very bad) to 10 (very good).

Statistical analysis

Analysis of Variance (ANOVA) was applied to the results obtained. The source of variation was the temperature of water. T-Tukey was applied as the test *a posteriori* with a level of signification of 95%. All statistical analyses were performed using the SPSS v.10.0 software package.

RESULTS AND DISCUSSION

Some physical parameters and other physico-chemical related to foam characteristics were not affected significantly with the water temperature (Table 1). However, in general, all physico-chemical parameters related to compound extraction increased significantly with the increase of water temperature. Similar evolution was reported in different coffee brews by other authors (Lingle, 1996; Clarke et al., 1987; Schomer, 1996). Appropriate extraction percentage (22%) (Lingle, 1996) was lightly exceeded by 96ºC and 98ºC EC samples which were perceived by the panel like more bitter and astringent EC's (Table 2). These results might indicate a little over-extraction.

88ºC EC samples were described as significantly less odour and flavour intensities and body, and not hot enough. All positive key odorant, except 2-methylbutanal and 2,3-butanedione, were significatively lower in 88ºC than in 92ºC EC samples (Figure 1), and pyrazines percentages were similar in both samples (Figure 2). Also, in general, positive and negative flavour notes were detected by smaller number of panel judges (Figure 3) because odour and flavour intensities were lower.

The highest positive key odorant percentages and the lowest negative key odorant percentages, except in hexanal and guaiacol, were obtained in 92ºC EC samples. Only significant ($p<0.05$) decreases in acetaldehyde and 3-methylbutanal were found when water temperature increased. By contrast, the pyrazines, which are usually associated with roasted and burnt flavour notes (Ho et al., 1993*),* were more extracted in 96ºC EC sample.

The positive flavour attributes (fruity, malty, fresh and straw) were perceived by a larger number of judges in the 92ºC and 96ºC EC samples. But, in the 96ºC EC samples, negative flavour attributes such as "burnt" were more often detected .

The 98ºC EC samples were defined with negative flavour notes such as "woody", "burnt" and "acrid".

Table 1. Physico-chemical results (mean; standard deviation)

	88ºC	**92ºC**	**96ºC**	**98ºC**
pH	5.3; 0.0[a]	5.4; 0.1[b]	5.4; 0.0[b]	5.4; 0.0[b]
Density (g/ml)	1.010; 0.000 [a]	1.010; 0.000 [a]	1.010; 0.000 [a]	1.010; 0.000 [a]
Viscosity (mN/m^2x s**)**	1.26; 0.06 [a]	1.25; 0.03 [a]	1.35; 0.11 [a]	1.29; 0.031 [a]
Surface Tension (mN/m)	49.33; 0.77 [a]	49.40; 2.06 [a]	49.62; 1.34 [a]	49.96; 1.03 [a]
Foam index (%**)**	12.1; 0.2 [a]	12.3; 0.1 [a]	12.3; 0.2 [a]	12.8; 0.2 [b]
Persistence of foam (min)	26.17; 0.98 [a]	28.17; 2.23 [a]	30.00; 4.56[a]	27.17; 3.12[a]
Total solids (mg/ml)	35.91; 0.50 [a]	39.22; 0.84 [b]	41.69; 0.95 [c]	41.62; 0.34[c]
Extraction (%)	19.2; 0.3 [a]	20.9; 0.5 [b]	22.2; 0.5 [c]	22.2; 0.2 [c]
Concentration (%)	3.6; 0.1 [a]	3.9; 0.1[b]	4.2; 0.1 [c]	4.2; 0.0 [c]
Total solids on filtrate (mg/ml)	34.66; 0.31 [a]	37.37; 0.70 [b]	39.10; 1.27 [c]	40.30; 0.22 [c]
Total lipids (mg/ml)	4.93; 0.03 [a]	5.15; 0.03 [b]	5.86; 0.24 [c]	6.06; 0.02 [d]
Caffeine (mg/ml)	2.02; 0.03 [a]	2.05; 0.03 [a]	2.09; 0.10 [a]	2.31; 0.13 [b]
Trigonelline (mg/ml)	0.81; 0.1 [a]	0.94; 0.02 [b]	1.15; 0.07 [c]	1.39; 0.02 [d]
Chlorogenic Ac. (5-CQA) (mg/ml)	1.01; 0.02 [b]	1.12; 0.02 [c]	1.01; 0.04 [b]	0.92; 0.02 [a]

In each row, different superscripts show significant difference ($p<0.05$) among different water temperatures.

In conclusion, the optimal water temperature of extraction in Colombian Espresso Coffees was 92ºC (86±2ºC extraction temperature) such as showed the best positive flavour attributes as well as the best key odorants profile, and overall quality. In addition, the extraction percentage was between 18% and 22%.

Physico-chemical, taste and mouthfeel parameters were not very useful for selecting the optimal temperature of extraction because the results of every EC sample were within normal ranges. Volatile and Sensory Flavour Profiles have been considered to be the most suitable methods most suitable for selecting the ideal water temperature for obtaining the EC with the best quality.

Table 2. Sensory Descriptive Analysis results (mean; standard deviation)

	88ºC	92ºC	96ºC	98ºC
Odour intensity	5.7; 0.9[a]	6.3; 0.8[b]	6.1; 1.0 [b]	6.7; 0.9 [c]
Body	4.7; 0.6[a]	5.5; 0.8 [b]	5.6; 0.7 [b]	6.0; 0.9[c]
Acidity	5.1; 1.4 [a]	6.0; 1.2 [b]	6.0; 1.4 [b]	5.9; 1.7 [b]
Bitterness	6.1; 1.5 [a]	6.3; 1.2 [ab]	6.6; 0.7 [ab]	6.8; 1.4 [b]
Astringency	5.7; 1.4 [a]	6.0; 1.4 [ab]	6.2; 0.8 [ab]	6.3; 0.7 [b]
Flavour intensity	6.0; 0.8 [a]	6.7; 0.8 [b]	6.4; 1.2 [b]	6.7; 1.0 [b]
Aftertaste intensity	5.8; 0.9 [b]	5.8; 0.9 [b]	5.3; 1.3 [a]	6.0; 0.9 [b]

In each row, different superscripts show significant difference ($p<0.05$) among different water temperatures.

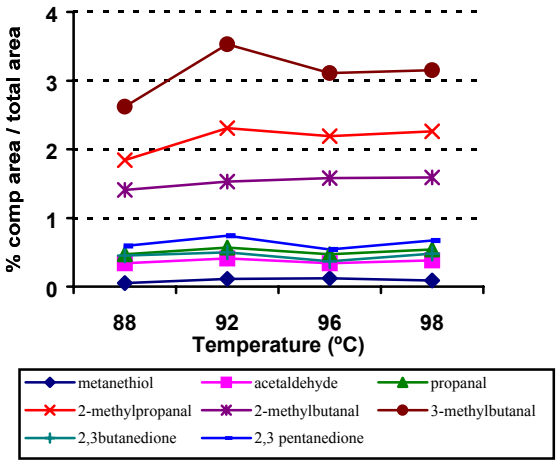

Figure 1. Positive key odorants results

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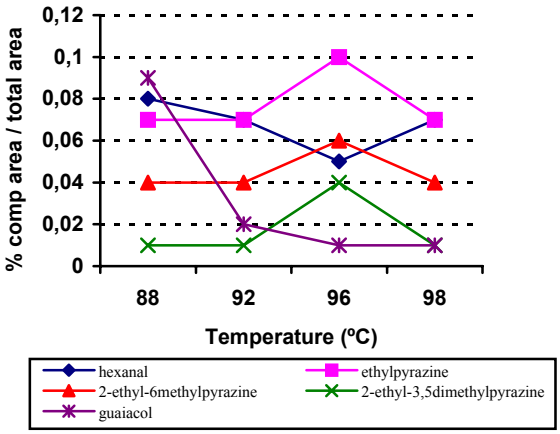
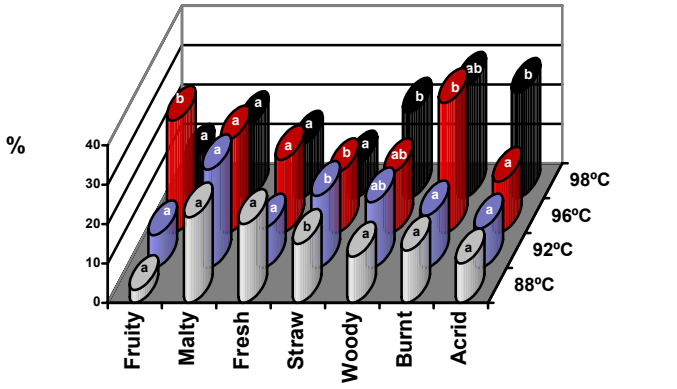
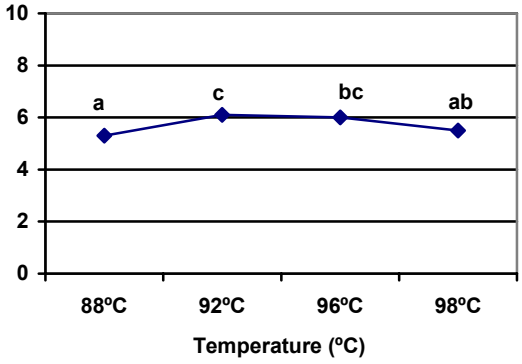


Figure 2. Negative key odorants results



In each flavour characteristics superscripts show significant differences ($p<0.05$) among different water

Figure 3. Sensory Flavour Profile results



Superscripts show significant differences ($p<0.05$) among different water temperatures

Figure 4. Overall Quality results

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Roasted and Ground Coffee in Nitrogen Gas Flushing Packages - II

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SUMMARY

In this paper it was evaluated the effect of the filling conditions in vertical flow pack equipment (with and without nitrogen injection), on the shelf-life of roasted and ground coffee. For this, one type of packaging material consisted of metallized polyester (PETmet) and linear low density polyethylene (LLDPE), (OTR of 0,57 cm^3(STP)/m^2/day at 25°C, 1 atm in dry conditions) were evaluated. After formation, filling and sealing of the packages, they were stored at 25±2°C/65±3%RH. A decrease of the residual oxygen concentration of the headspace was observed during the storage, probably caused by consumption of that gas in oxidation reactions. It caused sensorial changes in the product and limited its shelf-life. The results indicated that the shelf-life to the coffee was at least 6 months at 25°C/65%RH in nitrogen gas flushing packages (2 to 3% of residual oxygen in about 680 mL at 25°C and 0,92 atm), while in the system without nitrogen injection the shelf-life was 3 months.

INTRODUCTION

Roasted and ground coffee is susceptible to loss of quality caused by exposure to oxygen, moisture and high temperatures during storage. Oxidation of the coffee flavor compounds and oxidation of the lipid content of coffee are the main factors directly associated with the deterioration of typical fresh coffee flavor and the development of rancidity, a process also known as staling. In addition to accelerating this process of quality deterioration, high moisture levels are also associated with agglomeration problems and, at a later stage, microbial growth. (Cabral and Fernadez, 1992; Robertson, 1993). Another important factor that impacts coffee quality is the release, upon grinding, of carbon dioxide generated during roasting.

High quality roasted and ground coffee requires packaging systems that protect it from oxygen and moisture in order to preserve the initial coffee quality, better and for a longer time. Within this context, flushing packages with an inert gas or gas mixture is an alternative to the vacuum system and pillow packs currently used in Brazil.

A previously published study (Alves et al., 2000) evaluated the effect of the barrier properties of the packaging material on the shelf-life of coffee when filled in packages flushed with nitrogen.

This paper reports on the findings of a second stage of the same research project. In this second part, efforts concentrated on evaluating the effect of the residual oxygen content in the

headspace on the shelf-life of roasted and ground coffee, in packages made of a material with good oxygen barrier properties.

MATERIALS AND METHODS

Package

Experiments were carried out with the film structure that had presented the best gas barrier properties in the first part of this study (Alves et al., 2000). The structure characteristics of this material are shown in Table 1.

Table 1. Packaging material characteristics

Characteristics	
Composition	print/PET (polyester) - metallised high barrier/LLDPE (linear low density polyethylene)
Total thickness (μm)	68
OTR (cm^3(CNTP)/m^2/day) a 25°C, dry conditions and 1atm	0,57
WVTR (g water/m^2/day) a 38°C/90% RH-	1,13

OTR – oxygen transmission rate; WVTR – water vapor transmission rate

The packages were 16x29 cm in size and each package contained approximately 500 g roasted and ground coffee.

Product

Roasted and ground coffee with the following characteristics: degree of roast: medium; particle size: medium grind; initial moisture level: 3,00% on dry basis (IV= 2,87 to 4,30% d.b.).

Product stability during storage

The roasted and ground coffee was filled into packs of the metallised barrier material using a *flow pack* Bosch filling system, with and without injection of nitrogen. Part of the product flushed with nitrogen was separated to be used as control samples for sensory analysis.

All the product samples were stored at 25±2°C/65±3% RH, except for the control samples, which were stored at –18 ± 1°C. At regular intervals, the packages were evaluated as to the gas composition and total headspace gas volume, as well as seal integrity. At the same time, the roasted and ground coffee was submitted to sensory analysis.

Headspace gas composition measured in a PBI Dansensor, model Combi Check 9800-1 gas analyzing apparatus, using gas samples taken directly from the headspace through a silicone septum.

Total headspace gas volume direct reading at 25ºC and 0,92 atm. Each pack was opened after total immersion in a water bath. The gas from the headspace was collected in a measuring cylinder placed upside down over a funnel (Oliveira et al., 1996).

Seal integrity evaluated using a dye in a low surface tension solution (0,5% Rhodamina B in isopropanol).The dye solution was applied to the internal surface area of the seals. (Oliveira et al., 1996).

Sensory analysis. The samples of roasted and ground coffee were evaluated as to staling and compared to the frozen control samples (0 = no trace of staling and 9 = excessively stale) using a 10 cm non-graduated scale. The limit of acceptability in terms of stale coffee was set at rating 3,5.

RESULTS AND DISCUSSION

Total headspace gas volume

The total headspace gas volume of the packages (at 25°C and 0,92 atm) - mean value of six repetitions – was determined after 8 and 100 days storage. After 8 days, the total headspace gas volume was 687 mL (620-710 mL) and 613 mL (570-650 mL) for the packages with and without nitrogen flushing respectively. After 100 days, these values were 724 mL (710-750 mL) and 502 mL (430-630 mL) for the packages filled with and without nitrogen flushing respectively.

The total headspace gas volume of the packages flushed with N_2 remained practically constant throughout storage at 25°C due to the barrier properties of the packaging material. On the other hand, the packages filled without N_2 injection showed a slight decrease of the total gas volume, most probably due to uptake of O_2 by the product.

Headspace gas composition

Tests performed upon conclusion of the filling operation using measuring devices available at the coffee roasting plant (mean values of 3 packages) showed that the headspace of the packages flushed with N_2 contained 3,3% (3,3 a 3,3%) O_2, whereas those filled without N_2 injection contained as much as 11,9% (8,4 a 13,8%).

The results of the oxygen concentration test, carried out 5 days after processing and filling, showed that the total amount of initial residual oxygen had been consumed by the product in the packages flushed with N_2. The O_2 content in the packages filled without N_2 injection also decreased rapidly, although it took up to 40 days storage at 25°C/65% RH.

A previous study (Alves et al., 2000) reported that the major part of the initial O_2 content in the headspace of N_2 flushed packages was consumed after 15 to 30 days storage. Literature available on the subject mentions that the time required for oxygen depletion in a closed system varies between 3 and 6 weeks, depending on the residual oxygen concentration. (Cabral and Fernandes, 1982).

The O_2 level in the headspace of the packages filled without N_2 flushing dropped back from 21% to 11% due to the release of CO_2 by the product. The residual O_2 concentration in the nitrogen flushed packages was found to remain constant at about 3% in the form-fill machine of the roaster that collaborated with this study, a limitation which, under the current circumstances, cannot be eliminated due to the reduced number of gas injection nozzles (exclusively in the feeding hopper).

The CO_2 levels varied, on average, from 6,5 to 8,0% and 15,9 to 20,8% in the headspace of the packages filled with and without N_2 respectively. No trend towards reduction of the CO_2

levels was observed throughout the entire storage period, which is an indicative of the good gas barrier properties of the packaging material.

On average, the N_2 concentrations ranged from 92,0 to 93,5% and 78,7 to 86,0% in the packages filled with and without nitrogen injection respectively. The N_2 levels measured counterbalanced the oscillations that occurred in the concentration (%) of the other gases and increased proportionally over time due to O_2 uptake by the product.

Seal integrity

No penetration of dye solution was observed in any of the sealed areas of the packages filled with (48 units) and without N_2 injection (40 units), indicating that all the packages were hermetically sealed.

Sensory analysis

The results of sensory analysis of roasted and ground coffee during storage, as determined in this second study are presented in Figure 1.

Linear regression graphs were estimated on the basis of the data generated by sensory analysis of the coffee staling process, in packages with good barrier properties and filled with and without N_2 injection.

The linear equation that describes the development of staleness in the samples without N_2 flushing is: EV=1,550 + 0,021 X Days, adjustment coefficient: 62%. The time required for the samples to attain the limit of acceptability (rating 3,5) on the staling scale (0 – none to 9 – excessively stale) was calculated at 91 days (variation coefficient: 50 to 194 days). The reliability interval presented a wider range of variation due to the fact that the mean values relative to the storage periods were significantly scattered.

The linear equation that describes the development of staleness in the samples with N_2 flushing is: EV=1,092 + 0,022 X DAYS, adjustment coefficient: 79%. The time required for the samples to attain the limit of acceptability (rating 3,5) on the staling scale was calculated at 190 days (variation coefficient: 155 to 260 days).

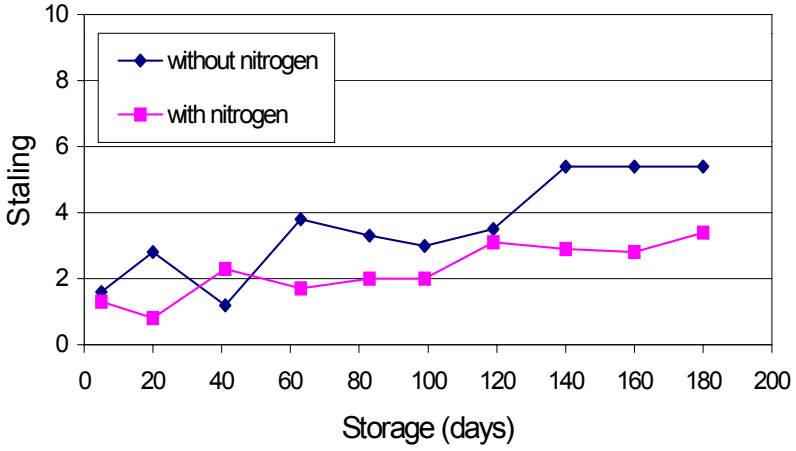

Figure 1. Staling of roasted and ground coffee, in different packaging systems, during storage at 25°C/65% RH

In a previously published study Alves et al. (2000) evaluated the effect of barrier properties of the packaging material on the shelf-life of roasted and ground coffee and reported a minimum shelf-life of 170 days at 25°C/65%RH. This finding confirms that gas flushing packs system preserves the quality of the product for about 6 months, provided that the storage and filling conditions are equivalent to those described in the study.

The achieved results indicate that the nitrogen gas flushing packs produces longer shelf-life in comparison to that obtained using a system without the injection of inert gas and provided that the packaging material used presents an oxygen transmission rate of about 0,5 cm^3(CNTP)/m^2/day.

CONCLUSIONS

Based on the findings of this study, it is possible to conclude that:

- Nitrogen gas flushing packs generates a longer shelf-life compared to that of filling systems **without** the injection of an inert gas.

- When stored under the conditions described in this study, the shelf-life of roasted and ground coffee in packages flushed with nitrogen is, at least, 6 months at 25°C/65%RH.

ACKNOWLEDGEMENTS

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Performance of Metal Cans in the Packaging of Soluble Coffees

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SUMMARY

Metal cans present an excellent performance concerning interactions between the product and the package as well as protection against oxidation, due to their impermeability. Therefore their mechanical performance becomes the main requirement when new specifications are needed. Material reduction has been strongly pursued by the packaging industry in order to reduce costs and the use of natural resources.

Alternative metal cans destined to the packaging of soluble coffee (100 g), composed of cans with two different body thicknesses and another one with reduction of the bottom thickness were studied as to the characteristics of the closure system (easy opening ends) and the mechanical performance. In relation to the closure system, the aperture forces of two brands of full opening ends and one of peel off ends were determined, as well as the residual thickness of the end score line of the full opening ends. For this parameter, a destructive and a non-destructive method were compared. The mechanical performance of the cans was evaluated in relation to the resistance to the punctual impact on different positions of the can, the free fall, the axial load, the vacuum and the transport simulation.

It was verified that the thickness of the body plays an important role in the resistance to the vacuum, that varied from 616,5 mbar to 836,2 mbar for body thickness of 0,15 mm and 0,16mm, respectively. The mechanical characteristics of the bottom material influenced the damage resulting from the free fall impact and the axial load resistance, which presented resistance up to 640,4 kgf, quite superior to the requested value during stacking. Some damages in the cans were observed after about 3000 km of transport simulation. The most frequent alterations were bottom and end abrasions.

Concerning the end performance, there was a linear correlation between the rupture force and the residual thickness of the score line in the first opening area. Full opening and peel off ends presented aperture average forces of (5,2-6,5) kgf and 2,2 kgf, respectively. The residual thickness determined by the non-destructive method presented statistically different results in comparison with the destructive method.

These results permit the conclusion that additional plate thickness reduction is still possible in the production of metal cans for soluble coffee.

INTRODUCTION

Metal cans have intrinsic characteristics that differentiate them from other types of package in several aspects, such as impermeability to gases, water vapor and odors, integrity, high mechanical resistance and protection against light. Thus, their natural characteristics thoroughly meet the requirements of soluble coffee protection.

Metal cans have been going through constant changes, especially in the reduction of the tin coating of the tinplate, due to economical and environmental requirements.

A thickness reduction implies that the mechanical resistance of the cans will be modified. This is controlled by the material hardness, the change of the can shape and the introduction of beads in the body.

The main parameters of the mechanical resistance of the cans are the resistance to the vertical load (necessary for the adequate stacking during distribution, especially in the storage), resistance to impacts that take place both in the can filling line and distribution, resistance to free fall impact (usual in the different steps of handling and distribution), resistance to internal positive or negative pressures and the resistance to vibrations and demands related to transportation.

The utilization of easy-open ends is mandatory for the packaging of several food products, especially soluble coffee, in order to satisfy more and more demanding consumers. Easy-open ends, however, have some limitations in the aperture force, that is, the necessary strength to break and remove the central panel. The aperture force is influenced by the design and residual thickness of the score line and by the characteristics of the material, which should be properly controlled in order to provide comfort and safety to users and to guarantee the can closure integrity until the product is consumed.

OBJECTIVE

To assess different alternatives of cans relative to the materials and types of easy-open ends in order to identify differences in their performance in function of such parameters of the can specification.

METHODOLOGY

Three samples of three-piece cans, identified as A, B and C, with nominal dimensions of 73 mm x 95 mm (diameter and height), produced by two Brazilian industries to pack 100 g of soluble coffee were studied. Cans were made of tinplate, had welded seam and easy-open end.

In relation to the closure system, the ends of cans A, B and C as well as a sample of peel off cans produced by another Brazilian industry were analyzed.

The cans were evaluated as to different parameters, separated in groups according to their characterization, the quality parameters of the easy-open ends and the mechanical and transport resistances, as per the procedures described in Dantas et al. (1996) and Bordin and Garcia (2000). Statistical analysis was based on the variance analysis applying the MSD method through the Statgraphics software, version 6.1.

RESULTS AND DISCUSSION

Table 1 presents the summarized characteristics of the three types of can whereas Tables 2 and 3 show the data of mechanical performance. Table 4 summarizes the characteristics of the ends relative to the easiness to open.

The resistance to vacuum in can C, with body thickness 5% higher in relation to cans A and B and very similar hardness, is 36% superior, showing an important influence of the thickness, although the higher end thickness may have had some influence as well. As to the free fall

impact, sample C presented the highest resistance, followed by sample A and finally B, although the three cans presented a high mean failure height corresponding to about 3 m. Free fall from this height is very unlikely.

Table 1. Main characteristics of the studied cans

		Sample A	Sample B	Sample C
Nominal dimensions (mm)	ϕ x h	73 x 95	73 x 95	73 x 95
Thickness (mm) (x±δx) [1]	Body	0,15 1± 0,0005	0,151 ± 0,0005	0,159 ± 0,0003
	End	0,188 ± 0,0005	0,188 ± 0,0005	0,208 ± 0,0003
	Bottom	0,166 ± 0,0005	0,188 ± 0,0005	0,179±0,0002
Rockwell hardness 30T (x±δx) [2]	Body	76 ± 0,20	76 ± 0,20	75 ± 0,091
	End	62 ± 0,18	62 ± 0,18	62 ± 0,18
	Bottom	73 ± 0,08	67 ± 0,21	74 ± 0,096

[1]Result of 25 determinations; [2]Result of 15 determinations; x±δx: mean ± standard deviation

Table 2. Resistance to vacuum, to free fall impact and to vertical load*

	Sample A	Sample B	Sample C
Resistance to vacuum (mbar) (x±δx)	616,5 ± 0,202	616,5 ± 0,202	836,2 ± 0,156
Resistance to free fall impact (cm) (x±δx)	H= 294 Ho = 300 s = 10,24	H = 288 Ho = 240 s = 82,16	H = 300 Ho = 300 S = 21,63
Resistance to vertical load (kgf) (x±δx)	569,5 ± 15,05	640,4 ± 19,84	572,5 ± 21,13

**Result of 20 determinations; H = mean height of failure (cm); (x±δx): Mean ± standard deviation; H_o = lowest height in which a failure occurred (cm); s = estimated standard deviation (cm)*

Table 3. Resistance to punctual impact on the can body, in width of deformation (cm)[1]

	Impact on the middle of the can height							
	Energy (cm/s)							
	Out of the weld				On the weld			
	100	140	180	220	100	140	180	220
Sample A[2]	-	4,0±0,10	5,0±0,11	5,8±0,04	Pontual	3,8±0,04	4,5±0,14	5,4±0,02
Sample C	-	3,6±0,05	4,4±0,15	5,7±0,03	Pontual	3,5±0,08	4,1±0,08	5,2±0,04
	Impact right below the double seam							
	Energy (cm/s)							
	100	140	180	220	260	280	300	320
Sample A[2]	-	2,4±0,06	2,7±0,05	3,0±0,04	3,2±0,05	3,3±0,05	3,6±0,06	3,8±0,06
Sample C	-	2,5±0,05	2,7±0,07	2,9±0,02	3,2±0,08	3,4±0,04	3,8±0,02	4,1±0,09

[1]Result of 5 determinations, in mean ± standard deviation; [2]Relative to samples A and B

The evaluation of the vertical load resistance showed that, although the alterations of the characteristics of cans A and B were concentrated on the bottom thickness, there was a significant difference in the mean resistance between the two samples. Sample C, with body and end thickness superior to samples A and B, presented very similar results to the first, with no statistical differentiation. This can be explained by the mechanical characteristics of the

metal plate, that is, the bottom higher thickness of sample B, that, combined with the production process by single reduced tin mill and the temper type (T65), have given some additional resistance to sample B double seam.

Table 4. Residual thickness and aperture force of the three samples of ends and aperture force of the *peel off* end [4]

Sample A			Sample B			Sample C		
Aperture force (kgf) [1]								
Rupture	**Tearing**		**Rupture**	**Tearing**		**Rupture**	**Tearing**	
2,88±0,03	5,21±0,05		3,09±0,04	6,34±0,05		2,12±0,05	6,52±0,13	
Sample A			**Sample B**			**Sample C**		
Residual thickness (mm) – Non destructive method[1]								
Position								
1 (Ring)	**2**	**3**	**1 (Ring)**	**2**	**3**	**1 (Ring)**	**2**	**3**
0,093 ± 0,0024	0,081 ± 0,0014	0,100 ± 0,0015	0,074 ± 0,0004	0,076 ± 0,0006	0,078 ± 0,0008	0,099 ± 0,0009	0,116 ± 0,0011	0,100 ± 0,0018
Residual thickness (mm) – Destructive method [2]								
Position								
1 (Ring)	**2**	**3**	**1 (Ring)**	**2**	**3**	**1 (Ring)**	**2**	**3**
0,060 ± 0,0009	0,060 ± 0,0018	0,082 ± 0,0018	0,062 ± 0,0000	0,069 ± 0,0013	0,063 ± 0,0009	0,076 ± 0,0018	0,088 ± 0,0018	0,075 ± 0,0013

[1] Result of 20 determinations, in mean ± standard deviation
[2] Result of 5 determinations, in mean ± standard deviation
[3] Result of 18 determinations, in mean ± standard deviation
[4] Rupture force (kgf) = 6,68 – 59,63 x residual thickness rupture point (mm); r^2 = 0,9994 (Destructive method)

The assessment of the resistance to punctual impact on the middle of the body showed some smaller damage on the side seam of the three samples in relation to the opposite position. Comparison among samples revealed that can C is less resistant than cans A and B.

On the impact close to the double seam, there was no constant difference of performance.

As far as the easy-open ends are concerned, the breaking strength of the three samples are statistically different at the error level of 5%, whereas the tearing strength presents differences only in sample A. The score line design in the three samples was also different. This was another factor of differentiation of the aperture force.

Figure 1 presents the results of the cans after the transport simulation for the period of 6 hours, corresponding to approximately 3000 km. Sample C had the lowest incidence of smashes, followed by samples A and B. The abrasion intensity on both ends was higher in sample C, followed by sample B. Can A showed the least influence of all. With regard to the abrasion on the body close to the ends double seam, sample C presented more damage than samples A and B, which were very similar

The paperboard box went through little alteration. Nevertheless its dimensions were not compatible with the can dimensions, although the package is utilized by an industry of soluble coffee in Brazil. Due to this fact, the movement and vibration of the cans during transportation was increased, thus resulting in intense abrasion. Therefore, the intensity of can damage in regard to abrasion was superior to the condition that would be obtained if a correct secondary package specification were utilized.

CONCLUSIONS

This study shows that it is possible to utilize tinplate of lower thickness to produce cans to pack soluble coffee. An example is the utilization of bottoms with thickness of 0.17 mm, considering that the effect of this lower thickness is offset by the hardness, resulting in a similar or superior performance in relation to the currently used plates. Additional thickness reductions of the body, end and bottom plates may be feasible.

Full open top ends made of tinplate present adequate aperture characteristics, but they are not protected against possible accidents resulting from incorrect or careless aperture procedure. Peel off ends with tinplate ring and the central part made of aluminum sealed to the tinplate present aperture force inferior to full open ends, resulting in a more comfortable and safe handling in relation to the first. This kind of can has been more and more used in cans of soluble coffee in Brazil as from 1999, when this technology was available for different food products.

As far as transportation is concerned, it is essential that the specification of the secondary package takes in consideration a geometric dimensioning so as to avoid unnecessary damages resulting from empty lateral space that allows excessive movements of the cans and the consequent damage to their appearance due to abrasion.

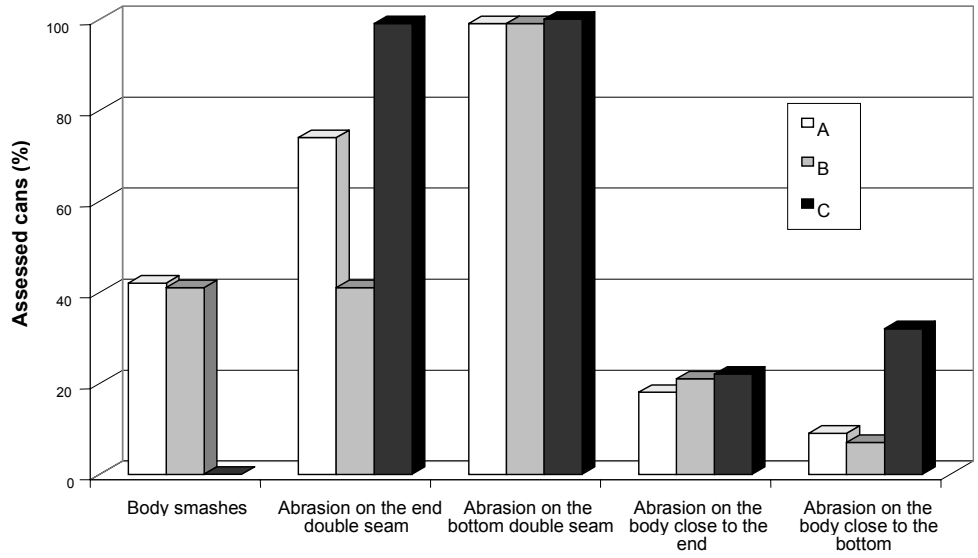

Figure 1. Results of the cans after the transport simulation for the period of 6 hours

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Coffee: Only Good Beans

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Many things have been said about coffee quality. Many programs and projects have been made with the intention to improve the taste and sensorial attributes to attend the expectations of more consumers, mainly the young adults. But none produced positive results. Unfortunately, with some exceptions, the majority of roasters focused on price and neglected quality.

Nothing important has been made to improve the standards of coffee quality. The table of types and defects is the same for 50 years, even if the technologies available and accessible can sort any kind of defected bean. Nowadays, capital defects are unacceptable and the coffee industry uses dark roasting to mask the problem. If coffee had to be sold before roasting, the consumer would accept none of these defects.

Daterra Coffee has adopted a radical approach transforming all the capital defects into bio-energy. As part of its ISO 14001 environmental certification, the residues from processing and the defects are compacted and transformed in briquettes reducing the use of wood to generate heat or decomposed to be used as natural coffee nutrient.

The use of capital defects is unfair to the consumer as well it works against the market, making coffee bitter and unpleasant. Consumers, mainly younger customers, demand mild and fresh coffees that do not accept any bad bean.

CHAPTER ONE

Currently quality concept and its 50 year history
Consequences for the consumer and the new rationale for the market

CHAPTER TWO

Agents against the “non capital defects” philosophy

- Roasters focused on price
- Low quality roasters
- Low level of information to coffee consumers
- Supermarkets price war

CHAPTER THREE

Agents on favor of “zero capital defects” concept

- Fine coffee roasters
- Consequent coffee leaders with quality conscience
- Strategists focusing market growth

New marketers for home roasting

CHAPTER FOUR

How to change this situation?

Adopting a new table of types and defects
Educate consumers
Developing new routes to use coffee beans sorted as capital defects

CHAPTER FIVE

Which impacts should be expected?

Increase on coffee quality beverage
Increase on coffee consumption, mainly the new comers
Balancing demand and world coffee production of good beans
New ways to market coffee

CHAPTER SIX

How to implement change?

ICO adoption of new concepts on defects accepted
Restrictions of capital defects on green coffee – visual and toxins
Information to consumers on quality and ecology
Developing a new global class coffee certification

CHAPTER SEVEN

Daterra strategy and actions

Harvesting defects have been stored to be utilized as bio-energy since 99/00
Researching new technology to develop new uses of non-perfect coffee beans

Highlights of the Workshop "Moisture Management for Mould Prevention"

G. VAN DER STEGEN, M. BLANC, R. VIANI

A lot of work about mould prevention in coffee production has been done in the recent years and good progress has been made since the previous mould prevention Workshop in Helsinki 1999. The Fact Finding Pilot Study (by Univ. of Surrey/UK and Tech. Univ. Denmark) plus further studies in several producing countries identified a number of critical points in the coffee production chain.

Coffee drying is one of the phases, which have a risk of mould growth and mycotoxin formation. Therefore adequate drying to uniformly low moisture levels plus avoiding of local wet spots, by e.g. inhomogeneous drying or rewetting or condensation, is crucial in prevention.

Efficient separation and discarding of husks is another crucial action. Studies have shown that the concerned moulds grow much more easily and produce far more mycotoxin on partially dried flesh of the coffee berry. Husks have been shown to contain by far higher levels of mycotoxin than the coffee beans.

Studies presented in this Workshop showed what the critical moisture levels in the coffee beans are with respect to mould growth and mycotoxin formation. The guidelines for GAP/GMP, first issued after the Nairobi 1997 Workshop, have been made more strict in this respect (new guidelines/Do's & Don'ts are attached*). Considering the vulnerability the drying cherry flesh/husks for mould growth, the critical moisture levels for this substrate are still a white spot.

Simple and cheap devices for (solar) drying of coffee can be of great help in improving drying practices including prevention of rewetting by rain or dew. A number of suggestions were presented during the Workshop and can be found in the reports of the individual presentations.

The national co-ordinators of the lead countries in the ICO/FAO Mould Prevention Project presented the progress of work and results obtained in their countries. Differences between the countries were clearly visible. Overall the progress achieved so far is really encouraging. The individual reports of each national co-ordinator are attached. These presentations plus the exchange of views enables also the other producing countries to take already their actions in enhancing the quality of their coffee. In this respect also the socio-economical factors governing coffee production and its quality in a country or specific area have to be taken into account.

In parallel to the work in producing countries a series of transport trials is being executed, about which was reported also in this workshop. Several studies about the effect of decaffeination and roasting have already been published and some more are in the pipeline. These show that during decaffeination of coffee ~3/4 of OTA is removed from the beans. A series of publications also shows that during roasting about 3/4 of the OTA is degraded (partly by physical removal with the chaff partly by other still not fully elucidated ways).

In the meantime the EU has issued regulation, which enforces by October 1, 2001 maximum limits for ochratoxin A on cereals and dried vine fruits. This regulation will be reviewed by end 2002, with a view to include a maximum limit for coffee, wine, beer, grape juice and cocoa products.

An update on development of mean OTA levels in R&G coffees at the retail level is given in the table below.

Table 1. Mean OTA levels in European R.&G. Coffees (in ppb, with number of samples between brackets)

Year of Sampling	France	Germany	Netherlands	Other EU countries	Total EU
1995	0.6 *(40)*	0.7 *(140)*	1.2 *(77)*	0.8 *(235)*	0.83 *(492)*
1996-'98	0.3 *(20)*	0.6 *(33)*	-	0.8 *(34)*	0.60 *(87)*
1999	*0.4 *(30)*	-	0.4 *(85)*	0.4 *(74)*	0.39 *(189)*
2000-'01	0.3 *(12)*	0.4 *(34)*	-	-	0.36 *(46)*
Overall	0.4 *(102)*	0.6 *(207)*	0.8 *(162)*	0.7 *(343)*	0.67 *(814)*

**Among the French data there is at the moment uncertainty about two samples with identical code, but one labelled "ground", reported to have quite different results. In above table the result for the ground sample is taken, as this difference likely relates to mycotoxin inhomogeneity.*

In the cup, as consumed, the mean level OTA for both R&G and instant coffee at normal dosing strength (40 g/l res. 15 g/l) is currently ~0.02 μg/l. This compares favourably to other concerned drinks.

All presentations given during this Workshop are individually included in these proceedings in order to enable all coffee producing countries to use the findings as much as possible for the enhancement of coffee quality through prevention of mould formation.

INFO ON MOISTURE METERS

Sinar Technology: www.sinar.co.uk; fax: +44 1525 371222;
KPM Aqua-Boy moisture meters: fax: +44 113 268 5054;
Wile moisture meters: www.farmcomp.fi/index.html;

further info also on: www.agric.gov.ab.ca/index.html and www.decagon.com/aqualab/

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***New Guidelines for GAP and GMP : Do's and Don'ts**

International Coffee Organization

Improve Your Coffee Quality By The Prevention Of Mould Growth

Good quality coffee receives bigger payments. In order to protect your revenues from coffee, it is essential that you provide only the best quality commodity available.

USE GOOD AGRICULTURAL PRACTICES TO ACHIEVE RESULTS:

1) During harvest

- The soil under the tree should be covered with a clean sheet of plastic during picking to avoid cherries getting contaminated by dirt or mixed up with mouldy cherries ("gleanings") from previous harvests.
- Cherries that have fallen to the ground are known to be susceptible to mould growth and therefore should not be used.
- Process fresh cherries as quickly as possible. Avoid storage of cherries, especially ripe and over-ripe ones, as any period of storage (in a bag or in a pile) increases the likelihood of mould growth.
- Do not dry on bare soil: Use trays or tarpaulins. Mould spores from previous lots are known to remain on the ground and this could result in clean cherries being contaminated during drying.
- The layer of drying cherries should be not more than 4 cm thick after the first 2-3 days of drying.
- Drying cherries must be regularly raked (5-10 times per day).
- Protect cherries during drying from rain and night dew.
- Avoid all re-wetting of partially dried and dried cherries, protecting them from moisture and rain.

2) During primary processing

- Site processing plant in a dry area, not in a swamp.
- Dispose of pulp from wet processing away from clean dry coffee. Compost it before using it as mulch in the field
- Keep equipment and facilities clean, separating residual partially processed material, and accumulation of dust and discarded material.
- Clean coffee from all husk material – more than 90% of mould comes from husks in sun dried cherries.
- Remove as many defects (husks, un-hulled cherries or mouldy beans) as possible.

- Use clean bags for storing and transporting cleaned dried beans.
- Keep separate cleaned dried beans from discarded material.
- Prevent recontamination by avoiding contact of clean green beans with dust, husks and dirty bags.
- Do not store cleaned, dry green coffee near rejects and husks.
- Processing should achieve a uniform green bean moisture content that is as low as feasible, but certainly not higher than 12,5%.

3) During transport and storage

- Cover bags during transport and storage to prevent re-wetting.
- Load and unload trucks or containers only in dry weather or under cover.
- Do not use damaged containers and prevent water leaks.
- Make sure that pallets or wooden floors of trucks and container are dry.
- Store coffee in well-ventilated and leak-proof warehouses. Store away from the walls.
- Cover bags/loose beans in container with waterproof or water-absorbent cover to prevent re-wetting of the top layer of coffee from condensation.
- Provide good quality control tests (including cupping) and ensure that they are adhered to, especially to check for moisture and defects.

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Influence of Water Activity on Mould Growth and Ochratoxin A Production in Coffee

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SUMMARY

The influence of water activity on mould growth and ochratoxin A (OA) production in raw coffee by *A. ochraceus* and *A. carbonarius* was evaluated. The adsorption isotherm of coffee beans determining the relation between water content during storage and the corresponding values for water activity was carried out. In the next step, coffee beans were inoculated with a mixture of *A. ochraceus* spores and stored in desiccators at 25ºC until reaching equilibrium relative moisture levels corresponding to coffee bean water activity (a_w) levels of 0.80, 0.87 and 0.95, respectively. The same procedure was carried out with a mixture of *A. carbonarius* spores using the same water activity at 25ºC. At a_w level of 0.80 OTA level was very low reaching a maximum level of 0.15 μg/kg and 0.40 for *A. ochraceus* and *A. carbonarius*, respectively after 21 days storage. At water activity above 0.80 (0.87 and 0.95) OA production was present. In a third experiment, coffee beans were inoculated with *A. ochraceus* and stored in desiccators at the same equilibrium moisture levels of the second experiment but at cycling temperatures of 25ºC for 12 h and 14ºC for 12 h. OA production was higher at cycling temperatures than at the 25ºC constant temperature.

INTRODUCTION

Ochratoxin A (OA) is produced by three main species *Penicillium verrucosum, Aspergillus ochraceus,* and *Aspergillus carbonarius.* Among *Aspergillus* species producing OA, the following species were found and isolated from several soils and coffees of São Paulo and Parana farms: *Aspergillus ochraceus, Aspergillus carbonarius* and *Aspergillus niger*(Taniwaki, et al., 1999; Urbano et al., 2000).

The influence of environmental conditions such as moisture content, temperature, incubation time and the nature of the substrate, may play an important role in the colonization by fungi and the amount of OA production in different grains. Coffee is harvested with different moisture content between 50-70% in ripe cherries, to 35-50% in raisins, and 16-30% in dry cherries. Moisture at the end of the drying process must be at or below 12% to prevent fermentations and mould growth (Illy and Viani, 1995). It was shown in previous studies that the presence of OA in coffee was normally a result of badly controlled harvesting procedures, and precarious drying and inadequate storage conditions, allowing for the proliferation of toxigenic fungi (Taniwaki et al., 1999; Urbano et al., 2000). However, the effect of re-wetting of coffee after it has been dried is little known; there is the possibility, though, that this could also be a cause of ochratoxin production, especially during storage and transport.

In the present study, the objective was artificially increase the moisture gain in raw coffee contaminated with *A. ochraceus* and *A. carbonarius* isolated in previous studies. The coffee was submitted to different water activity and temperature conditions in order to evaluate the fungal growth and ochratoxin A formation.

MATERIAL AND METHODS

Adsorption isotherm in raw coffee

Raw *Coffee arabica* from the experimental farm of the Instituto Agronômico de Campinas, Fazenda Santa Eliza, in Campinas (São Paulo, Brazil) was de-husked and sterilised by irradiation using Cobalt 60 at an intensity of 10 kGy at the Centro de Energia Nuclear na Agricultura-CENA/USP (Piracicaba, SP, Brazil).

The coffee was ground under asseptic conditions and its initial moisture content determined in a vaccum oven at 70ºC (AOAC, 1995). Five desiccators were prepared each containing one of the following saturated solutions: sodium chloride (a_w 0.75); ammonium sulphate (a_w 0.80); potassium chloride (a_w 0.84); barium chloride (a_w 0.90) and potassium sulphate (a_w 0.97). Three weighing bottles containing approximately 5 g of ground coffee were placed in each of the desiccators, which were maintained at 25ºC. Using an analytical balance, the weights were monitored constantly until the weights of each sample stabilised, accompanied by determinations of a_w using the Aqualab, model CX2, (Decagon, USA) by dew point method. Each determination was effected in triplicate.

Selection of ochratoxin producing isolates

The following strains of *A. ochraceus* and *A. carbonarius* isolated from raw coffee cultivated on different farms in Parana and São Paulo States of Brazil, and previously confirmed as potential producers of ochratoxin A were used: *Aspergillus ochraceus* CCT 7029 - isolated from raw coffee of Parana State origin; *Aspergillus ochraceus* CCT 7031 - isolated from raw coffee of Parana State origin; *Aspergillus ochraceus* CCT 7036 - isolated from raw coffee of Parana State origin; *Aspergillus carbonarius* CCT 6939 - isolated from raw coffee of Alta Paulista region; *Aspergillus carbonarius* CCT 6940 - isolated from raw coffee of Alta Paulista region; *Aspergillus carbonarius* CCT 6941 - isolated from raw coffee of Alta Paulista region.

Evaluation of ochratoxin A production by *A. ochraceus* and *A. carbonarius* in raw coffee

The saturated solutions used in this experiment were: ammonium sulphate (a_w 0.80); sodium and potassium tatrate (a_w 0.87); and lead nitrate (a_w 0.95). Coffee beans contaminated with 1 g of soil containing a mixture *A. ochraceus* (10^5 CFU/g) spores were placed in each desiccators at different relative humidities, and then incubated at 25ºC. In another experiment with an *A. ochraceus* mixture, coffee beans were incubated in the same water activity solutions as above, but the temperature was cycled at 14 and 25ºC for 12 h. In this experiment OA was analysed at 7, 14 and 21 days, after the coffee had reached the relative humidity equilibrium. The same procedure was carried out with *A. carbonarius* but only at 25ºC for 21 days.

Ochratoxin A analysis

The methodology for OA analysis was that proposed by Pittet et al. (1996), based on the use of the immuno-affinity column of Nakajima et al. (1990) for the clean-up step. The quantification was performed by HPLC in a Shimadzu LC-10VP system (Shimadzu

Corporation, Japan) set at 330 nm excitation and 470 nm emission with fluorescence detection.

RESULTS AND DISCUSSION

Figure 1 shows the isotherm of raw coffee at a temperature of 25ºC. It can be seen that at 12% moisture content, corresponding to an a_w of 0.75, the possibility of fungal growth is extremely remote, except in the case of xerophyllic species. In addition, it is unlike that toxigenic species would be able to develop under these conditions.

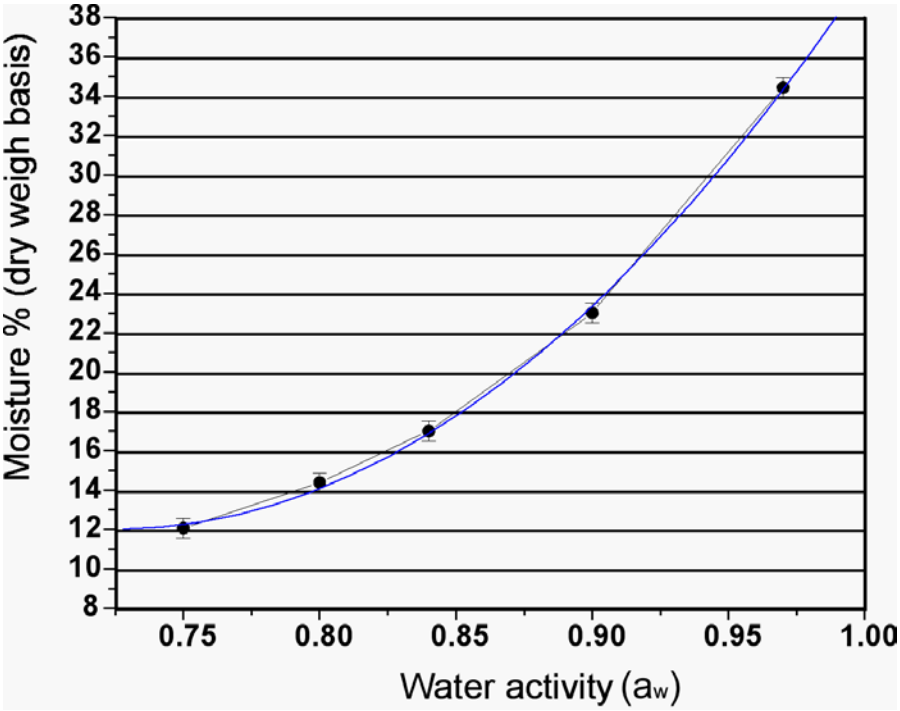

Figure 1. Adsorption isotherm of raw coffee at 25°C

The OA production by *A. ochraceus* in raw coffee at 25ºC with different water activity values after 21 days is shown in Table 1. Equilibrium of the water activity of the coffee samples and the solutions in the desiccators was reached after 44 days. This point was considered as zero time. At a_w of 0.80 there was very little growth and OA production was 0.15 µg/kg. At a_w of 0.87 and 0.95, OA was already produced before the coffee reached the desired water activity. This fact shows that OA is produced when a_w is above 0.80 and with enough water *A. ochraceus* can produce high OA levels in coffee, such as 7178 µg/kg. At cycling temperatures of 14 and 25ºC *A. ochraceus* produced higher OA levels than at a constant temperature of 25ºC, as shown in Table 2. But there was very little growth and OA production at a_w of 0.80.

Table 1. Production of ochratoxin A in raw coffee inoculated with *A. ochraceus* and kept at water activities of 0.80, 0.87 and 0.95 at 25ºC

Water activity (a_w)	Production of ochratoxin A (µg/kg)*	
	Zero (Equilibrium)	21 days
0.80	ND**	0.15
0.87	165.6	2499
0.95	2016	7178

**Mean of 3 repetition*
***Not detected*

Table 2. Production of ochratoxin A (OA) in raw coffee inoculated with *A. ochraceus* and kept at water activities of 0.80, 0.87 and 0.95 at cycling temperatures of 14ºC and 25ºC

	Production of OA (µg/kg)*	
Water activity (a_w)	**Equilibrium (0 days)**	**21 days**
0.80	ND**	0.35
0.87	794	3869
0.95	2307	8338

**Mean of 3 repetition*
***Not detected*

Table 3. Production of ochratoxin A in raw coffee inoculated with *A. carbonarius* and kept at water activities of 0.80, 0.87 and 0.95 at 25ºC

Water activity (a_w)	Production of ochratoxin A (µg/kg)	
	Equilibrium (0 days)	**21 days**
0.80	ND*	0.40
0.87	0.29	0.88
0.95	2.58	2.59
1.00	7.59	9.62

**Not Detected*

The OA production by *A. carbonarius* in raw coffee at 25ºC with different water activity values after 21 days is shown in Table 3. Equilibrium of the water activity of the coffee samples and the solutions in the desiccators was reached after 45 days which was considered as zero time. The maximum level of OA production was 9.62 µg/kg at a_w of 1.00 after 21 days. This fact suggests that at the studied conditions *A. carbonarius* does not produce OA as much as *A. ochraceus* in coffee, even with favourable conditions of water activity and temperature.

More studies are needed to better understand the influence of water activity for growth and OA production by toxigenic fungi in coffee.

CONCLUSIONS

Under the conditions studied these are the conclusions:

- Raw coffee is an adequate substrate for the growth of *A. ochraceus* and OA production.
- *ochraceus* produces higher OA levels than *A. carbonarius* in coffee with water activity at and above 0.87.
- To avoid OA production by *A. ochraceus* and *A. carbonarius,* coffee should be dried to water activity below 0.80 which corresponds to a moisture content of 14% (dry weight basis).

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The Drying Characteristics of Coffee Beans

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SUMMARY

Notwithstanding the importance of coffee as an international commodity, little work has been reported on the drying characteristics of green coffee beans. A programme of experimental work has therefore been carried out using a tray drier in order to investigate the mechanisms of moisture movement during the drying of coffee. The work has shown that the standard drying equation is valid over the initial drying period from the initial moisture content of 60 to 65% w.b. down to a moisture content of 30% w.b.. As expected the drying rate increased with increasing temperature, the rate constant in the initial drying period increasing from 0.00119 min^{-1} at 40°C to 0.0039 min^{-1} at 70°C. Separate measurements on the testa (outer skin) and nib (inner bean) moisture contents with time during drying are also used in order to gain an insight into the controlling mechanisms for moisture transfer during the drying process, this being detailed in the text.

INTRODUCTION

Much of the world's output of coffee is dried by open sundrying, but in order to reduce the amount of handling and to reduce spoilage, the trend is towards increasing use of mechanical driers. There is a variety of types of mechanical dryers available, (Mc Loy, 1959; Sivetz and Foote, 1963) but there is very little reported information on the drying characteristics of coffee beans. Possibly the most comprehensive evaluation of coffee processing is by Sivetz and Decroisier (1979), but even that work does not give details of drying characteristics.

A programme of work has therefore been devised to investigate the basic drying characteristics of coffee beans. The specific objectives of this study were to:

1. Generate single layer drying curves for coffee beans and to examine the effect of
2. changing the major variable, namely drying temperature, on these curves.
3. Establish whether the standard drying equation could be applied to these curves.
4. Obtain a better understanding of the mechanisms of moisture movement during the drying process.

In addition, a study was also carried out in order to evaluate the potential for the use of simple solar driers, particularly for small farmers, for drying coffee beans.

MATERIALS AND METHODS

Raw Material supply

Freshly harvested beans of the robusta variety were obtained for the study from a small scale coffee grower in the East of Trinidad.

Drying equipment and methods

A Lab Line Environette tray drier was used in the drying experiments. This was a fan blown drier of chamber size 2 m high by 0.6 m wide by 0.6 m deep, with temperature control to 0.25°C. The coffee was placed on a wire mesh tray in the drier during the experiments, this being weighed at intervals during drying using a digital top loading balance to determine the basic drying curve. The moisture content of the beans charged to the drier, as well as the final dried product, was measured using the Dean and Stark toluene distillation method. The equilibrium moisture content was measured by drying the rest of the material to constant weight. A series of experiments were carried out over a range of temperatures varying from 40°C to 70°C at intervals of 5°C.

In order to help gain an insight into the mechanisms of moisture movement during drying, an experiment was also carried out whereby beans were taken out of the drier at predetermined intervals, cut in half, and the inner bean carefully separated from the outer skin. The moisture contents of both the bean and the skin were then measured separately. This procedure was also carried out to look at the redistribution of moisture during a 'rest period' i.e. when the beans had been deliberately removed from the drier before completion of the process.

In addition, shrinkage characteristics were determined with time, by taking measurements on individual beans with a vernier caliper. These shrinkage characteristics were determined during the experiment at 70°C.

Field trials on solar drying were also carried out using a simple wire basket solar drier of the type reported by McGaw et al. (1986).

RESULTS AND DISCUSSION

Drying Characteristics

The initial moisture contents in the drying experiments varied from 58 to 64% w.b., and drying times to 12% w.b. increased from 17.5 hours at when drying at 70°C to as much as 80 hours when drying at 40°C as shown in Table 1. The drying curves are plotted in Figure 1 as fractional free moisture content against time for ease of comparison.

The application of the standard drying equation i.e:

$$(w - w_e)/(w_i - w_e) = a \exp(-kt)$$

where:

w is the moisture content at time t
w_i and w_e are the initial and equilibrium moisture contents
a and k are constants with k being referred to as the drying constant

to the results was tested by replotting them as the natural logarithm of the fractional free moisture content against time. This plot gave a linear relationship over the period from the initial moisture content down to a value of around 30% in each case, with the calculated drying constant, k, increasing from 0.0019 $mins^{-1}$ at 40°C up to a value of 0.0039 $mins^{-1}$ at 70°C. These results are also detailed in Table 1.

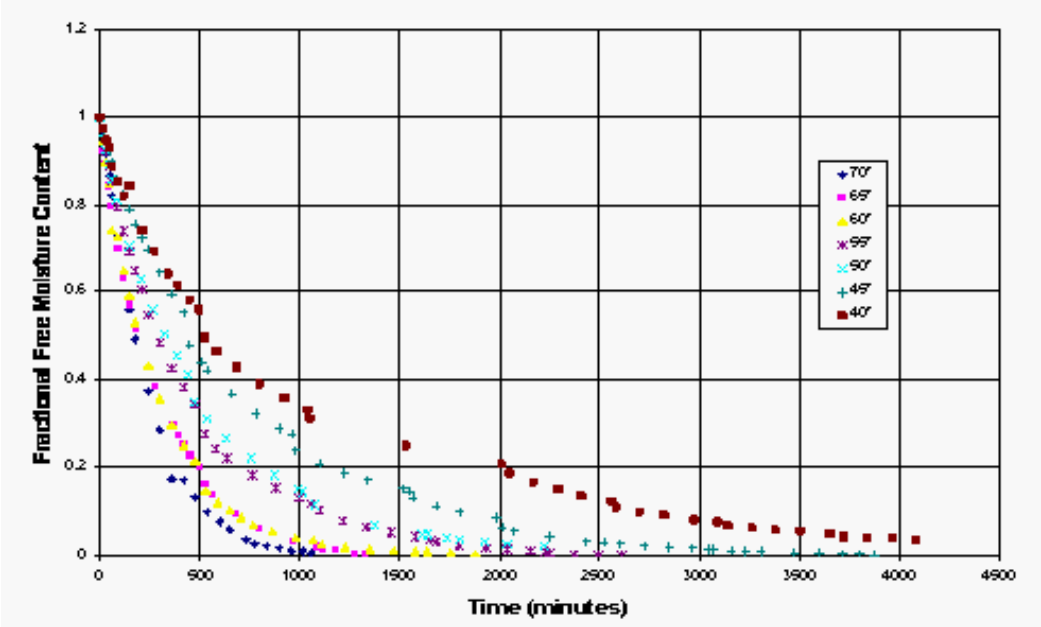

Figure 1. Fractional Free Moisture Content vs Time Curves

Table 1. Summary of Drying Results

Drying Temperature (°C)	Drying Times to 12% w.b. (hrs)	Equilibrium Constant ($mins^{-1}$)
70	17.5	0.00390
65	19.2	0.00318
60	24.2	0.00266
55	30.0	0.00228
50	45.8	0.00185
45	60.0	0.00127
40	80.0	0.00119

The results of the shrinkage experiment are shown in Figure 2, where it is seen that there was a sharp reduction in the size of the beans in the initial stages of drying, covering the period when the moisture content of the beans reduced from >60% to <50%. This took place in the first 3 hours of drying, over which period the actual reduction in size of the beans was measured to be 15.6%. Shrinkage continued thereafter at a much slower rate, as shown in Figure 2, with an overall size reduction of ~27.5%.

Distribution of Moisture between Testa and Nib

In order to obtain information on the mechanisms of moisture movement during drying, an experiment was also carried out whereby the moisture contents of the testa and the nib were measured separately on an hourly basis during drying. These results are detailed in Table 2. In Table 2, reference to 7* indicates that the coffee beans were taken out of the drier when the testa had effectively dried out. They were then left to stand in ambient conditions overnight, a period of about 12 hours, after which they were put back into the drier to complete the drying process. The results in the table clearly show that a redistribution of moisture took place within the beans during the 'rest period'.

In the second experiment where a ‘rest period’ was introduced, the drying temperature was lower i.e. 60°C and it was stared at an earlier period i.e. after 5 hours when the testa moisture content was still ~18% d.b. i.e. ~15% w.b. In this experiment however, the variation in testa

and nib moisture contents were measured at hourly intervals for the duration of the 'rest period'. These results are plotted in Figure 3 where it is seen that there was a steady rise in moisture contents of both the testa and nib up to the final value of the moisture content of the total bean ~43% d.b. i.e. ~29% w.b.. The overall bean moisture content only dropped slightly in the rest period. Observation of cut beans showed that in this period the free moisture located between the testa and the nib simply moved in the two directions until the space was effectively dry.

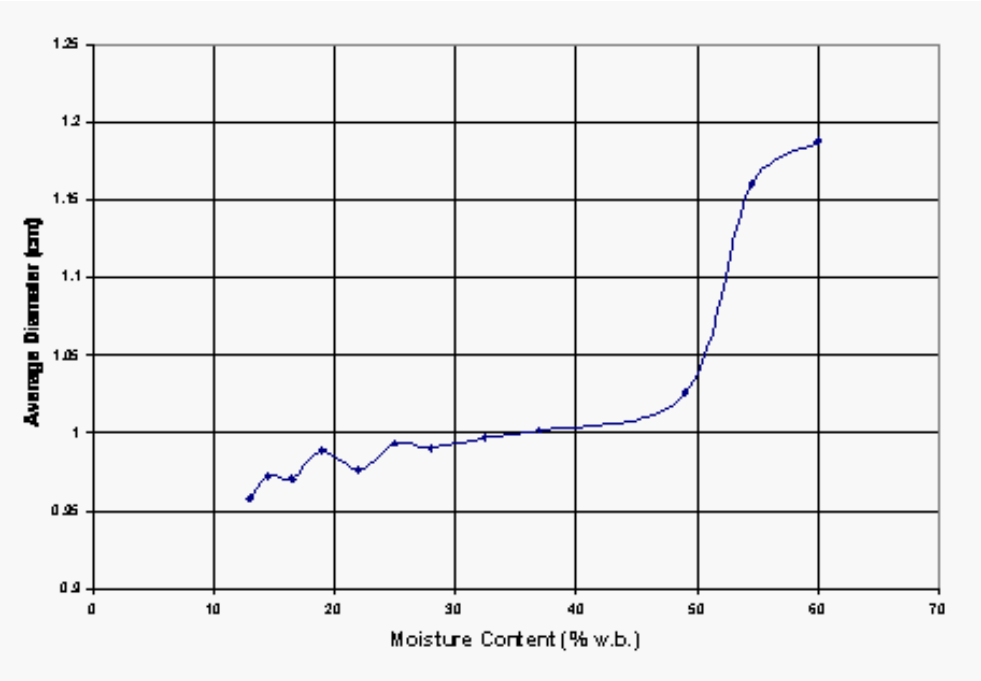

Figure 2. Graph of Size vs Moisture Content (at 70°C)

Solar Drying

Two tests were also carried out on a simple solar drier in order to see if this kind of drier, a wire basket drier, which is particularly appropriate for use by small farmers in the tropics, would be suitable for drying coffee. Since the sequence of drying in a solar drier incorporates the overnight 'rest period', it was predicted that these tests could also provide further data on the influence of introducing a break in drying on the overall drying curve.

These tests were carried out on an agricultural small-holding, with coffee being harvested from that site. Conditions during the tests were basically sunny but with an occasional shower. Maximum temperatures in the drier were just over 40°C around midday, with minimum night temperatures being ~18°C. The results are shown in Figure 4, where it is seen that drying to <12% w.b. was completed in about 5 days. Reference to Figure 4 shows that some moisture loss did in fact take place during the first night, but on subsequent nights there was moisture pick up as a result of the changing atmospheric conditions.

Implications of Experimental Results in Assessing the Mechanisms of Moisture Movement during Drying

Freshly harvested coffee beans have an overall moisture content of around 65% w.b.. In terms of the initial distribution of moisture throughout the beans, there is a film of moisture on the surface, both the testa and nib have moisture contents of around 30% w.b., with the rest of the moisture being located as free moisture between the testa and the nib.

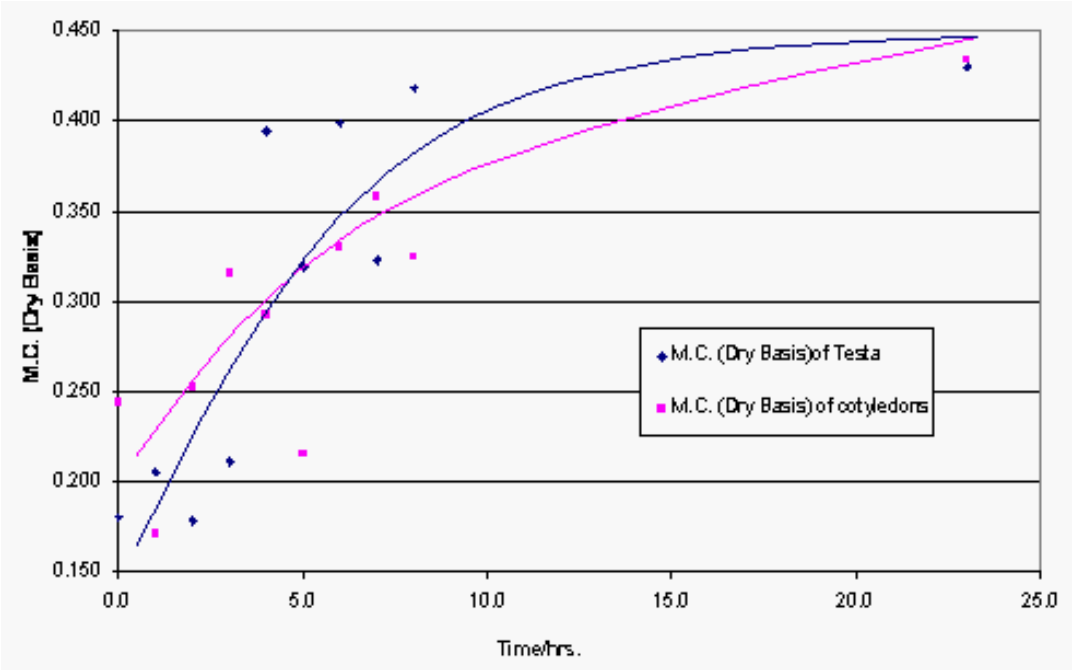

Figure 3. Graph of M. C. (Dry Basis) vs Time for the Testa and Cotyledons during the Rest Period

Table 2. Testa and Nib Moisture contents during drying at 70°C

DryingTime Hrs	Moisture Content of Testa % w.b.	Moisture Content of Nib % w.b.
0	28.8	26.8
1	25.1	25.7
2	21.2	24.7
3	17.1	25.0
4	12.4	26.0
5	5.2	29.9
6	3.3	30.1
7*	1.4	28.7
8	15.5	12.0
9	12.0	9.7
10	8.5	8.8
11	2.9	11.9
12	2.0	10.2

Visual observations of drying indicate that the free moisture on the bean surface dries off quickly, after which the testa begins to dry out. This is confirmed in Table 2, where it is seen that the testa moisture content reduces down to a value of ~1% or so in the first few hours of the operation. During this time the nib moisture content remains roughly constant at a figure of just below 30% wet basis. Referring back to the characteristic drying curve at the same temperature showed that the mean moisture content of the beans at the time at which the testa had dried out, had dropped to a figure of just about 28.5% w.b.; very similar to that of the nib. It may be inferred therefore that, not only had the testa dried out, but virtually all the free water between the testa and the nib had been removed. This was confirmed by visual observation of cut beans removed at this time of drying. In addition, the removal of the free water between the testa and nib would create conditions for shrinkage. This is shown in Figure 2 where it is seen that there was a sharp increase in shrinkage as the bulk of this free water was removed. In this initial period the standard drying equation was found to be valid, with the drying constant being a strong function of temperature as shown in Table 1.

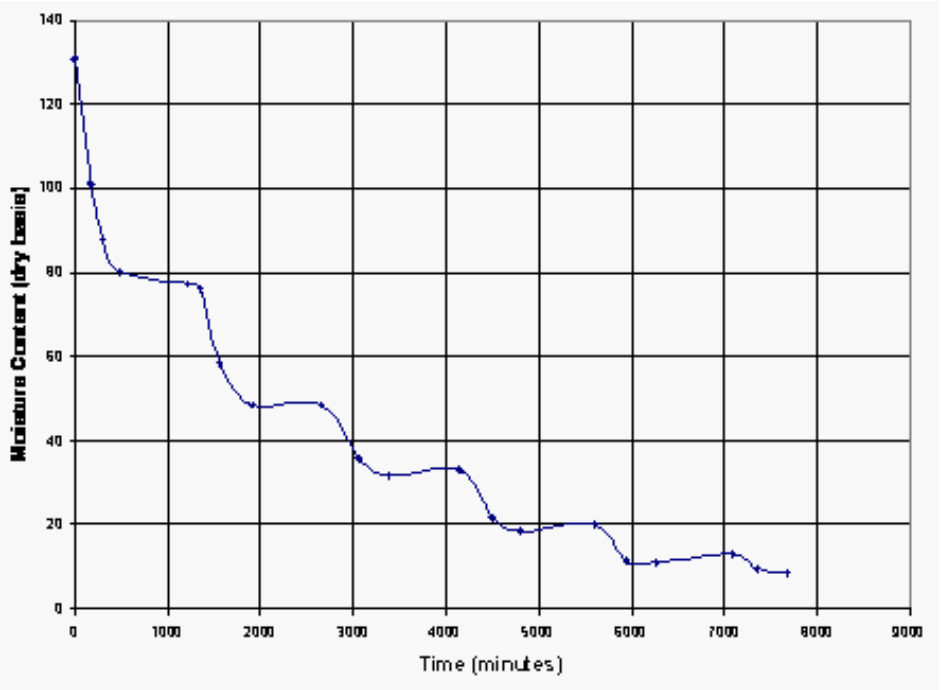

Figure 4. Graph of Moisture Content vs Time

The second period of drying may be explained simply as the removal of the moisture in the nib. Transfer would be by diffusion through the matrix of the nib to its surface. This will be followed by transfer through the testa, and ultimately from the testa surface into the stream of drying air. The standard drying equation was not valid in this period.

The introduction of a rest period allows for the redistribution of moisture in the beans, by virtue of the moisture gradient, but with only a small loss from the bean surface. This redistribution is clearly shown in Table 2 after the 7th hour of drying. The time of drying from 30% w.b. is reduced significantly if the 'rest period' is introduced. Reference to Figure 3 shows that the redistribution of moisture during the 'rest period' is by transfer of the free moisture from the gap between the testa and the nib into both of these parts of the bean.

The influence of the rest period is also shown in the drying curves from the solar drier, where reference to Figure 2 shows that, in the night period between day 1 and day 2, the moisture content dropped from around 80% d.b. to 77% d.b.. Further reference to Figure 2 also shows that when the drying starts again each morning, it is at a higher drying rate.

CONCLUSIONS

The work has:

a) Generated data on the drying characteristics of coffee beans, showing that the standard drying equation is valid down to a moisture content of 30% w.b., the drying constant, k, increasing from 0.00119 min^{-1} at 40°C to 0.0039 min^{-1} at 70°C.
b) Shown that drying is initially from the testa and by transfer of the moisture held between the testa and the nib through the testa to the drying air. The moisture content of the nib remains roughly constant in this period at a little less than 30% w.b.. Subsequently, the moisture in the nib is transferred out through the skin, the rate of drying being reduced significantly in this period.
c) Shown that the introduction of a 'rest period' after the above described initial period, allows the moisture in the nib to redistribute throughout the whole bean. This reduces

the time of application of drying air to the system significantly. It could therefore reduce the operating cost of an artificial drier.

d) Demonstrated that a simple solar drier, in a small farmer situation, can be used to dry coffee to give a satisfactory product.

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Development of Ochratoxin A (OTA) during Robusta (*Coffea canephora*) Coffee Cherry Drying, and Isolation of *Aspergillus carbonarius* Strains that Produce OTA *in vitro* on Coffee Cherries

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SUMMARY

The occurrence and formation of OTA in Robusta coffee was studied for five consecutive seasons under tropical conditions in Thailand. No evidence was found for the production of OTA in green coffee during controlled and ambient silo and bag storage conditions (Bucheli et al., 1998). In contrast, OTA formation was consistently formed during sun drying of coffee cherries in the pulp and parchment (husks) of the cherries (Bucheli et al., 2000). In replicated trials, dried coffee beans (green coffee) were shown to contain on average OTA concentrations that were approximately 100 times lower than those found in husks. OTA contamination of green coffee depended on cherry maturity, with green cherries being the least, and overripe cherries the most susceptible. Defects, and in particular the inclusion of husks, were the most important source of OTA contamination. OTA contamination occurred independently of whether cherries were placed during drying on concrete, bamboo tables or on the ground. Several strains of *Aspergillus carbonarius*, isolated from drying coffee cherries, were found to be potent OTA producers on γ-sterilized coffee cherries under laboratory conditions. A high relative humidity and a temperature of 25°C were optimal for OTA production, yielding up to 4800 μg/kg of OTA after two weeks of incubation. Our results suggest that better raw material quality, an appropriate drying and hulling procedure combined with a reduction of green coffee defects can effectively contribute to the reduction of OTA in green coffee.

INTRODUCTION

Ochratoxin A, a nephrotoxic and nephrocarcinogenic mycotoxin can be produced by several fungal species from the genus *Aspergillus* and by *Penicillium verrucosum* at an a_w of 0.85 and higher (Moss, 1996). It is most commonly found in cereals and cereal products, but a wide range of other commodities including green coffee have been reported as containing the toxin (Pohland et al., 1992). Little is known about the origin of OTA contamination in green coffee. A better understanding of the stage, the conditions and of the microorganisms that lead to OTA contamination of green coffee is needed for taking control measures to reduce OTA in coffee.

The present study summarizes various studies that were undertaken in Thailand and Switzerland to investigate the origin of OTA in green coffee. A comparative industrial storage trial of green Robusta coffee initiated in 1995 indicated clearly that OTA was present before storage, pointing to the possibility that harvesting and postharvest handling of coffee cherries could be the critical steps leading to OTA contamination (Bucheli et al., 1998). Preliminary screening of coffee materials from nine farms in Thailand in 1996 revealed that OTA was

produced on coffee cherries during sun drying. This initiated a series of experiments aimed at determining more precisely the fate of OTA under different conditions and steps of postharvest handling, and data were recently presented that support the hypothesis that the application of coffee cherry sun drying under tropical conditions can induce OTA formation on coffee cherries (Bucheli et al., 2000). In order to protect coffee cherries from OTA formation, it is also important to identify the moulds that are capable of producing this mycotoxin. *Aspergillus ochraceus* was assumed for many years as the main culprit. But *A. ochraceus* is not very frequently encountered in the tropics (Pitt et al., 1994), what makes its true significance questionable. Here we present data that indicate that *A. carbonarius* strains isolated from coffee cherries might be important producers of OTA in coffee cherries (Joosten et al., 2001).

MATERIALS AND METHODS

Plant Material

The green coffee and the coffee cherries used in this study were Robusta (*Coffea canephora* var. *robusta*). The coffee used for the storage trials was harvested between January and March 1995. All drying trials carried out at the Nestlé Coffee Buying Station (Sawi, Thailand) in 1997 to 1999 were performed with coffee cherries produced on three different farms.

Storage conditions and Sampling

Silo and bag storage were compared by storing 600 tons of green coffee each under air-conditioning (30°C, 60% RH), aeration, and nonaeration in three separate silos, at Chachoengsao factory (Nestlé Thailand). Bag storage consisted of 100 tons of green coffee kept under ambient warehouse conditions. Each month, samples of 5 kg were taken from the top and from the wall (top to bottom) of each silo. The corresponding bag sample was composed of aliquots (100 g each) taken from 50 different bags.

Coffee Cherry Drying Experiments

The effect of the drying method (ground, concrete and bamboo table) was tested in early 1997 at the Nestlé Coffee Buying Station, and on three different farms each using one of the drying methods. Drying experiments under optimal local conditions were repeated six times covering the main coffee harvesting period (December 1997 through February 1998). For each of the six trials, 150 kg of mostly ripe cherries were collected from two farms, located ca. 50 km from each other. The mixed material (300 kg) was dried separately on concrete (4 m^2) in 3 x 100 kg lots by spreading the coffee cherries in 2 to 3 cm thick layers, avoiding any rain during the drying period.

Sample Preparation

Coffee samples were kept frozen at –18°C before being air-freighted in dry ice in sealed plastic bags to Nestlé Research Center Lausanne, Switzerland. Samples produced in 1996 and 1997 were freeze-dried, and if necessary oven dried at 70°C, before being dehulled and separated into green coffee and husks (dried pulp and parchment). In 1998, 5 kg of dried cherries of each replicated trial were dehulled by cracking the fruits with a mortar, and separating husks from green coffee by shaking the material on a sieve. Dried cherries, husks and green coffee were milled in a mill operating with a sharp steel disk (Perten Laboratory Mill 3303).

OTA Analysis

Each sample was analyzed for OTA according to the method described by Pittet et al. (1996) and currently used by the industry (van der Stegen et al., 1997). The finely ground test portion was blended for 3 min with methanol/3% sodium hydrogen carbonate (50:50) and filtered. An aliquot of 4 ml filtrate was diluted to 100 ml with phosphate-buffered saline (PBS) and applied to an OchraTest immunoaffinity column containing a monoclonal antibody specific for OTA (Vicam Inc., Watertown, MA, USA). After washing with 10 ml of distilled water, the OTA was eluted with 4 ml of methanol and quantitated by reversed-phase HPLC with fluorescence detection. For samples showing OTA concentrations higher than 10 μg/kg, a confirmation of identity by methyl ester formation was carried out according to a procedure similar to that described by Nesheim et al. (1992).

Microbiological Studies

Thirty strains of *Aspergillus carbonarius* were isolated from ripe or drying coffee cherries during this study. Some of these strains were incubated on ripe cherries from Thailand, previously sterilized by means of γ-irradiation (10 kGy) using a Co^{60} source (Swiss Federal Institute of Technology, Lausanne). To adjust the water activity the sterilized cherries were transferred to desiccators in which the relative humidity (RH) was kept constant by the presence of a beaker containing demineralised water or saturated salt solutions. The following salts were used: KNO_3 (corresponding to ca 94% RH), $BaCl_2$ (ca 90% RH) and KCl (ca 84% RH).

RESULTS AND DISCUSSION

Impact of Storage

OTA was detected in all green coffee samples analyzed during the storage trials (Table 1). The observed differences in OTA contamination of silo- and bag-stored green coffee appeared to be directly linked to the amounts of defects (12% for bags versus 18% for silos). The substantial fluctuations in OTA content were likely due to raw material inhomogeneity, and not caused by microbiological growth during storage (Bucheli et al., 1998). The problem of inhomogeneity of OTA contamination had been described before (Blanc et al., 1998). These results indicated that OTA contamination of green coffee in Thailand is not the result of storage, but most likely linked to the conditions present during green coffee production.

Table 1. OTA content (μg/kg) of green coffee stored under different storage conditions

Storage condition	Month 0	Month 3	Month 5	Month 6	Average
Air-conditioned silo (top)	9.8	4.5	1.3	5.8	5.4
Air-conditioned silo (wall)	3.3	3.9	2.9	4.4	3.6
Aerated silo (top)	3	13.5	6.9	3.7	6.8
Aerated silo (wall)	2	2.2	2.9	4.4	2.9
Non-aerated silo (top)	3.5	2.2	1.4	1.8	2.2
Non-aerated silo (wall)	3	5.2	2.6	2.3	3.3
Bag storage	2.6	1.5	2.2	1.3	1.9
Average	3.9	4.7	2.9	3.4	

Farm Variability

Preliminary sampling on nine farms in the south of Thailand in 1996 indicated that OTA was formed during sun drying in the coffee cherry pericarp (pulp and parchment), the part of the cherry which is removed as husk in the dehulling process. In the samples from the farms, nonripe and ripe cherries contained only trace amounts of OTA in the green coffee (range nd-0.6 μg/kg; mean 0.3 μg/kg) and husk fraction (range 0.2-0.9 μg/kg; mean 0.4 μg/kg) (Bucheli et al. 2000).

Effect of Drying Methodology on OTA Production

Preliminary results obtained in 1996 indicated that the formation of OTA was not affected by the drying methodology used. To verify this observation, the kinetics of coffee cherry drying on concrete, most commonly used in Thailand, were compared in 1997 with drying on ground and bamboo table (Table 2).

Ripe coffee cherries of good quality were used in these experiments, which were basically free of OTA at the beginning of the drying process (day 0). Within the first 5 to 10 days of drying, OTA content increased in most of the husk samples (Table 2). However, its evolution was irregular, largely resembling the observed variability between farms (data not shown). Nevertheless, independent of the drying method used, green coffee had always at each drying stage very low OTA levels (average 0.2 μg/kg). Optimal raw material quality and careful drying were probably reasons for the consistent low contamination of green coffee in these experiments. The effect of ground, concrete and bamboo table drying on green coffee OTA contamination was also assessed during the 1997 harvest on three farms, each applying one of the three drying methods. On average, similar results were obtained for green coffee issued from concrete (4.3 μg/kg) and bamboo table drying (4.0 μg/kg), and lower values for ground drying (1.4 μg/kg). These observations indicate that the three drying methods can all potentially lead to OTA contamination of green coffee.

OTA Contamination in Defected Green Coffee

Industrial storage trials (Table 1) had indicated that OTA content of green coffee was possibly linked to defect count. OTA contamination of common green coffee defects was determined for green coffee delivered directly by farmers to Nestlé Coffee Buying Station (Table 3). Broken and infested beans, together with husks were the most important source of OTA contamination found in green coffee. The occurrence of 319 μg/kg of OTA in an aggregate husk sample of a public coffee dehuller demonstrated that husks are the richest source of OTA in green coffee.

Kinetics of OTA Formation during Cherry Drying

Comparison of drying methodology (Table 2) indicated that OTA formation on coffee cherries occurs frequently in the initial stages of drying. To substantiate this information, the evolution of coffee cherry drying and its impact on OTA formation were studied in 6 replicated trials between December 1997 and February 1998, covering the main coffee harvesting period in Thailand.

Table 2. Effect of coffee drying methodology (ground, concrete, bamboo table) on the OTA contamination of the green coffee and husk fraction of ripe cherries during sun drying

		Ground		Concrete		Bamboo table	
Drying (days)	Experiment	Green coffee	Husk	Green coffee	Husk	Green coffee	Husk
0	1	nd[a]	nd	nd	0.3	nd	nd
	2	nd	0.2	nd	0.9	nd	0.5
	3	nd	3.4	nd	5.6	-	-
5	1	1.1	19.1	0.4	29.9	nd	nd
	2	nd	3.0	0.2	2.9	1.6	34.1
	3	nd	41.3	0.2	220	0.2	10.1
10	1	0.4	5.8	0.3	15.8	nd	nd
	2	nd	1.5	nd	7.2	0.8	19.6
	3	0.2	11.7	0.8	124	0.2	0.9
15	1	0.2	15.6	nd	6.9	nd	nd
	2	0.5	3.3	nd	4.8	0.6	8.7
	3	nd	6.6	nd	31.9	0.6	2.4
20	1	-	-	nd	8.4	nd	0.3
	2	0.3	1.2				
	3	nd	11.6	0.2	16	nd	3.9
	average	0.2		0.2		0.3	

[a] *nd: not detected*

Table 3. OTA content (μg/kg) of four defected green coffee samples found in January 1997 in different deliveries of green coffee to Nestlé Coffee Buying Station (Sawi, Thailand)

Green coffee defect	1	2	3	4	average
Broken beans	11.7	4.7	4.3	7.3	7.0
Infested beans	15.7	8.2	9.4	5.3	9.7
Black beans	0.7	1.4	1.2	5.2	2.1
Partly black beans	8.1	2.9	3.5	1.9	4.1
Husks (Public dehuller)	319				
Husks (Factory)	35.6				

In these trials, initial cherry DM varied between 37 and 41%. Within 10 days of sun drying, cherry DM content reached 79 to 88%, and drying was normally completed upon 15 days. OTA formation in coffee cherries took place in all 6 replicated drying experiments (Table 4). OTA content was normally close to zero at the beginning of drying (day 0), confirming earlier results that ripe cherries are usually not contaminated with OTA at harvest. The rapidity and extent with which OTA appeared on cherries during drying was very variable. OTA increased rapidly within the first 5 days of drying in the first experiment, while it increased more steadily over the whole drying period in the other experiments. At the end of each drying experiment, a batch of 5 kg of dried cherries was separated into green coffee and husks, and analyzed for OTA. Concentrations of OTA in green coffee were on average 1.1% (range 0.4–1.7%) of those found for husks (Table 4). Together with the farm data obtained in 1996 on dried coffee cherries, which indicated an average green coffee concentration of approximately 4% of the concentration found in husks, the results presented here strongly suggest that OTA

formation takes place in the pericarp (pulp and parchment) of coffee cherries during sun drying. Possibly, the principal sources of OTA contaminated green coffee are damaged and overripe cherries susceptible to microbial growth during sun drying, and also the dehulling process, a dusty procedure where OTA contaminated cherries come into direct contact with green coffee.

Table 4. Evolution of OTA content (μg/kg) in six replicated coffee cherry drying trials

		Experiment					
Drying (days)		1	2	3	4	5	6
0	Cherries	0.7 +/- 0	0.4 +/- 0.1	0.5 +/- 0.3	-	3.9 +/- 1.3	49 +/- 80
5	Cherries	436 +/- 85	109 +/- 69	9.3 +/- 4.8	99 +/- 7	22 +/- 12	109 +/- 100
10	Cherries	256 +/- 81	189 +/- 113	62 +/- 57	109 +/- 81	68 +/- 106	174 +/- 38
15	Cherries	353 +/- 29	-	-	43 +/- 11	161 +/- 232	-
	Husks	-	190 +/- 63	88 +/- 20	162 +/- 23	77 +/- 18	407 +/- 278
	Green coffee	-	2.5 +/- 1.2	1.1 +/- 0.5	2.8 +/- 1.1	0.6 +/- 0.3	1.7 +/- 0.9
20	Husks	677 +/- 130					
	Green coffee	6.4 +/- 3.8					
G. coffee/husks (%)		0.9	1.3	1.3	1.7	0.8	0.4

Isolation of OTA Producing Moulds

As a cause and effect relationship between the ochratoxigenic *Aspergillus ochraceus* and OTA in green coffee has not been demonstrated (Mantle, 1998), other microorganisms or moulds might produce OTA on coffee. Interestingly, *Aspergillus niger* and *Aspergillus carbonarius* were shown to produce OTA under in vitro conditions (Heenan et al., 1998). These findings initiated attempts to isolate other OTA producing moulds from drying coffee cherries from Thailand. Six strains of *A. carbonarius* were identified that grew abundantly and produced a significant amount of OTA on γ-irradiated coffee cherries (Table 5), with the maximum being attained by strain M333 (930 μg/kg).

Growth of *A. carbonarius* on Coffee Cherries

Aspergillus carbonarius M333 was used for subsequent experiments in which the influence of temperature and a_w on OTA production was investigated (Table 6). It was found that far greater quantities of OTA could be produced on coffee cherries previously equilibrated at 100% RH. At the optimal temperature (25°C), 4810 μg/kg was detected after two weeks of incubation. At 20 and 30°C, OTA accumulation was still considerable (3380 and 2790 μg/kg,

respectively), but at 35°C only 7 µg/kg was produced. OTA production on coffee cherries was much lower at reduced water activities. At 0.94 a_w, 230 µg/kg was found after two weeks, although growth was apparently not inhibited under these conditions. OTA production was not detected at 0.90 a_w.

Table 5. Growth and OTA production of Aspergilli on coffee cherries

Species	Strain	Source	Growth[a]	OTA (µg/kg)
A. ochraceus	M75	Coffee, Brazil	+	nd[b]
A. carbonarius	M323[c]	Coffee, Thailand	++	280
A. carbonarius	M324[c]	Coffee, Thailand	++	650
A. carbonarius	M325[c]	Apples	-	nd
A. carbonarius	M326[c]	Tomatoes	++	115
A. carbonarius	CBS 110.49	Coffee	+	3
A. carbonarius	CBS 127.49	Air	++	410
A. carbonarius	M333	Coffee, Thailand	++	930
A. carbonarius	M334	Coffee, Thailand	++	180
A. carbonarius	M335	Coffee, Thailand	++	680
A. carbonarius	M336	Coffee, Thailand	++	920

[a] *++: good growth, +: moderate growth, +/-: slight growth, - : no growth*
[b] *nd: not detected (< 2µg kg^{-1})*
[c] *Identity confirmed by Centraalbureau voor Schimmelcultures, the Netherlands*

Table 6. Influence of temperature and water activity on growth and OTA production by *A. carbonarius* M333 on coffee cherries

Temperature (°C)	a_w	Growth[a]	OTA (µg/kg)
25	0.99	++	4810
25	0.94	++	230
25	0.90	+/-	nd[b]
25	0.85	-	nd
20	0.99	++	3380
27	0.99	++	4490
30	0.99	++	2790
35	0.99	+	7
37	0.99	+	nd

[a] *++: good growth, +: moderate growth,*
+/-: slight growth
-: no growth
[b] *nd: not detected (limit of detection, 2 µg kg^{-1}.*

CONCLUSIONS

This study provides data on the generation of OTA in Robusta green coffee that was obtained under field and experimental coffee cherry sun drying, and during industrial storage in Thailand. It is demonstrated that OTA is mainly produced during coffee cherry drying. The presence of low quality coffee materials such as damaged and overripe cherries is most likely one of the main parameters contributing to OTA development. Several strains of *Aspergillus carbonarius*, isolated from coffee cherries in Thailand, were found to be potent OTA producers on coffee cherries under laboratory conditions. High RH and 25°C were optimal for OTA production. The presented results indicate that the application of good coffee postharvest practices (Barel and Jacquet, 1994) should contribute to the reduction of OTA in green coffee.

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On the Activity of Fungi in Coffee in Relation to Ochratoxin A Production

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SUMMARY

Below are the main points of relative certainty and some related questions.

There are three OTA-producing fungi of significance that occur in association with coffee or coffee production systems: *Aspergillus niger, A. carbonarius* and *A. ochraceus* (and other Circumdati species).

Hydrophilic, non-OTA producers and seed homeostasis stabilise the commodity at high moisture content with respect to OTA contamination: does fermentation provide remediation? Is wet cherry safer than half-dry cherry?

Based on occurrence, *A. ochraceus* is the best-adapted coffee OTA-toxigen: is it a true coffee associate?

The most common, *A. niger,* has a low capacity for OTA production: is it an important quality degrader?

A. carbonarius has a narrower range of toxin-producing conditions suggesting that in most of it's OTA-producing range it would not compete successfully with hydrophilic coffee associates: is it an occasionally successful opportunist?

The balance of probability is that *A. ochraceus* is the source of OTA problems in coffee.

OTA toxigens are more common later in processing: is this *de novo* infection and if so what is the source and mechanism?

There is nothing so far to link any particular practice to **occurrence** of toxigenic fungi.

There is every reason to believe practice, when toxigenic fungi are present, is responsible for the development of OTA levels that constitute a problem: to what extent does growth and toxin production before harvest take place?

INTRODUCTION

In this paper the coffee production system is considered as an ecological system peculiar only in that man contributes greatly to its physical environment. This is true of all agricultural production systems. The objective is to assess our state of understanding as to how OTA arises in coffee, to be found in the finished product. By trying to synthesize what we know and using this to enunciate hypotheses along these lines, further research can be directed toward the most promising areas. The discussion, although dealing with a fully integrated system, will inevitably do so in terms of elements of the system. This is only because it is

impossible to write about a system (or even think about one!) in terms other than its parts. This is despite the fact that a system produces an outcome through the simultaneous interaction of all its components – dismantle the system and you dismantle the interaction, the nub of developing a predictive understanding.

Before moving to the detail, a few general points. It is clear that we are not necessarily seeking a single mechanism or scenario leading to OTA contamination of the coffee bean. It is also clear that we are looking at few potential OTA-producing fungi, not many, and that there is likewise a restricted range of fungi and other organisms these interact with and that all are adapted to conditions where coffee thrive and to each other. The climatic conditions we know to be fairly restricted, those where coffee grows, and the coffee plant and production methodologies further delineate a specific and relatively narrow set of what are sometimes called 'forcing functions'. This term refers to phenomena that are powerful or pervasive enough to bias outcomes. Things like the tides in estuarine systems, altitude in alpine systems or, in coffee production, the regime of the drying yard or interaction with the 'host' plant tissue.

To focus the discussion, the author proposes to identify the following elements of the system:

- Physical conditions of coffee production; climate + inputs + processing
- Associated organisms; micro-organisms + insects + other plants
- Nature of the host; plant immune system + commensal community + composition and structure of the tissue
- Properties of the toxigenic mould; physiological limits + success relative to the mixed community + distribution mechanism

Figure 1 relates fruit development, the agronomic part of the production chain, to subsequent methods of processing and to human influence on the system. The list is indicative rather than exhaustive but points out several husbandry issues that could be of consequence to the outcome. 'Rats' is included on the list only to remind us of the large collection of human-associated organisms that settle where man settles.

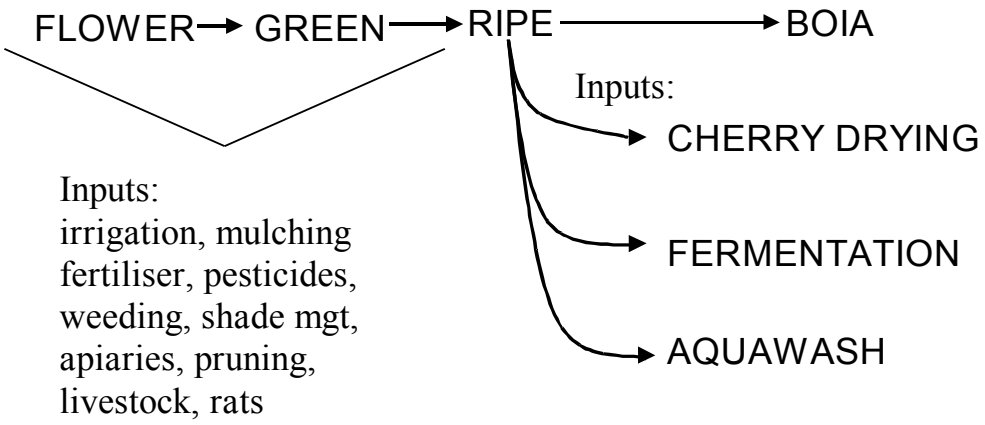

Figure 1. Relation of agrinomic and processing aspects of the coffee production ecosystem

COFFEE ASSOCIATES

Survey work in several coffee producing countries has shown there to be a fairly stable and uniform collection of organisms associated with the coffee fruit (Frank, 2000). There are

regional differences and these are usually greater than the international ones. The list includes *Candida edax, Cryptococcus album, Aureobasidium sp., Cladosporium sp. Fusarium stilboides, Penicillium brevicompactum, Kloekera apiculata,* in fermentation and coffee pathogens such as *Colletotrichum kahawae, Hemileia vastrix* as well as more general pathogens such as *Cercospora* and *Phoma.* As the coffee moves through the production system, through drying, the species change with *Aspergillus niger*, especially in robusta cherry coffee, *A. ochraceus, Eurotium ruber* and *E. repens* and *Wallemia sebi* all becoming sporadically common. However, the fungi common in the field also commonly retain dominance in the dried product.

The explanation for this shift in the community is partly the shift from wet to dry (water availability as a forcing function) in the presence of species adapted to high moisture and maladapted to drought but the diversity of outcomes can be taken as a proof that there are more delicate mechanisms at work as well. A methodological problem may also contribute to the rather fuzzy picture we have of this selection. It is impossible to reliably isolate relatively rare organisms by viable counting in the absence of selective media. Locating the first presence of rare species in a process is simply not possible. Hence, *A. niger* is almost never isolated in fresh cherries or the coffee rhizosphere but is probably the most common species in the beans of dried robusta cherries but we cannot say at what stage it enters the process. *A. ochraceus*, while not common, is regularly isolated from the seed of ripe fruit (it is also often present in the coffee rhizosphere) but the mechanism of the observed increase in **frequency** is not yet elucidated. It is also possible that these species are more common than the data indicate but are especially difficult to isolate from fresh fruit – perhaps our methodology gives a consistent underestimate. The confusion may well arise merely because we underestimate the strength of the selective pressure exerted by processing. In coffee fermentation, for example, *Kloekera apiculata* is almost never isolated from the cherry but is usually the only yeast isolated from fermentation liquor.

An extensive international survey failed to show any consistent correlation between contamination rates of the bean tissue, the mesocarp and the fruit surface (Frank, 2000). This is attributable to different species showing different degrees of adaptation to these niches such that some common species, *F. stilboides* for example, are equally likely to inhabit any of these niches while others, such as *Aureobasidium* and *Cladosporium* are much more common on the surface. *Candida edax* is more common, in relation to other fungi, in the mesocarp. What has not been possible to clarify is whether external contamination of the fruit followed by in-growth to the bean is the sole mechanism or even a mechanism of bean contamination. *A. ochraceus*, in particular, is more commonly recorded in the beans of ripe fruit but is rarely recorded from the other tissues so no correlation can arise here.

Little is known about bacteria but the picture is likely to be broadly similar to that of the fungi. At least one insect has an obligate relationship with coffee, the coffee berry borer and several others share coffee as a host with a limited list of tropical woodland trees. Insects such as the shot-hole borer, white stem borer and yellow-headed borer fall into this category. Polyphagous nematodes also can have an impact on coffee. Bacteria could be imagined to contribute to the nature of the niches they inhabit through excretion of extra-cellular enzymes or nutrient cycling, for instance, but it must be remembered that there are few bacteria that can grow at A_w values below 0.95. Boring insects clearly can breach mechanical plant barriers such as the bark, the fruit skin or the parchment so they could have a role to play in introducing fungi into areas in which fungi could grow were they able to gain access. No doubt there are symbiotic relations between insects and fungi but probably passive carriage of fungi on the exoskeleton or in the gut (many fungal spores can survive the insect gut) is most significant with respect to OTA production. It is important to note, however, OTA is not

primarily associated with coffee berry borer-effected beans and furthermore, the insects will most commonly transmit, aside from symbionts, what already is most common in its environment.

COFFEE AS HOST

The coffee fruit and bean can be considered in two aspects. Initially as a physiologically active entity with a developed 'immune' system which seeks a homeostatic condition in concert with commensal organisms. Latterly, once dead, as a substrate with a special chemical, physical and distributional composition not accurately characterized by the average. The coffee seed has no dormancy period and is clearly relatively fragile with respect to germability although a loss of germability does not necessarily mean a cessation of all physiological activity. A rapid and pronounced 'greening' response to physical injury, for example, can be observed even in re-hydrated stored beans, a response probably relating to chlorogenic acid conversion. With respect to chemical composition, there are demonstrable differences between *Coffea arabica* and *C. caneophora* and between cultivars of these species but the significance of these differences with respect to fungal growth and performance is not clear. The non-refractile carbohydrate of coffee is a poly-mannan comprising some 40% of the dry weight (Clarke and Macrae, 1989). Reducing sugars are very low and sucrose is by far the most important simple sugar or readily utilizable carbon source. This disaccharide comprises 3 to 4% of robusta and 6 to 8% of arabica dry weight. Little can usefully be said of the mineral and amino acid content other than their demonstrable adequacy for the plant tissue ensures that fungi can also thrive on its composition. Not all fungi will efficiently utilise lipids, the which comprise more than 10% by dry weight, particularly many of the isoprenoids, but the glycerides and their fatty acid composition are unremarkable. This area, along with that of the unusual poly-mannans, has a potential for specialists and generalists (the toxigenic organisms are probably generalists) to cooperate to achieve more efficient substrate utilization. The presence of an organism with a β-1,4 mannosidase or a benzyl oxidase would be expected to increase the activity of other saprophytes as well. Many phenolic metabolites such as phenylpropenoid derivatives and caffeine (Buchanan et al., 1981) are inhibitory to fungal growth and they may influence toxin production independent of their effect on fungal growth.

Although the nature of the substrate is undeniably significant, experience teaches that some sort of fungi will grow anywhere there is sufficient water. Thus water alone can be viewed as the controlling factor for mould growth. The selection of which moulds is complex. Once growth begins, the metabolic activity of the organisms can exert a degree of control on their immediate environment. Because the frame of reference for the germination of a fungal spore or the extension of hyphae is at the scale of a few micrometers, the details of water distribution rather than its average features must be understood. Methods of assessing water status in commodities, whether moisture or equilibrium relative humidity (erh) represent the average of a population of particles. Nuclear magnetic resonance (NMR) used for imaging (Figure 3) or the evaluation of water as regards its degree of binding (Figure 2) provides a technology for studying the distribution of water. Although the interpretation of these data in biological systems is not absolutely straightforward several aspects are clear enough to be helpful.

The pattern that emerges is one where the water is concentrated in a narrow band near to the surface and the inner tissues are dry. The centre cut, in particular, is a moist region. This is a rewetting pattern and the drying pattern induced by different drying methods would be a useful way to evaluate drying methodology with respect to particle-to-particle uniformity and the distributional aspects of the result.

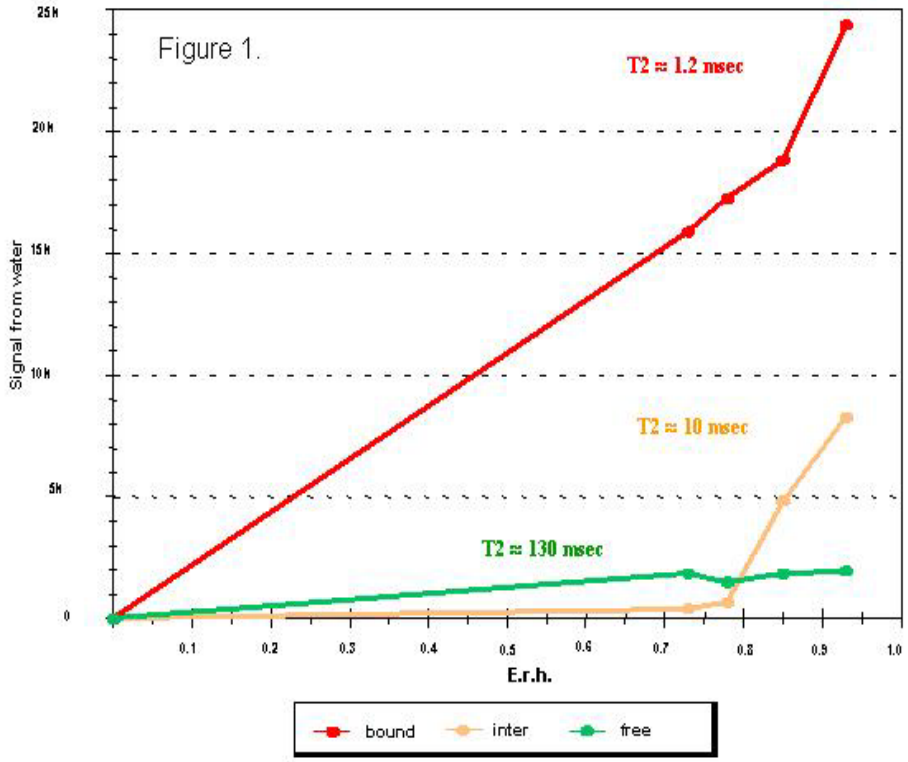

Figure 2. NMR water population data of re-hydrated dried beans equilibrated at various A_w values over H_2SO_4 solutions. Most of the additional water above 0.78 joins as the population with intermediate binding and corresponds to the moisture levels where fungal growth can take place. Means of five beans

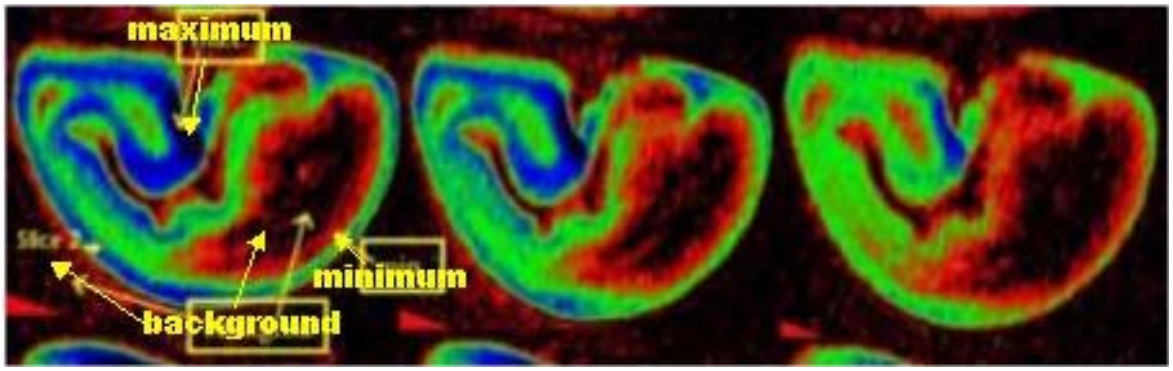

Figure 3. NMR images showing water distribution in a dry bean equilibrated at an A_w of 0.93 over an H_2SO_4 solution. Note that the tissues bounding the longitudinal slit are wet and the center of the cotyledons are dry. T_{echo} time increases from left to right meaning that the more constrained water is pictured left and the less constrained water is picture right

ECO/PHYSIOLOGY OF THE TOXIGENS

For a mycotoxin to accumulate in a commodity, a fungus capable of producing it has to be present and in conditions somewhat narrower than those permitting growth (Ominski et al., 1994). Growth of a toxigenic fungus does not necessarily equate with (significant) toxin production but toxin production requires growth. The issue of which fungi are the most important in the aetiology of a mycotoxin problem is reducible to questions of occurrence (=success in the face of competition) and production capacity in the commodity. A summary

of the situation is given in Table 1. The three producing fungi of significant occurrence are *Aspergillus niger, A. carbonarius* and *A. ochraceus*. The first becomes very common as the coffee is dried but rarely does it produce OTA and then in very small amounts. *A. carbonarius* is uncommon but a large proportion of strains are good OTA producers. Indications are (Pers. com. P.Bucheli) that this species ceases to produce at A_w levels around 0.92 and at temperatures above 35°C. It appears that the conditions appropriate for *A. carbonarius* to produce OTA strongly overlap those in which it could not successfully compete in the presence of fungi adapted specifically to high water availability. *A. ochraceus* is relatively common, more so as the commodity is dried, and about 80% of isolates are good OTA producers. The maximum temperature and minimum A_w are also much closer to the organism's growth limits, close to 40°C and A_w about 0.8. (Bacon et al., 1973)

Table 1. Current evaluation of the relative significance of the three OTA-producing species found in coffee.

OTA producer	Distribution	Physical limits	Production capacity
A. ochraceus	ubiquitous; not uncommon; fresh bean, rhizosphere, drying yard storage facilities	0.80 to 0.83 A_w <15 to ~40°C	**HIGH**
A. niger	ubiquitous; very common; drying yard, storage facilities	not known	**LOW**
A. carbonarius	not known; rare; drying yard, storage facilities	c.a. 0.92 to ~35°C	**HIGH**

COFFEE PRODUCTION SYSTEM

The coffee production system extends from the orchard to storage and transport or from the least controllable to the most controllable situation. In parallel it moves from the least easily studied aspect of the production system to procedures and practices that are more easily studied. Referring to the pre-harvest situation and alluding to Figure 1, we can say that there is a complex of influences centred on the coffee plant, its habitat, agronomic practices and associated organisms. In terms of outcomes, we know that *A. ochraceus* (along with several other species of the ochraceus group) can invade bean tissue by the stage of ripeness but it increases in incidence through processing. In the niger group early colonization of the bean is rare but fungi of this group can become very common by the end of drying so there must be one or more mechanisms of invasion between the orchard and the storage facility or our methods for isolating these species from fresh material are inadequate.

At this stage in the discussion it is important to make a point about how fungal occurrence is recorded. The methods employed to determine fungal presence in coffee beans gives an expression of **frequency**. Under some circumstances, frequency may be related to growth, in the sense of biomass, but it is not necessarily so. When the frequency of occurrence decreases it is an indication of death; an increase does not merely represent growth but introduction and growth. The method does not distinguish between a trace of growth and a mass of growth, both come out as '1'. Since direct plating of beans is preceded by surface sterilization, the counts of infected beans will measure the extent of penetration of the bean tissue by the fungus if, of course, the surface sterilization has been successful. The centre cut of coffee

adds a degree of uncertainty to this procedure but the harsh sterilization regime employed has given '0' contamination rates as well as 100% rates so clearly the method is basically sound.

Once separated from the tree, the conditions are more easily conceptualised except in the processing methodology where drying is allowed to take place on the tree, 'boia' in Figure1. There is no data available on the time-course of drying in this way but the supposition must be that drying takes place more slowly. In addition it could very well be that the continued attachment to the tree is significant and it is not clear when during senescence the vascular supply is broken. Otherwise, the cherries are either pulped, effectively eliminating the external fungi and diminishing the mesocarp fungi, or dried as they are. After pulping, the remaining mesocarp may be mechanically removed and the parchments dried or they may undergo fermentation. In the later case the evidence is that some fungi, yeasts in particular, can supplant other fungi in the bean tissue and then tend to die during drying, producing coffee with a very low infection rate.

The two most important interacting 'forcing functions' during drying, temperature and water availability, are illustrated as their average or gross changes in Figure 4. These two features are related to each other thermodynamically, changes in temperature change erh and evaporation and condensation cool and heat, and also show a complex interrelation as interpreted through a physiological system. The physiological response of an organism to a change in water activity (for example) will differ according to the prevailing temperature (also for example). Naturally, the substrate composition and the composition of the accompanying community will also have a major impact on how the fungus can respond thus axenic, artificial systems cannot be expected to reliably represent the *in situ* situation (Frank, 1998). At the micro-environmental level, the level at which a spore or small mycelium exists, temperature dynamics look quite different to Figure 4. There will be local heating on surfaces exposed to the sun with much lower values in the shade. The effective water status in this micro-environment is even harder to conceptualise in that fungus can alter their immediate environment both by generating water through respiration and by transporting it from a wetter area which it has colonized, to a dryer area. Translocation is one important aspect of the mycelial habit. Thermodynamically, the driest region, during drying, will be the outer surface since the rate-limiting step in coffee drying is undoubtedly migration of water through the bean tissues, not loss from the surface.

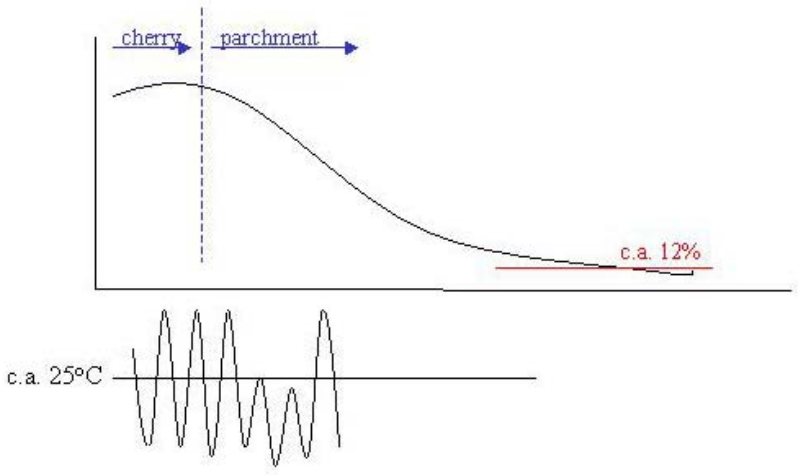

Figure 4. A generalized drying time-course showing diurnal changes in temperature on sunny and cloudy days. Cherries and parchments dry at about the same rate once the fruit skin ruptures after about two days. It is these sort of dynamics that the fungi 'see' though water relations at the micro-habitat level are too complex to speculate about

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CFC/ICO/FAO Mould Reduction Project in Selected Coffee-producing Countries

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SUMMARY

The status of the art in reaching the main objectives of the mold reduction project is reviewed:

I. Identification of optimal processing conditions, where practical operating practices have been refined, but where improvements, particularly in drying must still come.
II. Identification of OTA producing species and possible competitors, refining critical a_w windows; the main *Aspergilli* are now known, while the a_w windows still need to be refined for the different coffee products, and the question of the existence of ecological zones at different degree of risk must still be answered.
III. Identification of Critical Control Points [CCP] for mold infection and OTA formation in green coffee; important CCP already identified are the length of time between harvest and processing, and the length of time to reach an A_w of 0.80.
IV. Improvement of analytical and mycological laboratory facilities in the participating countries; information on the material and techniques needed is now available.
V. Development and field testing of codes of Good Hygienic Practice [GHP] and HACCP-based safety systems); the list of "Do's & Don'ts" is already a Code of GHP, while not enough information is yet available to finalize an HACCP-based safety system.

INTRODUCTION

The question of avoiding coffee contamination by ochratoxin A [OTA] during processing is being tackled since 1988 (Tsubuchi et al., 1988), when improved analytical techniques showed that this toxin is only partly decomposed during roasting (Viani, in print).

The four-year project is due to cover all types of green coffee production in all possible geographic areas (both arabica and robusta; all processing techniques). For this reason, the following seven countries were chosen for its implementation:

Brazil (mainly dry-processed arabica and robusta, Colombia (wet-processed arabica), Côte d'Ivoire (dry-processed robusta), India (all types of coffees both dry and wet processed), Indonesia (mainly dry-processed robusta), Kenya (wet-processed arabica) and Uganda (mainly dry-processed robusta).

The collaborating institutions in all the participating countries have signed the project implementation, and the first year plans have been agreed. India has officially started on January 1, 2001. Uganda continues from the previous two years project, financed by FAO.
The main objectives of the project are:

1. The identification of optimal processing conditions
2. The identification of OTA producing species and possible competitors, refining critical a_w windows
3. The identification of Critical Control Points for mold infection and OTA formation in green coffee
4. The improvement of analytical and mycological laboratory facilities in the participating countries
5. The development and field testing of codes of Good Hygienic Practice and HACCP-based safety systems

IDENTIFICATION OF OPTIMAL PROCESSING CONDITIONS

The list of operations likely to influence the risk of mold formation during processing has been further refined since the workshops of Nairobi in 1997 (Anon, 1997) and Helsinki in 1999 (Various Authors, 1999):

Harvest

- Cover the ground under the tree with a clean sheet of plastic during picking
- Do not pick cherries from the ground
- Process fresh cherries as quickly as possible
- Avoid storage of cherries before processing

Cherry drying

- Do not dry on bare soil: use trays, tarpaulins or concrete
- The layer of drying cherries should be not more than 3-4 cm thick after the first 2-3 days of drying
- Rake cherries regularly (3-5 times per day)
- Protect cherries on drying patio from rain and night dew by heaping them and covering up
- Avoid all re-wetting of partially dried and dried cherries, protecting them from moisture and rain
- Do not mix fresh cherries with partially dried ones

Primary processing of dry cherries

- Site hulling plant in a dry area, not in a swamp
- Do not process cherries at a moisture content higher than 13%
- Clean as much as possible away from coffee all husk material – in sun dried cherries more than 90% of mold comes from husks
- Remove husks and hulls, avoiding accumulation at the processing plant
- During grading, remove as many defects (husks, un-hulled cherries or moldy beans) as possible
- Keep equipment and facilities clean and in good working order, avoiding accumulation of dust and discarded material

Storage of dry processed coffee

- Use clean bags for storing and transporting cleaned dried beans

- Prevent recontamination by avoiding contact of clean green beans with dust, husks and re-used dirty bags
- Do not store cleaned, dry green coffee near rejects and husks

Wet processing

- Light cherries, separated during floatation, must either be processed carefully and fast or discarded
- Keep the pulper in good working order, adjusting it to avoid chipping beans or breaking parchment
- Keep the equipment (pulper, tanks, etc.) and facilities clean, sorting out residual partially processed material (skins, unpulped cherries, etc.)
- Dispose the pulp from wet processing away from clean drying parchment coffee on day of pulping
- Compost the pulp before using it as mulch in the field

Storage of wet processed coffee

- Store cleaned dried beans away from discarded material

Transport and storage of green coffee

- Protect bags during transport and storage to prevent re-wetting
- Load and unload trucks or containers only in dry weather or under cover
- Do not use damaged containers and prevent water leaks
- Cover bags/loose beans in trucks or containers with waterproof or water-absorbent cover
- Make sure that pallets or wooden floors in trucks and containers are dry
- Store coffee in well-ventilated and leak-proof warehouses away from the walls

Quality control tests

- Check moisture content at all steps in the trade line from farmer to exporter
- Check and discard defects known to increase contamination load (whole cherries, husks, moldy beans, dust)

IDENTIFICATION OF OTA PRODUCING SPECIES AND POSSIBLE COMPETITORS, REFINING CRITICAL A_W WINDOWS

Identification of OTA producing species

The mycological situation is now quite clear – The main *Aspergilli*, capable of producing OTA during coffee processing are:

A. ochraceus, *A. carbonarius*, *A. niger* (rarely), but a common species (Joosten et al., 2001; Heenan et al., 1998; Taniwaki et al., 1999). *A. citricus* and *A. lactocoffeatus*, and newly identified OTA-producing *Asperigilli* have been detected on rare occasions (Frank, personal communication).

Refining the critical A_w windows for different coffee products

The moisture content (expressed in dry basis) as a function of water activity curve for arabica and robusta green coffee beans, showing the typical sorption isotherm behavior in the lower activity region for foods in general is given in Figure 1 (it is fitted here to the model given by the GAB equation (Bucheli et al., 1998. van den Berg, 1985).

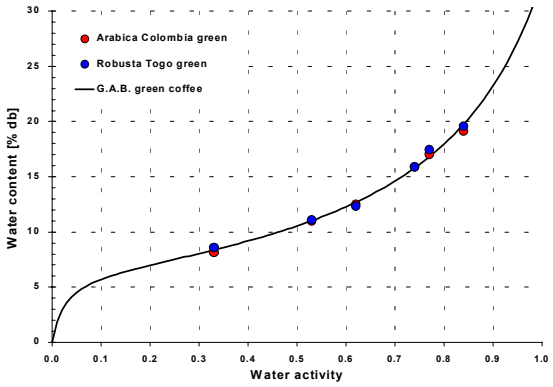

Figure 1. Sorption isotherms of arabica and robusta green coffee beans at 30°C [Courtesy G. Vuataz, Nestlé Research Center]

Other authors have found somewhat different relationships (Ayerst, 1965; Kulaba and Henderson, 1980; Urbano et al., in print). A standard procedure, reproducing as realistically as possible coffee processing, needs to be developed for the project.

The effect of A_w and temperature on growth and production of ochratoxin A [OTA] has been investigated on laboratory media and on coffee. The following data have been published for *Aspergillus ochraceus* and for *A. carbonarius* (Table 1).

Table 1. Values of A_w for growth and OTA production by *Aspergilli*

Mold	For growth		For OTA production			
	A_w		A_w		°C	
	Minimum	Optimum	Minimum	Optimum	Range	Optimum
A. ochraceus (Northolt et al., 1979)[i]	0.79-0.83	<0.99	0.83-0.87	0.99	12-37	depending on A_w[ii]
A. ochraceus (Bacon et al., 1973)[iii]				between 0,95 & 0,99	15-30	
A. ochraceus[iv]				0.92		
A. ochraceus (Patterson and Damoglou, 1986)	0.85	<0.97			12-37	25
A. ochraceus (Moss, 1996) [v]			ca 0.80			
A. carbonarius(Joosten et al., in print) [vi]			ca 0.90			25

I. On agar media in which the A_w had been adjusted by addition of sucrose or glycerol; germination times were of 0.5-2 days under optimum conditions for mold growth, increasing to 12-14 days under unfavorable conditions, and the effect of A_w on OTA production was found to be independent of substrate.
II. 31°C at A_w = 0.99, and 37°C at A_w = 0.95.
III. On poultry feed; *A. ochraceus* appears to produce OTA when near optimal conditions for conidial development are approached.

IV. See Kubala and Henderson, 1980: on a bread analog; some toxins may be produced at lower A_w than have been reported on synthetic media and, whenever possible, natural substrates should be used to investigate factors affecting mycotoxin production in foodstuffs.
V. The optimum pH for both growth and OTA production by *A. ochraceus* is around 5.6.
VI. On coffee beans.

Here again, a standard procedure, reproducing as realistic as possible coffee processing, needs to be developed for the project.

Indicative values of moisture for coffee products along the chain from the cherry to the green bean, show that there is a real risk of mold growth, particularly important for poorly dried cherries (Table 2).

Table 2. Approximate A_w values for coffee products

Coffee product	Moisture (%)	Approximate A_w
Fresh cherry	60-70	0.99
Dry cherry	14-20*	0.70-0.85
Parchment	14-18*	0.70-0.80
Green bean	10.5-11.5	0.50-0.60

(*) Risk of mold growth

It may be worth measuring A_w in coffee cherries and cherry constituents at different drying levels to refine the critical windows for mold proliferation.

The material balance in a fresh coffee cherry shows that most moisture is concentrated in the pulp and in the mucilage (Bressani and Braham, 1980) (Table 3).

Table 3. Material balance in a coffee cherry

Product	Fresh weight (g)	Dry weight (g)	Moisture (%)
Cherries	1000	345	65.5
Pulp	432	99	77.0
Mucilage	118	17	85.6
Hulls	61	41	32.0
Beans	389	191	51.0

The composition of pulp (Patterson and Damoglou, 1986), rich in reducing sugars, an important nutrient for *Aspergilli*, and of mucilage (Nigam, 2000) are given in Table 4.

Table 4. Composition of fresh pulp and mucilage

Component	In pulp (%)	In mucilage (%)
Moisture	76.7	85.0
Dry matter	23.3	15.0
• Protein	2.1	-
• Reducing sugars	12.4	-
• Total carbohydrates	-	7.0

Work is on-going in the project to understand the ecology of the *Aspergilli* found to infect coffee products, particularly to identify

- if there are differences in the risk of contamination for different geographical regions;
- possible competitors active at A_w's above 0.95,
- which is the optimal drying temperature to reduce the risk of mold proliferation, without spoiling coffee quality, for both cherries and parchment, as laboratory results ((Joosten et al., 2001; Taniwaki et al., 1999) indicate a drastic reduction in OTA production by *A. ochraceus* and *A. carbonarius* above 35°C.

IDENTIFICATION OF CRITICAL CONTROL POINTS [CCP] FOR MOLD INFECTION AND OTA FORMATION IN GREEN COFFEE

Important CCP have been identified and must now be refined:

- no more than a few hours between picking and beginning of processing of fresh cherries.
- no more than 4 days should be spent by coffee in the a_w window down to 0.80.

IMPROVEMENT OF ANALYTICAL FACILITIES IN THE PARTICIPATING COUNTRIES

Analytical laboratory facilities

Mold growth, with the risk of OTA formation, is a direct consequence of poor moisture control during green coffee production. It is, therefore, important that all estensionists involved in the project be equipped with portable rapid moisture analyzers.

Sophisticated and expensive HPLC analytical techniques are available for the detection of OTA down to less than 0.1 μg/kg in all coffee products (Pittet et al., 1996).

No rapid, inexpensive and sensitive field kit for OTA analysis is yet available, although commercial pre-purification columns are available for semi-quantitative analyses, and a cheap, rapid TLC method, sensitive to a few μg/kg, is now being finalized (Pittet, personal communication).

Any organism, wishing to introduce an analytical control of the OTA content of a lot of coffee, must also keep in mind that the question of green coffee sampling has not yet been satisfactorily answered.

Mycological laboratory facilities

A coffee mycology handbook, indicating the equipment and the techniques specifically developed for the project has been prepared (Frank, in print), and made available to all the participating countries.

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$$\mathbf{W = W_m K C A_w / (1 - C A_w) [1 + (K – 1)C A_w]}$$

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Studies on Mycoflora Association during Harvesting and on Farm Processing of Robusta Coffee in India

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SUMMARY

In recent years mycotoxin contamination in coffee particularly that of Ochratoxin-A (OTA) is drawing attention of consumers in the importing countries, as it is reported to be possible health hazard. India is one of the few coffee producing countries, that are involved in active research on mycotoxin in coffee ever since first report on OTA appeared in the Europe. Experiments were carried out at the Central Coffee Research Institute to study the associations of mycoflora in soil, air from drying yard and on-farm processing of coffee at estate level. In general, mould population was high on fresh fruits and their population reduced with reduction in moisture content. Yeast and *Fusarium* sp dominated on the surface of the fruits, mesocarp, fresh beans, which decreased during the drying process in both parchment and cherry preparations. *Cladosporium* sp was found in all stages of processing of cherry coffee. The OTA producing mould like *Aspergillus ochraceus* was absent in ripe & over-ripe fruits, parchment and pulper remnants, but its presence was observed in bulk cherry, gleanings, floats, soil and air sample from drying yard. The parchment and cherry prepared from normal fruits had better cup quality compared to those of defective samples like gleanings and floats.

INTRODUCTION

Coffee occupies a prime position among the plantation crops in India. India grows both arabica and robusta over an extent of 3,40,000 ha, almost in equal proportions producing nearly 2,92,000 MT of coffee per annum. The quality of coffee is influenced by several internal and external factors, among them microbial contamination is known to bringdown the quality of coffee. In recent times, the occurrence of Ochratoxin-A (OTA) in coffee is of great concern in coffee trade. OTA is a reported to be a nephrotoxic mycotoxin and suspected carcinogen (Boorman, 1989) and produced by moulds like *Aspergillus ochraceus, A. carbonarius* and *Penicillium verrucosum* in coffee. Levi et al. (1974) first reported the natural occurrence of OTA in coffee. Since then number of researchers have detected its presence in green and roasted coffee samples (Tsoubachi et al., 1984, 1998; Studer-Rohr et al., 1995). The knowledge on these fungi and their association during coffee production and processing is incomplete. In this paper mould and their association in robusta coffee during wet and dry processing were discussed in details.

MATERIALS AND METHODS

Selection of estate

The estate was chosen based on the frequency of *Aspergillus ochraceus* in soil and air during earlier study. The estate NC1-CKM is situated in Chikmagalur district of Karnataka, which is the major coffee-producing region in India. The estate comprises both arabica and robusta (~400 ac) with altitude of 3000 ft MSL with an average rainfall of 2500 mm and soil type of red laterite, with 60-70% shade of both cultivated and permanent shade trees. The estate produces coffee by both wet and dry method and this study is restricted to robusta coffee only.

Samples

Soil, cherries of different ripening stage, floats, gleanings, left out remnants from pulping unit, processed water, parchment and cherry from drying yard at different intervals and air flora from drying yard were collected for mycological examination.

Isolation of fungal flora

External fungi of cherries

Fifty cherries were washed in 100 ml of peptone water for 10 min in a platform shaker at 200 rpm. The washate was serially diluted to 10^{-4} dilution and the same was plated out on DG18 medium (Hocking and Pitt, 1980).

Internal fungi of cherries/beans

The cherries (100 Nos.) were collected from representative fruit lot and surface sterilized with 1% NaOCl solution for 8-10 min and rinsed thrice with sterile water. The sterilized cherries were plated on DG18 i.e. 10 cherries/ per plate.

Mesocarp mycoflora

The parchment (50 Nos.) with mucilage was hand- removed under aseptic condition and the pulp was transferred to 100 ml of 1% peptone water in a sterile polythene bag. The pulp was squeezed for 3-4 min and serially diluted to 10^{-4} dilution and the same was plated out in DG 18.

Soil, processed water and remnants from pulper unit

Standard serial dilution techniques was followed for enumeration of mycoflora from the above samples.

Air flora from drying yard

The Petri dishes containing DG18 media were exposed to air for 4 min in the experimental sites and incubated at room temperature for 5-7 days for mycological examination.

The slide culture technique (Riddel, 1950) was followed to study the morphological features of fungi. Identification of mycoflora was done as per the manual "Illustrated Genera of Imperfect Fungi" (Barnett, 1960; Raper and Fennel, 1965). All the mycological data presented in this paper were a cumulative observation made during single season

Cup quality evaluation

All the samples were analysed for cup quality at Quality Control Division, Coffee Board, Bangalore. The quality was determined by assessing raw, roast and liquor characteristics of the sample.

RESULTS

Soil and air flora

From the soil analyses conducted at the experimental sites, it was observed that *Cladosporium* sp (7×10^4) was found to be dominant followed by *Aspergillus* sp (6×10^4). *A. ochraceus* recorded a value of 2.5×10^4 and 0.5×10^4 in soil and air from drying yard respectively. Yeast was observed in the range of 5.5×10^4 and 3×10^4 in soil and air respectively. Other moulds like *Fusarium* sp, *Alternaria* sp, *Penicillium* sp and *A.niger* were also found to be associated with soil and air samples (Table 1).

Table 1. Soil mycoflora and air flora (drying yard)

Mycoflora	Soil (cfu x 10^4/ g)	Air flora from Drying yard (cfu/ 4 min)
A. niger	1.5	0.5
A. ochraceus	2.5	0.5
Aspergillus sp	6.0	7.5
Penicillium sp	2.0	1.0
Fusarium sp	1.0	0.5
Alternaria sp	-	3.0
Cladosporium sp	7.0	13.0
Yeast	5.5	3.0

Mycoflora associated with remnants from pulping unit

The data revealed that the remnants sample collected from pulping unit were predominantly of yeast (38.5-66.4%) followed by *Fusarium* sp (7.0-35.6%). The toxin producing mould like *A. ochraceus* was not recorded in the remnants, however *Aspergillus* sp was limited to 1.3-6.2% in left out fruits, cuts and bits and processed water. *Penicillium* sp was also commonly found distributed in samples in the range of 3.9-17.3% (Table 2).

Mycoflora associated with ripe and over-ripe fruits

Ripe and over-ripe fruits were found to be infested by to *Fusarium* sp (52% and 61.3%) followed by Yeast (24.1% and 21.7%). The mesocarp of both ripe and over-ripe fruits were found to be dominated by Yeast as 49.3% and 63.2% respectively as compared to other filamentous fungi (Table 3). The genus *Aspergillus* and *Penicillium* associations were commonly observed to be closer in beans (11.2 and 9%) obtained from over-ripe fruits compared to beans from ripe fruits.

Table 2. Mycoflora associated with samples of remnants in pulper unit

Mycoflora	Left out skins (10^4/g)	Left out beans (% infection)	Left out fruits (% infection)	Cuts & bits (% infection)	Processed Water (10^4/ml)
Yeast	36.8 (66.4)	65.4	38.5	67.3	26.5 (63.7)
Fusarium sp	15.3 (27.6)	30.7	35.6	23.4	2.9 (7.0)
Cladosporium sp	0.0	0.0	7.9	0.0	3.3 (7.9)
Aspergillus sp	0.0	0.0	6.2	1.3	1.7 (4.0)
Penicillium sp	3.3 (5.9)	3.9	11.7	8.0	7.2 (17.3)
A. ochraceus	0.0	0.0	0.0	0.0	0.0

Figures in parentheses are percentage distribution of mycoflora

Table 3. Mycoflora associated with ripe and over-ripe robusta fruits

Mycoflora	Ripe fruits			Over-ripe fruits		
	Surface (10^4/cherry)	Mesocarp (10^4/cherry)	Fresh beans (% infection)	Surface (10^4/cherry)	Mesocarp (10^4/cherry)	Fresh beans (% infection)
Yeast	9.2 (24.1)	24.9 (49.3)	91.5	11.3 (21.7)	28.0 (63.2)	20.7
Fusarium sp	19.8 (52.0)	6.1 (12.0)	2.7	31.9 (61.3)	3.7 (8.4)	54.8
Cladosporium sp	3.9 (10.2)	15.2 (30.0)	1.8	3.8 (7.3)	2.6 (5.9)	4.3
Aspergillus sp	2.1 (5.5)	1.8 (3.6)	4.0	2.4 (4.6)	2.9 (6.5)	11.2
Penicillium sp	3.1 (8.1)	2.5 (5.0)	0.0	2.6 (5.0)	7.1 (16.0)	9.0
A. ochraceus	0.0	0.0	0.0	0.0	0.0	0.0

Figures in parentheses are percentage distribution of mycoflora

Mycoflora associated with parchment and cherry coffee during drying

The Yeast was found to be dominant especially in both wet and dry parchment (75.3% and 64.1%) followed by *Fusarium* sp (16.2% and 26.2%). The *Aspergillus* sp and *Penicillium* sp were poorly represented on both wet and dry parchment. The OTA producing moulds *A. ochraceus* was found to be uncommon in both wet and dry parchment during drying

(Table 4). In cherry coffee drying, *Fusarium* sp was found to be common followed by yeast compared to other filamentous fungi, but its presence was subsequently reduced during drying. *Aspergillus* sp and *Penicillium* sp establishes their association with coffee beans with decrease of moisture in the coffee beans during the course of drying. The OTA producing mould *A. ochraceus* was observed in both fresh and dried fruits in the range of 0.5-1.3%. The frequency of other mould like *Mucor* sp, *Rhizopus* sp, *Alternaria* sp, *Aureobasidium* sp frequency were seemed to greatly vary with lower levels.

Table 4. Per cent incidence of mycoflora associated with robusta parchment and cherry during sun drying

Mycoflora	Parchment (Beans)			Bulk Cherry (Beans)		
	First day	Second day	Fourth day	First day	Fifth day	Eighth day
Yeast	75.3	64.4	64.1	26.0	22.9	16.2
Fusarium sp	16.2	18.7	26.2	44.8	44.4	23.6
Cladosporium sp	4.0	13.1	4.5	8.0	8.9	24.9
Aspergillus sp	4.5	3.8	2.3	11.8	13.3	15.9
Penicillium sp	0.0	0.0	2.9	8.8	9.4	18.1
A. ochraceus	0.0	0.0	0.0	0.5	1.20	1.30

Table 5. Per cent incidence of mycoflora associated with robusta floats and gleanings during sun drying

Mycoflora	Beans from Floats		Beans from Gleanings	
	First day	Eighth day	Initial	Final product
Yeast	40.6	23.3	31.5	5.0
Fusarium sp	31.2	29.7	40.5	15.0
Cladosporium sp	9.7	8.02	11.0	19.0
Aspergillus sp	8.8	20.1	9.5	22.0
Penicillium sp	9.6	17.4	5.0	11.0
A.ochraceus	0.0	1.24	0.5	1.5
A.niger	0.0	5.0	2.0	16.5

Mycoflora associated with floats

The surface mycoflora of floats was found to be dominated by yeast (40.6%) followed by *Fusarium* sp (31.2%) at initial stages of drying (Table 5). In dried floats, *Fusarium* sp (29.7%) was found to be dominant followed by yeast (23.3%). The *Aspergillus* and *Penicillium* sp association were found to be more pronounced on both wet an dry floats compared to ripe and over-ripe fruits. *A. ochraceus* was failed to establish itself in fresh floats. However, its presence was observed in dried floats (1.24%).

Mycoflora associated with gleanings

In general, the mould association with dropped fruits was found to be remarkably diversified compared to other samples (Table 5). Among various mycoflora *Fusarium* sp was found to be

closely associated with gleanings to an extent of 40.5% at initial stage and 15% in the final product. *A. niger* was recorded in the range of 2% and 16.5% in initial and final beans respectively, similarly *Penicillium* sp was observed in the range of 5% to 11%. The toxin-producing mould *A. ochraceus* was limited to 0.5% and 1.5% in initial and final stage of bean respectively, and its association was more pronounced in gleanings as compared to floats. The association of yeast was found to be depleting as the moisture levels in the bean went off, yeast recorded a higher value of 31.5% at initial stage but its presence was only 5% in the final bean.

Table 6. Cup quality evaluation

Sample details	Cup quality results
Robusta parchment	Good body, Fairly soft, slightly chocolaty, good acidity, FAQ+
Robusta cherry	Good body, over-ripe taste, FAQ
Robusta gleanings	Fair to good body, fair plus acidity, sourish, over-ripe, fruity, below FAQ
Robusta floats	Fair to good body, sourish, fair acidity, lacks flavour, thin liquor, woody fruity, below FAQ

Cup quality evaluation

The cup quality results of robusta parchment, cherry, gleanings and floats are presented in Table 6. Robusta parchment recorded Fair Average Quality plus (FAQ+) with cup rating of '5' followed by cherry coffee with cup rating of FAQ (4). Gleanings and floats recorded the rating of below FAQ (3).

DISCUSSION

Mould contamination in coffee is known to cause serious quality deterioration in field and processing level. At field level coffee has to pass through various steps in processing which favour fungal association. The present study examines the association of mycoflora particularly toxigenic mould like *A. ochraceus* at on-farm processing of robusta coffee in detail.

The occurrence of *A. ochraceus* was observed in soil and air sample from drying yard, but its presence was not observed in the wet processed coffee. The presence of *A. ochraceus* in coffee soil and air flora from drying yard was also reported earlier by Mick Frank (1999) and also in soil by Taniwaki et al. (1999). This restriction could be due to the proper sorting of fruits, pulping of fruits immediately after harvest, and washing immediately or after fermentation.

Interestingly the incidence of *A. ochraceus* was recorded in cherry samples probably due improper sorting of fruits i.e., drying of sound fruits in bulk with tree dried, split, sun scorched and over ripe fruits in the same drying yard. The presence of *Aspergillus* sp and *Penicillium* sp were observed in over-ripe fruits and pulper remnants but OTA producing mould like *A. ochraceus* was not observed in these samples. The mould *Aspergillus* sp and *Penicillium* sp were found to high on over-ripe, sun scorched and split fruits than sound fruits was earlier reported by Naidu et al. (1997).
The *A. ochraceus* incidence was observed in gleanings and floats in the range of 0.5-1.5% and 1.24% respectively. The incidence in gleanings is due to direct contact of cherries on the soil for a long time (usually fallen fruits are collected at the end of harvest in India), which facilitated the on soil infection. Generally floats are reported to be an un-sound fruits that is

improperly filled during the berry development, more over it is highly susceptible for any mould infection. Since tree dried fruits also comes under floats the infection rate in floats was found to be higher. Taniwaki et al. (1999) reported that OTA producing *A. ochraceus* appeared in cherries and raisins from trees in coffee from drying yard and from storage. In another study, Alves et. al. (1998) reported that in tree dried fruits *Cladosporium* was found to be the predominant fungus, whereas the *Penicillium* sp was most common fungi, the higher incidence of *A. niger* and *A. ochraceus* were also observed in processed coffee

In cup quality evaluation, robusta parchment recorded a better quality compared to, cherry but gleanings and floats recorded an inferior cup quality. The difference in quality is mainly because of processing of cherry as a bulk that includes ripe, over-ripe, half-ripe, greens and trees dried as a single lot and long contact of fruits with soil in case of gleanings and improper filling of fruits in floats respectively.

From this study some of the critical control points like soil, tree dried, floats and gleanings were identified as a source for toxigenic mould in coffee processing, further more studies are needed in coffee to identify code of Good manufacturing practices with respect to mould contamination.

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Mould Species Infestation during Sun Drying of Sound and Split Coffee Cherries

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SUMMARY

Drying procedures for robusta coffee commonly applied by farmers in Indonesia are sun drying of sound and split cherries. The second procedure is applied in some areas to shorten the drying time. An experiment on both procedures was conducted on a ground, a patio, and a plastic sheet with 3 and 6 cm thickness. Each treatment was conducted in 3 replications. The development of mould species during the drying courses were analyzed by the direct plating procedure on the DG-18 media. The dominant species observed in the beans were *Aspergillus niger*, *A. flavus*, *Penicillium citrinum*, and *A. ochraceus*. A few other minor species were also observed. Coffee splitting gave less contamination rate of some species particularly *A. ochraceus*. Fresh coffee cherries conditions especially its maturity determined the mould contamination status during the drying course. The contamination rate of mould species on drying treatments is discussed.

INTRODUCTION

People in coffee consuming countries have become more health conscious and the governments pay more attention to the content of the food they eat. The occurrence of mycotoxins, especially ochratoxin A (OTA) has recently become a great issue. Some countries have imposed such limits of OTA in coffee, like Italy (8 ppb for green coffee and 4 ppb for finish coffee products), Finland (10 ppb), and Greece (20 ppb). The European Commission has not imposed such limits for OTA in coffee, but various suggestions for limitation have been made. A recommendation (CE 22/12/1998 No. 26) by the commission suggested the member countries to apply such limits, with the reference of 5 ppb (Romani et al., 2000).

OTA is produced by some species of *Aspergillus* and *Penicillium*. One of them frequently found in coffee beans is *A. ochraceus* (Ismayadi and Zaenudin, 2000; Dharmaputra et al., 1999). OTA contamination of green coffee might be reduced by understanding the stage and conditions under which it is produced during its production and trading. Bucheli et al. (2000) reported that OTA contamination in green coffee had occurred at the sun-drying stage. It was also shown that OTA was accumulated mainly in husks and the concentration in dried coffee beans only about 1% of those found in husks.

Indonesian coffee is mainly consist of robusta type and produced by millions of farmers in small scale farms. The farmers harvest their coffee unselectively 2 to 4 times per season. The mixed cherries are dried under the sun on the ground, plastic sheets, or a concrete floor. There are two types of coffee cherries being dried i.e. normal (sound) cherries and split cherries. The first is more widely applied, while the split method is usually applied by farmers in East Java, and some farmers in southern Sumatera. Farmers use a simple splitting machine which is similar to the pulping machine. The split method is preferable since drying time is shorter.

Mycological aspect of coffee drying is very little known. The information concerning fungal development during the drying and conditions unsuitable for mould to grow and producing toxins is needed, in order to reduce the contamination rate of OTA in coffee beans. This study describes the mould development during sun drying of coffee cherries both in the normal form and the split form.

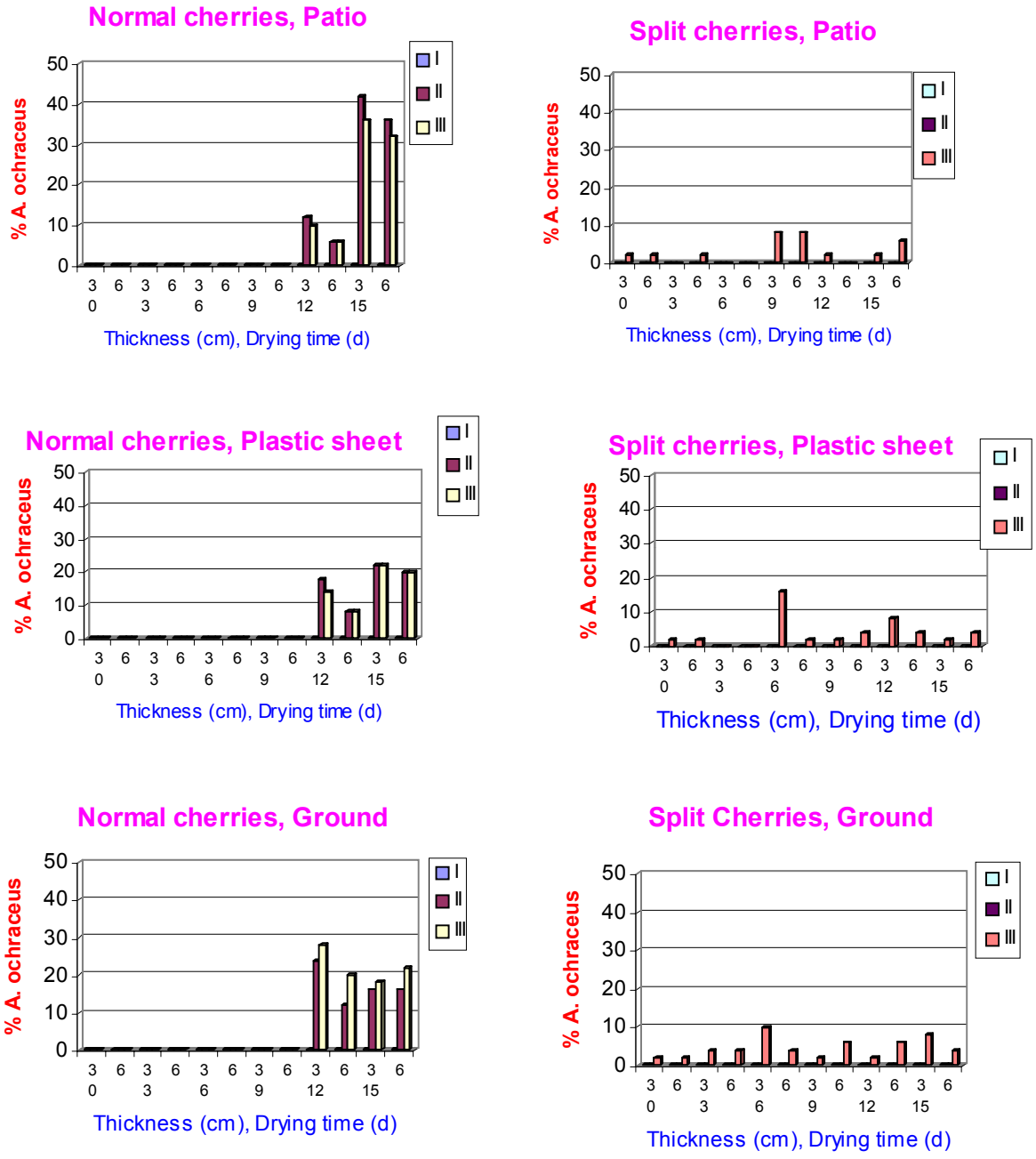

Figure 1. Contamination rates of *A. ochraceus* in coffee beans from normal and split cherries drying treatments

MATERIALS AND METHODS

Coffee cherries of robusta type was taken from Kaliwining Research Station and Gunung Pasang Estates surrounding Jember, East Java. The coffee from Kaliwining R.C. was mixed green and red cherries (experiment I and II), and the cherries from Gunung Pasang Estate were mainly (95%) the red ones (experiment III). The split coffee was prepared by using a motor driven splitting machine which is normally used by farmers in East Java.

Normal and split coffee cherries were sun dried on a concrete floor, plastic sheets, and hardened ground, 3 cm and 6 cm thick each. During drying the cherries were regularly turned 3 times per day, and covered with a plastic sheet at night. They were sampled every 3 days up to 15 days of drying. Mould contamination rate was determined by using the direct plating procedure of 50 beans on the DG-18 media and expressed in percent. Mould species was identified by the standard procedure (Samson et al., 1996).

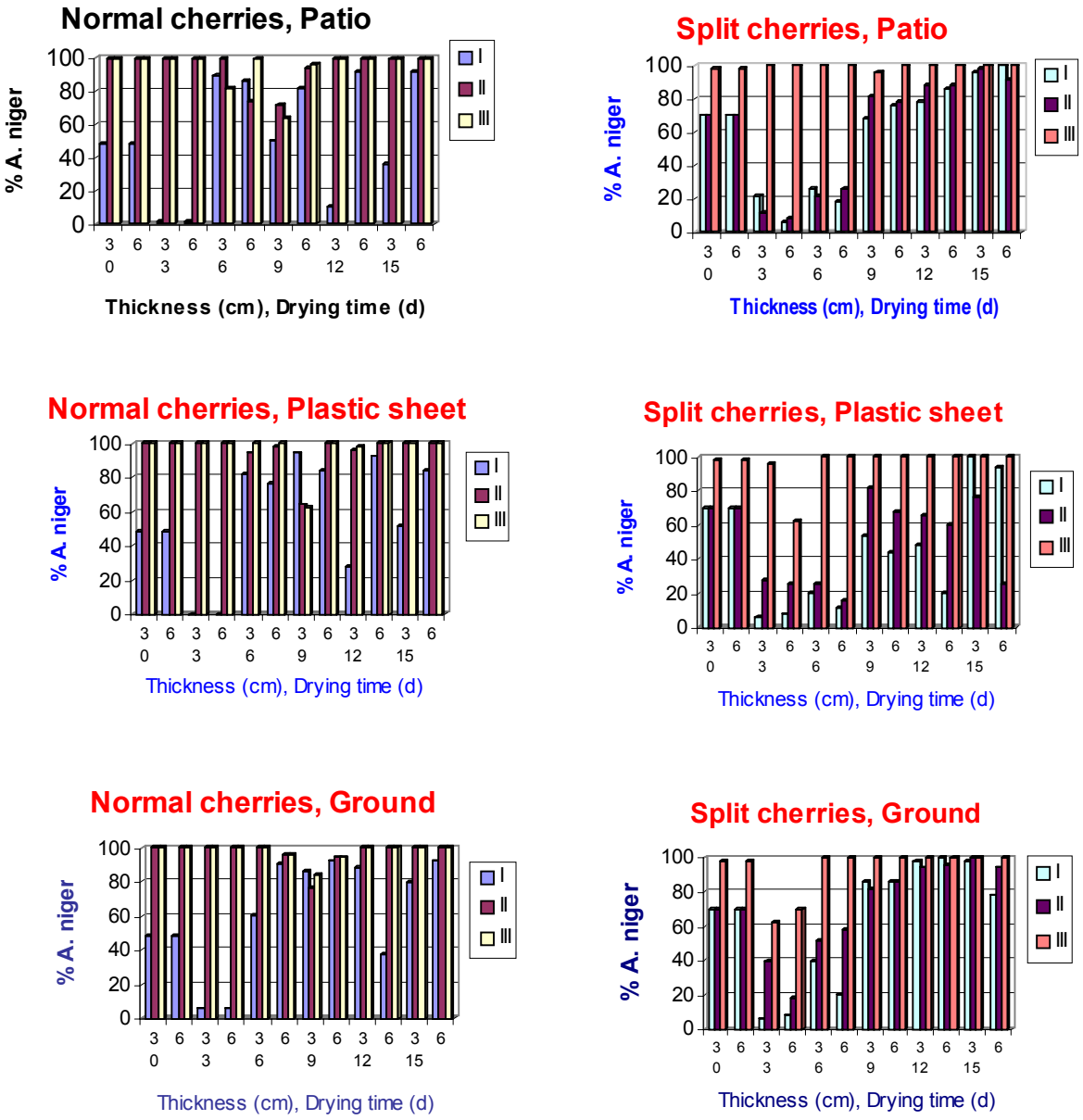

Figure 2. Contamination rates of *A. niger* in coffee beans from normal and split cherries drying treatments

RESULTS AND DISCUSSION

Dominant mould species infecting the cherries during sun drying process were *Aspergillus niger* group, *A. flavus* group, and *A. tamarii*. *A. ochraceus* group was infecting some samples at a lower level except for the red cherries (Figure 1). Some other minor species of genus *Penicillium* and *Aspergillus* were also encountered in the coffee beans (data are not mentioned).

Mould species were growing during the drying course, and the maximum growth was at 3 to 9 days, and became steady after that period. The growth was likely determined by the moisture status of the beans, which is higher at the early period

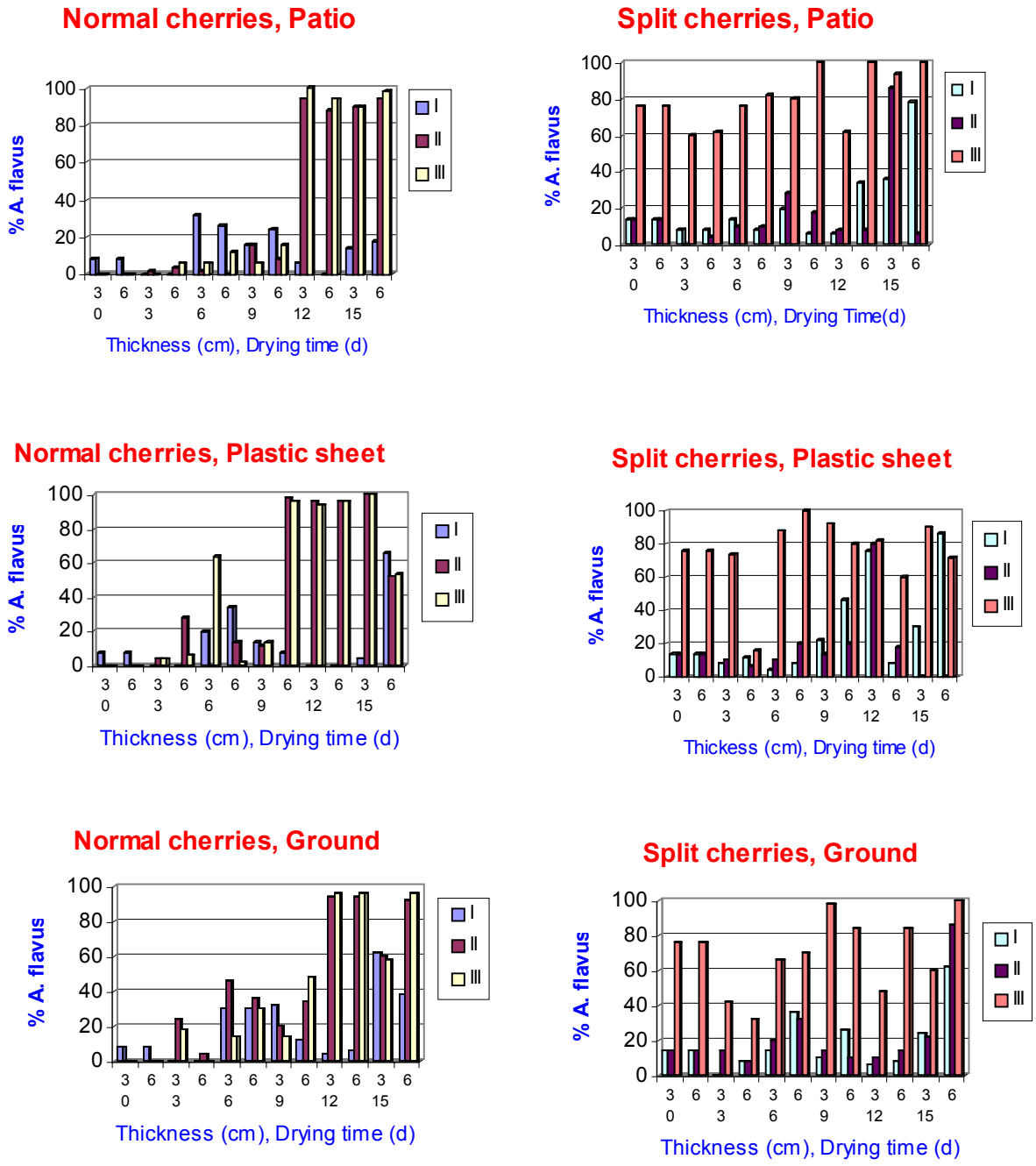

Figure 3. Contamination rates of *A. flavus* in coffee beans from normal and split cherries drying treatments

Contamination status of fresh cherries determined the contamination rate of the beans during the drying. As the moisture content was higher at the early stage of drying it made more suitable for moulds to grow and reached the maximum rate at the first few days of drying. In the final period of drying the contamination rate tended to be steady, due to the lesser moisture content. Thus the mould did not develop anymore.

A. ochraceus grew slower compared to other dominant species. A higher contamination rate was encountered in some samples after 12 days of drying (Figure 1). The maturity of coffee cherries determined the contamination rate, where more incidence occurred in the ripe (red) cherries (see experiment III). The last case was also reported by Bucheli et al. (2000) that the overripe cherries were more susceptible for OTA contamination compared to the green cherries. Ripe cherries have softer and more nutritious pulp which make moulds easily grow.

The mould contamination rate was likely independent to the drying methodology applied. However, coffee splitting tended to reduce contamination rate of *A. ochraceus* compared to the normal cherries. Bucheli et al. (2000) showed that drying of (normal) coffee cherries on concrete, bamboo tables, or ground gave no significant difference..

Since no OTA data had not been available, we could not determine to which titer of *A. ochraceus* that caused contamination of OTA in the beans. Hence, the OTA analysis is needed to confirm the relationship.

A. niger, the most dominant species, was contaminating the coffee beans at high rates (Figure 2). Splitting of coffee cherries likely reduced the contamination rate, except for the ripe ones (see replication III). The contamination was also independent to the drying method. Some strain of *A. niger* were reported capable of producing OTA (Abarca et al., 1994; Ono et al., 1995). However, our *in vitro* test to some *A. niger* isolates resulted none of them was capable producing OTA (unpublished data). Interaction of the species with other species especially *A. ochraceus* is still needed to be understood.

A. flavus was also a dominant species found in the coffee beans. The species steadily grew from the early drying up to 9th days (Figure 3). Coffee splitting did not influence the contamination rate, and it was independent to the drying method studied.

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Enhancement of Coffee Quality in Kenya by Prevention of Mould Current Status and Future Outlook

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SUMMARY

A survey was conducted to find out the likely risky areas in the production chain namely, the primary and secondary processing facilities and in green coffee warehouse of the marketing body. Results indicated that there were conditions such as leaking roofs, crevices in the tools and long storage periods that could favour growth of moulds. The recommended processing procedures, the survey and the activities proposed as Kenya's contribution towards the global project on coffee quality improvement thorough prevention of mould growth are described.

INTRODUCTION

Coffee is one of the most important agricultural commodities whose trading volume on the world market is second to petroleum. Much research has been performed to elucidate the factors that influence the cup quality. For instance, agronomists have put much effort in breeding, cultivation and optimizing the growth conditions of the coffee plant in order to produce high quality fruits (Clarke and Macrae, 1988; Bytof et al., 2000). Food chemists and technologists have investigated the process of coffee roasting and flavour generation. They are now able to put optimum control on the roasting process of green coffee in order to produce the desired quality of the product (Clarke, 1986). However, between harvest and roasting there are numerous steps of post harvest treatment namely, processing, storage and transport that also influence quality.

In Kenya, coffee contributes immensely to the economy. It is ranked the third most important agricultural export contributing 10.4% of total export earning. The coffee subsector employs 30% of the national labour force (Karanja, A M, Agric. Economist, personal communication). Coffee also plays a major role in the socio-cultural rites in some communities such as the Boran of Northern Kenya amongst who, marriage negotiations cannot start until the family of the prospective bridegroom presents a quantity of dried coffee cherries to the prospective bride's parents. The cherries are roasted and chewed.

Kenya produces mild Arabica Coffee of fine quality. The fine quality is a function of the inherent characteristics of the coffee cultivars, climatic and soil factors, field management and processing practices based on research recommendations. Coffee is grown in two climatically different regions namely, west and east of the Rift Valley. West of the Rift Valley experiences one extended rainfall season between March and September and has one coffee crop per year. East of the Rift Valley has two rainfall seasons namely, long rains (March to June) and short rains (October to November). The two rainfall seasons result in two coffee crops per year, the main crop resulting from the long rains flowering and the fly crop from the short rains flowering.

As a producing country, Kenya is concerned about fungal mediated quality deterioration and did collaborate in the European coffee industry pilot project on the mycology of coffee

production. The Kenya Coffee Research Foundation has developed and recommended coffee processing and storage procedures aimed at producing fine quality coffee and prevention of fungal growth. However, a survey conducted in 1998 covering primary and secondary processing and storage facilities revealed existence of conditions that might be conductive to fungal growth (Mmburu, Mason and Maina, unpublished).

This paper describes the recommended coffee quality enhancement procedures, the current processing practices, the findings of the survey and activities proposed as Kenya's component of the global project on coffee quality improvement through prevention of mould growth.

RECOMMENDED PROCEDURES FOR QUALITY ENHANCEMENT

Wet Processed Coffee

The stages in this method include harvesting, sorting, pulping, pregrading, fermentation, washing, drying and storage. The specific objectives accomplished at the various stages are described by Mburu (1995).

During harvesting, only ripe cherries judged by their uniform deep red colour should be picked, care being taken to prevent cherries from falling to the ground. When sorting to separate the unsound cherries e.g. under-ripe, over-ripe, dry, insect damaged, diseased, plant parts such as leaves and branches, contact with soil must be avoided by using clean materials such as tarpaulin.

Pulping of the sound cherries must be done on the day of the harvest. The pulper must be adjusted in such a way as to avoid nipping or crushing the coffee bean. The pulped coffee should be pre-graded on weight and size basis by means of an oscillating screen and water into firsts (those that sing thorough the screen), seconds (those in the water) and lights (those that float). The pulping machinery must be flushed with clean water after pulping.

Fermentation in order to degrade the mucilage which is done in concrete tanks must be completed within thirty six hours. Following fermentation, the parchment coffee should be washed then soaked for 16 hours. A final washing should be done after soaking. At this stage, parchment coffee has a moisture content of about 55%-wet basis. This has to be reduced by drying to 10.5% Wb (Mburu, 1995).

Dry Processed Coffee

In Kenya, only Robusta and the unsound Arabica coffee cherries separated from the red ripe ones during sorting are dry processed. The dried cherries are called buni. Drying should start on the day of harvest by spreading the cherries on drying tables, concrete surfaces or tarpaulin. The cherries should be covered at night and during wet weather. They should be dried to a moisture content of 12% wb. Storage of parchment and clean coffee should be done separately from buni. The store must be moisture and dust free. Coffee should be bagged and not in contact with concrete but on wooden pallets 15 cm away from any surface.

Despite the adequacy of the recommended procedures, the level of their adoption is low. This is attributed to cost and social-economic factors (Nyoro and Whitaker, 1986)

CURRENT PROCESSING PRACTICES

In Kenya, coffee is produced by large scale (Estates) farmers and small holder farmers

organized in co-operative societies. Primary processing of estates coffee takes place on the farms while the small holder farmers deliver their cherries to co-operative factories. In both, the coffee is west processed.

Following liberalization of the coffee industry, the minimum hectarage under coffee that qualifies a farmer to be licensed to operate a pulpery as been revised from four to two hectares. This has resulted in third category of farms called small or hand pulper based estates.

A survey aimed at finding out the critical points in the production chain on the three categories of farms, secondary processing facilities, and in green coffee warehouses of coffee mills and the Coffee Board of Kenya (CBK) which is the coffee marketing body in Kenya, was undertaken in 1998. Observations were made on conditions of process execution in respect to sanitation, conditions such as moisture; temperature and dust that may flavour mould growth, status of processing equipment, structures and adherence to recommended standard processing storage procedures.

Observations

During pregrading at the primary coffee processing factories, some pulp floated together with the lights. As these are not easy to separate when wet they were dried together. When dry the pulp is easily separated from the parchment. Drying tables were sagging, resulting in heaping of wet parchment coffee. Small estates using hand pulpers lacked skills and facilities such as fermentation tanks and drying tables. Their coffee was dried on various types of waterproof materials spread on the ground.

Dry processing by the smallholder farmer was observed to deviate from the recommended procedure: After sorting on each harvest day, the unsound (under-ripe, over-ripe, diseased, insect damaged and green) cherries are accumulated on the farm during the harvest season. Cherries from different harvest days are mixed and are not given attention during the harvest season. At the end of the harvest season, the coffee trees are stripped of the remaining cherries which include over-ripe, under-ripe, dry and green mature ones. These are mixed with those previously picked, and dried together.

Storage of parchment coffee at the co-operative primary processing factories was very poor, as many of the stores were improvisations. Some were damp and poorly ventilated. At one of the mills, coffee beans spilt on the floor were swept and heaped on the concrete floor. It may take several weeks before they are mechanically separated from the dust.

Storage conditions at the warehouses of the coffee millers and CBK were under natural ventilation. They lacked equipment for regulating internal environmental conditions of temperature and relative humidity. Cracks on the floor and walls and leaking roofs in some warehouses were observed. Films of dust were also observed except in the new mills. Storage of parchment and clean coffee was mainly in contact with concrete floors and walls contrary to the recommendation of wooden pallets 15 cm away from any surface. Some clean coffee lots were left uncollected from the CBK warehouses for years and were not quarantines from fresh stock. These observations indicate that there were critical points in the production chain at which growth of mould could occur.

KENYA'S COMPONENT OF GLOBAL PROJECT ON COFFEE QUALITY ENHANCEMENT BY PREVETION OF MOULD: FIRST YEAR ACTIVITES

In view of the diversity in the current production, processing and storage practices in Kenya, it I important to establish baseline information on the occurrence and types of OTA-produced fungi in the production and processing chain. The accomplishment of this objective will require detailed studies of the points hypothesized to be critical. The studies will involve sampling and isolation of OTA-producers fungi from selected sites.

This initial sampling will be base in Trans Nzoia District, representing the western climatic conditions and in Kirinyaga District, representing the eastern climatic conditions. The site will be farms of less than 20 acres, chosen with regard to geographic representation of the region and market position. Hence 10 co-operative farms and 10 small estates in each District will comprise the sampling sited.

Activities during the Main Harvesting Season

Agronomic and processing practices on the selected farms will be established by means of a questionnaire. Fresh cherries and coffee rhizosphere soil samples will be taken for microbiological analysis. Resampling will be on three farms of each type. Two will have shown evidence of harbouring OTA producing fungi and one hot, based on the initial analysis. Small estate samples of fresh cherries, parchment and buni will be taken for microbiological analysis.

The corresponding smallholder samples will be taken at their respective co-operative factories. Air samples at different orchard position and the drying yards at the farms and co-operative factories will be taken and analyzed.

Activities During the Fly Crop Harvest/Interharvest Period

In each District, one farm with evidence of OTA-producer fungi and one without will be selected. Fruit defects such as coffee berry disease and coffee berry borer damaged, over-ripe, fallen, split and mouldy will be collected and subjected to microbiological analysis. Samples containing OTA-producer fungi will be analyzed for OTA. Studies to investigate possible co-relations between fruit defects and occurrence of OTA-producer fungi will be carried out.

To Investigate transport, handling and storage, coffee samples (a few bags) will be taken from two known sources, one harboring OTA- producer fungi and one not. These will be subjected to microbiological analysis. Buni coffee sampling and analysis will be done throughout the chain to export.

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Toxigenic Mould Species Infestation in Coffee Beans Taken from Different Levels of Production and Trading in Lampung-Indonesia

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SUMMARY

Indonesian coffee is mainly produced by small scale farming system using a simple dry process procedure. The coffee product is dispatched to middlemen traders before being accepted by exporters. Some treatments are applied to the raw coffee beans before it is ready for export. An evaluation of mould species contamination of coffee beans taken from various levels of coffee chains was conducted. Its moisture content and physical quality were also observed. Mould species had contaminated the beans from early stage of production at the farmer level, and still existed at the last chain. The dominant species observed were *A. niger*, followed by *P. citrinum*, and *A. flavus*. *A. ochraceus* was present in a few samples. Mould contamination status was likely correlated with the handling procedure of each coffee sample. Coffee beans with a better quality had less contamination rate. Mould contamination status and moisture content of coffee beans samples taken from each level are presented. The handling procedure which gave less or no contamination of *A. ochraceus* needs to be resolved.

INTRODUCTION

Contamination of ochratoxin A (OTA) in coffee has recently become the main issue, since publications concerning the occurrence of OTA in coffee (Tsubouchi et al., 1984; Studer-Rohr et al., 1995; Nakajima et al., 1997; van der Stegen et al., 1997). More recently, Romani et al. (2000) reported that 106 out of 162 samples from various origins were positively containing OTA with concentration ranging from 0 to 48 ppb. It was also reported that African coffee samples were more contaminated with respect to other origin in terms of frequency and level of OTA. Nevertheless, some samples from Asia were also contaminated with the toxin.

The contamination of OTA in coffee is likely to have occurred in the early stages of its production. Experiments on storage of green coffee under tropical conditions proved that neither the growth and presence of OTA-producing fungi nor consistent OTA production was observed during the storage up to 8 months (Bucheli et al., 1998). Development of OTA in coffee was also proved to occur at the sun drying stage (Bucheli et al., 2000). Some mould species grew during the sun drying, including the OTA-producing *Aspergillus ochraceus* (Ismayadi et al., 2001). Appropriateness of drying, handling procedures and maturity of coffee cherries determined OTA contamination status of coffee product.

At present, Indonesian coffee plantations cover an area of 1.1 million ha consisting of about 95% small-holders, and 5% private and state owned plantations. More than 90% of which is robusta type. Millions of farmers are involved in the coffee production, with the acreage of about 0.6 ha in average. The small scale farming system applies simple procedure both in the

plantation management and coffee cherries processing. Mostly, they apply the dry processing method for robusta coffee and simple semi-wet method for arabica coffee.

Farmers sell their coffee as raw coffee beans (known as *"asalan"*) to one to two levels of local traders before finally coming to the exporters. Some final treatments to the raw coffee like re-drying, size grading, machine sorting of defectives, and polishing are conducted by exporters before it is suitable for export.

OTA can be produced by some fungal species of *Aspergillus* and by *Penicillium verrucosum* in certain conditions with a minimum a_w of 0.85 (Moss, 1996). Due to the open system of coffee processing, make it subject to be fungal infested and contaminated by their toxins at a suitable condition. Handling procedures of coffee at every stage of production and trading will determine the contamination status of mould and the toxins. Since various procedures are applied by farmers with different climatic conditions, it might cause various contamination status to the coffee product. This study describes the fungal contamination status of coffee samples taken from various stages of production at farmer level, local trading up to exporters level.

MATERIALS AND METHODS

Coffee samples were taken from farmers, middlemen, and exporters in Lampung Province. Most of the samples taken from farmers were still under processing, only 3 samples were ready for local trading types. All samples from middlemen were of the raw '*asalan*' coffee, and coffee samples from exporters were of raw and re-processed ones.

The moisture content was determined according to the standard oven method (SNI 01-2907-1999). Mould contamination level was determined by the direct plating procedure of 50 beans on DG-18 media and expressed in percentage. The fungal species were determined according to the standard procedure (Samson et al., 1996).

RESULTS AND DISCUSSION

The results of the mould analyses and moisture content determination of coffee samples were from farmers, middlemen and exporters are shown in Table 1 to 3. The moisture content of the samples varied from 16.53-18.47. The dominant fungal species found in the coffee samples were *A. niger*, *A. flavus*, *P. citrinum*, and *A. ochraceus*. Some other species were also encountered mostly in the genus of *Aspergillus* and *Penicillium*.

Farmers in Lampung (and other areas in southern Sumatera) process their coffee by the simple dry method. The unselected coffee cherries are dried on the ground, only few of them use a concrete floor or a plastic sheet. After a few days of sun drying. The drying time varies from 7 to 20 days depending on the climatic condition. Drying will be terminated when they judge it is dry enough to sell. Most farmers do not have a moisture tester. After drying the coffee is de-hulled with a motor-driven coffee huller. The coffee beans ar then locally traded as raw coffee (known as '*asalan*'). One to two levels of local traders are involved in the local trading. The trader mixes the accepted coffee and do minor treatments like sorting out of defectives and re-drying of wet beans.

Raw coffee samples taken from farmers and local traders mostly still had high moisture content (Tables 1-2). The average value was about 17%, and it was similar to the previous study (Dharmaputra et al., 1999). The relatively high moisture content of the raw coffee beans

is common in the local trading system. Farmers are not encouraged to produce raw coffee beans with less moisture content since no significant premium price is given.

Local traders usually sell the raw coffee beans to exporters in the port city (Bandar Lampung). Some treatments are normally done to the coffee i.e. re-drying using a drying machine, electronic sorting-out of defectives, size grading, polishing and packaging. By such treatments raw coffee beans normally becomes exportable coffee of grade 4 to grade 6. Only the coffee processed by the wet method yields a better grade. Beans' moisture content at the exporter's level were still high (Table 3), and it needs re-drying to meet the standard of 13%. Our previous study found that exportable coffee had moisture content of 12.6% in average (Dharmaputra et al., 1999).

Moulds contamination status was individual to each sample, and there was no specific correlation to the level of production and trading. It was proven that mould contamination had started at farmers' level, and it remained in the beans up to the exporters' level.

Some coffee samples consisted of mould-free beans at a low rate, and the frequency was higher to samples taken from farmers, while rare samples were found at the following levels. The quality of coffee samples containing mould-free beans was better compared to the highly contaminated samples, since the higher physical quality coffee had less moulds contamination. The condition might be achieved by a better handling of coffee during processing and storage (Bucheli et al., 1998; 2000). Processing conditions that led the high level of mould-free beans should be elucidated, hence could be used as a standard operating procedure (SOP) to be applied by farmers and traders.

Although, mould species had infested and grown at the first stage of coffee handling at the farmers' level, some farmers processed their coffee in a better way yielded better quality, less mould contamination especially *A. ochraceus*, the OTA producing species (Table 1). The two samples processed by the wet method had a higher proportion of mould-free beans, although some species were still present in the beans at a lower level. Moulds had infested coffee cherries and grown before the beans were dry. The wet process needs ripe cherries which are suitable to be de-pulped. However, ripe cherries are more susceptible to mould development (Bucheli et al., 2000; Ismayadi et al., 2001). The drying time of parchment coffee is faster compared to the whole cherries, providing less chance for moulds to grow. The separation of the pulp, which was proved to be the source of mould species and OTA, leads to less contamination in the beans.

As coffee maturity had determined the contamination status, where more OTA was observed in overripe cherries, compared to the green cherries; it is noted that processing of ripe and overripe cherries under the dry method may yield high contamination rate of moulds and OTA. The farmers in Lampung (and other areas) normally pick their coffee unselectively yielding a mixed of green and ripe cherries. They pick the coffee 2 to 4 times per season. Correlation of the coffee maturity with the contamination status in the mixed cherries is still needed to be elucidated, together with the (physical and cup) quality of the product.

The traditional drying on the ground might yield good and less mould contaminated beans, with few mould-free beans (Table 1). Bucheli et al. (2000) proved that drying coffee on the ground, a concrete and bamboo mats gave no significant difference to the OTA level in the coffee beans. However, drying on the ground made the beans dirty, and may yield high contamination and defects of the beans if it is not strictly controlled, especially at the moist condition.

The most dominant mould species found in the samples was *A. niger* group. It was reported that some strains of the species were capable of producing OTA (Abarca et al., 1994; Ono et al., 1995). *In vitro* test to some isolates of the species in their ability to produce OTA proved that no isolate capable producing OTA (Ismayadi, unpublished).

Other dominant species like *A. flavus* and *P. citrinum* may produce some toxins. The OTA-producing species, *A. ochraceus*, although contaminated at a lower rate could cause OTA contamination in the beans. OTA concentration in the samples had not been analyzed, hence we could not predict to which level of contamination of the species caused OTA accumulation in the bean.

Table 1. Moulds contamination of coffee samples collected from farmers in Lampung

Coffee samples	MC (%)	Mould-free beans (%)	Contamination level (%)				
			A. niger	*A. flavus*	***A. ochraceus***	*P. citrinum*	Other species
Dried on ground, 3 days	16.97	-	96	2	**-**	76	12
Dried on ground 3 days	16.94	6	92	-	**4**	10	12
Dried on ground 7 days	16.53	-	58	10	**72**	8	12
Dried on ground 15 days	16.95	-	98	-	**-**	12	18
Dried on ground 15 days	17.17	6	74	4	**-**	28	14
Dried on terpaulin, 15 days	18.47	8	56	4	**-**	28	20
Dried on concrete	17.05	-	80	30	**-**	96	12
Asalan-1-Wet processed	16.29	16	10	2	**4**	54	34
Asalan-2-Wet processed	16.50	20	16	-	**12**	54	8
Asalan DP	17.00	-	100	-	**16**	62	14

Since the fungal contamination had occurred at the first level of coffee production, it is better to provide such practices for the farmers and middlemen to produce better quality and OTA-free coffee. Farmers sell only low moisture and defective beans, providing a better coffee beans in local trading and export. Significant premium price has to be awarded to the farmers to produce the better coffee.

CONCLUSIONS

Mould contamination in coffee beans had started at the farmers' level, remains there up to the exporters' level. Handling of coffee at each stage determined the contamination status. Some samples collected from farmers contained some mould-free or free from A. ochraceus beans. The frequency samples with mould-free beans were reduced in the following levels. The production procedure yielding better, less contaminated or mould-free coffee beans needs to be elucidated.

Table 2. Moulds contamination of coffee samples collected from middlemen in Lampung

Coffee origins	MC (%)	Mould-free beans (%)	Contamination level (%)				
			A. niger	*A. flavus*	***A. ochraceus***	*P. citrinum*	Other species
Asalan-1, stored 1 month	17.08	-	100	-	**24**	-	-
Asalan-2, stored 1 month	17.32	-	94	4	**2**	46	12
Asalan-3, stored 1 month	17.51	-	98	-	**-**	26	4
Asalan-4, stored 1 month	17.91	-	100	6	**-**	8	-
Asalan-5, stored 1 month	17.69	-	66	4	**36**	52	12
Asalan-6 stored 3 months	16.68	-	100	-	**10**	56	80
Asalan-7, stored 3 months	17.83	34	40	-	**2**	20	32
Asalan-8, stored 6 months	16.76	-	100	42	**20**	10	2

Table 3. Moulds contamination of coffee samples collected from exporters in Lampung

Coffee origin	Moisture content (%)	Mould-free beans (%)	Contamination level (%)				
			A. niger	*A. flavus*	***A. ochraceus***	*P. citrinum*	Other species
Asalan-1	16.75	-	100	28	**6**	14	4
Asalan-2	16.70	-	90	2	**-**	34	8
Asalan-3	18.83	-	64	14	**30**	76	62
Asalan-4	17.71	-	78	14	**10**	60	14
Raw coffee for a polisher	16.74	-	96	4	**2**	30	12
After polished coffee (AP)	ND	-	76	50	**-**	6	10
Re-drying-1	16.70	-	94	36	**6**	36	8
Re-drying-2	16.39	2	84	2	**-**	54	18
Grade 4-1	17.23	-	92	6	**-**	10	10
Grade 4-2	18.36	-	20	2	**8**	78	68
Grade 4-3	15.88	-	100	8	**2**	24	14
Grade 4-4	16.69	-	82	8	**-**	6	54

Note: ND = not determined

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Mould and Ochratoxin A (OTA) Contamination in Coffee Samples from Four Districts in Uganda

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SUMMARY

Coffee samples (*C.canephora* and *C.arabica*) were collected from four coffee growing districts in Uganda. The samples were from cherries fallen on the ground under coffee trees, fresh ripe cherries from trees, green coffee beans from stores, dried coffee cherries and parchment coffee sampled from farmers drying yards. The coffee samples were dried on various surfaces to below 13% moisture content, surface disinfected by chlorine 0.4%, plated on Dichloran Rose Bengal Chloramphenicol agar at 25^{o}C for 7 days. Samples dried on raised trays and tarpaulins were generally less infected by the predominant moulds than those dried on bare ground. Isolates of *A.ochraceus* group, *A.niger* group, *Penicillium species,* like *verrucosum,* and *A.carbonarius* which were identified were inoculated on DRBC agar and their capability to produce OTA determined by Agar plug technique. Other portions of coffee samples were analysed for OTA contamination by Vicam Ochratest method and HPLC. OTA contamination was generally higher in dry processed coffee beans dried on the ground than wet processed coffee dried on trays. OTA levels in the samples were with in the range of 0-13 μg/kg.

INTRODUCTION

Uganda produces about 90% dry processed robusta coffee and 10% arabica coffee a majority of which is wet processed. Mould infestation has been implicated in both dry and wet processed coffees but the occurrence of both mould and OTA has mainly been found in dry processed than wet processed coffee.

In Uganda most farmers especially the low income earners with small coffee farms dry their coffee directly on bare ground in their homes. Others dry their coffee on papyrus mats, tarpaulins, raised platforms and a few dry on concrete or cemented floors. The method of drying adapted by the farmer highly influences the attack on coffee by moulds which may produce Ochratoxin A.

The objective of this work was to determine the common types of mould prevalent in coffee in Uganda, and also to establish those that are OTA producers. The relationship between the method of harvesting, drying and other coffee handling practices to the occurrence of mould and OTA production was investigated. Results from this work will be used in determining how best farmers and coffee dealers at all stages of the production chain should handle their coffee in order to prevent mould and OTA contamination.

MATERIALS AND METHODS

Sampling

Coffee samples were collected from farms, processing factories and stores in four coffee producing districts namely Mukono, Iganga, Masaka and Mbale. The type of samples collected were:

I. Robusta Coffee cherries fallen on the ground under coffee trees.
II. Fresh ripe robusta coffee cherries from trees.
III. Dry processed coffee beans from storage bags.
IV. Dried robusta coffee cherries from farmers drying yard.
V. Wet processed arabica coffee from farmers.

Sample Preparation

The ripe fresh robusta cherries were subdivided into equal proportions. One portion was separated into two parts and dried on raised trays and bare ground. Another portion of this fresh coffee sample was pulped, washed and the parchment was dried on raised trays and bare ground. All other samples were dried on raised trays including those which were found already being dried on the ground in the field. All the samples were sun dried to moisture content of below 13.0% which was determined by a moisture analyser Sinar 6060. Upon drying, the samples were sealed in khaki paper bags and taken to the Uganda National Bureau of Standards for mycological analysis. All samples for Ochratoxin A and quality analysis were hulled in the UDCA laboratory and the green coffee beans put in plastic sample bags and kept for later analysis.

Microbiological Analysis

The methodology used for isolation of fungi from coffee was based on the recommendation of the International Commission on Food Microbiology (ICFM; Pitt et al., 1992).

A minimum of 50 coffee cherries/seeds from each coffee sample were surface sterilised by immersion in 0.4% NaOCL for 2 minutes, after which the NaOCL was poured off and the samples rinsed three times in sterile distilled water. The coffee cherries/seeds were plated on DRBC (King et al., 1979) media (10 cherries/seeds per plate). The plates were incubated upright at 25^{o}C for 7 days according to the standard incubation regime for general fungal enumeration. After this incubation period, the plates were removed and the percentage of mould infection determined. Identification of the various genera was made possible by use of microscopy and a monograph of Raper and Fennel (1995).

The method for testing fungal mycotoxins was according to Filtenborg et al. (1983). Pure cultures of major genera observed on the plates were prepared by scooping a small portion of the mould using the blunt end of a sterile needle and inoculating it on pre-poured and set plates with DRBC media (DRBC; King et al., 1979). The cultures were incubated at 25^{o}C for 7 days.

Mycotoxin Analysis

Agar Plug Technique

One plug was cut out from the centre of each of the mould colony with a flame sterile stainless steel blunt needle (diameter 0.5 mm). The plugs were removed by using a flame sterilised pair of forceps and transferred to TLC plates. By means of a pasteur pipette, 2 drops of extraction mixture (Chloroform: Methanol, 2:1) was put on the agar plug. While still wet, the mycelium side of the plugs were gently pressed against the application line on the TLC plates and then removed. After drying the application spots, the TLC plates were placed in a developing tank containing the developing mixture (Toluene:Ethyl acetate:Formic acid, 5:4:1), and the plates were developed for an hour. The plates were then removed, dried in air for 5 minutes and then placed in a U.V light illumination cabin. The toxins were visualised in U.V light at 366 nm. The colour and R_f values of fluorescent spots were observed and compared with that from the OTA standard.

Ochratest-Vicam

The method used to analyse OTA in green coffee samples was based on the procedure recommended by Vicam in the Ochratest method. Each coffee sample (25 g) was blended with 50 ml methanol: 1% sodium bicarbonate (70:30 by volume) at high speed for 1 minute. The extract was filtered through a fluted paper into a clean beaker. To 10.0 ml of the filtered extract was added 40 ml of PBS/2% Tween-20 wash buffer and the mixture was shaken for thorough mixing. Two end caps were removed from the Ochratest affinity column and the column was then attached to the outlet of a 10 ml glass syringe barrel on a pump stand. The diluted extract was filtered through the glass microfiber filter into a glass syringe barrel using markings on the barrel to measure 10 ml. The filtered extract (10 ml) was passed through the Ochratest column at a rate of 1-2 drops/second (10 ml = 1.0 g sample equivalent). The column was then washed with 10ml of PBS/2.0% Tween-20 at a rate of 1-2 drops per second followed by 10 ml of distilled water at the same rate. The Ochratest column was eluted with 1.5 ml Ochratest eluting solution at a rate of 1 drop/second and all the sample eluate (1.5 ml) was collected in a glass cuvette. The cuvette was immediately placed in a pre-calibrated Fluorometer Vicam V1 series 4 to measure fluorescence and OTA concentration was read after 60 seconds. The assay range was 0-50 μg/kg and the limit of detection was 1.0 μg/kg.

HPLC

OTA analysis was performed at Nestle Research Laboratory by Mr. A Pittet.

RESULTS AND DISCUSSION

Microbiological and OTA Analyses

After incubation, each plate was read and the number of infected cherries/seeds counted and expressed as a percentage of the total number of seeds plated. Also differential counting of major genera was done and results recorded in Tables 1-5. The results showed that all the samples were mainly infected by *A.niger* group*, A.ochraceous* group and *Penicillium spp,* like *verrucosum,* and least infected by *A.carbonarius*. *A.carbonarius* was detected in only four samples; one coffee cherry sample and three green coffee bean samples. All these samples were from the stores in the field.

Dry robusta cherries fallen on the ground

All the coffee cherries fallen on the ground were found to be infected by *A.niger, A.ochraceus* and *P.spp.* and all tested positive for OTA tests. *A.niger* was the mould found with a very high incidence. No *A.carbonarius* was found. All the samples tested positive for OTA using both the Vicam Ochratest and HPLC. All the samples showed OTA levels less than 10 μg/kg. Sample MKK9 which showed very high levels of OTA had been rewetted during the drying period.

Table 1. Occurrence of OTA producing fungi in dry robusta cherries fallen on the ground

REGION/ SAMPLE	DRYING SURFACE	*A.ochraceus*	*A.niger*	*P.spp*	*A.carbonarius*	OTA(μg/kg)
IGK1	Trays	2(+)	80	0	0	N.D
MKK5	Trays	0	46(+)	8(+)	0	2.5
MKK9	Trays	0	52(+)	18(+)	0	5.4 400 HPLC
MSK9	Trays	0	44	20	0	9.2
MSK13	Trays	0	36(+)	4	0	9.3

(+) = the presence of OTA using the Agar Plug technique, N.D= Not detected

Fresh cherries dried on the ground and trays

Table 2

Shows dry processed fresh ripe coffee cherry samples which had been picked from trees and dried in raised trays and on bare ground. No *A.carbonarius* was detected in any of these samples. All the samples were infected by *A.niger* and least infected by *P.spp* but the incident of *A.niger* which was OTA producing was higher in samples dried on bare ground than those dried on raised trays. All the samples analysed for OTA showed amounts less than 4.5 μg/kg. A UCDA trial experiment of fresh cherries dried on trays (MKK8) and bare ground (MKK10) and pulped coffee dried on trays (MKP3) and on ground (MKP4) see Table 5 showed moderate infection by *A.niger* which was an OTA producer, and less infection by *A.ochraceus* and *P.spp*. All the samples dried on the ground showed higher levels of OTA contamination than the ones dried on trays.

Table 3

Above shows green coffee bean samples picked from the factory separator and bags at the coffee processing plant. All the samples were almost 100% infected by *A.niger* and 33% of its isolates were OTA producing. Three samples were moderately infected by *A.carbonarius* which indicated that *A.carbonarius* thrives in green coffee beans more than coffee cherries. *A.ochraceus* was isolated in 66.6% of the samples analysed whereas *P.spp* was isolated from three samples. Analysis of OTA in the samples revealed minimal amounts of OTA ranging from 2.3 to 5.2 μg/kg which pointed to the fact that hulling reduces the occurrence of OTA in coffee.

Coffee cherry samples were also taken from various sources especially those which were being dried on the tarpaulin, bare ground and from coffee which was being stored in bags (Table 4). Still in these samples, *A.niger* was the predominant fungi detected. Both *A.ochraceus* and *P.spp* were significantly low and *A.carbonarius* was detected in only one

sample. Even samples which were being dried on tarpaulin were highly infected by *A.niger* which pointed to cross contamination from either the processing plant or from the air. However most of the farmers could not reveal whether during the course of drying their coffee had experienced any rewetting or not. OTA analysis showed minimal OTA contamination of below 5.4 μg/kg except for two samples from Masaka which had been dried on the ground and stored in bags which had 9.1 and 13.0 μg/kg OTA respectively. It was also noted that generally the samples which were being dried in the drying yard had less OTA compared to the coffee which was already in store which showed that OTA production continues even during storage of improperly dried coffee cherries**.**

Table 2. Occurrence of OTA producing fungi in fresh ripe robusta cherries from trees

REGION/ SAMPLE	DRYING SURFACE	*A.ochraceus*	*A.niger*	*P.spp*	*A.carbonarius*	OTA (μg/kg)
MKK8	Trays	2(+)	16(+)	4	0	N.D (HPLC)
MKK10	Ground	0	56(+)	2	0	0.2 (HPLC)
MKK11	Trays	0	48	4(+)	0	N.D (HPLC)
MKK12	Trays	2	64	0	0	3.4
MKK15	Trays	0	30	0	0	N.D (HPLC)
MKK16	Ground	0	52	0	0	N.D (HPLC)
MKK17	Ground	16	84(+)	0	0	4.5
MKK19	Ground	2(+)	18(+)	10	0	3.9
MSK10	Trays	8(+)	30	8	0	1.2
MSK15	Ground	0	36(+)	0	0	3.6

(+) = the presence of OTA using the Agar Plug technique, N.D= Not detected

Table 5

Shows parchment coffee samples which include coffee found being stored in baskets, polythene bags, cemented floor and also coffee which was processed and dried on raised tray and the ground. Both *A.ochraceus* and *A.carbonarius* were not found in arabica parchment coffee analysed except for one sample found stored in bags which had 8.0% infection and another dried on a raised tray which had 98% infection. Only *A.niger* and *P.spp* were found in the coffee analysed. The effect of rewetting was envisaged in one robusta parchment coffee sample which was highly infected by the three major fungi isolated of which *A.ochraceus* and *P. verrucosum* were found to be OTA producers. OTA analysis by HPLC showed very low levels of OTA which meant that wet processing significantly reduces OTA production in parchment coffee. There were no significant differences between a sample of robusta parchment dried on the ground (MKP4) and the one dried on raised trays (MKP3) respectively although *A.niger* was more in coffee dried on bare ground than the one dried on trays. Both coffee samples showed low OTA amounts of 0.5 and 0.2 μg/kg respectively.

Green coffee beans sampled from processing plants and stores had *A.niger* which was the most predominant mould isolated especially from coffees which were being stored in bags while wet. Suprisingly, coffee cherries fallen on the ground under trees had the least infection rate by all the major mould genera. Also, no *P. spp* was found in green coffee beans apart from one arabica sample from Mbale (12%) and one robusta sample from Mukono district (90%). Though it is assumed that percentage infection decreases upon hulling, this was not

proved to be the case. It is in this regard that farmers need to continue to be sensitised to observe GAP, GMP and GHP at post harvest level.

Table 3. Occurrence of OTA producing fungi in dry processed coffee beans from storage bags

SAMPLE	DRYING/ STORE	*A.ochraceus*	*A.niger*	*P.spp*	*A.carbonarius*	OTA(µg/kg)
IGF1	Bags	30	100(+)	0	2	3
MBF1	Bags	0	92	0	0	3.4
MBF2	Bags	48	100	12	0	5
MKF1	Tarpaulin	0	100(+)	0	2	3.1
MKF2	Separator	44	100	90	0	2.6
MKF3	Bags	20	90	84	0	2.3
MSF1	Tarpaulin	4	100	0	14	3.3
MSF2	Tarpaulin	6	100	0	0	5.2

(+) = the presence of OTA using the Agar Plug technique, N.D= Not detected

Table 4. Occurrence of OTA producing fungi in dried robusta coffee cherries from yard

SAMPLE	FACTORY/ STORE	*A.ochraceus*	*A.niger*	*P.spp*	*A.carbonarius*	OTA (µg/kg)
IGK5	Tarpaulin	4	100	0	0	0.5 (HPLC)
MKK1	Tarpaulin	4	100	0	0	N.D
MKK2	Ground	2	80	2(+)	0	N.D
MKK3	Bags	6	100	0	0	4.2
MKK6	Ground	6	72	0	0	5.4
MSK2	Ground	4(+)	60	0	0	1.2
MSK3	Ground	6	64	0	0	9.1
MSK4	Bags	0	92	0	4	13
MSK12	Bags	2(+)	58(+)	0	0	3

(+) = the presence of OTA using the Agar Plug technique, N.D= Not detected

Table 5. Occurrence of OTA producing fungi in wet processed coffee

SAMPLE	DRYING/ STORE	*A.ochraceus*	*A.niger*	*P.spp*	*A.carbonarius*	OTA (µg/kg)
MBP2	Cemented Floor	0	78	26	0	4 4.7 (HPLC)
MBP4	Baskets	0	96	0	0	N.D
MBP5	Baskets	0	80	18	0	N.D
MBP6	Trays	0	68	2	0	N.D (HPLC)
MBP10	Trays	0	80	0	0	N.D (HPLC)
MKP1	Trays	0	82	28	0	N.D
MKP2	Trays	98(+)	98	100(+)	0	N.D (HPLC)
MKP3	Trays	0	14	0	0	0.2 (HPLC)
MKP4	Ground	0	46	0	0	0.5 (HPLC)

(+) = the presence of OTA using the Agar Plug technique, N.D= Not detected

Detection of OTA in coffee samples

Tables 1-5 showed coffee samples which have been analysed for OTA and the amounts found. Out of the 41 samples that have been analysed (15 samples by HPLC and 26 samples by Ochratest Vicam procedures respectively, it was noted that only one sample out of 13 positive ones had a significant amount of OTA 400 μg/kg (HPLC) which was believed to be due to rewetting because the same sample which was later analysed by Ochratest showed low level of 5.4 μg/kg. Very minimal concentrations of OTA were found in the other 12 samples analysed by HPLC and its occurrence was not significant in most of the samples pulped/un pulped whether dried on tarpaulins or bare ground. OTA by Ochratest procedure in coffee cherries from Masaka district ranged from 2.0-13.0 μg/kg and generally coffee cherries and green coffee from Mukono had least contamination. This is confirmed by the fact that the farmers and processors in Mukono adapted GAP/GMP practices earlier.

CONCLUSION

A. niger was the most predominant mould isolated in all samples from all districts. *A. ochraceous*, and *P. spp,* like *verrucosum,* were prevalent and *A. carbonarius* was least common. The prominence of *A. niger* could however have resulted from cross contamination during sampling and experimentation. All samples from those areas that practised GAP, GMP and GHP at farmer and processor levels showed less mould and OTA contamination. Also post harvest methods of handling coffee directly influence production of OTA in both pulped and un pulped coffee. Wet processing reduced mould contamination and OTA production more significantly both in arabica and robusta samples.

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Strategies to Guarantee the Quality of the Beverage in Colombian Coffees

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SUMMARY

Colombia has a great tradition as a coffee producing and exporting country and it is the world's premier producer of mild coffee. Today coffee represents 20% of the country's foreign exchange income. Although the quality of the exported coffee has always been assessed and controlled, by the coffee authorities, avoiding that poor quality coffees reach the foreign market, today Colombia's purpose is to enhance the coffee quality controls, in order to improve the competitiveness in the international trade market and to satisfy the consumers with a safe and high quality product. It is known that micro-organisms are involved in coffee processing but improper practices, mainly during the drying and storage steps can lead to spoilage, taints and growth of mycotoxin-producing filamentous fungi. Realising the importance of these factors, several research studies have been carried out in Colombia related to the impact of coffee processing practices and conditions on coffee quality, such as the immature harvested beans, over fermentation processes, storage of wet parchment coffee and inadequate drying. The relationship between OTA formation and quality of coffee depending on the moisture content of the coffee beans, and on the relative humidity and temperature of storage have been also considered. The latest results of these studies are now available to the coffee farmers and extension personnel in Technical brochures which emphasise the good coffee processing practices and the importance of carefully drying for the prevention of deterioration of coffee quality. Colombia is working on the transference of the technological knowledge to the farmers through recommendations, courses and advisory visits. This year it has been planned to begin a sampling work in selected coffee farms, representative of the kinds of farms in the regions of study, in order to know the microorganisms involved in coffee processing in Colombia, and also to identify the hazards and critical control points in coffee processing related to OTA formation.

RESUMEN

Colombia tiene una gran tradición como productor de café y es el primer productor de café suave. Actualmente el café representa el 20% de los ingresos externos del país. Aunque la calidad del café exportado ha sido siempre controlada por la autoridades, evitando que cafés de inferior calidad salgan al mercado, hoy el país tiene como propósito mejorar los controles de calidad con el fin de aumentar la competitividad del café en los mercados internacionales y satisfacer al consumidor con un producto de excelente calidad y seguro. Es conocido que los microorganismos hacen parte del proceso del café, pero prácticas inadecuadas, en particular durante el secado y almacenamiento pueden conducir al deterioro del producto, defectos y crecimiento de hongos filamentosos productores de micotoxinas. Teniendo en cuenta la importancia de estos factores se han realizado varios estudios en Colombia relacionados con el efecto de las prácticas y condiciones de proceso de beneficio en la calidad del café, tales como la cosecha de granos inmaduros, los procesos de sobrefermentación, el almacenamiento de café húmedo y el secado inadecuado del café. También se ha investigado sobre la relación

entre la formación de OTA y la calidad del café dependiendo del contenido de humedad del grano y de las condiciones de almacenamiento. Los resultados más recientes de estos estudios están ahora disponibles para el servicio de extensión y los caficultores en forma de boletines de avances técnicos, donde se enfatiza sobre la importancia de las buenas prácticas de beneficio y del secado cuidadoso, para prevenir el deterioro de la calidad del café. Colombia está trabajando en la transferencia del conocimiento a los caficultores por medio de recomendaciones, cursos y visitas de asesoría. Este año se ha planeado comenzar un muestreo en varias fincas seleccionadas, representativas de las regiones de estudio, con el fin de conocer los microorganismos presentes en el proceso de beneficio del café en Colombia, y también identificar los riesgos y puntos críticos de control en el proceso del café, en relación con la formación de OTA.

FULL REPORT

The Colombian coffee has been recognised world-wide as one of the best arabica coffees. The species *Coffea arabica* L., the geographic location and the climate of the Colombian coffee growing zones are favourable factors for the production of mild coffee of high quality. The methods used for controlling pests, as well as the type and care of the coffee processing and the storage conditions determine the quality characteristics of the Colombian coffee beverage.

During processing, the parts of the coffee fruit: beans, pulp and mucilage are separated, then the beans are washed and dried, with the purpose of conserving its physical, sensory and sanitary quality. In Colombia the wet processing method is used. It involves finger-picking of mature cherries, mechanical pulping without using water, and then a fermentation process in tanks, where the coffee beans are left between 12 and 16 hours to remove the mucilage layer, after this process the beans are washed. (*Over 97% of the coffee farmers use the fermentation process to remove the coffee mucilage. There is also a new system called "Becolsub" where pulping, removing of mucilage and washing are simultaneously done by using only 1 Litre Water per Kg parchment coffee*). The wet parchment beans are then mechanical or sun-dried to 10-12% moisture content. Most of the Colombian coffee is sun dried. Finally the parchment is removed from the coffee by hulling to obtain the green coffee, which is then selected by screening, sorted, packed and sold to buyers all over the world.

RECENT RESEARCH STUDIES ON COFFEE QUALITY

During the cultivation and processing about twenty-five defects can be produced, 80% of which are due to inadequate processing practices and non-suitable storage conditions. The quality of the harvested coffee can be deteriorated in few hours by bad processing practices, causing defects and economic losses. Realising the importance of these factors several research studies have been carried out in Colombia related to the impact of processing practices and conditions on coffee quality. The latest results of these studies are now available to the coffee farmers and extension personnel in Technical brochures and posters which emphasise the good processing practices and the importance of drying to prevent deterioration of coffee quality. Next some of these results will be summarised

Impact of immature beans on cup quality

Cup quality of coffee prepared with blends of green and ripe beans was evaluated. Blends with 0.5% to 15% green beans were wet processed. Processing included pulping without water, natural fermentation during 14 h or mechanical removal of mucilage, washing and sun drying. The presence of more than 2.5% green beans in blends, processed either by natural fermentation or by mechanical removal of mucilage, was demonstrated to affect coffee

quality, resulting in more than 30% rejection because of ferment, stinker, earthy and off-taints. Green beans do not pulp completely, causing black and fermented beans, which affect negatively, physical sanitary and organoleptical quality of coffee. The higher percentage of ripe coffee in a sample, the higher the scores in number of good quality cups. In Table 1 is shown the weight of the coffee fruit according to its maturity. It is to emphasise how immature and black beans weight half and a fifth as compared to ripe beans. Therefore not only bean and beverage quality will be affected by harvesting these beans but also harvest and parchment yields are reduced (Puerta, 2000).

Table 1. Weight of the coffee fruit according to its maturity (Puerta, 2000)

Degree of fruit maturity	Fruit Fresh Weight (g)	Fruit Dry weight (g)	Number of fruit per 100 g
Immature beans (green)	1.10	0.35	91
Semi-ripe beans	1.69	0.47	59
Ripe beans	2.06	0.61	49
Over-ripe beans	2.13	0.67	47
Black beans (fallen)	0.44	0.25	223

Influence of coffee processing on quality

Cup quality of beans processed by natural fermentation, mechanical removal of mucilage, without removal of mucilage; washing, without washing, well dried, kept as wet parchment coffee before drying, and processed by the dry method was analysed. Aroma, acidity, bitterness, body and global evaluation of beverage were measured by descriptive quantitative sensory analysis using a 9 point scale. The percentage of good and defective cups per treatment was calculated. The wet process, washing with clean water, and carefully drying produced the best beverages, in comparison to the other types and processing conditions. It was found that either ripe or immature coffee beans processed by the dry method presented stinker defect. Drying coffee with its mucilage produced dark and sticky beans, and aroma and taste like vinegar. The storage of wet parchment coffee affected the cup quality and earthy, overfermented and dirty flavours were perceived. It was demonstrated that by washing coffee after either natural or mechanical removal of mucilage, good quality cups with no strange flavours are obtained. It is concluded that coffee processing has a significant effect on beverage quality. Drying process is one of the most important stages of the processing in order to maintain quality (Puerta, 1999). Table2, Figure1.

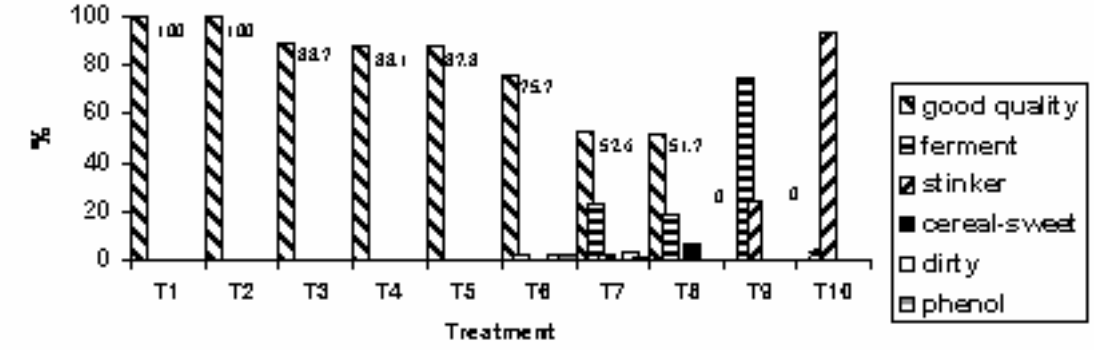

Figure 1. Coffee quality according to treatments (good quality or defects %) (Puerta, 1999)

Table 2. Description of treatments of studies on influence of coffee processing on quality (Puerta, 1999)

Treat	Processing method	Maturity	pulp removal	mucilage removal	washing	storage of wet coffee	drying
T1	wet	100% ripe	pulping	natural fermentation	clean water	no	sun
T2	wet	100% ripe	pulping	mechanical*	**	no	sun
T3	wet	65% ripe	pulping	natural fermentation	clean water	no	sun
T4	wet	65% ripe	pulping	mechanical*	**	no	sun
T5	wet	65% ripe	pulping	mechanical*	clean water	no	sun
T6	wet	65% ripe	pulping	mechanical*	clean water	2 days+	sun
T7	semi-dry	65% ripe	pulping	no	**	no	sun
T8	wet	65% ripe	pulping	mechanical*	**	2 days+	sun
T9	dry	100% ripe	no	no	no	no	sun
T10	dry	100% immature	no	no	no	no	sun

,Becolsub, simultaneously removal of mucilage and washing, +in buckets*

Causes of overfermented and stinker off-flavors

Several research studies have been carried out to understand the causes of overfermented coffee beans and beverage. (Puerta, 1995; Puerta et al., 1996; López et al., 1988; Federación, 1979). It is concluded that the following practices during processing are the cause or potentially risky:

- Harvesting of overripe and immature fruits.
- Delay in the beginning of processing longer than 8 hours. After picking, the pulp and mucilage of the coffee fruit begin a natural fermentation process, for that reason the beans must be processed immediately after harvested.
- Overfermentation processes: by leaving fresh depulped beans longer than 16 hours in the fermentation tanks. Depending on fermentation process duration, altitude and ambient temperatures the following cup grades will be obtained:
    - At the beginning of overfermentation (first hours) the beans may display good appearance, but the beverage taste like sour and ferment.
    - 24 hours aroma and flavor like vinegar, dirty, spotted parchment
    - 40 hours: wine-like, dirty parchment
    - 64 hours: fruit -like, fermented grenade-like
    - 70 hours: rancid-like, onion-like, discolored beans
    - After 70 hours: stinker, the bean tissues are affected, giving yellowish coloration, forming the sour and vinegar beans.
- By processing mixtures of coffee beans harvested from different days the quality deteriorates more quickly, and after 20 hours appears scent vinegar, and rotten fruit, after 40 hours 37% of the sample tastes like stinker.
- Incomplete fermentation processes or incomplete separation of mucilage by mechanical removal cause sour beans and stinker.
- By using waste water of processing for coffee washing
- Drying of good processed coffee beans with partially depulped beans.
- Drying of beans that have mucilage adhered to the beans.
- Berry borer damaged beans overferment more quickly due to the damage of the fruit.

Defects originated by inadequate drying

The drying is a method of preservation of foods. The water content must be reduced as soon as possible from the wet parchment coffee to avoid deterioration of the beans during the transport and storage. The moisture content of the beans is one of the most critical points to control in coffee processing. By drying the water activity of the beans decreases, thus avoiding the physical deterioration, the chemical decomposition and the deterioration of the beverage quality by micro-organisms, mainly fungi. The moisture content of the coffee should not exceed to 12%. If drying is not carefully carried out, both appearance and beverage characteristics will be affected. Inadequate drying, store under humid conditions, mixtures of coffees with different moisture contents, re-wetting of the coffee or interruption of the drying before the beans reach 12% are favourable conditions and bad practices to produce defects, such as: discoloured, dirty, faded beans, dirty, earthy, musty flavours or mycotoxins. (Archila, 1990; Puerta, 1995, 1999, 2000; Roa et al., 1999, 2000; International IUPAC, 2000; van der Stegen, 1997; Naidu et al., 1997; Federación, 1979).

Ochratoxin A studies

In order to know the natural fungi and the Ochratoxin A level in stored coffee, samples of good quality parchment coffee (CPS) and samples (green coffee) containing 5% weight of berry borer damaged beans (CA5%B) with moisture contents less than 10%, between 10 and 12% and above 12% were stored 12 months under the following conditions: <10ºC and <65% of relative humidity, 23-24ºC and 75-77% RH, 23-24ºC and >80% RH. Simultaneously the same type of samples was inoculated with 10^{11} spores/ml of *Aspergillus ochraceus* suspension. As check 100% good quality green coffee beans with 10 to 12% moisture content was stored under the same conditions. It was used the Ochratest method of Vicam. Fungi were analyzed monthly. *Penicillium spp.,* were the most predominant moulds in the samples followed by *Aspergillus spp., Rhizopus spp., Cladosporium spp., and Fusarium spp*. were also found. *Aspergillus ochraceus* remained during 12 months in every kind of sprinkled sample and under every storage condition, although in healthy green coffees the proportion was always lower. As most critical points for coffee quality and OTA formation were found the quality of the beans and their moisture content. OTA was not found in natural parchment stored below 10°C and 65% RH and moisture between 9 and 14%, nor in sprinkled with *A. ochraceus* parchment coffee under 10% and between 10-12% moisture, nor in CA%5B without sprinkling <10% moisture and stored below 10°C and 65% RH, nor in good quality beans of green coffee with or without sprinkling, moisture between 10 to 12% and storage conditions below 10°C and 65% RH. Considering every treatment and kind of stored sample the average OTA levels varied between 0 and 1.41 ppb and the maximum values between 0 and 1.92 ppb (1.92 for CA%5B). In average the OTA levels in natural samples (without sprinkling) varied between 0.13 ppb in good quality green coffee, 0.37 ppb in parchment coffee and 0.48 ppb in CA%5B; and in sprinkled samples between 0.23 in green coffee and 0.47 in both CA%5B and parchment coffee. The average highest OTA values were found in CA%5B (0.48 ppb), followed by parchment coffee 0.42 ppb. The average lowest values of OTA were found in good quality green coffee (0.18 ppb). It is to emphasise that good quality beans with 10-12% of moisture content displayed the smaller percentage of fungi colonisation and the lowest levels of OTA. Coffee samples stored below <10°C and <65% RH displayed the lowest levels of OTA (0 to 0.23 ppb (0.23 in CPS >12% moisture content)). It is concluded that good processing practices including separation and discard of defects, carefully drying and cool and dry storage conditions prevent deterioration of coffee by OTA. (Puerta, 2000; Acevedo, 1997; Puerta and Acevedo, 1996; Arango, 1998).

ASSISTANCE TO COFFEE GROWERS

Although the quality of the exported coffee has always been assessed and controlled, by the coffee authorities, avoiding that poor quality coffees reach the foreign market, today Colombia's purpose is to enhance the coffee quality controls in order to improve the competitiveness in the international trade market and to satisfy the consumers with a safe and high quality product. Therefore during the last years several lectures, meetings, workshops and other activities in order to give assistance to coffee growers about the impact of processing on coffee quality have been carried out, with the participation of the farmers, extension personnel, researchers and Federation authorities.

SURVEY ON QUALITY AND MOISTURE CONTENT OF THE COFFEE PRODUCED IN COLOMBIA

Since last year a survey to measure the moisture content and the cup quality of the coffee produced by farms has also being carried out. During last main harvest 4.636 samples taken from big coffee farms (size over 10 ha) including 14 producing zones were analysed. The results will let to identify specific failures in coffee processing in the farms. (Federation Nacional de Cafeteros de Colombia, 2000). Based on the results obtained through research, surveys and visits to the farms, the following good processing practices are nowadays available to the farmers and extension personnel in posters and technical brochures (Puerta, 2000, 2001; Federación, 2000; Roa et al., 2000).

GOOD PROCESSING PRACTICES TO GUARANTEE THE QUALITY OF THE COFFEE BEVERAGE

A method is not known to improve the quality in the post-harvest process, nor can be controlled all the variables involved with the formation of defects, like the climate and most of the environmental factors, but the producers, farmers, dealers and exporters can avoid the most of the defects, by means of the accomplishment of good practices before and during the harvest with a good management of the culture, opportune harvesting, good processing practices, cleanliness, carefully drying, suitable conditions for transport and storage of the beans. (Puerta, 2000, 2001).

Pest Management

- Avoid the use of chemical insecticides.
    - In this way are preserved
    - The coffee quality.
    - The farmer's health.
    - The ecological equilibrium.
    - Quality of soils, air and water.
    - The preference of the consumers by products that do not affect the environment.

Coffee harvesting

- Pick and process only ripe cherries.
- Avoid physical damage to the cherries.
- Do not process.
    - *Immature cherries* that produce ferment, stinker, grassy and pungent defects.
    - *Overripe cherries* that produce vinegar flavour and stinker and sour beans.

- *Black cherries* that produce pungent, burnt and carbony defects.
- *Gathered from the ground (fallen cherries)* that infest with fungi and micro-organisms the healthy coffee and risk the sanitary quality of the coffee.
- *Berry borer damaged cherries:* that may infest good coffee beans.

Coffee processing

- Keep in good working condition and cleanliness every equipment and device for coffee processing, including pulper, screen, desmucilaginator, tanks, dryer, trays, bags, warehouses of storage.
- Process coffee every day.
- Do not leave pulp, mucilage or coffee remainders in the equipment after processing.
- Do not allow the contact of the processing equipment and devices with animals, substances and materials different from the coffee and the water for processing.

Receiving of coffee cherry

- Use clean hopper.
- Avoid the siphon tank for receiving the coffee cherries, its use implies excessive water use and its pollution.
- Classify the good and ripe coffee by screening and sorting.
- Eliminate and discard stones, leaves and twigs.

Pulping

- Wash the pulper and eliminate any remainders of previous processes.
- Adjust the pulper to avoid semi-pulped and unpulped beans that affect the yield and quality.
- Separate completely pulp from cherries.
- Pulp berries immediately after harvesting and receiving.
- Do not leave the harvested berries longer than 6 hours without pulping and processing.
- Classify and separate berries that have not been pulped.
- Do not use water for pulping.
- Do not use water to transport the pulp and the pulped beans.
- Do not throw the pulp to the water sources.

Fermentation of mucilage

- Maintain the fermentation tanks clean.
- Leave the coffee beans in the tank for 15 to 16 hours, until mucilage has been transformed into soluble water products.
- Control the time of the fermentation process.
- Do not leave the beans in tanks longer than 16 hours to avoid overfermentation processes that affect negatively aroma and flavour and cause ferment, vinegar, onion, rancid and stinker tastes and defects.
- Do not mix in the fermentation tank coffees obtained from different days of harvesting.

Mechanical removal of mucilage

- Operate the equipment properly.
- Control that mucilage has been removed completely from the beans.
- Separate the beans that have mucilage still adhered to avoid ferment and stinker defects.
- Do not process immature beans.
- Do not process beans of previous harvests.

Washing

- Wash the coffee beans using clean and quality water.
- Wash completely the coffee beans until removing the compounds formed during the fermentation, to avoid vinegar, ferment and stinker defects.
- Classify and discard the defective beans.
- Separate foam and floats.
- Do not use dirty or wastewater to avoid faded, dirty, ferment or off-flavours.
- Do not use more than 1.5-L water by each Kg coffee to wash. In this way will be avoided pollution of water.

Drying of coffee

- Maintain patios, tables, trays, silos and devices in clean conditions.
- Keep the dryer in good operation conditions: air flow, fuel and control of drying temperature.
- Dry coffee immediately after washing.
- Stir coffee frequently a day, to obtain a uniform drying. (Every 4 hours during the first days sun drying and then every 12 hours or more frequently).
- Control layer thickness. (2 to 3 cm in sun drying).
- Control re-wetting during drying.
- Protect coffee against rain ensuring ventilation.
- Control that the final moisture content of the beans is between 10.5 and 12%.
- Do not store wet parchment coffee.
- Do not mix coffee with different moisture contents.
- Do not dry the good coffee with semi-pulped or unpulped beans.
- Do not dry coffee if mucilage remains on the beans; there is the risk of undesirable fermentation.
- Do not allow that animals put themselves in contact with the coffee.
- Control that the temperature of the beans is not higher than 43°C during drying.
- Interchange the airflow every 6 hours during drying in mechanical dryer.
- Do not allow the contamination of the coffee beans with smoke or combustion gases.
- Do not commercialise wet parchment coffee.

Storage

- Keep clean warehouses and transport vehicles.
- Store dry parchment coffee or green coffee in dry, cool and ventilated places.
- The best storage conditions are 65% of relative humidity and 15 °C or below. In this way the deterioration of coffee by moulds and mycotoxins is prevented.
- Control re-wetting of coffee during storage and transport.

- Do not store the coffee with chemical substances, fertilisers, feed, fuels or any other product that may contaminate to the coffee.
- Do not store parchment coffee together with green coffee or husks.

Disposal of by-products and waste water

- Treat the waste water using anaerobic reactors.
- Treat the pulp and the mucilage by composting, culture of edible mushrooms or any other process.
- Do not throw the liquid or solid by-products from the coffee processing to the water sources, without a previous treatment.

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Enhancement of Coffee Quality through Prevention of Mould Formation in Brazil

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SUMMARY

Brazil has the largest coffee production area of the world. Due to new investments, mainly in mechanisation and irrigation, yields are increasing. The number of bags produced per hectare rose from 10.59 in 1993 to 14.59 last year. This represents 19,410,000 beneficied bags of arabica type against 7,290,000 bags of robusta type.

Coffee is widely consumed all over the world. In order to guarantee consumer's safety it is necessary to evaluate possible hidden hazards in this product.

Mould formation on coffee beans results in a product of unacceptable quality. Some moulds produce toxins, which can be dangerous for human consumption. The principal is the ochratoxin A (OTA) which is nephrotoxic and possibly carcinogenic to humans.

During the last 5 years the research program of the Brazilian Ministry of Agriculture and Food Supply on coffee quality has invested in some aspects of fungi contamination and in the development of a methodology for OTA detection and increased analytical capabilities. The limit value imposed by the EU and suggestions made by the *Codex Alimentarius* inevitably led the Brazilian government to take some measures towards the monitoring of the coffee production chain.

Because of its large extension and different specific climatic conditions, Brazilian territory has been already being studied nation-wide in order to identify what zones are suitable for coffee production. In this way, a flowchart is necessary for each area in order to avoid hazard and identify control and critical points for mycotoxin formation.

The project "Enhancement of coffee quality through prevention of mould formation" (ICO/FAO Project) will contribute to improve coffee quality. Such a project will properly cause the enrolment of Brazilian universities and research centres with local extension and diffusion services reaching all Brazilian territory.

The possibility of contacting coffee experts from all over the world certainly will reverberate in all phases of the coffee production chain.

The focus of the program established by a conjoint FAO/EMBRAPA project has the objective of applying the concepts of the HACCP system in the identification and control of factors which increase the production of OTA in coffee, verifying possible differences and interactions in all the coffee chain. New strategies will be performed based on the knowledge obtained from the data produced during the project. Coffee sector personal will be trained in Good Agricultural Practices (GAP) and HACCP concepts by manual and videos. The recognition of the control and critical points and the GAP concepts in the coffee production

chain will result in steps for the better understanding of the factors to improve coffee quality by the different sectors with this aim: govern, technicians, producers, co-operatives and industries.

WHAT DOES COFFEE REPRESENT TO BRAZIL

Brazil has the greatest coffee production area in the world and yields are also increasing because of new investments, mainly in mechanisation and irrigation. The number of bags produced per hectare rose is almost 50% since 1993 and it represents 19,410,000 "beneficied" bags from *arabica* type against 7,290,000 from robusta type.

Brazil is unique in its ability to offer nearly every type and quality of coffee that the consumers may demand: Arabica or Robusta; natural cherries or washed; single origin or blends made to order; large volumes or specialities and also custom and niche products, such as organic.This diversity occurs because in Brazil coffee grows in different tropical latitudes, at different altitudes, and in unique soil and climate.

From 27 states there are six main coffee producing states (Paraná, São Paulo, Minas Gerais, Espírito Santo, Bahia and Rondonia), The states of Espírito Santo and Rondonia produce two varieties: arabica and robusta while the other state produce only arabica. They produce mostly natural processed coffee which is dried with the pulp intact in order to acquire natural sweetness. In addition, the newer pulped natural method also allows the sugar of the mucilage to add flavour and character to the bean. Washed coffees are produced in smaller quantities.

KNOWING BRAZIL

An example for such a variety is the state of Minas Gerais where the country's production is divided into 4 regions: mountainous, "**Sul de Minas**", with hand labor and mostly family owned small farms with a few of them producing washed coffee; "**Cerrado**", a savanna with well defined rainy summers and dried winters, in which plantations are large and mostly mechanised with some irrigation; "**Matas de Minas**", a very mountainous region, with hand labour and small family farms moving into high density planting and a small percentage of washed coffee and, finally, "**Chapada de Minas**"**,** a very small region with high plateaux and river cut gorges.

FLOW CHART'S OPERATION

In Brazil two processing systems are used. The principal and more ecological is known as dry system because it doesn't contaminate the water resources. Another one is washed system where the mucilage of cherries is taken in the water. To dry both processes can be done on the patio.The steps of beneficial, bag and transportation are the same as we can see in the flow chart.

BRAZIL AGRIBUSINESS

In Brazil coffee agribusiness depends on the network of suppliers of goods and services to operate efficiently. It includes research and development, extension services, production of inputs and equipment, financial services and logistics.

The FAO project will be the link among all sectors evolved on coffee chain. The focus for the program established by FAO/EMBRAPA project has the objective to apply the HACCP system to identify and control the factors that increase the production of OTA in coffee

growth, verifying the possible differences and interactions in all the coffee chain. Based on the data produced, new strategies will be performed.

REASONS FOR THIS PROJECT

Mould formation in coffee beans results in a product of unacceptable quality. Some moulds produce toxins, which can be dangerous for human consumption. The principal is the ochratoxin A (OTA) which is nephrotoxic and possibly carcinogenic to humans. Coffee is widely consumed all over the world but, in order to guarantee the consumers safety, its necessary to evaluate the hazards that may be hidden in this production.

FIGURES FROM BRAZIL

During the last 5 years, the research program of coffee quality from Brazilian Ministry of Agriculture and Food Supply has invested in some aspects of fungi contamination, methodology to OTA detection, the increase of analytical capabilities and the identification of the best areas to grow coffee.

The limit value imposed by the European Union and suggestions by the *Codex alimentarius* inevitably led the Brazilian government to carry on some attitudes to monitor the coffee chain production.

Study Area

Due to the Brazilian geographic extension some areas have been mapped for choosing the best site for coffee production. Each area needs to be worked on with special attention because of the specific climatic characteristics through of Brazilian Geographic Information System (GIS).

Further research will integrate the obtained results with temperature and relative air humidity in order to analyse the relationship between the intensity of mycotoxin contamination and climatic condition during the harvesting time. Such research will allow us to predict possible mycotoxin contamination levels based on environmental conditions.

Considering the available data for hazard determination in the final product, the application of the HACCPP system indicates that ochratoxin A is, in fact, coffee's most significant hazard. However, Brazil has continental dimensions and geographically different production areas, characterised by different **luminosity**, temperature, relative humidity and **seasonal**, all of which may interfere in the environmental **microbiota** with presence and incidence of fungi microbiota. Another aspect to be considered is the variation in the processing, transportation and conservation stages. Therefore, this significant hazard may vary according to the characteristics and origin of production, processing and storage.

REACHING THE TARGETS OF THE PROJECT

The activities intend to classify the coffee production flowcharts according to the production areas, with a view to verifying possible differences in the harvest, drying, transportation, and storage process.

This will enable us to identify which production areas present the most risks, as well as which stages of the process need to be controlled in order to avoid the appearance and development of fungi, including those which produce OTA, considering the production, harvest and

processing characteristics of each of those regions. These data shall be very useful for determining which geographical regions are mostly at risk, as well as for standardising the processing and storage stages, thus enabling application of the HACCP system based on real data.

RISKS TO THE CONSUMER'S HEALTH

Considering the previous activities we will collect data for a global study from environmental variables statistically organised, which will help us to understand the dynamics, as well as how and when the problems at issue will be controlled, constituting or not a risk for the consumers' health.

OCHRATOXIN A HOST INTERACTIONS

This proposed study consists of fungi quantitative analyses in beans that went through different drying processes, as well as the comparison of those quantities with the total fungi count and the number of toxigenic fungi that produce ochratoxin A. The results will be useful for the improvement of practices throughout the coffee chain.

WHAT WILL BE DONE FOR SANITARY ASSURANCE

The tools for the sanitary quality control of coffee in relation to ochratoxin A contamination will be made available, and the sampling plan and official analytic method will be the technological tools for the determination of ochratoxin A. They will be provided and established, enabling the setting of OTA contamination control parameters in the production chain, as well as the assessment of export-type-coffee contamination.

The sampling plan will be elaborated and implemented taking into account the maximum limits of OTA in coffee currently proposed by the European Union. The characteristics of the sampling plan, such as producer's and consumer's risks, bad lot, rejectable lot and critical level (control, tolerance and legislation) will be studied and defined according to an experimental statistical delineation and to a validated analytical methodology. The sampling plan will be made available by means of its official publication, as well as in sampling handbooks, videos and training actions.

HACCP IMPLEMENTATION SSYSTEM

Based on previous knowledge and on data obtained, problems connected to the contaminants will be evaluated and strategies to solve this problem will be proposed. Such proposals shall be analysed according to their effectiveness, throughout the experimental assessment of the control options. The validated options will serve as a basis for the refinement of the Hygienic Practices Basic Handbook.

This activity will be developed by a national team with the participation of specialists, so as to perfect the field activities. These activities include the training of consultants and multipliers, who will be responsible for the training of the field workers.

CONCLUSION

The FAO project will contribute to improving the coffee quality. It will cause the enrolment of universities and research centres from Brazil and local extension services/and it will reach all the Brazilian territory.

The possibility of contacting coffee experts from all over the world will certainly be reflected in all phases of coffee chain production.

The recognition of the critical points and the Good Agricultural Practices throughout the coffee chain production will result in the articulation with the government, technicians and producers.

PARTNERSHIPS

Those are some Brazilian Institutes and Universities that will participate in the project. Most of them are located near the coffee producers.

EMBRAPA , MAA, IAL, ITAL, EPAMIG, EMATER, UFV, UFLA.

The Role of Socio-economics in Preventing Toxinogenic Mould Contamination in Coffee

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SUMMARY

It is essential to acquire a microbiological understanding of toxinogenic fungi responsible for ochratoxin A formation if recommendations are to be made for reducing contamination risks to a minimum. Any such recommendations need to be passed on to producers, but decades of extension experience has shown that publishing technical documents, as well drafted as they might be, and training farmers is not enough to get them to change their habits. If such an approach is taken, the prevention objective of the coffee quality improvement project will not be achieved. Understanding the mechanisms leading to the adoption of a new technology by farmers requires knowledge of their constraints, in order to propose realistic new practices. A technical innovation usually has a good chance of being accepted if it is in response to a need expressed by users, provided it leads to further gain.

INTRODUCTION

Like many agricultural products, coffee does not escape the risks of contamination by toxinogenic moulds which, under certain conditions, can develop and produce ochratoxins, which are a danger to human health. Over the last few decades, consumers have become increasingly demanding when it comes to the quality of the foods they buy. Recent food safety problems, which have received a great deal of attention from the media, have strengthened the position of the political authorities in favour of fixing ever stricter sanitary standards. The European Union is planning to establish legislation fixing maximum acceptable OTA limits for a whole range of commodities, including coffee.

The project on the prevention of coffee contamination by toxinogenic moulds comprises two aspects: a) understanding contamination causes and conditions and b) proposing corrective measures, and having them applied by producers through a programme of training/dissemination of technical information. This calls for a compulsory change in farmer attitudes.

The experience acquired in a string of development projects over the decades has clearly shown that getting farmers to adopt a new technology comes up against numerous difficulties.

Between a ripe cherry and an exportable coffee bean, the risks of contamination by moulds exist throughout the chain, from producer to exporter and including traders and processors. Whilst it is widely accepted that the first stage of contamination is at the coffee plantation, the entire responsibility for such contamination cannot be placed exclusively on the shoulders of the farmer.

THE FAILURE OF TRADITIONAL EXTENSION SYSTEMS

Up to the end of the 70 s/beginning of the 80 s, it was accepted that agricultural progress – an increase in productivity, cost reductions, improvement of product quality – could only be achieved by disseminating the results of agricultural research. The example of sector development projects is typical (Figure 1)

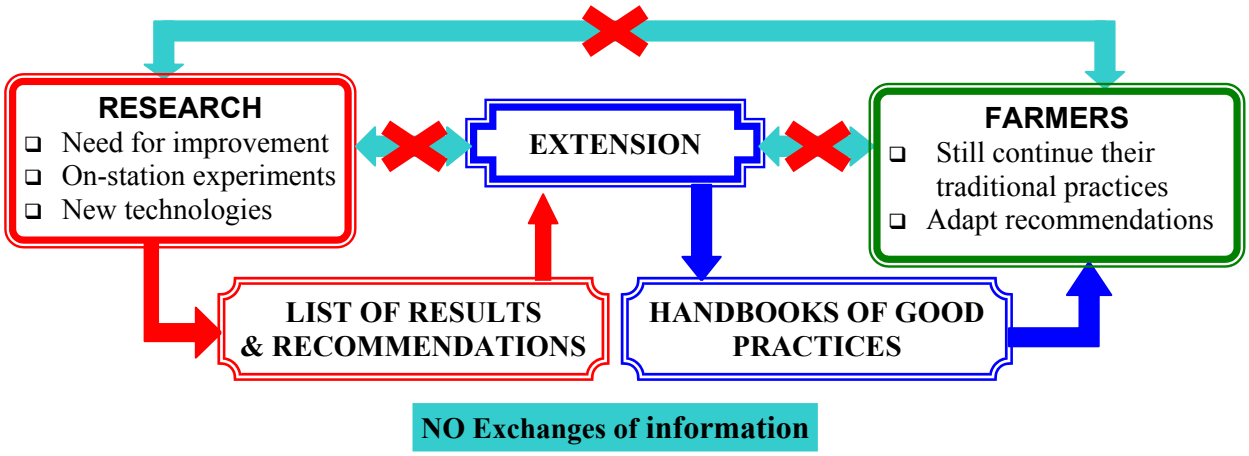

Figure 1. Pathways of recommendations proposed by research (old methods of technology transfer)

The research behind the recommendations

Agronomists at research stations have always striven to develop techniques making it possible to improve plantation productivity and reduce production costs. Economic calculations have shown that by strictly applying research recommendations, it was possible to improve the standard of living of farmers. It was thus that productivist schemes saw the light of day for coffee production, often moving further and further away from traditional farming practices.

Extension services

The dissemination of technical recommendations was entrusted to extension services, one of whose activities consisted in compiling the confirmed and undeniable results of research in an easily understandable form, in order to have them applied by farmers. The other work of the extension services consisted in visiting farmers and teaching them how to produce better. For major crops such as coffee or other cash crops, producing countries set up specialized structures. Thus came into being all the State-owned companies, which were intended to improve the production of the crops for which they were responsible.

The farmers

Farmers were the final link in the "Research-Extension-Producer" chain, and only had the choice of either agreeing or refusing to apply the extension recommendations. Their choice automatically classed them as good or bad farmers. Moreover, if some of them followed the extension advice, it was often because there were particular incentives. Once the major projects terminated, most farmers reverted to their traditional practices.

Analysis of why the failure occurred

Although this approach had a degree of impact, it was soon understood that progress was well below the initial expectations, apart from the rare exception. Moreover, it was usually found that the technical innovations were adopted during the life of the project, but were very quickly abandoned thereafter. For instance, an analysis of production figures in numerous countries over a decade or so clearly shows that production has stagnated, if not declined.

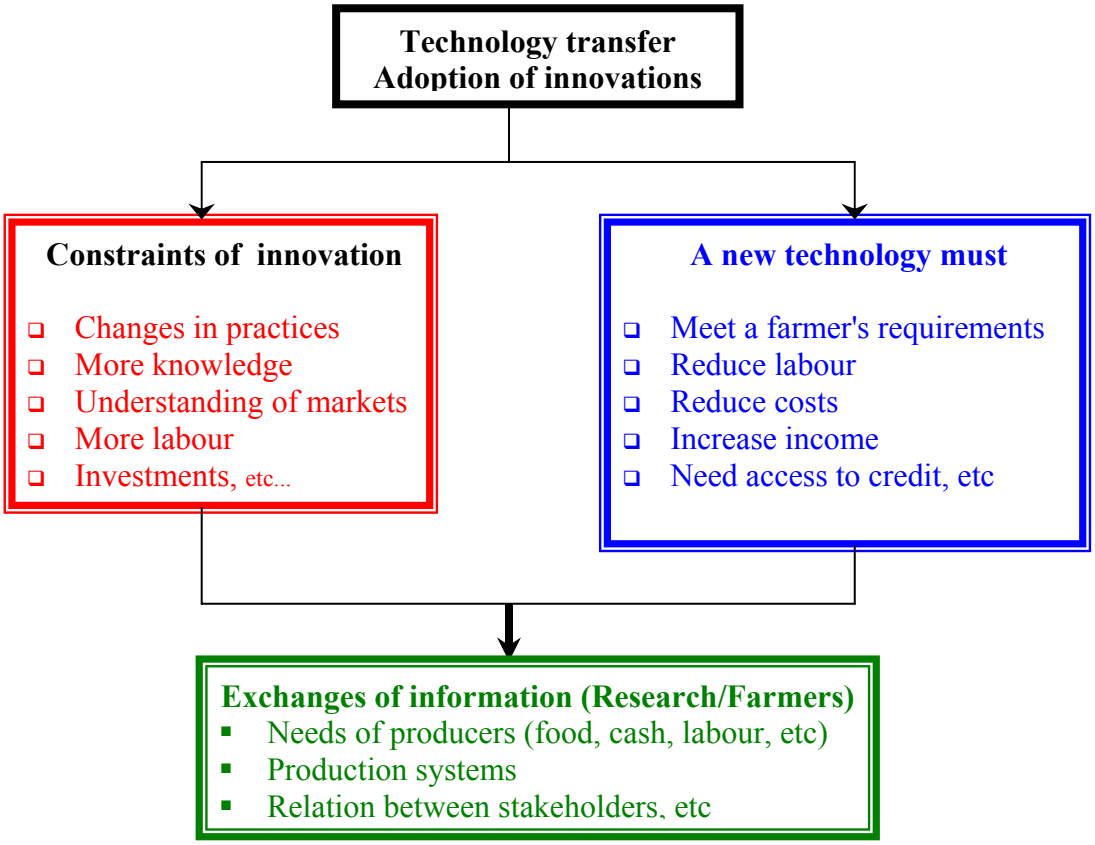

Figure 2. Extension and technology transfer

The first reaction was to say, on the one hand, that the extension services were not doing their job properly, or, on the other hand, that research proposed innovations that were too complicated or inappropriate, with each side feeling sure of its claims. New extension methodologies primarily based on on-farm experiments offered the advantage of getting researchers to leave their stations and establish relations with extension officers, and to review the ways in which technical information was passed on. Despite that, the adoption of new techniques by farmers remained very limited.

At the same time, economic sciences started taking an increasing interest in farmers and their farms. The first finding was the lack of communication between research and the users of that research, which meant that the recommendations proposed became increasingly distant from the daily concerns of the farmers. Socio-economics highlighted the large number of agronomic and socio-economic constraints faced by producers. They have the ongoing worry of managing the agronomic and economic risks on their farms. Lastly, socio-economics showed that technical innovation has some chance of being adopted if it satisfies a certain number of criteria other than technical criteria (Figure 2) and if it corresponds to a request expressed explicitly, or not, by the user.

PREVENTION OF OTA CONTAMINATION

The need for microbiological studies

All coffee extension manuals describe in detail how to go about harvesting and primary processing, in order to ensure that a top-quality product reaches the market. On the whole, this advice is more the result of years of practical experience than of in-depth research.

For more than thirty years, microbiological studies have also revealed the risks of quality deterioration when recommendations are not effectively applied. Inventories have been made of the microorganisms found on coffee.

The decision to introduce legislation has also shown that all the knowledge acquired was not enough for understanding and explaining the contaminations found in coffee: in particular, the origin of contaminations, the microorganisms responsible, the conditions for fungus growth, the conditions for ochratoxin A production, the risks of contamination throughout the commodity chain.

The role of socio-economics in prevention

From fresh berry to roasted coffee, there are many critical stages during which contamination risks occur. Whilst it is accepted that the greatest risks of contamination occur at the coffee plantation, from harvest to dry product, what happens between the farmer and the exporter and between the exporter and the roaster cannot be ignored.

It is the job of socio-economists to describe the behaviour of farmers and analyse their constraints, but also to detail the activities of the various stakeholders in the national commodity chains, and show the relations between the pressures exerted by those stakeholders with respect to each other.

The Ugandan and Kenyan commodity chains provide good examples of the situation, and of the grounds for looking at the overall functioning of the chain.

Case of the Ugandan commodity chain: robusta coffee is processed by the dry method. According to the initial information available, some farmers dry their coffee either on concreted areas, or on hard earth, others sell their coffee pre-harvest directly to a middleman, who harvests it and transports it to the nearest urban centre to dry it. Lastly, others prefer to sell their coffee semi-dried, but all are convinced that fresh cherry storage in heaps prior to drying accelerates drying. This farmer practice is clearly linked to a misunderstanding of the drying process, during which the risks of contamination need to be assessed, but it is also dictated by social and/or economic constraints. Although coffee fetches a lower price when sold pre–harvest, farmers must have other problems to face: they certainly need money urgently, and only a middleman can provide that. Under these conditions, how can farmer attitudes be changed? It is virtually sure that a handbook of good practices will have no impact.

Coffee is hulled in factories set up in the production zones, but it is only packed in Kampala. After hulling, the coffee mixed with shell particles is stored in old bags for several weeks, prior to washing and packing. Contamination risks are high during that phase.

Case of the Kenyan commodity chain: arabica coffee is only processed by the wet method, but a proportion of immature cherries has to be dried directly. These cherries are sent to the

cooperatives. Despite the precautions taken during storage, the risks of contamination between dry cherries and parchment coffee are not zero. During packing, even taking the necessary precautions, contamination always remains possible.

In addition, the pulping centres and packing units are used to processing sweepings and including them in low-grade coffees. In theory, this factor heightens contamination risks.

Do the probable corrective measures meet the criteria for their adoption?

Farmer requirements

It is clear that measures to improve quality, particularly with a view to preventing mould formation, will not tally with farmer requirements. All the more so in that such moulds do not have any known effect on yields, hence in theory they do not penalize farmers. Cup quality is affected, but coffee is primarily an export crop and is not consumed by the producers.

Reduction in labour requirements and production costs

It is not obvious that taking greater care during drying will reduce the amount of work on the farm. It will probably be necessary to turn the coffee more and protect it at nighttime or when it rains.

Switching from hard earth to a concreted area to reduce risks or from a concreted area to more sophisticated solar dryers calls for more or less substantial investment that farmers are very often unable to afford. Moreover, when credit systems exist, they are often not very effective and interest rates are high.

Income improvement

This criterion can be effectively applied, but it implies profound changes in the attitudes of the stakeholders and their relations with producers. Furthermore, price fluctuations are detrimental to an increase in "farm gate" prices.

Introducing a system of bonuses and discounts could be considered, but it needs to be fair and clearly understood by the farmers. Any such decision must come from the stakeholders in the national commodity chains and industrialists. However, the introduction of a discount system alone is bound to have a negative effect.

CONCLUSION

The role of socio-economics in a prevention project cannot be overlooked. It is essential for economic and biological sciences to be combined, in an attempt to propose recommendations that are acceptable, recommendations that follow on from the results of biological research (drying time, degree of drying, good post-harvest practices, etc.). It will be up to socio-economists to check that the constraints associated with new practices can be borne by farmers, but they must also propose corrective measures at all stages of the commodity chain.

Threatening not to purchase coffee containing ochratoxins involves certain risks. Firstly, it is virtually impossible to check the level of fungal or chemical contamination in every batch of coffee delivered by farmers. Only coffees reaching export can be checked. Secondly, exporters and buyers are going to try and overcome that risk by purchasing coffee at the lowest possible price, so as to keep any losses to a minimum, or they will only pay farmers

once they are sure that the coffee has been accepted in Europe. The third, and most unfavourable, hypothesis could be that coffee production will be abandoned, because agronomic risk management at the farm becomes too unwieldy, with very serious repercussions from both an individual and a national standpoint. Consequently, socio-economics must take an active role in drawing up the recommendations to be made, and not limit them to farmers only.

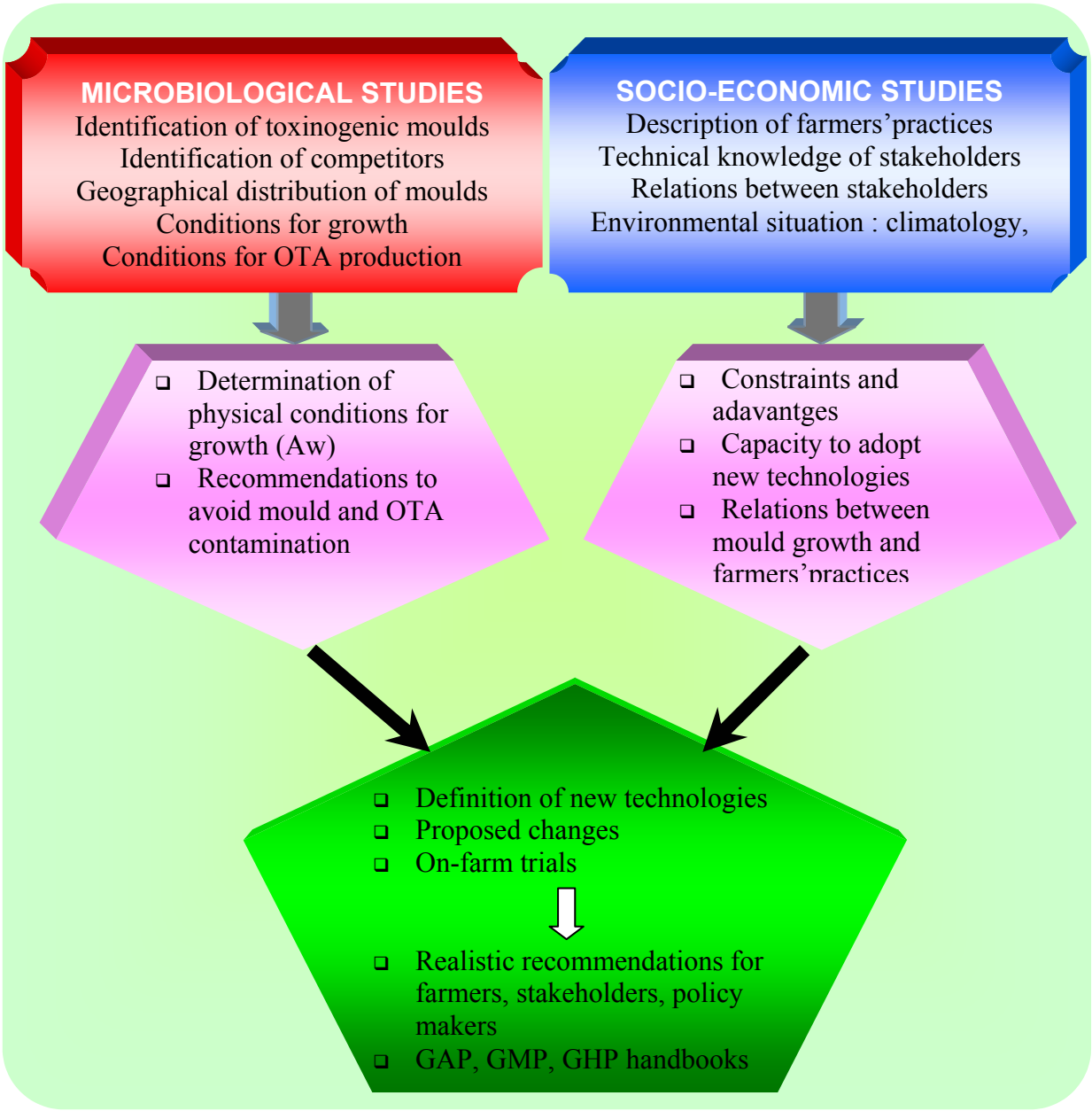

Figure 3. Microbiology and socio-economics in prevention

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Green Coffee Transport Trials

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SUMMARY

Several tests have been carried out to assess whether conditions favourable for mould growth and mycotoxin production might be encountered during the transportation of green coffee. Containers were equipped with probes to collect data on temperature and relative humidity.

The first trial was designed to assess the evolution of these parameters in the container headspace and within the coffee bags upon arrival in a North European harbour. Several periods of condensation were recorded in the headspace against the roof. Besides the condensation periods, long periods with a relative humidity above 85% have been recorded against the roof. This trial has given us a typical example of how condensation could damage green coffee beans at the top of the containers due to dramatic a_w increases.

The second trial allowed the evaluation of the risks of damage during the different stages of a shipment in an open liner. During the first leg of ground transport between the green coffee producing area and the port of embarkation there was a significant risk of damage due to dramatic temperature and relative humidity increases at the top of the bags. One short period of condensation was reached. No critical relative humidity was reached during the second leg of transport on the boat between the port of embarkation and a North European harbour.

A third trial was carried out with similar objectives to the second one but with coffee in bulk in a closed liner. Three different periods were evident during this trial: ground transport, with extreme conditions but without condensation, boat transport, with very stable conditions especially for the probe placed in the coffee and the storage period in the North European harbour with only small variations observed due to the summer conditions.

These three trials demonstrated that the most critical phases of the transport are the overland transport between the green coffee production site and the port of expedition and the storage period in a European harbour during the winter period. Some extreme conditions resulted in condensation of water on the coffee, increasing the risks of mould development. On the contrary, only slow and relatively small variations were observed during the sea transport phase in a closed container.

INTRODUCTION

The risk of mould development during green coffee transport depends mainly on water activity. During the different phases of green coffee transportation the relative humidity and the temperature in a container evolve depending on both external and internal conditions. Much practical expertise has been developed, mostly on optimal warehouse storage conditions. An ISO standard exists providing guidance to storage and transport. But the different phases of transport have not been scientifically documented nor has the expertise been made available within the coffee sector. The main objective of this study is to assess the

evolution of relative humidity and temperature. The results should allow us to identify whether critical conditions are created which alters coffee beans such as mould generation.

PARAMETERS MEASURED AND EQUIPMENT DEVICES

The key parameters to assess whether conditions are appropriate or at risk are relative humidity (HR) and temperature (T). Rotronic AGENT HT1 devices are used for recording HR and T. These instruments need an accurate calibration before being installed into a container.

Calibration procedure

An optimal complete calibration procedure requires 3 different temperatures and 5 different levels of relative humidity. Several hours are needed for careful equilibration. Each data logger must be checked before use in a trial. In our experiments we checked the logger at 25°C and 75.3% HR using NaCl. For eliminating the risk of drift, it is necessary to check calibration of instruments after finishing the trials.

Trials

Three tests have been carried out to assess whether conditions favourable for mould growth and mycotoxin production might be encountered during the transportation of green coffee.

Trial 1

The first trial was designed to assess the evolution of HR and T in container headspace and within coffee bags upon arrival at a North European harbour in winter. Five recorders were placed in a container according to the design shown in Figure1. The records were taken over a one month period from February 17 until March 18. Each data logger was set or recording one and HR every six minute. The data have been exported to Excel sheets to allow simultaneous plots.

Trial 2

The second trial was carried out to evaluate risks of damage during transport of a shipment in an open liner, which did not cover the parcel of coffee on top. Two very different phases of transport were considered:

- the 22 days terrestrial transport between place of harvesting and port of embarkment;
- the maritimal transport to Europe. This phase lasted 34 days.

Two data loggers were placed in the container, one on top of the coffee in middle of the container, the other 35 cm deep in coffee parcel

Trial 3

The third trial concerned bulk transport with all coffee wrapped in a closed liner. In this trial three data loggers were placed in the container, one on top of liner in middle of the container, the second 30 cm deep from the top, still in middle and the third on top of the coffee, inside the liner. The transport route was similar to that of the second trial. The terrestrial transport also lasted 22 days and maritime transport only 21 days. After arrival in European harbour, the container was left on the quay for a period of 9 days.

RESULTS AND DISCUSSION

Trial 1

Figure 1 shows the daily average values of T and HR records. This figure gives a view of the whole trial.

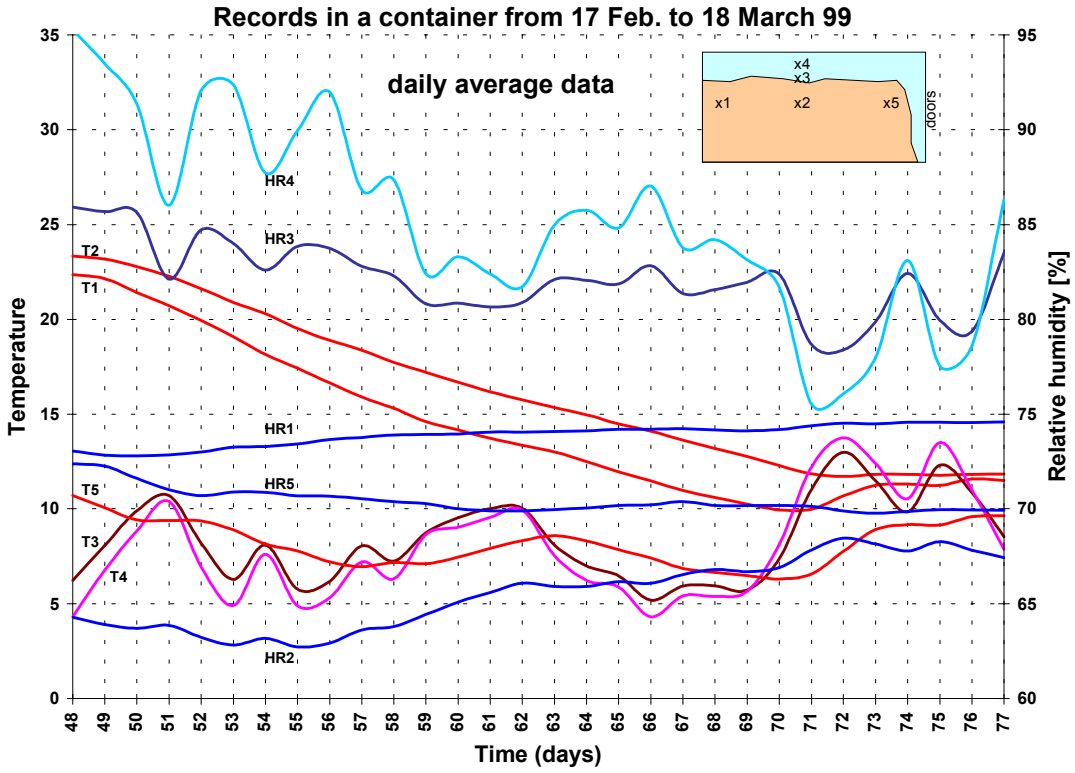

Figure 1. Daily average T and HR during storage in container

The following observations can be made on these data:

- The temperature between the bags, on rear of container (recorder N°1) regularly decreased during a period of 20 days (50 to 70, i.e. 19 Feb. to 11 March) from 22°C to 10°C; at the same time, the HR imposed by green coffee increases from 73% to 75%.
- The temperature between bags, in centre of container (recorder N°2) regularly decreased during the same period of time from 23°C to 12°C; while HR imposed by green coffee increased from 64% to 68%.
- The temperature between bags, on front (near the bottom) of container (recorder N°5) remained more or less constant and low during the same period of time from 9°C to 6°C; while HR imposed by green coffee remained at 70%.

These three observations confirm a large temperature gradient during the whole trial, a huge variation of Aw of coffee within the bags (min 0.62 and max 0.74 A_w), but no dramatic increase of HR at this level, between the bags.

- Above the bags, in middle of container (recorder N°3), the daily average temperature fluctuated between 5 and 13°C; while daily average HR imposed by temperature changes fluctuated between 86 and 78%.

- Against the roof, in middle of container (recorder N°4), daily average temperature fluctuated between 4 and 14°C; while daily average HR imposed by temperature changes fluctuated between 95 and 76%.

These last two observations confirm that HR of headspace may reach very high average values above 90%. More detailed observations will result from the analysis of data over a long period of time. The detailed data in Figure 2 did not show any daily cycle for recorders 1, 2 and 5 placed between the bags. It is the indication of a large inertia of heat exchange and water transfer between the coffee bags. On the contrary the detailed data show dramatic variations for recorders 3 and 4 placed above the bags or against the roof of the container.

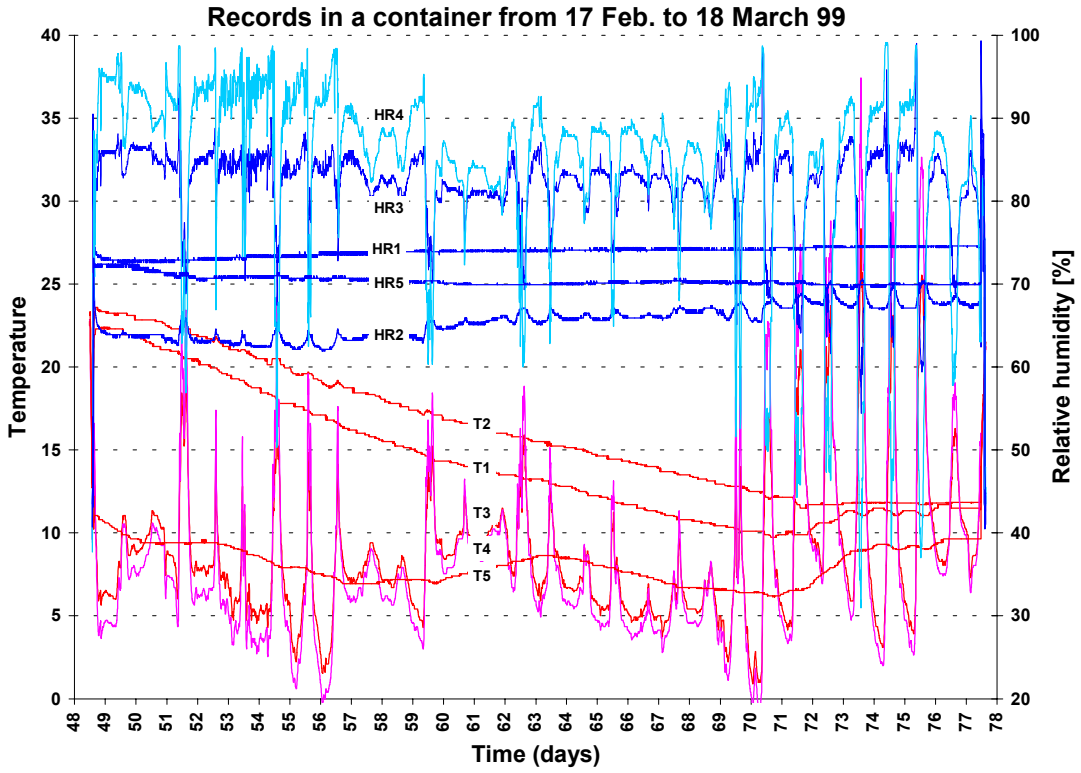

Figure 2. Detailed data

The values reached by HR4 indicate several periods of condensation and need more accurate investigation. One critical period is illustrated in Figure 3.

The condensation can be seen with both recorders 4, against the roof, and 3, on the top of the bags. Other similar condensation episodes were observed during the trial. Different relevant observations have to be discussed concerning the key phenomena:

Water content and temperature variations

Figure 4 shows a huge gradient of temperature existing at beginning of trial. The A_w's between 0.64 and 0.73 indicates large variations in water content, between 13.1 and 15.5% according to data from an averaged sorption isotherm. By sampling 20 bags in front of the doors an average water content of 13.8% was analysed.

The temperature gradient was slowly reduced during trial period but A_w's still indicate large water content variations (Figure 5), between 13.8 and 16.1%. The average water content in the bags in front of door is 14.4% by end of trials.

Water migration during the trial

From a theoretical point of view it is expected that the water will slowly migrate from regions of high A_w to regions of low A_w, and from regions of high temperatures to region of low temperatures. From the average data collected during this trial it is difficult to conclude about this water transport mechanism.

Condensation in headspace during trial

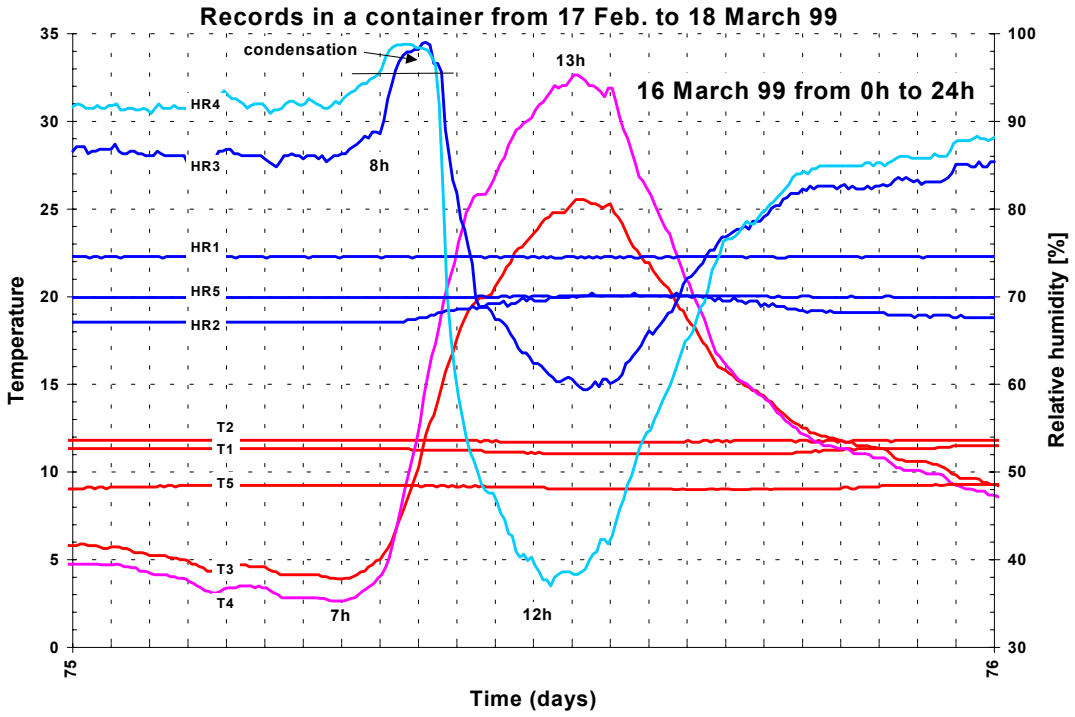

Figure 3. Example of a condensation period

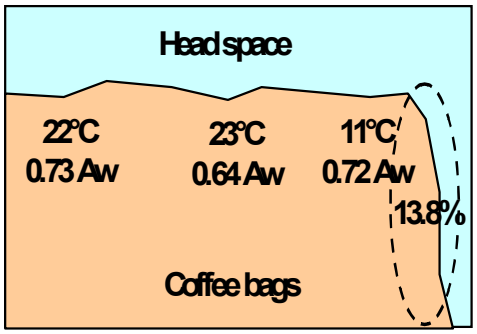

Figure 4. Average temperatures, Aw and water contents at beginning of trial

As container is closed, relative humidity is imposed by average Aw (about 0.70) of the beans at top of loading. Thus, in uniform temperature conditions, the HR in headspace would be about 70%. However, temperature in headspace changes dramatically during the trial, and especially between day and night. During first three weeks of the trial headspace temperature was below average temperature of beans near the top. Therefore the HR of headspace will always be above 70% and can reach 100%, depending on temperature. A total of 10 hours with condensation was recorded on 9 occasions. Such condensation may have produced uncontrolled flow of water drops on surface of bags. Very long periods with HR above 95%

have been recorded against the roof. Just above top of the bags (HR3) rather long periods (175h) with HR above 85% have been recorded. The recorded temperatures during the same periods were possibly high enough to have created conditions favourable to mould development and even mycotoxin generation.

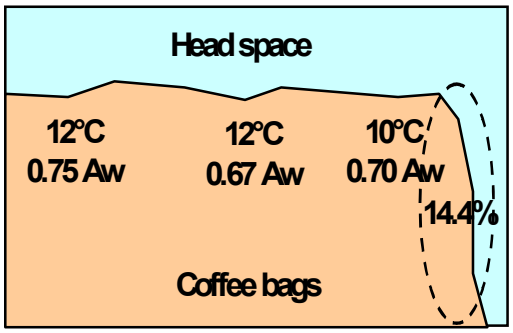

Figure 5. Average temperatures, Aw and water contents by end of trial

Trial 2

Figure 6 shows daily average T and HR during trial and Figure 7 full set of data.

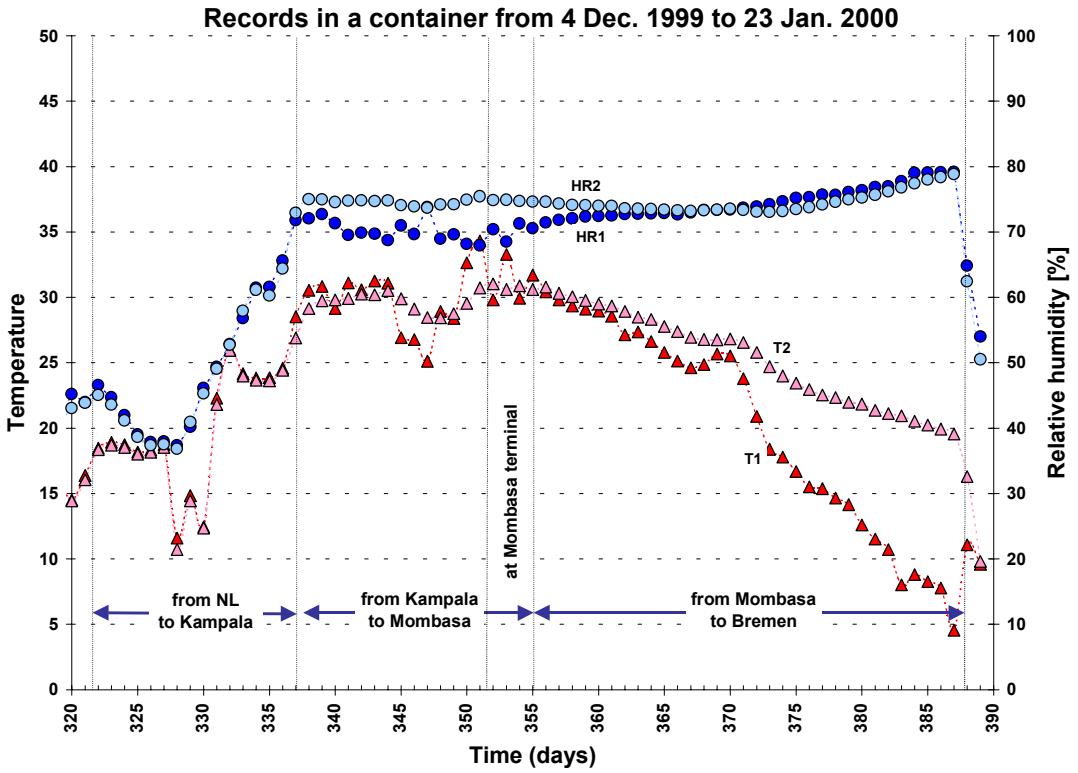

Figure 6. Trial 2. Daily average data

The different phases of transport are clearly illustrated in two previous figures. Two main very different steps can be identified: terrestrial transport between production site to port of embarkment and maritime phase to Europe. The data show dramatic daily cycles for the recorder N°1, indicating a large effect of surrounding conditions at top of coffee. Very small variations for recorder N°2 indicate large inertia of heat exchange and water transfer between

green coffee bags. Figures 8 and 9 show separately the evolution of T and HR during both phases of transport.

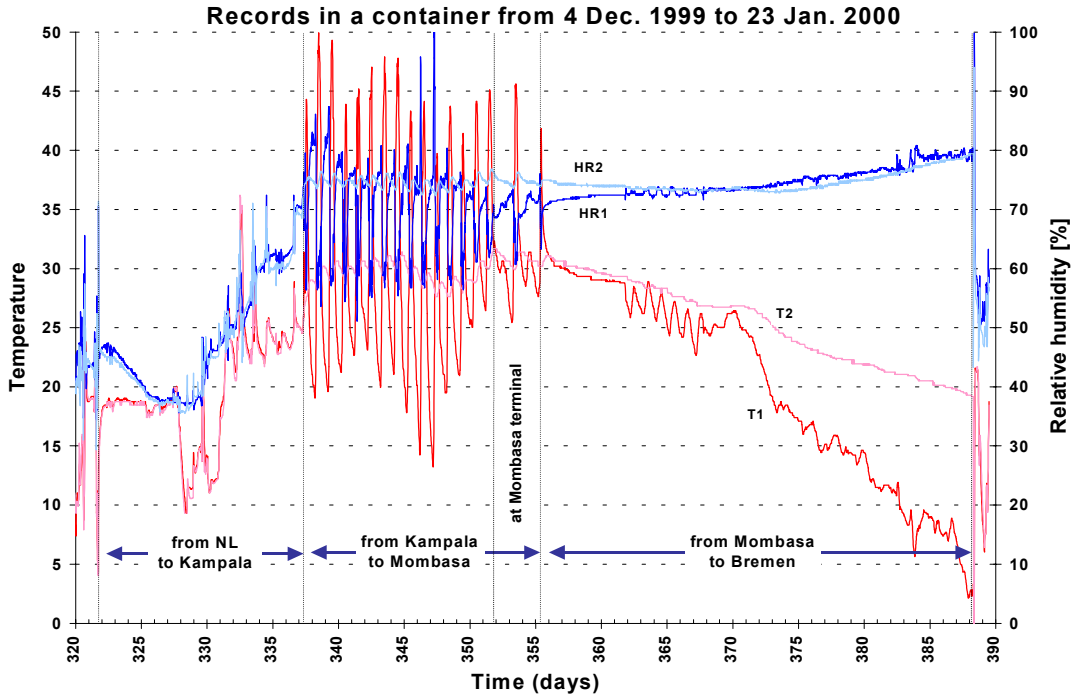

Figure 7. Trial 2. Full set of data

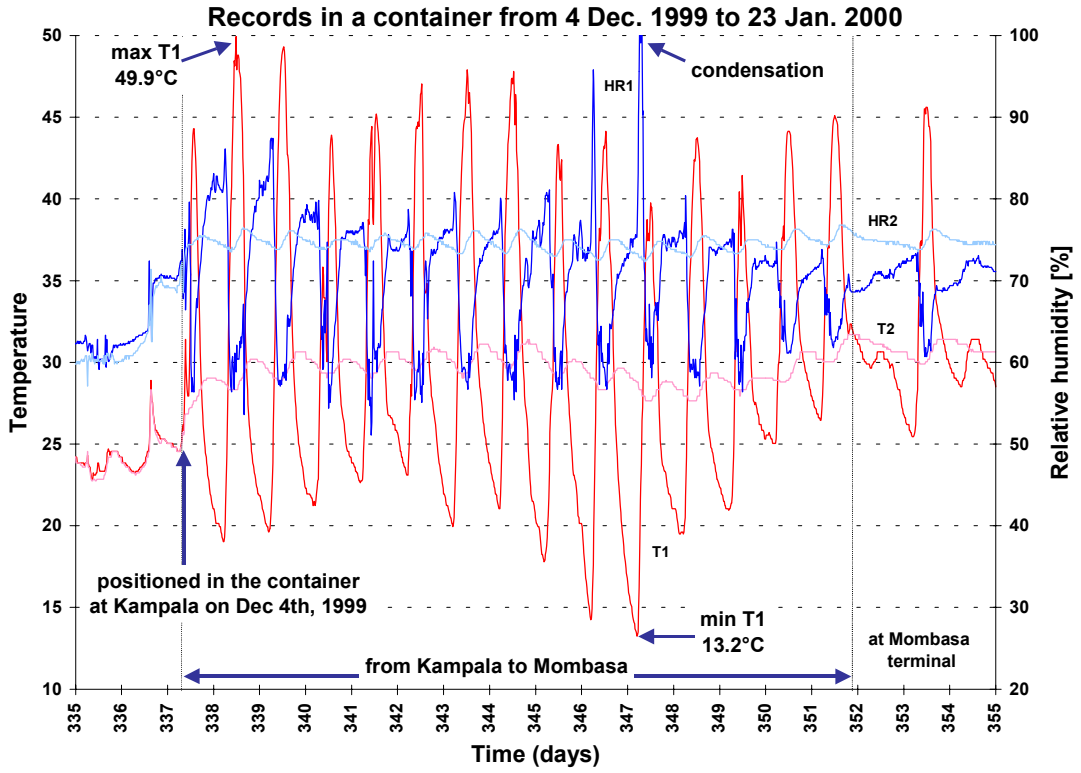

Figure 8. Trial 2. Terrestrial transportation phase

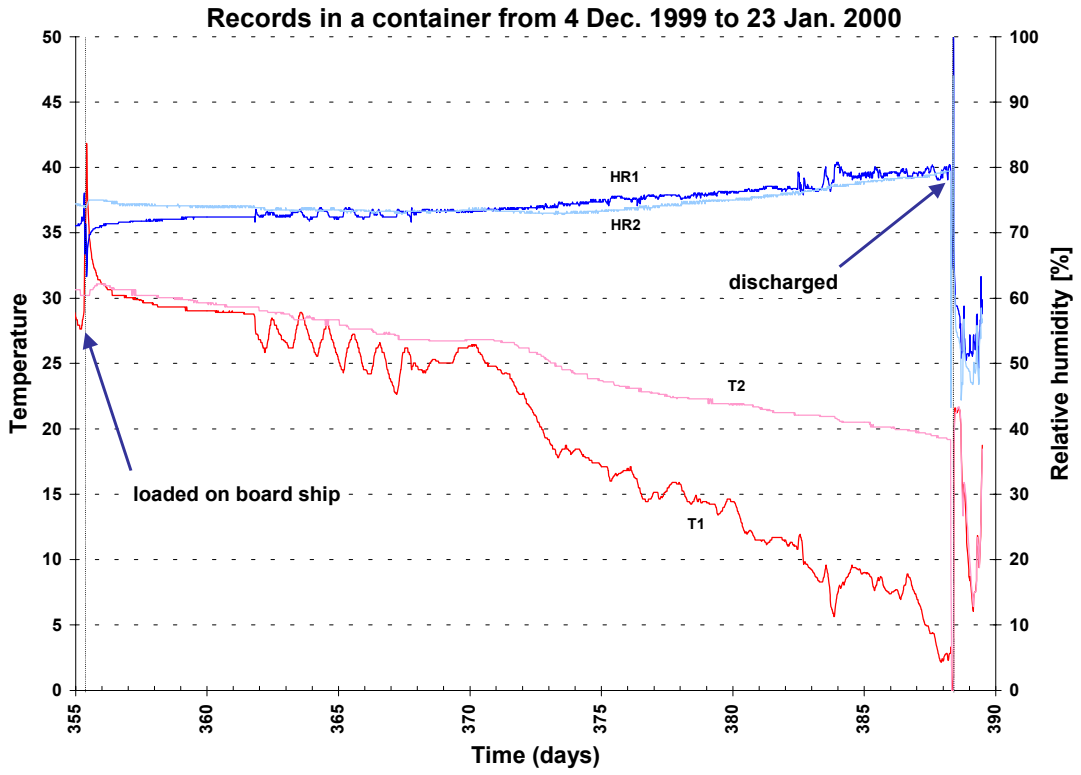

Figure 9. Trial 2. Maritime phase of transport

Trial 3

The results of this trial are quite similar to those of second trial but without condensation and less extreme conditions. As shown in Figure 10 a maximum of HR of 84.3% was recorded at top of container.

The container was left for a period of 10 days on arrival in Europe. Contrary to what was observed in first trial no condensation was recorded, probably due to summer conditions.

CONCLUSIONS

The results of these three transport trials made it possible to document different phases of green coffee transport between producing countries and Europe. Based on temperature and relative humidity records two phases of transport at risk for mould development and mycotoxins production were identified. Due to weather conditions external storage of a container in a north European harbour is the highest risk phase. The second phase at risk is the terrestrial transport between green coffee production area and port of embarking. The maritime leg of transport never showed suitable conditions for mould development or mycotoxins generation. Condensation under the roof at top of a container has been clearly observed during these phases at risk. Nevertheless, providing green coffee was dried below a critical moisture level, covering the coffee bags at the top or wrapping the bulk in a liner could very significantly reduce the risks.

Only one period of condensation was recorded during terrestrial transport but frequent periods with HR higher than 80% and a temperature of coffee at top close to 30°C shows that this phase of transport is higher risk. However the maritime phase never showed HR higher than

80% and the temperatures slowly decreased, making this phase much safer, provided no other incidents occur.

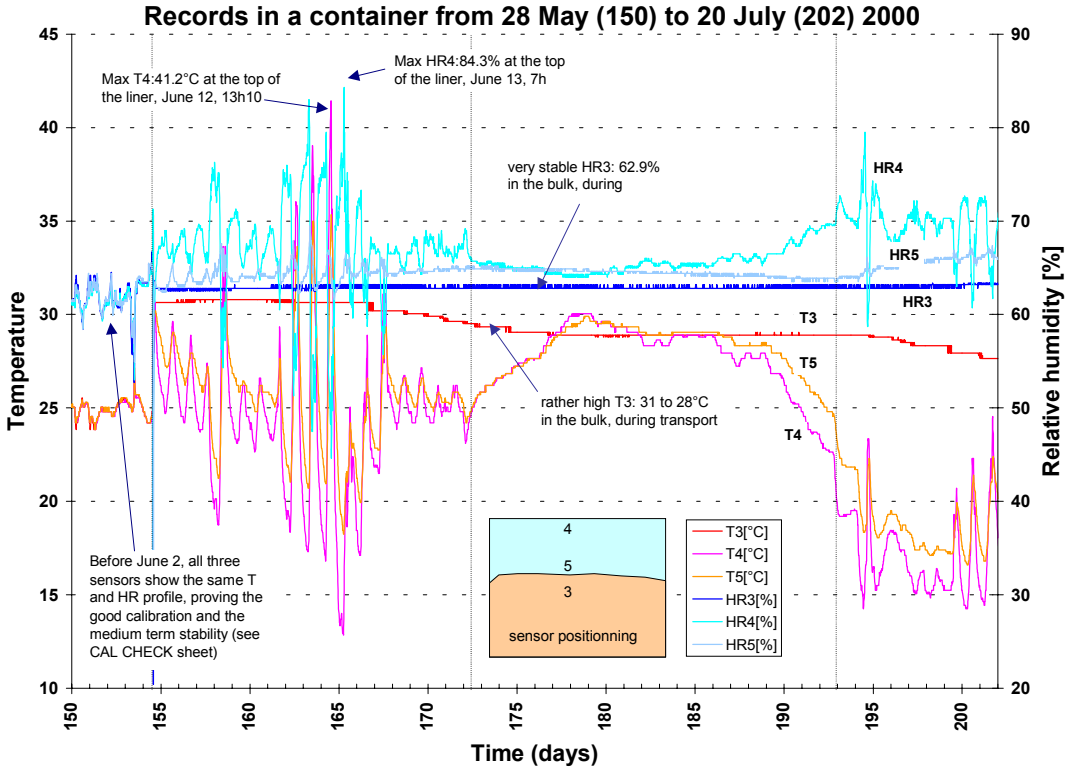

Figure 10. Trial 3. Full set of data

The Importance of Ochratoxin A in Foods: Report on the 56th Meeting of JECFA

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SUMMARY

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met at its 56th meeting in Geneva, Switzerland, in February 2001. This meeting was unique in that only mycotoxins were considered: aflatoxin M_1, fumonisins, some trichothecenes including deoxynivalenol and T-2 toxin, and ochratoxin A. Again unique in JECFA meetings, the work schedule dealt not only with toxicology, but with the whole range of factors influencing the estimation of human intake of these toxins, including the causal fungi and their occurrence, sampling plans, methods of estimation, and the occurrence of the toxins in foods around the world. This report will deal only with ochratoxin A, because of its potential importance in coffee. Information available at this date indicates that three major fungal sources of OTA exist in foods: a single *Penicillium* species, *P. verrucosum*, *Aspergillus ochraceus* and some related species, and *A. carbonarius*, together with the closely related *A. niger*. Information obtained by JECFA showed clearly that Europe is the major region of the world where OTA commonly occurs in foods. Within Europe, the major sources are cereals and cereal products, which accounted for ca 60% of OTA intake, wines (25% of intake), grape juice and coffee (5-7% each) and all other foods, less than 1% of the total intake. The fungal source of OTA in European cereals was considered to be *P. verrucosum*. The sources in the other foods were less clear, but species other than *P. verrucosum* were likely. The Committee concluded that at this time, no cases of acute intoxication in human populations due to intake of OTA have been reported. The Committee agreed that OTA is a kidney toxin in all animals species studied, and in some animals is a carcinogen of the kidneys, and has been observed to cause genetic toxicity. It is embryotoxic and teratogenic in rats and mice. However, the significance of the results of these studies for human toxicity remain unclear. The provisional tolerable weekly intake figure for OTA set by JECFA in 1995 (100 μg/kg body weight per week) was maintained at the current meeting. JECFA concluded that in Europe, consumption of cereals alone may cause heavy consumers to approach that figure for total OTA intake, but evidence was lacking that these levels existed elsewhere in the world. JECFA concluded that reduction in OTA intake was desirable, but that this was best accomplished by improved agricultural practices. Setting of maximum permitted levels of 20 or 5 μg/kg in cereals would have no significant influence on average OTA intakes.

JECFA

JECFA (the Joint FAO/WHO Expert Committee on Food Additives) is responsible for carrying out toxicological evaluations and risk assessments on all types of additives in foods, including ingredients, pesticides and mycotoxins. JECFA provides recommendations to the Committee on Food Additives and Contaminants (CCFAC) of the Codex Alimentarius Commission (Codex). Codex, a Commission under the World Trade Organization, in turn is responsible for recommencing limits for all types of food additives in foods in international trade. So JECFA has the primary responsibility for developing definitive toxicological and related information on food additives, which through Codex eventually becomes incorporated

into international food law.

THE JECFA 56th MEETING

The 56th meeting of JECFA was held in Geneva, Switzerland, on 6th to 15th February 2001. At the request of Codex, JECFA examined a range of mycotoxins in foods, specifically ochratoxin A, aflatoxin M, fumonisins, and some trichothecenes. In a major innovation, JECFA looked not only at toxicology, but also at a much broader range of issues, including causal fungi and their occurrence, methodology for detection and estimation, including sampling plans, occurrence in foodstuffs and intake by populations, and risk assessment. This approach resulted in the bringing together of experts in a wide range of disciplines, including mycologists, chemists, mathematicians and toxicologists. It was a unique gathering.

The remainder of this summary will be concerned with the one significant mycotoxin which may occur in coffee, ochratoxin A (OTA).

TOXICITY OF OCHRATOXIN A

A great deal of the JECFA meeting was focused on the toxicology of OTA. OTA has been shown to be nephrotoxic (toxic to kidneys) in all mammalian species tested. However large differences have been seen in sensitivity of the various species: pigs are more sensitive than rats, and rats than mice. OTA is also a carcinogen, but the doses needed to cause tumours are higher than those that can cause acute toxicity.

A few studies have reported that OTA can cause gene mutation in bacteria and mammalian cells, but most others did not. DNA damage can occur, and chromosomal and repair aberrations have been seen in mammalian cells and mice. It remains uncertain whether OTA reacts directly with DNA or via some active intermediate. OTA is genotoxic both in vivo and in vitro, but the mechanism of genotoxicity is unclear.

OTA can cross the placenta and is embryotoxic and teratogenic in rats and mice. It has also been shown to have immunosuppressive effects in a number of species. It inhibits the production of B and T lymphocytes. However both the immunological and teratogenic effects have been observed only at doses much higher than those that cause nephrotoxicity.

OTA has been found quite commonly in human blood samples, mostly from countries in the cool temperate area of the Northern Hemisphere. OTA appears to have a very long half life in blood. Despite this, no cases of acute intoxication in humans have been reported. For many years, attempts have been made to link OTA with the fatal human kidney disease Balkan Endemic Nephropathy, but no concrete evidence of the involvement of OTA has emerged. It seems likely that other nephrotoxic agents are involved.

In summary, while there is abundant evidence that OTA is a very toxic compound to all animal species, including man, the mechanism for toxicity remains unclear. The acute toxicity to kidneys is caused by lower doses than those which will cause cancer, or DNA disruption, or toxicity to embryos.

FUNGAL SOURCES OF OCHRATOXIN A

It has now become clear that OTA has several fungal sources, and that both geography and type of food determine which source is likely be responsible for OTA formation in a particular foodstuff.

The common source of OTA in cool temperate climates, i.e. Northern and Eastern Europe and Canada, is *Penicillium verrucosum*. This species grows only below about 30°C and is mostly associated with cereals and cereal products, and with meats, especially pig meats, of animals fed infected cereals. The presence of this species in coffee appears unlikely.

The original source of OTA, *Aspergillus ochraceus,* is widespread in warmer areas of the world, but is mostly associated with long stored foods, in warm temperate and subtropical areas. It appears to be a major source of OTA in coffee. Little evidence exists that *A. ochraceus* has an association with the coffee tree or coffee bean, and it appears to develop during drying and/or storage of green coffee beans. Several less well known *Aspergillus* species closely related to *A. ochraceus* also produce OTA, but it is still difficult to assess which of these, if any, have a role in OTA production in coffee.

It has been shown quite recently that *Aspergillus carbonarius* is also a major source of OTA. It appears to be most commonly associated with fresh fruit, especially grapes. It grows in hot climates, and because it has black spores and mycelium, appears to be ideally suited to growth in maturing and drying grapes. Hence it is considered to be responsible for the occurrence of OTA in grape juice, dried vine fruits and wines. It may occur to some extent in coffee and, like *A. ochraceus*, is believed to infect coffee beans during drying and storage.

Aspergillus niger is closely related to *A. carbonarius* and is much more common, perhaps occurring in a wider range of foods. However, few isolates of *A. niger* are known to produce OTA, and its role in production of this toxin in coffee is uncertain.

MAJOR FOODS CONTAINING OTA

The major food sources of OTA are shown in Table 1. The figures are averages based on information provided to JECFA for the 56th meeting: data are from the last 5 years and are very extensive. Most (85%) come from European sources. Smaller numbers of figures came from South America (7%) and North America (6%), Africa (1%) and Asia (1%). Care was taken to accept figures only where reliable and validated methods had been used, and effective sampling plans in place.

As noted above, cereals are the most important food source of OTA, but far as is known, only wheat and barley are affected, and these are only contaminated to a significant extent in Europe and Canada. Products derived from cereals, such as bread and pastries, and meats from animals fed on cereals in Europe, of which pigs are the most important, also contain lesser concentrations of OTA. On the basis of average levels of contamination, dried vine fruits, coffee, grape juice and wines are also important sources of OTA (Table 1).

ESTIMATES OF INTAKE BY HUMANS

Dietary figures from WHO standard diets were used to estimate weighted mean intakes of OTA by Europeans. Using this approach, the mean total intake of OTA was estimated to be about 45 μg/kg body weight per week (Table 2). The major sources of intake were cereals and wines, which contributed about 25 and 10 μg/kg body weight per week. Grape juices and coffee contributed 2-3 μg/kg and other foods, including dried fruits, beer, tea, milk, cocoa, poultry and pulses, contributed less than 1 μg/kg body weight per week. Thus, coffee, a relatively major potential source of OTA in the human diet on the basis of level of occurrence, is a much less important contributor on a percentage basis, estimated to be responsible for 5-6% of OTA intake in Europe. OTA in coffee is more an economic and socially perceived threat than a human health threat.

Information on OTA intakes outside Europe are not well documented. Intake levels of OTA around the world from coffee can be expected to be similar, as the commodity is widely grown under more or less similar conditions, and is widely traded. On the basis of current knowledge, coffee contributes a higher percentage of OTA intake in areas other than Europe, because cereals do not contain appreciable levels of OTA elsewhere, but the actual intake will vary relatively little, and more as a result of differences in diets than from other factors.

PREVENTION AND CONTROL

JECFA also discussed possible approaches to prevention and control of OTA in foods at the 56th meeting. In the case of coffee, it was agreed that most if not all OTA was formed in green coffee beans after harvest, during drying and storage. Therefore changes to agricultural practice and agronomic conditions during fruit formation were unlikely to play any real role in OTA reduction. However, harvesting practice is important, especially as the harvesting of cherries from the ground increases the probability of contamination with fungi capable of producing OTA. Contamination during picking and post-harvest transport, and from the drying areas, are also certainly of importance. Drying practice is a major factor in OTA formation. The advice widely given to farmers drying any commodity, that it is important to dry it quickly, and then keep it dry, certainly applies to coffee.

RISK EVALUATION

JECFA discussed at length the problems in evaluating the available toxicological information on OTA, and concluded that the data received since the last evaluation raised further questions about the mechanisms by with OTA causes renal toxicity and carcinogenicity. In particular, the mechanism by which it causes cancers remains unknown. In the absence of new information, the Provisional Tolerable Weekly Intake (PTWI) established at the last evaluation, 100 μg/kg body weight per week, was maintained. This PTWI includes a very large safety factor, mainly because the mechanisms for toxicity and carcinogenicity remain uncertain.

In assessing intakes in relation to the PTWI, JECFA noted that the intake of OTA by 95 percentile consumers (i.e. heavy consumers) of cereals in Europe may approach the PTWI from cereals alone. Clearly consumption of high levels of wine, or coffee, or both, by such high consumers of cereals would increase the OTA intake significantly. JECFA noted that individuals who consumed less than the PTWI of OTA were not at any appreciable risk; however, available information did not permit assessment of a quantitative risk for individuals whose intake of OTA exceeded the PTWI.

In asking JECFA to re-evaluate OTA, Codex had requested that the Committee consider the effect of setting the maximum permitted levels of OTA in foods at either 5 or 20 μg/kg. JECFA concluded that establishing either of these levels as a maximum would be essentially ineffective, as little food in international trade currently exceeded even 5 μg/kg OTA. JECFA concluded that the best way to reduce OTA intake lies not in regulation, but in efforts to lower overall contamination rates in the major food sources.

CONCLUSIONS

At the 56th meeting, JECFA stated again that OTA is a significant renal toxin in animals, and by implication, in man. Major fungi producing OTA are *Penicillium verrucosum*, which occurs mostly in cereals in Europe, *Aspergillus ochraceus* and several closely related species, which may occur in a variety of foods, including coffee, and *Aspergillus carbonarius* along

with some isolates of *A. niger*, with their major habitat in grapes and to a lesser extent in other fruit. *A. carbonarius* is perhaps of significance in coffee in some areas also.

Coffee is a major food for the occurrence of OTA, but the relatively low consumption of coffee beans (consumed almost entirely diluted as drinks) means that it is not a major source of OTA in the European diet, or so far as is known, in other regions of the world.

The occurrence of OTA in coffee is an economic or perceived problem, rather than a human health hazard. However, it must be emphasised that reduction in the incidence of OTA in coffee remains an important goal. This desirable outcome is unlikely to be reached by regulation, but by improvements in processing of green coffee beans, especially in drying and storage practice.

ACKNOWLEDGEMENT

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Table 1. Mean concentrations of ochratoxin A occurring in the European food supply

Commodity	Ochratoxin A (μg/kg)
Dried vine fruits	2.3
Cereals	0.94
Coffee	0.86
Grape juice	0.44
Wines	0.32
Cereal products	0.19

Table 2. Estimates of intake of ochratoxin A by an average individual in Europe[a]

Commodity	Average ochratoxin A intake, μg/kg per week	Percentage of intake
Cereals	25	60
Wines	10	25
Grape juice	2-3	5-6
Coffee	2-3	5-6
Pig meats	1.5	3
Dried vine fruit, beer, tea, poultry, cocoa, milk, pulses	< 1.0	2

[a]*Percentages are rounded figures*

Distribution of *Aspergillus ochraceus, A. niger* and *A. carbonarius* in Coffee in Four Regions of Brazil

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SUMMARY

This work investigated the distribution of ochratoxigenic fungi, and their ability to produce ochratoxin A (OTA), from coffee in four regions of Brazil. In total, 407 coffee samples were examined at different stages: mature (including immature) and overripe cherries from trees, overripe cherries from soil, and beans during drying and during storage on farm. Thirty six soil samples from coffee plantations were also analysed for the presence of ochratoxigenic fungi. The four regions studied were Alta Paulista (western area of São Paulo State), Sorocabana (southwest São Paulo State), Alta Mogiana (northeast São Paulo State) and Cerrado Mineiro (western area of Minas Gerais State), during the coffee harvest seasons of 1999 and 2000. Cherries and beans were surface disinfected with chlorine, plated on Dichloran 18% Glycerol Agar at 25ºC for 5-7 days, and analysed for the presence of *Aspergillus ochraceus, A. carbonarius* and *A. niger*. The potential for OTA production by fungal isolates was tested by the agar plug technique and by thin layer chromatography. In total, 872 isolates of these three species were taken from coffee samples from the different regions during this study. Although *A. niger* was the most common species found (549 isolates), only 3% produced OTA. *A. ochraceus* was also commonly occurring (269 isolates), with 75% being capable of OTA production. *A. carbonarius* was found (54 isolates) in only one region, Alta Paulista, which is very hot, and only from beans in the drying yard or in storage. However 77% of the *A. carbonarius* isolates were capable of producing OTA. Mean infection rates for cherries from the tree were very low, but higher in fruit from the ground, from the drying yard and from storage, indicating infection by toxigenic species after removal from trees. Infection rates from drying and storage were very variable, and these were reflected in variability in OTA analyses. This variability indicated a lack of quality control during the drying process on some farms studied.

INTRODUCTION

A major question in understanding the origins of ochratoxin A (OTA) in coffee remains the relative importance of the major fungal sources, and their geographic distribution. To date, few investigations have looked at this issue in depth. It is commonly assumed that the major source is *Aspergillus ochraceus* or closely related species more or less difficult to distinguish from *A. ochraceus.* The discovery during the past few years that *A. carbonarius* is also a common producer of OTA (Varga et al., 1996; Heenan et al., 1998) has prompted a more thorough investigation of this question. The main objective of this work was to study the distribution of fungi with the potential to produce OTA in coffee beans throughout the harvest and drying process. The investigation was carried out in four regions of Brazil where coffee is intensively grown.

MATERIAL AND METHODS

Coffee producing regions

The four Brazilian regions from which coffee samples were obtained were Alta Paulista (western area of São Paulo State), Sorocabana (southwest São Paulo State), Alta Mogiana (northeast São Paulo State) and Cerrado Mineiro (northeast Minas Gerais State). These are all major areas for coffee production.

Sampling

During the coffee harvest seasons of 1999 and 2000, over 400 samples of *Coffee arabica* fruit 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 & Pitt, 1980). The plates were incubated at 25ºC for 5 to 7 days. After incubation, plates were examined and representative colonies of *Aspergillus* species presumptively identified as *A. ochraceus* 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 the manual of Klich & Pitt (1988). Numbers of isolates identified as *A. ochraceus* or closely related species, *A, carbonarius* and *A. niger* were counted for each sample.

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 25ºC 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 60ºC 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 chloroform: ether: acetic acid (17:3:1) 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/4 mM 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).

RESULTS AND DISCUSSION

More than 400 samples of coffee beans from the 1999 and 2000 harvests were examined, and from them 872 isolates of *A. ochraceus* or closely related species, *A. niger* and *A. carbonarius* were obtained (Table 1). *A. niger* was the most common species found (549 isolates; 63 % of the three species). *A. ochraceus*, together with an unknown percentage of closely related species, was also common (269 isolates; 31%). *A. carbonarius* was much less common (54 isolates; 6 %), and was found in only one region, Alta Paulista, which is a relatively hot coffee growing area. Average infection rates of coffee fruit with these fungi at the various harvest and drying stages are also given in Table 1. It can be seen that infection rates overall were low. On average, *A ochraceus* infected less than 0.5 % of fruit on the tree. Rates increased during drying, and averaged 2.0 % in storage. Infection with *A. carbonarius*, averaging 0.5 %, was found only in samples from the drying yard and in storage.

Table 1. Infection of Brazilian coffee beans with fungi potentially capable of ochratoxin formation[a]

Stage	No. of samples	*A. ochraceus* (%)	*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

[a]*Total of 408 samples from four regions over two harvest seasons, 1999 and 2000*

When the 872 isolates were tested for the ability to produce OTA in culture, it was found that most *A. ochraceus* and *A. carbonarius* isolates were capable of toxin production (Table 2).

Although the figure for *A. carbonarius* (77 %) was lower than that found by Heenan et al. (1998), who reported 100 % capability, the figure for *A. ochraceus* (75 %) is much higher than has been reported previously. This finding may be due to the use of only fresh isolates, or perhaps to the presence of a more frequently toxigenic closely related species not taxonomically distinguished from *A. ochraceus* in this study. The perception that only occasional isolates of *A. niger* are potential OTA producers was confirmed, as only 6 of more than 500 isolates (3 %) was found to have this capacity.

Table 2. Percentages of isolates of fungi potentially ochratoxin A producers[a]

Species	No of isolates tested	Toxigenic on YES medium (%)
Aspergillus ochraceus	269	75
A. niger	549	3
A. carbonarius	54	77

[a]*Grown for one week at 25°C, tested by thin layer chromatography*

The low average infection rates found (Table 1) indicate little problem with infection by potential OTA producers, 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.

Table 3. Highest percentage infection in samples of Brazilian coffee beans with fungi potentially capable of ochratoxin formation[a]

Stage	*A. ochraceus* (%)	*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

[a]*Highest infections in 408 samples from four regions over two harvest seasons, 1999 and 2000*

However, infection by the more important species *A. ochraceus*, 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, 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 serious potential to produce 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.

Table 4. Ochratoxin levels in coffee beans at various stages

Stage in production (no. of samples)	Mean (µg/kg)	Range (µg/kg)
Cherries from tree (6)	<0.2	<0.2 - 0.4
Overripe from tree (16)	<0.2	<0.2
Overripe from ground (25)	2.0	<0.2 - 37
Drying yard (40)	2.1	<0.2 - 48
Storage (48)	3.4	<0.2 - 109

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.

CONCLUSIONS

In a study of fungi with the potential to produce OTA in Brazilian coffee, the major fungus likely to be responsible was found to be *A. ochraceus*. Of 269 isolates of this species, 75 % were found

to be capable of toxin production. *A. carbonarius* was much less common, but also had the potential to produce OTA in coffee, as must isolates examined were also toxigenic. Few cherries on the tree were infected with these species, indicating that their sources were likely to be soil, equipment and drying yard surfaces. Variability in infection rates of these toxigenic species was reflected in wide variability in OTA levels in samples from the drying yard and storage, indicating a need for improved quality control to reduce OTA levels in dried coffee. *A. niger* was more common than *A. ochraceus* or *A. carbonarius,* but possessed a much lower potential to make this toxin and is probably a relatively unimportant source of OTA in coffee.

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The Presence of Ochratoxin A in Coffee Due to Local Conditions and Processing in Four Regions of Brazil

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SUMMARY

This work was carried out with the main objective of investigating the relationship between the presence of ochratoxin A (OA) in coffee and local conditions and processing. Over four hundred coffee samples were examined at different stages: cherries and overripe from trees, overripe from the soil, the patio (drying yard) and barn storage. These four coffee regions were: Alta Paulista (western of São Paulo State), Sorocabana (Southwest São Paulo State), Alta Mogiana (northeast of São Paulo State) and Cerrado Mineiro (Western of Minas Gerais State), during the coffee harvest period of 1999 and 2000.

The average of ochratoxin A in 135 samples of cherries from trees, overripe from trees, overripe from the soil, patio and barn was 0.12, <0.2, 1.6, 2.1 and 3.4 μg/kg respectively. The highest OA contamination was found in coffee from patio and barn. Only one sample of coffee (floaters type and stored in a barn) presented an OA content of 108.5 μg/kg. The cause of such a high contamination was investigated with the farmer and several critical points were found during the drying process and local conditions such as: the farm being located in a region of hydrographic basin affected by heavy mist over the farm; the workers did not turn over the coffee as much as necessary; in most cases the coffee layer was very thick; the barn had moisture infiltration from the ceiling and floor; there was also the presence of birds in the barn and all these factors can favor fungal growth in dried coffee. Moreover, 36% of this coffee sample was contaminated with *Aspergillus ochraceus* a potential producer of OA. These factors may have contributed to such high OA levels in the coffee sample.

In Alta Paulista, coffee samples from patio and barn, which were visibly mouldy, presented ochratoxin A content in one these samples at level of 19.75 μg/kg. After investigation the reason found for this was that the coffee elevator was broken and the bottom with coffee became covered with water, remoistening the dried coffee again.

Alta Mogiana and Cerrado Mineiro are known as region producing good quality coffee, because of the dry climate and high altitude. OA was found is some samples although very sporadic. Other examples will be shown in this presentation correlating the presence of OA and farm conditions.

INTRODUCTION

Coffee can be infected by ochratoxigenic fungi at some stage in the coffee process and this can lead to the presence of ochratoxin A (OA). Fungal growth and toxin production may be affected by moisture content of the beans and other factors such as climate and geographical conditions in coffee growing areas, and associated with handling procedures. An understanding of these parameters can help to establish mechanisms to prevent the presence of OA in coffee.

The present work was carried out in order to investigate the relationship between the presence of ochratoxin A (OA) in coffee and local conditions and processing.

MATERIAL AND METHODS

Coffee producing regions

Four different producing regions consisting of two to three farms from each one, were selected characterized by beverage quality and climate conditions during harvesting:

- Alta Paulista: average-quality beverage, low altitude (<800 m), hot, rainy region (average of 21ºC and 47.1 mm/month rainfall).
- Sorocabana: low-quality beverage, low altitude (<800 m), cold, rainy region (average of 17.7ºC and 64.9 mm/month rainfall).
- Alta Mogiana: good-quality beverage, high altitude (800-1000 m), temperate, not too dry region (average of 19.5ºC and 29.5 mm/month rainfall).
- Cerrado Mineiro: good-quality beverage, high altitude (>1100m), temperate, dry region (average of 19.1ºC and 14.8 mm/month rainfall).

Sampling

During the 1999 and 2000 harvest periods, over 400 coffee samples (*Coffea arabica*) were collected at different stages of maturation and processing: cherries and overripe from trees, overripe from the soil, patio (drying yard) and barn storage.

Mycological analysis

Coffee beans were surface disinfected with 0.4% chlorine solution for 1 min (Pitt & Hocking, 1997). A total of 50 beans were plated directly (10 particles per plate) onto the culture medium Dichloran 18% Glycerol agar (DG18, Hocking and Pitt, 1980). The plates were incubated at 25ºC for 5 to 7 days.

Analysis of OA in coffee

Coffee samples were analyzed for OA 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 OA. After washing, the OA was eluted with high-performance liquid chromatography (HPLC) grade methanol and quantified by reverse-phase using a fluorescence detector. The detection limit of this method was 0.2 μg/kg. The mobile phase used was acetronitrile/4 mM sodium acetate with 0.5% acetic acid solution (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™ LC-18 (5 μm, 250 mm X 4,6 mm) column (Supelco Park, USA).

RESULTS AND DISCUSSION

Table 1 shows the percentage of infection by ochratoxigenic (*A. ochraceus* and *A. carbonarius*) species in coffee in four regions from the 1999 and 2000 harvests. The presence of ochratoxigenic species was higher in samples from soil (overripes), patio and barn.

Table 1. Percentage (%) of infection by ochratoxigenic (*A. ochraceus* and *A. carbonarius*) species in Brazilian coffee in 4 regions from the 1999 and 2000 harvests

Stage of maturation or processing	Alta Paulista		Sorocabana		Alta Mogiana		Cerrado Mineiro	
	Nº sample	% infection	Nº sample	% infection	Nº sample	% infection	Nº sample	% infection
Cherries from tree	12	0.6	22	2.0	18	0	3	0
Overripe from tree	17	1.0	17	0	20	0.6	3	0
Overripe from soil	14	6.0	24	5.0	20	0.8	5	0
Patio	37	12.0	42	0	33	3.2	16	0
Barn	38	5.0	30	5.0	28	1.4	9	4.0

The range of OA (μg/kg) detected in the coffee samples at different stages of coffee production is presented in Table 2.

Table 2. Range of ochratoxin A (OA) in coffee in four different region from the 1999/2000 harvests

Region	Nº of samples	Range of OA (μg/kg)							
		< 0.2	0.21-1.0	1.1-5.0	5.1-10.0	10.1-20.0	20.1-50.0	50.1-100	> 100
Alta Paulista	46	28	3	12	1	2	0	0	0
Sorocabana	41	30	4	3	2	0	1	0	1
Alta Mogiana	43	35	3	3	0	1	1	0	0
Cerrado Mineiro	5	3	2	0	0	0	0	0	0

Apart from 10 out of 135 coffee samples, all presented OA levels below 5 μg/kg. The highest OA levels were found in coffee from soil, patio and barn (Table 3), indicating that OA is a post-harvest contamination. Besides Cerrado Mineiro, other regions presented OA higher than 1 μg/kg.

An investigation of the relationship between the presence of OA in coffee and local conditions and processing was carried out. Table 4 gives a summary of the 10 cases where OA was found at 5 μg/kg level and higher. Each case was analyzed, as follows:

Cases 1 and 2

Samples from patio and barn, were collected from the same farm (farm1) located in Alta Paulista. *A. carbonarius* appeared in these coffee samples, which were visibly mouldy. OA content in these samples was 5.0 and 19.75 μg/kg in patio and barn, respectively. This region is hot, the average temperature being 21ºC, which may have favoured the growth of this species. After investigation the reason found for this was that the coffee elevator was broken and the dried coffee at the bottom became covered with water, thus rewetting it.

Table 3. Level of ochratoxin A (OA) contamination in different stages of coffee production (1999/2000)

Stage of coffee production	Nº of samples	Mean (μg/kg)	Range (μg/kg)
Cherries from tree	6	0.12	< 0.2 – 0.35
Overripe from tree	16	< 0.2	< 0.2
Overripe from soil	25	1,6	< 0.2 – 36.8
Patio	40	2.1	< 0.2 – 47.7
Barn	48	3.25	< 0.2 – 108.5

Table 4. Cases of ochratoxin A (OA) in coffee at high levels

Case	Region	Stage of coffee processing	% of infection of OA producing fungi	Level of OA (μg/kg)	Year of harvest
1	Alta Paulista	Patio	26% A. carbonarius	5.0	1999
2	Alta Paulista	Barn	6% A. carbonarius	19.7	1999
3	Alta Paulista	Patio	18% A. ochraceus	18.5	1999
4	Alta Paulista	Barn	Not found	9.6	2000
5	Sorocabana	Overripe from soil	4% A. ochraceus	6.2	2000
6	Sorocabana	Barn	Not found	8.0	2000
7	Sorocabana	Barn	36% A. ochraceus	108.5	2000
8	Sorocabana	Overripe from soil	12% A. ochraceus	36.76	2000
9	Alta Mogiana	Barn	2% A. ochraceus	10.3	1999
10	Alta Mogiana	Patio	Not found	47.7	2000

Case 3

A. ochraceus was found in 18% of coffee beans in one sample from patio, of farm 2 in Alta Paulista. The water activity was still enough for this species to grow.

Case 4

OA concentration was 9.6 μg/kg, although *A. ochraceus* was not found. The barn was built very close to the coffee dryer, which injects humid hot air into the coffee. The temperature of the coffee at the top was higher than 50ºC, which caused a warm humid condition for fungal growth and OA production.

Cases 5 and 6

OA was found in coffee samples from soil and barn (Farm 1, Sorocabana). The agricultural practice in this farm was very bad as the workers collected samples from raking the soil. *A. ochraceus* was found although at a low percentage of infection.

Cases 7 and 8

This farm (Farm2, Sorocabana) was badly affected by climate conditions of this region as well as poor practice. Only one sample of coffee (floaters type and stored in a barn) presented an OA content of 108.5 μg/kg. The cause of such a high contamination was investigated with the farmer and several critical points were found during the drying process and local conditions such as: the farm being located in a region of a hydrographic basin affected by heavy mist over the farm; the workers did not turn over the coffee as much as necessary; in most cases the coffee layer was very thick; the barn had moisture infiltration from the ceiling and floor; there was also the presence of birds in the barn and all these factors can favour fungal growth in dried coffee. Moreover, 36% of this coffee sample was contaminated with *Aspergillus ochraceus* a potential producer of OA. These factors may have contributed to such high OA levels in the coffee sample. Another sample from this farm collected from soil presented 12% of *A. ochraceus* infection and 36.76 μg/kg of OA.

Cases 9 and 10

Two samples, one from barn (1999 harvest) another from patio (2000 harvest) showed high OA concentration. This was surprising because Mogiana is known as a region with good coffee due to its high altitude, temperate climate and reasonable rainfall. However, when the cause of OA presence was investigated, it was found that the patio's size was very small for their coffee production, the coffee being put in one heap on the patio, waiting to be spread out to dry. There was also mist because the farm is located in a hydrographic basin. The time required to dry may last some days allowing for fungal growth. The ceiling was made of asbestos which heats the coffee and creates a higher temperature in the barn where the coffee is ideal for fungal development.

CONCLUSIONS

- A percentage of infection by ochratoxigenic fungi was lower in cherries and the overripe fruit stage on the tree;
- A percentage of infection was higher in overripe fruit found on the soil, patio and barn;
- The contamination range in the Cerrado Mineiro was less than 1 μg/kg;
- The main causes of a high contamination of ochratoxin were:
    - the rewetting of the dried coffee on the ground, patio and barn;
    - a very thick coffee layer;
    - the few times the coffee was turned over;
    - climate conditions
- However only nine samples were found with ochratoxin in over a hundred coffee samples examined at different stages.

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An Analytical Approach to Assess Ochratoxin A Contamination in Coffee

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SUMMARY

Several studies are underway in Brazil to develop and validate analytical methods for determination and quantification of ochratoxin A in green coffee, roasted and soluble coffee. A one-dimensional TLC method has been established (0.5 μg/kg). AOAC International has approved a Collaborative Study protocol for determining OTA in green coffee. Automation of a LC using a sample preparation system for determining OTA in green and roasted coffee has been fully accomplished. None of commercial kits for screening and quantification of ochratoxin A in green coffee evaluated so far have shown efficient for screening and quantification of OTA. Different ratios of particle size have been found for three different mills evaluated. Significant differences have been found in the OTA contamination level in green coffee sub samples obtained from different sub sampler mills

INTRODUCTION

Ochratoxin A (OTA) is a toxic secondary metabolite mainly produced by *Aspergillus ochraceus*, *A. carbonarius* and *A. niger*, and by *Penicillium verrucosum* in tropical and temperate climates, respectively (Mantle and Chow, 2001; Mantle, 2000). The toxin has been classified as a substance of the group 2B by IARC (1993) meaning the existence of sufficient evidence of its renal carcinogenicity to animals and possibly to humans.

To date the European Commission has discussed the possibility of laying down levels for OTA (3 μg/kg) in both roasted coffee beans and coffee products. The establishment of regulatory limits for ochratoxin A in coffee determines, for the coffee producing countries, the necessity of having in place accurate, precise and sensitive validated analytical methods and sampling plans to fulfil regulation requirements. Existing validated methods for green coffee (AOAC, 1998) cannot comply with the proposed regulation as well the non-existence of a validated sampling plan turns the assessment of OTA contamination of difficult approach. Specific and sensitive screening methods for ochratoxin A are well desired for monitoring and quality assurance of green coffee. The introduction of analytical quality assurance requirements such as ISO 17025 by some coffee importing countries may demand that laboratories demonstrate their capability by using validated analytical methods, having an intra-laboratory measurement control in place, tracing the reference materials and by taking part in proficiency schemes.

Several studies are underway in Brazil aiming at improving the sanitary quality of coffee produced in Brazil, concerning mould formation. This Laboratory has been sponsored by Brazilian Consortium for Research and Development of Coffee (CBP&D/Café) and the National Program for Control of Contaminants and Residues in Products of Vegetal Origin (PNCRV)/MA, to develop a number of studies in the area of method development and

validation, training and qualification, laboratory quality assurance and sampling aiming at assessing OTA contamination.

MATERIAL AND METHODS

Method validation has been carried out according to the IUPAC/AOAC/ISO (1988) and the method characteristics were determined in terms of recovery (R), relative standard deviation (RSD), linearity and limit of detection/quantification (LD/LQ). In the area of method development and validation, for regulatory purposes, preference has been given to methods using immunoaffinity column clean-up with liquid chromatography (LC) and thin layer chromatography (TLC) with densitometric and visual analysis. The application of a clean-up based procedure (Pittet et al., 1996) for one-dimensional TLC was investigated and the method characteristics were determined. Several preliminary tests were carried out to determine the suitable spotting and developing solvents as well as TLC plates (Santos and Vargas, 2001). A collaborative study protocol based on the method published by Pittet et al. (1996) – determining OTA by immunoaffinity clean-up and LC (Vargas et al., 1999) – was submitted to AOAC international. The automation of the immunoaffinity column clean-up and the determination step using a system for sample preparation ASPEC XL coupled with a LC system were carried out for green and roasted coffee and the linear correlation with the normal procedure was determined (Pittet et al., 1996; Vargas et al., 1999). The clean-up step of the LC method was changed – such as sample dilution, elution time to fit ASPEC XL. For screening purposes, commercially available immunoassay test kits were evaluated according to an intra-laboratory protocol and MA ordinance for test kits. The kits are evaluated for matrix effects, applicability, accuracy, precision, linearity, and correlation with an official method, limit of detection and false positive/false negative rate. In case the kit passes the evaluation above it is given a MA licence, which can be renewed every 2 year after further evaluation. The validation and method development were carried out under a quality assurance regime based on ISO 17025.

A sampling plan study is underway. Initially, tests have been carried out to evaluate the use of different commercially types of mills available in Brazil, regarding particle size, sub sampling, time of milling and sample heat during the grinding process. Green coffee samples ($n \geq 20$) were ground using 02 different types (type 2 and 3) of sub sampling mills (Figure 1) and each sub sample was analysed by LC and the results were statistically analysed (Friedman test and Wilcoxon test; $p < 0.05$) (Johnson & Bhattacharyya, 1986; Conover, 1980; SAS Institute Inc, 1985) regarding efficiency in sub sampling. Green coffee samples were ground in three different mills and were passed thoroughly by successive sieves of 14, 16, 20, 28, <28 mesh and the milling performance regarding particle size was determined for each grinder. Each fraction was subsequently analysed for OTA by LC (n=3) and the levels of contamination compared.

RESULTS AND DISCUSSION

An effective and reliable immunoaffinity clean-up based one-dimensional TLC method was established with a limit of detection of 0.5 µg/kg (Table 1). The LD determined are comparable to those published for LC (van der Stegen et al., 1997; Studer-Rohr et al., 1994). The in-house recovery rates and the RSD of the method, for both visual and densitometric analysis, were in good agreement with the performance characteristics considered acceptable for ochratoxin A in the range of 1-10 µg/kg (CEN 1999). The use of toluene-acetic acid (99:1, v/v) as spotting solvent associated with toluene-ethyl acetate-88% formic acid (6:3:1, v/v/v) and acetonitrile-methanol-water-glacial acetic acid (35:35:29:10, v/v/v/v) as developing solvents for both normal and RP TLC plates, respectively, was effective to resolve, separate and increase

the OTA sensitivity, eliminating the use of high toxic solvent such as benzene (AOAC, 1998; Levi, 1975). The linear correlation between LC and TLC was higher than 0.99 for both normal and reversed-phase TLC (Figures 2, 3, 4).

Type 1 mill Type 2 mill Type 3 mill

Figure 1. Type of mills used for evaluation of grinding power (particle size) of green coffee

A collaborative study harmonised protocol (Vargas et al., 1999) of the in-house validated method (Pittet et al., 1996) using immunoaffinity column clean-up with LC for determination of ochratoxin A in green coffee has been approved by AOAC International. The in-house method performance characteristics (Vargas et al., 1999) determined is shown on Table 1.

The automation of the immunoaffinity column clean-up and the determination step has been fully accomplished. The analysis of green and roasted coffee samples by ASPEC gave recoveries ≥ than 70% and RSD ≤20% and the linear correlation (r^2) between the two procedures was higher than 0.9 which are in good agreement with the recoveries published by Pittet et al. (1996) and the characteristics determined by our Laboratory (Vargas et al., 1999).

Table 1. Method Performance Characteristics: TLC - densitometry (normal and reversed phase) and LC

Method	Performance Characteristics				
	Linearity of calibration	Recovery (%)	RSD (%)	RSD TLC detection step	LD (μg/kg)
Normal phase TLC	>0.999 (0.04 to 84 ng)	83.7 to 104 [c] 98.4 (mean)	1.1 - 8.2 [c] 11.1-18.1 [d]	0.1 to 15[c] 2.6 to 17.1[d]	0.5
Reversed phase TLC [e]	>0.99 (0.42 to 84 ng	80-110 [c]	9.5 (mean spiked) 12.1 (mean naturally)	0.4 to 18.8 [d]	-
LC	>0.999 0.2-60 μg/kg	80 to 108% [a]	11 to 21[a,c] 10.5[b]	-	0.12

[a]determined by using spiked samples, in the range of 0.2 to 109 μg/kg, n=5; [b]determined by using a naturally contaminated green coffee test sample (5.23 μg/kg ± 0.55, n=42); [c]spiked sample; [d]naturally contaminated test sample; [e] C_{18} silica gel plate

Different percentages of particle size were obtained with the three type of mills (Figure 1). The highest percentage of larger particles (14 mesh) and smaller (<28 mesh) was obtained when type 1 and type 2 mill were used, respectively. Different levels of OTA contamination were found in the analysis of the green coffee fractions (14-28 mesh) obtained with the type 1 mill (Figure 5). The highest contamination was found in the fraction with the finest sample particle size (smaller than 28 mesh) indicating the influence of particle size in the contamination determined. The significant differences ($p<0.05$) for the levels of OTA contamination in green coffee sub samples were observed irrespective of type 2 and 3 sub

sampler mills (Table 2). The levels of contamination for the sub samples obtained from sub sampler A (Type 2 mill) were statically lower than those determined from sub sampler B; and the levels of contamination for the sub samples obtained from sub sampler A (type 3 mill) were statically lower than those determined from sub sampler B and C, being the results obtained for the sub samples from sub sampler B = sub sampler C (Table 2). The difference determined between the sub samplers may be due to a lack of homogeneous distribution of particle size inside the mill and, consequently, causing a non-homogeneous distribution of OTA contamination in the sub samples. Many difficulties have been faced during the grinding process such as the heat of the samples (40-60°C) and milling consuming time (30 min/kg) when types 2 and 3 were used.

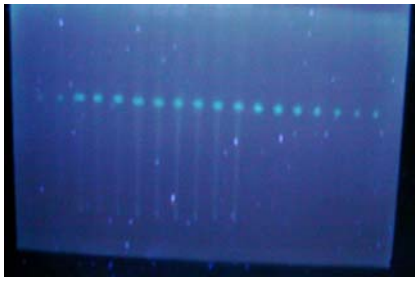

Figure 2. OTA standard and sample extracts spotted on normal TLC glass plates, 10 x 20, 0.25 mm thickness. Spotting solvent: toluene-acetic acid (99:1, v/v); Elution solvent: toluene-ethyl acetate-88% formic acid (6:3:1, v/v/v)

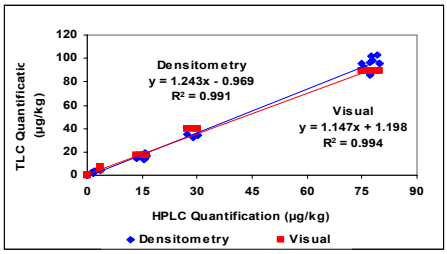

Figure 3. Coefficient of correlation (R^2) between LC and normal-phase TLC - visual and densitometric quantification for spiked green coffee samples

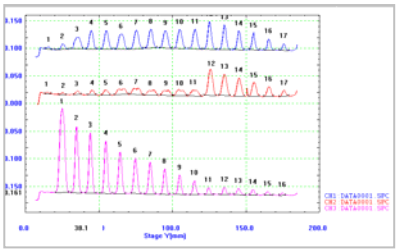

Figure 4. Densitograms of OTA standard and sample extracts spotted on normal TLC glass plates

None of commercially available immunoassays kits evaluated so far (RIDASCREEN®FAST; VERATOX®; VICAM, 1999) was efficient to evaluate OTA contamination in green coffee concerning accuracy, precision and false positive/false negative rate. Inconsistent results have been found even for non-contaminated samples (blank samples) making the usual bench control laboratory procedures worthless, indicating that matrix effects have been the a restraint for the use of the immunoassays for screening of coffee (example of matrix effect in Figure 6) especially when the proposed regulatory limits are very low and close to the

detection limits of the kits. In additional to the analytical difficulties, the high cost of these kits turns their use not attractive for developing countries.

Table 2. Descriptive and comparative analysis of OTA contamination levels in sub samples obtained from type 2 and type 3 sub sampler mills

Mill	Sub sampler	n	Descriptive measurements			
			Median	Mean	Sd	p
Type 2	*A*	30	0,24	0,44	0,74	**0,009**
	B	30	0,43	0,60	0,74	A < B
Type 3	*A*	21	2,44	3,78	4,99	**0,001**
	B	21	3,06	4,63	5,99	
	C	21	2,42	4,16	5,31	A < B = C

Note: *p for type 1 mill, test of Wilcoxon; p for type 2 mill, test of Friedman; p < 0.05; Sd: standard deviation*

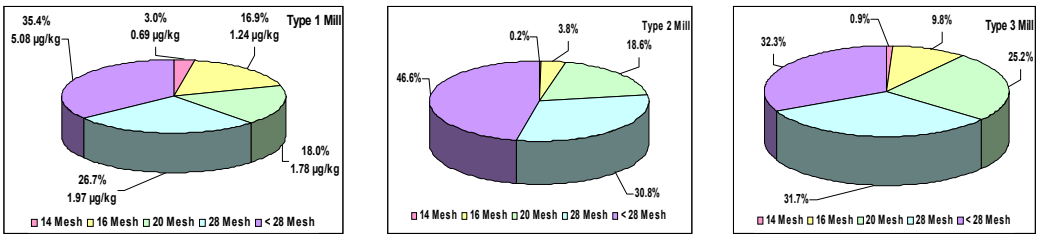

Figure 5. Percentage of particle size obtained for the 3 types of mill. OTA contamination level determined for the fractions of type 1 mill

CONCLUSION

The studies on method development and validation have contributed for the establishment of an official method for green coffee by LC. The performance characteristics of the TLC method, in terms of recovery, RSD and LD achieved in the present study make the method applicable in monitoring and survey programs especially in the coffee producing countries, and can comply with future regulation for OTA in coffee. The TLC method can be immediately available to all regular laboratories, without necessity of acquiring expensive equipment. The automation of the LC method allows a great number of samples to be analysed at once. There is a strong need for an inter-laboratory collaborative study of the TLC procedure to make it more widely available. Further studies should be carried out aiming at defining the most suitable mills for grinding coffee and defining a sampling plan to assess OTA contamination.

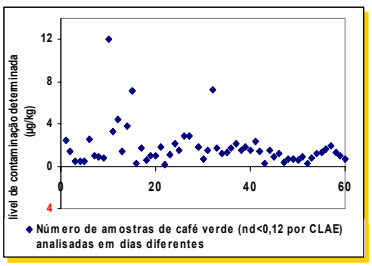

Figure 6. Levels of OTA contamination (false positives) determined for blank green coffee samples (nd < 0.12 µg/kg) by fluorometry (17)

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Preliminary Studies on the Destruction of Ochratoxin A in Coffee During Roasting

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SUMMARY

The presence of ochratoxin A (OTA) in coffee has been reported. The effect of roasting to reduce OTA levels in coffee was studied at different temperatures and periods of time. Around 7.5 kg of green coffee (*Coffee arabica*) was inoculated with 10^8 spores/ml of toxigenic *Aspergillus ochraceus* and incubated at 25ºC for 50 days. After this period the OTA content in coffee was determined according to the methodology of Pittet et al. (1996), and the concetration was 160.46 μg/kg. The coffee was submitted to irradiation with 10 kGy (Cobalt 60) to destroy the fungi. Three different treatments were used to obtain the following roasting point: middle, middle dark and dark, at temperatures around 200, 210 and 220ºC, respectively after 10 and 15 minutes. The average reduction was 22 and 48% of OTA at medium roasting point; 39 and 65% at dark medium roasting point and an average of 88 and 94% at dark roasting point after 10 and 15 minutes, respectively. The moisture values were 0.44 (200ºC), 0.30 (210ºC) and 0.30 (220ºC). These results showed that roasting can be effective to reduce OTA levels in coffee, depending on the temperature, time and equipment. However, to keep good quality coffee, it would be advisable to avoid that with a high OTA content; even roasting to reduce this toxin, the quality can be affected. Further studies are being carried out in order to evaluate the effect of filtering in these samples and the residual content of OTA in the beverage.

INTRODUCTION

Coffee is one of the most important agricultural product in Brazil, representing one of the greatest exchange values of the country. The harvest for the biennium 99/2000 was of 27.17 million sacks and the estimate for the 2000/2001 biennium is 28.9 million sacks, about 60% of which is destined for exportation.

Research is being carried out to evaluate the contamination of coffee by xerophyllic mycotoxin producing fungi, especially those producing ochratoxin A (OTA). This mycotoxin is a metabolite of fungi of the genus *Aspergillus*, mostly produced by *Aspergillus ochraceus* in Brazil. It possesses both carcinogenic and nephrotoxic potential.The maximum allowable limits for OTA vary from 1 to 5 μg/kg for baby foods, 2 to 50 μg/kg for cereals and from 50 to 300 μg/kg for animal feeds (Van Egmond, 1996). No limits for OTA have yet been stipulated in Brazil, but the European community has suggested limits of 4 ppb for roasted coffee and 5 ppb for raw coffee (Frank, 1999).

OBJECTIVE

Verify the effect of roasting on the reduction of the levels of ochratoxin A (OTA) using different time/temperature combinations.

MATERIAL AND METHODS

Coffee samples

A total of 7.5 kg of husked, unsorted Arabica coffee, screen 14, from the 1998 harvest, obtained from the Santa Elisa Experimental Farm of the Campinas Agronomic Institute, S.P., Brazil, was used in this trial.

Inoculation of the coffee with OTA producing fungi

Five strains of OTA producing *Aspergillus ochraceus* were chosen and reactivated in CYA medium at 25°C for 7 days for use in the artificial contamination of the coffee. After an intense growing phase, the spores were transferred to a test tube containing 40 ml phosphate buffer and count was carried out in a Neubauer chamber with the aid of a Zeiss microscope, obtaining an inoculum of 10^8 spores/ml.

Eleven glass jars, each containing from 600-700 g raw coffee were contaminated with the spore suspension, and a further 100 ml sterile distilled water added to increase the moisture content and promote fungal growth.

The coffee samples were incubated for 50 days at 25°C and they were irradiated with a dose of 10 kGy using a cobalt 60 source. The objective of the irradiation was to destroy the fungus before roasting the coffee.

Determination of Ochratoxin A

Ochratoxin was determined in the samples according to the methodology of Pittet et al. (1996), based on the use of immunoaffinity columns as described by Nakajima et al. (1990).

Determination of the moisture content and water activity (Aw) of the coffee

The Aw of the non-contaminated raw coffee (control) and of the contaminated and subsequently irradiated samples, were determined using the Aqua Lab apparatus (model 3TE). The moisture content was determined in a vacuum oven (National Appliance Company), using the methodology of the Instituto Adolfo Lutz, São Paulo, Brazil (1985).

Coffee Roasting

Trials were carried out at three levels of roasting, and at each level a nominal temperature was used with various times, as shown in Table 1.

The temperature of 200°C was considered as medium roast, 210°C as medium dark and 220°C as dark. A Probat pilot roaster (WERK, type RE1) with a capacity for 200 g per test was used. When the nominal temperature on the "display" of the roaster reached the desired value (220, 210 and 200°C), 200 g of coffee were introduced into the chamber and the time count started, with the temperature being registered every minute. The internal temperature of the roaster chamber was monitored with a thermocouple (Delta OHM, HD9214), in order to

construct heating curves of the process. The experiments were carried out in triplicate, resulting in 18 trials.

Table 1. Levels of roasting, temperature/time combinations

Degree of roast	Temperature(°C)	Time (min)
Medium	200	10 & 15
Medium/dark	210	10 & 15
Dark	220	10 & 15

RESULTS

Results of the % moisture and Aw determinations

The control coffee, which was not inoculated, presented a moisture content of 9.37% (mean of triplicates) and A_w of 0.64 (mean of duplicates). After fungal growth and sterilisation, the moisture content increased to 14.61 (mean of triplicates) and the A_w to 0.79 (mean of duplicates.

Table 3 shows the effect of roasting on the reduction of the moisture content, observing a gradual reduction as a function of the increase in temperature.

Table 3. Moisture content (%) of roasted coffee

Degree of Roast	Time (min)	Moisture (%)	Mean (%)
Medium	10	0.47	0.44
	15	0.40	
Medium dark	10	0.31	0.30
	15	0.29	
Dark	10	0.24	0.30
	15	0.35	

The analyses were carried out in triplicate.

Heating curves in the Roaster

The heating curves show the sequence of temperatures registered by the thermocouple every minute (triplicate measurements).

Figure 1 shows that in the trial in which the nominal temperature on the display was 200°C, the real temperature of the coffee during roasting, registered with the help of the thermocouple inserted into the roaster chamber, did not reach 200°C (graph c), the maximum value attained after 10 minutes processing being 183.9°C. The same occurred with a time of 15 minutes, reaching a maximum temperature of 194.9°C. In the trials at 210°C (graph b), the variation was smaller, reaching 210°C in 10 minutes roasting and 218°C in 15 minutes. At 220°C, (graph a), the temperature showed an increase of 4°C after 10 minutes (224°C) and of 16°C after 15 minutes, reaching 236°C.

In all the roasting trials registered by the heating curves (Figure 1), the objective was to reach a determined degree of roast, according to the desired characteristics, and in this respect, the pilot roaster was shown to be most efficient. As can be seen in Figure 1, at the medium roast

point (graph a), an average of 22.5% destruction of the toxin was observed after 10 minutes, and 48.14% after 15 minutes. At the medium dark roast point (graph b) at about 210°C, average reductions of 39.25% and 65.65% were obtained after 10 and 15 minutes roasting respectively. At the dark roast point (graph c) even greater levels of OTA destruction were obtained, 88.38% and 93.89% after 10 and 15 minutes respectively. It is important to mention that during the roasting process, the temperatures showed variations inherent to the actual process and to the pilot roaster.

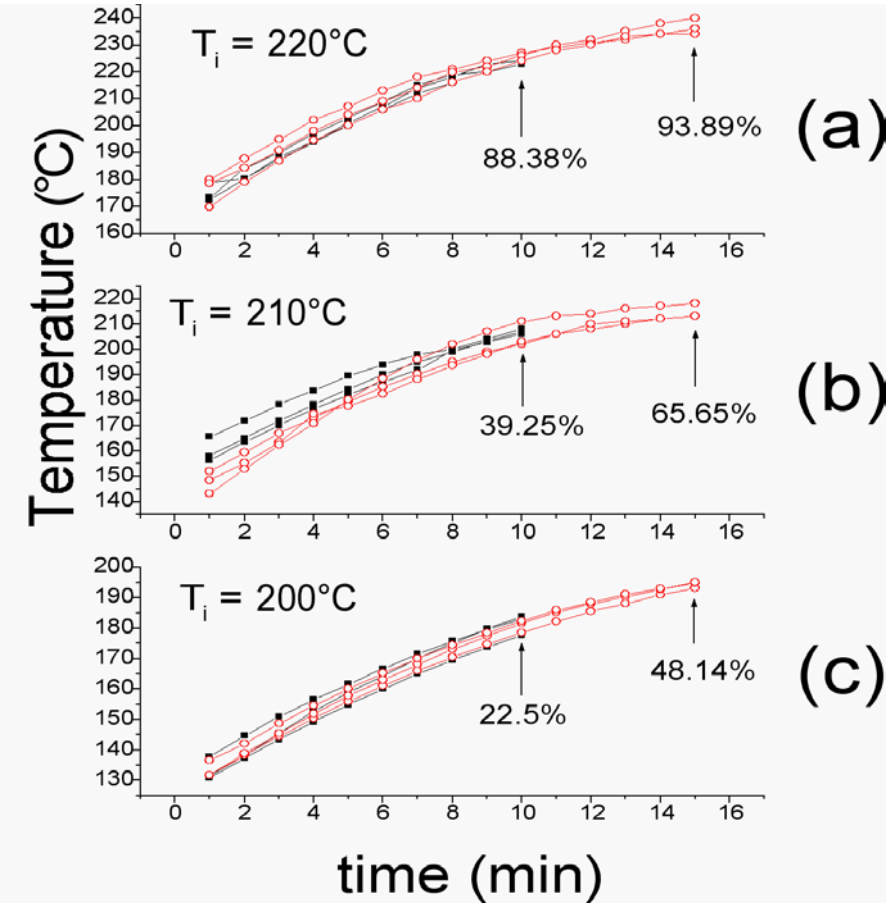

Figure 1. Heating curves: (T_i) = nominal roaster temperature (T_i) and % OTA destruction

OTA levels in roasted coffee

In the control coffee, the average level of OTA was 0.49 µg/kg and in the inoculated coffee, the average value was 160.5 µg/kg, this value being taken as the initial value in the roasting trials. As can be seen in Table 2, the levels of destruction varied, on average, from 38 to 48% at the nominal temperature of 200°C, from 39 to 65% at 210°C and from 89 to 94% at 220°C.

CONCLUSIONS

The results of this research show that roasting is an efficient procedure for the reduction of OTA levels in coffee, depending on the time/temperature combination. However it is quite difficult to estimate the degree of destruction of the mycotoxin as a function of the processing conditions, since there is no direct correlation between the nominal temperature registered on the equipment and the true temperature in the roasting chamber.

Factors such as type of equipment, thermostat sensitivity, dimensions of the roasting chamber, quantity and granulometry of the coffee and other factors, can affect the destruction of the mycotoxin with variable intensity, such that the results presented here are valid only under the specific conditions of these trials.

Further studies will be carried out to determine the effect of grinding of the roasted coffee on the OTA levels, as well as an evaluation of the effect of infusion on the toxin levels during beverage preparation.

Table 4. Levels of OTA(μg/kg) in the inoculated coffee before and after roasting and % OTA destruction

Coffee used	Level of OTA (μg/kg) (Triplicates)			Mean (μg/kg)	% OTA destruction
Control coffee	0.55	0.43	0.44	0.47	-
Inoculated coffee	151.6	182.5	147.3	160.46	-
Roasted coffee					
±200°C/10min	97.52	100.56	175.04	124.37	22.5
±200°C/15min	92.55	75.93	81.22	83.23	48.14
±210°C/10min	97.5	89.31	105.65	97.48	39.25
±210°C/15min	50.80	47.52	67.09	55.13	65.65
±220°C/10min	20.21	19.33	16.43	18.65	88.38
±220°C/15min	8.88	10.67	9.91	9.82	93.89

(-): Level of destruction not determined.

ACKNOWLEDGMENT

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A Process to Remove Mycotoxins from Green Coffee

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SUMMARY

Mycotoxin contamination in a lot of green coffee, especially by ochratoxin A (OTA), over the limit allowed by Italian law (Circ. Italian Ministry of Health, n.18 of 16/11/2000, which fixes the limit at 8 ppb for green coffee), prevents its introduction into the home market, resulting in a possible relevant economic loss. The process here described allows OTA removal from the green coffee bean using a physical method as the extraction with a suited solvent, for instance dichloromethane. With this system OTA concentration lowers well below the maximum limit of 8 ppb, then recovering the toxin immodified in the extraction solvent and in by-products, without forming eventual degradation products.

OTA contamination is eliminated by the solvent contact with the green coffee bean, and at the end of the process all the solvent is turned away, obtaining a product with good organoleptic properties. If the process is realised in an acid environment, OTA removal is enhanced and caffeine extraction lowered, optimising the process.

RÉSUMÉ

La contamination d'un lot de café vert par des mycotoxines, en particulier l'ochratoxine A (OTA) au-dessus de la limite établie par la législation italienne (Circ. du Ministère de la Santé, n.8 du 16/11/2000 qui prévoit la limite maximale de 8 ppb pour le café vert), en interdit la circulation sur le marché national, ce qui risque d'entraîner da graves conséquences économiques. Le procédé décrit ici permet d'enlever l'OTA des graines de café vert en utilisant une méthode physique: l'extraction par un solvant approprié, dans ce cas spécifique le chlorure de méthylène. Grâce à cette méthode, on réduit la concentration de la toxine largement au-dessous de la limite maximale admissible, et en même temps on la retrouve inaltérée dans le solvant d'extraction et dans les sous-produits dérivés, écartant ainsi tout risque de formation d'éventuels produits de dégradation.

La contamination par OTA est éliminée par le contact avec le solvant et au terme du procédé on ne trouve pas de résidus de solvant, et ainsi on obtient un produit fini qui garde de bonnes caractéristiques organoleptiques. Le milieu acide favorise l'élimination des OTA alors qu'il ralentit l'extraction de caféine, en optimisant le procédé en question.

INTRODUCTION

Mycotoxin contamination in a lot of green coffee, especially by ochratoxin A (OTA), is an important problem because of the possible adverse effect on human health (Petracco, 1998).

Therefore several countries are imposing strict rules against the importation of contaminated lots into their home market.

THE PROBLEM

- Some countries have already set a limit. Among these is Italy, where the limit is of 8 ppb (ng/g) for green coffee and 4 ppb for roasted coffee (Italian Ministry of Health, 2000).
- Lots presenting a contamination above this level must be rejected at the border, resulting in a relevant economic loss.
- European Commission has so far considered limits for the importation of contaminated coffee lots as unnecessary, nevertheless a final decision will be taken by December 2002 (Document SANCO/0453/00).
- The efforts carried out for prevention, both on cultivation of coffee plant and on crop processing, should produce a reduction of the risk caused by OTA; nevertheless, the existence of contaminated lots inside the coffee market is still a serious problem.

THE SOLUTION

- Highly desirable appears therefore a process apt to reduce any level of OTA contamination below detectability, or to extremely low concentration: this will allow suitable for safe human consumption lots otherwise rejectable.
- The use of chemicals, as for instance hydrogen peroxide and ammonium hydroxide, is not ideal for detoxification because they react with OTA to decompose its molecules, so leading to degradation products of unknown toxicity.
- In our process, a solvent extracts OTA without changing its chemical structure. Pilot plant tests showed OTA unmodified in process by-products. Laboratory tests showed OTA removal up to 100%.

DESCRIPTION OF THE PROCESS

The process is based on the physical extraction of OTA, as such, from the green coffee bean using a suited solvent, chosen by its particular affinity for OTA. This method acts not only on the outer layer, but also inside the green coffee bean.

The process, as developed and patented (Fabian, 1997), comprises the following stages:

- Introducing a load of OTA contaminated green coffee into a container in which the coffee is subjected to continuous mixing at convenient temperature conditions.
- Introducing wet steam into the container and upkeeping it for the time required to make the green coffee beans porous and permeable.
- Introducing the solvent suited to remove OTA, and drive it away immediately after it has removed the entire amount of OTA.
- Eliminating all traces of the solvent from green coffee and recovering it by distillation. After separating from the solvent all OTA content, which concentrates in the residues, return the clean solvent into the process.

EXPERIMENTAL

We analysed 127 samples of green coffee, most belonging to Robusta species. Each sample, taken according to the ISO sampling method (ISO 4072-1982 (E), 1982), was completely ground, homogenised and submitted to extraction, immunoaffinity clean-up and HPLC-spectrofluorimetry analysis as described by Studer-Rohr et al. (1995).

We found 85 samples positive, above detection limit of 0.2 ppb. Most of them presented a concentration below the limit of 8 ppb, but we evidenced several samples with a higher contamination, with two outliers over 30 ppb as can be seen in Figure 1.

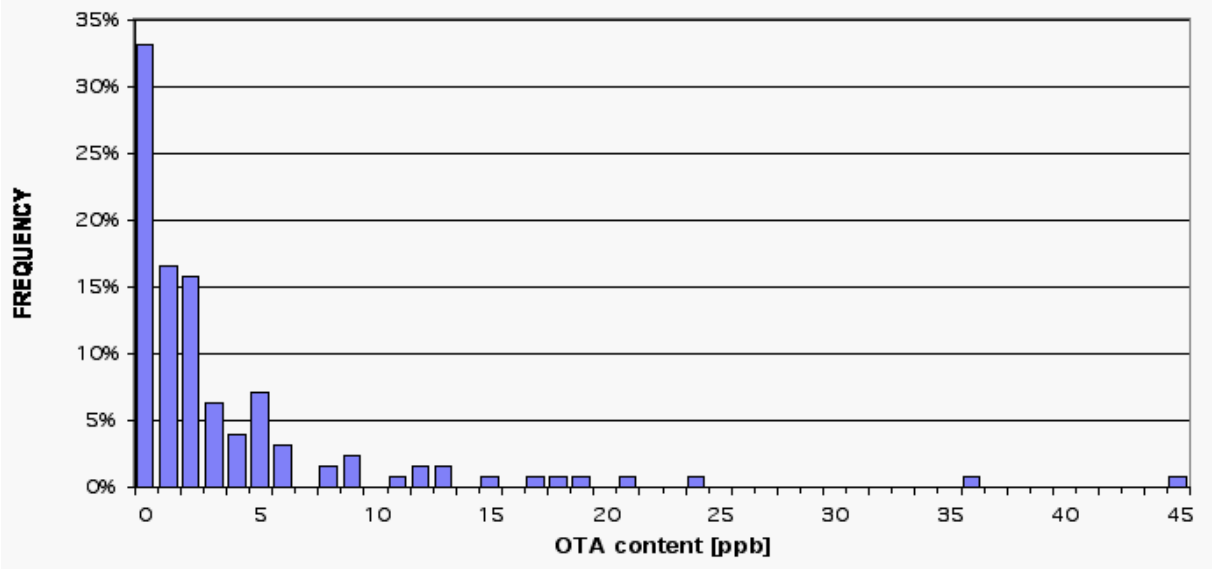

Figure 1. Contamination distribution of 127 analysed samples (85 positives)

Figure 2 shows OTA contamination of the 85 samples positive before our process, together with the OTA reduction percentage after the treatment.

For high OTA contamination the reduction has always been over 80%. The few cases of low reduction regard only samples with low OTA concentration, where sampling flaws may have affected the analysis

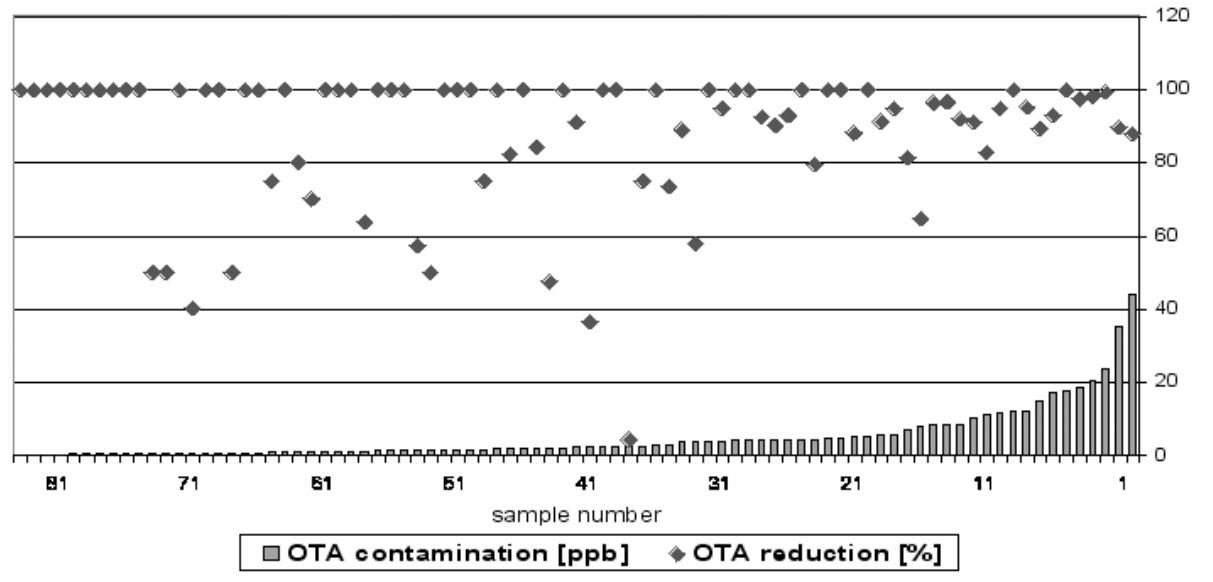

Figure 2. Contamination reduction in 85 positive raw coffee samples

IMPROVING THE PROCESS

At a laboratory scale, we made some tests to improve our process, with the purpose of minimising caffeine loss during the treatment while still extracting the whole OTA

amount.Best results were obtained using a solvent saturated with caffeine, or creating an acid environment.

Table 1 shows the results obtained treating a green Robusta coffee sample in a detoxification reactor. The extraction solvent utilised is a solution of formic acid in methylene chloride. For every test we repeated the process three times, reporting the average results.

Caffeine removal resulted modest, average of 27%. OTA reduction presented always high values, average of 80%. Further improvements are under study.

Table 1. Laboratory tests on 3 differently contaminated coffees, three-steps extractions with methylene chloride-formic acid 2%

		Caffeine				Ochratoxine A			
		in	out	removal	St.Dev.	in	out	removal	St.Dev.
Test	Iteration	[g/100g]	[g/100g]	[%]		[ppb]	[ppb]	[%]	
A	1	2,0	1,1	44		21,3	3,1	85	
	2	2,0	1,9	7		23,2	2,1	91	
	3	2,0	1,9	8		18,2	1,2	93	
	mean	**2,0**	**1,6**	**20**	21	**20,9**	**2,1**	**90**	4
B	1	2,9	1,9	34		15,3	1,7	89	
	2	2,3	1,8	19		13,2	1,4	89	
	3	2,3	2,0	12		11,9	0,9	92	
	mean	**2,5**	**1,9**	**22**	11	**13,5**	**1,3**	**90**	2
C	1	2,3	1,5	35		2,7	0,9	67	
	2	2,5	1,4	44		3,2	1,3	59	
	3	2,4	1,4	40		3,0	1,5	50	
	mean	**2,4**	**1,4**	**40**	4	**3,0**	**1,2**	**59**	8
3 LAB TESTS' AVERAGE:		**2,3**	**1,7**	**27**	16	**12,4**	**1,6**	**80**	16

ADVANTAGES OF THE TREATMENT

Utilising a proper solvent, for instance methylene chloride, shows the following advantages:

- It removes OTA selectively from the green coffee bean without interacting with aroma precursors of the product.
- No chemical reactions with the toxin occur, thus hindering the formation of potentially more toxic metabolites.
- Easy to drive away from green coffee after process, thanks to its low boiling point (39°C).

CONCLUSIONS

- With our process we can remove OTA from highly contaminated lots of green coffee, with a reduction above 80%.
- The coffee processed this way maintains its organoleptic properties without modifying cup quality.
- Caffeine content can be maintained at levels near to an unprocessed coffee.
- The bulk of OTA is recovered as such in the waxy by-products, to be disposed for non food/non feed utilisation.

- OTA-free processed coffee could be a way to eliminate the worry of up to 7% of global coffee production being prone to rejection, and eventually destruction, with great benefit for the least developed coffee producing countries' economy.

ACNOWLEDGEMENTS

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Ochratoxin A Production by *Aspergillus ochraceus* in Raw Coffee as Affected by Alternating Temperatures and Different Water Activity Values

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SUMMARY

The effect of changes in temperatures on ochratoxin A (OTA) production by *Aspergillus ochraceus* has not been studied extensively. These changes occur, mainly, during storage in farm barns and transport. The present work was carried out in order to evaluate the changes during day and night in storage of coffee in farm barns in São Paulo State. Two temperatures (14º and 25ºC) were chosen as the mean of day and night in different equilibrium relative humidity (ERH) conditions: 80, 87 and 95% for the production of OTA by *Aspergillus ochraceus* in raw coffee. Temperatures were cycled at 12 hour intervals. In similar conditions but without temperature cycling the coffee was maintained at 25ºC at these three relative humidities. Ochratoxin production was analysed at periods of 7, 14 and 21 days after the coffee had reached the equilibrium relative humidity. The water activity of coffee was checked with an Aqualab 3TE (Decagon) and the content of OTA in HPLC was quantified according to Pittet et al., (1996). At cycling temperatures, OTA production was 57.1%, 34.4% and 13.9% higher than the 25ºC constant temperature at water activity of 0.80, 0.87 and 0.95, respectively. Differences between cycling temperatures and constant temperatures were more critical in lower water activity values.

Further studies on the influence of inoculum vehicle on the production of OTA in coffee are being conducted. As well studies on OTA production by *A. carbonarius* in raw coffee with different water activity.

INTRODUCTION

The objective of this study was to determine the influence of alternating temperatures (14 and 25°C) and different values for equilibrium relative humidity (ERH) (80, 87 and 95%), on the growth of the mould *Aspergillus ochraceus* and its production of ochratoxin A (OTA). The temperature range was chosen based on annual data provided by agricultural technicians from farms in the State of São Paulo, Brazil. The study is justified by the fact that with some micro-organisms, rapid temperature changes can cause metabolic stress, resulting in greater or lesser toxin production. Similar studies with fumosins have already been carried out. The study also aims at predicting the behaviour of beans inside a container, contaminated with spores of *Aspergillus ochraceus*, on being submitted to the drastic temperature changes which normally occur during marine transport.

MATERIAL AND METHODS

Coffee

Arabica coffee beans, donated by the Agronomic Institute in Campinas, Brazil, were used for this study. The beans were sterilised by irradiation with a dose of 10 KGY at the Agricultural Centre for Nuclear Energy (CENA)/USP in Piracicaba, Brazil.

Strain selection and preparation of the Aspergillus ochraceus spore suspensions

Three strains were selected from previous experiments, isolated from coffee grown in the State of Paraná, Brazil. The strains were inoculated into malt extract agar (MEA) and incubated at 25°C for 5 days. The growing culture was transferred into a test tube containing 40 ml phosphate buffer pH 7,2 + 0.1% tween 80 and glass beads, and the suspension agitated in a vortex for 1 minute. A spore concentration of 10^7 CFU/mL was obtained.

Inoculation of *Aspergillus ochraceus* spores into the coffee

A 1 mL aliquot of spore suspension (10^7 CFU/mL) was inoculated into 10 g sterilised soil with a particle size of 300 μm. Subsequently 4 g of this mixture were added to 100 g of coffee. The coffee + inoculum was blender in a Wagner blender for 15 minutes. This procedure was repeated to a total weight of contaminated coffee of 1.5 kg.

Determination of moisture content and water activity in the polished raw coffee

The moisture content and water activity of the beans were determined initially and after reaching the equilibrium relative humidity by drying at 70°C (at vacuum) and by using the equipment Aqualab Cx2 (Decagon-USA), respectively.

Preparation of the saturated salt solutions for use in the dessicators, obtaining 3 different Equilibrium Relative Humidity (ERH) values

Saturated solutions of ammonium sulphate $(NH_4)_2SO_4$, sodium potassium tartrate $NaKC_4H_4O_6$ and lead nitrate $Pb(NO_3)_2$ were prepared, obtaining solutions of ERH 80, 87 and 95% respectively. The solutions were left in the dessicators for 7 days to attain equilibrium.

Preparation of the isotherm trials at alternating temperatures of 25 and 14°C and a constant temperature of 25°C, at 3 different equilibrium relative humidity values (80, 87 and 95%)

Plastic pots containing 25 g raw polished coffee were inoculated with a spore suspension (105 CFU/g) carried by soil. Five pots were placed in each dessicator (4 contaminated samples and one non-inoculated control). The dessicators were alternately placed in heating chambers at 25 and 14°C (12 hours at each temperature). The ERH values tested were 80, 87 and 95%, obtained using the saturated salt solutions. The non-inoculated pot was weighed at weekly intervals to determine the increase in moisture and the time needed for the coffee beans to reach equilibrium with the salt solution, based on the change in weight of the beans. Zero time was considered to be that when the beans reached the ERH of the salt solutions. Ochratoxin production was quantified after 0, 7, 14 and 21 days. All trials were carried out in triplicate.

With the aim of obtaining results showing the effect of temperature alternations on ochratoxin production, another trial was carried out in parallel under the same conditions but at a

constant temperature of 25°C. In this trial, the amount of ochratoxin was only quantified after 0 and 21 days.

Extraction and quantification of ochratoxin A in raw coffee beans

Ochratoxin was extracted by the method of Pittet et al. (1996). The residue was first dried in a current of nitrogen and then resuspended in 1 mL of 42% acetonitrile: 58% 4mM sodium acetate/acetic acid (19:1), which was the same solution as that used as the mobile phase in the HPLC determination. Quantification of the toxin was by high performance liquid chromatography (HPLC) (Shimadzu LC-10VP) using a fluorescence detector (excitation at 330 nm; emission at 470 nm).

PRODUCTION OF OCHRATOXIN A BY *ASPERGILLUS OCHRACEUS* AT ALTERNATING TEMPERATURES AND DIFFERENT VALUES OF EQUILIBRIUM RELATIVE HUMIDITY AND INCUBATION TIMES AFTER EQUILIBRIUM

The production of ochratoxin in the beans after reaching ERH values of 80, 87 and 95% respectively, at alternating temperatures (12 in 12 hours) of 14-25°C, is presented in Table 1

Table 1. The production of Ochratoxin A (ppb) by *A. ochraceus* in raw coffee at alternating temperatures (12 in 12 hours) of 14 and 25°C at different A_w values

	Ochratoxin (ng/g)				
	0 days	7 days	14 days	21 days	Control
Aw 0.80	ND*	ND	1.70	0.61	ND
	ND	ND	3.49	0.45	
	ND	ND	0.52	ND	
Mean	ND	ND	1.90	0.35	
Aw 0.87	955	1854	1907	4260	ND
	796	1347	1763	2999	
	631	1440	1778	4347	
Mean	794	1547	1816	3869	
Aw 0.95	2250	5483	7239	9253	6.71
	2273	7554	8917	9723	
	2399	7343	8443	6037	
Mean	2307	6793	8200	8338	

** Not detected (limit of detection 0.2ng/g).*

According to the results, it is evident that A_w is a limiting factor for the production of ochratoxin A, since with an A_w of 0.80, the production of ochratoxin was much lower than at 0.87 and at 0.95, principally the latter. It is important to note that when equilibrium was reached, large concentrations of ochratoxin had already been produced at A_w values of 0.87 and 0.95. The amount of ochratoxin found in the control at 0.95 (6.71 ppb) must be discounted from the values found in the samples, but does not significantly alter the result.

Table 2 shows the data for ochratoxin A production by *Aspergillus ochraceus* in coffee maintained at a constant temperature under the same conditions of ERH (80, 87, 95%) as in

the previous trial. The objective of this trial was to observe the effect of alternating temperatures on toxin production.

Table 2. Production of Ochratoxin A by *A. ochraceus* in raw coffee at a constant temperature of 25°C and different values for A_w, 0 and 21 days after equilibrium

	Ochratoxin A (ng/g)		
	0 days	**21 days**	**Control**
Aw 0.80	ND*	0.25	ND
	ND	<0.2	
	ND	ND	
Mean	ND	0.15	
Aw 0.87	154.78	3197	ND
	89.37	2056	
	252.63	2244	
Mean	165.6	2499	
Aw 0.95	2059	7544	6.58
	1749	6896	
	2239	7095	
Mean	2016	7178	

** Not detected (limit of detection 0.2ng/g)*

An analysis of Tables 1 and 2 shows variations in the differences in the production of Ochratoxin A due to the effect of alternating temperature, it being apparent that the effect of alternating temperature becomes more important at A_w values below 0.95. For example, at an A_w value of 0.87, the amount of ochratoxin at zero time of equilibrium was approximately 80% greater with alternating temperatures than at constant temperature, which is definitely significant. At an A_w value of 0.80 the differences were minimal, whereas at 0.95, there was a somewhat greater production of ochratoxin with alternating temperatures. This trial shows a tendency which could be interesting, since during marine transport of coffee, the temperature changes are much more drastic and occur at ERH values below 95%, usually between 70 and 85%.

CONCLUSIONS

Independent of the temperature studied, the concentrations of ochratoxin A encountered in the trials at ERH values of 87 and 95%, are very high, those obtained under alternating conditions of temperature being higher than those at constant temperature. With respect to toxin production, the trial comparing alternating temperatures with constant temperature was important under ERH conditions of 87%.

The production of ochratoxin A by *Aspergillus ochraceus* at ERH values of 80%, was not significant in any of the trials.

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Optimisation of the Inoculation of *Aspergillus ochraceus* in Coffee for Isothermal Studies Simulating Storage and Marine Transport of Raw Coffee

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SUMMARY

Isothermal study has been a useful tool to understand the behavior of grains in their environment such as relative humidity and temperature conditions as well as fungal growth. At initial stage of storage and maritime transport, coffee does not have the condition for most species of fungi to develop, except for xerophilic fungi. However, there may be situations in which the humidity inside the barns or inside the coffee containers is higher than the moisture of coffee, and for diffusion the moisture of coffee tends to equilibrate at a higher humiditiy. In case of transport in containers, humidity may also increase because of condensation caused by constant changes in temperature during the voyage. Nevertheless, the moisture gain is gradual and requires time to get to the equilibrium point necessary to reach optimal conditions for fungal growth and toxin production. The present study objective was to compare the traditional fungal inoculation technique, which consists of the direct application of water spore suspension (direct technique) of *Aspergillus ochraceus* in raw coffee versus techniques using solid vehicles (soil, ground coffee and starch) to carry the spores to coffee. Soil and ground coffee were tested at two sieve granulometries, 300 and 850 μm. Three parameters were evaluated: (i) changes in water activity of coffee beans after inoculation, (ii) homogeneity of spores in the vehicle (soil, ground coffee and starch) and (iii) homogeneity in adherence of vehicle to spores in coffee beans.

Soil showed itself to be the best vehicle for spores in relation to water activity changes, as compared to ground coffee and direct suspension. The water activity of coffee which was initially 0.654, increased to 0,669, 0.682 and 0.766 using soil, ground coffee and water as vehicles for spores, respectively. These higher values represented an increase in water activity of 2.4%, 4.3% and 17.1%, respectively. Starch was discharged from the beginning because it formed “grumos” after the inoculum had been added.

Soil and ground coffee at 300 μm sieve had a slight advantage in relation to homogeneity and uniformity of spores/vehicle over 800 μm sieve. Soil was chosen as the vehicle to inoculate spores of *A. ochraceus* due to its low changes in coffee water activity. Electronic microscopic observations showed the distribution and development of *A. ochraceus* inoculated in coffee beans by soil.

INTRODUCTION

In laboratory studies, the inoculation of spores is traditionally carried out in a direct manner, adding a certain volume of a concentrated spore suspension directly to the substrate or grain.

The objection to this method is the significant variation in water activity of the substrate or grain after inoculation, which can alter the growth of the organism and any possible toxin production. Thus in this study with coffee beans, the objective was to test various particulate carriers (soil, ground coffee and starch) to which the suspension was added before inoculation into the beans. The overall objective of the study was to simulate, as closely as possible, any increase in moisture content of the beans during storage in the silos and during marine transport in containers, since in these two situations, the beans are usually almost dry, with moisture contents of about 10- 12% and water activity values of about 0,64-0.68.

MATERIAL AND METHODS

Coffee

Arabica coffee was used for this study, donated by the Campinas Agronomic Institute. The beans were sterilised by irradiation using a dose of 10 KGY from a cobalt 60 source at the Agricultural Centre for Nuclear Energy (CENA/USP in Piracicaba, Brazil).

Carriers for the inoculation of Aspergillus ochraceus

- Laboratory grade starch powder (<100μm).
- Ground coffee (850 and 300 μm) – the coffee was ground and then sieved through the respective mesh.
- Red soil (850 and 300 μm) – collected on the farm of the Campinas Agronomic Institute and then sieved.

The carriers were sterilised at 121°C for 30 minutes, followed by dilution tests in culture medium to determine their sterility.

Spore suspension preparation

Producer strains isolated from coffee beans from the State of Paraná, were inoculated into malt extract agar plates (MEA) and incubated at 25°C for 5 days. Using a loop, colonies were placed in a test tube with 40 mL phosphate buffer pH 7,2 + 0.1% tween 80 and glass beads. A dilution containing 10^7 UFC/mL spores was prepared.

Methodology

The trials were carried out using the standard direct inoculation methodology as the control. The alternative methodologies using carriers (see item 2.2) were compared to the control with respect to:

- The water activity of the beans before and after spore inoculation
- The degree of homogeneity of the spores in the carrier.
- The degree of homogeneity of the spores in the beans

Direct method

This consists of adding 1 mL of a 10^7 CFU/mL spore suspension to 25 g of green coffee and mixing for 15 minutes in a Wagner blender.

Alternative method using soil (300 and 850 μm)

This consists of adding 1 mL of a 10^7 CFU/mL spore suspension to 10 g carrier (soil of 300 or 800 μm), mixing for 10-15 minutes and then adding 1 g of this mixture to 25 g raw coffee and mixing for a further 15 minutes in a Wagner blender.

Alternative method using ground coffee (300 and 850 μm)

Proceed as in item 2.4.2.

Alternative method using starch (mesh <100 μm)

Proceed as in items 2.4.2 and 2.4.3.

Determination of Water activity and moisture content

The equipment Aqualab Cx2 (Decagon-USA) was used to measure the water activity, with and without inoculum, and the moisture content was determined in a vacuum oven at 70°C for 24 hours.

Determination of the degree of homogeneity of the spores in the carrier

1 mL of a 10^7 CFU/mL spore suspension was added to 10 g of the respective carrier (soil, ground coffee, starch) with its respective mesh value. This suspension/carrier mixture was divided into 3 portions of approximately 3 g, and 27 mL 0.1% peptone water added to each. The similarity or not of the counts after dilution and plating, determined if the homogenisation of the suspension with the carrier had been adequate or not. For the direct method, 1 mL of spore suspension was divided into 3 parts of 0.3 mL and diluted with 2.7 mL of 0.1% peptone water, followed by dilution, plating and counting to see if the suspension had been well homogenised.

Determination of the degree of homogeneity of the mixture (suspension-carrier) in the raw coffee beans

1 g of the mixture (suspension-carrier) was added to 25 g polished green coffee beans. The new mixture was mixed in a Wagner blender for 15 minutes. Sixty beans were then plated in DG18 culture media (6 plates-10 beans/plate) using a flamed pincer. The plates were incubated at 25°C for 5 days.

RESULTS AND DISCUSSION

Table 1 shows the results obtained in the homogeneity test of the *Aspergillus ochraceus* spores suspension in the respective carriers, comparing uniformity with the direct method. The use of starch was not tested in any trial, since it formed large lumps in contact with the spore suspension, making its use impossible.

The colony counts showed little difference in uniformity in the case of homogenising with soil and coffee at 300 μm mesh, when compared to the direct method, the difference in one logarithmic cycle being logical since the suspension was diluted 10 times (1 mL suspension in 10 g carrier). With soil and coffee at 800 μm mesh, although not very different, the counts were somewhat lower but still in the range of 10^6. Thus it appears to be indifferent whether one uses mesh 300 or 850 or whether one uses ground coffee or soil.

Table 1. Colony counts using 2 different carriers for contamination and 2 mesh values, compared with direct inoculation

	CFU/mL*				
	Alternative Methods Carriers				
	Ground coffee		Soil		Direct method
	300 μm	850 μm	300 μm	850 μm	Coffee + suspension
Colonies					
1st	9×10^6	4×10^6	4.5×10^6	2×10^6	1.4×10^7
2nd	8×10^6	3×10^6	4.5×10^6	5.5×10^6	1.5×10^7
3rd	9×10^6	1.5×10^6	7×10^6	1.5×10^6	-

** Colony forming units per millilitre*

Table 2 shows the results of the homogeneity test with the incorporation of the suspension + carrier mixture in the raw coffee, as compared to the direct method.

Table 2. Percentage of contaminated beans using different carriers and mesh values. Comparison of the homogenisation of the contaminated beans as compared with the direct method

	Percentage of contaminated beans in the raw coffee %									
	Carriers								Direct method	
	Ground coffee				soil				Raw coffee + inoculum	
Carrier mesh	300 μm		850 μm		300 μm		850 μm			
% contaminated beans	100	100	50	60	100	100	100	100	100	100
	100	100	50	50	100	100	100	100	100	100
	100	100	30	60	100	100	100	100	100	100

According to the values shown in Table 2, the carriers soil and ground coffee at 300 μm, contaminated the same percentage of beans as soil at 850 μm. The carrier ground coffee at 850 μm, contaminated 50% less beans. This latter procedure was the only trial which did not show 100% efficiency in contaminating the beans, as compared to the direct method.

Table 3 shows the values for water activity of the raw coffee beans before and after inoculation with the different carriers and after direct inoculation.

According to the data shown in Table 3, the smallest variation in A_w occurs with soil (300 μm), ground coffee (300 μm) and the direct method, in that order respectively. It must be mentioned that the A_w was measured 24 hours after adding the suspension in the case of the carrier trials and directly on the raw coffee beans in the case of the direct method. With respect to water activity it is apparently interesting to use the alternative methods for the isotherm study in beans during storage and transport, since the variation in initial A_w is minimal. The differences obtained with the direct method were significant, not only from the actual values, but from the time that the bean did not pass through the gradual phases of incorporating moisture, thus acquiring a higher A_w in a shorter than normal time. The

influence of this on the growth of the organism and the production of toxin, will be the theme of future studies.

Table 3. Water activity values for the raw coffee beans before and after inoculation with the spore suspensions with carriers (soil and ground coffee) and by the direct method

Water activity Aw	[1]Raw coffee – no inoculum				Alternative methods ([1]coffee + carrier* + inoculum)			
			Direct method (Coffee + inoculum)		*Soil (300μm)		*Ground coffee (300 μm)	
	0.654	x=0.654	0.765	x=0.766	0.669	x=0.670	0.693	x=0.682
	0.654		0.766		0.670		0.671	

CONCLUSION

The alternative methods for the inoculation of *Aspergillus ochraceus* into raw coffee beans showed relative success, especially with respect to the water activity, in which the carrier soil at 300 μm mesh showed virtually no effect on the water activity of the coffee, whereas the direct method showed significant alterations. With respect to the homogeneity trials, the results were similar, with slight differences caused by the mesh of the carriers. This means that the use of carriers is viable in substitution of the direct method, especially in trials simulating contamination during the storage and transport of beans.

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Occurrence of Ochratoxin A in Raw Coffee for Export from Several Producing Regions in Brazil

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SUMMARY

The proposal of setting limits for Ochratoxin A (OTA) in coffee by the European Community has raised a need to verify the toxin levels in raw coffee exported by producing countries. The present survey investigated 37 samples of raw coffee prepared for export, from several producing regions in Brazil and two different harvests. In all, 97% of samples presented OTA concentration below 5 ng/g confirming the good quality of Brazilian raw coffee in respect to OTA contamination. The values found in this OTA investigation show a low occurrence of Ochratoxin A in a broad range of coffee producing regions, below the limits suggested for OTA in foods in the EU.

INTRODUCTION

Ochratoxin A is a mycotoxin produced by moulds such as *Penicillium verrucosum* and *Aspergillus ochraceus*, the latter found mainly in tropical regions (Moss, 1996). Cereals and cereal products are considered the main source of OTA in the diet. However, the toxin has been detected in other foodstuffs such as wheat, barley, meat, pork, beer and coffee (Jørgensen, 1998; Trucksess et al., 1999; Alldrick, 1996; Gareis, 1996). According to Taniwaki et al. (1999), *A. ochraceus* is the most frequent OTA producer in coffee growing regions in Brazil, although *A. carbonarius* and *A. niger* were also detected. The water activity (a_w) for OTA production by *A. ochraceus* is 0.85 (minimum) at an optimal temperature of 25°C (Moss, 1996).

Ochratoxin A inhibts protein synthesis both *in vitro* and *in vivo* through competition with phenylalanina. Its immunosuppressive action results in teratogenic and nephrotoxic effects. OTA was also found to increase lipid peroxidation *in vitro* and *in vivo*, which impacts cell and mitochondrial membranes (Dirheimer, 1996). Due to health concern, the Joint Expert Committee on Food Additives and Contaminants (JECFA) of the Food and Agriculture Organization (FAO) established a provisional tolerable weekly intake of 0.1 ng/g body weight (bw) (Speijers, 2000). Moreover, the European Commission's Scientific Committee for Food (SCF/EU) has been considering the proposition of regulatory limits for OTA in foods, including coffee.

The incidence of OTA in raw coffee has been previously reported (Tsubouchi et al., 1984; Micco et al., 1989). Studer-Rohr et al. (1994) analyzed 25 commercial samples of raw coffee from different companies and found 13 contaminated with OTA at levels ranging from 1.2 to 56 ng/g. In 1997, Nakajima et al. (1997) conducted an investigation in Japan with samples of

raw coffee from Asia, Africa and South America. In all, 30% of the samples were contaminated at levels between 0.1 and 17.4. None of the 4 samples from South America were contaminated. In 1998, Furlani (1998) described an investigation conducted by the Ministry of Agriculture in the United Kingdom determining OTA levels in 291 raw coffee samples from 27 countries. In all, 110 were contaminated with OTA at a maximum level of 27.3 ng/g. Trucksess et al. (1999) analyzed 19 raw coffee samples imported from South America in 1997 and found 9 contaminated at levels ranging from 0.1 to 4.6 ng/g.

Furlani (1998) conducted the first investigation on Brazilian raw coffee from 6 different producing regions in regard to the content of OTA. The results showed that 50% of *C. robusta* samples were contaminated at levels between 4.0 and 147.5, against 15.7% of *C. arabica* at levels ranging from 1.7 to 12.7 ng/g. Taniwaki et al. (1999), reported contamination in samples obtained in various producing regions between 0.3 and 19.75 ng/g.

This paper presents an investigation to determine the occurrence of OTA in Brazilian raw (green) coffee from several producing regions, two different harvests and exclusively prepared for export, anticipating possible regulatory limits to OTA by importing countries. This is the first report on the incidence of OTA in raw coffee exported from Brazil.

MATERIAL AND METHODS

Samples

A total of 37 samples of raw coffee exclusively intended for export were obtained from export companies in Santos, Brazil. The wideness of the sample origins comprised 2.5 million hectares of coffee growing area representing a production of 30 million 60 kg bags.

Samples of 1 kg were collected from coffee lots subsequent to the preparative process of screening (sorting by size), ventilation (sorting of broken and defective beans), color sorting (with an electronic machine) and cupping (detection of undesirable aromas such as “fermented” and “old”).

Determination of ochratoxin A by HPLC

The methodology of ochratoxin A analysis was that proposed by Pittet et al. (1996), based on the use of immuno-affinity column of Nakajima et al. (1990) for clean-up step. The quantification was performed by HPLC in a Shimadzu LC-10VP system (Shimadzu Corporation, Japan) set at 330 nm excitation and 470 nm emission.

Scanning Electron Microscopy (SEM)

Some samples were submitted to classification and its beans separated according to the official coffee classification into “sound”, “black” and “sour” in order to be observed separately under the SEM. Optically, sound beans had the typical green color and good appearance. Black ones, commercially known to be a result of fermentation during harvest or drying, presented a dark color. Sour beans, also a result of fermentation, presented a brown, glowing aspect.

Coffee beans were cut in half, de-fatted with acetone for 3 hours and placed in an incubator overnight. After sputtering with gold the beans were observed under the scanning electron microscope at 10 kV of acceleration.

Table 1. Composition of samples according to origin, harvest year, coffee variety, process (1) and growing conditions (2)

Sample Origin	Number of samples Harvest 1999/2000	Number of samples Harvest 2000/2001
State of São Paulo (Arabica)		
Araraquarense	1	2
Alta Paulista	1	2
Mogiana	1	2
State of Minas Gerais (Arabica)		
Sul de Minas	1	2
Cerrado	1	2
Zona da Mata	1	2
Bahia (Arabica)		
Wet process (1)	1	2
Dry process (1)	-	1
Espírito Santo		
Arabica coffee	1	2
Robusta coffee	1	2
Rondônia (Robusta)	1	2
Paraná (Arabica)	1	2
Government-stock (Arabica)	1 sample - harvest 1987/1988	-
Organic (Arabica) (2)	1	1

RESULTS AND DISCUSSION

Ochratoxin A

OTA content in 36 samples ranged from <0.2 ng/g to 0.85 ng/g with an average of 0.16 ng/g. One sample, excluded for average and range, reached a content of 6.24 ng/g. Water activity levels for all 37 samples ranged from 0.510 and 0.714 with an average of 0.614. In all, 20 samples or 54%, presented OTA concentration below the detection limit of 0.2 ng/g and 97% of samples showed OTA concentration below 5 ng/g (OTA limit suggested in the EU).

Scanning Electron Microscopy (SEM)

On the SEM the morphology of sound and sour raw coffee beans showed an organized endosperm: the cells contain their regular cytoplasm. The black ones showed an endosperm somewhat compressed and not well defined cellular contents. Its outer color was black and the endosperm structure was altered. There was no sign of molds, bacteria or yeasts in any of the observed beans.

CONCLUSION

The results show that Brazilian raw coffee for export has good quality in respect to OTA concentration. Although studies have already shown a low incidence of Ochratoxin A in Brazilian coffee (Furlani, 1998; Taniwaki et al., 1999; Urbano et al., 2000), the values found in this OTA investigation show a lower occurrence of Ochratoxin A in a broad range of coffee producing regions and below the limits suggested for OTA in foods in the EU. Procedures involved in export preparation such as screening, ventilation, color sorting and cupping might be effective on removing contaminated coffee beans.

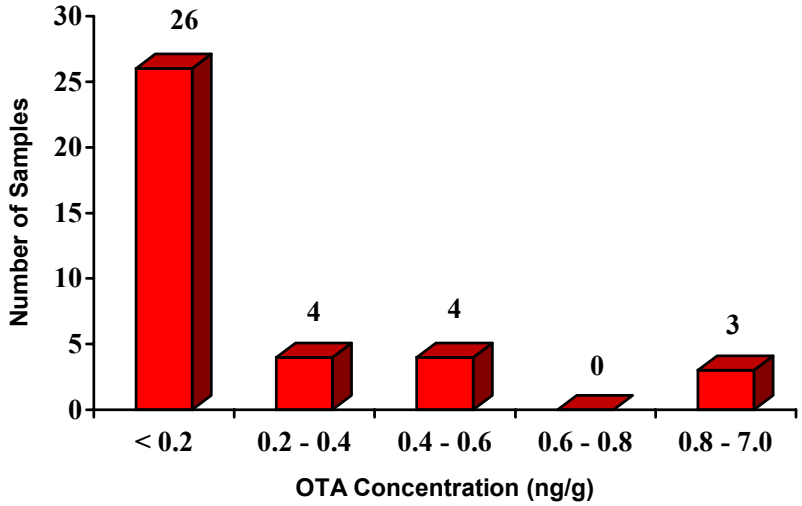

Figure 1. Data obtained from a lot of 37 samples of Brazilian export raw coffee from several producing regions.

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Toxigenic Potential of *Aspergillus Ochraceus* from Irrigated Brazilian Cerrado Coffee

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SUMMARY

The objective of this work was to identify points of contamination of Brazilian Cerrado coffee regarding the ochratoxin A (OTA) production by *Aspergillus ochraceus* and to test two methods to detect OTA production on the culture medium. Ninety-three samples of coffee from Minas Gerais, Bahia and Goiás States, collected during several phases of the production chain, were analyzed. Forty coffee grains composed each sample. The fungi cultures developed were isolated and evaluated according their macro and microscopic characteristics. The identification was performed using the key characteristics. From the analyzed samples 91% presented fungi contamination, 39% of this total identified as *A. ochraceus.* Two methods to evaluate the toxigenic potential for the OTA production were compared. The first one was by the OTA extraction in Agar-coconut medium and the other one by the agar plus impregnation directly on TLC plates. From the screening of thirty strains of *Aspergillus ochraceus* isolated, eighteen were ochratoxin A (OTA) producers. No difference was observed between the two methods utilized: both were capable to detect OTA.

INTRODUCTION

The agricultural expansion along the Brazil Central became an extension area of coffee production, in the cerrado region. The product of the cerrado area is characterized by good flavor coffee. One of the critical points to asssure the coffee quality is the control of the ochratoxin A (OA). So it is necessary to identify if the *A. ochraceus* is present on the coffee orchads and in which phases it infectes the green coffee, the cherry, or the grain. The objective of this work is to characterize some points of contamination on the production chain of Brazilian Cerrado coffee through the identification of the endogenous presence of AO, produced by *A. ochraceus.*

MATERIAL AND METHODS

The 93 samples of green coffee and grains were collected during several phases of the production chain. The *Coffea coanephora* and *C. arabica* used as sample were grown in three different states of Brazil: Minas Gerais, Bahia and Goiás. The approach to study of fungi mycobiota was in the identification of *Aspergillus ochraceus* strains that produced OA. Each sample was composed by 30 coffee grains. After sanitation with 2% sodium hypochlorite, the grains were incubated from 7 to 10 days on BOD at 25^{o}C. The fungi culture developed was isolated and evaluated regarding their macro and microscopic characteristics. The identification was performed using the keys characteristics described by Klich and Pitt (1988), and Samson et al. (1995).

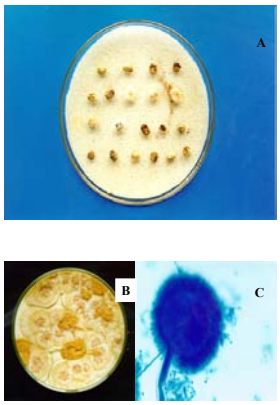
RESULTS AND DISCUSSION

The fungic contamination from Cerrado irrigated coffee was relevant (Mislivec et. al., 1983). The presence of *Aspergillus ochraceus* and *A. niger*, was detected in 91% of analyzed samples. 39% were identified as *A. ochraceus* (Table 1). *A. ochraceus* strains developed were identified according to macro and microscopics charactheristics (Figure 1). Tests to detect the ochratoxigenic potential were positives to 18 strains isolated.

No difference were observed when the two methods were compared, the OTA extraction in Agar-coconut medium and the other one by the agar plus impregnation directly on TLC plates, both were capable to detect OTA.

Table 1. Percentual of Contamination of *Aspergillus niger* and *A. ochraceus* from Brazilian Cerrado

ORIGIN	NUMBER OF SAMPLES	SAMPLES MOLDED(%)	CONTAMINATION (%)	
			A. niger	*A. ochraceus*
Araguari (MG)	42	100,00	53,40	42,80
Barreiras (BA)	24	83,30	12,50	45,80
Cristalina (GO)	20	80,00	25,00	20,00
São Sebastião do Paraíso (MG)	4	100,00	75,00	25,00
Mimoso do Oeste (BA)	3	100,00	33,30	66,70
TOTAL	**93**	**92,66**	**39,84**	**40,06**



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