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COFFEE AND HEALTH

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Introduction

Not too long ago, approximately until the mid-1980's, one could find in the weekly Current Contents listing of the world literature at least five article on coffee and another five on caffeine and health related issues. Most of this literature was suggesting, but not proving, that coffee was a health hazard for one of 15 diseases and complications and eight different cancer sites, none of which have ever been substantiated. During the past 3 to 5 years a drastic decrease in medical publications on coffee and health has occurred: some weeks and months go by when not a single paper is printed on these topics. How do we interpret this change? It has been my impression that for the many diseases of modern man for which no cause had been discovered, two suspected "causes" were used over and over again - either "stress" or coffee or both! Historically, it is difficult to understand why coffee/caffeine was alleged of causing so many different diseases in 18 organ sites of the human body. We are reminded of an editorial in the British Medical Journal: "What is it in man's devious make-up that makes him round on the seemingly more wholesome and pleasurable aspects of his environment and suspect them of being causes of his misfortunes? Whatever it is, stimulants of all kinds (and especially coffee and caffeine) maintain a position high on the list of suspicion despite a continuing lack of real evidence of any hazard to health" (Brit. Med. J. 1:1031, 1976).

From Allegations to Denials: Coffee is Vindicated

Of the fifteen metabolic, gastrointestinal, cardiovascular diseases and those of the prostate, breast, pregnancy and bones, all at one time or the other thought to be involved in a direct causal action or as a co-factor of coffee/caffeine, several were ruled out immediately. Among them, diabetes mellitus was shown unrelated to coffee, as Dr. Feinberg et al. had provided the first proof (see Appendix, Table 5).

Hyperuricemia and gout for a long time were a favorite target among anti-coffee groups. In conducting a brief anonymous questionnaire study among physicians in Switzerland in the 1960s, we were not surprised to find that close to 50% of 200 physicians would not recommend coffee drinking for their gout patients. The real villain of course is obesity, meat and alcohol consumption.

Cirrhosis of the liver should never have been seriously considered. However, a recent publication from the Kaiser-Permanente Insurance Group in California after studying 128,934 person's medical records came to a surprising conclusion - after confirming, of course, that alcohol remains the main cause of death from cirrhosis of the liver: "Cigarette smoking was independently related to risk of alcoholic cirrhosis, with cigarette smokers of a pack or more per day at trebled risk compared with lifelong nonsmokers. Coffee drinking, but not tea drinking, was inversely related to alcoholic cirrhosis risk, with persons who drank four or more cups per day at one-fifth the risk of non-coffee drinkers. This inverse relation between coffee consumption and risk of alcoholic cirrhosis was consistent in many subsets, including persons free of gastrointestinal disease. These data could mean that cigarette smoking promotes alcoholic cirrhosis and that coffee drinking might be protective" (Klatsky KL, Armstrong MA: Alcohol, Smoking, Coffee and Cirrhosis. Am J Epidemiol 1992;136:1248-1257).

The authors offered five possible explanations: (1) The inverse relation of coffee consumption to alcoholic cirrhosis is sufficiently strong that chance as the explanation is unlikely; (2) The inverse relation of coffee intake to alcoholic cirrhosis is unlikely to be due to confounding by the other related habits; (3) There is a well established relation of poor liver function to impaired caffeine metabolism. Reduced caffeine clearance has been proposed as a liver function test; (4) It is possible that, as cirrhosis progresses, those who drink more coffee might be more inclined to drink less alcohol; (5) A weaker relation of alcohol intake to risk of alcoholic cirrhosis in those who drank more coffee may suggest a protective effect.

Peptic ulcer is no longer an issue since people who smoke <u>and</u> drink coffee are more likely to develop peptic ulcers. However coffee drinking in the absence of smoking is rarely seen as cause of peptic ulcer, particularly since a bacterial etiology is being discussed lately. Hyperacidity and esophageal gastric acid reflux are seen in consumers of both, regular coffee and decaffeinated coffee. Therefore caffeine was effectively ruled out as causing hyperacidity. Premature ventricular beats were once believed to be related to the amount of coffee consumed. Dr. Ronald Prineas (J. Chron. Dis. 33:67-72, 1980) examined this question by age-adjusting the prevalence rate of PVC's in 7311 men 35-57 years of age in seven subgroups, no coffee, 1-2 cups, 3-4 cups, 5-6 cups, 7-8 cups, 9-10 cups, $\geq$11 cups per day. No coffee drinkers had 3.1% of men with PVC's and regular consumers of 7-8 cups per day had 3.2% with PVC's. Only among men drinking 9 cups and more, the prevalence of PVC's increased to over 5%.

The largest hypertension intervention study ever conducted in the United States provided final evidence that habitual coffee consumption including caffeine from many different sources (soft drinks, candy bars, brownies, analgesics, etc.) is unrelated to hypertension, myocardial infarction, stroke and cancer (Martin JB, Annegers TF, Curb JD, Heyden S, Howson C, Lee ES, Lee M: Mortality patterns among hypertensives by reported level of caffeine consumption. Prev. Med. 17:310-320, 1988). The 10,000 hypertensive men and women in this trial had selected to either use no coffee and no caffeine, or were consumers of little, moderate or high intake of both on a life-long basis. The most remarkable finding was the consistency of an absence of any association between different coffee-caffeine intake categories and all cause death rates, rates of stroke and CHD deaths <u>and</u> cancer death rates.

Among the eight cancer sites, at one time or the other mentioned to be linked to coffee was stomach cancer. The previously quoted editorial from the Brit. Med. J. (1976) makes reference to anticarcinogenic effects of caffeine in animal experiments. Beyond this observation, stomach cancer is the fastest disappearing cancer site in the Western hemisphere. The pancreas cancer - coffee relationship was literally albeit unintentionally invented by a team from Harvard University, Department of Public Health (Dr. MacMahon et al.) and represents a classical error in biomedical research: the mistake was to have chosen the wrong control patients for the cases with pancreas cancer. Controls consisted of patients with chronic gastrointestinal problems, from duodenal ulcers, Crohn's disease, diverticulosis and diverticulitis to ulcerative colitis, with other words patients who would be in no mood

to drink coffee, while the pancreas cancer patients enjoyed coffee until they fell ill. It took several long-term epidemiological studies to vigorously deny any association between coffee and pancreas cancer.

The breast cancer-coffee "story" was created by the same people who found a "causal linkage" between fibrocystic breast disease and caffeine (Minton and co-workers, Ohio State University). With the collapse of the latter - again, after appropriate studies of large numbers of women - the breast cancer and coffee allegation vanished. No scientific proof for a cause - and - effect relation between coffee and ovarian or prostate cancer or benign prostatic adenoma was ever presented.

Bladder and kidney cancer are found three times more often among smokers compared to non-smokers. Failure to account for the smoking habit among heavy coffee drinkers in the past has led to a number of articles assuming coffee to cause carcinogenesis in those two organs.

With colon cancer today being the most common cancer site, it is remarkable that not a single case-control study has ever mentioned coffee since 1966, when Higginson disproved a suspected association.

A favorite hypothesis of the anti-coffee lobby was to accuse coffee drinking during pregnancy of an adverse outcome of pregnancy. It took a major study of 12,208 pregnancies in non-diabetic, non-asthmatic women to disprove any connection. After controlling for smoking cigarettes during pregnancy, coffee drinking was unrelated to either low birth weight or short gestation (S. Linn et al.: No association between coffee consumption and adverse outcomes of pregnancy. N. Engl. J. Med. 306:141-145, 1982).

Osteoporosis and coffee consumption is an unlikely relationship - considering the influence of smoking among coffee drinkers on estrogen, causing early menopause compared to non-smokers.

From Vindication of Coffee to Actual Health Benefits

Table 1 reveals, beginning in 1983, a new trend in coffee research. For the first time, benefits of coffee-caffeine in humans are being discussed. In Table 2 a few more potential benefits are mentioned. Of considerable interest is the discussion of Timson on biological activities of caffeine: "Perhaps as a result of man's daily contact with the compound, which for many is in fact a drug of mild addiction, much interest has been taken in its affects on biological processes at all levels of organization from the molecular to the whole animal. It can be said with certainty that caffeine is mutagenic in micro-organisms, with less certainty that it may be mildly mutagenic in Drosophila and with near certainty that it is not mutagenic in mammals at least at the transitory peak levels achieved in normal human consumption. Although the question of caffeine's mutagenicity in mammals and man has been discussed in the past, and the available evidence suggests that caffeine is not mutagenic in mammals, yet there persists an almost subconscious feeling that it might, perhaps ought, to be mutagenic in mammals. This may in part be due to the well established mutagenicity in lower organisms and the clear demonstration of clastogenic activity in mammalian cells in culture. It is tempting to suggest that it may also be due to man's longstanding addiction to caffeine coupled with a feeling that such addiction should carry a penalty. However there are strong arguments against caffeine being mutagenic in man. In the first place caffeine is rapidly metabolized in mammals and mammalian cells and this is not the case in E.coli, which cannot demethylate the methylxanthines. Secondly caffeine has antimitotic activity against mammalian cells. Thirdly the long exposure of the human race to caffeine and other methylxanthines may have led to the evolution of some caffeine-resistance in man. The case for caffeine mutagenicity in man is therefore non-proven."

Finally, in Table 3 contradictory findings in coffee research are being blamed on the failure to distinguish between habitual coffee drinkers and caffeine-naive persons. Further, three diseases -

TAB 1: **SIX HEALTH BENEFITS OF COFFEE-CAFFEINE IN HUMANS**

systemic therapy for neonatal apnea	Curatolo PW Ann Int Med (98) 1983
effective as a topical treatment of atopic dermatitis	Curatolo PW Ann Int Med (98) 1983
a good source of potassium	Gillies ME Am J Clin Nutr (38) 1983
an analgesic adjuvant	Laska EM JAMA (251) 1984
an effective bronchodilator in young patients with asthma	Becker AB N Engl J Med (310) 1984
a booster of pain-free walking time for pts. with chronic stable angina	Piters KM Am J Cardiol (55) 1985
prevention of postprandial hypotension, particularly after breakfast	Onrot J N Engl J Med (313) 1985

TAB 2:

POTENTIAL BENEFITS OF CAFFEINE	
Increase of urinary Na+ excretion	Robertson et al. (1978) N Engl J Med 298 : 181
Caffeine's interaction with DNA suggests possible antineoplastic action	Timson, J. (1977) Mutat. Res. 47 : 1
Thermogenic effect (4 mg caffeine/kg body weight); metabolic rate increases	Jung et al. (1981) Clin. Sci. 60 : 527
Increase of minute ventilation in patients with COPD	Woodcock et al. (1981) N Engl J Med 305 : 1611

TAB 3: **CONTRADICTORY FINDINGS IN COFFEE RESEARCH**

Explanation: Failure to distinguish between habitual consumers and caffeine-naive persons. Beyond this central problem and after years of investigations, the conclusion is justified that caffeine is therapeutically ineffective in three conditions: artificial insemination of hypomotile sperm, childrens' minimal brain dysfunction and Parkinson's Syndrome

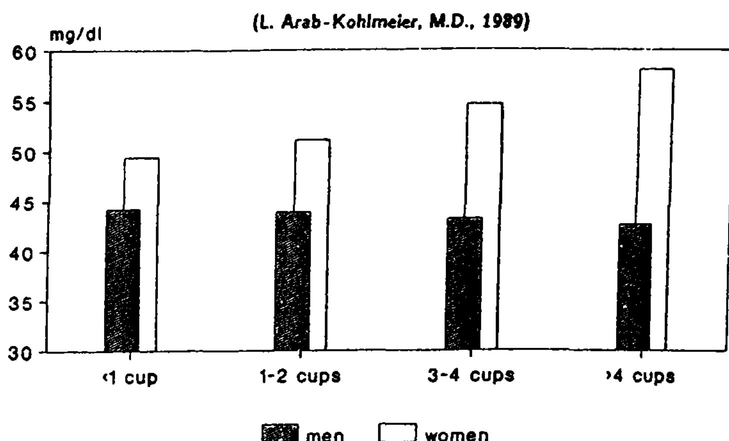
conditions are named in which coffee and caffeine at one time was expected to exert a positive influence. However, it is impossible to find such evidence.

Next, we will turn to the ever present coffee-cholesterol discussion, hoping to shed some new light on this old issue.

To some investigators, notably from Scandinavian countries, the coffee-cholesterol issue is still lingering on. A growing number of articles on the method of boiling (rather than filtering or percolating) coffee is thought to provide the answer to the discrepancies in results of the coffee-cholesterol association from different countries. To be more specific, a positive relationship between coffee consumption and increases in cholesterol levels was more often reported in female subjects compared to predominately negative reports in males. We have selected two reports, one a) an observational study of male and female coffee drinkers and b) an intervention study on females only. When HDL-cholesterol levels are significantly increasing (Fig. 1) with higher coffee consumption compared to a lower coffee ingestion, it must be expected to find total cholesterol levels increased. In contrast, among age-matched males, neither HDL nor total cholesterol levels changed with increasing coffee consumption.

FIGURE 1:

Adjusted mean levels of serum HDL cholesterol in men and women (age 18-24) according to coffee consumption*



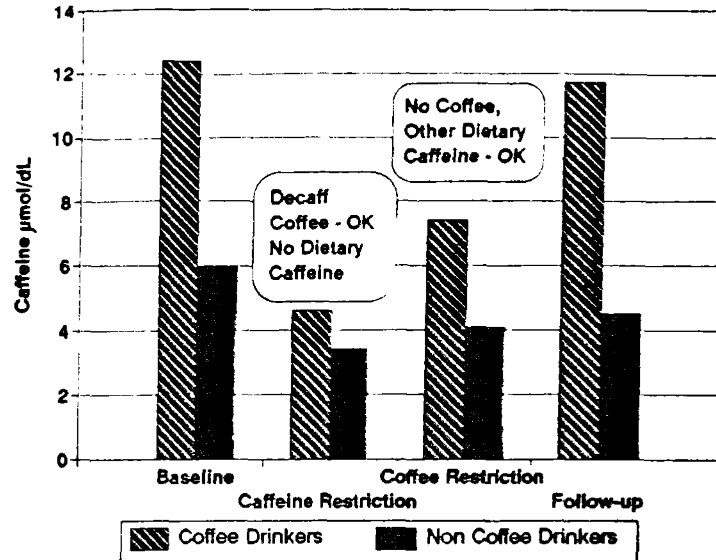
* adjusted for alcohol, BMI, physical activity, contraceptive use

Presented at the Workshop on Coffee, Plasma Lipids and Coronary Heart Disease. Goteburg/Sweden. 8-10 May, 1989.

Introduction

The possibility that coffee may increase cholesterol levels has created uncertainty among physicians. The confusion arose from cross-sectional studies, in which female coffee drinkers appeared to show a positive association more frequently than men. To clarify this relationship, we designed an intervention trial to reduce caffeine and coffee intake sequentially while measuring total cholesterol and the apolipoprotein A-I and B levels. We conducted the study among women who were coffee drinkers or not coffee drinkers. The trial spanned seven months with caffeine-free and coffee-free intervals.

FIGURE 2:



Sedor FA, Schneider KA and Heyden S: Effect of coffee on cholesterol and apolipoproteins, corroborated by serum caffeine levels. Am. J. Prev. Med. 7:391-396, 1991.

Participants

This report is based on the biochemical monitoring of 63 female non-smoking teachers assigned into two groups: persons who customarily drink regular coffee (RC) and persons who do not (NC). The NC group was consuming caffeine, which would be intentionally decreased in the study protocol. Each group represented a separate intervention study and was intended to be its own internal control. The study was composed of four periods. The first and last periods were the control periods, during which the participants in each group were to maintain their customary beverage consumption.

RC group

During the second period, intended to study caffeine withdrawal, we asked participants to eliminate caffeine from their diet, including caffeinated coffee and tea, carbonated soft drinks, chocolate and nonprescription medications. Caffeine-free varieties of coffee, tea, and carbonated beverages were allowed (No Caffeine, Decaff OK) (Figure 2).

During the third period, intended to study coffee withdrawal, the RC group was to abstain from any form of coffee or tea. They were free to resume intake of carbonated soft drinks containing caffeine, chocolate, and nonprescription medications (No Coffee, Caffeine OK).

NC group

During the second period, participants were to eliminate caffeine from their diet. Since this group did not drink any form of coffee, the restrictions were for tea containing caffeine, carbonated soft drinks, chocolate, and nonprescription medications. Caffeine-free varieties of tea and carbonated beverages were allowed (No Caffeine, Decaffeinated OK).

During the third period, the NC group was to abstain from any form of tea. They were free to resume the intake of carbonated soft drinks containing caffeine, chocolate, and non-prescription medications (No Coffee, Caffeine OK).

Each of the first three periods lasted four weeks. This time allowed for equilibration of lipid and apolipoprotein levels in response to the dietary changes. All participants recorded dietary histories each week. The final period lasted 16 weeks, and the participants continued to record dietary histories.

Non-fasting blood samples were collected initially and at the end of each period under standardized conditions. An internal laboratory study of ten healthy volunteers showed no significant differences between fasting and two-hour postprandial determinations of total cholesterol, apo A-I, and Apo B.

Results

Mean serum caffeine levels during the intervention protocol confirmed the participants' compliance (Figure 2). Caffeine levels for the RC group decreased 64% from baseline for the no-caffeine period and returned to 94% of baseline as caffeinated food and beverages were reintroduced. These differences were statistically significant ($P < .01$). The caffeine levels of the NC group obtained at the beginning of the study were significantly lower than those of the RC group. Samples from each study period demonstrated a similar pattern: an initial 46% fall from baseline and return to 26% below baseline. Except for these caffeine levels, no other differences between groups or intervals in lipids and apolipoproteins were observed to have statistical significance at the $P < .05$ level.

Initial total serum cholesterol levels were 4% lower in the NC group compared to the RC group and remained constant throughout the study in both groups. Importantly, the carrier lipoproteins were not influenced. Analysis of apo A-I demonstrated no significant difference between the four intervals or between the two groups, RC and NC. The levels of apo B were similarly unaffected by the dietary changes for each group.

Discussion

The distinction of this report lies in the measured serum caffeine levels. Serum caffeine concentrations were 50% lower at baseline in the NC group compared to the RC group. In response to the no-caffeine and no-coffee periods, the serum caffeine levels fell in both groups. However, in the follow-up period, when the NC group was asked to return to their customary drinking habits, caffeine remained below the baseline level. In the NC group, total cholesterol and apo B-levels and body mass index measured slightly lower than in the RC group; the differences were not clinically relevant. When the NC group became aware that carbonated beverages contained caffeine, some of them eliminated these beverages from their diet.

The teachers in our trial provided excellent dietary histories. The between-group caffeine ratios by history and by measurement agreed and, together with the comparability in fluid intake volumes, contributed to the credibility of the recordings.

Summary

Serum caffeine levels corroborated compliance with the dietary protocol. Analysis of the apolipoprotein levels confirms the absence of any influence of coffee on lipoproteins in normocholesterolemic persons. We observed no apparent causal association of coffee and caffeine consumption and cholesterol and apolipoproteins.

We realize that a small sample size may result in a limited power. However, given the negative results we obtained, a huge study group would have to be examined to reach the opposite conclusion that caffeine and coffee consumption does affect the serum cholesterol and apolipoprotein levels. We suggest that the occasional coffee-cholesterol association found in observational studies among persons with normal cholesterol levels may be due to chance.

Anyone following the literature closely will be impressed by the massive inconsistencies. We were among the first to point them out (Thelle DS, Heyden S, Fodor JG: Coffee and cholesterol in epidemiological and experimental studies. Atherosclerosis 67:97-103, 1987). Of some interest is a review of 30 years of animal experiments in rats, rabbits and rhesus monkeys (Table 4). Surprisingly, coffee feeding experiments in these animals failed to show any cholesterol raising effect; likewise, the expected effect of atherogenesis was not demonstratable in either aortas or coronary arteries. We therefore repeat our conclusion from 1987. A critical assessment of the published reports leads to the conclusion that the data are insufficient to warrant public health admonitions against coffee drinking. The inconsistencies point towards the influence of confounding and modifying factors including diet and brewing methods, which are not clear at this stage. In most populations the cholesterol raising effect is so small that it is of no public health concern. In the six years since the publication of this review no more debate was added with the exception of an occasional new study with essentially the perpetuation of the dilemma described. If indeed the brewing method was at the bottom of this cholesterol raising problem in certain Scandinavian countries then the problem can be solved by changing the preparation of the coffee. For the rest of the world it became a non-issue already many years ago.

TABLE 4: CHOLESTEROL-EFFECTS OF CAFFEINE TREATMENT IN EIGHT ANIMAL EXPERIMENTS IN THE PAST 30 YEARS

Finding	Reference
Rabbits: no difference in cholest. levels between animals with cholesterol feeding and those with caffeine in addition to cholesterol diet	Myasnikov Circulation (1958)
Neg. effect of caffeine on development of aortic atheromatosis, induced by cholesterol feeding	Czochra-Lysanowicz et al. Nadbitka Biuletynu (1961)
Nescafe fed by mouth, plus 1 g of cholesterol, (control group 1 g of cholesterol only): lower cholest. levels in caffeine-treated animals.	Heyden and Ruttner Pathol Microbiol (1966)
Atherosclerosis more extensive in cholesterol-fed rabbits (mean index 2.32) than in those simultaneously treated with caffeine (mean index 1.61)	Kedra et al. Pol Med J (1967)
Daily subcutan. inject. 0.22 ml caffeine benz.; both groups were fed cholesterol diet: Cholest. levels lower in experimental animals.	Heyden et al. J Chron Dis (1969)
No sign. diff. in degree of atheromatosis of the aorta between caffeine-treated & controls. A few controls showed more extensive atheromatosis; lower degree of coronary 'atherosclerosis' in caffeine-treated animals.	Heyden et al. J Chron Dis (1969)
Rhesus Monkeys: received 50% of fluids as coffee, no consistent coffee-induced changes in lipoproteins or in aortic fatty streaks.	Callahan et al. Am J Clin Nutr (1979)
Rats: serum cholest. rose acutely over 7 days of caffeine feeding but not after 25 days.	Haffner et al. Am J Epidemiol (1985)
Rats: Increase in fecal excretion of neutral steroids, "caffeine's pronounced hypolipemic effect".	Hostmark et al. Nutr Rep Internatl (1988)

SUMMARY

During the past decade a remarkable shift has been observed in reports concerning health effects of caffeine. Twenty years ago the medical literature used to culminate in allegations on entirely negative aspects of coffee consumption. They included a great variety of diseases, among them eight different cancer sites, heart attacks, strokes, hypercholesterolemia and hypertension, birth defects, gout, cirrhosis of the liver, diabetes, stomach ulcers and osteoporosis. We are now witnessing a total negation of all assumed and perceived cause and effect associations. Even more compelling appear reports on positive health effects of coffee-caffeine. They range from an analgesic adjuvant to systemic therapy for neonatal apnea and as an effective bronchodilator in young patients with asthma, from topical treatment of atopic dermatitis and a booster of pain-free walking time for patients with chronic stable angina, to the prevention of postprandial hypotension in elderly patients and as a good source of potassium. The vindication of coffee was to be expected but it was amazing that it took so long for common sense to return, and even more surprising that the many allegations emerged in the first place.

APPENDIX: TABLE 5

EFFECTS OF COFFEE ON GLUCOSE TOLERANCE

GLUCOSE LEVELS mg/dL

Minutes	Number of Subjects	Controls	Drinkers	p Value
0	23	80	79	N.S.
30	18	139	118	<0.01
60	23	122	99	<0.001
90	15	90	83	N.S.
120	23	77	73	N.S.
180	23	68	73	N.S.

L.J. Feinberg et al., Metabolism 17:91, 1968

INHIBITION OF ORAL CARCINOGENESIS BY ROASTED COFFEE BEANS AND ROASTED COFFEE BEAN FRACTIONS

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INTRODUCTION

Until recently most of the work on the cancer chemopreventive activity of coffee beans has concentrated on green coffee beans. Early studies at the University of Minnesota showed that a diet containing 20% green coffee beans (Colombian) could inhibit by approximately 60% the development of 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary neoplasia in rats (1,2). Further research at the University of Minnesota led to the isolation and identification of two antineoplastic agents (3,4). The two chemicals, kahweol and cafestol, are both plant oils or diterpenes that are structurally similar. The only difference in structure is that kahweol contains as additional double bond. Research with kahweol and cafestol (2) has shown that these chemicals can inhibit the development of carcinogen-induced mammary tumors in rats. These coffee oil constituents have also been shown to increase the activity of a major detoxifying enzyme, glutathione S-transferase, in laboratory animals (5). This enzyme facilitates the excretion of harmful compounds from the cell and eventually from the organism. The enhancement of the activity of this enzyme should help to protect the organism from the action of chemical carcinogens. Since kahweol is the more potent inducer of glutathione S-transferase activity, it is assumed that kahweol is the more potent inhibitor of chemical carcinogenesis.

Our laboratory has continued this research on the antineoplastic activity of green coffee beans (6-9). In each of these experiments, the hamster cheek pouch model for oral carcinogenesis was utilized. The data from these studies have shown the following:

1. Powdered diets containing 15-20% green coffee beans (Colombian) inhibited by 70-95% the development of DMBA-induced buccal pouch carcinomas (6,8,9).

2. Part of this inhibition in DMBA-induced neoplasia is due to the two diterpenoids, kahweol and cafestol (7).

3. Besides kahweol and cafestol, the results now indicate there are other cancer chemopreventive agents in green coffee beans (7-9). One or more of these as yet unidentified agents is in the defatted portion of the bean (8,9).

Taken together, these results indicate that the cancer chemopreventive activity associated with green coffee beans is more complex than originally thought. Instead of just two cancer chemopreventive agents, we are dealing with a complex mixture of antineoplastic agents. Some of these agents are in the oil; others are in the defatted portion of the bean.

As a continuation of our research, we decided to see what affect, if any, roasting might have on the cancer chemopreventive activity of coffee beans. During roasting, the beans are heated to 900°F and kept at this temperature for 16-17 minutes. This leads to obvious changes in color and size, as well as multiple changes in chemical composition. For example, the chemicals that give coffee its unique aroma are formed during the roasting process.

MATERIALS AND METHODS

Eighty female Syrian Golden hamsters (Lak:LVG) weighing 80-90 g were obtained from the Charles River Breeding Laboratories, Wilmington, Massachusetts. The animals were housed in wire-mesh cages of stainless steel in a temperature-controlled room (22°C) with a 12:12 hour light-dark cycle. Throughout the experiment, water and food were provided ad libitum.

After arriving the hamsters were given ten days to adjust to their new surroundings. During this time all of the animals were fed Purina Lab Chow (St. Louis, Missouri) specifically formulated for small rodents. The animals were then divided into four equal groups and placed on one of four diets. The hamsters in Group 1 remained on the Purina Lab Chow, while the animals in the other three groups received the same Purina Lab Chow supplemented with either 15% roasted coffee beans (Group 2), 12.75% defatted roasted coffee beans (Group 3), or 2.25% roasted coffee bean oil (Group 4). The roasted coffee beans (Colombian) and the two roasted coffee bean fractions (Colombian) were prepared at the Nestlé Research Centre, Vers-chez-les-Blanc, Switzerland. In the Colombian roasted coffee bean, the roasted coffee bean oil accounts for approximately 15% (w/w) of the whole bean. After the oil is removed, the residual fraction accounting for the remainder of the bean (85%) is the defatted roasted coffee bean. For the rest of the experiment, the animals remained on their respective diets.

The hamsters were given a week to adjust to the new diets. At the end of this week, 17 animals were selected from each group. The left buccal pouches of these animals were painted 3 times per week with a 0.5% solution of the carcinogen, DMBA (Sigma Chemical Co., St. Louis, Missouri). The DMBA was dissolved in mineral oil. The remaining animals, three per group, were used as controls. The left buccal pouches of these animals were painted 3 times per week with mineral oil. Camel hair brushes were used to apply the solutions to the pouches. Each application places approximately 0.05 ml of the solution on the surface of the pouch.

After a total of 36 applications, the hamster were sacrificed by inhalation of an overdose of carbon dioxide. The pouches were excised and the tumors were counted and measured (length, height and width). Since the tumors are exophytic and tend to be spherical in shape, the sum of the three measurements divided by six was used to calculate an average radius for each tumor. Using the formula for the volume of a sphere ($4/3\pi r^3$), an approximation of the volume of each tumor was determined. The sum of the volumes of all the tumors in a pouch was defined to be the animal's total tumor burden (10-12). Once the tumor data had been collected, the pouches were fixed in 10% formalin. The tissues were embedded in paraffin, processed by routine histological techniques, and stained with hematoxylin and eosin. The data from the macroscopic and microscopic observations were used to assess differences in tumor incidence, number, burden, and type. The Student's t-test was used to analyze the significance of the data. For each analysis, the data from one of the groups (2-4) treated with roasted coffee beans or one of the roasted coffee bean fractions were compared with the data for Group 1, the group on the normal diet. This procedure is identical to the procedure that was used in our earlier experiment with green coffee beans and the green coffee bean fractions (8,9).

RESULTS AND DISCUSSION

The data for tumor incidence is given in Table I. Three experimental animals died during the experiment. The cause of death in each case was due to respiratory problems. All of these hamsters were excluded from the study. At the end of the experiment, there were 16 hamsters in Groups 1, 2, and 3, and 17 hamsters in Group 4. The percentage figures for the number of tumor-bearing animals ranged from a low of 81% for Group 2 to a high of 94% for Groups 1 and 4. Multiple tumors were common in three of the four groups. The lone exception was Group 2. In this group, only 6 out of the 16 animals had 2 or more tumors. The figures for the other groups were 12/16 in Group 1, 11/16 in Group 3, and 13/17 in Group 4. The total number of tumors ranged from a low of 29 for Group 2, to a high of 70 for Group 1.

TABLE I. TUMOR INCIDENCE

Group	No. of Animals	No. of Tumor Bearing Animals	No. of Tumors
1	16	15 (94%)	70
2	16	13 (81%)	29
3	16	14 (88%)	45
4	17	16 (94%)	66

The data for average tumor number, burden and mass are given in Table II. From this table, it can be seen that the diet with whole roasted coffee beans, Group 2, reduced average tumor number by 60% and average tumor mass by 70%. Since tumor burden takes into account both number and size, the overall reduction in average tumor burden was 90%. A similar comparison between Groups 1 and 3 showed that the diet containing defatted roasted coffee beans reduced average tumor number by 35%, tumor mass by 75%, and average tumor burden by 85%. Comparing the data for Groups 1 and 4, it can be seen that the roasted coffee bean oil diet reduced average tumor number by 10%, tumor mass by 45%, and average tumor burden by 50%.

TABLE II. AVERAGE TUMOR NUMBER, BURDEN AND MASS

Group	Avg. No. of Tumors[a]	Avg. Tumor Burden[a], mm^3	Avg. Tumor Mass[b], mm^3
1	4.4 ± 0.7	238 ± 65	54.1
2	1.8 ± 0.4*	29 ± 15*	16.1
3	2.8 ± 0.6	38 ± 16*	13.6
4	3.9 ± 0.6	115 ± 33**	29.5

[a]Values are means ± S.E.
[b]Values for average tumor mass were calculated by dividing the values for average tumor burden by the corresponding values for average tumor number.
Statistically different from Group 1: *,$p<0.005$, **,$p<0.05$.

It is interesting to compare the results of this experiment to our earlier study with green coffee beans and the two green coffee bean fractions, defatted green coffee beans and green coffee bean oil (8,9). The data for average tumor burden for that experiment showed that diets containing 15% green coffee beans, 12.75% defatted green coffee beans, and 2.25% green coffee bean oil reduced average tumor burden by 70%, 55%, and 60%, respectively. In this experiment, the diets containing 15% roasted coffee beans, 12.75% defatted roasted coffee beans, and 2.25% roasted coffee bean oil reduced average tumor burden by 90%, 85%, and 50%, respectively. Comparing the two sets of data, it can be seen that roasting did not destroy to any significant extent the cancer chemopreventive activity associated with the coffee bean and the coffee bean fractions. The data even suggests that roasting may have increased the cancer chemopreventive activity in the defatted portion of the bean.

These results could have significance for humans. It is known that very little of the coffee oil actually reaches the coffee cup. In most forms of brewing, the oil is trapped by the filter. Most of the chemicals that enter the coffee cup come from the defatted portion of the bean. If these as yet unidentified cancer chemopreventive agent(s) in the defatted portion of the roasted coffee bean are soluble in hot water, then it is possible that they may be of benefit to humans. In this regard, it is interesting to note several recent reviews of epidemiologic studies on coffee and cancer suggest that coffee consumption may, in fact, lower the relative risk of colon cancer (13,14). The antineoplastic agent(s) in the defatted roasted coffee bean may be contributing to this protective effect.

Additional research on the cancer chemopreventive activity associated with the defatted roasted coffee bean is needed. As indicated, it is not yet known how much of this activity ends up in the coffee cup. Other obvious research objectives include the identity of the biologically active agent or agents, and the mechanism of action. In other words, how are these new antineoplastic agent(s) inhibiting the formation of DMBA-induced oral carcinomas? Another less obvious objective is the distribution of the active agent(s) in other parts of the coffee plant. It is possible that the stems, leaves, roots, or the pulp of the coffee cherry may contain the same cancer chemopreventive agent or agents and at higher concentrations.

ACKNOWLEDGMENTS

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SUMMARY

Whole roasted coffee beans, defatted roasted coffee beans, and roasted coffee bean oil were tested for cancer chemopreventive activity. It was found that the whole bean and the two roasted coffee bean fractions could inhibit the development of DMBA-induced oral carcinomas in hamsters by 50 to 90%. When compared to earlier studies with green coffee beans, defatted green coffee beans and green coffee bean oil, the data showed that roasting did not decrease the antineoplastic activity of the bean or the two coffee bean fractions. The data even suggests that roasting may have increased the cancer chemopreventive activity in the defatted portion of the bean.

RESUMEN

Se ha llevado a cabo un ensayo con granos enteros tostados, granos desgrasados tostados y aceite de granos tostados, todos ellos de café, para determinar su actividad quimiopreventiva contra el cáncer. Se encontró que el grano entero asi como las otras dos fracciones, podian inhibir el desarrollo de los carcinomas de baca inducidos por DMBA en hámsters con una efectividad de un 50 a un 90%. Al comparar los datos con estudios anteriores, realizados con granos sin tostar enteros, granos sin tostar desgrasados y aceite de granos sin tostar, se encontró que el tostado no disminuía la actividad antineoplástica del grano ni de las dos fracciones del grano de café. De hecho, los datos sugieren que el tostado puede incrementar la actividad quimiopreventiva contra el cáncer en la porción desgrasada del grano.

THE PRO- AND ANTIOXIDATIVE EFFECTS OF COFFEE AND ITS IMPACT ON HEALTH

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Introduction

Despite the abundance of sound toxicological data demonstrating the safety of coffee (1), controversy remains due to chemicals present in coffee which are mutagenic when assayed *in vitro* (2). Most of this mutagenic activity appears to be dependent upon hydrogen peroxide generation (2). Five different research groups have reported that freshly prepared instant coffees contain hydrogen peroxide at levels greater than 100 µM (3-7), while two other groups reported significantly lower levels (8,9). With the exception of the studies conducted by Tsuji et al. (7) and Rinkus and Taylor (9), detailed descriptions and validation of the analytical methods have not been reported. Therefore, we have utilized three independent analytical methods to measure hydrogen peroxide formation in instant coffee. All three methods demonstrate that hydrogen peroxide is slowly generated over time and that formation is dependent upon atmospheric oxygen.

The adventitious formation of hydrogen peroxide and the pro-oxidant activity of coffee *in vitro* appears to be attributed to polyphenolic thermal degradation products of chlorogenic and caffeic acid which reduce atmospheric oxygen in the presence of transition metal catalysts (10). However, we have discovered that polyphenols found in coffee also act as potent anti-oxidants and inhibit lipid peroxidation in model systems. Coffee is also strongly antimutagenic and inhibits hydroxyl radical mediated genotoxicity in bacterial mutation assays (11). Thus, coffee, like many antioxidants, displays both beneficial and detrimental effects *in vitro* which are highly dependent upon dose, atmospheric oxygen, and the biological and chemical endpoints used for assessment.

Long-term feeding studies in rodents given coffee as part of their daily diet have shown that coffee increases lifetimes with a concomitant decrease in spontaneous tumors (1,12,13). These beneficial effects may be attributed in part to the antioxidant activity of coffee. In this article we present some of the pro- and antioxidative effects of coffee *in vitro*. These findings illustrate the problems encountered with chemical and biological assays *in vitro* which may give conflicting results for polyphenolic compounds and beverages (1,2) that are not easily extrapolated to assess human health and safety.

Materials and Methods

Instant coffee powder (2.25 g) was added to 150 ml of distilled, deionized water and poured into a cooled 50 ml measuring flask. Coffee processing and fractionation of hydrogen peroxide was performed with Bond-Elut C-18 (500 mg) cartridges as described by Rinkus and Taylor (9) with minor modifications. The aqueous effluent from the cartridges were assayed for hydrogen peroxide using either homovanillic acid/horse radish peroxidase with fluorescence excitation recorded at 315 nm and emission at 425 nm (14), phenol red/horse radish peroxidase, or non-enzymatically with triiodide as previously described (9).

Mutation assays were performed with the Ames reversion test using tester strain TA102 as previously described by Minnunni et al. (15) except that the standard plate test was used. Revertants were normalized to 100% cell survival. The cell survival was determined by adding TA102 to a saturated culture of TA100 to give a final concentraton of approximately 5000 TA102 per ml of TA100. In addition to the culture of TA102 used to determine reversions, this mixed culture was used to determine survival by plating on minimal plates containing tetracyclin. The anti-mutagenic activity of coffee was shown by co-incubating instant coffee with 1 µmol t-butylhydroperoxide which induced 2100 revertants when assayed alone (normalized for 100% cell survival).

Ethyl linoleate (LAEE) was oxidized with Fentons reagent by a methods adapted from Tamura et al. (16) and lipid peroxidation was measured by measuring the malondialdehyde (MDA) relased following reaction with 2-thiobarbituric acid (TBA). The MDA-TBA adduct was measured by HPLC using tetrabutylammonium sulphate as an ion pairing reagent (17).

Results

Freshly prepared instant coffee contains small amounts of hydrogen peroxide (< 50 µM). However, incubation of the brew over one hour at 37 °C in an open flask results in a time dependent increase in hydrogen peroxide and reached 400 µM. The kinetics of hydrogen peroxide formation was similar for all brands tested (N=4) and was independent of caffeination. Hydrogen peroxide levels were similar when measured by each of the three methods and these values are comparable to those reported by several other investigators (2-7). Hydrogen peroxide generation is dependent upon atmospheric oxygen. When coffee was incubated at 37 °C over one hour under an atmosphere of argon, there was little evidence of hydrogen peroxide formation (Figure 1). However, the peroxide content in the control increased from *ca.* 30 to 275 µM. Purging the sample with oxygen during incubation resulted in a significant increase in hydrogen peroxide which could already be detected at T_0 at levels approaching 300 µM. Thus, oxygenation has a pronounced influence on hydrogen peroxide formation.

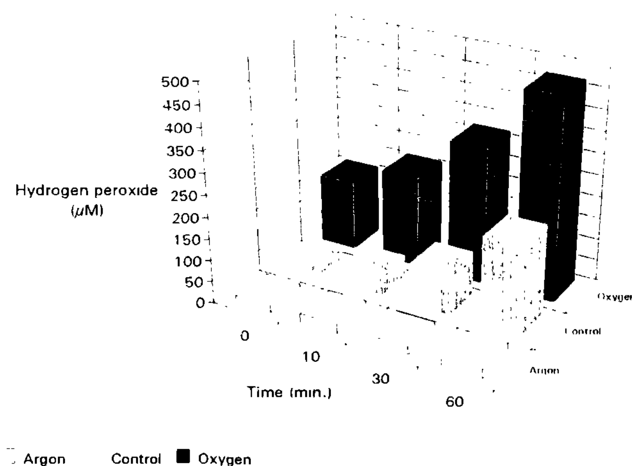

Figure 1. Hydrogen peroxide formation in instant coffee as a function of time and atmospheric oxygen

A dose-dependent increase in both toxicity and mutation were induced when coffee was assayed above 10 mg per plate with *Salmonella typhimurium* (Figure 2). However, these effects were abolished when catalase was added to the incubation mixture. Similar findings were reported by Nagao et al. (2). These results demonstrate that hydrogen peroxide is primarily responsible for the *in vitro* biological activity of coffee.

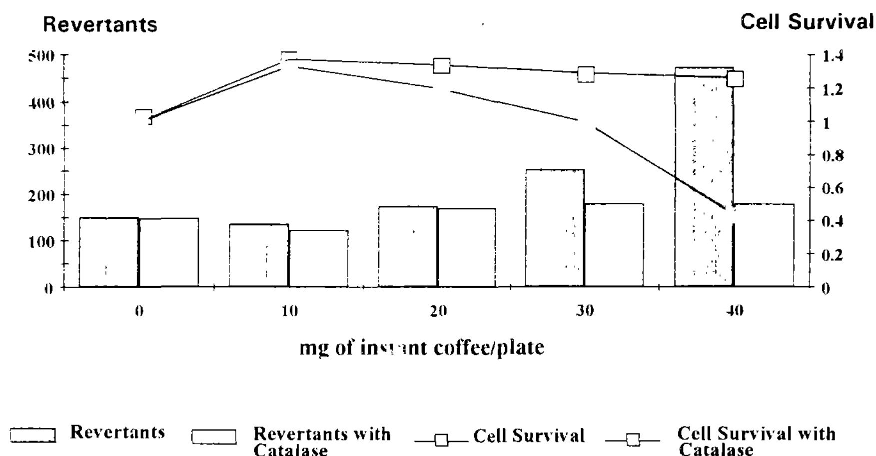

Figure 2. Genotoxicity of coffee in presence and absence of catalase

Potent anti-mutagenic effects of coffee could be seen in the same assay when coffee was incubated at lower dose levels (Figure 3) in combination with tert-butylhydroperoxide, an organic hydroperoxide, which causes radical damage to DNA (15). Under these assay conditions coffee was a potent anti-oxidant and suppressed both the mutagenicity and toxicty induced by this peroxide. Antimutagenic effects of coffee were seen at the lowest dose tested (2.5 mg) which indicates that on a per weight basis the antioxidative potency of coffee approaches that seen for rosemary extracts (15)

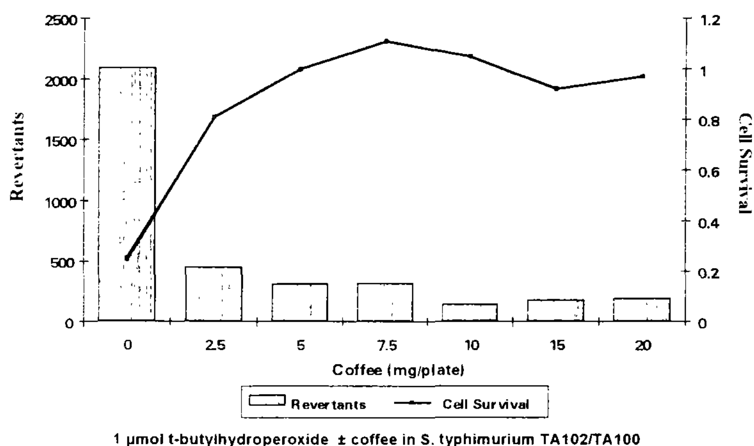

Figure 3 Protective effect of instant coffee against t-butylhydroperoxide genotoxicity

Instant coffee brew also inhibits the lipid peroxidation of ethyl linoleate in a model system using MDA formation as an endpoint (Figure 4). At the lowest dose tested (10 µg of instant coffee/ml incubation), the antioxidant effects of instant coffee were comparable to vitamin E. The anti-oxidant effect was dependent upon dose and at concentrations above 100 µg/ml, instant coffee was a pro-oxidant. In contrast, vitamin E displayed antioxidant activity at all doses tested.

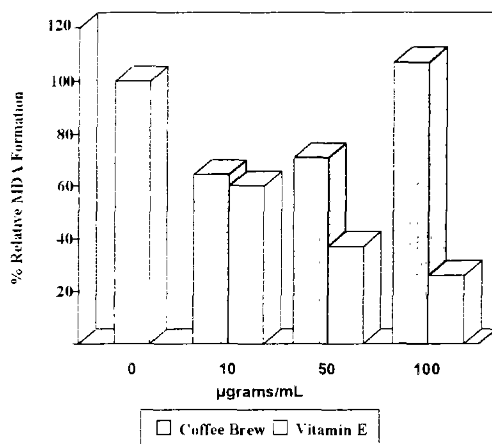

Figure 4. Antioxidant effects of instant coffee and vitamin E in ethyl linoleate lipid peroxidation

Discussion

There is an abundance of sound toxicological data which demostrates that coffee is safe when consumed in moderation (1). The safety is supported by long term feeding studies which show that coffee has no adverse effects when given as part of the daily diet and actually decreases spontaneous tumor development (12,13). However, numerous studies *in vitro* have shown that coffee possesses mutagenic activity (1-3,5,6,8). This mutagenic activity is abolished by catalase and this indicates that much of the genotoxicity is caused by hydrogen peroxide (2,3,11). Hydrogen peroxide is also generated in other beverages such as tea and wine and has been implicated in their direct-acting mutagenicity (6,18). The formation of hydrogen peroxide in all of these beverages occurs through an auto-oxidation process where phenolics reduce atmospheric oxygen in the presence of transition metal catalysts (10). Notably, the oxidative damage induced by these beverages *in vitro* and their biological relevance for human health has not been adequately evaluated. Hydrogen peroxide is continuously formed in our bodies as a by-product of metabolic processes and humans possess a battery of defense mechanisms for protection against oxidative damage (19,20). Consequently, the biological data obtained *in vitro* must be interpreted with caution.

We have examined other chemical and biological endpoints to determine if coffee can also serve as an antioxidant. Our findings reveal that coffee inhibits lipid peroxidation, free radical DNA damage and mutagenesis. These results are highly dependent upon concentration, atmospheric oxygen and the endpoints used for measurement. Consistent with these findings, polyphenols have also been shown to have a protective role in cytotoxicity induced by hydrogen peroxide *in vitro* (21). Thus, coffee, like many antioxidants, has a dual role *in vitro* and can exert both beneficial and detrimental effects.

Investigations on cancer risk in humans have not provided any strong evidence that coffee intake is correlated with increased risk for any of the common cancers (22,23). In fact, there is evidence that coffee consumption has a protective effect against colorectal cancer (23,24). Studies of coffee and lower urinogenital tract or bladder cancer have given conflicting results, with both positive and negative associations reported (24). In many of the studies which found positive associations between coffee intake and cancer risk, the researchers have stressed that there may be confounding factors such as cigarette smoking. However, a recent report by Viscoli et al. (25) which summarized the case-control studies on bladder cancer and coffee drinking concluded that "the best available data do not suggest a clinically important association between the regular use of coffee and development

of cancer of the lower urinary tract in men or women". Thus, additional studies are warranted to better evaluate the pro- and antioxidant effects of coffee. For instance, elucidating the mechanism of the antioxidant and anticarcinogenic activities of coffee should aid in assessing the significance of biological results obtained *in vitro* and *in vivo* and consequently provide additional evidence that moderate coffee consumption does not present a health risk and in addition, may have beneficial effects.

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Abstract

Despite the abundance of sound toxicological data demonstrating the safety of coffee, controversy remains due to the presence of chemicals present in coffee which are mutagenic when assayed *in vitro*. Much of this biological activity appears to be attributed to hydrogen peroxide and methylglyoxal which are also produced in our bodies as by-products of metabolic processes. We have investigated hydrogen peroxide formation in prepared instant coffee and elucidated mechanisms for its formation. Based upon model systems, hydrogen peroxide formation appears to result from polyphenolic thermal degradation products of chlorgenic and caffeic acid and formation is catalyzed by transition metals. Although it is clear that coffee causes oxidative damage *in vitro*, we have also seen that coffee displays anti-oxidant activity and acts as a free radical scavenger. At low doses, coffee can suppress the *in vitro* mutagenicity of oxidants such as tert-butyl-hydroperoxide and can also inhibit lipid peroxidation and malondialdehye formation. Thus, coffee, like many anti-oxidants has both beneficial and detrimental effects *in vitro* and these effects are dose dependent. Two long-term feeding studies in rodents that were given coffee as part of their daily diet have shown that animals given coffee had longer lifetimes and a concomitant decrease in spontaneous tumors. These benefical effects may be attributed in part to polyphenols in coffee which served as anti-oxidants. Thus, the results obtained from biological assays *in vitro* and their implications on health and safety should be interpreted with extreme caution. Future studies will be performed *in vivo* to further evaluate the pro- and anti-oxidant effects of coffee.

En dépit de l'abondance des résultats en toxicologie démontrant que le café est un produit sûr, la controverse existe toujours en raison de la présence dans le café des produits chimiques qui sont mutagènes lors d'essais in-vitro. Cette activite biologique peu apparemment être en grande partie attribuée à l'hydrogène peroxyde et au methylglyoxal, lesquels sont egalement produits dans l'organisme comme intermédiaires des processus métaboliques. Nous avons étudié et élucidé le mécanisme de la formation d'hydrogène peroxyde dans le café instantané. En se basant sur des systèmes de modélisation, la formation de l'hydrogène peroxyde apparaît comme résultant de la dégradation thermique des acides chlorogenique et cafféique en produits polyphenoliques, et les métaux de transition sont des catalyseurs de cette réaction. Comme il est clair que le café provoque des dommages oxydatifs in-vitro, nous avons également vu que le café montre un pouvoir antioxydant et agit comme un capteur de radicaux libres. A faibles doses, le café peut supprimer in-vitro l'activité mutagénique des oxydants tels que le ter-butyl-hydroperoxyde, il peut aussi inhiber la peroxidation des lipides et la formation de malonaldéhyde. Cependant, le café, comme beaucoup d'antioxydants, a aussi bien des effets bénéfiques que nuisibles in-vitro, ces effets étant dépendants de la dose. Deux études nutritionnelles à long terme chez les rongeurs qui ont reçu du café comme composant de leur diète quotidienne ont démontrés une plus grand longévité et une concomitant baisse de tumeurs spontanés. Ces effet bénéfique pourraient être attribué d'une part à des polyphenols du café, qui agissent comme antioxydants. Par conséquent, des résultats obtenus des essais biologiques in-vitro et leurs implications sur la santé et sécurité doivent être interprété avec la plus grande prudence. Des futures études in- vivo vont être entrepris pour évaluer des effets pro- et antioxydant du café.

CAFFEINE CONSUMPTION AND METABOLISM IN PREGNANT WOMEN DURING THE THIRD-TRIMESTER PREGNANCY

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INTRODUCTION

Caffeine is a widely consumed natural ingredient of many foodstuffs and beverages, although individual consumption patterns show large variations. Chronic caffeine exposure in humans has not been associated with long term ill effects. Several studies have examined the effects of caffeine exposure on mothers, fetuses and newborns during and after normal pregnancies (Kirkinen et al., 1983; Martin and Braaken, 1987; Fenster et al., 1991; Linn et al., 1982; Barr and Streissguth, 1991). While the results of these studies have differed, they have not demonstrated significant and consistent impact on the maternal or perinatal outcomes which were considered. This finding might result from the lack of sensitivity in the study methodologies used and / or from the large, uncontrolled individual differences in the behavior, nutritional status and metabolic capacity of the patients involved.

Caffeine metabolism and elimination appears to be impaired during normal pregnancy (Aldridge et al. 1981; Knutti et al. 1981; Brazier et al. 1983). As a result, caffeine levels in the maternal and fetal compartments can become quite elevated when compared to those in normal nonpregnant individuals. Therefore, any efforts which attempt to relate the effects of maternal caffeine intake throughout pregnancy must account for the altered disposition of caffeine which occurs in pregnancy.

Our current study differs from those previously cited in a number of ways. First, all previous studies evaluated acute effects following maternal intake of a standard caffeine dose; in the present work, caffeine was not administered during the observation period. Next, we evaluated the fate of caffeine and its metabolites in maternal plasma and urine every two weeks. The metabolic capacity for caffeine was assessed with urinary ratios of caffeine metabolites (Kalow and Tang, 1993), and determinations of plasma levels of caffeine, paraxanthine, theophylline and theobromine. Caffeine half-life was also calculated. These biochemical parameters might be expected to constitute a better estimate of caffeine exposure for the fetus than caffeine intake by dietary inventory, since large, individual differences in their plasma levels might occur irrespective of reported intake. The most important goal of this study was track and compare the course of caffeine metabolism in normal third trimester pregnancy women until delivery or attainment of 40 weeks' gestation.

METHODS

Subjects

Twenty non-smoking pregnant women were enrolled at the Medical College of Georgia Hospitals and Clinics. They had a normal gestation without medical or obstetric complications and received no medication except the administration of prenatal vitamins. From the dietary questionnaires (Fenster et al., 1991) completed at enrollement and at the end of the study, the patients daily caffeine consumption was evaluated at the end of the study. They were classified as high consumers when caffeine intake was higher than 500mg/day and low consumers when caffeine intake was lower than 200mg/day.

Experimental Protocol

Observation of fetal behavior as described in detail in a previous paper (Devoe et al., 1993) was performed at 30 weeks' gestation and was repeated every two weeks, at 32, 34, 36, 38 and 40 weeks. For each observation, patients were asked to bring to the hospital their urine collected over the previous 24 hours. After an hours rest, maternal blood samples were taken at the beginning and end of each fetal behavioral observation which was conducted for 2 hours.

HPLC Analysis of Caffeine Metabolites

Urine samples (0.3ml) were saturated with 120mg ammonium sulfate and extracted with 6ml chloroform-isopropanol (9:1,v/v). The two phases were vortexed for one minute and the organic phase was separated by 10 minutes centrifugation at 2000rpm. The organic extract was then evaporated to dryness at 40°C with a stream of dry nitrogen. The dried residue was solubilized in 0.5ml of 0.05% acetic acid solution and 0.1ml was injected on a HPLC. The same extraction procedure was applied for plasma samples except that ammonium sulfate saturation was not necessary and that extraction and centrifugation times were reduced to 30 seconds and 5 minutes, respectively. The internal standard used to calculate extraction recovery was 7-Ethyl-theophylline. Caffeine, paraxanthine, theophylline and theobromine were separated on a reverse phase column (Nucleosil 5-C18, Macherey-Nagel) and the separation was obtained isocratically with a solution of 0.05% acetic

acid containing 35% methanol. The flow rate of the eluant was fixed at 1ml/minute and the metabolites were detected in UV at 272 nm. All the urinary metabolites: caffeine, dimethylxanthines, monomethylxanthines, dimethyluric acids, monomethyluric acids and 5-Acetylamino-6-formylamino-3-methyluracil (AFMU) were separated on the same column but using a gradient described in **Figure 1**. The concentrations of these metabolites were calculated from standard calibration curves.

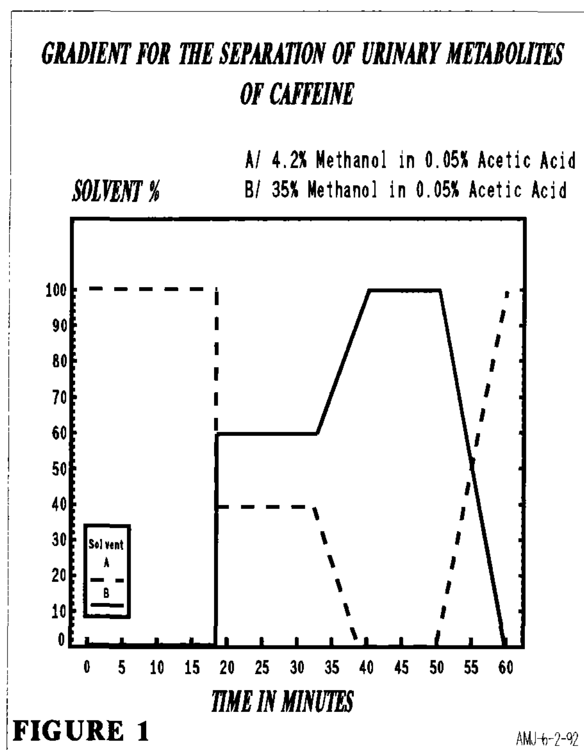

FIGURE 1

OBSTETRIC CHARACTERISTICS OF THE STUDY POPULATION

	High Caffeine	Low Caffeine	Significance
Number of Patients	10	10	--
Maternal age (years)	23.8±5.6 (17-31)	26.2±7.2 (18-37)	p=0.001
Gestational age at birth (weeks)	38.9±1.2 (37-40)	39.1±1.0 (37-40)	p=0.10
Caffeine Intake (mg/day)	564±15 (238-944)	70±68 (0-195)	p=0.0002

TABLE 1

RESULTS

Table 1 shows the obstetric characteristics and mean daily caffeine intake, estimated from dietary questionnaire, of the high (10 subjects) and the low (10 subjects) caffeine consumers during pregnancy. Mean maternal age was significantly higher in the group of low caffeine consumers. Individual caffeine intake ranged widely in both high (238-944mg/day) and low (0-195mg/day) caffeine groups. According to dietary questionnaires, patients did not apparently change their patterns of caffeine consumption during the period of the study.

During the third trimester, the mean plasma caffeine concentrations differed significantly between the high and low caffeine groups (**Figure 2a**). However, some subjects in the low caffeine group showed higher maternal plasma caffeine concentrations than subjects in the high caffeine group (**Figure 2b**). The highest maternal plasma caffeine concentration noted was 6mg/l. **Figure 2b** also

shows that important intra-subject variations occur in plasma caffeine concentrations, especially for subjects in the high caffeine group. These important differences could be explained by the daily variations in caffeine consumption which cannot be detected with routine dietary questionnaires but which can be estimated from the total metabolites excreted in a 24 hour urine sample. Plasma concentrations can also be modified by the well-recognized impairment of caffeine metabolism during pregnancy. Again, a single measurement of plasma caffeine levels cannot assess this metabolic alteration as well as can be accomplished through the estimation of caffeine half-life.

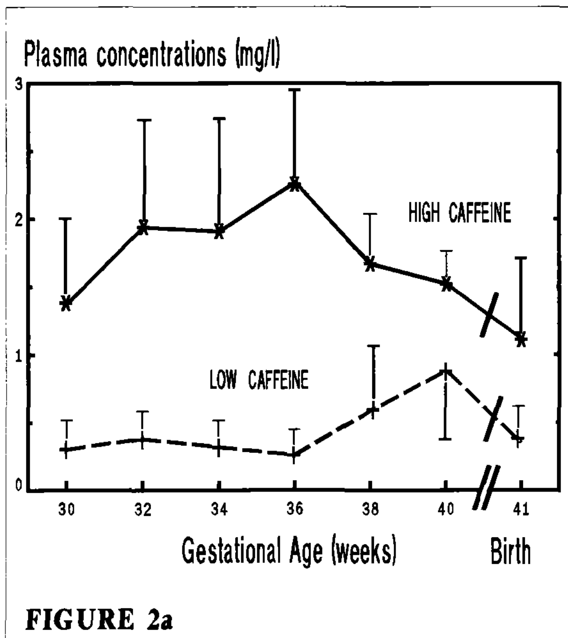

FIGURE 2a

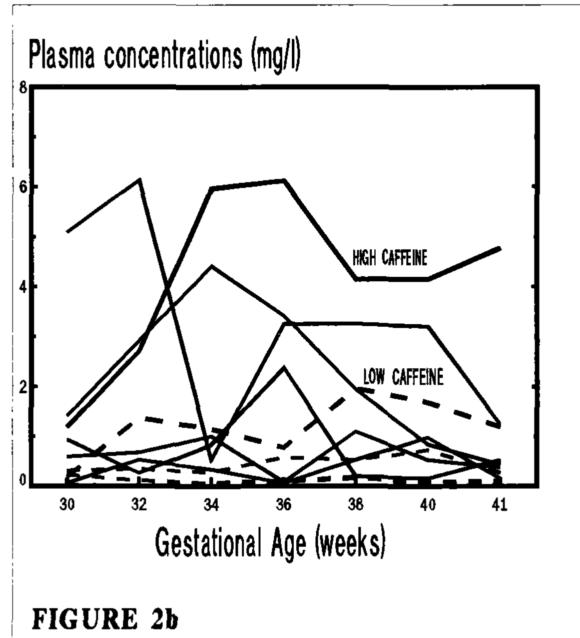

FIGURE 2b

Caffeine half-lives, calculated from the two maternal plasma samples obtained before and after the fetal behavioral test are shown in **Figure 3.** Mean caffeine half-life increased from 7.4 hours at 30 weeks' gestation, peaked at 11-12 hours at 36-38 weeks' gestation and returned to 6.5 hours at 40 weeks' gestation. **Figure 3** also shows caffeine half-lives in two patients whose initial values were similar at 30 weeks' gestation (3 hours 15 minutes vs 3 hours 57 minutes). Patient 9 was typical of most pregnant women, and her caffeine half-life increased to more than 15 hours. However, patient 2 showed no significant changes in caffeine half-life, ranging from 2 hours to 4 hours throughout the study period. Therefore, while most pregnant women exhibit a prolongation in caffeine half-life, we have a first, unique case report for unchanged caffeine metabolic capacity in a pregnant woman. Such wide individual variations indicate clearly that specific characterization of subjects' metabolism of caffeine is critical when behavioral or epidemiologic studies of caffeine are undertaken in pregnancy.

Caffeine intake on the day preceding the fetal behavioral study was estimated through the quantitative analysis of total caffeine and caffeine metabolites in 24 hour urine sample. Simultaneous presentation for a typical high groupe patient's caffeine intake as determined by urinary excretion, caffeine half-lives and plasma caffeine concentrations is shown in **Figure 4.** In spite of impaired caffeine elimination due to increased caffeine half-life, caffeine plasma concentrations in this patient remained at low levels due to reduced caffeine intake. Such results suggest that most patients in the high caffeine intake group may have reduced their caffeine intake as caffeine elimination was prolonged with increasing gestational age.

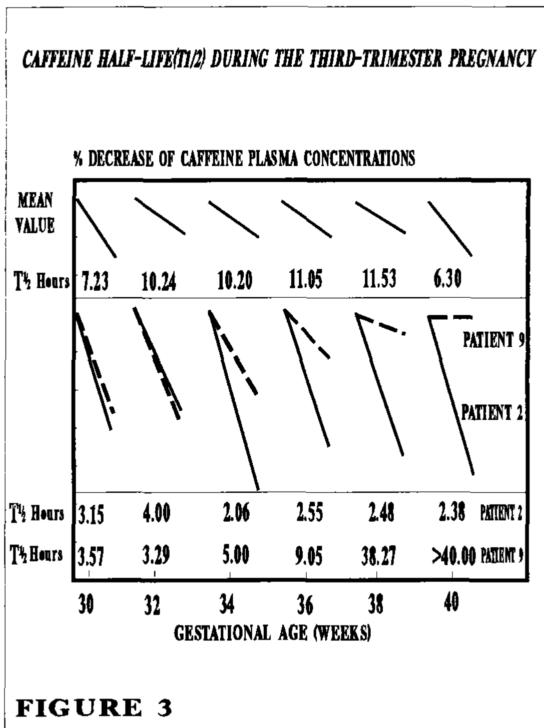

FIGURE 3

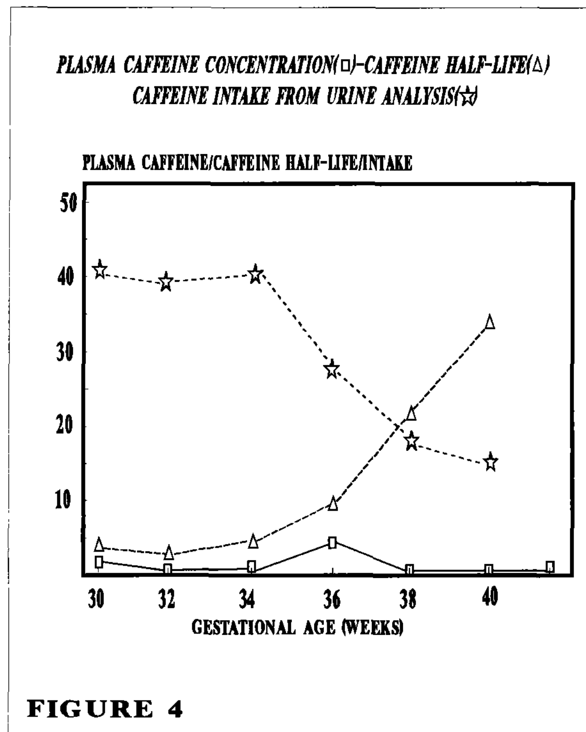

FIGURE 4

The ratios of urinary metabolite concentrations were shown to represent indexes of acetylation phenotypes (AFMU/1MX), 3 and 7-demethylation (paraxanthine/caffeine), xanthine oxidase (1methyluric acid/1methylxanthine) and microsomal P-450 8-hydroxylation (1,7-dimethyluric acid/paraxanthine) (Arnaud, 1993). These indexes exhibit large changes in some patients while remaining stable in other patients during the third-trimester pregnancy according to the modification of caffeine metabolism observed. The impairment of caffeine metabolism was more marked in patients with a low index of caffeine 3-demethylation. A 12 fold inter-individual difference in this index was recorded in this study and one of the highest value was found in the patient who did not exhibit any change in her caffeine half-life during the whole pregnancy (**Figure 5**).

DISCUSSION & CONCLUSIONS

Most previous studies of the effects of caffeine intake on pregnant women and their infants have been investigated using responses to a single administration of a high dose of caffeine (McGowan et al.,1987; Salvador and Koos,1989). While this approach confers good control over the dose given and the plasma levels obtained, it does not account for the capacity of subjects to metabolize caffeine or the effects of chronic caffeine ingestion throughout pregnancy.

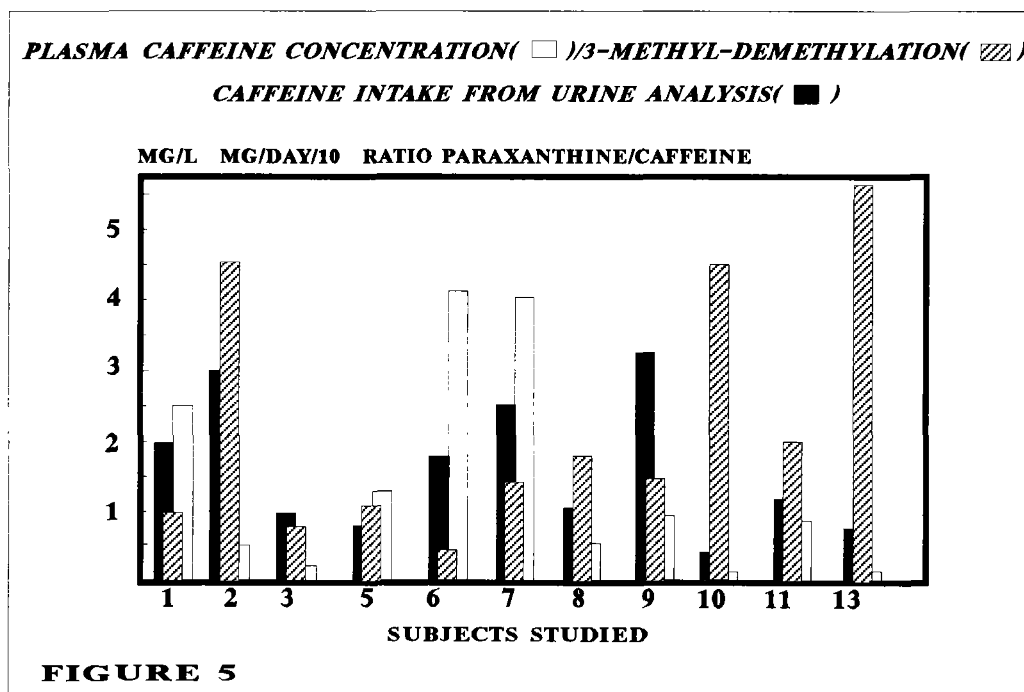

FIGURE 5

This study is the first to report caffeine consumption during the last trimester of pregnancy using a dietary questionnaire as well as the plasma and urinary analyses of caffeine and its metabolites. In previous studies, the reliability of self-reporting has been questioned. Kennedy et al (1991) found no significant differences between plasma caffeine levels for categories of young, healthy volunteers according to reported low, intermediate or high caffeine intake. Better correlations of estimates of caffeine intake were obtained by measurements of caffeine and / or paraxanthine in saliva from afternoon samples (taken at 5:00 P.M.) (James et al.,1988; James et al.,1989). From the results of our study, showing large individual variations in the metabolic capacity of caffeine and the continuous change of its metabolism during the third trimester pregnancy, it is important that physiological studies on caffeine will include in the futur, more accurate evaluation of caffeine intake as well as the metabolic characterization of the patients studied. After a first comparative analysis of the fetal behavior in the two consumer groups, it will be interesting to reanalyse again the data in relation to the plasma levels found during the observation period. A better correlation

would suggest that plasma levels are more important than long-term caffeine ingestion on the behavioral effects studied.

A major question raised by studies such as ours is the relative risk for fetal health associated witn the plasma caffeine levels observed in our patients. The highest value which we determined (6mg/l), after an overnight fast can be extrapolated back to a plasma concentration of 14 mg/l at 5:00 P.M. the preceding day, assuming a typical caffeine half-life of 12 hours. This value falls within the therapeutic range of 8-20 mg/l recommended for the treatment of neonatal apnea. Further, Aranda et al (1980) found no adverse neonatal effects associated with plasma levels as high as 50 mg/l. We can conclude that caffeine intake, even for those pregnant women whose daily consumption is highest and whose metabolic capacity is most impaired, would be unlikely to result in excessive or hazardous fetal plasma caffeine levels. Our data also suggest that some women will decrease their caffeine intake during the third trimester, either intentionally or unintentionally. Should any pregnant woman wish to control her caffeine plasma levels, while retaining her enjoyment of coffee or tea, substitution of decaffeinated beverages would be recommended.

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ABSTRACT

A longitudinal cohort study of 20 normal third-trimester pregnancies was performed on two groups of pregnant women categorized by dietary history as "high" caffeine consumers (> 500mg/day) and as "low" caffeine consumers (< 200mg/day). Between 30 and 40 weeks biweekly 2-hour continuous ultrasonographic observations of fetal heart rate, breathing activity as well as eye,trunk and extremity movements were conducted. Maternal caffeine and methylxanthines plasma levels were analyzed before and after each session and 24 hour urinary caffeine metabolites were identified and quantified. From these data, acetylation phenotypes, indexes of 3-methyl demethylation and caffeine half-lives were calculated. Results on fetal behavior have already been described (Am. J. Obstet. Gynecol.,1993,168,1105-1112) and this study reports the metabolic characterization of these pregnant women.

Prolonged caffeine half-lives, above 10 hours, were observed for all women except one showing values from 2 to 4 hours for the 7 sessions. This unchanged half-life can be explained by the very high index of caffeine demethylation observed in this subject.

Low plasma levels are found in very low caffeine consumers while a better prediction of plasma level is obtained in the high consumer group and also in several subjects of the low consumer group when the intakes as well as the capacity of caffeine metabolism of the subjects are considered.

In spite of extremely large individual variations in caffeine intake and metabolism in these women during the last trimester of pregnancy, some subjects showed a decreased intake when caffeine half-lives were prolonged to maintain or even decrease plasma concentrations. However, such a control does not seem to be effective for some individuals.

In conclusion, large variations in caffeine intake and metabolism are observed in women during the last trimester of pregnancy. To establish any correlation between caffeine and some physiological effects, plasma or saliva caffeine levels and urinary metabolites must be analyzed to eliminate the inaccuracy in the evaluation of dietary intake and to estimate the individual variability in caffeine metabolism and clearance. Although safe plasma caffeine levels were

observed in this study, pregnant women are advised to drink decaffeinated coffee and beverages to have a better control of caffeine plasma level while enjoying their dietary habit

RESUME

Une étude longitudinale de cohorte a été effectuée sur 20 femmes au cours des trois derniers mois de grossesse. Celles-ci étaient classées d'après leurs habitudes alimentaires, en deux groupes de forte (> 500mg/jour) ou de faible consommation (< 200mg/jour). Entre la 30ème et 40ème semaine, et chaque deux semaines, l'observation du rythme cardiaque, de la respiration ainsi que des mouvements des yeux, du tronc et des extrémités a été réalisée pendant 2 heures, à l'aide d'ultrasons. Les analyses des concentrations plasmatiques maternelles de la caféine et de ses métaboliques ont été obtenues avant et après chaque observation clinique et la totalité des métabolites urinaires de la caféine excrétés le jour précédent, ont été identifiés et quantifiés. A partir de ces résultats, les phénotypes d'acétylation, les indexes de 3-méthyl déméthylation et les demi-vies ont été calculés. Les résultats sur le comportement du foetus ont déjà été publiés (Am. J. Obstet. Gynecol., 1993,168,1105-1112) et cette étude présente les caractéristiques métaboliques de ces femmes enceintes.

Une prolongation de la demi-vie de la caféine au delà de 10 heures a été observée chez tous les sujets à l'exception d'une femme dont les valeurs se sont maintenues entre 2 et 4 heures pour les 7 observations cliniques. Cette surprenante constatation peut être expliquée par l'indexe très élevé de la déméthylation identifié chez cette femme.

De très bas niveaux de caféine sont trouvés dans les échantillons plasmatiques des très faibles consommateurs. Pour les forts consommateurs ainsi que pour une importante proportion de faibles consommateurs, une meilleure estimation des valeurs plasmatiques est obtenue en prenant en compte, en plus de la consommation, les capacités individuelles de métabolisme des sujets.

En dépit de l'extrême variation de la consommation de caféine et de son métabolisme chez chaque sujet, durant le dernier trimestre de gestation, il a été observé une diminution de la consommation lorsque la demi-vie de la caféine était prolongée, ce qui avait pour effet de maintenir ou même de diminuer les concentrations plasmatiques. Ce contrôle semble toutefois absent chez certains sujets.

En conclusion, de très grandes variations de consommation et de métabolisme de la caféine ont été observées chez des femmes durant le dernier trimestre de gestation. Afin d'établir des corrélations entre la caféine et des effets physiologiques, il est indispensable d'analyser les concentrations plasmatiques ou salivaires de la caféine ainsi que ses métabolites urinaires, qui expliquent les variations individuelles observées et permettent de corriger les évaluations imprécises des apports évalués par des questionnaires. Bien que les concentrations plasmatiques de caféine trouvées dans cette étude ne présentent aucun risque pour le foetus, on ne peut que recommander aux femmes enceintes de consommer du café et des boissons décaféinés afin de maintenir au plus bas les niveaux plasmatiques tout en préservant le plaisir que leur apporte leurs habitudes alimentaires.

OCHRATOXIN A (OTA) IN GREEN AND ROASTED COFFEE BEANS

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Introduction

The mycotoxin ochratoxin A (OTA) derives its name from Aspergillus ochraceus, the mould from which it first was isolated. It is the main toxic component in cultures of this mould, yet it is also produced by other ubiquitously found moulds such as in various other strains of Aspergillus and Penicillium. The fact that OTA is produced by a variety of moulds, many of which are also found on corn, maize and cereal crop in more temperate climates such as Western Europe (Hamilton et al., 1982), as well as the suggestion of a link between OTA and human Balkan Endemic Nephropathy (BEN) (DFG, 1990), resulted in an increased request for a better database on the presence of OTA in foodstuffs and a demand to characterise the potential health risk associated with the daily exposure of humans to OTA. One of the foodstuffs that had gained attention with regard to contamination with OTA is coffee. OTA has been detected in green and roasted coffee beans (Cantafora et al., 1983, Tsubouchi et al., 1987 and 1988, Micco et al., 1989). Yet inconsistent results have been published with respect to the influence of the roasting process on the OTA content and the transfer of OTA into the coffee brew Steigmeier et al., 1991). It is the intention of this paper not only to present new data regarding the contamination of green coffee beans with OTA and the effects of the roasting process on the OTA content in roasted coffee beans and the corresponding coffee brew, but also to attempt a preliminary risk assessment of OTA in the light of every day coffee consumption.

Toxicology and Carcinogenicity of OTA

OTA consists of a dihydroisocumarin moiety linked through its 7-carboxyl group by an amid bond to one molecule of L-β-phenylalanin (Fig. 1). The toxicity of OTA is characterised primarily by its high nephrotoxicity. Indeed, OTA induced nephrotoxic effects in all mammalian species tested so far (pig, chicken, mouse, rat, dog), although the sensitivity of the different species varied (Kuiper-Goodman and Scott, 1989). Also regarding the kinetic data big differences among the various species were observed (Hagelberg et al., 1989).

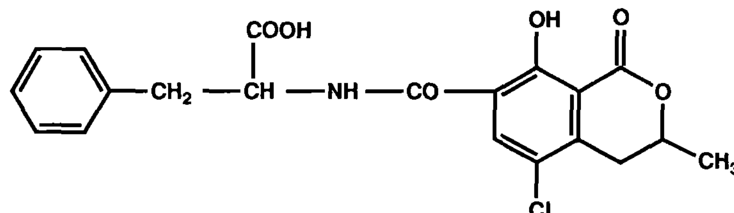


Fig.1: Ochratoxin A

The ochratoxigenic nephropathy of pigs is a well known and often observed disease (Krogh et al., 1976). A disease of poultry related to OTA contaminated feed as well as outbreaks of a similar disease involving the death of a high number of turkeys have been reported in the USA and were causally related to OTA contaminated corn (Harwig et al., 1983). Of interest with regard to human risk assessment is the carcinogenicity (Tab. 1) in absence of mutagenicity and the nephrotoxicity of OTA.

Table 1: OTA induced kidney tumors (adenomas + carcinomas) in 2 year mouse and rat bioassays

Mouse C57BL/6JxC3H	(Bendele et al.,1985)					
	Adenomas			Carcinomas		
OTA (µg/kg bw)	0	120	4800	0	120	4800
male	0/50	0/47	26/50	0/50	0/47	14/50
female	0/50	0/47	0/50	0/50	0/47	0/50

Rat F344	(Boorman, 1989, Technical Report Series No. 358)							
	Adenomas				Carcinomas			
OTA (µg/kg bw)	0	21	70	210	0	21	70	210
male	1/50	1/51	6/51	10/50	0/50	0/51	16/51	30/50
female	0/50	0/51	1/50	5/50	0/50	0/51	1/51	3/50

According to the male rat data, OTA must be regarded as one of the most potent non-genotoxic carcinogens known to date. Male rats presented with a ten-fold higher renal carcinoma incidence than females and more importantly, this at a OTA concentration of 210 µg OTA/kg bw per day for 52 weeks (Boorman, 1989). In addition one is struck by the fact that despite receiving the enormous dose of 4800 µg OTA/kg bw per day for almost their life time female mice do not produce renal tumors, yet male mice present with a renal tumor incidence of 80% under comparable conditions (Bendele et al., 1985).
Induction of renal tumors specially in male rats by non-genotoxic compounds has recently been linked to an increased level of the male rat specific urinary protein $\alpha_{2\mu}$-globulin (Swenberg et al., 1992, Dietrich and Swenberg, 1991). As humans do not synthesise this urinary protein, it was recently ruled by the EPA Risk Assessment Forum (EPA, 1991) that tumor data in male rats in conjunction with increased level of $\alpha_{2\mu}$-globulin are not to be considered as a basis for human cancer risk assessment. In view of the fact that OTA is non-genotoxic, binds to a protein of 20kD size, and induces the highest incidence of renal tumors in male rats, the question was asked whether OTA potentially increases the renal tumor incidence via the $\alpha_{2\mu}$-globulin mechanism. Male and female rats were treated orally with 1mg OTA/kg bw per day or with 150mg d-limonene/kg bw per day for 7 days. As depicted in table 2 OTA did not induce increased cell proliferation in either sex of rats and neither was there an accumulation of $\alpha_{2\mu}$-globulin in OTA treated male rats, whereas the contrary was the case in the positive control treated with d-limonene (Rásonyi et al., 1993). Therefore we concluded that the P2 renal tubulus cells are not a target for OTA and that the male rat urinary protein $\alpha_{2\mu}$-globulin has no role in the OTA induced renal tumors of male rat.

Table 2: Cell proliferation of the proximal tubulus cells (P2 segment) and $\alpha_{2\mu}$-globulin accumulation in kidney of OTA and d-limonene treated male and female F344 rats.

	P2 cell proliferation	
	Male	Female
Control	7.0 ±1.2%	7.0 ± 0.7%
d-limonene	20.0 ± 6.6%*	22.0 ± 5.0%*
OTA	7.0 ± 1.9%	6.0 ± 2.0%

	$\alpha_{2\mu}$-globulin - immunohistochemistry	
	Male	Female
Control	1-2	0
d-limonene	4-5	0
OTA	2	0

(Grade scale for area and intensity of $\alpha_{2\mu}$-globulin immunostaining: 0 absent; 1 present; 2 mild; 3 moderate; 4 severe; 5 markedly severe)
*significantly higher ($p<0.05$ two-way ANOVA) than the corresponding controls and OTA exposed group

OTA was suggested as one of many causative agents or even the causative agent in the etiology of Balkan Endemic Nephropathy (BEN) and the also increased incidences of human urothelial tract tumors in the same area (Anonymous, 1990). Table 3 depicts some of the etiologic features of BEN as well as some facts supporting or contradicting the notion that OTA may be the causative agent. The suggestion that OTA may be a factor involved in the urothelial tract tumors observed in the endemic areas of BEN is also quite problematic. In male rats and mice, OTA induced renal epithelial tumors of

the proximal region, whereas urothelial tumors are presented in humans. As the mechanism involved in the induction of rodent renal tumors is unknown to date, it is difficult to make an extrapolation to the human situation.

Table 3: Some facts relating OTA to the etiology of BEN (Radovanovic et al., 1989)

Epidemiological Features:

- the disease is known to exist in only three countries of south-eastern Europe (Bulgaria, Romania and Ex-Yugoslavia)
- endemic locations are situated in the vicinity of a river, often in flooded areas;
- clustering of cases within families is one of the most prominent features of the disease;
- there is a mosaic like topographical distribution of cases, with spared households even in the most affected locations;
- an overt clinical picture is absent in children and adolescents: incidence begins to rise in the third decade of life and is proportional to age
- the mortality risk from BEN is not significantly different for males and females
- tumors of the upper urothelium may be even 100 times more frequent in endemic areas than in neighbouring non-endemic areas
- BEN is associated with a rural life, while the native urban population has been spared;
- immigrants to an endemic area may develop the disease after sufficiently long (usually over 20 years) exposure time
- early emigration from an endemic area (in childhood) may prevent the occurrence of BEN

Supporting facts:

- the human pathology is a diffuse fibrosis as it is in the ochratoxigenic porcine nephropathy
- OTA occurs in food of the affected area
- nephropathy villages are topographically predisposed to damp storage conditions and therefore to more mouldy food
- OTA is found in the human sera of people living in the endemic area
- in the affected area there is a very high incidence of urinary tract tumours
- OTA is carcinogenic to rats and mice

Contradictory facts:

- differences in the amount of OTA in foodstuffs from BEN endemic and control villages were not significant
- non impressive and/or inconsistent differences have been obtained by comparison of blood samples of the different areas
- porcine nephropathy was so far not demonstrated in BEN endemic areas
- BEN is not known in parts of the world where OTA affects pig husbandry
- the occurrence of OTA is ubiquitous whereas BEN is only known in one single area of the world.

Occurrence in food

In view of the situation presented above it is important to have a rough estimate of the daily human OTA intake via food. Table 4 represents an overview of some of the food products analysed for OTA contamination and the resulting estimated daily intake of OTA calculated for Germany.

Table 4: Ochratoxin A in food

Type of food	number of samples	%contaminated	mean or range in µg/kg	Reference**
Bread and cereals	1100	10	3.8*	DFG, 1990
Bread and cereals	380	38	0.1 - 1.5	BGA, 1991
Cereals and pasta	77	47	0.5	CLA, 1992
Cereals and corn	31	22	0.2	Tox, 1992
Corn and corn products	1540	5	77.6*	DFG, 1990
Corn and corn products	11	45	0.6	CLA, 1992
Sausages (pork meat)	325	18	0.1 - 0.2	DFG, 1990
Estimated daily intake of OTA via food: ≈ 100 ng/day				**(DFG, 1990)**

* mean of the contaminated samples
**DFG: Deutsche Forschungsgemeinschaft, Germany; BGA: Bundesgesundheitsamt, Germany; CLA: Cantonal Laboratory of the Canton Aargau, Switzerland; Tox: Institute of Toxicology, Switzerland

Occurrence in coffee

As different food items are contaminated with OTA quite frequently, the question was asked what proportion of commercially available green coffee beans are contaminated with OTA and to what extent this contamination could contribute to the daily intake of OTA. From the analysis of 25 commercial green coffee bean samples (table 5), 13 contained detectable amounts of OTA. The arithmetic mean of these samples was 3.5µg/kg (a value of 0.25µg/kg was taken for negative samples, the sample with 56µg/kg was not included since this sample showed a spoiled appearance). Since only a very restricted number of samples were analysed, this figure is by no means representative. Further studies are needed to determine the average contamination level in coffee.

Table 5: Concentrations of OTA in 25 commercial green coffee samples

Origin or type of coffee	total samples	positive samples (> 0.5µg/kg)	µg OA/kg
Columbia	5	3	1.2; 9.8; 9.9
Santos	2	1	7.4
Brasil	3	2	2.0; 4.0
Central america	1	0	
Robusta	3	2	2.2; 3.6
Ivory Coast	2	2	9.9; 56.0
Kenia	3	0	
Guatemala	1	0	
Costa Rica	1	0	
Tanzania	1	1	2.2
New Guinea	1	0	
Zaire	1	1	17.3
unknown	1	1	11.8

The results obtained with HPLC (High performance liquid chromatography) were confirmed by GC-MS (Gas chromatography-mass spectrometry). In Fig. 2 three GC-MS chromatograms are depicted, showing a standard, a highly contaminated sample and a low contaminated sample.

Fig. 2 A: Mass spectrum at retention time 1353": standard, 10ng OTA methylated (corresponding to 500µg OTA/kg coffee

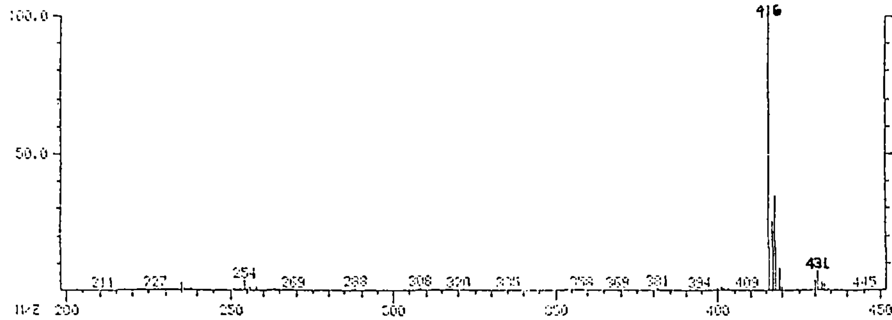


Fig. 2 B: Mass spectrum at retention time 1371": green coffee with 300µg methylated OTA/kg coffee

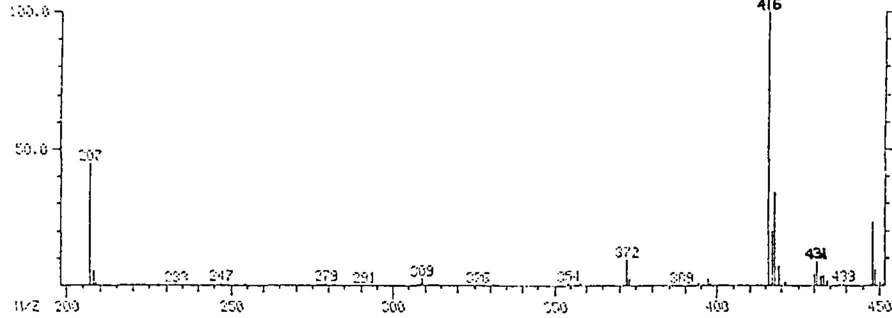


Fig. 2C: Multiple ion detection chromatogram: green coffee with 1.5µg methylated OTA/kg coffee; tracks of the ions 416, 418, 431, 433

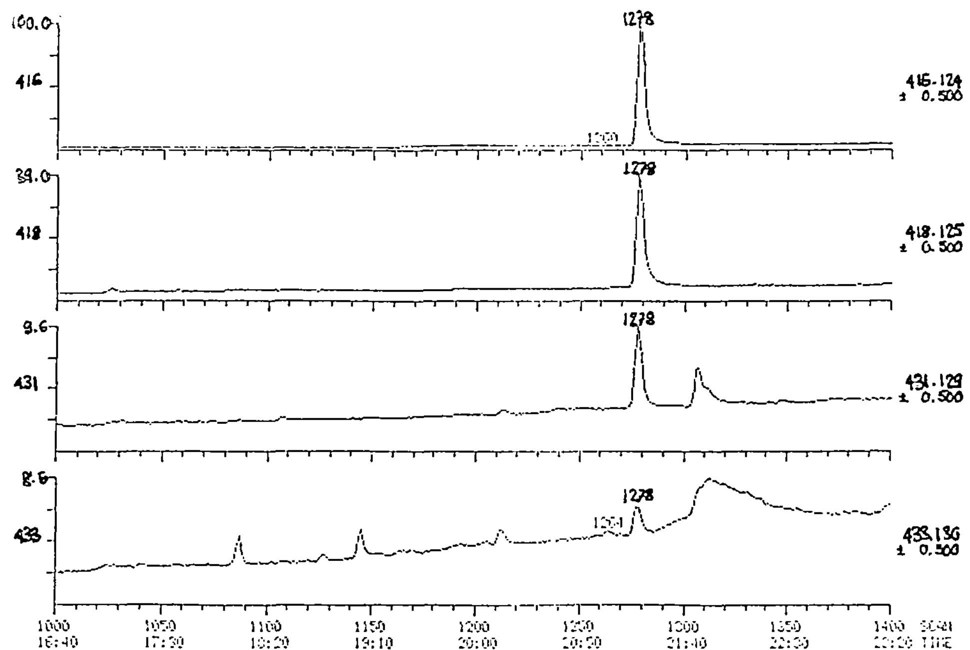
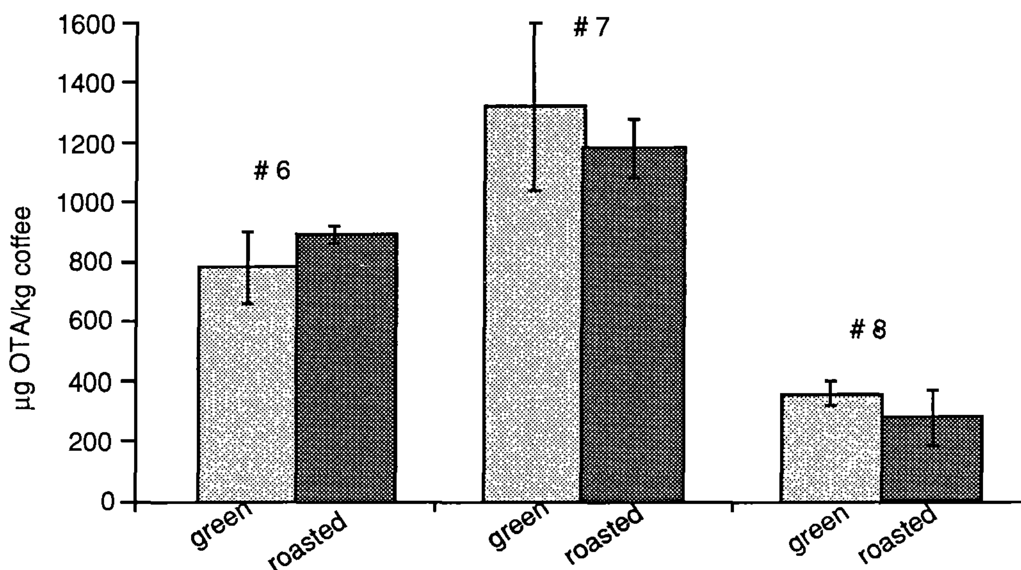


Fig. 3: Destruction of OTA during the roasting process in three highly naturally contaminated coffee samples

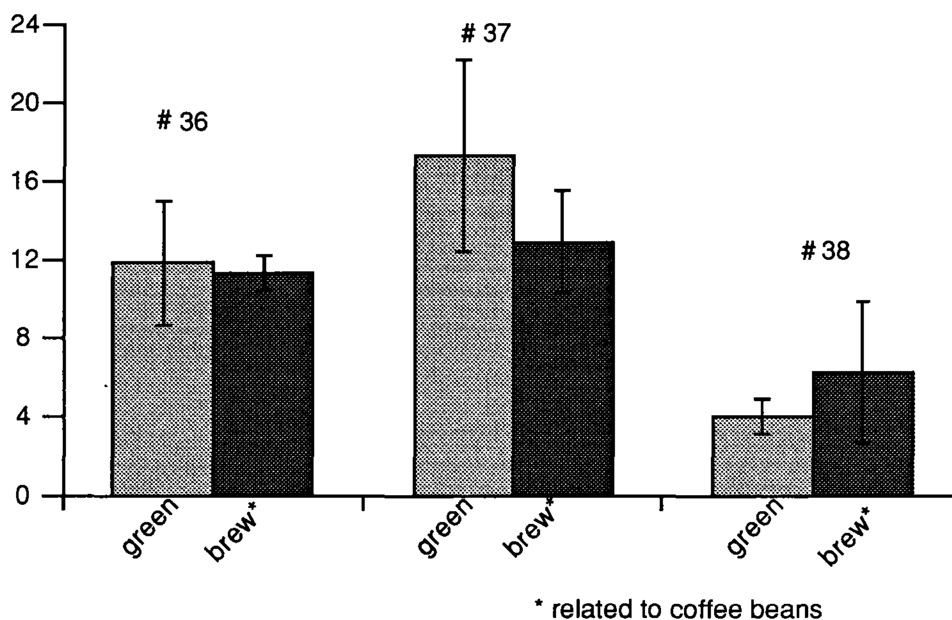


Sample No.	OTA in green coffee [µg/kg][a)]	OTA in roasted coffee[b)] [µg/kg][a)]
No. 6	780 ± 120	890 ± 30
No. 7	1320 ± 280	1180 ± 100
No. 8	360 ± 40	280 ± 90

a) Arithmetic mean of 4-6 aliquots of 30 g ± standard error of the mean
b) The values are all based on the weight of green coffee beans.

Roasting of rejected samples containing high natural levels of OTA, had no effect on final levels of OTA in roasted beans (Fig. 3), giving an indication of OTA's high heat stability. However, OTA is very inhomogenously distributed within coffee beans indicated by the high standard error of the mean. Our results reveal no apparent difference between the OTA levels of green and roasted beans. For analytical reasons these studies on destruction had to be performed with highly contaminated non-commercial samples. Extrapolation of these results to low contaminated samples is not a priori possible. A different behaviour can not be excluded. Due to the analytical error at these low levels a large number of samples would have to be examined.
Similarly, the carry-over of OTA from green beans via roasted coffee into the brew showed no obvious differences between the OTA levels of green beans and the coffee brew (Fig. 4).

Fig. 4: Carry-over of OTA from green beans into the coffee brew



Sample No	OTA in green coffee [µg/kg]a)	OTA in coffee brewb) µg/kg]a)
No. 36	11.8 ± 3.2	11.3 ± 0.9
No. 37	17.3 ± 4.9	12.9 ± 2.6
No. 38	4.0 ± 0.9	6.2 ± 3.6

a) Arithmetic mean of 4-6 aliquots of 30g or 150ml ± standard error of the mean
b) The values are all based on the weight of green coffee beans.

Risk evaluation

From our preliminary data, the estimated daily intake of OTA via coffee is about 25-100ng OTA, based on an assumed daily coffee consumption of 25g (about 3-4 cups) and a contamination level of 1-4µg/kg. The daily intake of OTA via food was previously estimated to be about 100ng (DFG, 1990). The total daily intake via coffee and food is, therefore, estimated to be about 2-3ng/kg bw/day. This value can be compared to the Provisional Tolerable Weekly Intake of the Joint FAO/WHO Expert Committee on food additives (1990) of 112ng/kg bw/week or 16ng/kg bw/day. However, this value accounts for renal damage only and does not take carcinogenicity data into consideration.
Kuiper-Goodman and Scott (1989) calculated a tolerable daily intake of OTA with respect to the carcinogenicity based on the data of Boorman (1989) either with a NOEL/safety factor approach or with a mathematical low-dose extrapolation: with an experimentally observed NOEL for tumors at 21µg/kg bw/day and a safety factor of 5000 they proposed an estimated tolerable intake of 4.2ng/kg bw/day for humans;

while mathematical linear extrapolation to a lower 95% confidence level for a life time tumor risk of 1:10^6 ("virtually safe dose", VSD) resulted in a VSD of 0.2ng/kg bw/day.
At present it is difficult to comment on the carcinogenic risk of human exposure to OTA at low dose levels as only very limited data exists on the mechanism of OTA and no kinetic data for humans are available. Especially the use of a linear dose-effect extrapolation without a threshold must be challenged since so far no evidence for a genotoxic action of OTA is available. If, however, this most conservative approach of a linear high to low dose extrapolation is applied, a cumulative life time risk of approximately 15 tumors/10^6 can be calculated from the preliminary data of the total OTA intake. If this figure is transformed into yearly incidences, OTA contributes only 0.5/10^6 cases to the yearly background renal tumor incidence of 65/10^6 and 37/10^6 in males and females respectively.
Nevertheless, as OTA is known as a mycotoxin with carcinogenic, nephrotoxic, teratogenic and immunotoxic properties the exposure to OTA should be kept to a minimum. Efforts to reduce the contamination should be undertaken.
In order to achieve a better estimate of the degree of OTA contamination in coffee, as well as to assess the overall contribution of coffee consumption to human OTA intake, analyses of a large number of commercial coffee samples and processed coffee products are necessary.

Summary

Ochratoxin A (OTA) is a carcinogenic mycotoxin which is produced by predominantly two ubiquitous fungal species (e.g. Aspergillus and Penicillium). Several toxicity studies with OTA indicated the kidney as the primary target organ. In a 2-year feeding study with rats OTA was shown to induce renal carcinomas already at low doses (e.g. 70µg/kg bw - 30% tumor incidence). OTA is found in foodstuff, predominantly in cereals but also in coffee beans. In commercial green coffee beans OTA was detected in 13 of the 25 samples analysed in our preliminary and by no means representative study. Roasting (250°C, 150 sec) of highly contaminated green coffee beans resulted in a negligible reduction of the OTA level. OTA was also found to be elute almost completely into the brew. Further studies are needed to establish the behaviour at low cantamination level. Our very preliminary results suggest, therefore, that regular coffee consumption may contribute to human OTA exposure.

Zusammenfassung

Ochratoxin A (OTA) ist ein kanzerogenes Mycotoxin, welches vor allem von zwei ubiquitären Schimmelpilzsorten produziert wird (Aspergillus und Penicillium). Mehrere Toxizitätsstudien zeigten, dass die Niere als Hauptzielorgan am stärksten von Ochratoxin A geschädigt wird. In einer 2-Jahres Fütterungsstudie mit Ratten induzierte OTA Nierentumore bereits im tiefen Dosisbereich (70µg/kg KG - 30%Tumor Inzidenz). OTA kann vor allem in Cerealien, aber auch in Kaffeebohnen nachgewiesen werden. In einer ersten, nicht repräsentativen Studie konnte in 13 von 25 kommerziellen grünen Kaffeeproben OTA detektiert werden. Rösten (250°C, 150 sec) von hoch kontaminierten Kaffeeproben hatte auf den OTA-Gehalt keinen Einfluss. Auch konnte gezeigt werden, dass OTA praktisch vollständig in das Kaffeegetränk übergeht. Ob allerdings dasselbe Verhalten auch bei niedrigem Kontaminationsgrad gefunden wird, müssen weitere Studien zeigen. Auf Grund unsere noch unvollständigen Resultate muss der Schluss gezogen werden, dass der Kaffeekonsum einen Beitrag zur Belastung des Menschen mit OTA leisten kann. Weitere systematische Untersuchungen von repräsentativen kommerziellen Mustern sind zur besseren Beurteilung der Situation erforderlich.

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DOES CAFFEINE DEPENDENCE EXIST ?

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Recently (1990), Holtzman [7] proposed considering caffeine as a model drug of abuse. In order to evaluate whether the main arguments for such a hypothesis, regular use in the majority of the population, excessive use in a few subjects, and the appearance of withdrawal headache in some subjects, are a sufficient basis for this proposal, the present article reviews first the psychobiology of reward and then some recent caffeine experiments in humans.

I. Psychobiology of reward

On the subjective level, reward means pleasure and satisfaction. On an objective level, it is manifested by the frequency of acts that produce pleasure and satisfaction. For a more detailed introduction to the biological basis of reward, the reader can be referred to a book edited by Liebman and Cooper [8].

The **neurophysiological substrate** of the reward system was discovered in the late 50's. It was seen that rats permanently implanted with electrodes into appropriate brain regions vigorously use a lever which allows them to deliver electric currents into the brain. The main particularities of this phenomenon can be described as follows. The phenomenon is obtained within about 25% of the brain volume, and the frequencies of this electrical self-stimulation vary between about 50 to 5000 stimulations per hour, depending on the electrode localization. The stimuli need to be short (about 0.5 sec), as the "pleasure" induced by the currents rapidly changes into discomfort. When the current is turned off, the animals cease rapidly to press the lever, and extinction is therefore very rapid. Negative withdrawal effects have never been reported. Upon introduction of an experienced animal into the experimental cage, lever pressing hardly starts immediately, but, after a first current train, which is often delivered by the experimenter as a "priming

reward," lever pressing starts at a mostly regular pace. The behavior has therefore also been compared to the phenomenon of eating salted peanuts. Even without hunger, each peanut induces appetite for the next one, but no particular withdrawal problems arise when the peanut box is taken away.

The **neurochemistry** of this behavior has been studied with a great number of different methods. One of them uses the procedure of brain dialysis in the intact and awake animal with permanently implanted minitubes. This procedure allows the measurement of the turnover and release of specific neurotransmitters in distinct brain regions. With this method, it was seen that rewarding behavior is accompanied by the release of dopamine into three distinct brain regions. Dopaminergic activation of the prefrontal cortex appears to be necessary to organize the specific reward memories and expectations. Dopaminergic activation of the striatum is critical for the organization of the goal-directed behavior involved in rewarding acts. Dopamine release into the nucleus accumbens was found to be critical for the organization of the experience of reward.

A further support for the concept of the dopaminergic nature of the reward system of the brain comes from the anatomical organization of the system. Most of the cell bodies of the dopamine neurons are rather densely packed in the ventral midbrain. From there the axons travel forward in a rather compact bundle before they spread out for the described target areas where the axon terminals release dopamine. It is precisely this compact bundle which is subject to the most intensive electrical self-stimulation.

These views have been supported by a number of behavioral experiments. After an animal is treated with dopamine antagonists, it will deliver only a few bursts of current into the brain and then cease lever pressing as if "all the fun were gone." Food getting and drinking are accompanied by dopamine release into the mentioned areas, and selective neurotoxic lesions of the dopamine system cause general apathy, loss of eating, drinking, and exploration.

Such observations raise, of course, the question as to whether pharmacological substances that in some way activate the dopamine system might induce reward and constitute thereby a potential for dependence. This has in fact been proposed and is by now widely accepted. The rationale has been outlined by Wise [11].

The main argument is that excitation of the self-stimulation areas, direct dopaminergic activation as well as eating or drinking all involve activation of the motor system in a way that appears as a psychomotor stimulation. Psychomotor stimulation, as opposed to pure motor activation, has to be seen in this context as an activation which is goal directed and adapted to the particular situation.

The list of pharmacological substances that produce both psychomotor activation and measurable dopaminergic activation includes the opiates, cocaine, amphetamine, barbiturates, benzodiazepines, cannabis, phencyclidine, and nicotine. The same also holds for caffeine, as reviewed recently by Bättig and Welzl [1].

In fact, all these substances can more or less easily induce self-application by humans, whereas there are hardly controlled reports of self-application of dopamine antagonists.

The **active self-application** of psychoactive substances by laboratory animals has been studied widely in the past years. For systemic administration, intravenous catheters were used in most cases, and, in analogy to electrical self-stimulation, the animals are given

the opportunity to receive "shots" by pressing a level. Alcohol and morphine have also been offered as drinking liquids.

Using this technique, psychoactive substances can be grouped into three categories.

Opiates, cocaine, and amphetamine, the "hard drugs" are self-injected easily by experimental animals when the substances are offered at a "fixed rate," which means that every bar press delivers a shot. With alcohol, this appears to be the case only for animals that are genetically selected for their alcohol proneness. Opiates, cocaine, and amphetamine can therefore be considered as "strong reinforcers." Tolerance to the rewarding effects develops rapidly, and as a consequence, the number of self-injections increases gradually. With cocaine and amphetamine, there appears in parallel a sensitization rather than tolerance to the toxic effects, so that fatalities due to intoxication occur earlier and more frequently than with the opiates.

The extinction of such behavior upon discontinuation of the delivery of the substances is very slow, and it has been observed that cocaine-habituated animals may continue several thousand times to press a lever after substance delivery has been shut down. Reducing the single doses produces increased bar pressing for all three hard drugs (up-titration), whereas increasing the doses hardly reduces bar pressing.

All other dopaminergic substances, as far as they have been tested, reveal a distinctly different pattern of effects. As reviewed by Griffiths and Woodson [6], caffeine was hardly self-injected by experimental animals. In this respect it resembles nicotine, which also is hardly self-injected if one uses the fixed-rate technique. With nicotine, it has been demonstrated, however, that reliable self-injection can be obtained by using a "time-out procedure." With this procedure, animals have to press the level several times until they get an injection, and each injection is followed by a "time-out period," during which the substance cannot be obtained. The interpretation of this result remains under discussion, because similar procedures have also been used successfully to "teach" monkeys to deliver themselves painful electric shocks. The question therefore remains open whether the delivery of the substance or the time-out period constitutes the reward.

In any case, most dopaminergic substances other than the hard drugs are at best very weak reinforcers. In addition to the dependence of self-application on particular schedules of reinforcement, there is rapid extinction upon discontinuation, efficient "down-titration" for increases of the single doses, and no "up-titration" for the lowering of the single doses.

A more direct support for the dopaminergic concept underlying drug taking is presented by studies that investigated whether animals would self-inject the substances into critical brain regions. This has been successfully done so far with cocaine, amphetamine, and opiates, as reviewed by Wise [11]. Amphetamine and cocaine are self-injected only into the nucleus accumbens, and there it inhibits the reuptake of dopamine into the axon terminals so that its activity in the synaptic clefts is prolonged. The opiates are self-injected only into the ventral midbrain, where the cell bodies of the dopamine neurons are situated. These cell bodies are directly activated by the opiates, and as a result, they fire increasingly so that more dopamine is released from the axon terminals in the different target areas. By the same mechanism, probably also alcohol, nicotine, and most other dopaminergic substances increase firing of dopamine neurons, whereas the reuptake inhibition in the target areas seems to be specific for amphetamine and cocaine.

A third group of psychoactive substances includes the antagonists of the dopaminergic system or substances that have no, perhaps not even indirect, effects on the dopamine

systems. Prominent examples would be antipsychotic and antidepressive substances. For these, self-application has never been demonstrated, not even when using "time-out" schedules.

The **negative reinforcement** of drug taking is an important element in popular beliefs about opiate addiction. Negative reinforcement consists in the development of the regular use of a substance, because it terminates or postpones negative feelings or pain of any sort. In contrast to the unitary, dopaminergic, positive reward system, different negative states are organized in different brain systems and this holds for pain states or the feeling of hunger, etc. This has been demonstrated particularly for the opiates. Endogenous opiates play a decisive role in the modulation of pain through the periaqueductal grey area of the midbrain. Injection of morphine into this area produces pronounced analgesia and tolerance, and a withdrawal syndrome upon discontinuation of the injections. Most remarkably, however, attempts to induce active self-injection of opiates into the periaqueductal grey area have failed. On the other hand, the already mentioned vigorous self-injection of the substance into dopamine areas does not produce analgesia, and discontinuation does not result in withdrawal syndromes.

This favors the idea that drug taking may be exclusively mediated through the positive effects of the substances. In favor of this view, it may be mentioned that aspirin which is taken to terminate pain has so far not created a "drug problem." Clinical observations also support the view that morphine withdrawal syndromes constitute more an "inconvenient side-effect" than the central element of morphine seeking.

II. Coffee and caffeine

Although caffeine has psychostimulant effects, whether these are the specific reasons for coffee consumption remains less clear than proposed by Holtzman [7], who considered caffeine as a model of drug abuse. The **behavioral criteria of dependence**, as used in modern research, are manifold and are to be considered in this context in part in a rather global and in part in a more detailed fashion. Tolerance, with rapidly increasing needs for higher and more frequent doses, as is characteristic for the hard drugs, is certainly absent for caffeine.

Resistance to extinction, which is so great for hard drugs that users engage in criminality to obtain the substances, also appears to play at most a negligible role for coffee and caffeine.

Pathological psychoactivation, such as is obtained with the hard drugs, alcohol, and cannabis, is missing with caffeine. Instead, low to moderate doses have beneficial effects on mental performance, as reviewed by Bättig and Welzl [1].

Titration behavior for caffeine is also unknown.

Negative effects on health have so far also not been documented.

A rather high regularity of intake, however, is certainly present for caffeine intake, but increases of the doses are absent.

Withdrawal phenomena have for a long time been recognized, consisting mainly in headache and its side-effects on general well-being.

There remain thus two aspects to be considered in more detail.

Perception of the effects of caffeine would be an important aspect of dependence if it could be demonstrated for the low doses ingested daily. Evans and Griffiths [2] recently presented a study done in five subjects. These were first trained to discriminate between the effects of placebo capsules and capsules that contained 300 mg caffeine. The discrimination was learned on the average in about a dozen sessions up to the criterion of 80% or more correct guesses. After this task was learned, it was tested whether lower and higher doses would also be correctly identified. This was, however, reliably the case only for doses that were at least as high as the training doses. The assessment of different parameters of mood showed that recognition of caffeine was mainly secondary to increased reports of feeling jittery, nervous, and shaky. In a few subjects, placebo recognition was further secondary to feelings of fatigue.

That feelings induced by the absence of caffeine would be easier to detect than those induced by the presence of caffeine was quite convincingly demonstrated in an experiment by Griffiths et al. [3] a few years earlier. Six very heavy coffee drinkers were followed-up in the research ward over alternating periods of regular and decaffeinated coffee. Although the subjects were allowed to drink coffee ad lib, they consumed consistently about the same number of cups. During the first two days with decaffeinated coffee, however, there were significant decreases for vigor, feeling stimulated, and liking the coffee as well as increases of headache and fatigue.

A more extended field study has been carried out recently at our laboratory. After a run-in period of three days with the habituated coffee, the 120 subjects were divided into three groups. A first group received for the following nine days caffeinated instant coffee, a second one decaffeinated instant, and a third one caffeinated instant every third day and decaffeinated instant for the other days. The measures included saliva caffeine, cardiovascular parameters, and physical activity, all of which are reported on in the contribution by Höfer and Bättig in this volume. An electronic diary was used to assess and store different behavioral ratings, among these, several ones related to the perception of coffee.

The quality of the taste dropped when the subjects had to switch after the initial run-in period to instant coffee, but the presence or absence of caffeine showed no effect.

The stimulating effect of caffeine was also rated lower for instant than for the habituated filter or espresso coffee. The manipulation of caffeine affected these ratings more modestly at the level of borderline significance.

Subjective strength of coffee was hardly affected for the all-day means of the ratings, but comparing the ratings made after breakfast with those made before dinner showed that the second measurement reflected the caffeine manipulations better than the first one.

A single rating for caffeine content was made in the evening before dinner. It also showed some modest response to the caffeine conditions, with higher ratings for caffeinated than for decaffeinated coffee. These observations suggest that a modest perception of caffeine in coffee is made only several hours after drinking the coffee.

In this respect, it is also of interest to consider the parameters that were not significantly affected by caffeine. These included blood pressure, perception of work load, stress feelings, muscle and joint pain, different sleep parameters, and eating behavior.

Liking coffee and its effects is another aspect which merits closer consideration. It can be tested in several ways.

Griffiths and Woodson [5] tested whether subjects would prefer caffeine or placebo capsules. For each dose, the subjects had a series of opportunities to choose. Out of 11 subjects, only three reliably preferred caffeine against placebo at the lower dose range of 100 and 200 mg caffeine. On the other hand, nearly all subjects preferred caffeine less frequently the higher the doses. Only two subjects preferred the highest dose of 600 mg to placebo. This argues again for the concept that undesired effects of higher doses are increased and that such perceptions decrease intake.

In a later experiment, Griffiths and colleagues [4] tested in a rather direct approach how much work subjects would do to receive regular coffee, decaffeinated coffee, placebo capsules and caffeine capsules, all at the dose level of 100 mg caffeine. Toward this goal, the subjects were offered each day 10 occasions to work for such rewards. The duration of ergometer bicycling invested was similar for the days when reward consisted in caffeinated, decaffeinated coffee or in caffeine capsules, but it was much lower on the days when placebo capsules were offered. This can be taken as evidence that both coffee per se with or without caffeine and caffeine in capsules were similarly liked.

In the field study done at our laboratory, subjects were free to drink as many cups as they wanted. Remarkably, however, the average number of cups remained between about 5 and 5.5 for all periods of the experiment, unaffected by the switch to the instant preparation and unaffected by caffeine.

On the basis of such results, it appears justified to consider how the consumption of caffeine could be categorized within the wide group of psychoactive substances that are more or less regularly consumed.

Addiction, dependence, habit, or pleasure? A definition of addiction was proposed in 1964 by the U.S. Surgeon General [9]. It included the elements of compulsive need, increase of the dose, strong psychic and possibly physical dependence on the effects as well as negative effects both on the individual consumer and on the society. Among these criteria, only the headache withdrawal syndrome could be considered, but this syndrome did not go along with an increased need for coffee, and it was highly transient.

In the same report, a definition of habit was also offered, and it included a not compulsive desire for use to induce improved well-being, little or no increase of the doses, some degree of psychic dependence, and if negative health effects, then on the individual user only, but not on the society. These elements would fit coffee consumption, with the exception of the withdrawal aspects. For these aspects, the coffee habit is clearly differentiated from the tobacco smoking habit. Whereas smoking cessation produces hardly any physical withdrawal symptoms but strong increases in craving or psychic desire, nearly the opposite is seen for stopping caffeine intake, which does not result in craving for coffee, but can produce headache, which, however, also is not accompanied by increases in consumption.

A third term, "dependence," was proposed in 1988 in a later publication by the U.S. Surgeon General [10], which was tailored mainly to the habit of tobacco smoking. It includes highly controlled and compulsive, but not increasing use. Psychoactive effects including beneficial ones were considered as an additional element in contrast to the more conventional approach of considering only negative effects, such as are obtained with the hard drugs, alcohol, and cannabis, as undesirable. A third element was seen in drug

reinforced behavior such as inhaling a low nicotine cigarette more deeply than one of higher nicotine content.

An element that is not covered by any of these concepts is sensory reward by taste when habitually drinking caffeinated beverages. This, although so far only poorly investigated scientifically, could well be crucial, in addition to the elements which characterize the syndrome of a habit. If this were verified, an acceptable definition of regular coffee consumption would state it as a "habitual Genussmittel consumption." "Genussmittel" is a term existing unfortunately in German only, and it means "substance of enjoyment."

In this sense, the initially outlined principles of the psychobiology of reward should be remembered. It states that consummatory acts are rewarding. Thus not food but eating is the reward-maintaining ingestive behavior. Similarly, not caffeine but rather the act of coffee drinking appears to be the main reward in caffeine-habituated subjects.

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Summary

Psychobiological research has shown that dopaminergic activation organizes reward through the nucleus accumbens, reward memories and expectation through the prefrontal cortex, and goal-directed (rewarding) activity through the striatum. Dopamine antagonists have no reward properties, but direct and indirect dopamine agonists do. Among them, the "hard drugs" (opiates, cocaine, amphetamine) produce increasing and toxic self-administration and are "strong reinforcers." All others are light to moderate reinforcers (barbiturates, alcohol, cannabis, phencyclidine, benzodiazepines, nicotine, caffeine). With caffeine, perception of the effects is poor, coffee drinking does not adapt to the caffeine content, but consumption is highly regular. Coffee consumption does not meet the criteria of addiction or dependence, but those of a habit for sensory and consummatory pleasure.

Résumé

La recherche psychobiologique a démontré que les activités dopaminergiques organisent la récompense et satisfaction par le nucleus accumbens, des mémoires et d'expectations de récompense par le cortex préfrontal et des activités psychomoteures de récompense par le striatum. Les substances antagonistes de la dopamine ont aucune propriété de récompense. Les agonistes directs ou indirects de la dopamine par contre ont de telles propriétés. Parmi eux, les "drogues" (opiates, cocaine, amphétamine) induisent une consommation s'accroissante et toxique. Tous les autres dopamine agonistes (barbiturates, alcool, cannabis, phencyclidin, benzodiazepines, nicotine, caféine) ont des propriétés de récompense modérées ou même faibles. Pour la caféine, la perception subjective des effets est pauvre, la consommation de café ne s'adapte que peu à la teneur en caféine, mais la consommation est assez régulière. La consommation de café ne remplit ni les critères d'addiction ou de dépendence, mais plutôt ceux d'une habitude particulière de s'offrir des plaisirs de goût par la consommation.

EFFECTS OF LOW DOSES OF CAFFEINE IN COFFEE ON HUMAN PERFORMANCE AND MOOD

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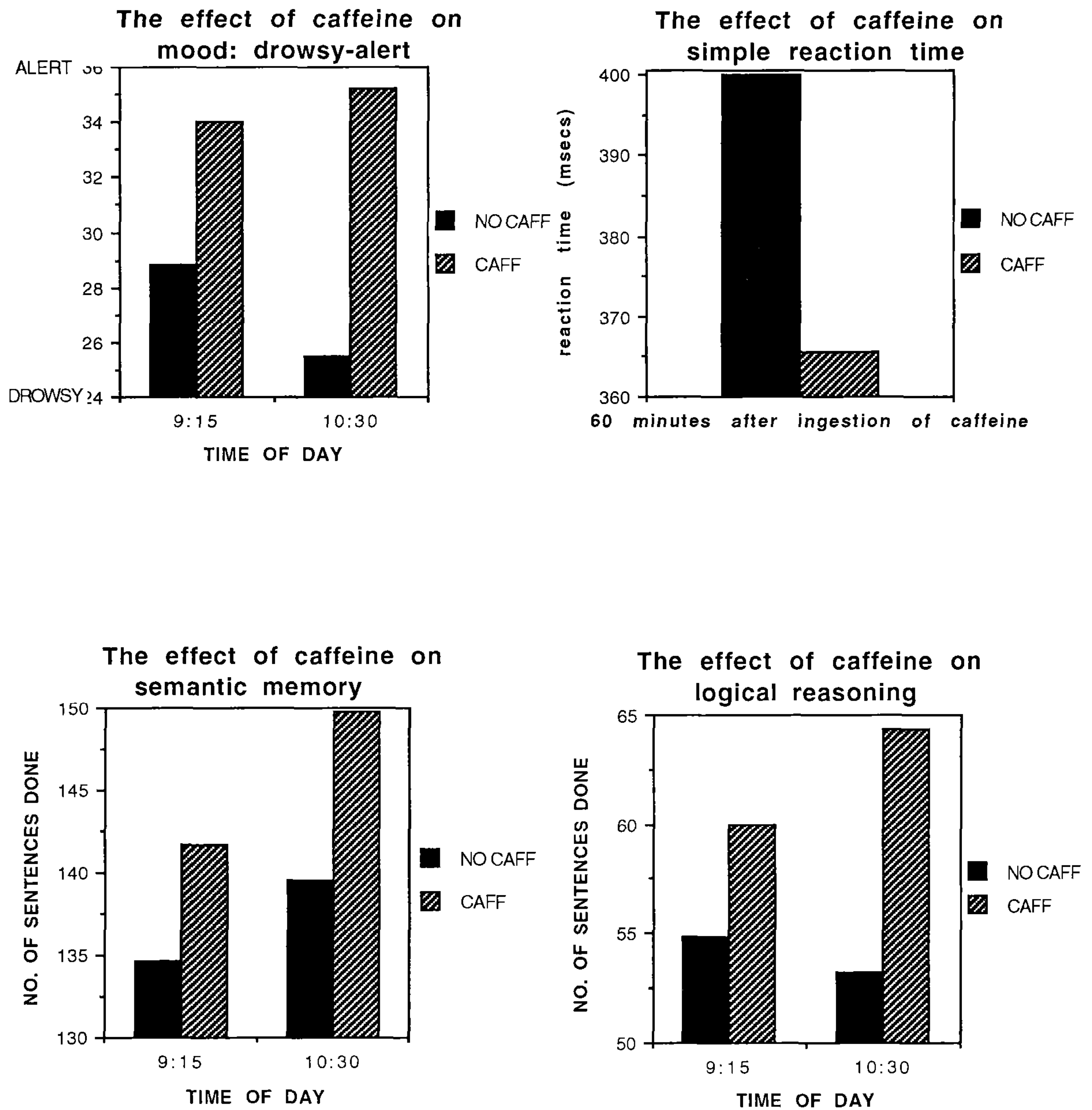
Beneficial effects of caffeine consumption can be traced back to the Tang Dynasty (AD 618-907) where it was believed that drinking tea could prolong life. The coffee bean was also known to have stimulating effects, and Arab goat herders in the 15th century noticed that their goats became more active after chewing the red berries of the coffee plant (Gould et al., 1984). Pietro della Valle (cited by Lieberman, 1992), writing in the 17th century, reported that coffee "prevents those who consume it from feeling drowsy. For that reason, students who wish to read into the late hours are fond of it."

In the last twenty years psychologists have carried out systematic studies of the effects of caffeine on mental functions and mood. Experiments have often used quite large doses of caffeine (over 200 mg) and the results suggested that ingestion of caffeine may increase psychomotor speed (Swift & Tiplady, 1988), reduce both simple and choice reaction time (Clubley et al., 1979; Smith et al., 1977), improve the ability to maintain attention (Lieberman, 1988) but have few effects on short or long term memory (Roache & Griffiths, 1987). The subjective reports of mood also suggest that caffeine has a stimulatory effect, with alertness and vigour being increased following the ingestion of caffeine (Leathwood & Pollet, 1983). Results from studies using high doses of caffeine have also demonstrated that anxiety and tension may increase (Loke, 1988).

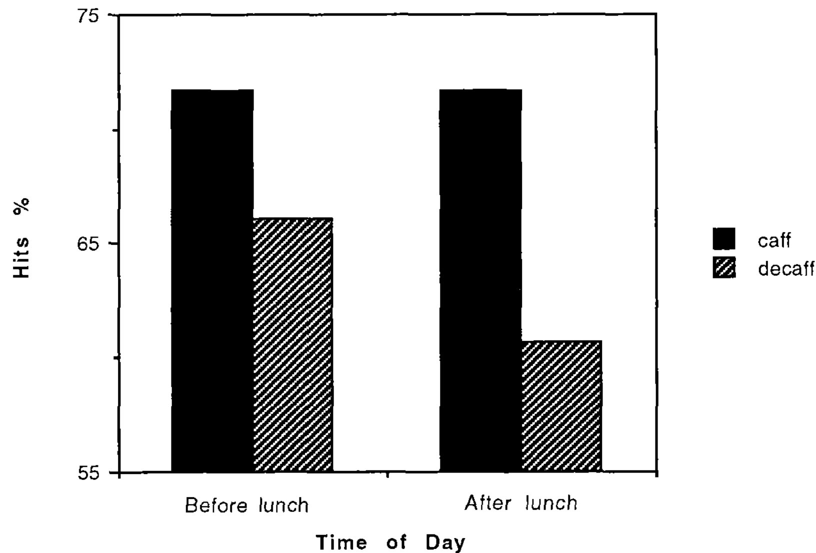
These behavioural effects of caffeine are quite robust when doses in the region of 200-250 mg are used. Indeed, the following figure shows effects we have obtained in our laboratory using such doses. These results demonstrate that caffeine improves reaction time, vigilance and subjective alertness. Furthermore, we have demonstrated that memory tasks involving speed are also improved following consumpton of caffeine. For example, subjects given caffeine carried out logical reasoning tasks more quickly than those in the decaffeinated condition. Similarly, retrieval of information from general knowledge is faster following ingestion of caffeine. These effects have been observed at a variety of different testing times across both the day and night (Smith et al., in press). A major aim of the present study was to determine whether such effects are produced by smaller doses of caffeine in coffee.

Figure 1: Effects of high doses of caffeine on alertness, reaction time and semantic memory and logical reasoning tasks.



Smith et al., (1990) demonstrated that caffeinated coffee prevented the post-lunch dip in alertness and sustained attention (see Figure 2). Another aim of the present study was to determine whether such results could be demonstrated with smaller doses of caffeine.

Figure 2: Effects of caffeine and lunch on the Bakan vigilance task



A number of experiments have demonstrated that the personality dimension of impulsivity is important in modifying the effects of caffeine on behaviour. Studies by Revelle and his colleagues (Anderson & Revelle, 1982; Revelle et al., 1976) suggest that caffeine enhances the performance of high impulsives but impairs that of low impulsives (when testing occurs in the morning). Smith et al., (1991) also found that it was the high impulsives who gained most benefit from consumption of caffeine. A final aim of the present study was to examine whether caffeine /impulsivity interactions were observed at lower doses.

METHOD

Subjects were tested on 3 consecutive days. On the first day subjects were practiced at the performance tests and completed questionnaires assessing personality (introversion/extraversion; impulsivity; sociability; neuroticism; trait anxiety; morningness), recent mood (anxiety, depression, perceived stress, positive and negative mood), and food and caffeine intake. The weight and height of the subjects was also recorded at this time. On the second and third days each subject was tested 3 times; once in the late morning, once in the early afternoon (90 minutes after the start of lunch) and then finally in the late afternoon (3 hours after the start of lunch). On one of the days subjects were given lunch and on the other they abstained from eating. The order

of lunch conditions was counterbalanced across subjects. At the end of the lunch (and at the corresponding time on the no lunch day) the subjects were given a 150 ml cup of decaffeinated coffee. Half the subjects had caffeine (1.5 mg/kg body weight) added to this. The caffeine manipulation was carried out double blind.

Subjects:

46 female subjects took part in the study. Another aim of the study was to examine the effects of dieting and dietary restraint. 14 of the subjects were currently on a diet, 16 had high levels of dietary restraint (as measured by the Dietary and Restraint Questionnaire) and 16 low dietary restraint. The subjects were paid £36.00 for participating in the study.

Nature of the meal:

All subjects were given a standard lunch consisting of orange juice, a cheese salad roll, packet of crisps and a chocolate bar.

Procedure:

At the start of each session blood pressure and pulse were recorded. Subjects then rated their mood using 18 visual analogue scales with bi-polar adjectives (e.g. Drowsy/Alert; Tense/Calm) at the end (after Herbert et al., 1976). Subjects then completed a series of performance tests, the order of which was counterbalanced across subjects. Data from the following tasks are reported here:-

Memory tasks

Free recall task: the subjects were shown a list of 20 words presented at a rate of one every 2 seconds. At the end of the list the subject had 2 minutes to write down (in any order) as many of the words as possible. Subjects were shown a different list at each test session.

Delayed recognition memory task: at the end of the test session subjects were shown 40 words which consisted of the 20 words shown at the start plus 20 distractors. The subjects had to decide as quickly as possible whether each word had been shown in the original list or not.

Logical reasoning task: this test was developed by Baddeley (1968) and the subjects were shown statements about the order of the letters A and B followed by the letters AB or BA (eg. A follows B: BA). The subjects had to read the statement and decide whether it was a true description of the order of the letters. If it was, the subject pressed the T key on the keyboard, if it wasn't, they pressed the F key. The sentences ranged in syntactic complexity from simple active to passive negative (eg. A is not followed by B). Subjects completed as many as possible in 3 minutes.

Semantic processing task: this test, developed by Baddeley (1981), measures speed of retrieval of information from general knowledge. Subjects were shown a sentence and had to decide whether it was true (eg. canaries have wings) or false (eg. dogs have wings). The number completed in 3 minutes was recorded, as was the accuracy of responding.

Psychomotor/Attention tasks

Variable fore-period simple reaction time task: in the variable fore-period simple reaction time task a box was displayed on the screen and at varying intervals (from 1-8 secs) a square appeared in the box. The subject had to press a key as soon as the square was detected. This task lasted for 3 minutes.

Five choice serial response task: in the five choice serial response task five keys were arranged in a pentagon and there was another key in the middle. When the subject pressed the central key one of the outer keys lit up and the subject had to press that key then press the central key again which led to the next key being lit up. Subjects were only allowed to respond with the fore-finger of their dominant hand. This task lasted for 3 minutes and the number of responses made, number of errors and number of gaps (occasional long responses) were recorded.

Repeated digits vigilance task: in this task subjects were shown three digit numbers on the screen at the rate of 100 per minute. Each number was normally different to the preceeding one but occasionally (8 times a minute) the same number was presented on successive trials. Subjects had to detect these repeats and respond as quickly as possible. The number of hits, reaction times for hits, and false alarms were recorded. The task lasted for 3 minutes.

At the end of the performance tasks the subjects rated their mood again.

RESULTS

Statistical analyses

Analyses of covariance, with the pre-lunch scores as covariates, were performed on the data. The between subject factors were caffeine condition, diet category and time of testing (half the subjects were tested at: 11.00, 13.30 and 15.30 the others at: 12.00, 14.30 and 16.30). The within subject factors were lunch/no lunch days and time of day (early v late afternoon). Task parameters (eg. minutes doing the task) were also included as within subject factors.

Main effects of caffeine

(a) *Cardiovascular function*

There were no significant effects of caffeine on either pulse or blood pressure.

(b) *Mood*

Prior to starting the performance tests subjects in the caffeine condition felt significantly more alert and energetic. After the tests those in the caffeine condition felt significantly more alert, stronger, more clear-headed, more well-co-ordinated, more energetic, more quick-witted, more proficient and more interested. The alertness data are shown in Figure 3.

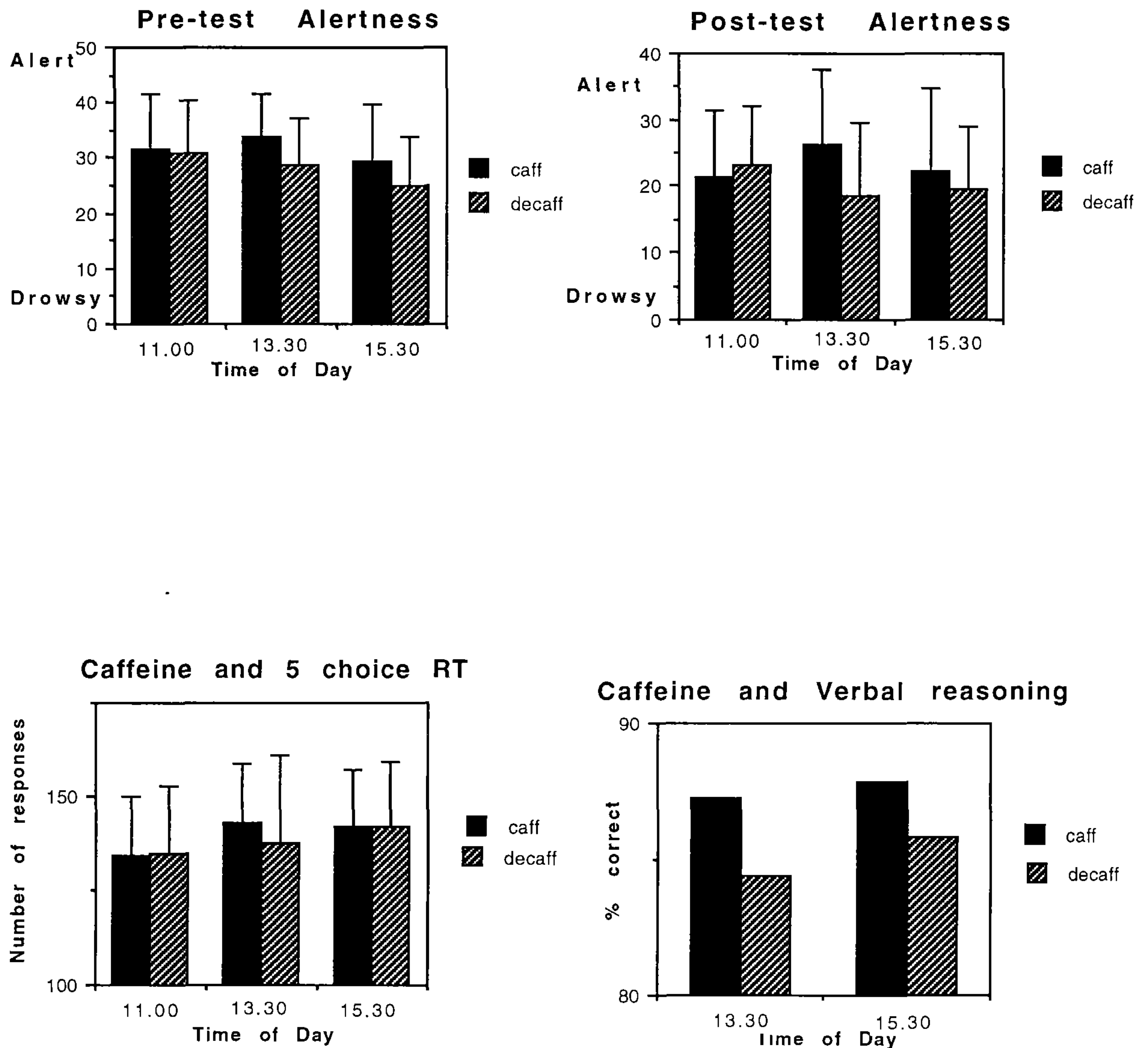
(c) *Performance*

There were no significant effects of caffeine on the free recall, recognition memory, semantic memory, simple reaction time or repeated digits vigilance tasks. However, those in the caffeine condition made significantly more responses in the five-choice serial response task, and also performed the logical reasoning task more accurately than those in the de-caffeinated condition. These effects of caffeine are shown in Figure 3.

Caffeine and time of day

The effects of caffeine on mood after performing the tests was especially beneficial in the early afternoon testing session. Similarly, the effect of caffeine on the number completed in the five choice serial response task was greatest at this time, whereas this time of day was associated with the lowest level of performance in the de-caffeinated group. These

Figure 3: Effects of low doses of caffeine on alertness, psychomotor speed and verbal reasoning.



effects are shown in Figure 3. These effects of caffeine were apparent on both days when lunch was consumed and when the subjects abstained from eating.

Caffeine and Impulsivity

Only one interaction between caffeine and impulsivity was significant and this was in the analysis of the free recall data. The high impulsives given caffeine recalled more words than the high impulsives in the decaffeinated condition. The opposite pattern of effects was seen in the subjects with low levels of impulsivity. However, this effect must be treated with caution as, given the large number of analyses carried out, it could represent a chance effect.

DISCUSSION

The present study clearly demonstrates that low doses of caffeine, similar to those frequently consumed by large numbers of people, have beneficial effects on mood, psychomotor performance and the ability to think logically. However, other aspects of mental functioning were not significantly altered following ingestion of caffeine. This may reflect several things. First, low doses of caffeine may influence these functions but a more powerful design (large number of subjects) is required for such effects to achieve significance. Secondly, certain types of performance may be more sensitive to contextual factors associated with caffeine consumption, such as the regular caffeine usage of the subjects, the time of day of administration and other background conditions. Further research is clearly required to determine the impact of such factors. It is, of course, also possible that low doses of caffeine will not influence certain types of behaviour at all. Such a conclusion, however, is premature until further studies with low doses of caffeine are conducted.

The present results showed that the effects of caffeine were especially pronounced in the early afternoon testing session. This confirms previous findings obtained with higher doses of caffeine. The effects of caffeine were similar on days when lunch was consumed and when the subjects abstained from eating, which suggests that either caffeine influences the endogenous component of the post-lunch dip rather than the meal-related part, or that it has a more global effect which is independent of these but more easily detected when alertness is reduced.

There was little evidence of the effects of caffeine being greater in high impulsive subjects and this, perhaps, reflects the time of day at which the testing was carried out. The beneficial effect of caffeine seen in high impulsive subjects has been shown to be largely restricted to the morning. Indeed, opposite effects have been reported later in the day, so a pattern of no difference between low and high impulsives given caffeine in the middle of the day could reflect this changing caffeine/impulsivity effect over the course of the day. Alternatively, the caffeine/impulsivity effect may only be obtained at higher doses and further research is needed to resolve this issue.

Overall, the present results show that the low doses of caffeine consumed by many people will have beneficial effects on mood and performance. The precise profile of these effects now needs to be determined in further research which considers the contextual factors associated with caffeine consumption.

SUMMARY

An experimental study of the effects of low doses of caffeine (1.5 mg/kg body weight) on mood and mental performance was carried out. 46 female subjects were assigned to either the caffeine or de-caffeinated condition, the caffeine manipulation being double-blind. The caffeine was administered in a 150 ml cup of de-caffeinated coffee, given

after lunch (or at a similar time when no lunch was consumed). Baseline mood and performance data were recorded prior to the caffeine manipulation and the tests were repeated again in the early afternoon and late afternoon. The results showed that caffeine increased alertness and improved psychomotor speed and the accuracy of verbal reasoning. The effects of caffeine were greater in the early afternoon than late afternoon and these effects were observed on days when lunch was consumed and when the subjects abstained from eating. There was little evidence of the effects of caffeine being different in low and high impulsive subjects.

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EFFECTS OF CAFFEINATED VS. DECAFFEINATED COFFEE : A BLIND FIELD STUDY

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The aim of the present study was to evaluate the effects of decaffeinated and caffeinated coffee on physiological, behavioral and subjective parameters in habitual coffee consumers under field conditions.

We will focus on caffeine abstinence effects of selected variables and the time course of these effects. The time course will be considered for continued caffeine abstinence as well as for successive abstinence periods which are sepraratec by intermittent single days with caffeine consumption. A second focus will be the effects of the onset of caffeine consumption, i.e. the effects during the intermiitent days with caffeine consumption.

MATERIALS AND METHODS

Design

The field study lasted 12 complete days. The subjects were randomly assigned to three groups with different, blind treatment: After 3 days on habitual coffee (baseline), the subjects received for 9 days either regular instant coffee, an intermittent regime with 2 days decaffeinated and 1 day regular instant coffee (repeated 3 x), or decaffeinated coffee.

Subjects

One-hundred-and-twenty healthy volunteers participated in the study, i.e. 20 males and 20 females in each group. Subjects were between 20 and 45 years old, habitual coffee consumers with 4 to 10 cups per day, non-smokers, in self-reported good health, especially normotensive. Some sample characteristics are summarized in Table 1; differences between the treatment groups were not obtained.

Table 1: Sample characteristics (mean ± SD, percent)

Variable	Males	Females	Significance of Sex effect
N	60	60	
Age (years)	31.0 ±7	32.4 ±7	n.s.
Height (cm)	177.6 ±5	166.4 ±6	***
Weight (kg)	75.2 ±9	60.9 ±8	***
SBP (mmHg) [1]	121.1 ±11	107.6 ±9	***
DBP (mmHg) [1]	74.9 ±10	67.9 ±9	***
Heart rate (bpm) [1]	70.1 ±10	74.5 ±8	*
Oral contraceptives (%)	0	17	
Coffee consumption (cups/day)	5.6 ±2	5.8 ±2	n.s.
Cup size (0.1l)	1.7 ±0.5	1.8 ±0.5	n.s.

[1] Cardiovascular variables refer to traditional arm assessment in sitting position in the lab at the end of the study
Significance level: *** $p<.001$, * $p<.05$; n.s. not significant

Parameters

Electronic Diary

The subjects had to complete an electronic diary (Organizer II, Psion, England) six times per day at predefined occasions. The electronic diary asked for subjective well-being, e.g. whether the subject felt at the moment awake, sleepy, dull - these three items were combined to 'wakefulness', for headaches and for other variables. The questions were to be answered on analogue-like scales from 'not at all' to 'very much' with a range from 1 to 20.

Cardiovascular parameters

At the same times, the subjects had to measure three times their blood pressure (BP) and heart rate (HR) with a finger measurement device (HEM-815F, Omron, Japan) and to type the results into the electronic diary. These data were later corrected for outliers within each triplet of measures.

Motor activity

Motor activity was assessed continuously with an activity monitor (Gaehwiler, Switzerland) which was to be worn on the wrist of the non-dominant arm. These data were summarized, i.e. averaged for the time intervals between the measurement entries on the electronic diary. The possible range is from 0 to 253.

Statistics

The presented figures refer to daily means for the three treatment groups. The statistical results are based on full-factorial analyses of variance (sex, treatment, day, measurement); in order to compare the experimental phase or parts of it with the corresponding baseline period, contrast analyses were computed (Rosenthal & Rosnow, 1985). For all reported F-values, the degrees of freedom are df=1,1254; the F-values are given in the following order: for the decaff group: decaff days vs. baseline, initial decaff days (d4-6) vs. baseline; for the intermittent group: decaff days vs. baseline, caffeine days vs. baseline.

RESULTS

Subjects' compliance with the experimental regime was good, as is demonstrated by the dramatically different concentrations of caffeine in saliva on the days with decaffeinated coffee (Fig. 1) despite stable coffee consumption over time, independent of the caffeine content.

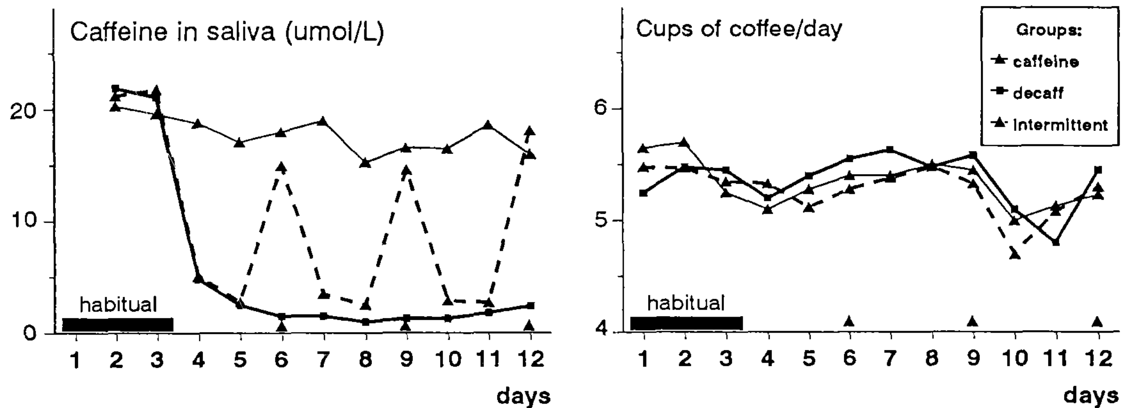

Fig. 1: Compliance with experimental regime: daily means of caffeine concentration in saliva and of coffee consumption. Triangles on the x-axis indicate the caffeine days for the intermittent group (dashed line).

Headache was increased in the decaff group for the period with decaffeinated instant coffee, especially during the first 2-3 days (cf. Fig. 2; $F=13.53$, $p<.001$; $F=52.41$, $p<.001$). In the intermittent group, too, headache was increased on the decaff days ($F=82.19$, $p<.001$), but the effect weakened from one abstinence period to the next. On the intermittent caffeine days, headache normalized to baseline level ($F=0.01$, n.s.).

Wakefulness was decreased during the first 2-3 days of caffeine abstinence (cf. Fig. 3; $F=3.50$, $p<.10$; $F=16.16$, $p<.001$). In the intermittent group, wakefulness was decreased during caffeine abstinence ($F=38.60$, $p<.001$), with a weakening trend of this effect from one abstinence period to the next. On the intermittent caffeine days, in contrast, wakefulness was increased relative to the baseline level ($F=11.40$, $p<.001$).

Motor activity was decreased during caffeine abstinence (cf. Fig. 4; $F=8.70$, $p<.01$; $F=11.62$, $p<.001$; $F=27.90$, $p<.001$), both in the continued and the intermittent group, with no clear tendency for weakening or tolerance over time. On the intermittent caffeine days, motor activity normalized to baseline level ($F=1.34$, n.s.).

Heart rate was increased during caffeine abstinence (cf. Fig. 5; $F=30.28$, $p<.001$; $F=12.51$, $p<.001$; $F=13.30$, $p<.001$)), both in the continued and in the intermittent group. There is no tendency of this effect to weaken over the observed period. On the intermittent caffeine days, heart rate remained at the increased level of caffeine abstinence ($F=7.30$, $p<.01$; vs. decaff days: $F=0.28$, n.s.).

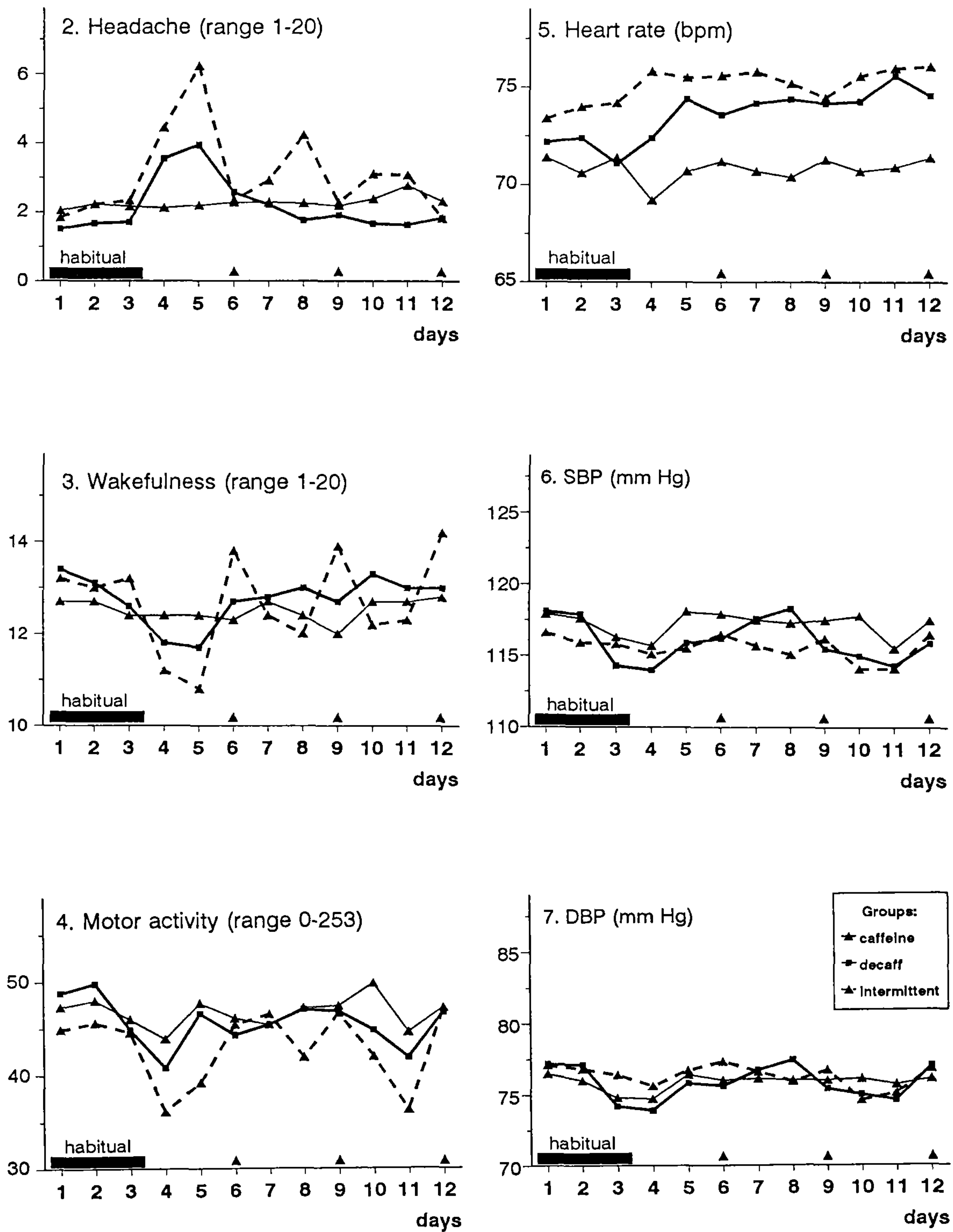

Fig. 2-7: Dependent variables: daily means for the 3 treatment groups. Triangles on the x-axis indicate the caffeine days for the intermittent group (dashed line).

Systolic and diastolic blood pressure were not affected by the caffeine content of the instant coffee (cf. Fig. 6 and 7; ANOVAs: F(day x treatment) < 1.50, n.s.).

DISCUSSION

The reported field study aimed to evaluate the effects of decaffeinated vs. caffeinated coffee on subjective, behavioral and physiological parameters. It was shown that caffeine abstinence is associated with increased headache, decreased wakefulness and motor activity and with increased heart rate. The duration of most of these abstinence symptoms is restricted to 2-3 days; this is supported by the fact that the abstinence symptoms weaken over successive abstinence periods, thus indicating a quick development of tolerance to caffeine abstinence. There is one exception to this rule: The increased heart rate which is observed under caffeine abstinence shows no return to baseline level during the whole observation period of 9 days. Furthermore, heart rate level does not seem to be normalized by single days of caffeine consumption. An explanation might be that the persistence of the increased heart rate on the intermittent caffeine days is associated with the increased wakefulness; motor activity, however, is not increased on these days but reaches only baseline level.

The lack of caffeine abstinence effects on blood pressure in this study is concordant with other field studies which reported no or at best weak effects, and the weak ones mostly reached significance only for selected time periods or selected measures (Bak & Grobbee, 1990, 1991; Burr et al., 1989; Denaro et al., 1991; MacDonald et al., 1991; Rosmarin et al., 1991; Van Dusseldorp et al., 1989, 1991).

The present field study further showed that the onset of caffeine consumption after intermediate abstinence resulted in increased subjective wakefulness, but had no further effects with respect to the reported variables. The stimulating effect of caffeine is well-known from laboratory studies; under natural field conditions, however, it can be confirmed only for habitual coffee consumers.

In summary, caffeine abstinence causes moderate and mostly transient withdrawal effects. Furthermore, there seems to be no titration for caffeine, i.e. coffee consumers don't tend to consume more coffee when the coffee contains less caffeine. Thus, caffeine might be seen as a minor reason for coffee consumption. The different time course of the abstinence effects, the interindividual differences in these effects as well as the possible reasons for coffee consumption merit further investigation.

Summary: The effects of regular vs. decaffeinated coffee on cardiovascular parameters, motor activity, and subjective well-being were investigated in a field study over two weeks. The subjects assessed six times per day blood pressure, heart rate (Omron HEM-815F), wakefulness and headache (range 1-20) on a portable mini-computer (Psion Organizer II); motor activity was measured continuously (range 0-253). The first 3 days served as baseline (habitual coffee), during the following 9 days subjects received caffeine containing instant coffee (N=40), decaffeinated instant coffee (N=40), or an intermittent regime (2 days decaff, 1 day caff, repeated; N=40). Blood pressure was not affected by the caffeine dosage. Decaffeinated coffee resulted in increased heart rate (diff 2 bpm), decreased motor activity (average 40, diff 4), decreased wakefulness (average 13, diff 1), and increased headache (average 2, diff 1). On the intermittent caffeine days, heart rate remained at the increased level of the decaffeinated days, activity and headache normalized to baseline level, and wakefulness reached higher values than on baseline days (diff 1). In part

the effects of caffeine abstinence were transient, i.e., more pronounced in the first 2-3 days. The results show that the onset of caffeine abstinence as well as the onset of caffeine consumption affects subjective, behavioral and physiological parameters. These effects seem to have differing time courses.

Résumé: Les effets de café normal vers décaféiné sur des paramètres cardiovasculaires, l'activité corporelle et le bien-être subjectif ont été étudiés par une étude sur le terrain pendant deux semaines. Les sujets ont rapporté six fois par jour leur pression sanguine, leur pouls (Omron HEM-815F), leur vigilance et leur mal de tête (marge 1-20) sur un mini-calculateur portable (Psion Organizer II), l'activité corporelle étant mesurée continuellement (marge 0-253). Les premiers 3 jours ont servi de 'baseline' (café habituel), pendant les 9 jours suivants les sujets ont reçu du café soluble avec caféine (N=40), du café soluble décaféiné ou un régime intermittent (2 jours caf, 1 jour décaf, répété; N=40). La pression sanguine n'a pas été influencée par le dosage de caféine. Le café décaféiné resultait en un pouls augmenté (diff 2 bpm), une activité corporelle diminuée (moyenne 40, diff 4), une vigilance diminuée (moyenne 13, diff 1) et un mal de tête augmenté (moyenne 2, diff 1). Pendant les jours intermittents avec caféine, le pouls est resté au niveau élevé des jours décaféinés, activité et mal de tête se normalisant au niveau de 'baseline', et la vigilance a atteint des valeurs plus hautes que pendant les jours 'baseline' (diff 1). En autre, ces effets de l'abstinence étaient transients, c'est à dire, plus prononcés pendant les premiers 2-3 jours. Les résultats indiquent que le début de l'abstinence de caféine comme le début de la consommation de caféine influencent des paramètres subjectifs, comportementaux et physiologiques. Les effets semblent avoir des cours du temps différents.

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DOSE-EFFECT RELATIONSHIPS BETWEEN CAFFEINE AND VARIOUS PSYCHOPHYSIOLOGICAL PARAMETERS

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In the present study we investigated the dose-effect relationship between caffeine and mental performance, EEG, cardiovascular and subjective parameters.

Most of the studies on the effects of caffeine on different parameters of mental performance have been done either with single doses or with restricted manipulation of the doses. As an exception, Lieberman and coworkers compared in 1987 the effects of 0, 32, 64, 128, and 256 mg caffeine on a large battery of mood and performance measures. They obtained improvements for some of the tasks only (auditory vigilance and four-choice reaction-time), and this with all tested dose levels without any dose-effect interaction. Possible reasons for this failure to detect a dose effect interaction might be seen in the procedure, which involved several hours of testing after a single dose, and in the absence of pre-/post-administration comparisons.

In order to reduce possible sources of variance, the present study involved the comparison of pre-/post-administration performance in a cross-over design, in which the same subjects were exposed to different dose levels. The task selected for this study, the subject-paced rapid information processing task, has been found to be facilitated by caffeine in several experiments of our lab (Bättig and Buzzi 1986; Hasenfratz et al. 1991 and submitted) when these experimental conditions were respected. However, in a recent experiment on stress and caffeine, which necessitated a between-subject comparison, improving effects of caffeine were obtained in a marginal fashion only (Hasenfratz and Bättig 1992).

In this task the subjects had to press the response key as rapidly as possible after the detection of a target. A target was a sequence of three consecutive odd or even digits in a sequence of pseudorandomly presented digits (1 - 8). The initial presentation rate was 90 digits/min. Thereafter the inter-digit interval decreased in steps of 33 msec after each

correct response (hits) and increased in identical steps after each error (commissions and omissions). In this way the difficulty level remained constant for each subject and the processing rate was taken continuously as the main performance parameter.

A second aspect, which merits attention, is the fact that physiological measurements have been considered only occasionally in past studies on caffeine and performance. A goal of the present study was therefore to assess several dimensions of caffeine action simultaneously. This involved the simultaneous measurement of cardiovascular, EEG and mood parameters.

As the RIP task also represents a mental stressor, a further aspect of the study was to investigate interactions between caffeine and this stress in a dose-dependent fashion. As a second stressor, the cold pressure task was included in the design. It was chosen because it is known to elicit pressor effects primarily through sympathetic, α-adrenergic activation (Allen and Crowell 1989; France and Ditto 1992), in contrast to the RIP task which as mental task appears to produce pressor effects primarily through sympathetic, ß-adrenergically mediated increases in cardiac activity (France and Ditto 1992; Hasenfratz and Bättig 1992, 1993).

METHODS

The <u>subjects</u> were 20 female non-smoking regular coffee drinkers with a mean age of 33.4 years, they all reported being in good health and drinking 6.4 cups of coffee/day. They were selected responders to an advertisement in a local newspaper and their fee consisted of a fixed sum plus an efficiency bonus, which was based on their performance in the RIP task. All subjects were required to abstain from caffeine-containing foods and drinks for 12 hours preceding the experimental session.

According to a 2 x 4 cross-over <u>design</u>, the subjects participated in a training session and four subsequent experimental sessions. An experimental session consisted of a pre- and a posttreatment part, both of which were identical. They included 1 min for the cold pressor task and 20 min for the RIP task with 5 min rest phases before and after each phase.

The physiological parameters were continuously recorded and the subjective ratings were assessed at the beginning of the session, before and after the treatment phase, and at the end of the session.

RESULTS / DISCUSSION

The probably most interesting result of the present study is that although effects of caffeine were observed within each parameter category, the dose-effect curves for the different parameters were rather heterogeneous, as the data suggested some positive, some negative, and in some cases no apparent dose-effect relationships.

Increasing effects with increasing doses were observed for dominant EEG frequencies of the α and ß bands, for wakefulness and the coffee ratings strength and stimulation and slightly also for anxiety. As an example **Figure 1** shows the results for the dominant α frequency. The EEG parameters were assessed during each 5-min rest phase three times before and three times after the beverage (1, 4 = before cold pressure, 2, 5 = after cold pressure/before RIP, 3, 6 = after RIP). Whereas there was no pre/post change for the placebo beverage (broken line) there was a marked increase with the highest dose (6 mg). When the mean pre/post differences are computed, a positive dose effect relationship is suggested.

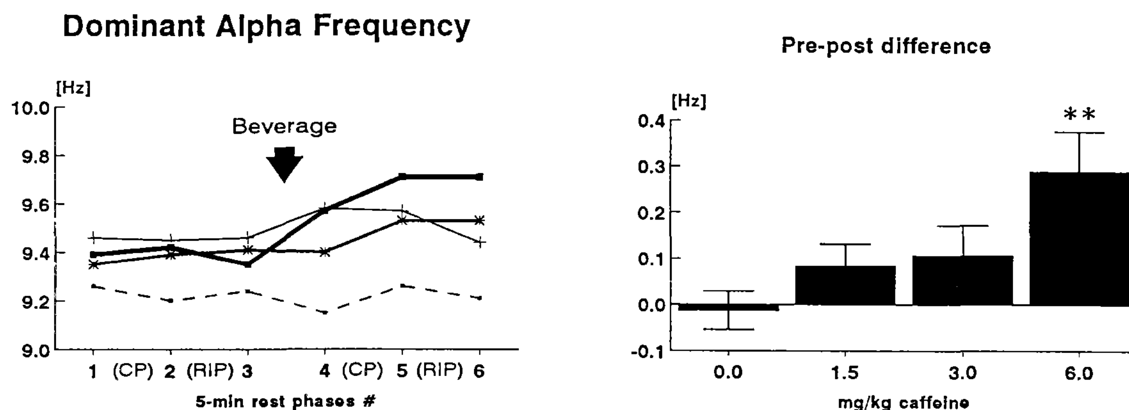

Figure 1. Dominant α-EEG frequency: rest phase means (left panel) and mean pre- to posttreatment difference (± S.E.) (right panel) for the four caffeine doses. (- -·- -) 0.0 mg/kg; (—+—) 1.5 mg/kg; (—*—) 3.0 mg/kg; (▬▬▬) 6.0 mg/kg caffeine. Significant differences from 0.0 mg/kg (paired t-test) are indicated (** $p < 0.01$).

Very similar pictures were obtained for the dominant ß frequency, for wakefulness, and the coffee ratings activation and strength.

No apparent dose-effect relationships were seen for the effects on reaction time and motor activity. As shown in **Figure 2**, the mean pre/post decrease in reaction time was greater and similar for all caffeine doses than for the placebo beverage. A very similar picture was also obtained for motor activity, where the pre- to posttreatment increase seen with the placebo beverage was similarly suppressed by all caffeine doses.

For reaction time, a similar result was obtained by Lieberman and colleagues. Although all administered doses of caffeine decreased the reaction time, there was no dose-effect relationship. They suggested that the slope of the dose-effect function is too shallow relative to the ability of the task to resolve differences between the doses of caffeine administered. For the effects on activity, a similar conclusion can be drawn.

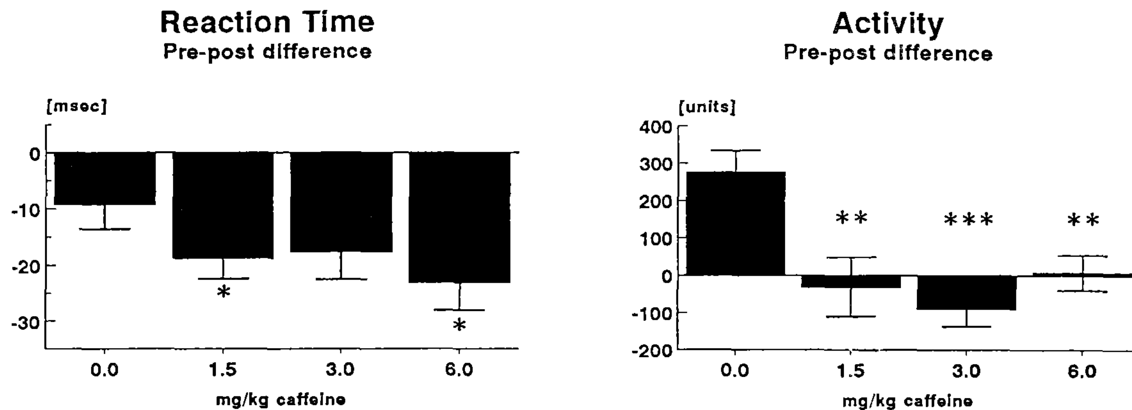

Figure 2. RIP reaction time and motor activity: mean pre- to posttreatment differences (± S.E.) for the four caffeine doses. Significant differences from 0.0 mg/kg (paired t-test) are indicated (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

Surprisingly, negative dose-effect relationships were obtained for RIP processing rate and blood pressure. The greatest pre- to posttreatment increase in processing rate (**Figure** 3) was obtained with the lowest dose, whereas the effect of the highest dose did not differ significantly from that of the placebo beverage. Very similar results were also obtained for systolic and diastolic blood pressure.

However, a comparison of performance effects between different studies is rather difficult, as the tasks used vary considerably. Lieberman et al. (1987), who investigated the effects of different doses in eight performance tests, found improvements for only two parameters (four-choice reaction time, correct detections in an auditory vigilance task), and this in a dose-independent fashion. Frewer and Lader (1991) used 250 mg and 500 mg caffeine to investigate the effects on three different tasks. Their results were inconsistent in that for one parameter (hit rate in a rapid information processing task) the higher dose, for another parameter (hit probability in a continuous attention task) the lower dose showed the greater beneficial effect. This not only stresses the great dependence on the task used but also questions the existence of any dose-effect relationship. The results of the present study, which used a task that has previously been shown to be sensitive to caffeine, suggest that there may be an individual optimal dose which might have beneficial effects. With overdosing, on the other hand, the effect would vanish.

A similar conclusion can be drawn for the effects on blood pressure. A result by Sharp and Benowitz (1990), which would fit into this picture, is that a positive association between serum-caffeine concentration and blood pressure was found only in infrequent but not in habitual caffeine users. As the subjects of the present study were habitual caffeine users, the negative dose-effect relationship is not contradictory to the results of these authors.

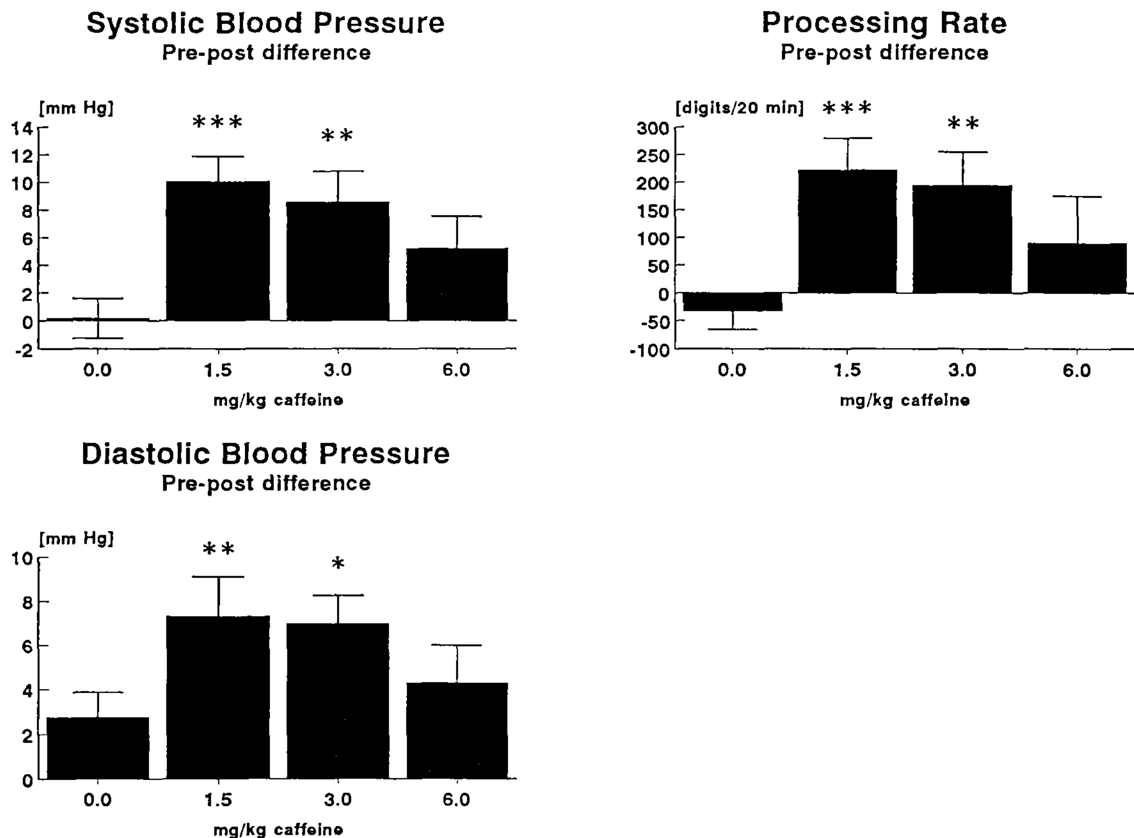

Figure 3. Blood pressure and RIP processing rate: mean pre- to posttreatment differences (± S.E.) for the four caffeine doses. Significant differences from 0.0 mg/kg (paired t-test) are indicated (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

Although the stress reactions to the cold pressure task were generally greater than those to the RIP task, they were similarly additive to the effects of caffeine, as suggested by the lack of significant interactions, a result which is in agreement with earlier studies, especially with respect to blood pressure (France and Ditto 1988, 1992; Lane 1983; Lane and Williams 1985; Lane et al. 1990; Pincomb et al. 1988).

In conclusion, whereas for the EEG parameters and subjective ratings rather positive dose-dependent effects were observed, this was not the case for mental performance parameters, blood pressure and activity. Thus, it can be suggested that doses higher than the minimal effective dose seem to be disadvantageous for those parameters which benefit from caffeine. Generally, the dose-response functions seem to be rather shallow and heterogeneous. Further, for the various parameters the dose range within which they are sensitive to caffeine is different, and the caffeine doses which might have beneficial effects

on parameters of performance are rather small, approximating those found in caffeine-containing beverages.

Summary
Most of the studies reported so far on the effects of caffeine on different parameters of mental performance have been done either with single doses or with restricted manipulation of the doses, and physiological measurements have been considered only occasionally in past studies on caffeine and performance. In the present study we investigated the effects of 0.0, 1.5, 3.0 and 6.0 mg caffeine/kg body weight on various psychophysiological parameters in 20 female coffee drinkers. Caffeine, as compared to placebo, generally increased mental performance, electrocortical arousal and blood pressure and decreased reaction time and physical activity. However, whereas a positive dose-effect relationship was suggested for electrocortical arousal and subjective ratings of alertness, activation, and strength of coffee, a negative one was suggested for mental performance and blood pressure. No clear dose-effect relationship was seen for the decreases in reaction time and motor activity. Thus, these results suggest that the dose-effect curve is rather flat and the individual critical caffeine doses needed to produce positive effects are rather smaller than those generally used in previous experiments.

Résumé
Les études sur les effets de la cafféine sur différents paramètres de la performance mentale rapportées jusqu' aujourd'hui, n'ont utilisé qu'une ou qu'un petit nombre de doses et des fonctions physiologiques n'ont été considerées que rarement. Dans l'étude actuelle nous avons examiné chez 20 femmes, régulières consommatrices de café, les effets de 0.0, 1.5, 3.0 et 6.0 mg cafféine/kg sur différents paramètres. Comparée avec le placébo, la cafféine a augmenté la performance mentale, l'excitation électrocorticale et la tension artérielle, et a baissé les temps de réaction et l'activité physique. Tandis qu' une relation positive entre la dose et l'effet a été trouvée pour l'excitation électrocorticale et les jugements subjectifs de la vigilance, de l'activation et de l'efficacité du café, une relation négative a été trouvée pour la performance mentale et la tension artérielle. Quant à la diminuation des temps de réaction et de l'activité physique on n'a pas pu trouver une relation systématique entre la dose et l'effet. En conclusion, ces résultats indiquent que la courbe de l'effet de dose est plutôt plate et que la dose nécessaire pour produire des effets positifs sur des fonctions mentales varie individuellement et se trouve à un niveau plus modeste que rapporté dans la majorité des études précédentes.

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EFFECTS OF CAFFEINE CONSUMPTION ON ENERGY METABOLISM AND HEART RATE IN LEAN AND OBESE WOMEN

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Introduction

Although coffee is the most popular beverage consumed in industrialized societies, it is surprising to note that the number of studies performed on the thermogenic effect of caffeine in humans is somehow limited (1-6). The early studies date back from the first half of this century (7-11) and the few subsequent investigations have primarily reported thermogenic responses over a short period of time (1-4, 6). In addition, no data is available in overweight subjects during a 24-hour period.

The aim of the present study was to explore the magnitude of coffee-induced thermogenesis (i.e. the increase in heat production consecutive to coffee consumption) as well as the effect of coffee intake on lipid, carbohydrate and protein utilization assessed over a 24 hour period in both lean and overweight women.

Material and Methods

Ten lean and 10 obese non-smoker women, aged from 20 to 35 years participated in this study. Their physical characteristics are shown in Table 1. The obese women were selected on the basis of a body mass index (BMI) greater than 25 kg/m^2 and a percentage body fat > 30% of body weight. The women were moderate coffee drinkers (2 to 4 cups of coffee per day).

Table 1

CHARACTERISTICS OF THE SUBJECTS

Group	Lean	Obese
Age (years)	24.0 ± 1.0	25.0 ± 1.2
Weight (kg)	62.4 ± 3.1	80.2 ± 2.6
Height (cm)	167.3 ± 1.7	169.5 ± 2.3
BMI (kg/m2)	22.3 ± 1.0	28.0 ± 0.9
Body fat (5)	27.6 ± 1.9	36.1 ± 0.8

In order to assess 24 h energy expenditure and substrate oxidation, each women spent one day in a large whole body respiration chamber (12) on two occasions: on the first occasion they drank coffee with caffeine and on the other occasion decaffeinated coffee. The cups of coffee were drunk at breakfast, lunch and dinner as well as in the middle of the morning and in the middle of the afternoon. The caffeinated coffee contained 3.73% caffeine (Nescafé, Nestlé) whereas the decaffeinated coffee contained only 0.063% caffeine. Each subject was randomly allocated to one of the two treatments using a double-blind crossover design. The total caffeine dose ingested by each subject was calculated from actual body weight for the lean women (20 mg/kg·d) and from the predicted "normal" body weight (calculated from a BMI of 22.5 kg/m^2) for the obese women.

Two periods of exercise of half an hour duration (walking on a motor driven treadmill at 4 km/h, 5 & 10 % elevation) were performed by the subjects. Spontaneous physical activity was measured by a radar (13). Continous heart rate recording was performed during day-time by means of a portable device. Following the night in the chamber, basal metabolic rate BMR was measured by indirect calorimetry using a ventilated hood system (14).

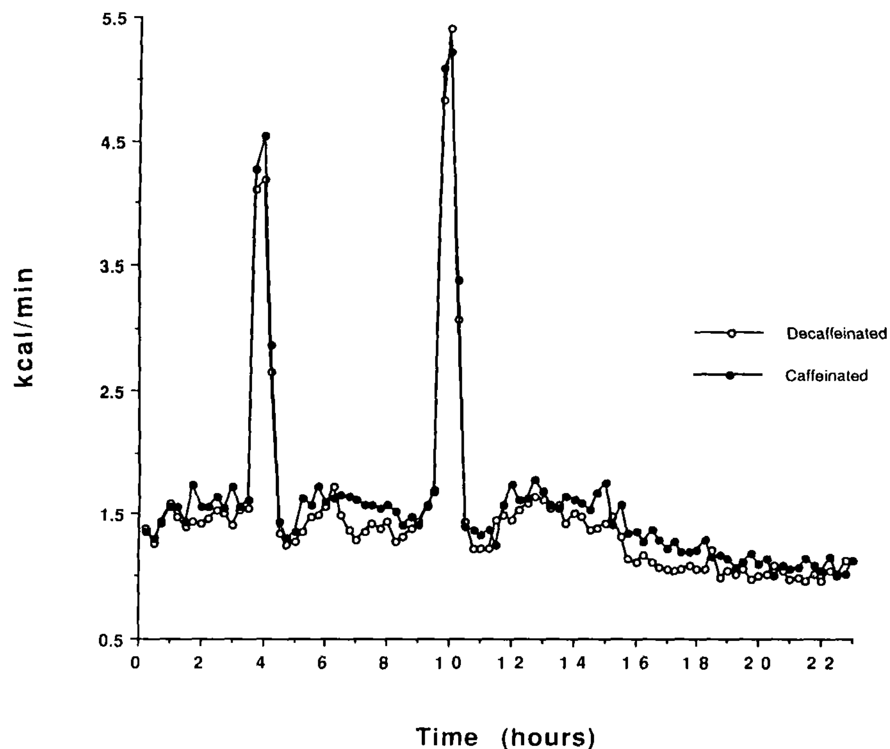
During both tests, energy intake in the respiration chamber was identical. It was calculated for each women on the basis of the predicted basal metabolic rate (BMR) of each subject with a 55% increment to take into account postprandial thermogenesis and the cost of physical activity (1.55 x BMR). The diet consisted of usual food and contained 50% carbohydrate energy, 32% lipid energy and 18% protein energy. The meals were prepared by a trained dietitian.

Twenty-four hour urinary collections partitioned into a day-pool and a night-pool were performed. The rates of nitrogen, epinephrine, norepinephrine, theobromine, theophylline, paraxanthine and caffeine were measured. In addition, a saliva sample was collected in the late afternoon for methylxanthines determination.

Results

Twenty-four hour energy expenditure was significantly increased by caffeinated coffee consumption in lean (164 ± 28 kcal/day, i.e. a rise of 7.6 ± 1.3%) and in obese subjects 124 ± 53 kcal/day, i.e. a rise of 4.9 ± 2%, $p < 0.005$). In lean women, the thermogenic effect of caffeine was observed both during day time and night time whereas in obese subjects the rise in energy expenditure occurred primarily during day time. The pattern of change in 24 EE throughout the study period in the control group is shown in Figure 1. No difference in spontaneous physical activity (as measured by the radar system) was observed among the two groups of subjects or between the two treatments.

Figure 1



- Energy expenditure during the 24 hour test in **lean** women. Each point represents a 15 minute measurement period.
- o decaffeinated tests; • caffeinated tests.
- In **obese** women, the stimulation of energy expenditure was less important and less prolonged during the night than in **lean** women.

Basal metabolic rate, measured on the following day in postabsorptive conditions, was not influenced by caffeine consumption in both groups of subjects. In addition, caffeine consumption did not affect the energy cost of walking (Table 2).

Table 2

Energy expenditure (EE) during exercise and rest

	Exercise kcal/min		Basal EE kcal/min	
	lean	**obese**	**lean**	**obese**
Decaffeinated	5.11 ± 0.35	6.78 ± 0.34	1.01 ± 0.02	1.12 ± 0.03
Caffeinated	5.40 ± 0.39	6.78 ± 0.30	1.04 ± 0.03	1.13 ± 0.03
Difference	NS	NS	NS	NS

The overall 24 h respiratory quotient (RQ) was nearly identical with both treatments in obese and lean subjects (Table 3). This was also the case for the RQ measured during treadmill exercise. By contrast, in postabsorptive conditions, the morning following the caffeinated coffee consumption, a modest decrease in RQ - which was statistically significant during BMR and on the borderline of statistical significance during sleep - was observed in both groups of women.

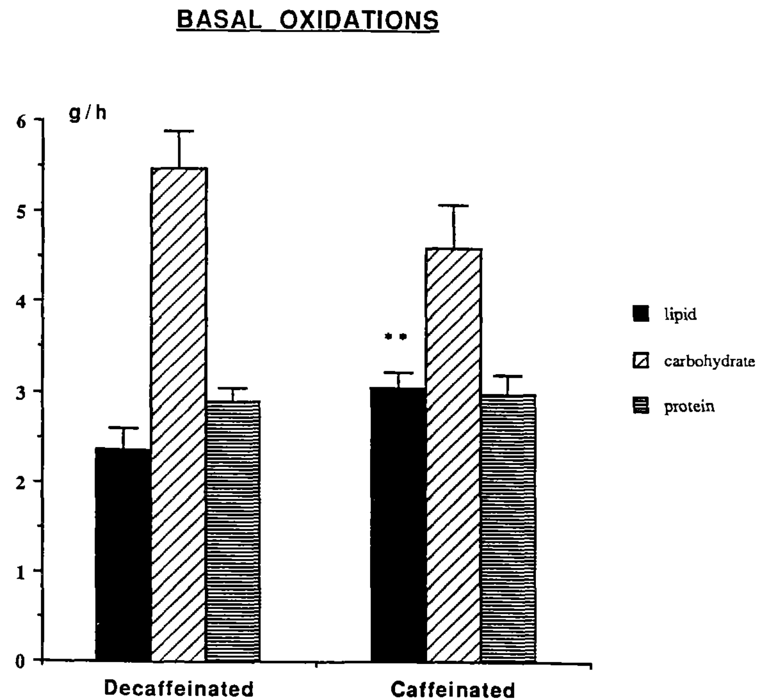
Table 3

Respiratory quotient (RQ)

	24 hour RQ		Exercise RQ		Basal RQ	
	lean	**obese**	**lean**	**obese**	**lean**	**obese**
Decaffeinated	0.84 ± 0.01	0.83 ± 0.01	0.92 ± 0.01	0.96 ± 0.01	0.85 ± 0.01	0.83 ±0.01
Caffeinated	0.84 ± 0.01	0.83 ± 0.01	0.91 ± 0.01	0.94 ± 0.01	0.83 ± 0.01	0.80 ± 0.01
Difference	NS	NS	NS	NS	p=0.008	p=0.02

Expressed in terms of substrate oxidation, the lower RQ induced by caffeinated coffee consumption reflected an increased postabsorptive lipid oxidation in response to caffeine which was drunk the previous day (Figure 2).

Figure 2



- Substrate oxidation measured during the basal metabolic measurements on the day following coffee consumption in the **lean** women. There was no stimulation of energy expenditure secondary to caffeine at this time but an enhanced fat oxidation. The changes in carbohydrates and protein oxidations were not significant. Values are mean ± SEM.

Despite high doses of caffeine, the caffeinated coffee consumption did not show any effect on heart rate: during the day time, the heart rate was nearly identical with both treatments (Table 4). This was also the case during the exercise period.

Table 4

Heart rates

	Diurnal beats/min		Exercise beats/min	
	lean	**obese**	**lean**	**obese**
Decaffeinated	81 ± 3	80 ± 3	122 ± 4	129 ± 4
Caffeinated	80 ± 3	79 ± 3	124 ± 5	128 ± 5
Difference	NS	NS	NS	NS

The rate urinary catecholamine excretions were measured both during the day- and night-time spent in the chamber. Coffee consumption increased the rate of urinary epinephrine excretion during day time in both groups (Table 5). This could partly explain the thermogenic effect of caffeine observed. The slight rise in urinary norepinephrine excretion observed with caffeinated coffee was not statistically significant. The urinary excretions of urinary methylxanthines are also shown in Table 5. Approximately 2% of the administered caffeine dose were excreted in urine over a 24-h period. The amount of theobromine, theophylline and paraxanthine excretions in urine was substantially higher in obese subjects when compared to lean women, although this difference was not statistically significant for paraxanthine.

Table 5

Diurnal urinary catecholamines excretion

	Epinephrine (µg/h)		Nor epinephrine (µg/h)	
	lean	**obese**	**lean**	**obese**
Decaffeinated	0.53 ± 0.05	0.61 ± 0.10	2.28 ± 0.15	2.66 ± 0.24
Caffeinated	0.80 ± 0.06	0.83 ± 0.09	2.39 ± 0.18	2.83 ± 0.33
Difference	p=0.007	p=0.04	NS	NS

Urinary methylxanthines excretion (mg/d)

Group	Theobromine	Theophylline	Paraxanthine	Caffeine
Lean	9.9 ± 1.74	2.5 ± 0.2	36.2 ± 5.4	25.6 ± 4.7
Obese	16.6 ± 2.7	5.1 ± 0.7	51.5 ± 6.4	24.3± 2.8
Difference	p=0.03	p=0.002	NS	NS

Discussion and conclusion

The study demonstrates that the consumption of coffee containing caffeine results in a significant thermogenic response which should not be neglected in real life situation.

The thermogenic effect of caffeine primarily takes place during the course of the day, namely in the postprandial phase, after the ingestion of coffee, whereas the lipolytic effect of caffeine is delayed and was observed only several hours after the last meal and the last coffee consumption, namely in the postabsorptive conditions the next morning. It is possible to obtain an estimate of the effects of each cup of coffeee consumption on the magnitude of thermogenesis: if one divides, in the lean group, the excess energy expenditure induced by caffeine consumption by the number of cups of coffee consumed during this study, one gets a value of 17 kcal of energy expended per cup of coffee (containing typically 100 mg of caffeine). If one adds to the thermogenic effect the lipolytic effect of caffeine obtained in fasting conditions, the excess energy expended and lipid burned out may play a non-negligible role in the daily energy balance of man.

These two effects were of smaller magnitude in obese as compared to lean women. The heart rate pattern observed during the day was not affected by caffeinated coffee consumption. Obese women exhibited a higher urinary excretion of methylxanthine (theobromine, theophylline) than lean subjects.

The present study suggests that in addition to its thermogenic effect, caffeine could also contribute to stimulate the rate of lipid oxidation in a postabsorptive subject. This appears particularly interesting since the capacity to oxidize endogenous lipids in fasting conditions represents an important factor in body weight regulation. In this context, the ingestion of caffeinated coffee may have a positive impact in the maintenance of body weight. However, it should be pointed out that the relative contribution of coffee to weight regulation is relatively modest, as compared to the influence of the composition of food intake on body weight. Recent evidence suggests that the proportion of lipid in the daily food intake is an important determinant of body weight in humans.

Summary

The magnitude of coffee induced thermogenesis and the influences on substrate oxidation were investigated in 10 lean and 10 obese healthy women, over two 24-hour period in a respiratory chamber. On one occasion they consumed coffee and on the other occasion, the same amount of decaffeinated coffee. The magnitude of thermogenesis and its duration were smaller in obese (4.9 ± 2.0%) than in lean subjects (7.6 ± 1.3%). It was prolonged during the night only in lean women. The stimulation of energy expenditure was mediated by a concomitant increase in both lipid and carbohydrate oxidation. On the following day, in postabsorptive basal conditions, the thermogenic effect vanished, but there was a significant increase in lipid oxidation in both groups. The magnitude of this effect was however blunted in obese women.

Résumé

L'ampleur de l'augmentation de la thermogenèse induite par la consommation de café ainsi que l'effet sur l'utilisation des substrats ont été étudiées auprès de 10 femmes minces et 10 femmes obèses pendant deux périodes de 24 heures passées dans une chambre respiratoire. Au cours du premier test, elles ont consommé du café caféiné et du café décaféiné au cours du second. L'ampleur de la thermogenèse et sa durée étaient moins élevées chez les obèses (4.9 ± 2.0%) que chez les sujets minces (7.6 ± 1.3%). L'effet thermogénique du café était prolongé pendant la nuit chez les femmes minces seulement. La stimulation de la dépense énergétique était expliquée par une augmentation simultanée de l'oxidation des lipides et des glucides. Dans des conditions basales postabsorptives (le lendemain du test) l'effet thermogénique avait disparu, mais on constatait une augmentation significative de l'oxidation des lipides dans les deux groupes. Cependant, l'ampleur de cet effet était significativement plus faible chez les femmes obèses.

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ITALIAN STYLE COFFEE AND SERUM CHOLESTEROL

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Introduction

The first experiment on coffee consumption and blood cholesterol level was described by Egede-Nissen on 1970 in hypercholesterolaemic subjects; although the observation was scarcely controlled, the author suggested that subjects abstaining from coffee reduce their serum levels of total cholesterol by 17% (1).
The strongest and positive association between coffee consumption and serum cholesterol is reported in the Tromsø Study, carried out in the Scandinavian countries (2), where the coffee is widely consumed as boiled coffee.
To clarify the relation between brewing methods and blood cholesterol, Bak (3), analyzed 24 cross-sectional studies and observed that the beta-coefficient of total cholesterol per cup of coffee was fourfold higher in populations who drank boiled coffee than in populations drinking filtered coffee. Bak's observation was confirmed by recent experimental studies (4-9).
Fried et al (10) reported a significant positive association between consumption of filtered normal coffee and both LDL-cholesterol and HDL-cholesterol. On the contrary, Superko et al (11) found an increase of LDL-cholesterol and Apo-lipoprotein B in subjects consuming decaffeinated filtered coffee, without increase in drinkers of normal coffee. A factor present in the *Robusta* coffee species, usually utilized for the preparation of decaffeinated coffee was suggested to be responsible for the difference between caffeinated and decaffeinated coffee. In the Israeli Cordis study, the consumption of 'mud' coffee (a type of Turkish coffee) and of the instant coffee, resulted in an increase of serum total cholesterol and LDL-cholesterol (12). The study was not controlled for milk and milk products consumption.
Finally, Zoch et al (13) claimed that the cholesterol-raising factor is contained in the lipid-rich fraction of coffee, as separated after boiling.
On the basis of these considerations, we carried out the present study with the aim to evaluate if the Italian coffee (based on filtration) might induce the changes in blood lipids observed in humans when coffee is brewed by boiling.

Methods

Eighty-four young adults were selected among physicians recruited for army service as Officers in the Health Military Corps, in the Army Medical School of Florence (Italy). After a 3-week baseline (habitual coffee consumption) subjects were randomly allocated for 6 weeks in three groups of coffee consumption: "espresso"; "mocha"; and no coffee, but tea. The 6-week intervention was followed by a 6-week switch back, during which subjects come back to their habitual consumption. The size of each group was established in about 30 subjects.
The phases of the study are reported in fig 1.

Figure 1: Study calendar

Baseline					
Clin. examin.	Anthropometry	Blood			
Blood pressure	Food freq..	Life style			
Blood					
Intervention					
Blood	Blood	Blood	Blood	Blood	Blood
Coffee	Coffee	Coffee	Coffee	Coffee	Coffee
	Anthropometry	Food freq..	Life style	Anthropometry	Food freq..
Switch back					
	Blood		Blood		Blood
1st	**2nd**	**3rd**	**4th**	**5th**	**6th**
week					

During the intervention the subjects were asked to maintain their habitual "life style" and "dietary habits". The sample was composed of habitual coffee consumers and the tea group (abstaining from coffee) was considered as experimental group. Eleven blood samples were collected for each subject (2, 6, and 3 in the baseline, the intervention, and the switch back period, respectively) and analyzed in the same occasion.
Basic anthropomety and blood pressure (systolic and diastolic) were measured.
Basic clinical examination and clinical history were collected, as well as a self administered questionnaire concerning smoking habits, alcohol and drugs use, and physical activity.
The dietary history was asked to the subjects three times, using an "ad hoc" constructed semi-quantitative food frequency questionnaire to rank intake levels. Coffee consumption and added milk were daily auto-recorded during the 6-week intervention period.
Espresso coffee was prepared using a professional machine (at a pressure of about 9 bar and at the temperature of 90-94 °C). Mocha coffee was brewed by a mocha coffee pot, which consists of three parts. Water is placed in the lower portion and ground coffee is placed above this in a metal filter; the boiling water, under the slight pressure created by the steam, rises through the filter and the bed of ground coffee and passes through a tube into the upper portion of the coffee machine.
Usually 6 g of finely ground (medium to dark roasted) coffee were used per cup. All coffee used was a mixture of *Arabica* species, available on the Italian market. Tea was a brand of regular tea.

Results

No subject interrupted the study; only 7 subjects missed the last blood collection of the switch back for logistic reasons. During the intervention, only one subject of the tea group declared the consumption of one cup of mocha coffee.
The habitual alcohol use, smoking habits and physical activity of subjects are shown in table 1.

About 74% of subjects drink alcoholic beverages, mostly wine and beer; 34% smoke, on average 10-20 cigarettes per day; and more than 90% has a regular physical activity for about 20 minutes per day, out of the regular military training. The mocha group showed the higher percent of alcohol drinkers, the lower proportion of subjects performing physical activity, and the lower use of drugs, but no significant differences were observed among groups (Chi-square).

Tab 1. Description of the three study groups: Percent of subjects with the features

	Whole sample	Espresso	Mocha	Tea
Number of subjects	84	28	28	28
Alcohol consumers (%)	74	68	82	73
Smokers (%)	34	39	39	23
Physical activity				
never (%)	9	4	14	8
minutes/day	20	21	18	21
Drug users (%)	10	14	4	12
Hypertension in family (%)	38	36	39	38
Hyperlipidemia in family (%)	13	12	12	19

No significant difference was observed by Chi-square

The dietary habits of the three study groups during the intervention are reported in table 2.

Tab 2. Dietary pattern, limited to energy, coffee, fatty acids, milk and dairy products, of the three experimental groups during the intervention period (mean ± SD).

	Espresso	Mocha	Tea
Energy (kcal)	2207±526	2371±494	2411±505
Coffee (cup/day)	3.1±1.2	2.8±1.1	0
SFA (g/day)	38.1±9.6	35.4±5.3	44.0±8.7
MUFA (g/day)	44.7±9.7	39.2±7.1	51.6±10.5
PUFA (g/day)	12.6±4.8	10.5±2.8	10.5±2.3
Milk (g/day)	95±104	137±108	126±120
Dairy products (g/day)	116±91	124±84	107±66

No between groups effect was observed by ANOVA at 95%

No significant differences among groups were observed by ANOVA, even though the tea group showed a higher intake of fatty acids. The mean coffee consumption during intervention was 3.1 and 2.8 cups/day/per person for espresso and mocha group, respectively.

Figures 2 and 3 show the point by point values of total cholesterol and LDL-cholesterol during the study. Trends were rather comparable, the values during the baseline and intervention periods being almost similar. Although during the switch back period both total and LDL-cholesterol tend to gradually increase, suggesting a change in the determinants of lipid profile, no significant differences by factorial ANOVA were found among groups. We explain this increase with the change in life style (and possibly in dietary pattern) due to the end of the training at the military medical school and the return home for a 2-week holiday.

Figure 2: Point by point serum cholesterol levels (nmol/L) in the three experimental groups (mean±SE).

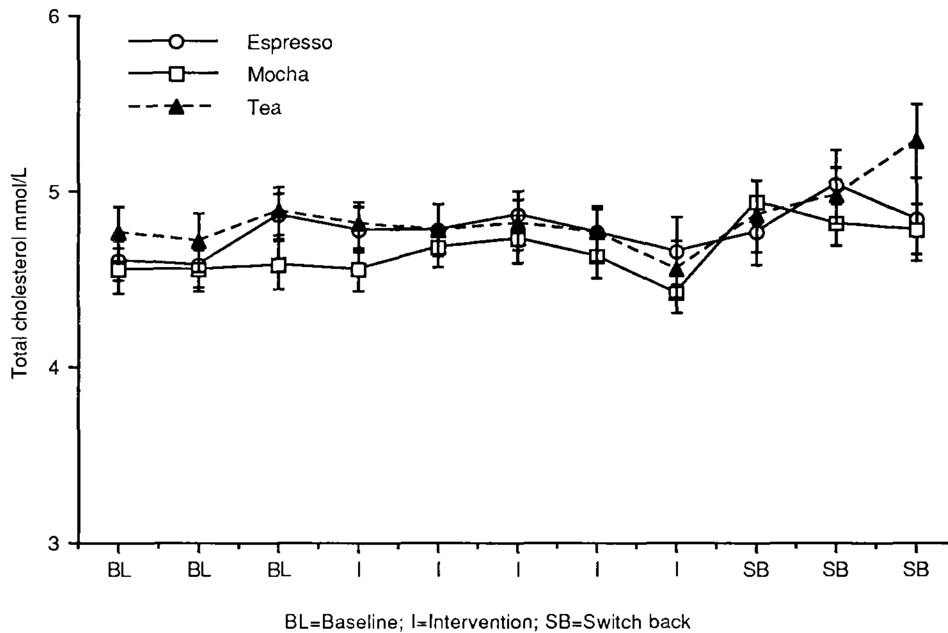
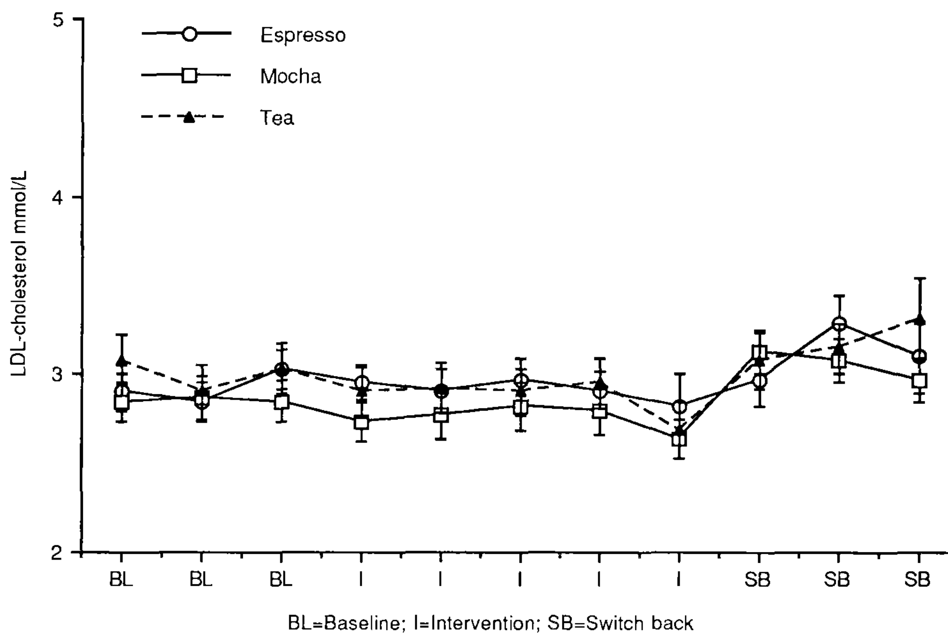


Figure 3: Point by point LDL cholesterol levels (nmol/L) in the three experimental groups (mean±SE).



Discussion

Our results demonstrated that the Italian coffee does not determine changes in blood lipids and lipoproteins. Neither the restriction of coffee intake (tea group) altered the lipidemic profile of subjects, as hypothesized in the design of this study. The small differences between the baseline and intervention periods were not significant, and without biological relevance.

As hypothesized by Zoch et al (13), the factor in coffee responsible for the increase of blood cholesterol should be searched in the lipid-rich soluble fraction of the boiled coffee that, as reported by Ahola et al (14), is retained by the paper filter.

In our study neither espresso nor mocha affected the serum lipid profile, suggesting that the "increasing cholesterol" factor does not pass through the metal filter of the coffee machine or is not extracted from the ground coffee. This could be also due to the very short contact-time between coffee and hot water in the espresso and mocha preparations.

The boiling method requires 10 or more minutes of direct contact of grounded coffee and boiling water, while the methods based on filtration require a shorter time of contact between coffee and hot water (20-100 seconds).

In conclusion our results reinforce the hypothesis that the filtered coffee does not act on blood lipids, even though the paper filter is not present, and, thus, that brewing method of coffee could be a determinant factor in regulating blood lipid levels.

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Abstract

The purpose of this study was to evaluate if the Italian coffee preparations (based on filtration) might induce the changes in blood lipids observed in humans when coffee is brewed by boiling method. Eighty-four young adults, after a 3-week baseline, were randomly allocated in three regimes of coffee consumption: espresso, mocha, and no coffee, but tea. The 6-week intervention was followed by a 6-week switch back. Total cholesterol and LDL-cholesterol were measured 11 times during the study. Dietary pattern, alcohol consumption, smoking habits, drug use, and anthropometric data were also recorded. No relevant and significant change of serum total cholesterol, HDL-cholesterol and LDL-cholesterol was observed between baseline and intervention in all groups.
The results indicate that coffee brewed in the Italian way does not alter blood levels of total cholesterol and LDL-cholesterol, reinforcing the hypothesis that filtered coffee does not affect blood lipids.

Key words: Espresso and Mocha coffee, brewing methods, serum cholesterol, intervention study.

Resume:

Cette étude avait pour but d'évaluer si le café italien (préparé par filtration) a les mêmes effets sur les lipides hématiques que le café preparé par ébulition.
Quatre vingt quatre adultes , àpres trois semaines de ligne de base (consommation habituelle de café), ont été divisés par tirage au sort en trois groupes consommant exclusivementsoit du café express, soit du café moka soit du thé. Les six semaines d'intervention ont été suivies par trois semaines à la ligne de base. Le cholesterol total et LDL ont été mesurés à 11 reprises au cours de cette experience. Les apports alimentaires, la consommation d'alcool, de cigarettes, de médicaments et les données anthropomètriques ont été enregistrées. Dans aucun des groupes une difference significative n'a été mise en evidence entre le niveau de base et la période d'intervention.
Ces resultats suggèrent que le café infusé à la manière italienne n'a pas d'effet sur le cholesterol total et LDL, confirmant l'hypothèse selon laquelle le café filtré ne modifie pas les lipides hématiques.

Mots cles: Café express, café moka, méthodes d'infusion, cholesterol hématique, intervention

EFFECTS OF COFFEE OIL ON PLASMA CHOLESTEROL IN THE RAT

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Introduction

Cross-sectional studies within populations have demonstrated that consumption of "Scandinavian-type" boiled coffee is associated with increased serum cholesterol levels in man (1-7). Controlled clinical trials have confirmed the cholesterol raising effect of boiled coffee and demonstrated that the method of preparation of coffee is critical in that drip-filtered coffee or paper-filtered boiled coffee had no effect on serum cholesterol (8-11). The lipid fraction isolated from boiled coffee was shown to elevate serum cholesterol (12) suggesting that the causative agent(s) responsible for the hypercholesterolaemic effect of boiled coffee resides in the lipid fraction which is retained in the filter paper during filtration of the brew (13,14). Analysis of the lipid content of different brewed coffees supports this hypothesis since "Scandinavian-type" boiled coffee can contain about 60-160 mg coffee oil per cup, filtered coffee contains about 1-7 mg coffee oil per cup (15). Instant coffee is similar to filtered coffee with 1.8-6.6 mg coffee oil per cup (15).

The major components of the coffee lipid fraction are triglycerides (which comprise about 90% of the coffee lipids) sterols, phospholipids and diterpene alcohol esters (16). It has been proposed that the cholesterol-raising factor(s) is a non-triglyceride, non-saponifiable component of this lipid fraction (12-14).

The availability of a suitable animal model would greatly facilitate the identification of the causative agent(s) responsible in the coffee lipid fraction for the hypercholesterolaemic effect of boiled coffee and for delineating the mechanisms involved. It was recently reported that boiled coffee consumption in place of drinking water by male Syrian hamsters fed on a high-fat diet produced an increase in plasma total cholesterol levels (17). However, another group reported that freeze-dried boiled coffee incorporated into the diets of either hamsters or gerbils had no influence on serum cholesterol levels (18). The reasons for this apparent difference in responsiveness to boiled coffee are unknown and illustrates the difficulties in finding an appropriate animal model system to study the mechanisms underlying the hypercholesterolaemic effects of coffee lipids. Although the rat is usually considered an unsuitable model for studying certain aspects of lipoprotein metabolism we report here for the first time that consumption of coffee oil produces an increase in plasma cholesterol levels in this species. This effect was

accompanied by alterations in other parameters which suggest that the liver may be the target organ for the hypercholesterolaemic effects of coffee lipids.

Materials and Methods

Male and female Sprague-Dawley rats (3 weeks of age) were obtained from Iffa Credo S.A. (L'Arbresle, France). The animals were individually housed and fed certified laboratory chow (Nafag 890, Nähr und Futtermittel AG, Gossau, Switzerland) as basal diet. Following 11 days on basal diet the animals were randomly allocated to four groups (A-D), each comprising 10 males and 10 females. Group A received basal diet into which 2.5% of a 50:50 mixture of corn and palm oils (reference oil) were incorporated; Group B received basal diet containing 0.1% coffee oil and 2.4% reference oil; Group C received basal diet containing 0.5% coffee oil and 2% reference oil; and Group D received basal diet into which 2.5% coffee oil was incorporated. The corn-palm oil mixture was used as reference oil since its fatty acid composition closely matched that of the coffee oil. The coffee oil used was extracted from spent coffee grounds and can be considered to be representative of the lipid component of boiled coffee (15). Chemical analysis demonstrated the coffee oil to contain about 7.9% unsaponifiable matter.

The test diets were provided *ad libitum* and food and water consumption, and body weights were measured at regular intervals throughout the study. After 28 days on the test diets the animals were fasted for 20 hours, anaesthetised and blood was collected from the abdominal aorta. The livers were excised, weighed, fixed in Bouins and embedded in Paraplast paraffin wax. Sections were stained with Haematoxylin and Eosin prior to histopathological evaluation. Plasma total cholesterol (19) and bilirubin (20) were determined using a Cobas Bio automated analyser (Roche Diagnostica). Serum protein profiles were determined by electrophoresis on cellulose acetate in a Beckman Microzone cuvette and evaluated using a Beckman Appraise densitometer. The results were analysed using Dunnet's t-test.

Authorisation for animal experimentation was obtained from the Cantonal Veterinary Service, Vaud, Switzerland and experimentation was performed in conformance with Swiss federal and cantonal laws on animal protection, and in accordance with the ethical guidelines of the Swiss Academy of Science and Medicine.

Results

The test diets produced no abnormal behaviour and the animals remained in good health throughout the study period. A significant reduction of body weight gain was observed for group D animals throughout the course of the study and this was reflected in the terminal body weights (Table 1). Liver weights in both sexes of group D animals were significantly increased ($p<0.01$). (Table 1). When expressed as a ratio of body weight liver weights in both groups and D were found to be significantly increased ($p<0.01$). Food and water intake was significantly reduced in animals of group D during the first week of treatment, but were comparable to controls (Group A) thereafter (data not shown). The mean daily intake of coffee oil calculated on the basis of weekly food consumption and body weight was 99, 515, 2500 mg per kg body weight for males, and 100, 494, 2470 mg per kg body weight for females, for groups B, C and D respectively.

Plasma total cholesterol was significantly increased in male animals of group D and was also slightly but not significantly increased in males of groups B and C compared to controls (Table 2). In contrast females were much less responsive to the effects of coffee oil on plasma cholesterol. Plasma bilirubin levels were greater ($p<0.01$) in the coffee oil groups C and D for males, and for females for group D compared to control animals fed the reference oil (Table 2). Group C and D males also showed a marked alteration in their serum protein electrophoretic profile with an increase in the beta globulin fraction accompanied by a corresponding decrease in the alpha-1 globulin fraction ($p<0.01$). In contrast in females of groups C and D, the decrease in alpha-1 globulins was reciprocated by an increase in the albumin fraction (Table 3).

Table 1. Body weight and liver weights in Sprague Dawley rats fed diets containing coffee oil

	Males			
	A	*B*	*C*	*D*
Coffee Oil (mg/kg bw/day)	0	96	515	2500
Body weight (g)	317 ± 21	314 ± 25	305 ± 27	288 ± 24*
Liver weight (g)	9.9 ± 0.9	10.1 ± 1.0	10.5 ± 1.0	13.4 ± 1.7**
Liver/Body weight	3.1 ± 0.15	3.21 ± 0.15	3.45 ± 0.23**	4.64 ± 0.30**
	Females			
	A	*B*	*C*	*D*
Coffee Oil (mg/kg bw/day)	0	100	494	2470
Body weight (g)	221 ± 19	217 ± 21	215 ± 16	205 ± 14
Liver weight (g)	6.8 ± 0.7	6.6 ± 0.7	7.2 ± 0.7	8.1 ± 0.8**
Liver/Body weight	3.08 ± 0.11	3.04 ± 0.13	3.36 ± 0.18**	3.96 ± 0.27**

Mean values ± standard deviations.
Mean values significantly different from controls: *P<0.05, **P<0.01

Table 2. Plasma cholesterol and bilirubin in Sprague Dawley rats fed diets containing coffee oil.

	Males			
	A	*B*	*C*	*D*
Total cholesterol (mmol/l)	1.62 ± 0.37	1.89 ± 0.30	1.97 ± 0.33	2.59 ± 0.32**
Total bilirubin (mmol/l)	1.12 ± 0.40	1.19 ± 0.35	1.64 ± 0.30**	2.65 ± 0.33**
	Females			
	A	*B*	*C*	*D*
Total cholesterol (mmol/l)	2.13 ± 0.35	2.14 ± 0.37	2.25 ± 0.45	2.44 ± 0.49
Total bilirubin (mmol/l)	1.26 ± 0.31	1.25 ± 0.32	1.38 ± 045	2.04 ± 0.71**

Mean values ± standard deviations.
Mean values significantly different from controls: *P<0.05, **P<0.01

Table 3. Serum protein profile in Sprague Dawley rats fed diets containing coffee oil.

	Males			
	A	*B*	*C*	*D*
Albumin (%)	49.9 ± 2.5	49.1 ± 1.8	49.2 ± 2.0	50.5 ± 1.8
Alpha-1 globulins (%)	24.4 ± 2.9	24.4 ± 1.7	21.3 ± 1.7**	16.8 ± 1.4**
Alpha-2 globulins (%)	8.1 ± 0.6	8.2 ± 0.8	8.8 ± 0.6	9.7 ± 0.8**
Beta globulins (%)	15.5 ± 0.8	15.9 ± 1.2	18.4 ± 1.3**	21.0 ± 1.4**
Gamma globulins (%)	2.1 ± 0.5	2.4 ± 0.5	2.3 ± 0.6	2.0 ± 0.5
	Females			
	A	*B*	*C*	*D*
Albumin (%)	50.1 ± 2.3	51.2 ± 1.8	52.8 ± 2.6*	54.9 ± 2.5**
Alpha-1 globulins (%)	24.3 ± 1.5	23.2 ± 1.2	22.1 ± 1.8*	19.3 ± 2.6**
Alpha-2 globulins (%)	7.0 ± 0.6	7.1 ± 0.8	7.0 ± 0.8	7.5 ± 0.6
Beta globulins (%)	15.8 ± 1.3	15.8 ± 0.9	15.7 ± 1.6	16.1 ± 1.0
Gamma globulins (%)	2.9 ± 0.6	2.8 ± 0.6	2.5 ± 0.7	2.2 ± 0.7

Mean values ± standard deviations
Mean values significantly different from controls: *P<0.05, **P<0.01

No major deliterious alterations in liver histopathology were noted. However, there was a dose-dependent increase in the incidence and severity of bile duct hypertrophy with coffee oil treatment for both sexes (Table 4).

Table 4. Bile duct hypertrophy in Sprague Dawley rats fed diets containing coffee oil.

	Males				Females			
	A	*B*	*C*	*D*	*A*	*B*	*C*	*D*
Absent	5	4	0	0	9	4	2	0
Minimal (multifocal)	5	3	4	1	1	4	6	5
Very slight (generalised)	0	3	6	1	0	2	2	5
Slight (generalised)	0	0	0	7	0	0	0	0
Slight/moderate (generalised)	0	0	0	1	0	0	0	0

Values shown are the number of animals showing finding (n=10 per group per sex)

All of the coffee oil induced effects were found to be reversible on withdrawal of the test diets (data not shown).

Discussion

Coffee oil incorporated into the diet of Sprague-Dawley rats produced a dose-dependent increase in plasma cholesterol levels, predominantly in male rats. At 2.5% coffee oil in the diet there was a 60% increase in total cholesterol ($p<0.01$) while at 0.5% there was a 22% increase, the latter not reaching statistical significance Equivalent doses of coffee oil for humans based on energy intake would be 14.5 and 2.9 g/day respectively (Table 5). Interestingly in recent clinical studies in humans the administration of coffee lipids at 2 and 3 g/day produced increases in serum cholesterol of about 13% and 25% respectively over initial levels[1]. A previous clinical study in humans with 1.3 g coffee lipids per day reported an increase in serum cholesterol of 16% and 23% after 3 and 6 weeks respectively (12). Thus on an energy intake basis the male rat appears to be of similar sensitivity to the hypercholesterolaemic effects of coffee lipids as humans.

Table 4. Extrapolation of Coffee Oil Exposure - Human vs Rat

Parameter	Man	Rat
Body Weight (kg)	70	0.3
Food Intake:		
(g/day)	500	25
(g/kg body weight/day)	7.14	83.3
(MJ/day)	10	0.43
Coffee Oil Intake:		
(Standardised to energy intake)		
(g/day)	2	0.086
(g/MJ)	0.2	0.2
(g/kg diet)	4	3.44

In addition to its effects on plasma cholesterol levels, coffee oil also produced a dose-dependent increase in plasma bilirubin and an alteration in the serum protein profile. Again there was a clear sex difference in effect indicating an association with the effect on plasma cholesterol. Plasma bilirubin levels are controlled by the liver and increased levels may reflect a decreased biliary clearance. Taken together with the effects of coffee oil on liver weight and bile duct hypertrophy these results implicate the liver to be a target organ for the effects of coffee lipids. The altered serum protein profile is also likely to be mediated through effects on the liver. The increase in alpha-2 and beta-globulins at the expense of alpha-1 globulins may reflect an alteration in the plasma lipoprotein profile and this is the subject of current investigations.

The effects of coffee oil on serum protein profile and plasma bilirubin occur at levels lower than those which produce a significant effect on serum cholesterol. This provides an indication that the hypercholesterolaemic effects of coffee lipids may be secondary to their effects on mechanisms within the liver, for example effects on biliary clearance or apoprotein production or uptake.

In conclusion, the rat model described appears to be predictive for the human response to coffee lipids and may provide a useful tool to delineate the mechanisms involved in coffee-lipid induced hypercholesterolaemia. The liver

[1] R.P. Mensik et al. (submitted for publication).

appears to be the primary target for the coffee lipids and the effects on blood cholesterol may be secondary to effects on this organ.

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Abstract

The consumption of "Scandinavian-type" boiled coffee has been associated with elevated serum cholesterol levels in man. This effect has also been demonstrated after consumption of coffee oils. At the present time a reliable animal model to study the mechanisms responsible for this effect has not been established. We eport here for the first time that ingestion of coffee oil at high doses produces elevated plasma cholesterol in Sprague Dawley rats and that on an energy intake basis this species is of similar sensitivity to coffee lipids as humans. The effects of coffee lipids on plasma cholesterol in the rat appear to be secondary to their effects on the liver. This study demonstrates this model to be predictive for the human response to coffee lipids and is useful for delineating the mechanisms involved.

Résumé

La consommation de café préparé à la façon Scandinave a été associée à des concentrations sanguines de cholestérol élevées chez l'homme. Le même effet a été démontré après la consommation d'huiles de café. A présent il n'existe pas de modèle animal établi et sûr pour l'étude des méchanismes responsables de cet effet. Nous présentons ici le premier rapport qui démontre que l'ingestion d'huile de café à hautes doses produit une hypercholestérolémie chez le rat Sprague-Dawley et que, calculé sur la base de la consommation d'énergie, cette espèce montre une sensibilité aux huiles de café comparable à celle de l'homme. Chez le rat, les effets des huiles de café sur la concentration sanguine du cholestérol semblent être secondaires par rapport à leurs effets sur le foie. Notre expérience démontre que ce modèle laisse prédire la réaction humaine aux huiles de café et qu'il est utile pour l'étude des méchanismes impliqués.

« FACTEURS DE CONFUSION » DANS LES ÉTUDES ÉPIDÉMIOLOGIQUES SUR LA CONSOMMATION DE CAFÉ

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Durant les trentre dernières années, une grande partie de la recherche épidémiologique a été consacrée à l'étude des risques pour la santé associés au tabagisme, à la consommation de boissons alcoolisées et à celle du café. En comparaison avec les nombreux autres "facteurs d'environnement faisant l'objet de travaux épidémiologiques," il s'agit d'habitudes de consommation "adoptées" par une part importante de la population plutôt que "subies" comme peuvent l'être la pollution atmosphérique ou (souvent) une exposition professionnelle. Les limites de l'observation épidémiologique dans l'établissement de risques sont, ainsi que sous les verrons, encore plus grandes dans ce cas puisque la méthodologie s'écarte davantage du modèle expérimental dans lequel l'exposition au facteur d'environnement est attribué.

Dans les trois cas, les consommations concernent des xénobiotiques chimiquement très complexes ayant, chez l'homme, des effets physiologiques bien établis et dont certains effets toxiques (globaux ou d'un composé majoritairement actif) ont pu être mis en évidence chez l'animal, à des doses généralement très supérieures à celles communément observées dans les populations humaines. C'est en particulier le cas du café et de la caféine. Il est cependant important sur le plan de la santé publique, d'être en mesure d'apprécier quantitativement les risques associés aux expositions réelles au delà de la mise en évidence d'une simple signification statistique. A condition bien entendu que les risques calculés soient bien ceux associés à l'exposition c'est-à-dire qu'ils soient "sans biais".

Il n'est pas dans notre propos de discuter l'ensemble des travaux épidémiologiques portant sur les effets de la consommation de café ni même de recenser les biais potentiels (ou avérés) dans les résultats obtenus. De nombreuses revues générales permettent de faire le point dans chaque domaine de la pathologie d'où il ressort qu'une augmentation des risques pour la santé chez les forts consommateurs de café par rapport aux non consommateurs n'est retrouvée que de façon inconstante et les risques relatifs observés sont le plus souvent inférieurs à 2 [1]. Dans cette situation, l'analyse du "confounding" ("confusion"? en Français) joue un rôle particulièrement important dans la discussion des résultats, bien que sa méthodologie soit loin d'être entièrement mise au point ainsi que le montre encore la littérature récente [2]. Nous nous contenterons ici d'évoquer le rôle possible de certains facteurs de confusion dans l'estimation des risques associés à la consommation de café à partir d'exemples de travaux portant sur le cancer du pancréas et la reproduction chez la femme.

Rappelons que bien d'autres sources de biais existent en épidémiologie analytique, que l'étude effectuée soit de type cas-témoins, de cohortes ou de l'une de leur variante possible. Elles peuvent être réunies en deux catégories [3] :

- Les erreurs de mesure (de l'exposition) et de classification (malades/non malades) dont les conséquences sur le sens et l'importance du biais ne sont pas évidentes bien que l'on puisse s'attendre en général à une sous-estimation de l'intensité de la relation facteur-maladie.

- La séléction différentielle des sujets de l'étude (par exemple des cas et des témoins) qui, bien entendu, peut à priori introduire des biais dans l'un ou l'autre sens.

L'examen de la littérature dans les deux domaines pathologiques précédents, conduit à penser qu'aucune étude publiée n'échappe totalement à ces sources de biais, même dans le cas des études de cohortes dont la méthodologie, cependant, présente beaucoup d'avantages sur le plan théorique.

Schématiquement un facteur de confusion est à la fois en relation causale (ou présumée telle) avec la maladie et associé au facteur d'exposition, sans en être une conséquence [4]. On conçoit aisément qu'une partie, voire la totalité du risque observé avec le facteur d'exposition ne soit que le reflet du rôle du facteur de confusion. Le biais ainsi introduit est une surestimation du risque relatif d'autant plus forte que l'intensité du lien du facteur de confusion avec la maladie, d'une part et avec le facteur d'exposition, d'autre part, est importante.

L'analyse des effets de confusion ne peut évidemment être réalisée qu'à partir de facteurs connus ou suspectés par des hypothèses physiopathologiques ou des résultats antérieurs. La situation idéale est évidemment de pouvoir proposer un modèle de confusion unique et simple qui suffirait pour expliquer l'ensemble des observations déjà faites dans un même domaine et qui serait confirmée par les études ultérieures.

C'est ainsi qu'en 1984, KINLEY et McPHERSON [5] suggèrent que l'association du café avec le risque de cancer du pancréas observée dans leur étude cas-témoin pour des consommations récentes puisse refléter un état particulier de soif chez les malades en évolution, les conduisant, dans certains environnements culturels, à une consommation supérieure de café. Dans ce cas, le facteur de confusion ("la soif") est une conséquence de la maladie, entrainant une augmentation de l'exposition. Moins d'attention semble aujourd'hui accordée à ce facteur de confusion possible car, en particulier, l' élévation de la prise générale de boissons chez les cas n'a pas été retrouvée dans les études récentes [6]. Une démarche du même type a conduit plus récemment STEIN et SUSSER [7] à proposer que des facteurs hormonaux pourraient à la fois être le reflet d'un risque d'avortement et de prématurité et permettre une consommation supérieure de café par la femme enceinte (absence de nausée). Ce modèle clairement exposé par LEVITON à votre dernier Congrès [8] ne semble pas avoir encore reçu de confirmation épidémiologique.

Les deux exemples précédents illustrent clairement le fait qu'il ne peut exister de liste pré-établie de facteurs de confusion propres à l'étude des effets sur la santé d'une habitude de consommation comme celle du café et qu'il y aura toujours place, parallèlement au progrès des connaissances biologiques, à des modèles de confusion ad-hoc, spécifiques de chaque domaine physiopathologique et dont il conviendra de vérifier le bien-fondé.

Cependant, ainsi que nous le soulignions dans notre introduction, l'usage du café, comme celui du tabac et des boissons alcoolisées est adopté "spontanément" par une partie de la population et les forts consommateurs peuvent présenter des caractéristiques (comportementales, biologiques, psychosociales ...) qu'il est légitime d'évoquer comme possibles facteurs de confusion des risques liés à cet usage. Il peut paraitre étrange, à première vue, de reprendre, au sujet de la consommation de café, un ancien débat définitivement tranché dans le cas du tabagisme. Trois arguments de nature différente nous semblent devoir le justifier : biologique (l'évidence obtenue par l'expérimentation animale est sans commune mesure dans le cas du tabac et du café) - méthodologique (l'intensité des risques associés au tabac, contrairement au café, est telle que sa confusion par des caractéristiques individuelles des fumeurs est inenvisageable) - enfin épidémiologique (de fait, plusieurs études ont montré que le comportement tabagique joue effectivement un rôle de facteur de confusion pour la consommation de café et non l'inverse).

C'est ainsi que le tabagisme, considéré actuellement comme un facteur causal du cancer du pancréas [9] est associé de façon dose-dépendante à la consommation de café [10]. Il joue donc un rôle de confusion pour ce dernier facteur d'exposition. Il ne semblerait pas possible aujourd'hui de rapporter les résultats d'une étude sur ce sujet sans prendre en compte le tabagisme dans l'analyse, contrairement à plusieurs travaux publiés dans le passé. De la même façon, le tabagisme et la consommation d'alcool durant la grossesse jouent un rôle de facteur de confusion évident dans la relation entre la consommation de café et le poids de naissance des enfants [11].

Cependant la prise en compte d'un facteur de confusion ne permet en aucune façon de s'assurer que le biais qu'il crée soit supprimé. La mesure du facteur est entachée d'une erreur qui conduit généralement au maintien dans l'analyse d'un biais résiduel. Dans le

cas de la mesure de facteurs de consommation, l'erreur peut provenir de la difficulté d'estimer une consommation ancienne (tabac et cancer du pancréas) ou d'une sous-déclaration de consommation actuelle du tabac en particulier chez la femme enceinte (effet "MORRISON" décrit par LEVITON [8]. Une étude prospective Danoise importante (12 000 femmes enceintes) observe un risque relatif élevé d'enfants prématurés et/ou de petit poids de naissance (de l'ordre de 2) chez les mères buvant plus de 3 tasses de café par jour seulement lorsqu'elles sont non fumeuses [12] ; les auteurs eux-mêmes soulignent le rôle possible d'un biais de déclaration du tabagisme à l'origine de ce résultat. Enfin, la qualité de l'ajustement statistique sur le facteur de confusion que représente le tabagisme, doit être également discutée dans chaque cas et, qu'il s'agisse d'exposition chronique (cancers) ou actuelle (grossesse) l'analyse stratifiée chez les fumeurs et les non fumeurs [13] n'est vraisemblablement pas suffisante pour éliminer le biais de confusion.

Au delà du tabagisme et de la consommation d'alcool, les caractéristiques individuelles des consommateurs de café et ou de caféine, propres à jouer un rôle de facteur de confusion ont été peu recherchées d'une manière systématique en dehors du sondage effectué par SCHREIBER et al [10] dans un échantillon de la population adulte Américaine des deux sexes. Les liaisons observées sont dans l'ensemble faibles et les auteurs concluent que les effets de confusion possibles sont vraisemblablement négligeables dès lors que la consommation de tabac est prise en compte. Plusieurs remarques néanmoins peuvent être faites.

S'agissant de caractéristiques comportementales ou psychosociales il est probable que les liaisons sont d'intensité différente selon la situation socio économique et les habitudes culturelles des groupes de population ...

La possibilité d'interactions entre les caractéristiques individuelles et le niveau de la consommation de café n'a pas été évoquée par les auteurs, bien que l'on puisse imaginer qu'une certaine typologie alliant activité professionnelle, éducation, scores de stress et de personnalité, comportement alimentaire ... puisse mieux caractériser le fort consommateur de café. Il resterait à montrer que les individus ayant cette typologie présentent des risques indépendamment de leurs consommations de tabac, d'alcool et de café. Il ne semble pas que des recherches de ce genre aient actuellement été entreprises. Dans les populations de femmes enceintes [14], il est remarquable que les fortes consommatrices de café présentent un ensemble de caractéristiques particulières (âge, éducation, activité professionnelle, parité, antécédents d'avortement ...) qui pourraient à priori jouer un rôle de confusion quant à l'issue de leur grossesse. Il apparait cependant peu vraisemblable que leur prise en compte, en plus de la consommation de tabac et d'alcool, modifierait les conclusions des auteurs, montrant une augmentation du risque de retard de croissance intra utérin (risque relatif de 2,9) chez les consommatrices de plus de 300 mg de caféine par jour.

En conclusion, au delà de facteurs potentiellement de confusion mettant en jeu des mécanismes particuliers à certains processus physiopathologiques, l'examen de la littérature montre que seules les consommations de tabac et d'alcool peuvent être considérées comme des facteurs généraux de confusion établis dans la mise en évidence de risques pour la santé associés à la consommation de café ou de caféine. L'utilisation de

marqueurs biologiques permettant une appréciation plus objective des consommations dans les études épidémiologiques est fortement recommandée afin de mieux cerner l'effet de confusion qu'elles introduisent. Enfin peu de travaux ont étudié le comportement alimentaire et les dimensions psychosociales des consommateurs de café en interaction avec leurs risques de santé.

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Les études épidémiologiques d'observation ne permettent pas d'établir la preuve causale d'une association entre un facteur d'exposition et un risque particulier pour la santé : elles constituent cependant très souvent une voie d'approche indispensable pour l'étude de facteurs d'environnement (xénobiotiques en particulier) en confrontation avec les données acquises sur leurs effets biologiques et toxicologiques. Parmi les nombreuses difficultés méthodologiques rencontrées dans ces études, la recherche des effets "de confusion" dûs à l'association du paramètre étudié avec des facteurs déjà connus ou suspectés de la maladie est décisive pour interpréter correctement un risque observé. Les recherches épidémiologiques effectuées depuis une vingtaine d'années sur les effets potentiels de la consommation de café, en particulier dans le domaine de la reproduction et de certains cancers illustrent ces difficultés.

CONFOUNDING EFFECTS IN EPIDEMIOLOGIC STUDIES ON COFFEE

Epidemiologic studies do not enable us to establish the causality of an association between a factor of exposure and a particular risk for health. Nevertheless, they generally represent the only way for studying environmental factors (chiefly xenobiotics) in relationship with health, in parallel with the data acquired on their biological and toxicological effects. Among numerous methodological difficulties, looking for "confounding effects" due to the association of the parameter under study with already known or suspected disease factors, is essential for a proper interpretation of an observed risk. The epidemiologic work performed in the last 20 years on the potential effects of coffee consumption, particularly in the field of reproduction and for some cancers, illustrates these difficulties.

COFFEE, CAFFEINE AND THE COUPLING BETWEEN CEREBRAL BLOOD FLOW AND ENERGY METABOLISM

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INTRODUCTION

Coffee and caffeine have well known stimulant effects on the central nervous system, that are reflected by increased alterness, wakefulness and motor activity as well as stimulated neuronal activity (for review, see 1). Many of the effects of caffeine and other methylxanthines on the central nervous system can be related to their antagonism at the level of adenosine receptors (1,2-5). Therefore, the effects of methylxanthines could be of particular importance with respect to the key role for adenosine in coupling cerebral blood flow to energy metabolism (6-8). Indeed, cerebral energy metabolism (i.e. oxygen and glucose utilization) and blood flow are closely interrelated (9-12) such that changes in the activity of the brain lead to parallel changes in cerebral energy metabolism and blood flow (12-14).

In the present paper, we will review the effects of coffee, caffeine and other methylxanthines on cerebral blood flow and energy metabolism in both animal and man and try to integrate these data in the well known effects of the methylxanthines on behavior. Moreover, we will discuss on the possible adverse side effects of therapeutic doses of caffeine and theophylline as used in the treatment of asthma and apnea of prematurity on brain function. Indeed, methylxanthines are able to simultaneously increase cerebral energy metabolism and decrease cerebral blood flow.

EFFECTS OF METHYLXANTHINES ON CEREBRAL ENERGY METABOLISM

The effects of methylxanthines on cerebral energy metabolism have only been explored in rats. All studies have been performed by means of the quantitative autoradiographic [^{14}C]2-deoxyglucose method of Sokoloff et al (15) which allows the simultaneous visualization of functional activity in discrete areas of the brain of conscious animals. This technique also permits the identification of neuronal pathways affected by a pharmacological agent which is very useful for relating behavioral effects to the central action of a drug.

General effects on cerebral energy metabolism

The stimulant effects of caffeine on the central nervous system are associated with increases in local rates of cerebral energy metabolism. Administration of an acute dose of 10 mg/kg caffeine or continuous perfusion of methylxanthine at a rate of 0.30 mg/kg/min induce a general 15% increase over control values in the rates of local cerebral glucose utilization; this increase is particularly prominent in monoaminergic cell groupings, such as the dopaminergic substantia nigra and ventral tegmental area, the serotoninergic medial and

dorsal raphe nuclei and the noradrenergic locus coeruleus. Caffeine increases also the rates of energy metabolism in the structures of the extrapyramidal motor system (caudate nucleus, globus pallidus, sensory-motor and cerebellar cortex) and in numerous thalamic nuclei (motor, limbic and sensory relays, as well as in some limbic areas such as the hippocampus (*Figure 1* and refs. 16-22).

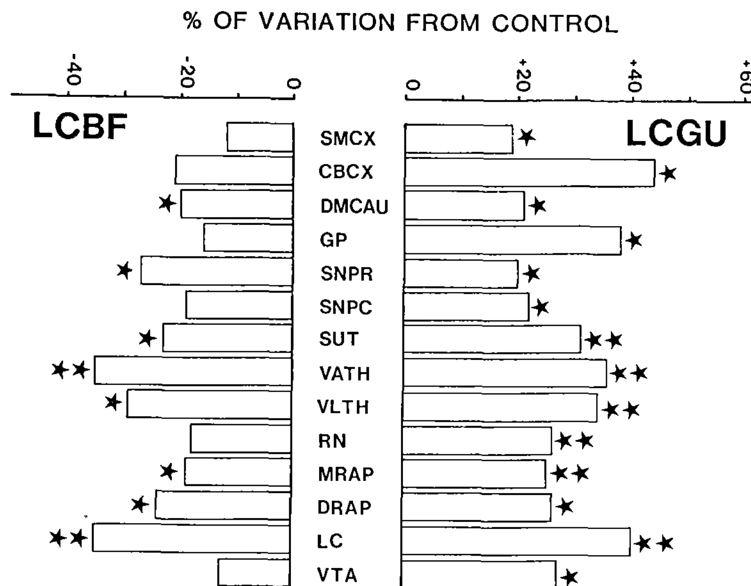

Figure 1: Effects of the acute administration of caffeine (10 mg/kg) on local cerebral glucose utilization (LCGU) and local cerebral blood flow (LCBF) represented as % of variation from controls in selected brain areas.
Abbreviations: SMCX: sensory-motor cortex, CBCX: cerebellar cortex, DMCAU: dorsomedian caudate nucleus, GP: globus pallidus, SNPR: substantia nigra pars reticulata, SNPC: substantia nigra pars compacta, SUT: subthalamic nucleus, VATH: ventroanterior thalamus, VLTH: ventrolateral thalamus, RN: red nucleus, MRAP: medial raphe, DRAP: dorsal raphe, LC: locus coeruleus, VTA: ventral tegmental area.
** $p < 0.05$, ** $p < 0.01$, statistically significant differences from control.*
Drawn from data from Nehlig et al (19,33).

The cerebral metabolic increases induced by acute caffeine exposure are well correlated to changes in cerebral electrical activity recorded in discrete areas of the brain. Indeed, caffeine is able to increase firing rates of cortical, reticular formation and locus coeruleus neurons, as well as to decrease the firing rate of neurons in the medial thalamus and caudate nucleus (1,17). These differential actions of caffeine in discrete brain areas show that caffeine is able to excite the central nervous system at many levels including the reinforcement of inhibitory influences which also consume energy (1,17).

Correlation with locomotor activity

There is a very good correlation between the nature of the structures in which glucose utilization is significantly increased by caffeine and the well-known behavioral effects of the methylxanthine. Indeed, glucose utilization is particularly increased in most areas of the extrapyramidal motor system (1-22) which probably reflects the stimulant effects of the methylxanthine at similar doses (10 mg/kg) on locomotor activity and general behavior (1). The methylxanthine seems capable of activating the nigrostriatal pathway, resulting in decreased electrical activity in the caudate nucleus subsequent to stimulation of dopamine release by the nigrostriatal nerve endings (1). Moreover, this effect is dose-dependent and caffeine, at a dose of 10-50 mg/kg has been shown to antagonize the akinesia induced by catecholamine depletion in mice (1).

Interactions with the sleep-wake cycle

Likewise, the well-known interaction of caffeine with sleep translates into increases in energy metabolism in structures known to control the sleep-wake cycle, such as the mesencephalic reticular formation, locus coeruleus and raphe nuclei (1,16-22). The lowering of thalamic electrical activity correlates also well with sleep disturbances induced by caffeine (1). In the cat, 10 mg/kg of caffeine, which is the dose used in most of the studies reported here in the rat, produces an activation of the cortical EEG similar to the activity produced by direct stimulation of the reticular formation, a structure which plays a central role in vigilance and awakening (1). Moreover, caffeine reduces serotonin availability at postsynaptic receptor sites which elicits a reduction in the sedative effect of the amine on activity and has repercussions on sleep mechanisms and motor function (1).

Correlations with other psychostimulant effects of caffeine

Caffeine increases also glucose utilization in the hippocampus (16,17,19,21,22) together with elevating the excitability of the structure *in vitro* (1). High doses of caffeine provoke electrical modifications in the hippocampus similar to those that are recorded during generalized seizures. The highly stimulant effect of caffeine on that cerebral structure shows the importance of the limbic system in the development of the convulsant and anxiogenic effect of the methylxanthine (1). Caffeine also selectively inhibits dopaminergic neurons of the ventral tegmental area. The latter effect of caffeine on the limbic system would explain not only the action of the substance on attention and vigilance, but also the clinical observations of exacerbation of schizophrenic symptoms. Finally, in rodents, caffeine modifies concentrations and rates of cerebral utilization of noradrenaline, dopamine and serotonin, especially in some structures belonging to the limbic system. If similar limbic effects exist in man, they could have important clinical consequences and could theoretically predispose some individuals to the beneficial psychological effects linked to the absorption of coffee (1).

Dose-dependent effects of caffeine on brain energy metabolism

After the administration of very low (0.1 mg/kg) to low (1.0 mg/kg) doses of caffeine, glucose utilization is unchanged in the brain as a whole and increases only in scattered areas. Within the structures most sensitive to caffeine are mainly dopaminergic areas such as the caudate nucleus, ventral tegmental area, habenula and paraventricular nucleus of the hypothalamus (16). Thus, at these low doses, caffeine already stimulates several dopaminergic systems, both the mesostriatal, mesolimbic, incertohypothalamic and tuberohypophyseal systems. The effects of caffeine on hypothalamus might be a reflection of the endocrine stress syndrome induced by high doses of caffeine (23) and underline the sensitivity of the central endocrine system to the methylxanthine.

Cerebral energy metabolism and the tolerance to caffeine

The acute administration of 10 mg/kg of caffeine increases rates of glucose utilization to the same extent whether the rats have been previously exposed to saline or to 10 mg/kg/day of caffeine for two weeks. Indeed, in both groups of animals, glucose utilization is similarly increased in all brain areas by a new injection of 10 mg/kg of caffeine. The increase in glucose utilization is especially prominent in monoaminergic cell groupings and most areas of the extrapyramidal motor system. Moreover, energy metabolism in locus coeruleus and caudate nucleus remains significantly increased 5-6 hrs after the last injection of caffeine in the chronically methylxanthine-exposed group and does not increase further when an acute dose of caffeine is given to these animals. In substantia nigra compacta and dorsal raphe, glucose utilization is also significantly higher in the chronically exposed rats than in controls 5-6 hrs after the last injection of caffeine, but an additional injection of caffeine increases further glucose utilization in those two areas. Thus, cerebral energy metabolism does not seem to develop tolerance to the stimulant effects of methylxanthine. Moreover, a few areas appear to be very sensitive to caffeine effects. These are the nigrostriatal pathway involved in the control of motor activity, and raphe nucleus and locus coeruleus involved in the control of the sleep-wake cycle (17).

EFFECTS OF METHYLXANTHINES ON CEREBRAL BLOOD FLOW

Methylxanthines such as caffeine or theophylline induce vasodilatation, except in the central nervous system where they raise cerebrovascular resistance; this actually contributes to a reduction in cerebral blood flow. The cerebral vasoconstrictive properties of methylxanthines have been demonstrated in both humans (24-32) and animals (20-22,33,34). In rats, caffeine induces a decrease in local cerebral blood flow, mainly in the areas where it increases metabolism, i.e., in monoaminergic cell groupings, motor and limbic systems, and in the thalamus (*Figure 1* and refs 20-22,33). A recent study with positron emission tomography in humans showed a 30% decrease in whole brain blood flow after an intravenous dose of 250 mg of caffeine with no

showed a 30% decrease in whole brain blood flow after an intravenous dose of 250 mg of caffeine with no interregional differences (31). Several other studies have shown a 20-30% cerebral blood flow decrease induced by 250 mg of caffeine in man (29,32,35). The absence of interregional differences in the extent of decrease of cerebral blood flow induced by caffeine has also been reported previously (29). The value of cerebral blood flow before caffeine exposure strongly affects the extent of decrease in cerebral blood flow induced by caffeine, suggesting that some regulatory mechanism may prevent decreases below some minimum tolerable level, which could be 30 ml/100 g/min (31). In that study the decrease of cerebral blood flow was accompanied by a significant increase in subjective anxiety ratings in response to caffeine (31). Likewise, in another study, the level of anxiety was also increased by 250 mg of caffeine in control subjects, but surprisingly caffeine did not affect anxiety levels in patients with anxiety disorders, although the methyulxanthine induced cerebral blood flow decreases in both groups (32).

Methylxanthines are very frequently used in the treatment of idiopathic apnea in the preterm infant (36). In newborns, the effects of methylxanthines on cerebral blood flow are contradictory. In some studies, the administration of 6-10 mg/kg theophylline or aminophylline in newborn (37) and premature infants (38-40) induces reductions in cerebral blood flow up to 21%. The latter study shows however that the decrease in cerebral blood flow velocity, assesssed by Doppler ultrasonography, is not accompanied by adverse effects on cerebral function (visual evoked potentials) in stable, preterm infants (40). Conversely, many other studies show that blood flow velocity is not affected by methylxanthines, even at the quite high common loading dose of 20 mg/kg caffeine used to treat apnea in preterm infants (41-44).

However, it is not known whether the decrease in cerebral blood flow is directly related to the methylxanthine administration or secondary to the decrease in arterial pCO_2 induced by the drug (45). Indeed, hypocapnia has been recorded after methylxanthine administration in both preterm neonates (40,46) and in adult animals (16-20) and humans (24,26,30,31). However, in most human adult studies, the decrease of cerebral blood flow induced by caffeine has been shown to be independent from hyperventilation and decrease in arterial pCO_2 (28,29,31). Indeed, a decrease in pCO_2 of much greater magnitude than the one recorded after the administration of caffeine is required to alter equally cerebral blood flow (26). However, in neonates, it has been shown that the decrease in cerebral blood flow can be avoided when pCO_2 is monitored and maintained at a constant level (47), which shows the greater sensitivity of the immature brain to hypocapnia.

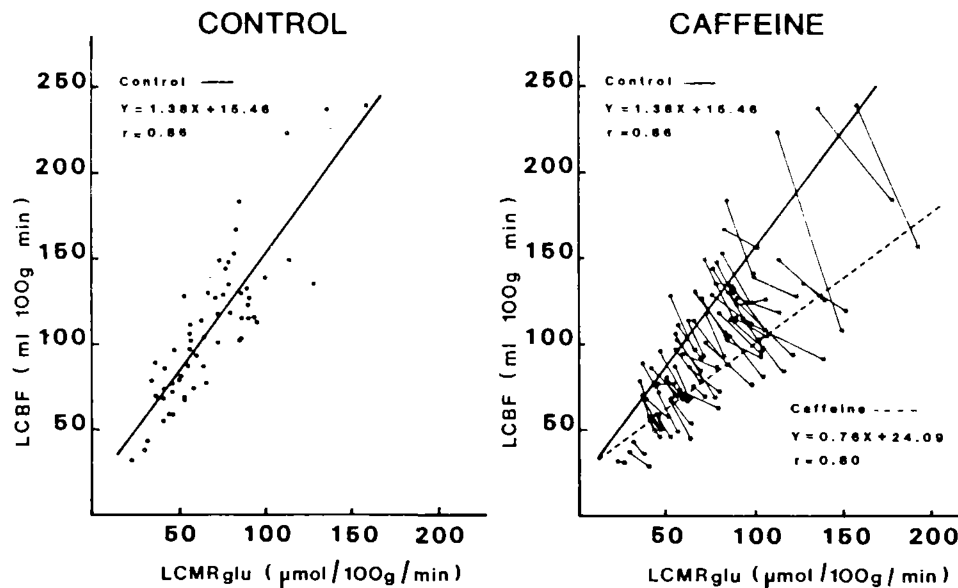

Figure 2: Effects of the acute administration of caffeine on the relationship between local cerebral metabolic rates for glucose (LCMRglu) and local cerebral blood flow (LCBF). The slopes of the regression lines, continuous for control and dashed for caffeine-exposed rats, are calculated from 61 brain structures. The lines between the points represent the extent of change induced by caffeine in each cerebral structure. The decrease in the slope of the regression line is indicative of the marked hypoperfusion induced by the administration of 10 mg/kg of caffeine. Drawn from data from Nehlig et al (19,33).

COUPLING BETWEEN CEREBRAL BLOOD FLOW AND METABOLISM

In most situations, cerebral blood flow and glucose utilization are closely coupled in all cerebral regions, so that modifications in cerebral activity elicit parallel changes in cerebral glucose utilization and blood flow (7-11). In general, changes in cerebral blood flow are the consequence of variations in cerebral energy metabolism (12-14). Contrary to the majority of pharmacological agents to which man is frequently exposed, caffeine has the property of inducing cerebral hypoperfusion accompanied by simultaneous increase in glucose utilization (*Figure 1* and refs 16-22); in other words, it resets the level of coupling between cerebral blood flow and energy metabolism (*Figure 2*). Methylxanthines thus seem to modify the regulating mechanism between cerebral blood flow and cerebral metabolism. Although this mechanism is not yet well understood, adenosine, with which methylxanthines compete, is known to be one of the modulators of regulation in the relationship of blood flow to metabolism in the central nervous system (6,7). Indeed, theobromine, a weaker adenosine antagonist than caffeine or theophylline, has only minor effects on cerebral blood flow and metabolism whereas propentophylline, an adenosine uptake blocker, induces the reverse effect, i.e. an increase in cerebral blood flow and a decrease in glucose utilization. Thus, several xanthine derivatives can differently reset the relationship between metabolism and blood flow in the brain (48).

CAFFEINE, CEREBRAL BLOOD FLOW AND PATHOLOGY

In adult rats, rabbits and dogs, theophylline has been shown to significantly attenuate, reduce the duration or even block the increase in cerebral blood flow recorded during moderate and severe hypoxia (49-55), whereas dipyridamole and papaverine, inhibitors of adenosine uptake, have the opposite effects (54,55). Conversely, theophylline produces no significant effect on hypercapnia-induced increase in cerebral blood flow (49,50). These results confirm the specificity of action of theophylline and other methylxanthines on hypoxia-induced adenosine release (49-56). Likewise, theophylline significantly reduces the hyperemia observed during seizures in adult animals, prevents tissue hyperoxia and enhances brain damage. These data strongly suggest that adenosine is partly responsible for the increase in cerebral blood flow recorded during seizures and has neuroprotective effects (57).

Finally, the acute administration of caffeine in rats accelerates ischemic damage consecutive to stroke whereas chronic administration of the methylxanthine protects the brain against ischemic damage, probably through the increase of the number of adenosine receptors (58). In man, chronic consumption of caffeine is inversely related to the risk of fatal and non fatal stroke (59). The advice of a recent paper is that the trick would be to drink enough coffee to increase the number of central adenosine receptors, but also to be able to stop the intake of drinks containing the methylxanthine when a stroke happens, in order to prevent caffeine to antagonize the beneficial effects of adenosine at the cerebral level (60).

CONCLUSION

In conclusion, it appears that, in adult humans and animals, methylxanthines widely increase cerebral energy metabolism and decrease cerebral blood flow. These substances are thus able to reset the level of coupling between cerebral blood flow and energy metabolism inducing a relative hypoperfusion of the brain at a constant metabolic level, which may be deleterious in some pathological situations. Moreover, these data stress the important regulatory role of adenosine in the mechanism of coupling between cerebral blood flow and energy metabolism.

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Summary

COFFEE, CAFFEINE AND THE COUPLING BETWEEN CEREBRAL BLOOD FLOW AND ENERGY METABOLISM

The effects of coffee and caffeine on cerebral energy metabolism and blood flow have not been studied in great detail. In adult animals, caffeine induces a generalized increase in the rates of cerebral glucose utilization. This increase is mainly prominent in all monoaminergic cell groupings as well as in most structures belonging to the extrapyramidal motor system and in thalamic relay nuclei. These increases in cerebral glucose use are well correlated with the known effects of caffeine on cerebral electrical activity, locomotor activity and the sleep-wake cycle, and with other psychostimulant effects of caffeine. Moreover, cerebral energy metabolism does not seem to develop a tolerance to the stimulant effects of caffeine.

Caffeine and theophylline incerease cerebrovascular resistance and decrease cerebral blood flow. The vasoconstrictive properties of caffeine have been shown in both men and animals. Caffeine decreases mostly cerebral blood flow in regions where energy metabolism is simultaneously markedly increased. Thus, caffeine resets the level of coupling between cerebral blood flow and metabolism by inducing a relative hypoperfusion at a given metabolic level. However, the decrease of cerebral blood flow consecutive to caffeine is independent of the pCO_2 decrease in adults whereas it can be avoided in newborn and premature babies if the partial pressure of CO_2 in arterial blood in maintained in the physiological range.

In conclusion, methylxanthines are able to change the regulatory mechanism which couples cerebral blood flow and metabolism levels. This effect may be mediated by adenosine which represents one of the main factors involved in the dynamic coupling between cerebral blood flow and metabolism, i.e. between delivery and use of oxygen and metabolic substrates.

Résumé

CAFE, CAFEINE ET COUPLAGE ENTRE LE DEBIT SANGUIN ET LE METABOLISME ENERGETIQUE CEREBRAL

Les effets du café et de la caféine sur le métabolisme énergétique et le débit sanguin cérébral n'ont fait à ce jour que l'objet d'un nombre limité d'études. Chez l'animal adulte, la caféine induit une augmentation généralisée de l'utilisation cérébrale de glucose. Celle-ci est particulièrement marquée dans tous les groupements cellulaires de monoamines, dans les structures du système extrapyramidal moteur et dans de nombreux relais thalamiques. Ces augmentations de l'utilisation cérébrale de glucose sont bien corrélées aux effets connus de la caféine sur l'activité électrique cérébrale, l'activité locomotrice et le cycle veille-sommeil ainsi qu'aux effets psychostimulants de la méthylxanthine. De plus, le métabolisme énergétique cérébral ne semble pas développer de tolérance aux effets stimulants de la caféine.

Par ailleurs, caféine et théophylline augmentent la résistance cérébrovasculaire et diminuent le débit sanguin cérébral. Les propriétés vasoconstrictrices de la caféine ont été mises en évidence à la fois chez l'homme et chez l'animal. La caféine diminue plus particulièrement le débit sanguin cérébral dans les régions où elle augmente le métabolisme de manière marquée. La caféine réajuste donc le niveau de couplage entre débit sanguin et métabolisme cérébral en induisant une hypoperfusion relative à un taux métabolique donné. Toutefois, la réduction de débit sanguin cérébral est indépendante de la baisse de pCO_2 induite par la caféine chez l'adulte et semble pouvoir être évitée si la pression partielle en CO_2 du sang artériel est maintenue à un niveau physiologique chez le nouveau-né à terme ou prématuré.

En conclusion, les méthylxanthines semblent pouvoir modifier le mécanisme de régulation assurant le couplage entre débit sanguin et métabolisme cérébral, peut-être par leur action au niveau des récepteurs de l'adénosine. L'adénosine est en effet un des facteurs intervenant dans le couplage dynamique entre débit sanguin et métabolisme cérébral, donc entre apport et utilisation de l'oxygène et des substrats métaboliques.

AN OVERVIEW OF THE MAILLARD REACTIONS RELATED TO AROMA GENERATION IN COFFEE

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The thermal generation of aromas has long been a topic of interest in flavor research. An insight into the chemistry and mechanisms underlying the generation of these aromas in food systems has become necessary for the development of high-quality products for the food and beverage industry. The Maillard reaction has been traditionally responsible for the generation of roasted, toasted, or caramel-like aromas as well as the development of brown colors in foods. The pathways of this well-known reaction can be divided into three stages. The first stage is a condensation between a carbonyl group of a reducing sugar and an amino group to form a Schiff base. This Schiff base rearranges to Amadori compound (1-amino-1-deoxy-2-ketoses) or a Heynes compound (2-amino-2-deoxy-1-aldose) depending on whether the carbonyl sugar was an aldose or ketose, respectively. The Amadori and Heynes compounds are colorless and without flavor. Amadori and Heynes compounds in the intermediate Maillard stage undergo β-elimination and enolization to produce α-dicarbonyl intermediates. These α-dicarbonyls are labile and can react by a number of pathways to generate the characteristic Maillard flavors. The final stage consists of polymerization of intermediates to form brown melanoidan pigments.

The vast majority of the mechanistic chemistry of the Maillard reaction was determined by investigating the interactions of reducing sugars and free amino acids in simple model systems. The chemical characteristics and reactivity of both peptide- and protein-bound amino acids have not been studied to an appreciable extent.

Maillard Reactions of Proteins

Studies of the Maillard chemistry of intact proteins focuses for the most part on glycosylation of the ε-amino group of lysine (Watkins et al., 1985; Alaimo et al., 1992). Since the α-amino groups of the amino acid monomers are chemically included within the polypeptide chain, their reactivity is hindered dramatically. Therefore, the nature of the amino acid side chains endogenous to the protein itself is most important when considering the Maillard reaction of these macromolecules. As mentioned previously, the ε-amino group of lysine possesses the ability to partake in the initial stage of the Maillard reaction. Protein-bound lysine can act as an "activator" to induce sugar degradation and produce a variety of nonnitrogen-containing heterocyclic compounds such as furans and pyrans (see Figure 1). This is accomplished through the formation of an unstable "bound" Amadori product which then generates a variety of dicarbonyl fragments leading to aroma formation.

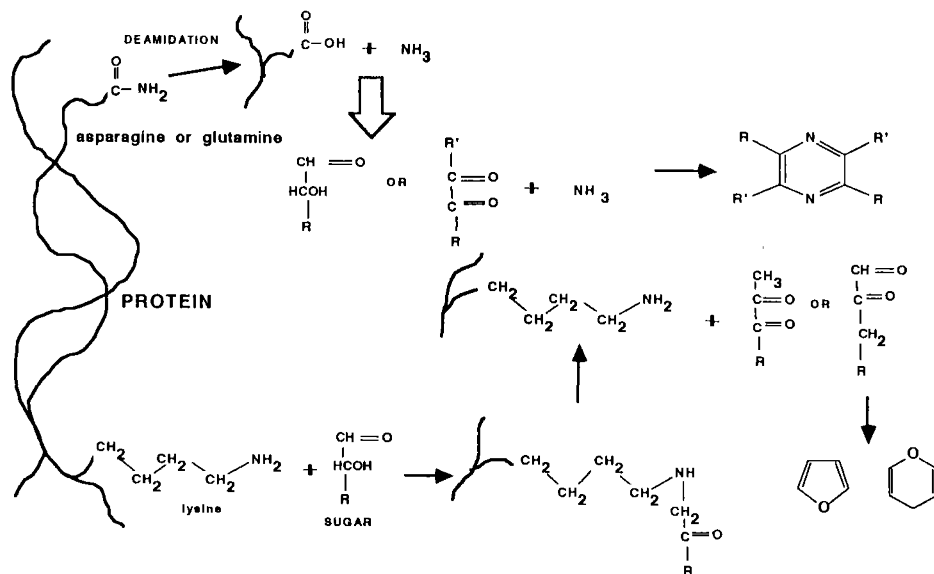

Figure 1. The participation of proteins in the Maillard reaction.

Another reaction that proteins have been shown to undergo is spontaneous deamidation of asparagine and glutamine residues in the presence of heat and moisture to form aspartic and glutamic acids resulting in the liberation of a molecule of ammonia (Wright, 1991) (see Figure 1). This free and reactive ammonia molecule can then enter into a series of reactions resulting in its combination with various sugar degradation products leading to the formation of amino carbonyls which produce pyrazines

Contribution of Deamidation Reaction to Pyrazine Formation in a Glutamine Model System

Pyrazines are nitrogen-containing heterocyclics that are potent characteristic flavorants found in a wide range of raw and processed foods (Maga, 1992). In coffee, 79 members of this family were found (Flament, 1991). Pyrazines are usually associated with the generation of roasted and burnt flavor notes. These unique and desirable sensory properties make pyrazines essential to the food industry (Maga, 1982). As shown in the past, both glutamine and asparagine have been shown to produce considerably more pyrazines than do their corresponding acids when heated with reducing sugars (Koehler et al., 1969). This seems to suggest that the amide nitrogen, possibly through deamidation, is available to contribute to amino/carbonyl interactions leading to pyrazine generation. In order to clarify the participation of deamidation reaction in pyrazine formation, we used a glutamine with a labelled ^{15}N isotope at the amide side chain and a ^{14}N at the α-amino group to investigate the relative contribution of the α-amino nitrogen and the amide nitrogen to pyrazine formation.

50 G. of wheat starch, 500 mg of glucose, and 100 mg of L-glutamine/or L-glutamine-^{15}N (amide-^{15}N) were mixed with 500 ml deionized water and then freeze-dried. The solid mixture was further placed in the upper level of a desiccator; a Pyrex dish containing 20 ml of deionized water was placed in the lower level to adjust moisture content of the samples back to 12-14%. The moisture content of the samples was measured according to the AOAC air over method . The samples were then transferred into a reaction vessel and heated at 180^0 C for one hour.

The heated sample (1 g) was packed in the center of a glass tube and the silanized glass wool was placed on the two ends of the tube. 1 µl of 1.001 mg/ml deuterated toluene was spiked into the tube as the internal standard. The tube was further sealed in a Scientific Instrument Services (SIS) solid sample purge-and-trap apparatus (Ringoes, NJ) and the volatiles were purged with nitrogen at a flow rate of 40 ml/min to a silanized

glass-lined stainless steel desorption tube (4.0 mm i.d. x 10 cm length). This desorption tube was from Scientific Instrument Services, Inc. (Ringoes, NJ) and consisted of 3 cm bed volume of Tenax-TA adsorbent and 3 cm bed volume of Carbotrap adsorbent. This volatile isolation was carried out at 80°C for one hour. The desorption tube prepared from the isolation procedure was connected to an SIS model TD-1 Short Path Thermal Desorption unit. This desorption unit was interfaced into a Varian 3400 gas chromatograph coupled with a Finnigan MAT 8320 high-resolution GC-MS. The volatiles trapped in the desorption tube were then thermally desorbed into the GC at 220°C for 5 minutes and analyzed by GC-MS.

The pyrazines that were identified from heating isotope-labelled glutamine with glucose in the dry system included pyrazine, methylpyrazine, ethylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethyl-pyrazine, vinylpyrazine, and 2-ethyl-5-methylpyrazine. The relative contributions of amide and α-amino nitrogen to pyrazine formation are shown in Figure 2. From this figures it is obviously revealed that more than half of the pyrazines consisted of ^{15}N nitrogen atoms that came from the amide chains of glutamine. These data demonstrated that deamidation did happen and could participate in the pyrazine formation. Similar results were also investigated by Bohnenstengel and Baltes (1992). They found that in the asparagine and glucose mixtures, predominantly nitrogen-containing heterocycles (pyrazines, pyridines, pyrroles) were formed, whereas the mixtures of aspartic acid/glucose yielded furans and aliphatic carbonyls. These results further implied that ammonia from deamidating the side chains of glutamine more easily reacted with dicarbonyl than the α-amino groups of glutamine. This was reasonable because the α-amino groups could not directly react with dicarbonyl like ammonia and had to proceed through Strecker degradation to generate pyrazines. These results supported that deamidation has a profound effect on pyrazine generation.

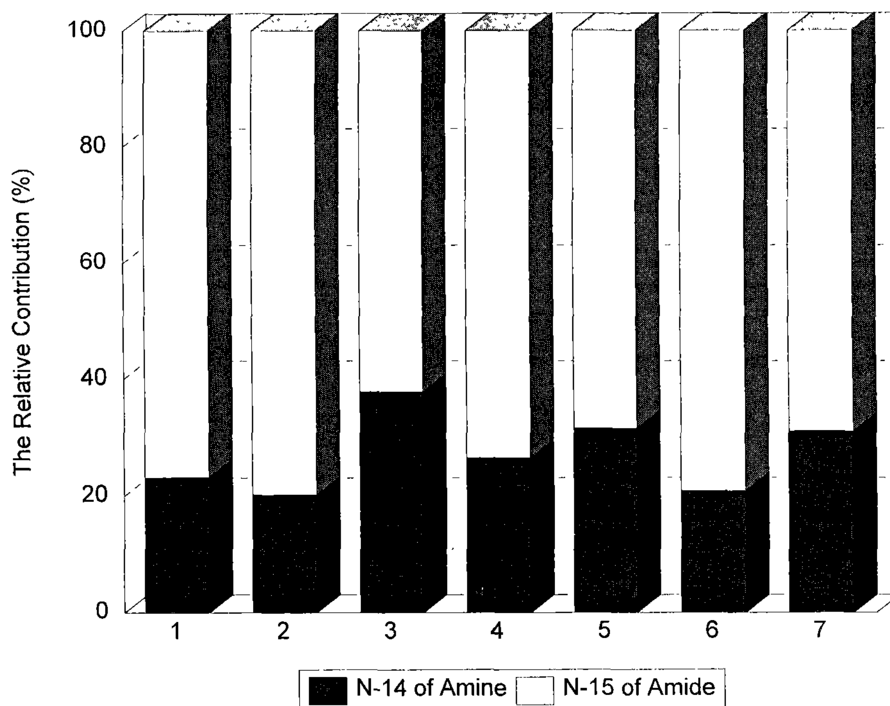

Fig 2. The relative contribution of amino and amide nitrogen to pyrazine formation in the reaction of labelled glutamine with glucose from the dry system. 1 = pyrazine, 2 = methylpyrazine, 3 = 2,5- & 2,6-dimethylpyrazine, 4 = ethylpyrazine, 5 = 2,3-dimethylpyrazine, 6 = vinylpyrazine, and 7 = 2-ethyl-5-methylpyrazine.

Effect of Deamidation Reaction on Pyrazine Formation in Peptide Model System

Maillard reactions of dipeptides, Ala-Gln and Ala-Glu with glucose, were studied in an aqueous solution of pH 8 at 180°C. Both dipeptides generated significant amounts of pyrazines. Figure 3 shows the distribution of major pyrazines in these two systems. It is interesting to observe that Ala-Asn generated higher amounts of unsubstituted pyrazines and methylpyrazine, and Ala-Asp produced more higher substituted pyrazines such as 2,5-dimethylpyrazine and 2,5-dimethyl-3-ethylpyrazine. It should be noted that among pyrazines, 2,5-dimethyl-3-ethylpyrazine has been reported by Tressl (1989) to be one of the most significant contributors to coffee flavor. Shibamoto (1977) has demonstrated that the reaction between ammonia and sugar produced simply substituted pyrazines such as methylpyrazine and ethylpyrazine. It is, therefore, reasonable to explain that Ala-Asn can release ammonia upon heating which in turn will lead to the formation of more pyrazine and methylpyrazine.

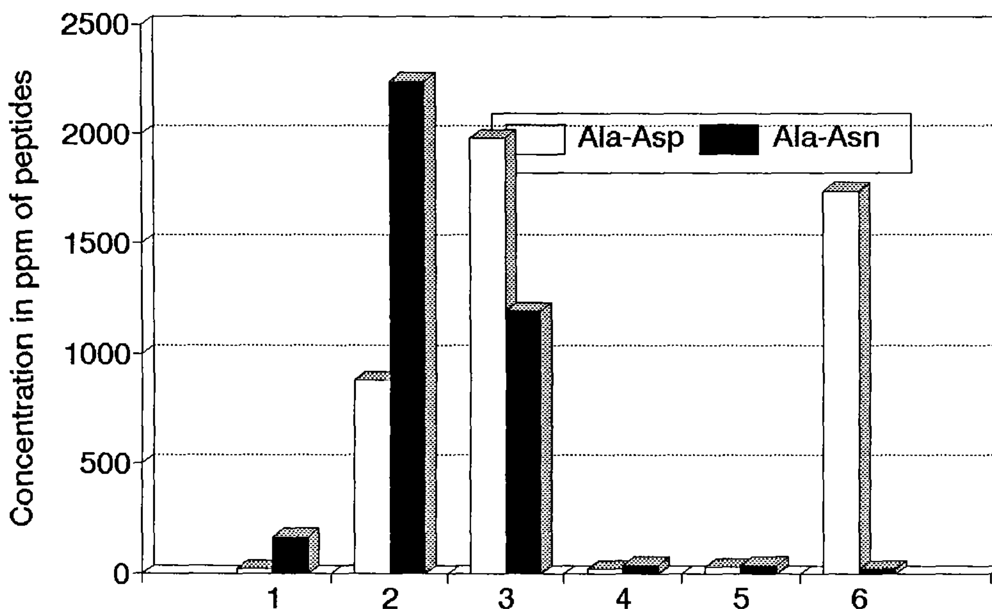

Fig 3. The contribution of selected pyrazine formed in the Maillard reaction of glucose with dipeptides, Ala-Asp and Ala-Asn in an aqueous solution. 1 = pyrazine, 2 = methylpyrazine, 3 = 2,5-dimethylpyrazine, 4 = ethylpyrazine, 5 = 2,3-dimethylpyrazine, 6 = 2,5-dimethyl-3-ethylpyrazine.

Maillard Reaction Compounds in Coffee Aroma

Besides pyrazines, a large number and wide variety of flavor compounds are formed via the Maillard reaction in coffee. We generally break these compounds into heterocyclics and nonheterocyclics. At one time, it was thought that the nonheterocyclic volatiles, primarily Strecker aldehydes, were most important in providing the Maillard browning flavors. However, as methodologies advanced, the heterocyclic volatiles were discovered and it was recognized that the heterocyclics were of greatest flavor significance (Reineccius, 1990).

The classification system presented by Nursten (1980) for volatiles formed as Maillard products is of particular interest since the origin of each class of volatiles is implied. Table I places examples of volatile compounds identified in coffee in three classes as presented by Nursten.

Table I. Examples of Maillard aroma compounds reported in coffee

Class	Selected Compounds in Coffee
1. "Simple" sugar dehydration/fragmentation products:	
a. Furans	2-furfural
	5-methyl-2-furfural
	2,5-dimethyl-4-hydroxy-3(2H)-furanone
	2-methyl-5-ethyl-4-hydroxy-3(2H)-furanone
b. Pyrones	2-methyl-3-hydroxy-4H-pyran-4-one (maltol)
	6-methyl-3,5-dihydroxy-2,3-dihydro-4H-pyran-4-one
c. Cyclotenes	3-methyl-2-hydroxy-2-cyclopenten-1-one
d. Carbonyl compounds	2,3-butanedione
	2,3-pentanedione
	2,3-hexanedione
e. Acids	acetic acid
2. Simple amino acid degradation products:	
a. Aldehydes	2-methylbutanal
	3-methylbutanal
b. Sulfur compounds	methanethiol
	dimethyl sulfide
	dimethyl disulfide
3. Volatiles produced by further interactions:	
a. Pyrroles	2-pyrrolecarbaldehyde
	2-acetylpyrrole
b. Pyridines	2-methylpyridine
	2-acetylpyridine
c. Pyrazines	2-(2-furyl)pyrazine
	tetramethylpyrazine
d. Oxazoles	2,4-dimethyloxazole
	2,4,5-trimethyloxazole
	2-ethyl-4,5-dimethyloxazole
	2-phenyloxazole
e. Thiazoles	2,4,5-trimethylthiazole
	2,4-dimethyl-5-ethylthiazole
	2-acetyl-4-methylthiazole
f. Thiophenes	2-thiophenecarbaldehyde
	2-acetylthiophene
	thiopheno[3,2-b]thiophene

The furans are particularly important in quantity and quality for coffee flavor, with 99 members identified. Alkyl- and alkenyl-substituted furans do not present any significant sensory interest. On the other hand, furfural, a typical caramelization product of sugar has a characteristic toasted penetrating odor (Flament, 1991). Other sugar degradation products, such as cyclotene and 2,5-dimethyl-4-hydroxy-3(2H)-furanone have been known as chemicals giving a caramel-like or burnt sugar aroma. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone also known as furaneol, has a very low odor threshold (0.04 ppb) and contributes caramel, sweet and coffee-like aroma qualities to fresh coffee. It is known that furaneol is unstable in aqueous solution at ambient temperature (Shu et al., 1988). It is expected that the concentration of furaneol in coffee will decrease if freshly prepared coffee is allowed to stand for a few hours.

As indicated by Shibamoto (1991), the major role of furans in coffee aroma is that they produce the keynote aroma chemicals of roasted coffee upon secondary reaction with sulfur-containing compounds. Furfurylthiol has been known as one of the most important keynote chemicals in roasted coffee. It is interesting to note that in a recent study on a model reaction of cysteine and inosine 5'-monophosphate, furfurylthiol and its related compounds, di-2-furfuryl sulfide and di-2-furfuryl disulfide were identified as the major compounds generated (Zhang and Ho, 1991).

The mechanisms for the formation of heterocyclic compounds in coffee have been reviewed by Shibamoto (1991). The sensory properties of those compounds also have been extensively reviewed by Flament (1991).

Quantification of Volatile Compounds in Roasted Coffee Beans by Combined Absorbent Trapping, Short Path Thermal Desorption GC-MS

Numerous methods have been used to quantify the volatile components in complex food matrices (Hartman et al., 1993). We have studied the use of combined adsorbent trapping-short path thermal desorption gas chromatography-mass spectrometry as an simple and reliable method to identify and quantify the volatiles of roasted coffee beans.

Vacuum packed Columbian undecaffinated roasted coffee beans were purchased from a local supermarket. Roasted coffee beans were ground in a benchtop grinder with dry ice to prevent the generation of the artifacts. After passing through a 20 mesh sieve, 1 g of coffee powder was packed in the center of a glass tube and the silanized glass wool was placed on the two ends of the tube. The volatiles were isolated and analyzed by the combined absorbent trapping, short path thermal desorption GC-MS in the same manner as described in the section of glutamine model system.

Table II lists the compounds identified as well as their concentrations in the roasted Columbian coffee beans. Figure 4 shows the gas chromatogram of the roasted coffee beans. From the table, we can see that 39 out of 69 compounds identified are Maillard reaction products. Three major compounds, 2-furfural, 3-furfural, 5-methyl-2-furfural and 2-furfuryl alcohol are furanoids.

Table II. Volatile compounds identified in roasted Columbian coffee

Peak No.*	Compound identified	Conc. (ppm)
1	2,3-butanedione **	1.78
2	2-methyl-3-buten-2-ol	0.06
3	3-methylbutanal **	0.27
4	2-methylbutanal **	0.46
5	3-methyl 3-buten-2-one	0.07
6	3-methyl 2-butanone	0.10
7	2,3-pentanedione **	0.73
8	2,2,3-trimethylpentane	0.05
9	2-hydroxy-1-penten-3-one	1.43
10	3-hydroxy-3-penten-2-one	1.66
11	3-methyl 2-cyclopenten-1-one	0.71
12	phenol	0.20
13	1-methylpyrrole **	1.11
14	2,3-dihydro-4-methylfuran **	0.39
15	pyrazine **	2.82
16	pyridine **	4.12
17	isoamyl alcohol **	0.43
18	3-methylthiophene **	0.30
19	acetic acid **	9.77
20	2-ethoxypropane	3.80
21	hexanal	0.53
22	3-methyl 2-pentanone	0.61
23	dihydro-2-methyl-3(2H)furanone **	9.67
24	2-furfural **	22.55
25	unknown (Mr=94)	5.57
26	methylpyrazine **	6.56
27	3-furfural **	33.30
28	tetrahydro-2-methyl-2-furanol **	0.25
29	glycol diacetate	19.53
30	2-furfuryl alcohol **	63.52
31	2,5-dimethylpyrazine **	9.30
32	2,3-dimethylpyrazine **	2.48
33	tetramethyfuran **	1.34
34	2,5-hexanedione	0.74
35	benzaldehyde	1.26
36	5-methylfurfural **	26.58
37	1-(acetyloxy)-2-butanone	4.58
38	isovaleric acid **	0.96
39	pentanoic acid	0.47
40	2-methylbutanoic acid **	1.15
41	furfuryl acetate **	9.69
42	1-methyl-2-formylpyrrole **	1.14
43	2-ethyl-5-methylpyrazine **	0.95
44	2-ethyl-6-methylpyrazine **	7.53
45	2-ethyl-3-methylpyrazine **	1.14
46	3,4-dihydro-2,5-furandione **	0.49
47	2,2'-bifuran **	0.17
48	3,4-dimethyl-2,5-furandione **	0.17
49	1-methyl-3-(1-isopropyl)cyclohexane	0.14

Table II. Continued

Peak No.*	Compound identified	Conc. (ppm)
50	isopropenylpyrazine **	1.26
51	2,2'-methylenebifuran **	0.97
52	2,6-dimethyl-3-ethylpyrazine **	3.81
53	2-methoxyphenol	2.11
54	2,5-dimethyl-3-ethylpyrazine **	0.37
55	2-ethyl-4-methylphenol	0.32
56	linalool oxide (2)	0.47
57	linalool	0.73
58	2-methyl-5-propionylfuran **	0.19
59	tetramethylbenzene	0.28
60	2-methyl-5-(1-propenyl)pyrazine **	0.09
61	2-heptylfuran	1.18
62	methyl salicylate	0.26
63	alpha-terpineol	0.12
64	2-methyl cinnoline	0.20
65	2-phenyl-2-butenal **	0.18
66	difurfuryl ether **	0.50
67	1-hydroxy-2-acetyl-4-methylbenzene	1.20
68	isoeugenol	0.12
69	beta-damascenone	0.17

* Peak No. refer to that shown in Fig. 1
Mr. : Molecular Weight
** generated from Maillard reaction

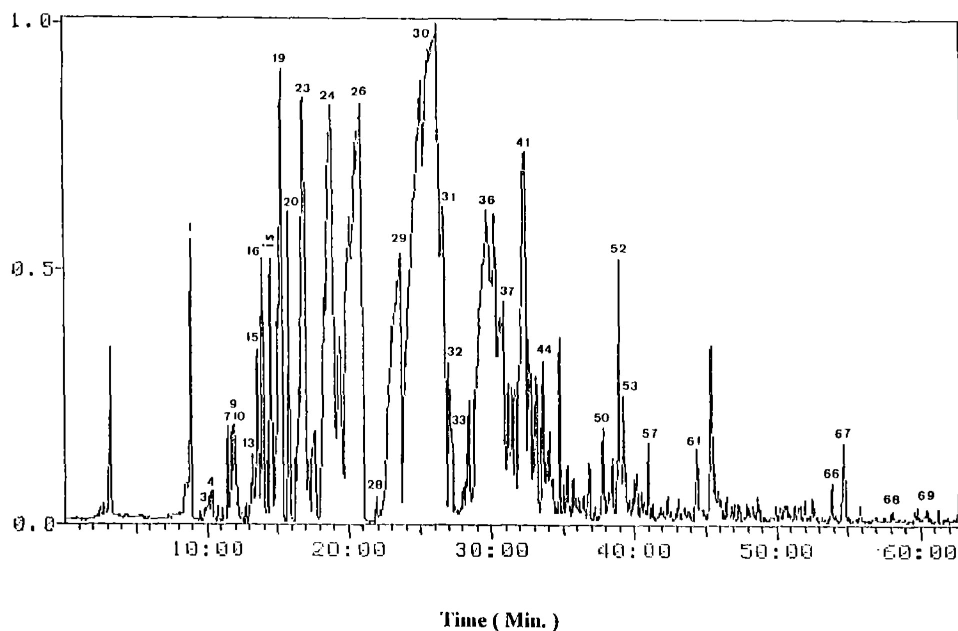

Figure 4. Gas chromatographic profile of roasted Columbian coffee beans

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THE ANALYSIS OF SULFUR COMPOUNDS IN COFFEE AROMA BY SULFUR CHEMILUMINESCENCE DETECTION/GAS CHROMATOGRAPHY

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INTRODUCTION

Coffee aroma is a complex mixture of volatile compounds. Over 800 compounds have been identified and described in the literature. Of these, sulfur compounds are extremely important to the aroma of coffee products. By themselves, these compounds are usually irritating and foul smelling. However, their proper concentration and blend in coffee aroma are required for the full body characteristics of roast coffee. The threshold concentrations for these compounds[1,2] can be as low as 1 pg/l of air. Technological advances such as the advent of capillary gas chromatography (GC) and then gas chromatography mass spectroscopy (GC/MS) have greatly enhanced the knowledge of these compounds in coffee. Shibamoto[3] has summarized the historical development of coffee volatile analysis.

Several types of detectors are employed to analyze sulfur containing compounds. The simplest technique is the use of flame ionization. Flame ionization detectors (FID) lack both sensitivity and selectivity for the analysis of most sulfur compounds. Flame photometric detectors (FPD) have been used as a selective and sensitive detector for sulfur, but lacks a linear output. Mass spectroscopy (MS) is the current method of choice because it is selective, sensitive and can identify unknown compounds. Its disadvantages are that it requires an extensive capital layout and an operator specially trained in this technique. Chemiluminescence (CL) is a good substitute for both MS and FPD because it is selective, sensitive, the output is linear, simple to operate and relatively inexpensive.

Early applications of CL measured trace levels of gasses in the atmosphere[4,5]. The basic theory for the CL reaction is described as the oxidation of a compound that can

produce an electronically excited state in its reaction product. When the excited state relaxes a small fraction undergo CL. The reactions have been summarized by Hutte, Sievers and Birks[6] as either ozone generated, halogen generated or nitrogen generated CL. Ozone generated CL is used in today's commercial CL detectors. In this reaction sulfur, carbon or nitrogen monoxide (XO) are oxidized to the dioxide with ozone (1). The products of the reaction are dioxides and oxygen. The dioxide forms an electronically excited state. Most are thermally quenched. Only a few undergo radiative relaxation(2). In a second reaction olefins and other hydrocarbons CL directly with ozone (3). The design of a sulfur chemiluminescence detector (SCD) must take into consideration all of these reactions plus the limited amount of excited SO_2^* produced.

Ozone Generated

$$XO + O_3 \rightarrow XO_2^* + O_2 \quad (1)$$

$$XO_2^* \rightarrow XO_2 + h\nu \quad (2)$$

or

$$C{=}C + O_3 \rightarrow OxidizedProducts + h\nu \quad (3)$$

Despite these restrictions the detection of sulfur by CL is quite selective and sensitive. In this paper, operating conditions are evaluated to determine the best performance of the SCD for the routine analysis of sulfur compounds in headspace coffee aroma.

Experimental

Materials

The standard sulfur compounds, dimethyl sulfide, carbon disulfide, thiophene, dimethyl disulfide, 2-furfuralthiol and 4-methylthiazole (internal standard), were purchased from Aldrich Chemical Company. Standard mixtures were prepared between 0.07 and 50 ng/µl in 1:1 diethyl ether:petroleum ether. Hydrocarbon standard mixture C7 through C15 is used to derive the retention index values for 2-furfuralthiol and 4-methylthiazole. Soluble and roast commercial coffee samples were purchased locally.

Apparatus

Standard mixtures and coffee samples were analyzed using a Hewlett Packard HP5890 with an FID and a Sievers 355 SCD. A J&W DB-5 60m, 0.32mm ID, 1.0 µm film column was used for the separation. The average linear velocity of the carrier gas was 28 ml/sec. The SCD has three adjustment controls that can regulate the pressure and temperature conditions of the burner chamber. Each has been set at the factory for optimum sensitivity and selectivity of sulfur. The specifications require the burner

pressure to be 200 torr at a temperature of 780 °C. The ozone reaction chamber has a maximum pressure of 30 torr. The actual SCD burner conditions for this experiment is within the specification ranges, the burner chamber pressure and temperature of 190 torr at 790 °C, and the pressure in the ozone reactor at 7 torr. The flow of hydrogen and air to the burner is 100 ml/min @ 80 psig and 20 ml/min @ 80 psig, respectively. The ozone generator requires air at 60 ml/min. @ 6 psig. It is not recommended to change these conditions. The FID detector requires air at 430 ml/min. @ 60 psig and hydrogen at 30 ml/min. @ 60 psig. Both headspace and liquid samples were injected into the system using a Chrompack Cryofocus injector. A Chrompack CP-Sil 5CB, 20 cm, 0.53 mm ID, 5 μm film is used for the trap.

Analytical Procedure

Packaged roast coffee samples are punctured, brought to atmospheric pressure with air and sealed with a sticky septum. After a one hour equilibration at ambient room temperature, a 2 cc sample volume is injected into the cryofocus injector. The soluble coffee is not equilibrated with air. The 2 cc soluble coffee headspace samples are immediately injected. Peak identification and quantity is determined by external standard analysis. The separation is performed using the following operating conditions for the Chrompack Cryofocus injector and HP5890 gas chromatograph.

Cryofocus Injector

Top Zone Temperature	110 °C
Trap Temperature	-150 °C
Trap Holding Time	2 minutes
Trap Injection Temperature	180 °C
Trap Injection Time	5 minutes
Vent Flow	5 ml/minute

Gas Chromatograph

Initial Temperature	20 °C
Hold Time	10 minutes
Ramp Rate	10 °C/minute
Final Temperature	160 °C
Final Hold Time	11 minutes
Post Column Split	33% FID, 67% SCD

Results and Discussion

To evaluate the linearity of the SCD, several mixed standards in 1:1 diethyl ether-petroleum ether were analyzed. A summary of the results are in Table I. The detector has a linear output for 4 orders of magnitude. Carbon disulfide has the lowest detection limit, 3 pg. The detection limit is defined as 3 times the noise. Based on the concentration of sulfur in carbon disulfide, approximately 2 pg of sulfur can be detected. Other compounds are less sensitive because they contain stoichiometrically less sulfur.

Table I: SCD Performance

Compound	RI	Low Standard (ng)	High Standard (ng)	Correlation	Detection Limit (ng)	Sulfur Detection Limit (ng)
Dimethyl Sulfide		0.08	31.4	0.999	0.008	0.004
Carbon Disulfide		0.06	22.2	0.999	0.003	0.002
Thiophene		0.06	22.3	0.999	0.020	0.008
Methyl Disulfide	753	0.06	22.2	0.997	0.015	0.010
2-Furfuralthiol	916	1.04	104.8	0.992	0.1	0.028
4-Methylthiazole	823	0.06	24.7	0.997	0.060	0.020

The specificity for detecting sulfur over that of carbon is greater than 10^7. This is illustrated in Figure 1a and 1b. The two chromatograms were obtained from a 1 µl injection of the lowest concentration mixed standard. Note that the solvent is not detected by the SCD in Figure 1a. In Figure 1b, the FID, the solvent peak eliminates the possible detection of the sulfur compounds. Benner and Stedman[7] developed an SCD utilized halogenated hydrocarbons to stabilize the signal from the detector. To determine whether chlorinated hydrocarbons solvents have an effect on the sensitivity of the standards, a 10:45:45 methylene chloride-petroleum ether-diethyl ether was compared to standards in solvent without methylene chloride. The results are in Table

Table II: The Effect of Methylene Chloride Solvent on Detection of Standards

Compound	Concentration ng	Area w/ o $MeCl_2$	Area w/ 10% $MeCl_2$	% Change
Dimethyl Sulfide	.78	27000	26000	-3.7
Carbon Disulfide	.55	34000	39000	+14.7
Thiophene	.57	7600	8000	+5.3
Methyl Disulfide	.59	11000	11000	0.0
4-Methylthiazole	.61	3000	2800	-6.7

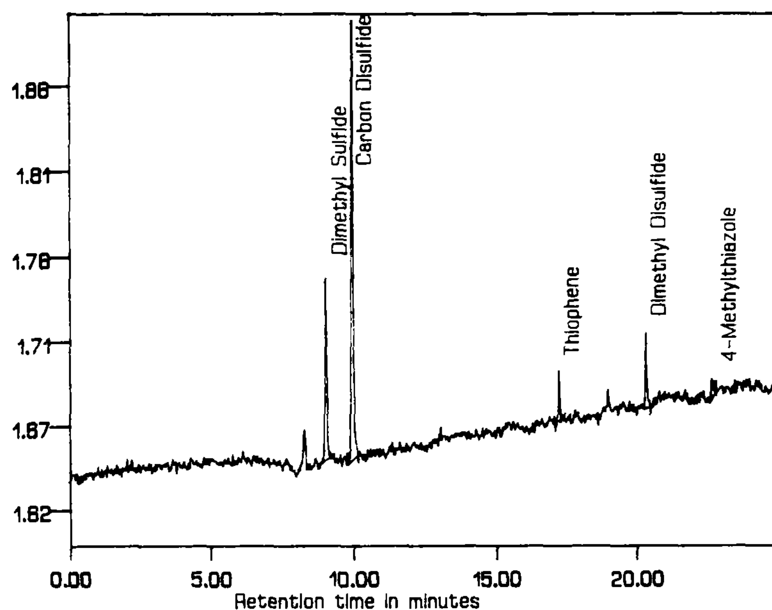


Figure 1a: An SCD chromatogram of a 1 µl injection of a low standard containing 0.08 ng of dimethyl sulfide, 0.06 ng of carbon disulfide, thiophene, methyl disulfide and 4-methylthiazole.

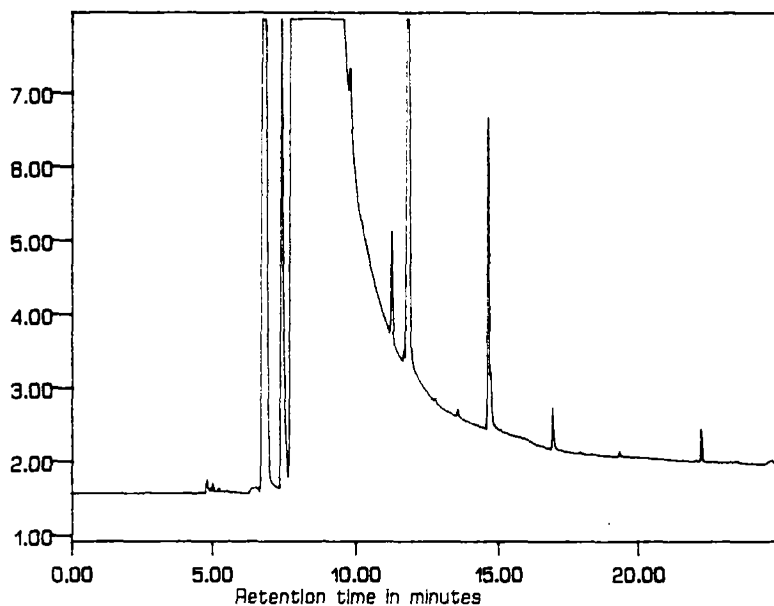
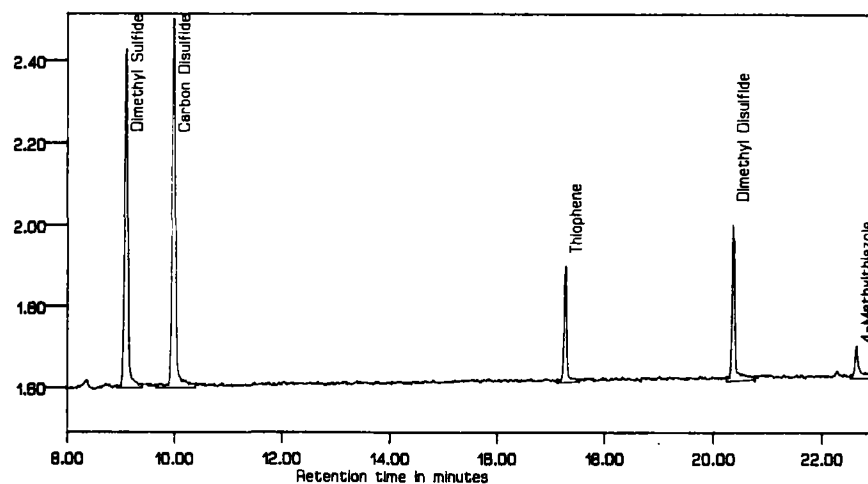


Figure 1b: An FID chromatogram of a 1 µl injection of the same standard as above.

(a)



(b)

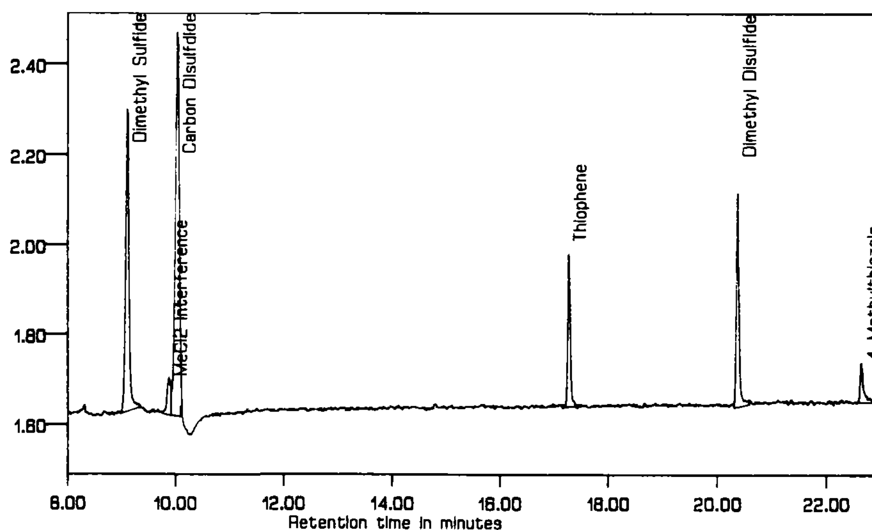


Figure 2: Chromatogram (a) is an analysis of a 1 µl injection of a mixed standard containing 0.8 ng of dimethyl sulfide, 0.6 ng of carbon disulfide, thiophene, methyl disulfide and 4-methylthiazole dissolved in 1:1 diethyl ether-petroleum ether. Chromatogram (b) is the same standard dissolved in 10:45:45 methylene chloride-petroleum ether-diethyl ether.

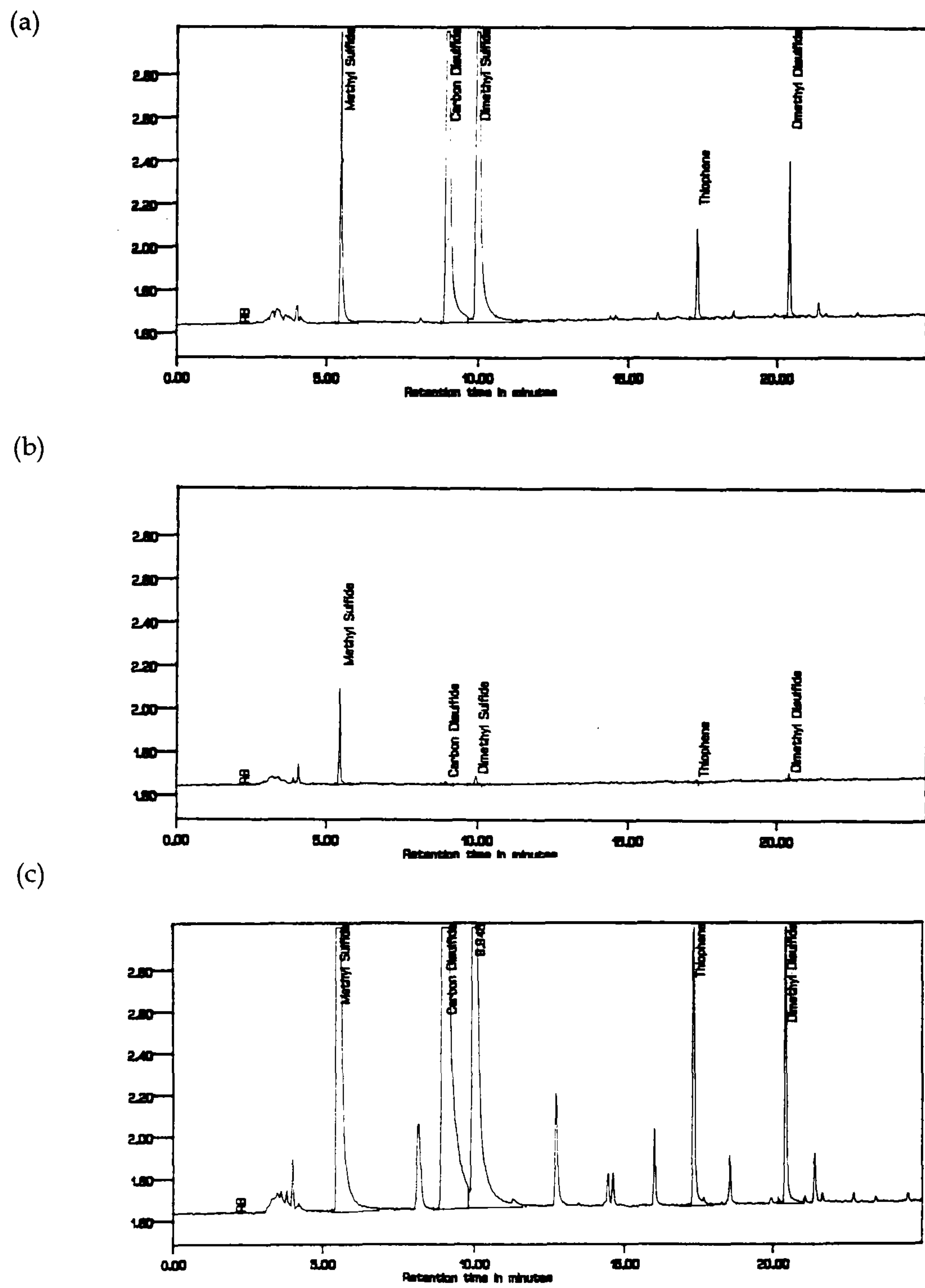


Figure 3: Cryofocus headspace analysis of (a) R&G, (b) unaromatized soluble coffee and (c) aromatized coffee.

II and Figure 2.Although there were small variations in the area count for each of the standards, most were within a 2σ variability for that standard at that concentration. Carbon disulfide was the only peak affected by the solvent. The high concentration of methylene chloride causes an observed chemiluminescence and then quenching which overlaps the retention time for carbon disulfide. It is important to select the correct solvent for dissolution of the sample.

In Figure 3, three samples, a commercial R&G and two soluble coffee products, are analyzed using SCD cryofocus headspace analysis. The chromatograms illustrate the wide concentration range that can be monitored within the linear range of the detector. The concentration of dimethyl sulfide, carbon disulfide thiophene and dimethyl disulfide are in Table III. The results are measured in ng of compound per ml of headspace. The technique is simple, rapid and reproducible.

Table III: SCD Head Space Analysis of Various Coffees

	R&G		Soluble Aromatized		Soluble Non-Aromatized	
Compound	Average ng/ ml	SD ng/ ml	Average ng/ ml	SD ng/ ml	Average ng/ ml	SD ng/ ml
Dimethyl Sulfide	3.83	0.10	21.59	0.46	0.013	0.001
Carbon Disulfide	2.81	0.15	6.46	0.03	0.017	0.006
Thiophene	0.73	0.01	3.57	0.05	0.011	0.002
Methyl Disulfide	0.79	0.03	2.74	0.03	0.038	0.008

Conclusion

The analysis of sulfur aroma compounds by chemiluminescence detection has proven to be very useful for the headspace analysis of coffee products. The technique is extremely sensitive and selective. This is borne out by the fact that as little as 2 pg of sulfur can be detected and that the carbon fraction of the molecule is at least 10^7 less sensitive than sulfur. The presence of halogenated hydrocarbons, such as methylene chloride, at high concentrations concurrently eluting with a sulfur-containing compound can cause error. Compared with FPD, SCD has a much broader dynamic linear range. The detector is simple to operate and can easily be substituted for an FID detector. Unlike a MS detector, the SCD cannot identify compounds without a standard, but the cost is substantially less, and its operation and maintenance is simpler. SCD is a positive addition to the analyst's arsenal of instrumentation in elucidating the complex chemistry of coffee.

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Summary

Coffee aroma is a complex mixture of volatile compounds. Over 800 compounds have been identified and described in the literature. Of these, sulfur compounds are extremely important to the aroma of coffee products. Today there are several techniques, such as mass spectroscopy, flame photometry and chemiluminescence, available to the chemist for the analysis of these compounds. This paper discusses the theory, selectivity, sensitivity, operating conditions, advantages and application of the sulfur chemiluminescence detector.

Résumé

L'arôme de café est un mélange complexe de composés volatils. Plus de 800 composés ont été identifiés et décrits dans la littérature. Parmi ceux-ci, les composés du soufre sont extrémement importants pour l'arôme des produits du café. De nos jours les chimistes disposent de plusieurs techniques comme la spectroscopie de masse, la photometrie à flamme et la chimioluminescence pour l'analyse de ces composés. Cet exposé discute la théorie, la sélectivité, la sensibilité, les modes d'opérations, les avantages et les applications de la chimioluminescence avec un détecteur de soufre.

QUANTIFICATION OF 2-METHYLISOBORNEOL IN ROASTED COFFEE BY GC-MS

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1 INTRODUCTION

The major coffee species commercially available are Coffea Arabica and Coffea Canephora var. Robusta. They differ considerably in bean size, price and consumer preference. Robusta beans are smaller with an uneven surface and contain a large share of discolored beans. The lower price for Robusta reflects the excessive supply in the market. With regard to the taste characteristics, Robusta has a harsh, earthy, musty and cocoa-like character, whereas, Arabica exhibits a milder and more fruity flavor which is preferred in parts of Europe and in the US.

The characterization of the chemical differences in the volatile fraction has been a large field of investigation in the past. Tressl et al., 1978 and 1978a, reported that the content of phenol and phenol derivatives is markedly higher in Robustas than in Arabicas and that some carbohydrate degradation products, e.g. 2-hydroxymethyl furfural, 2,5-dimethyl-4-hydroxy-3(2H)-furanoneas well as maltol and its derivatives are predominant in Arabicas. Comparing the headspace aroma profiles, sulfur compounds were found to occur in higher amounts in Robustas (Gutmann et al., 1977; Nurok et al., 1978). Recent findings indicate that only a relatively small number of volatile compounds contribute to the flavor of roasted coffee (Holscher et al., 1990). Blank et al., 1992 and 1992a, identified and quantified the potent odorants of Arabica and Robusta coffee using aroma extract dilution analysis. Their results indicate that Arabica coffee contains higher amounts of enol-oxo compounds whereas, pyrazines and phenolic compounds are predominant in Robustas.

Recently, the question was posed, whether the Robusta flavor could be traced back to one specific Robusta aroma impact compound. Using the GC/sniffing technique, an *earthy, musty* smelling region was detected, which was absent or at least much weaker in aroma extracts obtained from Arabica coffees. After several enrichment and preseparation steps, the *earthy*-smelling compound was identified as 2-methylisoborneol (MIB) by comparison of retention data, mass spectra and sensory properties with synthetic MIB (Vitzthum et al., 1990). At present, the quantification of MIB in roasted coffee requires time consuming fractionation by HPLC and 2-dimensional GC-MS and gives only semi-quantitative data. Therefore, the objective of this work was to improve the methodology to quantify MIB in roasted coffee on a routine base.

2 EXPERIMENTAL

2.1 Sample Material

Green coffee samples were roasted to a medium degree of roast with a fluidized bed roaster for 180 sec. Prior to analyses the roasted samples were stored at -50°C. Robusta coffees were taken from Indonesia, Conillon, Ghana, Zaire, Uganda, The Cameroons and Ivory Coast and for comparison Arabicas were taken from Colombia, Kenya, Brazil and El Salvador. For roasting kinetics, Indonesian Robusta was roasted to six different degrees of roast reaching from a very light to an espresso-type roast.

2.2 Sample Preparation

An overview of the analytical procedure is given in Figure 1. 100 g ground roasted coffee and 0.1 μg deuterated MIB, synthesized according to Wood and Snoeying, 1977,were suspended in 1l distilled water. Simultaneous distillation/extraction (SDE) was carried out for 2h using 100 ml diethylether/pentane (1+1; v/v) as solvent (Schultz et al., 1977; Silwar, 1982). After drying over anhydrous sodium sulfate, the extracts were carefully concentrated by gentle distillation with a Vigreux-column to about 0.3 ml. The aroma extract obtained by SDE was fractionated by column chromatography on silica gel 60. Fraction 1 was eluted with diethylether/pentane (6+94; v/v) and discarded. Fraction 2 was obtained by elution with diethylether/pentane (10+90; v/v) and concentrated to about 0.5 ml. 2 μl of this concentrate was applied to GC-MS.

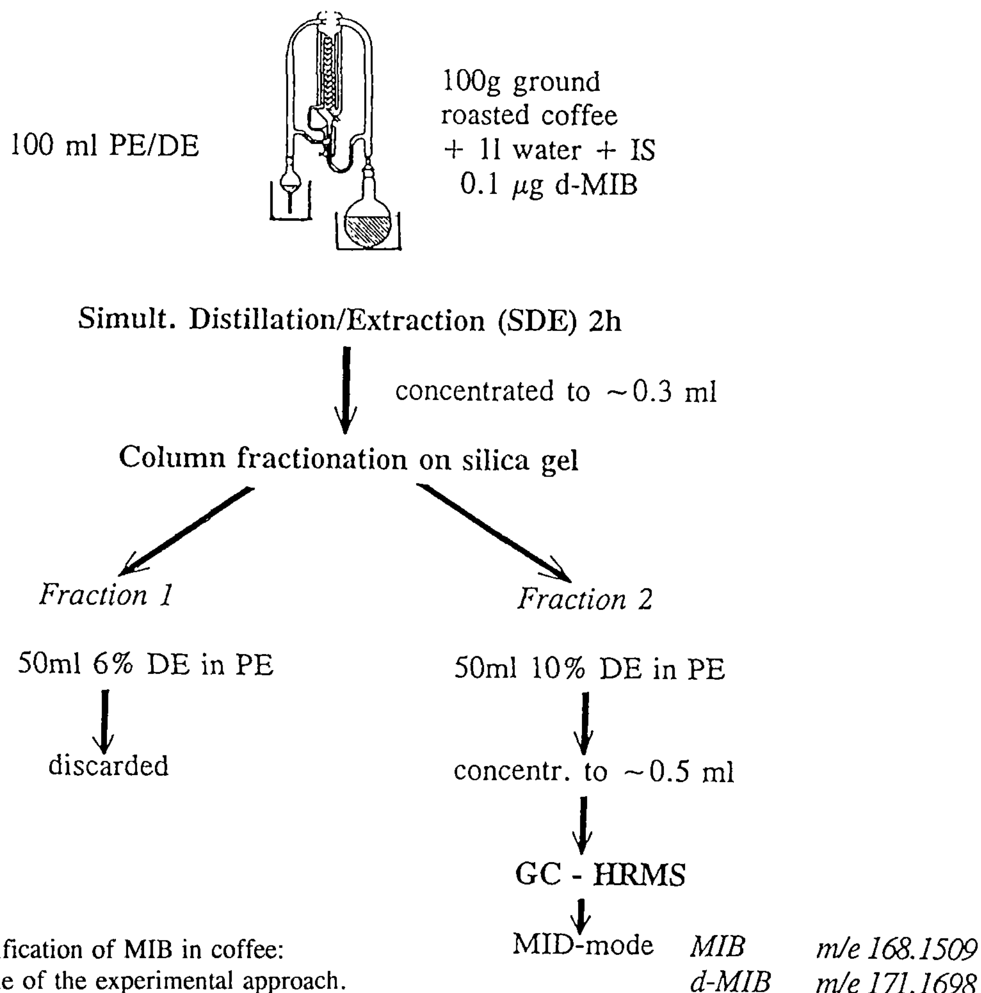

Figure 1: Quantification of MIB in coffee: Scheme of the experimental approach.

2.3 Gas Chromatography

Samples were injected using a temperature programmable injection system (60°C to 200°C; 12°C/sec) into a HP 5890 II gas chromatograph. Separation was performed on a DB-WAX column (60m x 0.25 mm; 0.25 μm film thickness; temperature profile: 35°C hold for 1 min, 40°C/min to 60°C, 3°C/min to 220°C, hold for 10 min). Helium was used as carrier gas at a flow rate of 1-2 ml/min.

2.4 Mass Spectrometry

GC-MS and GC-MS/MS experiments were performed on a Finnigan MAT 95 Q tandem mass spectrometer using the GC conditions as in section 2.3. Mass spectra were generated at 70 eV in the electron impact mode, emission current was 1.0 mA, the ion source was held at 220°C and the resolution was 3000. Quantification was done according to the internal standard method in the Multiple Ion Detection (MID) mode. The selected precise masses were m/z 168.1509 for the target peak of MIB and m/z 171.1698 for the internal standard d-MIB. For MS/MS experiments air was used as collison gas at a pressure of 4-6x10^{-5} mbar; the collison offset voltage was -50 eV. Synthetic MIB was introduced via probe inlet at a source temperature of 100°C.

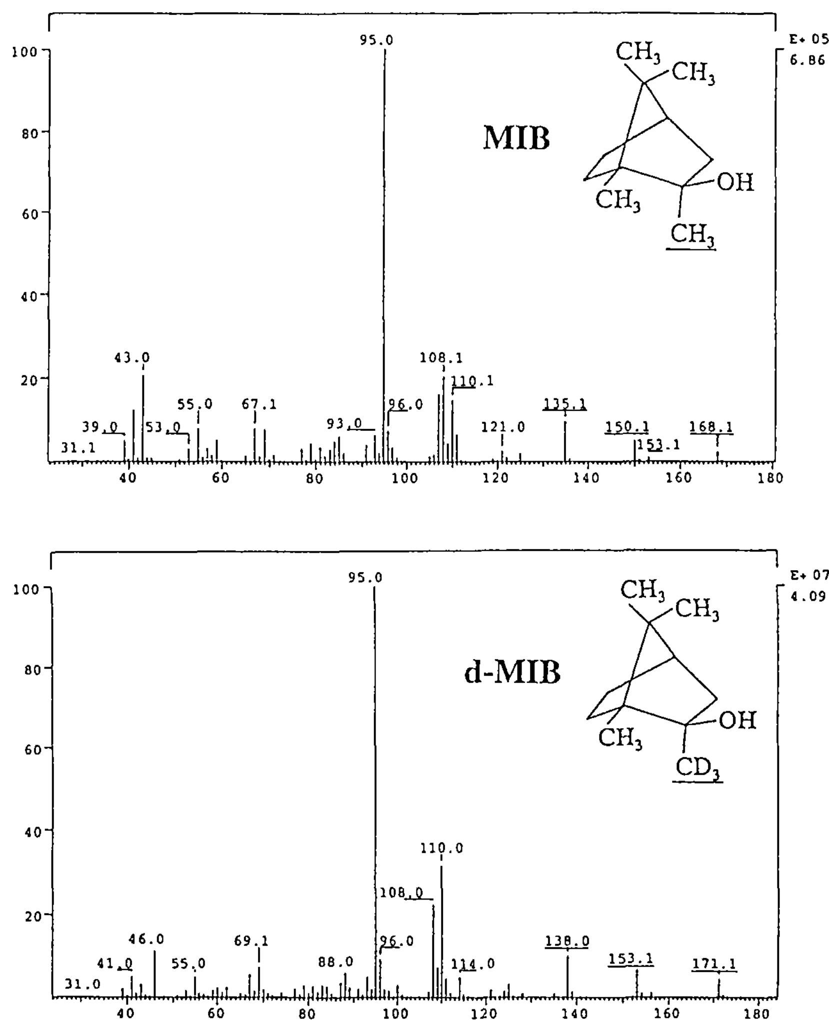

Figure 2: Mass spectra of MIB and d-MIB.

3 RESULTS AND DISCUSSION

MIB was described as an *earthy* and *musty* smelling compound by numerous authors, mainly as an off flavor causing agent in drinking water. The odor threshold of MIB in water is in the range of 2 to 5 ng/l (Persson, 1979; Ito et al., 1987). In coffee beverages, the value was found to be approximately 5 ng/l (Vitzthum et al.,1990). Hence, a quantitative analytical test method requires an extremely low detection limit and a high selectivity. This goal only can be achieved by highly sophisticated spectroscopic techniques. SDE has proven to be a suitable and convenient tool for the isolation of MIB from complex matrices like roasted coffee (Vitzthum et al., 1990). Deuterated MIB was added as an internal standard prior to isolation of the volatile fraction. The use of a stable isotope for internal standardization was first reported by Sweely et al., 1966, and has now been widely applied for the analysis of trace components in aroma research (Schieberle and Grosch, 1987). The deuterated MIB matches all chemical and physical properities of authentic MIB and therefore compensates all losses during sample preparation. The mass spectra of MIB and deuterated MIB derivative are given in Figure 2.

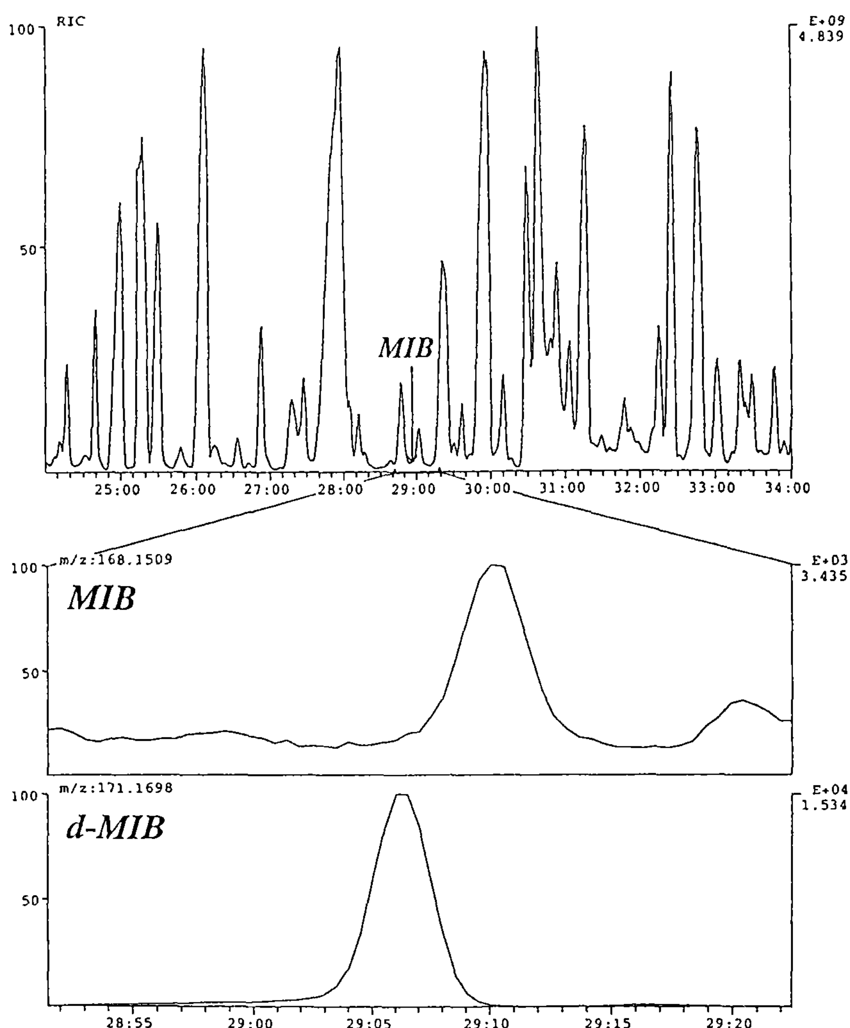

Figure 3: Cuts from a total ion chromatogram and MID mass tracks of a subfraction of a roasted Indonesian Robusta aroma extract.

Total aroma extracts obtained from coffee are complex mixtures containing more than 1000 volatile compounds at extremely broad concentration levels ranging from ppm to ppt. Therefore, the quantification of trace levels of MIB can only be achieved by prefractionation. Under the chosen conditions of column chromatography on silica gel, most of the volatile complex is removed. Further selectivity and sensitivity was achieved by using high resolution mass spectrometry in the MID mode. MID monitors the intensities of preselected characteristic ions. In the present case, the molecule ions, 168 and 171, respectively, were found to give the highest accurracy. Consecutively, the gain in sensitivity was about a factor of 1000 compared to the overall technique. A very high precision was accomplished by running the MID-mode by continous introduction of perfluorokerosene (PFK) as a reference. Using this procedure, the chromatogram showed only two peaks, which could be quantified easily (Figure 3). The relative standard deviation was about 13% and the lower quantification level 20 ng/kg.

To confirm the authenticity of the ion chosen for quantification, MS/MS experiments were performed. Therefore, the daughter ion MS/MS spectra of the selected parent ion m/z 168.1 (the molecule ion of MIB) were obtained of MIB in coffee extract and of synthetic MIB for reference (Figure 4).

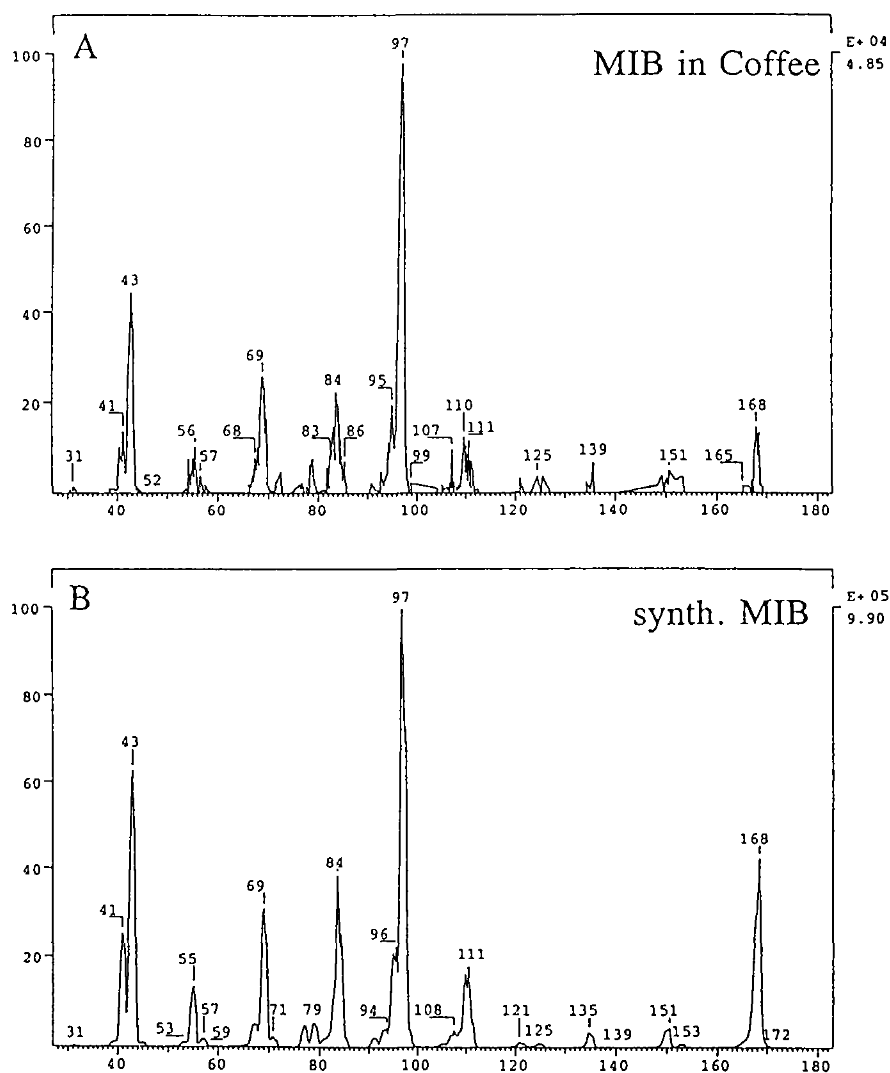

Figure 4: Daughter ion MS/MS spectra of the parent ion 168.1 of MIB in coffee (A) and synthetic MIB (B).

Figure 5 shows the degradation of MIB during roasting in Indonesian Robusta, which was roasted to 6 different degrees of roast at variable temperature and constant time. The resulting graph follows an exponential decay against roasting temperature characterized by a relatively strong reduction during early stages of roasting whereas, the decrease slows down during darker roast. Nevertheless, MIB is clearly detectable even in espresso-type roasts.

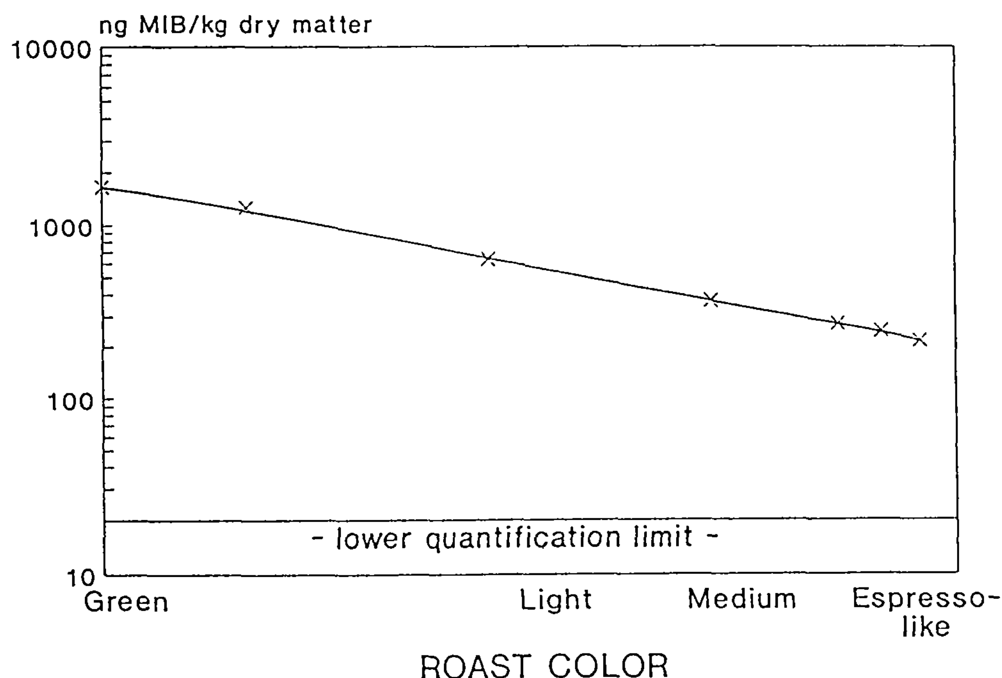

Figure 5: Degradation of MIB during roasting.

Applying the technique mentioned above, various single strains of roasted Robusta and Arabica coffee samples were monitored for the overall MIB content. The results are summarized in Table 1. MIB levels in the Robusta coffees were consistently higher than in the Arabica coffees by an order of magnitude.

Robusta	MIB-level [ng/kg dry matter]	Arabica	MIB-level [ng/kg dry matter]
Indonesia	310	Brazil	<20
Uganda	185	El Salvador	$\ll 20$
Conillon	120	Colombia	$\ll 20$
Ghana	430	Kenya	$\ll 20$
Zaire	240		
Ivory Coast	200		
The Cameroons	120		

Table 1: Concentration of MIB in various roasted Robusta and Arabica coffees.

4 CONCLUSIONS

* Quantification of MIB in roasted coffee could be improved and facilitated significantly using a deuterated internal standard in combination with high resolution MS.

* A substantial amount of MIB is destroyed during roasting according to a first order kinetics. However, MIB is clearly detectable in espresso-type roasted coffee.

* MIB was quantified in various roasted Robusta and Arabica roasted coffees. The levels in Robusta are at least 10-fold higher than those in Arabica.

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SUMMARY

The development of an improved test method for 2-methylisoborneol (MIB), one of the key aroma compounds contributing to the characteristic earthy and harsh flavor of Robusta coffee is described. The quantification of MIB in a complex matrix like roasted coffee could be achieved on a routine base. Simultaneous distillation/extraction was applied for isolation of the volatile compounds followed by column chromatography on silica gel. MIB was quantified via high resolution GC-MS using deuterated MIB as internal standard. The authenticity of the mass fragment chosen for quantification was confirmed by tandem mass spectrometry. Typical values of MIB in Robusta were at least 10-fold higher than in Arabica coffee.

RÉSUMÉ

Le dévelopement de la méthode améliorée pour déterminer le 2-méthylisobornéol (MIB), l'un des composés clés, responsables de l'arôme typique "terreux" du Robusta, est décripte. La quantification routinière de MIB dans la complexe matrixe de café torréfié fût réalisé. Distillation/extraction simultanée est appliqué pour l'isolation des composés volatils, suivie d'une chromatographie à colonne au gel de silice. MIB a été estimée au moyen d'une haute résolution GC/MS en utilisant MIB deuteriée en temps que standard interne. L'authenticité de la masse fractionnée choisie pour la quantification a été confirmée par MS/MS expérimente. MIB a été trouvé en quantité 10 fois plus élevée pour les cafés Robustas que, dans les café Arabicas.

QUANTIFICATION OF POTENT ODORANTS IN COFFEE

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Introduction

Aroma extract dilution analyses have shown that 2-furfurylthiol (I), 2-ethyl-3,5-dimethylpyrazine (II) and 2,3-diethyl-5-methylpyrazine (III) contribute strongly to the roasty and earthy odour notes of roasted coffee [1-4]. Furthermore, 2-methylisoborneol (IV) exhibiting an "earthy, musty" sensorial impression has been suggested [5] to be one of the aroma key compounds of Robusta coffee.

As recently discussed [6] an accurate quantification of odorants being trace components of a food can be performed by using the corresponding odorants labelled with a stable isotope as internal standards. Apart from small isotope effects, the labelled standards have chemical and physical properties identical to those of the odorants to be quantified. There-

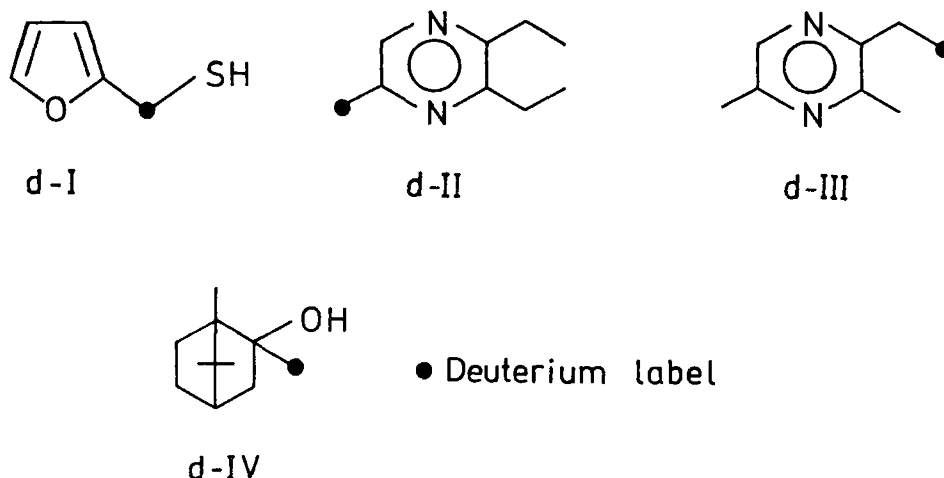

Figure 1. Chemical structure of labelled odorants used as internal standards in the stable isotope dilution assays of odorants I to IV.

fore, the analyte and its internal standard are equally enriched by the purification procedure.

The quantification of the odorants I to IV in Arabica and Robusta coffee samples by using the corresponding odorants labelled with deuterium as internal standards (Figure 1) is reported in this paper.

Methodology

The syntheses of the deuteriated internal standards d-I, d-II and d-III have been earlier described [7, 8]. Compound d-IV was prepared by a Grignard reaction of [^{2}H]-methylmagnesium iodide with camphor [9]. The analytical procedure for the quantification of the odorants I, II and III is summarized in Table 1. Compound IV was isolated by simultaneous distillation and extraction (SDE) from green coffee (500 g).

Table 1. Analytical procedure for the quantification of compounds I, II and III

- The sample (40-120 g) was 3 times extracted with diethyl ether saturated with water. The solvent used for the first extraction was spiked with the internal standards d-I, d-II and d-III.
- The combined extracts were concentrated and the volatiles were distilled off in vacuo from the nonvolatile material.
- The volatile fraction was freed from the acids by extraction with aqueous sodium hydrogen carbonate.
- Column chromatography on Silica Gel; elution with pentane/diethyl ether (95 + 5, v/v, 120 mL, fraction 1), (50 + 50, v/v, 120 mL, fraction 2) and diethyl ether (240 mL, fraction 3).
- Capillary GC of fractions 1 (compound I) and 3 (compounds II and III).
- Mass chromatograms in the chemical ionization mode.

Results and Discussion

The concentrations of furfurylthiol and of the two pyrazines were determined in samples of roasted Robusta and Arabica coffee (Table 2). The Robusta coffee contained with 2 mg/kg somewhat more of the furfurylthiol than the Arabica with 1.7 mg/kg. In the case of the two pyrazines the difference between the two kinds of coffee was larger, as their levels in the Robusta were approximately twice as high as in the Arabica sample.

Table 2. Odorants I, II and III in roasted Robusta and Arabica coffee (powder)

Odorant	Robusta[a] (µg/kg)	Arabica[b] (µg/kg)
2-Furfurylthiol (I)	1956	1708
2-Ethyl-3,5-dimethylpyrazine (II)	824	492
2,3-Diethyl-5-methylpyrazine (III)	233	112

[a] The samples of Robusta and Arabica coffee were from Indonesia and Colombia, respectively.

A coffee brew of 1 L was prepared from 54 g of the coffee powder. After addition of the internal standards to the brew the pyrazines were extracted and quantified (Table 3). As to expect the brew obtained from the Robusta coffee contained higher levels of both pyrazines than the brew from the Arabica coffee. The extraction yield was calculated on the basis of the concentrations of the pyrazines in the powders. As detailed in Table 3 in brackets 60 to 64 percent of the two pyrazines occurring in the coffee powders were extracted by the hot water.

Table 3. 2-Ethyl-3,5-dimethylpyrazine (II) and 2,3-diethyl-5-methylpyrazine (III) in coffee brews[a]

Odorant	Robusta (Indonesia)		Arabica (Colombia)
		μg/L	
II	26.6 (60 %)[b]		16.8 (63 %)
III	8.0 (64 %)		3.7 (61 %)

[a] Coffee powder (54 g) on a coffee-filter was extracted with hot water (1 L).

[b] The extractcion yield of the odorant is presented in ().

The extraction yield of the 2-furfurylthiol was higher and lay in the range of 90 percent (Table 4). This result was obtained when the odorant I was extracted (method A) or when it was distilled off in vacuo from the brew, which had been cooled to room temperature, and by extraction of the distillate (method B). However, when the furfurylthiol was isolated by SDE (method C), the yield was much higher: 215 μg/L were found after 1.5 hour of SDE and 240 μg/kg after 3 hours of SDE. The conditions used for SDE, in particular the boiling of the sample, were more drastic than the conditions in the methods A and B. We assume that precursors, which are present in the brew, are degraded at the relatively high temperature of SDE with formation of 2-furfurylthiol.

Table 4. 2-Furfurylthiol in coffee brew[a]

No.	Method[b]	Concentration (μg/L)	Extraction yield (%)
1	A	92	87
2	B	97	92
3	C (1.5 h)	215	204
4	C (3 h)	240	227

[a] Powder of Robusta coffee (54 g) on a coffee-filter was extracted with hot water (1 L).

[b] Method A: Liquid/liquid extraction, Method B: Distillation in vacuo, Method C: simultaneous distillation and extraction.

The stability of 2-furfurylthiol during storage at -35°C and at room temperature was compared in Arabica and Robusta coffees (Table 5). After 40 days at -35°C the Arabica sample contained 1.7 mg/kg of 2-furfurylthiol. Storage at room temperature lowered the concentration, although the sample was vacuum-packed. Only 0.32 mg/kg of the thiol was found after 40 days. The thiol was also not stable in the Robusta sample, the concentration decreased from 2.0 mg/kg to 0.79 mg/kg. In the second experiment with the Robusta coffee the vacuum package was opened and the sample was poured into an Erlenmeyer flask which was sealed and then stored for 40 days. Under these conditions the coffee was in contact with air. However, the result (Table 5) indicates that the effect was not very important, as the concentration of the thiol decreased during storage approximately as rapid as in the vacuum-packed samples.

In conclusion, 2-furfurylthiol is not stable in ground coffee, when the sample is stored at room temperature. This result does not confirm earlier findings [10] according to which the concentration of 2-furfurylthiol increases during the storage of coffee. As the de-

Table 5. Stability of 2-furfurylthiol (I) during storage of roasted coffee

Sample	I (mg/kg) after 40 days at -35°C	at +22°C
Arabica (Colombia)[a]	1.73	0.32
Robusta (Indonesia)[a]	2.0	0.79
Robusta (Indonesia)[b]	2.05	0.58

[a] The sample was vacuum-packed.

[b] The vacuum package was opened and the sample was poured into an Erlenmeyer flask which was sealed and then stored.

crease in the thiol affects negatively the aroma, it is very suitable as an indicator substance for storage defects of coffee.

2-Methylisoborneol (IV) was determined in four samples of green coffee (Table 6): 1.28 µg/kg and 0.74 µg/kg were found in two Robusta samples. In contrast, the two Arabicas contained lower levels: 420 ng/kg were found in the sample of Arabica from Colombia and 80 ng/kg in that from Santos.

Table 6. Concentration of 2-methylisoborneol (IV) in green coffee

Sample	IV [ng/kg]
Robusta (Ivory coast)	1280
Robusta (Indonesia)	740
Arabica (Colombia)	420
Arabica (Santos)	80

Conclusion

On the last ASIC conference Blank et al. [3] reported a first set of stable isotope dilution assays for the quantification of odorants contributing singificantly to the coffee flavour: sotolon, furaneol, diacetyl, 2,3-pentandione, ß-damascenone and 3-mercapto-3-methylbutylformate are examples. This list is extended by the four odorants discussed here. More methods are now available for the objective determination of flavour differences in coffee samples.

Summary

2-Furfurylthiol (I), 2-ethyl-3,5-dimethylpyrazine (II), 2,3-diethyl-5-methylpyrazine (III) and 2-methylisoborneol (IV) were quantified in coffee samples by stable isotope dilution assays. Samples of a freshly roasted Robusta coffee contained (mg/kg) 2.0 (I), 0.82 (II) and 0.23 (III). In a sample of Arabica coffee containing 1.7 mg/kg (I) the levels of (II) and (III) were about half as high as in the Robusta coffee. The concentration of (I) decreased during storage of ground coffee at room temperature. Approximately 90 percent of (I) and 65 percent of (II) and (III) in the powders were extracted by the preparation of a coffee brew. Two samples of green Arabicas (420 and 80 ng/kg) were lower in IV than two Robustas (1280 and 740 ng/kg).

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CHARACTERIZATION OF UNSATURATED ALDEHYDES IN GREEN COFFEE

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1 INTRODUCTION

The volatile aroma of roasted coffee is mainly generated during roasting. Nevertheless, certain volatile components are present already in green coffee. On the one hand, these substances contribute to typical green coffee odor. On the other hand, they may contribute to origin dependent flavor or off flavor characteristics in the corresponding roasted coffee [1-6]. The aim of this work was to elucidate certain compounds of green coffee that are probably generated via autoxidation of the unsaturated fatty acids [7,8]. This issue required the investigation of their sensory properties and their chemical structures. The identification and structural elucidation of unsaturated aldehydes was carried out by means of chromatographic and spectroscopic methods.

2 EXPERIMENTAL

2.1 Sample Preparation

100g of ground green Colombian coffee and 1l of distilled water were placed in a 2l round bottom flask. The isolation of the volatile fraction was carried out by simultaneous distillation/extraction with a modified apparatus according to reference [9] for 2 hours using a mixture of 100 ml of *n*-pentane/diethyl ether (1:1, v/v) as solvent. The organic extract was dried over anhydrous sodium sulfate and concentrated to 1ml by means of a *Vigreux*-column (250 x 10mm).

2.2 Gas Chromatography

Gas chromatographic separation was performed on a HP 5890 II gas chromatograph equiped with a DB-Wax capillary column (60m x 0.25mm; 0.25μm film thickness). Helium was used as carrier gas at a flow rate of 1-2 ml/min; temperature program: 35°C for 2 min, then 40°C/min to 60°C, then 3°C/min to 215°C, hold for 15

min. A sample volume of 3μl was injected via a temperature programmable injection system (60°C to 200°C; 12°C/sec) and GC sniffing was performed by splitting the GC effluent (in a ratio 1:1) to a flame ionization detector and a sniffing port.

2.3 Hyphenated Spectroscopic Techniques

Mass spectroscopy was performed on a *Finnigan* MAT 95 Q and mass spectra were generated at 70eV in the electron impact mode. Fourier Transform Infra Red (FTIR) spectra were recorded by a *BIO-RAD Tracer* interface using the direct deposition technique in combination with the 'FTS45' FTIR spectrometer. The transfer line was held at 250°C and the *slide* (see below) was cooled down to -196°C with liquid nitrogen. The GC experimental conditions were the same as in section 2.2.

2.4 Isolation of (*E*,*Z*)-2,4-Decadienal and NMR Spectroscopy

(*E*,*Z*)-2,4-Decadienal occuring as an impurity in synthetic (*E*,*E*)-2,4-decadienal (Aldrich, Steinheim, Germany) was isolated by means of GC effluent cryo-trapping using a preparative *Gerstel* 'Multi Column Switching' system (Gerstel, Mühlheim, Germany). The isolate was eluted with $CDCl_3$ and NMR spectroscopy was carried out using a *Bruker* AC-250 MHz spectrometer (512 scans, aquisition time ca. 17 min).

3 RESULTS AND DISCUSSION

The elucidation of the volatile complex of roasted coffee has been a large field of investigation during the last decades. However, only a relatively small number of papers deal with the volatile fraction of green coffee. This may be a consequence of the fact that coffee is not used in the green state but only after roasting. Some years ago, the typical green coffee odor could be related to the occurrence of 2-methoxy-3-*iso*propyl- and 2-methoxy-3-*iso*butyl pyrazine [1] and furthermore a variety of saturated and unsaturated aldehydes was identified [10,11]. These compounds are known to be breakdown products of lipid oxidation. Their occurrence in green coffee is not surprising due to the fact that the total lipid content in green coffee amounts about 13-15%. More than one half of the fatty acid complex is formed by linoleic acid. Two GC peaks of a green aroma extract attracted special interest (peak A and B in Figure 1). At the sniffing port the odor descriptions were distinctively different: peak A exhibited a *metallic* aroma note as opposed to peak B which possessed a *fried/oily* odor impression. However, mass spectra were similar and indicated the presence of isomeric 2,4-decadienals.

Mass Spectrometry

The mass spectra of peak A and B are shown in Figure 1. At the first glance the mass spectra of peak A and B are identical. The comparison of sensory impression, mass spectral and retention data of peak B with literature data and reference substance confirmed the chemical structure of peak B as (*E*,*E*)-2,4-decadienal. Recently, (*E*,*E*)-2,4-Decadienal has been identified in green and roasted coffee [6, 12]. It arises from autoxidation of unsaturated fatty acids [7,8]. The general fragmentation pattern of 2,4-decadienals is illustrated in Figure 2. As a result of a easy shift of the aldehyde proton along the conjugated double bonds the base peak is the fragment ion m/z 81 that arises from a rearranged molecule ion with a 'ketene-like' structure. Therfore, the normal 'allyl' cleavage between carbon atoms C6 and C7 leading to the fragment ion m/z 95 does not result in the base peak. The similarity of the mass spectra of peak A and B is a clear evidence for different orientations of the double bonds (*E*/*Z* isomers) because of the conditions of mass spectrometry. Fundamentally, four *E*/*Z* isomers of 2,4-decadienal are possible (Figure 3) however, the comparison of retention data of peak A with the literature [13] reveals the presence of (*E*,*Z*)-2,4-decadienal. In order to confirm this hypothesis, infra red spectroscopy was performed; mass spectroscopy is not capable of distinguishing between *E*/*Z* isomers.

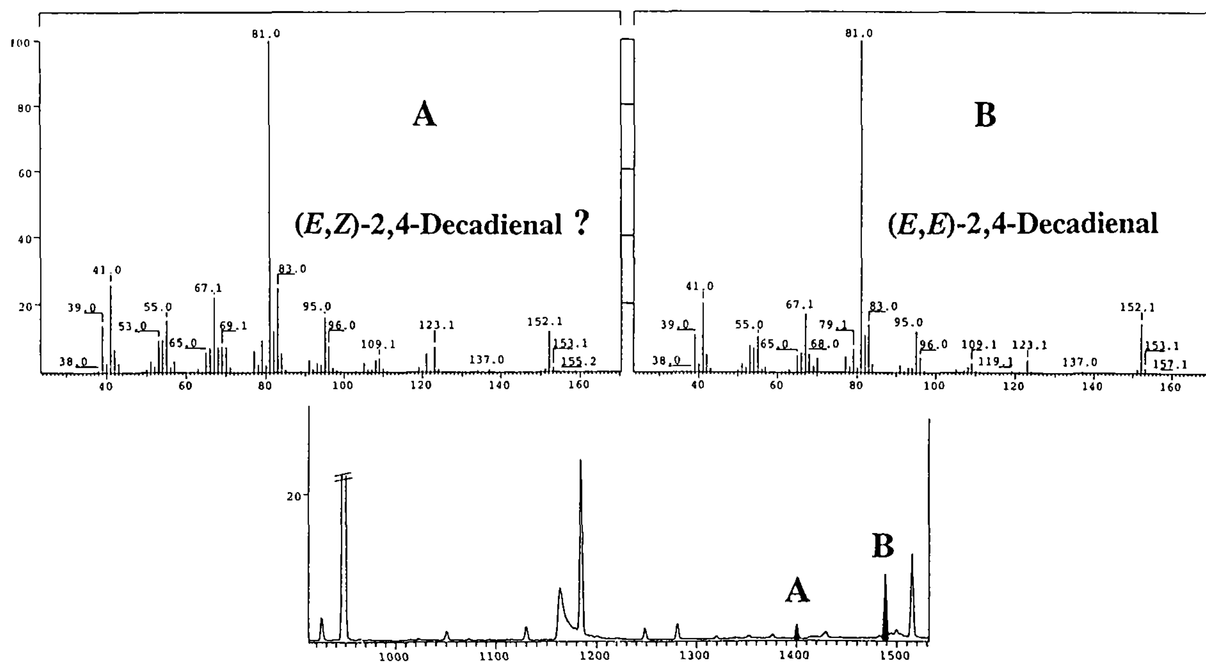

Figure 1: Cut from a total ion chromatogram of a green coffee aroma extract and mass spectra of isomeric 2,4-decadienals.

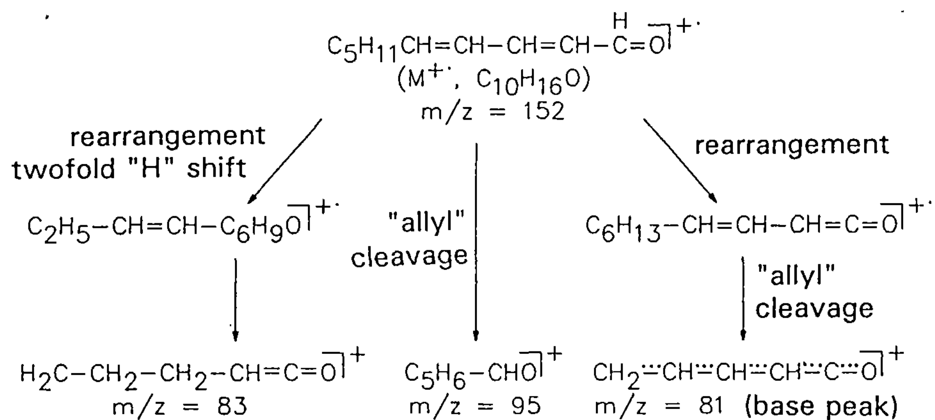

Figure 2: Fragmentation pattern of 2,4-decadienals.

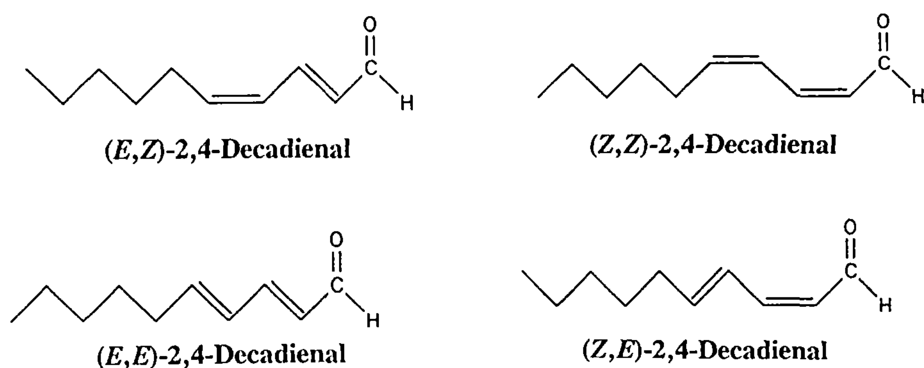

Figure 3: Isomeric 2,4-decadienals.

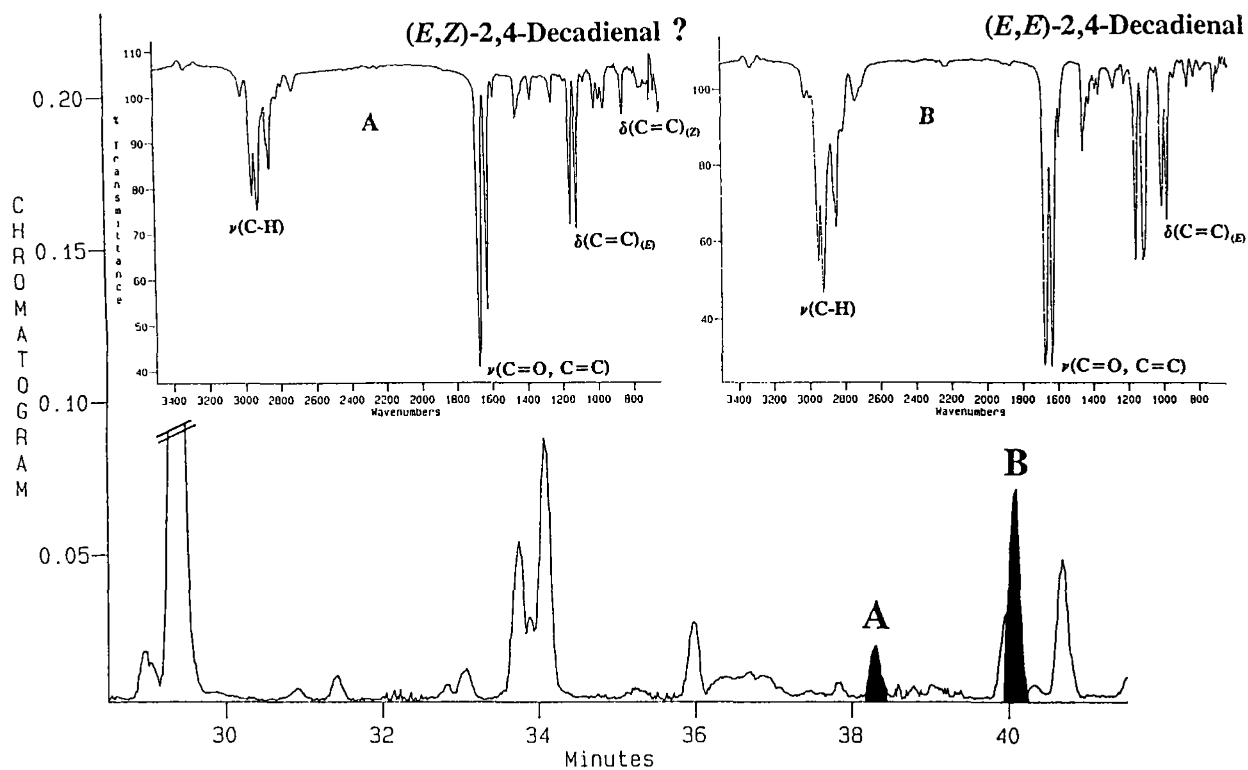

Figure 4: Cut from a Gram-Schmidt chromatogram and FTIR-spectra of isomeric 2,4-decadienals.

FTIR-Spectroscopy

The major drawback of conventional GC-FTIR *lightpipe* systems is the lack of sensivity. The newly introduced direct deposition technique using the so called *Tracer* interface has overcome this disadvantage [14,15]. The GC effluent is cryo-trapped onto a moving 60mm x 30mm zinc selenide slide which is cooled with liquid nitrogen. The detection limit for strong infra red absorbers is about 50 picogram which comes close to GC-MS conditions [16]. The sensitivity of the *Tracer* system is therefore about 100-fold lower compared with conventional GC-FTIR systems. That is the reason why FTIR spectra can be monitored on-line from the GC effluent without any prefractionation and enrichment steps. Figure 4 shows a Gram-Schmidt chromatogram of the same green aroma extract. The differences in the peak pattern between the total ion and the Gram-Schmidt chromatogram are due to the different response factors depending on compound specific structural peculiarities.
Some selected assignments of the absorption bands of the two isomeric 2,4-decadienals to the type of vibration are given in Figure 4. As expected, the spectra are quite similar from wave number 3500 to 1300 because isomeric double bonds scarcely effect vibrations caused by the aliphatic part of the molecule and strength of bonds. The most important differences are to be seen in the fingerprint region below wave number 1100. After normalizing the region of the CH vibrations in the range of 3050 to 2700 cm^{-1}, it is obvious that the $\delta_{o.o.p.}$ absorption band for the (*E*) double bond exists for the decadienal isomer at 994 cm^{-1} and that this band has roughly half the intensity as in the spectrum of the (*E*,*E*)-2,4-decadienal (992 cm^{-1}). Furthermore, the $\delta_{o.o.p.}$ absorption band for the (*Z*) double bond occurs at 862 cm^{-1} and one can assume that the structure of this decadienal isomer is (*E*,*Z*)- or (Z,*E*)-2,4-decadienal.

Preparative Enrichment of (*E*,*Z*)-2,4-Decadienal, NMR Spectroscopy

Generally, the confirmation of a chemical structure runs into problems if the authentic reference substance is difficult to obtain in a pure state. In the present case, it was found that the commercially available synthetic (*E*,*E*)-2,4-decadienal contains a substantial impurity of an isomeric compound having similar retention data and organoleptic properties as the substance B. Therefore, an attempt was made to isolate this impurity by automatic GC effluent cryo-trapping in the milligram range.

(*E*,*E*)-2,4-Decadienal				(*E*,*Z*)-2,4-Decadienal			
δ [ppm, $CDCl_3$]	Multi-plicity	Assignment, Integral	$^3J_{HH}$ [Hz]	δ [ppm, $CDCl_3$]	Multi-plicity	Assignment, Integral	$^3J_{HH}$ [Hz]
9.49	d	H^1 (C<u>H</u>O); 1H	H^1H^2 = 7.85	9.59	d	H^1 (C<u>H</u>O), 1H	H^1H^2 = 7.85
6.03	dd	H^2; 1H	H^2H^3 = 15.2	6.13	dd	H^2; 1H	H^2H^3 = 15.2
7.04	dd	H^3; 1H	H^3H^4 = 10.0	7.42	dd	H^3; 1H	H^3H^4 = 11.2
6.22-6.29	m	H^4, H^5; 2H		6.25	t (br.)	H^4; 1H	H^4H^5 = 11.0
2,17	qa	H^6; 2H		5.99	dt	H^5; 1H	H^5H^6 = 7.8
1.42	qi	H^7; 2H		2.32	qa	H^6; 2H	
1.18-1.35	m	H^8, H^9; 4H		1.0-1.6	m	H^7, H^8, H^9; 6H	
0.85	t	H^{10}; 3H		0.89	t	H^{10}; 3H	

Table 1: NMR spectral data of (*E*,*E*)-2,4-decadienal and (*E*,*Z*)-2,4-decadienal; chemical shifts [ppm, $CDCl_3$], assignments (derived from ^1H,^1H-COSY NMR spectra), coupling constants [$^3J_{HH}$] (d = duplet, dd = double duplet, t = triplet, qa = quartet, qi = quintet, m = multiplet; br. = broad)

The structural confirmation of this isolate was performed via NMR spectroscopy and the compound was identified as (*E*,*Z*)-2,4-decadienal. For the determination between *E*/*Z* isomers the most important tool is the $^3J_{HH}$ coupling constant. Therefore, it is obvious that the protons H^2H^3 in the NMR spectrum of (*E*,*Z*)-2,4-decadienal are sited in an *E*- and H^4H^5 in a Z-configuration $^3J(H2H3)$ = 15.2 Hz, $^3J(H4H5)$ = 11.0 Hz (Table 1). As a result of the smaller coupling constant $^3J(H4H5)$ the multiplicity of the signal for H^4 is similar to a triplet because of the similar values of $^3J(H3H4)$ = 11.2 Hz and $^3J(H4H5)$ = 11.0 Hz. In comparison to that one finds a double dulplet for the protons H^2 and H^3 because of the more different values of $^3J(H2H3)$ = 15.2 Hz and $^3J(H3H4)$ = 11.2 Hz. The overall appearance of the NMR spectrum of the (*E*,*E*)-2,4-decadienal demonstrates the differences between the two isomers (Table 1). The assignment of the NMR signals to the corresponding protons of the two isomers (*E*,*E*)- and (*E*,*Z*)-2,4-decadienal was achieved by using the $^1H,^1H$-COSY NMR spectra. With the authentic reference material, peak B could be clearly identified as (*E*,*Z*)-2,4-decadienal via comparison with sensory properties, retention and spectroscopic data.

4 CONCLUSIONS

* Two unsaturated aldehydes isolated from green coffee and exhibiting different odor characteristics were identified as (*E*,*E*)-2,4-decadienal and (*E*,*Z*)-2,4-decadienal.

* Both compounds are well known breakdown product of the autoxidation of unsaturated fatty acids.

* The structural confirmation could only be achieved by a combination of various chromatographic and spectroscopic techniques especially by the use of direct deposition GC-FTIR and NMR.

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SUMMARY: It is known from literature that a number of carbonyl compounds occur in green coffee. These compounds are well known as breakdown products of unsaturated fatty acids which form a major part of the lipid complex. The objective of this work was to characterize some volatile carbonyl compounds which exhibited metallic and fried/oily odor notes, respectively, at the sniffing port. The volatile fraction was isolated by means of simultaneous distillation/extraction. Characterization and identification of the compounds of interest were carried out by GC effluent sniffing and GC-MS. Isomer specification was achieved either on-line by direct deposition GC-FTIR or off-line after enrichment by automatic preparative GC and subsequent NMR. By this combined analytical approach two unsaturated aldehydes were identified as (E,E)-2,4-decadienal and (E,Z)-2,4-decadienal.

RÉSUMÉ: D'après la littérature il est reconnu qu'un nombre de composés carbonyles se trouvent au sein du café vert et que ceux-ci sont en général reconnus comme étant les produits résultant de la décomposition des acides graisseux non-saturés qui sont éléments du complexe graisseux. Le but de ce travail a été de caracterisés certains composés légers carbonyles volatiles qui donnent certaines odeurs métallique et de friture/huileuse, qui peuvent être senties l'olfactométrie. La fraction volatile a été isolée par distillation/extraction simultanée. La caractérisation et l'identification de ces composés intéressants a été établi à la sortie du CPG des odeurs et par CPG-SM. Les spécifications isomérique ont été atteintes soit par mesures simultanées au CPG (on-line) par mise en place directe CPG-IRTF, ou séparément (off-line) après enrechissement par CPG-préparé automatique et, suivi par RMN. Deux aldéhydes ont été identifiés en tant que (E,E)-2,4-decadienal et (E,Z)-2,4-decadienal.

STALING OF ROASTED COFFEE : VOLATILE RELEASE AND OXIDATION REACTIONS DURING STORAGE

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INTRODUCTION

Staling of roasted coffee is a phenomenon resulting from a series of degradation reactions which are not still completely understood. Although roasted coffee is considered a long shelf-stable product, it is affected by certain physical and chemical changes during storage which can lead to its rapid quality loss.
Changes in flavour and organoleptic properties of roasted coffee during storage are generally attributed to the loss of volatiles and to the development of oxidation reactions. Moisture content, oxygen availability as well as storage temperature seem to strongly affect the staling rate of roasted coffee (Radtke-Granzer and Piringer, 1991; Cros and Vincent, 1980). Nevertheless it must be pointed out that, while several attempts have been made to relate headspace volatile concentration and sensory properties of coffee brew, very few data are available about the "nature" of the oxidation reactions involved in staling of roasted coffee. Vitzhum and Werkhoff (1979) observed that the ratio between two headspace volatile compounds (2-methylfuran and 2-butanone) (M/B) could be successful in relation to the flavour rating scale. A good relationship between headspace volatile concentration and sensory assessments was also found by Ito et al., (1983) for roasted ground coffee samples stored in different pakaging conditions; results showed that significant volatile loss was detected after about 4 mounths of storage. Kallio et al., (1990) observed that the ratio of several pairs of compounds present in coffee headspace increased with storage time and they could be used as indicators of product quality.

Although free radicals are formed during the roasting process, the measurement of free radical evolution during storage by electron spin resonance (ESR) can be a suitable tool to evaluate the staling degree of roasted coffee; Baesso et al., (1990) found that the ESR intensity decreased during storage and the rate of change was strictly related to storage conditions. Hinman (1991) observed that coffee showed a significant oxygen consumption just after roasting; it is well known that Maillard reaction products exhibit oxygen scavenging properties (Anese et al., 1993), nevertheless the decrease of free radical concentration suggests that radicalic reactions such as lipid oxidation could be involved in staling of roasted coffee.
The aim of this research note was to study the influence of some storage conditions such as temperature and oxygen avaliability on volatiles and carbon dioxide release and on lipid oxidation reactions in roasted coffee during storage.

MATERIALS AND METHODS

Green coffee beans (Coffea Arabica) were dark roasted in an industrial plant (PACS, Bologna). Just after roasting part of coffee beans were ground in a laboratory mill. Roasted coffee had a moisture and lipid content of 2.93 % and 9.74 % (on wet basis) respectively.
Just after roasting samples of whole beans and ground coffee were exposed to the air in a glass container and stored at 4, 25 and 40 °C. Ground coffee samples, previously subjected to an industrial degassing process, were packed under vacuum in polyethylene bags and then conditioned at the same storage temperatures. At regular intervals, over a period of about 70 days for volatile and carbon dioxide release and 250 days for lipid oxidation, samples of roasted beans and ground coffee were removed for headspace analysis and for the evaluation of rancidity.

Headspace volatile and carbon dioxide analysis

A quantity of 2.5 g of roasted beans or ground coffee were put in 20 ml capacity vials which were subsequently sealed with butyl septa and metallic caps (Dani Italy). The vials were than conditioned at room temperature for at least 24 hours before analysis.
- Headspace carbon dioxide analysis: determination of headspace carbon dioxide concentration followed the method described by Massini et al., (1990). A Dani 3200 gas-chromatograph equipped with a thermal conductivity detector and a 2mxemm ID glass packed column filled with Parapack Q 80-100 mesh was used. The operating conditions were as follows: column temperature, 200 °C; injector and detector temperature, 200 °C; carrier gas (He) flow rate 20 mL/min. The headspace volume injected was 0.5 mL using a precision sampling syringe (Dynatech, USA), provided with pressure lock A-2 and a gas volume capacity of 0-1 mL. The chromatograms were recordered and analysed using a Varian 4290 integrator. Data were

expressed as a percentage of carbon dioxide present in the headspace in equilibrium with the sample.
- Headspace volatile analysis: a Varian mod. 3700 gaschromatograph equipped with flame ionization detector and with a glass 2mx2mm ID column filled with Carbowax 20M (6.6%) on Carbopack B 60-80 mesh was used. The gaschromatographic conditions adopted were: column temperature 80 °C, injector and detector temperature 200 °C, carrier gas (N_2) flow rate 20 mL/min. The headspace volume injected was 0.5 mL using a precision sampling syringe as described above. The chromatograms were recordered and analysed using a Shimadzu CR 1B integrator. Data were expressed as total peak area of volatiles present in the headspace in equilibrium with the sample.

Evaluation of peroxide value.
Lipid extraction was carried out using chloroform at room temperature in order to avoid any oxidation reactions during sample preparation. A volume of 100 mL of chloroform was added to 15 g of roasted groung coffee. The suspension was stirred for 2 hours at room temperature and than filtered through Whatman n. 1 filter paper. Coffee fat matter was obtained by evaporating chloroform by a Rotavapor Buchi Mod. RI10 at 25 °C.
Rancidity was determined through peroxide value measurements according to AOAC (1980). The analysis was carried out on samples of about 1 g of coffee fat matter. Data are expressed as mEq of peroxides for kg of fat matter.

Data analysis
All the data reported in this paper are the average of at least three repetitions. The coefficients of variation, calculated for all the measurements taken as the ratio of the standard deviation and the mean value, were lower than 5% for CO2 analysis, 7% for volatiles and 4 % for peroxide value.

RESULTS AND DISCUSSION

Volatile and carbon dioxide release from roasted coffee beans exposed to air as a function of storage time are plotted in figures 1 and 2.
Values of volatile total peak area and CO2 % at time 0 are referred to samples taken immediately after roasting/cooling of coffee beans. In both cases storage temperature seems to strongly affect headspace volatile and CO2 concentration; the rate of volatile and CO2 release increased as the storage temperature increased.
First order kinetic constants of volatile and CO2 release, calculated from linear regression analysis of data, and their correlation coefficients (r^2) are reported in table 1.

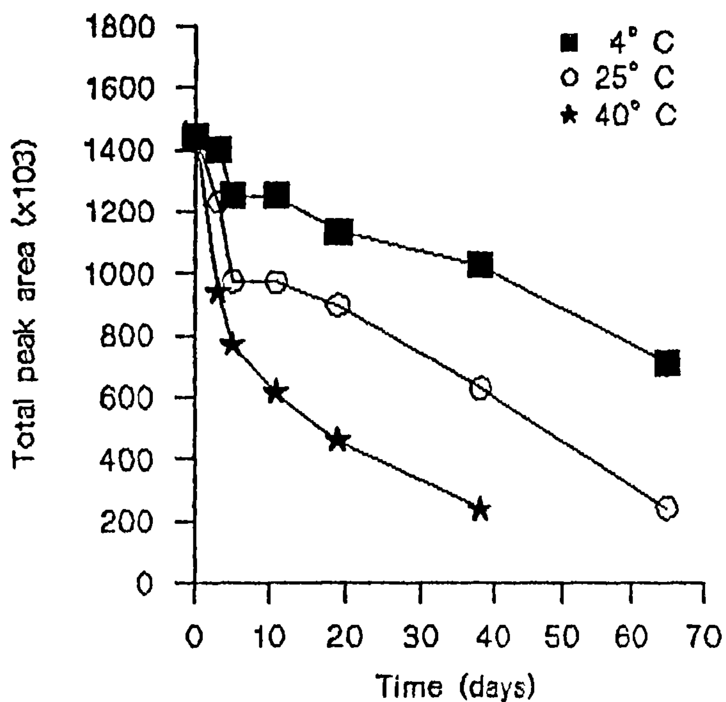

Figure 1: Changes in headspace volatiles, expressed as total peak area, of roasted coffee beans stored at 4, 25 and 40 °C in presence of air.

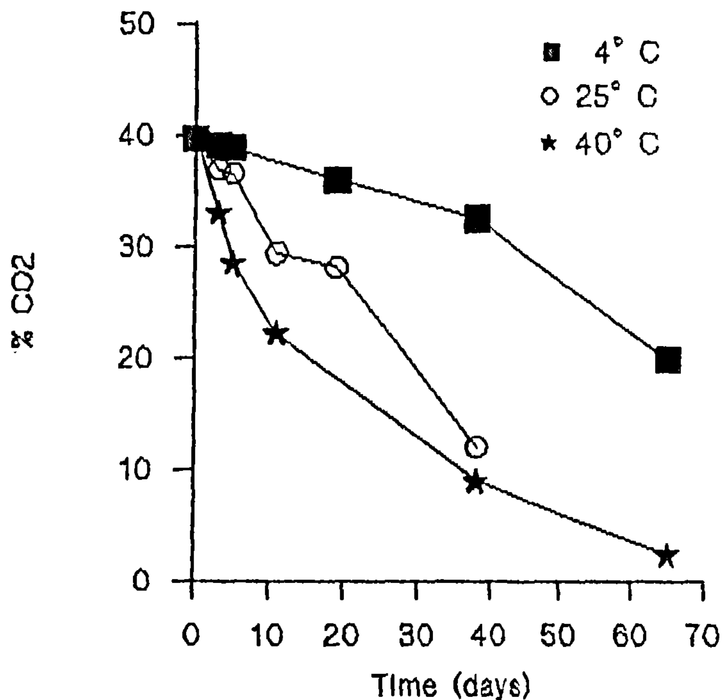

Figure 2: Changes in headspace carbon dioxide, expressed as percentage, of roasted coffee beans stored at 4, 25 and 40 °C in presence of air.

Table 1: first order rate constants of volatile and carbon dioxide release of roasted coffee beans stored at 4, 25 and 40 °C.

Storage Temperature (°C)	Volatiles k (days^{-1})	r^2	Carbon Dioxide k (days^{-1})	r^2
4	0.9 10^{-2}*	0.961	0.9 10^{-2}**	0.987
25	2.3 10^{-2}*	0.960	4.2 10^{-2}*	0.967
40	3.7 10^{-2}*	0.990	4.1 10^{-2}*	0.992

*: $p<0.001$; **:$p<0.01$

It is interesting to note that the kinetic constants of volatiles and CO2 release resulted quite similar; it indicates that the steady evolution of carbon dioxide was always related to a similar behaviour of volatile compounds. This was true also for ground coffee which is generally subjected to a degassing process before packaging.
Figures 3 and 4 show the evolution of volatiles and CO2 present in the headspace as a function of time of ground coffee samples stored at 4, 25 and 40 °C.
If as values at time 0 were taken those observed for coffee beans just after roasting/cooling, a dramatic decrease in the headspace volatile and CO2 concentration was detected within the first few days of storage; in fact, after three days, the residual percentages of volatiles present in the headspace were 50, 35 and 18% for storage temperatures of 4, 25 and 40 °C respectively. As regards CO2 evolution, the residual gas percentages present in the headspace in the same storage conditions were 63, 37 and 33%. The initial rapid decrease in headspace volatile and CO2 concentration due to grinding seems to follow a zero order kinetic; nevertheless the rate constants were not calculated because few experimental data were available in the linear range of the kinetic.
The evolution of volatiles of ground coffee samples stored under vacuum in polyethylene bags is shown in figure 5. It can be noted that, in our experimental conditions, a slight decrease in the volatile total peak area during storage was observed; these results suggest that in this case a certain permeability of the packing material was present. It is interesting to observe that the value of total peak area of samples at time 0 (kept immediately after filling the ground coffee into the packages) was quite lower than that observed for just roasted coffee beans. This is due to the degassing process of ground coffee carried out before packaging. First order rate constants of volatile release of ground coffee samples stored under vacuum at 25 and 40 °C are shown in table 2.

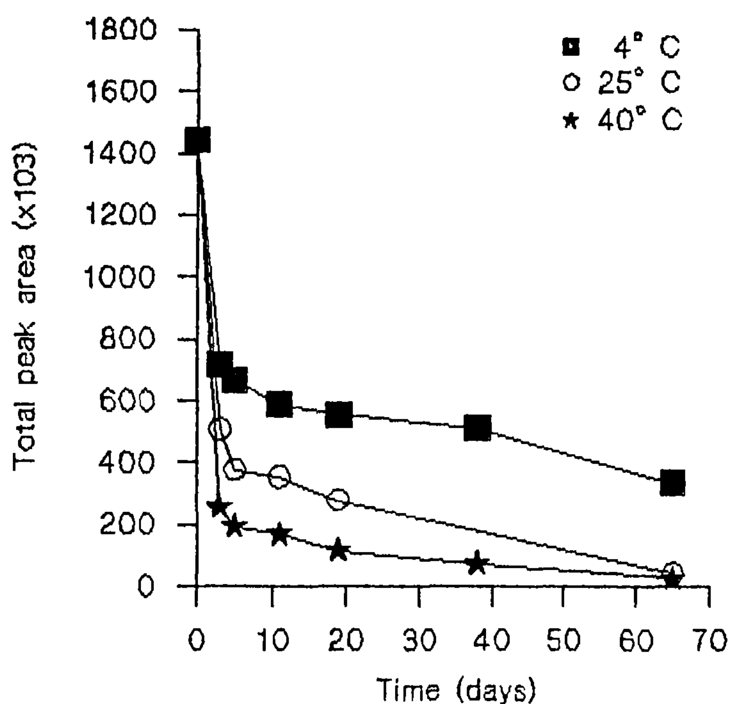

Figure 3: Changes in headspace volatiles, expressed as total peak area, of ground coffee samples stored at 4, 25 and 40 °C in presence of air.

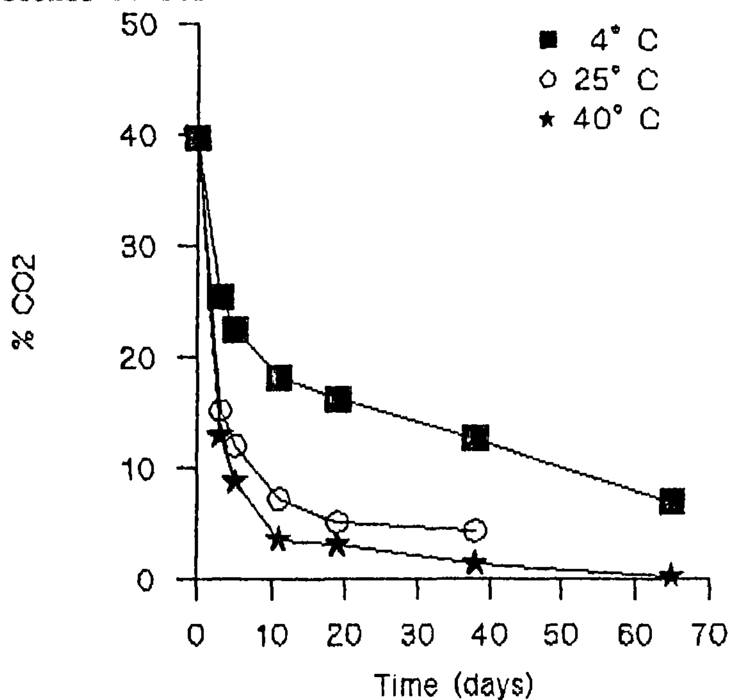

Figure 4: Changes in headspace carbon dioxide, expressed as percentage, of ground coffee samples stored at 4, 25 and 40 °C in presence of air.

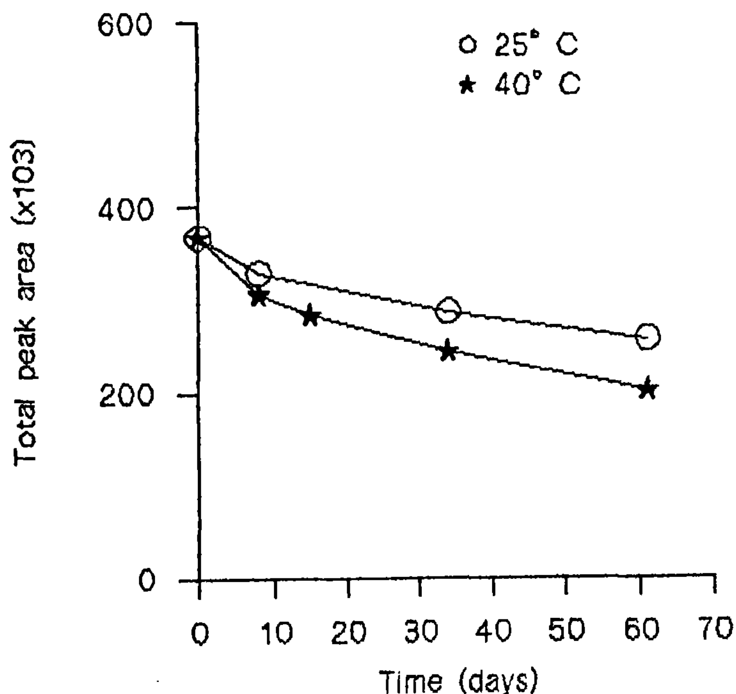

Figure 5: Changes in headspace volatiles, expressed as total peak area, of ground coffee samples packed under vacuum and stored at 25 and 40 °C .

Table 2: first order rate constants of volatile loss of ground coffee samples stored under vacuum at 25 and 40 °C.

Storage Temperature (°C)	k (days^{-1})	r^2
20	0.4 10^{-2b}	0.995
40	0.7 10^{-2a}	0.996

a: $p<0.001$; b: $p<0.01$

As expected the rate constants resulted five times lower than those observed for roasted coffee beans exposed to the air.
Changes in peroxide values of ground roasted coffee samples stored at 25 °C under vacuum or exposed to the air are shown in figure 6.

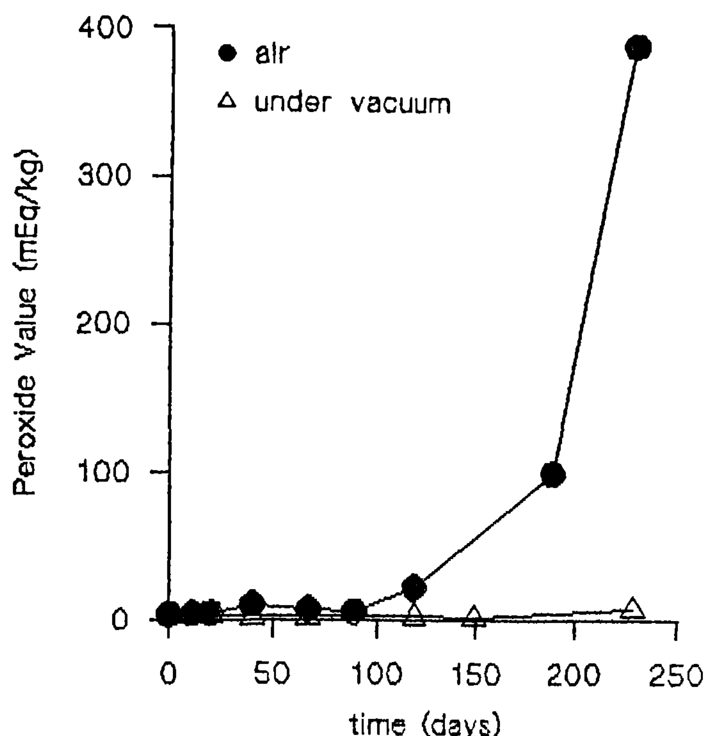

Figure 6: Changes in peroxide values of ground coffee samples stored at 25 °C packed under vacuum or exposed to the air.

It can be observed that ground coffee samples exposed to the air showed a dramatic increase of the peroxide index after four mounths of storage while no changes were observed for coffee samples packed under vacuum. Lipid oxidation of roasted coffee resulted quite slow and strictly dependent on oxygen availability. Nevertheless it must be pointed out that the degree of lipid oxidation of green coffee beans before roasting is of great importance for the quality of roasted coffee (Nicoli et al., 1993).

CONCLUSIONS

The rate of volatile and carbon dioxide release from roasted coffee resulted quite similar indicating that the steady evolution of carbon dioxide is always related to an equal behaviour of volatile compounds. Results confirmed that volatile and carbon dioxide release was enhanced by grindind and by the increase of storage temperature.
Lipid oxidation of roasted coffee resulted quite slow and strictly dependent on oxygen availability. Although this reaction did not affect the quality of packed roasted coffee, it can enhance staling after package opening as well as during home use and storage.

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SUMMARY

Although roasted coffee is a long shelf-stable product it is affected of some physical and chemical changes during storage which can lead to its rapid quality loss. Staling of roasted coffee during storage is generally attributed to the loss of volatiles and to the development of oxidation reaction. Changes in headspace volatile and carbon dioxide concentrations as well as in peroxide value of roasted coffee samples during storage were studied. The rate of volatile and carbon dioxide release from roasted coffee resulted quite similar and strongly influenced to storage temperature. Lipid oxidation was observed only after four mounths of storage and its development was strictly dependent of oxygen availability.

RIASSUNTO

I fenomeni di invecchiamento del caffe tostato nel corso della conservazione sono generalmente attribuiti alla perdita di sostanze volatili e allo sviluppo di reazioni di ossidazione. In quato lavoro e` stata studiata la cinetica di rilascio delle sostanze volatili e dell' anidride carbonica in campioni di caffe` tostato in funzione di diverse condizioni di conservazione. La velocita` di rilascio delle sostanze volatili e` risultata paragonabile a quella dell'anidride carbonica e fortemente influenzata dalla temperatura di conservazione. L'ossidazione della frazione lipidica e` risultata una reazione relativamente lenta se confrontata al rilascio di sostanze volatili e strettamente dipendente dalla disponibilita` di ossigeno.

STATUS OF RESEARCH IN THE FIELD OF NON-VOLATILE COFFEE COMPONENTS

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Introduction

The main non-volatile components of coffee are carbohydrates, lipids, proteins or melanoidins, chlorogenic acids, minerals, alkaloids and aliphatic acids. In this paper the progress made in chemistry and analysis of these and some other compounds will be reviewed. The technology and the chemical behaviour of the pure substances are not subject of this paper.

Carbohydrates

The chemical structures of the insoluble carbohydrates have been investigated by Bradbury and Halliday [1,2]. Berger et al. [3] determined fructose and glucose in soluble coffee with enzymatic methods after acid hydrolysis to detect adulterations by chicory, cereals and malted barley.

The best method for determination of soluble carbohydrates seems to be HPLC with a pulsed amperometric detector [4]. But there have been excellent results with HPLC and post-column detection, with enzymatic analysis or with GC of TMS derivatives. The main purpose was to detect adulterations in soluble coffees. It was found that elevated levels of total xylose, total mannitol, of free sugars and of psicose are indicators for coffee husks, mannose, maltose and total glucose for maltodextrins, sucrose and total glucose for (caramelized) sugar [5-8]. The contents of inositol were determined [7]. Silwar and Lüllmann [9] determined 5 monosaccharides and sucrose in 12 Arabica and 8 Robusta green coffees of 13 countries. The total sugar contents and the amounts of sucrose and mannose were higher in Arabica samples, whilst reducing sugar contents and rhamnose levels were higher in Robustas. Maltose was present in one Robusta sample only.

Lipids

The lipid fraction of coffee is mainly composed of triglycerides, but more interesting are the diterpenes, the sterols and the carboxylic acid-5-hydroxytryptamides. The most interesting work has been done about 16-O-methoxycafestol by Speer et al. [10-14]. This substance is characteristic of

Robusta coffees and therefore the percentage of Robustas in a commercial mixture of roast and ground coffee or in soluble coffee may be calculated. Methoxycafestol is usually determined by HPLC. HRGC has also been applied. A decomposition product of cafestol has been tentatively identified [15]. Quantitation of cafestol and kahweol in a variety of Arabica, Robusta and Arabusta samples have been published [10]. The behaviour of the latter substances during steam treatment and roasting was investigated [16]).

Mariani and Fedeli [17] separated 14 sterols in 16 Arabica and 14 Robusta green coffees by GC after silylation. The two species could be differentiated with 15 % error by analysis of 24-methylenecholesterol, sitostanol and Δ^5-avenasterol.

A rapid HPLC method for the determination of carboxylic acid-5-hxdroxytryptamides has been published [18]. The German Institute of Standards (DIN) checked a similar method and concluded that it was not suitable as a standard method due to the poor stability of the calibration standards [19].

The formation of secreted coffee oil at the surface of the dark roasted beans was studied [20,21]. This formation takes place above a roasting loss of about 18 % and increases during storage up to 45 days. No oxidation of this oil occurs during a storage of 50 days at 30 °C in air if the coffee is not irradiated by ultraviolet light.

Amino acids

The proteins of coffee have not been investigated themselves thoroughly in the last years, but there has been a very extensive study of the free and bound amino acids in green and roasted coffee [22,23]. Several differences between species, varieties and countries of origin have been found. No free amino acids could be detected after roasting for 5 min at 220 °C. Nehring [24,25] analysed the total D- and L-amino acids after acid hydrolysis. From the ratios of the enantiomers of alanine, leucine, phenylalanine and glutamic acid a multiple correlation equation could be derived, by which the organic roasting loss can be calculated. This calculation method is independent of roasting temperature, roasting duration, roaster type, variety of the coffee and of steam pre-treatment. It can be applied to a single coffee bean [26].

Melanoidins

Most of the proteins and a lot of the carbohydrates react during roasting forming melanoidins. The analysis of melanoidins has been performed in the working group of Steinhart, who reported in Paipa [27-29]. Differences were found between normal and pre-steamed roasted coffee. The structure of the melanoidins has been investigated by Heinrich and Baltes [30] using Curie point pyrolysis followed by GC/MS. They found 97 fragments of phenols, carbocyles, N-heterocycles, benzoids and furanoids. Bound caffeine and bound chlorogenic acid were found in several fractions.

Minerals

The binding of minerals, especially of iron, zinc, calcium and magnesium, to these melanoidins has been investigated. Jackson and Lee [31] stated that iron, calcium and magnesium were highly soluble in instant coffee, about 45 % of the first being in soluble complexes. There was no significant difference between regular and decaffeinated coffee. Zinc could not be detected. Asakura et al. [32] investigated the zinc chelating compounds in instant coffee. Three fractions could be separated, the main fraction being melanoidins with intermediate molecular mass. Nevertheless the chelating activity of the melanoidins was supposed to be weak in contrast to the activity of complexes with low molecular weight organic acids.

The binding properties of roasted barley malt has been studied [33]. In raw barley malt, about 60 % of the calcium and magnesium content, 30 % of the zinc and only 10 % of the iron content is hot water soluble. With increasing degree of roast, the percentages of soluble calcium and magnesium increase steadily to about 90 % in dark roast, while the percentages of soluble zinc and iron

increase to about 50 and 20 %, resp., in a light roast, then decrease to about 30 and 10 %, resp., in a dark roast. These suggests, that the four cations, especially iron and zinc, are bound in raw malt by substances, which are destroyed during roasting. These are most likely organic acids or cell wall constituents. In roasted malt, iron and zinc seem to be bound by melanoidins. About 40 % of the soluble zinc is bound by high molecular substances.

Chlorogenic acids

These acids are most remarkable among the acids of coffee, since they are by far prevailing in green coffee. A new acid, 3-caffeoyl-4-feruloylquinic acid has been detected in Robusta beans [34]. Clifford and Jarvis [35] studied the contents of the chlorogenic acids and of 17 chlorogenic acid-like substances in 42 green Robustas and found several indications for the geographic origin. Clifford et al. [36] studied the increase of chlorogenic acids, caffeine and trigonelline during the maturation of the fruit and found, that only the contents of chlorogenic acids increase considerably about 25 - 30 weeks after flowering.

Furthermore, there had been some contributions to the analysis. A comparison between the photometric method of Häusermann and Brandenberger and the HPLC has been made [37,38]. The values according to both methods are equal in green coffee, but with increasing degree of roast the photometric method gives increasing higher values than the HPLC [37,38]. 5 - 9 % of the interfering substances are of high molecular mass and could be chlorogenic acid residues in the melanoidins, the rest may be low molecular mass decomposition products of chlorogenic acid with free o-dihydroxy-groups [39]. Humphrey and Macrae [40] recommended the derivative spectroscopy at 325 nm for a simple and rapid analysis of coffee/chicory-mixtures. Hughes and Thorpe [41] developed a GC determination of 5-CQA, quinic acid and sucrose. The method of choice is HPLC. The German Institute of Standards (DIN) adopted a HPLC standard method in 1992 [42].

Very interesting are the changes of the chlorogenic acids during storage, steaming and roasting. Schünemann [43] determined the contents in discoloured raw beans and found a decrease in these contents with increasing green to black olour (Fig. 1) and a correlation with the brightness (Fig. 2).

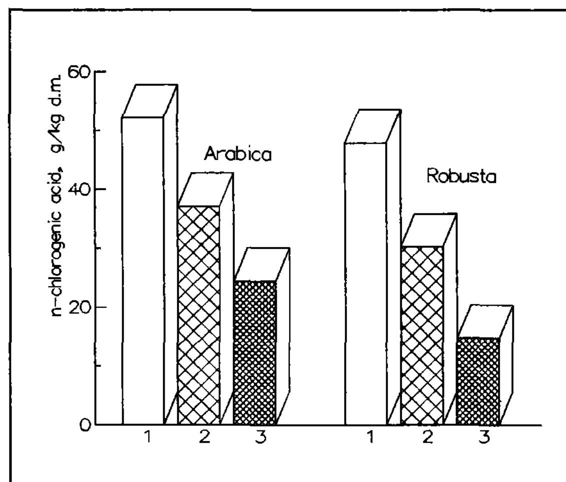

Fig.1 Contents of 5-CQA in normal and discoloured raw coffee beans (Cameroun Arabica, Ivory Coast Robusta). 1= normal beans, 2= greenish discoloured beans, 3= black discoloured beans

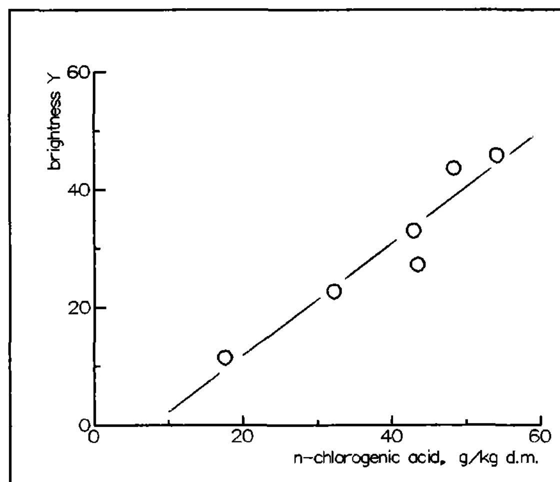

Fig. 2 Correlation between brightness of raw coffee beans (Haiti Arabica) and content of 5-CQA

Clifford et al. [36] determined the contents, also of scopoletin, in fluorescent beans and stated that although these beans contained on average some 5,5 % less of each chlorogenic acid, most likely the fluorescence is due to chlorogenic acids exposed to the surface of the beans.

Dibert et al. [44-46] presented extensive equilibrium and kinetic data for the extraction of chlorogenic acids and oil from green coffee. Purdon and McCamey [47] used the 5-CQA / caffeine ratio for the determination of roast colour and type of beans, Hughes and Thorpe [41] the contents of 5-CQA and sucrose and the QA/5-CQA ratio in order to distinguish between commercial brands. Scholz-Böttcher analysed the various isomers of quinic acid and quinide, which are formed during roasting, and found also a correlation with the roast colour [48-50]. Held [51] studied the changes during steaming of green beans. As expected, isomerisation occurs, and therefore the contents of 3-CQA and of 3,4-DCQA increase, while the others decrease (Fig. 3, 4). The degradation correlates to some extend with the amount of water sorbed by the beans (Fig. 5).

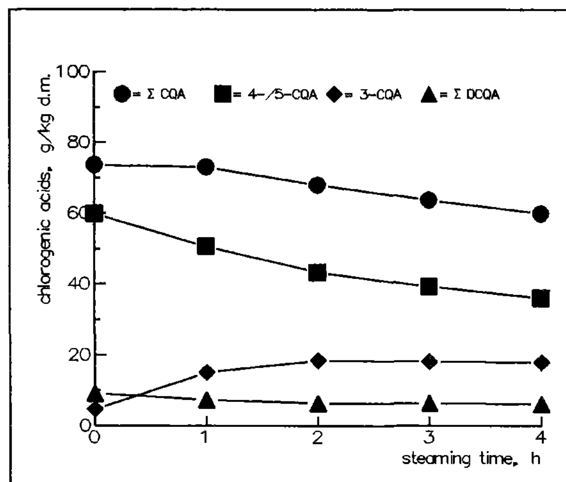

Fig.3 Changes of chlorogenic acids content in raw Columbia Arabica beans during steaming

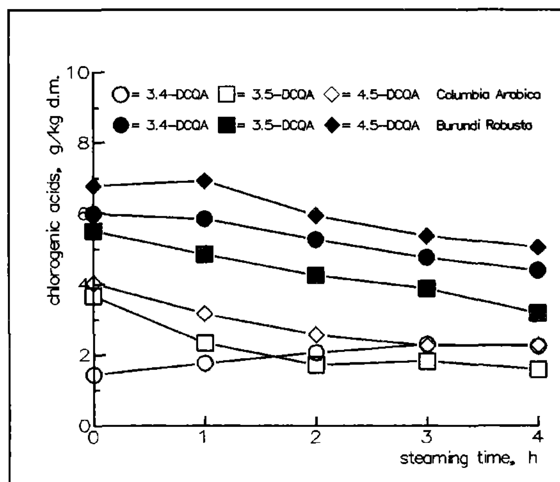

Fig.4 Changes of dicaffeoylquinic acids content in raw coffee beans during steaming

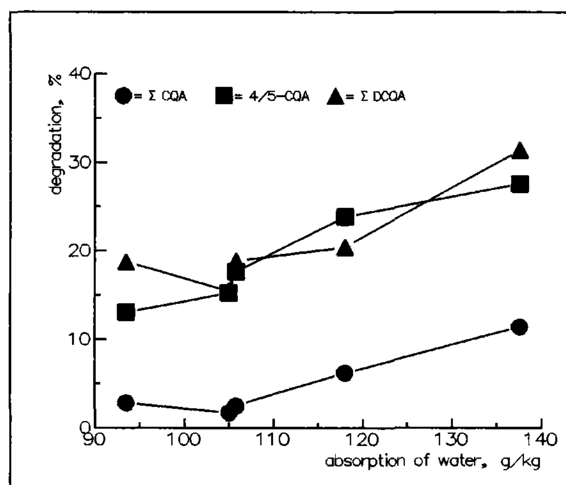

Fig.5 Degradation of chlorogenic acids in different varieties of raw coffee beans after steaming for 2 h

Remarkable are also two publications on the taste of pure 5-CQA. It was stated that this acid tastes not bitter [52], but astringent [53]. The astringency proved to be of the same nature as tannic acid or tannin, but of a weaker response.

Other acids

Besides the two papers presented at the Montreux meeting [54,55], only a few papers have been published. Dalla Rosa et al. [56] confirmed our results [55] that the pH of the beverage decreases with storage time and with temperature. They studied in addition colour changes and correlation with sensory scores by regression equations. Heinrich [57] identified benzoic acid and Hughes and Thorpe [41] identified 22 acids in roasted coffee by GC/MS and GC/IR. Among these acids, pimelic, phthalic, suberic, sebacic, ascorbic, iso-ferulic, dodecandioic and sinapinic acids have not been described in coffee as yet. They have not been determined quantitatively. Barlianto [58] identified and determined 64 acids in roasted chicory (48 new) and 60 (47 new) in roasted barley malt. Most of them were trace acids, a lot of them formed from carbohydrates, probably by Maillard reaction. Bähre and Maier [59] confirmed the presence of many of these acids in coffee. Franz [60] analysed phytic acid and some of its decomposition products (inositol tri- to pentaphosphates) in roasted malt (Fig. 6, 7).

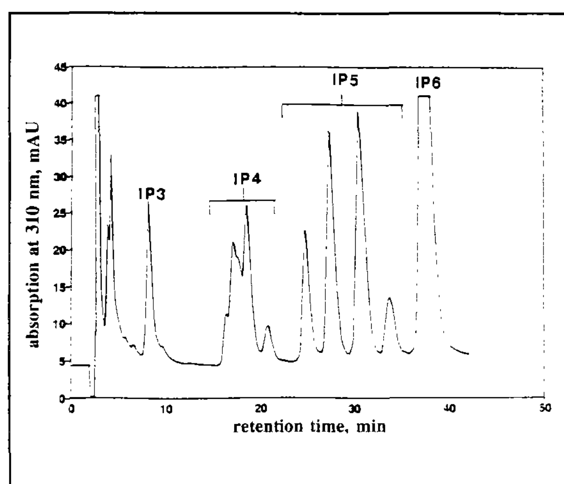

Fig.6 HPLC chromatogram of phytic acid and its degradation products in roasted barley malt. IP=Inositol-phosphate. The number denotes the number of phosphate residues.

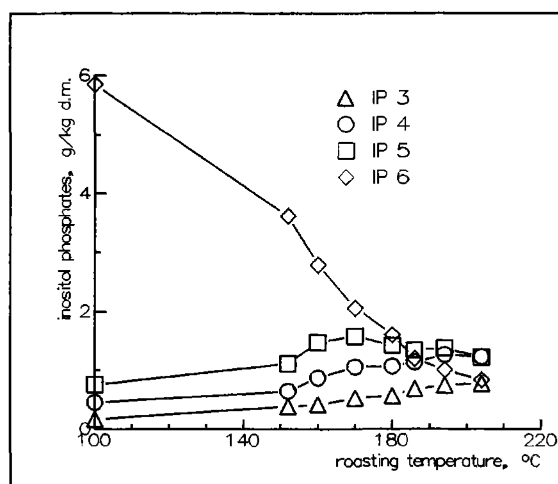

Fig.7 Changes of the contents of phytic acid and its degradation products in a roasted barley malt series. For IP see Fig.6.

Alkaloids

Papers about the metabolism and distribution in coffee plants have been published [61,62]. Le Pierres et al. [63] studied the variability of the contents of caffeine in different green coffee beans. It was concluded that in order to have a precision of 95 % it is necessary to take 400 beans from a sample for a determination. Danho et al. [64] used the isotopic analysis of ^{2}H, ^{13}C and ^{15}N to discriminate between natural and synthetic caffeine and between coffees of African and American origin. Gennaro and Abrigo [65] determined caffeine and theobromine simultaneously in coffee beverage without clean-up by HPLC on RP-18 with octylamine orthophosphate as ion pair reagent, Kazi [66] used a C-18 cartridge for clean-up. The ISO method [67,68] uses treatment with heavy magnesium oxide (90°C, waterbath) and clean-up on a phenyl modified silica solid phase extraction column followed by HPLC on RP-18 with methanol/water as eluents. A similar rapid method was proposed by the German Institute of Standards (DIN) [69]. It works without the purification column and has a better precision, but gives somewhat higher values, especially with decaffeinated coffees.

Trugo and Macrae [70] studied the degradation of trigonelline and sucrose in Arabica and Robusta coffee, and the formation of nicotinic acid. They used HPLC with a mass detector for trigonelline and sucrose, reversed phase HPLC for caffeine, and ion-pair reversed phase HPLC for nicotinic acid. These methods proved to be more rapid and simple than traditional methods and of adequate precision and accuracy. Mazzafera [71] used another HPLC method (ODS-Hypersil; NaOAc 5%, pH 5) following the same clean-up to determine the contents of trigonelline in various coffee species. In Arabica, Robusta and Liberica beans, he found higher values than all other preceding investigators.

Stennert and Maier [72] compared several extraction and clean-up methods for the determination of trigonelline and caffeine by HPLC and TLC. The most suitable proved to be HPLC in connection with hot water extraction without additions. A silica phase and pure water as mobile phase was used. The determination of trigonelline by TLC, followed by extraction and photometry, is possible with a similar precision, while the in-situ photometry was worse.

Trace components

Coffee is very low in contaminants, such as pesticides, mycotoxins, cancerogens, and mutagens. Relating to pesticide residues and mycotoxins, surveys have been given at the San Francisco meeting [73,74]. The destruction of aflatoxin B1 in artificially contaminated green coffee beans during roasting ranges from 90 to 100 % [75]. The situation with reference to cancerogens has been reviewed [76], and it was stated that in case of benzo[a]pyrene the residues in normal roasted coffee seems to depend above all by the amounts in green coffee and that a decrease during roasting takes place. This has been confirmed for a green coffee with 0.9 μg/kg benzo[a]pyrene by Klein et al. [77]. They concluded that there are very low contents of polycyclic aromatic carbohydrates in roasted and instant coffees of the four greatest suppliers in Germany.

The presence of traces of N-nitrosopyrrolidine in two Canadian instant coffees and one finely ground coffee has been confirmed [78]. There seem to be no other nitrosamines eventually in coffee.

Otherwise, coffee may contain antimutagenic substances. One or some of these present in decaffeinated instant coffee may inhibit N-nitrosamine formation in humans [79]. In green coffee, kahweol and cafestol and other agents prevented buccal pouch carcinomas in the hamster cheek [80]. Another antimutagenic substance may be 5-hydroxymethylfurfural (HMF) [81,82]. Kanjahn [83] analysed the contents in 3 series of roasted coffee (Arabica, Robusta and a blend) and 20 commercial blends. HMF is formed at rather low degrees of roast.

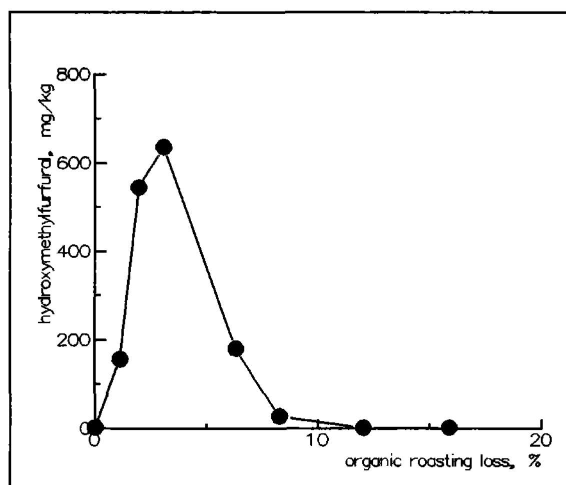

Fig. 8 shows as a typical example the contents (calculated on green coffee d.m. basis) after roasting the blend. The maximum content, calculated on roasted coffee d.m. basis, was 656 mg/kg. In instant coffee, the contents are higher. In commercial products in the seventies 1100 to 3600 mg/kg were found [84].

Fig.8 Changes of hydroxymethylfurfural contents in an Arabica blend during roasting

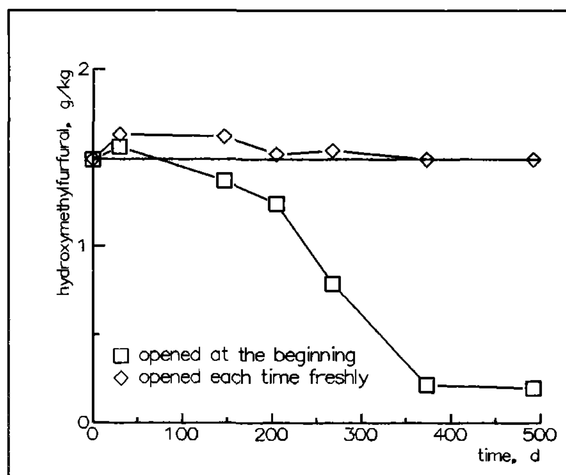

Fig. 9 shows a new experiment [83] with commercial instant coffees of the same origin, tightly closed in jars as usual. If every jar was opened at the day of the analysis, the content was nearly constant over 500 days, but if one jar was opened and repeatedly analysed, the contents decreased, presumable by oxidation.

Fig.9 Changes of hydroxymethylfurfural contents in a commercial soluble coffee during storage at 20°C

Kanjahn [83] detected furfural with the same HPLC system developed for the HMF determination. These results are contradictory to those of Mohr and Wichmann [85] who stated that they are lower in "pre-treated, stomach-friendly" coffees. Kanjahn analysed 8 normal, 8 pre-treated, 2 decaffeinated and 2 natural mild coffee commercial blends and found no differences in the contents of furfural, these ranking from 80 to 160 mg/kg. Only in espresso the content was lower, about 50 mg/kg. In 10 normal commercial instant coffees, the contents were 10 to 90 mg/kg, but one decaffeinated "stomach-friendly" sample possessed about 300 mg/kg. Therefore, the lower furfural content is not suited as an indicator of pre-treatment.

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Summary

An overview of the most important developments in coffee chemistry and analysis during the last years is given. Included is new research work on carbohydrates, diterpenes, alkaloids, minerals as well as amino, chlorogenic and other acids. Especially stressed are the formation of minor acids and the decomposition of phytic acid during roasting, the decomposition of chlorogenic acids during steaming and the chemical determination of the degree of roast.

MATERIAL BALANCE ON FREE SUGARS IN THE PRODUCTION OF INSTANT COFFEE

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INTRODUCTION

The manufacture of instant coffee from green coffee involves a number of processing steps. These generally include blending, roasting, extraction, flavor preservation, concentration, and drying. Each step of the manufacturing process contributes to one of the numerous physical and chemical changes which occur during the entire process. Of particular interest for this work are the chemical changes which occur during the roasting and extraction processes, particularly with respect to the levels of free sugars found in the resulting instant coffee.

It is well established that the roasting process destroys more than 90% of the free sugars and that the monosaccharides of arabinose, mannose and galactose are formed during extraction (1).

Recently, it has been reported that processing variables such as blend variations, roasting conditions, and extraction conditions can contribute to the observed variation in the level of free sugars (2). However, previous work did not quantify the magnitude of the changes observed in free sugars as a result of the roasting and extraction processes.

CURRENT WORK

Realizing that the free sugar profile and the level of the polyhydric alcohol mannitol varies considerably from the green coffee bean to roasted coffee to instant coffee (Table 1), this work was aimed at quantifying the material balance of these compounds.

Table 1

CARBOHYDRATE PROFILES Green Coffee, Roasted Coffee, Instant Coffee						
	Green		Roasted		Instant	
Mannitol	0.027%		0.017%		0.037%	
		% of total		% of total		% of total
Sucrose	3.09 %	[96.2]	0.08 %	[81.5]	0.16%	[9.2]
Glucose	0.043 %	[1.3]	0.001 %	[1.0]	0.038%	[2.2]
Fructose	0.049 %	[1.5]	0.007%	[7.1	0.360%	[20.7]
Mannose	0.018 %	[0.09]	0.006%	[6.1]	0.377%	[21.7]
Arabinose	0.0004%	[0.01]	0.0042%	[4.3]	0.6663%	[38.3]
Galactose	0.00%	[0.0]	0.00 %	[0.0]	0.137%	[7.9]
Total Free Sugars	3.2104%		0.098%		1.7383%	
(Average data on 21 samples of green coffee, roasted coffee, and instant coffee.)						

As can be seen in Table 1, the level of mannitol decreases from the green coffee bean (0.027%) to the roasted coffee bean (0.017%), implying that mannitol is not totally thermally stable at roasting temperatures. In the instant coffee, the mannitol level (0.037%) is higher than in the initial green. The detailed material balance will show how this level is achieved.

In green coffee, sucrose comprises 96% of the total free sugars. Upon roasting, 97% of the total free sugars are destroyed, while sucrose remains predominant at 82% of the free sugars. In the instant coffee, fructose, mannose, and arabinose now make up 80+% of the free sugars.

In this study, a detailed material balance was conducted through both the roasting and extraction processes on mannitol and the six free sugars of sucrose, glucose, fructose, mannose, arabinose, and galactose. This material balance shows how these compounds are either preserved, destroyed, or created as a result of the roasting and extraction processes.

ANALYTICAL METHODS

The determination of mannitol and the six free sugars was accomplished by analysis with a gas chromatograph. Extract samples were freeze dried to remove water prior to the analysis. All samples were extracted with dimethyl sulfoxide to solubilize the sugars and the solution was silated with tri-sil concentrate. This caused the free sugars to form a volatile complex which was analyzed by direct injection into a gas chromatograph. The percent relative standard deviation for this method had been determined to be 2.52% for mannitol and 1.49% for total free sugars (2).

RAW MATERIALS/EXPERIMENTAL WORK

Two basic studies were carried out. In the first study, Brazil Arabicas and Brazil Robustas were blended prior to roasting. The roasted beans were ground and 35 grams of the ground coffee was placed into a laboratory autoclave (Parr Instrument Company, Model Number 4522M, 2000 ml). The coffee was extracted in two stages using 700 ml of distilled water for each stage. Fresh extraction was carried out at 90°C for 20 minutes. The grounds were decanted, another 700 ml of water was added, and hydrolysis extraction was carried out at 190°C for 20 minutes. The fresh extract and the hydrolysis extract were combined, well mixed, cryofied, and freeze dried prior to analysis. The results of this study were used to quantify the combined effects of the roasting and extraction processes.

In the second study, a blend of Brazil Arabicas and multiple sources of Robustas were blended prior to roasting. Again, the roasted coffee was ground and extracted in two stages. For this study, the fresh extract and the hydrolysis extract were kept separate and analyzed for free sugars.. The results of this study were used to quantify the effects of fresh extraction and hydrolysis extraction.

RESULTS

In the first study, a total of 21 autoclave runs were made using 21 different green coffee blends. Mannitol and free sugar values for the green coffee, roasted coffee, and instant solids were determined for each run. The average value for each compound was calculated across all 21 runs in order to determine the material balance.

Key to calculating the material balance is to understand the roasting loss and the extraction yield and the effect that those losses have on the reported percentages. For this study, the roasting loss was measured at 15% (i.e. 15% shrinkage) and the extraction yield was calculated as 45.53% based on the weight of roasted coffee. To simplify the material balance calculations, a basis of 100 grams of green coffee was used. This yielded 85 grams of roasted coffee [100 grams * (1-15%)] and 38.7 grams of instant coffee (85 grams * 45.53% yield).

Table 2

MATERIAL BALANCE: ROASTING AND EXTRACTION

	GREEN COFFEE		ROASTED COFFEE			INSTANT COFFEE		
	%	Mass	%	Mass	% of Green Mass	%	Mass	% of Roasted Mass
Mannitol	0.027	(.027 g)	0.017	(.014 g)	53.5	0.037	(.014 g)	100
Sucrose	3.09	(3.09 g)	0.08	(.068 g)	2.2	0.16%	(.062 g)	91.1
Glucose	0.043	(.043 g)	0.001	(.0009 g)	2.0	0.0038	(.0147 g)	1634
Fructose	0.049	(.049 g)	0.007	(.006 g)	12.1	0.360	(.1393 g)	2322
Mannose	0.028	(.028 g)	0.006	(.0051 g)	18.2	0.377	(.1459 g)	2861
Arabinose	0.0004	(.0004 g)	0.0042	(.0036 g)	892.5	0.6663	(.2579 g)	7162
Galactose	0.00	(0.0 g)	0.00	(0.0 g)	N/A	0.137	(.053 g)	N/A

As shown in Table 2, there is a loss of mannitol during roasting as indicated by the ratio of the mass of mannitol in the roasted coffee to the mass of mannitol in the green coffee. This ratio of 53.5% indicates nearly half of the initial mannitol is destroyed in roasting. In extraction, all of the mannitol in the roasted coffee is recovered as indicated by the ratio of the mass of mannitol in the instant coffee to the mass in the roasted coffee, i.e. 100%.

The free sugars of sucrose, glucose, fructose, and mannose are significantly reduced during roasting. Arabinose, while at a low level in the green coffee, is seen to increase by a factor of 9 upon roasting. Galactose is undetectable in both the green and the roasted coffee.

In extraction, sucrose is observed to be almost totally preserved with 91% of the sucrose in the roasted coffee extracted. The levels of glucose, fructose, mannose, and arabinose are all significantly increased with factors ranging from 16 to 71. All of the galactose in the instant coffee is created in extraction.

In the second study, three runs were made in the autoclave using three separate blends of Arabicas and Robustas. The fresh extract (90°C) was decanted and analyzed separate from the hydrolysis extract (190°C). In order to calculate the material balance, the fresh yield was determined to be 17.22% based on the roasted coffee. The hydrolysis yield was calculated as 28.56%. To simplify the material balance, a basis of 100 grams of roasted coffee was used. This resulted in a basis of 17.33 grams of fresh solids and 28.56 grams of hydrolysis solids.

Table 3

MATERIAL BALANCE IN EXTRACTION

	ROASTED COFFEE		FRESH SOLIDS			HYDROLYSIS SOLIDS		
	%	Mass	%	Mass	% of Roast Mass	%	Mass	% of Roast Mass
Mannitol	0.011	(.011 g)	0.043	(.007 g)	63.6	0.009	(.003 g)	27.3
Sucrose	0.0373	(.0373 g)	0.1443	(.025 g)	67.0	0.0539	(.0154 g)	41.3
Glucose	0.001	(.001 g)	0.003	(.0005 g)	50.0	0.047	(.0134 g)	1342.3
Fructose	0.0059	(.0059 g)	0.0220	(.0038 g)	64.6	0.5392	(.1511 g)	2561.0
Mannose	0.0069	(.0069 g)	0.031	(.0054 g)	77.9	0.5029	(.1436 g)	2081.6
Arabinose	0.0030	(.003 g)	0.0299	(.0052 g)	172.7	0.9781	(.2893 g)	9311.5
Galactose	0.00	(0.0 g)	0.00	(0.0 g)	N/A	0.1279	(.0365 g)	N/A
	Free Sugars - Total Mass			.0399 g			.6393	

Total Free Sugars = .6792 (5.9%: fresh extraction; 94.1% hydrolysis extraction)

As shown in Table 3, 63.6% of the mannitol in the roasted coffee is extracted at 90°C. The remainder of the mannitol extracts during hydrolysis so that 99.9% of the mannitol in the roasted coffee is finally extracted. Sucrose behaves similarly--67% of the sucrose is extracted in the fresh solids and the remainder in the hydrolysis. The total mass of sucrose in this study exceeded 100%. This is possibly attributed to the variation in the analytical method and the limited number of autoclave runs.

Glucose, fructose, and mannose partially extract in the fresh stage while arabinose is created in the fresh stage extraction. No galactose is seen in the fresh extract as no galactose appears in the roasted coffee.

In the hydrolysis stage, glucose, fructose, mannose, and arabinose are all created in significant amounts as indicated by the ratio of these sugars in the instant coffee to the roasted coffee. The galactose in the instant coffee completely comes from hydrolysis.

Table 4 summarizes the observations made for roasting and extraction. Roasting is seen to destroy all sugars except arabinose, which is created, and galactose, which is not present. Extraction preserves virtually all of the mannitol and sucrose while significant levels of the other free sugars are created.

Table 4

MATERIAL BALANCE SUMMARY

CARBOHYDRATE	ROASTING	EXTRACTION
Mannitol	Destroyed (46%)	Preserved (100%)
Sucrose	Destroyed (98%)	Preserved (91%)
Glucose	Destroyed (94%)	Created (1700%)
Fructose	Destroyed (88%)	Created (2300%)
Mannose	Destroyed (82%)	Created (2900%)
Arabinose	Created (890%)	Created (7200%)
Galactose	None Present	Created (Infinite)

PREDICTION CORRELATIONS

As a final part of this study, green coffee samples and the instant coffee produced from those samples for 17 industrial runs were analyzed. The level of the carbohydrate in the spray dried powder was correlated to the level of the carbohydrate in either the green coffee or the roasted coffee (in the case of arabinose). From this analysis, correlation equations were developed which are shown in Table 5.

Table 5

PREDICTION CORRELATIONS FOR INDUSTRIALLY PRODUCED POWDERS

Carbohydrate	Correlation
Mannitol	1.73 * (% in green)
Sucrose	0.066 * (% in green)
Glucose	2.04 * (%in green)
Fructose	9.76 * (% in green)
Mannose	19.52 * (% in green)
Arabinose	191.23 * (% in roasted)
Galactose*	[0.12-0.18%]
Total Free Sugars	0.635 * (% in green)

* Galactose cannot be correlated to either green or roasted coffee--observed values in this study ranged from 0.12-0.18%.

As can been seen in Table 5, mannitol, sucrose, glucose, fructose, mannose, and total free sugars can be predicted in the instant coffee based on the level in the green. Arabinose can be predicted based on the level of arabinose in the roasted coffee. Galactose cannot be predicted. Rather, values ranging from 0.12%-0.18% were observed during these studies.

SUMMARY

A laboratory autoclave was used to conduct a material balance on mannitol and six free sugars in the roasting and extraction of coffee. Roasting was observed to generally destroy these carbohydrates to some degree. Fresh extraction removed a percentage of each compound while the hydrolysis extraction was responsible for the creation of certain sugars.

Correlation factors were developed which can aid in estimating the percentage of these compounds one would expect in the instant coffee based on the level measured in either the green or roasted coffee.

RESUME

Un autoclave de laboratoire a été utilisé pour effectuer un bilan-matières sur le mannitol et six sucres libres lors de la torréfaction et de l'extraction du café. L'observation a révélé que la torréfaction détruit généralement ces glucides dans une certaine mesure. Une extraction fraîche a retiré un pourcentage de chaque composé tandis que l'extraction par hydrolyse a causé la création de certains sucres.

Les facteurs de corrélation qui été développés peuvent aider à estimer le pourcentage de ces composés que l'on pourrait s'attendre à trouver dans le café soluble en fonction du niveau mesuré dans le café vert ou dans le café torréflé.

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FATTY ACIDS IN COFFEE

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The two most important coffee species, Coffea arabica and Coffea canephora var. robusta, contain between 7 and 17 % fat. The lipid content of Arabica coffee beans averages some 15 %, whilst Robusta coffees contain much less, namely around 10 % lipid.
Three-quarters of these lipid constituents are fatty acids. More than 23 different fatty acids have so far been identified by various authors; ten of these are unsaturated and five are odd-numbered.

For the most part the fatty acids are to be found in the combined state; most are esterified with glycerol in the triglycerides, some 10 % are esterified with the diterpenes cafestol and kahweol, and a small proportion - about 2 % - is to be found in the sterol esters [1].
But uncombined - i.e. free - fatty acids also occur.

Whereas the distribution of the fatty acids in the triglycerides and diterpene esters has to some extent already been investigated [2; 3], the data on the free fatty acid content of coffee are inadequate and there are no data at all on the nature and composition of the uncombined fatty acids [4; 5; 6; 7].
The reason for this is that the free fatty acids are expressed by the acid value, a common but indirect determination procedure used in the analysis of fats.
But for the free fatty acids in coffee this method is only very approximate, for it includes not only the free fatty acids themselves but other acid compounds as well. This means that the total content of the free fatty acids is not accurately stated.
Above all, this indirect method does not, of course, make it possible to differentiate between the individual fatty acids.

Our aim was therefore to determine the free fatty acids directly; this was to be done by isolating the free fatty acids from the other lipid constituents such as triglycerides, diterpene esters etc.

We achieved such isolation by means of gel permeation chromatography, using the following method (Fig. 1):

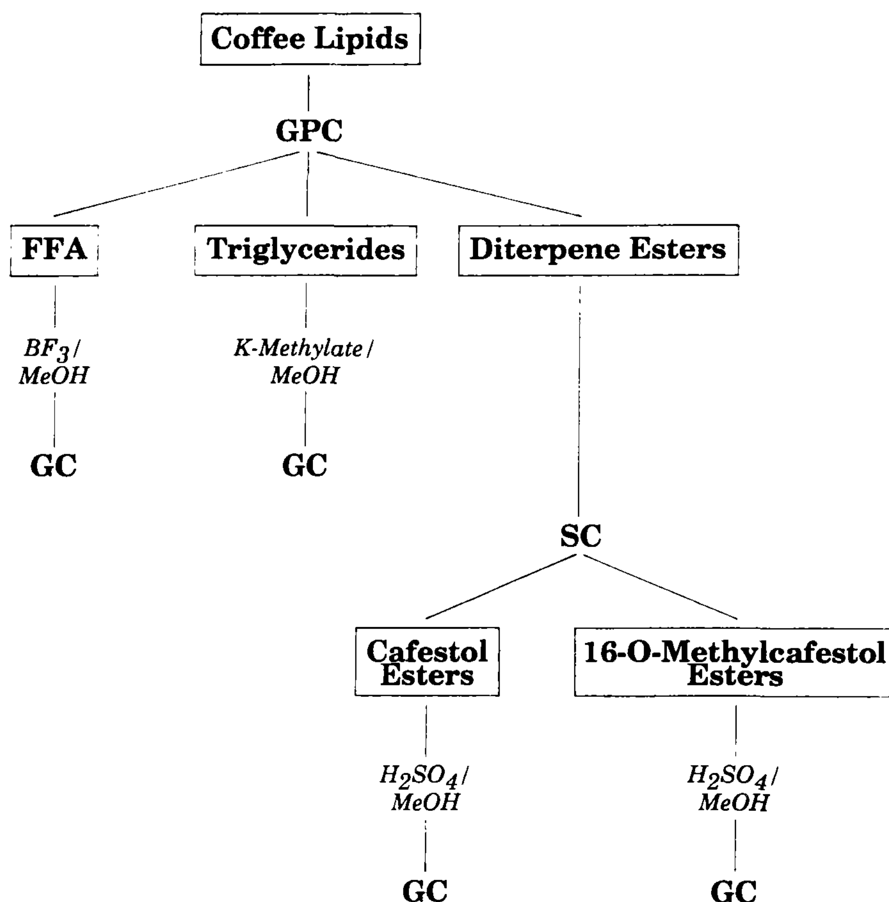


Fig. 1. Analytical scheme for determination of free fatty acids and fatty acids combined in triglycerides and diterpene esters

The coffee lipids are first extracted from the ground coffee with tertiary butyl methyl ether, that is with a relatively polar solvent.
With the aid of gel permeation chromatography the coffee lipids can be divided into individual fractions: a fraction containing the free fatty acids, a fraction with the triglycerides and a further fraction containing the diterpene fatty acid esters.
The individual fatty acids are determined by capillary gas chromatography as methyl esters. For this, the free fatty acids are converted with BF_3/methanol and the triglycerides are transesterified with potassium methylate.

Eight Robusta coffees and four Arabica coffees grown in different areas were analysed. The values for content of the free fatty acids determined and stated in Fig. 2 are in relation to the lipid content of the coffee; for the Robustas analysed they lie between 1 g and 2.7 g per 100 g lipid. The fluctuations are relatively large, even for coffee of the same provenance, as shown by the two samples from Indonesia.
At 1 to 1.5 g per 100 g lipid the Arabica coffees all had a somewhat lower free fatty acid content than the Robustas.

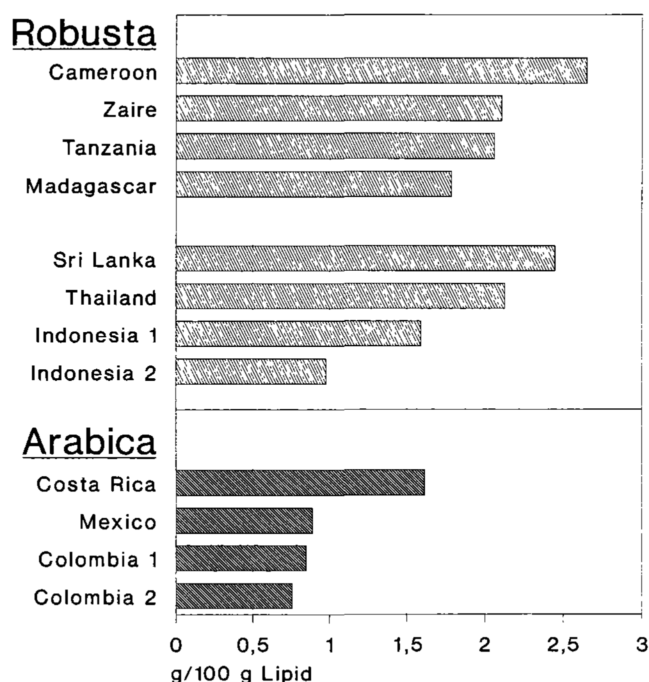
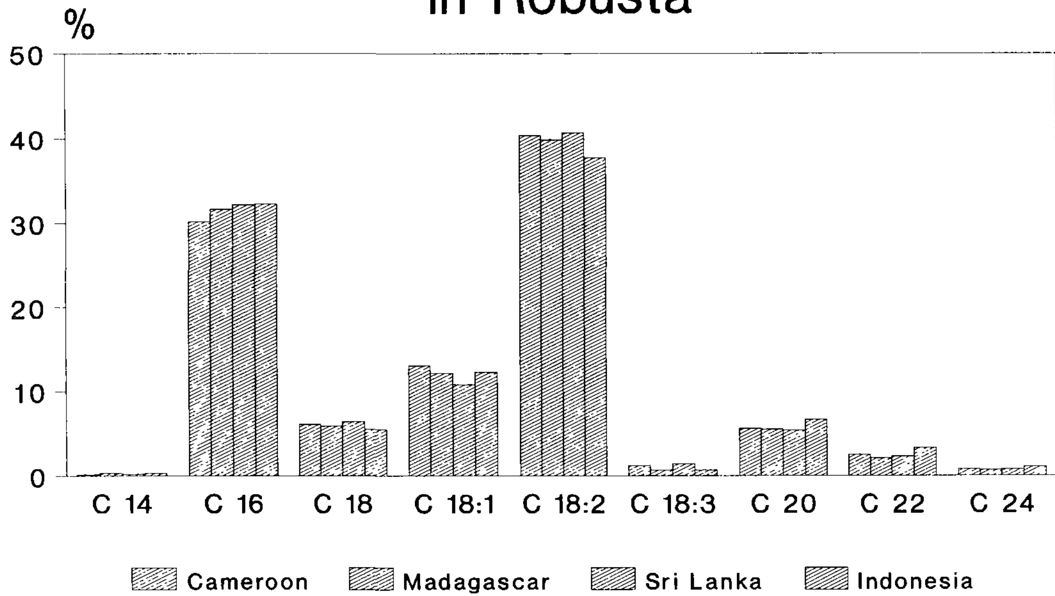


Fig. 2. Content of free fatty acids in green Robusta and Arabica coffees

By direct determination via the methyl esters it is now possible to give information on the individual free fatty acids and the percentages in which they are distributed in the two species of coffee.
Fig. 3 shows this for four Robusta and four Arabica coffees. Nine different free fatty acids were detected which - and this is especially apparent - are very uniformely distributed in the Robusta and Arabica coffees respectively.
In both coffees the main fatty acids are $C_{18:2}$ and C_{16}. It was also possible to detect large proportions of C_{18}, $C_{18:1}$, C_{20} and C_{22}. There were no more than traces of C_{14}, $C_{18:3}$ and C_{24}.

Free Fatty Acids

in Robusta



in Arabica

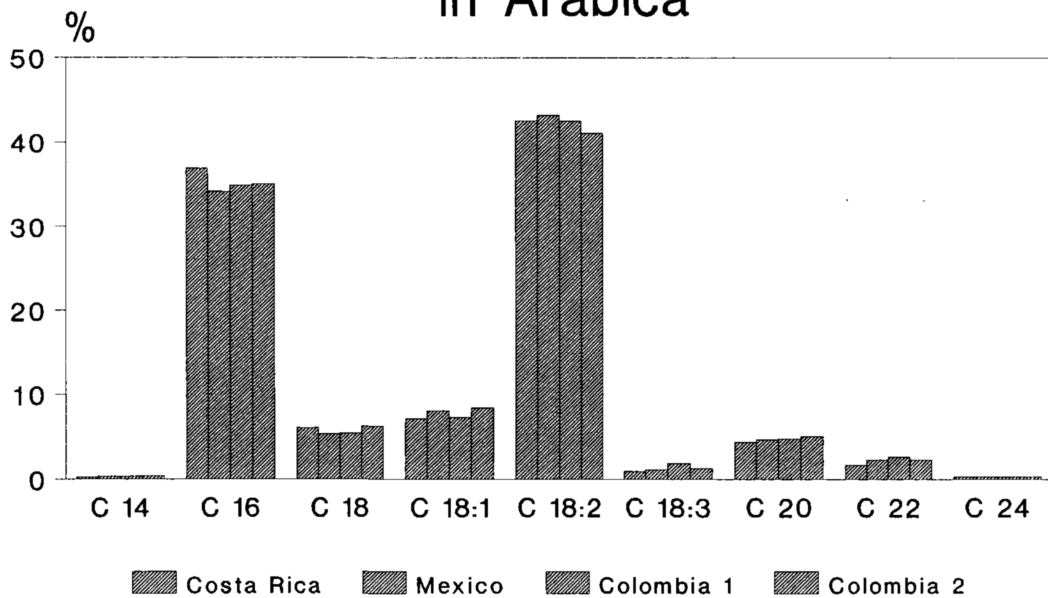


Fig. 3. Distribution of the free fatty acids in green Robusta and Arabica coffees

With this mode of presentation, differences between Arabica and Robusta only become visible on closer scrutiny - that is, when their stearic acid and oleic acid content is compared. While the proportion of stearic acid is noticeably smaller than that of oleic acid in the Robustas, the percentages of these two acids in the Arabica coffees are almost equal.

This difference in content becomes even more obvious when the gas chromatograms are compared (Fig. 4.). In the right-hand chromatogram the two peaks marked with "No. 4" and "No. 5" are the same height, but in the case of the Robusta coffee the oleic acid peak is twice as high as the stearic acid peak. This difference in stearic / oleic acid content is to be found in all eight Robusta coffees analysed.

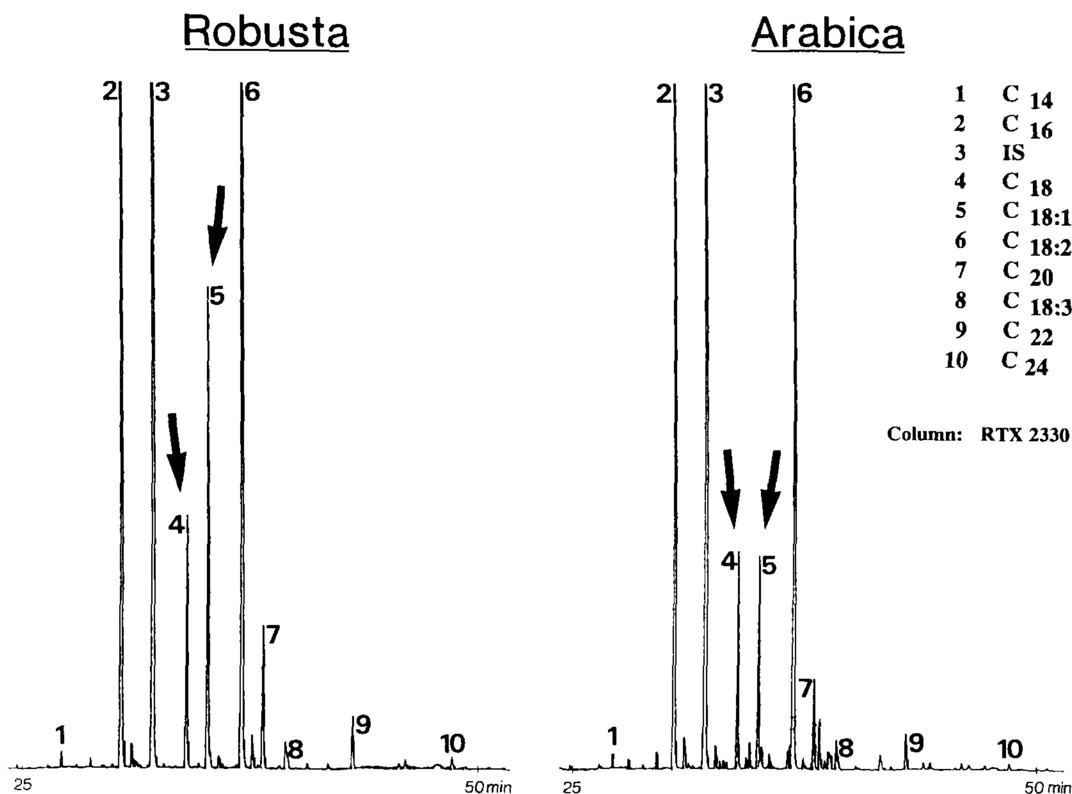


Fig. 4. GC chromatograms of **A** Robusta coffee, **B** Arabica coffee

The coffees analysed so far have all been green coffees. The question now is: does the free fatty acid content change as a result of roasting?

To answer this question a green Robusta coffee from Madagascar was roasted at various temperatures between 242 and 264 °C for 2½ minutes in each case and then analysed.
The results can be seen from Fig. 5.
This shows plainly that the content of free fatty acids has decreased by about 20 %. On the other hand the different roasting temperatures clearly did not have a significant effect on free fatty acid content; this is shown by the almost equal height of the columns.
Moreover, there were scarcely any changes in the distribution of the individual fatty acids. Only the linoleic acid content decreased slightly as the roasting temperature increased.

Using this series of roastings I would now like to demonstrate the difference in content between the directly determined free fatty acids and those determined indirectly via the acid value. Whilst the difference is only about 360 mg for green coffee, it becomes steadily greater as the roasting temperature increases; at the last roasting stage the difference is 1300 mg.

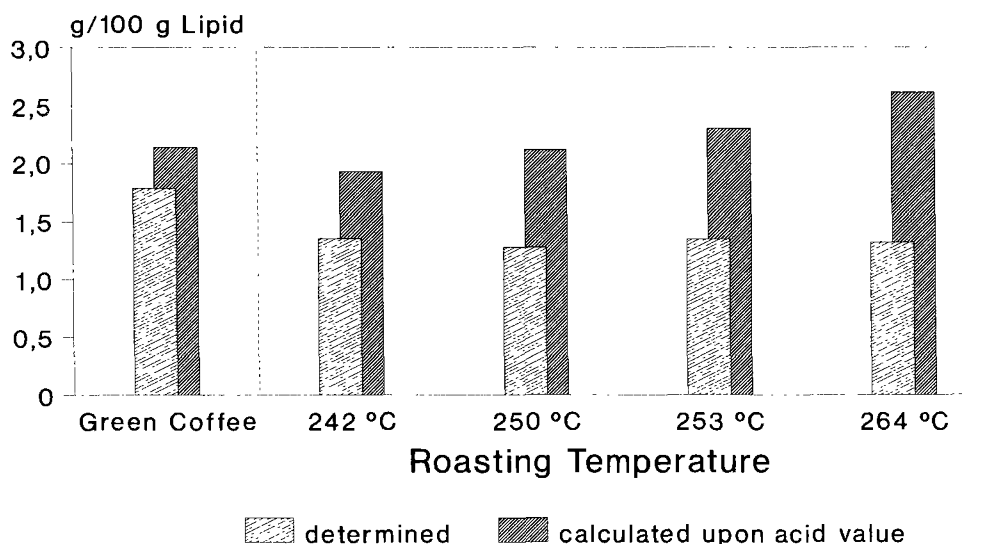


Fig. 5. Content of free fatty acids in green and roasted coffee

This can be explained by the fact that more and more acid compounds are released as the temperature rises. These are primarily phenolic degradation products of the chlorogenic acids that considerably distort the results for the free fatty acids. This is clear evidence of the advantage of the new, direct method of determining free fatty acids.

Isolation of the free fatty acids by gel chromatography had also provided us with a fraction containing the triglycerides only and a further fraction with only the diterpene esters; this awakened our interest in investigating distribution of the fatty acids in the triglycerides and diterpene esters for possible differences.

In the triglycerides the same nine fatty acids were found as described above. These had already been detected by FOLSTAR et al. [3]. But traces were now found of other fatty acids: these included $C_{16:1}$ and $C_{20:1}$ and also the odd-numbered fatty acids C_{15}, C_{17}, C_{19}, C_{21} and C_{23}. This was the first time that C_{19} and C_{21} had been detected in coffee (CI mass spectra s. Fig. 8).
Distribution of the main fatty acids in the triglycerides was very similar to that of the free fatty acids.

The third fraction resulting from the gel chromatographic system described above contains the diterpene esters. To distinguish the cafestol esters from the 16-O-methylcafestol esters it is no problem to separate them from each other using disposable silica gel columns.
The esters are then each transesterified and analysed by gas chromatography. Here, however, transesterification cannot be carried out with potassium methylate as in the case of the triglycerides; steric effects make it necessary to use H_2SO_4 / methanol.

The distribution of the most important fatty acids esterified with cafestol and 16-O-methylcafestol [8] is shown in Fig. 6.
Here the dominant fatty acid is clearly palmitic acid, followed by linoleic acid. Oleic acid and stearic acid are found in quantities between 17 and 10 %; there are traces only of linolenic acid and arachidic acid.
Distribution of the fatty acids in the two diterpene esters tends to be very uniform.

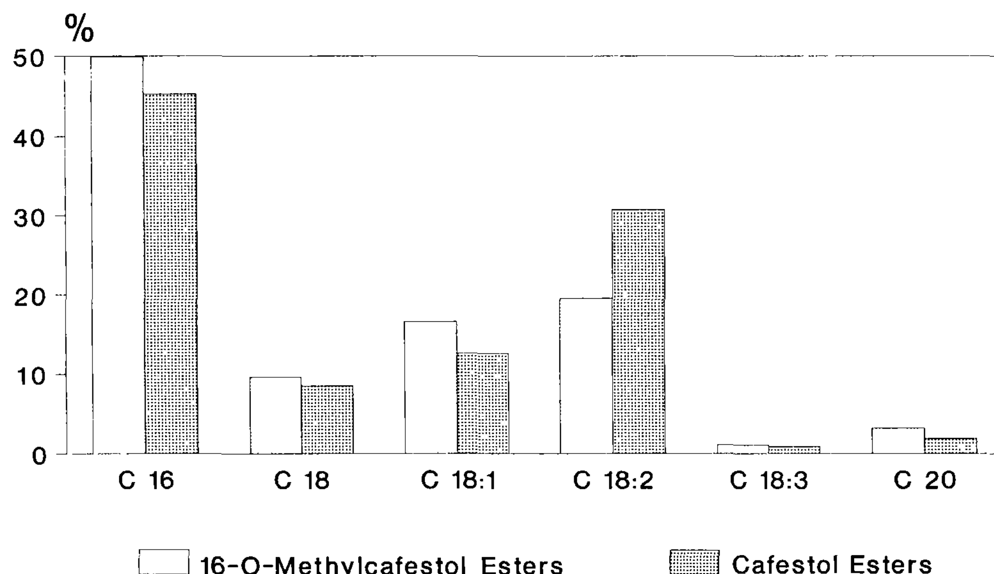


Fig. 6. Distribution of the main fatty acids in diterpene esters

If the distribution of the fatty acids in the triglycerides and that of the free fatty acids is added to Fig. 6., the result is a very different distribution pattern (Fig. 7.).

We have just seen that the main component of the fatty acids in the diterpene esters is palmitic acid, followed by linoleic acid. In the case of the triglycerides and free fatty acids exactly the reverse applies, the biggest percentage is linoleic acid, followed by palmitic acid.

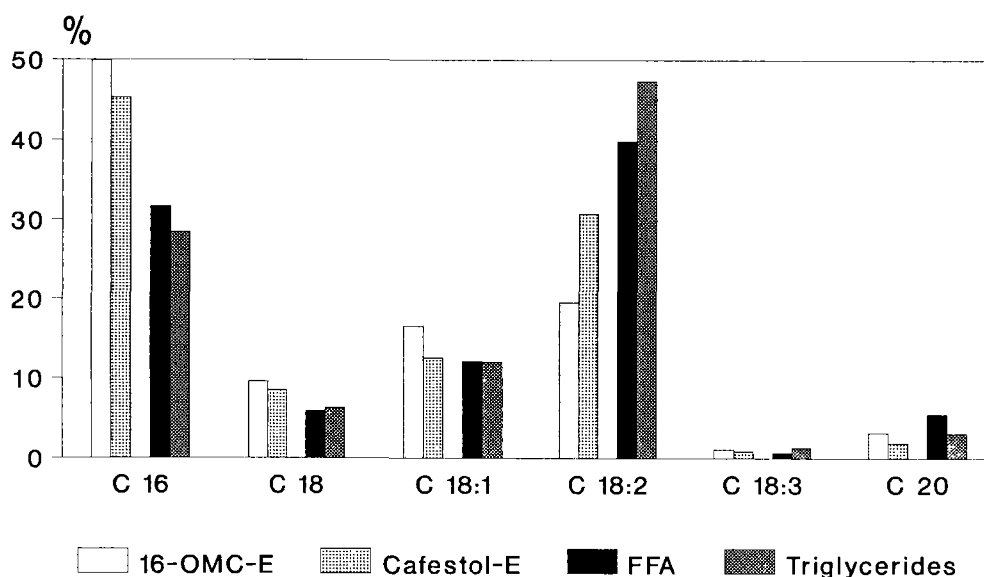


Fig. 7. Distribution of the main fatty acids combined in triglycerides and diterpene esters and of the uncombined

In this way it was possible to demonstrate that both the presence of the individual fatty acids and their distribution in the lipid constituents triglycerides, diterpene esters and free fatty acids varies very considerably.

These investigations are continuing.

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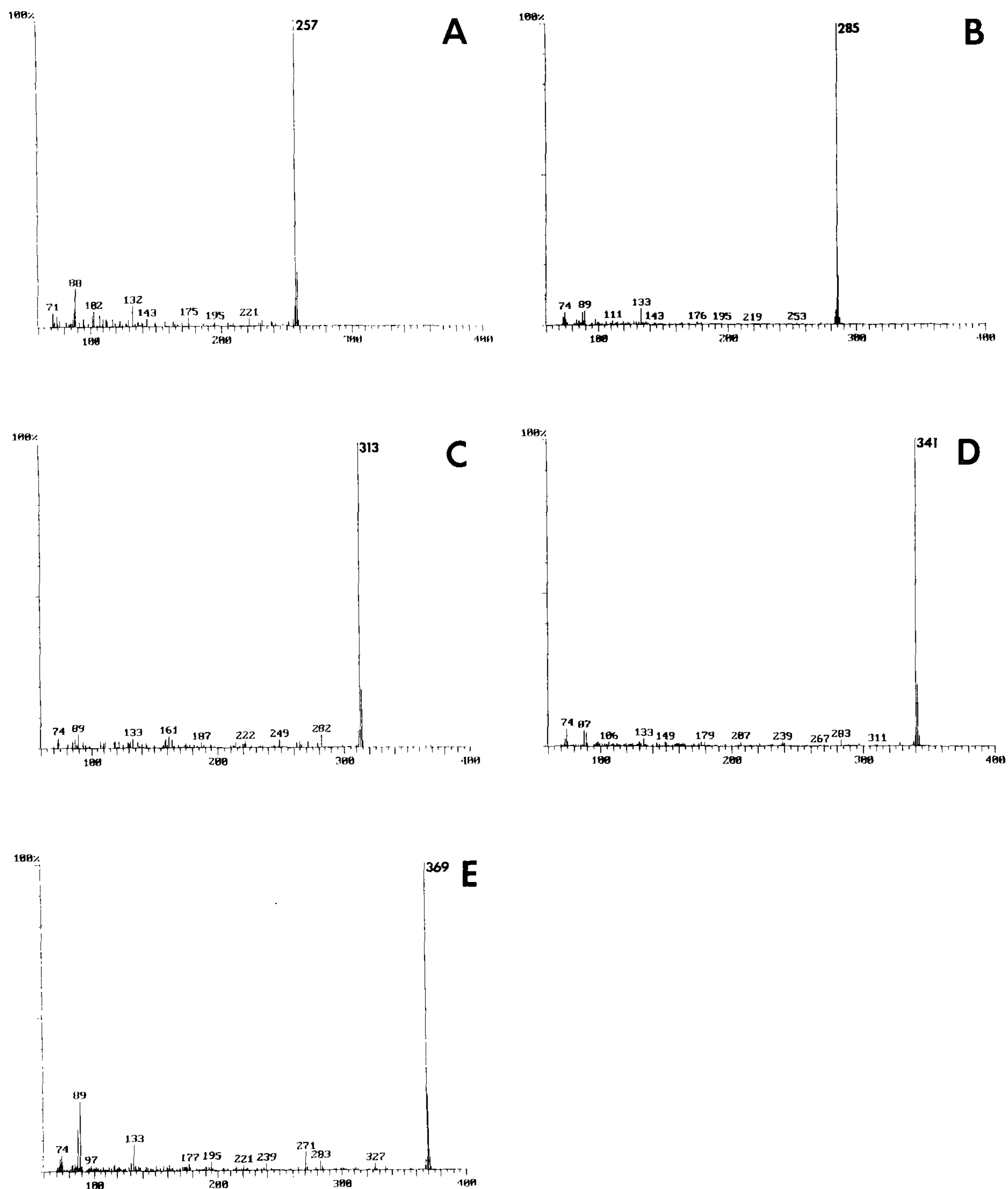

Fig. 8. CI mass spectra of the odd-numbered fatty acids detected in triglycerides
A C_{15} **B** C_{17}
C C_{19} **D** C_{21}
E C_{23}

Summary

Using gel permeation chromatography, a method has been developed of isolating the triglycerides, diterpene esters and the free fatty acids of coffee from each other into three separate fractions.
In this way it is possible to determine the free fatty acids and the fatty acids bound both in the triglycerides and in the diterpene esters independently of each other.

Information is given on the free fatty acids - in terms of both overall content and percentage distribution - in several Robusta and Arabica coffees.
A series of roastings shows that the overall free fatty acid content analysed by the new, direct method is considerably lower than that determined via the acid value.

The distribution of the fatty acids bound in the triglycerides corresponds to that of the free fatty acids, whereas the fatty acids of the cafestol and 16-O-methylcafestol esters have a different distribution pattern.

Zusammenfassung

Es wurde ein gelchromatographisches Verfahren entwickelt, das die Triglyceride, Diterpenester und freien Fettsäuren des Kaffees voneinander abtrennt und jeweils in einer Fraktion erfaßt.
So ist es möglich, die freien Fettsäuren und sowohl die in den Triglyceriden als auch die in den Diterpenestern gebundenen Fettsäuren getrennt voneinander zu bestimmen.

Die freien Fettsäuren - ihr Gesamtgehalt und ihre prozentuale Verteilung - sind für einige Robusta-und Arabica-Kaffees angegeben.
An einer Röstserie wird gezeigt, daß der nach der neuen, direkten Methode analysierte Gesamtgehalt an freien Fettsäuren gegenüber dem über die Säurezahl ermittelten Gehalt deutlich niedriger liegt.

Die Verteilung der in den Triglyceriden gebundenen Fettsäuren entspricht der der freien Fettsäuren. Ein hiervon abweichendes Verteilungsmuster ergibt sich dagegen für die Fettsäuren der Cafestol- und 16-O-Methylcafestol-Ester.

MELANOIDINS IN COFFEE. SEPARATION AND CHARACTERIZATION BY DIFFERENT CHROMATOGRAPHIC PROCEDURES

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1. INTRODUCTION

Roasting of coffee beans causes profound changes in the bean contents, leading to the formation of typical aroma and browning substances. Both the Maillard reaction and caramelization, non-enzymatic browning reactions that take place during the roasting process, are characterised by their melanoidin end products, which are polymeric, yellow to dark brown substances. Although, according to the literature, brewed coffee contains 15% melanoidins by dry weight, they are the least studied ingredients in roasted coffee [15].

Up to now, the reaction mechanisms leading to the formation of melanoidins are only partially cleared up. Sample reaction systems made it possible to isolate a few pre-melanoidin compounds and to detect parts of the mechanism [1, 12]. However, attempts to isolate browning substances in pure form from roasted coffee have remained unsuccessful.

Over the past years some information as to the chemical nature of the water soluble melanoidins in roasted coffee could be achieved. It is generally assumed that melanoidins are polydispersed substances of more or less high molecular masses with aliphatic and aromatic molecule segments and negatively charged groups [3]. Based on their similar chemical and physical characteristics, a kinship to humic acids has been proposed [13, 14]. Peptides, amino acids, polymeric and monomeric carbohydrates as well as phenolic compounds have been isolated from melanoidin fractions [15]. How these individual components are joined to one another remains to be answered.

As little is known about the physiological effects of melanoidins. They possibly have a stimulating effect on the stomach intestinal tract, thereby causing irritations in some persons [4, 5, 17]. This stomach irritating effect is reported to be substantially diminished by treating the coffee beans with steam prior to roasting [6, 16].

To date, it has not been possible to substantiate any significant chemical difference between the so called mild coffees and other coffees. It is feasible that steam treatment causes changes in the browning substances. However, as long as their exact composition and structure remain unknown, speculation as to their physiological effects is not possible. An important research goal, therefore, should be the identification of these substances.

The structure determination in the classical sense is not possible at present because melanoidins are only obtainable as mixed fractions. Instead, identification should entail the separation of the melanoidin fractions into various sub-units using a number of methods and characterising these sub-units. This should enable the unravelling of structural elements and repeating sub-units.

In the present paper, melanoidins dissolved in a hot water extract of roasted coffee are isolated, then further separated and characterised. For reasons discussed above, both normal and steam treated coffees are studied in order to ascertain the differences, if any, in their corresponding melanoidin fractions.

2. MATERIALS AND METHODS

Table 1 summarises the coffee samples studied.

Table 1: *coffee samples studied*

coffee species	steamed
Ethiopia arabica	no
Ethiopia arabica	yes
Indonesia robusta	no
Indonesia robusta	yes

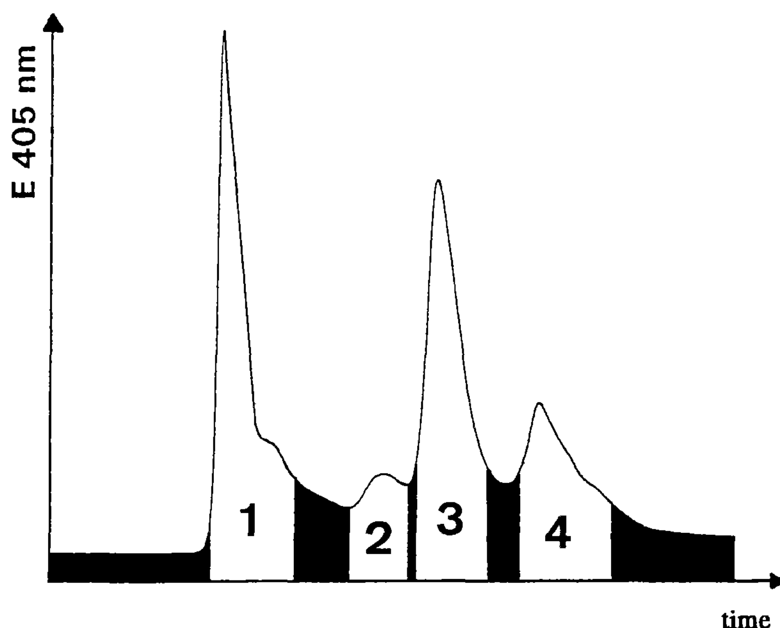
The following analytical procedure was used: a hot water extract of each roasted coffee sample was separated into a number of brown coloured fractions using gel filtration chromatography (GFC). The individual fractions were then lyophilised, characterised using various chemical and physical methods and further separated via thin layer chromatography. The thin layer chromatographic fractions were themselves eluted, lyophilised and characterised.

The first separation step for the melanoidins dissolved in the hot water extract was GFC on sephadex gel (the conditions used as well as a sample chromatogram are shown in Figure 1). Four coffee melanoidin fractions (CMF) were thus obtained.

The lyophilised solutions of the four fractions differed in colour, smell, consistency and solubility. The molecular masses of the four fractions were determined using high pressure exclusion chromatography (HPEC). The application of this method for coffee melanoidin fractions has been described in the literature. Results, however, were often unsatisfactory due to the fact that secondary separation effects influence the elution behaviour of the sample and that of the calibration standards differently. The chromatographic conditions applied were therefore chosen under the following considerations: a salt-containing eluting solvent was used to minimise ion occlusion, ion exclusion and absorption effects. Such effects between sample and gel matrix present a problem especially for water based HPEC because the water soluble polymers are polyelectrolytes or highly polar compounds. The type and concentration of ions in the eluting medium directly influence the conformation of the polymers and thereby influence their elution behaviour.

Figure 1: *gel filtration chromatography*

column:	XK-50/60 (Pharmacia Ltd.)
gel:	Sephadex G-25 fine; 5,0 cm i.d. x 55 cm l
eluent:	5,5 ml dist. water/min
detection:	405 nm
sample:	25 ml aqueous coffee extract (1:6)



The choice of suitable calibration standards for the determination of molecular masses of melanoidins is difficult because it must be made from compounds from other substance classes. Sodium-polystyrene sulfonates seem to be a suitable choice because they exhibit characteristics similar to melanoidins that are often responsible for the observed interferences: they are polymeric, anionic compounds containing both aliphatic and aromatic structural groups. To insure a separation free from interferences, pullulanes, polymeric and non-ionic aliphatic calibration standards, were studied in parallel.

An ultrahydrogel column (from the company Waters) and a phosphate buffer (pH 11) were used in order to depress secondary separation effects. Molecular masses of the four CMF determined from a calibration curve are summarised in table 2.

Table 2: *Molecular masses of coffee melanoidin fractions related to sodium-polystyrene sulfonate calibration standards*

Coffee Melanoidin Fraction (CMF)	Molecular Mass
1	63 100 - 12 600
2	5 750
3	5 750 - 1 510
4	3 630

The molecular masses of the CMF listed to the right in table 2 are for the four fractions of all coffee samples because they are not dependent upon coffee type or treatment.

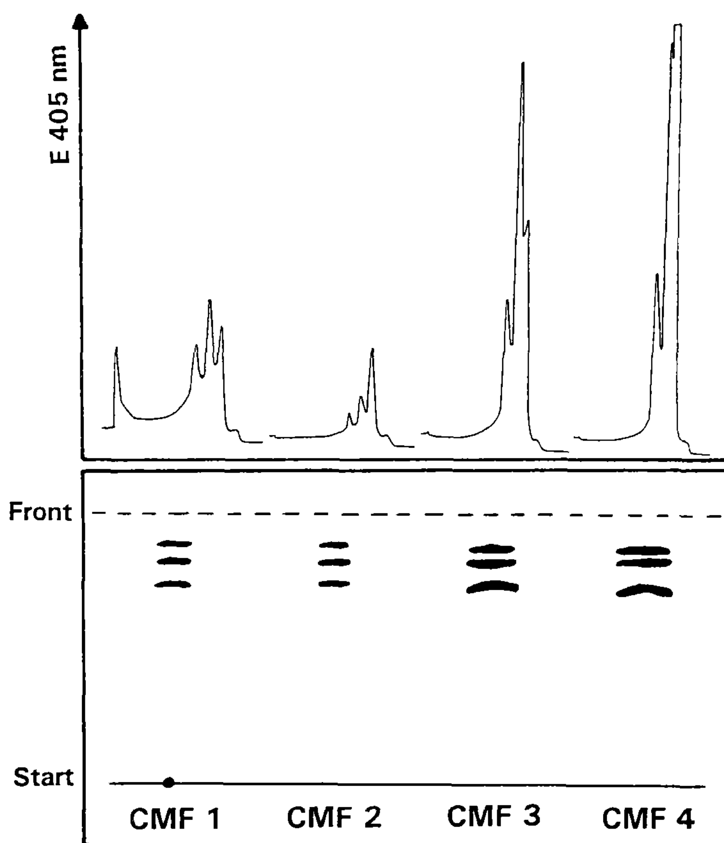
It is not possible to compare these results with literature values except with values obtained from identical CMF. Molecular masses for CMF isolated in a similar manner, determined with water based HPEC were between >100.000 and 1.000 D. Somewhat lower values were obtained from melanoidins that were permethylated prior to the HPEC analysis to reduce undesirable interactions between the sample and gel matrix. These values are similar to those in table 2, which is a further indication of a successful suppression of undesirable separation effects in the chromatographic system presented here.

To further separate and characterise the coffee melanoidin fractions a thin layer chromatographic technique was developed. Such attempts at separating melanoidins with thin layer chromatography have been reported for instance by GANSSMANN [7] and KÄSSER [8]. They were unsuccessful at obtaining a clean separation into spots or zones with their chromatographic systems. The elution was incomplete, forming brown trails in the direction of the mobile phase.

Therefore, the thin layer chromatographic studies were performed similarly to those developed by KHAIRY in 1980 for humic acids [9]. Using silica gel as the stationary phase and a mobile phase of 70 parts 25% ammoniac solution to 30 parts 1-propanol allowed the separation of the CMF into a maximum of four brown coloured bands.

Figure 2 depicts the separation of the four fractions obtained after GFC on sephadex gel under the conditions described above. The corresponding densitograms are shown at the top of the figure. One may readily see that part of the first CMF remains at the starting point. Differences between the individual fractions are ascertainable, however, no differences between treated and untreated coffee can be discerned.

Figure 2: *Thin layer chromatography of the four CMF*



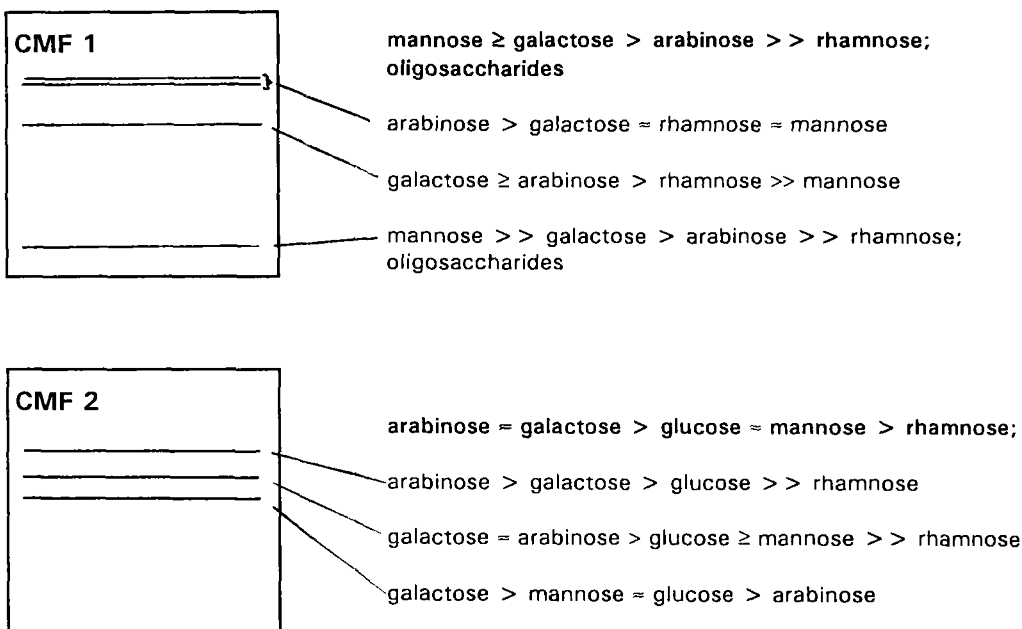
The following considerations could explain the successful separation: KHAIRY and ZIECHMANN [10, 11] based their thin layer chromatographic studies on a hypothesis from ZIECHMANN [19], according to which humic substances and non-humic substances (e.g. carbohydrates, peptides, etc.) form a so-called 'humic substance system' through secondary valent bonding. Reproducible separation of these systems is, according to KHAIRY [9], only possible after breaking up the secondary valent bonds with alkaline solutions. Applying this model to the CMF, it is feasible that in addition to covalently bonded individual components (amino acids, sugars, phenolic compounds etc.) forming smaller units, these in turn can be bound to one another via secondary valent bonds thereby forming larger conglomerates. According to this, the zones obtained with thin layer chromatography represent small subunits of the starting fraction. In order to isolate substances eluting as bonds preparative layer chromatography (PLC) was used.

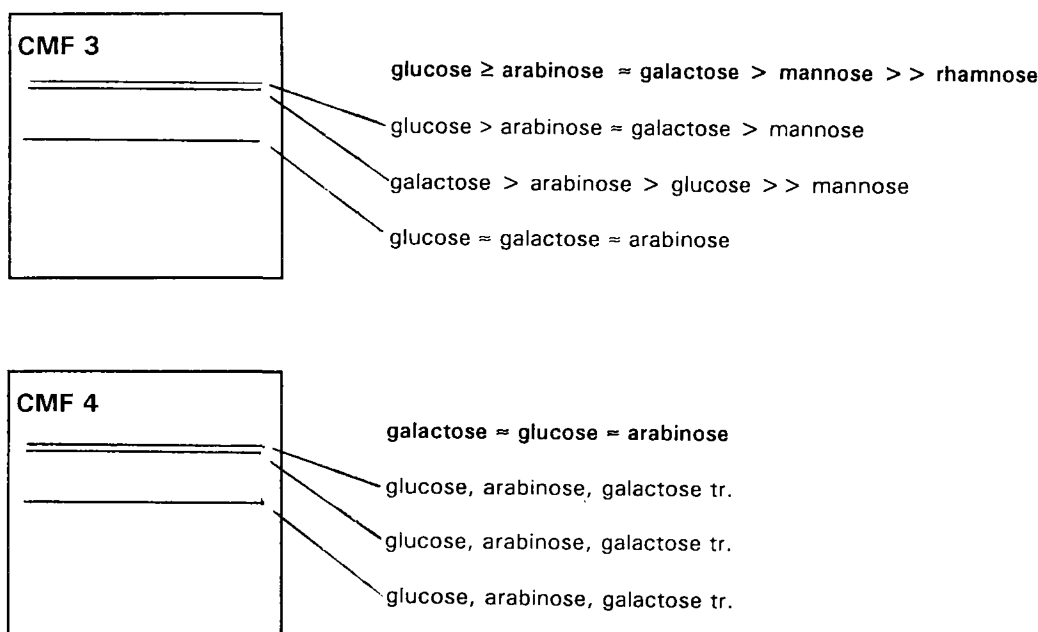
A total of seven or eight brown coloured fractions were isolated from the aqueous roasted coffee extracts using column chromatography and PLC. These fractions were analysed for carbohydrates using thin layer chromatography following acid hydrolysis and after forming derivatives using gas chromatography. The carbohydrates were investigated due to the fact that carbohydrates comprise 50% dry weight of unroasted coffee and surely play a key role in the Maillard reaction and caramelisation.

3. RESULTS AND DISCUSSION

Qualitatively the same carbohydrates were detected in all of the coffee samples. Quantitative differences were so small that they will not be discussed further. Figure 3 shows a representative example of the carbohydrate distribution in the individual CMF 1 to 4 from untreated Ethiopia Arabica after PLC separation.

<u>Figure 3</u>: *Preparative thin layer chromatography of the coffee melanoidin fractions (CMF) 1 - 4 (Ethiopia Arabica, untreated)*





A large decrease in carbohydrates was found after acid hydrolysis of the CMF from 1 to 4 in all the coffee samples. In the first CMF mannose, galactose and arabinose were the dominating carbohydrates besides smaller amounts of rhamnose and glucose. Following the PLC separation, almost all of the mannose was recovered in the lower most fraction, whereas the other carbohydrates were distributed in the upper fractions.

The first CMF differed from all other CMF's in its PLC separation in that a band remained at the starting point, containing a high amount of mannose. Following methylation analysis, a 1,4-linked mannan having little galactose in its side chains was identified. This is possibly part of the β-1,4-linked mannan isolated from unroasted coffee by BRADBURY and HALLIDAY [2]. THALER and ARNETH [18] found that water insoluble mannan is partially transformed to a water soluble form upon roasting. BUTTLE also identified a polysaccharide of this type in a coffee melanoidin fraction of high molecular mass [3].

The dominant carbohydrates in the second CMF were arabinose, galactose and glucose besides small amounts of mannose and rhamnose. The arabinose content in the PLC fractions increased in the direction of the mobile phase, whereas the galactose content decreased.

The dominant carbohydrates in the third CMF were arabinose, galactose and glucose as well as small amounts of mannose and rhamnose. As was observed in the second CMF, an increase in the arabinose content in the direction of the mobile phase was found.

Galactose, arabinose, glucose, mannose and rhamnose were detected in the fourth CMF. No noticeable distribution of the carbohydrates over the PLC fractions was observed.

4. SUMMARY

The water soluble melanoidins from two Arabica and Robusta coffees, where one sample of each was steam treated, were separated, isolated and characterised using chromatographic methods.

The melanoidins were separated into four brown coloured fractions using GFC. The molecular masses of these fractions were then determined using HPEC. The HPEC system used here was able to suppress the influence of secondary separation effects through the choice of appropriate system parameters.

The first successful separation of CMF into a number of zones using thin layer chromatography was achieved. All four fractions obtained from GFC were further separated into three or four fractions. The success of the thin layer chromatographic separation is compared to results and structural models proposed for humic substances and their applicability to the CMF is discussed.

The results of the carbohydrate analysis in all of the chromatographically separated fractions were presented using a roasted coffee sample as a representative example.

ZUSAMMENFASSUNG

In 2 Arabica- und 2 Robustakaffees, von denen jeweils eine Probe wasserdampfbehandelt war, wurden die wasserlöslichen Melanoidine mit Hilfe chromatographischer Verfahren aufgetrennt, isoliert und charakterisiert.

Über Gelfiltrationschromatographie (GFC) wurden die Melanoidine in 4 braun gefärbte Fraktionen aufgetrennt, die hinsichtlich ihrer Molekularmassen über Hochdruckausschlußchromatographie (HPEC) untersucht wurden. Das vorgestellte HPEC-System war dadurch gekennzeichnet, daß durch die Wahl geeigneter Systemparameter ein Einfluß sekundärer Trenneffekte auf die Trennung unterdrückt werden konnte.

Es gelang erstmals eine Auftrennung von Kaffee-Melanoidin-Fraktionen (CMF) mittels Dünnschichtchromatographie in mehrere Zonen. So konnten alle 4 über GFC erhaltenen Fraktionen in 3 bzw. 4 weitere Fraktionen aufgetrennt werden. In Zusammenhang mit der dünnschichtchromatographischen Trennung wurde ein für Huminstoffe aufgestelltes Struktur-Modell auf die CMF übertragen und diskutiert.

Die Untersuchungen aller auf chromatographischem Wege erhaltenen Fraktionen auf Kohlenhydrate wurden exemplarisch an einer Röstkaffeeprobe dargestellt.

RÉSUMÉ

Les mélanoidines solubles dans l'eau de deux cafés Arabica et Robusta, un échantillon de chacun traité avec de la vapeur, furent separés, isolés et caractérisés selon les méthodes chromatographiques.

Les mélanoidines furent séparés en quatre fractions colorées en brun par la chromatographie de filtration au gel (GFC). Ensuite, les masses moléculaires de ces fractions furent déterminées par HPEC. Le système HPEC employé ici était capable de supprimer l'influence des effets de séparation sêcondaire par le choix de paramètres de système appropriés.

La première séparation réussie de fractions de mélanoidines de café dans plusieures zones fut acheveé par chromatographie en couches légères. Toutes les quatres fractions obtenues par GFC furent encore séparées en trois ou quatre fractions.

Le succès de la séparation par chromatographie en couches légères est comparé aux résultats et modèles structurales proposés pour matières humique. Leur application aux fractions de mélanoidines de café est discutée.

Les résultats de l'analyse de carbohydrates dans toutes les fractions séparées par chromatographie furent présentés en employant un échantillon de café torrefié à titre d'example.

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ÉTUDE DES CHANGEMENTS CHIMIQUES DANS LE CAFÉ SOUS FORME DE BOISSON PENDANT L'EXTRACTION ET LA CONSERVATION

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INTRODUCTION

Le café est une boisson complexe et très instable, qui doit ses propriétés de goût, d'arome et de saveur, à une certaine quantité de substances, qui sont extraites du café grillé et moulu et qui restent inaltérées dans la boisson pour un très court instant. Après l'extraction, en effet, des modifications physico-chimiques, qui ont lieu dans le café, altèrent les qualités organoleptiques du produit à une vitesse supérieure quand la température de conservation augmente.
A ce propos, on peut dire que l'acidité est un facteur prépondérant pour le goût du café et , à travers la mesure du pH, il est possible d'évaluer l'acceptabilité du produit.
Da Porto et ses collaborateur (1991) ont étudié l'évolution de l'acidité de la boisson en fonction du degré de torréfaction du café. Dans cette étude on a observé que le café le plus acide est celui qui est obtenu avec une torréfaction moyenne.
En outre, Dalla Rosa et al. (1986), ont enquêté sur l'évolution du pH dans la boisson de café pendant la conservation à quatre températures différentes. Après 50 jours, le produit conservé à la température de 4°C a été jugé encore acceptable; à 40°C, au contraire, l'acidité de la boisson a dépassé le niveau d'acceptabilité (pH de 4,8) déjà après 30 jours.
Notre but était de decouvrir le rôle de certains acides organiques sur l'augmentation de l'acidité titrable et donc sur la diminution dans le temps de la qualité du café.

MATERIAUX ET METHODES

L'expérience a été conduite avec un café torréfié à l'italienne. L'extraction a été effectuée par le contact direct poudre/solvant dans un matras fermé, soumis à une agitation et à une température constantes de 85°C pendant 10 minutes. Après quoi, on a filtré le café pour le séparer de la poudre.

Dans toutes les extractions on a utilisé de l'eau déionisée avec un pH de 6,39. Pour le vieillissement, la boisson a été conservée dans un thermostat à 40°C constants.
Sur les échantillons on a effectué les déterminations analitiques suivantes:
- pH, avec pHmètre Crison MicropH 2001;
- acidité titrable, par titrage avec NaOH N/10 jusqu'à pH 7 et pH 9;
- pourcentage de substances solubles, en fourneau à la température de 105°C pour 8 heures;
- acide clorogénique, par la méthode spectrophotométrique (méthode officielle A.O.A.C., modifiée), avec un spectrophotomètre Perkin Elmer 550 SE UV/VIS;
- acide lactique, malique et citrique par la méthode enzymatique (kit Boehringer Mannheim, Allemagne).

RESULTATS ET DISCUSSION

Dans cette recherche nous avons étudié avec une attention particulière les acides organiques qui passent dans le café sous forme de boisson avec le solvant. On a déterminé, en premier lieu, le pH de 5 fractions de café prélevées progressivement pendant la sortie de l'extrait et, sur les mêmes fractions on a déterminé l'acidité titrable au pH 7 et au pH 9.
Sur la figure 1 nous pouvons voir que la première et la deuxième fraction contribuent d'une façon déterminante à l'acidité totale de la boisson.

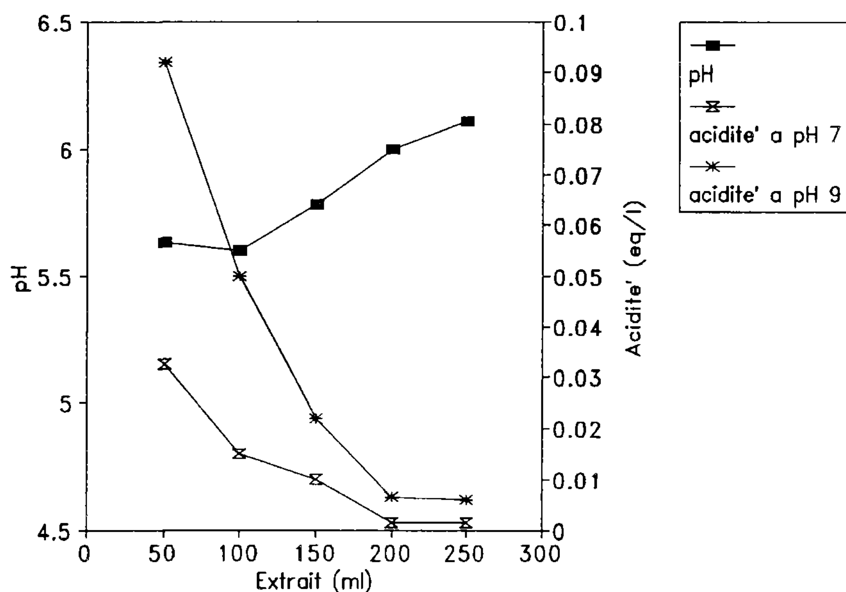

Figure 1: Acidité du café sous forme de boisson divisé en 5 fractions de 50ml, pendant la sortie de l'extrait, titrée à pH 7 et à pH 9 et pH de chaque fraction.

Par conséquent, on a suivi la modalité d'extraction de certains acides organiques à travers leur cinétique.
Dans l' histogramme de la figure 2 on peut observer les quatre acides organiques considérés, c'est à dire les acides clorogénique, malique, citrique et lactique, contenus dans chaque fraction de breuvage.

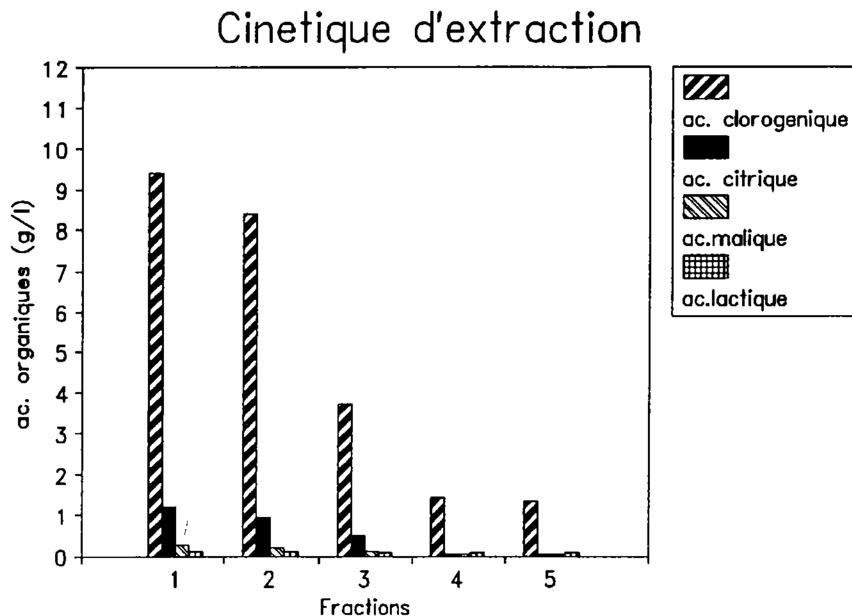

Figure 2: Quantité (g/l) des acides clorogénique, malique, citrique et lactique, dans 5 fractions de café, prélevées pendant la sortie de l'extrait.

Il est évident que la majeur partie de ces acides passe dans la boisson avec les deux premières fractions. Nous avons pu confirmer ce que l'intuition nous avait fait comprendre en observant la figure 1.

La cinétique d'extraction appartenait au premier ordre pour tous les acides considérés: de même que les substances solubles (Nicoli et al., 1987), les substances volatiles (Severini et al., 1988) et la caféine (Nicoli et al., 1987).
Le tableau 1 réporte les constantes de vitesse (k ml^{-1}) pour les acides clorogénique, malique, citrique et lactique, avec les correspondant coefficients de correlation (r). La valeur de "p" a été dans tous les cas de 0.001.

Tableau 1

	Ac.Clorog.	Ac.Mal.	Ac.Citr.	Ac.Lact.
k (ml^{-1})	0.0024	0.0025	0.0031	0.0002
r	0.950	0.979	0.974	0.945

Le dosage des mêmes acides a été effectué, en outre, pendant le vieillissement du café à 40°C. Le contenu global de chaque acide considéré a été exprimé en fonction du temps de conservation à 40°C (fig. 3).

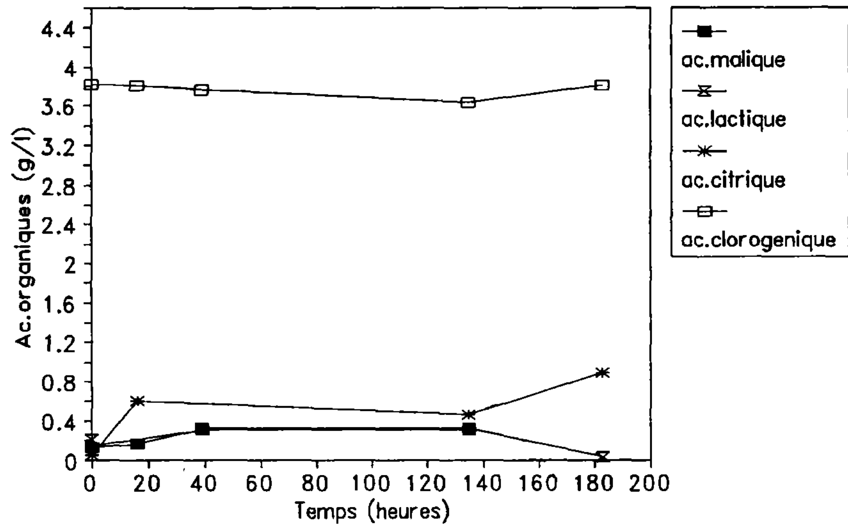

Figure 3: Modification de la quantité (g/l) des acides clorogénique, malique, citrique et lactique, présente dans le breuvage pendant la conservation a 40°C.

On peut voir que la quantité présente dans le café des quatre acides ne change pas au cours du temps, malgré la haute temperature. Au contraire (fig.4), le pH diminue régulièrement jusqu'au niveau d'acceptabilité.

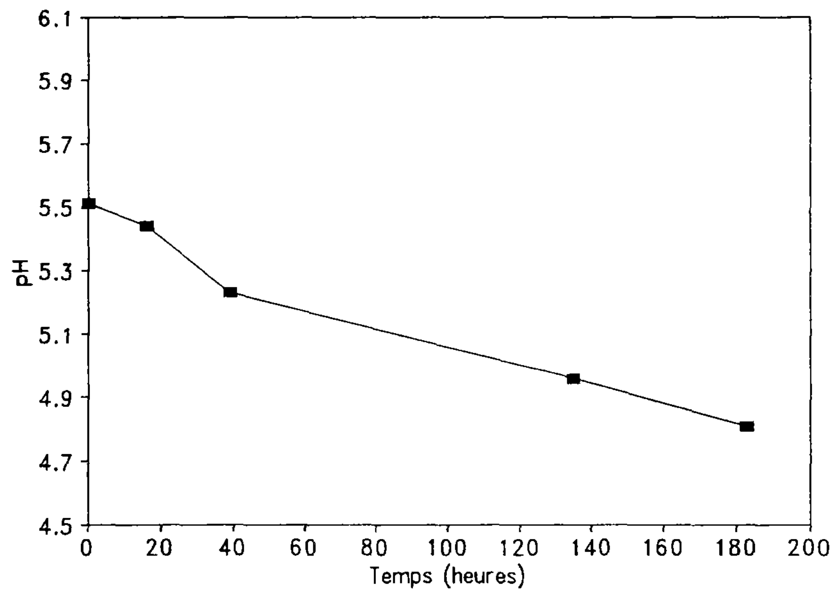

Figure 4: Diminution du pH du café pendant le temps de conservation à 40°C.

La contribution de ces quatre acides à l'acidité titrable à pH 7 a été calculée en additionant les équivalents pour litre dans la boisson de chaque forme non-dissociée et partiellement dissociée des acides. Le calcule a été réalisé en prenant en considération que, à pH 7, ces acides sont titrés presqu'a 99%, sauf pour la troisième fonction de l'acide citrique, qui est titré pour 80%.
En construisant les deux courbes de l'acidité titrée et de l'acidité calculée par la contribution des acides que nous avons déterminés, nous pouvons observer que ces acides ne contribuent que faiblement à l'acidité titrée(figure 5).

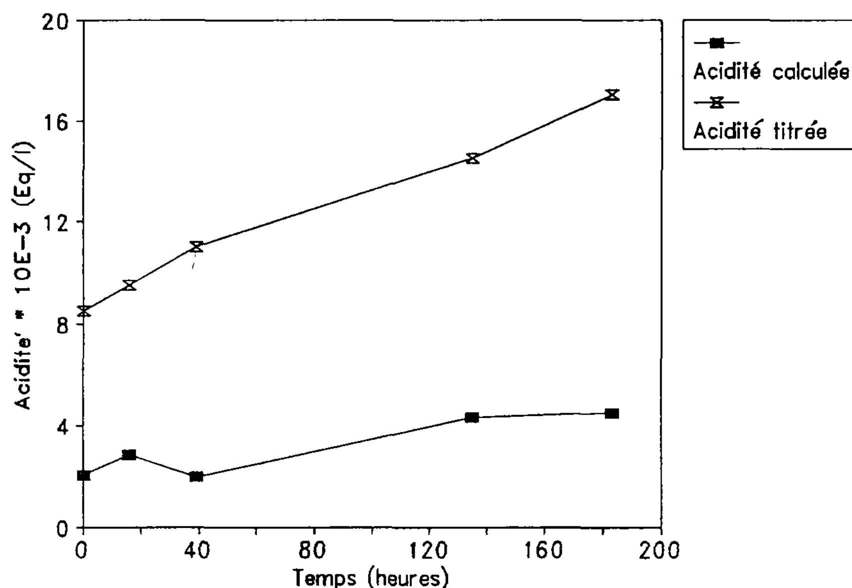

Figure 5: Acidité titrée e acidité calculée par la contribution des acides clorogénique, malique, citrique et lactique, en fonction du temps de conservation à 40°C.

CONCLUSION

A travers notre expérience nous avons vu que la détérioration du café n' est pas due à l'augmentation des acides clorogénique, malique, lactique et citrique.
Nous pouvont formuler l'hypothèse que d'autres substances, qui dérivent, peut-être, de la réaction de Maillard, ou qui dérivent d'autres réactions (Maier et al., 1984), pendant qu'on passe le café, sont responsables de l'altération de ce breuvage.

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RIASSUNTO

In questo lavoro di ricerca e' stata studiata la cinetica di estrazione di alcuni acidi organici, quali l'acido clorogenico, l'acido malico, l'acido citrico e l'acido lattico. La bevanda ottenuta da un caffe' tostato all' italiana e' stato poi conservato alla temperatura di 40°C per osservare le modificazioni chimiche dovute all' invecchiamento della bevanda. In particolare si e' potuto notare che la diminuzione di qualita' del caffe' e la sua acidificazione durante la conservazione non dipendono dall' incremento degli acidi clorogenico, malico, citrico e lattico.

RESUME

La cinétique d' extraction de certains acides organiques, c'est à dire les acides clorogénique, malique, citrique et lactique, a été étudiée pendant la preparation du café sous forme de boisson. Le café obtenu a été conservé à la température de 40°C de façon à étudier les changements dus au vieillissement de la boisson. Nous avons vu que l'altération du café n' est pas due à l'augmentation des acides clorogénique, malique, lactique et citrique.

GENERATION OF FURFURYL MERCAPTAN IN CYSTEINE-RIBOSE MODEL SYSTEMS IN RELATION TO ROASTED COFFEE AROMA

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INTRODUCTION AND BACKGROUND:

In 1926 Reichstein and Staudinger (1) reported the presence of furfuryl mercaptan (Fur-SH) or 2-furfurylthiol in roasted coffee. In 1944 Giral and Fernandez synthesized furfuryl mercaptan from furfural and confirmed its importance to the odor of coffee (2).

Tressl found that the level of Fur-SH in roasted Robusta coffee is about double that of Arabica coffee, ranging from about 1000-2000 ppb in Arabicas to about 2000-3800 ppb in Robustas (3).

Several workers have reported the organoleptic importance of this compound in coffee aroma. Tressl and Silwar (4) consider it to be either a positive impact component or off-flavor component depending upon concentration. It was perceived to have a character of freshly roasted coffee at concentrations of 0.01-0.5 ppb, while at levels of 1 to 10 ppb it had an aroma of "stale coffee with a sulfury note." Holscher and Steinhardt (5) used GC sniffing and aroma dilution analysis techniques (ADA) to study the organoleptic importance of separated GC peaks. They reported furfuryl mercaptan to be one of the more important odor components in roasted coffee. Recently Blank, Sen and Grosch (6) also used ADA to compare the potent odorants of a roasted and ground Arabica coffee to the brew prepared from this coffee. The sensory impact of furfuryl mercaptan was found to decrease by about 1/4 in the brew relative to the ground coffee which they attribute to either lower solubility in the brew or degradation by the hot water during brewing.

Model Systems:

Studies have demonstrated that furfuryl mercaptan may be a significant product in model systems containing both a reactive sulfur source and a pentose sugar or furfural (a sugar degradation product).

The study of the cysteine/xylose model system under aqueous conditions at 180° C and pH 5 action led Tressl to propose a mechanism for the production of furfuryl mercaptan via dehydration and reduction of the 3-deoxpentosone which is a known Maillard reaction product (7). Farmer, Mottram and Whitfield (8) reported Fur-SH formed in a cysteine/ribose model system heated for 1 hour at 140°C and pH 5.7. Shibamoto investigated the formation of sulfur and nitrogen containing compounds upon heating an aqueous model system consisting of furfural, hydrogen sulfide and ammonia at 100° C for 2 hours. Furfuryl mercaptan was identified as a prominent but not the major component in the reaction mixture (9). The pH of the system was not reported.

Coffee Precursors:

Primary precursors of furfuryl mercaptan in green coffee may be free or polymeric forms of pentose sugars (ribose, arabinose, xylose) and a sulfur source. Hexose sugars may also be a precursor upon fragmentation.

The major pentose sugar in coffee is arabinose which is a component of the highly branched arabinogalactan polysaccharide located in the cell wall. The arabinogalactan structure was characterized by Bradbury and Halliday (10). They reported that the level of polymeric arabinose in Arabicas and Robustas ranges between 3.4% and 4.1% on a dry basis. The high concentration of the terminal arabinose residues on the branches suggests that arabinose would be susceptible to liberation or degradation during roasting. While arabinogalactans are often covalently associated with protein in plants, their investigation failed to demonstrate this in coffee.

Bradbury and Haliday also found low levels of polymeric xylose (~0.2%) in green coffee (10). Tressl et al. (11) reported trace amounts of free ribose (e.g. 0.05%-0.07%) among the reducing sugars in green Arabica and Robusta coffee, while some free arabinose was found upon roasting.

Thaler and Arneth found that about 60% of the polymeric arabinose is lost upon the roasting Arabica green coffee, decreasing from 1.7% in the green to about 0.68% (12).

Tressl et al reported no free cysteine or methionine among the free amino acids of Robusta and Arabica coffee (11). Thaler and Gaigl found that cysteine content of the protein fraction of green coffee ranged from about 2.9% to 3.9% in Arabica and Robusta coffee and was found to drop significantly to a range of about 0.14% to 0.76% (13, 14). The protein bound methionine decreased to a smaller degree upon roasting. Thaler reviewed the macromolecular structure of coffee and indicated a considerable amount of the protein is combined "with a polysaccharide complex as structural protein in the cell wall" (15).

In summary, potential pentose precursors in coffee are small amounts of free ribose and a larger pool of polymeric arabinose; a potential sulfur source may be protein bound cysteine although other sulfur sources may also play a role. The coffee cell wall may be the site of both the carbohydrate and protein precursors.

Reaction Kinetics:

Data on the kinetics of Maillard reactions are not extensive. It is well known that cooking food under different conditions can result in a variety of flavors.

Reineccius (16) attributed these differences to reaction kinetics and specifically the activation energy for a specific reaction. The activation energy determines how temperature affects the rate of formation of a product. Reactions with low activation energies are generally favored at lower temperatures, while reactions with higher activation energies are generally favored at higher temperatures. Leahy and Reineccius examined the kinetics of formation of some alkyl pyrazines (17). A high correlation was found between the formation of pyrazines and reaction time indicating pseudo zero order kinetics. Activation energies for the formation of various pyrazines were found to range from 27 to 45 kcal/mole.

High temperature short time kinetics of a Maillard reaction between a proline/glucose model system were recently investigated at temperatures ranging between 160° C and 220° C and reaction times ranging 0.25 min and 5 min. It was found that one compound, 5-acetyl-2,3-dihydro-1H--pyrrolizine, was the major product and also formed by pseudo zero order kinetics. It was determined that this compound had a relatively high energy of activation of 45 kcal/mole (18).

Roasting Process:

The roasting of coffee is a kinetic process. The flavor of roasted coffee is highly dependent upon time and temperature. The roasting of coffee has been typically carried out at temperatures ranging from about 200° C to 260° C and times ranging from about 90 sec to about 20 min. The change in some volatile constituents of roasted coffee with roasting time has been demonstrated by Gianturco (19). It was observed that some well known coffee volatiles increase to maximum and then decrease with roast time. Included were three compounds clearly of carbohydrate origin: 5 methyl furfural, furfural, and furfuryl alcohol. Very little is known about the kinetics of flavor reactions under roasting conditions.

It is the purpose of this study to investigate the kinetics of the generation of furfuryl mercaptan under simulated coffee roasting conditions. As a preliminary experiment the level of furfuryl mercaptan was measured in coffee after roasting for various time intervals. Reaction kinetics of a ribose/cysteine model system was also determined using a continuous flow reactor under coffee roasting time/temperature conditions. Ribose/cysteine was chosen because of the greater information available in the literature. Arabinose, a stereoisomer of ribose, would have very similar chemical properties and would have been equally valid for examination in this study.

EXPERIMENTAL SECTION

Coffee Bean Roasting:

One pound batches of green Colombian coffee beans were roasted for various times ranging from six to sixteen minutes in a Jabez Burns coffee roaster.

Coffee Bean Analysis:

Three grams of roasted and ground coffee beans were combined with 5.0 gm of water in a 50 mL pear shaped flask. The sample was indirectly steam distilled and 8 mL condensate collected. The aqueous phase was extracted in a Mixxor as described by Parliment (20). Samples were analyzed by gas chromatography in a Hewlett Packard model 5890 gas chromatograph using a 60m x 0.32 mm i.d. column coated with a 1 micron film thickness of DB-5 liquid phase. A Sievers model 355 sulfur chemiluminescent detector was employed to detect the sulfur containing species. (Greater details on the Sievers detector can be found in the paper by G. Cohen in this Symposium).

Reaction Conditions in Continuous Flow Reactor:

1.2 gm cysteine and 1.5 gm ribose were combined in 100 mL of deionized water (pH= 3.75). The solution was pumped through a 1.0 mL sample loop (1/16 inch stainless steel tubing) which was immersed in a temperature controlled silicone oil bath. The reaction products were immediately cooled by immersing the connecting tubing in a cooled water bath. The products then passed through a 550 psi check valve and were collected for analysis. The pump permitted various flow rates ranging from 0.1 to 9.9 mL/min and thus residence or reaction times ranging from 10 min to about 0.1 min. Two experimental methodologies were conducted. In the first, the oil bath temperature was held constant and flow rates were varied to give reaction times ranging from 0.50 min to 5 min. This provided average rate constants. In the second methodology, the reaction time was held at 1.0 min and the oil bath temperature was varied from 180° C to 220° C. The second methodology provided single point rate constants for product formation (relative GC/MS counts/min) over a range of temperatures. Both sets of data proved useful in determining in Arrhenius activation energy for compound formation. The overall equipment and procedure has been described in detail previously (18).

Most of the studies were conducted at an initial pH of 3.75, yielding a final pH of 4.3, since this approximates the coffee system. In one set of experiments the pH was reduced to 2.0 with phosphoric acid.

Analysis of Model System:

The products of reaction (8 mL) were transferred to a 10 mL Mixxor. One gram sodium chloride was added and the aqueous phase extracted with 0.75 gm diethyl ether (containing 1 mg ethyl nonanate per 10 mL diethyl ether as internal standard). This procedure has been previously described by Parliment (20).

The etheral extracts were analyzed via GC/MS. A Varian model 3700 gas chromatograph was used with a 15m x 0.32 mm i.d. fused silica column coated with a 1 micron film of DB-5. Oven conditions were: 5 min at 60° C then 5° C/min to 230° C and a final hold of 10 min. The column effluent passed through an open split interface into a Finnigan model 705 Ion Trap Mass spectrometer. The identifications were achieved by comparison of the generated spectra to those of the NBS Library Compilation or to published spectra and relative concentrations of the products were determined using the Ion Trap quantitation program.

RESULTS AND DISCUSSION

Figure 1 is a plot of the generation of Fur-SH versus roasting time in

minutes in the Jabez Burns roaster. It is seen that the generation of Fur-SH increases with roast time. In the early stages of the roast (up to about 9 min) we find that very little generation of Fur-SH occurs. It is known that little reaction occurs in the early part of the roast cycle or the drying phase because moisture is leaving the green coffee and the internal bean temperature remains low. As the bean temperature rises, flavor development begins (21).

Figure 2 is the generation of Fur-SH versus time at temperatures of 190° C, 200° C and 210° C. The generation of Fur-SH is linear with time. This data is consistent with a zero order reaction kinetics model. Labuza has found that most food reactions follow either pseudo zero order or first order kinetics (22). The formation of many Maillard reaction products appear to follow pseudo zero order kinetics (16, 17). The rate constants and correlation coefficients were generated from the slope of the regression equation for the data using the Lotus Freelance program and are given in Table 1 below:

Table 1. Average rate Constants for Formation of Furfuryl Mercaptan At 190° C, 200° C and 210° C

Temp (°C)	Rate Constant (k)	Corr. Coeff. (r^2)
190	1.35	0.992
200	2.55	0.962
210	14.29	0.994

Figure 3 is the generation of Fur-SH at 220° C. The generation of Fur-SH is seen to increase to a certain level, and appears to reach a maximum between 1 and 2 minutes, and decrease again. This suggests that Fur-SH either decomposes or reacts further to produce other products. There may be an optimum time/temperature range for the formation of Fur-SH in roasting. This finding is consistent with the work of Schirle-Keller and Reineccius describing the reaction kinetics for the formation of oxygen containing heterocyclic compounds in a glucose/cysteine model system (23). They found that "some compounds need a certain level of energy to be formed, but too much will result in their degradation to other compounds and they disappear from the chromatogram."

Figure 4 is the Arrhenius plot [log rate constants versus 1/Temperature (Kelvin)] for activation energy of formation (E_a) of Fur-SH at pH 3.75. The rate constants were obtained at 190° C, 200° C and 210° C (see Table 1 and Figure 2). The (E_a) determined was 52.6 kcal/mole (r^2= 0.931) and was calculated from the slope of line generated by regression analysis from the following relationship:

$$E_a = (-2.303)(1.987 \text{ cal/degree mole})(\text{slope of line}).$$

The E_a for Fur-SH was also determined by the second procedure by measuring the single point rate constants over a range of temperatures and at pH 3.7 and pH 2.0. The Arrhenius plot is given in Figure 5.

FIGURE 1
GENERATION OF FURFURYL MERCAPTAN IN COFFEE VERSUS ROAST TIME

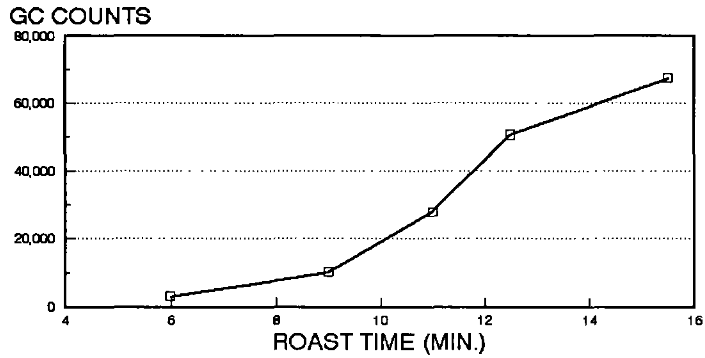


FIGURE 2
GENERATION OF FURFURYL MERCAPTAN VERSUS TEMPERATURE (190C, 200C, AND 210C)

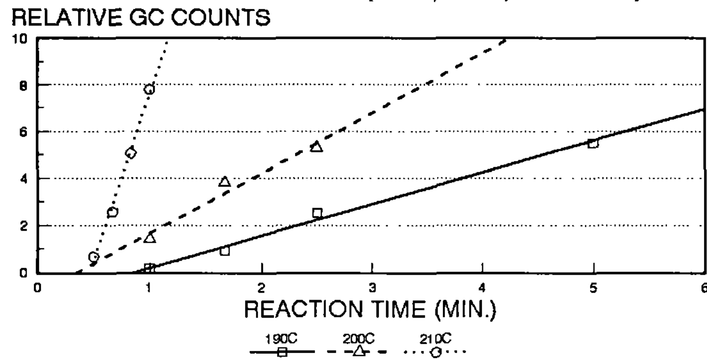


FIGURE 3
GENERATION OF FURFURYL MERCAPTAN AT 220C

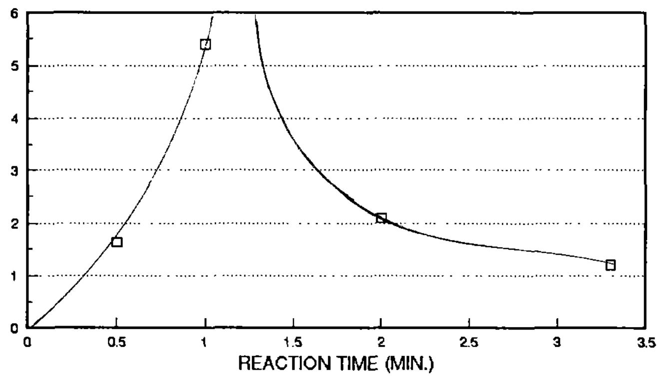


FIGURE 4-ARRHENIUS PLOT
FOR FURFURYL MERCAPTAN AT pH 3.75
(BASED ON k CONSTANTS AT 190C, 200C, AND 210C)

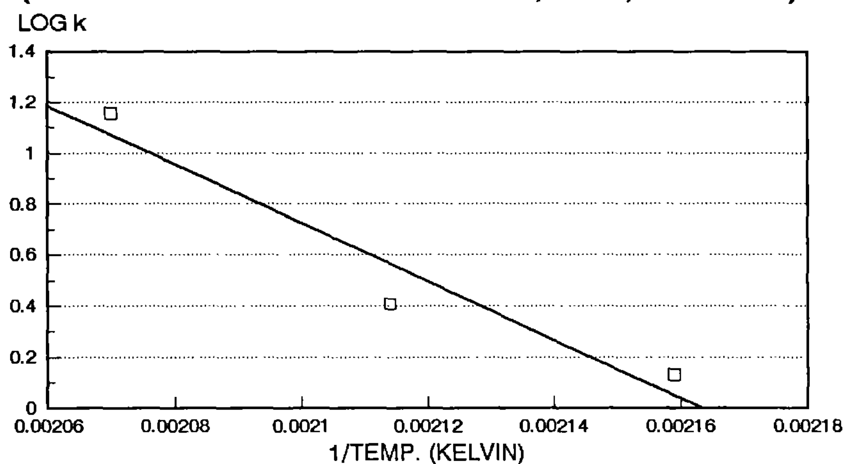
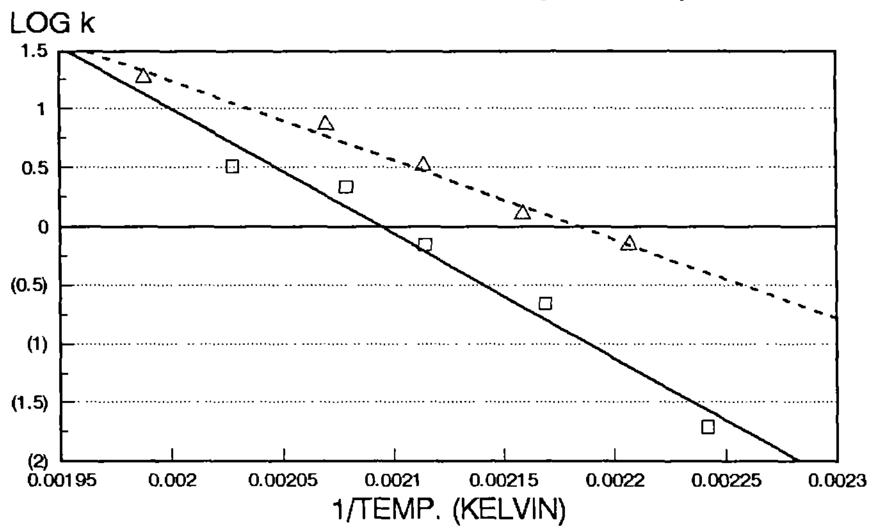


FIGURE 5- ARRHENIUS PLOT
FOR FURFURYL MERCAPTAN AT pH2 AND pH 3.75



The activation energies are summarized in the Table 2 below:

Table 2 Activation Energies for Formation of Fur-SH at pH 3.75 and pH 2.0 (kcal/mole).

pH	Methodology	E_a Fur-SH	Corr. Coef. r^2
3.75	1	52.6	0.931
3.75	2	48.6	0.965
2.0	2	31.0	0.986

The values of the E_a for Fur-SH formation determined at pH 3.75 by the two methods compare very well. The average for the two methods is 50.6 kcal/mole. As can be seen the activation energy decreases about 30% as the pH is lowered which suggests a pH dependency in the reaction mechanism and the formation is favored at lower pH conditions.

The presence of furfuryl alcohol and furfural in the reaction mixture suggests that these may be intermediates in the formation of Fur-SH. Additional research such as evaluating kinetics of Fur-SH formation with these suspected intermediates is currently underway and will be presented at the National American Chemical Society Meeting, Chicago, Illinois, Aug. 1993.

SUMMARY AND CONCLUSIONS:

A coffee roast study demonstrated that the level of furfural mercaptan increased with roast time. A study of the ribose/cysteine model system under various roasting time/temperature conditions was performed. Using a continuous flow reactor, it was demonstrated that furfural mercaptan increases linearly with reaction time consistent with pseudo zero order kinetics. The energy of activation for the formation of furfuryl mercaptan was determined to be about 50 kcal/mole at pH 4. The relatively high energy of activation for furfuryl mercaptan, relative to other compounds, suggests that its formation would be favored at the high temperature conditions of coffee roasting. The literature suggests that the coffee cell wall may be source of carbohydrate and protein bound precursors for furfuryl mercaptan.

RÉSUMÉ ET CONCLUSIONS:

Une étude de la torréfaction du café a démontré que le niveau de furfuryl mercaptan augmente en relation avec le temps de torréfaction. Une étude d'un modèle d'un système de ribose/cysteine à différentes conditions de temps et de température de torréfaction, dans un réacteur à écoulement continu, a démontré que le furfuryl mercaptan augmente de façon linéaire par rapport au temps de réaction selon une vitesse de réaction d'ordre pseudo-zéro. L'énergie d'activation pour la formation du furfuryl mercaptan a été déterminée en utilisant un réacteur à écoulement continu comme ayant une valeur de 50 kCal/mole à un pH de 4. La valeur

favorisée à des conditions de torréfaction à hautes températures. La littérature suggère que les membranes des cellules de café peuvent être la source d'hydrates de carbone et de protéines servant de précurseurs pour le furfuryl mercaptan.

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ELECTRONIC NOSES. PRINCIPLES, APPLICATIONS AND OUTLOOK

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INTRODUCTION

Sensory analysis, relying on the human sense of smell, is still the primary method for evaluation and quality control of odours and flavours in a wide range of industrial sectors. These include the food industry, the beverage industry and the personal products and perfume industries. In all of these industries there are therefore significant potential benefits, in terms of improved quality control and improved process control, which could be obtained if sensory analysis could be supplemented by analytical techniques for measuring and characterising odours. Of course some analytical techniques, such as the use of gas chromatography with mass spectroscopic detection (GCMS), do exist which can be used to characterise the composition of mixtures but these are complex, expensive and do not directly address the problem of odour or flavour analysis.

We have been working since the early 1980s on the development and applications of an artificial olfactory system, or electronic nose. The project is a collaboration between research groups in the Engineering department at the University of Warwick and the Chemistry department at the University of Southampton and brings together expertise in sensor design and fabrication, electronics, signal processing and pattern recognition techniques. The aim of our research is to design instruments which mimic the human olfactory system. To do this it is necessary to know something of the mechanism and function of the olfactory system and to identify the general features which are believed to be important for its operation. In the next section we briefly review these before going on to describe electronic noses and their operation. We conclude the article with some examples of the applications of electronic noses and a forward look at this technology.

CHEMICAL SENSES AND FLAVOUR

The sensation of flavour is due to the concurrent stimulation of all our chemical senses, together with the integration of the signals from the component senses by the higher brain centres. In humans there are three main chemoreceptor systems: gustation (the sense of taste), olfaction (the sense of smell) and

the trigeminal sense. Taste is used mainly to detect non-volatile chemicals which enter the mouth while the sense of smell is used to detect volatile compounds. Receptors for the trigeminal sense are located in the mucous membranes and the skin and are important in the detection of irritants and chemically reactive species. In the perception of flavour all three senses have a role but by far the most significant part is played by the sense of smell.

The sensation of smell arises from the stimulation of the olfactory neurones, the individual receptor cells located high up in the olfactory epithelium, by odorant molecules. Whilst odours can be simple or complex, a distinction based on the nature of the stimulus rather than the quality of the sensation, most natural flavours and odours are complex in that they are the result of a mixture of many tens, and possibly many hundreds, of different individual volatile chemicals, or odorants. These individual odorants are typically small hydrophobic, organic molecules containing one or two functional groups and with masses upto approximately 300 Da. The relationship between the physico-chemical properties of the odorant molecules and their odour has been discussed by several workers [1,2]. Whilst it is clear that size, shape and polar properties of the molecule determine its odour properties the rules which govern this are only poorly understood. Consequently the classification of odour type [3] is empirical and the number of distinct descriptors required (the dimensionality of the problem) is not well established.

The mammalian olfactory system makes use of a large number of non-specific receptors which show broad patterns of response. Typically, in the human olfactory epithelium there are about 50 million primary receptor cells. These primary receptors are about 1 μm in diameter and end in a conspicuous dendrite which protrudes into the mucus layer covering the epithelium. This dendrite has between 5 and 20 cilia which radiate out into the mucus layer, presumably in order to increase the sensing area of each cell. The primary neurones send their signals to secondary cells located in the olfactory bulb. There is a marked convergence in the information processing at this stage with between 1,000 and 20,000 primary receptor cells synaptically connected to each secondary call. This suggests that the secondary cells play an important role in processing and integrating the information; a view which is consistent with the observation that, while the primary receptor cells are non-specific in their responses to odorants, the secondary cells respond to distinct odorant categories [4]. The secondary cells, in turn, pass signals on to higher cells which are involved in the processing of the olfactory response. These interactions are reminiscent of processing in the visual system [5] - the brain appears to process the information from the olfactory neurones as an odour image.

COMMERCIAL ODOUR MEASURING INSTRUMENTS

Currently there are a number of commercial instruments available for odour monitoring. These vary in price and sophistication from laboratory based instruments to portable monitors. In all cases these instruments are based on the use of a single, non-specific, gas sensor which is used to measure the total odour intensity. The different devices are based on the use of a metal oxide thermal sensor, a metal oxide conductimetric sensor, or a bilayer lipid membrane coated mass sensor, Table 1. The promotional literature for each device describes a range of applications in fragrance monitoring, the food industry and environmental monitoring. These instruments have only one sensor they can be used to quantify odour intensity but they cannot be used to identify and characterise odours or to make subtle distinctions between different odours. For these latter applications more sophisticated systems, "electronic noses", using arrays of sensors linked to suitable multivariate statistical techniques of data analysis are required. Such systems will be commercially available in the near future.

TABLE 1. Commercial instruments for odour measurement.

Instrument	Supplier	Sensor type	Operating temperature	Comments
Portable odor monitor	Sensidyne Inc. (USA)	Pellistor	300-500 °C	Hand-held instrument with LCD display built-in micro air pump. Responsive to various aromatic, odorous compounds.
XP-329 portable odor level indicator	New Cosmos Electric Co. (Japan)	Pellistor	300-500 °C	Hand-held instrument with LCD display and built-in micro air pump. Responsive to various aromatic, odorous compounds.
Toyo SF-105 series fragrance sensor	Europhor Instruments SA (France)	Coated piezo-electric crystal	ambient	Portable instrument available with optional suction kit for air and gas sampling and optional sensor for use in water.
Alabaster-UV	Europhor Instruments SA (France)	Metal	300-500 °C	Laboratory instrument with digital display and chart output. Sample is placed in a measurement chamber which incorporates ozone generator to remove residual odours.

ELECTRONIC NOSES

A number of groups around the world are actively engaged in research on artificial olfactory systems and their applications to problems in flavour and odour measurement. In each case the general features of the approach are the same, the use of an array of non-specific gas sensors linked to hardware and software to acquire and process the data, Figure 1. In this section we discuss each of the components of an electronic nose in turn, beginning with the sampling method. The particular optimum combination of technologies depends upon the type of sample and the problem to be addressed.

Sample presentation:
The choice of sampling method and sample presentation obviously depends upon the particular application, the form of the odour sample and the instrumentration to be used. Nevertheless it is important to give consideration to this aspect of the problem as it can have an important bearing upon the quality and reproducibility of the results to be obtained. Two obvious approaches are the use of a static headspace system or a flow injection system. In the static headspace system the sensors are either placed into a closed volume with a measured quantity of the sample or are placed in a closed volume of clean air into which a measured volume of the odour is injected. This type of approach has the advantage that the absolute amount of sample is well controlled and so measured differences in odour intensity are related to differences in odorant concentration and release. In addition it is relatively easy to control the temperature of the measurement: temperature is an important variable because it can affect both the release of odour from the sample and the response of the individual sensors. In flow injection systems a carrier gas (frequently clean air) is used to carry the odour from the sample to the sensor array. It this respect the arrangement is more like the mammalian system in which the flow of air carries the odour to the olfactory epithelium. Depending upon the design and complexity of the flow injection system this may allow control of the odour concentration reaching the

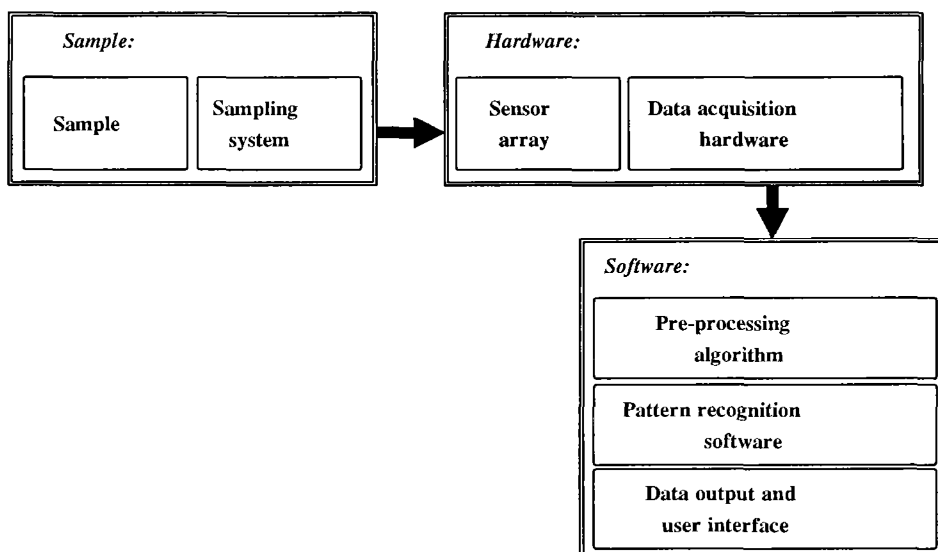

Figure 1. Block diagram of an electronic nose.

sensor array. The flow-rate and form of the sample are also likely to be important considerations in this type of system.

Sensor array:
Various different types of gas sensor have been used to make arrays for application in electronic noses and these are summarised in Table 2. The basic requirements for the individual sensor elements are that they should respond to a range of simple, volatile organic molecules. In addition the sensors must have broadly overlapping sensitivities: if all the sensor elements in the array respond in an identical manner the array will be unable to discriminate between odours. It is also important that the sensitivity of the sensor elements be matched to the typical concentrations of volatiles to be measured; in practice this means sensitivities at the ppm and sub-ppm level.

Practical considerations dictate that the response times of the sensor elements be in the range of seconds to minutes in order to obtain an acceptable measurement time. This constraint applies to both the "on" and "off" responses since it is also essential that the array recovers after exposure to the test sample in order to be ready for use with the next sample. Implicit in this is the requirement that the sensors show reversible responses - the exposure of the array to one sample should not affect its response to subsequent samples.

In order to make devices which can be replicated it is essential that the individual sensor elements be reproducibly manufactured so that they have reproducible response characteristics. A particular problem to be faced in the development of an electronic nose is the problem of calibration and training. For a large sensor array this represents a significant investment in time and effort and for commercial applications it is essential that the calibration and training data derived from one array be portable to other, nominally identical, arrays so that replicate instruments can be constructed without the need to calibrate each instrument individually.

TABLE 2. Examples of Sensor Arrays

Sensor type and number	Principle of operation	Application	Ref.
Commercial tin oxide (Figaro)	Change in conductivity when operated at high temperature (ca. 300 °C). Sensitive to combustible organics.		
8 element		Classification of 47 compounds by their odour class (eg. minty, ethereal, pungent, etc.).	[6]
6 element		Discrimination of 5 types of whiskey.	[7]
12 element		Coffee blends and roasts.	[8]
Coated quartz crystal resonator	Measurement of mass change on adsorption of species into coatings		
6 element		Discrimination of different alcoholic beverages. Data analysed by artificial neural net.	[9]
Lipid coated multi-channel electrodes	Measurement of shift in membrane potential		
8 element		Use to measure taste (sweet, sour, sugar, bitter 'umami'). Data analysis by PCA.	[10]
Large area FET with various metals	Measurement of photocapactiative current		
Equivalent to 324 element array		Used to produce "odour images" for simple vapours (ethanol, ammonia etc.).	[11]
Amperometric electrochemical sensors	Measurement of catalytic current		
4x4 element		Portable device for toxic vapour identification.	[12]
Polymer chemoresistors	Measurement of change		
12 element		Used to discriminate different beers.	[13]
20 element		Various applications.	[14]

In addition to these essential features there are a number of other properties of the sensor elements which, whilst not essential, are highly desireable. For instance, it is desireable that the sensors be stable and not subject to large drifts in their response. It is possible to alleviate some of the problems caused by drift by suitable signal processesing and pattern recognition techniques [15] but it is desireable, if possible, to minimise the problem to start with. It is also desirable to have sensors with inherently linear, or near-linear, responses since the majority of pattern recognition methods are linear.

Finally there are several features which are desireable from the practical viewpoint. First, the devices should not be too large so that sample volumes are not too great. Second, the power consumption of the sensors should, if possible, be low so that the device is portable. Third it is desireable that the fabrication process used to make the sensors be compatible with the maunfacture of arrays comprising a reasonable number of sensor elements.

Of the various sensor types listed in Table 2 our work has concentrated on the use of arrays of metal oxide conductimetric sensors and on the use of arrays of conducting polymer chemoresistors [16].

Hardware and pre-processing algorithms:
Associated with the sensors it is obviously essential to have suitable hardware to control the sensors and acquire the data from them. This may include measurement of other parameters, such as temperature, flow rate or humidity, as appropriate.

Having acquired the data from the sensor array it is important to give some thought to pre-processing the data before feeding it into the pattern recognition software. Various pre-processing parameters have been used including difference models [17] where the sensor parameter used is the difference in resistance (or conductance) in the sample and in air (R_{sample}-R_{air}), relative models [18] in which the ratio of the resistance (or conductance) in air and the sample is used (R_{air}/R_{sample}), and fractional difference models [19] using the fractional resistance (or conductance) change ($\{R_{sample}$-$R_{air}\}/R_{air}$). In addition log parameters are sometimes used to linearise the non-linear concentration response of some gas sensors. The choice of optimal pre-processing method depends on the type of sensor and its response characteristics.

Pattern recognition:
The output from the pre-processor is fed into the pattern recognition software. There are two basic types of pattern recognition architecture [20,21]; supervised and unsupervised. In supervised pattern recognition systems, the pattern recognition module attempts to separate out the known odour inputs into distinct classes or groups. This supervised pattern recognition system learns the input patterns, or paradigms, and associates them with particular sample types (eg. particular coffee types). In unsupervised pattern recognition schemes, the pattern recognition software attempts to identify the sample by comparision of the input pattern with a knowledge base, previously derived by supervised pattern recognition techniques. Figure 2 summarises the two approaches.

There are a range of different pattern recognition techniques which can be used [for a review see 20 and 21]. These include classical linear pattern recognition methods such as principal component analysis [22], feature weighting [23], Euclidean cluster analysis [22] and discriminant function analysis [8] as well as techniques based on the use of artificial neural networks [24].

Resume:
The ability of an electronic nose to sense odours depends on the choice of sampling method, the sensor type and number, the pre-processing method chosen and finally the pattern recognition techniques selected. From this it will be clear that there is no such thing as "the electronic nose" rather there are a whole range of application specific electronic noses (ASENs) which can be put together by assembling and selecting the basic component parts and which can be designed and tailored towards particular applications and problems. We will now move on to descibe some of these applications and the results obtained.

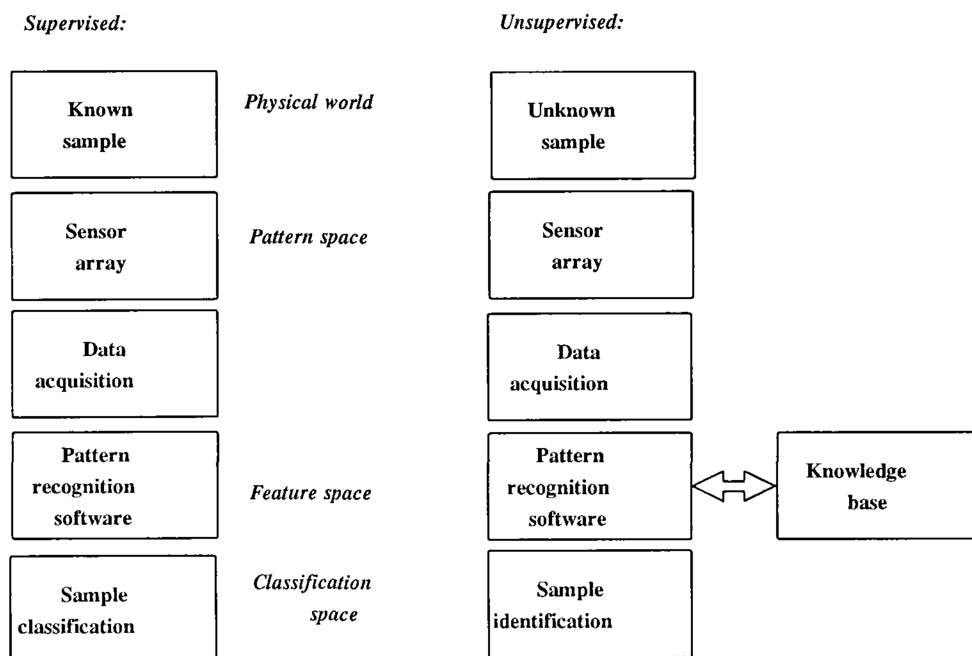

Figure 2. Generalised pattern recognition architectures.

APPLICATIONS OF ELECTRONIC NOSES

The number, and range, of applications of electronic noses continues to increase. In Table 2 we have given just a few representative examples of sensor arrays and their applications. In this section we consider our results for some application areas in more detail.

Coffee Odour:

Several groups have looked at the application of gas sensor arrays to the discrimination of coffees and coffee odours. These studies have made use of arrays of metal oxide [8,25,26] or conducting polymers [13,27] resistance sensors. Aishima used an array of 6 commercial metal oxide gas sensors (Figaro) in a static system to discriminate four different coffee samples comprising a freeze dried instant coffee, a spray dried instant coffee, a ground coffee sample of *Coffea arabica* and a ground coffee sample of *Coffea robusta* [25]. In a subsequent study the same array was used to discriminate the aroma of different varieties of coffee beans (6 varieties of *Coffea arabica* and 2 varieties of *Coffea robusta*) and to discriminate 5 different roasting levels for a given variety [26]. Using hierarchical cluster analysis he was able to distinguish the 8 varieties and the different roasting levels.

Gardner *et al.* [8] have reported similar results using an array of 12 commercial metal oxide sensors (Figaro) in a static system. In this case they studied three different commercial coffees and the effect of roasting level on one particular coffee. Using discriminant function analysis they were able to distinguish the different coffees at the 90% confidence level, figure 3, and to see the effects of roasting time on the pattern of responses from the sensors. Subsequent studies comparing the use of a 12 sensor metal oxide array with a 12 sensor conducting polymer array in a static system to discriminate Brazilian and Columbian coffee samples [27] showed that better results could be obtained using the polymer array, figure 4. This difference in the performance of the two types of sensor array arises because the polymer sensors are responsive to different chemical features of the odorant molecules which make up the coffee aroma and because the responses of the conducting polymer sensors to the coffee odours are less strongly correlated than the metal oxide responses. This illustrates the need to optimise the choice of sensors for the particular problem.

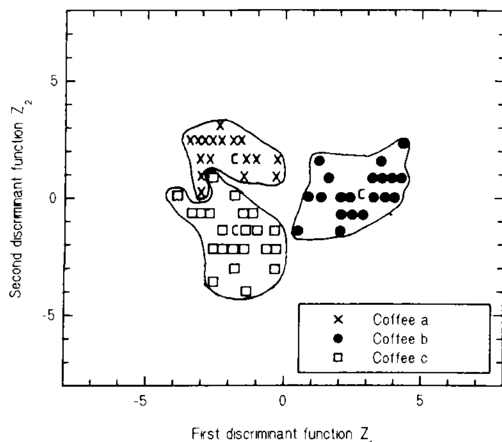

Figure 3. Results of discriminant function analysis and classification of three commercial coffee samples obtained using a 12 element metal oxide array (from [8]).

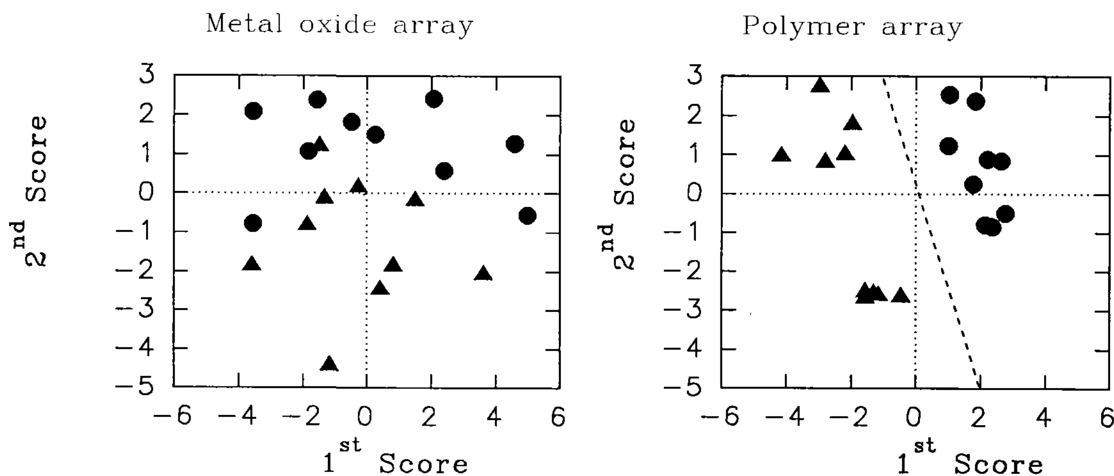

Figure 4. Principal components analysis of autoscaled relative conductance data from 12 element arrays of metal oxide and conducting polymer sensors for the discrimination of Brazilian (▲) and Columbian (●) coffee. The dotted line indicates a hyperplane classification (from [27]).

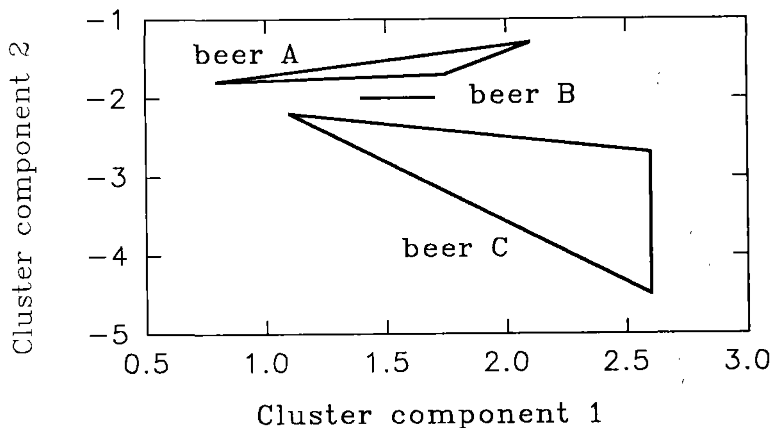

Figure 5. Cluster graph of the response of a 6 element polymer array to samples of three different commercial beers (from [13]).

Beer flavour:
Commercial beer production is a batch process and so inter-batch quality monitoring and control is a significant concern. Beer flavour is a complex problem because of the large number of flavour chemicals present, some with low (ppb) flavour thresholds, in the presence of high concentrations of water and ethanol. Studies of beer flavour show that there are over 100 separately identifiable flavour elements of which 39 or so are present in most beers. We have been involved in the study of beer flavours and beer flavour monitoring using an arrays of conducting polymer gas sensors [13]. We have used theese arrays to distinguish 3 different commercial brands of beer using cluster analysis with high degree of success, figure 5. In addition we have been able to identify tainted beer and to distinguish this from the control with an 80% success level.

FUTURE PROSPECTS

The results obtained to date by various groups, including our own, have clearly shown that arrays of non-specific gas sensors linked to suitable pattern recognition algorithms can make useful distinctions on the basis of odour or flavour in the case of beer, coffee and other foodstuffs and beverages. The prospects for the application of this technology are very good and we can expect the first commercial multi-sensor instruments to become available in the near future.

At the same time there are a number of areas which should now be addressed in order to take the technology forward. First, the design and fabrication of sensors and sensor arrays. The sensors which are presently available are comparatively crude and much scope exists for their improvement. The combination of micromachined sensor arrays with low power CMOS circuitry should lead to small, low cost, portable electronic noses. Improvements in the design and operation of the sensors will lead to better performance which will translate into improved discrimination and reliability of electronic noses. Second, improved methods for sample presentation so that more reproducible odour sampling is possible. Third, the development of multivariate calibration routines to be used with these arrays. Fourth, the development of suitable user interface to present the multivariate data in a form which is appropriate and helpful for the operator.

SUMMARY

Arrays of non-specific gas sensors linked, through suitable hardware, to pattern recognition software can be used to discriminate complex odours from foodstuffs and beverages and to make distinctions between different coffee samples and roasting levels. The design of these sensor array devices, or "electronic nose", depends upon the selection and optimisation of the sample presentation, choice of sensor type and number of sensors, selection of suitable pre-processing methods and choice of pattern recognition technique. The optimum configuration of the system depends on the particular application and problem addressed.

RÉSUMÉ

Des matrices de détecteurs non spécifiques de gaz, connectées au moyen d'instruments appropriés à un programme de reconnaissance de formes, sont utilisées pour séparer de complèxes odeurs émanant de produits alimentaires et pour distinguer différents échantillons de café ainsi que différents niveau de grillage. La conception de la matrice de capteurs, le nez électronique, depend de la sélection et de l'optimisation de la présentation de l'échantillon, du choix du type de capteurs et de leur nombre, de la sélection de méthodes de prétraitment des signaux et enfin du choix de l'algorithme de reconnaissance des formes. La configuration optimale du système depend de l'application choisie et du problème à résoudre.

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DÉTERMINATION DE LA CAFÉINE ET DE LA MATIÈRE SÈCHE PAR SPECTROMÉTRIE PROCHE INFRAROUGE. APPLICATIONS AUX CAFÉS VERTS ROBUSTA ET AUX CAFÉS TORRÉFIÉS

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I INTRODUCTION

A l'heure actuelle, il n'existe pas de méthodes rapides de dosage de la caféine. Les méthodes courantes utilisées sont basées soit sur la spectrométrie UV (1,2,3) soit sur la chromatographie en phase gazeuse (4) ou liquide (5,6,7).

Ces méthodes, même si les techniques analytiques ont progressé ces dernières années, nécessitent toujours plusieurs étapes : extraction, purification et analyse, étapes qui exigent toutes une durée relativement longue, impliquant l'impossibilité d'obtenir un résultat dans un délai très court.

La spectrométrie par réflexion dans le Proche Infrarouge est une technique rapide, non destructive, demandant une préparation simple de l'échantillon et qui est déjà appliquée à de très nombreux dosages : détermination de la teneur en matière sèche, matière grasse, protéine, sucre, etc.. dans les céréales ou dans des graines entières.(8,9,10,11)

Son application au dosage de la caféine et de la matière sèche dans les fèves de café est plus récente et seuls quelques auteurs (12,13) ont travaillé sur ce sujet.

Notre étude a pour but :

- d'établir des équations d'étalonnage applicables au café vert ROBUSTA et à tous les mélanges de cafés torréfiés (ARABICA/ROBUSTA) quelle que soit leur origine géographique.

- de présenter les résultats de fiabilité de la spectrométrie par réflexion dans le Proche Infrarouge par rapport aux méthodes physico-chimiques de référence.

II MATERIEL ET METHODES.

1) ECHANTILLONS.

Cafés verts.

Nous avons utilisés des échantillons de cafés verts (n=231) *Coffea robusta* originaires majoritairement de Côte d'Ivoire (n=189) et d'autres pays comme l'Indonésie, Togo, Ouganda, Centre Afrique, Gabon, Brésil et Vietnam.(n=42). Leur teneur en caféine varient de 1,5 à 4,3 %.

Cafés torréfiés.

Les échantillons de café torréfié (n=155) sont :

- des échantillons commerciaux.
- des échantillons préparés au laboratoire en particulier pour les mélanges ARABICA / ROBUSTA.

Leur teneur en caféine varient de 0,7 à 3,3%.

2) APPAREILLAGE.

Les mesures de spectres dans le proche Infrarouge sont effectuées avec un appareil NIRSystems 4500 PERSTORP couplé avec un ordinateur INTEL i386 302-20 ; l'ensemble du système est exploité par le logiciel ISI205.

Analyses Proche Infrarouge :

Les mesures d'absorption dans le proche Infrarouge sont obtenues par réflexion entre 1300 et 2400 nm sur les différents échantillons, mis sous forme de poudre. La caféine pure présente une bande d'absorption comprise entre 2160 et 2320 nm avec un maximum à 2250 nm.

3) ANALYSES CHIMIQUES : METHODES DE REFERENCE.

Pour établir les équations d'étalonnage, tous les échantillons ont été analysés en utilisant deux méthodes chimiques de référence :

Détermination de la teneur en matière sèche.

La matière sèche des cafés est déterminée d'après la norme AFNOR NF V 05-202.

Détermination de la teneur en caféine.

La teneur en caféine des différents cafés verts et torréfiés est déterminée par la méthode publiée par VITZTHUM (4). Cette méthode est basée sur une :

- extraction de la caféine en milieu basique (MgO).
- détermination quantitative par chromatographie en phase gazeuse. Cette détermination est effectuée par un détecteur NP-FID.

III RESULTATS ET DISCUSSIONS.

DETERMINATION DES EQUATIONS D'ETALONNAGE.

Les équations d'étalonnage sont développées à l'aide du logiciel ISI205.

A) Café vert ROBUSTA.

Trois séries d'équations d'étalonnage correspondant à la matière sèche et à la caféine ont été développées à partir de 231 échantillons analysés chimiquement et constituant trois fichiers:

- échantillons toute origine. (Equations I)
- échantillons même origine (Côte d'ivoire). (Equations II)
- échantillons sélectionnés. (Equations III)

Les équations III sont développées à partir d'un fichier constitué de 70 échantillons sélectionnés de manière statistique et ayant pour origine la Côte d'Ivoire. La sélection est effectuée en imposant une limite (H=0,6) aux "distances de Mahalanobis" dans l'algorithme permettant de structurer le fichier et de sélectionner les échantillons.

Les conditions de calcul concernant le traitement mathématique appliquées à la méthode de régression MPLS sont résumées par le Tableau I.

Tableau I : Conditions mathématiques utilisées par la méthode de régression MPLS.(C. ROBUSTA).

Conditions spectrales	Dérivée	Intervalle	Lissage	Nbre de termes de l'équation
1300-2400 nm	2	4	4	7

Le traitement mathématique est effectué à partir de la dérivée seconde des spectres enregistrés et exprimés en fonction de Log1/R. La dérivée est calculée tous les 4 nm de même que le lissage. Le nombre de termes de l'équation est fixé à 7.

Les caractéristiques statistiques des équations établies à partir des différents fichiers sont résumées Tableau II.

Tableau II : Caractéristiques statistiques des équations obtenues à partir des différents fichiers.

ECHANTILLONS TOUTE ORIGINE (I)

CONSTITUANTS	ECH.	MOY.	SEC	R^2	SEVC	1-RV
MAT. SECHE	219	93,18	0,10	1,00	0,12	1,00
CAFEINE	223	2,66	0,10	0,95	0,11	0,94

ECHANTILLONS MEME ORIGINE (II)

CONSTITUANTS	ECH.	MOY.	SEC	R^2	SEVC	1-RV
MAT. SECHE	177	93,57	0,10	1,00	0,10	1,00
CAFEINE	186	2,69	0,10	0,95	0,11	0,94

ECHANTILLONS SELECTIONNES (III)

CONSTITUANTS	ECH.	MOY.	SEC	R^2	SEVC	1-RV
MAT. SECHE	67	93,20	0,10	1,00	0,13	0,99
CAFEINE	69	2,59	0,08	0,97	0,11	0,94

Echantillons : Nombre d'échantillons sélectionnés à partir du fichier pour établir les équations d'étalonnage.

SEC : Erreur standard de calibration. (Erreur de la différence entre la valeur obtenue par la méthode de référence et la valeur obtenue par la méthode Proche Infrarouge).

SEVC : Erreur standard de validation.

1-RV : Grandeur assimilable au coefficient de détermination R^2.(RV est le rapport de la variance expliquée sur la variance totale).

Les caractéristiques des équations II sont très proches de celles développées à partir du fichier complet.(Equations I). Pour les équations III elles sont comparables à celles des équations précédentes. Le résultat est même sensiblement amélioré pour la caféine, (R^2=0,97 et SEC=0,08) ce qui semble logique car la population des échantillons est plus homogène et de même origine.

Les performances des équations développées peuvent être évaluées par rapport aux différents fichiers par les données obtenues :

- déviation standard. (SD)
- coefficient de détermination.(R^2)
- erreur standard de prédiction. (SEP)

en comparant les valeurs prédites par les équations à celles obtenues par les méthodes de référence.

EQUATIONS I : Les résultats obtenus par cette régression sont résumés Tableau III .

Tableau III : Performances des équations I établies par rapport au fichier toute origine.

CONSTITUANTS	**ECH.**	**METH.**	**MOY.**	**SEP**	**SD**	**R^2**
MAT. SECHE	**231**	**REF.**	**93,11**		**1,81**	
		NIR	**93,12**	**0,14**	**1,80**	**0,99**
CAFEINE	**231**	**REF.**	**2,65**		**0,45**	
		NIR	**2,65**	**0,10**	**0,44**	**0,95**

Les résultats obtenus sont excellents pour la détermination de la matière sèche (SEP=0,14, R^2=0,99). Pour la caféine, les résultats sont bons (SEP=0,10, R^2=0,95), avec des valeurs de SD comparables entre les deux méthodes.

Les équations des droites de régression sont les suivantes :

Mat. sèche : Y=1,00X-0,24

Caféine : Y=0,99X+0,01

EQUATIONS II : Les performances des équations II vérifiées à partir du fichier Côte d'Ivoire et du fichier toute origine sont résumées tableau IV.

Tableau IV : Performances des équations II établies par rapport au fichier Côte d'Ivoire et au fichier toute origine.

CONSTITUANTS	ECH.	METH.	MOY.	SEP	SD	R^2
MAT. SECHE	189	REF.	93,51		1,72	
	CI	NIR	93,52	0,14	1,71	0,99
	231	REF.	93,11		1,81	
	FTO	NIR	93,12	0,16	1,80	0,99
CAFEINE	189	REF.	2,68		0,45	
	CI	NIR	2,69	0,10	0,44	0,95
	231	REF.	2,65		0,45	
	FTO	NIR	2,65	0,11	0,43	0,94

D'après le Tableau IV, les performances des équations II établies à partir du fichier Côte d'Ivoire sont comparables à celles des équations précédentes I établies à partir du fichier toute origine. Les valeurs de SEP obtenues par les équations I et II par rapport au fichier toute origine sont pratiquement identiques pour la matière sèche (0,14 et 0,16) et la caféine (0,10 et 0,11). Il en est de même pour les valeurs de SD : (1,71 et 1,80) pour la matière sèche et (0,44 et 0,43) pour la caféine. De plus ces valeurs de SD sont comparables à celles obtenues par les méthodes de référence.

Les équations des droites de régression sont les suivantes :

Fichier Côte d'Ivoire :

Mat. sèche : Y=1,00X-0,37

Caféine : Y=1,00X+0,01

Fichier toute origine :

Mat. sèche : Y=1,00X-0,14

Caféine : Y=1,00X

EQUATIONS III : Les performances des équations III par rapport aux trois fichiers existants sont résumées Tableau V.

Tableau V : Performances des équations III établies par rapport au fichier constitué d'échantillons sélectionnés, au fichier Côte d'Ivoire et au fichier toute origine.

CONSTITUANTS	ECH.	METH.	MOY.	SEP	SD	R^2
MAT. SECHE	70	REF.	93,05		2,03	
	SEL	NIR	93,05	0,14	2,00	1,00
	189	REF.	93,51		1,72	
	CI	NIR	93,51	0,14	1,71	0,99
	231	REF.	93,11		1,81	
	FTO	NIR	93,12	0,16	1,79	0,99
CAFEINE	70	REF.	2,59		0,47	
	SEL	NIR	2,59	0,08	0,46	0,97
	189	REF.	2,68		0,45	
	CI	NIR	2,66	0,12	0,43	0,93
	231	REF.	2,65		0,45	
	FTO	NIR	2,63	0,13	0,42	0,92

Dans le cas de la matière sèche, les performances de l'équation III calculée à partir du fichier constitué d'échantillons sélectionnés sont pratiquement identiques aux performances de l'équation I testée sur le fichier global et aux performances de l'équation II testée sur le fichier Côte d'Ivoire.

Pour la caféine, les performances de l'équation III sont légèrement inférieures à celles des équations I et II dans les mêmes conditions mais restent cependant très acceptables.

Dans ces conditions expérimentales, les équations des droites de régression sont les suivantes :

Fichier échantillons sélectionnés :

Mat. sèche : $Y=1,01X-1,05$

Caféine : $Y=0,99X+0,02$

Fichier Côte d'Ivoire :

Mat. sèche : $Y=1,01X-0,70$

Caféine : $Y=1,02X-0,03$

Fichier entier :

Mat sèche : Y=1,01X-0,58

Caféine : Y=1,02X-0,04

B) Cafés torréfiés.

Deux série d'équations d'étalonnage ont été développées à partir d'un fichier comprenant 155 spectres d'échantillons de cafés torréfiés analysés, de composition variable en café ARABICA et ROBUSTA. Les teneurs en caféine varient de 0,57 à 3,27%.

- Equations I développées à partir du fichier complet : Tous les échantillons sont pris en compte.

- Equations II développées après structuration du fichier et sélection des échantillons : Ces équations sont développées à partir de 34 échantillons du fichier global, sélectionnés de manière statistique par le logiciel ISI205.

Les caractéristiques statistiques des équations I et II obtenues à partir des fichiers sont résumées Tableau VI.

Tableau VI : Caractéristiques statistiques des équations I et II obtenues à partir des fichiers.

FICHIER COMPLET (I)

CONSTITUANTS	ECH.	MOY.	SEC	R^2	SEVC	1-RV
MAT. SECHE	150	96,85	0,10	0,99	0,14	0,98
CAFEINE	148	1,86	0,06	0,99	0,08	0,98

FICHIER ECHANTILLONS SELECTIONNES (II)

CONSTITUANTS	ECH.	MOY.	SEC	R^2	SEVC	1-RV
MAT. SECHE	34	97,54	0,11	0,99	0,22	0,97
CAFEINE	34	2,05	0,08	0,99	0,13	0,97

Les caractéristiques statistiques des équations I sont excellentes pour les deux constituants et en particulier pour la caféine.(R^2=0,99 et SEC=0,06). Il en est de même pour les caractéristiques des équations II malgré le petit nombre d'échantillons sélectionnés.

Ces résultats sont confirmés par les performances de ces équations testées sur le fichier global. Les résultats sont résumés Tableaux VII et VIII.

Tableau VII : Performances des équations I établies par rapport au fichier global. (cafés torréfiés).

CONSTITUANTS	ECH.	METH.	MOY.	SEP	SD	R^2
MAT. SECHE	155	REF.	96,86		1,27	
		NIR	96,86	0,13	1,26	0,99
CAFEINE	155	REF.	1,86		0,59	
		NIR	1,86	0,07	0,59	0,99

Les équations des droites de régression sont les suivantes :

Mat. sèche : Y=1,00X-0,09

Caféine : Y=1,00X+0,01

Tableau VIII : Performances des équations II calculées à partir d'échantillons sélectionnés et testées sur le fichier global. (cafés torréfiés).

CONSTITUANTS	ECH.	METH.	MOY.	SEP	SD	R^2
MAT. SECHE	155	REF.	96,86		1,27	
		NIR	96,86	0,14	1,28	0,99
CAFEINE	155	REF.	1,86		0,59	
		NIR	1,84	0,13	0,59	0,95

Les équations des droites de régression sont les suivantes :

Mat. sèche : Y=0,99X+1,14

Caféine : Y=0,98X+0,06

Les résultats sont excellents pour la détermination de la matière sèche (R^2=0,99 et SEP=0,14) et comparables à ceux obtenus avec l'équation I établie à partir du fichier global. Pour la caféine, les résultats sont légèrement inférieurs (R^2=0,95 et SEP=0,13) par rapport à ceux obtenus précédemment(R^2=0,99 et SEP=0,07) mais restent très acceptables.

IV CONCLUSION.

La détermination des teneurs en matière sèche et en caféine sur cafés verts ROBUSTA et sur cafés torréfiés par la spectrométrie par réflexion dans le Proche Infrarouge est possible et les résultats obtenus peuvent être considérés comme fiables. Les valeurs obtenues de SD par cette technique sont identiques ou comparables, dans tous les cas, à celles obtenues par les méthodes de référence.

Les résultats de cette étude ont apporté des améliorations par rapport à ceux déjà publiés.

Ces améliorations ont deux origines possibles :

- dans le cas du café vert, nous avons utilisé uniquement des cafés verts de l'espèce *Canephora* (Variété *Robusta*) alors que les travaux précédents ont été effectués à partir d'échantillons de cafés de deux espèces différentes *Arabica* et *Canephora*.

- dans le cas des cafés torréfiés, la gamme des teneurs en caféine de nos échantillons est plus restreinte, de 0,57 à 3,27%, par rapport celle utilisée dans l'étude précédente qui variait de 0,01 à 3,6% ; la présence d'échantillons de cafés torréfiés à faible teneur en caféine (cafés décaféinés) n'améliore pas les performances des équations d'étalonnage.

De ces constatations, il en résulte que, pour obtenir des résultats fiables, il faut travailler avec des courbes d'étalonnage spécifiques aux produits.

RESUME

A l'heure actuelle, il n'existe pas de méthodes de dosage rapides et fiables de la caféine et de la matière sèche sur cafés verts et torréfiés. L'utilisation de la spectrométrie par réflexion dans le Proche Infrarouge, technique rapide, non destructive et demandant une préparation simple des échantillons, a permis d'établir des courbes d'étalonnage applicables aux cafés verts ROBUSTA et à tous les mélanges de cafés torréfiés (ARABICA/ ROBUSTA), quelle que soit leur origine géographique.

Les résultats peuvent être considérés comme fiables et les valeurs de déviation standard (SD) obtenues par cette technique physico-chimique sont comparables à celles trouvées lors de l'utilisation des méthodes chimiques de référence. Les coefficients de détermination R^2 varient de 0,95 à 0,99 suivant les équations développées.

L'utilisation de courbes d'étalonnage spécifiques aux produits considérés permet d'obtenir une meilleure précision des mesures.

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QUELQUES ACIDES PHÉNOLS PARTICULIERS DES FÈVES DE CAFÉIERS SAUVAGES MALGACHES ET AFRICAINS

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INTRODUCTION

L'expression du métabolisme secondaire chez les végétaux peut être considérée comme le résultat d'un processus de différenciation. Ainsi, les composés phénoliques qui constituent l'un des groupes les plus importants et les plus diversifiés des produits secondaires sont-ils étudiés entre autres comme des marqueurs de la diversité biochimique des espèces. Bien que les flavonoides soient les plus utilisés (Harborne 1984), les dérivés des acides phénols ont permis par exemple récemment de préciser la classification chez le genre *Vitis* (Boursiquot et al. 1986)
Les études chemiotaxinomiques du sous genre *Coffea* sont limitées malgré le nombre élevé d'espèces connues, et celles incluant les souches sauvages de la région malgache demeurent très fragmentaires. Elles concernent en particulier les lipides (Chassevent et al. 1974), les purines (Ornano et al. 1967, Chassevent et al. 1974, Charrier & Berthaud 1975, Clifford et al. 1991, Rakotomalala et al. 1992), la trigonelline (Ornano et al. 1967), les terpènes (Chassevent et al. 1967) et les acides chlorogéniques (Chassevent et al. 1974, Colonna 1979, Anthony et al. 1989, Clifford et al. 1989a). Cependant, ces études n'ont porté que sur un nombre très limité de caféiers et ne concernent pratiquement que des mesures de teneurs globales.

Afin de préciser la diversité biochimique des caféiers (Rakotomalala 1992), nous avons effectué une analyse systématique des dérivés hydroxycinnamiques des graines de fruits matures de 56 populations de caféiers sauvages de la région malgache (*Mascarocoffea*) et de 14 échantillons représentant 9 espèces africaines (*Eucoffea*).
Nous présentons ici, les résultats concernant la caractérisation de ces composés.

MATÉRIEL ET MÉTHODES

Matériel végétal : graines de fruits matures de 56 populations malgaches représentatives des 7 séries botaniques (*Garcinioides*, *Humblotianae*, *Mauritianae*, *Millotii*, *Multiflorae*, *Subterminales*, *Verae*)

définies par Chevalier (1938) et Leroy (1967) et de 14 échantillons représentant 9 espèces africaines (*canephora, arabica, kapakata, liberica, congensis, racemosa, sessiliflora, pseudozanguebariae, eugenioides*).

Extraction : 1 à 2 g de café vert moulu sont extraits 4 fois successivement par 25 ml d'éthanol 80%, pendant 1 h sous agitation magnétique à température ambiante. Après évaporation de l'alcool, la phase aqueuse est lavée par l'éther de pétrole (40-60) puis extraite par l'acétate d'éthyle selon Fleuriet & Macheix (1972). La phase organique est évaporée à sec et le résidu repris par 10 ml de méthanol et analysé par CLHP.
Hydrolyse alcaline : par l'hydroxyde de tétraméthylammonium (TMAH) selon Clifford (1989b) pendant 5 min (condition ménagée) ou 90 min à température ambiante. Les esters méthyliques obtenus sont analysés par CLHP.
L'hydrolyse classique par NaOH 2N (concentration finale) à température ambiante n'a été employée que rarement, elle conduit en effet à des résultats quantitativement très variables.
Hydrolyse acide : par HCl 2N (concentration finale) à 100°C pendant 1 à 2 h. Réalisée en milieu méthanolique, l'hydrolyse conduit à l'accumulation des esters méthyliques des acides phénols comme dans le cas de l'hydrolyse alcaline.

Analyses chromatographiques : chromatographe Varian 5000, colonne LiChrospher 5μm RP18, gradient de 5% à 75% de méthanol dans H_3PO_4 2mM à 100% de méthanol en 35 min, débit 1 ml min^{-1}, détecteur à barrette de diodes HP 1040A.
Couplage CLHP-SM : chromatographe HP 1050, spectromètre HP 5989A MS engine, interface particle beam, mode EI à 70 eV, station Unix HP 98785. Le gradient linéaire de méthanol dans l'eau est adapté à l'analyse.
Couplage GC-MS : chromatographe HP 5890, colonne OV1, 50 m x 0,25 mm, He 1,5 ml min^{-1}, injecteur 280°C, four de 80°C à 150oC (4°C/min), détecteur HP-MSD 5971, station Unix HP 98785.

Composés témoins :
Esters quiniques : acides chlorogénique et dicafeylquinique, acide férulyl-5-quinique, monoesters quiniques de l'acide p-coumarique (isolés de la pomme par A. Fleuriet).
Acides libres : caféique, férulique, isoférulique, cis et trans o-coumarique, p- coumarique, cinnamique, sinapique, 4-méthoxycinnamique, 3,4,5-triméthoxycinnamique, etc ...
Esters méthyliques : férulate, cinnamate, caféate. Les autres esters ont été préparés par estérification en milieu acide dans le méthanol.
Autres : coumarine, acide trans 2-β-glucosyloxycinnamique.

RESULTATS

Caféiers cultivés

Les profils chromatographiques de *C. canephora* déterminés à 280 et 324 nm (fig 1) sont tout à fait représentatifs, en ce qui concerne les composés majeurs, de ceux obtenus avec les caféiers originaires du continent africain. Sur les 18 composés caractérisés, 9 représentent plus de 90% (aire) du mélange.
Composés identifiés
L'acide caféyl-5-quinique (acide chlorogénique - pic 3) est le composé le plus important du mélange (35 à 55% de l'aire totale). Les acides caféyl-3-quinique (pic 1) et caféyl-4-quinique (pic 2) ont été identifiés par comparaison de leurs temps de rétention et de leurs spectres UV avec les isomères obtenus par transestérification (TMAH) de l'acide chlorogénique.
Les acides férulyl-5-quinique (pic 5), dicaféyl-3,4-quinique (pic 6), dicaféyl-3,5-quinique (pic 7) et dicaféyl-4,5-quinique (pic 8) ont été identifiés par comparaison (tr, UV) avec des composés témoins.

Le caféyltryptophane (pic 9) a été caractérisé à partir des données chromatographiques et spectrales décrites par Morishita et al. (1987).
Composés présumés
C. canephora : par analogie avec les résultats de Morishita et al. (1986) et de Trugo & Macrae (1984), et par la similarité de leurs spectres UV avec celui de l'acide férulique, les pics A et C ont été respectivement attribués aux acides férulyl-3-quinique et férulyl-4-quinique. Les pics F, G et I sont supposés correspondre aux acides cafeoyl-féruloyl-quinique et avec beaucoup de réserve H pourrait correspondre au caféoyltryptophane.
C. arabica : le pic C de même temps de rétention que chez *C. canephora* présente un profil spectral identique à celui de l'acide p-coumarylquinique. Par comparaison avec des extraits de pomme, le pic C a été attribué à l'acide p-coumaryl-5-quinique.

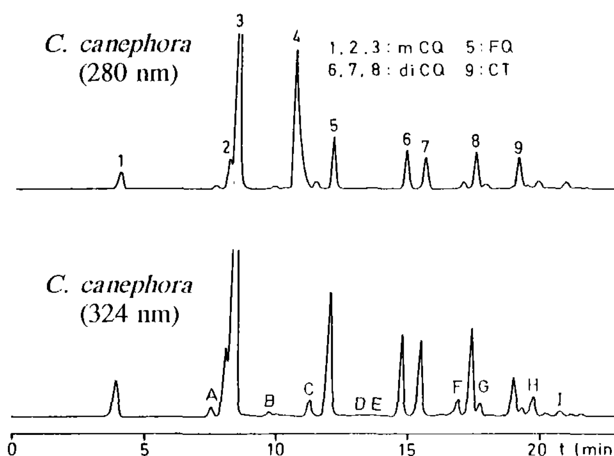

Fig. 1 - Profil chromatographique de l'extrait phénolique brut de *C. canephora* (identification dans le texte).

Caféiers sauvages malgaches et africains

Les caféiers malgaches analysés présentent dans l'ensemble des profils chromatographiques très diversifiés et souvent très complexes (fig. 2). L'analyse des spectres UV montre qu'un pic même symétrique correspond fréquemment à un mélange de composés. L'analyse de la fraction phénolique après hydrolyse acide ou alcaline (fig. 3) permet de mettre en évidence la présence d'acides phénols autres que les acides caféique et férulique décrits dans la littérature (Poisson 1977, Van der Stegen & Van Djuin 1980, Clifford 1985, Clifford et al. 1989a). L'acide chlorogénique est présent dans tous les échantillons alors que nous n'avons pas pu mettre en évidence la présence de dérivés féruliques dans une dizaine de populations.

Acide p-coumarique
Les dérivés de l'acide p-coumarique ont été identifiés tels quels ou caractérisés par la formation de l'ester méthylique correspondant après hydrolyse alcaline.
L'acide p-coumaryl-5-quinique est le composé majeur (fig. 4) de *C. tsirananae* (comparaison avec les isomères des acides p-coumaryl-quiniques extraits de la pomme, accumulation de p-coumarate de méthyle après hydrolyse acide en milieu méthanolique ou alcaline par le TMHA).
Largement distribué chez *Mascarocoffea*, mais généralement en faible quantité, ce composé est accompagné de ses isomères 3- et 4-quinique, très difficiles à mettre en évidence de part la complexité des chromatogrammes.

Acide sinapique
La présence de dérivés sinapiques n'a été confirmée qu'indirectement par identification du sinapate de méthyle après hydrolyse (H+/MeOH, TMHA). Cet ester dont le profil spectral UV est facilement repérable (fig 5) a été caractérisé chez de nombreuses populations, mais nous n'avons jamais pu mettre en évidence les esters sinapiques initialement présents dans l'extrait phénolique brut.

Acide 4-méthoxycinnamique
L'ester méthylique de cet acide a été mis en évidence par couplage GC-MS de l'hydrolysat (TMAH) de

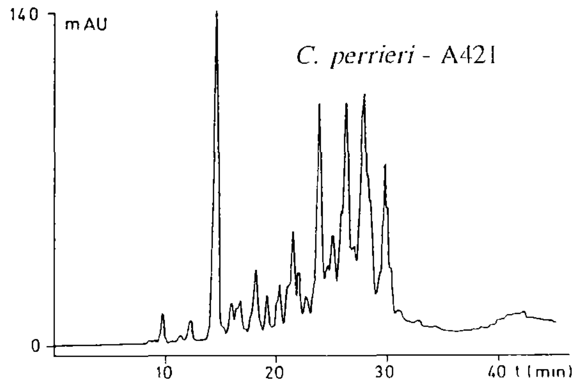

Fig. 2 - Profil chhromatographique de l'extrait phénolique brut de *C. perrieri* A421.

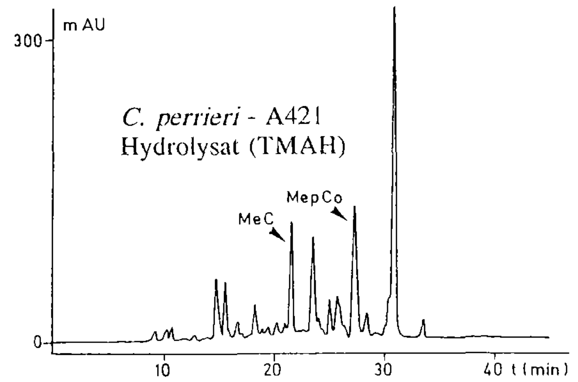

Fig. 3 - Profil chromatographique de l'extrait de A421 après hydrolyse (TMAH).

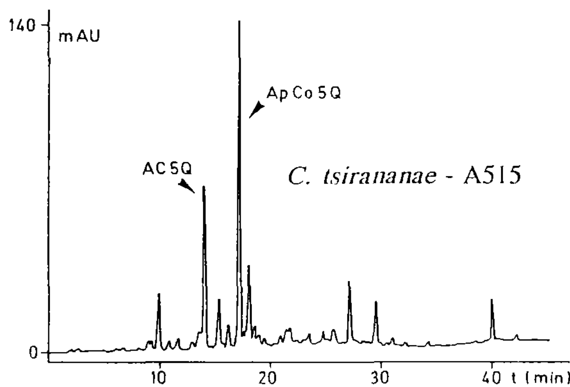

Fig. 4 - Profil chromatographique de l'extrait phénolique brut de *C. tsirananae* (voir texte).

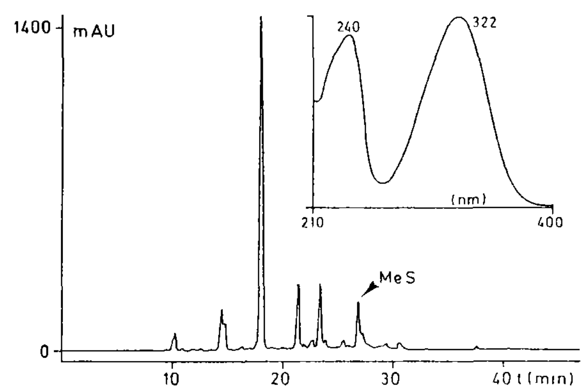

Fig. 5 - Caractérisation du sinapate de méthyle dans l'hydrolysat de *C.andrambovatensis.*

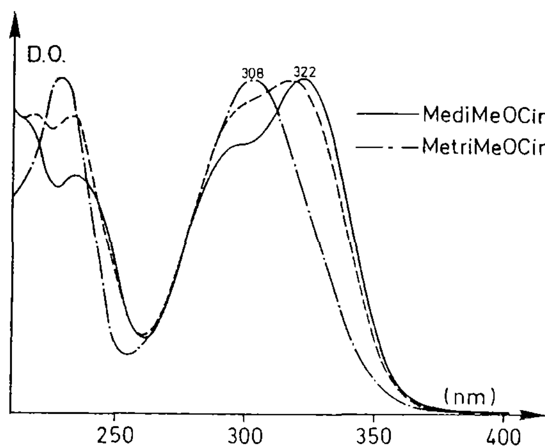

Fig. 6 - Spectres d'absorption des esters méthyliques des acides di et triméthoxy cinnamiques et de leur mélange.

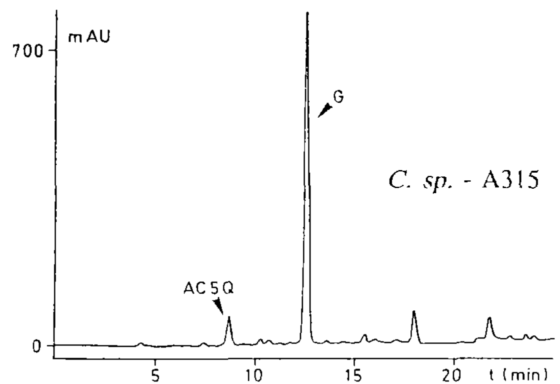

Fig. 7 - Profil chromatographique de l'extrait phénolique brut de *C. sp.* A315.

3 échantillons [m/z : 161 (100), 192 (98), 133 (54), 118 (17), 89 (17), 77 (14)]. Le spectre UV de ce composé est identique à celui du p-coumarate de méthyle.
Nous avons vérifié à partir de témoins d'acides libres et d'acide chlorogénique que les conditions d'hydrolyse et d'analyse ne conduisent pas à des réactions de méthoxylation. Ainsi, sous réserve de confirmation ultérieure, des dérivés p-méthoxycinnamiques sont considérés comme présents dans les extraits de ces caféiers.

Acide 3,4-diméthoxycinnamique
Présents dans une dizaine de populations, l'ester méthylique de cet acide est par exemple trouvé en grande quantité dans l'hydrolysat (H+/MeOH, TMAH) de *C. augagneuri* A519. Sa structure a été confirmée par couplage GC-MS et LC-MS [m/z : 222 (100), 191 (88), 91 (47), 147 (44), 105 (37), 207 (23)].

Acide 3,4,5-triméthoxycinnamique
La caractérisation après hydrolyse de l'ester méthylique de l'acide 3,4,5-triméthoxycinnamique est délicate. En effet, bien que trouvé dans 16 populations, il n'est présent en quantité importante que dans *C. perrieri* ou il est toujours associé avec l'ester de l'acide 3,4-diméthoxycinnamique dont il est difficile de le séparer par chromatographie. Cependant son spectre de masse [m/z : 252 (100), 237 (56), 221 (52), 177 (21), 209 (14), 149 (13)] le rend facilement distinguable du dérivé diméthoxylé.
Dans nos conditions, l'analyse par couplage LC-MS conduit à un spectre résultant de la somme des spectres des dérivés di et triméthoxylés. La sélection d'ions spécifiques permet cependant de caractériser individuellement chacun des dérivés. De même, le spectre UV du mélange des deux esters (fig 6) correspond à la somme des spectres UV des deux composés témoins.

Acide o-coumarique
Trouvé en abondance dans la serie *Multiflorae*, la caractérisation du β - glucoside de l'acide trans o-coumarique ou mélilotoside (composé **G**) a été effectuée comme suit sur l'échantillon A315 (*C. sp*). pour lequel le composé G est largement prédominant (fig. 7).
1) L'hydrolyse de **G** (purifié par CLHP préparative) par la β - glucosidase conduit à la formation de glucose et de l'acide trans o-coumarique. l'hydrolyse par l'α - glucosidase est inopérante.
2) L'hydrolyse alcaline (OH^- et TMAH) n'affecte pas le composé **G**, il ne s'agit donc pas d'un ester glucosidique. Par ailleurs, le spectre UV montre un léger déplacement batochrome (+ 8 nm) indiquant que le groupement carboxyle est libre.
3) L'hydrolyse acide en milieu aqueux fait apparaître après 15 min l'acide trans o-coumarique (56%) et la coumarine (44%). Après 3 heures de réaction, l'acide libre disparait totalement au profit de la coumarine, laquelle est accompagnée des produits de dégradation thermique du glucose (5-hydroxyméthyl furfural et furfurol).
4) L'hydrolyse acide en milieu méthanolique conduit après 1h 30 min à la formation du trans o-coumarate de méthyle (51%) et de la coumarine (49%).
5) Toute tentative d'analyse par spectrométrie de masse en couplage (GC ou LC) ou en introduction directe en mode EI (et même CI^+ ou CI^-/méthane) conduit à l'obtention du spectre de la coumarine.
En fait, les hydrolyses chimiques et la spectrométrie de masse impliquent un traitement thermique entraînant la rupture de la liaison O-glucose et la cyclisation spontanée de l'acide cis o-coumarique (via l'isomérisation trans - cis de l'acide o-coumarique.
6) Le profil chromatographique des extraits de *Melilotus alba* et *Melilotus officinalis* indique la présence de 2 composés majeurs correspondant respectivement au mélilotoside et à la coumarine. La co-chromatographie avec l'extrait phénolique de A315 montre que le composé G se confond avec le mélilotoside et possède les mêmes caractéristiques UV.

La présence de ce glucoside dans les caféiers est remarquable. Chez les légumineuses, la destruction de la cellule met en contact l'isomère cis (formé par photoisomérisation) du mélilotoside avec l'enzyme spécifique cis β - glucosidase pour produire l'acide cis o-coumarique qui cyclise spontanément pour donner la coumarine (Oba et al. 1981, Alibert et al. 1982, Rataboul et al. 1985). Chez les caféiers , malgré la destruction inévitable des structures cellulaires lors du broyage, nous n'avons jamais détecté dans les extraits phénoliques bruts ni l'acide o-coumarique ni la coumarine sous forme libre. Ce phénomène pourrait s'interpréter par l'abscence de la cis β - glucosidase ou par sa présence sous forme inactivée (Brown 1981).

CONCLUSION

Si le complexe phénolique des caféiers cultivés est très homogène, la diversité des dérivés hydroxycinnamiques de l'ensemble des formes spontanées analysées est tout à fait remarquable. En effet si la présence de dérivés de l'acide p-coumarique apparaît comme naturelle, ces dérivés étant considérés comme les plus fréquents chez les végétaux (Bate-Smith 1956), celle des dérivés de l'acide sinapique est beaucoup plus rare bien que leurs teneurs puissent être élevées (Macheix et al. 1990) et celle des dérivés méthoxycinnamiques est rarissime (Forest & Ray 1972).

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Résumé

QUELQUES ACIDES PHENOLS PARTICULIERS DES FEVES DE CAFEIERS SAUVAGES MALGACHES ET AFRICAINS.

L'analyse systématique des dérivés hydroxycinnamiques a été effectuée sur les graines de fruits matures de 56 populations de caféiers sauvages de la région malgache (*Mascarocoffea*) et de 14 échantillons représentant 9 espèces africaines (*Eucoffea*).
Le profil chromatographique (CLHP) de la fraction phénolique totale est généralement complexe et les acides phénols sont dans la majorité des cas caractérisés sous forme d'esters méthyliques (obtenus par hydrolyse : H^+/MeOH, TMAH) par comparaison de leurs temps de rétention, spectres UV et spectres de masse (GC-MS, LC-MS) avec des témoins commerciaux.
Les acides férulique et p-coumarique sont largement représentés dans les échantillons, l'acide caféique l'étant dans tous. Chez *Mascarocoffea*, les acides sinapique et 4-méthoxycinnamique sont en faibles concentrations relatives dans plusieurs peuplements alors que les acides o-coumarique, 3,4-diméthoxycinnamique et 3,4,5-triméthoxycinnamique sont, quand ils sont présents, les composés phénoliques majeurs du mélange.
Les acides phénols sont de bons marqueurs de la diversité biochimique des caféiers.

Sumary

PECULIAR PHENOLIC ACIDS FROM WILD MADAGASCAN AND AFRICAN COFFEES.

An extensive study of hydroxycinnamic derivatives was carried out on beans of mature fruits from 56 populations of wild Madagascan coffee (*Mascarocoffea*) and from 14 samples representing 9 African coffee species (*Eucoffea*).
The HPLC profiles of the raw phenolic extracts were generally complicated, so phenolic acids were in most cases transformed into their methyl esters (obtained after hydrolysis : H^+/MeOH, TMAH). These esters were characterized by comparison of their retention times, UV and mass spectra (GC-MS, LC-MS) with those of authentic commercial samples.
Ferulic and p-coumaric acids were found in most samples and caffeic acid in all samples. In *Mascarocoffea*, sinapic and 4-methoxycinnamic acids were at low relative concentrations in some populations, whereas o-coumaric, 3,4-dimethoxycinnamic and 3,4,5-trimethoxycinnamic acids were, when present, the main phenolics.
Phenolics are good markers of the biochemical diversity within coffee germplasm.

FREE RADICAL SCAVENGING REACTIONS IN COFFEE

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Introduction

The formation of stable free radicals during the roasting of coffee beans is well known (Troup *et al*, 1989); some of these free radical centres are found in green beans, but the majority are formed during the roasting process. These free radicals are, however, considered to be of little direct biological significance because of their great stability.

One potentially beneficial property of free radicals is their ability to scavenge other, perhaps more reactive and therefore potentially toxic, free radicals through an annihilation reaction:

$$A\cdot \quad + \quad B\cdot \quad \rightarrow \quad AB$$

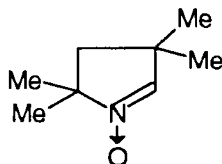
Examples of reactive free radicals are superoxide ($O_2\cdot^-$) and hydroxyl ($HO\cdot$) both of which have been linked to degenerative processes in humans (Halliwell, 1987). Such free radicals are generated during the digestion of food and then interact with the other dietary components and the gut lumen. Conversely, the stable free radicals in coffee and other charred polysaccharides might react with other dietary free radical scavengers, thus reducing the ability of the body to control the reactions of metabolically-generated free radicals.

Chemical species with unpaired electrons which include transition metal ions as well as organic free radicals can be detected, and in some cases identified, by the technique of electron paramagnetic resonance (EPR) spectroscopy. Some free radicals have very short lifetimes or spectral characteristics which make their detection difficult; in such cases the technique of "spin trapping" is used. A spin trap is a molecule which combines with reactive free radicals to form a stable free radical which can then be detected by EPR.

The present study represents an initial attempt to investigate the potential roles of the soluble solids of coffee as free radical scavengers or in the deactivation of other free radical scavengers. The free radical scavenging ability of coffee was investigated by measuring the extent to which it can compete with a chemical spin trap for the superoxide free radical. In addition the ability of coffee to deactivate other free radical scavengers was investigated by using EPR to compare the amounts of ascorbyl radical in solutions of ascorbic acid with and without added coffee.

Experimental

Materials

Samples of roasted and green beans were donated by James Aimer Co.Ltd., Dundee; a common proprietary brand of instant coffee was also used. The spin trap TMPO (3,3,5,5-tetramethyl-1-pyrroline N-oxide) 

, xanthine (99-100%) and xanthine oxidase (buttermilk, 0.5 I.U./mg) were purchased from Sigma (Poole, UK) and used without further purification. Ascorbic acid (99+%) was purchased through Aldrich (Gillingham, UK).

Antioxidant Assay

Competition experiments versus the nitrone spin trap, TMPO, were adapted from an assay, utilising xanthine / xanthine oxidase, which follows the formation of $O_2{\cdot}^-$ spectrophotometrically (Rice-Evans et al, 1991). To this end, cytochrome c (the indicator) was excluded from the assay and replaced with TMPO (0.06M). The products of superoxide addition to the spin trap were monitored by EPR.

Prooxidant Assay

Thermal generation of the ascorbyl radical in the range 300 - 350K was investigated with solutions of ascorbic acid (0.5M) in K_2HPO_4 / KH_2PO_4 at pH7.4 in the absence and presence of instant coffee (10mg/ml).

EPR spectroscopy

EPR spectra were recorded on a Bruker ESP 300E spectrometer operating at X-band (*ca* 9.5GHz) frequencies. Solid samples of ground coffee beans and instant coffee were placed in 4mm o.d. quartz tubes, solutions in a standard aqueous flat cell (Wilmad, Buena, NJ, USA) of approximate dimensions 40 x 4mm. Temperatures were controlled throughout in a constant temperature accessory operating at 295K unless otherwise stated (Bruker Spectrospin, Coventry). Other relevant spectrometer settings are indicated in figure captions.

Results and Discussion

(i) Free Radicals in coffee

The EPR spectra of raw and roasted ground Robusta beans are presented in Figure 1. The spectra provide little information on the nature of the free radical species present because of the overlapping signals from numerous carbon and oxygen-centred free radicals which result in a broad, almost isotropic, singlet. Upon roasting, the signal increased in intensity approximately 2-4 fold (Figure 2) but retained the same overall shape.

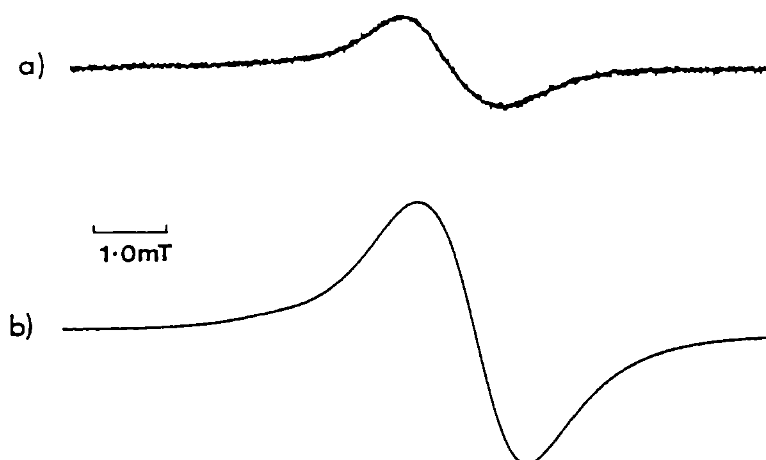


Figure 1 *EPR spectra of ground raw (a) and roasted (b) Robusta beans*
Centre field, 348.0mT, sweep width, 10.0mT, microwave power, 1mW, microwave frequency, 9.78GHz, modulation amplitude 0.285mT, modulation frequency, 100kHz.

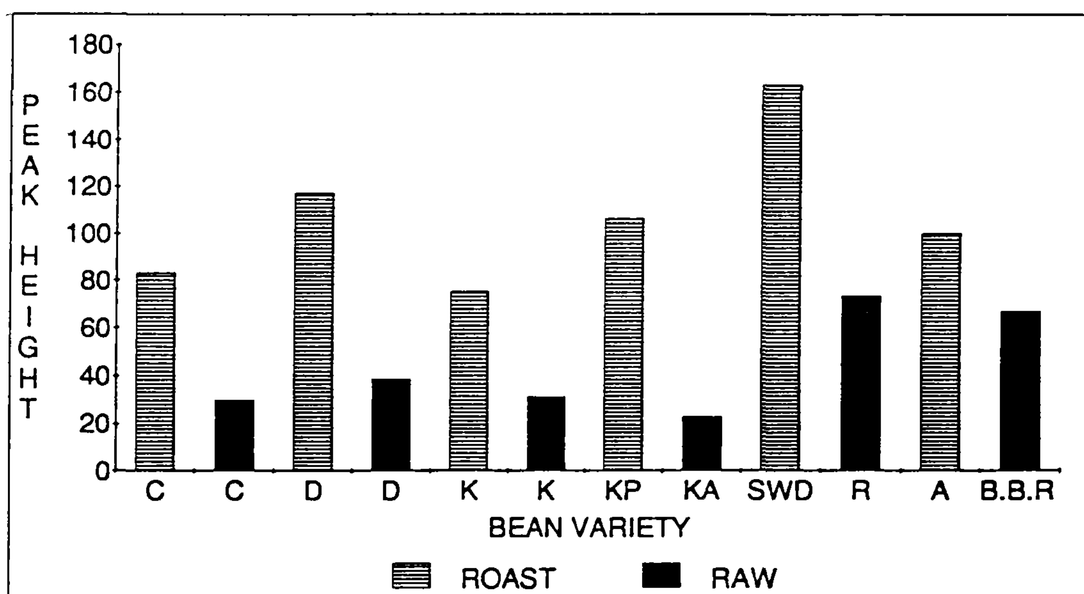


Figure 2 *EPR peak to peak signal intensity of the free radical component of green and roasted beans. C - Columbian; D - Decaffeinated; K - Kenya; KP - Kenya Peaberry; KA - Kenya AB; SWD - Swiss Water Decaffeinated ; R - Robusta; A - Merchant's blend; BBR - Bold Bean Robusta*

After aqueous extraction of roasted Robusta beans at 20°C, the free radical remained associated primarily with the solid component, with very little in the aqueous phase, but after extraction with boiling water most of the free radical activity was in the aqueous phase. Dry instant coffee, frequently prepared (in part) from Robusta beans, has a similar free radical content to roasted beans and aqueous solutions have spectra similar to that of the dry, roasted beans (Figure 3).

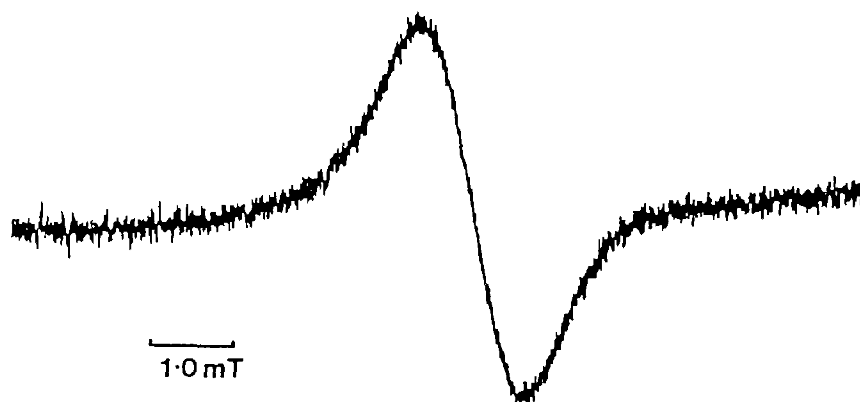


Figure 3 *EPR spectrum of an aqueous solution of instant coffee*
Centre field, 348.5mT, sweep width, 5.0mT, microwave power, 5.0mW, microwave frequency, 9.79GHz, modulation amplitude, 0.50mT, modulation frequency, 100kHz.

(ii) Reaction of coffee with antioxidant free radical scavengers

The reaction of one of the major dietary antioxidants, vitamin C (ascorbic acid) with solutions of instant coffee has been investigated over a range of temperatures. Ascorbic acid was selected for these experiments partly because the ascorbyl radical which is formed by the one electron oxidation of ascorbate, has a distinct EPR spectrum (Figure 4, insert) but also because vitamin C is regarded as the most important water-soluble vitamin with antioxidant properties. Figure 4 depicts the effect of increasing temperature on the intensity of the ascorbyl radical EPR spectrum from a 0.5M aqueous solution of ascorbic acid with and without the presence of 10mg/ml instant coffee. The increase in ascorbyl radical formation in the presence of coffee above 320K demonstrates that coffee possesses some prooxidant activity.

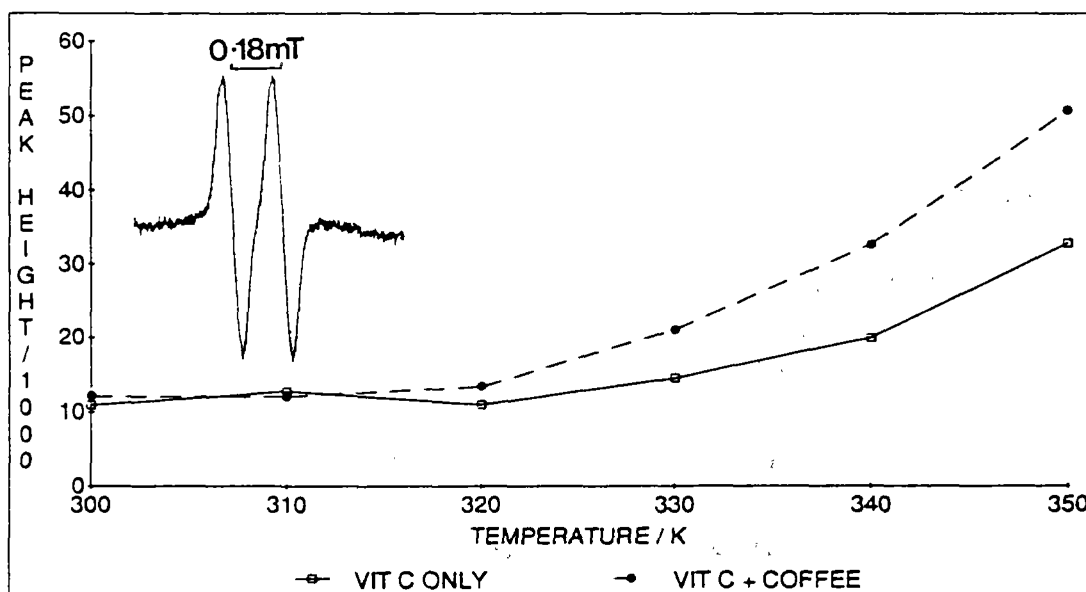


Figure 4 ***Effect of instant coffee on the temperature-dependent formation of ascorbyl radical from ascorbic acid.***

(iii) Free radical scavenging properies of coffee

The potential antioxidant or free radical scavenging properties of coffee have been investigated through its reaction with the superoxide radical anion, $O_2\cdot^-$. Superoxide is frequently formed in oxidative reactions in biological systems; it is reactive and generally has a short lifetime in aqueous solution so its presence is detected by the EPR spectrum of its spin adduct with TMPO whose development with time is shown in Figure 5.

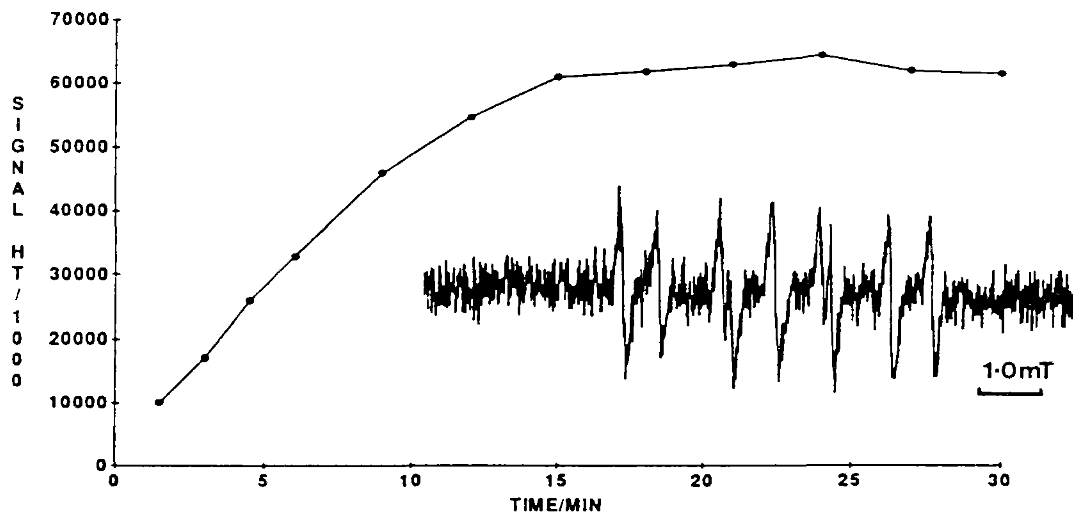


Figure 5 *Generation of the TMPO-$O_2\cdot^-$ adduct by xanthine / xanthine oxidase.*

If coffee is an effective free radical scavenger, it will compete with TMPO for superoxide and a diminution in the EPR signal of the TMPO adduct will result. The effect of adding solutions of instant coffee to give final concentrations from 0-1% is shown in Figure 6 where it can be seen that there is a progressive decrease in the EPR signal with increasing coffee concentration (coffee is thus acting as a free radical scavenger).

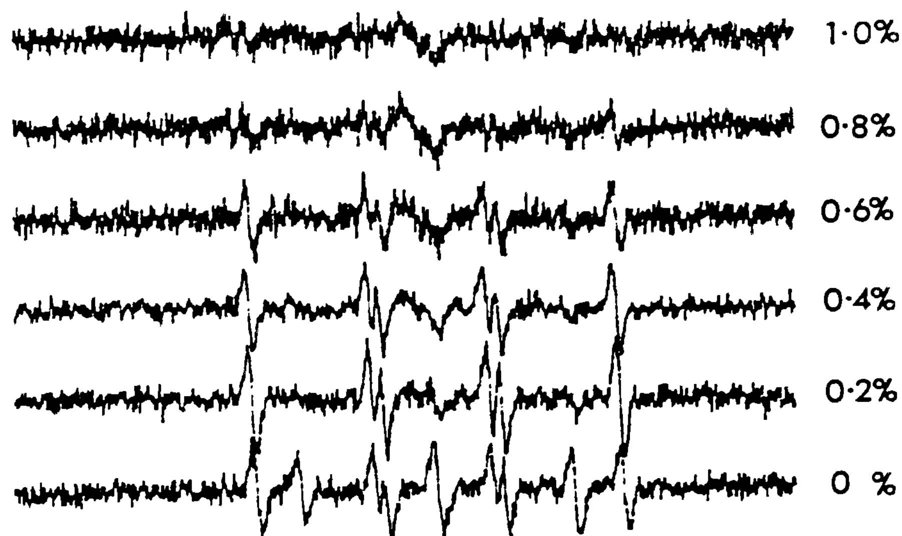


Figure 6 ***Effect of addition of differing amounts of a coffee solution to the preformed TMPO-$O_2\cdot^-$ adduct***

Addition of the coffee either at the start of the experiment, or after the 15 minutes incubation period necessary to maximise the TMPO-adduct signal, before recording of the EPR spectrum, had no effect on the magnitude of the observed signal, indicating that the $O_2\cdot^-$ TMPO adduct is scavenged at least as effectively as superoxide itself.

The additional three peaks seen in the absence of coffee are due to an oxidation product of TMPO which is always present in commercial preparations (Thornalley,1984; Evans *et al*,1985). It is interestng to note that coffee scavenges this adduct, TMPO-X, in preference to either $O_2\cdot^-$ or the TMPO-$O_2\cdot^-$ adduct.

Conclusions

Both pro- and antioxidant properties of coffee have been identified in the present study. Temperature-dependent formation of the ascorbyl radical is observed to be a function of the coffee concentration suggesting a prooxidant role of one or more components of instant coffees. That this only manifests itself at temperatures > 320K suggests that it is unlikely to be of great significance, but may discourage the wary from simultaneous taking of one's daily vitamin C supplement with a nice hot cup of coffee! The antioxidant (free radical scavenging) properties of coffee are, however, considered to be of greater significance, since these are observed at physiological temperatures and lower.

Summary

Free radical formation during the coffee roasting process is associated with the charring of polysaccharide components. Although these free radicals are water-soluble and can be detected by EPR spectroscopy in aqueous solutions, their high stabilities suggests that they have no great biological significance. Prooxidant properties of coffee have been observed through the enhanced formation of the ascorbyl radical in solutions of ascorbic acid at temperatures > 320K. In contrast, antioxidant (free radical scavenging) properties are observed at ambient temperatures, at which coffee solutions are able to scavenge effectively adducts of the TMPO spin trap and possibly also the superoxide radical anion.

Sommaire

La formation des radicaux libres durant la torréfaction du café est associeé à la carbonisation des composantes polysaccharides. Ces radicaux libres sont solubles dans l'eau et sont discernables par la technique de RPE en solution aqueuse; cependant, ils sont très stables et n'ont donc pas une grande importance biologique. Les qualités prooxydantes du café ont été observées par l'augmentation de la formation du radical ascorbyl dans des solutions d'acide ascorbique aux températures > 320K. Au contraire, les propriétés antioxydantes (le nettoyage des radicaux libres) ont été observées aux températures ambientes. A ces températures, les solutions du café peuvent nettoyer effectivement les adduits du capteur de spin TMPO, et peut-être aussi l'anion radical superoxide.

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TRACE METAL PROFILES OF GREEN COFFEES

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Introduction

Green coffee beans contain a very wide range of metallic elements, varying from potassium at levels of around 2% to many trace elements, such as cobalt and chromium, at levels well below one part per million(1). The concentrations of metallic elements found in coffee plants, and in coffee beans, may be controlled by the requirements of the plant and therefore may be held quite constant irrespective of the concentrations of that element in the immediate environment. Thus, levels of the macroelement potassium are relatively constant in a wide range of green coffees, even when subjected to fertilization regimes with high levels of potassium. Some of the microelements, such as manganese, also play a rôle, as enzyme co-factors, and differences between species as to their requirements for such elements are to be expected. Differentiation of arabica and robusta coffees on the basis of their manganese contents is well established (2). In addition to those elements for which there is a well defined biochemical rôle in the growing plant, or indeed for those elements for which such a rôle might be postulated, coffee contains a wide range of microelements whose presence seems more fortuitous. The levels of these elements are more likely to be influenced by their concentration and chemical state (availability) in the soil.

The "health" of a coffee plant will be affected if it is unable to acquire sufficient amounts of an essential element, either due to low concentration or availability in the soil or possibly disease. Alternatively the plant may suffer if it receives a toxic dose of a particular element. The effect on coffee quality due to such deficiencies (or indeed excesses) is unknown as is the actual elements and their levels which are required for the production of coffee of optimum quality.

Trace elements may also have a rôle to play during coffee processing. For example many enzymes are involved in wet processing, such as phenoloxidase, and their activity will be influenced by the presence of metallic elements. It is also possible that transition metal ions, such as copper, will act as catalysts for lipid oxidation during roasting, and subsequent storage of roast coffee, leading to rancidity.

It would appear, therefore, that there are a number of ways in which metallic elements could influence the quality of coffee, either directly during growing or during

subsequent processing/storage. Also it is highly likely that the levels of some elements will be indicative of the growing plants' microenvironment and could therefore provide a means of differentiating coffees of different geographical origins.

Several studies have been published showing data for metallic elements in green coffee using neutron activation analysis(3) or atomic absorption spectro-photometry(4).

These methods are somewhat laborious, the former requiring an activation source (reactor) and the latter being only a single element technique. This present study looks at the potential of more recent multielement techniques, namely inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) for the determination of trace metal profiles in green coffee.

Method

The green coffee samples used in this study are listed in Table 1, they were analysed as is with moisture contents around 10%.

Table 1 Green Coffee Samples Used in Trace Metal Study

Region	Code	Municipality	Sensory Score (Merit)
Central	159	POCOS DE CALDAS	1
	161	POCOS DE CALDAS	1
	165	BOTELHOS	5.5
	168	POCOS DE CALDAS	3
	105	BOTELHOS	3
	171	POCOS DE CALDAS	5
Central South East	198	CACHOEIRA DE MINAS	1
	167	CAMPESTRE	4
	6	S. RITA DO SAPUCAI	
	73	S. RITA DO SAPUCAI	
	50	CARMO DE MINAS	
	187	CARMO DE MINAS	
North West	97	PATROCINIO	5.5
	228	PATROCINIO	6
	95	PATROCINIO	6
	123	ARAGUARI	5.5
	140	ARAGUARI	2
	93	PATROCINIO	2

The samples were briefly ground in a small coffee grinder to assist the subsequent digestion. Extensive grinding was not carried out to avoid contamination from the grinder blades.

Samples for ICP-OES were digested in concentrated nitric acid (0.5g coffee in 10ml) in a pressure controlled PTFE bomb in a CEM microwave digestion system (MDS 2100) for two hours. The subsequent clear solutions were diluted to 50 ml with water.

Several multielement standards were prepared in 20% nitric acid to match the sample matrix. Quantitation was achieved by external calibration. Blanks were recorded with acid which had passed through the digestion procedure to check for contamination. Detection wavelengths for the various elements are shown in Table 2. The instrument was a Perkin Elmer Plasma 40.

Samples for ICP-MS were boiled in nitric acid (1g coffee in 10ml) in PTFE beakers until near dryness. Hydrogen peroxide (1ml) was added and the samples reheated with nitric acid (5ml) and again allowed to go to near dryness. The residues were diluted to 100ml

and spiked with indium (100 µg ml^{-1}) as an internal standard. The instrument used was a Finnigan MAT SOLA ICP-MS.

Table 2 Detection Wavelengths used in ICP-OES

Element	Wavelength (nm)
Molybdenum	202.030
Zinc	213.856
Phosphorus	214.914
Lead	220.353
Aluminium	226.916
Barium	233.527
Iron	238.204
Boron	249.773
Manganese	257.610
Chromium	267.716
Magnesium	279.553
Calcium	315.887
Copper	324.754
Strontium	407.771
Potassium	769.896

Statistical Manipulation of Data

Multielement methods, such as ICP-OES and ICP-MS, produce a vast amount of data which are difficult to manipulate and interpret manually. In this present evaluation all of the data sets were subjected to principal component analysis (PCA) using the SIRIUS software package (TV Karstang and OM Kvalheim).

In the trial sets of samples each duplicate was treated as an individual sample so that the subsequent separation on PCA would give a measure of reproducibility. All the data were subjected to autoscaling (ie standardization of variables) prior to PCA, to avoid undue influence of the major elements. The results are presented in the form of score plots, showing the calculated principal components, and loadings plots, showing the contribution of each element to the differentiation afforded by the principal component in question.

Results and Discussion

ICP-OES Data

The elemental composition of the six coffees used as a trial set, analysed in duplicate, are shown in Table 3. In addition to the 12 elements reported attempts were also made to determine molybdenum, chromium and lead. In the cases of molybdenum, and chromium the levels were very near the detection limit of the instrument and very poor replication was obtained. The values for lead were subject to a large interfering peak, probably a secondary line of another element present at much higher levels, and this gave rise to unrealistically high results. The absence of significant amounts of lead was subsequently confirmed by ICP-MS.

PCA of the 12 elements reported in Table 3 showed a high level of differentiation between the samples and also a reasonable degree of replication Figure 1. The corresponding loadings plots confirmed that many of the elements were contributing to the differentiation and that it would not be possible to handle the data in a univariate manner Figures 2 and 3. The major contributors to PC1 were manganese, copper, strontium and barium. Samples from the central region (159, 161 and 165) were clearly differentiated from the others and indeed the samples from the other regions were also well separated (see Table 1). However, the duplicates were not very satisfactory and in many cases this was thought to be due to interferences from other elements. The lack of robustness of the method was subsequently confirmed by analysis of further batches of samples which lead to somewhat different degrees of differentiation.

Table 3
Elemental Composition of Green Coffees Determined by ICP-OES (mg kg^{-1}, as is)

Element	97a	97b	159a	159b	161a	161b
K	18400.0	19800.0	18200.0	19300.0	18600.0	17700.0
P	2220.0	2250.0	2230.0	2300.0	2210.0	2040.0
Mg	2030.0	2290.0	1920.0	2110.0	1940.0	1790.0
Ca	362.0	407.0	333.0	368.0	315.0	367.0
Fe	42.5	42.0	33.6	23.2	25.7	28.8
Mn	19.2	23.5	39.2	53.6	43.7	37.7
Cu	15.0	16.5	14.1	15.6	15.7	13.0
Al	99.7	74.2	52.2	51.6	51.0	87.7
Zn	10.5	8.6	7.8	12.0	7.3	8.0
B	9.7	11.3	9.4	9.6	9.9	15.1
Sr	3.7	4.2	14.5	16.2	12.8	14.2
Ba	6.6	4.1	6.8	7.3	5.1	8.0

Element	165a	165b	198a	198b	228a	228b
K	18700.0	18300.0	15200.0	16600.0	19300.0	18700.0
P	2210.0	2180.0	2270.0	2240.0	1960.0	1900.0
Mg	2040.0	2250.0	2160.0	2250.0	2050.0	2020.0
Ca	376.0	418.0	380.0	389.0	353.0	344.0
Fe	28.4	26.5	60.2	38.6	33.1	35.6
Mn	40.0	40.9	61.0	59.9	16.9	14.7
Cu	15.7	16.9	15.3	15.6	18.0	17.9
Al	55.1	54.3	110.2	82.5	55.5	58.4
Zn	7.4	8.4	9.1	11.0	9.3	12.7
B	10.2	12.3	6.9	6.6	9.1	11.0
Sr	10.0	14.5	6.3	6.2	3.3	3.1
Ba	7.5	8.1	6.0	4.6	2.7	3.1

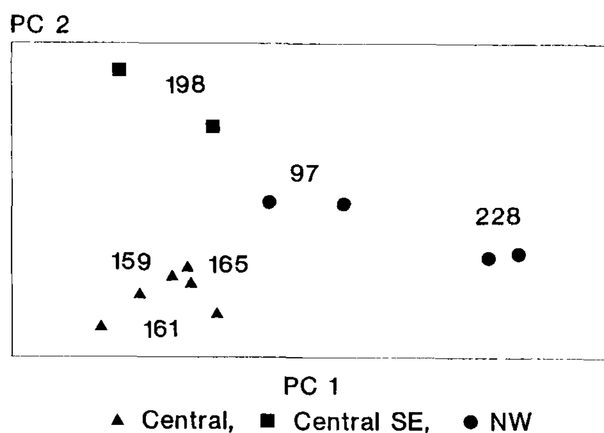

Figure 1. Score plot (PC1 vs PC2) for ICP-OES data from Table 3.

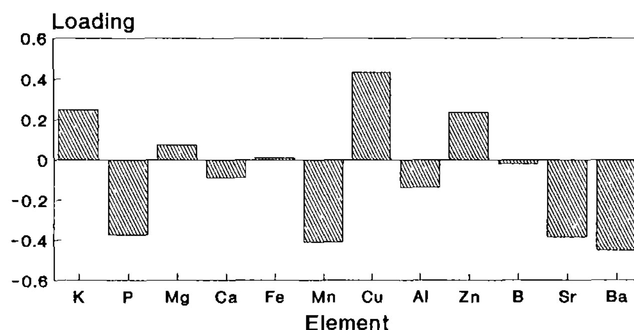

Figure 2. Loadings plot for PC1 corresponding to Figure 1.

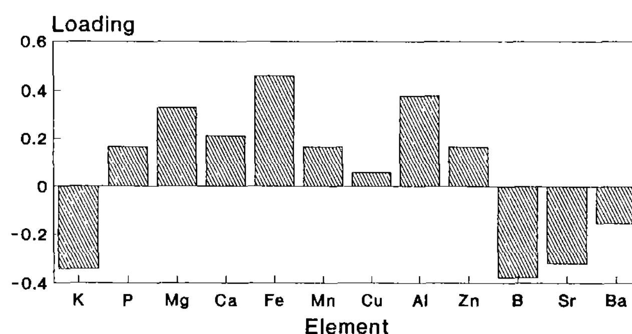

Figure 3. Loadings plot for PC2 corresponding to Figure 1.

Table 4
Elemental Composition of Green Coffees Determined by ICP-MS (mg kg^{-1}, as is)

Element	73a	73b	123a	123b	95a	95b
Mg	1790.69	1820.00	1919.59	1780.87	2405.99	2590.43
Ca	386.30	383.56	388.50	385.35	592.30	546.45
Sc	1.25	1.11	1.22	1.06	1.48	1.55
V	0.04	0.02	0.03	0.03	0.04	0.02
Cr	0.38	0.33	0.29	0.32	0.45	0.49
Mn	23.95	25.00	25.33	25.23	15.88	17.69
Fe	29.91	32.00	28.87	24.63	37.58	34.25
Co	0.09	0.09	< 0.01	< 0.01	< 0.01	< 0.01
Cu	3.15	3.19	2.67	3.09	3.00	3.48
Zn	3.51	3.09	3.96	3.99	4.47	4.41
Rb	44.65	45.49	34.16	33.42	7.23	6.50
Sr	2.24	2.46	2.50	2.14	3.30	3.06
Mo	0.31	0.42	< 0.01	< 0.01	0.12	0.09
Ag	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Cd	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Sb	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Cs	0.12	0.14	0.12	0.14	< 0.01	0.01
Ba	4.70	4.06	1.68	1.53	2.60	2.43
Hg	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pb	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

Table 4 (contd)
Elemental Composition of Green Coffees Determined by ICP-MS (mg kg^{-1}, as is)

Element	171a	171b	105a	105b	50a	50b
Mg	1587.23	1404.35	1389.63	1563.91	1367.83	1327.83
Ca	238.70	243.28	260.95	232.12	461.02	332.26
Sc	0.94	0.98	0.40	0.39	1.56	1.80
V	0.05	0.02	0.02	0.01	0.04	0.02
Cr	0.38	0.43	0.16	0.11	0.68	0.53
Mn	24.56	23.45	21.24	19.08	20.15	19.91
Fe	22.89	25.75	22.41	25.16	24.75	24.66
Co	0.19	0.13	0.37	0.25	0.09	0.08
Cu	2.53	3.09	3.73	4.03	2.10	2.20
Zn	4.63	4.30	4.98	5.07	3.65	3.44
Rb	48.01	48.50	40.64	42.75	15.22	13.63
Sr	11.95	13.09	2.86	2.74	1.73	1.42
Mo	0.10	0.08	0.07	0.06	0.07	0.06
Ag	< 0.01	0.01	< 0.01	0.01	< 0.01	< 0.01
Cd	< 0.01	< 0.01	< 0.01	< 0.01	0.08	< 0.01
Sb	< 0.01	< 0.01	< 0.01	< 0.01	0.01	< 0.01
Cs	0.27	0.29	0.16	0.14	0.01	0.00
Ba	4.18	4.66	3.88	3.65	4.06	4.29
Hg	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pb	< 0.01	< 0.01	0.30	0.07	0.03	< 0.01

ICP-MS

In view of the success of ICP-OES as a means of differentiating samples, albeit with poor reproducibility and repeatability, it seemed worth pursuing the use of alternative techniques, hopefully less subject to interference. A second trial set of samples was selected from those available and analysed using ICP-MS for the 20 elements shown in Table 4. Potassium was not recorded as its high level was outside the measurement range. Similarly data for silver, cadmium, antimony, mercury and lead were not incorporated into the PCA as they were all around the detection limit of the technique. Results of the PCA are shown in Figure 4 with the corresponding loadings plots in Figures 5 and 6. As with ICP-OES there is clear differentiation between the samples, but additionally in this case there is much better reproducibility between the duplicate samples. PC1 provides significant discrimination between the samples and indeed also appears to provide some separation on the basis of their geographical origin. With reference to the loadings plot in Figure 5 it is again apparent that many elements are making a significant contribution to this discrimination.

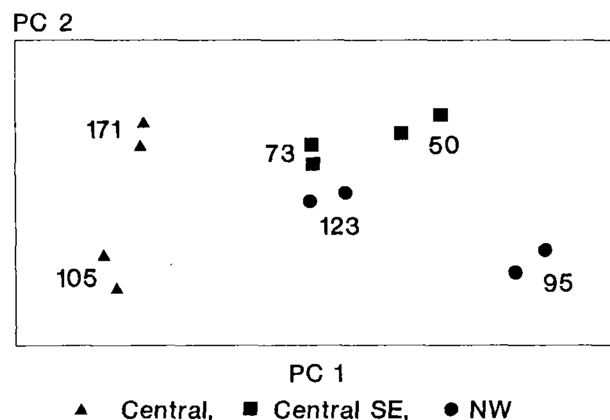

Figure 4. Score plot (PC1 vs PC2) for ICP-MS data from Table 4.

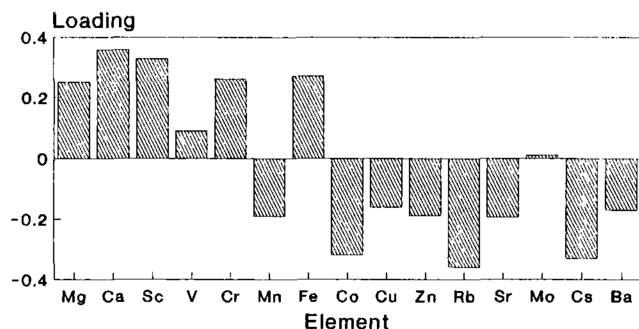

Figure 5. Loadings plot for PC1 corresponding to Figure 4.

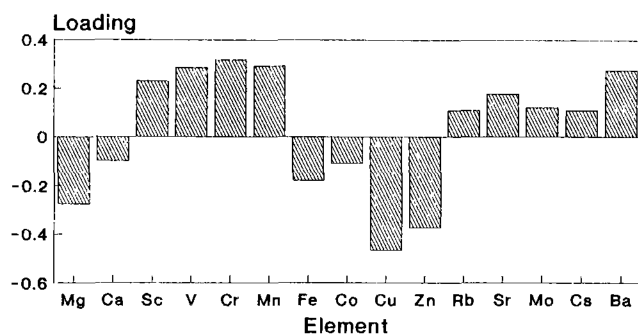

Figure 6. Loadings plot for PC2 corresponding to Figure 4.

The investigation was then extended by studying a further 12 samples, (Table 5) using the same elemental data as above. However, in this case only single determinations were made on account of the good proven reproducibility. The PCA shown in Figure 7 again shows significant differentiation between the samples, although the samples are not well grouped on the basis of geographical origin.

The statistical analysis could be extended to the combination of the data sets from Tables 4 and 5, ie providing a set of 18 samples. However, PCA is not an ideal technique for such a process as any slight shifts in operating conditions between the batches will lead to a separation on the basis of sample batch as well as that due to inherent sample differences. Indeed when the two data sets were combined such a separation was observed due to confirmed shifts in the sensitivities for vanadium and chromium and possibly for other elements. Alternative statistical techniques, such as cluster analysis, would be more appropriate. Some global statistics for the ICP-MS data for all the samples in Table 1 are shown in Table 6.

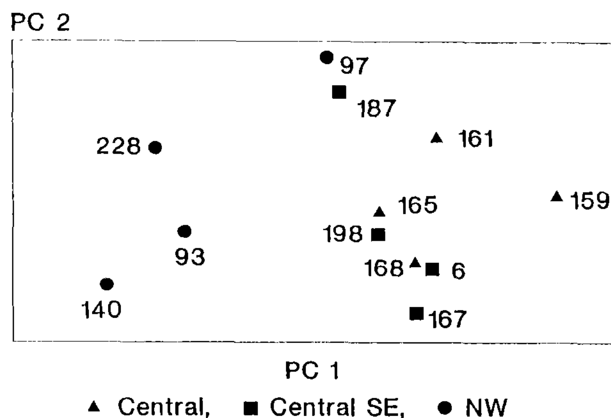

Figure 7. Score plot (PC1 vs PC2) for ICP-MS data from Table 5.

Table 5
Elemental Composition of Green Coffees Determined by ICP-MS (mg kg^{-1}, as is)

Element	159	97	198	161	165	168
Mg	1732.27	1445.52	1923.98	1608.76	1687.84	1754.28
Ca	331.17	206.91	258.40	238.50	220.94	245.99
Sc	0.25	0.29	0.60	0.73	0.62	0.66
V	0.14	0.08	0.07	< 0.01	0.07	0.04
Cr	0.21	* 3.23	0.58	0.24	0.27	0.66
Mn	38.43	12.76	46.73	34.92	31.78	34.72
Fe	19.42	23.00	18.20	18.46	18.81	20.33
Co	0.10	< 0.01	0.17	< 0.01	0.10	0.16
Cu	3.15	2.75	3.12	2.63	4.49	3.55
Zn	1.46	4.88	3.46	3.91	8.61	6.66
Rb	40.10	12.04	17.28	37.13	33.92	42.41
Sr	8.84	3.96	2.83	7.81	8.34	10.91
Mo	0.98	0.50	0.29	0.13	0.15	0.15
Ag	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Cd	< 0.01	0.02	< 0.01	< 0.01	< 0.01	< 0.01
Sb	0.21	< 0.01	0.28	0.83	< 0.01	< 0.01
Cs	< 0.01	< 0.01	0.18	< 0.01	< 0.01	< 0.01
Ba	1.25	0.15	0.18	0.77	1.55	0.83
Hg	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pb	< 0.01	< 0.01	0.20	< 0.01	0.12	< 0.01

Table 5 (contd)
Elemental Composition of Green Coffees Determined by ICP-MS (mg^{-1}, as is)

Element	140	93	167	228	6	187
Mg	2128.67	2105.95	1803.86	1864.75	1892.40	1654.59
Ca	240.15	258.32	242.15	194.25	273.72	196.22
Sc	0.63	0.71	0.61	0.77	0.63	0.43
V	0.20	0.17	0.23	0.06	0.13	0.05
Cr	0.50	0.52	0.48	0.39	0.35	0.17
Mn	24.43	14.61	39.92	11.29	25.40	23.72
Fe	31.27	27.68	21.05	23.46	22.37	17.97
Co	0.01	< 0.01	0.25	< 0.01	0.03	0.10
Cu	4.63	4.04	3.89	4.74	3.26	3.27
Zn	15.61	4.05	6.07	6.54	4.69	5.62
Rb	15.45	14.04	38.11	6.33	41.47	21.92
Sr	1.29	1.85	9.77	0.57	10.67	1.39
Mo	0.16	0.21	0.12	0.23	0.09	< 0.01
Ag	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Cd	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Sb	0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Cs	0.04	0.01	< 0.01	0.01	0.04	0.03
Ba	0.08	0.20	1.21	0.02	2.42	0.14
Hg	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pb	< 0.01	< 0.01	0.01	0.02	< 0.01	< 0.01

* Eliminated as outlier in PCA

Table 6
Variable Statistics for ICP-MS Data

Element	Mean	Minimum	Maximum	SD
Mg	1772.97	1327.83	2590.43	313.30
Ca	306.56	194.25	592.30	108.43
Sc	0.86	0.25	1.80	0.43
V	0.07	0.01	0.23	0.06
Cr	0.39	0.11	0.68	0.15
Mn	25.01	11.29	46.73	8.85
Fe	24.79	17.97	37.58	5.31
Co	0.10	0.01	0.37	0.10
Cu	3.32	2.10	4.74	0.71
Zn	5.04	1.46	15.61	2.66
Rb	29.18	6.33	48.50	14.84
Sr	4.90	0.57	13.09	4.00
Mo	0.18	0.01	0.98	0.21
Ag	0.01	0.01	0.01	0.00
Cd	0.01	0.01	0.08	0.01
Sb	0.06	0.01	0.83	0.18
Cs	0.07	0.01	0.29	0.09
Ba	2.10	0.02	4.70	1.68
Hg	0.01	0.01	0.01	0.00
Pb	0.04	0.01	0.30	0.07

Correlation with Sensory Quality

Overall merit scores, determined by sensory evaluation, for the samples are shown in Table 1. Attempts were made to correlate these scores with the elemental data produced, both on a univariate and multivariate basis. No clear correlations were established although there is some evidence to show that a high level of manganese may be associated

with reduced quality as shown in Figure 8. The precise reasons for this observation, if indeed it is true, are unclear.

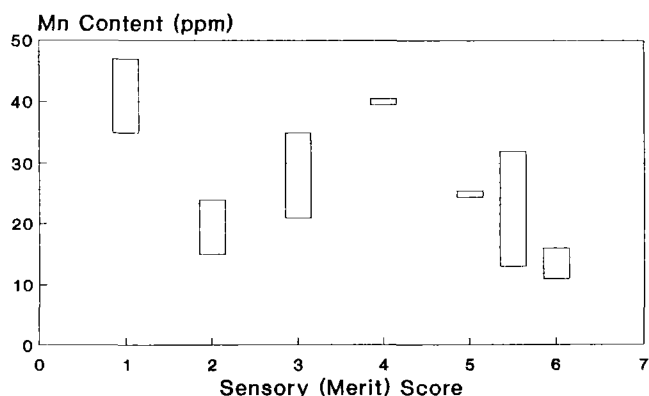

Figure 8. Correlation of sensory (merit) score with manganese content.

Conclusion

Recent advances in multielement techniques allow rapid acquisition of trace metal profiles. However, in the case of coffee samples, there are still problems to be overcome with reliable sample preparation methods and the presence of interferences from other elements. The techniques provide a high degree of discrimination between samples, especially when coupled with multivariate techniques. However, correlation between trace metal profiles in coffee beans and the plants microenvironment is less well established as indeed is the possible significance of these elements in relation to product quality.

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SUMMARY

Trace elements play an important rôle in the health of coffee plants and may therefore affect the quality of beans. Additionally some elements, eg transition metals, may influence changes during roasting and storage.

The present study shows the value of modern multielement techniques, ICP-OES and ICP-MS, in providing elemental profiles. The data allow clear differentiation between green coffee samples and suggest some correlation with geographical origin. There is also some evidence to suggest that the concentrations of some elements, eg manganese, may be correlated with quality.

Les éléments à l'état de trace jouent un rôle important pour la sauté des plantes de café, et peuvent ainsi affecter la qualité des grains. Par ailleurs, certains éléments, comme les métaux de transition, peuvent être la cause de changements pendant la torréfaction et l'emmagasinage.
La présent étude montre la valeur de techniques modernes multi-élémentaires, ICP-OES et ICP-MS, qui fournissant des profils élémentaires. Les données montre une nette différenciation entre les différents échantillons de cafés verts, et suggèrent l'existence d'une corrélation avec leur origine géographique. On peut également mettre en évidence une corrélation entre la concentration en certains éléments, comme par example le manganèse, et la qualité du café.

ANÁLISIS POR MEB DE LA POROSIDAD DEL CAFÉ TOSTADO

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INTRODUCCION:

En el proceso de tostación de café (calentamiento seco a temperaturas superiores a los 200°C) la presión interna de los gases, primero de agua libre vaporizada y posteriormente de los gases de pirólisis, provoca un aumento del volumen del grano, fundamentalmente a partir de los 210 °C que es cuando su material estructural alcanza la temperatura de transición vitrea (Mahlmann, 1984). La reducción de peso por pérdida de agua, CO_2 y volátiles, así como el aumento de volumen de los granos hacen que disminuya la densidad y aumente la porosidad de la estructura del grano. Se ha observado que estos cambios son dependientes del tiempo o grado de tostado, llegándose a estabilizar a partir de un determinado momento (Massini,1990).

La superficie interna de los granos de café tostado y de las particulas del café molido es extremadamente heterogénea debido a la presencia de macro y microporos. La caracterización estructural del producto es muy útil y en muchos casos necesaria para el estudios y caracterización de los fenómenos de difusión y adsorción de los gases producidos durante el tostado o presentes en el ambiente. Se ha visto que la atmósfera interna de los granos de café es afectada por la variedad de la muestra y la temperatura de tostado, así como por la reducción de tamaño de partícula durante el molido.(Saleeb, 1975). Por otro lado, el deterioro del café está muy afectado por la exposición de la superficie interna (porosidad) al oxigeno, agua, CO_2, etc.; por lo tanto la microestructura del grano juega un papel muy importante en el deterioro del producto debido a que en ella es donde se alojan estos gases y desde donde son liberados o retenidos.

La porosidad del café ha sido analizada por distintos autores. Radtke (1979) ha obtenido valores de porosidades de 38% para café con cafeína y 49% para café descafeinado. En los últimos años la microscopía electrónica de barrido (SEM) ha sido aplicada en varios estudios de las propiedades físicas del café para poder obtener un mejor conocimiento de su estructura. (Burguin, 1969; Saleeb, 1975; Amorim, 1976; Dentan, 1977; Kazi y Clifford, 1985; Puhlman, 1986; Duplatre, 1987). Amorim (1976) por medio de análisis de imagen de micografias

obtuvo distribuciones de frecuencias del grosor de la pared celular, y valores de porosidad que van de 49.7 a 63.8 %. Por otro lado Kazi y Clifford (1985) midieron celulas de café tostado en un intervalo de 15 a 48 μm dependiendo del tipo de café, siendo más pequeñas (15 - 39 μm) en los cafés con tueste regular y mayores (15 - 48μm) en cafés tostados con alto desarrollo.

El objetivo de este trabajo es cuantificar la porosidad del cafe tostado a diferentes temperaturas, así como analizar algunas características morfogeométricas estructurales, observables por SEM, que son útiles para el estudio de los fenómenos de transporte de materia en el café en grano o molido. Fenómenos que a su vez son determinantes de la velocidad de deterioro del producto.

MATERIAL Y METODOS:

MATERIAL:

Las variedades de café estudiadas pertenecen a las dos especies que en niveles comerciales son las más interesantes: Coffea Arabica Linn. y Coffea Canephora Pierre ex Foechner también conocidas como arabica y robusta respectivamente. En arabica se seleccionó un café de Colombia, y en robusta uno de Uganda, ambos criba 18.

METODOS:

PREPARACION DE LAS MUESTRAS:

El tostado del café se realizó en un equipo de tambor rotatorio de la marca TecAIRE S.A. con control de temperatura en el centro del cilindro. Las muestras (25g) fueron tostadas a 2 temperaturas diferentes 220°C y 295°C hasta el mismo grado de tostado, establecido en base al color. Este se midió en un espectrocolorímetro MINOLTA CM 1000, determinándose las coordenadas CIELab referidas al observador 10° e iluminante D65. Las muestras se caracterizaron en cuanto a su pérdida de peso y humedad. Esta última se determino por valoración Karl Fischer (Scholz, 1988) en un equipo Mettler DL-18.

Después de tostadas las muestras fueron molidas. Se utilizó un equipo de molienda comercial de la marca FAEMA S.A. ajustando la separación de las muelas en el número 4, llegando a un grado de molido similar al de los cafés comerciales. El tamaño de particula se caracterizó por tamizado en atmósfera de nitrógeno, con una serie de tamices C.I.S.A. de 19.5 cm de diametro y de tamaño de malla comprendido entre 0.125 y 1.0 mm.

La muestras molidas o en grano fueron desgasificadas en una estufa de vacío a 110°C y a una presión de 30 mbar hasta peso constante.

DETERMINACION DE DENSIDADES:

Se midió la densidad aparente para el café en grano y molido. Para ello se tomaron muestras pesadas y se colocaron en una probeta de vidrio de 25 ml. Se midió el volumen que ocupaban los granos o las partículas después de su vibración en un vibrador C.I.S.A. durante 15 min, (a partir de ese tiempo el volumen de las muestras en la probeta no cambió significativamente). Se calculó la densidad como la media de seis repeticiones para cada muestra.

La densidad de los granos se midió con un picnómetro para sólidos. Se utilizó vaselina desaireada. Se colocaron los granos de café dentro del picnómetro con vaselina evitando la incorporación de burbujas de aire (Lerici, 1980). Se calculó la densidad como la media de seis repeticiones para cada muestra.

La densidad real se determinó por el Método de Day (Mohsenin,1970). Se midió la presión manométrica en un sistema integrado por dos cámaras de volumen conocido, conectadas por una llave. En una de ellas, de volumen calibrado (V_2), se coloca la muestra, en cantidades variables, midiendose en cada caso la presión del sistema (P_1) después de evacuar una determinada cantidad de aire de la cámara (V_1), con la llave de conexión cerrada, y la

presión resultante de la expansión del gas en el sistema de cámaras, al abrir la llave de conexión (P_3). El cociente (P_3-P_1)/P_3, corregido con factores de calibrado del equipo mide el volumen hueco en la cámara que contiene la muestra (ε). De la representación de ε frente al cociente masa de muestra/V_2 se obtiene la densidad real haciendo $\varepsilon = 0$ en la ecuación de la regresión obtenida.

Los ensayos se realizaron por triplicado para cada muestra y el ajuste de los puntos experimentales se realizó conjuntamente.

MICROSCOPIA ELECTRONICA DE BARRIDO:

Las muestras de café tostado, una vez desgasificadas, fueron directamente metalizadas con oro. Se utilizó un metalizador POLARON E 6020 a vacío menor que 10^{-2} usando corriente de ionización de 20 mA durante 60 segundos. Se utilizó un microscopio electrónico de barrido ISI DS-130, equipado con un haz de electrones de 20KV de potencial acelerador para las observaciones.

ANALISIS DE IMAGEN:

Una vez obtenidas las micrografías se realizo en las mismas e l análisis de imagen con un equipo formado por una videocamara SONY blanco y negro SSC-M350CE y un ordenador IBM-PC 386 con Perception Image Analysis System de SYNOPTICS LTD, por medio del cual se han obtenido las medidas de area,perimetro, diametro mayor. diametro menor y factor de forma.para cada uno de los elementos analizados.

Para realizar éste análisis se elaboraron plantillas que facilitaron la delimitación de las formas y areas de cada uno de los poros observables de las micrografías. Estas plantillas fueron captadas directamente con la camara y sometidas al análisis por medio de Perception.

RESULTADOS Y DISCUSION.

1.- ESTUDIO DE LA POROSIDAD:

Las muestras estudiadas presentaron un mismo grado de tueste, correspondiente a una pérdida de peso de 17.0 ± 0.9%. Las coordenadas de color de las mismas fueron L* = 24.8±0.7, a* = 7.5±0.4, b* = 7.8±0.3, variando la diferencia de color (ΔE) entre ellas de 0.78 a 1.03. La humedad final de las muestras también fue uniforme (2.5 ± 0.8 %). El café molido presentó un tamaño medio de partícula de 0.48±0.08 mm.

Al hablar de la porosidad (ε) del café habría que distinguir entre la fracción de poros interna (εi) de los granos y la fracción de huecos entre granos (εe), como consecuencia de un determinado empaquetamiento. A su vez, en el café molido puede establecerse también esa distinción (εi y εe) ya que las partículas de café siguen conservando una fracción de poros internos (Clo y Voilley, 1983).

Para evaluar estas porosidades se ha determinado la densidad real (del sustrato sólido café) mediante el método de Day, la densidad de los granos de café (incluyendo sólo los poros internos) y las densidades aparentes del café en grano y molido (incluyendo poros internos y externos en ambos casos).

En las determinaciones por el método de Day de las diferentes muestras tostadas los puntos experimentales fueron ajustados linealmente para cada muestra con un nivel de significación estadística del 99%. Para comparar el comportamiento de las diferentes muestras se ha realizado un análisis de la covarianza sobre los ajustes finales de los datos. Este permite detectar influencia de la temperatura de tueste sobre la densidad real del café de Uganda, ya que las rectas obtenidas en el método de Day difieren significativamente, ($\alpha < 0.01$). Por otro lado se comportan de forma diferente ($\alpha < 0.05$) los cafés de distinto origen aunque estén tostados a la misma temperatura.

La densidad real de las distintas muestras se determinó por sustitución de $\varepsilon = 0$ en las ecuaciones ajustadas. La tabla # 1 presenta los valores de densidades obtenidos para los distintos cafes. Se puede observar un valor ligeramente superior de la ρ real de los robustas frente a los arábicas, a las dos temperaturas de tueste.

Las densidades aparentes (ρa) obtenidas para los granos y el molido, así como la densidad de los granos (ρG) se pueden observar en la Tabla #1. Un análisis de la varianza permite establecer que no se presentan diferencias estadisticamente significativas en ρa y ρG entre los cafés tostados.

A partir de la densidad de los granos tostados (CT) y verdes (CV) y del porcentaje de pérdida de peso en el punto final de tueste se ha determinado el aumento relativo de volumen:

$$\Delta V_r = \frac{\rho a(CV)}{\rho G(CT)} \left(1 - \frac{\Delta mT}{100}\right) - 1$$

Este es 0.73 y 0.71 para los cafés de Colombia y 0.33 y 0.62 para los cafés de Uganda tostados a 220°C y 295°C respectivamente. Esto denota un mayor hinchamiento del grano en la variedad arábica, con independencia de la temperatura de tueste, mientras que el grado de hinchamiento de los granos del café robusta es altamente sensible a la temperatura. Lo cual concuerda con lo encontrado también en otros cafés de distintos orígenes (Londoño,1992).

Utilizando estas densidades se llega a la determinación de la porosidad del café (Tabla #2). No se observan diferencias entre las porosidades totales determinadas para las distintas muestras de café tostado, tanto molido como en grano. La porosidad externa del lecho de granos es superior a la del molido, como cabe esperar de la reducción de tamaño de partícula. La εe en el lecho de café molido se ha determinado asumiendo que las densidades de las partículas de café son iguales a las de los granos, lo cual implica la igualdad en la porosidad interna (ε). Esto es cierto en la medida que las partículas conservan la microestructura del grano lo cual puede confirmarse al considerar sobre la microfrafias por SEM del café (Fig. 2 y 3) el área correspondiente a un círculo de diametro igual al diametro medio de las partículas molidas. Una partícula de este tamaño tiene del orden de 10 unidades celulares a lo largo de su diametro.

Tabla #1 Densidades relativas del Café Molido y en Grano

	ρ real	ρa (CM)	ρa (CG)	ρG
C. 220	0.92±0.05	0.40±0.01	0.33±0.04	0.66±0.16
C. 295	0.89±0.09	0.40±0.01	0.28±0.02	0.67±0.13
U. 220	0.96±0.07	0.44±0.02	0.32±0.02	0.76±0.26
U. 295	0.98±0.07	0.43±0.01	0.31±0.01	0.64±0.07

ρ real = Densidad real.

ρa (CM) = Densidad aparente del café molido.

ρa (CG) = Densidad aparente del café en grano.

ρG = Densidad del café en grano.

La porosidad interna referida al volumen efectivo de los granos ε' calculada a partir de ρG y ρ real, están estre 25 y 30 %. Aunque los cafés de Uganda tienen un valor de ε' ligeramente superior a los de Colombia, el error standar de cada valor no permite considerar que las diferencias sean significativas estadísticamente.

La porosidad de los granos obtenida por medio de el análisis de imagen de las microfrafías de SEM (ε'' en Tabla #2) corresponde a una porosidad superficial que incluye fundamentalmente huecos celulares y pequeños poros en las paredes de las células, observables por SEM. Para cada muestra si midieron entre 700 y 861 poros de varias micrografías. Se puede observar que los valores de la porosidad de los granos obtenidos por SEM son muy similares a los obtenidos por el método de Day en las muestras de café robusta, aunque son algo superiores para las

muestras de café arábica. Respecto al método de Day hay dos fuentes de error que llevarían a una estimación de ε' por defecto, por un lado la determinación de la densidad real puede quedar afectada por la presencia de moléculas extrañas adsorbidas al sustrato, por otro lado en la determinación de la ρG los poros de gran tamaño pudieron quedar embebidos de vaselina.

Los valores obtenidos por análisis de las micrografías son más coherentes con los obtenidos para otros cafés por Radtke (1979), que están entre 38 y 47 %, y son todavía inferiores a los calculados, también por análisis de micrografías, por Amorim (1976), que oscilan entre 50 y 64 %. Estos resultados demuestran que los valores encontrados parecen estar muy afectados por la técnica de medida.

Tabla #2 Porosidad del café tostado.

	Café molido		Café en grano		Porosidad del grano	
	ε	εe	ε	εe	ε'	ε''
C. 220	56±8	39±29	64±15	50±29	28±10	43±1
C. 295	55±13	40±23	65±18	58±23	25±7	38±5
U. 220	58±10	42±26	71±15	58±10	30±15	37±8
U. 295	56±9	33±15	68±12	52±9	35±9	36±3

ε = porosidad total en el lecho de café.

εe = porosidad externa en el lecho de café.

ε' = porosidad interna por el Método deDay.

ε'' = porosidad interna por Análisis de Imagen.

2.- ANALISIS DE LA MICROESTRUCTURA.

a) Análisis cualitativo:

La fig. 1 permite apreciar morfología típica del grano de café o cotiledón de la semilla en las muestras tostadas. Se puede observar la estructura enrollada formada por una capa celular más externa, y una capa acelular ó mucílago central (MC) que se extiende prácticamente a lo largo de la mitad de la anterior. El mucílago central, contiene principalmente hemicelulosa y un poco de pectina, pero nada de celulosa, y en el se inserta el embrión (Burguin, 1969). Así mismo, la zona interior del plegamiento de la estructura está ocupada por el tegumento argentado central (TAC). Tanto el mucílago central como el TAC aparecen más alterados por el tostado que la zona celular y correponden a zonas con grandes grietas

Las paredes celulares presentan las típicas estructuras nodales y se encuentran atravesadas por numerosos canales, (plasmodesmos) que enlazan unas células con otras (Dentan, 1977) y que no son observables por SEM. Las células, sin espacios intercelulares (Dentan, 1977), se observan prácticamente vacias, aunque en alguna aparecen pequeños restos del contenido celular presente en el café verde. Este es inicialmente coagulado durante el tostado y progresivamente destruido en las reacciones de pirólisis. Sin embargo las paredes celulares aparecen solo parcialmente alteradas (fig 2 y 3).

Aunque la observación cualitativa de las micrografías no parece evidenciar grandes difrencias en el grado de hinchamiento de las células de las distintas muestras consideradas, el análisis de la variación relativa del volumen de los granos si que ofrece diferencias, como se comentó anteriormente. Esto puede explicarse por la formación de grandes burbujas en el grano, ubicadas fundamentalmente en la zona del mucílago central y del tegumento argentoso central, como puede apreciarse en las figuras 1a y 1b para los cafés de Colombia y Uganda, las cuales aparecen más anchas cuanto mayor es la temperatura de tueste.

Por otro lado la formación de grandes huecos está asociada también con la unión de varias cavidades celulares, por ruptura de sus paredes, sobre todo en la parte externa del grano. Esto puede observarse en la figura 2, donde se aprecia la mayor intensidad de destrucción estructural del café arábica en esta zona. En general puede observarse (Fig 2 y 3) que la destrucción de las paredes celulares es superior en el café arábica que en el robusta, como han apuntado otros autores (Puhlman, 1986), observándose una mayor cantidad de poros en las paredes del primero

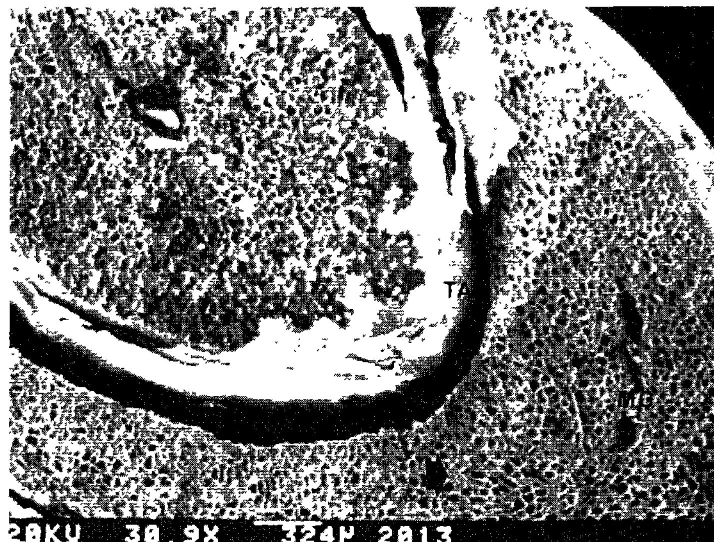
b) Analisis morfogeométrico:

Mediante análisis de imagen de las micrografías de SEM se han medido las dimensiones de poros de cada una de las muestras de café. La Tabla 3 muestra los valores medios y la moda de los parámetros morfométricos área, perímetro, diametro mayor y menor y factor de forma de los poros medidos. Así mismo las fig. 4 y 5 muestran la distribución de tamaños del diametro mayor de los poros y de su factor de forma, respectivamente, para cada café. El número de elementos medidos para cada muestra oscila entre 700 y 860. El factor de forma es la medida de la circularidad de un poro. Este varia dentro de un intervalo de 0 a 1, donde 1 es un circulo perfecto y 0 una linea recta. Es obtenido a partir del area y el perímetro del objeto.

Tabla #3 Caracteristicas morfogeométricas del café tostado.

		Colombia 220	Colombia 295	Uganda 220	Uganda 295
Area (μm^2)	m	427	445	459	509
	M	12	12	15	15
Perimetro	m	77	78	82	83
(μm)	M	27	17	52	17
Diametro	m	23	24	24	25
esférico	M	2	2	2	2
Factor de	m	0.66	0.67	0.68	0.72
Forma	M	0.77	0.77	0.77	0.77
Diametro	m	26	27	29	29
mayor (μm)	M	12	2	32	22
Diametro	m	16	17	18	19
menor (μm)	M	12	7	12	2

Se ha comparado las distribuciones de los distintos parámetros morfométricos de las muestras de cafe mediante un análisis de Kolmorogov-Smirnov. Esto permite afirmar que no hay diferencias estadísticamente significativas entre las distribuciones de los parámetros de los distintos cafés, a excepción de la distribución de áreas de poros donde se observan valores mayores en los cafés de Uganda, a la vez que la mayor temperatura de tueste parece inducir también mayores áreas de poros en ambos tipos de café. Esto está en coherencia con los valores de la difusividad del vapor de agua encontrados en estas mismas muestras de café (Ortolá et al. 1993) que son ligeramente mayores en los cafés de Uganda y aumentan también con la temperatura de tostado. Para estas mismas muestras se observaron velocidades de desgasificación también en coherencia con estos resultados (Bogotá, 1992). Probablemente las mayores presiones internas de gas que se producen durante el tostado a altas temperaturas son responsables del incremento en el área de los poros.



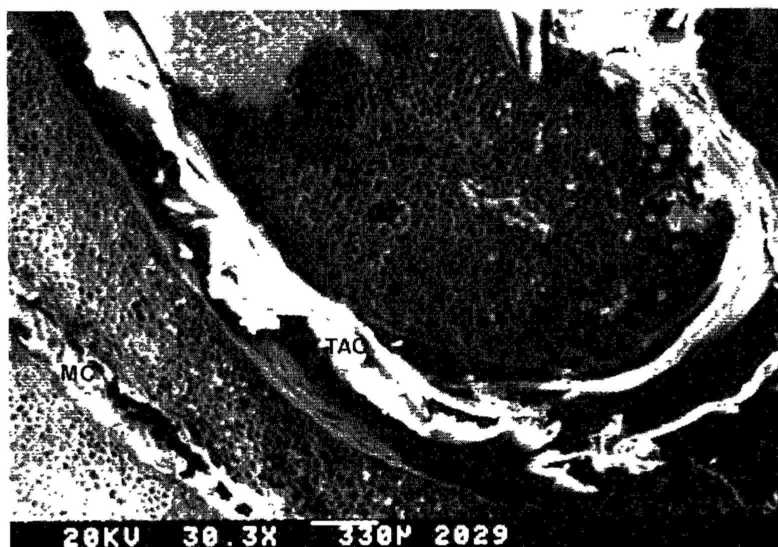
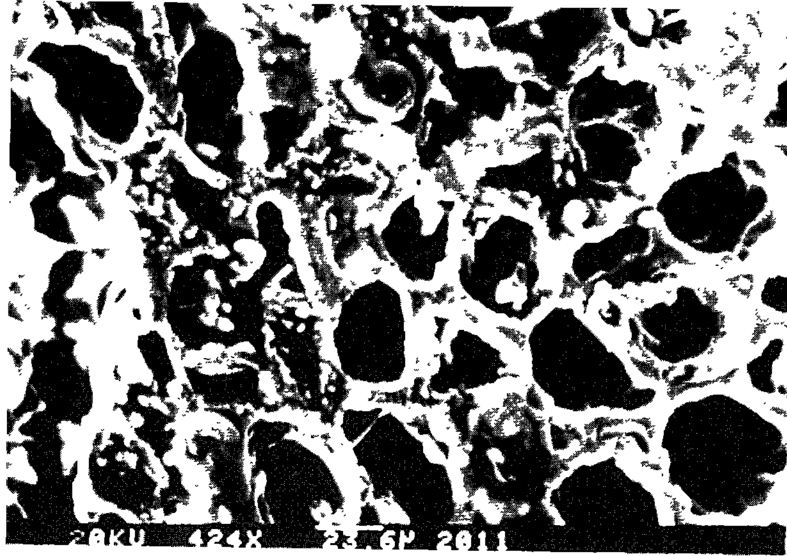


Fig #1 Micrografías a pocos aumentos del corte transversal
de a) café arábica tostado a 220°C
b)café robusta tostado a 295°C
MC = mucilago central
TAC = tegumento argentado central
➧ = material celular



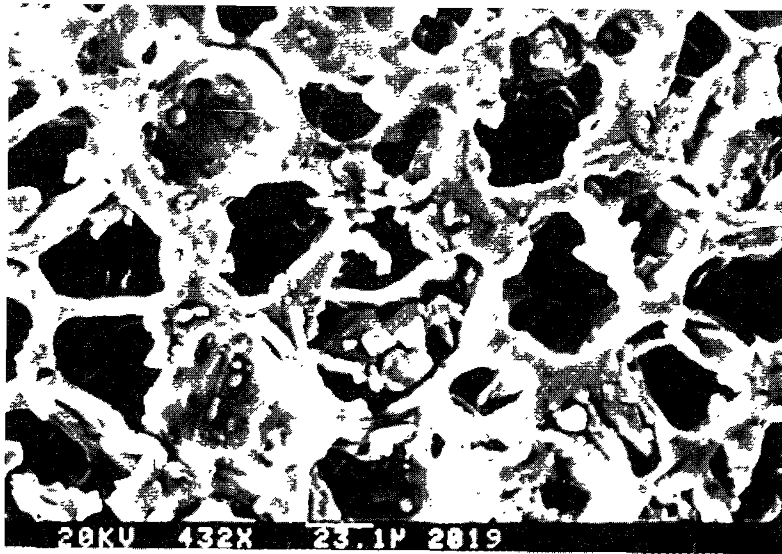
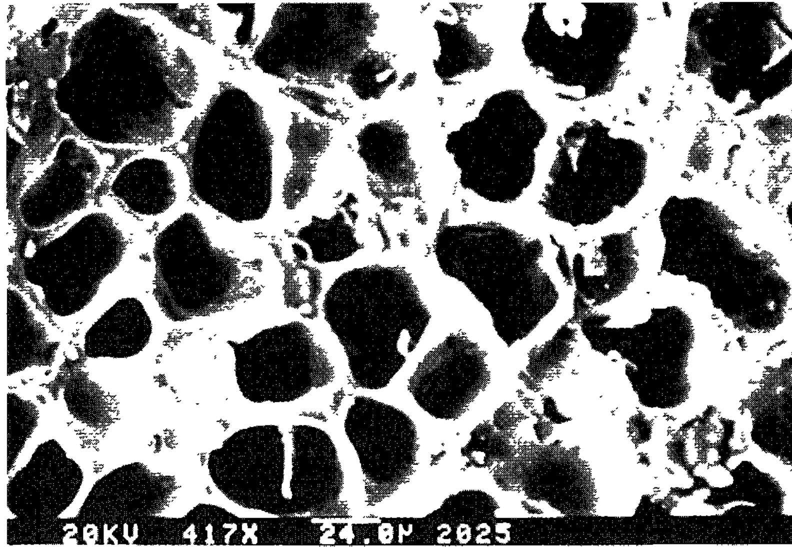


Fig #2 Micrografías de café arábica tostado a
a) 220°C zona externa del grano
b) 295°C zona central del grano



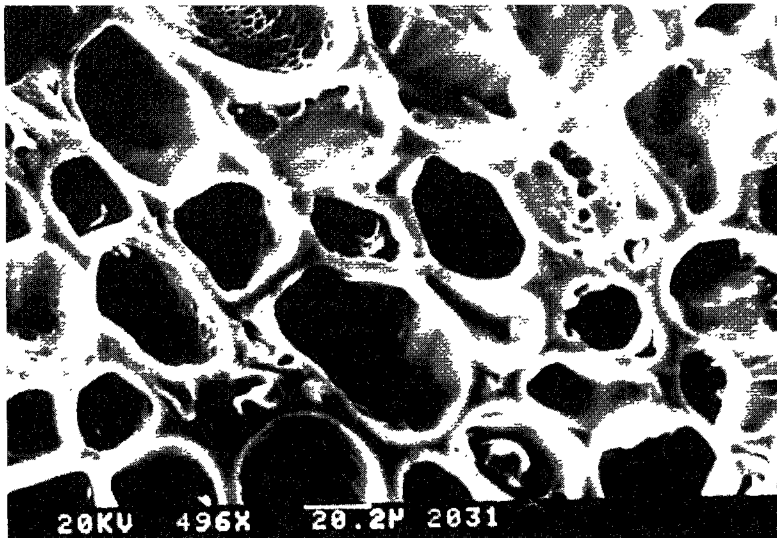


Fig #3 Micrografías de la zona central
del café robusta
a) tostado a 220°C
b) tostado a 295°C

Fig. #4 Distribución de Diámetros

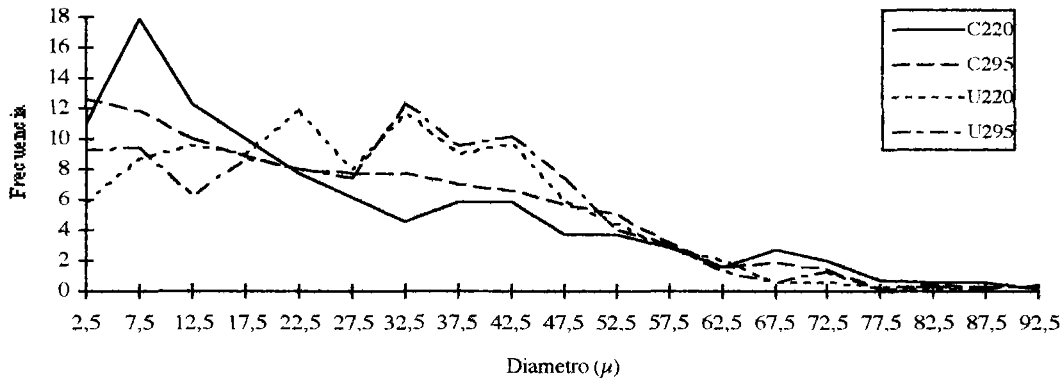
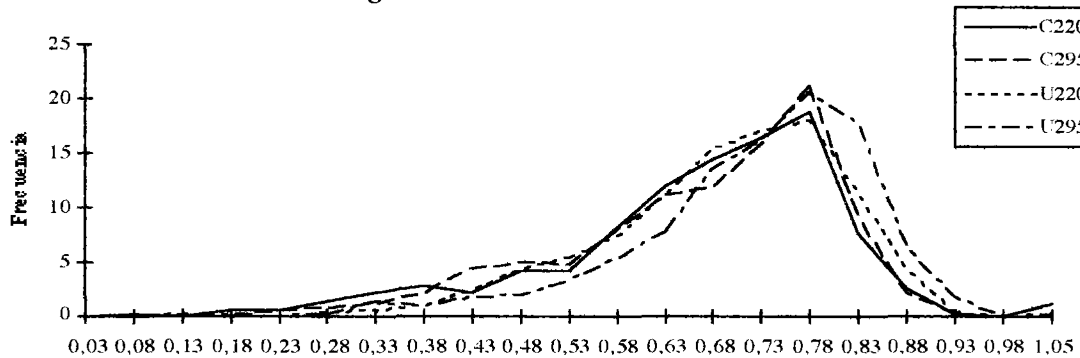


Fig. #5 Distribución del Factor Forma



Por otro lado, es de destacar el hecho de que los poros con diametro menor al medible sobre las micrografías obtenidas no son cuantificados en el análisis. Esto no es importantte en la evaluación de la porosidad total de las muestras, ya que estos constituirían una fracción muy pequeña del volumen de huecos dado su pequeño tamaño, sin embargo pueden tener un papel muy importante en los proceos de difusión y adsorción de gases. Por un lado pueden inducir la condensación capilar del gas para determinada presión del mismo y por otro pueden dificultar la difusión de las moléculas del gas a través de los huecos de la estructura sólida.(Ortolá et al. 1993) Esto es especialmente importante en el caso de las partículas y granos de café cuya microestructura se ha visto que está integrada fundamentalmente por una gran asociación de células sin espacios intercelulares, cuya única interconexión son los plasmodesmos de tamaño del orden de los Å. (Dentan, 1977) y los poros de diferente tamaño formados en las paredes celulares durante el tostado. La circulación de los gases en el interior del sólido tiene que ocurrir ,pues, de célula a célula por pequeños canales. Esta es la razón por la que procesos como la liberación completa del CO_2 son súmamente lentos, sobre todo para el café en grano donde las moléculas de gas de la zona interna han de atravesar un grán número de células hasta llegar a la superficie.

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SEM ANALYSIS OF POROSITY OF ROASTED COFFEE.

ABSTRACT.

Arabica and robusta green coffee beans from Colombia and Uganda were roasted at two different temperatures, controling weight loss and colour, as roasting indexes. The different coffee samples were analyzed using SEM (scaning electron microscopy) in order to determine physical changes during coffee roasting.

The micrographs were studied by image analysis, obtaining porosity values (ε) for each sample and porous size distribution.

ε values have been compared with the ones obtained by Day's method. No significant differences were found betwen them for robusta coffee (around 30% of ε). However Colombia samples showed ε higher values when is determined by image analysis than those determined by Day's method.

ANALYSE MICROSCOPIQUE ELECTRONIQUE DE BALAYAGE DE LA POROSITE DU CAFE TORREFIE.

RESUME

Des grains de café verd des variétés arabica et robusta, de Colombie et Uganda, furent torréfiés a deux températures différentes, en controlant la perte de poids et la couleur come indice de la torréfaction. Les différents échantillons de café furent analizés en utilizant la microscopie électronique de balayage, ceci afin de déterminer les changements physiques survenus durant le processus de torréfaction du café.

Les microphotographies furent étudiées par analyse d'image, s'obtenant des valeurs de porosité (ε) pour chaque échantillon et distribution de taille des pores.

Les valeurs de ε furent comparées avec les résultats obtenus par la méthode Day. Aucune différence significative ne fut trouvée pour la variété robusta (proche a 30%). Par contre les échantillons de colombie présentent des valeurs de ε plus élevées par analyse d'image comparé aux résultats obtenus par la méthode de Day.

CONTROL CINÉTICO Y TERMODINÁMICO DE LAS INTERACCIONES AGUA-CAFÉ TOSTADO

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INTRODUCCION

Uno de los aspectos más importantes de la tecnología del café tostado son los problemas de estabilidad del producto, siendo el contenido en humedad del café decisivo para determinar la intensidad con que se produce el fenómeno deteriorativo (Quast & Teixeira Neto, 1976). El papel de la humedad del café en las reacciones deteriorativas no ha sido descrito en forma precisa. El agua puede participar en las reacciones deteriorativas como reactivo o como elemento movilizador de solutos (Labuza, 1971) favoreciéndolas según su "disponibilidad", expresada en términos de su actividad de agua (aw). La relación existente entre el contenido en agua del café y los factores temperatura y humedad relativa ambiental, es muy importante para conocer el comportamiento durante el almacenamiento y predecir la estabilidad. Esta relación se encuentra establecida en las isotermas de adsorción de agua del café tostado y molido que ha sido poco estudiada (Gane, 1950; Ayerst, 1965; Lewichi et al., 1973; Quast y Texeira, 1976; Hayakawa et al., 1978; Weisser, 1986) y la información no es muy clara. Por otro lado, llama la atención la ausencia de datos cinéticos (difusividad) para la hidratación del producto.

Generalmente se recomienda que el café tostado y molido, (dependiendo del contenido de oxígeno o del grado de vacio del envase), no tenga una humedad superior al 4-5 % (p/p) para obtener un período de vida media, conservando su calidad, hasta 18 meses a una temperatura del orden de 25 °C. Este valor es algo superior al contenido de humedad (b.s.) de la capa monomolecular estimado con la ecuación de BET por Hayakawa et al. (1978) (3.73% a 20 °C , y 2.2 % a 30 °C). La humedad de la monocapa se ha estimado que es el contenido de agua más seguro para prevenir las reacciones de enranciamiento en productos con grasa (Labuza, 1976).

El objetivo del presente estudio consiste en determinar las isotermas de adsorción de agua y la difusividad de este componente en cafés de diferentes especies, tostados a alta y baja temperatura.

MATERIALES Y METODOS

Materia prima

Las variedades de café estudiadas fueron: Coffea Arabica Linn. de orígen Colombia y Coffea Canephora Pierre ex Foechner de Uganda, ambos de la criba comercial 18.

Métodos

PREPARACION DE LAS MUESTRAS

Tostado: se realizó en un equipo con tambor rotatorio y control de temperatura en el centro del cilindro.modelo Tec 250 Especial C, de la casa Tec-Aire S.A., a 220 °C y 295 °C

Molienda: se efectuó en un molino comercial, de muela troncocónica con separación regulable de la firma FAEMA S.A., ajustando la separación de muelas al número 4.

Desgasificación: se realizó en una estufa de vacío a 110 °C y 30 mbar de presión, hasta peso constante.

CARACTERIZACION DE LAS MUESTRAS

Distribución de tamaños de partícula: se analizó, en atmosfera de nitrógeno, utilizando una serie de tamices vibratorios C.I.S.A. de 19.5 cm de diámetro, con tamaño de malla comprendido entre 0.125 y 1.0 mm. El tiempo de vibración para cada análisis, hasta la situación de equilibrio, fue de 20 min.

Medida de color: se utilizó un espectrofotómetro MINOLTA CM 1000 con esfera integradora y ventana de exposición de 8 mm de diámetro. Se determinaron las coordenadas CIELab con el sistema de referencia iluminante D_{65} / observador 10°.

Determinación de la humedad: se determinó mediante valoración Karl Fischer. El método se basa en la extracción a reflujo del agua contenida en aproximadamente 1 gramo de café molido, con 50 ml de metanol en ebullición durante 30 min. (Scholz, 1988). Durante la extracción, se determinó volumétricamente el contenido en humedad de la muestra, en un equipo titulador Karl Fischer DL-18 Metler, con precisión del 0.3%.

DETERMINACION DE LAS ISOTERMAS DE ADSORCION DE AGUA

Se ha utilizado el método estático estandarizado en el proyecto COST 90 (Spies & Wolf, 1983; Wolf et al., 1984). Muestras de aproximádamente 2 gr. de café desgasificado, fueron colocadas en pequeños botes twist-off (diámetro 40 mm y altura 48 mm). Estos se acondicionaron por duplicado en distintos recipientes de vidrio de cierre hermético, provistos de un frasco con una solución salina saturada o de ácido sulfúrico con diferentes niveles de actividad de agua a 25 °C (Tabla 1) (Rockland & Nishi, 1980; Labuza et al., 1985; Resnik et al., 1985), para mantener constante y a niveles conocidos, la húmedad relativa interna de cada una de ellos.

TABLA 1. Actividad de agua para las distintas disoluciones

DISOLUCIONES	a_w (t = 25 °C)	DISOLUCIONES	a_w (t = 25 °C)
Bromuro de Litio	0.064	Carbonato Potásico	0.430
Acido Sulfúrico 21.67 N	0.065	Nitrato Magnésico	0.536
Cloruro de Litio	0.113	Cloruro Cúprico	0.675
Acido Sulfúrico 18.11 N	0.117	Cloruro Sódico	0.753
Acetato Potásico	0.225	Cloruro Potásico	0.845
Cloruro de Magnesio	0.330	Nitrato Potásico	0.925

Las muestras de café molido se mantuvieron en una habitación termostatada a 25 °C hasta que alcanzaron peso constante (equilibrio entre su contenido de humedad y la humedad relativa en el interior de los recipientes herméticos). Alcanzado el equilibrio se determinó la humedad de las muestras por el método Karl Fischer descrito anteriormente.

DETERMINACION DEL COEFICIENTE DE DIFUSION DEL AGUA

Se hizo pasar un flujo de aire (6 10^5 Kg/hr m2), con ayuda de una bomba, por el interior de tres frascos lavadores de gases, de capacidad 125 ml, adaptados en serie. Los dos primeros contenían una disolución de $MgCl_2$ saturada (aproximadamente 25 ml) y el tercero lana de vidrio, que actuaba como filtro del aire. Al final de éste fueron colocadas dos placas filtrantes (diámetro 40mm y porosidad de la placa N° 0) en las cuales se colocaron muestras de aproximadamente 1 g de café, tapándose con lana de vidrio para evitar pérdidas en la fluidización de la muestra. Se hicieron medidas de peso de las mismas en balanza analítica, y de humedad por el método Karl Fischer a distintos tiempos hasta 13 horas, tiempo en que las muestras alcanzaron una humedad similar a la de equilibrio.

El experimento se llevó a cabo en una cámara termostada a 25 °C.

RESULTADOS Y DISCUSION

Las muestras estudiadas presentaron un mismo grado de tueste, correspondiente a una pérdida de peso de 17.0 ± 0.9%. Las coordenadas de color de las mismas fueron L* = 24.8±0.7, a* = 7.5±0.4, b* = 7.8±0.3, variando la diferencia de color (ΔE) entre ellas de 0.78 a 1.03. La humedad final de las muestras también fue uniforme (2.5 ± 0.8 %). El café molido presentó un tamaño medio de partícula de 0.48±0.08 mm, siendo la distribución de tamaños la dada en la tabla 2.

TABLA 2. Distribución del tamaño de las partículas

Diámetro med.(mm)	fracción másica(%)
0.900	5.32 ± 0.04
0.715	20.51 ± 0.12
0.565	28.10 ± 0.16
0.450	25.08 ± 0.15
0.360	13.26 ± 0.06
0.285	4.29 ± 0.01
0.225	4.22 ± 0.12
0.180	1.22 ± 0.00

Isotermas de adsorción.

La humedad de las muestras desgasificadas y molidas, expuestas a temperatura y humedad relativa constante hasta equilibrio, se evaluó por el método Karl Fischer y no a través de la variación de peso durante su equilibrado a una humedad dada.La razón se basa en que la variación de peso corresponde no sólo a perdida o ganancia de agua, sino también de volátiles, sobre todo CO2. Así mismo la desgasificación de las muestras no comporta el secado completo, existiendo, por tanto, una humedad residual en las muestras colocadas en las cámaras de humedad controlada.

Las isotermas de adsorción de los diferentes cafés molidos, se muestran en las fig. 1 y 2. Estas presentan una forma sigmoide, con dos mesetas, característica de productos porosos en las que, además del mecanismo de adsorción, que actua a bajos valores de aw, se presenta a humedades mayores un mecanismo de condensación capilar (Clarke y Macrae, 1985). Corresponden a una isoterma tipo IV de la clasificación de Brunauer (Weisser, 1986). El rápido ascenso de la isoterma, previo a la segunda meseta, comienza en todos los casos para humedades de alrededor de 6.5 % (aw = 0.536), lo que se corresponde según la teoría de condensación capilar aplicando la ecuación de Kelvin (ec.1) a un radio de poros de 16 Å.

$$r = -2\sigma V / R T \, Ln\, a_w \qquad (1)$$

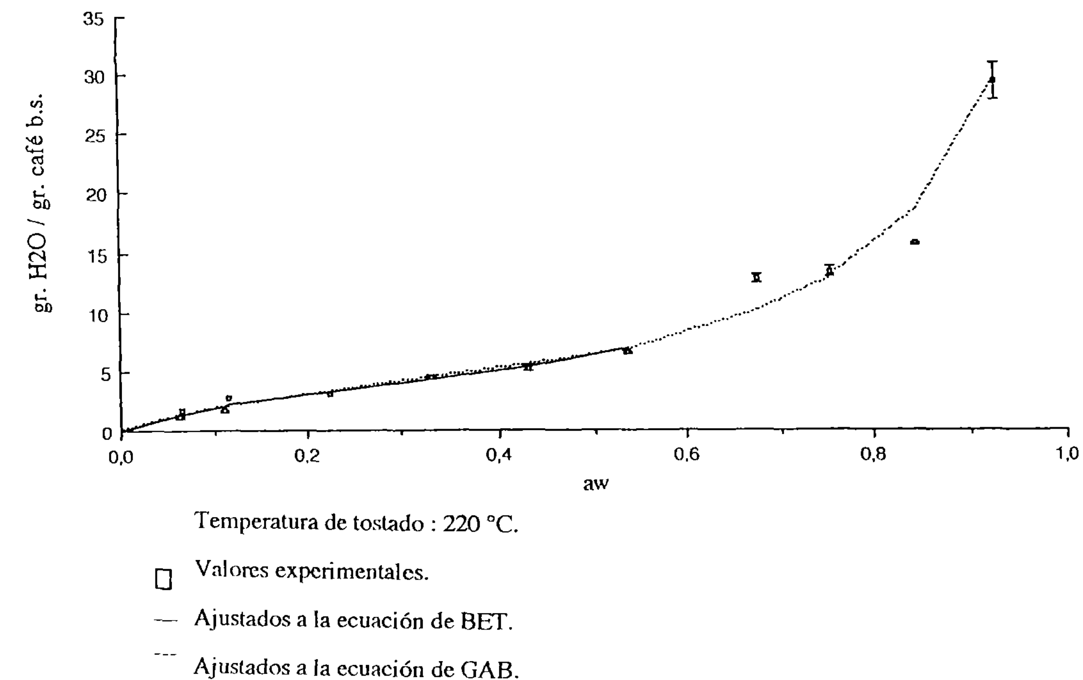
donde, σ: Tensión superficial del agua.
V: Volumen molar del agua.
r: radio de los poros.

La isoterma alcanza la segunda meseta (saturación capilar de los poros) para valores de aw = 0.675 (r = 27 Å) en todas las muestras de café lo que parece sugerir una distribución de tamaños de microporos similar en todos los casos. Por otro lado, los valores de la humedad de saturación capilar (W_{SC}) determinados en el punto con aw = 0.845 corresponden a un 15 y 17.5 % para el café molido arábica y robusta respectivamente. Esto parece indicar que la fracción de poros asociada con la condensación capilar es mayor en las muestras de robusta. Como resumen, parece que el fenómeno de condensación capilar se produce en poros de tamaño muy pequeño (16 Å ≤ r ≤ 27 Å), inferior al tamaño de las cavidades celulares del café tostado (Gutiérrez, 1992) y que corresponderían más bien a pequeños canales de las paredes celulares, presentes ya en la estructura del café verde -plasmodesmos- (Dentan, 1977), o generados en los procesos de pirólisis. La presencia en el café tostado de microporos de estas dimensiones ha sido citada como responsable de fenómenos de condensación capilar de CO_2 (Saleeb, 1975).

Para las dos variedades de café, la isoterma vuelve a recuperar un claro sentido ascendente, para aw > 0.845, probablemente debido a que comienzan a ser responsables de la depresión de la aw los fenómenos de disolución.

Los datos experimentales, para aw ≤ 0.536, se han ajustado al modelo de BET. Sus parámetros, Wm (valor de la humedad en la monocapa) y C (constante, relacionada con la energía de adsorción), han sido estimados con una regresión lineal y optimizados con una regresión no lineal (α<0.01 en todos los casos). Se ha considerado aw ≤ 0.536, ya que fuera de esta zona el modelo tiene sentido físico dudoso (Iglesias et al., 1976; Toupin et al., 1983). Los valores encontrados se registran en la tabla 3. Los datos de los parámetros del modelo de BET, Wm y

Fig. 1 : ISOTERMAS CAFE COLOMBIA.

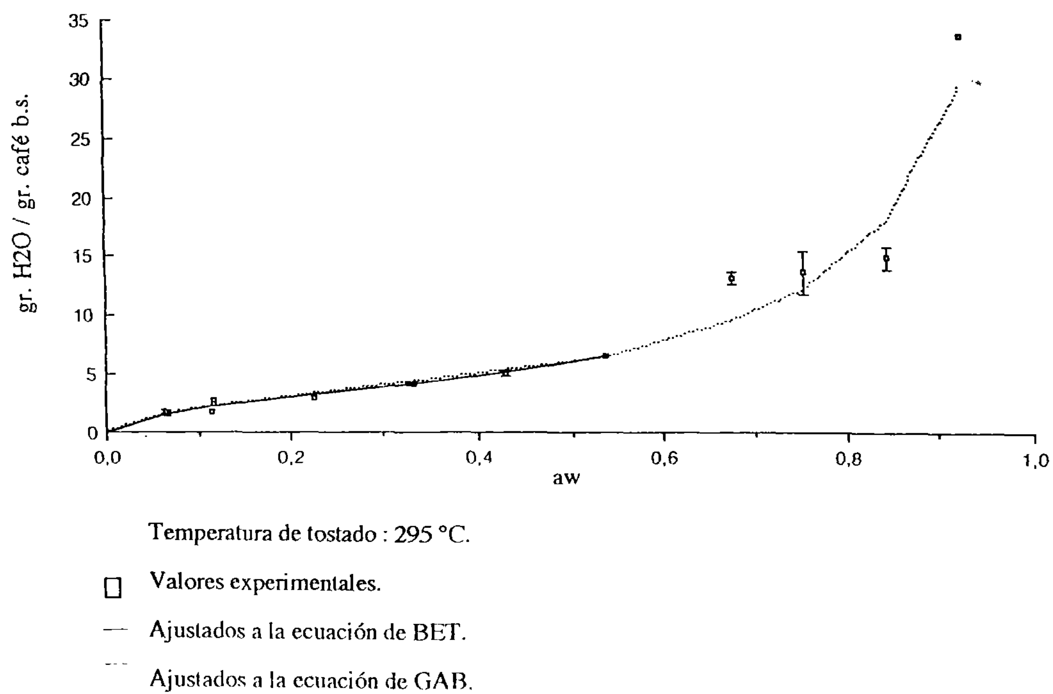


Temperatura de tostado : 220 °C.

□ Valores experimentales.

— Ajustados a la ecuación de BET.

--- Ajustados a la ecuación de GAB.



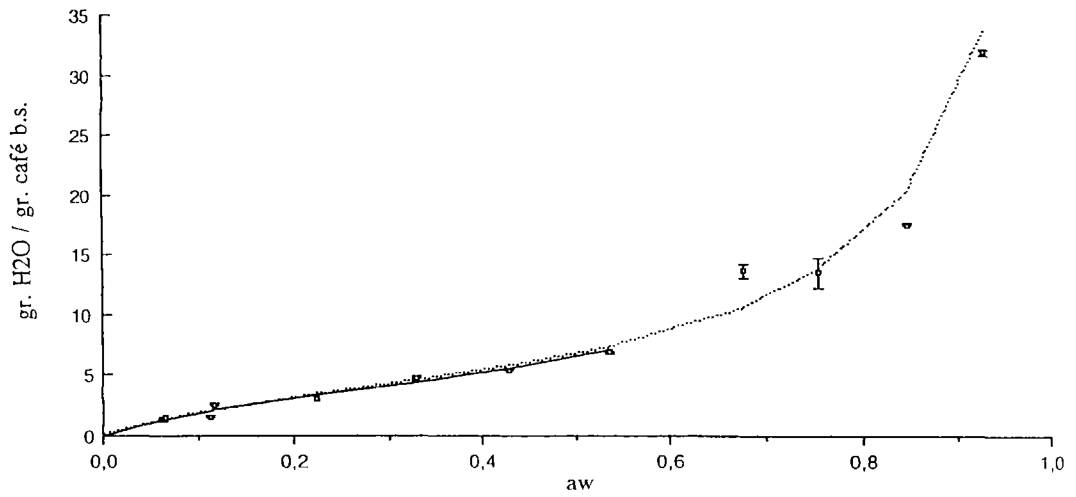
Temperatura de tostado : 295 °C.

□ Valores experimentales.

— Ajustados a la ecuación de BET.

--- Ajustados a la ecuación de GAB.

Fig. 2 : ISOTERMAS CAFE UGANDA.

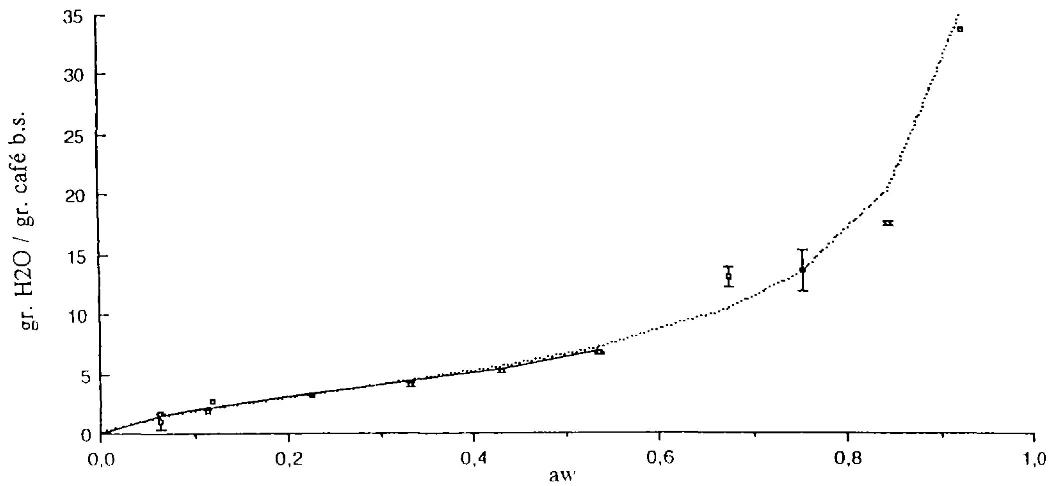


Temperatura de tostado : 220 °C.

□ Valores experimentales.

---- Ajustados a la ecuación de BET.

--- Ajustados a la ecuación de GAB.



Temperatura de tostado : 295 °C.

□ Valores experimentales.

__ Ajustados a la ecuación de BET.

--- Ajustados a la ecuación de GAB.

C son del mismo orden que los determinados por Hayakawa et al. (1978) y Weisser (1986) para café tostado y molido. No se observan diferencias estadísticamente significativas en los valores de Wm y C de las diferentes muestras, aunque parece apreciarse un valor de C ligeramente mayor en las muestras tostadas a altas temperaturas, probablemente por un menor grado de tueste en el interior del grano, lo que implicaría una mayor presencia de sustancias hidrofílicas. A su vez estos valores parecen también algo superiores en el café arábica que en el robusta, para una misma temperatura de tueste, posiblemente debido al hecho de que los cafés arábica presentan después de tostados, una cantidad mayor de polisacáridos que los café robusta (Trugo, 1985). En las figuras 1 y 2 se observa esto cualitativamente ya que los cafés de la especie arábica presentan en su isoterma un punto de inflexión ligeramente más pronunciado, siendo más alto el valor de C cuando más acentuado se presenta este punto en las isotermas. Este punto de inflexión corresponde al fín de la monocapa y comienzo de la disminución del calor diferencial de adsorción (Salwin, 1959; Díaz et al., 1976).

TABLA 3. Parámetros de la ecuación de BET[1], para café tostado molido.

Parametro / Muestra	Colombia 220 °C	Colombia 295 °C	Uganda 220 °C	Uganda 295 °C
Wm	3.5 ± 0.3	3.3 ± 0.2	3.7 ± 0.3	3.5 ± 0.3
C	9 ± 3	12 ± 4	8 ± 2	10 ± 3
So	123 ± 11	116 ± 7	130 ± 11	123 ± 11
r^2	0.946	0.953	0.953	0.949

[1]. Obtenidos por regresión no lineal para $a_w \leq 0.536$.

s_o. Superficie específica (m^2/g de café). Calculada como $S_o = 3.5 \times 10^3 W_m$. (Labuza, 1978).

El modelo de GAB, recomendado por el programa COST 90 como el mejor modelo teórico con fines predictivos para alimentos, en un amplio intervalo de aw (Van den Berg, C., 1984; Wolf, W, Spiess, W. E. L., Weisser, R. B., 1984; Labuza et al., 1985), también se ha ajustado a los datos experimentales mediante una regresión lineal múltiple ($\alpha<0.01$ en todos los casos). Los datos obtenidos por este análisis se registran en la tabla 4 . Del análisis de los parámetros obtenidos se llega a las mismas conclusiones que para el ajuste del modelo de BET.

TABLA 4. Parámetros de la ecuación de Gab[2], para café tostado molido.

Parámetro / Muestra	Colombia 220 °C	Colombia 295 °C	Uganda 220 °C	Uganda 295 °C
Wm	3.96	3.70	4.01	3.89
C	7.19	9.51	6.73	7.06
K	0.92	0.95	0.95	0.96
r^2	0.630	0.707	0.618	0.631

[2]. Obtenidos por regresión lineal múltiple para $a_w \leq 0.925$.

Se ha comparado la bondad del ajuste de cada modelo (BET y GAB) a cada muestra mediante un test F sobre el cociente de los cuadrados medios residuales de cada modelo, no observándose en ningún caso diferencias significativas. Atendiendo a esto se considera un modelo u otro según la finalidad: el modelo de GAB con fines predictivos, y el modelo de BET en el análisis físico de los parámetros. Sin embargo, hay que tener en cuenta que, pesar de la alta significación estadística del ajuste del modelo de GAB en las isotermas estudiadas, éste no permite predecir el comportamiento en la zona donde ocurre la saturación capilar comentada anteriormente. Por tanto, para humedades relativas (HR) comprendidas entre 0.536 y 0.925 los valores de humedad del café predichos tienen un cierto error por defecto o por exceso, según que HR sea mayor o menor que 0.75 aproximadamente, tal como puede observarse en las fig 1 y 2.

Coeficiente de difusión del agua.

El estudio de la cinética de humectación del café, llevado acabo en la forma descrita en material y métodos, permite obtener curvas humedad/tiempo como la que se da en la fig 3 para el café de Colombia tostado a 220°C. La curva presenta tres zonas que ponen de manifiesto un comportamiento diferente del café en tres etapas. En la primera (zona A) que se extiende hasta unos 90 minutos el producto gana humedad regularmente hasta alcanzar entre un 2 - 3 % de contenido en agua. A partir de este momento, el café entra en otra fase en que el proceso de humectación es muy lento y además la ganancia de agua es muy irregular lo que se manifiesta en la gran dispersión obtenida para las medidas de humedad. Finalmente, después de cerca de 13 h, la muestra alcanza la humedad de equilibrio (4.5 %) con la humedad relativa del ambiente (HR= 0.332), establecida a partir de las isotermas, siendo

ya en este punto menor la dispersión de la medida. Esto parece evidenciar una humectación en tres etapas, tal como se indica en la Fig. 3.

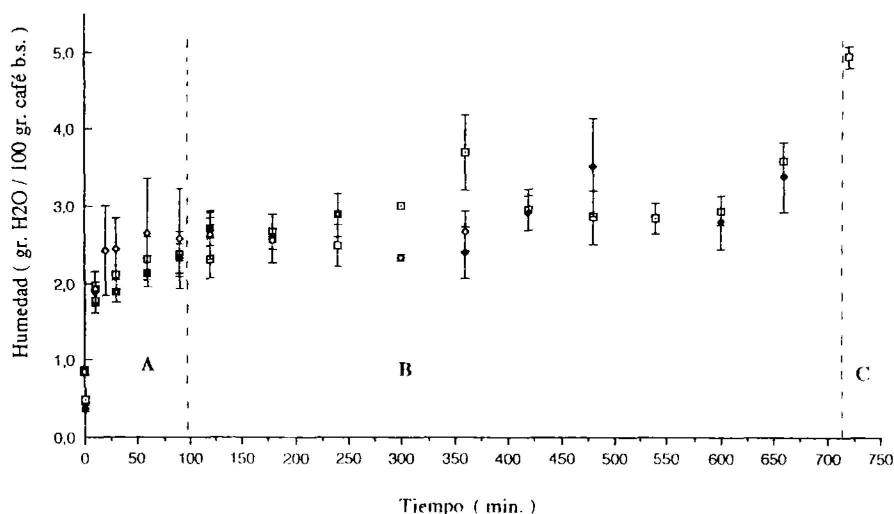


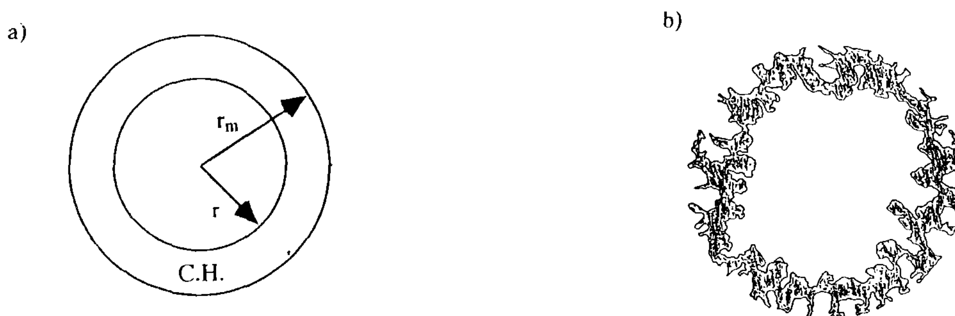
Fig. 3 : CAFE COLOMBIA. 220 °C.

Es de destacar que cuando la muestra llega al final de la etapa A la medida de su aw en un equipo DECAGON proporciona un valor igual a la humedad relativa del ambiente (0.332), aunque el contenido medio en humedad de la muestra es inferior al de equilibrio. Esto permite suponer que al menos la superficie de las partículas posee una humedad igual a la de equilibrio, aunque el interior de las mismas esté seco debido a que haya algún impedimento a la difusión de moléculas de agua. Esto supondría admitir que, al finalizar la etapa A, las partículas estarían rodeadas de una "capa hidratada" (CH) que rodea un "core" seco (fig 4a). A partir de la humedad media (W_m), humedad de equilibrio (W_e) y radio medio de las partículas (r_m) se puede calcular el espesor de la CH, planteando un balance en términos de concentración volumétrica de agua en la partícula. Este permite llegar a la ec. 2, donde r es el radio de la esfera interna que define el espesor de la CH (r_m-r).

$$(r_m^3-r^3)/r_m^3 = W_m/W_e \qquad (2)$$

La relación r/r_m que se obtiene para los cafés estudiados es de 0.78. Por otro lado, estudios microestructurales (Gutierrez, 1993) permiten establecer que el radio medio de las partículas de café consideradas equivale a 5 unidades celulares, por lo que se deduce que el espesor de la CH sería el correspondiente a una capa monocelular alrededor de la partícula de café (fig. 4b). La microestructura de las partículas de cafe corresponde a un aglomerado de células casi totalmente vacias y conectadas por poros muy pequeños. Esto apoyaría la hipotésis de la CH: las moléculas de agua se difundirían primero a la capa de células más externa en contacto directo con el ambiente donde existe una buena convección (relación entre la reistencia externa e interna a la difusión próxima a cero). En la superficie interna de las células, las moléculas de agua quedarían adsorbidas inicialmente hasta formar la capa monomolecular y multicapas. El paso a la siguientes células más internas está muy impedido. Por un lado el pequeño diámetro de los poros que las comunican permite suponer que en estos se da un mecanismo difusional de tipo Knudsen mucho más lento y altamente dependiente del tamaño de los poros. Por otro lado el nivel de humedad de equilibrio de la muestra, muy próximo a la humedad de la capa monomolecular hace pensar que el número de moléculas adsorbidas en multicapas y por tanto con mayor movilidad difusional es relativamente pequeño. En resumen, a partir de la hipótesis planteada, se podría decir que el fenómeno de hidratación del interior de las partículas de café tiene una gran energía de activación por lo que requiere tiempos muy largos, al igual que ocurre con la liberación del CO_2 . La gran energía de activación convierte la hidratación del "core" interno en un proceso controlado por el caos molecular, lo que justifica la dispersión en las medidas de humedad (próximas a la humedad de la capa monomolecular) en la zona B de la curva de humectación. Por otro lado parece que cuando la monocapa de toda la supercicie interna de la partícula ha sido cubierta (final de la zona B) el producto alcanza rápidamente la humedad de equilibrio al desaparecer uno de los impedimentos a la difusión.

Fig. 4 :



Se ha determinado la difusividad del agua en la capa hidratada de las partículas de café con los datos de la zona A de la curva, asumiendo un mecanismo de difusión molecular en esta etapa. La tabla 5 presenta los resultados experimentales humedad / tiempo, utilizados para el cálculo. La humedad media ha sido corregida mediante la ec. (2), asumiendo que toda el agua presente en la muestra está concentrada en la CH. Los datos han sido ajustados mediante la ecuación (3), considerando:
- Distribución de humedad inicial homogénea en toda la C.H.
.- Espesor de la capa, igual 1/10 del diámetro medio de las partículas.
.- Resistencia exterior a la transferencia de materia despreciable.
.- Concentración de humedad en la interfase constante e igual a la humedad de equilibrio con la humedad relativa del aire.
.- El sistema isotrópico y difusividad constante.

$$[(W_\sigma - W_t) / (W_\sigma - W_o)] = [8 / \pi^2] \exp(-D \pi^2 t / a^2) \qquad (3)$$

donde: W_σ : Humedad de equilibrio
W_t: Humedad a un tiempo t de la CH
W_o: Humedad inicial
a : Espesor de la CH.

TABLA 5. Humedad (g agua/100 g café m.s.) de las muestras determinada por el método Karl Fischer, a diferentes tiempos de humectación.

Tiempo (min)	Colombia 220 °C	Colombia 295 °C	Uganda 220 °C	Uganda 295 °C
0	0.58	1.10	0.74	1.15
10	1.86	1.68	1.76	1.12
30	1.97	1.90	1.53	1.62
60	2.88	2.46	2.73	1.74
90	2.34	2.34	3.14	2.00

La tabla 5 presenta los valores obtenidos de difusividad (m^2s^{-1}) con el error evaluado para estas determinaciones, así como el coeficiente de determinación del ajuste lineal de la ecuación (3). En todas las muestras, llama la atención el pequeño valor de la difusividad, del orden de 10^2 veces más pequeño que el encontrado en otros productos alimentarios para el agua en fase líquida (Guarda, 1988). Esto sólo puede entenderse si también la mayor parte de las células externas de las partículas se comunican con el ambiente a través de poros muy pequeños y poseen superficie interna activa a la adsorción, donde las moléculas de agua van quedando ligadas y por tanto con menor movilidad.

Por otro lado, se observan valores ligeramente más altos de difusividad en las muestras tostadas a mayor temperatura. Igualmente los cafés robusta presentan valores más altos. Esto es concordante además con la mayor

facilidad, observada en estas muestras a la desgasificación. El área media de los poros (Gutierrez, 1993), que es mayor en las muestras con difusividad más alta, podría justificar este hecho.

TABLA 6. Difusividad aparente del agua en las distintas muestras	
Muestra	Difusividad ($m^2 s^{-1}$) x 10^{-12}
Colombia 220 °C	0.25 ± 0.12
Colombia 295 °C	0.33 ± 0.17
Uganda 220 °C	1.29 ± 0.58
Uganda 295 °C	1.50 ± 0.91

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RESUMEN

Se ha estudiado el comportamiento frente a la adsorción y difusión de agua, a 25 °C, de café tostado y molido de las especies Arábica de Colombia y Robusta de Uganda, tostados a 220 y 295 °C. El grado de tueste (dark roast) se controló a través de la pérdida de peso y el desarrollo del color.

Para la determinación de las isotermas se ha utilizado el método estandarizado COST 90, en el intervalo $0.064 \leq aw \leq 0.925$. Las isotermas experimentales (tipo IV de la clasificación de Brunauer) fueron ajustadas mediante la ecuación de BET para $aw \leq 0.536$. No se observaron diferencias significativas en el contenido de humedad de la capa monomolecular (3.5 ± 0.3.gr agua / 100 gr m.s.). La condensación capilar, para $aw > 0.675$ tiene lugar en poros de 16 - 27 Å de radio.

Los coeficientes de difusión fueron muy pequeños, presentando impedimentos estéricos a las moléculas de agua. Además, un control cinético durante la rápida hidratación del café parece dejar al producto con un menor contenido en humedad que el esperado por el análisis de las isotermas.

ABSTRACT

Adsorption and diffusion behaviour of water, at 25°C, in ground roast coffees have been studied. Arabica and Robusta varieties from different origins, roasted at 220 and 295 °C were considered. Degree of roast (dark roast) was controlled through weight loss and developed colour.

Standarized COST method, over the range $0.064 < aw < 0.925$, were used for isotherme determination. Experimental isotherms (type IV as clasified Brunauer) has been fitted by BET equation for $aw < 0.520$. No significant differences were observed in monolayer moisture content (3.5 ± 0.3.gr water / 100 gr dry coffee). Capillary condensation, for $aw >$ o.675 , occurs in pores of 16 - 27 Å in radius.

Water diffusion coefficients were very small, showing sterical impediments to water molecules. Also, a kinetic control during the fast hydratation of coffee seems to lead the product to lower moistures content than the expected from the isotherms analysis.

DISCRIMINATION DE CAFÉS
À L'AIDE D'UN NEZ ÉLECTRONIQUE

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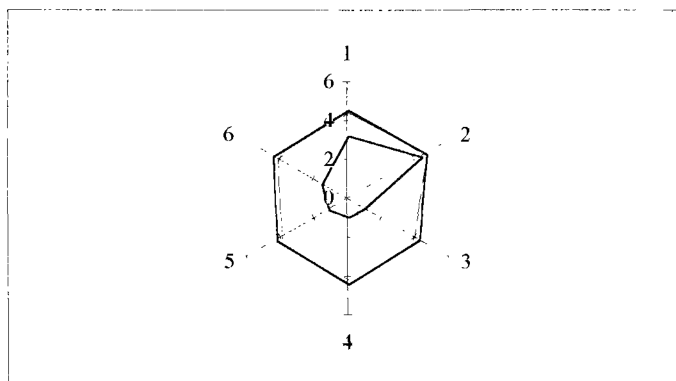
Les capteurs en oxyde métallique du FOX 2000 sont utilisés pour reconnaître la qualité organoleptique de différents grains de cafés. La qualité d'un café est généralement déterminé par des goûteurs experts qui se basent sur différents paramètres tels que l'arôme. La pyrolyse et la formation de composés phénoliques et soufrés déterminent en grande partie les caractéristiques finales du café. La chromatographie gazeuse (GC) est une méthode commune de contrôle des composés volatils du café. A côté des méthodes analytiques traditionnelles permettant la séparation de toutes les molécules du headspace du café. il existe une forte demande pour une méthode de contrôle rapide comme les techniques multi-capteurs et les méthodes de reconnaissance de forme permettant d'évaluer la qualité et l'intensité globale d'un arôme à partir de l'expérience olfactive d'un panel de personnes.

La mesure des composés hydrocarbonés, des carbonyles, des esters et des composés soufrés (thiophènes, sulfides...) sont les indicateurs chimiques les plus couramment utilisés dans ce domaine. Les capteurs SX24 et SX25 ont une bonne sensibilité pour les dérivés soufrés et aminés. Des différences de 4 volts sur une échelle de 0-5 dcv sont ainsi obtenues pour certaines qualités de café ; ces valeurs sont corrélées avec des intensités aromatiques plus fortes dans le cas des robusta. D'une façon similaire, il est possible d'utiliser des conditions identiques (30 secondes d'analyse, génération du head-space à température ambiante, 120 ml/mn comme débit-flux d'air). De bons résultats sont aussi obtenus pour différentes teneurs en humidité parce que les molécules aromatiques du café sont plus réactives que les molécules d'eau. En conséquence, l'humidité n'affecte pas les performances du modèle de reconnaissance.

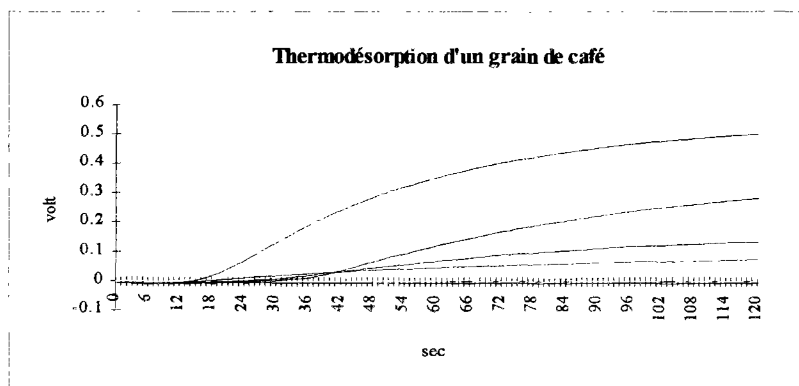


EMPREINTES DE DIFFERENTES QUALITES DE GRAINS DE CAFE (*)
(*) valeurs absolues des 6 capteurs du FOX 2000 sous forme de représentation en étoile (RADAR)

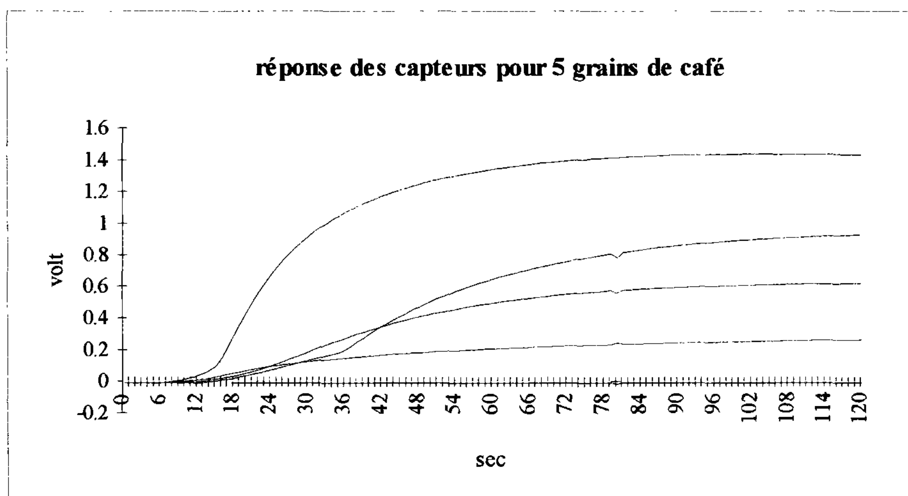
Ceci confirme les applications potentielles d'un nez électronique pour le contrôle-qualité et le contrôle de process en ligne dans l'industrie du café (1).

L'espace de tête (headspace) d'un grain de café a été mesuré par couplage d'un headspace dynamique TEKMAR LSC 2000 au FOX 2000. Nous voulions ainsi savoir si un nez électronique multi-capteurs était capable d'être utilisé à la place d'un GC pour reconnaître et mesurer l'intensité d'arôme dégagé par des grains de café.

Un grain de café a été introduit dans le réceptacle en verre de l'headspace dynamique et purgé pendant 15 mn à température ambiante avec de l'air pur reconstitue. La pompe interne du nez électronique a été éteinte et le flux de gaz vecteur a été ajusté à 6 ml/mn. De l'air pur a été utilisé à la place du gaz vecteur habituel car les capteurs en oxyde métallique du nez électronique répondent également à l'hélium et à l'azote.

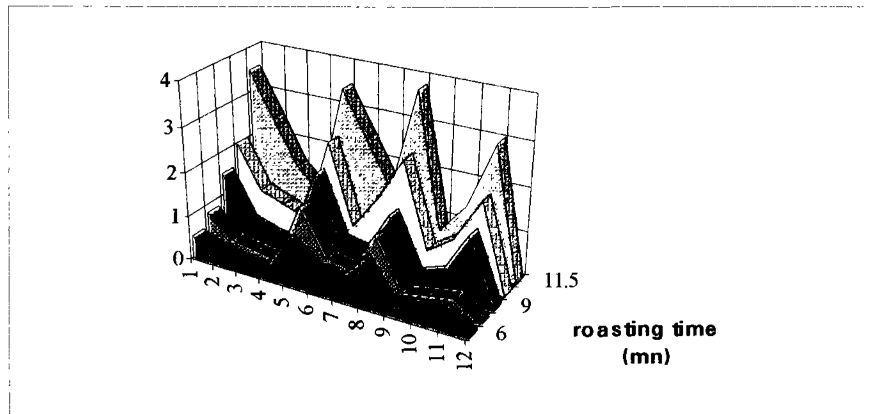


Les composés volatils piégés sur un tube en ténax sont désorbés thermiquement à 100 °C et injecté dans le nez électronique via le tube d'entré du FOX 2000. Pour une telle opération, la ligne de transfert du préconcentrateur a été directement connectée au nez électronique par introduction du capillaire dans le tube du FOX 2000. Les réponses des capteurs ont été enregistrées pendant 2 mn avec un maximum de réponse de 0.6 Volt pour le capteur n°3. Ensuite, la même expérience a été répétée en introduisant 5 grains de café au lieu de 1. L'empreinte de l'odeur reste bien évidemment identique entre les 2 expériences.



Cette empreinte est obtenue par comparaison des conductances relatives normalisées des capteurs pour être indépendant de la concentration. Cependant, quand on étudie les réponses absolues des capteurs, la concentration en arôme et la concentration en volatiles totaux ont augmenté. La réponse du capteur n°3 atteint ici 1.5 Volt et témoigne de l'augmentation de l'intensité de l'odeur.

Nous avons ainsi montrer qu'il est possible d'utiliser un nez électronique couplé à de l'analytique traditionnelle (préconcentrateur de composés volatils). L'utilisation courante du FOX 2000 sera cependant dirigée vers du contrôle/qualité ou du contrôle en ligne. De nombreuses publications font ainsi état d'excellents résultats obtenus pour le suivi des processus de torréfaction et de mélange. L'appareil a ainsi été adapté et couplé à un logiciel de réseau de neurones pour décider du temps optimum pour obtenir la meilleur qualité d'arôme lors de la torréfaction.



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RECOVERY OF BENEFICIAL COFFEE AROMAS FROM THERMAL HYDROLYZATES

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Introduction

Coffee manufacturers continue to strive to produce instant coffees that have the quality of fresh brewed coffee. Soluble products lack the aromatic level and character of fresh brewed coffees primarily because of high temperature processing and water removal via evaporation and drying. Over the years, processors have developed means of capturing coffee aromas and incorporating them into the dried product to enhance their aromatic character. Examples include steam aromas (1), grinder gas aromas (2), condensates from evaporation of extracts (3), etc. There has also been a trend over the years to increase the extraction yields, initially by increasing temperatures within conventional percolation systems and more recently by subjecting spent grounds from percolators to further high temperature hydrolysis, to generate additional soluble solids (4).

The production of high quality instant coffees from such severe processing seems paradoxical. Fractionation techniques have been used to recover the more beneficial aromatics and remove the less desirable, non-coffee like aromas from thermal hydrolyzates. Examples include evaporative stripping (5), and multistage condensation (6), which separate aromas on the basis of relative volatility or boiling point. The aromatic character of soluble coffees can be improved and enhanced using these processes but they are neither very selective nor efficient in the recovery of beneficial coffee aromas.

Gustafson et al. (7) have demonstrated the ability of high surface area, non-polar resins to adsorb organic substances, and commercial applications that use this type of adsorbent generally remove undesirable organic substances such as pesticides (8), phenolic compounds (9), etc., from an aqueous system. This paper describes a technology that separates undesirable volatile organics and recovers specific desirable aroma components from thermal hydrolyzates via contact with a polystyrene-divinylbenzene adsorbent (10). The purified aroma from the process can be used to provide an enhanced aroma character for coffee products.

Experimental Methods

A thermal hydrolyzate was generated using partially extracted (43% dry basis) roast and ground coffee, composed of a 50% arabica and 50% robusta blend. The partially extracted grounds are slurried in water to a 10% concentration and subjected to a temperature of 220°C for 8 minutes in a plug flow reactor.

The high temperature slurry is then flashed into a vessel at atmospheric pressure and the vapors, composed of water vapor and coffee aromas, are condensed. Because of the dilute nature of the aroma compounds, the condensate is concentrated further using distillation to achieve an organics concentration of about 3% in water.

Separation and recovery of aromas in the distillate is carried out on a single glass column using a polystyrene-divinylbenzene, microporous adsorbent. This resin is approved for removal of organic substances from aqueous foods, according to specifications defined by the U.S. Food and Drug Administration (11). The adsorbent used in the following experiment is in accordance with those specifications. The adsorbent is slurried with water and added to the column, and is also back washed with additional water from the bottom of the column, to remove any air bubbles that might be present within the column. The slurry is allowed to settle and the excess water drained from the bottom of the column, leaving a water level of about 15 cm. above the top of the adsorbent bed.

The distillate recovered from the thermal hydrolyzate is then fed down flow through the bed, maintaining an aqueous level of about 30 cm. above the adsorbent. Effluent from the bottom of the column is collected until breakthrough of a strong non-coffee aroma is detected. The purified aqueous aroma is then added to a coffee extract, prior to spray drying, to produce a soluble coffee product with an enhanced aroma character. Figure 1 shows a simplified diagram of the process flow, and Table 1 lists operating conditions used for the adsorbent column.

Table 1
Adsorbent Column Operating Conditions

Column:	Glass
Height (m)	2.1
Diameter (cm)	10.2
Adsorbent:	Polystyrene-divinylbenzene
Supplier	Bio-Rad (SM-4)
Mesh size (U.S. screen #)	-20 +50
Weight (kg)	4.5
Adsorbent bed dimensions:	
Height (cm)	76.2
Diameter (cm)	10.2
L/D	7.5
Aroma feed rate (cc/min)	40
Superficial Velocity (cm/hr)	29.3
Residence time (hr)	2.6
Operating temperature (°C)	20

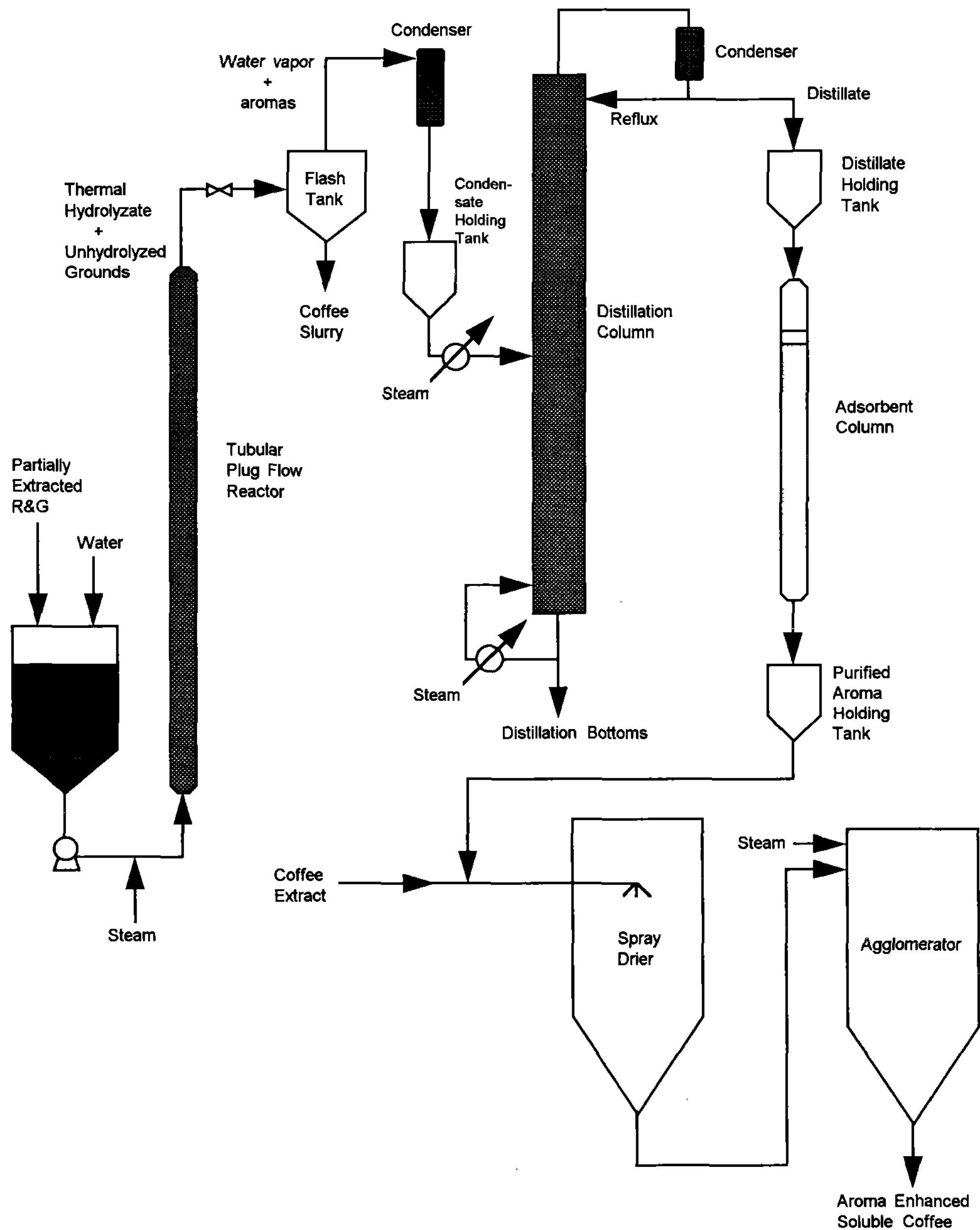


Figure 1
Simplified Process Flowsheet
Condenser
Water vapor
+
aromas
Flash
Tank
Thermal
Hydrolyzate
+
Unhydrolyzed
Grounds
Conden-
sate
Holding
Tank
Coffee
Slurry
Steam
Condenser
Distillate
Reflux
Distillate
Holding
Tank
Distillation
Column
Adsorbent
Column
Tubular
Plug Flow
Reactor
Partially
Extracted
R&G
Water
Steam
Distillation Bottoms
Purified
Aroma
Holding
Tank
Steam
Coffee
Extract
Spray
Drier
Agglomerator
Steam
Aroma Enhanced
Soluble Coffee

Analytical Methods

Coffee streams were analyzed for their volatile aroma composition using both Purge and Trap (Unicon Series 840) and direct injection to a capillary column (Chrompack CP-Sil 5 CB, 0.32mm i.d., 1.2u film thickness, 50 m. length), using GC oven conditions of 0°C start, 6 min. hold, rate of 6°C/min. to 200°C. A three way post column split was used with flame ionization, flame photometric, and nitrogen/phosphorous detection. Data in this paper is reported based on the response of an external standard, 4-methylthiazole.

Results and Discussion

1. Generation of coffee aromas during thermal hydrolysis

Thermal hydrolysis of partially extracted coffee grounds produces relatively large amounts of volatile aromas. The total level of aromas generated under the specific conditions as stated previously, are more than twice the level that exists in the starting roast and ground coffee. The quality, however, is very non-coffee like and considered to be objectionable by expert coffee tasters. Aroma generation is due to Browning and Maillard reactions under the high temperature extraction conditions (12) since reducing sugars and amine precursors are present in sufficient quantities. Even though these thermal hydrolyzates are of a poor coffee quality, there are still high levels of beneficial aromas present, based on analytical measurements.

The amounts of total and selected aromas were quantified in the starting roast and ground coffee, an extract that achieves a dry basis yield of 43%, and aromas recovered from the thermal hydrolyzate via a flash condensate, using Purge and Trap GC analysis. Results are shown in Table 2.

Table 2
Aroma Generation from Thermal Hydrolysis
100 kg. Roast & Ground Coffee Basis

	Roast & Ground	Extract (43% yield)	Thermal Flash
Total Volatiles (g)	156.0	204.5	403.9
Acetaldehyde (g)	2.5	12.5	68.7
Diacetyl (g)	3.3	5.5	13.8
3-Methylbutanal (g)	7.8	7.7	100.6
Furfural (g)	5.5	43.5	25.8

2. Recovery of beneficial coffee aromas

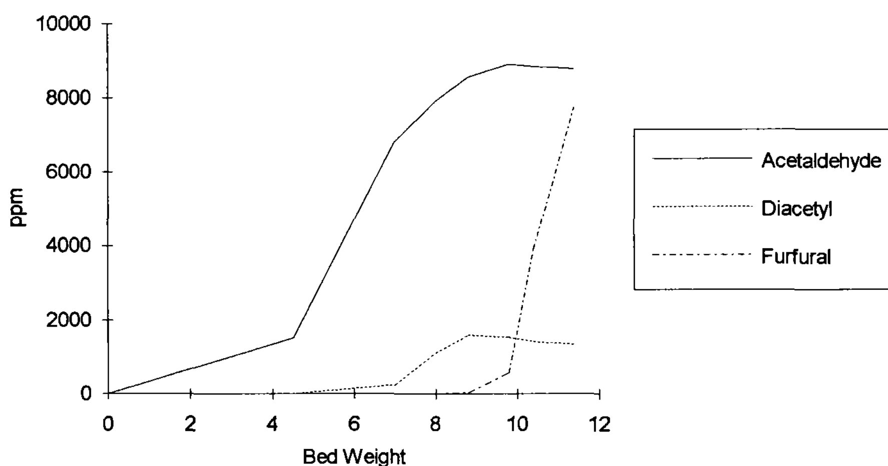
Large quantities of acetaldehyde and diacetyl are present in the thermal aroma stream. These compounds impart fruity/winey and buttery characters respectively to coffee, flavor notes that are associated with the quality of Arabica coffees. Selective fractionation of these two coffee aromas can be achieved using a non-polar adsorbent.

Using operating conditions as set forth in Table 1, the concentrated thermal aroma stream is fed down flow through the resin bed. Effluent from the bottom of the column is collected in aliquots referred to as bed weights, i.e., the weight of the adsorbent bed used in this experiment (4.5 kg.). The first two bed weights are nearly void of coffee aromas and can be discarded. A fruity aroma is detected near the end of the second bed weight and becomes very strong in bed weights 3-6. This character is due to the composition of acetaldehyde as determined by GC/MS analysis. At bed weight 7, the fruity character begins to become faint and the character of the effluent changes to a buttery type, due to the composition of diacetyl as determined by GC/MS.

Within 30 minutes of the start of the operation, a yellow band appears at the top of the column. It moves down the column at about 4 cm. per hour and gradually widens to a width of about 15 to 20 cm. at the bottom of the column. This yellow band is the concentrated diacetyl fraction of the feed stream. After the band exits the column, the character of the effluent changes again, to a strong, musty, somewhat dill-like character.

The breakthrough of the off note corresponds very closely to, but is not due to the presence of furfural. Furfural can therefore be used as an indicator of the off note in determining the point of breakthrough. If the effluent is collected until the breakthrough of furfural occurs, the character of the combined bed weights is predominantly buttery, with a slight fruity/winey background note. If a predominantly fruity/winey character is desirable, collection can be stopped at an earlier time in the cycle. Figure 2 shows concentrations of acetaldehyde, diacetyl, and furfural in the effluent at various stages of the cycle. Breakthrough was determined at the end of bed weight 9.

Figure 2
Determination of Breakthrough



At the conclusion of the operation the remaining liquid is drained from the column. The adsorbent is backwashed with water and discharged from the bottom of the column. Alternately the column can be regenerated using an organic solvent.

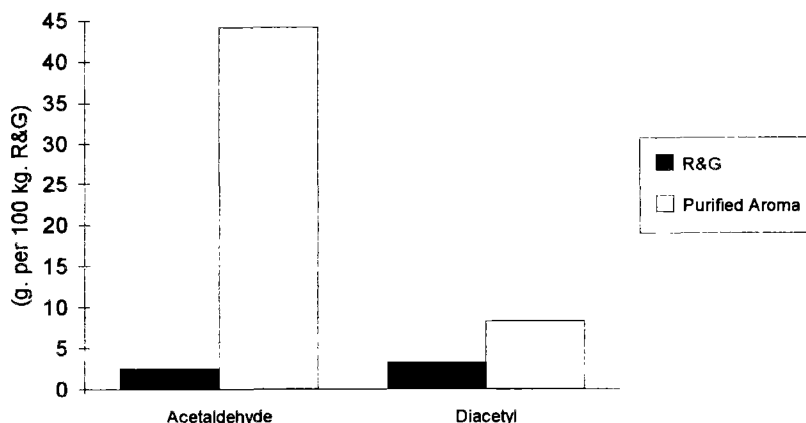
Concentrations of total and selected aromas in the feed stream and the purified aroma, as well as corresponding recoveries, are shown in Table 3. The process is very selective, for recovery of acetaldehyde and diacetyl. These two compounds account for 84% of the total organics present in the purified aroma stream.

Table 3
Recovery of Aromas

	Feed (ppm)	Product (ppm)	Recovery (%)
Total Volatiles	31,200	8220	26
Acetaldehyde	8417	6480	77
2-Methylpropanal	1432	21	1
3-Methylbutanal	3325	2	<1
2-Methylbutanal	1150	0	<1
Furan	6	0	<1
2-Methylfuran	6	0	<1
Diacetyl	934	449	48
2,3-Pentanedione	313	10	3
Furfural	8863	46	1
Nitrogenous compounds	156	25	16

The quantities of acetaldehyde and diacetyl that are recovered from this process are much greater than that which exist in the starting roast and ground coffee. Figure 3 shows the levels of acetaldehyde and diacetyl in 100 kg. of the starting roast and ground coffee and the purified aroma, generated from this quantity of coffee. Acetaldehyde quantities are more than seventeen times greater, and diacetyl 2.5 times greater than the levels in roast and ground coffee.

Figure 3
Quantities of Aromas Recovered



3. Production of aroma enhanced soluble coffee products

The purified aroma can be added to soluble coffee extracts before the spray drying step to provide an enhanced flavor and cup aroma. For example, the aroma is added to a coffee extract as the sole aroma source at 0.07 grams of aroma per gram of soluble coffee solids, then dried to produce an enhanced aroma product. This add back level represents less than the available amount of purified aroma from the process material balance. Low add back levels give marginal flavor benefits, and high levels produce strong, buttery flavors that are not reminiscent of coffee. Alternately the purified aroma can be combined with other aromas recovered from the soluble coffee process and added to the extract before drying. The greatest benefit can be obtained by enhancing the aroma character of spray dried coffees so that the level of acetaldehyde is from 50-125 ppm dry basis and the level of diacetyl is from 10-40 ppm dry basis. The best compositional level will depend upon the presence of other coffee aromas in the product. The purified aroma can also be used to naturally enhance other coffee products such as roast and ground coffee, liquid coffees, or flavored coffees, to deliver a unique, yet still coffee-like flavor and aroma.

Compared to commercially available spray dried/agglomerated coffees, the levels of acetaldehyde and diacetyl can be increased several fold in the finished product. These levels are similar to or even higher than which exist in commercial freeze dried coffees. One advantage of this technology is its ability to produce cost advantageous spray dried coffees with a better freeze dried type quality. The aroma quality of freeze dried products can be further enhanced as well. Figures 4 and 5 show the composition of acetaldehyde and diacetyl in the enhanced spray dried/agglomerated product compared to several commercially available spray dried/agglomerated and freeze dried coffees.

Figure 4
Acetaldehyde Composition

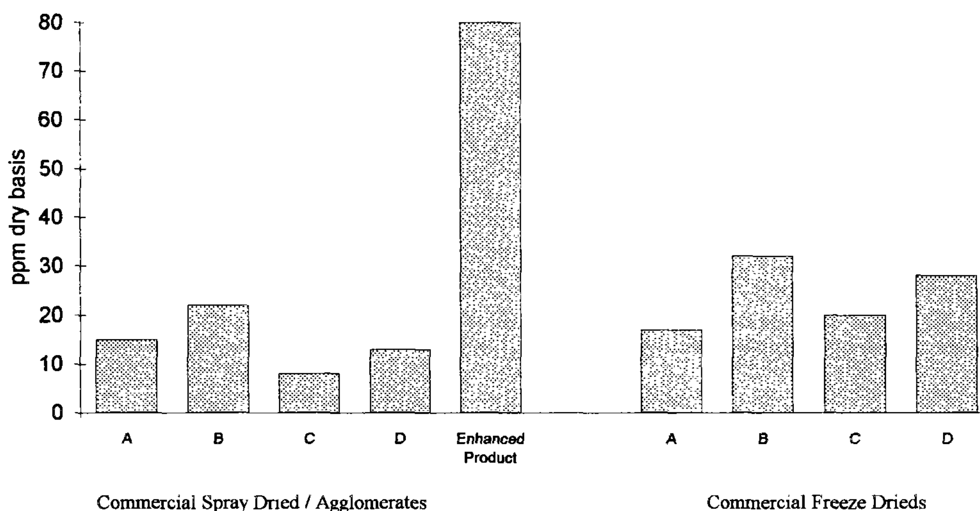
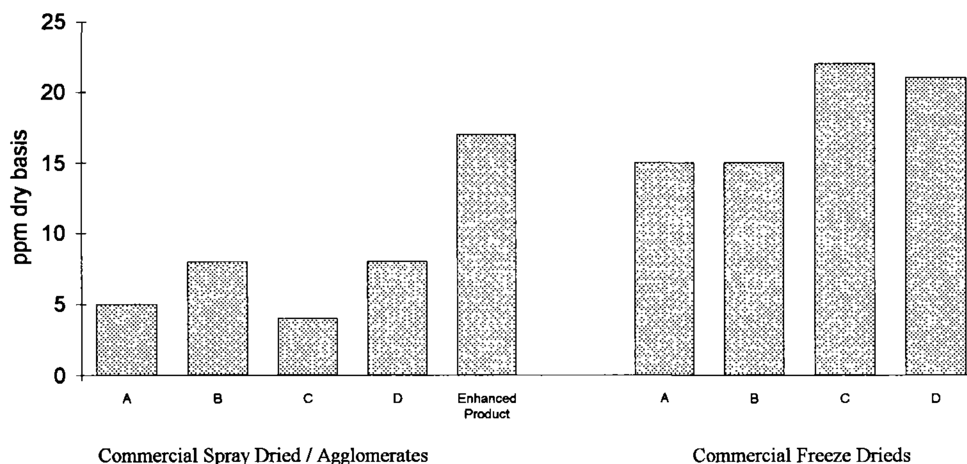


Figure 5
Diacetyl Composition



In addition to the quality advantages, this technology also offers cost advantages since soluble solids are generated via thermal hydrolysis of partially extracted coffee grounds. The enhanced product referred to in Figures 4 and 5 incorporates these additional soluble solids to achieve an overall dry basis roasted yield of 64%. This is significantly greater than typical commercial soluble yields of about 50%. Even with the dilution effect of the additional soluble solids, this technology provides sufficient recovery of beneficial aromas to enhance the aromatic character of soluble products.

Conclusions

High temperature extraction conditions, used in the manufacture of soluble coffees, produce a very poor, non-coffee like, extract quality. Large quantities of volatile aromas are generated during thermal hydrolysis, some of which are responsible for the non-coffee like quality, but other beneficial aromas are also present based on GC/MS analyses. Selective recovery of beneficial coffee aromas such as acetaldehyde and diacetyl, which are present but masked by the off odors, is possible via contact of the aromas with a non-polar, microporous adsorbent. The more non-polar aroma components are preferentially adsorbed and the more polar, water soluble components are eluted to produce a dramatically improved quality. The purified aroma possesses a predominantly buttery, slightly fruity/winey character that can be added to a coffee extract prior to drying to produce an enhanced coffee flavor and cup aroma.

Acknowledgments

The authors would like to thank William Rowan, Edmund Mofford, and Dr. Fredric Kleiner for their contributions to the engineering design and pilot scale installation of the process and to Edward Civinskas for his contributions to the development of the analytical methodologies and product analyses.

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Summary

Beneficial coffee aromas such as diacetyl and acetaldehyde can be recovered from thermal hydrolyzates that contain other off-flavors/aromas. A condensate recovered from the evaporation of such extracts is passed through a bed of a non-polar, microporous adsorbent and the effluent collected until the presence of off aromas is detected. Furfural can be used as a marker for the off aromatics. The effluent can be used to enhance the top notes of traditional coffee products, or produce new, unique coffee flavors.

Résumé

Des arômes bénéfiques du café, tel le diacétyl et l'acétaldéhyde peuvent être récupérés dans les hydrolyzats thermiques contenant d'autres saveurs/arômes non-désirables. Le condensé provenant de l'évaporation de tels extraits est diffusé à travers un lit d'adsorbant microporeux non-polaire et l'effluent est accumulé jusqu'à ce que la présence d'arômes indésirables soit détectée. Le furfural peut être utilisé comme marqueur pour les arômes non-désirables. L'effluent peut être utilisé pour relever les meilleures saveurs des produits de café traditionels ou pour la production de nouvelles saveurs de café.

CO_2 SORPTION IN ROAST AND GROUND COFFEE

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INTRODUCTION

The degassing of roast and ground coffee is a practical problem in coffee processing and package design. The most common package type, the vacuum bag, requires a terminal pressure below about 300mbar to retain package appearance and texture through distribution. There are numerous other coffee package types where control of terminal pressure is significant as well: vacuum cans, where the sound of pressure relief is a consumer cue for freshness, positive pressure packages, where the same is true, but the pressure difference to the atmosphere must be controlled to avoid coffee spraying, and in non-rigid package designs, where the package itself may have a pressure limit.

Several reports describe the rate and amount of CO_2 release from roasted coffee [1-3]. These studies can be of use in determining the effects of particle size, temperature and other processing conditions on the rate of CO_2 loss. There are however few reports of the equilibrium between CO_2 and roast and ground coffee in the literature. Saleeb [4] reported sorption isotherms of CO_2 on coffee at the sublimation point of CO_2 (194.7°K). He described the initial, low-pressure parts of these isotherms as involving physical adsorption. However, the equilibrium at ordinary temperatures of packing and storing have not previously been reported. The studies described here will show the relationship between CO_2 content and package pressure at equilibrium. With this information, package pressure can be related to the package specifications and to degassing time for any combination of package and product.

In addition, isotherms were measured at three different temperatures (2, 21, and 35°C); with these data, the heat of sorption was calculated, and from it the effect of temperature variation during shipping can be estimated.

METHODS

Sorption isotherms were determined by measuring separately the headspace and sorbed CO_2 in vacuum cans of coffee, each with about 1L of internal volume. A range of equilibrium pressures was generated

by varying the fill weights of the cans from 50g to 300g. Headspace CO_2 partial pressure was measured by gas chromatography, and the total amount of headspace CO_2 estimated by using this pressure and the void volume of the can. This in turn was measured using the skeletal density of the coffee as determined by helium pycnometry. The sorbed CO_2 was measured following measurement of the headspace partial pressure by sweeping with nitrogen a 50-g sample of R&G coffee removed from the can while refluxing in 250mL of water. The measurement scheme is shown in Figure 1. The gas stream is dried by the reflux condenser and by the desiccant, and CO_2 is quantitatively adsorbed on the Ascarite tube and determined gravimetrically. That this procedure discriminates between sorbed and headspace CO_2 is shown in Figure 2. In these samples, duplicate measurements of headspace and sorbed CO_2 were made on a set of samples 1 hour after packing and after one day. The material balance for these samples was nearly perfect.

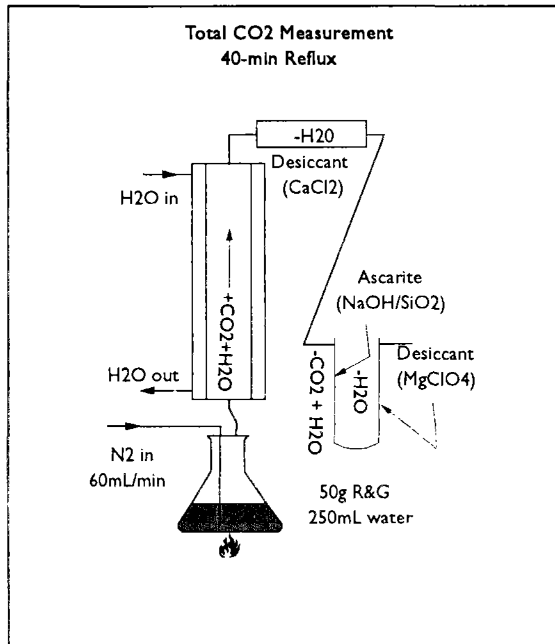

Figure 1

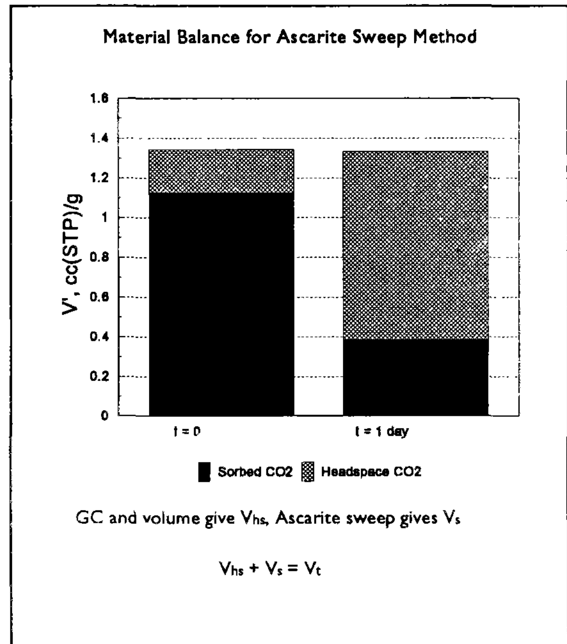

Figure 2

The coffee used in these experiments was roasted in a fluidized-bed roaster. Materials and roasting conditions are shown in Table 1.

Table 1
Roasting Conditions

Coffee Type	Roasting Time, sec	ρ(WB), g/cm^3
Colombian - short	110 - 120	0.33 - 0.35
Colombian - medium	320 - 330	0.33 - 0.39
Colombian - long	700 - 760	0.38 - 0.43
Colombian Decaff - short	110 - 115	0.32 - 0.37
Brazil - short	110 - 120	0.30 - 0.32

RESULTS

Isotherms measured on these samples all appeared linear, as typified by the results shown in Figure 3.

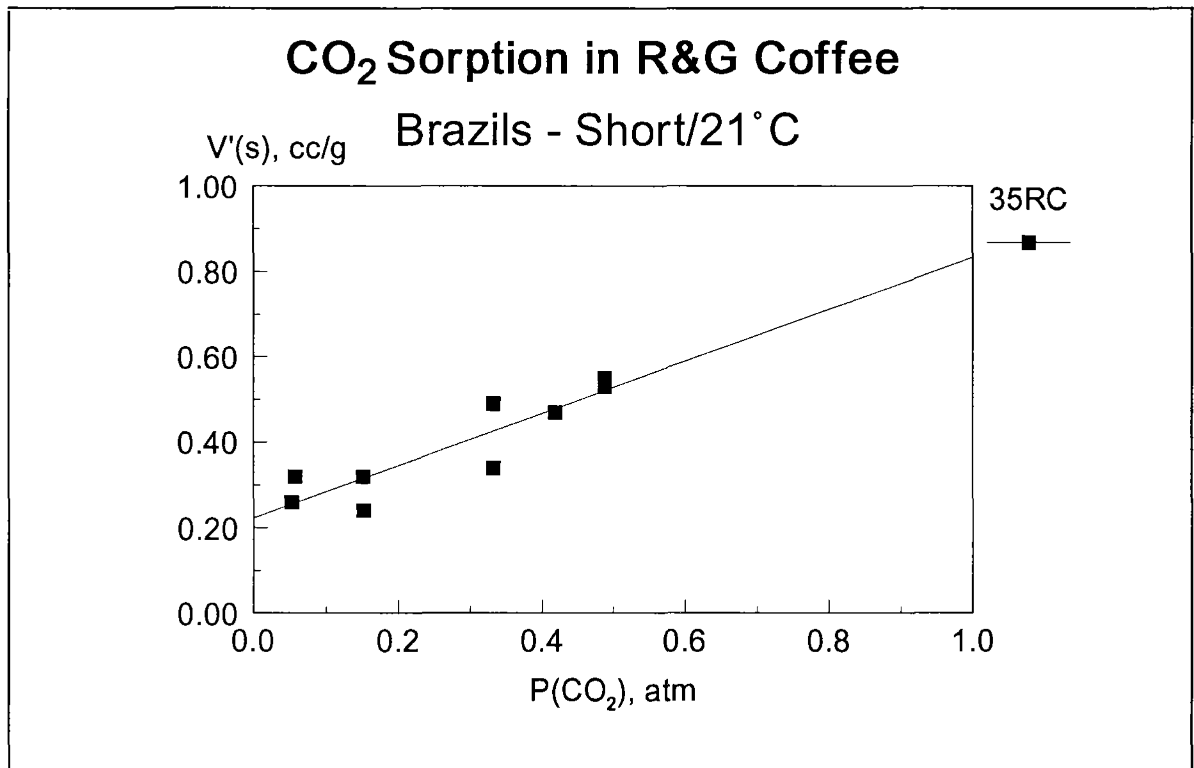

Figure 3

Data from all of the isotherms were fitted to straight lines according to the equation

$$V'_s = V'_{s,o} + kP(CO_2) \tag{1}$$

where V'_s = volume of CO_2 sorbed per gram
and $P(CO_2)$ = partial pressure of CO_2, atm

The results are shown in Table 2.

The most striking thing about these isotherms is that they are so nearly superimposible. The relatively narrow range in the parameters is not believed to be significant, which is obvious from a consideration of Figure 4. The other important feature is that all isotherms show a finite intercept, with a value of about 0.20cc(STP)/g, and slopes are in the range 0.6 - 0.9 cc(STP)/g.atm.

In addition to these isotherms at 21°C, isotherms were also measured for Colombian - short samples (50RC) at 2 and 35°C. The results of these measurements are shown in Figure 5. In this case, the isotherms varied considerably in both slope and intercept, the isotherms at lower temperatures having higher intercept and slope values than those at higher temperatures.

Table 2
Isotherm Parameters, 21°C

Bean Type	Roast Time	Roast Color	$V'_{s,o}$, cc/g	k, cc/g.atm
Colombian	short	35	0.14	0.62
Colombian	short	50	0.10	0.87
Colombian	short	65	0.17	0.53
Colombian	medium	35	0.25	0.75
Colombian	medium	50	0.10	0.96
Colombian	medium	65	0.21	0.65
Colombian	long	35	0.17	0.86
Colombian	long	65	0.21	0.70
Colombian-Dcf	short	35	0.14	0.63
Brazil	short	35	0.22	0.61

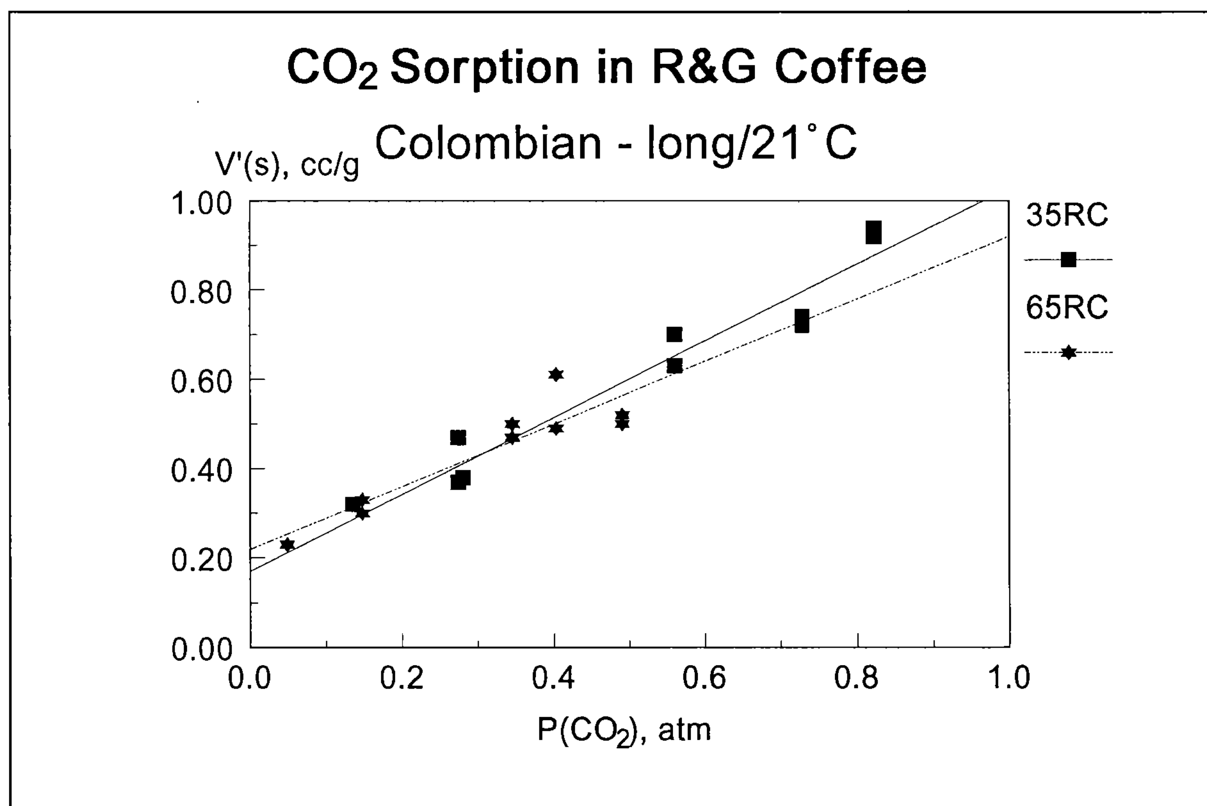

Figure 4

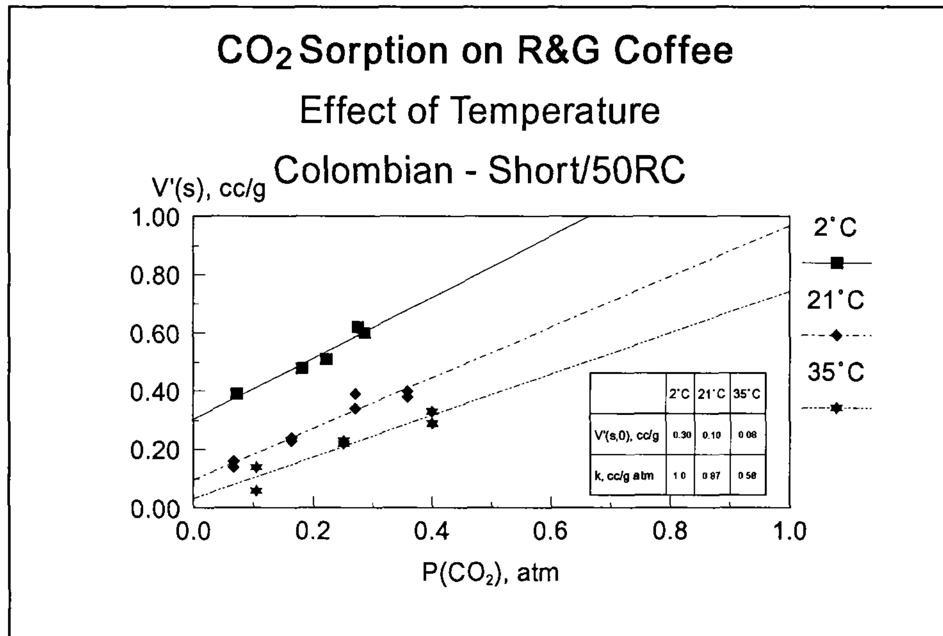

Figure 5

Table 3
Temperature Dependence of Isotherm Parameters

	2°C	21°C	35°C
V'(s,o), cc/g	0.30	0.10	0.08
k, cc/g.atm	1.0	0.87	0.58

DISCUSSION

Characterization of Coffee Particles -- Low-temperature CO_2 isotherms [4] show two regimes in CO_2 uptake. At the very lowest pressures, very small pores or pores with extremely small openings are filled. As the pressure increases, condensation begins, and progressively larger pores will condense CO_2 as the relative pressure increases, according to the Kelvin equation. The temperature of these measurements is very near to the critical temperature of CO_2 (about 34°C), and no condensation should be observed at any pressure. However, physical adsorption will still take place on the surface of the coffee particularly in the extremely fine pores where the interaction with the CO_2 molecules is so much stronger because of the geometry. The puzzling aspect of these data is the persistent and reproducible adsorption at very low pressures, and the regular variation of the $V'_{s,o}$ and k parameters with temperature. The usual interpretation of this type of feature in a high-temperature isotherm is a very high-energy sorption in a small number of sites, which are filled at very low pressures, followed by a Henry's Law type of adsorption at more ordinary energies over the pressure range of the experiment. The high-energy interaction could only result from the presence of pores with dimensions a small multiple of that of the CO_2 molecule itself.

Saleeb's [4] results show at much lower temperatures a residual adsorption of about 0.1cc/g, which he

ascribed to normal physical adsorption. There was some variation among the different coffee samples in his study, and the residual low-pressure adsorption of about 0.2cc/g in this study must be viewed as being quite similar. This translates to a specific area of about $1m^2/g$.

The heat of adsorption can be calculated from the isotherms using the formula

$$\frac{q_{st}}{R}=T^2\left[\frac{d\ln P}{dT}\right]_{V'_s} \tag{2}$$

Using these isotherms and measuring from the 2 and 35°C isotherms at a coverage of about 0.4cc/g the calculated result is an isosteric heat of about 8000 cal/mol, quite a bit higher than the heat of vaporization of CO_2, which is about 2200cal/mol. This is a very reasonable value for a heat of adsorption in a microporous system.

The coincidence among the isotherms for all coffee types at all roast colors evaluated is quite interesting. Sorption at the pressures and temperatures used in this study takes place primarily in the very smallest pores, consistent with finite intercepts observed. Changes in the large-pore structure across degree of roast are well-known, as are changes with roasting time at a fixed degree of roast. These differences are observed in the form of density differences seen in Table 1. If the sorption seen at the lowest pressures in this study does result from micropore sorption, then it suggests that there is an underlying matrix of microporous material which does not suffer significant change in size and total volume over the range of roasting variables used in this study.

Practical Applications -- The CO_2 isotherm has utility in calculating terminal pressure in coffee packages, either from previously measured total CO_2 volumes (which can be related to degassing time), or when changing weight to volume ratios. The basic relationships among net weight, package volume, CO_2 content and terminal pressure will be set out below.

If the internal package volume is V, then the package void volume is

$$V_{void}=V-[\rho\cdot wt] \tag{3}$$

where ρ is the skeletal density of the coffee from helium pycnometry
and wt = the weight of the coffee in grams

The total CO_2 volume is V_{total}, expressed in cc(STP). The total volume divides itself as

$$V_{total}=V_{hs}+V_s \tag{4}$$

where V_{hs} is the amount of CO_2 in the void volume of the can is cc(STP)
and V_s is the amount of CO_2 sorbed on the coffee

In units of cc(STP), the amount of CO_2 in the headspace is

$$V_{hs}=P \cdot V_{void}\left(\frac{273}{298}\right) \tag{5}$$

if P is expressed in atm. In the same units, the amount sorbed is

$$V_s=wt\left[V'_{o,s}+kP\right] \tag{6}$$

Then combining the headspace and sorbed CO_2 and setting equal to the total CO_2, we have

$$P=\frac{V_{total}-\left[V'_{o,s} \cdot wt\right]}{V_{void}\left(\frac{273}{298}\right)+k \cdot wt} \tag{7}$$

Equation 7 may be rearranged to cover any changes in the product net weight and package volume; once the total CO_2 content is established at any point in the process, substitution can be effected by making the substitution

$$V'_{total}=wt \cdot V_{total} \tag{8}$$

then re-writing Equation 7 as

$$P=\frac{\left[V'_{total}-V'_{o,s}\right]}{\frac{V_{void}}{wt}\left(\frac{273}{298}\right)+k} \tag{9}$$

Using this equation and setting the CO_2 content of the coffee at the time of packing (V'_{total}) constant, the terminal pressure can be calculated for different net weights and package void volumes using the isotherm parameters.

SUMMARY

Sorption isotherms for CO_2 on R&G coffee have been measured. The isotherms are linear, and no dependence on degree or speed of roast or coffee type was observed. All isotherms at 21°C showed a finite adsorption at very low pressure, equivalent to an area of about $1m^2/g$. Both the intercept and slope of the isotherm change on changing the temperature. These results show the persistence of the very fine micropore structure of the roasted coffee through the roasting process, despite the opening up of the large pores. The isotherm parameters can be used to calculate terminal pressure from the package volume and net weight if the total CO_2 content is known.

RESUME

Les isothermes de sorption du CO_2 dans le café moulu ont été mesurées. Les isothermes sont linéaires et aucune dépendence quant à la vitesse ou le degré de torréfaction ou le type de café n'a été observée. Toutes les isothermes à 21°C ont démontré un niveau fini d'adsorption à très basse pression, équivalent à une surface de $1m^2/g$. La pente et l'ordonnée à l'origine de l'isotherme change quand la température est modifiée. Les résultats démontrent la persistance de la structure microporeuse très fine du café torréfié durant la torréfaction, malgré l'élargissement des pores les plus grandes. Les paramètres des isothermes peuvent être utilisés pour calculer la pression résiduelle à partir du volume du paquet et de la masse nette si le contenu total en CO_2 est connu.

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ESPRESSO COFFEE BREWING DYNAMICS : DEVELOPMENT OF MATHEMATICAL AND COMPUTATIONAL MODELS
or
Dynamics of Fluid Percolation through a Bed of Particles Subject to Physico-chemical Evolution, and its Mathematical Modelization

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1. INTRODUCTION

Preparing a cup of espresso coffee is an operation that finally can be performed by anyone: nowadays there are special products and coffee machines for home usage that enable even the less experienced, through a simple and easy-to-learn sequence of operations, to achieve in their own home a result that so far was obtainable only in coffee bars by means of professional equipment: namely a good cup of this typical Italian beverage [1].

The quality of the brew in the cup, however, is not always the one desired, mainly since the final result is determined by many distinct elements (Fig.1): water contained in a tank must be forced up by a pump (which increases pressure) and pass through a heat exchanger (which increases temperature). At the end, the water flows through a dose of ground coffee, which has the form of a compact cake.

The dynamics of this complex phenomenon that pours all the characteristic substances of espresso coffee [2] into a cup can be defined in other terms as percolation of a liquid through a compacted bed of particles.

In the present work, we have set out to study the fluid dynamics of percolation and to explain it by means of mathematical and computational models.

2. THE EXPERIMENTAL APPROACH

The scientific method begins with the objectification of a phenomenon by placing it under experimental observation and by collecting measurement data.

An intuitive approach to the study of percolation consists in gathering fractions of brew at constant rates of time. A more efficient way employs modern flow, pressure and temperature continuous sensors as well as data-acquisition computer techniques. This method enables us to obtain discharge curves as a function of time: these curves constitute the basis for a classical semiempirical approach consisting in drawing a law from a series of experimental data (Fig.2).

But obviously by doing so we limit ourselves to considering the process as some sort of a "black box", without examining the deeper mechanisms of the phenomenon. On the other hand, however, our main objective is to develop a prediction model that is able to transform a set of independent variables (the input: p, T, m) into one or more dependent variables (the output: V, t and their combination Q= V/t).

3. DEVELOPMENT OF A DETERMINISTIC MODEL

A common approach of classical hydraulics would consist in applying the law of Darcy [3] to the black box. This law states:

> the loss of pressure along a pipe is proportional to the flow rate of the liquid through it.

Its equation is: $\Delta p = R\,Q$

where R stands for the hydraulic resistance of the entire system.

But two observations that lie in clear contradiction with this equation emerge from experimental curves:

1. flow is not constant at a constant pressure drop, but displays an initial transient and then decreases in time until it reaches an apparently asymptotic value (dependent on T)

2. the mean flow is not proportional to the pressure applied, but increases with pressure until reaching a certain value, and then it remains constant, or even decreases.

The latter observation is particularly important as it indicates the system's tendency to self-adjust itself in contrasting pressure variations. This may result in cups that are apparently (hydraulically) similar, but produced by completely different systems and thus potentially having a different taste!

At this point a question arises as to whether the anomalous pressure dependence is to be put down to the chemical reactions of extraction, triggered by high temperatures in the vicinity of the boiling point of water (100°C).

After all, well 20% of the original weight of the ground coffee passes into the cup, if determined as dry substance: this might result in a variation of the structure of the bed.

In order to find an answer to this question, we used the coldest water possible (practically T = 4°C) in our experiments. Results revealed that the asymptotic non-monotonic behaviour persists also under such conditions (Fig.3).

The following is an equation [4] that approximates the experimental behaviour of Q as function of time, excluding the initial transient peak:

$$Q = a + be^{-ct}$$

By multiplying the equation by e^{ct}, it can be written as:

$$Q\,e^{ct} = a\,e^{ct} + b$$

which, by taking first derivative on both sides of the equation, yields:

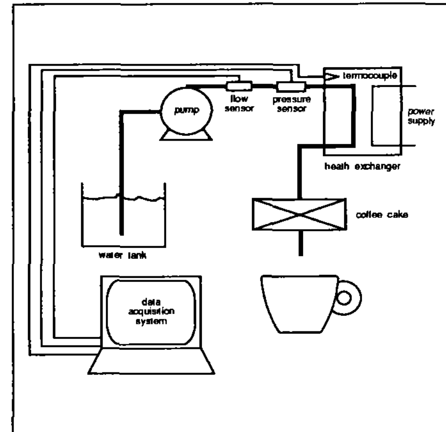

Fig.1 Espresso flowsheet

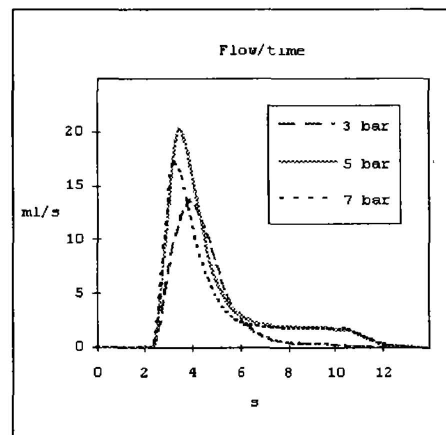

Fig.2 Discharge curves

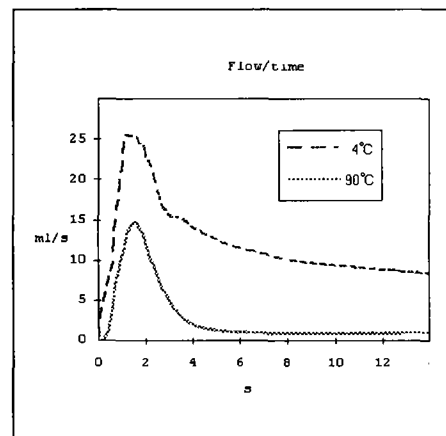

Fig.3 Flow dependence on T

$$e^{ct}\, dQ/dt + Q\, c\, e^{ct} = a\, c\, e^{ct}$$

After simplification we get the following equation:

$$dQ/dt + Q\, c = a\, c$$

On the other hand, literature also mentions models of deformable filter beds [5] which are of great importance to the study of underground waters. The dependence of the flow on pressure in these models is of the following type:

$$\Delta p = m\, Q + n\, dQ/dt$$

that is

$$dQ/dt + m/n\, Q = \Delta p/n$$

As can be seen, the latter equation has the same form as the one we obtained, at constant Δp.

The equation may be integrated by multiplying both members by $e^{m/n\, t}$

$$e^{m/n\, t}\, (dQ/dt + m/n\, Q) = e^{m/n\, t}\, \Delta p/n$$

and subsequently by integrating both members

$$\int_0^t (dQ/dt\; e^{m/n\, t} + m/n\, Q\, e^{m/n\, t})\, dt = \int_0^t e^{m/n\, t}\, \Delta p/n\, dt$$

It is interesting to observe that the left-hand member is itself a derivative

$$\int_0^t d/dt\, (\, Q\, e^{m/n\, t}\,)\, dt = \int_0^t e^{m/n\, t}\, \Delta p/n\, dt$$

hence

$$Q\, e^{m/n\, t} - Q_{(t=0)} = \Delta p/n\; n/m\; (e^{m/n\, t} - 1)$$

Then, by properly rearranging the equation, it is possible to formulate the following type of relaxation law

$$Q = \underbrace{1/m\, \Delta p}_{a} + \underbrace{(Q_o - 1/m\, \Delta p)}_{b}\; e^{-\underbrace{m/n}_{c}\, t}$$

which is formally identical to the experimental law observed initially.

4. HYPOTHESIS FOR A STRUCTURAL MODEL

The flow's exponential dependence from time suggests that the filter bed displays a variable geometry. As no form of modification occurs in the external geometry, at least after the short initial transient, it is likely to assume that some form of modification does take place inside the bed, to which we have turned our attention.

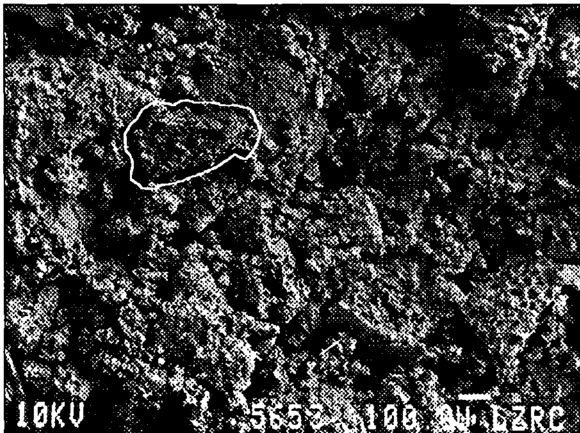

Fig.4 Electron microscopy of the coarse

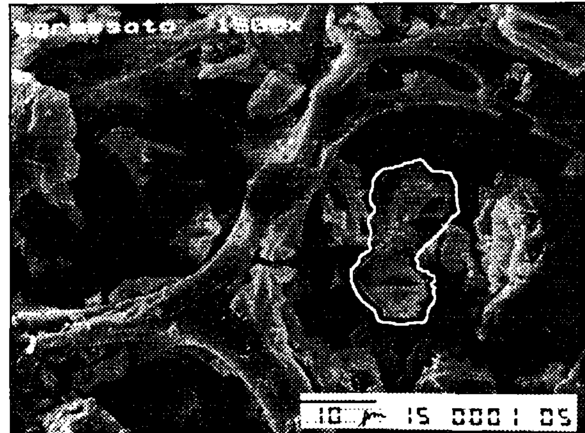

Fig.5 Electron microscopy of the fines

Electronic microscopy shows the structure of the ground coffee cake: it consists of coarse particles (Fig.4) which contain fragments of cell walls called fines (Fig.5). In such a setting, it is possible to hypothesise that the fines migrate and eventually accumulate somewhere in the bed.

A convincing although indirect confirmation of this hypothesis comes from a series of tests on the reversal of percolation direction. These tests were made by percolating cold water through an overturnable extraction chamber (Fig.6).

The direct discharge curve (Fig.7) reveals the same behaviour as previously, which remains unchanged even when the pumping device is arrested momentarily.
The percolation chamber is then rotated, and percolation resumes after the pumping device has been switched on, with the flow that has consequently reversed its direction. Surprisingly, now the flow increases: this effect can be explained if we assume a counter-migration of particles to take place.

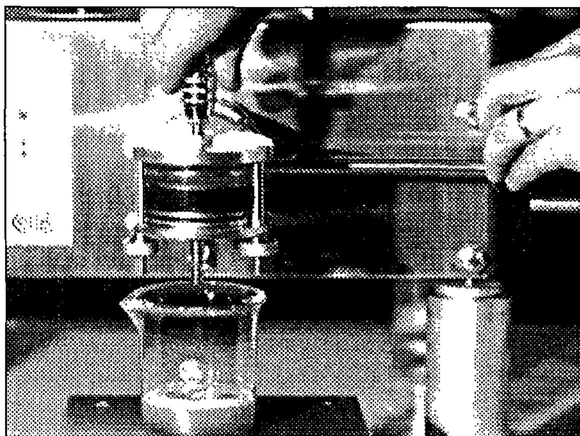

Fig.6 An overturnable percolation setup

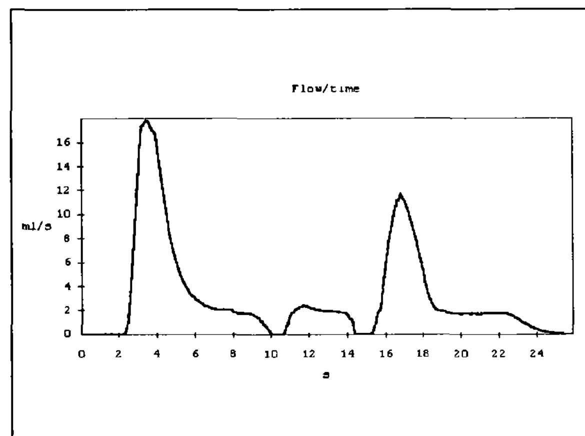

Fig.7 Direct/inverse discharge curve

Now we shall try to imagine a possible law [6] that provides an explanation for this form of experimental behaviour: let us resort to Darcy's law once again, this time by applying it in a differential form on a pressure field:

$$Q = k\ \partial p/\partial z$$

Let us suppose that k, the hydraulic conductivity, is not a constant but a function of two new variables, F and M, which we shall soon define:

$$k = k\ (F,M)$$

Let us imagine that this hypothetical system (Fig.8) is constituted by a skeleton composed of irremovable coarse particles and that water flows through it. The flowing liquid drags along the smaller fragments we called fines.

The following quantities are the essential characterising parameters of the system:

D	diameter of the original bed
L	height of the original bed
ε	porosity of the original bed
$Q(t)$	volumetric flow of discharge
Q/ε	axial speed of the liquid
$p(z,t)$	local pressure

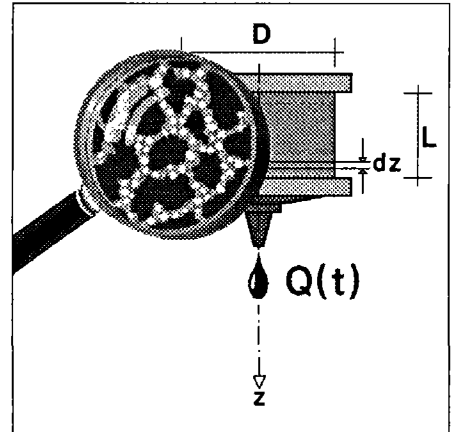

Fig.8 System's hypothesis

The first of the three phenomena we are trying to incorporate in the model is the formation of a bottom layer densely packed with fine particles.

This formation could possibly take place according to the following dynamics:

> the increase rate in the thickness of the packed layer is directly proportional to the concentration of the moving fines and to their speed, while it is inversely proportional to the difference between the maximum concentration allowable in the layer (saturation) and the concentration of the moving fines.

The relevant equation is:

$$dh/dt = \frac{\alpha\, Q/\varepsilon\, F}{[S - (F + M)]}$$

where

$h(t)$	thickness of the packed layer
α	reduction factor of the speed of the migrating fines in relation to the speed of the liquid
S	saturation concentration of the fines in the packed layer
$F(z,t)$	concentration of the moving fines above the packed layer
$M(z,t)$	concentration of potentially removable fines in the skeleton.

The second phenomenon concerns the material balance of the fine particles:

> the quantity of fines in a given elementary layer of the bed evolves in time as a consequence of the release of new fine particles by the skeleton, and of the dynamic inflow and outflow of moving fines.

The equation is:

$$\partial F/\partial t + \partial/\partial z\, (\alpha\, Q/\varepsilon\, F) = - \partial M/\partial t$$

The third phenomenon is the release, which we assume to be irreversible, of fine particles by the skeleton:

> the rate of release of new fine particles by the skeleton is proportional to the concentration of fines in the skeleton itself (or rather to the fraction of fines ready to be stripped) as well as to the flow of the stripping liquid.

Its equation is:

$$\partial M/\partial t = -\beta Q\ [M(t) - \Theta(Q)]_+$$

where

$\Theta(Q)$ threshold concentration of irremovable fine particles in the skeleton, assumed to be dependent on the speed of the liquid
β release coefficient
$[x]_+$ notation meaning:

x when x > 0
0 when x < 0

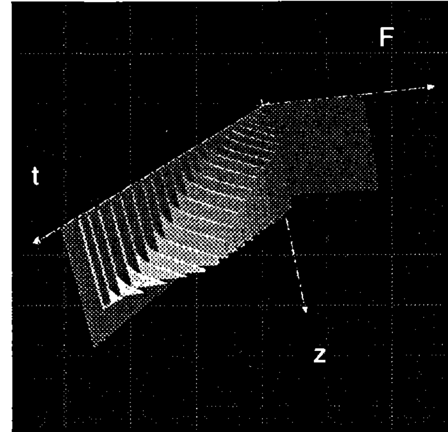

Fig.9 Numerical solution plot

This model constitutes a stimulating step in explaining the dynamics of percolation. However, finding an exact analytical solution to the relevant differential equation system still remains a difficult, if not impossible, task. On the other hand the numerical solution of the system (Fig.9) requires, to be viable, finding appropriate approximations and identification of coefficients.

Only that way could the model serve as a prediction tool. As a matter of fact, modelization may be employed to its best and fullest use when it is applied to practice. In the case in point, the prediction tool could be applied most usefully if predicting the behaviour of the real system from the input variables values, without requiring the experiments to be performed physically.

It should be recalled that our objective is to develop a "black box" that is able to transform a set of input variables (i.e. p, T, pH, hydraulic R) into output variables related to the cup (i.e V, t, ρ, η, % extracted, foam, etc.).

The content of the box is not important in itself, as long as the results are consistent. Indeed, the very equations could be replaced by a computer programme capable of calculating their solutions in numerical form.

5. PROPOSAL FOR A QUALITATIVE MODEL

It certainly appears evident from the present description that the problem under examination involves a high degree of complexity, due to the variability of the cake's geometry in time and in space. Such a degree of complexity is likely to arouse the fear that the computational work necessary to solve it is excessively cumbersome, if we consider the current solution techniques based on finite differences and the applicable computer technologies.

The computer has been employed in this third approach not only to solve the classical differential equation systems numerically, but actually to replace them completely.

An aid to the simulation of complex systems is offered [7] by a particular field of artificial intelligence that studies physical phenomena by a heuristic approach, namely an approach coming from experimental and qualitative observation[8].

The following discussion has been referred to in the literature [9] as "molecular ontology", which aims to develop a modelization of physical phenomena by breaking them down into their single constituent elements (in the present case coffee particles, elementary volumes of water) and by describing their reciprocal interaction from a local point of view. These interactions together build up the system's overall behaviour, called emerging behaviour.

This model is well suited in modeling problems characterised by a high degree of similarity (Brownian movements, fluid flows) and in which the boundary conditions are not completely defined. Computational procedures involved in this model are not particularly burdensome.

The hypothesis originates from a conceptual reference pattern [10] in which purely quantitative aspects, simulated by a particular class of "cellular automata" [11], are integrated with qualitative aspects of molecular ontology.

Mathematician Von Neumann stated [12] that cellular automata may constitute a valid alternative to differential equations.

Cellular automata may be conceived as a stylised universe, where space is represented by a uniform lattice. Each of its nodes, or cells, corresponds to a variable that defines its state.

Time progresses at discrete steps, and the laws of such universe are defined by simple common functions, called rules. At each step these rules allow determining the new state of a cell by taking into consideration also the state of neighbouring cells.

The common functions of this system are local and uniform, and their application causes the system to evolve dynamically and thus to display an overall behaviour that may even be extremely complex (Figg.10 and 11).

Later authors [13] developed a particular class of automata called "FHP lattice gas automata".

This class of automata gave positive results in the modelization of fluid dynamics problems, showing a definite correspondence with the Navier-Stokes' classical equations. It is possible to overlap this set of deterministic and quantitative rules of behaviour with other qualitative and non-numerically-defined rules, drawn from expert knowledge.

In our percolation model [14], the following elements or elementary "agents" have been identified:

- minimum cluster of water molecules
- non-movable particles constituting the skeleton
- particles susceptible of being mobilised by the percolating fluid, namely the fines.

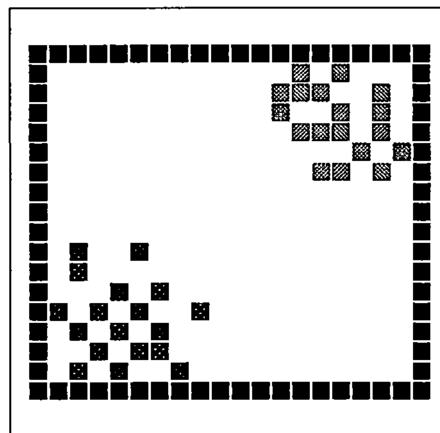

Fig.10 Brownian motion: start

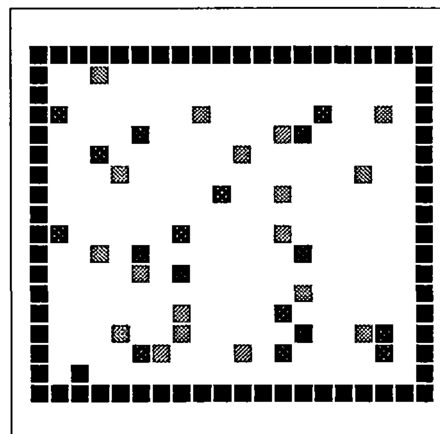

Fig.11 Brownian motion: end

As explained previously, this is a hybrid model of cellular automata, as, along with quantitative rules of fluid dynamics, also other qualitative rules are applied, which describe the transport phenomena of solid particles as well as the rearrangement of the cake. Each individual agent is associated with a state and with a set of local rules of behaviour, somehow similar to the messages that are exchanged among players during a football game. In Fig.12 a few of the applied rules, in the form of LISP-like computer language, are shown.

```
Free propagation Rule
(if
 ((out_particle_right)
then
  (move particle_right)).

Two particles collision Rule
(if
 ((in_particle_right)(in_particle_left))
then
(random((out_particle_up_right)(out_particle_down_left))
      ((out_particle_down_right)(out_particle_up_left)))).

Local block translation Rule
(if
 ((>abs_val_force
threshold)(<force_y0)(>force_x0)(empty down_right)
then
  (move block down_right))
if
 ((abs_val_force threshold)(<force_y0)(>force_x0)
  (abs_val_force_y abs_val_force_x)
   (occupied down_right))
then
(move block down)).
```

Fig.12 Example of LISP-like rules for simulation of percolation

The lattice representing the "playing field" may be empty, or may be taken up by various fixed structures so as to simulate walls arranged in different positions. The simulation result can be represented by visualising the fluid speed vectors, whose envelope produces the flow lines.

As far as our coffee cake is concerned, the simulations produced up to now (Fig.13) have been obtained by an entire night's work of a powerful PC, despite the fact that the simulation is set in only two dimensions. We simulated a bed of partially movable particles having a given possibility of migrating and accumulating on a critical section which simulates the metal filter. It is interesting to note the development of micro vortexes, and to follow the trajectories of the stripped particles.

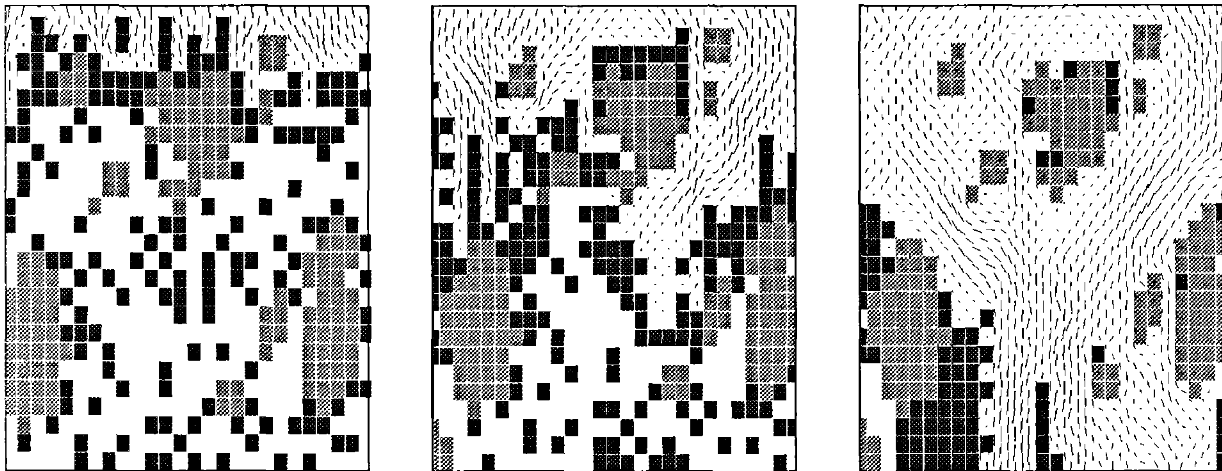

Fig.13 A choice of subsequent stages of coffee cake percolation simulation

Finally, a calculation program integrates the number of particles leaving at a given time and converts them into a diagram (Fig.14): the abscissa represents time, also defined as number of simulation periods, while the ordinate means the exiting particles computation, comparable to an instantaneous flow. In spite of the low number of agents and the two-dimensional topology, the result is similar to the experimental one.

In the future, this tool may prove to be useful in simulating different modes of percolation in order to identify the initial optimal parameters (pressure, grind, coffee type). The tool should be expanded to a tridimensional topology and the size of the lattice could be enlarged. However, this requires highly powerful computers possibly with a parallel architecture.

6. CONCLUSION

The objective set out in the present research was the development of a prediction tool that allows experiments to be conducted "soft", that is without requiring the physical handling of the very objects (coffee, water, machines).

Indeed, a totally experimental approach, that means a design of experiment considering all the above-mentioned variables, would lead to an excessively time-consuming work-plan.

"Soft" results, however, must be consistent with those produced by "hard" trials. Otherwise, the model, be it mathematical or computational, will have to be improved until it becomes "perfect", asymptotically speaking.

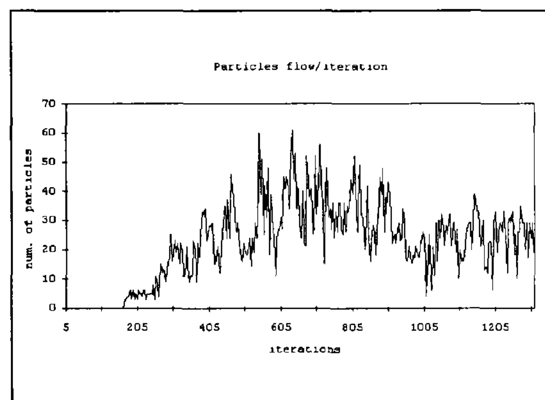

Fig.14 Simulated discharge curve

This calls for the contribution of various branches of science which, by mutual integration, will allow reaching a better understanding of facts which, though belonging to our everyday life, seem to be simple only apparently.

7. ACKNOWLEDGEMENTS

The present piece of work is the outcome of a choral effort of many people. The authors feel specially obliged towards mathematicians Prof. Fasano, Prof. Primicerio and Dr. Baldini of the University of Florence and towards information scientists Dr. Bandini, Prof. Cattaneo, Dr. Tarantello and Dr. Rigotti of the University of Milan. They are grateful also to Prof. Niezgodka of the University of Warsaw for generating tridimensional imaging and to Mr. Cargnelli for directing the educational audio-visual based on the present scientific report.

8. VIDEO PRESENTATION

This article was presented at the Montpellier 1993 ASIC Congress by a videotape recording, which has been transferred into a VHS tape, either PAL or NTSC standard, available at cost price in English or Italian version writing to:

ILLYCAFFE' SpA - via Flavia, 110 - 34147 TRIESTE - Italy
attention Dr. Marino Petracco
phone ++39 40 3890397 - fax ++39 40 3890 490

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ESPRESSO COFFEE BREWING DYNAMICS:
DEVELOPMENT OF MATHEMATICAL AND COMPUTATIONAL MODELS

Preparing an espresso coffee cup is a common act of apparent simplicity, hiding a lot of complex phenomena which use to lead to unexplainable variations in the cup's aspect and appeal.

It may be scientifically defined as "Fluid Percolation through a Bed of Particles Subject to Physico-chemical Evolution".

An attempt is made to interpret experimental data, setting out differential equations of the classic hydrodynamics and showing how an insight into the coffee grounds is needed to explain the outfit's behaviour.

Difficulties in trying to resolve in exact way complicated differential equations systems may be overcome by numerical solution, thanks to modern computers. A newer trend in computer science is called "naïve physics", where neither the solutions nor the equations themselves are obtained by classical deduction. Instead, local rules of behaviour are defined into a computer program and a graphical simulation of the phenomena is obtained step by step.

DYNAMIQUE DE LA PREPARATION DU CAFE ESPRESSO:
MODELISATION MATHEMATIQUE ET PAR ORDINATEUR

La préparation d'une tasse de café espresso est une action apparemment très simple, mais elle cache beaucoup de phénomènes complexes qui provoquent des variations inexplicables dans l'aspect et le charme même de la boisson.

On pourrait la définir comme "Ecoulement d'un fluide à travers d'un lit de particules, sujet à une évolution physico-chimique".

On a essayé d'interpréter des données expérimentales, jusqu'à produire des équations différentielles du type classique en hydrodynamique, et on a montré qu'il nous faut un examen de l'intérieur de la couche de café moulu pour expliquer le comportement du système.

Les difficultés de la résolution analytique exacte de systèmes compliqués d'équations différentielles peuvent être surmontées par solution numérique sur ordinateur. Une nouvelle discipline de science de l'information, qui s'appelle "naïve physics", n'essaie pas de résoudre ni même de poser des équations en utilisant le raisonnement déductif classique: au contraire on y établit des règles locales de comportement dans un logiciel d'ordinateur, et on y obtient pas à pas une simulation graphique du phénomène étudié.

ESPRESSO-KAFFEE ZUBEREITUNG DYNAMIK:
ENTWICKLUNG VON MATHEMATISCHEN UND COMPUTERSIMULIERTEN MODELLEN

Anscheinend ist die Zubereitung einer Tasse Espresso-Kaffees sehr einfach, aber Sie verbirgt mehrere komplizierte Phänomene, die unerklärbare Veränderungen in dem Aussehen und in der Magie der Tasse verursachen.

Man kann Sie wissenschaftlich beschreiben als "Flüssigkeits Perkolation durch eine Teilchenschicht, die durch eine chemische und physikalische Evolution bedingt ist".

Man versuchte die Experimentdaten anzudeuten, in dem man Differentialgleichungen der klassischen Hydrodynamik ansetze. Man zeigte wie notwendig es ist, einen Blick in die Kaffeepulverschicht zu werfen um das Verhalten des Systems zu erklären.

Man kann die Schwierigkeiten der Lösung von Systemen komplizierter Differentialgleichungen durch numerische Computerlösung überwinden.

Eine neue Tendenz in der Computerwissenschaft, die "Naïve Physics" heißt, kann die Gleichungen durch Deduktivmethode weder lösen noch aufstellen. Statt dessen, werden lokale Verhaltensregeln in einem Computerprogramm angesetz, und man erhält schrittweise eine graphische Simulation des Phänomens.

INSTANT COFFEE WITH NATURAL AROMA BY SPRAY-DRYING

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1.BACKGROUND

Instant coffee is made by processing coffee beans through the following steps: roasting, grinding, extraction, concentration, drying and packing. As the result of recent technological improvements in extraction, concentration(freeze concentration, reverse osmosis), recovering and adding aroma, it has become possible to produce high quality extract. The method of drying coffee has a significant impact on those characteristics which are considered important for instant coffee: flavour, moisture, color, density, solubility, particle size distribution and powder flowability.

Freeze drying and spray drying are the most frequently used methods for producing instant coffee. With freeze drying, concentrated coffee extract is frozen and then milled, and the granules so obtained are sifted to ensure uniform size.The next step is vacuum drying. Because the moisture sublimates, there is very little change in the aroma characteristics caused from heating or oxidation. However, there is some aroma losses due to the necessary long exposure time. The instant coffee produced by this method is rather expensive because of the high costs for both equipment and energy.
Spray drying is the other major processing technique. Concentrated coffee extract

is atomized inside a drying chamber where the water is removed by contact with air at temperatures ranging from 200 to 300 ℃ . Multi-nozzles are often used in this spraying technique to pressure-atomize the extract, generating concentrated coffee extract droplets that will be dried to the final powder. Better energy efficiency and controllability of physical properties for instant coffee production are achieved with this method. This technique permits large scale production and results in a product with low density and good flowabilty. Because of the relatively simple equipment, production costs are low. The disadvantages are the aroma losses and the caramel flavor imparted to the product.

2. INTRODUCTION

Improvement of aroma retention has been shown in a comparison of conventional high temperature spray dried product and freeze dried product. Freeze dried product had 17% more low boiling point volatiles and 75% more high boiling point volatiles than conventional spray dried product (1). There is also a report that aroma retention of spray dried product can be improved by increasing the soluble solids content and temperature of the liquid, keeping a high temperature and a low relative humidity in the drying air, and ensuring a larger droplet diameter (2). Being somewhat sticky, instant coffee tends to adhere to the chamber walls. When this occurs, taste quality deteriorates due to longer exposure to the high temperatures; also, scorched particles become mixed with the powder causing further quality deterioration. In conventional high temperature spray drying chambers, a portion of this spray may extend outside the downward hot air flow in such a way that some powder will adhere to the chamber ceiling and wall. Furthermore, due to the relative low volume of hot air, the particle residence time inside the chamber is prolonged, creating conditions which may overheat the product.
To perform the low temperature spray drying, a single nozzle with a spray angle that fit the air vent opening was selected for use. As the drying phenomenon occurs almost instantaneously, it was possible to adjust the hot air flow within a certain range, as equired. The hot air vents were constructed so as to direct the air flow in a manner that significantly reduces the amount of powder coming in contact with the chamber ceiling and wall.
In Fig.1, the pattern of liquid and air flow distribution in the spray-drying chambers used for this experiment is shown; a comparison of the various characteristics of each dryer is shown in Table 1.

Fig - 1 Comparison of flow of liquid and hot air

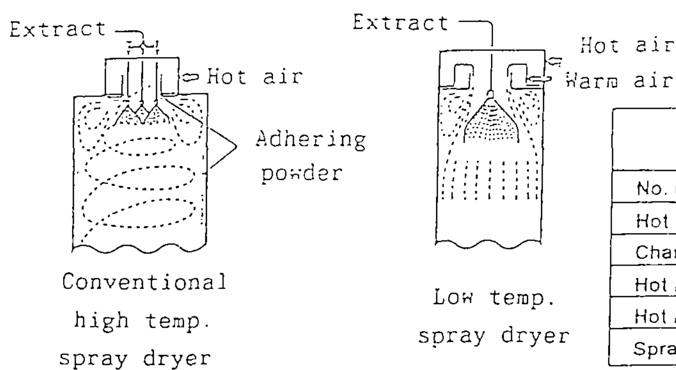


Table - 1 Comparison of spray dryers

	Conventional high temp. dryer	Low temp. dryer
No. of nozzle	4	1
Hot Air Temp.	200~300℃	130~180℃
Chamber Temp.	110~130℃	90~100℃
Hot Air Volume	low	high
Hot Air Flow	swirling	rectified
Spray Pressure	30kg/cm2	30kg/cm2

This paper compares the aroma retention of product made with the single nozzle low temperature spray-drying method , to that of product made using both the conventional high temperature spray-drying method and the freeze-drying method.

3.METHOD AND MATERIAL

3.1.Materials

For this comparison, a blend of Arabica (70%) and Robusta (30%) coffee beans were roasted, ground and extracted, according to current industrial techniques. The resulting extract was split into two parts. One was freeze concentrated (T.S.30%), then dried using three methods: conventional high temperature spray drying (hot air at 270℃), low temperature spray drying (hot air at 140, 160 and 180 ℃), and freeze drying. The second part was thermally concentrated (T.S.43%) and spray dried using conventional high temperature spray drying (hot air at 270℃), and low temperature spray drying (hot air at 140℃).

3.2.Analytical method of aroma

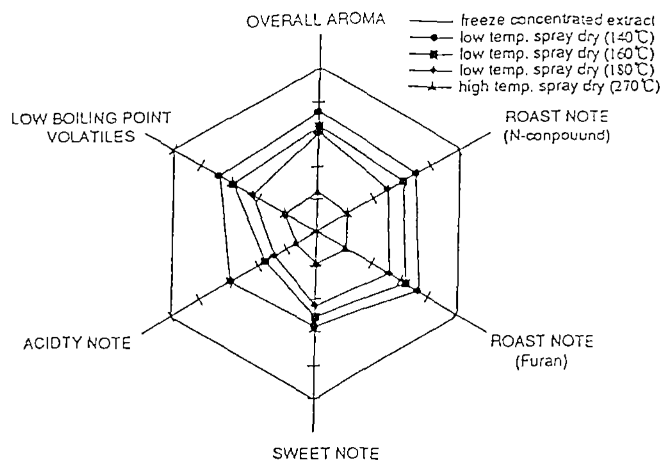
The materials, concentrated coffee extract or powder, were pre-treated either by adsorption onto TENAX, or by purge and trap techniques. After this, the volatiles were quickly heated to 200℃ and automatically injected into a gas-chromatograph equipped with flame ionization detector.

4. RESULTS AND DISCUSSION

4. 1. Aroma retention comparison

The mentioned analytical procedure was applied to compare aroma retention of extract obtained by freeze concentration with that of products dried using the methods below:

(a) conventional high temperature spray drying
(b) low temperature spray drying



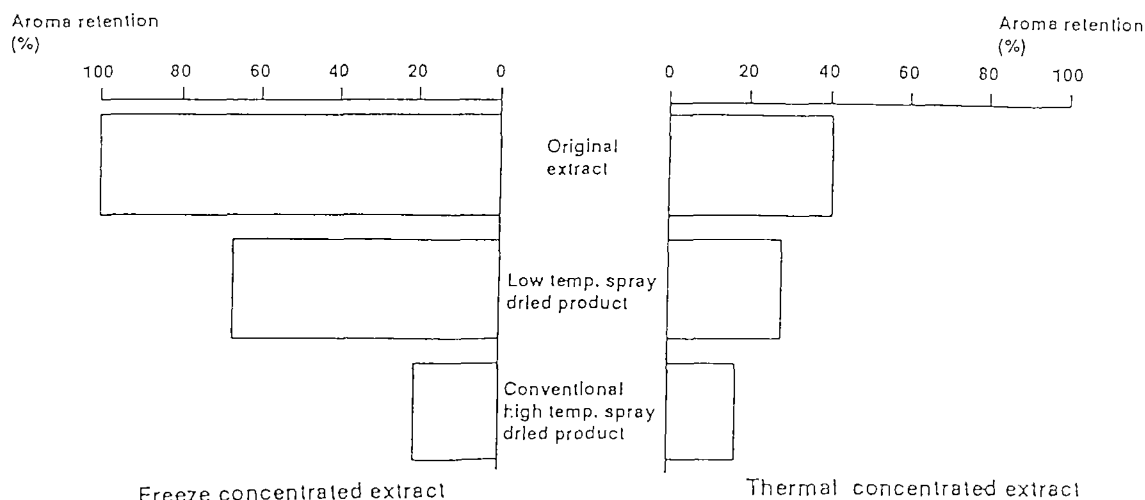
The overall aroma profile obtained by gas-chromatography is presented in Fig. 2 for various inlet drying air temperatures, using freeze concentrated extract as an index (equal to 100). Results show that aroma retention is much greater for the instant coffee produced by spray drying at low temperatures than for the product dried at high temperatures. Evaluation by experienced tasters indicated that the conventional high temperature dried product lacked body and had a caramel and burnt/over-cooked aroma. Conversely, the low temperature spray dried product did not have such characteristics, and the flavour was very close to the original extract. The reason for the over-cooked aroma is thought to be high air temperatures scorching of product which reduces the amount of low boiling point volatiles, resulting in an imbalanced.

The aroma retention at low drying temperatures was higher. Air flow rectification inside the chamber, and better cooling devices at the conical bottom, are some features that might also have contributed to the significant reduction of powder adhering to the chamber wall.

4.2.Effect of concentration method on aroma retention

To compare the difference in aroma retention between different extract concentration methods, freeze concentrated and thermal concentrated extracts were dried at 140 ℃ and 270 ℃ respectively, using the low temperature drying tower type and the conventional drying tower type. Fig.3, shows the aroma retention of products produced under the above-mentioned conditions.

Fig-3 Comparison of aroma retention - by concentration methods



The freeze concentreted extract was indexed as 100%. Thermal concentrated extract was 40%.The low temperature dried product had the higher aroma retention for extracts concentrated by either heating or freezing process compared to the other method. The aroma retention level of the low temperature spray dried product is higher than that of the thermal concentrated extract. From these results, it is thought that because aroma is lost during high temperature spray drying.Thermally concentration also cause significant aroma loss. Using high quality raw materials and extract, production of powder with a high degree of aroma retention could be effectively achieved through combination of freeze concentration and low temperature spray drying, it is speculated.

4.3. Effect of drying method on aroma retention

In order to compare the differences in aroma retention between drying methods, two portions of freeze concentrated extract were dried by freeze drying and spray drying at 140 ℃ . The so-obtained powders were analysed and compared with the original extract (the extract was indexed as 100). The results are shown in Fig. 4.

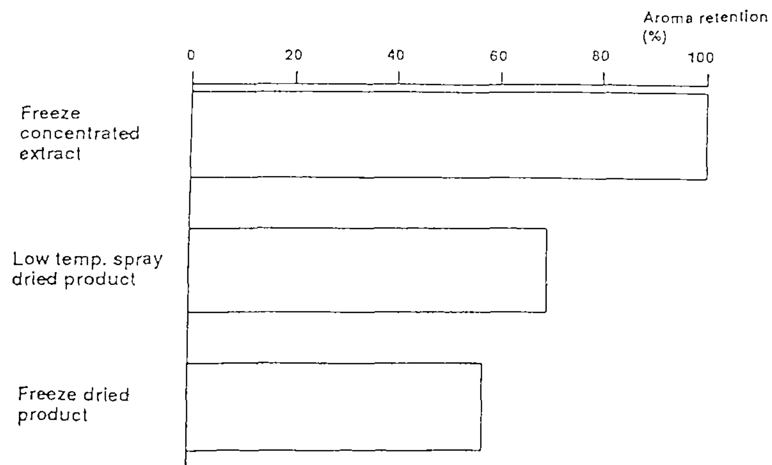


Fig-4 Comparison of low boiling point volatiles - by drying method

Aroma retention for low temperature spray dried product and freeze dried product were 69% and 57%, respectively. It is thought that the aroma retention level of the freeze dried product is lower than that of low temperature spray dried product because of the large aroma loss resulting from the long vacuum drying time needed for freeze drying. This long time is required because the maximum concentration level that can be achieved by freeze concentration is less than that of thermal concentrated extract. From the results obtained, it can be concluded that low temperature spray drying is also effective for drying extracts with low solids concentration, demonstreted in the tests much by better aroma retention.

5.CONCLUSIONS

1.Qualitywise, the product spray dried in the low temperature drying chamber has proved to have much more natural taste, being closer to the original extract independent of whether such extract was thermally or freeze concentrated. It also lacks caramel and burnt/over-cooked flavour notes that are present in the powder produced by the conventional high temperature spray drier.

2.Aroma retention in the product made by the low temperature spray drying method is superior to that obtained in the product made by the conventional high temperature spray drying method.

3.Even when compared with an equivalent freeze dried coffee, the powder obtained by low temperature drying exhibits better aroma retention. Particularly, the retention was greater for an extract with low solids content, such as freeze concentrated coffee extract.

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SUMMARY

Recently, for the purpose of preserving aroma,freeze concentrating and freeze drying have been applied to coffee extract to produce an instant coffee powder of higher quality. However, one drawback is that such drying is very expensive. On the other hand, although the cost of the original high temperature spray drying is quite low, this method most adversely affects the quality.
To overcome these weak points, aroma retention of product made with a single nozzle low temperature spray drying system has been studied. Qualitywise, the product spray dried in the low temperature drying chamber has proved to have much more natural taste, being closer to the original extract independent of whether such extract was thermally or freeze concentrated. It also lacks caramel and burnt/over-cooked flavour notes that are present in the powder produced by the conventional high temperature spray dryer. Aroma retention in the product made by the low temperture spray-drying method is superior to that obtained in the product made by the conventional high temperature spray-drying method.

HIGH YIELD COFFEE TECHNOLOGY

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INTRODUCTION

High yield coffee products have become well accepted in the U.S. coffee industry over the past few years. The ability to roast fewer beans, but give consumers nearly the same volume and number of brew cups has been a marketing success. Just a few years ago, the standard retail roast & ground coffee container held 16 oz. of coffee. Today, the standard is 13 oz., and there are products on the store shelf with net weights as low as 10.5 oz. These coffees continue to nearly fill the old 16 oz can, and in some cases give the same number of cups as 16 oz. of coffee. The technology required to deliver quality, reduced density, high yield coffee products typically involves high temperature, short time (fast) roasting. Fast roasting, in this report is intended to mean roast times less than 3 minutes at temperatures from 200-500 degrees C. The operational result of fast roasting can be a greatly expanded or "puffed" bean with higher extractable brew solids. Fast roasting can be done in large multi-stage roasters, such as the Probat, Thermalo, Jetzone, etc. with very high heat inputs.

As coffee roasters seek greater yields or move to lower and lower densities, via faster roasting, uneven roasting increasingly becomes a problem. This shortcoming can result in a high degree of roast variation within the roaster, such as tipping and burning of the outer edges of the bean. However if tipping and burning can be controlled, an improved high yield, reduced density coffee can be produced. Folgers has improved this technology by developing proprietary pre-drying/roasting technology. Roasting pre-dried beans with low moisture produces a whole roast with less color variation, higher extractable solids, and more density development. This report will describe the work and implications of roasting beans with low moisture in a fast roasting environment. We believe that moisture has a role in "puffing" roasted beans, and density development, however it may not be the major factor. A physico-chemical model will be described which relates bean moisture to chemical degradation reactions, which we believe is the driving force for flavor and density development in high yield coffee products.

EXPERIMENTAL

Materials

Green coffee used in this work was typical Central American arabica or Brazilian robusta coffee or their blends.

Chlorogenic Acid was purchased from the Aldrich Chemical Company of Milwaukee, Wisconsin USA.

Equipment

The TGA/FT-IR consisted of a Nicolet 60X Fourier Transform Infrared spectrometer equipped with a heated flow cell interface in the main sampling compartment. A mercury-cadmium-tellurium (MCT-A) nitrogen-cooled detector was used. A Mettler thermogravimetric analyzer (TGA) was connected to the flow cell by a heated glass transfer line that runs from the bottom of the TGA furnace to the inlet of the flow cell. The furnace was purged with nitrogen, which carries the evolved gases to the heated flow cell for detection. The DSC consisted of a Mettler TA4000 Thermal analyzer which was used to measure heat capacity of ground green coffee samples as a function of temperature.

Coffee Pre-drying

Drying experiments were done on a Wenger Model 4220 Belt Dryer. A typical batch of 300 pounds was dried to less than 5% moisture at a temperature of 70°C to 120°C. Drying time varied from 2-6 hours depending on the temperature.

Batch Roasting

A Thermalo Model 23R roaster, manufactured by Jabez-Burns was used to batch roast pre-dried beans. The roaster was operated with an inlet air temperature of 200-425°C, for 1-3 minutes at a burner rate of 1-3 million BTU's / hour.

Continuous Roasting

A Jetzone Model 6452 fluidized bed roaster manufactured by the Wolverine Corporation was used to conduct continuous roasts. The Jetzone was operated with an inlet air temperature of 260-315°C for 15-60 seconds at a typical burner rate of 2.4 million
BTU's / hour.

Roast Color

Roast color was determined with a Hunter Photoelectric Color Difference meter using the "L" value (0-100 scale).

RESULTS AND DISCUSSION

Raw coffee beans typically arrive at processing plants with 10-12% moisture, and several technical reports implicate this moisture in the puffing of coffee beans. Sivetz (1) has reported that the larger than normal puffing of new crop beans may be related to the higher than average moisture level of 10-15%. Tea & Coffee Trade Journal (2) in the April 1986 issue reported that the production of carbon dioxide and expansion of water vapor during the roast produces internal pressures which account for puffing. Wrigley (3) in his book "Coffee", also gives a similar account. Finally, in U.S. patent 4, 737,376, issued to Brandlein et. al.(4) in 1988, teaches that high internal bean moisture promotes hydrolysis and allows the beans to remain more pliable during roasting. This allows for greater puffing during roasting. We also believe that moisture is important in the roasting process, but is less so for puffing. An analysis of the roasting process (fig. 1) shows that as much as one-third of the roasting time or one-half the energy is required to simply drive moisture from the bean.

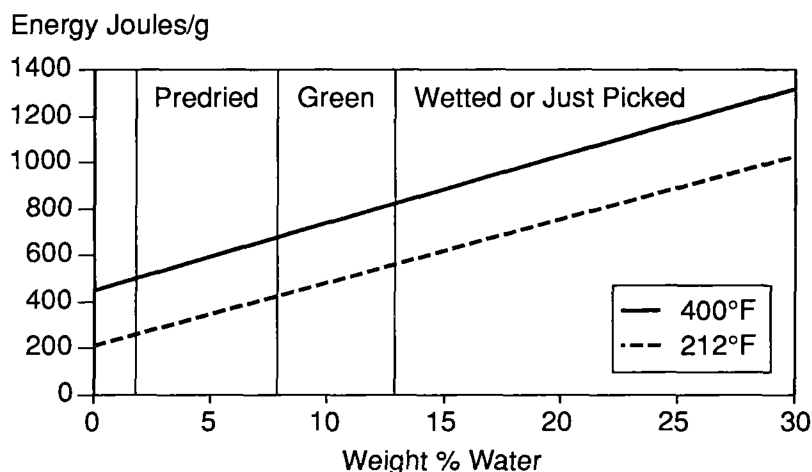

Figure 1

However this step is an essential prerequisite for the important roast flavor development reactions to occur. Until this phase is complete, little roast flavor development or expansion occurs (fig. 2).

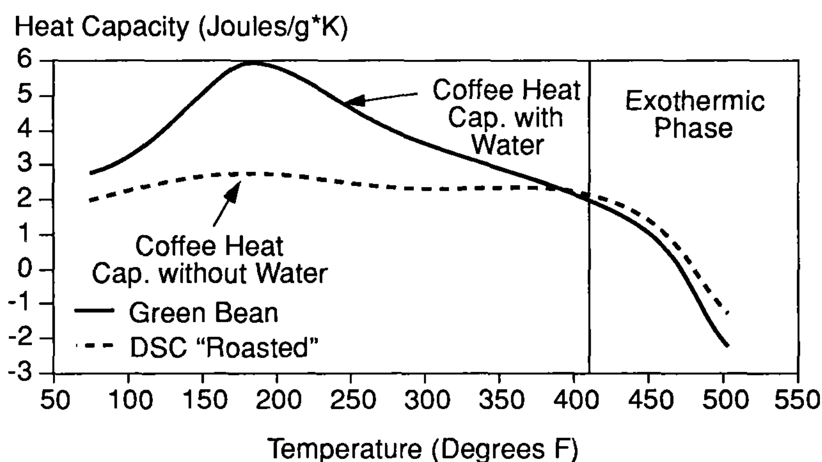

Figure 2

A description of the variables which control roasting is contained in three equations (1-3) which can be derived from basic heating equations found in engineering textbooks. They will not be discussed in detail, but are shown because they provide a basis for understanding the physical and chemical changes occurring during the dewatering of green coffee beans and subsequent pyrolytic development.

Convection: $t_{c,1} = a \cdot \rho \cdot C_p/h$ (1)

Conduction: $t_{c,2} = a^2 \cdot \rho \cdot Cp/k$ (2)

Conduction plus Evaporation: $t_{c,3} = C_p(T_{out}-T_{in})/L \cdot a^2 \cdot \rho \cdot Cp/k$ (3)

t_c	= characteristic time	L	= latent heat of vaporization of H_2O
a	= bean thickness	ρ	= apparent density
Cp	= heat capacity	$T_{out/in}$	= temperature outside or inside the bean
h	= heat transfer coefficient	k	= thermal conductivity

The equations describe the characteristic time constants, t_c, for the fundamental heating mechanisms experienced by the bean, i.e., convective and conductive heating, and conductive heating plus evaporation. We think they offer a valid description of the heating process occurring in the roaster. The various heating mechanisms are controlled by the bean thickness, density, heat capacity, thermal conductivity, heat transfer coeffficient, and/or the bean temperature. The model-picture generated from understanding these equations is one of moisture seeking to exit the bean, while roaster heat seeks to penetrate to the bean core. Consequently there is a water vapor/CO_2 front moving from the bean core to the surface, while an evaporation front moves from the surface to the core (fig. 3).

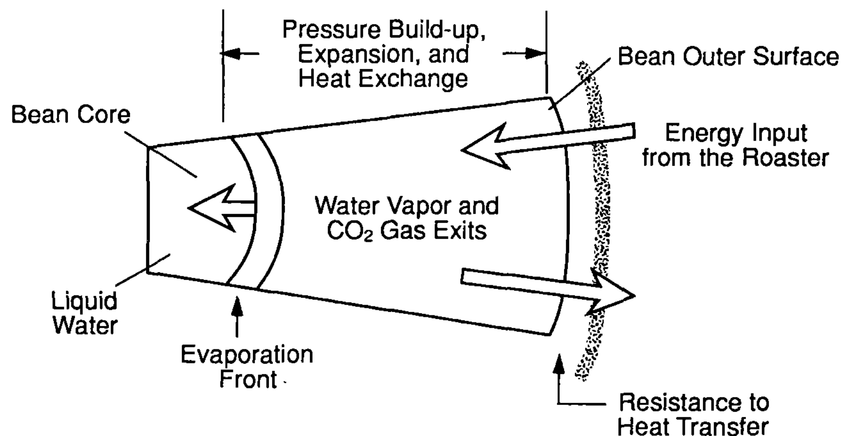

Figure 3

A brief summary of the physical forces controlling the roasting process then are:

1. heat conduction into the bean
2. water evaporation from the bean
3. bean structure impedes water & CO_2 loss
4. internal pressure build-up
5. bean expansion

CHLOROGENIC ACID PUFFING MODEL

To this point, we have devoted a lot of attention to the role of moisture in bean puffing. However a thermal analysis of coffee beans undergoing the roasting process revealed some interesting findings. If we accept the Tg reported in the Brandlein patent, the bean dewatering phase occurs too early to be a major factor in bean expansion (fig. 2). Another consideration discussed in the literature is the role of decomposing carbohydrates as a source of CO_2. The major evolution of carbon dioxide should occur near the beans' Tg, if CO_2 is the driving force for bean expansion. A review of the decomposition temperatures (5) of the carbohydrates associated with green beans does not fully account for the expansion, although some may play contributory roles. Our studies indicate that the decomposition of chlorogenic acid, and it's sharp evolution of CO_2 very near the Tg better fits the puffing model. Thermal gravimetric analysis, differential scanning calorimetry and FTIR of pure chlorogenic acid (fig. 4) show that a great volume of carbon dioxide is evolved very near the beans' glass transition point. Note the position of the heavy line which marks the Tg of coffee relative to several thermal events. The loss of CO_2 is accompanied by a significant weight loss in the TGA which coincides with consecutive exothermic and endothermic spikes in the DSC. The FTIR confirms the evolution of CO_2.

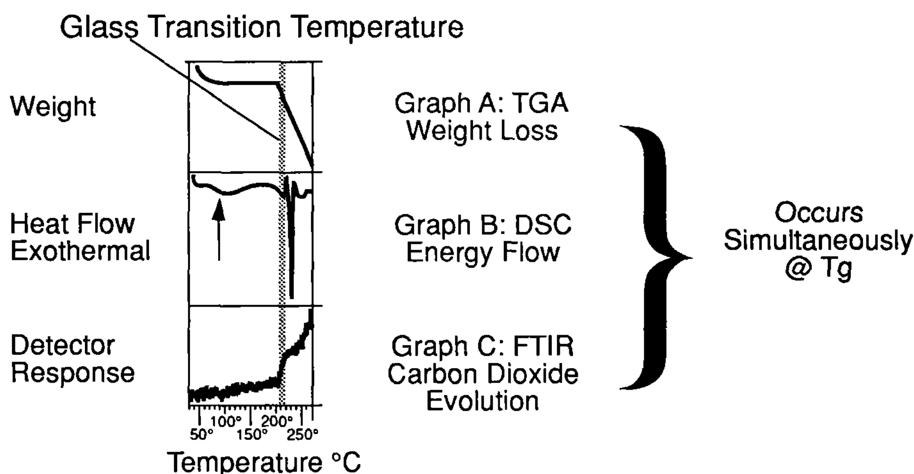

Figure 4

The important point is that all of these events occur very close to, or at the beans' glass transition temperature. A simple emerging model for puffing then, is that a key step in puffing is bean dewatering, followed by rapid heat transfer to the bean core. As the core temperature rises, chlorogenic acid degrades, sharply ejecting CO_2 and building internal pressure just as the bean reaches the Tg, where it is most pliable. Additionally, a concerted "puff" of CO_2 will have more impact than the same amount released slowly. We speculate that this event is an important factor in puffing and density development. This implies that if puffing, or low density is the desired outcome, injecting heat rapidly into the bean core via high temperatures is desirable. This was confirmed by model results from a "puffing indicator" (fig. 5).

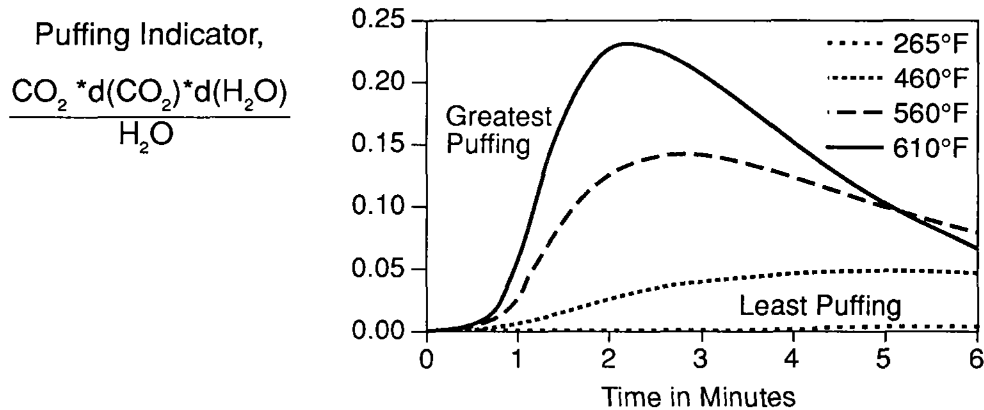

Figure 5

The puffing indicator attempts to describe the relationship between the rate of evolution of CO_2 and steam, while recognizing how moisture impedes rapid heat flow. The model results demonstrate that the ability to rapidly increase roasting temperatures while avoiding uneven roasting can have a dramatic impact on puffing.

PRE-DRYING TECHNOLOGY

This roasting work and the chlorogenic acid puffing model are the basis for a process invention designed to produce uniformly roasted, high yield, low density coffees. We have discovered that reduced density coffee can be produced from green beans with less than 10% moisture without the usual tipping and burning and thus more uniform roast color. This discovery is contrary to prior thinking that high levels of moisture and the resulting steam expansion in the bean during rapid roasting is responsible for the puffing and density development.

A key finding from this work is that one way to rapidly move heat to the bean core is to move the dewatering step outside the roaster. A "pre-drying" step is used to reduce the moisture level of green coffee below 5% which can be accomplished by a variety of heating techniques such as heated air, heated surfaces, microwave, dielectric, radiant heat or freeze drying. However the preferred method is heated air drying. The drying step must be conducted under gentle enough conditions to avoid premature roast related reactions. A set of drying curves established on a Model 42200 Wenger Belt Dryer under 300 pound batch conditions (fig. 6) with convective air @ 0-70% moisture and temperatures from 70°C to 120°C can require about 2-6 hours.

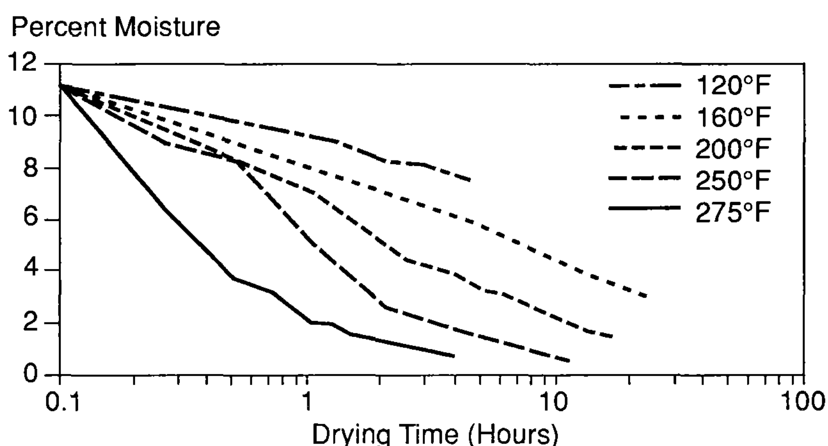

Figure 6

The time required to achieve target moisture will be a function of air distribution, air velocity, air temperature, relative air humidity and the initial moisture content of the green beans. Slow drying using conventional drying units can be easily fitted into existing commercial roasting lines. Warehouse type drying can also be performed in large rooms or storage silos. The coffee may even remain in the shipping bag provided air is free to flow in and out of the bag, e.g. a coarse weave burlap bag. After the green coffee beans have been uniformly pre-dried, and the moisture profile has been equilibrated, they are ready for roasting.

The pre-dried the beans can then be fast roasted to any desired Hunter L (5) value, although 14-25 may be preferred. Fast roasters suitable for use with this technology can utilize any method of heat transfer, however forced convective heat transfer is preferred. Typically the pre-dried beans are charged to a bubbling bed or fluidized bed roaster where a hot air stream is contacted with the bean. The fast roasting is done at temperatures of 200-425°C for roast times of 1 to 3 minutes. A typical batch fast roast, using for example a Thermalo Model 23 roaster charged with 100-300 pounds of dried beans takes 1-3 minutes for roasting at a burner rate of 1-3 million BTU's/hour. A typical continuous fast roast, using a Jetzone fluid bed roaster is operated with an inlet air temperature of 260-315°C and a residence time of 15-60 seconds at a burner rate of about 2.4 million BTU's/hour. As soon as the desired roast color is reached, the beans can be quenched by ambient air or water. The roast coffee beans of the present invention have a whole roast tamped bulk density from 0.28 to 0.38 g/cc. If quench water is used, the amount is carefully controlled so that minimal water is absorbed, typically less than 6%. The resulting ground coffee beans have a ground tamped bulk density of 0.25 to 0.39 g/cc.

SUMMARY

We have described a physico-chemical model which implicates the degradation of chlorogenic acid and its sharp evolution of carbon dioxide near the glass transition as a key factor in bean puffing. Basic heating equations, modeling work with supporting experimental data have been used to develop a proprietary pre-drying/roasting technology. The primary benefit of this technology is improved high yield coffees with better roast uniformity. The process invention involves moving the drying phase of roasting outside the roaster, and fast roasting the resultant beans. This technology should facilitate the development of more aggressive fast roasting process conditions and even lower density coffees.

RÉSUMÉ

Nous avons décrit un modèle physico-chimique qui implique que la dégradation de l'acide chlorogénique et son évolution très nette du gaz carbonique près de la transition vitreuse constituent un facteur déterminant du gonflement des grains. Des équations de chauffage de base et un travail de modélisation s'appuyant sur des données expérimentales ont été utillsés pour développer une technologie propriétaire du préséchage/de la torréfaction. Le principal avantage de cette technologie est l'augmentation du rendement des cafés avec une torréfaction plus uniforme. L'invention du procédé nécessite le transfert de la phase de séchage de la torréfaction en dehors du torréfacteur et la torréfaction rapide des grains en résultant. Cette technologie devrait faciliter le développement de conditions de torréfaction rapide plus aggressives et produire des cafés à la densité encore plus faible.

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The Folgers Coffee Company

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OPTIMUM FERMENTATION PROTOCOLS FOR ARABICA COFFEE UNDER ETHIOPIAN CONDITIONS

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INTRODUCTION

In the washed coffee production, final quality, among other factors, is greatly dependent upon the fermentation process. This was well demonstrated by many workers in the field though some argue that there is still incomplete knowledge for what occurs to the beans during the process giving rise to differences in final quality (1, 8, 11). The primary object of fermentation is to breakdown the mucilage covering the parchment so that it may be washed off without difficulty which otherwise makes drying or handling difficult. The mucilage that sticks to the parchment when the skin is torn off by pulping is the inner half of the skin tissue and consists of a mass of delicate thin walled cells stuck together by glue. The glue is the pectic substances naturally formed by polymerization of the hexose hydroxyl sugars forming the protopectin chains (9, 11). The degradation of mucilage is a natural process caused by the action of enzymes present within the coffee cherry and is a function of temperature, coffee variety, its geographic and cultural origin, picking standards, natural microflora and others (1, 8, 11).

Ethiopia, as a center for Coffea arabica L., is the pot of extreme heterogeneity and is endowed with edaphic and climatic variablities to possess enormous ranges of coffee mix (4). Here coffee grows between altitude range of 500m and 2200m (2, 6). The early wet processing plants in the country were established in the early fifties and research in this field was started some two decades ago at Jima-Melko. Many important results that have ensued were used in the development of the industry but the program was interrupted eventually (1, 3). However, there were changes in the coffee husbandary; wet processing plants increased in number and by ecological coverage. And there were reports of congestion of factories and quality problems (5). For this reason, the study reported in this paper was carried out across regions.

EXPERIMENTAL

1. Sample preparation

Red coffee cherries used to conduct the trials were obtained from respective farms at Jima-Melko Research Centre, IAR, and Bebeka and Limu Kosa farms of the Coffee Plantation Development Corporation (CPDC). At Melko, typical factory bulk containing all range of beans was used. Proportional mix and separate individual samples of the Coffee Berry Disease (CBD) resistant cultivars were used for the test at the CPDC sites. Pulping was

The main coffee areas in Ethiopia. (Source:(4))

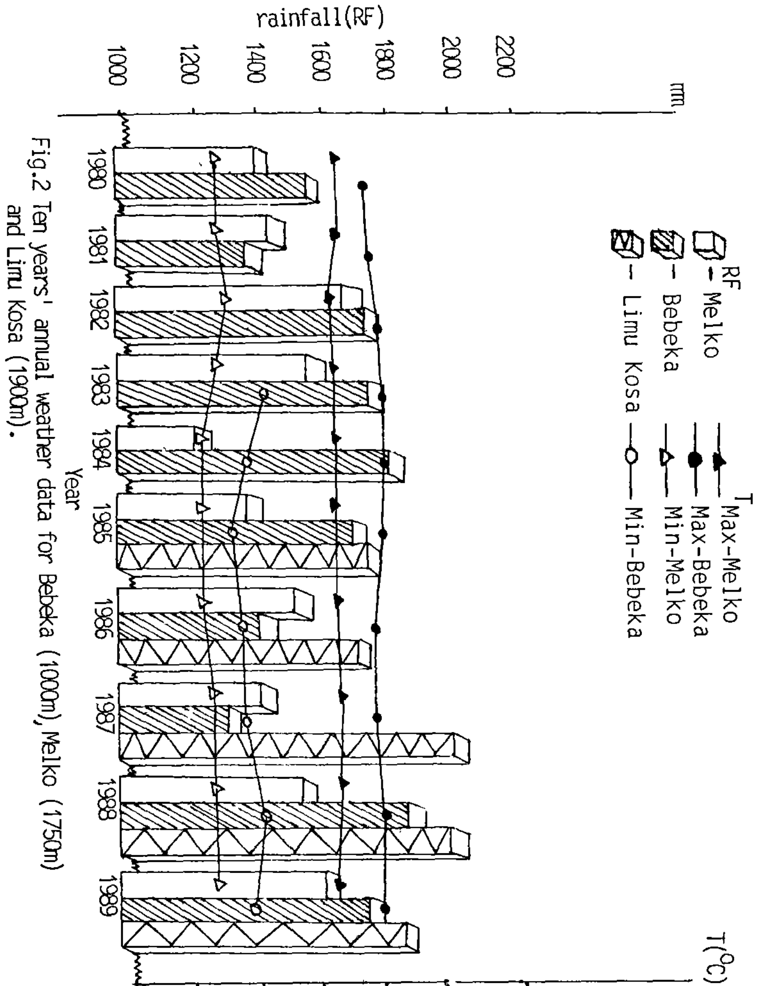

Fig.2 Ten years' annual weather data for Bebeka (1000m), Melko (1750m) and Limu Kosa (1900m).

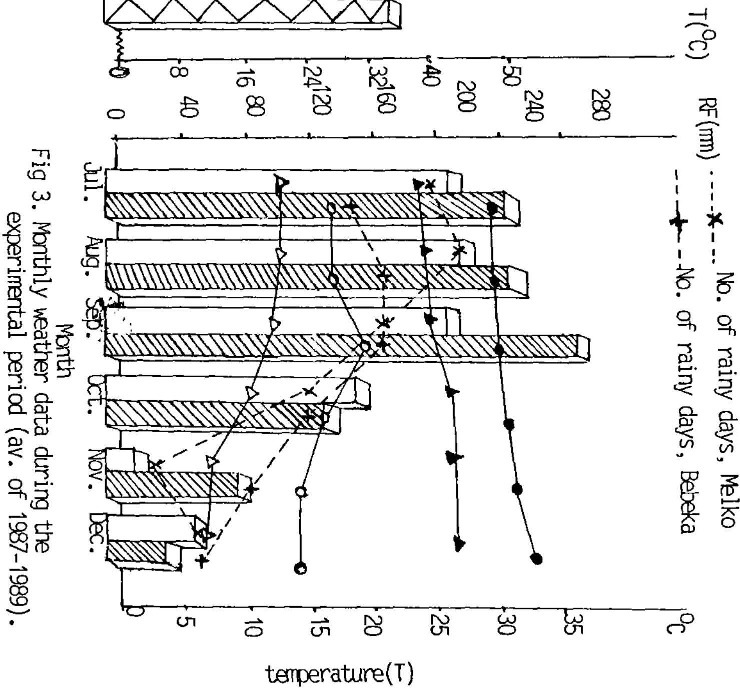

Fig 3. Monthly weather data during the experimental period (av. of 1987-1989).

done by disc pulper. Ten litre capacity plastic cylinderical bowls were used as a fermentation receptacle for small size samples. Mucilage breakdown was checked at every three hours interval from pulping and judged complete when the parchment can be washed clean. Simultaneous checking was done on coffee fermenting in commercial tanks. Fermenting coffee was washed at half time and soaked for the second part. The samples were dried by standard sun/shade method.

2. Quality analysis

Coded samples were submitted to trade liquorers in Addis Ababa, and making blind test at panel they reported the result in the standard coffee classification report and added comments on commercial aspects. Number of samples tested were 786.

Table 1 Terms used for the assessment of the raw, roast and liquor quality

Raw		Roast			Liquor		
Colour	Quality	Type	Centre cut	Quality	Acidity	Body	Flavour
Bluish	Fine	Brilliant	White	Fine	Pointed	Full	Fine
Greyish-blue	Good/ Fine	Bright	Normal	Good/ Fine	Medium	Medium	Good/ Fine
Grey-green	Good	Ordinary	Irregular	Good	Light Medium	Light medium	Good
Greenish	Fair/ Good	Dullish	Brownish	Fair/ Good	Light	Light	Fair/ Good
Greyish	FAQ	Dull		FAQ	Lacking	Lacking	FAQ
Brownish-green	Fair			Fair			Fair
Brownish	Poor/ Fair Poor			Poor/ Fair Poor			Poor/ Fair Poor

3. Statistical

Two methods of fermentation, under-water (wet) and two-stage (dry and soak), were tested at six different fermentation times (24,36,48,64,72 and 96 hours) in four replications at Bebeka and seven fermentation times (24,36,48,64,78,94 and 110 hours) in three replications at Melko and Limu Kosa. A split plot arrangement, method as main plot and fermentation time as sub plot was used for the purpose of statistical analysis.

RESULTS AND DISCUSSION

1. Mucilage degradation

At Melko where the farm consists of National and International collections, beans ranging from small round to long beans were included and have shown great variablity for fermentation. The CBD resistant cultivars included in the test were dominantly of the short to intermediate types (Table 2). Generally, the mucilage of short beans was light, transparent and easily eroded, and that of long beans was dense, fibrous and hard to remove (Table 2). On the other hand, though Ethiopian cropping season is of comparatively short, altitude viz a viz temperature seems to have played a major role in determining the fermentation condition (Fig 1,2,3 and Table 2).

The main feature of mucilage break down at Bebeka was that there was no delayed fermentation. Fermentation was found well advanced or completed by the first morning after pulping (about 16 hours); the only exceptions being the post rain early picking seasons (Fig 3, Table 2). At Melko, under normal factory condition, fermentation was complete at about 40 hours, which is in agreement with the previous work (1). Due to cool humid weather in the highlands of Limu, mucilage degradation usually requires long time. Complete fermentation was achieved at about 60 hours or more (Table 2). In some dull weather

conditions, small reminants of intact mucilage which persists for several days was observed. This indicates occurrence of slow fermentations which may be similar to that reported by Gamble et al (7).

Table 2 Time for mucilage breakdown (ranges in hours) and bean characteristics of some CBD resistant cultivars.

Cultivar	Limu Kosa	Bebeka	Bean mucilage appearance	Bean length group
741	30-48	24-36	Straw look, medium	intermediate
744	40-72	36-48	luxurious,but irregular	long
7440	36-57	24-40	dull, rich	intermediate
7454	36-64	24-40	dull, rich	intermediate
74110	24-40	15-24	light	short
74112	24-40	15-24	light, strawish	short
74140	24-40	15-24	light, attractive	short
74158	30-48	24-36	transparent, medium	short
74165	24-40	15-24	bright, lean	short

2. Quality

Improvement on the raw quality due to fermentation was achieved at all locations. The treatment differences were significant and also highly significant for some specific tests (Fig 4A, Table 3). No significant drop in the raw quality was found up to highest time tested, though the gain is not significant beyond 64 hours at Melko and Bebeka, and 78 hours at Limu Kosa. In the liquorers' report, the best grey blue and grey green beans were recorded in this optimum range, and foxy beans indicative of under-fermentation were reported at lower fermentation time (Table 4).

In general, no marked improvement, on roast quality was observed beyond 48 hours, though some ordinary and irregular roasts were reported up to 64 hours (Fig 4B, Table 3). At Bebeka, the treatments failed to show significant difference on this component of quality. Though the idea that an improvement in raw appearance favourably affects the roast appearance (11) is agreeable results of this study have shown that the effect on roast, if any, is limited to the initial, comparatively shorter, fermentation period.

Significant effect of fermentation on liquor quality was up to fermentation time of 48 and 64 hours (Fig 4C, Table 3). Earlier, it was shown that the improvement brought about by diffusion during the fermentation process is limited (1). This idea was supported by the results that the treatment differences are significantly better at lower fermentation time only. Foul cups were recorded at about 96 hours at Bebeka. Sour cups were unanimously reported at higher fermentation times at all locations and depression on flavour was reported in this range (Table 4). Though no record was made in this study, the risk of onion flavour was reported at prolonged fermentation time (1,10).

On the overall (Table 4), optimum quality was achieved somewhere at 64 hours at Melko, 78 hours at Limu Kosa and lower total fermentation time of about 48 hours at Bebeka.

The underwater method was superior in all aspects of quality (Table 4). The treatment differences due to the method on raw quality were significant at all locations (Fig 4A). This method was more favoured in the lowlands than in the highlands. This is clearly demonstrated by significant treatment differences in all raw, roast and liquor quality at Bebeka (Fig 4A, B,C). At Limu Kosa the gain due to this method over the dry and soak is only about half a class or nil (Fig 4C).

The fermentation procedure which results in best coffee qualities as well as rapid and more controllable fermentation is desirable to avoid congestion at the factory and eliminate the possibility of occurrence of deletereous off flavours and taints. The mucilage characteristics of different coffees has been shown to affect timing of fermentation. Given heterogeneity of Ethiopian coffee and its speciality markets, detailed study in this trait is further demanded. In the foregoing discussion, it has been demonstrated that blind adherence to fixed fermentation time is not satisfactory in that it may lead to under-or over-fermentation in varying climatic conditions and coffee types.

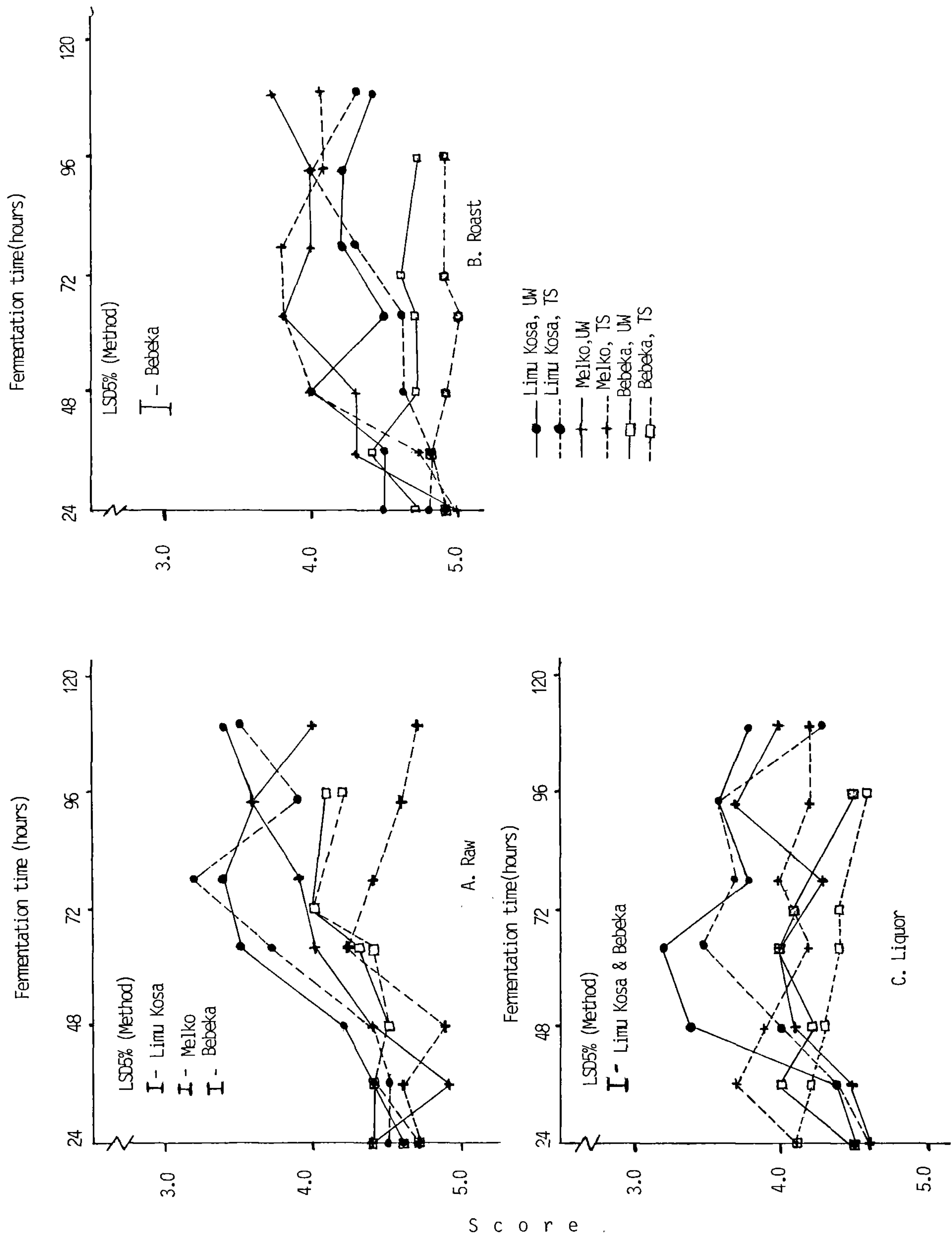

Fig.4 Mean scores of the three quality components.

Table 3 Composite quality data for raw, roast and liquor at Limu Kosa, Melko and Bebeka.*

Score	Limu Kosa			Melko			Bebeka		
Time (hrs)	raw	roast	liquor	raw	roast	liquor	raw	roast	liquor
24	4.9c**	5.1b	5.1d	4.6bc	5.0c	4.6b	4.6c	4.8	4.3ab
36	4.9c	5.1b	4.9cd	4.7c	4.5bc	4.5b	4.4bc	4.6	4.1a
48	4.8c	4.8ab	3.9a	4.6bc	4.1ab	4.1ab	4.5bc	4.8	4.3ab
64	4.1b	5.0ab	3.9a	4.1a	3.8a	4.0ab	4.3abc	4.9	4.2a
72/78	3.8a	4.7a	4.2ab	4.1a	3.9a	4.3ab	4.0a	4.7	4.2a
94/96	4.2b	4.6a	4.1a	4.1a	4.1ab	4.0ab	4.2ab	4.8	4.5b
110	3.9ab	4.6a	4.6bc	4.4ab	3.9a	4.0ab	-	-	-

* Lower score denotes better quality

**Mean separation in a column by DMRT. Means followed by common letter are not significantly different at 5% level.

Table 4 Coffee quality, average of some of the test samples and some defect scores*

Location	Limu Kosa		Melko		Bebeka		Defect***	
Time	Method		Method		Method			
(hrs)	UW**	TS	UW	TS	UW	TS	foxy	Sour
24	Fair+	Fair+	Fair	Fair	Fair+	Fair+	2.5	0.5
36	FAQ-	FAQ-	FAQ-	FAQ-	FAQ-	FAQ-	2.0	0
48	FAQ-	FAQ	FAQ	FAQ	FAQ	FAQ-	1.3	0
64	FAQ	FAQ	FAQ+	FAQ	FAQ-	Fair	0	0
72/78	Fair/Good-	FAQ+	FAQ	FAQ	Fair	Fair	0	0
94/96	FAQ+	FAQ+	FAQ	FAQ	Poor/Fair	Poor/Fair	0	1.0
110	FAQ+	FAQ	Fair+	Fair+	-	-	0	2.0

** UW = under-water TS = two-stage

*+ or -, indicates marginally above or below the quality.

*** 3- highest 2- intermediate 1- slight 0- not detected

Any time in the range of, below 24 hours, 24 to 48 hours, 48 to 72 hours, or above 72 hours of mucilage degradation will be washed at the first, the second, the third, or after the third day from pulping, respectively. The three stage tanks presently used at washing stations in the country can accommodate all the ranges for normal flow of coffee with the exception of the last range. As a guide, washed coffee producing factories may arbitrarily be grouped altitudinally as 1200m and below, 1200m-1500m, 1500m-1800m and above 1800m for varying fermentation practices, and this was extrapolated from the study.

The series of comparatively large experiments has shown good relation between the fermentation conditions and coffee qualities. However, it is worth re-mentioning here worry of kulaba (8) in that the subjective method of assigning classification has costed much time and energy in attaining reliable results. This time-honored system of quality testing need be supported objectively and scientifically.

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ABSTRACT

The fermentation process which provides the mild character for the washed coffee is known to be a function of multitude of factors. Ethiopia, as a center for (Coffea arabica L.) is the pot of extreme hetegeneity and climatic variablities. To identify fermentation condition across locations, two methods (Under water and Two-stage) and seven fermentation times (24, 36, 48, 64, 78, 94 and 110 hours) were considered in preparing samples for quality test. Mucilage degradation progress was recorded during fermentation. Scoring values used in coffee classification report and commercial descriptive standards were used to determine and catagorize coffee quality. Quality was tested for individual releasesd CBD resistant cultivars and bulk factory acquistions at Bebeka (1000m), Melko (1750m) and Limu (1900m), all representing different reputed regions of Ethiopian coffee. Significant and ideal total fermentation period at Limu and Melko were 78 and 64 hours respectively (at P = 0.05). However, at Bebeka it was significant under 48 hours at similar probability. Underwater fermentation condition was better than the Two-stage at all regions.

SOLIBILITIES OF CAFFEINE, THEOPHYLLINE AND THEOBROMINE IN WATER AND THE DENSITY OF CAFFEINE SOLUTIONS

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Abstract

Caffeine, theophylline and theobromine are important ingredients of coffee, tea, maté, guarana and cacao, respectively. Although very similar in molecular constitution, they differ nevertheless, widely in many physical properties. The solubilities of the three compounds, for instance, are very different. The solubilities of these purines in water have been measured over a wide temperature range using different experimental techniques. The effect of pH on solubility and crystallisation behaviour has also been studied. The exact knowledge of these properties is important for extraction processes of the purines (e.g. recovery from CO_2 decaffeination process solutions, water decaffeination) or from waste materials as well as for separation and purification techniques or pharmaceutical applications. Furthermore, the density of aqueous caffeine solution along the saturation line has been determined with precision. This enables continuous concentration measurement of process solutions by an in-flow density meter.

A NOTE ABOUT THE USE OF MICROCUTTING AND THE SETTING UP OF AN *IN SITU* MICROGRAFTING TECHNIQUE FOR *COFFEA ARABICA*

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Abstract. *In vitro* microcutting is a well known technique which has been applied to coffee for more than 20 years. It can be considered as :

-True-to-type vegetative multiplication, and in this case it represents a tool for breeding programs for example.

-Mass propagation technique, at an industrial level, only if the current technique is improved.

Those aspects are interesting, but raise different practical and economical problems:

-The *ex vitro* acclimatization is costly and difficult

-The feasability of micrografting a microcutting on rootstocks resistant to nematodes may be questionned.

We describe here two simple techniques which would allow to overcome these two problems at a cheaper cost.

Résumé.

La technique de microbouturage, bien connue et appliquée au caféier depuis plus de vingt ans, peut être considérée comme :

- Technique de multiplication végétative conforme; c'est alors un outil pour les programmes de sélection par exemple.

- Technique de multiplication de masse, industrielle ou commerciale, sous réserve d'aménagements de la technique telle qu'elle est connue actuellement.

Ces perspectives sont intéressantes, mais soulèvent différents problèmes pratiques et économiques:

- Sortie *ex vitro* délicate et coûteuse

- Faisabilité du greffage de microboutures sur du materiel résistant aux nématodes.

Nous décrivons deux techniques simples qui permettent de surmonter ces deux problèmes à moindre coût.

INTRODUCTION

The vegetative multiplication of *C. arabica* is necessary to conserve genetic diversity or in order to fix hybrid structures obtained within a breeding program.

The *in vitro* microcutting technique for coffee has been known for several years (Custers, 1981). It consists of producing plantlets from dormant axillary buds under aseptic conditions. The utilization of a controlled environment and of phytohormones allows a multiplication rate of 6 new shoots every 8 weeks (Dublin, 1984).

The acclimatization step is now thoroughly refined (Berthouly, 1991), but is costly and sometimes difficult when the surrounding conditions are not perfect.

Also, in order to confer to *C. arabica* a certain tolerance to soil nematodes (especially to *Pratylenchus,* which is a big problem in Central America), it is necessary to graft the microcuttings onto a *C. canephora* seedling rootstock. This has not yet been described in the literature.

In this study, we describe two simple techniques in order to overcome these problems at a lower cost.

Ex vitro acclimatation

Material and methods.

Coffea arabica microcuttings cultivated on a multiplication medium are separated from the mother shoots and are induced to root in non sterile conditions.

The rooting solution contains in 75 mg/l IBA[1] + 50 mg/l NAA[2].

The microcuttings remain in the solution about 15 hours (overnight) and are then transferred to ice-cube trays with perforated bottoms which contain a sterile potting mix of soil, sand and coffee pulp (3:1:1) as described by Berthouly (1991). The trays, with dimensions of 36 cm x 10 cm x 4 cm are placed in shaded paropagators where relative humidity can be controlled by progressively opening the cover.

Results

After two weeks, roots start to appear. After one month, the rooted and acclimatized microcuttings can be transferred to polyethylene bags containing the same potting mix, where they can develop before being transplanted toin the field.

An average of 100% rooting can be obtained when the following conditions are observed :

- microcuttings at least two cm high and with three pairs of leaves are used.
- propagators are in a good sanitary condition at the time of acclimatization
- very progressive diminution of the relative humidity
- application of a light salt solution every two weeks

Discussion

The method here described uses some points already known (like the potting mix composition), but also presents some new aspects :

- an easily executed method
- a reduction in costs (less mix used, economy of time)
- a better control of the environmental conditions in the propagators
- no predator damage, as is often the case when the cuttings are directly acclimatized in the nursery
- an easy distribution to the different users (shipping of the trays)

Perspectives

We want to make trials with deeper trays in order to allow the plantlets a longer time before shipping

In vivo micrografting

Background

A recent work (Anzueto, 1993) indicates that there is no resistance source to the nematode *Pratylenchus spp.* in the *Coffea arabica* species.

A method currently used (in Guatemala and Brazil) to circumvent this very aggressive nematode is the

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[1]***IBA = Indolebutyric acid***

[2]***NAA = Naphtaleneacetic acid***

hypocotyledonary graft onto a *C. canephora* seedling rootstock as described by Reyna (1966).

The purpose of our work was to verify whether this technique could be applied to microcutting scions.

Material and methods.

The rootstocks are seedlings of CATIE's T3561 clone, and the graft is performed when the plantlets are at the "small soldier" or "butterfly" stage.

The scions are microcuttings from the Catuai variety with sizes ranging from 0.8 cm to 2.5 cm.

Microcutting bases are cut diagonally,a slightly longer slot is made on the rootstock and the two parts are assembled with a thin strip of "Parafilm^R".

The grafted plants are then transplanted in a potting mix which has been previously disinfected with a fungicide ("Vitavax" = carboxin + captan), and are placed in a shaded area.

The "Parafilm^R" strips are removed after 20 days.

The plants are acclimated 30 to 40 days after grafting.

Two kinds of grafts were tested:

- terminal slot
- lateral slot

Results and Discussion.

Grafting is virtually 100% successful for both treatments. However, the lateral slot graft is preferable since the scion grows faster.

This work demonstrates that it is possible to graft microcuttings onto *Pratylenchus spp.*-tolerant rootstocks.

This technique constitutes an alternative to the *ex vitro* acclimatization process for *C. arabica* microcuttings.

Prospects.

Couturon (1976) set up an *in vivo* method for grafting zygotic embryos onto *C. arabica* and *C. canephora* rootstocks. Similarly, we want to try grafting young stages of somatic embryos onto nursery seedlings. This would considerably reduce the costs related to the *in vitro* phase.

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HIGH FREQUENCY INDUCTION AND REGENERATION OF MULTIPLE SHOOTS FROM HYPOCOTYL SEGMENTS OF *COFFEA ARABICA*

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INTRODUCTION

Coffee is an important cash crop to the economy of many developing countries, including Tanzania. The susceptibility of commercially-grown cultivars to the large number of races of coffee leaf rust (*Hemileia vastatrix*), coffee berry disease (*Colletotrichum coffeanum*) and other diseases limit the profits. Biotechnological methods have potential in the development of trees resistant to these diseases, but depend on the efficient micropropagation techniques for the clonal multiplication of large numbers of genotypes. There have been reports of successful *in vitro* culture of shoot apical meristems (Kartha *et al.* 1981; Zok 1985; Berthouly *et al.* 1988); axillary buds (Dublin 1980; Sondahl *et al.* 1984) and somatic embryogenesis (Sondahl & Sharp 1977; Neuenschwander & Baumann 1992). The later methods are currently used for the multiplication of cultivated species of *C. arabica* and *C. canephora*.

In this paper we describe the efficient regeneration of multiple shoots from hypocotyl segments of three genotypes of *Coffea arabica*.

MATERIALS AND METHODS

Seeds from three genotypes of *C. arabica*, N_{39}, Hybrido de Timor (VCE1594) and Rume Sudan (VC510) were used. Surface sterilization of glasshouse-germinated seedlings tended to produce latent microbial contamination. Therefore, seeds were surface-sterilized and incubated on 90 mm triple vent Petri dishes containing saline sucrose agar as described by Sondahl *et al.* (1984). Seeds free from contamination were transferred on to a germination medium containing half-strength B5 inorganic salts and vitamins (Gamborg *et al.* 1968), sucrose 20 g l^{-1}, BA 0.23 mg l^{-1}, IAA 0.44 mg l^{-1}, activated charcoal 2.5 g l^{-1} and agar 8 g l^{-1}. The pH was adjusted to 5.6 prior to autoclaving. The seeds were incubated at 26°C in darkness for 4 to 5 weeks. Germinated seeds were transferred to sterile containers containing the same medium, but without hormones and incubated for 3 weeks under diffuse light (12 h photoperiod) at 26°C. Hypocotyls of 5-8 cm were harvested and cut into 2 mm segments. In 90 mm Petri dishes, the segments were placed vertically on a medium containing 20 ml solidified MS salts (Murashige & Skoog 1962), B5 vitamins, cysteine-HCl 78.8 mg l^{-1}, sucrose 30 g l^{-1}, supplemented with 0.2, 1.0, 2.0, 4.0 and 8.0 mg l^{-1} of benzyl amino purine (BA) in diallel combinations with 0.2, 1.0, 2.0 and 3.0 mg l^{-1} of indole-3-butaric acid (IBA). The pH of the media was adjusted to 5.6 before autoclaving. Each treatment consisted of 10 explants and was replicated three times. The cultures were kept at 26°C with 12 h photoperiod provided by cool fluorescent lights. Explants were subcultured on to fresh medium every 4 weeks until formation of

multiple shoots.

RESULTS AND DISCUSSION

Hypocotyl segments of *C. arabica* represent convenient explants for the initiation of cultures and they have considerable potential for regenerating shoots without an intervening callus phase. Whole plants were easily regenerated from these shoots. The growth responses of the three genotypes are shown in Table 1. After 4 weeks of culture, explants on media with high auxin levels formed calli. At higher concentrations of BA, in conjunction with IBA 0.2 and 1.0 mg l^{-1}, multiple shoot regeneration occurred from hypocotyl segments at 100% frequency after 8 to 10 weeks (Fig. 1). There were differences ($P<0.05$) between genotypes in their production of multiple shoots per segment. The highest number of shoots per segment was achieved on explants cultured on a medium with 8 mg l^{-1} IBA (Fig. 2). Shoots were harvested and transferred to a rooting medium containing half-strength MS salts supplemented with 0.2 mg l^{-1} IBA without sucrose (Kartha *et al.* 1981).

The technique described here can be useful in the micropropagation of selected genotypes and in *Agrobacterium*-mediated transformations.

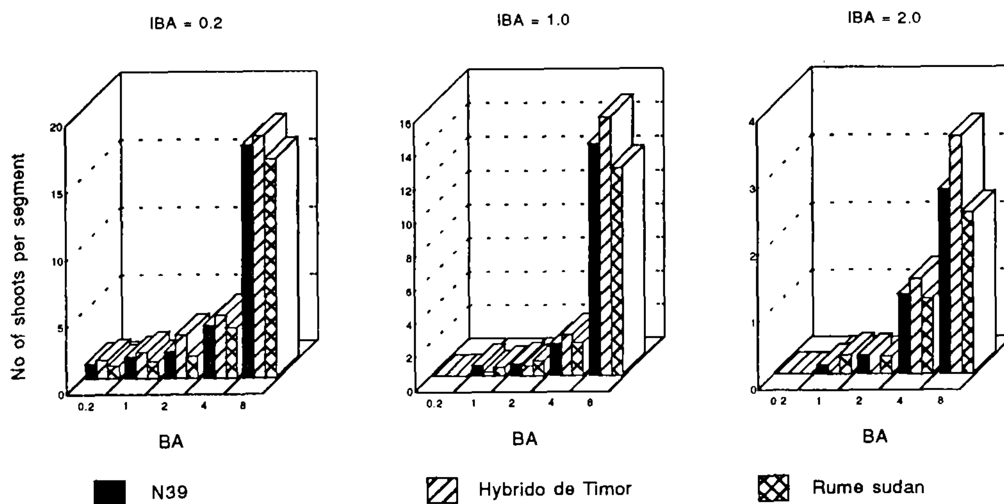
Table 1. Effect of BA x IBA (mg l^{-1}) on hypocotyl segments of *Coffea arabica* genotypes

IBA \ BA	0.2	1.0	2.0	4.0	8.0
0.2	S	S	S	MS	MS
1.0	C	CS	S	MS	MS
2.0	C	CS	CS	CS	CS
3.0	C	C	C	C	C

S - single shoots; C - calus; CS - callus + single shoots; MS - multiple shoots

Fig. 1. Regeneration of multiple shoots from hypocotyl segments

Fig. 2. Influence of IBA and BA (mg 1-1) concentrations on the production of shoots per segment



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SUMMARY

An efficient tissue culture system for high frequency of induction and regeneration of multiple shoots from hypocotyl explants of *Coffea arabica* have been developed by manipulation of culture media and selection of explants. Explants grown on basal medium (MS salts + B_5 vitamins) containing combinations of 8 mg l^{-1} BA and 0.2 mg l^{-1} IBA regenerated shoots at 100% frequency. The upper segments were more regenerative than the lower segments of the hypocotyl.

MICROPROPAGATION OF SUPERIOR GENOTYPES OF *COFFEA ARABICA* L.

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Introduction

The genetic improvement of coffee by conventional methods is an extremely slow process due to the long life-cycle of the plant. Through this process one can take more than 30 years in order to obtain a variety able to be spread in coffee farms.The potencialities of ***in vitro*** tissue culture can be used in different fields of coffee research, namely on improvement programmes. There are superior genotypes of ***Coffea arabica L.*** obtained by conventional breeding programmes resistants to coffee rust (***Hemileia vastatrix*** Berk. & Br.) and to Coffee Berry Disease - CBD (***Colletotrichum coffeanum*** Noack sensu Hindorf) which need to be multiplied in large quantities and to be comercially evaluated in early generations, in a clonal orchad.
The conventional methods of propagation are very slow and expensive and the improved seeds of ***C.arabica*** superior genotypes, are insufficient to satisfy the farmer's demands.Therefore, the development of methods of assexual reproduction of ***C.arabica*** is an urgent necessity.
Different approaches have been considered for ***C. arabica*** micropropagation:
1) Meristem and axillary bud culture inducing multiple shoot development, 2) Nodal culture by breaking down the apical dominance and inducing the development of arrested orthotropic and plagiotropic buds, 3) Somatic embryogenesis and 4) Induction of adventitious shoot development.
Recent advances in these approches will be presented and discussed.

Apical meristem and axillary bud culture

Coffee plant present one apical meristem and each axil leaf has 4-5 dormant orthotropic buds and two plagiotropic buds. The plagiotropic buds only start its development from the 10th -11th node.
The apical meristem culture and the culture of dormant buds, both orthotropic and plagiotropic, gave rise to plantlets which can be used as initial explant for coffee micropropagation.
Using the potentialities of apical and axillary bud culture, we attemped to obtain large scale multiplication in the cultivars Catimor, Catuai, Caturra, Geisha, Matari, etc., by culturing meristem in a medium composed by Murashige & Skoog (MS,1962) Mineral salts, supplemented with Gamborg's (B-5) vitamins (Gamborg ***et al.*** 1968), benzilaminopurine (BAP) or 6-furfuriloaminopurine (Kin), indol-3-acetic acid (IAA) and acetic acid (NAA) in different concentrations.
Different factors interfeering in the meristem differentiation, plant regeneration and induction of multiple shoots were studied (Carneiro & Ribeiro, 1989a, 1990).
In the different studied cultivars, the number of multiple shoot induced was different.We noticed the importance of the genotype in the success of the technique. The number of shoots obtained varied from 7 to 19 plantlets per initial shoot in an average of 9 plantlets/ shoot, what means 9^{6} shoots at the end of one year.

Nodal culture

Each coffee node has 8-10 dormant buds. The enhancement of these buds, by breaking down the apical dominance, gives high rates of coffee plant multiplication. There are, however, important constraints in this technique, namely the dificulty to discontaminate the initial material, the oxidation and the break down of apical dominance, which is very important in coffee. Working with cv.Caturra, we obtained a great improvement concerning to the levels of contamination (41.86%) and oxidation (4.65%) and high rates of plant multiplication(Carneiro & Ribeiro, 1989a). The cv.Caturra nodes were cultured in MS medium supplemented with myo-inositol,nicotyinic acid, pyridoxine-HCL, thiamine-HCL, L-cysteine-HCL, IAA and BAP. The break down of apical dominance was achieved in more or less 3 weeks with 50 um of BAP and 0.5 um of IAA.
The primary shoots obtained by development of the arrested buds were cultured in multiple shoot inducing medium (Carneiro & Ribeiro, 1989b) composed by MS mineral salts supplemented with B-5 vitamins, BAP, IAA in different concentrations and ascorbic acid.
As far as break down was concerned, improvement was achieved when we used nodes of plants regenerated ***in vitro*** by meristem culture or by apical bud culture as initial explant. In these cases, we broke down the apical dominance in 8-10 days (Ribeiro & Carneiro, !989).
Working with cv. Caturra, we have obtained an average of 8.65 primary shoots per node and an increasement of number of shoot in 2nd subculture.
In ideal conditions maintaining this level of multiplication we could obtain very high rates of multiplication. In studies carried out recently in cvs.Catuai, Catimor, S. 4 Agaro, etc., we obtained an average of primary shoots superior to 10 plants/node which means that trough careful and timely transfer it will be possible to obtain at the end of 1 year 10^6 plants/node.

Induction of adventitious shoot development

Adventitious shoot development is an alternative way of coffee micropropagation.Shoots are originated in tissues located in areas other than leaf axils or shoot tips.
Adventitious shoot development is one rare phenomenon in woody plants. It is possible to induce it ***in vitro***, in media of determinated composition and rich in cytokinins.
In coffee, Dublin (1980a, 1980b; Saleil, 1982) obtained some results working with Arabusta and with some clones selected at Ivory Coast.Carneiro (1990), obtained for the first time, good results with ***C.arabica*** cultivars,Caturra,Geisha and Càtimor.
We screened different culture media and explants like hypocotyl, internode and pieces of leaves belonging to different ***C. arabica*** genotypes.
The levels of multiplication were not very high. Indeed, the maximum of shoots per explant obtained, 5-6, was found in cv. Caturra and cv. Catimor showed a very low aptitude to produce adventitious shoots.
An important correlation between the aptitude to neoformation and the genotypes was found in the different studied cultivars of ***C. arabica*** . We also found differences in this aptitude according to the levels of isolation and type of explant.
The number of propagules could be increased by subdividing and reculturing the ***in vitro*** derived shoots, or they can be transfered directly to the rooting phase.

Somatic embryogenesis

The phenomenon of somatic embryogenesis, i. e., the initiation and development of embryos from somatic tissue, was first recognised in coffee plants by Staritsky (1970) who was successful in inducing somatic embryogenesis and plant regeneration in soft internodes of ***C. canephora.***
Sondahl and Sharp (1977a) established with seccess, cultures of mature leaves of ***C. arabica*** cv. Bourbon, in medium containing MS mineral salts supplemented with thiamine-HCL, L-cysteine-HCL, myo-inositol, sucrose and different combinations of Kinetin and diclorophenoxiacetic acid (2,4-D). This "induction medium" was considered to be ideal for induction of high frequency of somatic embryogenesis. Secondary cultures are established by subculturing 45-50 days old tissue into a conditioning medium, containing half strength MS salts, except Kno_3 which was added 2 x concentrations, sucrose ,Kin and naphthalene acetic acid (NAA) under a 12 hours light period.
In the secondary cultures, the authors caracterized two sequences of morphogenesis, low frequency somatic embryo (LFSE) or direct embryogenesis and high frequency somatic embryo (HFSE) or indirect embryogenesis. The first one is observed after 13-15 weeks and the second one after 16-19 weeks.
We successfully induced direct somatic embryogenesis (Carneiro, 1987) in internodes of two ***C. arabica*** genotypes, Geisha and DK1/6 in 7-8 weeks, in presence of high level of cytokinin and absence of auxin. Marques (1990) referred to a HFSE in a single medium based in a modified Sondahl and Sharp's medium with high level of a cytokinin and also in absence of auxin.

Neuenschwander **et al.** (1990) described a alternative pattern of somatic embryo-development in suspension cultures of ***C. arabica***. They named this system, Self-Controlled Somatic Embryogenesis (SCSE). In this system large number of uniform embryos mature spontaneously in suspensions, without the need of changing the culture conditions. SCSE is highly synchronized. One gram of primary tissue produces about 600 well shaped somatic embryos reaching the size of zygotig embryos.

Rooting

Rhizogenesis is perhaps the most difficult and expensive phase of micropropagation. the success of the technique is closely linked to the percentage of survival of newly formed plantlets and with the ability of its root system to acclimatization to the autotrophic conditions. The rooting in woody plants is very difficult and still one of the major constraints of its ***in vitro*** micropropagation.
In the previous works developed in coffee micropropagation, this important stage of the process was negleted.
At CIFC, an intensive study has been carried out aiming to establish one pratical method of ***in vitro*** and ***in vivo*** rooting. In the experiments carried out, we studied factors like concentrations of mineral salts, levels of sucrose, effect of activated charcoal, growth regulators, namely auxins, IAA, indol butyric acid (IBA), NAA cytokinins, Zeatin, Kinetin, BAP and media consistency.From the different studies, we could report the favorable effect of the reduction of mineral salt concentrations to half or to a third. This fact occurred, probably due to a decreased requirement, of the coffee plantlets, for nitrogen in this phase of micropropagation. We also verified that the high levels of sucrose originated rooting inhibition. The activated charcoal plays an important role as adsorvent of toxic products namely cytokinins of the previous stages of micropropagation.The cytokinins, Zea, Kin, and BAP induced rooting in cv.Caturra.In this case the results suggest, that the cytokinins seem to behave as a stimulant of rooting in the absence of exogenous auxins (Carneiro, 1990).

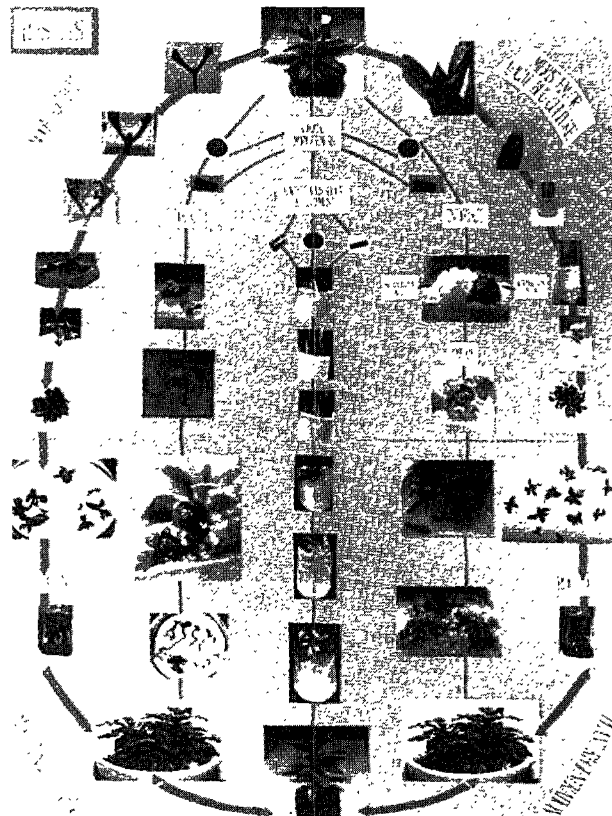

Fig.1. Schematic representation of four ways of ***in vitro*** propagation of ***Coffea arabica*** L. cultivars.

Discussion

Modern improvement programme for coffee, would take advantage of standard breeding methods and ***in vitro*** techniques.

The different pathways referred above and schematicaly represented in Fig.1. for assexual reproduction of ***Coffea arabica*** are an important tool in coffee improvement programmes. As it happens in all the techniques, the four proposed systems present advantages and limitations.

The fundamental problem that a coffee breeder has to face is the genetic stability of the final product which is the seed. Any alteration produced at the level of chromosome during the processes of coffee micropropagation, could interfeer in the beverage quality.However, minor variations could be tolerated, since this fact does not introduce alterations in the agronomic value of the final product. Therefore, the choice of the best way of micropropagation is very important.

The apical meristem and axillary bud culture and the nodal culture are the safer techniques of coffee micropropagation, which allow the obtention of plants true-to-type.By using the technique of induction of somatic embryogenesis, we could obtain very high rates of propagules at a low price. However, the obligatory passage through a significatevly long phase of indifferentiated callus could be a source of important variations, namely somaclonal variation.

The induction of adventitious shoot development, is also one efficient technique that may result in a loss of morphogenetic potential of the tissue or in an increase in genetic variability. The rates of multiplication obtained by this methodology are not very high, resulting in an increased price per propagule.

In conclusion, the results already obtained are promising and we can consider the coffee plant as a Model Plant to which, it is possible to apply any process of in vitro micropropagation aiming the breeder's goal, i.e., the maintenance of the genetic stability or the obtention of genetic variability.

Summary

Coffee is one of the most important products in the world's market. The genetic improvement of coffee plants by conventional methods is an extremely slow process due to the long-life-cycle of the plant. Using this process one can take more than 30 years to obtain one variery able to be spread in coffee farms. The potencialities of *in vitro* tissue culture can be used in different fields of coffee research, namely in improvement programmes. The *in vitro* micropropagation is therefore the unique procedure which allows the production of large amouts of coffee superior genotypes with resistance to coffee rust and to Coffee Berry Disease (CBD), in a short period of time and its evaluation to commercial value on a clonal orchad. Different approaches have been considered for *C.arabica* cultivars micropropagation: meristem and axillary bud culture inducing multiple shoot development, nodal culture by the break down of apical dominance inducing the development of arrested orthotropic and plagiotropic buds, induction of adventitious buds and somatic embryogenesis. Recent advances in these approaches will be presented and discussed.

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COFFEA ARABICA PROTOPLAST CULTURE : TRANSFORMATION ASSAYS

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INTRODUCTION.

Plant cell cultures are suitable systems in which to study the synthesis, transformation and degradation of secondary metabolites. A loss of secondary productivity has often been related to the morphological dedifferentiation of cells *in vitro*. Many attempts are being made to manipulate the metabolism of the cells in order to increase the yield of the interesting metabolites (Bramble *et al.*, 1991). The recent developments in plant molecular biology and the success of plant cell transformation techniques (*Agrobacterium tumefaciens - rhizogenes*) have opened numerous perspectives to establish high producing plant cell lines. The most widely used procedure to obtain high producing plant cell cultures is to isolate single cell clones selected after protoplast transformation and culture.

In this study coffee cell has been chosen as a model cell line. Coffee protoplasts have been isolated from different explants but best results concerning the plant regeneration where obtained when embryogenic cells are used (Söndhal *et al.* 1980, Schöpke *et al.* 1987, Acuna and De Pena 1991). However, to select high-producing transgenic plant cells, the basic protoplast isolation and culture conditions should be as optimal as possible, and are described in this paper.

Indeed, high rates of callus formation from protoplasts are necessary to allow efficient recovery of transformed cell lines. Recently, Spiral *et al.* (1993) have reported efficient transformation of coffee cells by *Agrobacterium rhizogenes*. In this paper, preliminary results are presented concerning the transformation of coffee cells by different strains of *Agrobacterium tumefaciens*.

MATERIAL AND METHODS.

Plant cell culture : Suspension cultures of *Coffea arabica* were established from roots via callus cultures and were generously given by Francereco (France). Callus tissues were cultured on a Gamborg B5 (Gamborg et *al.*, 1968).

Isolation and culture of protoplasts : Cells from culture in exponential phase (5 days after subculture) were preplasmolysed in K3 salts (Nagy and Maliga, 1976) containing sucrose 0.5M for 1 hr (110 rpm). 1 gram of preplasmolysed cells were collected and transferred in 10 ml of the enzymatic solution (cellulase R10 1%, macerozyme R10 0.8%, driselase 0.5%). Incubation was occured in the dark, at 30°C under low shaking (50 rpm) for 15 hrs. Viable protoplasts were collected after centrifugation at 45 g for 7 mn. Their density and their viability was estimated in a heamocytometer (Huang *et al.*, 1986). Protoplasts were cultured at 24°C in 5ml liquid medium at a density of 2 10^5 protoplasts ml^{-1} (P ml^{-1}). The various cell regeneration media tested in this study included MS (Murashige and Skoog, 1962), K3 (Nagy

and Maliga, 1976), Gbg and PRL4 (Gamborg et *al*., 1968). All this media were supplemented with sucrose 0.5M, BAP (6-benzylamino purine)1.0 mg l^{-1}, NAA (naphtalene acetic acid) 1.5 mg l^{-1}, kinetine 0.1 mg l^{-1}. After 7 days of culture, protoplasts were diluted by a factor 2 and after 15 days of culture, the culture medium was changed. The initial and final plating efficiency (IPE, FPE) was calculated after 15 and 30 days of culture respectively.

Transformation techniques : The binary plasmid (pTDE4, PGS, Gent Belgique) containing the gene construct (β-glucuronidase under the control of the 35S promoter of cauliflower mosaic virus, and the neomycin phosphotransferase under the Pnos promoter) is transferred by conjugation into different strains of *Agrobacterium tumefaciens* (C58C1Rif^R(pGV2260), C58C1Rif^R(pMP90), AGL1(pTiBo542).
5 days old coffee cells were cocultivated with a suspension of *Agrobacterium*. After 7 days, the cells were washed to eliminate most of the bacteria and placed on culture medium containing antibiotics to kill bacteria (cefotaxim 500 µg ml^{-1}) and to allow the selection of transformed cells (paramomycin 20 µg ml^{-1}). Histochemical staining of β-glucuronidase activity was occured following the method of Jefferson (1987).

RESULTS AND DISCUSSION.

Culture of protoplasts.

The protoplasts were cultured in media suitable to their reforming their cell walls and to their dividing to form colonies and calluses. The protoplasts obtained from 5 day old suspensions were cultivated at a density of 2 10^5 P ml^{-1} in various media (K3, MS, Gbg, PRL4) supplemented with cystein (50 mg l^{-1}), BAP (1 mg l^{-1}), NAA (1.5 mg l^{-1}), kinetine (0.1 mg l^{-1}), BSA (0.1%) and casein (250 mg l^{-1}). The best plant cell wall regeneration and cell division are observed when coffee protoplasts were cultured in MS and K3 media. Plating efficiencies of 14.8 and 13.8% are recorded for these two media respectively, while only 8.7% and 5.7% are obtained when Gbg or PRL4 media are used as culture media respectively. Gbg medium which is the optimal culture medium for *Coffea arabica* cell culture itself doesn't allow cell wall formation and can't initiate first cell division.

The protoplasts obtained for 5 day old suspension were cultured at different concentrations in K3 medium.

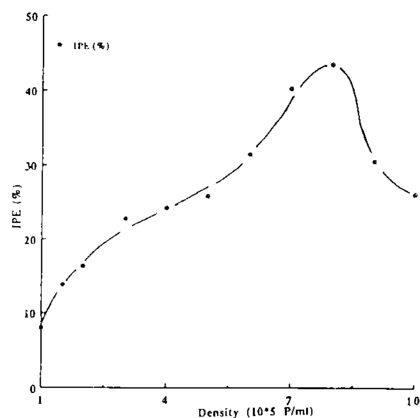

Fig.1. Effect of protoplast density on the viability of the cells at 15 days (IPE).

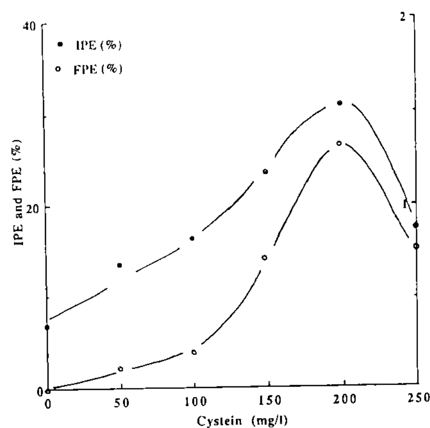

Fig.2. Effect of cystein on the viability of the cells at 15 days (IPE : •) and at 30 days (FPE : o).

The optimal viability of the cells are recorded for a relatively high density of protoplasts (8 10^5 P ml^{-1} : Figure 1).
The presence of cystein seems to be necessary in the culture medium to maintain high viability of the cells. A plating efficiency of 30% is recorded when 200 mg l^{-1} of cystein is present in the culture medium (Figure 2). The coffee cells generally contain a great amount of phenols, polyphenols and aromatic derivates that are trapped by cystein.The best growth and survival of coffee cell colonies are achieved in culture media containing hormones. The coffee protoplasts require an exogeneous supply of cytokinins (BAP and kinetine) and auxin (NAA) for the initiation and maintenance of substained divisions. The optimization of the exact concentration of each hormones must be achieved to increase the divisions of the cells. In our case, the sequences of culture seems also to be of great importance to improve division frequency.

Transformation of coffee cells.

The use of *Agrobacterium* mediated plant transformation systems is now firmly established and is becoming routine for model systems such as tobacco and petunia. Recent reports have shown that different species (*Coffea arabica*) can be transformed and regenerated (Spiral *et al.*, 1993).
Different *Agrobacterium* strains which contains a Ti plasmid depleted of its T-DNA (C58 containing pGV2260 or pMP90, AGLI) can be used as an acceptor strain for the binary vector (pTDE4). The *Agrobacterium* Ti plasmid pGV2260 is derived from the pTi B6S3 vector carrying the octopine synthase. The Ti plasmid pMP90 is derived from the pTi C58 vector carrying the nopaline synthase. The AGLI strain is derived from EHA101 strain containing the Ti plasmid pTiBo542 that confers hypervirulence of the strain. Our results show that only *Agrobacterium* C58 containing the Ti plasmid pGV2260 or pMP 90 can transform the coffee cells.

Strain of *Agobacterium tumefaciens*	Expression of GUS gene (%)
C58 (pGV2260)	0.05
C58 (pMP90)	0.06
AGLI (pTiBo542	_
Control	_

Table 1 : Transformation of coffee cells with differents *Agrobacterium* strains.

The expression of the β-glucuronidase is also observed in these two cases. These preliminary results must be carried on to determine the optimal conditions for an efficient transformation of coffee cells.

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SUMMARY.

The biosynthesis of the secondary metabolites seems to be related to the differentiation of the cells or plant organs such as shoots or roots. Many efforts have been made to improve the productivity of the plant tissue cultures. A recent development to overcome those difficulties is the genetic transformation of plant cells with *Agrobacterium rhizogenes* or *tumefaciens* to introduce useful traits or to establish high-producing lines. The must widely used procedure to obtain high producing plant cell cultures is to prepare single cell clones by preparing protoplasts. The prepared protoplasts are plated at low density (2 10^5 p/ml) and allowed to grow into callus culture which subsequently are selected for their productivity before their use in bioreactor.

Coffee cells has been chosen as a model cell line because the biosynthesis of the secondary metabolites is well characterized. Moreover, it's possible to isolate biochemically intact protoplasts. However, high rates of callus formation from protoplasts are necessary to allow efficient recovery transformed cell lines. Identification of limiting factors (auxine and cytokinin requirements, sugar, cystein, BSA, light, ...) should facilitate the development of a culture procedure to obtain high rates of callus formation.

If genetically engineered plant cell cultures have to be used for the production of secondary metabolites, the expression of the introduced gene has to be stably maintained over a long periods of subcultured. In order to obtain funtionnal transgenic cell lines the optimization of the transfer DNA protocol (Agrobacterium strain, explant lines, bacterial concentration, cocultivation period) must be achieved. The expression of a chimeric GUS gene is used as a model to establish conditions for both the transformation and for efficient and stable expression of foreign genes.

ÉTUDE DE L'INFLUENCE DE TENEURS RÉDUITES EN OXYGÈNE SUR DEUX PROCESSUS DE CULTURE *IN VITRO* DU CAFÉIER (*COFFEA ARABICA*) : L'EMBRYOGENÈSE SOMATIQUE ET LE MICROBOUTURAGE

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OBJECTIFS

Pour le microbouturage : L'objectif était de prolonger la conservation du matériel végétal en laboratoire en ralentissant son métabolisme. Cette technique aurait pour avantage un gain de place (par rapport aux collections *in situ*) et un nombre d'intervention sur les boutures réduit.

Pour l'embryogenèse somatique : L'objectif était d'évaluer le rendement embryogène des cals placés dans les conditions atmosphériques les plus proches de celles de la germination d'embryons zygotiques, c'est à dire à teneur réduite en oxygène.

INTRODUCTION

L'influence d'hypoxie relative (12% et 9%) a été étudiée sur deux techniques de multiplication végétative *in vitro* du caféier (*C. arabica*) en vue d'optimiser ces processus : le microbouturage et l'embryogenèse somatique. L'hypoxie a été provoquée par l'intermédiaire d'un "oxyreducer".

RESULTATS

Pour le microbouturage: la baisse de la teneur en oxygène semble ralentir la croissance des microboutures; néanmoins le reprise de croissance après les traitements est normale. En outre, le faible stockage de proline, en considération de la quantité que le caféier est susceptible d'accumuler, nous laisse à penser que l'agression subie par la plante n'est pas majeure.

Pour l'embryogenèse somatique: Lors de l'embryogenèse somatique directe du *C. arabica* une faible baisse du pourcentage d'oxygène (12%) stimule la formation d'embryons. Cette formation peut être éventuellement favorisée par un apport d'ATP, produit en réaction à cette modification de l'environnement.

Par contre, il apparait qu'une hypoxie trop prononcée (9%) bloque le processus embryogène. Dans le cas de l'embryogenèse somatique indirecte, la réduction de la teneur en oxygène quelle qu'elle soit (9% ou 12%) ne semble pas favorable au développement embryogène.

CONCLUSIONS

Pour le microbouturage: La durée du stockage devrait être augmentée (un an) et les hypoxies devraient être plus prononcées (inférieures à 9%) pour confirmer les résultats.

Pour l'embryogenèse somatique : Le processus d'embryogenèse somatique directe devrait être testé avec des teneurs de l'ordre de 12% à 20,9% pour vérifier qu'une faible réduction d'oxygène est favorable à la formation d'embryons. L'objectif serait de déterminer le niveau d'hypoxie pour lequel la production d'embryons serait optimale.

Lors de l'embryogenèse somatique indirecte, la baisse de la teneur en oxygène semble néfaste mais une intervention ponctuelle et à des moments précis au cours de la formation embryogène pourrait améliorer les résultats.

RESUME

L'effet de différentes teneurs en oxygène a été étudié sur deux processus de culture *in vitro* du caféier: le microbouturage et l'embryogenèse somatique. Des microboutures déjà établies ont été multipliées sur un milieu de Murashige et Skoog contenant 4.44 μM de BAP. Ces microboutures ont servi aux diverses expériences et leurs feuilles ont été prélevées afin d'être mises en induction d'embryogenèse somatique selon deux méthodes : un procédé indirect, avec formation de cal, mettant en jeu une séquence de deux milieux différents, et un procédé sur un milieu unique, dit de Yasuda, permettant l'apparition des embryons somatiques sur le limbe de l'explant d'origine avec très peu de cal.

Les teneurs réduites (12% et 9%) en oxygène ont été obtenues par l'intermédiaire d'un appareil, l'"Oxyreducer", qui, à l'aide d'une sonde, teste la concentration en oxygène à l'intérieur d'une enceinte de culture et l'ajuste et la maintien au niveau désiré (généralement plus bas), par injection d'azote.

Le suivi du développement des microboutures, évalué par leur hauteur, a permis de mettre en évidence une réduction de croissance notoire, mais non rédhibitoire, pour 9%, constituant une possibilité de conservation à moyen terme.

Egalement, la réponse embryogène des explants foliaires (abondance du cal, nombre d'embryons formés par explant, abondance de la réaction) a été modifiée par des teneurs réduites, ce qui se rapproche des conditions physiologiques observées dans la graine pour un embryon zygotique.

De plus, un marqueur de stress, la proline, a été analysé pour les microboutures. La capacité du caféier à accumuler cet acide aminé montre sa bonne résistance au stress induit par la diminution de la teneur en oxygène.

ABSTRACT

The effect of reduced oxygene concentrations was studied on *in vitro* culture of coffee (*C. arabica*), through two processes : microcutting and somatic embryogenesis.

Already established *C. arabica* var. Icatu microcuttings were multiplied on a Murashige and Skoog medium supplemented with 4.44 μM BA. Those microcuttings were used for several experiments and their leaves were eventually removed in order to be submitted to somatic embryogenesis induction through two methods : an indirect technique, with abundant callus formation through a sequence of two different media, and a method involving a single medium (Yasuda's medium) allowing direct somatic embryogenesis on leaf blades.

Reduced levels of oxygene (9% and 12%) were obtained through a device called 'Oxyreducer' which testes the oxygene concentration in a culture chamber, through a probe and regulates it by N_2 injections from a pressure tank.

The monitoring of microcuttings development through height and weight measurement showed a significant reduction in growth at 9% O_2 without loss of viability. This phenomenon can constitute an efficient kind of medium-term preservation.

Also, the embryogenic response of the leaf explants was modified by low O_2 concentrations as shown by callus reduction and higher average number of somatic embryos. In this case, lowering the oxygene concentration allows the somatic embryo to be in conditions physiologically similar to that of the zygotic embryo in the seed.

ZYGOTIC AND SOMATIC EMBRYO CRYOPRESERVATION IN COFFEE (*Coffea arabica, C. canephora* and Arabusta)

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Abstract

Coffee can successfully be conserved in the field but this method is very expensive and presents high risks of loosing some material through biotic or abiotic events. Therefore we investigated the possibilities of conserving zygotic and clonal (somatic) embryos in liquid nitrogen (LN; at a temperature of -196°C).

First, several pretreatments designed to avoid chilling injury were evaluate on both zygotic and somatic embryos of *C. arabica*, var. catimor and *C. canephora* var. Robusta. Those genotypes were chosen due to their responsiveness in producing embryogenesis. Cultures were initiated by placing 1.5 cm^2 leaf sections on induction medium. Somatic embryos appeared after 10 weeks.

For example, prolonged exposure to high sucrose levels caused the embryos to develop more normally after the cold treatment.

Three cryopreservation protocols were tested.

Treatments included either rapid freezing by direct immersion in LN or slow freezing at either 0.5, 0.8 or 1°C/mn to -40°C prior to immersion in LN. Samples were either slow-thawed for 30 mn or rapid-thawed in a 40°C water bath.

Results from some of these experiments are very promissing. The embryos survived freezing but did not germinate directly. Instead,they produced embryogenic calli and somatic embryos. Robusta embryos showed the higher percentage of survival (71%).

Résumé :

Les caféiers peuvent facilement être conservés sous forme de collections au champ, mais cette méthode est onéreuse et représente des risques élevés de perte de matériel par cause biotique ou abiotique. Nous avons donc recherché la possibilité de conserver des embryons zygotiques et clonaux (somatiques) dans de l'azote liquide ("LN"; à une température de -196°c).

Tout d'abord, plusieurs prétraitements destinés à éviter les dégats dûs au froid ont été essayés à la fois sur les embryons somatiques et zygotiques de *C. arabica* var. catimor et *C. canephora* var. Robusta. Ces génotypes ont été choisis pour leur bonne capacité embryogène. Les cultures sont initiées en plaçant des sections foliaires de 1.5 cm^2 sur un milieu d'induction. Les embryons somatiques apparaissent après 10 semaines. Par exemple, une culture

prolongée sur de fortes concentrations en saccharose a permis un meilleur développement des embryons.
Trois protocoles de cryoconservation ont été essayés. Les traitements ont consisté en, d'une part, une congélation rapide par immersion directe dans LN, ou, d'autre part en une congélation lente à des rythmes de 0.5, 0.8 ou 1°c/mn jusqu'à -40°c avant immersion dans LN. Les échantillons ont été soit décongelés lentement pendant 30 mn, soit rapidement par trempage dans un bain-marie à 40°c. Les résultats de certaines de ces expériences sont très prometteurs. Les embryons ont survécu à la congélation, mais n'ont pas germé directement. Au contraire, ils produisent des cals embryogènes, puis des embryons somatiques. Les embryons de Robusta ont montré le meilleur pourcentage de survie (71%).

INTRODUCTION

Coffee can successfully be conserved in the field but this method is very expensive and presents high risks of loosing some material through biotic or abiotic events. Therefore we investigated the possibilities of conserving zygotic and clonal (somatic) embryos in liquid nitrogen (LN; at a temperature of -196°C).

MATERIAL AND METHODS

For somatic embryos, two genotypes were used : *Coffea arabica* var Catimor and *Coffea canephora* var Robusta. All the embryogenic cultures were started from leaves from microcuttings cultivated on a standard multiplication medium. Cultures were initiated by placing 1.5 cm^2 leaf sections on induction medium (Yasuda *et al*, 1985). Somatic embryos appeared after 10 weeks.

Three cryopreservation protocols were tested: 1) the protocol reported by Bertrand-Desbrunais *et al*,(1988) which utilizes a culture pretreatment on increasing concentrations of sucrose followed by infiltration with DMSO; a protocol (Dereuddre *et al*, 1990) in which embryos are first placed in alginate beads prior to dehydration in air; 3) finally, a protocol developped at CATIE (Abdelnour-Esquivel *et al*, 1992) involving an air-dehydration pretreatment followed by a rapid freezing in liquid nitrogen and a rapid thawing. Treatments included either rapid freezing by direct immersion in LN or slow freezing at either 0.5, 0.8 or 1°C/min to -40°C prior to immersion in LN. Samples were either slow-thawed for 30 min or rapid-thawed in a 40°C water bath.
Preliminary experiments were also conducted to observe the effects of cryoprotectants such as sucrose (up to 0.75M), DMSO (5, 10 and 15%) and others, on germination of the embryos.

Regarding zygotic embryos, we experimented three different genotypes : *C. arabica* var Caturra, *C. canephora*, and the hybrid arabusta (*C. arabica* x *C. canephora*). Three different stages of maturity were used: embryos from green fruits (ca. two months before harvest), from yellowing fruits (ca. four days before full maturity) and from red mature fruits. The freezing protocol has been previously described (Abdelnour-Esquivel *et al*, 1992).

RESULTS

Zygotic embryos. Most of the results have been previously reported. We observed a 100% germination in the untreated control, and the rate of survival with or without a LN treatment varied according to the moisture content. We also observed a large genotypic effect; *C. canephora* presented a significantly lower survival rate after freezing. Gemination of surviving embryos occured and fully developped plantlets were obtained with all species.

Somatic embryos. Somatic embryos have been obtained directly from leaf discs, with very little or no callus formation. We have been successful in cryopreserving somatic embryos of *C. canephora* but not of *C. arabica*. The first signs of growth appeared between the 5th and the 7th week after freezing. With three replications of the same experiment, it is obvious that there is a large difference between replications. The regrowth percentage at 15 weeks is 72% in one replication and 36% in the other two. Another important difference in response between treatments is the speed of regrowth. Direct immersion into LN was the worst treatment. Embryos pretreated with partial dehydration showed the most rapid regrowth.
We found that the factors that affect success in cryopreservation, as measured by regrowth percentage are : 1) somatic embryo quality and 2) variations in the cooling process inherent to the device used.

DISCUSSION - CONCLUSIONS

For zygotic embryos, we successfully cryopreserved material from *C.arabica, C. canephora* and from the interspecific hybrid arabusta. Plantlets developed directly from those frozen embryos. A simple procedure was used, involving partial desiccation and direct immersion in liquid nitrogen.

For somatic embryos, lack of success with *C. arabica* may be due to the inadequacy of both the somatic embryogenesis medium and the recuperation medium. During the process of somatic embryogenesis, many abnormal embryos are produced from this line and the germination percentage is very low. There is also some evidence of vitrification, a physiological disorder.

Several other trials are under evaluation. We are re-evaluating some embryogenic culture protocols as well as several *C. arabica* genotypes reactivity.

It should therefore be possible to have access to long-term preservation of coffee genetic resources through cryopreservation.

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CRYOPRESERVATION OF APICES OF *COFFEA RACEMOSA* and *COFFEA SESSILIFLORA* USING THE ENCAPSULATION/DEHYDRATION TECHNIQUE

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INTRODUCTION

The present study aimed at setting up a cryopreservation technique for apices sampled on *in vitro* plantlets. Apices appeared as the best candidates for long-term preservation of coffee genetic resources. Indeed, genetic characteristics of each species are preserved since plants are multiplied *in vitro* by microcuttings. Moreover, direct growth recovery of apices after cryopreservation is a garantee for the trueness to type of regenerated plants.
Cryopreservation experiments were performed using the encapsulation-dehydration technique. Indeed, encapsulation-dehydration appears as the only freezing technique likely to ensure both high survival rates and reproducible results in the case of apices (Dereuddre, 1992).

MATERIALS AND METHODS

Plant material
This study was carried out with *in vitro* plantlets of two coffee species originating from eastern Africa : *Coffea racemosa* and *C. sessiliflora*.

Micropropagation
Plantlets were cultured on an MS solid medium supplemented with sucrose (40 $g.l^{-1}$) and benzyl amino-purine (BAP, 0.3 $mg.l^{-1}$), according to Bertrand-Desbrunais (1991). They were maintained at 27 ± 1°C under a light intensity of 50 $\mu E.m^{-2}.s^{-1}$ PAR, with a photoperiod of 12 hrs light / 12 hrs dark.

Production and excision of shoot tips
Plantlets were cut into stem segments comprizing two nodes. They were cultured for three weeks on the same basal medium supplemented with BAP (0.5 $mg.l^{-1}$) and naphtalene acetic acid (NAA, 0.2 $mg.l^{-1}$) in order to induce the development of axillary shoot tips. One mm long shoot tips comprizing 2 to 4 leaf primordia were then excised and placed for recovery in Petri dishes on the same medium, for 24 hrs.

Encapsulation
Shoot tips were suspended in liquid medium devoid of calcium and supplemented with alginic acid (30 $g.l^{-1}$). Encapsulation of apices was achieved by dropping this solution into a liquid medium supplemented with $CaCl_2$ (100mM), for alginate polymerization.

Pregrowth and dehydration
Alginate beads, containing 1 to 3 shoot tips, were placed for 1 to 10 days in liquid medium supplemented with various sucrose concentrations. In some experiments, beads were transferred dayly in media with increasing sucrose concentrations. After preculture, alginate beads were dehydrated for various periods (0 to 6 hrs) in the air current of a laminar flow cabinet.

Freezing and thawing
Beads were frozen rapidly by direct immersion in liquid nitrogen (-196°C) where they were kept for a minimum of 20 minutes. They were rewarmed slowly by placing them at room temperature under the laminar flow for 2-3 minutes.
Recovery
Encapsulated shoot tips were then placed in Petri dishes containing solid medium. Survival was estimated after 3 to 4 weeks. Apices were considered surviving when leaf expansion and/or growing and callusing was noted. Apices showing signs of recovery were extracted from the beads and placed on the satndard emdium (BAP 0.3 $mg.l^{-1}$).

RESULTS

For both species, survival was generally high for sucrose concentrations comprized between 0.1 and 0.75 M, whatever the pregrowth duration (Table 1). When preculture was performed with 1 M sucrose, survival dropped whatever the pregrowth duration.
In all conditions growth recovery of shoot tips was much more rapid with *C. racemosa* than with *C. sessiliflora*. With both species, increasing sucrose concentrations and pregrowth durations considerably slowed down growth recovery.

Table 1: Effect of sucrose concentration and pregrowth duration on the survival of shoot tips of *C. racemosa* and *C. sessiliflora*.

	C. racemosa					*C. sessiliflora*				
	1 day	3 days	5 days	7 days	10 days	1 day	3 days	5 days	7 days	10 days
0.1 M	17/17	14/15	15/15	12/15	12/15	15/15	15/15	15/15	14/15	15/15
0.3 M	17/17	13/15	14/15	8/15	12/15	15/15	15/15	15/15	15/15	15/15
0.5 M	17/17	14/15	10/15	12/15	13/15	15/15	15/15	15/15	14/15	15/15
0.75 M	17/17	11/15	9/15	6/15	13/15	15/15	15/15	12/15	11/15	15/15
1 M	13/17	7/15	7/15	6/15	4/15	6/15	14/15	8/15	7/15	6/15

In all pregrowth conditions experimented, survival of control shoot tips decreased in line with increasing desiccation periods (Table 2). However, dehydration appeared to be less damaging after a 10-day pregrowth period.
Survival of cryopreserved apices was obtained with both species after different pregrowth and desiccation periods. After desiccation, growth recovery of some surviving control and cryopreserved apices occurred in the form of callusing only, whereas other apices turned green and developed directly into plantlets. Cryopreservation considerably slowed down the growth recovery intensity of surviving shoot tips, which could be observed 3-4 weeks after thawing only.
Progressive increase of sucrose concentration during pregrowth did not modify the survival rate of cryopreserved apices of *C. racemosa*. However, it allowed to considerably reduce the delay in growth recovery of cryopreserved apices.

Table 2: Effect of preculture conditions and dehydration duration on the survival of control (-LN) and cryopreserved (+LN) shoot tips of *C. racemosa* and *C. sessiliflora*.

	C. racemosa								*C. sessiliflora*							
	3 days				10 days				3 days				10 days			
	0.5 M		0.75 M		0.5 M		0.75 M		0.5 M		0.75 M		0.5 M		0.75 M	
	- LN	+ LN	- LN	+ LN	- LN	+ LN	-LN	+LN	- LN	+ LN	- LN	+ LN	- LN	+ LN	- LN	+ LN
Dehydration																
0 h	10/10	0/10	10/10	0/8	8/8	0/7	7/7	0/8	7/7	0/8	7/7	0/8	7/7	0/8	7/7	0/8
1 h 30	10/10	0/10	10/10	0/8	8/8	0/7	7/7	0/8	6/7	0/8	5/7	0/8	7/7	0/8	7/7	0/8
3 h	8/10	0/10	7/10	0/8	8/8	0/7	6/7	0/8	6/7	0/8	3/7	0/8	5/7	0/8	7/7	**1/8**
4 h 30	3/10	**1/10**	8/10	**1/8**	6/8	0/7	2/7	0/8	3/7	0/8	3/7	0/8	3/7	0/8	7/7	**1/8**
6 h	2/10	**2/10**	4/10	0/8	6/8	0/7	3/7	0/8	1/8	0/8	4/7	**1/8**	3/7	0/8	5/7	**2/8**

CONCLUSION

This preliminary study demonstrated that cryopreservation of shoot tips of two coffee species, *C. racemosa* and *C. sessiliflora*, is possible using the encapsulation/dehydration technique. However, additional experiments are necessary in order to increase the survival rates and to improve the recovery pattern of apices. Higher survival rates may be obtained by modifying some parameters of the process. Callusing of apices may be limited or suppressed by refining the hormonal balance of the recovery medium. An histological study is presently carried out in order to observe the damages caused to apices by the successive steps of the cryopreservation process. In a second step the improved process should be experimented with other coffee species. In conclusion, cryopreservation of coffee genetic resources may be foreseable in a not too distant future using encapsulation/dehydration of apices.

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SUMMARY

As regards seed storage behaviour, coffee is classified as recalcitrant since seeds remain viable for very limited periods only. Genetic resources are thus conserved as field collections. *In vitro* collections are being set up which will allow medium-term conservation of germplasm. For long-term storage, cryopreservation (liquid nitrogen, -196°C) is the only method currently available. In the case of apices, cryopreservation experiments were performed using the encapsulation/dehydration technique.
Apices sampled on *in vitro* plantlets of *Coffea racemosa* and *C. sessiliflora* were encapsulated in alginate beads, precultured for various durations in liquid medium with high sucrose concentration, partially desiccated under the laminar flow and immersed rapidly in liquid nitrogen. Survival of cryopreserved apices was noted after pregrowth and desiccation periods.
These preliminary experiments indicate that cryopreservation of apices of coffee is possible. Optimal conditions for each step of the process are being sought. They will then be experimented with various coffee species.

RESUME

Pour la conservation des graines, le caféier est classifié comme récalcitrant puisque les semences de cette espèce ne restent viables que pendant des durées très limitées. Les ressources génétiques de cette espèce sont donc conservées sous forme de collections en champ. Des vitrothèques sont en cours de mise en place afin de permettre leur conservation à moyen terme. Pour la conservation à long terme, la cryoconservation (azote liquide, -196°C) est la seule méthode disponible actuellement. Avec les apex, les essais ont été réalisés en utilisant la méthode d'encapsulation/déshydratation.
Des apex prélevés sur des vitroplants de *Coffea racemosa* ont été encapsulés dans des billes d'alginate, prétraités en milieu liquide à forte concentration en saccharose pendant des durées variables, partiellement déshydratés puis immergés rapidement dans l'azote liquide. La survie d'apex cryoconservés a été obtenue pour différentes durées de prétraitement et de dessiccation.
Ces essais préliminaires indiquent que la cryoconservation d'apex de caféiers est possible. Les conditions optimales de chaque étape du procédé sont actuellement recherchées. Elles seront ensuite expérimentées avec d'autres espèces de caféiers

TRANSIENT EXPRESSION OF β-GLUCURONIDASE FOLLOWING BIOLISTIC DELIVERY OF FOREIGN DNA INTO COFFEE TISSUE

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INTRODUCTION

Genetic engineering may offer an important contribution to breeding of coffee. Possible applications are the use of genes for insect resistance (*Bacillus thuringiensis* genes), use of male sterility for production of F1 hybrids, and gene manipulation for cafein-free coffee seeds. Techniques as *Agrobacterium*-mediated transformation[2,8,11] and electroporation[3] have been studied with variable succes. The availability of an efficient callus induction and regeneration method from suspension cultures, makes it accesible to apply the biolistic method.

This report describes the first demonstration of transient expression of the GUS marker gene (β-glucuronidase) following biolistic delivery of foreign DNA into different types of coffee tissue (leaves of microcuttings, somatic embryos and suspension cultures).

MATERIALS AND METHODS

Plant material.

Microcuttings: Sterilized orthotropic nodes obtained from greenhouse grown plants were cultured at 27°C, at photoperiod of 12 hours a day and subcultured each two to three months by transfer on fresh modified MS medium. Young leaves of the microcuttings were used for the biolistic experiments by placing them abaxial surface down on a solidified callus induction medium[4] (MS salts half strength, 2,4-D 0.5 mg/l, IBA 1 mg/l, 2-iP 2 mg/l, sucrose 30 g/l). A one day pretreatment was applied using the same medium with sucrose concentration increased to 100 g/l. This osmotic pretreatment has shown to be stimulatory for transient expression of introduced genes into plant material[9,12,13] (van Boxtel, unpublished data).

Suspension cultures: Sterilized young leaves obtained from greenhouse grown plants were cut in small pieces of one cm^2 and placed on callus induction medium (described above). The explants were cultured in dark at 27°C in small glass yars (100 ml). After one month, a primary callus was formed and the explants were transferred to solidified expression medium[4] (MS salts half strength, 2,4-D 1 mg/l, BA 4 mg/l), and cultured at low light intensities. Two to three months later friable callus that had been developped on the explants was transferred to 50 ml of liquid cell culture medium[4] (MS salts half strength, 2,4-D 1 mg/l, kinetin 1 mg/l) in 250 ml erlenmeyers. The suspensions were cultured at 27°C (indirect lighting) on a gyratory shaker at 100 rpm, until a stable suspension was obtained after 2 months of initiation, and subcultured each 12-15 days. Suspensions were spread out over a filter paper using a Buchner funnel and placed one day on a modified MS

medium (sucrose 100 g/l) before biolistic treatment.
Somatic embryos: Somatic embryos could be obtained two months after transfer of embryogenic suspensions into a modified MS medium containing 5 mg/l BA. The (im)mature embryos were transferred to liquid MS medium (sucrose 100 g/l) and agitated at 100 rpm five hours before biolistic treatment.

Plasmids.
Plasmids pCH1 and pBMCV 102120k were obtained from the Laboratoire de Biologie Cellulaire, INRA, Versailles, France. Plasmid pUBQ1 was provided by J. Callis, Dept. of Biochemistry and Biophysics, Davis, California, USA. Plasmid pP1-mini-T1 was a gift from B. Lescure, Laboratoire de Biologie Moleculaire des Relations Plantes-Microorganismes, CNRS-INRA, Toulouse, France. Details of plasmids are shown in Fig 1.

Particle gun device.
The device used for our experiments is a powder driven gun[14], modified by F. Quetier and co-workers of the Laboratoire de Biologie Moleculaire Végétale, Univerité Paris XI, Orsay, France. HC100 tungsten particles, which have a mean diameter of approximately 1.0 μm (METABAP, Paris), were coated with plasmid DNA using calcium/spermidine precipitation[6]. About 2 μl of the DNA/particle suspension was loaded onto the macroprojectiles, thus theoretically containing 10 μg DNA and 2.5 mg tungsten particles per shot. The target cells were placed 180 mm below the macroprojectile stopping plate and bombarded with a single shot under partial vacuum of 40 mbar.

Postbombardment handling.
After bombardment, the Petri plates were placed at 25°C with indirect light for two days. Plant material for histochemical GUS assays was stained with 1 mg/ml 5-bromo-3-chloro-3-indolyl-β-D-glucuronic acid solution following an overnight incubation at 37°C, and decolored with alcohol 95°.
For selection procedure, bombarded leaf explants and somatic embryos were placed on callus induction medium, containing 100-200 mg/l of kanamycin. After 1 month, callus formed on the explants was transferred to callus expression medium. Developped (secondary) embryos were tested on their transgenic character two months after the last transfer, by X-gluc staining.

RESULTS AND DISCUSSION

1. Endogenous GUS expression. Light bluish staining after X-gluc incubation has sometimes been observed in control treatments of suspension cultures containing embryogenic structures (proembryos, globular embryos or mature somatic embryos), and can thus be considered as endogenous GUS expression. This endogenous expression in embryogenic structures appears to be different from real GUS expression, as being lighter coloured and more diffused. Such has never been observed in cell suspensions or leaf explants. Endogenous GUS expression in plant material is a phenomenon that has been observed also in other species[7].
2. Cell suspension cultures. Although an increase of transient expression of the GUS gene in coffee suspension cultures could be obtained by an osmotic treatment of 0.35 M of sucrose, the absolute level remained nevertheless low (up to 100 blue spots per plate). Moreover, regeneration of bombarded suspension cultures under kanamycin selection in liquid or solid medium, could not be achieved.
3. Somatic embryos. Very high levels of GUS expression were obtained 10 days after bombardment of somatic

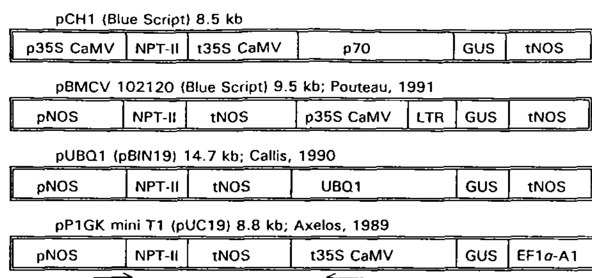

Fig. 1. Schematic diagram of the chimeric gene constructs used in the transient expression experiments.

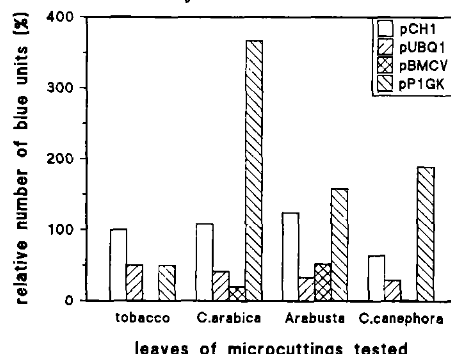

Fig. 2. GUS assays on microcutting leaves of tobacco and coffee, bombarded with the chimeric GUS constructs, described in Fig. 1. Relative number of GUS expressing units (pCH1 on tobacco = 100%) are the mean of 3-9 treatments.

embryos. Intensely stained areas, especially at the root apex, where fast growing secondary embryos are formed, were considered as real GUS expression. Tests to verify presence of stably transformed plantlets are to be carried out soonly.

4. Leaf explants. The wounding of coffee leaves induced by biolistic treatment (intrusion of tungsten particles) can be responsible for callus formation. The possibility exists that a touched cell, containing tungsten/plasmid, is at the base of induced callus. Actually, a study is being realized to verify this possibility. Four chimeric gene constructs have been tested on leaves of 3 coffee species (Fig. 2). The average amount of blue spots on tobacco leaves, when using the pCH1 construct, which varied between 50 and 500 per plate, was used in each experiment as internal standard (100%). Until now, best results were obtained on *C. arabica* leaves using the EF1α-A1 promoter from *Arabidopsis thaliana*[1]. Besides an increase in blue events per bombarded plate (up to 1300), also their intensity could be improved. This facilitates the recognition of blue spots on coffee leaves producing normally large quantities of polyphenoles during X-gluc incubation. Primary callus and proembryos, developped on bombarded leaves, have been tested using the fluorimetric and the histochemical GUS assay. No fluorescence could be observed after MUG-incubation. Using X-gluc-incubation, light bluish colored young embryos could be observed.

As a conclusion, the present report shows that the transient expression system using high-velocity microprojectiles provides valuable information concerning gene expression in coffee. The readily detectable expression of the GUS gene in coffee leaves supports the way for genetic engineering of this important crop.

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Summary. Aiming at the obtention of transgenic coffee plants with resistance to insects, a viable transformation method is under development. The availability of an efficient callus induction, a suspension initiation and regeneration method, permits the use of the biolistic method. Transient expression of the β-glucuronidase gene was best detectable and most frequent observed in leaves of coffee microcuttings, whereas coffee somatic embryos showed endogenous GUS expression in control treatments. Highest GUS activity was observed in *C. arabica* leaves when the GUS gene was placed under control of the EF1α-A1 promoter of *Arabidopsis thaliana*, in comparison to 3 other promoters. The use of this promoter opens perspectives in obtaining stably transformed coffee plants. Plantlets regenerated under kanamycin selection will soon be tested for their transgenic character.

Résumé. Une méthode fiable de transformation génétique, ayant pour but l'obtention de plantes transgéniques de caféier résistantes aux insectes, est étudiée. La disponibilité d'une méthode efficace pour l'induction du cal, l'initiation des suspensions de cellules embryogènes et de leur régénération, nous a mené à développer une méthode biolistique. Parmi les tissus testés, l'expression transitoire du gène de la β-glucuronidase était la mieux détectable et la plus fréquente dans des feuilles issues de microboutures, tandis que des traitements témoins d'embryons somatiques ont montré une expression endogène du gène GUS. Le niveau le plus élevé d'activité GUS a été observé dans des feuilles de *C. arabica* en utilisant le gène GUS sous contrôle du promoteur EF1α-A1 d'*Arabidopsis thaliana*, en comparaison à 3 autres promoteurs. L'utilisation de ce promoteur ouvre des perspectives pour l'obtention des caféiers transformés d'une façon stable. Des plantules régénérées sous sélection de kanamycine seront testées pour leur caractère transgénique.

HAPLOID CELL COLONY FORMATION FROM MECHANICALLY ISOLATED MICROSPORES OF *COFFEA ARABICA*

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INTRODUCTION

The main significance of haploids in coffee breeding is their use in interspecific hybridization. Selected di-haploids from a heterozygous diploid *Coffea* species can be used to hybridize with *C. arabica* (2n = 4x = 44), resulting in homogenous hybrids. Following haplo-diploidization, a one-step-selection can be made instead of a long pedigree selection, since in haploids all hereditary traits are expressed. Di-haploid plants from *C. arabica* were obtained by polyembryony (Dublin and Parvais, 1975). *In vitro* methods include the culture of intact anthers (Sharp *et al*, 1973; Ascanio, 1987; Raghuramulu, 1989) and of isolated microspores (Carneiro, 1992). Masses of embryogenic single haploid cells offer a target for genetic transformation (Neuhaus *et al*, 1987) an for *in vitro* selection. This report describes the successful induction of mechanically isolated microspores from *C. arabica* to undergo the sporophytic pathway resulting in division and haploid cell colony formation.

MATERIALS AND METHODS

Plants of *C. arabica* cv. Catuai and Catimor were grown in the coffee collection of the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Costa Rica, under field conditions. Flower buds containing microspores, of which the majority were in the mid- to late-uninucleate stage, were collected and precultured at 4°C for two days. The flower buds were surface sterilized for 15 min in a 6% sodium hypochlorite solution and rinsed three times in sterile deionized water. Mechanical isolation of microspores was performed on anthers after excision from the buds or by whole bud maceration with a microblender. In the first technique, anthers were cut along the longitudinal axis and put in a drop of wash medium (WM) on a 250 μm sieve. The WM consisted of a half strength Murashige and Skoog medium supplemented with 6 or 12% sucrose and (mg/l) thiamine.HCl (2), meso-inositol (100), pyridoxine.HCl (1) and nicotinic acid (1). The microspores were carefully squeezed out of the anthers, with the knob of a scalpel and filtered through the first sieve and a second one with a pore size of 48 μm. After collecting and washing the microspores in microspore medium (MM), by two centrifugations, they were resuspended in MM, distributed to multiwell plates and cultivated at 27°C in the dark. The MM was identical to the WM but was supplemented with (mg/l) 2,4-D (1), BAP (1); or with a combination of NAA (0.5), 2,4-D (0.5) and kinetin (0.5) used to regenerate plants from coffee

protoplasts (Spiral and Pétiard, 1991) or contained no homones. Androgenetic reponses were measured by counting the number of divided microspores at day 19-20. Media replacement and osmotic pressure reduction occured at 3-week intervals. The determination of microspore developmental stages was performed using DAPI (4',6-diamidino-2-phenylindole), a DNA-specific stain. The viability of microspores was evaluated with fluorescein diacetate.

RESULTS

Manual microspore isolation from excised anthers proved to be superior to whole bud maceration, which requires the use of an antioxidant due to the rapid browning of bud tissue remnants. An early androgenetic reponse was marked by a 3- to 5-fold volume increase in 30-40% of the microspores. Up until day 15, a portion of them had been swelling more and had lost the round shape due to starch accumulation. They did not divide and slowly degraded, though some still remained after two months of culture. Five days after culture initiation the first bright microspores of medium size were observed, and within three more days, a few of them had divided equally or inequally. By day 8 the first multicellular structures containing more than four cells had been formed. By day 10, the number of bright microspores capable of division had increased. Further cell colonies were formed continuously and reached the highest density at day 19-20, when a few two- three- and four-cell-structures could still be found. The formed cell colonies contained about 64 cells and remained within the exine, which did not break open, preventing further development of the cell colonies. Media replacement combined with osmotic pressure reduction did not stop the subsequent degradation of the multicellular structures. Up until day 17-20, the same relative number of cell colonies occurred in MM with 6% or 12% sucrose. For a longer culture time (32 days) the higher initial osmotic pressure proved to be more favourable for cell colony maintenance. No hormones are needed until cell colony formation. BAP was detrimental to microspore division. Neither 2,4-D nor the combination of NAA, 2,4-D and kinetin added to the MM at culture initiation resulted in more divisions or in a stimulation of further development of cell colonies into calli and androgenetic embryos. The microspore method allows the rapid observation of effects caused by modifications in the MM or culture conditions. In addition, the oxidation of anther tissue can be avoided. This method represents the first step in an efficient, single haploid cell regeneration system which can be used for doubled haploid lines production and for genetic transformation, and which can already be used to test the androgenetic potential of various coffee species.

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Abstract

Mechanically isolated microspores of *C. arabica* were induced to undergo androgenesis resulting in haploid cell colony formation. The isolation was performed on anthers after excision from the buds precultured for two days and prior to culture initiation. Microspore cultures were initiated on a half strength Murashige and Skoog medium with 6-12% sucrose. Low sugar concentration and the addition of various growth regulators did not increase the number of responding microspores. The microspore developmental stage suitable for androgenesis proved to be the mid- till late uninucleate. An androgenetic response was early marked by a 3 to 5-fold volume increase of 40% of the microspores. Microspore division started at day 5. At day 12 to 15, the stationary microspore suspension was found to contain microspores with the completed first equal or unequal division as well as cell colonies resulting from further divisions of either the generative or the vegetative cell. This represents the first step to establish an efficient, single, haploid cell regeneration system for coffee which may be used for doubled haploid line production allowing the selection for recessive traits and the use of the large amount of genetic variation through meiotic recombination.

Résumé

Des microspores isolées de façon mécanique de *C. arabica* ont subi une induction androgénétique formant ainsi des colonies de cellules haploïdes. L'isolement a été réalisé sur des anthères isolées de boutons floraux ayant été cultivés deux jours avant l'initiation de la culture. Les cultures de microspores ont été initiées sur un milieu de Murashige et Skoog dilué de moitié contenant de 6 à 12% de saccharose. De faibles concentrations en sucre et l'addition de régulateurs de croissance variés n'ont pas amélioré le nombre de microspores réactives. Il a été montré que le stade de développement de la microspore le plus adéquat se situe entre les stades uninucléés médian et tardif. Une réponse androgénétique est marquée par une augmentation de volume de 3 à 5 fois pour 40% des microspores. La première division est observée au 5ème jour. Après 12 à 15 jours, la suspension stationnaire de microspores contient à la fois des cellules ayant complété leur première division, symétrique ou asymétrique selon le cas, ainsi que des colonies cellulaires résultant de divisions ultérieures de la cellule 'générative' ou 'végétative'. Cette recherche représente la première étape pour établir une régénération efficace à partir de cellules haploïdes de café, ceci pouvant être utilisé pour la production de lignées haploïdes-doublées permettant ainsi la sélection de caractères récessifs et l'utilisation d'une variabilité génétique étendue grâce à la recombinaison méiotique.

DIRECT SOMATIC EMBRYOGENESIS IS GENOTYPE SPECIFIC IN COFFEE

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Introduction

The coffee plant cycle (seed to seed) may take three years to complete, such an evaluation of yield potential from a progeny should require six years. Considering a minimum of six cycles in a selection program to achieve an acceptable homozygosity level, it may take more than 30 years for a breeder to release a new coffee variety. Coffea arabica is a tetraploid self-pollinated crop, the most cultivated species in Brasil (90 %), producing high coffee cup quality. The main breeding goals for this species are: (1) high yield, (2) nematode tolerance against Meloidogyne incognita, (3) disease resistance against the leaf rust (Hemileia vastratix), and (4) insect resistance against the leaf miner (Perileucoptera coffeella).

C. canephora is a diploid and self-incompatible species, being used as a source of resistance to the leaf rust and nematodes. This species is cultivated at warmer and low altitude areas and comprises about 10% of plantings in Brasil. Therefore, cloning C. canephora mother plants of high agronomic value is worthwhile to achieve uniform populations rapidly. LC 2258 is a high value mother plant because it is resistant to leaf rust and to nematodes, being used as a rootstock for C. arabica cultivars to achieve tolerance to M. incognita (Gonçalves et al, 1990).

Somatic embryogenesis, with "high and low frequency" was described in the seventies by Sondahl & Sharp (1977), Sondahl et al (1979) for coffee, using a system with a primary "conditioning medium" followed by a secondary "induction medium". Embryogenic callus was directly obtained by Yasuda et al (1985) from C. arabica leaf explants in the presence of belzyladenine, without passing by "conditioning medium". However, direct embryogenesis was described by Dublin (1981) as a primary event consisting of embryo formation without passing the callus phase. Embryos were also directly obtained by Hatanaka et al (1991) from leaf explants of C. canephora in the presence of BAP, 2-Ip or kinetin.

The process of direct embryogenesis is more interesting because it takes less time to regenerate plants. Dublin (1981) suggested this process to be less prone to somaclonal variation. Somaclonal variation can appear in calli regenerated plants, as reported for other species (Larkin and Scowcroft, 1981). Indirect embryogenesis may take eight months to produce plantlets, and the direct process could considerably reduce this time. In this paper we report that direct embryogenesis is affected by the plant genotype.

Materials and methods

Leaf explants from C. canephora LC 2258 C116 and from the interspecific hybrid H24601 (F_1) between C. arabica and C. canephora were used initially in the experiments. The fourth to second leaf pairs were harvested and washed in running water with commercial detergent, then disinfected with NaOCl 1.3 % for 30 minutes and rinsed three times with sterile water. The leaves were then incubated overnight in a sealed plastic box with sterile wet paper, being disinfected again as before. Mother plants from different genotypes (Tables 3, 3), showing field tolerance to the nematode M. incognita, were also evaluated for the capacity of direct embryogenesis.

Disinfected leaves were cut, with leaf main veins were removed and 1.0 cm^2 square explants were placed in medium plates with half MS salts, 650 uM of cysteine, 20 g/l of sucrose, 4.0 g/l of bacto agar, and pH 5.5. Five days later, the explants were evaluated for contamination and oxidation percentage. Healthy explants were placed in a medium for embryogenic induction (Sondahl et al. 1985), but hormones were zeatin, kinetin and 2-iP at 0, 10, 50, and 100 uM; and adenine at 0, 10 and 50 uM, using a factorial combination in a randomized complete block design with five replications. The experiments were installed in a room with temperature of 25^{0}C, photoperiod of 14 hours, illuminated by cool white fluorescent light with 4000 lux, and evaluated every 30 days, grading 0 for none and 4 when the explant borders were totally crowned with hundreds of embryos, otherwise indicated.

Results and discussion

Direct embryogenesis (DE) was observed as a white crown of embryos surrounding explant edges from 60 to 90 days after plating. Globular callus was observed at the cut explant borders four weeks after plating, but turning to hundreds of embryos later on. They were observed in leaf explants of C. canephora with higher frequency in the treatment zeatin 10 uM and kinetin 50 uM with adenine 50 Um (Table 1). However, embryos were not formed in vein cuts, but normal callus were formed instead. Direct formation of embryos was observed more consistently for the hybrid H24601 in the treatment zeatin 50 Um. Apparently there was a synergistic adenine effect with kinetin and 2-iP, but not for zeatin. Moreover, the embryo formation frequency was lower for H24601 than for C. canephora.

Coffee DE can be obtained in medium without auxins, but in the presence of cytokinins, as indicated by Dublin (1981). We did not succeed using his protocol and we did experiments (unpublished) using different varieties of C. arabica, C. canephora, variables as cytokinins and auxins, whole or half MS and combinations in basic medium MS. From those experiments, we noted that (1) auxins inhibited DE, as already observed by Dublin (1981); (2) it was necessary relatively high doses of cytokinins (10 uM or higher) to get DE, (3) there was a genotypic effect, as C. arabica leaf explants yielded very few direct embryos as compared to C. canephora. In those experiments, we observed embryos growing directly from the leaf explants of C. canephora between 60 and 90 days from plating. Embryos were also directly obtained from leaf explants of C. canephora in the presence of BAP, 2-iP or kinetin by Hatanaka et al (1991). Adenine was indicated to be a metabolic precursor of cytokinins (McGaw, 1988) and was reported by Dublin (1981) to affect embryogenesis in coffee. Our results did not clarify the relevance of adenine as affecting DE. However, adenine alone was not able to induce embryogenesis (Table 1), an indication that it was not used nor was metabolized to a functional cytokinin.

Sondahl and Sharp (1977) established a system for plant regeneration, by callus induction from leaf explants, then induction of embryos in a secondary medium. Sondahl et al (1984) later called their two kinds of secondary embryo formation events, "high frequency somatic embryo" (HFSE) as DE, and "low frequency somatic embryo" (LFSE) as indirect embryogenesis. Embryogenic callus was directly obtained by Yasuda et al (1985) from C. arabica leaf explants in the presence of belzyladenine, bypassing the inducting primary culture. DE was described by Dublin (1981) as a primary event consisting of embryo formation without passing the callus phase and without using auxins for callus induction.

Table 1. Number of replications with explants crowned with embryos, regenerated from leaves of Coffea after 120 days in culture.

		C. canephora				H24601			
		Adenine (uM)				Adenine (uM)			
Hormone	(uM)	0	10	50	Total	0	10	50	Total
Control		0	0	0	0	0	0	0	0
Kinetin	10	0	1	1	2	0	0	0	0
	50	2	1	3	6	0	0	0	0
	100	0	0	2	2	0	0	0	0
	Total	2	2	6	10	0	0	0	0
2iP	10	0	1	1	2	0	0	1	1
	50	1	2	1	4	0	1	0	1
	100	0	1	1	2	0	0	0	0
	Total	1	4	3	8	0	1	1	2
Zeatin	10	3	2	2	7	0	0	0	0
	50	1	1	1	3	1	1	1	3
	100	0	1	1	2	1	0	1	2
	Total	4	4	4	12	2	1	2	5
Total		7	10	13	30	2	2	3	7

Embryos were also directly obtained by Hatanaka et al (1991) from leaf explants of C. canephora in the presence of BAP, 2-iP or kinetin. We did not use the "conditioning medium", but only primary medium including zeatin as hormone, observing a kind of globular callus four weeks later, then a crown o embryos after eight to eleven weeks. Zygotic embryos can be macroscopically observed around 16 weeks after fertilization (Mendes, 1941; Mendes et al, 1954), having a size around of three millimeters. Thus, the somatic direct embryos are formed faster than normal zygotic embryos.

Seven mother plants, 2291 MI, 2264 MI, 2291 C66, 2258 C106, 2258 C212 AD, 2258 C55 and 2258 C44 with field tolerance to the nematode M. incognita were initially evaluated for the capacity of DE, using same conditions but hormone was only zeatin 10 uM. A strong plant genotype effect was observed, such explants from 2291 and 2264 mother plants yielded almost 100 % of DE, while those from 2258 C55 and C44 did not. The same experiment was repeated eight months later including five new mother plants of interest (Table 2), with 40 replications each. In the first evaluation 35 days after plating, results confirmed previous observations of globular like callus. A crown of white embryos was visually identified 60 days later. Results additionally did not indicated association among internal explant contamination, oxidation and DE, but explants from 2258 C106 oxidated in a highest proportion, as it occurred in the experiments with seven mother plants, an indication that explant oxidation was associated with specific plants. Evaluations at 92, 132, 153 and 190 days confirmed first observations.

Therefore, the genotypic effect was evident, and it is likely that there may be genes that favors DE, which can be induced by zeatin, or repressed by auxins. DE was reported also for strawberry (Nehra and Stuschnoff, 1989), indicating a strong variety effect. In this case auxin was used along with cytokinin, without inhibiting effects. Thus, the auxin cytokinin balance model may work differently for different species.

Nematode tolerant mother plants of commercial interest could be micropropagated via DE to nematode infested areas from the western region of the São Paulo State and other regions of Brasil, where coffee was eradicated due the presence of M. incognita. Besides, clones could be produced to be ideally evaluated for tolerance to races of M. incognita. The tolerance evaluation would be accurate if clones are used instead of heterozygous coffee seedlings, as it is being done. Being C. canephora genetically self-incompatible, producing progenies 100 % heterozygous, the identification of different and compatible nematode tolerant mother plants should allow the formation of clonal nurseries to produce tolerant hybrid plants.

Table 2. Leaf explants from mother plants of C. canephora, showing contamination, oxidation and percentage of explants graded with direct embryogenesis at 10 and 19 weeks after plating.

Mother plants	Contam.	Oxid.	Grades[a,b] 1	2	3	4	Grades[a,c] 1	2	3	4
	%	%	%	%	%	%	%	%	%	%
2291 MI a	17.5	3.0	6.1	15.1	78.8	0.0	3.1	0.0	90.6	6.2
" " b	27.5	65.5	48.3	41.4	10.3	0.0	18.0	18.0	64.0	0.0
2264 MI	10.0	0.0	0.0	13.9	86.1	0.0	0.0	3.0	94.0	3.0
2291 C66 AD	15.0	8.8	11.8	64.7	23.5	0.0	0.0	34.0	66.0	0.0
2258 C55 a	42.5	4.3	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
" " b	40.0	0.0	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
2258 C106	10.0	94.4	100.0	0.0	0.0	0.0	91.0	9.0	0.0	0.0
2258 C126 a	17.5	39.4	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
" " b	2.5	20.5	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
2258 C212 a	7.5	0.0	100.0	0.0	0.0	0.0	73.0	27.0	0.0	0.0
" " b	0.0	0.0	100.0	0.0	0.0	0.0	31.0	64.0	5.0	0.0
2258 C44	0.0	0.0	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0
Average	15.8	19.5								

a: Maximum and no DE, grades 4 and 1, respectively; b: ten weeks; c: 19 weeks

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Summary

Direct somatic embryogenesis was evaluated in coffee from 12 mother plants of Coffea canephora and from an interspecific hybrid (F1) between C. arabica and C. canephora. C. canephora is self-incompatible and diploid, whereas C. arabica is self-compatible and tetraploid. The former is a source of nematode and leaf rust resistance, being also used as rootstock for C. arabica cultivars as a mean of nematode control. Direct somatic embryogenesis could be used as a rapid and convenient method to micropropagate agronomically valuable plants of C. canephora.

Results of experiments indicated that direct embryogenesis was genotype specific and was achieved by using Zeatin 10 uM, the best treatment for C. canephora. No association was observed among embryogenesis rate, oxidation and the level of contamination. Low frequency of direct embryogenesis was observed for the hybrid, and the highest for mother plants of C. canephora, but eight plants were not responsive. Some of those mother plants associate nematode and rust resistance, being of high value for rootstock micropropagation and cropping in nematode infested areas.

ADENINE METABOLISM AND THE BIOSYNTHESIS OF THEOBROMINE AND CAFFEINE IN DEVELOPING LEAVES OF COFFEE PLANTS

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INTRODUCTION

In tea and coffee plants, there are several lines of evidence to indicate that purine alkaloids, namely caffeine and theobromine, are synthesized from purine nucleotides via xanthosine [1]. However, only a few investigations have been carried out to examine the relationship between purine metabolism and the biosynthesis of these purine alkaloids, and most of such studies have been performed with tea plants [2-5]. In the present study, we first determined the levels of purine alkaloids and then we examined the overall metabolism of [8-^{14}C]adenine in young, freshly emerged coffee leaves at different stages of development.

MATERIALS AND METHODS

Plant materials — Young leaves from 1-year-old coffee plants (*Coffea arabica* L.), obtained from the Takii Seeds Company, Tokyo, were used for the experiments. Positions of leaves used are shown in Figure 1.

Analysis of purine alkaloids — Analysis of purine alkaloids was performed by HPLC as described in our previous paper [2].

Administration of labelled compounds — Segments of surface-sterilized coffee leaves (100 mg fr. wt.) were incubated in 2 ml of sterilized medium which was composed of 30 mM potassium phosphate buffer (pH 5.6), 10 mM sucrose and 4.5 μM [8-^{14}C]adenine (sp. act. 2.04 MBq/μmol) in a 30 ml-Erlenmeyer flask with a centre well that contained 0.1 ml of 20% KOH. After 4 and 24 hrs of incubation on a horizontal rotary shaker at 27° C, 20 μl of 6% perchloric acid were injected into the main compartment of each flask and incubation continued for a further 5 min to complete elimination of $^{14}CO_2$ from the medium. The leaf segments were washed with water and were immediately frozen with liquid nitrogen and used for analysis of labelled metabolites.

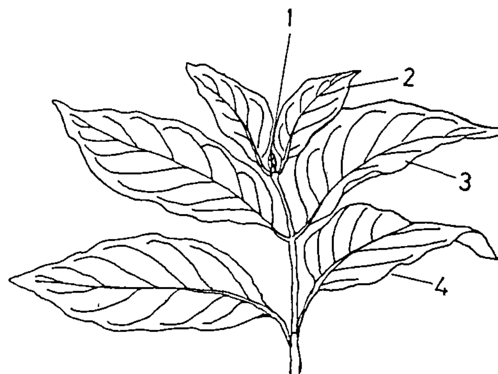

Fig. 1. Designation of leaves of coffee *(Coffea arabica L.)* plants.

Analysis of labelled metabolites — Extraction and fractionation of the perchloric acid-soluble compounds and nucleic acids were performed as described in our previous papers[2, 6]. The perchloric acid-soluble compounds were fractionated by TLC on cellulose plates, and autoradiograms were made by exposing plates to X-ray film for approximately 3 weeks. Labelled compounds were identified by co-chromatography with standard unlabelled compounds.

RESULTS

Figure 2 shows the levels of theobromine and caffeine in leaves 1 and 2. For comparison, leaves of these compounds in similar young leaves of tea plants (leaf 1, first leaf from the shoot apex; leaf 2, second leaf from the apex) are also shown in the Figure. In both coffee and tea leaves, theobromine and caffeine were found but no theophylline was detected. The level of theobromine was slightly higher than that of caffeine in leaf 1 of coffee plants. By contrast, the level of caffeine was higher than that of theobromine in leaf 2 of coffee plants and in both leaves 1 and 2 of tea plants. In tea seedlings, theobromine was observed only in young leaves and it was not detected in aged leaves [2].

Metabolism of [8-^{14}C]adenine by leaves 1 and 2 of coffee plants is shown in Figure 1. In leaf 1, about half of the total radioactvity that was taken up by the leaf segments was located in salvage products, namely nucleotides and nucleic acids, 4 hrs after the administration of the labelled compound.

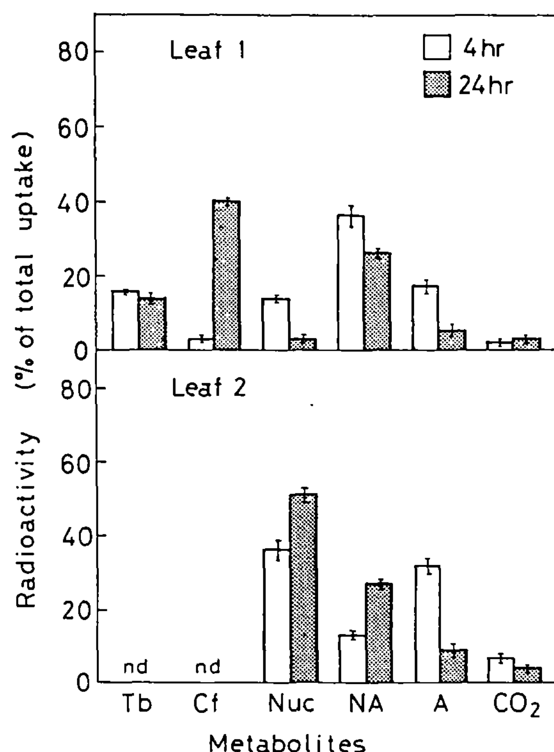

Fig. 3. Metabolism of [8-^{14}C]adenine in the leaf 1 and leaf 2 of coffee plants. nd, not detected. Small amounts (9-12% of total uptake) of radioactivity were located in five unidentified spots, as visualized by autoradiography.

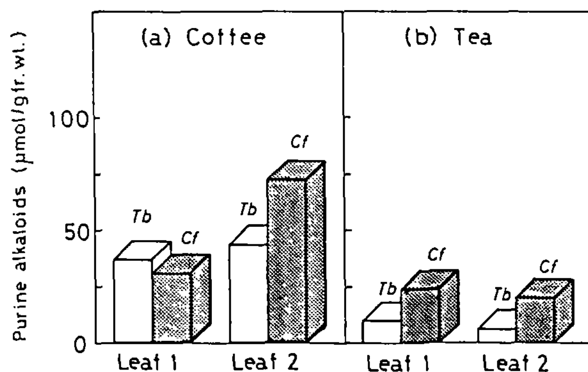

Fig. 2. Levels of theobromine and caffeine in the leaf 1 and leaf 2 from 1-year-old coffee plants (a) and 4-month-old tea plants (b). No theophylline was detected.

Theobromine (16%) and caffeine (3%) were also labelled during this period. After 24 hrs, more than half of the radioactivity was distributed in purine alkaloids, namely caffeine (40%) and theobromine (14%). The radioactivity in nucleotides decreased during the periods from 4 to 24 hrs. Only 2% (4 hr) and 3% (24 hr) of the total radioactivity was released as $^{14}CO_2$. By contrast, in leaf 2, no incorporation of radioactivity from [8-^{14}C]adenine into theobromine or caffeine was found during the 24 hr that followed administration of the labelled precursor (Fig. 3). Most of the radioactivity was retained in nucleotides and nucleic acids. These compounds contained 49% and 78% of total radioactivity at 4 and 24 hrs after administration of the precoursor, respec-tiv ely. It is noteworthy that the rate of release of $^{14}CO_2$ from [8-^{14}C]adenine was low in leaf 2 just as it was in leaf 1.

DISCUSSION

In higher plants, almost all exogenously supplied adenine and adenosine is salvaged by adenine phosphoribosyltransferase and adenosine kinase, and AMP is formed [7]. As a result, we can easily follow the metabolic fate of adenine nucleotides that have been prelabelled with [8-^{14}C]adenine or adenosine.

In plants, catabolism of AMP is initiated by the reaction catalyzed by AMP deaminase, and the purine ring is completely degraded by the conventional catabolic pathway, via allantoin and allantoate, with CO_2 and NH_3 being produced as the final products of degradation [7]. In addition to the catabolic pathways, several plants including coffee plants, utilize xanthosine, an intermediate in the catabolism of purine nucleotides, for the synthesis of purine alkaloids [1].

Although evience for the details of the pathway from AMP to xanthosine has not been obtained, our present results clearly demonstrate the conversion of adenine to caffeine in young coffee leaves. We did not detected the incorporation of radioactivity into any intermediates in the pathway from AMP to xanthosine. However, this failture is also probably a result of the small size of the pools of these metabolites.

The results of the present study also indicate that the biosynthesis of caffeine from adenine occurs only at the very early stages of leaf development. These results are consistent with the findings that the level of caffeine increased at the very early phases of development of coffee leaves, reaching a maximum when the leaves were fully opened [8]. Similar age-dependent biosynthesis of caffeine has been reported in fruits of coffee [9], and in the leaves [2, 3,5], flowers [4] and fruits [10] of tea plants and leaves of mate plants [11]. In opened leaves (leaf 2) of coffee plants, the capacity for the biosynthesis of caffeine was almost completely absent. One of the most probable limiting factors in the synthesis of caffeine is the activity of *N*-methyltransferases. In tea leaves, strict seasonal variations in the activities of these enzymes have been observed [5].

The physiological significance of the biosynthesis of caffeine in very young leaves of coffee plants is unclear. Friscknacht et al. [8] proposed that the synthesis of caffeine is aimed at the prevention of predation by animals.

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SUMMARY

Concentrations of theobromine and caffeine were 38 and 32 μmol/g. fr.wt., in the first leaves (leaf 1), and 45 and 74 μmol/g fr. wt. in the second leaves (leaf 2), respectively. No theophylline was detected in leaves at either position. [8-^{14}C]Adenine taken upby segments of leaf 1 and leaf 2 was converted to nucleotides and nucleic acids. However, conversion of [8-^{14}C]adenine to theobromine and caffeine was found only in leaf 1. More than half (54%) of the radioactivity taken up by leaf 1 was distributed among purine alkaloids. The extent of degradation of adenine nucleotides by the conventional purine catabolic pathway was low in both leaf 1 and leaf 2.

7-GLUCOSYLADENINE, A NEW ADENINE METABOLITE IN COFFEE CELL SUSPENSION CULTURES

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Introduction

When suspension-cultured cells of *C. arabica* are fed with [^{14}C] adenine, most of the radioactivity is incorporated into purine alkaloids (7-methylxanthine, theobromine, caffeine), into xanthosine and, depending on the exogenous adenine concentration, into a new adenine derivative. The synthesis of the latter is induced by adenine. We isolated this substance from coffee cells fed with 1 mM adenine. After crystallization, it was identified by X-ray analysis as 7-D-glucopyranosyladenine. For metabolic studies [8-^{14}C] 7-glucosyladenine was prepared by in vivo synthesis from [8-^{14}C] adenine.

Experimental

Suspension-cultured cells

The cell line used had been established according to [1] from primary callus cultures of *Coffea arabica*. The suspension cultures were cultivated in a commercially available Murashige-Skoog (M&S) medium (4.71 g/L; Flow Laboratories) supplemented with 30 g/L sucrose, 10 mg/L L-cysteine, 1 mg/L thiamine, 1 mg/L 2,4-D and 0.2 mg/L kinetin. The pH was adjusted to 5.7-5.8 with 1N KOH. Cells were subcultured (10 g fresh wt / 60 ml medium) every 2 weeks and kept in 250 ml Erlenmeyer flasks in the dark at 27°C on a gyratory shaker at 90 rpm.

Isolation of 7-glucosyladenine

Cells (135 g) in stationary phase were added to 135 ml M&S medium containing 2 mM adenine (to give an average adenine concn of 1 mM) and cultured for 5 days. Then the cells (200 g fresh wt), harvested by sieving, were mixed in a centrifuge tube with HCl (8.62 µl concd HCl/g cells to give a final concn of about 0.1 N HCl), frozen and thawed up, and then extracted by sonication at RT for 30 min. Cell residues were removed by centrifugation at 25'000 g. The resulting supernatant (about 150 ml) was purified in two separate runs using a cation exchange column (Dowex 50 W X8 50/100, H^+ form; 1.5 x 16 cm). After application of 75 ml, the column was rinsed with 150 ml water. Elution was done with 300 ml 1 N NH_4OH. After solvent evaporation, the residue was dissolved in 2 ml water and subjected to preparative HPLC (ODS Hypersil, 5µm,

column 20 x 250 mm and precolumn 20 x 50 mm) with 13.5 ml/min 3% methanol. The UV detector was set at 254 nm and the fractions containing the glucoside (RT 22.3 min) of two runs were combined and dried. The co-eluting cis-diols guanosine and inosine were removed by binding them to a boronate gel column (Affi-Gel® 601, Bio-Rad Laboratories) at pH 8.8. Therefore, the residue was dissolved in 2 ml starting buffer (0.25 M NH_4Ac, pH 8.8) and applied onto the column (4 ml bed vol) previously equilibrated with buffer. The effluent containing the non-binding 7-glucosyladenine was collected during elution with 20 ml buffer. Most of the buffer could be evaporated under reduced pressure, however, to remove all of it, rechromatography on the system mentioned above was necessary, but this time with 5 % MeOH as solvent. Analytical chromatography revealed an impurity with the absorption maximum at 210 nm still present in the preparation. Therefore, the residue obtained after the second preparative chromatography was dissolved in as little hot water as possible. After a few days colorless needles crystallized. They were collected and washed several times with cold MeOH. From 200 g cells (fresh wt) 5-10 mg 7-glucosyladenine could be isolated.

In vivo synthesis of [8-^{14}C] glucosyladenine
400 µl conditioned medium was mixed with 100 µl [8-^{14}C] adenine (22 µCi, 49 µCi/µmol). Then 1.5 g cells were added to give a final adenine concn of about 224 µM. After 24 h the cells were extracted by freezing, thawing up and sonicating for 10 min at 4°C. After removing cell residues by centrifugation at 12'000 g (4°C) for 3 min the extract was centrifuged through a filter (Ultrafree-MC, 10'000 NMWL; Millipore). 1 ml of this cryosap was mixed with 1 ml 0.25 M NH_4Ac, pH 8.8 and purified on the boronate column (see above). The run through containing the glucoside was lyophilized and dissolved in 200 µl H_2O. Purification was done by analytical HPLC and multiple injections (25 µl). The fractions containing 7-glucosyladenine were combined and the solvent was evaporated. 7-Glucosyladenine was redissolved in 1 ml H_2O. HPLC combined with on-line radioactivity measurement (see below) showed that 2.25 µCi of [8-^{14}C] 7-glucosyladenine were isolated with a specific activity of about 50 µCi/µmol.

Analytical HPLC was performed on a Nucleosil C18 column (5 µm, 250 x 4 mm, precolumn 4 x 4 mm) with 50 mM ammonium phosphate, pH 3.8 [A] and MeOH/MeCN (1:1) [B] at a total flow of 1 ml/min and by the following gradient (% B in A): 0-5 min (0), 5-14 min (0-3), 14-25 min (3-20), 25-30 (20). Parameters were controlled by a Hewlett Packard liquid chromatograph HP 1090 equipped with diode array detector set at 254 nm. Column temperature was 40°C and injection volume 25 µl.

Radioactivity was monitored on-line by a liquid radioactivity detector (Flo-One / Beta, A-200; Canberra Packard) after HPLC separation and mixing with 1 ml/min scintillation cocktail (Pico-Aqua, Canberra Packard).

Crystallographic measurements were performed on a Rigaku AFC5R diffractometer with graphite-monochromated MoK_α radiation and a 12 kW rotating anode generator.

Results

Colourless needle crystals having approximate dimensions of 0.05 x 0.12 x 0.40 mm were obtained from a solution in water. The X-ray diffraction analysis has established the structure of 7-D-glucopyranosyladenine ($C_{11}H_{15}N_5O_5 \cdot H_2O$, formula weight 315.29) shown in Fig. 1. The crystals are enantiomerically pure (optical active), however the absolute configuration of the molecule has not yet been determined. The crystal lattice also contains water as solvent molecule in a 1:1 ratio with the main compound. There is a complex 3-dimensional network of intra- and intermolecular hydrogen bonds (see Fig. 2). For more details see X-ray structure report.

7-Glucosyladenine is chemically stable in acidic (0.1 N HCl) as well as in basic (0.1 N NaOH) solutions. λ max in water is at 270 nm (ε 10'960), in 0.1 N NaOH at 270 nm (ε 11'040) and in 0.1 N HCl at 272 nm (ε 15'840) as shown in Fig 3. Hydrolysis into adenine and glucose is achieved by boiling for 1 h in 1 N HCl.

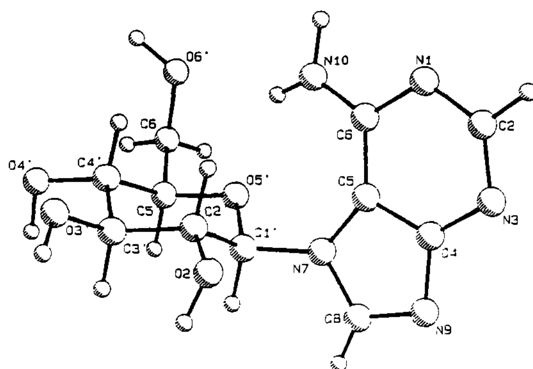

Fig. 1
Molecular structure of 7-glucosyladenine.

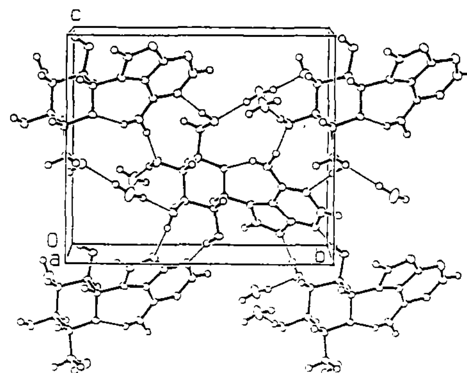

Fig. 2
Packing plot of 7-glucosyladenine.

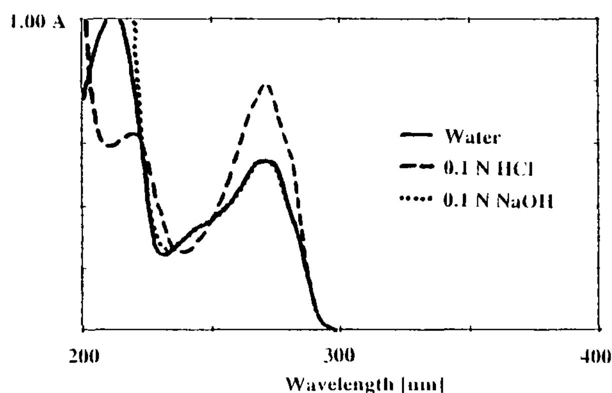

Fig. 3
UV spectra of 7-glucosyladenine at different pH.

N-7-glucosides of purines are particularly unusual. They are known from the cytokinin metabolism. Exogenous zeatin and BAP are inactivated by conversion to 7- and 9-β-glucopyranosides [2]. 7-glucosyladenine was first described in radish cotyledons by Tao et al. [3]. After incubation with 5 mM [^{3}H] zeatinriboside they detected, in addition to the principal metabolite 7-D-β-glucopyranosylzeatin, significant radioactivity in a unknown substance. It was identified as 7-D-β-glucopyranosyladenine by cochromatography and cocrystallization with authentic 7-β-D-glucopyranosyladenine prepared according to Cowley et al. [4]. Unlabeled glucosyladenine was prepared by supplying a high concentration of adenine to excised radish cotyledons.

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Summary
A novel compound was isolated from suspension-cultured cells of *C. arabica* growing in the presence of 1 mM adenine and was identified as 7-glucopyranosyladenine. The high specific activity of the isolated 7-glucosyladenine after feeding [8-^{14}C] adenine signifies a very close metabolic relation to the precursor adenine and points to a small or absent glucoside pool. Preliminary results indicate that 7-glucosyladenine is a salvage form of exogenous adenine.

TOWARD THE CONSTRUCTION OF A GENETIC MAP IN COFFEE

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Construction of a genetic map of the coffee genome based on molecular markers has been initiated. First efforts are focused on *Coffea canephora* which presents the advantages of being a diploid and highly polymorphic species. The generated information will be extended to other species including the allotetraploid species *C. arabica*.

The segregating population selected for linkage analysis consists of 100 doubled haploid genotypes (DH) derived from the heterozygous clone IF 200. DH were developed using haploid embryos occurring spontaneously in association with polyembryony (Couturon and Berthaud, 1982), and are characterised by a strict homozygoty which leads to considerable advantages for linkage analysis and agronomic evaluation.

The DH population is being evaluated for agronomic and technological characteristics (adaptation to industrial processing, quality). To overcome the important inbreeding depression exhibited by the DH genotypes, most part of the evaluation is carry out on top-crosses involving the different DH genotypes and a common homozygous parent tester (HD 160-02). Important traits such as yield, susceptibility to leaf rust, bean size and fruit maturation have been scored ; first observations showed large genetic variability within the DH population (Lashermes *et al.*, 1993a).

Both restriction fragment length polymorphism analysis (RFLP) and random amplified polymorphic DNA assay (RAPD) are performed in order to find polymorphic DNA markers. For RFLP analysis, genomic and cDNA clones originated from arabusta libraries (Duchateau and Paillard, 1993) as well as short repeat oligonucleotides (microsatellite DNA) are used as probe. RAPD involves primers constituted by arbitrary decamer oligonucleotides (Lashermes *et al.*, 1993b).

A large screening of genomic probes and primers for detection of polymorphism within the DH population, and between IF 200 (parental clone of DH's) and DH 160-02 (homozygous parent tester) is being done. A considerable polymorphism has been observed. Concerning RAPD, 40 % of the tested primers detected polymorphism between IF 200 and DH 160-02, and 11 % within the DH population. Segregations of

polymorphic markers observed on a large number of DH genotypes were in agreement with mendelian expectations.

The development of this genetic linkage map will bring important informations on coffee genome and chromosomal organisation, and may open up interesting aspects of crop evolution. High density DNA marker map offers the most direct approach for genetic analysis of quantitative traits, and may eventually form the basis for identifying and cloning genes of agronomic interest.

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SCREENING OF ARABICA COFFEE COLLECTIONS FOR BEBEKA ENVIRONMENT

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Abstract

Two hundred eight coffee (Coffea arabica L.) collections of four groups were evaluated for yield and some growth characters. Considerable differences were observed between collections and between years in most groups. Year by collection interactions were also significant. Yield distributions were skewed to the higher classes in most groups. The average yield of the top selections from group I, II, III and IV were respectively 16.9, 5.0, 9.3 and 10.8 Qts of clean coffee/ha and all together 16 selections have been found yielding 10 to 19 Qts/ha. The genetic advances through selection were 0.9, 1.5, 2.2 and 2.1 Qts/ha while the broad sense heritabilities were 19, 76, 50 and 45 percent for group I, II, III and IV, respectively.

The top selections exhibited good growth performance. The correlation between yield and the growth characters were highly significant. Furthermore, in this study, selection for a particular locality appeared to be more successful if the search is largely focused on collections from vicinities or other areas having similar environment to the area in question.

STUDIES ON THE INHERITANCE OF THE S_H3 RESISTANCE FACTOR TO COFFEE LEAF RUST

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INTRODUCTION

Generally S_H genes are dominant and condition total susceptibility to compatible races and specific resistance to incompatible races (Rodrigues et al, 1975). Thus a plant with S_H3 factor would be susceptible to virulence
factor v3. Virulence of compatible races to S_H3 has been reported as complex, both in the field and in the laboratory (Kushallapa and Eskes, 1989).

This may have been responsible for the effective field resistance of varieties possessing the gene in India, before evolution of more virulent races. The present study envisages elucidating the heritability of the S_H3 resistance factor.

MATERIALS AND METHODS

Parents and F2 hybrids derived from reciprocal crosses between S1934 (S_H3, S_H2?, S_H5) and AB3 (S_H5) were screened with race II (v5) and isolate H1, attacking S1934 in the island of Java.
Previous screening with race I (v2,v5) indicated that the SH2 factor is absent in the F2 and thus in S1934. Therefore, the consitution of S1934 would be SH3,SH5.

Leaf disks (2cm diameter) were made from 169 hybrid plants and from 10 plants each from Caturra, S1934 and AB3 and inoculated with 10µl droplets of *Hemileia vastatrix* spore suspension with 900 to 1200 spores/droplet (Leguizamon, 1985). Leaf disks were incubated in obscurity for 48 hours, then placed in climatic chamber (12 hrs photoperiod, 24 - 26 °C) for resistance studies.

Latency Period (LP) was recorded as the duration taken for 50% of the infected sites to sporulate. Reaction Type was assessed using the 0 to 9 scale devised by Eskes and Toma-Braghini (1981). These parameters are widely used for assessment of disease resistance (Eskes, 1983; IRRI, 1979).

RESULTS AND DISCUSSION

The segregation of Reaction Type was not affected by the reciprocal cross (ie. S1934 x AB3 or AB3 x S1934): A 3:1 segregation was observed both ways thus the single analysis of all hybrids was possible.

S1934 is a derivative of Kents and S26 and thus it may possess the genes S_H2 and S_H3 inherited from the respective ancestors. AB3 and S1934 both possess the S_H5 gene in a homozygote state. All plants susceptible to race II were also susceptible to isolate H1. However variable Reaction Types to H1 were observed in F2 plants resistant to race II (Table 1). Thus virulence of H1 is more complex than can be explained by one resistance gene.

Latency Period was 21 days for both the control varieties and F2 hybrids susceptible to race II (Table 2). A slight but negligeable variation was observed within each test for susceptible plants.

Plants with intermediate resistance showed longer Latency Period.

The observation of variable Reaction Types can best be explained from two hypotheses:

FIRST HYPOTHESIS: based on classifying reaction types into three groups:

VR/R : Very Resistant to Resistant (0 to 2)
MR/MS: Moderately Resistant/Susceptible (3 to 8)
S: Susceptible (9).

It gives a repartition of: 43 VR/R : 87 MR/MS : 39 S,
which follows a 1:2:1 segregation. Thus the S_H3 factor is partially dominant in relation to H1.

SECOND HYPOTHESIS: by classifying Reaction Types into 4 categories:

VR (0, 1) : MR/R (2 to 5) : MS (6 to 8) : S (9).

Under these categories F2 hybrids are repartitioned as follows:

27 VR : 68 MR/R : 35 MS : 39 S

Repartition of VR:MR/R:MS is 1:2:1 thus suggesting segregation for a single partially dominant factor interacting with the S_H3 factor.
Dominant stage of this factor (modifying gene) would induce VR (very resistant) reactions to the v3 gene. The heterozygote and the homozygote recessive genotype of this factor induce R to MR and MS reactions respectively. Plants fully susceptible to H1 lack this gene.

Table 1: Reaction Types observed in F2 population (169 plants) inoculated with race II and isolate H1.

RACE/ ISOLATE	REACTION TYPE									
	0	1	2	3	4	5	6	7	8	9
II	129									40
H1	14	13	16	12	29	11	7	13	15	39
1ST HYP.	43			87						39
2ND HYP.	27		68				35			39

0 = NO INFECTION, ... 9 = ABUNDANT SPORULATION

Table 2: Comparison of Latency Period (LP) between reaction types in F2 descendance.

REACTION TYPE	RACE II			ISOLATE H1		
	MEAN	S.D.	C.V.	MEAN	S.D.	C.V.
4, 5	-	-	-	33.6	6.01	18 %
6, 7	-	-	-	26.6	6.01	23 %
8, 9	21.9	3.36	15 %	21.7	5.01	23 %
9 (*)	19.0	0.53	3 %	19.0	1.03	5 %
9 (**)	21.3	2.41	11 %	19.3	1.50	8 %

MEAN: Days S.D.: Standard Deviation C.V.: Coefficient of Variation
CONTROLS: (*) CATUAI (**) AB 3

CONCLUSION

After studying segregation of the S_H3 factor in F2 hybrids, two hypotheses have been advanced to explain the variable Reaction Types usually observed due to S_H3. In both cases the SH3 resistance seems more complex than can be explained by one dominant gene. This complex nature may be related to the relative durability of this resistance under field conditions.

This study was conducted in CIRAD laboratory, Montpellier, France, in 1991.

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ÉVALUATION D'HYBRIDES DE *COFFEA ARABICA* L. EN VUE D'OBTENIR DES VARIÉTÉS RÉSISTANTES À LA ROUILLE ORANGÉE ET À L'ANTHRACNOSE DES BAIES

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INTRODUCTION

Deux maladies fongiques graves affectent la caféiculture: la rouille orangée dans la plupart des grands pays producteurs et l'anthracnose des baies qui sévit essentiellement dans les pays africains.

Cette dernière maladie peut entraîner des pertes de récolte de 80%. Les traitements chimiques se révélant à la fois coûteux et d'une efficacité limitée, la recherche de variétés résistantes semble opportune.

MATERIEL ET METHODES

Les stations de recherche de l'IRA du Cameroun sont riches de plusieurs collections de caféiers comprenant entre autres les variétés prospectées en Ethiopie par la mission ORSTOM/IRCC de 1966, et des variétés exploitées commercialement. Des séries d'observations ont été menées afin d'évaluer les caractéristiques agronomiques et les niveaux de résistance de ces différentes variétés vis-à-vis des pathogènes.

Au sein de ces populations, on a pu sélectionner un certain nombre d'individus possédant des capacités de production équivalentes à celles des variétés commerciales et/ou des niveaux de résistance intéressants. (Tableau 1)

Un programme d'hybridations a été réalisé afin de créer des variétés regroupant les caractéristiques favorables de chacun des parents, et a conduit à la mise en place de deux essais d'hybrides à la station de recherche de Foumbot:

-un essai d'hybrides diallèle triangulaire à 7 géniteurs avec autofécondation

-un essai d'hybrides divers

Tableau 1: **CRITERES DE SELECTION DES PARENTS**

Variétés	**Productivité**	**résistance HV**	**résistance CBD**
Ja	*	*	*
Ca	*		
Bo2	*	*	
Dg10			*
Il3	*		*
Kf9			*
Et1	*	*	*
Et3			*
Et5		*	
Et7	*	*	*
Et13		*	*
Et29		*	*
Et30	*	*	*
Et33	*	*	*
Et41	*	*	*
Et56		*	*
Et59	*		*

RESULTATS

Les productions individuelles ont été contrôlées pendant 4 années consécutives.

Dans le cas de l'hybride diallèle avec autofécondation, comprenant 2 variétés commerciales (Caturra et Java), et 5 variétés non commerciales (Dg 10, Il 3, Et 3, Et 7, Et 56), on constate une certaine vigueur hybride (Tableau 2):

-hybrides entre variétés non commerciales:
   3 hybrides sont significativement supérieurs à chacun de leurs parents.

-hybrides entre variétés non commerciales et Caturra:
   tous les hybrides sont supérieurs à chacun des parents

-hybrides entre variétés non commerciales et Java:
   aucun des hybrides n'est supérieur à chacun des parents

Le meilleur hybride a produit plus de 250% du Caturra

Dans le cas de l'essai d'hybrides divers, 14 hybrides ont eu une production significativement supérieure à celle du Java.

Les observations réalisées lors de fortes attaques de rouille, ont montré que certains hybrides (Ca x Et 3, Ca x Il 3) ont été très peu atteints alors que le Caturra l'a été gravement.
La situation de moyenne altitude (1000 m) de la station n'a pas permis d'observer d'attaques d'anthracnose conséquentes et de constater les types de réaction *in-situ* des caféiers.

Tableau 2:

variétés et hybrides	kg md/ha:résultats non significatifs	kgmd/ha:résultats significatifs
Ca x Dg 10		>Ca ,>Dg 10
Ca x Il 3		>Ca ,>Il 3
Ca x Et 3		>Ca ,>Et 3
Ca x Et 7		>Ca ,>Et 7
Ca x Et 56		>Ca ,>Et 56
Ja x Dg 10	<Ja	>Dg 10
Ja x Il 3	<Ja ,>Il 3	
Ja x Et 3	>Ja	>Et 3
Ja x Et 7	=Ja	>Et 7
Ja x Et 56	<Ja ,>Et 56	
Dg 10 x Il 3	>Dg 10,>Il 3	
Dg 10 x Et 56	<Dg 10,<Et 56	
Il 3 x Et 56	>Il 3 ,>Et 56	
Et 3 x Dg 10		>Et 3,>Dg 10
Et 3 x Il 3	>Et 3 ,>Il 3	
Et 3 x Et 7		>Et 3,>Et 7
Et 3 x Et 56		>Et 3,>Et 56
Et 7 x Dg 10	>Et 7 ,>Dg 10	
Et 7 x Il 3	>Et 7 ,<Il 3	
Et 7 x Et 56	>Et 7 ,>Et 56	

CONCLUSIONS ET PERSPECTIVES

L'analyse des résultats de production a permis la sélection d'un certain nombre d'hybrides.

Dans le cadre des divers projets de collaboration (IRA, CIRAD, CIFC) des tests en laboratoire sont entrepris afin de déterminer les valeurs réelles des hybrides vis-à-vis des agents pathogènes.

La multiplication végétative *in-vitro* des hybrides prometteurs est en cours pour la mise en place de parcelles d'essais de comportement.

RESUME:

Les stations de l'Institut de Recherche Agronomique (IRA) du Cameroun possèdent plusieurs collections comprenant environ 350 introductions de caféiers arabica issues de diverses prospections (ORSTOM/IRCC,...).

Plusieurs années d'observations ont permis de repérer des variétés présentant des bons niveaux de résistance aux deux maladies fongiques principales affectant les caféiers: la rouille orangée et l'anthracnose des baies.

Un programme d'hybridation a été lancé afin d'obtenir des plantes possédant à la fois de bons caractères agronomiques (productivité, architecture, adaptabilité, résistance à la sécheresse) et des niveaux de résistance satisfaisants vis-à-vis des agents pathogènes.

Deux essais d'hybrides ont été mis en place en 1986 à la station de recherche IRA de Foumbot en zone de moyenne altitude (1000 m). Il s'agit:
- d'une part d'un essai d'hybrides diallèle triangulaire à 7 géniteurs avec autofécondation,
-d'autre part d'un essai d'hybrides divers.

Des notes ont été données à la suite d'observations visuelles (taux de croissance, architecture, production individuelle, vigueur, ...), et les récoltes ont été mesurées pendant 4 années consécutives.

L'analyse des résultats a montré que certains hybrides possèdent des caractéristiques agronomiques intéressantes et des potentialités de production supérieures à celles des variétés commerciales.

Des tests précoces de résistance à l'anthracnose des baies sur les descendances d'hybrides sont en cours.

ABSTRACT:

The stations of the Institute of Agronomic Research in Cameroon (IRA) have several collections comprising approximately 350 arabica coffee varieties originating and collected from various explorations (ORSTOM/IRCC,...).

Many years of observations have revealed certain varieties with a high level of resistance to the two principal fungal diseases of coffee plants: coffee leaf rust and coffee berry disease.

A breeding program was launched in order to obtain plants which show positive agronomic characteristics (productivity, architecture, adaptability, resistance to drought), and at the same time, satisfactory levels of resistance to the pathogen agents.

Two experimental plots were set up in 1986 at the IRA station in Foumbot, which is located in a mid altitude zone (ie 1000 m). They comprise:
- in the first part an incomplete diallel cross of 7 parents with self-pollination,
-and in the second part, an experiment with various different hybrids.

Marks were given after visual observations (growth rates, architecture, individual yields, vigour,..), and the harvests were measured for 4 years.

The analysis of the results shows that certain hybrids posses interesting agronomic characteristics and production potentials superior to existing commercial varieties.

Early testing for resistance to coffee berry disease on the hybrids' descendants is ongoing.

RÉSISTANCE DE *COFFEA* SPP. À LA MINEUSE DES FEUILLES DU CAFÉIER, *PERILEUCOPTERA COFFEELLA* (GUÉRIN-MÉNEVILLE, 1842) (*LEPIDOPTERA - LYONETIIDAE*)

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1. INTRODUCTION

La chenille mineuse des feuilles du caféier est un des plus importants ravageurs de **Coffea arabica** dans les principaux pays producteurs spécialement au Brésil, dans certaines parties de l'Amérique Centrale et en Afrique de l'Est (PARRA, 1985; ARANDA DELGADO, 1986; WAIKWA & MATHENGE, 1977). Les pertes de production sont souvent très élevés (ALMEIDA, 1973) et sont dues à la diminution de la photosynthèse (MAGALHAES, 1964) en raison de la chute prématurée des feuilles (CROWE, 1964; WALKER & QUINTANA, 1969).

La lutte chimique est très efficace, mais couteuse. Toutes les variétés de **Coffea arabica** sont sensibles à cet insecte (MEDINA FILHO et al., 1977); par contre, différents niveaux de résistance ont été déjà vérifiés chez quelques espèces de **Coffea.** VICENTE-CHANDLER et al., (1968), notent la résistance de **C. stenophylla** à l'insecte. D'autres espèces comme **C. liberica, C. dewevrei, C. eugenioides, C. salvatrix** et **C. racemosa** (MEDINA FILHO et al., 1977) ont été aussi citées comme résistantes à **P. coffeella.** Ainsi l'utilisation de variétés résistantes doit passer par une hybridation de **C. arabica** avec des espèces sauvages. Ceci a été initié au Brésil avec **C. racemosa.**

L'objectif de cette étude est l'évaluation de la résistance de quelques espèces de caféiers africains et malgaches.

2. MATERIEL ET MÉTHODES

Élevage des insectes. Perileucoptera coffeella (Guérin - Méneville, 1842) (Lepidoptera - Lyonetiidae) a été élevé selon la méthode décrite par KATIYAR & FERRER (1968) sur jeunes caféiers de l'espèce **C. arabica,** en insectarium à une température de 30° ± 1°C, humidité relative de 70 ± 10% et photopériode de 16 heures.

Matériel Végétal. Les semences ou plantes de la plupart des espèces testées comme **C. tetragona, C. perrieri, C. tsaratananae, C. resinosa, C. jasminoides, C. vatuvavyensis, C. dolichophylla, C. bertrandi, C. millotii, C. farafaganensis** e **C. bonnieri**, toutes d'origine Malgache, ont été fournies par l'ORSTOM; **C. humilis** était disponible en serre au CIRAD et **Psilanthus travancorensis** provenant de Ceilan a été obtenu de l'Inde. La variété Catuaí Vermelho, CH2077-2-5-81 de **C. arabica** originaire du Brésil, a été utilisée comme témoin sensible à l'insecte.

Méthodologie d'évaluation. Des feuilles de la première à la cinquième paire de feuilles détachées de chacune des espèces étudiées, ont été introduites dans les cages d'élevage. Le lendemain trois disques porteurs d'oeufs ont été prélevés de chaque paire de feuille. Les disques ont été placés dans des boîtes plastiques, sur des éponges humides, maintenues à une température de 30° ± 1°C et photopériode de 16 heures. Quatre oeufs par disque ont été maintenus jusqu'à l'éclosion des larves, deux entr'elles sont alors éliminées. Les paramètres biologiques observés sont: la durée d'incubation des oeufs, la durée des phases larvaires et de chrysalide, leur viabilité, la durée totale du cycle et le nombre d'adultes obtenus à partir de 100 oeufs. La résistance des plantes est notée selon une échelle de 0 à 4, où 0 correspond à l'absence de développement larvaire et 4 au développement complet.

3. RESULTATS ET DISCUTIONS

La variation pour la durée d'incubation des oeufs n'est pas était significative: en moyenne 4,5 jours. La viabilité des oeufs est supérieure à 85%, à l'exception de celle observée chez **C. perrieri** qui est de 73%.

Il existe des différences notables entre espèces dans la durée et la viabilité de la phase larvaire. La durée de développement varie de 8,5 jour chez **C. arabica** à 21,3 jour chez **C. tetragona**, étant de 11,2 jours chez **C. bonnieri** et de 14,2 jour chez **C. bertrandi**. La viabilité larvaire est très faible pour la plupart des espèces; elle est nulle chez **C. resinosa** et **C. farafanganensis** et très élevée pour **C. arabica, C. perrieri** et **C. travancorensis**.

La durée de la phase de chrysalide est de l'ordre de 6 jours pour la plupart des espèces, mais relativement plus élevée pour **C. jasminoides**. Leur viabilité est supérieure à 50%, à l'exception de celles observées chez **C. tetragona** et chez **C. millotii**.

La durée totale du cycle de développement est de 26,2 jours à 29,6 jours. Elle est la plus courte pour les insectes élevés sur **C. bonnieri** (25,4 jours) et **C. arabica** (25,8 jours) et la plus longue sur **C. tetragona** (35,6).

Dans l'échelle de sensibilité de 0 à 4 points, le niveau 4 représenté par **C. arabica** caractérise un matériel très sensible à la mineuse. Le **C. travancorensis** avec une note de 3,29 peut être considéré comme sensible, au même niveau que **C. canephora**. Le note de **C. vatuvavyensis** (1,90) représente une sensibilité moyenne. Les espèces **C. perrieri, C. dolichophylla, C. humilis, C. bertrandi, C. millotii, C. bonnieri, C resinosa** et **C. tsaratananae** sont considérées comme résistantes (0,80 à 1,53). Enfin les espèces **C. tetragona, C. jasminoides** et **C. farafanganensis**, sont très résistantes ou pratiquement indemnes.

Chez quelques espèces, une certaine variabilité intraspécifique a été mise en évidence malgré le nombre réduit d'individus, comme dans le cas de **C. vatuvavyensis** où deux plantes sont moyennement sensible; une résistante et une sensibles. Cette variabilité s'oppose à la grande homogénéité présenté par **C. arabica**.

Jusqu'à présent, environ une trentaine d'espèces de caféier ont été évaluées et aucune relation précise ne peut être observée entre le niveau de résistance à **P. coffeella** et l'origine géographique des espèces africaines. Dix des onze espèces malgaches évaluées dans cette étude se montrent résistantes ou très résistantes et une moyennement espèce sensible (**C. vatuvavyensis**). Des quinze espèces africaines évaluées dans cette étude ou antérieurement, une est très sensible (**C. arabica**), deux sont sensibles (**C. canephora** et **C. congensis**) les autres espèces étant généralement très résistantes. Les deux espèces indiennes (**P. travancorensis** et **P. bengalensis**) se sont montrées sensibles

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Resumé. P. coffeella est un des ravageurs les plus importants de la culture du café. Les pertes de production sont très élevées et sont surtout la conséquence de la chute prématurée des feuilles. Tous les cultivars connus de **C. arabica** sont sensibles à ce ravageur. Différents niveaux de résistance ont déjà été signalés chez les espèces sauvages de **Coffea**. Dans cette étude, plusieurs espèces africaines ont été évaluées pour leur résistance. La durée et la viabilité de chacune des phases du développement des insectes élevés sur disques de feuilles de différents âges ont été observées, ainsi que la réaction de chaque espèce de **Coffea** à l'infestation, à travers une échelle de 0 a 4 points. La durée du développement larvaire et la viabilité de ce stade constituent le paramètre biologique présentant les plus grandes différences selon les espèces de **Coffea**. Chez **C. arabica** les larves ont une durée de développement de 8,6 jours et un pourcentage de viabilité de 91,7. Chez **C. tetragona** la durée de développement larvaire est de 21,3 jour. Chez **C. resinosa** et **C; farafaganensis**, toutes les larves sont mortes au cours de cette phase. L'échelle d'évaluation des dégâts permet de classer les diverses espèces de caféiers évalués en cinq niveaux de sensibilité. Le premier niveau est représenté par **C. arabica** avec une moyenne de 4 points, caractéristique d'un matériel très sensible; le cinquième niveau (0) est représenté par les espèces **C. tetragona, C. jasminoides** et **C. farafaganensis** considérées comme les plus résistantes. Les autres espèces occupent des positions intermédiaires. Une certaine variabilité intraspécifique a été mise en évidence pour quelques espèces; cette variabilité s'oppose à la grande homogénéité présenté par **C. arabica.**

Abstract. P. coffeella is one of the most important coffee pests. Crop losses induced by this insect are mainly a consequence of leaf shedding. All cultivars of **C. arabica** are highly susceptible to this pest, but different levels of resistance can be found within diploid coffee species. In the present study resistance of several african species was evaluated. Duration and viability of different developmental stages of the insect were observed in coffee leaf disks as well as severity by using a 0 to 4 point scale. The most important différences between coffee species were observed for the duration and viability of the larval phase. For **C. arabica**, the duration of larval development is 6 days with 91.7% viability. With **C. tetragona** this duration is 21.3 days and with **C. resinosa** or **C. farafaganensis** all larvae die. The severity measurements using the 0 to 4 point scale, allow for a classification of coffee species in five levels of susceptibility. The most resistant species tested are **C. tetragona, C. jasminoides** and **C. farafaganensis. C. arabica** is the most susceptible species, the other species are intermediate. A certain variability for resistance within diploid species has been observed, which contrasts with the homogenous susceptibility of **C. arabica** cultivars.

PROGRESS ON BREEDING FOR RESISTANCE OF ARABICA COFFEE TO LEAF RUST (*HEMILEIA VASTATRIX* B. ET BR.) IN INDONESIA

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INTRODUCTION

Arabica coffee (*Coffea arabica* L.) was introduced to Java by the Dutch East Government in 1699 (Cramer, 1957). This introduction was followed by the establishment of coffee estates in the island. Due to the excellent quality of Arabica coffee from Java, the so-called "Java Coffee" had become popular in the world coffee market.

By 1876 *H. vastatrix*, the notorious leaf rust disease, attacked Arabica coffee in Indonesia (Cramer, 1957). The infection was followed by a rapid epidemy of the disease, and it destroyed the cofee plantations in a short period. Two decades later the production of Arabica coffee in Java dropped significantly (Cramer, 1913; Schweizer, 1929). While the coffee production was declining, a research program on breeding for resistance to leaf rust established by the Planters' Association.

In order to overcome the problem of leaf rust a number of resistant species of the genus of *Coffea* were introduced to Java, e.g. *C. liberica* and *C. excelsa* in 1877 (Cramer, 1913). In 1900 *C. canephora* var. *robusta* was also introduced to Java.

Presently, Robusta dominates coffee growing areas in Indonesia, so the country is wellknown as the biggest Robusta producer. Robusta, however, also accupies highland areas which is actually more favorable for Arabica.

Arabica coffee growing areas in Indonesia at the moment only reaches 75.000 ha with the total production of 43.000 tons annually. That is why, the government intends to increase Arabica coffee growing by converting Robusta grown in the highland.

In order to support the program, breeding activities to find Arabica coffee varieties resistant or tolerant to leaf rust and having good quality as well as high yielding have been intensified since the end of 1970's. The progress of program execution will be discussed briefly in this paper.

PROGRESS ON BREEDING FOR RESISTANCE TO LEAF RUST

Leaf rust is an important disease of Arabica coffee in Indonesia, especially on Typica and Caturra varieties. Till the end of 1970's, however, research activities dealing with the effort to control the disease has not been intensively carried out.

Up to now *H. vastatrix* does not merely attack the susceptible varieties in Java, but the fungus also severely attacks Arabica coffee outside the island. Generally, the disease intensity is affected by the altitude, e.g. the lower altitude has the higher intesity. AB7, a susceptible Java Typica type, showed 83,6 % of disease intensity at 650 m a.s.l. compared to 54,1 % at 1200 m a.s.l. (Mawardi & Hulupi, 1992).

Program on breeding for resistance of Arabica coffee to leaf rust in Indonesia was initiated in 1900. That was due to the decline of coffee productivity attributed to the lack of seed selection. The discussion at a Planters' Conference led to a proposal to start a company for producing improved seed (Cramer, 1957).

Firstly, the Pasoemah variety of *C. arabica* was found on the Pasoemah by the estate manager, in a field planted in 1903,

as regularly high producing. The seedlings were uniform, densely branched and productive (Cramer, 1916 *cit.* Cramer, 1957).

The Pasoemah variety was then introduced to Java in 1912. In 1915 the new variety proved much more productive than the common Arabica but even so it did not suffer from over bearing. Since then the Pasoemah variety, further improved at "Besoekisch Proefstation", presently IRICC, became a favorite variety of *C. arabica* under the name of Blawan-Pasoemah, or BLP. It was known to be resistant to leaf rust and over bearing (Cramer, 1917). By now, however, the best practical variety of BLP 12 had been severely attacked by *H. vastatrix* and was sensitive to over bearing dieback.

In 1928 Abyssinian coffee was introduced to Java. The first crop was obtained in 1932. In an experimental field, it surpassed the other kinds by its very vigorous growth and high resistence to leaf rust (Cramer, 1957). Unfortunately, the recommended varieties of AB3 and AB4 were also attacked by *H. vastatrix* though not as severe as that of BLP series.

In addition to the improving disease resistance by seed selection and by the introduction of more resistant species, there is still a third device for fighting *Hemileia.* This is the use of hybrids with increased resistance.

In Java, the first report on the Kalimas hybrid *(C. liberica* x *C. arabica)* mentions its resistance to *H. vastatrix* while Arabica and Liberica were both heavily attacked by the fungus. There was no spot on the Kalimas hybrid's leaves. However, the original hybrid free at Kalimas which acquired the reputation of being free from *H. vastatrix* early as 1890 and gave a high yield in 1898, suffered heavily in the beginning of July 1898 (Kramer, 1899 *cit.* Cramer, 1957).

The breakdown of resistance is assumed to be the effect of new races formation. As postulated by the CIFC (1971) resistance of *coffee* sp. to *H. vastatrix* is determined by compatibility between resistance gene(s) in the host and virulence gene(s) in the pathogen. A number of physiological races had been identified in Indonesia as mentioned in Table 1.

Due to the susceptibility of BLP and AB series to leaf rust, the "Besoekisch Proefstation" introduced a number of S-lines from India in 1957. A trial to evaluate their resistance to leaf rust and productivity was conducted as soon as the seeds were received. The experiment was carried out at the Ijen highland, East Java, consisted of primary and secondary trials.

Among the introduced S-lines, S288 and S795 showed high yielder and resistant to leaf rust. However, they have high percentage of elephant beans especially during the dry period. Selected progenies of S795 has lower percentage of elephant beans than that of S288 or original S795. Thus, the selected S795 is still feasible to be recommended to the farmers.

At the same period of the introduction of S-lines, a number of Ethiophian Arabicas were also introduced to Java. Coffee seedling were received from the USDA, Maryland (USA) among which USDA 230762 showed moderate resistance to leaf rust and was productive. Up to now, the variety is still recommended to the farmers. The type of USDA 230762 is similar to Agaro. Resistance genes of USDA 230762 has been postulated as SH_4, SH_5 (Bettencourt, 1981).

Table 1 : Identified physiological races of *H. vastatrix* in Indonesia

Races	Identified on Varietas	Location	Authors
I ($v_{2,5}$)	BLP, AB, USDA 230731 other Typica ?	Java, Timor	Goujon (1971), Rodrigues *et al.*(1975), Rodrigues (1985) *
II (v_5)	BLP, AB, USDA 230731 other Typica ?	Java, Timor	Goujon (1971), Rodrigues *et al.*(1975), Rodrigues (1985) *
III ($v_{1,5}$)	BLP, AB, other Typica ?	Java, Timor	Goujon (1971), Rodrigues *et al.* (1975)
XV ($v_{4,5}$)	BLP, AB, other Typica ?	Java, Timor	Goujon (1971), Rodrigues *et al.* (1975)
XVII ($v_{1,2,5}$)	USDA 230731	Java	Rodrigues (1985) *
XXII (v?)	Typica ?	Timor	Goujon (1971)
XXV (v?)	Typica ?	Timor	Goujon (1971)
XXVI (v?)	Typica ?	Timor	Goujon (1971)
XXIX (v?)	Typica ?	Timor	Rodrigues *et al.* (1975)
XXX (v?)	Typica ?	Timor	Rodrigues *et al.* (1975)
Is.KJ.91 ($v_{3,5}$)	S-line progenies	Java	Mawardi (1991) **

Notes : * Result of uredospores identification from Java.
** Inoculation done in the laboratory of Phytopatology IRCC, CIRAD.

S1934 was introduced to Java in 1970. The variety showed short internodes, dark narrow leaves and resistant to leaf rust. S1934 is more productive than S795.

S795 and S1934 are the F_3 and F_4 progenies of S288 x "Kents", respectively (Srinivasan & Vishveshwara, 1978). S288 has been postulated to carry the dominant factors of SH_3 and SH_5 for resistance to leaf rust whereas "Kents" has the factors of SH_2 and SH_5 (Bettencourt & Carvalho, 1968 *cit.* Ramachandran & Srinivasan, 1979)

In the early 1960's S288 and S795 were crossed with Typicas. The progenies showed resistant to leaf rust and were productive. A number of individual F_1 were propagated by cuttings to develop elite clones. However, progeny test was also conducted continuously to develop elite varieties. F_3 improved seeds have been recommended to the farmers, especially for marginal soil.

Planting materials carrying resistance factor of SH_3 showed higher rust in the middle of 1980's. As mentioned in Table 1, virulence gene v_3 was identified in the isolated spores of Is.Kj.91.

In order to find planting materials having durable resistance to leaf rust, breeding program to combine resistance factors carried by S-Lines and "Hibrido de Timor' has been carried out. The progeny of (AB7 x S1934) x Catimor C-1662 showed good habitus (semi-dwarf) and was resistant to leaf rust as well as productive. The percentage of elephant beans is lower than that of S1934.

In order to find planting materials having durable resistance to leaf rust, breeding program to combine resistance factors carried by S-Lines and "Hibrido de Timor' has been carried out. The progeny of (AB7 x S1934) x Catimor C-1662 showed good habitus (semi-dwarf) and was resistant to leaf rust as well as productive. The percentage of elephant beans is lower than that of S1934.

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SUMMARY

Breeding for resistance of Arabica coffee to leaf rust *(H. vastatrix)* has been intensified in Indonesia since the end of 1970's. The activities consist of selecting mother trees and introducing resistant varieties as well as creating new genotypes by crossings.

Concerning the population of physiological races of *H. vastatrix* found in the Java island and outside Java in the decade of 1970's, the SH_3 had been considered the main gene to improve the resistance on traditional varieties. At the moment, however, the varieties containing SH_3 gene and their progenies were attacked by the fungus, some of which showed a severe symptom.

In order to find Arabica coffee varieties with durable resistance, some crosses to combine SH_3 gene with other genes of Hibrido de Timor or their progenies had already been made. The progenies containing SH_3 gene with the genes derived from Hibrido de Timor showed dwarf type, resistance to *Hemileia vastatrix* and high yielding capacity.

RESEARCH ON ORGANIC ARABICA COFFEE FARMING IN INDONESIA

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INTRODUCTION

Arabica coffee growing area in Indonesia at the moment covers 75,000 ha belonging to government estates, private estates as well as smallholders. The main growing areas of the coffee are located in Gayo highlands (Aceh), Tana Toraja (South Sulawesi), Ijen plateau (East Java), and Kintamani (Bali). Due to the problem of leaf rust *(H. vastatrix)*, the farmers do not only grow Arabica on the highlands but also plant Robusta coffee.

Most of the farmers cultivate Arabica coffee traditionally without using any fertillizers and pesticides or they grow coffee organically which management is still very poor. To improve the yield and to achieve sustainable farming, a research program on organic coffee has been conducted at Gayo highlands. The research is aimed to achieve better management of organic farming system of Arabica coffee to meet the standard of organic agriculture according to IFOAM (1992).

EVALUATION ON ARABICA COFFEE FARMING

A survey to describe the problems on Arabica coffee cultivation at the farmers level dealing with organic agriculture standard has been carried out in the Gayo highlands, district of Aceh Tengah. The survey consisted of 270 farmers as sample in 9 subdistricts.

Recently, the total coffee area in Aceh Tengah reaches 62.000 ha, that consists of 85 % Arabica and 15 % Robusta. Most of them are planted on fertile soil and some on virgin soil.

Old planting is low yielding, because the farmers grow coffee traditionally with low-input technology. Most of the farmers use local varieties which are susceptible to leaf rust and berry borrer. Yet, only 1,0 % of them control the pest and disease. The other pests and diseases are not considered as important as leaf rust and berry borrer, so they never control them. Noxious weeds are sometimes controlled by using herbicides.

Since the farmers grow coffee on fertile soil, only 1 % of them apply inorganic fertillizer while 31 % put organic material. Inorganic fertillizer is not applied directly to the coffee, but usually to the annual intercrops e.g. vegetables, food crops, etc.

In order to prevent soil erosion, several farmers start making terraces, especially on the steep slope (> 30 %). A number of farmers (47%) do not do soil tillage.

The main shade tree is *Leucaena glauca*, which is at the moment severely attacked by the jumping lice.

Only 25 % of the farmers have applied good pruning system on coffee.

RESEARCH ON ORGANIC COFFEE FARMING

Regardless the poor management, Gayo highlands has produced organic coffee. Better management is expected to increase coffee yield and sustainability of the farming system. To improve organic cultivation with good management, especially in Gayo highlands a research program has been established.
The progress of the research activities are as follows :

Soil nutrition and management :

- Research on *Azolla* sp. showed that the aquatic ferns are potential as compost. They are symbiosis with Anabaena as nitrogen fixation agent. The C/N ratio of fresh Azolla is 8 - 10 %. The farmers can easily multiply them in the pond with the water depth 5 cm and apply 50 kg TSP/ha.
- Research on endomicorrhyzae *(Gigaspora margarita)* showed that micorrhyzae can improve rock phosphate availability besides reducing the damage of coffee seedling to nematode attacked.
- Research on composting process by using local material showed that the low C/N ratio (13,9) was achieved after 3 months.
- Research on soil prevention by reducing erosion rate is carried out by terracing. On the terrace side vetiver grass *(V. zizanioides)* is planted to reduce run-off. The grass does not only reduce run-off but also produces biomass for mulching and serves as a source of compost material. Preliminary observation showed that terracing reduces erosion rate.

Pest and disease control :

- The main pests and diseases of Arabica coffee in Indonesia are berry borrer, nematodes, leaf rust, and root diseases. The research is emphasized to develop integrated control without using any pesticides.
- Application of *Beauveria basiana* at the farmers level showed that the fungus was able to reduce the damage from 25,90 % to 11,75 %. Sanitation is also recommended to minimize source of infestation.
- The use of *Trichoderma sp.* is being studied intensively to control root diseases, especially *R. lignosus.*
- Cu spraying and tolerant varieties are also being studied to control leaf rust.

Shade and Pruning :

- Multiplication by cuttings of several varieties and clones of seedless *Leucaena* having a good canopy for shade and tolerant to jumping lice is studied intensively. The result showed that the highest percentage of rooted cuttings was achieved by the use of green cuttings rather than the green-brown and the brown ones.

Improvement of Plant Materials :

- Research is aimed to obtain varieties and composit variety of coffee resistant to leaf rust, as well as clone or varieties of *Leucaena* sp. tolerant to jumping lice.

Organisation :

- Besides technical aspects, organisation plays an important role in producing organic coffee. Record on several aspects concerning organic coffee should be made on each level such as farmers as the source of raw material (cherries) up to processor (factory). When the organisation is properly run the inspection of organic coffee can be done easily to obtain sertification. This research program also covers organisation research to produce organic coffee.

Implementation :

- By intensive extension services the farmers start to adopt several techniques such as e.g. integrated maintenance of cofee without using any pesticides, herbicides, and synthetic fertillizer components, making terraces, practicing good pruning and applying compost in the effort to produce organic coffee.

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SUMMARY

Arabica coffee growing areas in Indonesia at the moment covers 75.000 ha. The main growing areas are located in the highlands of Gayo (Aceh, Sumatera), Tana Toraja (South Sulawesi), Ijen Plateau (East Java), and Kintamani (Bali). The government intends to increase Arabica production by converting Robusta coffee grown in the highlands.

Most of the Arabica coffee produced by smallholders is grown without using any inorganic synthetic materials to increase soil nutrition or to control pests and diseases. The traditional farmers normally produce less than 500 kg green coffee/ha/year.

In order to increase Arabica coffee yield at the traditional farmers' level a research program on organic coffee farming has been established in Gayo highlands. The research is mainly aimed to create a sustainable farming system at the farmers' level by applying components of organic agriculture.

Some components of organic coffee farming studied on the program above consist of improvement of plant materials, the use of legumes as shade trees as well as to prevent soil erosion, and research on biofertillizer in addition to biological control of major pests and diseases. This paper will discuss the highlight of the research progress and some preliminary implementation at the farmers' level.

RELATIVE PERFORMANCE OF THREE COFFEE CULTIVARS UNDER VARYING SHADE LEVELS

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ABSTRACT

Three coffee cultivars (75227, 74165, 74158) were planted under varying light regimes estimated using LI COR 776 point quantum sensor. Split plot design with four replications within the shades (main plot) was used to subplant the coffee cultivars (subplot). Biochemical constituents, yield and yield components at seedling and maturity stages were determined using standard protocols. Significantly higher ($p<0.05$) yield and yield components were obtained at both stages between 50-70% light regimes. Among the coffee lines 75227 out performed the other two cultivars with regards to metabolite synthesis at seedling and yield at maturity. Hence, biochemical and dry matter synthesis at seedling stages are efficiently correlated determinants to predict mature coffee performance at a later stage.

AVANTAGES ET INCONVÉNIENTS DE L'UTILISATION DES DIVERS MODES DE COUVERTURE DANS LES CAFÉIÈRES AU BURUNDI

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Introduction

Une grande partie des zones de cultures du caféier Arabica au Burundi sont caractérisées par une faible pluviométrie et une longue saison sèche. Dans d'autres parties, de fortes pentes nécessitent de recourir à un dispositif anti-érosif efficace pour éviter l'entraînement des terres.

Problématique du paillage au Burundi

L'application du paillis telle qu'il est actuellement recommandé au Burundi, c'est à dire en conservant une épaisseur toujours supérieure à 10 cm, n'est pas facile à réaliser et entraîne des contraintes lors de son exécution. On notera en particulier la diminution de la disponibilité en matériaux de paillage dans les zones de forte pression démographique du fait de la disparition progressive des jachères.

Le prélèvement de quantités importantes de matière végétale pour réaliser le paillage entre en concurrence avec les autres activités de l'exploitation telles que l'élevage ou les cultures vivrières. L'utilisation des résidus de récolte et des feuilles et stipes de bananiers conduit à un transfert de fertilité des parcelles vivrières et des bananeraies vers les caféières.

Le manque de ressource en matériel de paillage conduit les caféiculteurs à négliger ce thème cultural. Ils laissent alors la couverture naturelle envahir leurs plantations ou pratiquent un sarclage superficiel du sol au risque d'en altérer la fertilité et d'entraîner leur érosion. D'autres caféiculteurs sont amenés à acheter le paillis qui est devenu insuffisant dans leur propre exploitation.

Du fait des difficultés rencontrées par les cultivateurs burundais pour pailler leurs caféiers, des techniques alternatives pour la couverture du sol sont recherchées.

Etude des alternatives au paillage total

Une première méthode consiste à réduire les quantités de paillis apportés en n'appliquant le paillis qu'une ligne sur deux et en alternant les applications une année sur deux. Les autres méthodes font appel aux cultures intercalaires de légumineuses.

Les productions moyennes des caféiers cultivés en association avec divers types de couverture du sol sont comparées entre elles. Le *Themeda Gigantea* a été utilisé comme source de paillis.

Dans les conditions habituelles de culture des caféiers, c'est à dire sans apport d'engrais, les caféiers paillés produisent globalement plus que ceux qui sont associés à d'autres cultures. Par contre, si l'on apporte de l'engrais azoté (urée) aux caféiers, cette différence disparaît. Ceci montre l'effet concurrentiel de la culture associée vis à vis du caféier.

Si l'on prend en compte le bénéfice calculé à partir des recettes provenant de la vente des récoltes de café et des coûts de production, on constate alors que l'utilisation du paillis pour assurer une épaisseur de plus de 10 cm après décomposition sur toute la surface cultivée tel que recommandé actuellement ne permet qu'un bénéfice net qui est significativement plus faible que les autres traitements exception faite de la couverture naturelle qui concurrence fortement les caféiers. En effet, malgré un bon rendement des caféiers, ce traitement nécessite d'apporter près de 30 tonnes de matière verte par hectare et requiert une grande quantité de main d'oeuvre.

Le bénéfice le plus élevé est obtenu avec le paillage alterné grâce à une économie de 50 % sur les matériaux de paillage ainsi qu'une économie très importante en main-d'oeuvre. L'utilisation d'une couverture du sol par Desmodium est aussi une méthode permettant un bon rapport.

Dans les essais sur sols pauvres, une concurrence entre les deux plantes associées a été observée. Mais elle a pu être compensée par un apport d'engrais azoté aux caféiers. (Cfr tableau 1 ci-dessus). Il faut cependant noter que les légumineuses avaient été cultivées sans avoir préalablement amélioré le sol pour leur donner les conditions favorables à leur croissance et leur permettant de fixer l'azote atmosphérique.

Les nouveaux essais devront utiliser des variétés sélectionnées du point de vue des quantités de biomasse produites et de leurs capacités à fixer l'azote afin d'éviter toute concurence avec les caféiers.

Choix des plantes qui peuvent être associées aux caféiers

La plante utilisée en association avec les caféiers doit conserver les autres avantages du paillis. C'est à dire avoir un rôle antiérosif, produire une forte biomasse permettant le paillage des caféiers et elle ne doit pas concurrencer les caféiers pour les éléments minéraux et pour l'eau, en particulier dans les régions à longues saison sèche.

Dans les nouveaux essais, trois légumineuses ont été retenues. Le *Leucaena leucocephala* et le *Flemingia congesta* qui possèdent tous deux un enracinement profond et pivotant, ce qui limite à la fois les concurrences hydrique et nutritionnelle vis à vis des caféiers et permet de les utiliser en lutte antiérosive. De plus, ces deux plantes acceptent d'être fréquemment taillées. La troisième plante est le *Desmodium intortum* qui produit plus de biomasse et permet une couverture complète du sol. Cependant, il ne pourra pas être recommandé dans les régions à longue saison sèche car on a pu observer que, lors de la reprise de la saison des pluies, son enracinement étant situé au-dessus de celui du caféier, il capte toute l'eau des premières pluies, ce qui rallonge d'autant le stress hydrique des caféiers.

Etude pour lever les contraintes liées aux légumineuses

Pour donner à la légumineuse les conditions nécessaires pour la rendre capable de croître facilement et de fixer l'azote, une correction du sol adaptée aux besoins de la plante est nécessaire. Cette correction a été évaluée dans un essai comparant la croissance du *Leucaena leucocephala* K28 et du *Desmodium intortum* dans un sol corrigé ou non par un amendement dolomitique et phosphaté.

L'effet hautement significatif des apports de dolomie et de phosphate sur l'inoculation a ainsi pu être mis en évidence. On observe que l'inoculation ne se montre efficace que si elle est associée à un amendement dolomitique ou phosphaté. Les deux effets semblent même cumulatifs.

Des observations complémentaires en champ ont révélés que le Leucaena et le Flemingia présentent un intérêt antiérosif certain mais nécessitent d'être inoculés tandis que le Desmodium semble s'accomoder des souches existantes dans le sol

Conclusion

Le paillage des caféiers est une pratique indispensable dans les conditions de sol et de climat du Burundi. Parmi les effets les plus importants, on retiendra la protection du sol contre l'érosion et l'évaporation en saison sèche d'une part et l'apport de matière organique et d'éléments nutritifs d'autre part. Cependant cette pratique est laborieuse et provoque un transfert de fertilité qui nuit aux autres cultures.

Pour pallier au manque de ressources en matériaux de paillage, on peut proposer la réduction des quantités apportées de moitié par rapport aux quantités actuellement recommandées. Cette réduction présente l'avantage de permettre une économie financière.

La culture intercalaire de légumineuses n'intéresse actuellement pas les planteurs de caféiers du fait de la concurrence observée entre les plantes associées. Cette technique peut cependant devenir intéressante si l'on donne à la légumineuse les conditions favorables à sa croissance. Les premiers résultats montrent que la concurrence qui existe peut être levée par une correction du sol appropriée.

Des études sont en cours pour trouver des variétés productives et facile à noduler et pour estimer les doses et fréquences des corrections (chaulage, apport de phosphate) à effectuer.

Résumé

Les conditions de culture du caféier Arabica au Burundi rendent indispensable l'emploi du paillis (fortes pentes, longue saison sèche, sols pauvres). Cependant, les ressources en paillis se font de plus en plus rares et des alternatives doivent être envisagées. Parmi celles-ci, les légumineuses de couvertures présentent une solution intéressante mais avec des inconvénients qui ont été évalués dans des essais sur centre et en milieu rural (faible biomasse produite, concurrence vis à vis des caféiers). Des solutions pour les résoudre sont étudiées (choix de variétés productives, techniques culturales adaptées).

Abstract

The use of mulch in coffee plantations is required in Burundi conditions (steep slopes, length of dry season, leached soils). But the usual sources of mulch (grasses) are getting more and more hard to find and other mulching materials are searched. Among those, leguminous plants present many advantages but also inconvenients which were evaluated in trials (little quantities of mulch produced, concurrence with coffee trees). Solutions to solve those problems are studied (Choice of producing varieties, amelioration of cultivation techniques).

COFFEA ARABICA L. SUITABLE AREAS IN ETHIOPIA

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INTRODUCTION

This investigation was a part of the National Crop Suitability Study, which was conducted in 1988-89 in Ethiopia. Its objective was to set the environmental requirements for coffee production, with low to medium inputs level, and then shows the potential areas on the map of the country. For this study altitude, temperature, soils and rainfall were taken as factors affecting coffee growth, development and production.

Altitude : Coffee is observed to be growing from 550 in Gambella to 2 500 meters above sea level (MASL) in Wello. However, the major production areas lie between 1 200-2 000 MASL as observed in practice. Eventhough weed is a limiting factor, still in few humid places below 1 000 MASL, in the South Western region, coffee grows. The optimum temperature for coffee seed germination is 30° C during the day and 23° C at night, but for mature coffee the optimum temperature would be 24° C and 20° C during the day and at night respectively; for each 1° C above 24° C the photosynthetic rate would decrease by 10% and become nil at temperatures greater than 30° C. The day temperature which does not exceed over 32° C and not less than 7° C at night is found suitable for coffee production in Kenya. Extreme temperature and large fluctuations between day and night have usually an adverse effect. Shade greatly modify the surrounding temperature; with reasonable canopy, the temperature may be reduced by 10° C compared to the surrounding air. In a similar way, as altitude increases, the temperature decreases, that is for every 160 meters increase in altitude, the temperature decreases by 1° C. The mean annual range of 14-20° C is considered cool and found suitable for general crop growth in Ethiopia.

Soils : In high rainfall areas, coffee can grow successfully in shallow soils but a remarkable crop yield is reduced with excessive rainfall or unusual long dry season. Regarding soil types, Nitosols, Acrisols, Luvisols, Cambisols, Phaeozems, Fluvisols, Regosols, Andosols and Histosols were quoted to be suitable for coffee production.

Rainfall : The minimum required depends on frequency of cloud cover, soil type, air temperature, relative humidity, wind speed and the amount of radient solar energy arriving at the evaporating surface. Thus in Kenya where the annual distribution is bimodal, the minimum amount of rainfall required is 890 mm. However,the minimum requirement for the areas which get their annual rainfall in one season is 1,145 mm. Two to four consecutive months with less than 50 mm of rain is important for the initiation of flower buds. Generally a rainfall which is accompanied by cloud, high relative humidity is conclusive for optimum coffee production in Ethiopia.

MATERIALS AND METHODS

Altitude (topographic), soils, rainfall maps which were provided by the National Committee for study of land suitability for crop production, were used. Forty coffee demonstration sites in seventeen subdistricts in four regions were studied in relation to known coffee types performances; based on this study an altitude of 1 500-1 800 MASL was rated highly suitable and less than 1 500 and above 1 800 MASL rated moderately and marginally suitable (see table 1). Regarding temperature it was assumed to have been taken care in altitude and not considered for the final result. Based on seventeen subdistricts observation the annual rainfall of 1 300 mm and above was rated highly suitable and less than 1 300 mm was rated in catagories of moderately and marginally suitable ranges. Regarding soils, 215 coffee growing subdistricts major soil types were studied : from this study Nitosols (N), Acrisols (A), Luvisols (L), Cambisols (B), Lithosols (I), Phaeozems (H), Chromic vertisols (Vc), Fluvisols (J), Andosols (T) were identified to be suitable for coffee growing in their descending order of frequency of occurance. For the purpose of this paper all soil types were considered equally suitable for coffee production if altitude, temperature and rainfall are conclusive.

Table 1 : Major environmental requirements and their ranges of suitability for coffee production in Ethiopia

Environmental requirement	Unit	Ranges of suitability		
		Highly	Moderately	Marginally
Altitude	MASL	1500-1800	1200-1499 1801-2000	900-1199 2001-2200
Rainfall	mm	1300 and above	1100-1299	900-1199
Soils	FAO	NALVCBIHJT		

SUMMARY

Maps of the country in the scale of 1:2,000,000 were drown for altitude, rainfall and soils; then these maps were superimposed to locate the highly, moderatly and marginally suitable areas and the land area was measured by digital planometer.

Results :

Highly suitable areas	12,530,980 hectars
Moderately suitable areas	5,462,084 hectars
Marginally suitable areas	5,114,054 hectars
Total land areas	23,107,118 hectars

Discussion :

The results obtained were more or less similar to what has been observed in practice in coffee growing regions of the country.
The information could be used to advise the farmers on the possibility of using the areas which have similar environments for coffee production, and also the type of modifications to be made for moderately and marginally suitable areas to bring them to the next favorable stage to improve production. Moreover it assists to plant each coffee type in an areas similar to its origin for successful adaptation and subsequent production.

If marginal areas are excluded, there would be a land areas of 17,994,064 hectars suitable for coffee production; but currently there is approximately 321,048 hectars under coffee production. This study shows the existence of a potential land area of about 17,672,916 hectars which could be used for coffee production.

SEASONAL FLUCTUATIONS IN LEAF NUTRIENT CONTENTS OF FERTILISED AND UNFERTILISED ARABICA COFFEE IN PAPUA NEW GUINEA

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INTRODUCTION

Good estimates of the fertiliser requirements of coffee are obtained from nutrient balances based on anticipated yields. Coffee leaf analytical data should then permit improved, site-specific modifications to generalised fertiliser recommendations. However, despite standardised sampling and analytical procedures, coffee leaf nutrient levels have often appeared to be poorly correlated with actual nutrient requirements. This may be because seasonal fluctuations in leaf nutrient contents have been underestimated, and insufficient attention has been given to the phenological time when the leaf samples were collected.

This study aims to illustrate the seasonal fluctuations in leaf nutrient contents in coffee in Papua New Guinea, and to explain them in terms of the annual crop development cycle, thereby facilitating the interpretation of leaf analytical data, and improving subsequent fertiliser programmes.

METHODS

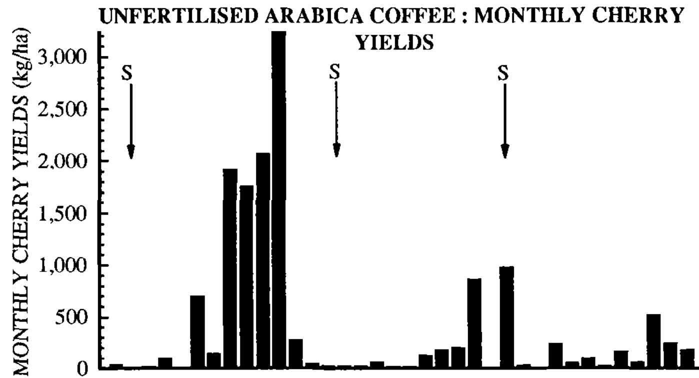
Leaf samples were collected at monthly intervals for three years (October 1987 - September 1990) from 17 unfertilised, shaded, smallholder coffee gardens, and 13 fertilised, shaded and unshaded, plantation coffee blocks in the Eastern and Western Highlands Provinces of Papua New Guinea (altitudes 1500-1700 masl, mean annual rainfall 2000-2500 mm).

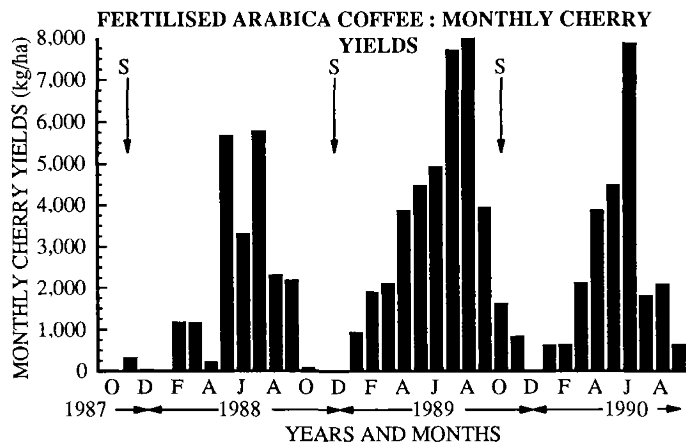
On each occasion, at least 40 leaves were collected from each site - two pairs from each of 10 trees. The leaves sampled were the third pairs from the growing tips of primary branches midway between the ground and the top of the trees. Leaves were collected equally from all four quadrants (N,S,E and W) of the trees.

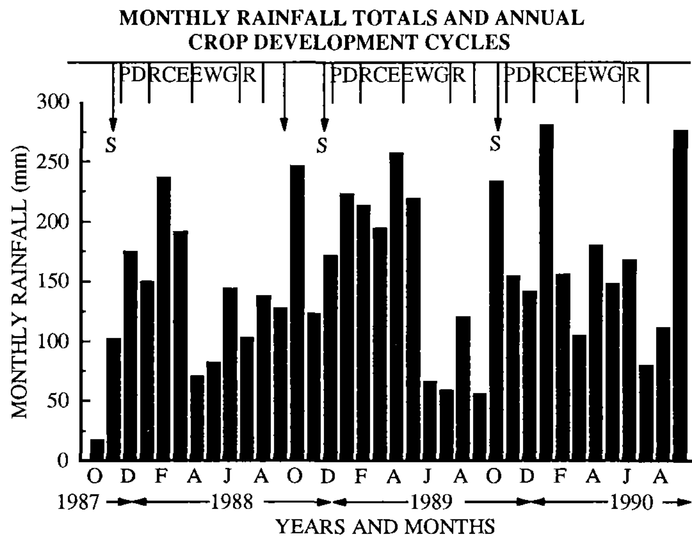
Samples were washed in cold water, dried for 24 hours at 60°C, ground, and analysed for total N (Kjeldhal), P, K, Ca, Mg, Na, S, Zn, B, Fe, Mn and Cu (nitric/perchloric acid digestion).

RESULTS

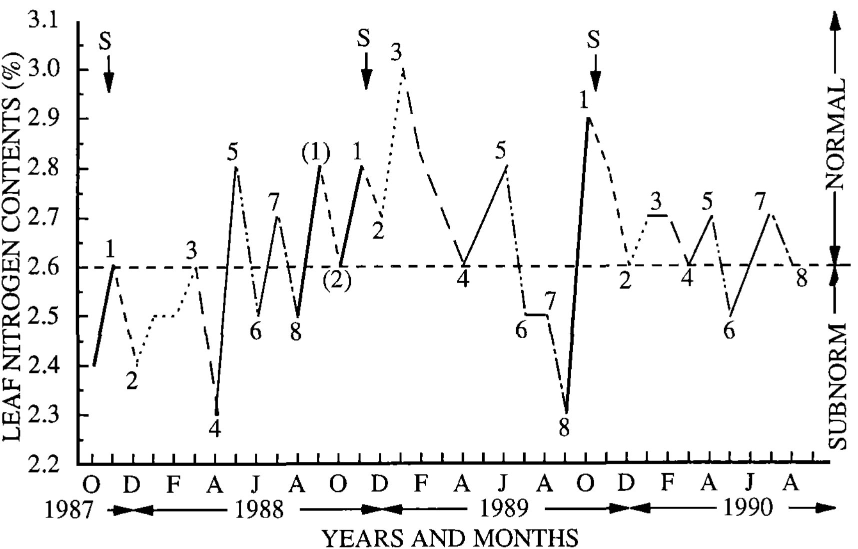
Although the seasonal fluctuations of each of the 12 nutrients listed above were examined, the results are

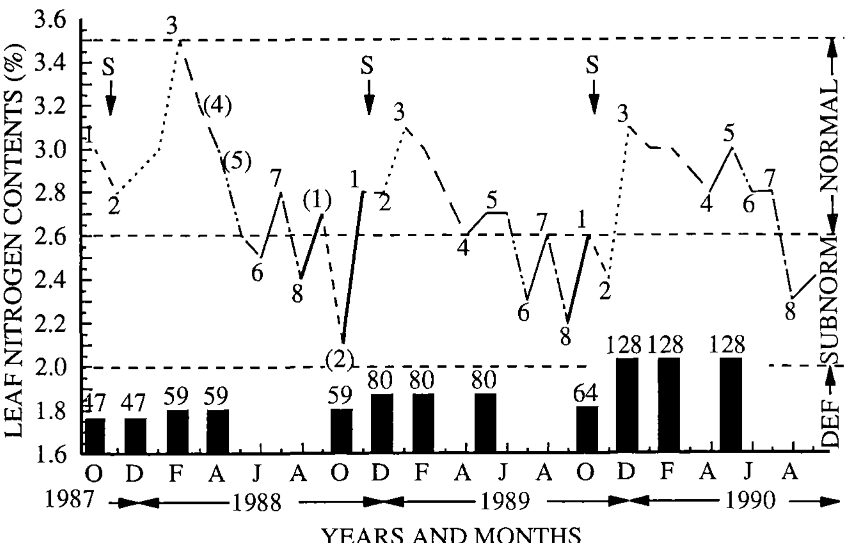






NOTE : Recorded at Aiyura Highlands Agricultural Experiment Station





ANNUAL COFFEE CROP DEVELOPMENT CYCLE AND LEAF N CONTENTS

S =	Rainfall stimulus to flowering (breaks flower bud dormancy)
———	Soil nitrogen flush following rains
1 =	Pre-flowering leaf N peak due to soil nitrogen flush
- - - -	Flowering (7-10 days after stimulus)
2 =	Trough in leaf N due to flowering
········	Pinhead dormancy phase (PD) of 7 weeks - low N demand
3 =	Pre-season leaf N peak due to low N demand during PD, and increased N uptake from OM as rain increases
— — -	Period of rapid cherry expansion (RCE) and endosperm formation (13 weeks) - high demand for N
4 =	Trough in leaf N due to demands of RCE
5 =	Leaf N peak as demand falls at end RCE
-—··—···	Endosperm weight gain (EWG) phase of 12 weeks - high N demand
6 =	Trough in leaf N due to demands of EWG
7 =	Leaf N peak as demand falls at end EWG
-—·—·	Final ripening (R) phase of 6 weeks - high N demand
8 =	End of season trough in leaf N following demands of final ripening phase

illustrated here by the nitrogen fluctuations from two locations - an unfertilised shaded coffee garden and a nearby fertilised unshaded coffee block.

Unlike most other coffee-growing countries, Papua New Guinea does not have a clear dry season, although in most years a less wet period does occur during the May-September period (see bar chart of monthly rainfall totals). Therefore, under PNG conditions, some coffee ripens during most months of the year. However, if a clear rainfall stimulus to flowering can be identified, yields are seen to peak 8-9 months later (see bar chart of monthly cherry yields).

Beginning with the rainfall stimulus, the annual crop development cycle may be summarised as shown on the rainfall bar chart, and described in the key.

The seasonal fluctuations in leaf nitrogen contents from the unfertilised and fertilised sites are related to the annual crop development cycles in the two line graphs, as explained in the key.

CONCLUSIONS AND RECOMMENDATIONS

1. The seasonal fluctuations of most nutrients are considerable, often ranging from subnormal (or even deficient) levels, to normal (or even excessive) levels in a single year. It is therefore difficult to interpret leaf analytical data without due regard to the phenological time of sampling, and an understanding of the seasonal fluctuations in leaf nutrient contents.
2. The changing demands of the trees at different stages in the annual crop development cycle are shown to explain the seasonal fluctuations in leaf nutrient contents.
3. Thus, provided the time in the crop development cycle when the samples are collected is known, a more realistic interpretation of the analytical results is possible.
4. Although fertiliser applications generally increase the overall leaf nutrient contents, they do not remove their seasonal fluctuations. Some peaks and troughs however may be enhanced or reduced by fertiliser applications.
5. Suitable times for leaf sampling appear to be at the end-of-season low, when the main crop has been harvested, and/or at the pre-season high, as rapid cherry expansion commences.

OTHER NUTRIENTS

1. Seasonal fluctuations in leaf N, P, K, S, Zn and B contents follow a similar pattern, although response times to the changing demands of the trees at different stages in the crop development cycle may vary.
2. Leaf Fe and Mn contents are closely related to the rainfall pattern, the leaf contents of both nutrients falling as rainfall increases.
3. Leaf Mg and Ca contents are strongly influenced by an antagonism with leaf K contents, particularly when one or more of the nutrients is at a high level. When all three are at normal or lower levels, their seasonal fluctuations are related to the crop development cycle.

SUMMARY

The study aims to illustrate, and explain, seasonal fluctuations in coffee leaf nutrient contents under PNG conditions. Leaf samples were collected, at monthly intervals, for 3 years, from 30 sites, and analysed for total N, P, K, Ca, Mg, Na, S, Zn, B, Fe, Mn and Cu contents.

Leaf N contents, from unfertilised and fertilised coffee, are used to illustrate seasonal fluctuations of leaf nutrient contents, which are shown to be considerable, but well explained by the annual crop development cycle. Fertiliser applications increase leaf nutrient contents generally, but do not remove their seasonal fluctuations.

Interpretation of leaf analytical data is thereby facilitated, provided the phenological time of sampling is known. Recommended times for collecting leaf samples are as rapid cherry expansion begins, and/or after the main crop has been harvested.

THE EFFECT OF TIME OF HARVESTING ON YIELD AND HARVEST QUALITY IN ROBUSTA COFFEE

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INTRODUCTION

The ripening period of coffee between September and January coincides with the peak period for harvesting cocoa, the principal export crop of Ghana. Farmers are therefore likely to leave their coffee cherries unharvested until they turn black, despite advice to the contrary (Ampofo and Osei-Bonsu, 1988). Coffee cherries harvested at different stages of ripening affect the yield and the quality of the produce (Coste 1992a). Siebers (1981) in Ghana attributed "smelly cup" quality to a high proportion of black berries at harvest whilst the "sour" quality was due to green unripe berries some of which may be immature (Osei-Bonsu et al, 1989). However in Togo (Osei-Bonsu, 1989) and Cameroun (Osei-Bonsu and Oppong, 1991) coffee is harvested at 60-80% ripe. The experiments discussed here were designed to investigate the merits and economics of sequential red-ripe coffee harvesting (Wrigley, 1988) and single one-off harvesting as practised in some countries for robusta coffee.

MATERIALS AND METHODS

The plot of coffee used in these studies at the Cocoa Research Institute of Ghana was of improved robusta materials rejuvenated in 1990. Sequential harvesting (four times) of ripe coffee cherries was compared with three treatments in which all the coffee was picked when 60%, 80% or 100% ripe from 15 tree plots under which polythene sheets have been spread to enable retrieval of dropped berries. Data collected included time taken to pick dropped coffee berries before and after harvest as well as the time taken for the actual harvesting operation and the weights of these fractions. A sample of 5kg of the harvested crop was separated into black, red, yellow and green cherries and each fraction separately weighed.

RESULTS AND DISCUSSION

Before harvesting at any stage other than 100% ripe, a 6% loss as a result of berry drop is incurred. Approximately 10% of the coffee crop was

lost when berries were left on the trees to 100% ripe stage (Table 1).

Table 1: Components of yield in coffee harvesting

	Sequential Harvesting 4 times		Single harvest at 60%		80%		100% Ripe	
	Kg ha^{-1}	Prop.	Kg ha^{-1}	Prop.	Kg ha^{-1}	Prop.	Kg ha^{-1}	Prop.
Dropped berries before harvesting	450.0	0.056	391.9	0.064	701.8	0.062	451.6	0.104
Harvested berries	7100.0	0.879	5363.7	0.879	9410.4	0.875	3613.0	0.833
Dropped berries during harvesting	527.8	0.065	347.1	0.057	721.5	0.062	271.2	0.063

This represented about 4% more coffee berries dropping to the ground before the harvesting which may not be retrieved. Another source of lost yield (6%) was from berries dropping to the ground during the harvesting operation.

About 70% of the time was actually engaged in picking approximately 85% of the cherries from the trees and sequential harvesting of coffee demanded more time than single harvest operations at any stage of ripening (Table 2). In respect of output rating, it was more efficient to pick berries in a single harvest operation (6 - 7kg/hr.) than to do

Table 2: Duration of operations and efficiency rating as affected by stage of ripening

Operations	Single Harvest at 60% Ripe Time/ha (hrs.)	RE* (kg.hr^{-1})	80% Ripe Time/ha (hrs.)	RE* (kg.hr^{-1})	100% Ripe Time/ha (hrs.)	RE* (kg.hr^{-1})	Sequential Harvesting 4 time Time/ha (hrs.)	RE* (kg.hr^{-1})
Picking dropped berries before harvest	143.7	2.73	280.5	2.41	328.7	1.37	263.9	1.71
Harvesting from trees	754.0	7.11	1204.0	7.26	575.3	6.28	1385.2	5.13
Picking dropped berries after harvest	136.2	2.55	1195.4	3.48	145.7	1.86	221.3	2.38

$$*RE = \text{Relative Efficiency} = \frac{\text{Quantity of berries picked}}{\text{No. of hours taken for operation}}$$

selective picking (5kg/hr.) as was done in the sequential harvesting. Similarly, more time was required to pick berries from the litter as the relative efficiency of this operation is low. Cherry harvest quality tended to depend on the stage of ripening and the frequency of harvest operations (Table 3).

Table 3: Effect of stage of ripening on the quality of cherry harvest

Berry Grade	Single Harvest at 60% Ripe	80% Ripe	100% Ripe	Sequential Harvesting 4 times
	%	%	%	%
Black	11.3	24.2	27.1	12.8
Red	45.0	31.4	71.5	76.4
Yellow	5.0	5.7	1.4	8.9
Green	35.7	38.7	0	1.9

Over 70% red-ripe cherries was obtained at 100% ripe or by sequential harvesting, whereas about 35 - 39% of the harvested produce was still green when harvested at 60-80% ripe and will, therefore, eventually affect yield and cup quality (Coste, 1992b; Siebers, 1981). However, leaving cherries till 100% ripe may result in loss through berry dropping before harvest or berries turning black on the trees and also affecting cup quality thereby. Sequential harvesting therefore appears to be the best operational option except that it is expensive (Ampofo and Osei-Bonsu, 1988; Wrigley, 1988). A better determination of the stage of ripening at 80% may reduce the green berry fraction, increase the red cherry proportion and increase operational efficiency of harvesting robusta coffee.

SUMMARY

Experiments on harvesting coffee at the Cocoa Research Institute studied the effects of the frequency of harvesting and the stage of coffee crop ripening on the efficiency and quality of harvest. Up to 12% of the crop drop and can be lost before or during the harvest operation. A further 4% crop can be lost by leaving coffee till 100% ripe. Whilst the black berry portion of the crop increases (up to 27%) by delaying harvesting, as much as 38% of the crop can be picked green by improperly assessing the stage of ripening. The highest proportion of red-ripe cherries is always obtained by sequential harvesting or at 100% ripe. However the economics of sequential harvesting suggests that it is inefficient and a better criterion for determining the stage at which to harvest other than at 100% ripe may have to be found.

Resumé

Des expériences sur la récolte du café a l' Institut de Recherche sur le Cacao ont étudié les effets de la fréquence de la récolte et du stade de la maturité du café sur l'efficacité et la qualité de la récolte.

Le pourcentage de la récolte peut baisser de 12% et être perdu avant ou pentant l' opération. En laissant le café mûrir jusqu' à 100%, on peut en perdre 4% de plus. Alors que la part de baie noire de la culture augmente (jusqu' à 27%) en retardant la récolte, on peut cueillis jusqu' à 38% de baies vertes si on calcule in-correctement le stade de maturité. La plus grande proportion de baies rouges est toujours obtenue par récolte séquentielle ou a 100% de maturité. Cependant l'économie de la récolte séquentielle suggère que ce procédé est inefficace et qu' il faudrait trouver un meilleurcritère pour déterminer le stade auquel on récolte, autrement qu' à 100% de maturité.

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The authors are sincerely grateful to Messrs. L. K. Akuffo, J. N. Lartey and the other Technical staff of CRIG Sub-station, Bunso for their assistance in running this trial. Many thanks are also due to Mrs. Esther Ankrah for pains takingly preparing this paper. This paper is published by kind permission of the Executive Director, Cocoa Research Institute, Tafo.

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ANTHRACNOSE DES BAIES DU CAFÉIER ARABICA AU CAMEROUN : ESTIMATION DU DÉLAI DE CHUTE DES BAIES MALADES

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Introduction

La sensibilité des baies au CBD varie au cours de leur développement. C'est entre le 2ᵉ et le 4ᵉ mois après floraison que les jeunes baies vertes sont le plus sensible. C'est à ce moment que l'on observe les symptômes caractéristiques de la forme active de la maladie (lésions déprimées de couleur brun-foncé, envahissant progressivement toute la baie et entraînant sa chute). En fin de campagne, on observe une nouvelle période de sensibilité, mais c'est la forme "scab", sans réel impact économique, qui sévit. Comme il y a peu d'indications sur le délai entre l'apparition des symptômes et la chute, nous avons tenté, par l'analyse des corrélations entre baies malades et chutes, de l'estimer.

Matériel et méthodes

Ces données ont été recueillies lors d'une étude d'évaluation de nouveaux produits fongicides en plantation paysanne. Pour chaque produit, 100 arbres ont été tirés aléatoirement. Sur chaque arbre, 5 branches ont été observées toutes les semaines entre le 2ᵉ et le 4ᵉ mois. A chaque passage ont été relevés 1) le nombre total de baies, 2) le nombre de baies présentant des symptômes. Nous avons ainsi pu analyser la corrélation entre le nombre de baies malades à une semaine donnée et les chutes observées ultérieurement.

Résultats

I. Evolution des effectifs

Cf. graphes 1 et 2.
Le traitement à l'EXP 02314 A a permis de sauvegarder 2 fois plus de baies que sur le témoin.

II. Estimation du délai de chute

A. Témoin non traité
Cf graphe n°3

Entre la 11e et la 16e semaine, on peut expliquer plus de la moitié (de 50 à 62%) des chutes par le nombre de baies malades à la semaine précédente. La majorité des baies atteintes doit
donc chuter avec un délai de l'ordre d'une semaine. On n'observe pas de chutes notables de baies atteintes depuis plus de 2 semaines. A partir de la 17e semaine, en cumulant les chutes sur 2 à 3 semaines suivant l'observation de baies malades, on peut expliquer de 50 à 68% des chutes. Le délai moyen, au cours de cette période, peut être estimé à 3 semaines +/- 1. Surtout vers la fin des observations, on observe des corrélations très hautement significatives entre les chutes et les effectifs de baies malades de semaines éloignées (jusqu'à 5 semaines).

B. Traitement à l'EXP 02314 A (traitement le plus efficace).

Cf graphe n°4
On peut estimer le délai moyen de chute, entre la 10e et la 16e, à une semaine environ. Mais on note des corrélations très hautement significatives avec des baies atteintes depuis 2 semaines dès la 12e semaine et depuis 5 semaines dès la 16e semaine. A partir de la 17e semaine, les chutes ne peuvent être corrélées préférentiellement avec les baies malades d'une semaine donnée. On observe au contraire des corrélations très hautement significatives avec les baies malades de nombreuses semaines antérieures y compris de semaines très éloignées (il existe encore une corrélation significative à $P<0.05$ entre les chutes enregistrées à la 23e semaine et les baies malades à la 11e).
Vers la 17e semaine, les chutes proviennent très probablement de baies atteintes depuis 1 à 5 semaines; et depuis 1 à 8 semaines à la fin de nos observations.

C. Chutes précoces entre la 8e et la 10e semaine.

Lors des premières semaines de comptages, on enregistre des chutes qui ne peuvent être expliquées par nos observations. Ces chutes peuvent avoir une cause physiologique ou être dues à des insectes. Cependant le fait que 1) ces chutes sont beaucoup plus importantes sur le témoin que sur les arbres traités et 2) que l'on observe des corrélations plus fortes et plus tôt sur le traitement à l'EXP 02314 A, laissent supposer que ces chutes importantes seraient causées,au moins en partie, par le CBD mais évoluant tellement rapidement que les comptages hebdomadaires ne permettent pas de noter la présence de symptômes; soit parce que l'évolution est très rapide en raison du faible volume des baies, soit parce que la visualisation de symptômes est très difficile.

<u>Conclusions</u>

Cette étude permet de proposer le modèle suivant pour le délai symptômes-chutes:
- moins d'une semaine jusqu'aux chutes de la 10e semaine
- de l'ordre d'une semaine jusqu'à la 16e semaine
- augmentation progressive jusqu'à 4 semaines vers le 6e mois, avec une part non négligeable de baies atteintes depuis 5 semaines.

Le meilleur traitement fongicide a:
- diminué très probablement de manière importante le nombre de baies infectées
- ralenti nettement le développement du champignon dans les baies infectées (le délai peut atteindre 8 semaines voire plus).

Résumé

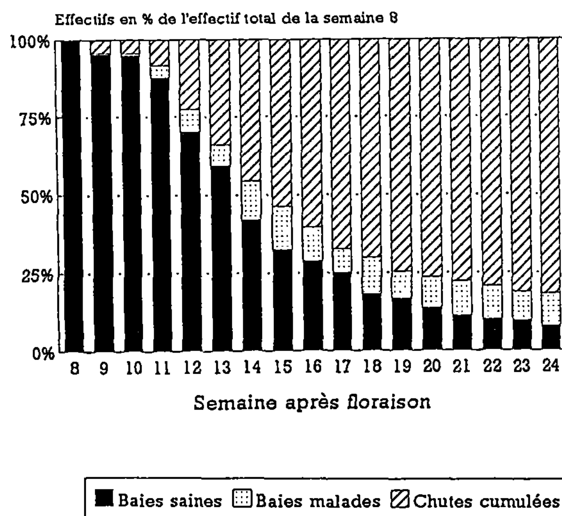
L'anthracnose des baies du caféier arabica (Coffee Berry Disease ou CBD), due à Colletotrichum coffeanum Noack sensu Hindorf, est une maladie localisée, jusqu'à maintenant, au seul continent africain. Sous des climats d'altitude frais et humides, cette affection peut provoquer des pertes de production de l'ordre de 80%; c'est le cas dans certaines parties de la caféière arabica camerounaise, constituée presque exclusivement de variétés très sensibles.

Les nombreux génotypes sauvages présents dans les collections camerounaises constituent une source potentielle d'une gamme étendue de résistance; toutefois, c'est la lutte chimique qui est actuellement toujours largement utilisée. La connaissance de l'évolution au cours du temps des populations de baies saines et malades est importante, aussi bien pour la compréhension des processus de résistance que pour l'appréciation du mode d'action de molécules fongicides. Lors de cette étude, nous avons tenté d'estimer le délai moyen entre l'apparition des symptômes et la chute des baies.

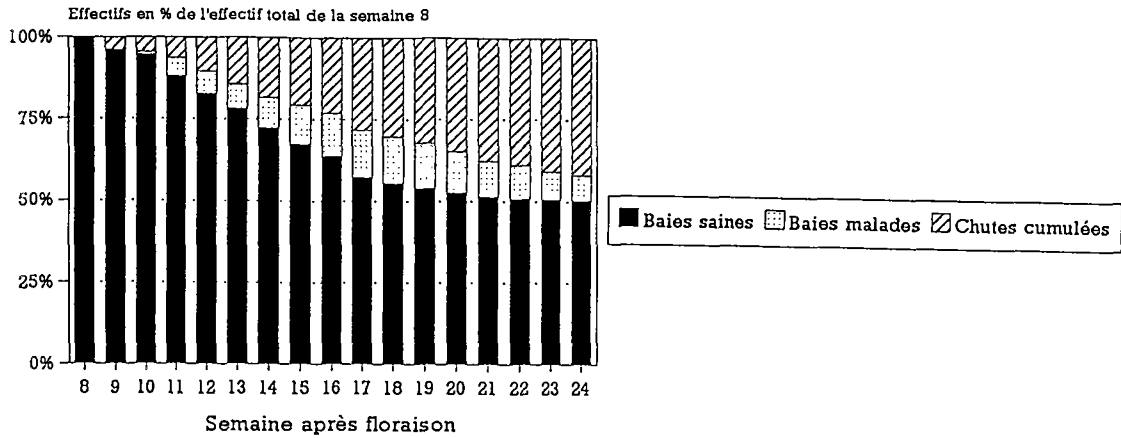
Chaque semaine, nous avons relevé le nombre de baies saines et atteintes de CBD. Le délai symptôme-chute a été estimé par corrélation entre le nombre de baies malades à une semaine donnée et les chutes observées les semaines suivantes.

Ce délai semble varier au cours de la saison. Dans le cas de traitements avec une molécule fongicide, il ressort que l'un des modes d'action serait de freiner le développement du pathogène ayant infesté les baies.

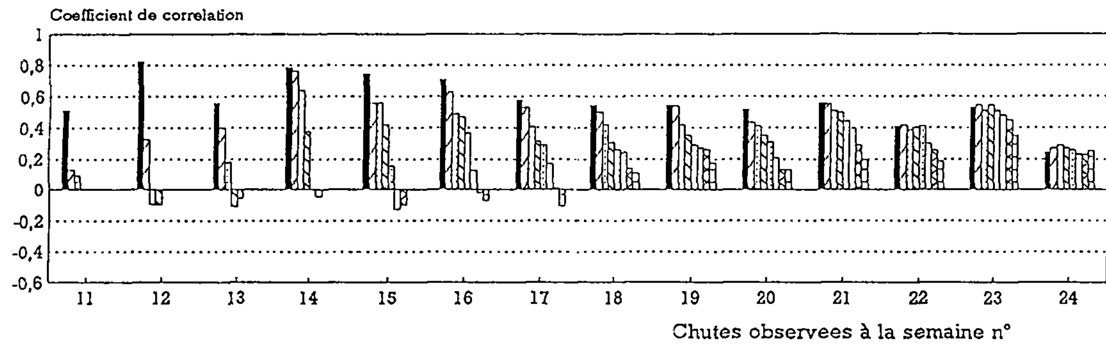
Graphique n°1: Evolution des baies; témoin non traité



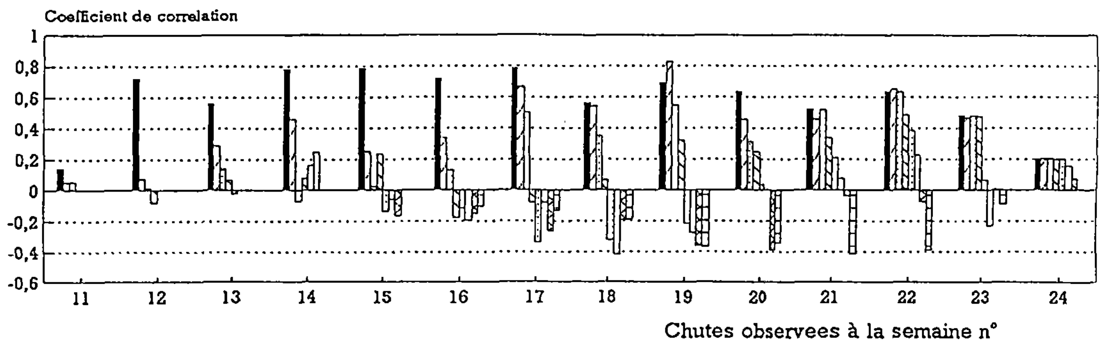
Graphique n°2: Evolution des baies; traitement à l'EXP 02314 A



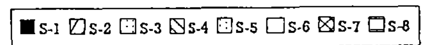
Graphique n°3: Corrélations chutes-baies malades; témoin non traité



Graphique n°4: Corrélations chutes-baies malades; traitement à l'EXP 02314 A



Corrélation avec les baies présentant des symptômes à la semaine



RECHERCHE DE TESTS PRÉCOCES DE SÉLECTION DE *COFFEA ARABICA* RÉSISTANTS À L'ANTHRACNOSE DES BAIES (CBD) SUR MATÉRIELS *IN VIVO* ET *IN VITRO*

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La relance de la production de *Coffea arabica* ne peut s'envisager au Cameroun qu'avec la diffusion de plants sélectionnés, notamment pour la résistance à l'anthracnose des baies ; la lutte chimique contre cette maladie étant toujours contraignante et coûteuse.

A partir de l'évaluation au champ depuis plusieurs années, du comportement de différentes lignées en collection, dix génotypes présentant des niveaux variés de sensibilité vis à vis du CBD ont été repérés.

SENSIBLES	MOYENNEMENT RESISTANTS	RESISTANTS
Et 2	Et 21	Et 33
Et 19	Et 1	Et 3
Et 16	Et 28	Java
Caturra		

(Et : génotypes issus d'une prospection ORSTOM.IRCC en Ethiopie)

Différents protocoles d'inoculations artificielles ont été mis en place à partir de 6 isolats de *C. coffeanum*.

MATÉRIEL *IN VIVO*

- sur baies détachées : inoculation (suspensions de conidies) d'une goutte (10 μl) à 0-10^3-10^5-10^6 spores/ml. T : 20° C et 26° C.

- sur hypocotyles : stade : 45 jours après semis (stade clou ou cotylédonnaire)
  * pulvérisation de suspensions de conidies à 10^6 spores/ml à 25° C et 20° C. Lecture à 30 jours.
  * pulvérisation de filtrats issus de culture en milieu liquide des isolats de *C. coffeanum*. Mélange v/v/v/v filtrats j+6, j+14, j+21, j+30. T : 25° C et lecture à 30 jours.

MATÉRIEL *IN VITRO*

- sur hypocotyles d'embryons zygotiques maintenus en survie en conditions stériles sur papier filtre (T:26° C, humidité saturante) :

* dépôt d'une goutte d'inoculum (suspension de conidies à 10^6 spores/ml), et lecture à 6 jours.

* immersion dans le filtrat pendant 15 mn après dessication partielle 15 mn sous hotte à flux laminaire, et lecture à 6 jours.

RÉSULTATS

Les inoculations sur baies détachées, placées à 20° C ont montré que les niveaux d'attaques obtenus au laboratoire peuvent être corrélés à ceux observés au champ.

Sur hypocotyles de plantes *in vivo*, une température de 25° C ne permet pas une expression suffisante des symptômes ; les expériences sont reprises actuellement à 20° C.

Par contre, *in vitro*, en six jours, avec des isolats pathogènes, des symptômes allant de légères nécroses à une nécrose totale de l'hypocotyle pour les génotypes moyennement résistants à sensibles a permis, sur de petits effectifs de comparer et de corréler pour un certain nombre de génotypes les réactions au laboratoire et les niveaux de sensibilité observés au champ.

Les nécroses obtenues avec les filtrats sont, pour l'instant trop importantes. Une expérimentation permettant d'évaluer les temps de dessication et d'immersion optimaux, ainsi que la concentration idéales est en cours.

RÉSUMÉ

À partir de l'évaluation du comportement au champ de 10 génotypes, des graines récoltées sur ces lignées, présentant divers niveaux de résistance à l'anthracnose des baies, ont été utilisées pour effectuer des inoculations artificielles sur les baies détachées (suspension de conidies) et sur les hypocotyles (suspensions de conidies, filtrats issus de culture en milieu liquide de *C. coffeanum*).

En parallèle, une collection d'embryons zygotiques, issus de graines de la même gamme de sensibilité vis à vis du CBD, a été mise en place afin de développer des tests d'inoculations artificielles sur hypocotyles similaires à ceux sur plantules *in vivo*.

Les inoculations de suspensions de conidies sur les baies détachées placées à 20° C et sur les hypocotyles de plantules *in vitro* maintenus en survie en conditions stériles à 25° C, ont donné en six jours les résultats les plus encourageants.

SUMMARY

Seeds harvested from 10 genotypes which had shown different levels of resistance to coffee berry disease (CBD) during field evaluation, were used in the laboratory to inoculate artificially the berries and the hypocotyles of the seedlings sewed, with suspensions of conidia and filtrates derived from liquid culture of *C. coffeanum*.

At the same time, a collection of zygotic embryos developed from seeds of the same range of sensibility to CBD has been produced in order to develop similar tests of inoculation than plantlets *in vivo*.

Inoculations, with suspensions of conidia, on detached berries at 20° C and on hypocotyles of zygotic embryos maintained under steriles conditions *in vitro* have given encouraging results in six days.

GROWTH OF THE COFFEE ORANGE RUST *HEMILEIA VASTATRIX* IN HOSTS AND NONHOSTS

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INTRODUCTION

Studies of host-parasite coevolution have designated as "hosts" only those species on which a given fungus is found to develop and reproduce on at least one plant, among those sampled (Anikster & Wahl, 1979). The biotrophs, such as the rusts, have a restricted range of hosts, frequently being limited to certain cultivars of a single plant species (Ride, 1985; Luke *et al.*, 1987). During successful infections, rusts go through complex development stages (Mendgen et al., 1988) and failure to grow or differentiate at any one of those stages is associated with the expressions of resistance of the infected plants (Heath, 1981, 1982;Ride, 1985). Thus, in resistant cultivars of host species, with some exceptions, the incompatible races of rust fungi stop growing usually after the formation of one to several haustoria (Heath, 1974; Rijo & Rodrigues, 1978; Niks,1983;Rohringer & Heitefuss, 1984; Martins *et al.*, 1985; Tiburzy *et al.*, 1990). On the contrary, nonhost plants (i.e. plant species for which a given rust fungus is not a pathogen) tipically react preventing the formation of the first haustorium by rust fungi (Heath, 1974;Niks, 1983; Wood & Heath, 1986; Hoppe & Koch, 1989). In the present study we compare histologically the stages of pre-penetration and postpenetration of the coffee orange rust on leaves of a resistant host and different nonhosts

MATERIAL AND METHODS

Infected material. Host plants of *H. vastatrix* (*Coffea arabica* L.-CIFC 110/5), and the nonhost species *Ixora javanica* L., *Vigna sinensis* L., *Phaseolus vulgaris* L. and *Arachis hypogaea* L.were maintained in the greenhouse conditions. The leaves (lower epidermis) were inoculated with uredospores of *H.vastatrix* (culture 1427-race II) according to the technique described by D'Oliveira (1954-57) and D'Oliveira & Rodrigues (1961).

Microscopic observations: 1) Germination "*in vivo*" and appressoria formation - following the technique described by Silva *et al.* (1985), the percentages of germination "*in vivo*" and of appressoria formed over and outside the stomata were evaluated; 2) Fungus colonization process - it was observed in cross section of infected leaf fragments made with a freezing microtome, stained and mounted with cotton blue in lactophenol (Rijo & Rodrigues, 1978). At different times after inoculation, in 200 infection sites the growth stages of the fungus reached (stage 1 = appressorium; stage 2 = penetration hypha; stage 3 = anchor; stage 4 = haustorial mother cell (HMC)) were evaluated. In each infection, the lenght of all the hyphae within the leaves was measured with a micrometric eye-piece; in order to obtain the mean value, 50 infections per plant were considered as a minimum in each day of observation. The number of haustoria per infection (HMC stage) was also evaluated. The microscopic observations were made with a microscope Leitz Dialux 20.

Statistical analysis. It was accomplished with variance analysis (ANOVA) and arcsine transformation was performed for percentage data. If a significant difference was found, Scheffe's test was used for mean comparisons.

RESULTS

Germination and appressoria formation

About 24 hours after the inoculation, the percentage of germinated uredospores was similar in the resistant host *C.arabica* and in the nonhost *I. javanica, V. sinensis, P.vulgaris* and *A. hypogaea* (Table 1). The same was observed regarding the percentage of appressoria formed over the stomata, the sole exception being the nonhost *A.hypogaea* where that value was significantly lower (Table 1). On the other hand, the percentage of uredospores which differentiated appressoria outside the stomatal areas was very much reduced and identical in the different plants under study (Table 1).

Table 1. Percentage of germinated uredospores "in vivo" and of appressoria formed (over and outside stomata) by *H. vastatrix* (culture 1427) on leaves of host and nonhost plants, 24h after inoculation.

Plant species	Germination %	Appressoria	
		over stomata (%)	outside stomata (%)
C. arabica (H)*	63.00 a	53.75 a	3.25 a
I. javanica	61.00 a	47.50 a	4.00 a
V. sinensis	58.50 a	37.00 a	4.75 a
P. vulgaris	56.00 a	36.50 a	3.75 a
A. hypogaea	51.00 a	9.75 b	3.25 a

* (H) represents the host specie
Values in the same column followed by different letters differed significantly according to Scheffe's test P ≤ 0.05

Fungus colonization

The plants of *C. arabica* resistant to the fungus and the nonhost *I.javanica* were those which presented stronger similarity regarding hyphal growth at the postpenetration stage. In fact, at precisely 24 hours after the inoculation, it was not possible to distinguish these species when the different stages of fungus growth and hyphae length within the leaf tissues where analysed (Table 2). The same was observed when the haustoria formation was studied (Table 3). Thus, in both species, the percentage of appressoria that maintained full was lower than 25%, what means that more than 75% of penetrations took place. The subsequent growth of the fungus ceased in different stages the most representative of which was the stage of HMC in general with 3 haustoria (in the subsidiary cells and first layer of the spongy parenchyma) - (Fig.1 and 2).

In *V. sinensis* and *P. vulgaris* 40% of the formed appressoria remained full or, in other words, there was 60% penetrations. These values were significantly lower than those observed with the host *C. arabica* and the nonhost *I. javanica.* However, these four species did not differentiate from each other from the first and second days after the inoculation, when the hyphal growth and the stages of fungus growth were evaluated (Table 2). In *V. sinensis* and *P.vulgaris* the infections that penetrated aborted in different stages (Figs.3 and 4) with more frequency in the stage of HMC with formation of 3 haustoria, similarly to what happened with *C. arabica* and *I. javanica* (Tables 2 and 3).

In *A. hypogaea,* the fungus presented a behaviour different from that observed in the other species under study (Tables 2 and 3).In *A. hypogaea* most of the appressoria remained full (88 % at 4 and 7 days after inoculation) and only 1% of the reduced number of penetrations reached the stage of HMC without haustoria formation (Fig.5).

The hyphal growth of *H. vastatrix* in the studied species (Fig. 6) was based in the mean growth values of the hyphae as shown in Table 2. As it was already referred, the penetrations of the fungus in both host and nonhosts ceased growth in different stages, showing senescent hyphae particularly evident from the third day after inoculation.

Table 2. Fungal growth (culture 1427), in different times after inoculation, in host and nonhost leaves

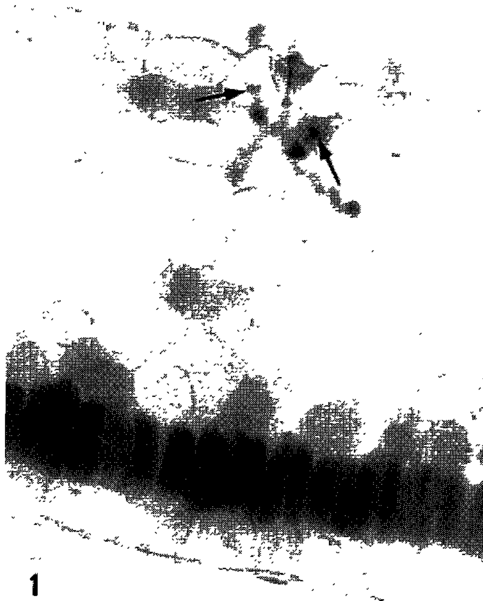
Days after inoculation	Plant species	Frequency (%) of fungal growth stages: Apressorium 1	Penetration hypha 2	Anchor 3	HMC 4	$\bar{x}_w$ [@]	Hyphal length (µm) inside tissue $\bar{x} \pm$ SD[#]
1	*C. arabica* (H)*	33.0	45.5	21.0	1.0	1.90 a	30.3 ± 18.3 a
	I. javanica	31.5	37.5	29.0	2.0	2.02 a	38.6 ± 16.6 a
	V. sinensis	60.5	30.0	9.5	0.0	1.49 b	31.4 ± 24.9 a
	P. vulgaris	62 0	23.5	10.5	4.0	1.57 b	37.1 ± 16.5 a
	A. hypogaea	90.0	9.5	0.5	0.0	1.11 c	11.4 ± 5.0 b
2	*C. arabica* (H)*	24.0	31.0	20.5	24.5	2.46 a	41.7 ± 22.2 a
	I. javanica	24.5	22.5	23.0	30.0	2.59 a	48.1 ± 20.8 a
	V. sinensis	40.0	18.5	24.5	17.0	2.19 a	46.6 ± 24.1 a
	P. vulgaris	41.0	20.0	21.0	18.0	2.16 a	45.6 ± 23.3 a
	A. hypogaea	89.5	9.0	1.0	0.5	1.13 b	12.5 ± 6.8 b
3	*C. arabica* (H)*	24.0	26.0	19.0	31.0	2.57 a	53.2 ± 39.5 a
	I. javanica	22.5	17.5	21.0	39.0	2.77 a	55.7 ± 26.7 a
	V. sinensis	40.0	18.0	20.0	22.0	2.24 a	53.4 ± 24.0 a
	P. vulgaris	40.0	18.5	18.5	23.0	2.25 a	52.9 ± 25.1 a
	A. hypogaea	89.0	9.0	1.5	0.5	1.14 b	14.0 ± 8.6 b
4	*C. arabica* (H)*	24.0	25.5	18.0	33.5	2.63 a	55.9 ± 46.2 a
	I. javanica	22.5	15.0	20.0	42.5	2.83 a	61.9 ± 25.1 a
	V. sinensis	40.0	18.0	16.5	25.5	2.28 a	58.1 ± 29.0 a
	P. vulgaris	40.0	18.0	18.5	23.5	2.26 a	54.7 ± 26.9 a
	A. hypogaea	88.0	9.0	2 0	1.0	1.16 b	14.3 ± 10.1 b
7	*C. arabica* (H)*	22.5	25.5	18.0	34.0	2 64 a	59.3 ± 56.0 a
	I. javanica	22.0	15.0	18.5	44.5	2 86 a	63.8 ± 46.5 a
	V. sinensis	40.0	17.5	17.0	25.5	2.28 a	59.5 ± 33.3 a
	P. vulgaris	40.0	18 0	18.0	24.0	2.26 a	56.5 ± 25.1 a
	A. hypogaea	88.0	8.0	3.0	1.0	1.17 b	15.4 ± 13.4 b

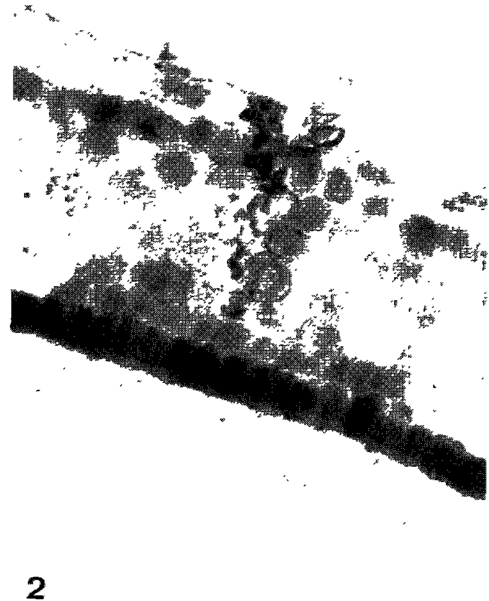
* (H) represents the host specie.
@ $\bar{x}_w$ = weighted average.
# $\bar{x}$±SD = mean ± standard deviation.
Values in the same sub-column followed by different letters differed significantly according to Scheffe's test at P≤0.05.

Table 3. Haustoria formation of ***H. vastatrix*** (culture 1247) in host and nonhost leaves, 7 days after inoculation

Plant species	Maximum stage growth (frequency)	% HMC with at least one haustotrium observed	N° of haustoria more frequently observed
C. arabica (H)*	HMC (> 24-25 %)	97.1 a	3
I. javanica	HMC "	96.6 a	3
V. sinensis	HMC "	52.9 b	3
P.vulgaris	HMC "	47.9 b	3
A. hypogaea	HMC (1%)	0.0 c	0

*(H) represents the host specie.





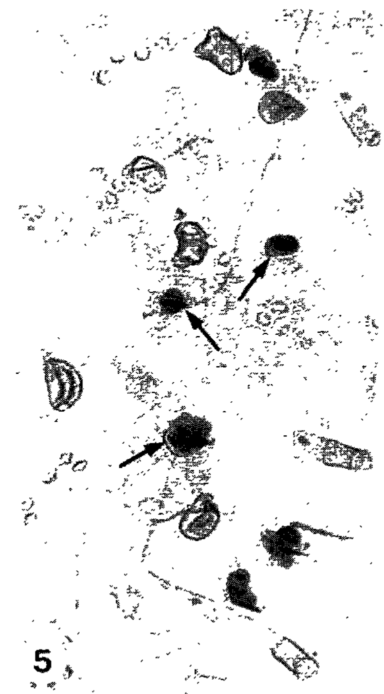


Fig 1. ***C. arabica*** - Senescent mycelium with haustoria (arrows) - (x 400); Fig.2. ***I. javanica*** - Senescent mycelium in the mesophyll (x 250); Fig. 3. ***V. sinensis*** - Senescent mycelium in the first layers of the mesophyll with a visible haustorium (arrow) - (x 250); Fig. 4. ***P. vulgaris*** - Two infection sites with mycelium (surface view) - (x 570); Fig. 5. ***A. hypogaea*** - Appressoria (arrows) over the stomata on the leaf surface (x 250).

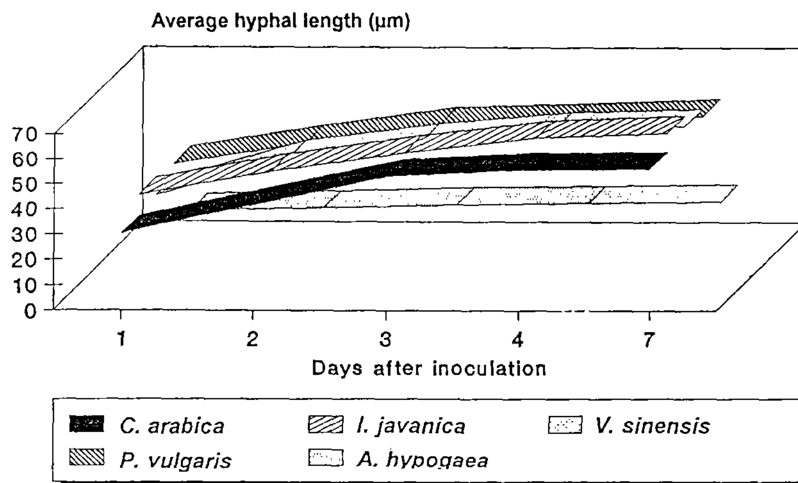


Fig. 6. Hyphal growth of *H. vastatrix* (culture 1427) in host and nonhost leaves.

DISCUSSION AND CONCLUSIONS

Several studies indicate that the defense mechanisms that influence the pre-penetration stages of the rusts have stronger expression in the nonhosts. In fact, considering that each rust should be more adapted to the leaf surface of the host species, it might happen that in the nonhosts they find unfavofable conditions to the uredospore germination and appressoria formation which might be formed in the wrong site (Heath, 1974, 1977; Wynn, 1976; Luke *et al.*, 1987). In the present work, the precent germination of uredospores of *H. vastatrix* was similar in the host plants of *C. arabica* resistant to the rust and in the different nonhosts. The same happened in relation to the percentage of appressoria formed over the stomata with exception of the nonhost *A. hypogaea* where this value was much lower.Regarding the appressoria not formed over the stomata, their percentage values were always very reduced and similar in the hosts and nonhosts. Heath (1977) and Ride (1985) defend that the lesser rust development of the rusts on the nonhost leaf surfaces, although may be important, it does not explain tottaly the resistance of the nonhosts. In fact, in all the nonhost rust interactions occur at least some attempts of penetration by the pathogen (Ride, 1985) and in opposition to the host plants, the defense mechanisms of the nonhosts act before the formation of the first haustorium (Leath & Rowell, 1966; Heath, 1974, 1977; Niks, 1983; Hoppe & Koch, 1989). In the present study, in the resistant host most of the appressoria lead to penetration of the leaf tissues but the fungus stoped its growth with higher frequency in the stage of HMC with formation of 3 haustoria in different cells.

It is worth of mention that the fungus presented in the nonhosts *V. sinensis, P. vulgaris* and particularly in *I. javanica* a behaviour similar to that of the host *C. arabica* what suggests that the defense mechanisms of these species acted mainly after the formation of the first haustorium. Thus, among the studied species only in *A. hypogaea* the fungus aborted in incipient stages of infection, previously to the haustoria formation, getting closer to what is generally referred as a characteristic of the nonhosts resistance expression. The most restricted growth of the fungus, both in the pre-penetration and postpenetration stages allows to conclude that the defense mechanisms of *A. hypogaea* acted more quickly than in the resistant host and remaining nonhosts.

ABSTRACT

The germination, appressoria formation and growth of *Hemileia vastatrix* in leaf tissues of resistant *Coffea arabica* plants as well as in nonhost species such as *Ixora javanica*, *Vigna sinensis*, *Phaseolus vulgaris* and *Arachis hypogaea* were studied.The germination ability of the fungus was good on host and nonhost and the percentage of apressoria formation over the stomata was equally good with exception of *A. hypogaea* where it was significantly lower. The percentage of full appressoria was lower in *C.arabica* and *I. javanica* (< 25%), followed by *V. sinensis* and *P. vulgaris* (40%) and by *A. hypogaea* (88%). In the host resistant plants and in *I. javanica, V. sinensis* and *P. vulgaris* the fungus ceased growth in different stages of infection with higher frequency in the stage of haustorial moyher cell (HMC) with 3 haustoria. In *A. hypogaea* only 1% of the infections reached the stage of HMC but without haustoria formation. The most restricted growth of the fungus in *A. hypogaea* suggests a quicker expression of resistance in this species than in the resistant hosts and remaining nonhosts.

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CHEMICAL CONSTITUENTS OF UREDOSPORES OF *HEMILEIA VASTATRIX*

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INTRODUCTION

Coffee-growing has been affected by many problems, one of the most serious being infection by orange rust, *Hemileia vastatrix* B. &Br.. Knowledge of the chemical constituents of coffee rust may be of value in developing strategies for the protection of the coffee plant from this fungus. Cell-cell recognition among host cells and pathogenic microorganisms depends probably on their surface composition.

Although it is well known that steroids are required for growth and sexual reproduction of many species of fungi (Hendrix, 1970), little is known about their role in host-parasite interactions between fungi and higher plants.

Cell-wall composition of *H. vastatrix* was studied in order to understand the plant-fungus association.

DISCUSSION

A common response of plants, often associated with disease resistance, is localized and rapid death of plant cells at the infection site. This reaction, referred to as hypersensitive response (HR), includes the accummulation of phytoalexins (Michael and Kuc', 1987). In *Hemileia vastatrix* interactions, different races of the pathogen can be distinguished by the type of response they elicit in different coffee cultivars.

It has been suggested (Kuc', 1982) that fungal metabolites, even in cultivar pathogenic races, can elicit resistance. Bostock *et al.* (1981) demonstrated that the polyunsaturated C20 fatty acids eicosapentaenoic and arachidonic acids extracted from *Phytophthora infestans* elicit the accummulation of high levels of sesquiterpenoid stress metabolites that are considered phytoalexins.

In cell walls isolated from uredospores of *H. vastatrix*, it was found (Maxemiuc-Naccache and Dietrich,1981) lipids are the predominant wall components, along with polysaccharides, proteins and phosphate. Since sterols are

important components of all membrane lipids (Thompson, 1965), it was of interest to determine their identity in host and parasite so as to follow changes in sterol composition of the host-parasite complex during disease development and test lipid fractions of rust uredospores for biological activity.

We have already reported (Tavares, 1985) the presence in uredospores of *H. vastatrix* some oxygenated fatty acids, including 9,10-*cis*-epoxyoctadecanoic acid, in agreement with previous reports by Tulloch (1962), and treo-dihydroxyoctadecanoic, which could arise at least in part from hydrolysis of the epoxy acid (Tulloch, 1963), and smaller amounts of hexadecanoic, octadeca-9,12-dienoic and octadecanoic acids.

Further study of the chemicals of this rust fungus yielded a complex mixture of sterols, whose structures were determined by spectroscopic methods and comparison with literature data (Rubinstein, 1976; Knights, 1967; Djerassi, 1978; Yokoyama, 1975). In race I uredospores the major sterol is 24-ethyl-5α-cholesta--7,24(28)(*Z*)-dien-3β-ol (**1**), while in race III the predominant sterol is 24-ethyl-5α-cholesta-7,22(*E*)-dien-3β-ol (**2**).

Traces of **2**, 24-methyl-5α-cholest-7-en-3β-ol (**3**) and 24-ethyl-5α-cholest--7-en-3β-ol (**4**) were identified in race I uredospores, while race III contained traces of both **3** and **4**, but not **1**.

Both compounds **3** and **4** have been identified in other rust fungi (*Basidiomycetes, Uredinales*) (Turner, 1983; Weete, 1980). Jackson and Frear (1968) identified the Δ^7 and $\Delta^{7,24(28)}$ sterols in flax rust (*Melampsora lini*) uredospores and small amounts of the $\Delta^{5,7}$ isomer. Lin *et al.* (1972, 1974) identified the $\Delta^{7,24(28)}$ C_{29} sterol as the main sterol of *Uromyces phaseoli* uredospores, together with smaller amounts of the corresponding Δ^7 C_{29} isomer.

Weete (1977) proposed that rust uredospores could fall into one of two types, according to sterol composition: one type containing predominantely 24-ethyl-5α-cholest-7-en-3β-ol (**4**), the other 24-ethyl-5α-cholesta-7,24(28)--dien-3β-ol (**1**).

This is the first time sterols **1**, **2**, **3** and **4** are reported in *H. vastatrix* uredospores. Ours is the first report of the occurrence of 24-ethyl-5α-cholesta-7,22(*E*)-dien-3β-ol (**2**) in rust fungi. Although previous reports (Weete, 1974) refer the presence of a diunsaturated C_{29} sterol in two rust species (*Puccinia graminis* var. *tritici* and *P. striiformis*), the exact location of the double bond was not determined.

The most significant differences between rust spores and other spores is the abundance of Δ^7 C_{29} sterols and the absence of ergosterol in the rusts (Weete, 1980).

Although these compounds are minor sterols of some higher plants, the fact that the "typical" higher plant sterols cholesterol, campesterol, stigmasterol and sitosterol are absent from the spores suggests that these rust sterols are true fungal products. This is further confirmed by their absence in healthy leaves of the host-plant, the same being observed for the major fatty acids isolated from rust.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES. - Melting points were determined on a Reïchert Thermovar melting point apparatus and are uncorrected. Nmr spectra were recorded in $CDCl_3$ solution on a Brucker C_XP (300 MHz) spectrometer using TMS as internal standard. Ir spectra were recorded Perkin-Elmer 298 spectrophotometer in KBr. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Mass spectra were determined with a Kratos MS-25 RF spectrometer at 70 eV. For GC-MS a Carlo Erba Mega HRGC 5160 gas chromatograph fitted with a 26 m x 0.32 mm Chrompack CP Sil 5 0.12 µm film thickness capillary column was used with the following temperature program: 190°C (10 min), 240°C (4°C/min, 5 min), 265°C (5°C/min, 5 min), 285°C, (5°C/min, 30 min). Tlc was carried out on precoated Si gel 60 F254 sheets (Merck) and detection was achieved by spraying with 5% ethanolic phosphomolibdic acid followed by conc. H_2SO_4 and heating at 100°C. Solvents were purified and dried by standard methods. Petrol refers to the fraction b.p. 40-60°C and ether refers to diethyl ether. Extracts were dried over anhydrous sodium or magnesium sulphate.

MATERIAL. - Leaves of *Coffea arabica* L. cv. Caturra (CIFC 1637) were infected with uredospores isolates 1285 and 995 respectively race I and III of *H. vastatrix*. On susceptible plants in optimum conditions of temperature and light, initial symptoms appear as a light green clorotic 10 to 15 days after innocculation and uredospores are produced from them approximately two weeks later.

Uredospores of *H. vastatrix* races I and III were collected from infected leaves and stored in capsules of gelatin, in a dessicator at 0-5°C and 50% humidity.

EXTRACTION. - Uredospores of *H. vastatrix* (3.15 g race I; 3.23 g race III) were extracted successively at r.t. with solvents of increasing polarity (pet. ether, CH_2Cl_2, MeOH), to afford complex mixtures of mono-, di-, tri-glycerides and carotenoids. The uredospores residues after extraction were treated with 1N methanolic NaOH for 24 h at r.t.. The mixture was filtered and the filtrate, upon removal of MeOH, extracted with CH_2Cl_2 (3 x 20 ml). The combined extracts were dried and concentrated to dryness at reduced pressure to give residues (21.9 mg, 0,7% fr.w. for race I; 32.8 mg, 1.0% fr.w. for race III), which were mainly composed of sterols.

The aqueous phase was neutralised with 1N HCl and extracted with CH_2Cl_2 (3 x 20 ml). The combined extracts were dried and concentrated to dryness at reduced pressure to give residues (114.3 mg, 3.6% fr.w. for race I; 104.6 mg, 3.3% fr.w. for race III), which were mainly composed of fatty acids.

ISOLATION AND IDENTIFICATION OF FATTY ACIDS. - Extracts rich in fatty acids were subject to fractional crystallization to afford *treo*-9,10-dihydroxyoctadecanoic and *cis*-9,10-epoxyoctadecanoic.

treo-9,10-Dihydroxyoctadecanoic (71.2 mg; 1.9% fr.w.), white crystals from AcOEt/MeOH, m.p. 92-98°C [Tulloch (1960) 95-96°C], $[\alpha]^{20}$ +2.36 (CH_2Cl_2, *c* 0.081) [Tulloch (1960) $[\alpha]^{20}$ +1.61], υ_{max} 1720 (C=O) cm^{-1}; ^{1}H-NMR δ 0.88 (3H, *t*, C-CH_3), 1.28 (20 H, 2*s*, CH_2), 1.48 (4H, *m*, C<u>H</u>$_2$-CH(OH)-CH(OH)-C<u>H</u>$_2$), 1.63 (2H, *m*, β-CH_2), 2.34 (2H, *t*, α-CH_2), 1.92 (2H, *s*, 2xOH, exch. D_2O), 3.41 (2H, *s*, C<u>H</u>(OH)-C<u>H</u>(OH)), m/e 332, 259, 215, 155, 147, 129, 103, 73; identical with an authentic sample.

cis-9,10-Epoxyoctadecanoic (19.4 mg, 0.53% fr.w.), white crystals from AcOEt/pet.ether, m.p. 53-56°C, $[\alpha]^{20}$ + 11.1 ($CHCl_3$, *c* 0.09) [Tulloch (1960) $[\alpha]^{20}$ +23.5 (MeOH, *c* 0.5)], υ_{max} 1690 (C=O) , 845 (CH-CH) cm^{-1}, ^{1}H-NMR δ 0.88 (3H, *m*, C-CH_3), 1.27, 1.32 (20 H, 2*s*, CH_2), 1.50 (4H, *m*, C<u>H</u>$_2$-CH-CH-C<u>H</u>$_2$), 1.57-1.68 (2H, *m*, β-CH_2), 2.35 (2H, *t*, α-CH_2), 2.88 (2H, *m*, CH-CH), m/z 312, 199, 187, 171, 155, 153, 143, 139, 127, 109, 83, 74, 55, 41; identical with an authentic sample.

GC/MS analysis of the mother licquors showed the presence of hexadecanoic, octadeca-9,12-dienoic and octadecanoic, present in identical % in both race I and III.

<u>Hexadecanoic</u>, m/z 270 (M^+), 241, (M^+-29), 239 (M^+-31), 227 (M^+-43), 199, 185, 171, 157, 143, 129, 115, 101, 97, 87, 74, 73, 59, 57, 55, 43.

<u>Octadeca-9,12-dienoic</u>, m/z 294 (M^+), 263 (M^+-31), 220 (M^+-74), 165, 151, 137, 123, 109, 95, 81, 74, 67, 55, 43, 41.

<u>Octadecanoic</u>, m/z 298 (M^+), 267 (M^+-31), 255 (M^+-43), 241, 227, 213, 199, 185, 171, 157, 143, 129, 101, 97, 87, 85, 74, 71, 69, 67, 57, 55, 43, 41.

ISOLATION AND IDENTIFICATION OF STEROLS. - Extracts rich in sterols were subject to fractionation by cc and tlc with CH_2Cl_2-MeOH (90:1, v/v) to afford 24-ethyl-5α-cholesta-7,24(28)(Z)-dien-3β-ol (2.5 mg, 0.07% fr.w.) for race I and

24-ethyl-5α-cholesta-7,22(E)-dien-3β-ol (2.3 mg, 0.07% fr.w.) for race III.

24-Ethyl-5α-cholesta-7,24(28)(*Z*)-dien-3β-ol (**1**) (2.5 mg, 0.07% fr.w.), ^{1}H-NMR δ 0.53 (*s,* 18-CH_3), 0.80 (*s,* 19-CH_3), 0.93 (*d,* 21-CH_3), 0.85 (*d,* 26-CH_3), 0.82 (*d,* 27-CH_3), 0.88 (*d,* 29-CH_3), 1.04 (*m,* 28-CH_3), 2.75 (*m,* 25-CH); ei/ms m/z 412 (M^+), 314, 271, 255, 231; ei/ms after silylation m/z 484 (M^+), 386, 346, 379, 255, 213.

24-Ethyl-5α-cholesta-7,22(*E*)-dien-3β-ol (**2**) (2.3 mg, 0.07% fr.w.), ^{1}H-NMR δ 0.55 (*s,* 18-CH_3), 0.80 (*s,* 19-CH_3), 0.93 (*d,* 21-CH_3), 0.84 (*d,* 26-CH_3), 0.81 (*d,* 27-CH_3), 0.80-0.87 (*m,* 29-CH_3), 1.03 (*d, J*=6,3, 21-CH_3); ei/ms m/z 412 (M^+), 397, 369, 351, 309, 300, 299, 282, 273, 271, 255, 252, 246, 231, 229, 213; ei/ms after silylation m/z 484 (M^+), 469, 441, 394, 379, 372, 357, 343, 255, 229, 213.

GC/MS analysis of the mother licquors showed the presence of traces of 24-methyl-5α-cholest-7-en-3β-ol and 24-ethyl-5α-cholest-7-en-3β-ol in both races I and III.

24-Methyl-5α-cholest-7-en-3β-ol (**3**), ei/ms m/z 400 (M^+), 385, 367, 309, 299, 300, 271, 255, 231; ei/ms after silylation m/z 472 (M^+), 457, 382, 367, 345, 343, 318, 255, 229, 213.

24-Ethyl-5α-cholest-7-en-3β-ol (**4**), ei/ms m/z 414 (M^+), 399, 396, 384, 355, 340, 299, 273, 271, 255, 253, 246, 245, 231, 299, 223, 213; ei/ms after silylation m/z 486 (M^+) 471, 396, 381, 345, 303, 267, 255, 299, 213.

SUMMARY:

Occurrence of sterols 24-ethyl-5α-cholesta-7,24(28)(*Z*)-dien-3β-ol (**1**), 24-ethyl-5α--cholesta-7,22(*E*)-dien-3β-ol (**2**), 24-methyl-5α-cholest-7-en-3β-ol (**3**), and 24-ethyl--5α-cholest-7-en-3β-ol (**4**) is reported for the first time in *H. vastatrix* uredospores. This is also the first report of the occurrence of (**2**) in rust fungi. That these rust sterols are likely to be true fungal products is suggested by their absence in healthy leaves of the host-plant.

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DEVELOPMENT OF STRATEGIES TO CONTROL COFFEE LEAF RUST IN PAPUA NEW GUINEA

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Abstract

Coffee Leaf Rust became established in the main coffee areas of Papua New Guinea in 1986. As a prerequisite to the development of a chemical control strategy, the epidemiology of coffee leaf rust was monitored at seven locations on the Highlands of Papua New Guinea at altitudes ranging from 1,410 m to 1,880 m.

The pattern of the disease development was similar for all sites. The disease incidence was low during the October to February period and thereafter increased to reach a maximum in May/June/July, after which time the disease decreased.

The maximum disease incidence decreased with increasing altitudes. This was particularly apparent at sites higher than 1,600 m above sea level where the level of the disease did not warrant the application of fungicides.

The severity of coffee leaf rust at different altitudes was shown to be correlated with temperature. Monthly mean temperatures of 15°C or below in January or February significantly suppressed disease development in May/June. This data was used to design experiments to develop strategic spray schedules. Data from two seasons research showed disease is effectively controlled by three applications of copper oxide in January, February and March.

Introduction

Papua New Guinea is an island in the South Pacific with a population of approximately 3.5 million people. The country relies on coffee as a major export crop which contributes to the both the local and national economies.

Most of the arabica coffee is grown on the Highlands extending from Yonki in the east to Mendi in the west at altitudes ranging from 1,200 m to 1,900 m.

In 1986 a new and widespread outbreak of coffee leaf rust was discovered which rapidly spread throughout the Highlands. Prior to this, coffee leaf rust was not considered endemic in Papua New Guinea.

A research program was established to study the development of the disease throughout the Highlands and also to develop cost effective methods for disease control.

Materials and Methods

1. *Epidemiology*

Detailed measurements on the development of coffee leaf rust and the associated influence of weather on the disease were made at monthly intervals.

Experiments were established at seven locations at altitudes ranging from 1,410 m to 1,880 m. Seasonal development of the disease was monitored on 24 selected branches at each site, and correlated with the rainfall and temperature data recorded at each site.

2. *Control of Coffee Leaf Rust*

Based on the information from the disease development studies, copper oxide treatments were applied to coffee trees in a factorial experiment in January, February, March, April and May at three sites in the main coffee growing areas of Papua New Guinea.

The incidence of coffee leaf rust was monitored at monthly intervals to establish the most effective programme to control the disease.

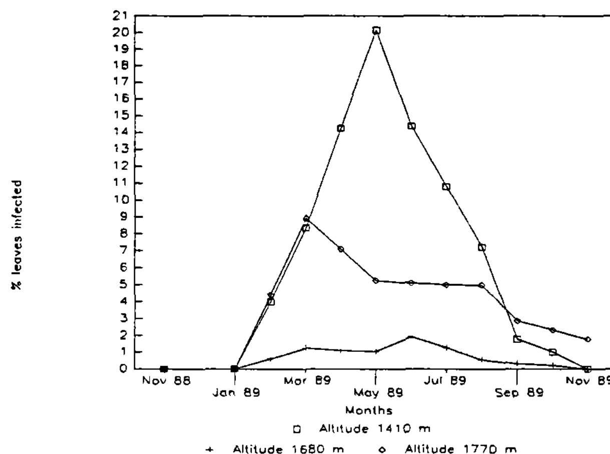
Results and Discussion

1. *Seasonal Development of the Disease*

The pattern of the development of the disease was similar for all areas with the maximum incidence of the disease in May/June/July. However, the severity of the disease varied at different sites (Figure 1).

Figure 1

The seasonal development of Coffee Leaf Rust at three altitudes on the Highlands of Papua New Guinea.



2. *Effect of Altitude on Coffee Leaf Rust*

Coffee grown at higher altitudes was less severely infected with coffee leaf rust than that grown at lower altitudes (Figure 1).

As a result of this study it was established that it is unnecessary to spray coffee with fungicides to control coffee leaf rust at locations higher than 1,600 m above seas level.

3. *Effect of Temperature on Coffee Leaf Rust*

The effect of altitude on the development of coffee leaf rust is associated with the effect of temperature on the disease. Temperatures below 15°C in January and February are a major determinant in the subsequent development of coffee leaf rust (Table 1). There is a significant correlation between the monthly mean minimum temperatures below 15°C in January and February and the maximum incidence of the disease five months later.

Table 1

Correlations between maximum disease incidence (MDI) and mean minimum monthly temperatures (M.MinT), 4 & 5 months preceding MDI.

		M.MinT linear	M.MinT logistic
$mMDI_{(t-4)}$	$100r^2$	45.80	56.40
	F	12.81 **	7.05 **
$mMDI_{(t-5)}$	$100r^2$	56.70	67.40
	F	14.09 **	7.90 *

[a] $100r^2$ = Percent variance accounted for
[b] F = Variance ratio; statistically significant $p \leq 0.05$ (*) and $p \leq 0.01$ (**)

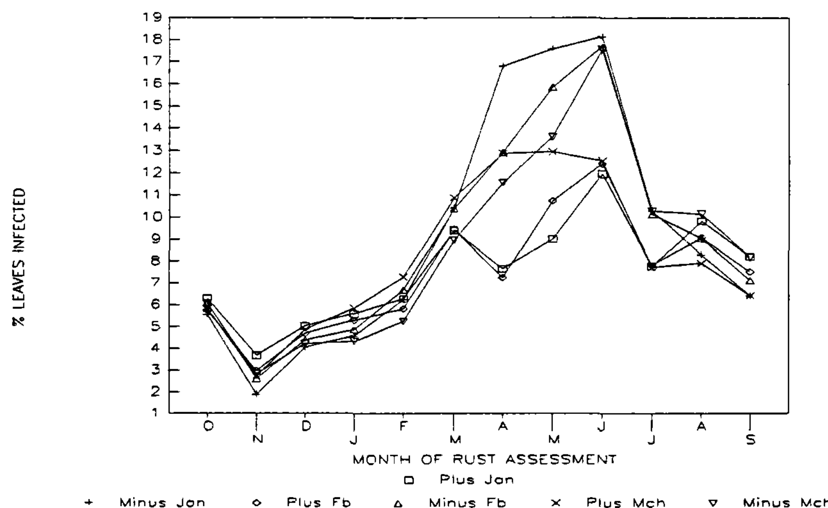
Note: There is no significant correlation between MDI & the M.MinT 0, 1, 2, 3 & 6 months prior to MDI.

4. *Development Of Chemical Control Strategies*

The main effects of sprays applied in January, February and March significantly reduced the incidence of coffee leaf rust (Figure 2). The main effects of the application in April and May did not significantly reduce the incidence of coffee leaf rust until August.

Figure 2

The Incidence of Coffee Leaf Rust Following the Foliar Application of Copper Oxide as a Factorial Experiment in January, February, March, April and May. Main Effects of the Treatment in January, February and March.



Data in Figure 2 confirms the results of the epidemiology study in which the conditions in January and February influenced the development of the disease later in the season.

When the effect of the actual treatments are examined, a single application of copper oxide in January, February or March reduced the incidence of coffee leaf rust (Table 2).

The most effective treatment was when copper oxide was applied on three occasions in January, February and March (Table 2).

Table 2

The Incidence of Coffee Leaf Rust Following a Single Application of Copper Oxide to the Foliage of Coffee Trees in either January, February or March, two Applications in either January & February, January & March, or February & March, and three Applications in January February & March.

Treatment	Month in which incidence of Coffee Leaf Rust was recorded											
	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP
Nil	3.18	1.33	3.71	5.69	8.37	13.85	24.58	38.37	29.67	16.11	9.89	10.12
J	16.62	7.59	8.28	7.75	8.31	11.73	9.51	7.49	10.37	7.42	12.38	14.14
F	13.72	1.46	9.4	5.43	4.42	10.35	15.83	8.45	13.32	6.88	13.29	9.27
Mch	12.19	2.02	3.4	3.18	4.5	6.87	12.37	12.27	14.61	9.58	8.8	4.62
J & F	9.36	4.44	5.47	5.92	3.19	7.25	8.52	12.31	13.3	7.56	14.21	15.79
J & Mch	10.85	2.83	4.96	4.03	6.63	9.48	5.86	5.68	10.05	3.61	17.76	11.24
F & Mch	6.87	2.89	1.05	3.11	4.34	5.95	14.35	20.26	13.25	8.17	8.48	10.61
J & F & Mch	12.69	4.89	3.97	6.44	2.78	5.25	4.27	4.64	3.79	4.43	10.62	10.27
LSD ($P \leq 0.05$)	*10.62*	*4.33*	*6.85*	*5.5*	*6.42*	*9.54*	*11.78*	*14.92*	*12.5*	*7.21*	*8.87*	*6.18*

Acknowledgements

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LE PATHOSYSTÈME « *COFFEA ARABICA*/PATHOGÈNES FONGIQUES/ENVIRONNEMENT » EN NOUVELLE-CALÉDONIE : INTER-RELATIONS À DIFFÉRENTES ÉCHELLES SPATIALES ET ANALYSE DU RISQUE ÉPIDÉMIQUE

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1 - OBJECTIFS

L'ambition du projet réside dans une analyse de la dynamique des épidémies fongiques considérée sous l'angle d'une étude globale du "pathosystème". L'objectif du programme consiste à analyser les inter-relations entre les trois composantes de ce pathosystème pour comprendre son fonctionnement, c.à.d. pour déterminer le calendrier d'apparition des maladies (rouille orangée, anthracnose, cercosporiose, ...) et leur sévérité. Dans sa phase initiale, le programme repose sur les opérations suivantes :

- identification de sites représentatifs des conditions de caféiculture traditionnelle et caractérisation de l'environnement dans chacun d'entre eux.
- suivis épidémiologiques mensuels
- interprétation statistique des données ainsi obtenues.

La finalité de cette approche réside dans l'élaboration d'un outil épidémiologique moderne (aide à la décision par prévision du risque épidémique), permettant une gestion raisonnée de l'environnement agricole.

2 - METHODOLOGIES

En 1992, le dispositif sur le terrain était composé de parcelles réparties sur 11 sites choisis au sein de plantations traditionnelles de caféiers en fonction de leurs caractéristiques géographiques et de premières observations épidémiologiques réalisées en 1991. Dans chaque parcelle, 10 arbres ont été choisis le long d'un transect médian ; 4 rameaux par arbre ont été marqués au niveau du premier noeud portant des feuilles. Les relevés épidémiologiques ont été effectués selon le principe du suivi "feuille à feuille" (Kushalappa et al. 1983, Avelino et al. 1992) qui consiste à décrire en détail la structure initiale de chaque rameau (nombre de noeuds, position des feuilles, etc.) afin d'appréhender, tout au long du cycle, le devenir des feuilles considérées individuellement (dans le cas présent, le patrimoine initial est de plus de 10 000 feuilles). Une échelle de notation définit des niveaux de gravité (de 1 à 5) en fonction du nombre de lésions par feuille et de leur étendue. L'état sanitaire des rameaux et des baies, et la production ont été également notés.

L'environnement de chaque site a été défini par les paramètres suivants : localisation géographique, situation topographique, exposition aux vents dominants, densité de plantation, état général de la culture, pluie (fréquence + intensité quotidienne), température, insolation, durée d'humectation foliaire, humidité du sol à 20 et 50 cm, lumière incidente au travers de la canopéee. Les caractéristiques édaphiques du sol de chaque parcelle ont été prises en compte : pH, texture du sol, CEC, bases échangeables, fertilité, etc.

La gestion de l'ensemble de ces données phytopathologiques et environnementales a été réalisée avec la base de données "ORACLE". Les analyses statistiques ont été effectuées avec la bibliothèque de logiciels "ADE 3.4" créée par Chessel & Dolédec, 1992, (Université de Lyon 1).

3.SIGNATURES EPIDEMIQUES

Au terme de l'enquête de 1992, les résultats obtenus mettent en évidence une grande diversité de situations tant au niveau des signatures épidémiques, que des caractéristiques environnementales.

La rouille est sans conteste la maladie la plus représentée sur le Territoire. Les niveaux de sévérité des attaques varient sensiblement puisqu'en fin de cycle, certaines parcelles sont complètement défoliées alors que d'autres en revanche, présentent des niveaux d'infestation très faibles. La distribution des trois pathogènes se caractérise également par une mosaïque de cas. Selon les parcelles, on observe soit essentiellement la rouille, soit l'anthracnose et la cercosporiose (mais pas de rouille), soit des combinaisons deux à deux. Dans l'ensemble, les dégâts majeurs (défoliation puis mortalité de rameaux) sont enregistrés dans les sites fortement atteints par la rouille.

La variabilité enregistrée confirme la pertinence du choix des sites d'étude et autorise de ce fait l'analyse du couplage spatio-temporel entre les données épidémiologiques et les données pathologiques afin d'en déterminer les inter-relations.

4. ANALYSE DES CORRELATIONS PATHOLOGIE/ENVIRONNEMENT

La co-structure des données environnementales et pathologiques a été mise en évidence à l'échelle des sites (analyse des valeurs moyennes) et des parcelles (analyse des écarts aux moyennes). La variabilité "inter-sites" rend compte de 75% de la variabilité des paramètres environnementaux et de 63% de celle relative aux données pathologiques.

L'ordination "inter-sites" simultanée de ces deux types de tableaux révèle un positionnement des parcelles en axe 1 positif, pour celles qui sont le plus concernées par la rouille, la défoliation, la mortalité des rameaux et en axe 2 négatif, pour celles qui sont plus infestées par l'anthracnose et la cercosporiose. Cette représentation identifie également des sites dont les "signatures" sont particulières (ILP, YAT). Le niveau des dommages subis par les parcelles est corrélé à cette ordination par certaines caractéristiques du milieu : relief élevé, forte amplitude de température, mauvaise texture du sol, pH favorable au caféier, fertilité réduite.

L'ordination "intra-site" met en évidence l'incidence d'autres paramètres environnementaux comme l'ombrage et l'exposition au vent sur le positionnement statistique des différentes parcelles d'un même site.

5. PREVISION DU RISQUE

Basée sur les analyses précédentes, une discrimination des parcelles groupées par niveaux de rouille ou d'anthracnose a été réalisée en fonction des données environnementales. Le pouvoir prédictif lié à cette discrimination a été testé sur chacune des parcelles, après exclusion du modèle du site correspondant. Ce test met en évidence le caractère significatif de la liaison environnement/pathologie puisque respectivement pour la rouille et pour l'anthracnose, 61 et 65% des parcelles sont ainsi correctement classées.

Dès lors, le modèle inspiré par ces analyses autorise de bonnes estimations du développement des épidémies sur caféier en Nouvelle-Calédonie.

6. CONCLUSION

Les résultats obtenus en Nouvelle-Calédonie confortent la mise en oeuvre, à partir de Nouméa, d'un programme diversifié, à dimension régionale. En effet, ces méthodologies et/ou les résultats obtenus seront extrapolés et validés dans d'autres écosystèmes de la zone Pacifique afin d'y appréhender le comportement des pathogènes. Sur ces bases, une collaboration s'est engagée avec les partenaires suivants :

* Papouasie Nouvelle Guinée : Coffee Research Institute (et antenne CIRAD) situé dans les Highlands.
* Vanuatu : implantation CIRAD à Esperitu Santo et plantations traditionnelles de l'île de Tanna.
* Polynésie française : ORSTOM/Services Territoriaux de la Protection des Végétaux, station de Papara.
* Indonésie : Department of Plant Pathology, Faculty of Agriculture, Malang University (East Java).

Les investigations en cours, ou à venir, concernent :

* l'étude fine des processus dynamiques à différentes échelles (parcelle, arbre, rameau),
* la mise en évidence des facteurs déclenchants, c.à.d. qui régissent les diverses phases des processus épidémiques,
* la validation des préconisations dans l'espace et dans le temps,
* la quantification (à l'aide de sondes à DNA) de l'hétérogénéité génétique des souches infectantes de chaque agent pathogène,

* le test, sur plantules, du pouvoir pathogène des souches infectantes.

Ces deux dernières actions de recherche visent à estimer la part de variation qui est due, dans la variabilité des signatures épidémiques, à l'agent pathogène lui-même.

RESUME

L'objectif de ces recherches concerne l'intégration simultanée de paramètres épidémiologiques et environnementaux afin de déterminer les facteurs majeurs régissant la dynamique des maladies (rouille, anthracnose, cercosporiose) affectant le caféier. La finalité de l'étude réside dans l'élaboration d'un outil épidémiologique permettant une aide à la décision par la prévision du risque épidémique dans des sites à vocation caféicole.

L'observation mensuelle et la quantification de l'état sanitaire de caféiers dans 28 parcelles réparties en 11 sites différents, l'enregistrement des données environnementales (climat, sol, etc.), la gestion de ces données par la Base "ORACLE", sont les étapes méthodologiques précédant les analyses de données effectuées avec le logiciel "ADE 3.4"(Chessel & Dolédec, 1992, Univ. Lyon).

La co-structure des données pathologiques et environnementales a été mise en évidence à l'échelle des sites (analyse des valeurs moyennes) et des parcelles (analyse des écarts aux moyennes). L'ordination "inter-sites" simultanée des deux types de tableaux révèle un classement des parcelles entre les plus concernées par la rouille, la défoliation, la mortalité des rameaux et celles qui sont plus infestées par l'anthracnose et la cercosporiose ; ce phénomène est corrélé à certaines caractéristiques du milieu (pH, structure du sol, relief, écarts de T°). L'ordination "intra-site" met en évidence l'incidence d'autres paramètres environnementaux (ombrage et exposition au vent) sur la dynamique épidémique. Enfin, un modèle inspiré par ces analyses autorise de bonnes estimations du développement des épidémies en Nouvelle-Calédonie.

Ces premiers résultats confortent la mise en oeuvre, depuis 1992 et à partir de Nouméa, d'un programme régional d'épidémiologie sur caféier. Des investigations sont en cours pour : i) étudier les processus dynamiques à différentes échelles, ii) mettre en évidence les facteurs environnementaux régissant les diverses phases du processus épidémique, iii) valider ces préconisations tant au plan temporel que spatial (comparaisons avec les sites de Papouasie, Indonésie, Vanuatu, iv) estimer le niveau de variabilité des souches infectantes.

SUMMARY

The pathosystem "*coffea arabica*//fungal pathogens/environment" in New-Caledonia : inter-relations at various scales and epidemic risk analysis.

The objectives of these investigations deal with the integration of epidemiological and environmental parameters in order to identify and to rank the main factors that influence the dynamic of fungal epidemics (rust, anthracnosis and cercosporiosis) on coffee.

The monthly readings of the individual pathological status of coffee plants located in 28 plots (scattered within 11 differents sites), the monitoring of weather characteristics (climate, soil, etc.), the data management using the "Oracle" baseare the preliminary steps of statistical analyses performed with the ADE 3.4 software (Chessel & Doledec 1992, Univ. Lyon 1).

The co-structure of pathology and environment data was demonstrated at both site (averaged values analyses) and plot (differences with means) scales. The simultaneous ordination of the two kind of tables indicates a classification of the plots from the more concerned by rust, leaves falling, branches decay to those that were heavily infested by anthracnosis and cercosporiosis. This phenomenon could be correlated to certain environmental variables such as pH, soil structure, topography and variation of temperature. The intra-site ordination reveals that others parameters such as shade and predominant winds exposure have to be considered for explaining the epidemic development in New-Caledonia.

These first results reinforce the development of a regional research programme on coffee pathogens epidemiology. Further investigations are to be done concerning the detailed dynamic process of the epidemics, the identification of disease launching factors, the validation of the spatial and temporal simulations in various areas of the Pacific zone, and the genetic variability of the causal pathogens.

STATUT PHYSIOLOGIQUE DES FEMELLES COLONISATRICES DE *HYPOTHENEMUS HAMPEI* (FERR.)

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INTRODUCTION

L'activité de dispersion des femelles de *Hypothenemus hampei* (Ferr.) présente, tant sur le terrain qu'au laboratoire une distribution dans le temps. Une dispersion maximale se produit autour de la huitième heure d'une photophase de 12 heures (Giordanengo, 1992). Les femelles qui quittent les baies d'émergence ont été nommées **colonisatrices** (Giordanengo, 1992).

La détermination du statut physiologique de ces femelles colonisatrices est très importante pour connaitre l'homogénéité des populations de ces femelles particulières et pour comprendre le comportement de colonisation de la plante hôte et la rencontre des sexes. Nous rapportons ici un test simple et rapide permet de savoir si les femelles sont vierges ou fécondées quand elles quittent les cerises, ceci par observation microscopique de la spermathèque après dissection.

METHODOLOGIE DE DISSECTION

Les scolytes proviennent des fruits infestés naturellement prélevés dans les caféières de Colombie. Les cerises de café et les insectes ont été maintenus dans une chambre climatisée à 25±1°C avec une humidité relative de 80% et une photopériode de 12 heures.

La dissection comporte les quatre points suivants:

1- Ecartement et enlèvement des ailes postérieures et antérieures.
2- Ouverture de l'abdomen face dorsale.
3- Prélèvement de la spermathèque
4- Observation de la spermathèque dans du liquide physiologique au microscope optique (40x10)

RESULTATS

Les résultats obtenus apparaissent sur la figure 1

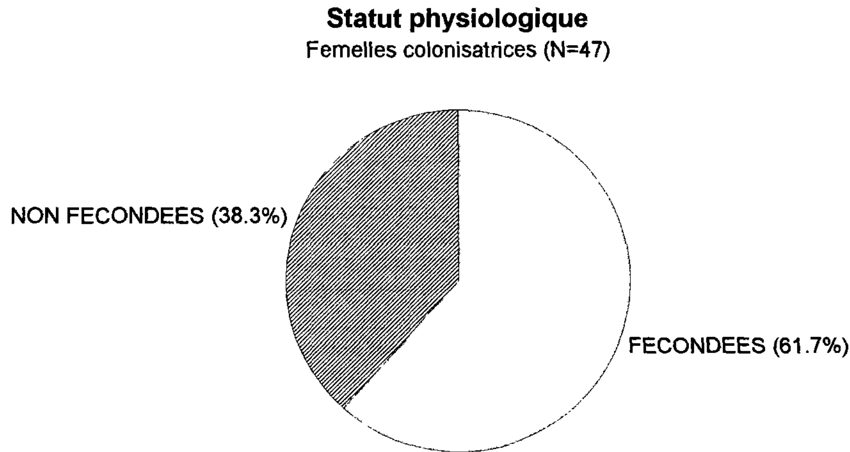

FIGURE 1: Répartition des femelles fécondées et vierges.

CONCLUSIONS

Ces résultats obtenus au laboratoire montrent clairement l'hétérogénéité des femelles colonisatrices, en ce qui concerne leur état physiologique. Bien que le plus grand pourcentage corresponde à des femelles fécondées (61,7 %), il est important de souligner que la proportion de femelles non fécondées (38,3 %) n'est pas négligeable.

Ceci suggère qu'il pourrait y avoir des accouplements de femelles en dehors de leur baie de naissance contrairement aux affirmations selon lesquelles l'accouplement se ferait entre frères et soeurs et se déroulerait uniquement dans les baies de naissance (Baker, 1984).

Désormais s'impose l'étude de la signification biologique de telles sorties de la baie sans fécondation préalable; en particulier la relation possible avec la réception de messages phéromonaux.

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RESUME

Une méthode rapide et valable pour déterminer le statut physiologique des femelles qui quittent les baies d'emergence est décrite. Elle est basée sur l'observation de la spermathèque de l'insecte au microscope optique. La présence ou l'absence de spermatozoïdes dans la spermathèque permet d'établir l'état physiologique des femelles dites colonisatrices.

DYNAMIQUE DE SORTIE DE *HYPOTHENEMUS HAMPEI* EN PRÉSENCE DE CERISES VERTES

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I. INTRODUCTION

Le scolyte du café *Hypothenemus hampei* provoque des pertes économiques considérables chez la quasi totalité des producteurs de café. Les taux d'infestation varient de 40 à 80 % en Afrique et de 20 à 60 % en Amérique du Sud (1 & 2).

A l'intersaison, on trouve sur les arbres des cerises de café sèches scolytées et des cerises vertes immatures. Cette période est cruciale pour la dynamique ultérieure des populations du scolyte du café car les drupes sèches constituent un réservoir de réinfestation qui peut être très important, une seule cerise peut en effet abriter plus d'une centaine de femelles.

Les facteurs physiologiques ou abiotiques qui induisent la sortie de ces femelles (dites alors colonisatrices) sont mal connus. La période d'activité de la femelle s'échelonne de 10 h à 18 h avec un maximum entre 14h et 16h (3). Pendant cette phase d'activité, les cerises de café sont visuellement et olfactivement attractives pour les femelles colonisatrices (3,4 &5).

Nous avons donc étudié, au laboratoire, les fréquences de sortie de ces femelles colonisatrices en fonction de la luminosité, de la pression atmosphérique ambiante et de la proximité ou non de cerises vertes.

II. MATÉRIEL & MÉTHODE

II.1. Dispositif de sortie

Une boîte obscure à la lumière (A) est reliée, par un tube transparent (B), à une boîte translucide (C). La boîte (A) contient des cerises sèches alors que la boîte (C) peut contenir des cerises vertes. Le tube de liaison (B) est grillagé à son extrémité (C). Attirées par la lumière pendant leur phase d'activité, les femelles se déplacent de (A) vers (C), sans pouvoir pénétrer dans cette dernière boîte et entrer en contact des cerises.

II.2. Déroulement chronologique des essais 1, 2 & 3

- Récolte des cerises sèches sur le terrain et vérification de leurs taux d'infestation.
- Disposition des cerises sèches (n = 5 à 13) dans les boîtes (A) du dispositif expérimental, et de deux cerises vertes dans les boîtes (C).
- relevé journalier des femelles sorties et renouvellement des cerises vertes vers 17h30.
- En fin d'expérience, dissection des cerises sèches pour dénombrer la population de femelles résiduelles.

Les essais 1, 2 & 3 se sont déroulés à 27°C et à une humidité relative de 90%. Le dispositif

de sortie est positionné face à une fenêtre et subit donc les variations d'intensité lumineuse naturelle. Sur l'ensemble de ces 3 essais, 12200 sorties de femelles colonisatrices ont été totalisées.

II.3. Mesure indirecte de la fécondation des femelles colonisatrices

Le pourcentage de fécondation des femelles colonisatrices (sorties en présence ou en l'absence de cerises vertes) a été mesuré indirectement, par l'observation de la viabilité des pontes. Chaque femelle testée est introduite dans une logette contenant un peu de milieu artificiel d'élevage. Les femelles n'ayant pas pondu dans un délai de deux mois (ou ayant produit des oeufs stériles) sont considérées comme "vierges". Ces observations ont été effectuées à température et luminosité ambiantes sur un total de 512 femelles testées. Le milieu d'élevage utilisé au laboratoire depuis 1989 permet à toute femelle fécondée de produire une ponte viable (6).

III. RÉSULTATS (Fig 1)

Les sorties présentent des variations journalières importantes liées à l'insolation et les insectes sortent de la boîte (A) plus abondamment en présence de cerises vertes qu'en l'absence de ces cerises. En moyenne 35% de sorties supplémentaires ont été observées en présence de cerises vertes. Cependant, les variations journalières, en partie induites par l'insolation, restent bien plus importantes que les variations de sorties induites par la présence ou l'absence de cerises (Fig 1).

Part contre, sur la durée de nos essais, la mesure de la pression atmosphérique n'a pas permis d'établir de corrélation avec les sorties.

Environ 95% des femelles testées ont donné une descendance. Ce pourcentage est constant, en présence ou en l'absence de cerises vertes. Après la mise sur milieu artificiel, une période de 3 à 50 jours est nécessaire pour obtenir la première ponte.

IV. CONCLUSION

Ces expérimentations confirment que les femelles colonisatrices possèdent un phototropisme positif (3) et montrent que leurs sorties des cerises sèches dépendent de l'insolation ainsi que de la présence ou non de cerises vertes. Deux hypothèses indépendantes ou liées peuvent expliquer les variations de sorties observées :

1). les stimuli lumineux et olfactifs pénétrant dans la boîte (A) par l'orifice du tube (B) favorisent le changement comportemental de simple "*femelle fécondée vivant à l'intérieur d'une graine*" à celui de "*femelle colonisatrice*".

2). une fois les insectes sorties des cerises, les stimuli lumineux et olfactifs présents dans la boîte (A) ainsi que les stimuli visuels et olfactifs présents dans le tube B optimisent le déplacement de (A) vers l'extrémité opposée de (B) et limitent le nombre de retour de (B) vers (A).

L'augmentation des sorties de femelles colonisatrices en présence de cerises vertes confirme l'importance des stimuli olfactifs ou/et visuels (3, 4 et 5) dans la dynamique de colonisation des baies de café.

Des études comportementales sont actuellement en cours, à l'ORSTOM de Nouméa, pour établir les rôles respectifs de la vision et de l'olfaction.

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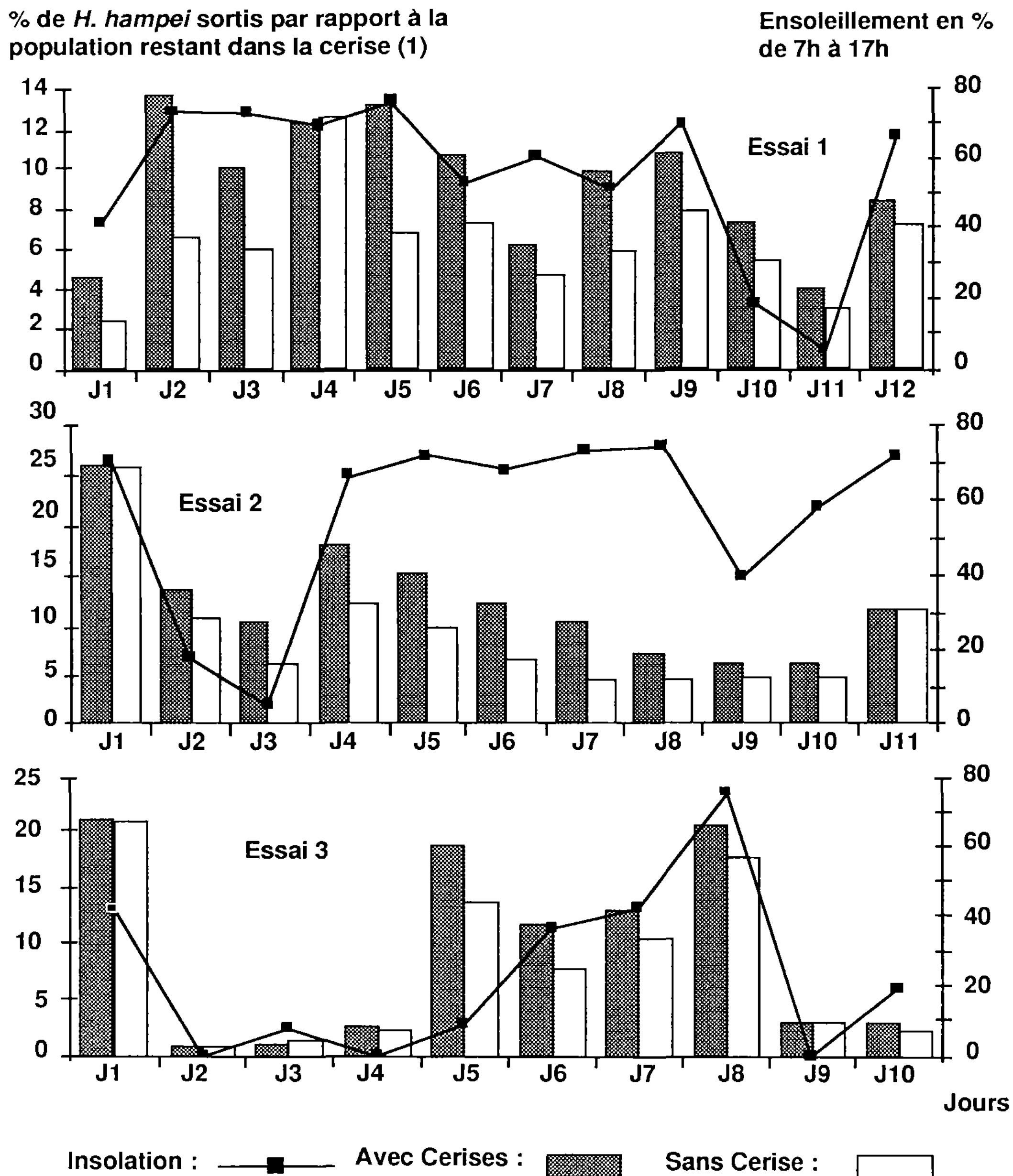
Résumé : Le scolyte du café, *Hypothenemus hampei*, passe la majeure partie de son cycle à l'intérieur des cerises de café. La phase la plus critique pour ce ravageur est donc la période où les adultes femelles se déplacent à la recherche d'une nouvelle cerise. A l'intersaison, on trouve sur les arbres des cerises sèches abritant une population parfois très importante, et des cerises vertes immatures. Les facteurs abiotiques et physiologiques qui conditionnent la sortie des femelles de ces cerises non récoltées ont été peu étudiés. Trois expérimentations ont été conduites afin d'apprécier les stimuli qui interviennent dans la sortie des cerises par les femelles dites colonisatrices.

Dynamique de sortie (en %) et insolation Fig 1



(1) Calcul du % de sortie :

$$\% \text{ sortie au jour n} = \frac{\text{EffJn}}{\sum_{n \text{ à } N} \text{EffJn} + \text{Fcd}} \times 100$$

Avec :
EffJn : Effectif des sorties au jour n
N : durée de l'essai en jour
Fcd : Femelles adultes vivantes trouvées lors de la dissection des graines à la fin des essais

Le dispositif expérimental a permis d'observer que la sortie des femelles est en relation avec l'insolation. Nous avons également constaté une augmentation de la proportion de femelles colonisatrices sorties en présence de cerises vertes. Ceci confirme l'existence de stimuli olfactifs et/ou visuels qui interviendraient dans le déplacement de *H. hampei* vers les cerises vertes.

Abstract : The coffee berry borer, *Hypothenemus hampei,* spends most of its life cycle within coffee berries. The most critical period for this pest is thus when females move from one plant or berry to another. Dry unharvested berries, which at times contain large numbers of borers, could be an important source of infestation of the rare green berries present during the post-harvest season. Abiotic and physiological factors affecting the emergence of adult females from dry berries have been little studied. Three experiments were carried out in order to examine the effects of light and other stimuli on emergence of adults from dry berries. A positive correlation between light and adult emergence was established. Increased emergence was observed in the presence of green berries, confirming that visual and/or olfactory stimuli from theses berries play a role in the emergence or post-emergence movement of *H. hampei.*

PRINCIPALES ESPÈCES DE COCHENILLES DU CAFÉIER ET LEURS ENNEMIS NATURELS EN AMÉRIQUE CENTRALE

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Les cochenilles comptent actuellement parmi les ravageurs les plus dommageables pour la caféiculture en Amérique Centrale. De graves infestations de Planococcus citri (RISSO) sur la partie aérienne des caféiers, survenues au Nicaragua durant les années 80 (CALDERON et al., 1989) ont montré que des espèces naturellement bien contrôlées, pouvaient devenir de redoutables ravageurs lorsqu'elles étaient placées dans des conditions écologiques particulières. Les attaques du système racinaire causées par Rhizoecus americanus (HAMBLETON) au Guatemala (HERNANDEZ, 1965), Geococcus coffeae GREEN au Salvador (GONZALEZ, 1980) et plus récemment les dégâts provoqués par Dysmicoccus bispinosus BEARDSLEY (GARCIA & al., 1990; GARCIA, 1991) au Guatemala, ont permis de confirmer l'importance économique des espèces souterraines qui, jusque-là, avaient présenté un intérêt limité.

OBJECTIFS:

1. Montrer la diversité des espèces de cochenilles communément rencontrées sur caféier en Amérique Centrale.
2. Présenter les principales espèces dans leur milieu.
3. Décrire les dégâts et rechercher les causes des infestations de Planococcus citri (RISSO), Dysmicoccus bispinosus BEARDSLEY et Geococcus coffeae GREEN.
4. Mettre en évidence l'importance des ennemis naturels pour la régulation des populations.

1. CLASSIFICATION DES ESPECES ET REPARTITION GEOGRAPHIQUE

PSEUDOCOCCIDAE

Dysmicoccus bispinosus BEARDSLEY (GUA, SAL, HON, NIC)*
Dysmicoccus brevipes (COCKERELL) (GUA, SAL, CR)*
Geococcus coffeae GREEN (GUA, SAL, NIC, CR)
Paraputo sp. (GUA)
Planococcus citri (RISSO) (GUA, SAL, HON, NIC, CR)
Planococcus halli EZZAT & McCONNELL (GUA)
Pseudococcus elisae BORCHSENIUS (GUA, SAL)
Pseudococcus sp. aff. *elisae* BORCHSENIUS (SAL)
Pseudococcus longispinus (TARGIONI & TOZZETTI) (SAL)
Puto antioquensis (MURILLO) (GUA)
Rhizoecus americanus (HAMBLETON) (GUA)
Rhizoecus coffeae LAING (CR)
Rhizoecus nemoralis (HAMBLETON) (SAL)
Rhizoecus setosus (HAMBLETON) (NIC)
Rhizoecus sp. (NIC)

*
GUA = Guatemala
SAL = El Salvador
HON = Honduras
NIC = Nicaragua
CR = Costa Rica

COCCIDAE

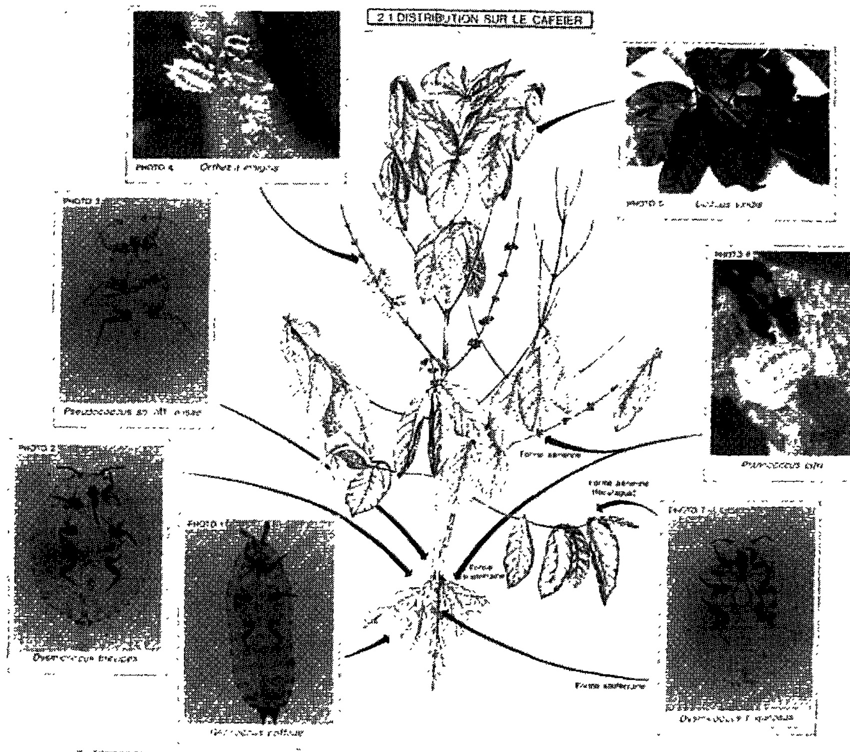
Coccus viridis (GREEN) (SAL, NIC)
Pulvinaria sp. (NIC)
Saissetia coffeae (WALKER) (NIC)
Toumeyella liriodendri (GMELIN) (GUA)

ORTHEZIIDAE

Orthezia insignis BROWNE (NIC)
Orthezia sp. (NIC)

2. LES COCHENILLES DANS LEUR MILIEU

2.1. DISTRIBUTION SUR LE CAFEIER (Photos 1 à 7).



2.2. AUTRES PLANTES HOTES

Les cochenilles du caféier peuvent coloniser des plantes de couverture et des arbres d'ombrage à l'intérieur d'une même caféière (Tableaux. 1 et 2). De cette façon se constituent des sources quasi permanentes qui assurent la pérennité des espèces.

ESPECES DE COCHENILLES	PLANTES DE COUVERTURE	ARBRES D'OMBRAGE
Dysmicoccus bispinosus	Solanum nigrum	Inga fissiolyx I. xalapensis
Dysmicoccus brevipes		Inga xalapensis
Geococcus coffeae	Borreria alata	
Paraputo sp.		Inga fissiolyx
Planococcus citri	Synedrella nodiflora Bidens pilosa Melanthera nivea	Inga fissiolyx Solanum bansii
Planococcus halli	Galinsoga parviflora Melanthera nivea	
Pseudococcus elisae	Ipomea tiliacea I. trifida	
P. longispinus	Cyperus ferax Commelina diffusa	

TABLEAU 1 : Cochenilles du caféier rencontrées sur d'autres plantes au Guatemala, d'après GARCIA (1991).

ESPECES DE COCHENILLES	ARBRES D'OMBRAGE
Dysmicoccus bispinosus	Inga sp.
Dysmicoccus brevipes	Inga sp.
Planococcus citri	Gliricidia sepium
Pseudococcus eliae	Musa sp.

TABLEAU 2 : Cochenilles du caféier rencontrées sur arbres d'ombrage au Salvador et au Nicaragua.

2.3. ASSOCIATION AVEC LES FOURMIS

Les relations de "mutualisme" observées depuis longtemps chez les homoptères sont particulièrement marquées chez les cochenilles. Ces relations sont complexes et peuvent présenter, selon les espèces et selon le milieu écologique, différents degrés de dépendance.

Quels sont les bénéfices attribués aux cochenilles?

- la protection contre les ennemis naturels (principalement chez les espèces aériennes),

- le nettoyage des colonies encombrées par les excrétions de miellat dont l'accumulation favorise le développement de fumagine (Capnodium sp.),
- le transport des colonies vers d'autres sites.

Quels sont les bénéfices attribués aux fourmis?
- la consommation du miellat comme supplément alimentaire,
- l'élevage des cochenilles rendue possible par leur situation de dominance sur ce groupe d'insectes.

Au Guatemala, les associations localisées sur le système racinaire du caféier ont été observées par GARCIA (1991). Chez les associations enregistrées, Solenopsis geminata (FABRICIUS) est la fourmi la mieux représentée; viennent ensuite Crematogaster stollii FOREL, Camponotus planatus (F. SMITH) et Camponotus abdominalis (FABRICIUS).

3. LES PRINCIPALES INFESTATIONS D'IMPORTANCE ECONOMIQUE

3.1. INFESTATIONS DE Planococcus citri AU NICARAGUA

Les premières attaques de forte intensité ont été observées en 1984 dans les fermes "Santa Margarita" et "Las Carolinas" près de San Marcos, dans la région de Carazo. Jusqu'en 1988, de nombreuses infestations ont été signalées dans cette même région, au coeur de la zone caféière. Dans les parcelles les plus touchées, les pertes de récoltes/mz * furent estimées à plus de 75%.

Au cours d'infestations massives, les colonies de P. citri forment des manchons autour des tiges et des glomérules, provoquent la chûte des fleurs et des fruits et détruisent feuilles et bourgeons. Les dégâts sont accentués avec l'envahissement par la fumagine, de tous les organes du caféier.

Pourquoi de telles infestations? et comment furent-elles contrôlées?

1976: Mise en place du Programme National d'Erradication de la Rouille Orangée du caféier "Hemileia vastatrix BERK. et Br."

1980-1983:
- Elimination de l'ombrage (déforestation); mise place de variétés à croissance rapide et à port bas (caturra et catuaï); culture de haute technicité.
- Prolifération de la mineuse des feuilles "Leucoptera coffeella GUER. MEN." principalement durant la saison sèche.
- Traitements insecticides massifs et répétés.
- Contrôle de la mineuse et **ELIMINATION DE LA FAUNE UTILE** (prédateurs, parasitoïdes).

1984-1988: Prolifération de P. citri. Nouvelles applications d'insecticides.

1989-1990: Diminution des traitements pour des raisons économiques; traitements par "foyers d'infestation" chez quelques producteurs; utilisation de produits plus sélectifs.

1991-1993: Disparition des infestations de cochenilles; **RETOUR DE LA FAUNE UTILE ET EQUILIBRE RETROUVE.**

* 1 mz = 0.7 ha

3.2. INFESTATIONS de Dysmicoccus bispinosus AU GUATEMALA ET DE Geococcus coffeae AU SALVADOR

3.2.1. D. bispinosus

L'importance des attaques de D. bispinosus dans la caféière guatémaltèque a été rapportée pour la première fois par GARCIA et al. en 1990. Parmi les espèces souterraines recensées, D. bispinosus semble incontestablement la mieux implantée. Sa répartition géographique coïncide avec la bande caféière allant du sud-est au sud-ouest du pays.

D. bispinosus attaque principalement la racine pivotante. Cette espèce est généralement associée à un champignon du genre Bornetina dont le mycélium, plaqué autour de la racine, offre une excellente protection aux populations et leur permet de se développer rapidement.

Les dégâts provoqués par D. bispinosus sont irreversibles: la dégradation progressive du cortex racinaire engendre la dégéneréscence des organes aériens du caféier qui se manifeste par le jaunissement et la chûte des feuilles, le déssèchement des rameaux et la forte diminution de la fructification.

3.2.2. G. coffeae

G. coffeae est une espèce souterraine très commune au Salvador. Sa répartition géographique n'a pas encore été définie avec précision. Cette espèce se développe sur le chevelu racinaire, principalement à proximité de la base du tronc et dans un espace compris entre 0 et 10 cm de profondeur.

Les dégâts causés par G. coffeae ne sont pas aussi intenses que ceux provoqués par D. bispinosus. Dans la plupart des cas ils se manifestent chez la plante par une perte de vigueur plus ou moins prononcée.

3.2.3. Origine des infestations de D. bispinosus et G. coffeae

Les études préliminaires de bioécologie de D. bispinosus et les connaissances acquises sur G. coffeae depuis plusieurs années n'ont pas permis de déterminer l'origine des infestations de ces ravageurs.

Actuellement, plusieurs axes de recherche sont envisagés:

- étude des relations entre le développement des populations et les facteurs pédologiques (types de sols) et climatique (température et précipitations).
- étude des facteurs physico-chimiques de la rhizosphère pouvant exercer une influence sur les populations.
- rôle et importance de la biocoenose (microorganismes, champignons et organismes animaux).
- rôle et importance des peuplements végétaux (mauvaises herbes, plantes de couverture, plantes répulsives, etc).

4. IMPORTANCE DES ENNEMIS NATURELS DE COCHENILLES

Dans l'agro-écosystème constitué par la culture du caféier, les insectes prédateurs et parasitoïdes jouent un rôle fondamental dans la régulation des populations de cochenilles, principalement les espèces aériennes. Au Nicaragua, les différents spécimens récoltés ont été prélevé soit sur des colonies de cochenilles isolées, soit dans de micro-foyers d'infestation localisés sur deux ou trois caféiers (Tableaux 3 et 4).

A l'échelle de la Région centraméricaine, la récolte et l'identification de la faune auxiliaire sont en cours. On sait déjà que de nombreuses espèces doivent compléter la liste commencée au Nicaragua.

PREDATEUR FAMILLE ET ESPECE	STADE ACTIF LARVE	ADULTE	HOTE
COCCINELLIDAE			
Azya luteipes	+	+	Pseudococcidae
			Coccus viridis
Chilocorus cacti L.	+	+	Pseudococcidae
Heperapsis centralis MUSL.	+	+	Pseudococcidae
CHRYSOPIDAE (espèces indéterminées)	+	+	Planococcus citri
CECIDOMYIIDAE (espèces indéterminées)	+		Orthezia insignis
	+		Planococcus citri
DROSOPHILIDAE Pseudiastata pseudococcivora SABROSKY	+		Orthezia insignis

TABLEAU 3 : Prédateurs de cochenilles récoltés au Nicaragua

PARASITOIDE INTERNE FAMILLE ET ESPECE	HOTE
DROSOPHILIDAE Gitona brasiliensis LIMA	Orthezia insignis
FAMILLE INDETERMINEE (espèce indéterminée)	Orthezia insignis

TABLEAU 4 : Parasitoïdes de Pseudococcidae récoltés au Nicaragua

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ÉTUDE DE LA DISTRIBUTION DE *PRATYLENCHUS* SP. DANS UNE PLANTATION DE CAFÉIERS AU GUATEMALA

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1 - INTRODUCTION

L'échantillonnage a été réalisé sur une parcelle d'un hectare composée de 50 lignes de 98 caféiers (densité 1m x 2m) *Coffea arabica*. Deux caféiers par ligne ont été tirés au hasard et un dénombrement de *Pratylenchus sp.* par gramme de racine a été effectué sur les 100 arbres ainsi échantillonnés. La pente de la parcelle expérimentale est de 5 à 8 % dans sa moitié supérieure et de 8 à 12 % dans sa partie inférieure. Les lignes de caféiers sont disposées en courbes de niveau. Ces observations permettent l'étude de la distribution du nématode et a pour objectif la définition d'une procédure d'échantillonnage utile à toute expérimentation sur *Pratylenchus spp.* La position de chaque arbre échantillonné est repérée par un numéro de ligne et un numéro d'ordre sur la ligne. Cette information permet l'étude de la répartition spatiale du nématode sur la parcelle et peut amener à affiner la méthode d'échantillonnage si une régionalisation de la répartition de ces espèces est mise en évidence.

2 - RESULTATS

2.1 - Distribution observée

La distribution de *Pratylenchus sp.* et les principaux paramètres statistiques relatifs à cette distribution sont présentés dans le tableau I.

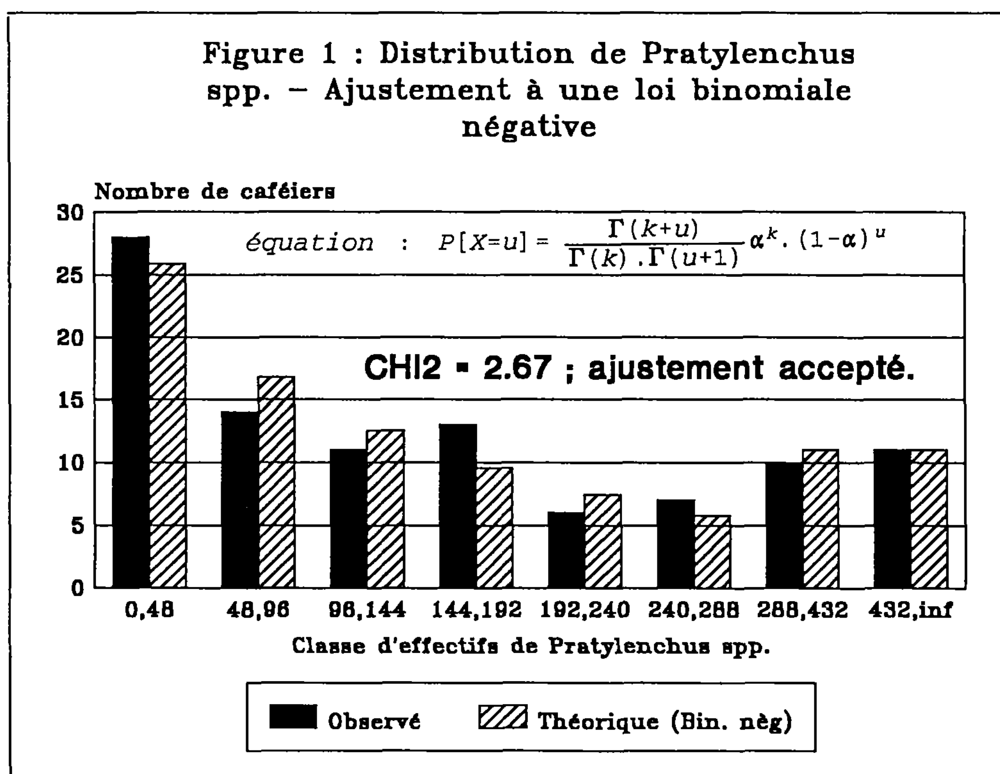
La distribution observée est très dissymétrique ; les classes à faibles effectifs de *Pratylenchus sp.* sont fortement représentées. Par ailleurs, cette distribution est caractérisée par une variance très supérieure à sa moyenne (41668,5 >> 188,5), ce qui implique qu'il y a surdispersion, c'est à dire que la distribution de cette espèce est très agrégative.

Tableau I : Distribution de *Pratylenchus sp.*

Classes	Nombre de caféiers	Paramètres statistiques
[0 , 48[	28	
[48 , 96[	14	Effectif : 100
[96 , 144[	11	
[144 , 192[	13	Moyenne = 188.54
[192 , 240[	6	
[240 , 288[	7	Variance= 41668.51
[288 , 336[	2	
[336 , 384[	4	Ecart-type = 203.11
[384 , 432[	4	
[432 , 480[	1	Minimum = 0
[480 , 528[	1	
[528 , 576[	1	Maximum = 958
[576 , 624[	2	
[624 , 672[	1	Coefficient de
[672 , 720[	2	variation = 107.7 %
[720 , 768[	0	
[768 , 816[	2	Médiane = 125
[816 , 864[	0	
[864 , 912[	0	
[912 , ∞ [	1	

2.2 - Ajustement à une loi binomiale négative

La loi binomiale négative a souvent été utilisée pour modéliser les nombres d'insectes par plante (ANSCOMBE F.J., 1949 ; BLISS C. I., 1956 ; CHESSEL D., 1978), car elle permet de rendre compte des phénomènes agrégatifs. Plusieurs écritures de cette loi sont possibles, celle de ANSCOMBE F.J. (1950) sera retenue et l'ajustement est présenté dans la figure 1. Les tests χ^2



permettent d'accepter cet ajutement au risque 5% .

2.3 - Transformation des variables pour d'éventuelles comparaisons

L'inférence statistique classique n'est pas possible avec les effectifs bruts, car la distribution n'est pas normale. Par conséquent, pour comparer l'efficacité de différents traitements sur les populations du nématode, deux méthodes peuvent être préconisées :

- tests non paramétriques :
  - . KRUSKAL et WALLIS, (1952); pour des dispositifs randomisés.
  - . FRIEDMAN, (1937) ; pour des dispositifs en blocs.
- analyses de variance et tests de comparaisons multiples de moyennes sur des données transformées.

2.3.1 - Choix d'une transformation

Différentes études ont été réalisées pour déterminer les transformations adéquates en fonction des distributions observées (BARTLETT M.S., 1947 ; ANSCOMBE F.J., 1948 ; TUKEY J.W. , 1957). Plusieurs transformations ont été testées sur les données présentées dans cette étude. La transformation : $\sqrt{k}.\text{Argsh}[\sqrt{(x+0,5)/k}]$ a été retenue, car elle minimise le coefficient de variation et permet d'avoir une distribtion quasi-normale.
Le tableau II présente les différents paramètres statistiques obtenus avec cette transformation.

TABLEAU II: Paramètres statistiques avec et sans transformation

Transformation	Moyenne	Var	e.t.	CV(%)	β_1	β_2
sans transf.	188.54	41668.5	204.13	108.27	1.64	5.39
$\sqrt{k}.\text{Argsh}[\sqrt{\frac{x+0.5}{k}}]$	2.77	0.59	0.77	27.77	-1.20	4.20

avec β_1 : coef. de symétrie, (= 0 pour une loi normale)
β_2 : coef. d'aplatissement, (=3 pour une loi normale)
Argsh : fonction inverse du sinus hyperbolique
k : coefficient de dispersion de la loi binomiale négative

2.3.2 - Taille d'échantillonnage

Il est possible de calculer des tailles d'échantillons pour mettre en évidence des différences entre deux traitements avec une précision donnée.

Soient : α : le risque de première espèce fixé par l'expérimentateur
t_α: la valeur théorique du test de Student associé
D : la différence en % de la moyenne à fixer par l'expérimentateur

Si les variances des deux traitements sont homogènes, de variance commune, il faut que le nombre de répétitions par traitements (n) soit choisi de telle sorte que :

$$D = (t_\alpha.CV.\sqrt{2})/\sqrt{n} \qquad \text{d'où} \quad n = 2.(CV/D)^2.t_\alpha$$

Par exemple, si l'expérimentateur souhaite mettre en évidence une différence entre traitements de l'ordre de 10% de la moyenne générale, un minimum de 62 répétions par traitement sera nécessaire.

2.4 - Etude de la répartition spatiale

La distribution étant fortement agrégative, il s'agit maintenant d'étudier la répartition spatiale afin de détecter une échelle d'hétérogénéité ou un éventuel gradient. Dans cette perspective, deux modèles d'analyse de variance seront développés : le modèle hiérarchique et le modèle de GREIG-SMITH (1952). Pour appliquer ces modèles, la parcelle étudiée est divisée en

blocs constitués d'un nombre de lignes allant de 1 à 24: 2 blocs de 24 lignes, 4 blocs de 12 lignes, 8 blocs de 6 lignes, 16 blocs de 3 lignes et 48 blocs de 1 ligne, constituant ainsi 5 facteurs emboîtés (hiérarchiques).

Dans le modèle hiérarchique, les tests comparent les carrés moyens des facteurs (2r) aux carrés moyens des facteurs (r) de niveaux inférieurs, alors que dans le modèle de GREIG-SMITH, les carrés moyens des facteurs sont tous comparés au carré moyen résiduel. Les analyses suivant ces deux modèles sont présentées pour les effectifs transformés de *Pratylenchus sp.* (Tableau III).

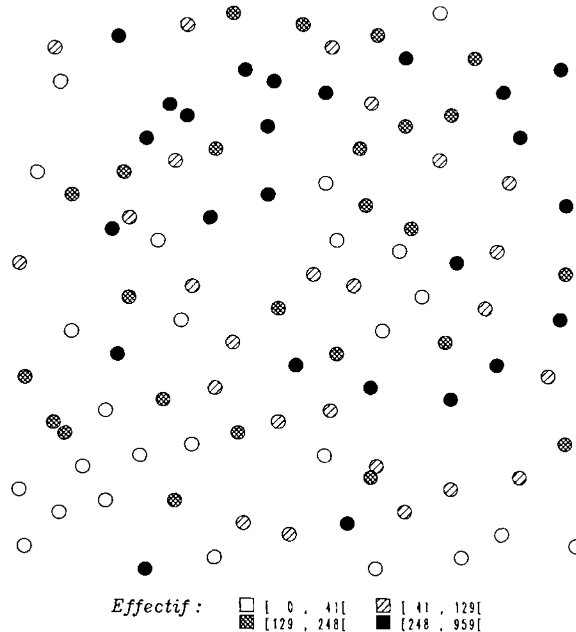
Tableau III : Etude de l'hétérogénéité spatiale de *Pratylenchus sp.*, données transformées.

Source de variation	ddl	SCE	hiérarchique F	hiérarchique α(%)	GREIG-SMITH F	GREIG-SMITH α(%)
Blocs de 24 lignes	1	4.24	1.91	30.15	9.73	0.31
Blocs de 12 lignes	2	4.45	2.86	16.92	5.11	0.98
Blocs de 6 lignes	4	3.11	0.70	61.11	1.78	14.75
Blocs de 3 lignes	8	8.84	2.37	4.00	2.53	2.18
Blocs de 1 ligne	32	14.95	1.07	40.66	1.07	40.66
Résiduelle	48	20.92				

Les analyses des données transformées permettent de définir des tailles de blocs à utiliser dans le cas de dispositifs expérimentaux ayant pour but de comparer divers traitements. Dans cet optique, des blocs de 3 lignes seraient préférables. Des études similaires sur d'autres parcelles devraient permettre de confirmer ces résultats, mais il est possible que les hétérogénéités observées dépendent en partie de la structure de la parcelle étudiée (pente, gradient, etc...). Dans l'attente d'autres résultats, il est proposé, pour des expérimentations concernant l'espèce étudiée, de constituer des blocs tels qu'ils sont définis précédemment, avec un nombre total de caféiers échantillonnés par bloc et par traitement de 3 à 4.

Enfin, une cartographie de la parcelle étudiée en fonction des effectifs du nématode est présentée en figure 2, elle permet d'avoir une représentation spatiale de la distribution de *Pratylenchus sp.*

Figure 2 : Répartition spatiale de *Pratylenchus spp.*



3 . CONCLUSION

La distribution de *Pratylenchus sp.* est fortement agrégative dans la parcelle étudiée et s'ajuste très bien à une loi binomiale négative.

Ces ajustements nous ont conduit à adopter une transformation de variable de type $\sqrt{k}.\mathrm{Argsh}[\sqrt{(x+0,5)/k}]$ pour tenter de rendre les distributions normales afin d'envisager l'utilisation de la statistique inférentielle classique et de définir des tailles d'échantillons en fonction des objectifs visés.

L'étude de la répartition spatiale a permis de mettre en évidence une échelle d'hétérogénéité au niveau de la distribution du nématode considéré, mais cette structure spatiale doit être confirmée sur d'autres parcelles pour qu'elle puisse être utilisée dans la définition d'une procédure d'échantillonnage générale. Dans l'attente d'autres résultats, le dispositif expérimental et la procédure d'échantillonnage pour l'espèce *Pratylenchus sp.*, dans le cas d'une comparaison de plusieurs traitements, devraient être les suivants (pour une parcelle d'environ 50 lignes):

- parcelle divisée en blocs constitués de 3 lignes de caféiers
- échantillonnage de 3 à 4 caféiers par bloc et par traitement pour le dénombrement des nématodes

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RÉPARTITION SPATIALE DES DÉGÂTS CAUSÉS PAR LES SCOLYTES (*HYPOTHENEMUS HAMPEI* FERR.) DANS UNE PARCELLE DU SALVADOR
Implications sur l'échantillonnage

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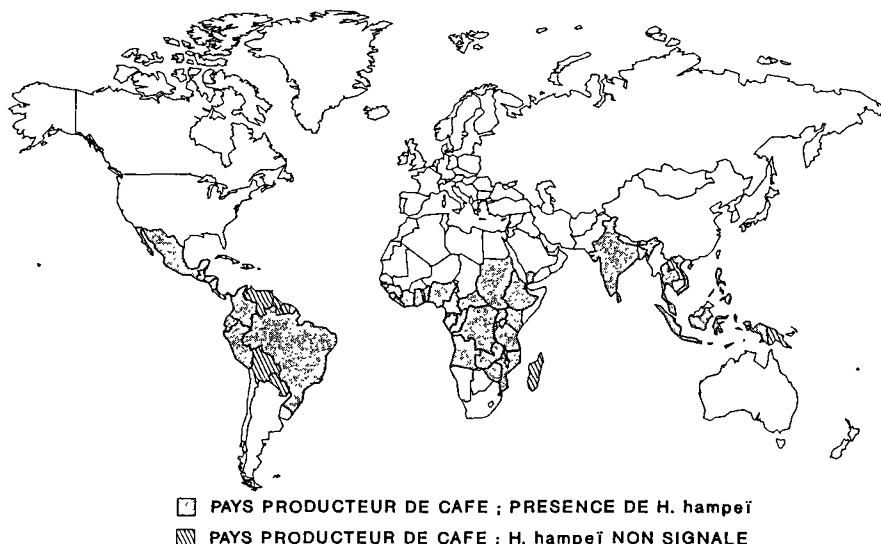
INTRODUCTION

Le scolyte des drupes du caféier, *Hypothenemus hampei* Ferr, est un déprédateur du caféier qui cause d'importantes pertes économiques. Il provoque des chutes de jeunes fruits et induit une diminution de la qualité de la production par la présence de grains perforés. Originaire d'Afrique, il s'est rapidement répandu dans presque toutes les aires de culture du caféier (figure 1).

Figure 1 : Répartition des scolytes dans le monde



La lutte contre cet insecte exige une bonne connaissance des niveaux de population au cours de l'année, soit pour décider de l'opportunité d'un traitement et contrôler son efficacité, soit pour gérer au mieux l'introduction éventuelle de parasitoïdes. Mais, du fait de la distribution agrégative du scolyte et de la grande hétérogénéité des plantations, ces niveaux de population sont très difficilement estimables.

Différentes méthodes d'échantillonnage ont été proposées, mais aucune ne semble répondre efficacement aux problèmes posés. Cette étude, réalisée à partir de données exhaustives sur une parcelle du Salvador, permet d'avoir une vision plus précise de la distribution des scolytes par arbre. A partir de ces informations, des améliorations des procédures d'échantillonnage seront proposées.

MATÉRIEL ET MÉTHODES

Les données résultent d'un prélèvement exhaustif réalisé au Salvador dans une caféière expérimentale. La parcelle de 2500 m^2, relativement plane, est composée de 21 lignes espacées de 2,50 m comportant chacune 40 caféiers disposés tous les 1,25 m environ. Les caféiers sont des *C. arabica* de la variété Bourbon, cultivés en multicaule. Le recépage s'effectue à des ages différents selon les arbres, en fonction de leur architecture.

Les fruits sains et perforés ont été dénombrés sur chaque arbre non recépé, ce qui a permis de calculer la production totale et le taux d'attaque par arbre. La position de chaque arbre a été repérée par un numéro de ligne et un numéro d'ordre sur la ligne. Les données ont été récoltées sur trois semaines, du 28 juillet au 17 août 1988, soit environ deux à trois mois après le début de la fructification, ce qui correspond à la période où un traitement insecticide éventuel serait le plus efficace. Compte tenu du peu d'évolution du taux d'attaque à cette période (BOURBON-MARTINEZ O., 1989), on peut considérer qu'il s'agit d'une "photo instantanée" de la parcelle.

Outre les méthodes classiques de la statistique descriptive, cette étude fait appel à des techniques de description spatiale (cartographie, semi-variogrammes) et de modélisation de champs aléatoires sur un réseau. Enfin le calcul de l'intervalle de confiance sur l'estimation des taux d'attaque se fait en utilisant la méthode du Bootstrap.

RÉSULTATS

ÉTUDE DE LA DISTRIBUTION OBSERVÉE DU TAUX D'ATTAQUE

La distribution observée du pourcentage d'attaque (nombre de fruits perforés par arbre / nombre total de fruits sur l'arbre) est dissymétrique (le coefficient de dissymétrie de Fisher est de 1,27). La variance est très supérieure à la moyenne ($1,78\ 10^6 >> 1699$) ce qui indique qu'il y a surdispersion. L'ajustement à une loi de Poisson, caractéristique d'un phénomène aléatoire, est donc impossible. Cette forte surdispersion pourrait s'expliquer par un caractère agrégatif du scolyte et par le fait, qu'étant peu aptes aux vols longs, les femelles pondent dans une zone écologique favorable non loin de leur zone de naissance. Un phénomène d'allélochimie, impliquant une attirance préférentielle des scolytes par des arbres déjà attaqués, pourrait également expliquer le caractère agrégatif des attaques.

Le pourcentage d'attaque (considéré comme une variable discrète) est modélisé par une loi binomiale négative (RÉMOND F. et al., 1993). L'ajustement est accepté par les tests χ^2 et D_{max} au risque de 5 % avec les paramètres:

$$\alpha = 0{,}0538 \ ; \ k=0{,}377$$

Les moyennes des lois binomiales négatives ne convergent que très lentement vers des lois normales. La méthode de COCHRAN (1977) permet de déterminer le nombre minimal d'arbres (n_m) pour que la normalité soit vérifiée si on néglige les effets des moments d'ordre supérieur à 3, suivant la formule : $n_m > 25 \ . \ G_1^2$, avec G_1 = coefficient de dissymétrie de FISHER. Selon cette méthode, le nombre minimal d'arbre à échantillonner pour pouvoir faire une approximation normale dans une procédure d'échantillonnage est très grand (>306) .

ÉTUDE DE LA RÉPARTITION SPATIALE

*** Étude des relations entre arbres voisins**

Le logarithme du pourcentage d'attaque (après élimination des arbres non attaqués) semble pouvoir être relié aux logarithmes des pourcentages d'attaque des arbres voisins par une relation du type :

$$\ln (P_{x,y}) = \sum_{i=-1}^{1} \sum_{j=-1}^{1} \alpha_{i,j} \ln (P_{x+i,y+j}) + N(0,\sigma)$$

Il s'agit d'un modèle de type markovien au second ordre (Guyon, 1993) qui devrait permettre d'expliquer entre 30 et 50 % de la variance initiale par les voisins, le complément étant modélisé par une loi normale. Le logarithme du pourcentage d'attaque est donc composé d'une partie aléatoire et d'une tendance (foyers) (figures 2 et 3).

L'étude des semi-variogrammes montrent que les arbres sont tous corrélés entre eux à l'échelle de la parcelle et que les corrélations sont positives pour deux arbres situés à moins 10 m de distance.

Figure 2 : Répartition des résidus (partie aléatoire)

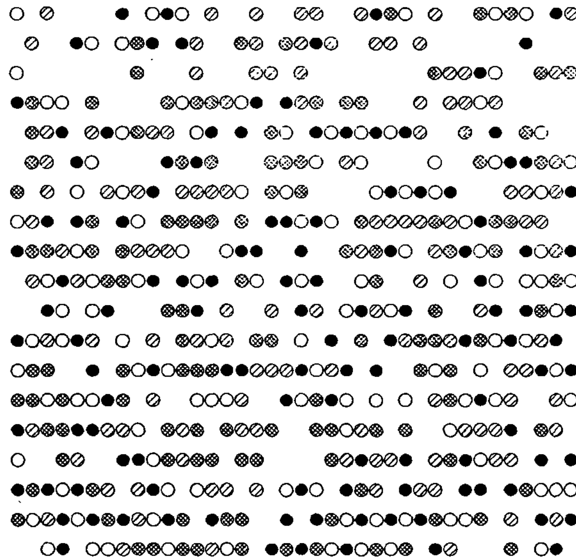
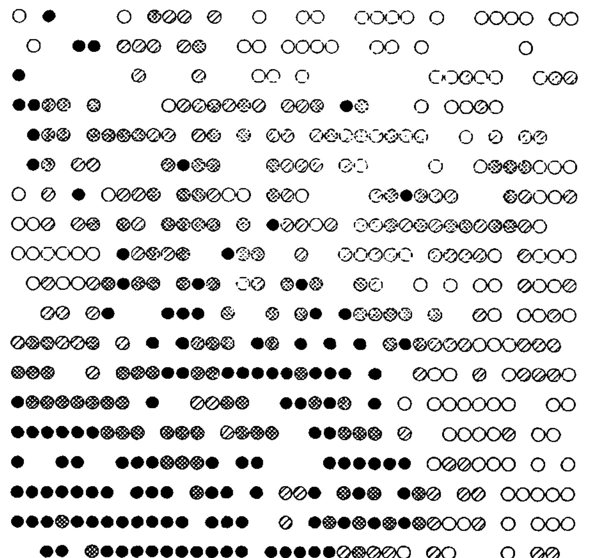


Figure 3 : Répartition des foyers (moyenne géométrique)



ÉTUDE DES MÉTHODES D'ÉCHANTILLONNAGE

La loi de la moyenne des taux d'attaque n'est pas calculable explicitement car l'indépendance entre les arbres n'est pas vérifiée. De plus le calcul d'une loi binomiale négative nécessite la connaissance de deux paramètres. Il est donc nécessaire de faire des études préalables pour les estimer.

Plusieurs techniques d'échantillonnage ont été envisagées : aléatoire, stratifié et systématique. Compte tenu des corrélations positives entre arbres, l'échantillonnage stratifié devrait donner des résultats meilleurs que l'échantillonnage aléatoire. L'échantillonnage systématique est plus efficace, au moins à une dimension, lorsque les variations sont linéaires (VAILLANT et al., 1990. Ce type de sondage a l'avantage d'être simple à mettre en oeuvre et donne des pécisions au moins aussi bonnes qu'un sondage aléatoire s'il n'existe pas de variations cycliques dans les caféières. Des simulations ont été réalisées sur la parcelle étudiée. Les intervalles de confiance sur la moyenne du taux d'attaque sont calculés par la méthode du Bootstrap pour les deux premiers types d'échantillonnage (figures 4 et 5).

Figure 4 : INTERVALLE DE CONFIANCE SUR
LA MOYENNE DU TAUX D'ATTAQUE PAR ARBRE
- BOOTSTRAP -(ECHANTILLONNAGE ALEATOIRE)

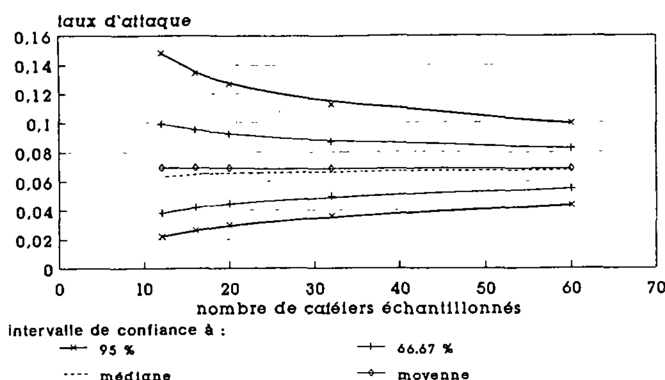
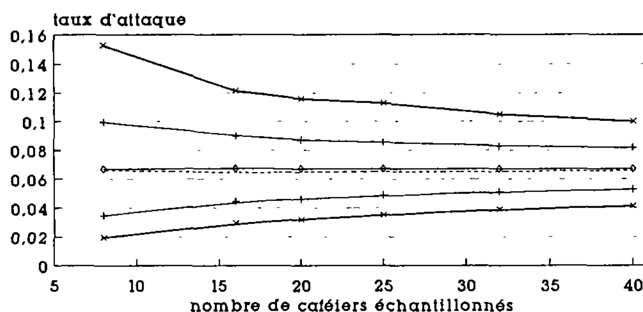


Figure 5 : INTERVALLE DE CONFIANCE SUR
LA MOYENNE DU TAUX D'ATTAQUE PAR ARBRE
- BOOTSTRAP - (1 CAFEIER PRELEVE/STRATE)



Les intervalles de confiance sont dissymétriques. La stratification entraîne un gain de précision relativement net. Il semble qu'il soit intéressant d'échantillonner sur au moins 15 arbres (pour le taux d'attaque moyen sur la parcelle) ou 20 arbres (pour la moyenne du taux d'attaque par arbre). Cela permet d'obtenir ainsi un gain de précision important.

L'échantillonnage systématique ne peut pas être étudié selon la même méthode. Tous les cas possibles de sondage systématique ont été examinés pour plusieurs pas d'échantillonnage. Les résultats sont les suivants :

☞ pour un taux d'échantillonnage de : 1/16 (4x4) , les moyennes varient de : 5,25 % à 7,5 %
1/25 (5x5) 3,95 % à 13,5 %
1/36 (6x6) 2,80 % à 9,7 %

Les résultats obtenus sont donc très variables en fonction du choix du premier arbre à échantillonner. Pour éviter un biais, il vaut mieux choisir un plan d'échantillonnage équilibré par rapport aux bords de la parcelle. Il est probable que les résultats obtenus sous cette condition soient plus proches de la véritable moyenne. Cependant, plus la parcelle sera grande, plus le nombre d'arbres échantillonnés sera grand, et moins le résultat dépendra d'un arbre ayant un taux extrême ou de l'équilibre du plan d'échantillonnage par rapport aux bords de la parcelle. Des économies d'échelle seront donc sûrement possibles pour les caféières plus importantes.

CONCLUSION

La distribution des attaques par arbre du scolyte des drupes du caféier, *H. hampei* Ferr., est très agrégative puisqu'elle s'ajuste à une loi binomiale négative. La distribution des attaques dans l'espace n'est pas homogène. Les dégâts se répartissent selon un gradient à partir d'un foyer. A l'échelle de la parcelle, les arbres sont tous corrélés entre eux.

L'utilisation de la méthode du bootstrap a permis de vérifier que l'échantillonnage stratifié est meilleur que la prise d'arbres au hasard sur la parcelle d'étude (on obtient une précision équivalente avec environ deux fois moins d'arbres échantillonnés). Toutefois, la précision reste relativement faible, même en sondant intégralement 40 arbres sur 800.
L'échantillonnage systématique semble donner de bons résultats, mais cela reste à confirmer, notamment à l'aide d'un modèle de type markovien.

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RÉSUMÉ

La répartition spatiale des dégâts causés par les scolytes (*Hypothenemus hampei* Ferr.) a été étudiée sur une parcelle du Salvador. Les pourcentages de fruits attaqués par arbre présentent une distribution de type binomiale négative et sont répartis selon un gradient. Ils présentent néanmoins de très fortes variations entre arbres voisins, notamment en raison des différences de production.

Il faudra donc échantillonner sur un nombre d'arbres supérieur à celui donné par une approximation normale. Cependant des économies d'échelle importantes seront possibles pour les grandes parcelles.

Trois types de sondage ont été comparés sur cette parcelle : aléatoire et par strate (en utilisant la méthode du bootstrap) et systématique (par des simulations). L'échantillonnage stratifié permet un gain de précision significatif par rapport à l'échantillonnage aléatoire. L'échantillonnage systématique est simple à mettre en oeuvre et semble également donner de bons résultats ; mais il doit être étudié dans diverses situations écologiques avant d'être proposé.

LABORATORY AND SMALL-SCALE FIELD TESTS OF FOUR INSECTICIDES AGAINST DEFOLIATING CATERPILLARS OF *EPICAMPOPTERA STRANDI GLAUCA* HAMPS. (LEPIDOPTERA : DREPANIDAE) ON COFFEE IN GHANA

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INTRODUCTION

Two Epicampoptera species, E. strandi glauca (Hamps.) and E. ivoirensis (Watson), are serious defoliators of both young and old coffee in Ghana. When leaves are scarce, damage may spread to the bark of green shoots (Padi, 1985a). Serious caterpillar outbreaks occur mainly in April-July and September-November during the rainy season (Padi et al., in press) and chemical control is often rendered inffective since the chemicals are washed off by rain before they have enough time to kill. All the five caterpillar instars usually occur together in outbreaks although one stage often predominates at any given point in time. Damage is most serious and extensive at the late 4th and early 5th instar stages (Padi, in preparation) and can lead to dieback, crop loss and even death of trees (Le Pelley, 1968). A number of insecticides have previously been tested and found effective against 4th and 5th instar caterpillars of Epicampoptera spp. in Ghana (Padi, 1985b; 1986) but some of them did not have a quick initial kill whilst others are no longer in production.

The present study was aimed at determining the most effective of four chemicals, Folimat (500g/l Omethoate + 25g/l Cyfluthrin), Karate, Ofunac and Delfin (Bacillus thuringiensis) against 1st-5th instar caterpillars of E. strandi glauca with the view to selecting those which have a quick initial kill and are more likely to kill the caterpillars before being washed off by rain.

A. LABORATORY SCREENING:

MATERIALS AND METHODS

The Four insecticides were tested against 1st-5th instar caterpillars of E. strandi glauca at the manufacturers' recommended dosages of 0.4% for Karate 25 EC, 0.8% for Ofunac 40 EC, 1.5% for Folimat and 0.5% for Delfin. For each larval instar, an insecticide treatment involved twenty-five caterpillars placed in a circular plastic container 80 mm high and of 110mm

basal diameter, containing five fresh Coffea canephora leaves to serve as food. There were five replicates per treatment for each of the five caterpillar stages. The contents of each container was sprayed with the appropriate insecticide solution using a small hand sprayer. The control treatment which was also replicated twice was sprayed with distilled water. After treatment, each container was covered with a piece of very fine mesh held in place with a rubber bung. To ensure adequate coverage of insecticide solution (or distilled water), four squirts of the liquid were applied to the contents of each container and any excess liquid was drained off.

Caterpillar mortality was recorded hourly for six hours and, subsequently, at 24 and 48 hours. The data was corrected using Abbott's Formula.

RESULTS AND DISCUSSION

All the four chemicals tested were more effective against younger (1st-3rd instar) than older (4th-5th instar) caterpillars (Table 1). The results also show that although all the chemicals, except Delfin, effected 100% mortality of 1st-3rd instars within the first 3-4 hours, the same did not apply to 4th and 5th instars except in the case of Folimat and Karate (4th instars only) (Table 1). It will, therefore, be necessary to test Ofunac on 4th and 5th instars and Karate, on 5th instars only, at slightly higher concentrations in order to determine the lowest dosage that would effectively control all the five instar stages within the first 3-4 hours after treatment. Delfin had a poor initial kill on all caterpillar stages and performed poorly against the 4th and 5th instars.

B. SMALL-SCALE FIELD TRIAL:

MATERIALS AND METHODS

Based on the results of the laboratory tests, 0.4% Karate and 0.8% Ofunac were selected for a small-scale field trial when an outbreak of *Epicampoptera* occurred on a 2 ha improved *C. canephora* plot (Plot PX2) at Cocoa Research Institute of Ghana (CRIG), Tafo. Folimat, regrettably, had to be dropped from further tests, upon advice from its manufacturers, because it was considered too expensive for pest control on coffee which is a relatively unimportant cash crop in Ghana.

There were two 0.4 ha replicate plots per treatment. Spraying was done using motorised knapsack mistblowers. Caterpilar mortality was assessed on 100 trees randomly selected at the centre of each plot. Assessment was done 1 hr, 24 hrs, 1 week and 2 weeks after treatment.

RESULTS AND DISCUSSION

Karate was effective against all instars within 1 hr after treatment whilst Ofunac was effective 24 hrs but not 1 hr after treatment (Table 2). One week after treatment, no caterpillars were recorded on both Karate and Ofunac treated plots. Two weeks after treatment, however, 360 and 420 first instar caterpillars were recorded on the Karate and Ofunac plots, respectively. These must have originated from pupae which were present but unaffected by treatment. *Epicampotera* pupae are wrapped up in rolled leaves and are out of reach from contact insecticides. For an effective control of old caterpillar outbreaks, therefore, it would be necessary to apply two treatments two weeks apart.

CONCLUSIONS

Karate emerged as the best chemical, both in the laboratory and in the field, for its quick initial kill of 1st-4th instar caterpillars. It is, however necessary to develop a reliable method for detecting outbreaks at an early stage when the caterpillars are predominantly in the early instar stages and are more vulnerable and cheaper to control. This will also ensure that outbreaks are controlled before the plants suffer extensive damage by older caterpillars.

For a quicker and more effective control of 4th and 5th instar caterpillars, it would be necessary to test both Karate and Ofunac at slightly higher concentrations than those recommended by the manufacturers.

ACKNOWLEDGEMENT

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SUMMARY

Four insecticides, Karate, Folimat, Ofunac and Delfin, were tested at the manufacturers' recommended dosages in the laboratory against 1st - 5th instar caterpillars of *Epicampoptera strandi glauca* Hamps., a serious defoliator of coffee in Ghana. There were five replicates per treatment and 25 caterpillars per replicate. Subsequently, Karate and Ofunac were compared in a small-scale field trial on a 5 ha plot during a caterpillar outbreak. There were two o.2 ha replicate plots per treatment and caterpillar mortality was assessed on 100 trees ransomly selected at the centre of each plot.

Folimat emerged as the best insecticide in the laboratory test but was excluded from the field trial upon advice from the manufacturers for being uneconomical for pest control on coffee in Ghana where coffee is a minor cash crop. Karate was better than Ofunac and Delfin for its quick initial kill of 1st - 4th instar caterpillars. Two weeks after treatment, reinfestation by 1st instar caterpillars occurred on both the Karate and Ofunac treated plots. It was concluded that two teratments, two weeks apart, will be required for the effective control of the caterpillars on coffee. It was also considered necessary to develop a reliable method for detecting outbreaks at an early stage when caterpillars are more vulnerable to insecticides.

Table 1. Results of laboratory tests on four insecticides against *Epicampoptera strandi glauca* hamps. (1 = Folimat; 2 = Karate; 3 = Otunac; 4= Delfin; figures corrected to the nearest whole number)

Time after	Corrected % mortality (mean of 5 replicates each of 25 caterpillars)																			
	1st instars				2nd instars				3rd instars				4th instars				5th instars			
treatment	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1 hour	100	100	55	20	100	95	35	20	98	90	30	60	95	85	50	70	60	40	35	5
2 hours	100	100	88	52	100	95	60	40	98	95	75	75	100	98	80	70	100	75	75	10
3 hours	100	100	96	85	100	100	90	90	100	100	90	75	100	100	85	70	100	80	75	15
4 hours	100	100	98	92	100	100	100	100	100	100	100	75	100	100	90	70	100	80	80	20
5 hours	100	100	98	95	100	100	100	100	100	100	100	90	100	100	90	75	100	80	85	20
6 hours	100	100	98	100	100	100	100	100	100	100	100	100	100	100	100	75	100	80	85	25
24 hours	100	100	98	100	100	100	100	100	100	100	100	100	100	100	100	90	100	90	100	60
48 hours	100	100	98	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	90

Table 2. Results of small-scale field test on effectiveness of Karate (0.4%) and Ofunac (0.8%) on Epicampoptera strandi glauca Hamps.

% mortality (mean of 2 replicates)

Time after treatment	0.4% Karate 1st-3rd instar	0.4% Karate 4th-5th instar	0.8% Ofunac 1st-3rd instar	0.8% Ofunac 4th-5th instar
1 hour	100.0	97.8	92.7	75.5
24 hours	100.0	100.0	100.0	100.0

THE FERMENTATION OF FRESH COFFEE PULP FOR USE IN ANIMAL FEED

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INTRODUCTION

Coffee pulp represents 29% of the coffee fruit on a dry weight basis. This material is frequently dumped in water courses causing serious environmental problems (Braham & Bressani, 1980).

Attempts to incorporate this byproduct into animal feed have so far been unsuccessful since levels above 10% impair the growth rate. This impairment may be due to the caffeine content (Clifford & Ramírez-Martínez, 1991*a*; Bressani & Braham, 1980) or to the presence of low molecular mass phenols and tannins. Soluble and insoluble condensed tannins are present in coffee pulp (Clifford & Ramírez-Martínez, 1991*b*) and since tannins complex with proteins this may account for the negative nitrogen balance which results (Mehansho *et al.*, 1987).

The use of silage for animal feed is of considerable economic interest and coffee pulp appears to be an ideal raw material for silage production if the inhibitive effect of the tannins could be overcome. Previous studies have shown the ability of *Lactobacillus plantarum* to modify the tannins of sun dried coffee pulp when incubated at 30°C with some added nutrients. This paper reports the use of *L. plantarum* to reduce the tannin content of fresh coffee pulp, thus eliminating the drying process and utilising the sugars naturally present.

MATERIALS

Fresh coffee pulp was provided by the Coffee Experimental Station in Campinas and the culture of *L. plantarum* by the Institute of Food Technology (ITAL) in Campinas. All other reagents were standard items from reputable commercial sources.

METHODS

Extraction of acetone-soluble tannins: The method of Clifford *et al.* (1991*a*) was used without the addition of formic acid. *Porter's test for proanthocyanidins:* The method of Porter *et al.* (1985) was used. *Dimethylaminocinnamaldehyde (DMAC) test for proanthocyanidins:* The method of Price *et al.* (1978) was applied using 0·1% DMAC and 4% concentrated hydrochloric acid.

Fermentation by L. plantarum: Fresh coffee pulp (10%, 20% and 30% m/v) was homogenized in water and 50 ml aliquots incubated at 32°C for up to eight days with and without a 1% addition of a 24 hr suspension culture

of *L. plantarum* in MRS medium, and with and without 1% additions of glucose, sucrose or lactose. The fermentation process was followed by measuring pH changes. Transformation of the tannins was determined on the supernatants and on the acetone-soluble material extracted from the silage solids by means of Porter's test and the DMAC test.

RESULTS AND DISCUSSION

pH changes

The changes in pH value show the progression of the fermentation. As lactic acid is produced so the pH value drops. For silage to be stable the final value must be below pH 4·2 to inhibit the metabolism of putrefactive bacteria. In all trials the pH value initially dropped below pH 4·0 with or without the addition of *L. plantarum* and with or without the addition of any sugars, indicating that there was no inhibition of *L. plantarum* by pulp solids up to a concentration of 30%. However in trials with 10% and 20% pulp and no added sugars the pH subsequently rose above pH 7·0 after 120 and 168 hours of incubation respectively. This indicated the presence of naturally occurring organisms in the coffee pulp which either metabolise lactic acid or develop at low pH values and produce basic metabolites, thus neutralizing the acid. These organisms became dominant after the initial dominance of the lactic acid producers. This pH rise did not occur with 30% pulp as substrate, indicating inhibition of the pH-elevating flora by some component of this more concentrated homogenate of coffee pulp.

Without the addition of *L. plantarum* a similar sequence was observed, but the falls and rises in pH value were erratic indicating the necessity to inoculate with *L, plantarum* to ensure standardization of the reactions and assure the fall in pH value. The addition of sugars also seemed to ensure no subsequent rise in pH value. Sucrose and glucose were more effective than lactose and sucrose was chosen for further experimentation due to its lower cost.

Changes in the tannin fractions.

Modification of the tannin fractions was followed by using the DMAC and Porter's tests to analyse the supernatants and acetone-extractable tannins remaining in the fermented pulp solids. McMurrough & McDowell (1978) and Delcour & De Varabeke (1985) both showed that on a weight basis when compared to flavan-3-ol monomers, oligomers produce progressively less colour with increase in the degree of polymerisation. Although substitution by DMAC can occur at positions 6 and 8 of the flavan-3-ols, position 8 is favoured sterically. Since there is a marked reduction in the colour produced by dimers compared to (+)-catechin, it was concluded that only the "upper" terminal residue had reacted and that steric factors were impeding access to the "lower" residues in the oligomers. However Delcour & De Varabeke (1985) also suggested that the reaction with the "upper" residue of a dimer could be influenced by the stereochemistry of the "lower" residue, and thus there would be no simple inverse relationship between the intensity of the colour produced with DMAC and the degree of polymerisation even for pure compounds.

Coffee pulp contains 0·19-0·86% db of the monomeric flavan-3-ols (Ramírez-Martínez, 1989) and their presence would obviously interfere in the determination of tannins with DMAC. Porter's reagent has the advantage of not reacting with the monomeric flavan-3-ols. Porter *et al.* (1986) have demonstrated that each monomer in an oligomer is converted more or less efficiently to the corresponding anthocyanidin pigment by oxidative depolymerisation, with the exception of the "lowest" unit, which is released unchanged. However, the anthocyanidin pigments are labile and the amount of pigment released is not stoichiometric. In contrast to DMAC Porter's reagent produces more colour per unit weight of tannin with increase in molecular mass, although the increase declines exponentially with increase in molecular mass. For these reasons both methods of analysis were employed, and although it is recognised that neither method will give absolute values for the tannin content, it is hoped that some useful between-treatment comparisons can be made.

An examination of the DMAC data shows that with no added sugar, the larger the percentage of pulp the greater the percentage reduction in DMAC-reactive tannins in the supernatant after 192 hours fermentation (17, 40 and 63% reductions for 10, 20 and 30% pulp respectively). However the situation was the inverse for the residues with 74% reduction at 10% pulp and 47% reduction at 30% pulp. With added sucrose, the percentage reduction in supernatant tannin was greater with 10% pulp than with 30% pulp (62% and 35% respectively), but for the corresponding residues the situation was again the inverse with 93% reduction at 30% pulp and only 78% at 10% pulp. Thus it can be seen that there is a considerable reduction in the content of DMAC-reactive tannins as a result of fermentation by *L. plantarum*, the reductions being greatest with 30% pulp and added sucrose, this

addition also preventing subsequent pH rise with lower pulp concentrations.

An examination of the Porter's data, after allowing for the interference of the added sucrose when analysing the supernatants, indicated a reduction in the contents of Porter's-reactive tannins, both in the supernatants and in the residues, which was independent of pulp concentration and/or sucrose addition (32-34% in the supernatants and 91-93% in the residues).

Integrating the colorimetric data with those for the changes in pH value leads to the conclusion that 30% pulp with 1% added sucrose and inoculation with *L. Plantarum* is optimal for reducing the tannins contents and ensuring stability of the silage.

CONCLUSIONS

It was concluded that *L. plantarum* could be used to ferment coffee pulp producing a reduction in tannins content of up to 90% in the residue, thus making it more acceptable for use in animal feed. The use of natural fermentation is not recommended at pulp concentrations below 30%. The addition of 1% sucrose is recommended to prevent subsequent rises in pH value.

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SUMMARY

This paper demonstrates the use of *L. plantarum* in reducing the tannin content of fresh coffee pulp for incorporation in animal feed. The importance is shown of incubation time and pulp concentration, also the beneficial effect of added sugar.

RÉSUMÉ

Ce travail demontre l'utilsation du *L. plantarum* dans la reduction du contenu de tannines dans la pulpe fraiche de café pour l'utiliser en silo. L'importance du temps d'incubation et de la concentration de la pulp est établié ainsi que l'effet benefique de l'addition de sucre.

DÉGRADATION DE LA CAFÉINE PAR DEUX CHAMPIGNONS FILAMENTEUX

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Résumé

La pulpe de café est un sous-produit du café fruit (50% poids humide). Elle est riche en matières azotées et en sucres, mais contient aussi des substances antiphysiologiques (caféine, polyphénols, ...). Nous avons isolé des champignons filamenteux (Penicillium et Aspergillus) capables de se développer sur la pulpe et de dégrader spécifiquement la caféine présente. Ces observations constituent des résultats originaux, car rares sont les micro-organismes aptes à se développer en présence de caféine, voire à la dégrader. Nous exposons ici les travaux préliminaires de recherche sur la physiologie de deux champignons cultivés sur un milieu synthétique à base de caféine, prise comme source d'azote. L'accent est plus particulièrement mis sur la caractérisation des premiers intermédiaires obtenus au cours de la dégradation de la méthylxanthine, à l'aide d'une technique par HPLC.

ANALYTICAL CHARACTERIZATION OF COFFEE CARBOHYDRATES

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Coffee contains two main extractable polysaccharides (arabinogalactans, galactomannans), the molecular characteristics of which have not yet been totally elucidated[1,2,3,4]. Moreover coffee origin, roasting and extraction conditions may influence carbohydrate structure and properties. This work described a comprehensive analytical approach for the study of carbohydrate characteristics obtained under various conditions.

MATERIAL

Samples consisted in green (**G**) and roasted (**R**) coffee extracts obtained by leaching ground coffee (0.4-0.8 mm) with distilled water under two different sets of conditions: 95°C-1h (**95**) and 180°C-15min (**180**). Coffees of two origins were studied: Colombian Arabica (**C**) and Togo Robusta (**T**).

METHODS

Carbohydrates structure was studied by combination of analytical methods as described in **Figure 1**.

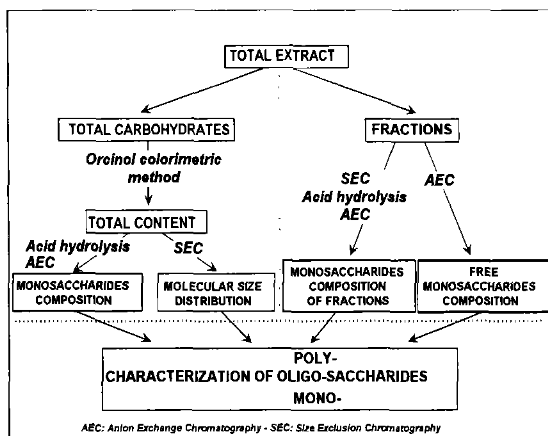


Figure 1: *Analytical approach for the study of coffee carbohydrates.*

The **total carbohydrate** content was determined with the sulphuric acid-orcinol method. **Monosaccharide** compositions were determined using a DIONEX Anion Exchange Chromatography (AEC) system equiped with a PA100 column and a pulsed electrochemical detector. Chromatographic conditions included the use of distilled water as eluent (1mL/min) and postcolumn addition of NaOH 0.1N (0.5mL/min). The **molecular weight distribution** was investigated by Size-Exclusion Chromatography (SEC) with two Pharmacia Superose 6 and 12 columns combined in series.

Elution was performed in KOH 0.1N (0.5mL/min). Continuous and specific detection of carbohydrates was achieved with the sulphuric acid-orcinol method. **Fractions** were obtained either by SEC or by filtration on C18 cartridges. Their carbohydrate characteristics were analyzed as previously described.

RESULTS

Coffee origin did not appear to influence significantly carbohydrates characteristics. Therefore only the results concerning the Colombian coffee are presented hereafter. **Figure 2** presents the carbohydrate content and the monosaccharide composition of the different coffee extracts obtained with Colombian coffee. Roasting and extraction temperature affect both quantitatively and qualitatively the carbohydrates composition of the extract. **Figure 2** also presents the chromatographic profiles of the four extracts obtained with the Colombian coffee. The molecular size distribution of extractable coffee carbohydrates (MW range: 200-1 000 000) is greatly influenced by the extraction conditions.

***Figure 2**: Carbohydrate content (2a), monosaccharide composition (2a), and chromatographic profiles (2b) of carbohydrates from Colombian coffee extracts obtained in various conditions.*

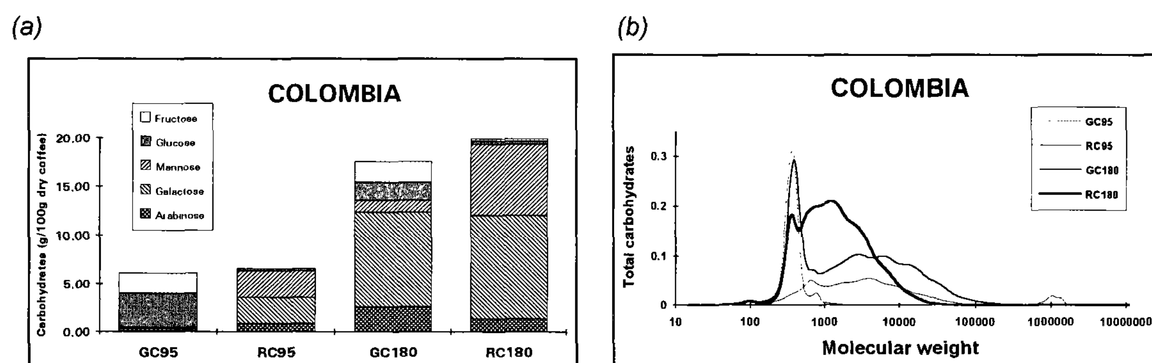


Figure 3 presents the chromatographic profiles combined with the monosaccharide analysis of size-excluded fractions for the four Colombian coffee extracts. The characteristics of arabinogalactans and galactomannans are affected by roasting and extraction temperature.

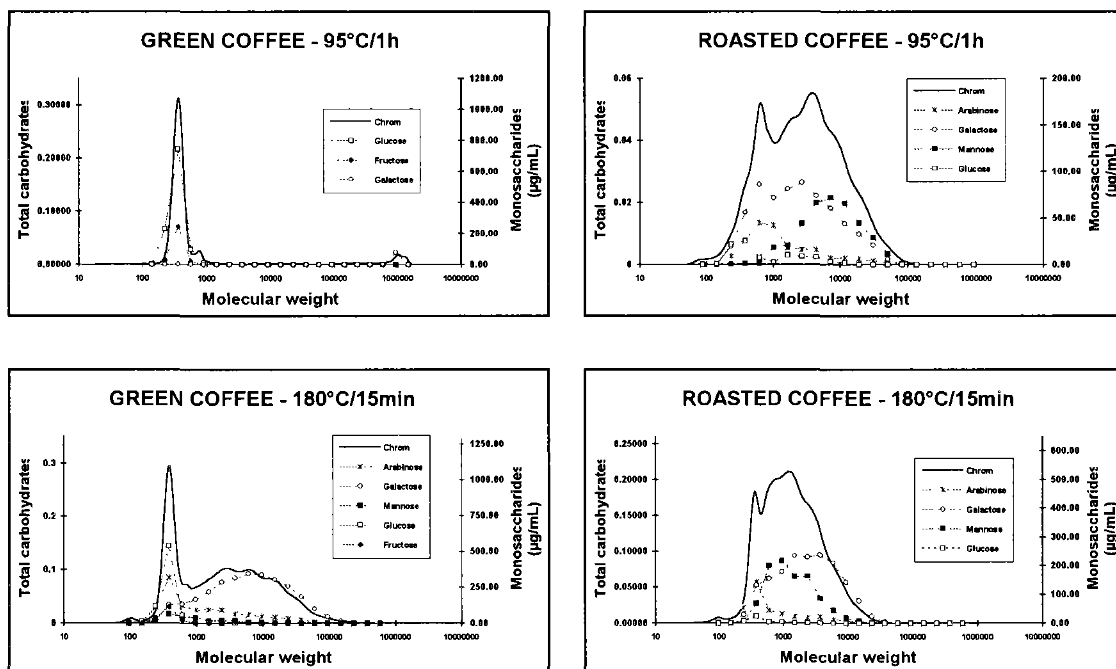
DISCUSSION

The molecular characteristics of extracted arabinogalactans and galactomannans differ and evolve differently according to the extraction parameters.

Arabinogalactans are only substantially released from ground coffee at high extraction temperature (>95°C). Roasting have a double effect on arabinogalactan structure. First it decreases its molecular weight range from 200-200 000 (green coffee) to 200-50 000 (roasted coffee). Secondly it reduces the arabinose substition degree of galactan chains from 1 ARA / 7 GAL (green coffee) to 1 ARA / 12 GAL (roasted coffee). Arabinose and arabinogalactan branchings are preferentially released either as free monosaccharides or as small oligosaccharides (degree of polymerization <6). Arabinose is furthermore thermally decomposed.

By contrast, roasting is essential to **Galactomannan** solubilization. High temperature of extraction improves galactomannan solubility but narrows its molecular weight distribution range from 800-80 000 (extraction at 95°C) to 200-20 000 (extraction at 180°C). Galactose substitution degree of mannan chains could not be determined with this analytical conditions.

Figure 3: *Combination of chromatographic profiles and monosaccharide composition of carbohydrates from Colombian coffee.*



SUMMARY

An analytical approach was developed to characterize quantitatively and qualitatively the different carbohydrate components. The characteristics of extractable coffee carbohydrates were studied as a function of coffee origin, coffee roasting, and extraction conditions. Main carbohydrates species were described in terms of molecular size and monosaccharide composition, the characteristics of which was found to depend on all extraction parameters.

RESUME

Ce travail présente une méthode analytique permettant la caractérisation qualitative et quantitative des différents glucides extraits du café. Les influences de l'origine végétale, de la torréfaction et des conditions d'extraction sur les caractéristiques moléculaires des glucides ont été étudiées. Les principales familles glucidiques sont décrites en terme de taille moléculaire et de composition en oses. Leurs caractéristiques montrent une dépendance certaines par rapport à l'ensemble des paramètres d'extraction.

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THERMAL STABILITY OF 2-METHYLISOBORNEOL IN ROBUSTA COFFEE

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Introduction

MIB (2-methylisoborneol), a potent odorous compound was recently identified in roasted Robusta coffee at sub-ppb levels and assumed to be one of the key compound responsible for the typical Robusta flavour [1]. In another study however, MIB was found to be readily removed from Robusta green beans by application of steam treatment [2], indicating that MIB might not be thermally stable. In view of these apparently contradictory findings, a stepwise investigation was initiated: First, MIB was quantified in a range of green coffees (study 1). Then, the effect of steam heating or roasting on MIB content was investigated (study 2). Finally, the impact of MIB on coffee brew flavour was assessed by sensory evaluation (study 3).

Experimental

Materials *Study 1*: The green coffee investigated were Ivory Coast, Uganda, Zaire, Togo, and Indonesian for Robusta, and Colombia (batch1) for Arabica.

Study 2: Green beans, steam heated green beans (2 hours at 133°C/3 bar), and medium roasted beans were analysed. The samples were Robusta Cameroon and Arabica Colombia (batch 2)

Study 3: Brews were prepared with medium roasted Arabica (Colombia batch 1, 5% in hot water), and spiked with MIB synthesised according to N.F.Wood & al. [3].

Isolation of MIB in coffee samples 40g of ground coffee were put with 150ml of water in a micro steam distillation extraction device containing 2ml of methylenechloride. After 2 hours extraction, the organic fraction was gently concentrated to 0.5ml under nitrogen.

Analyses of SDE extracts Green coffee extracts (study 1) were directly analysed by capillary GC-CI-MS HP5890 / Finnigan TSQ 700: Splitless injection; elution on DBWAX (60m x 0.25mm, film 0.25um): 50°C to 220°C at 5°/min; CI reagent gas ammonia; source temperature, 180°C. Heat treated coffees extracts (study 2) were first fractionated by preparative capillary GC in order to enrich the MIB-containing fraction. Gerstell instrument, column HP1 20m x 0.53mm; elution 60°C-10°C/min-160°C-30°C/min-220°C; internal standard n-butylhexanoate. Thirty injections were collected together at -50°C and then injected in a GC/MS HP 5971 (EI mode). GC conditions were similar than for study 1.

Results

The MIB content found in different green coffees is reported in Table 1. MIB levels in Robusta coffees cover a broad range of concentrations, including low ppt values as reported by Vitzthum for Arabica coffees (1). After heat treatment, no MIB could be detected, even in the case of a relatively high initial content of MIB found in one particular batch of Colombia coffee (see Table 2 and Figure 1). These results contrast with those obtained by Vitzthum who quantified MIB in roasted samples.

Table 1: MIB content of green coffee

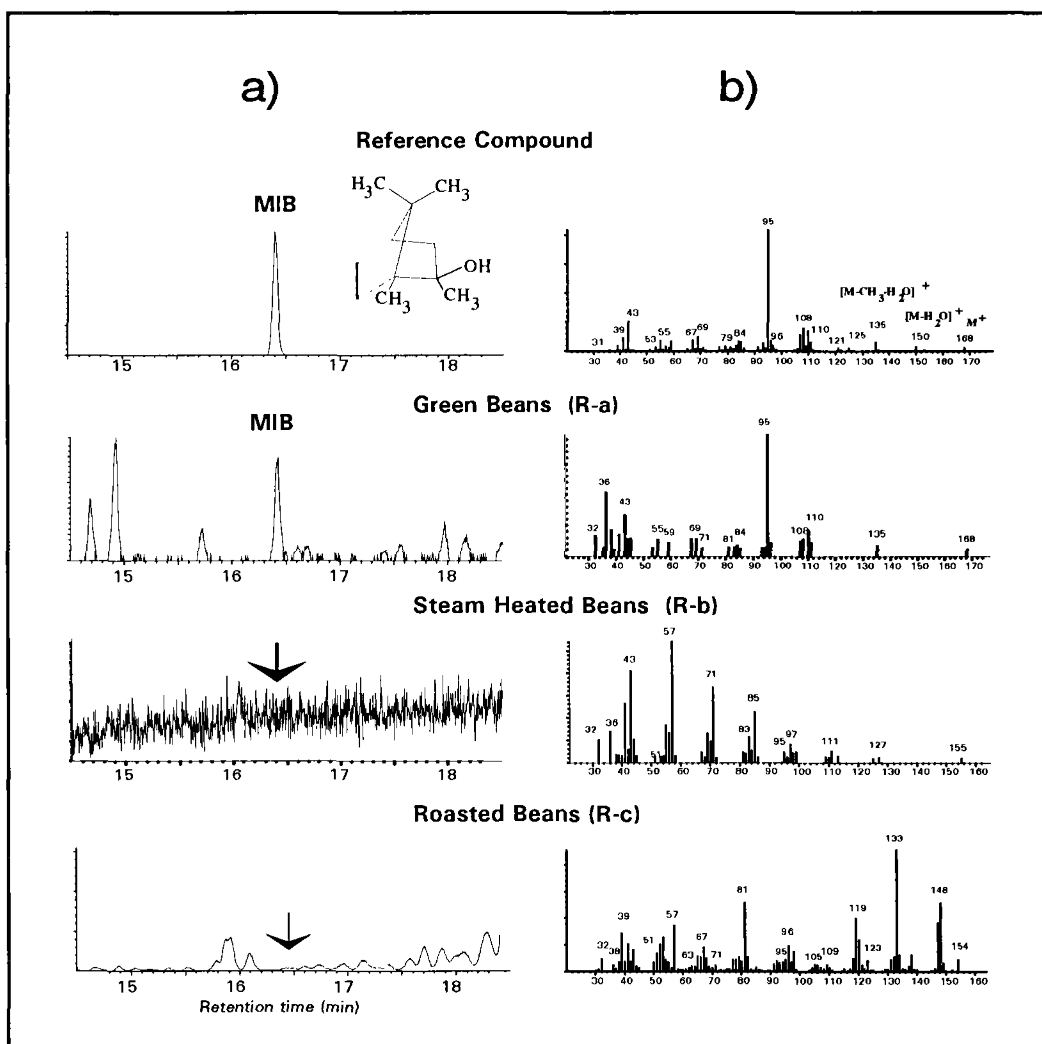
Variety	Origin	MIB ppb
Robusta	Ivory Coast	0.30
	Uganda	0.12
	Zaire	0.05
	Togo	0.03
	Indonesian	0.02
Arabica	Colombia (batch 1)	<0.005

Table 2: MIB in heat treated coffees

Origin	Sample		MIB ppb
Cameroon	green	R-a	0.3
	steam-heated	R-b	nd
	roasted	R-c	nd
Columbia (batch 2)	green	A-a	2.2
	steam-heated	A-b	nd
	roasted	A-c	nd

(nd : not detected)

Figure 1: GC-MS analysis of cumulated fractions from Robusta coffee samples
a) Selected ion chromatogram of m/e 95 b) EI spectra recorded at MIB retention time



In the brew, the sensory impact of MIB was assessed using triangular tests on Colombia coffee spiked with MIB. Results are presented in Table 3. No typical Robusta character were reported by the panel.

Table 3 : Sensory impact of MIB in coffee brew

Dosage MIB ppb	triangle resolved	Sensory character
0.010	1 / 5	bitter, acrid
0.025	3 / 5	bitter
5.0	4 / 5	musty, earthy

Conclusions

MIB concentrations in green coffees cover a broad range (0.005 to 2 ppb). Higher MIB levels are not necessarily specific to Robusta coffees. MIB is completely removed from coffee by steam heating or roasting. In a brew, the Robusta flavour character is not related to the presence of MIB.

Summary

Methylisoborneol (MIB), the alleged Robusta flavour key compound, was quantified by GC-MS in a range of green coffee. Higher levels of MIB were found in Robusta green coffees (0.03-0.3 ppb), as well as in one batch of Colombia Arabica (2.2 ppb). MIB was completely removed from coffee by steam heating or roasting.

Résumé

Le méthylisobornéol (MIB) décrit comme l'un des composés clés de la note Robusta, a été quantifié par GC-MS dans différents café verts. Les plus hautes teneurs ont été trouvées dans des cafés verts Robusta (0.03 - 0.3 ppb) ainsi que dans un lot de Colombie (2.2 ppb). Le méthyl-isobornéol n'est plus détecté dans le café vert traité à la vapeur ou dans le café torréfié.

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LIPIDS IN THE COFFEE BREW

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Introduction

In coffee beans different lipid constituents are to be found: inter alia triacylglycerides [1, 2], free fatty acids [3], and 16-O-methylcafestol esters [4]. Investigations into the transfer of these constituents from the coffee powder into coffee brews prepared by different brewing methods were of special interest.
In the research reported here, the lipids in boiled coffee (Scandinavian style), in espresso and in filter coffee were analysed.

Methods and Materials

Green coffee - Madagascar Robusta - was roasted for 2½ minutes at 253 °C and ground to different grades of fineness: coarse for boiled coffee, very fine for espresso, fine/medium for filter coffee.

Preparation of boiled coffee: 25 g coffee powder, 500 ml water;
preparation of espresso: 30 g coffee powder, 200 ml water;
preparation of filter coffee: 25 g coffee powder, 500 ml water

The ground coffee and the coffee brews were analysed according to the analytical scheme (Fig. 1).

Results

The total lipid content of the boiled coffee (Scandinavian style) is by far the highest with 2.2 % related to the ground coffee, lower for espresso (0.4 %) and lowest for filter coffee (0.2 %) (see Fig. 1). These data are equivalent to 1.1 g total lipids per liter in boiled coffee brew, 0.6 g/l in espresso and 0.08 g/l in filter coffee brew.

Furthermore, coffee powders of different grinding grades were each prepared according to the three brewing methods. The lipid content in boiled coffee is stated in Fig. 3. The finer the grinding grade of the coffee powder, the more lipids were extracted into the brew. In the case of the espresso coffee exactly the reverse applies (Fig 4). In the three filter coffee brews no differences were observed.

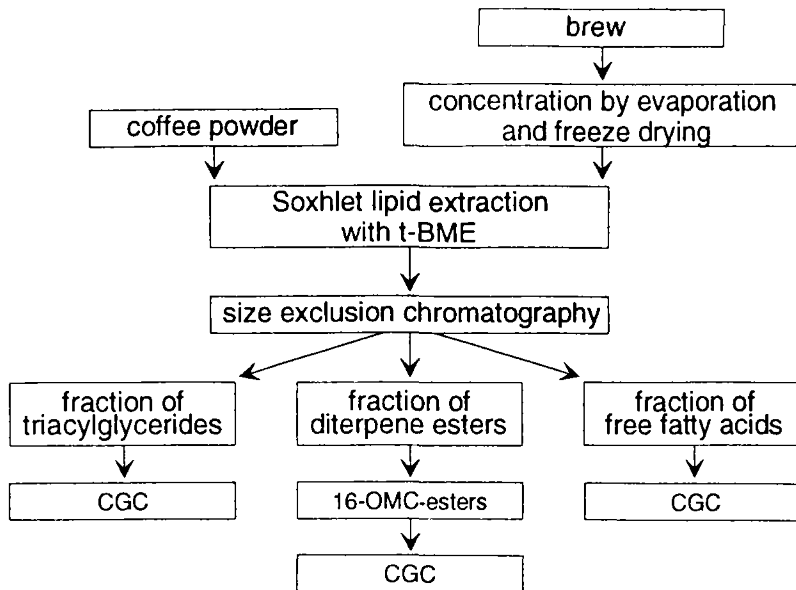

Fig. 1

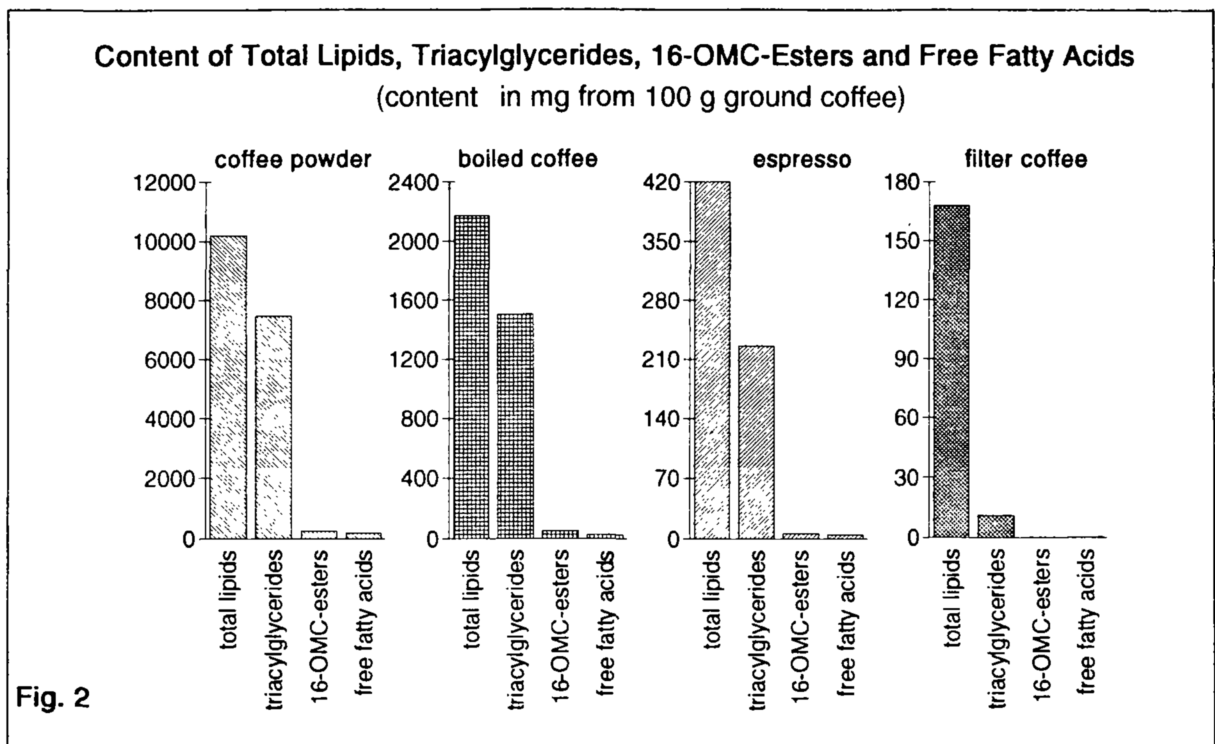

Fig. 2

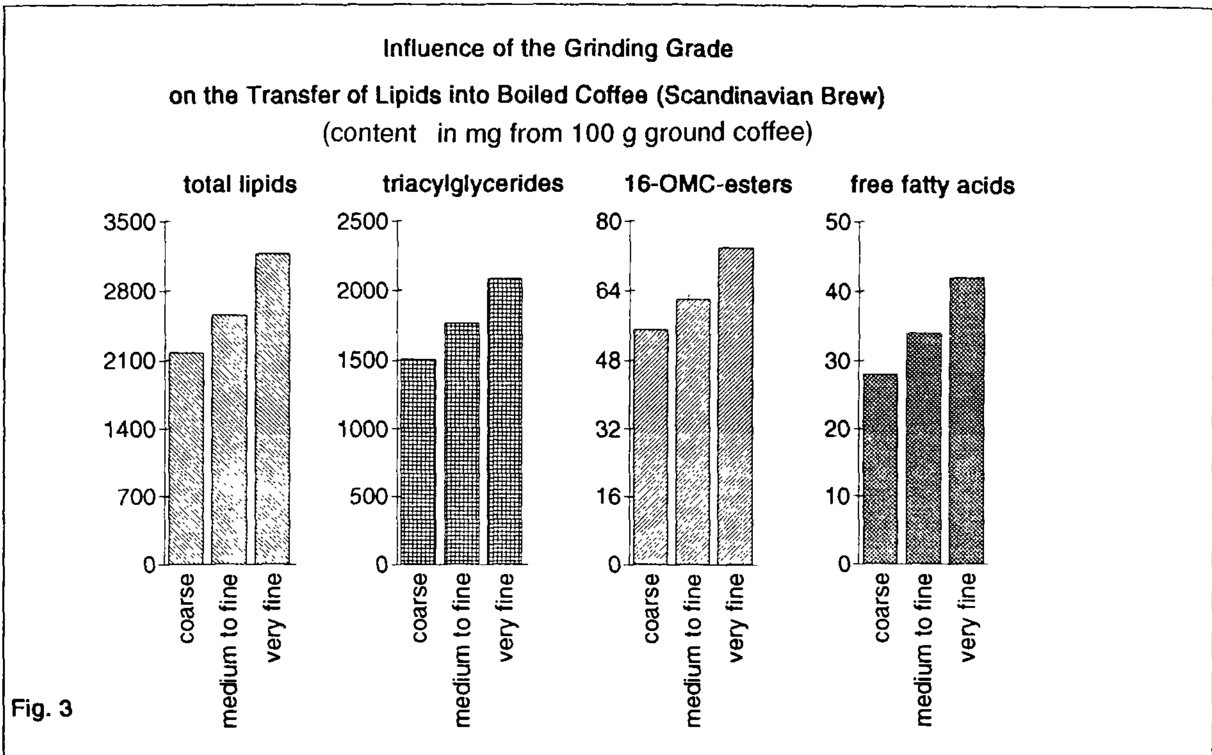

Fig. 3

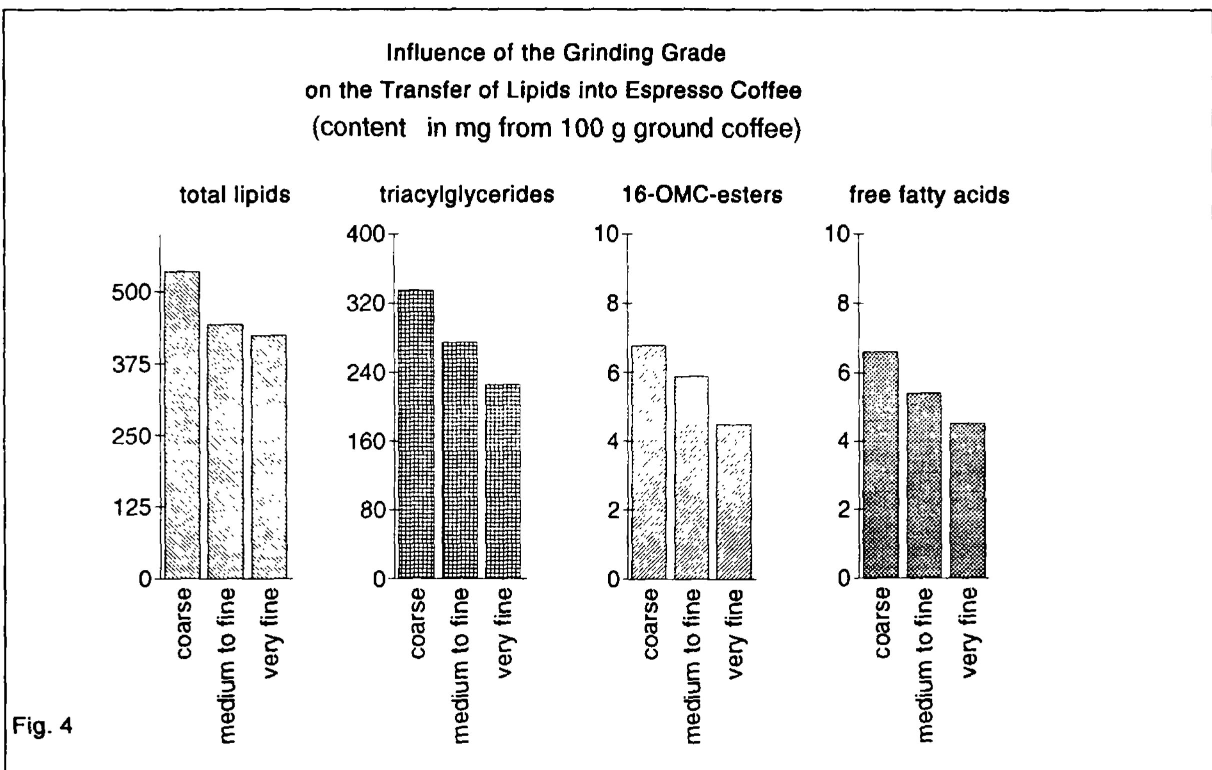

Fig. 4

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Summary

The transfer of coffee lipids from the coffee powder into the coffee brews (boiled coffee, espresso, filter coffee) was investigated for the total lipid content, the triacylglycerides, free fatty acids, and 16-O-methylcafestol esters. Depending on the brewing method the grinding grade of the roasted coffee had a different influence on the transfer.

Zusammenfassung

Die Übergänge der Kaffeelipide vom Kaffeepulver in die Kaffeeaufgüsse (Aufguß skandinavischer Art, Espresso, Filterkaffee) wurden am Beispiel des Gesamtlipidgehaltes, der Triacylglycerine, freien Fettsäuren und 16-O-Methylcafestolester untersucht. Der Mahlgrad des Röstkaffees hat je nach Zubereitungsart unterschiedlichen Einfluß auf die Menge der im Kaffeeaufguß enthaltenen Lipidbestandteile.

THE AROMA COMPOSITION OF THE COFFEE BEVERAGE. QUANTITATIVE DETERMINATION OF STEAM-VOLATILE AROMA CONSTITUENTS

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INTRODUCTION AND OBJECTIVE

Roasted coffee is one of the foods which is especially "rich in aroma". Almost 800 individual components have been discovered to date (1). Some years ago we investigated the qualitative and quantitative composition of the steam-volatile aroma complex of roasted coffee with the following results (2):

- Depending on roasting conditions and blend, the steam-volatile aroma complex (excluding acids) of medium-roasted Arabica coffees amounts to 700 - 800 ppm (2).
- Approx. 170 components in the ppm-range (1 - 150 ppm) and 70 components in the ppb-range (1 - 500 ppb) make up 85 to 95 % (by weight) of the aroma complex.
- Heterocyclic aroma constituents represent 80 - 85 %, and of these, furans (38 - 45 %), pyrazines (25 - 30 %), pyridines (3 - 7 %) and pyrroles (2 - 3 %) make up the majority.
- Sulfur-substituted furans (0,4 %), thiophenes (0,4 %), thiazoles (0,15 %) and oxazoles (<0,01 %) were determined in considerably lower concentrations.
- Approx. 3 to 5 % of the aroma complex consist of aliphatic components and the same percentage of aromatic components, whereas the concentration of alicyclic components is <0,5 %.

It is well known that Arabica and Robusta coffees differ considerably in their aroma composition (3,4):

- Robustas show (due to their high content of free amino acids, of chlorogenic acids and of 3-feruloylquinic acid) significantly higher

concentrations of pyrazines, phenols and phenol ethers than Arabicas. Direct correlations were established between individual amino acids of green coffee and aroma compounds which are formed during roasting.

- Arabicas contain (due to their high sucrose content) considerably higher amounts of steam-volatile furans, HMF and some aliphatic sugar degradation products than Robustas. Furthermore, Arabicas and Robustas differ in their composition of N-alkylpyrroles, N-furfurylpyrroles, pyridines, thiazoles, sulfur-substituted furans and aliphatic sulfur compounds.

All the above data have been established for roasted coffee beans, not, however, for the coffee beverage, which has received comparatively little attention.

The O B J E CT I V E of this investigation was to determine the qualitative and quantitative composition of the steam-volatile aroma complex in brewed Arabica and Robusta. The results are intended as a basis for evaluating the contribution of individual aroma constituents to the total aroma impression.

MATERIALS AND METHODS

ROASTED COFFEE & COFFEE BREW

Arabica Coffee (Kenya) and Robusta Coffee (Indonesia) were roasted on a Jetzone lab-scale roaster for 3 min. to the same colour value of 11,0.

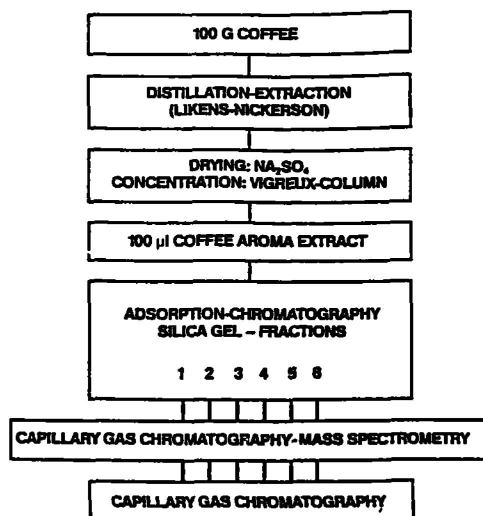
The beans were ground and brewed under the same conditions. 60 grams were used to brew on litre of coffee.

SAMPLE PREPARATION AND INSTRUMENTAL ANALYSIS

The following samples were analysed:

- ground roasted coffee
- coffee brew
- coffee grounds

The volatiles of these products were analysed as shown in the block scheme (2).



RESULTS

As Fig. 2 illustrates the aroma of roasted coffee is a complex mixture of components with widely varying concentrations. In order to identify and quantify main and trace components, the aroma concentrate was separated by adsorption chromatography on silica gel into six fractions according to increasing polarity of the components and the fractions further analysed by capillary gas chromatography mass spectrometry.

Once the steam-volatile components have been profiled by GC/MS, precise GC methods can be applied for routine analysis. The identification is based on the calculation of Kovats indices, and the quantification on the internal standard method. At the beginning of the analysis 10 suitable steam-volatile substances are added to the coffee, so that each fraction of the aroma extract contains 1 to 2 internal standards for quantification of individual aroma components. Using this method, about 400 components can be detected and of these about 170 aroma components in the ppm-range (1 ppm to 150 ppm) and about 70 aroma components in the ppb-range (1 ppb to 500 ppb) can be positively identified and quantified.

Fig. 2 shows the distribution of the coffee aroma extract during fractionation on silica gel. The following results were obtained:

FRACTION 1:

- represents 0,8 - 1,2 % w/w of the steam-volatile aroma and contains approx. 75 components:
  aliphatic, alicyclic and aromatic hydrocarbons, alkyl- and alkenylfurans, aliphatic sulfides, di- and trisulfides, and some unknown substances

- It is not surprising that the greatest amount of this fraction remains in the coffee grounds since most of the components are not or only partially water-soluble. Only 7 % are found in the brewed coffee (Fig. 3). A considerable part of fraction 1 evaporates during brewing.

FRACTION 2:

- represents 1,3 - 1,8 % w/w of the steam-volatile aroma and contains approx. 100 components:
  N-alkyl- and N-furfurylpyrroles, condensed furans, kahweofuran, homokahweofuran, sulfur-substituted furans and other S-heterocyclic compounds, 3-phenylfuran, and unidentified components

- Some of the components of fraction 2 are more soluble in water, so that 20 - 25 % w/w of this fraction are transferred to the brewed coffee (Fig. 4).

FRACTION 3:

- represents 3,5 - 5,5 % w/w of the steam-volatile aroma and contains approx. 60 components:
  aliphatic, aromatic and heterocyclic aldehydes, ketones, diketones, esters, ethers and phenolethers, pyrrol, indol, 2-phenyl-2-alkenales, and compounds with unknown structures
  30 - 70 % w/w of fraction 3 are transferred to the brewed coffee. Arabica and Robusta differ considerably in their transfer rates (Fig. 5).

FRACTION 4:

- represents 20 - 25 % w/w of the steam-volatile aroma and contains approx. 45 components:
  furfural, 5-methylfufural, phenol and alkylphenols, oxalkylthiophenes, N-furfuryl-2-oxalkylpyrroles, and some unidentified compounds
- 60 - 75 % w/w of this fraction are transferred to the brewed coffee. Fig. 6 illustrates the different transfer rates of Arabica and Robusta.

FRACTION 5 :

- represents 30 - 40 % w/w of the steam-volatile aroma and contains approx. 110 components:
  furfurylalcohol and other heterocyclic alcohols, aliphatic and aromatic alcohols, thiazoles, oxazoles, furanones, alkyl-, furyl- and cyclopentapyrazines, oxalkylpyrroles,

hydroxyketones, and some compounds with unknown structures
- Fig. 7 shows the different transfer rates of Arabica (approx. 50 %) and Robusta (approx. 80 %).

FRACTION 6:

- represents 30 - 40 % w/w of the steam-volatile aroma and contains approx. 15 components:
  pyridine and alkylpyridines, pyrazine and its methyl derivatives
  Robusta permits more of fraction 6 (Fig. 8) to be transferred to the brewed coffee (80 %) than Arabica (60 %).

TOTAL AROMA TRANSFER

- Our investigation of Arabica (Kenya) and Robusta (Indonesia) showed (Fig. 9) that Robusta transferred more steam-volatile aroma (75 %) from the roasted beans to the brewed coffee than Arabica (55 %). One litre of brewed coffee contains approx. 25 - 50 mg of steam-volatile aroma (without acids), i. e. a cup (150 ml) contains approx. 3,5 - 7,5 mg.

- The various groups of components or the individual aroma constituents differ considerably in their rate of transfer from the bean into the beverage depending on their watersolubility and their concentration in the roasted coffee. This is very important, because Arabica and Robusta differ (due to their aroma precursors) considerably in their aroma composition.

A detailed report on these results is in preparation.

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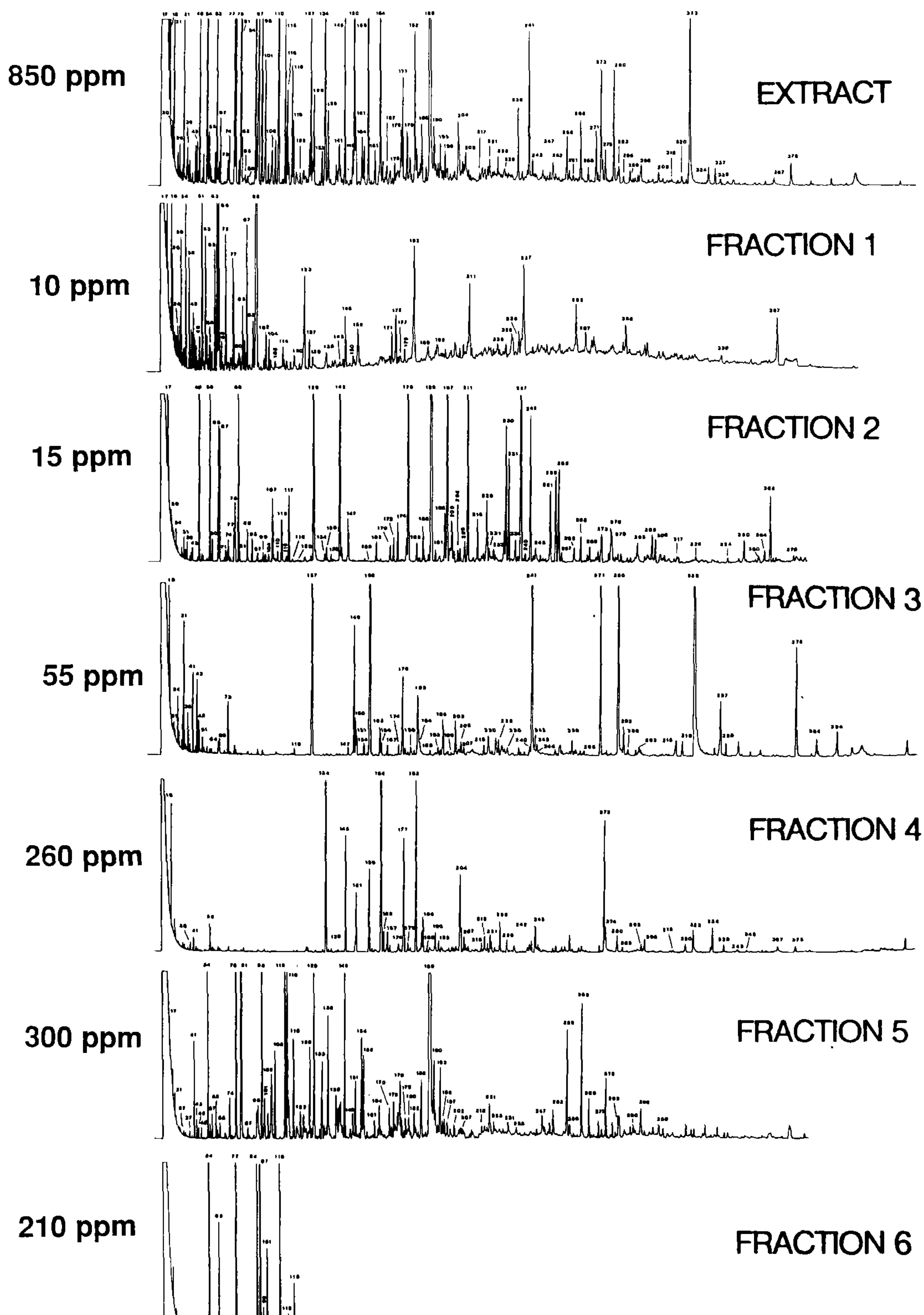
Fig. 2 : Distribution of Arabica (Kenya) coffee aroma extract during fractionation on silica gel.



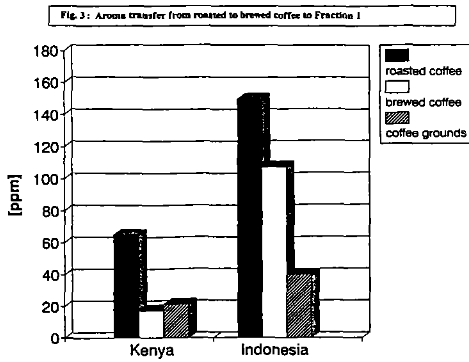


Fig. 3: Aroma transfer from roasted to brewed coffee to Fraction 1

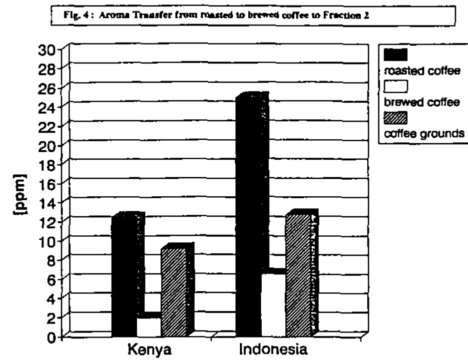


Fig. 4: Aroma Transfer from roasted to brewed coffee to Fraction 2

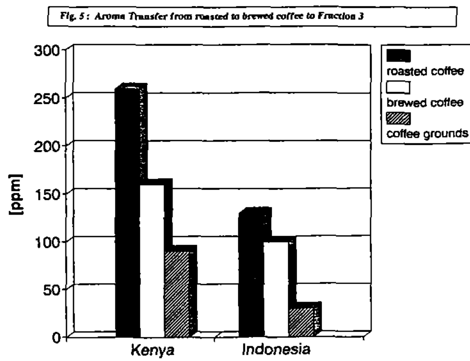


Fig. 5: Aroma Transfer from roasted to brewed coffee to Fraction 3

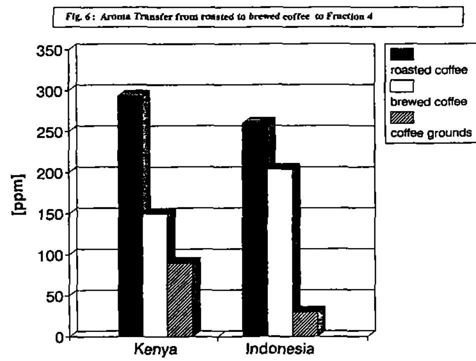


Fig. 6: Aroma Transfer from roasted to brewed coffee to Fraction 4

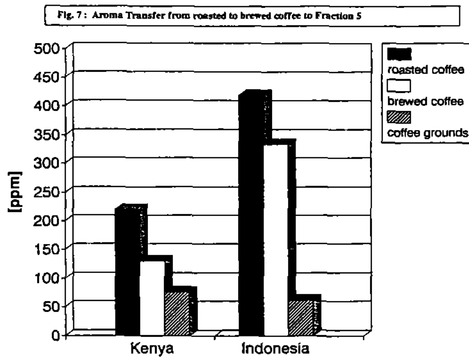


Fig. 7: Aroma Transfer from roasted to brewed coffee to Fraction 5

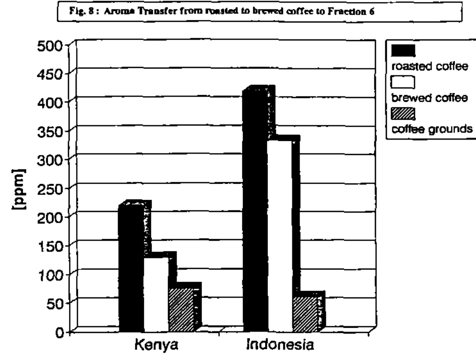


Fig. 8: Aroma Transfer from roasted to brewed coffee to Fraction 6

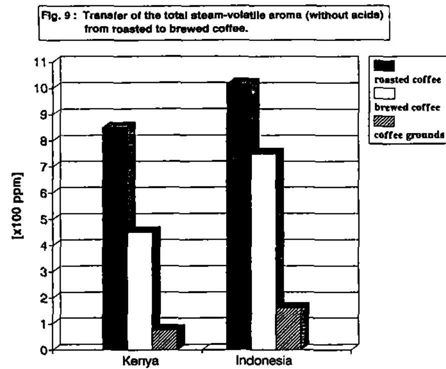


Fig. 9: Transfer of the total steam-volatile aroma (without acids) from roasted to brewed coffee.

DEHYDROCAFESTOL AND DEHYDROKAHWEOL : TWO NEW ROASTING COMPONENTS IN COFFEE

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Discovery and identification

A striking feature of the HPLC chromatogram of the unsaponifiable matter during determination of the diterpenes cafestol and kahweol in coffees [1] is two signals that occur only in the roasted coffee sample and not in the sample of green coffee (Fig. 1) [2].
By recording the EI mass spectra (Fig. 3) it was possible to identify dehydrocafestol and dehydrokahweol, which are evidently formed during roasting by dehydration of cafestol and kahweol (Fig. 2).
In the case of dehydrocafestol the exact position of the double bond that had thus come about was determined by analysing the ^{1}H-NMR spectra (Fig. 4).

Formation of dehydrocafestol as a function of roasting conditions

Two Robusta coffees with greatly differing cafestol content were each roasted for three minutes at different temperatures and then analysed. It was found that as the roasting temperature increased the cafestol content steadily fell and the dehydrocafestol content rose at practically the same rate (Fig. 5).
When the ratio of cafestol content to dehydrocafestol content is determined at the various roasting temperatures, the figures for the two coffees are found to be more or less equal at the same stage of roasting although the diterpene content differs.
The ratios therefore seem to depend solely on the degree of roasting and not on the cafestol content in absolute terms.
This connection is to be substantiated by further investigations.

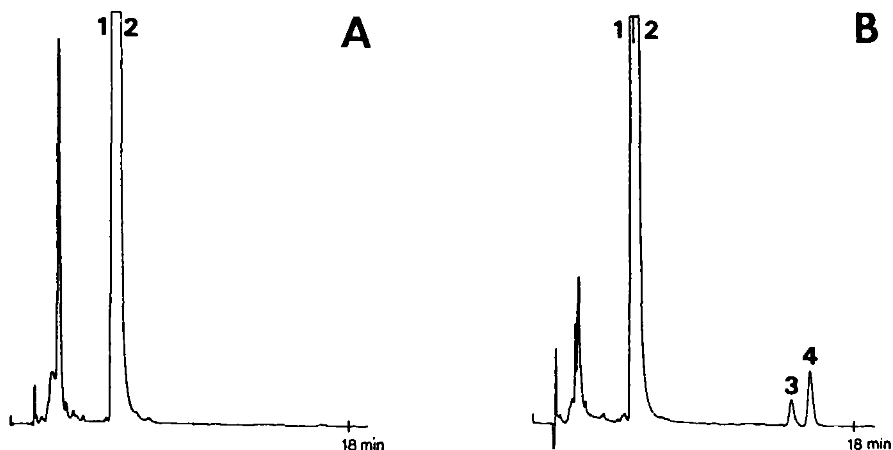

Fig. 1 AB. HPLC-chromatograms of Colombian Arabica
A green coffee, **B** same coffee roasted (270 °C, 3 min)
1 Kahweol, **2** Cafestol, **3** Dehydrokahweol, **4** Dehydrocafestol
Column: Hypersil ODS 5
Mobile phase: Acetonitrile/H_2O 60/40
Detection: 220 nm

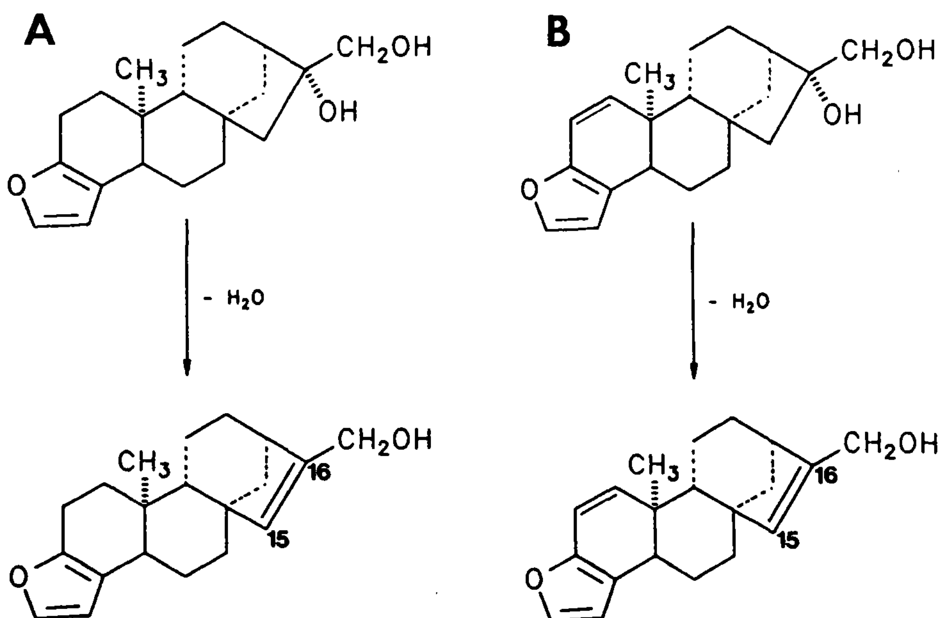

Fig. 2 AB. Structures of **A** Cafestol / Dehydrocafestol, **B** Kahweol / Dehydrokahweol

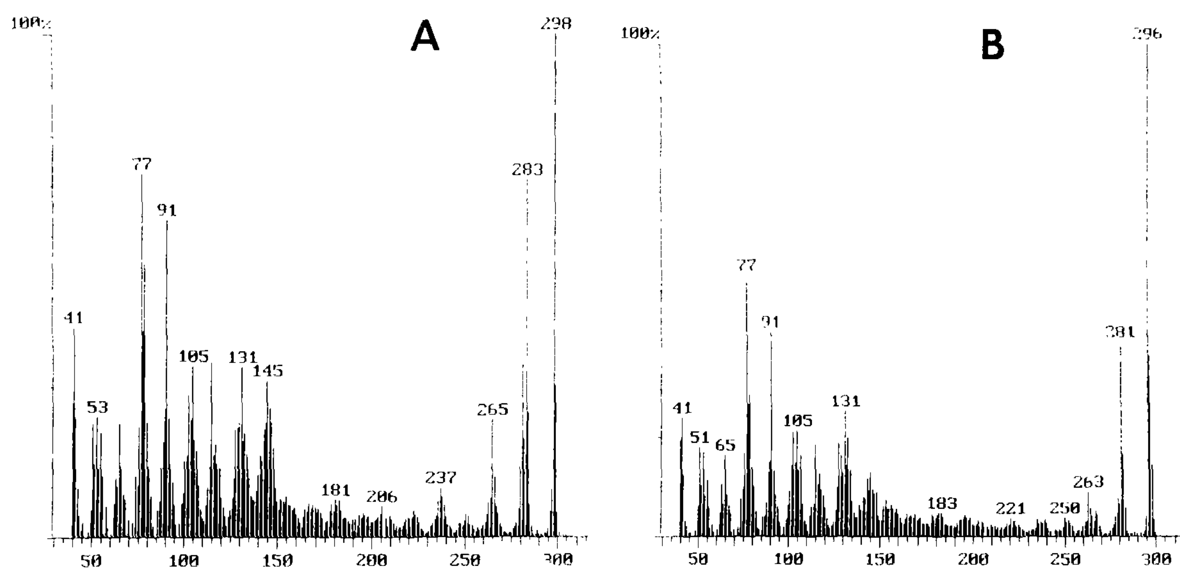

Fig. 3 AB. EI mass spectra of **A** Dehydrocafestol, **B** Dehydrokahweol

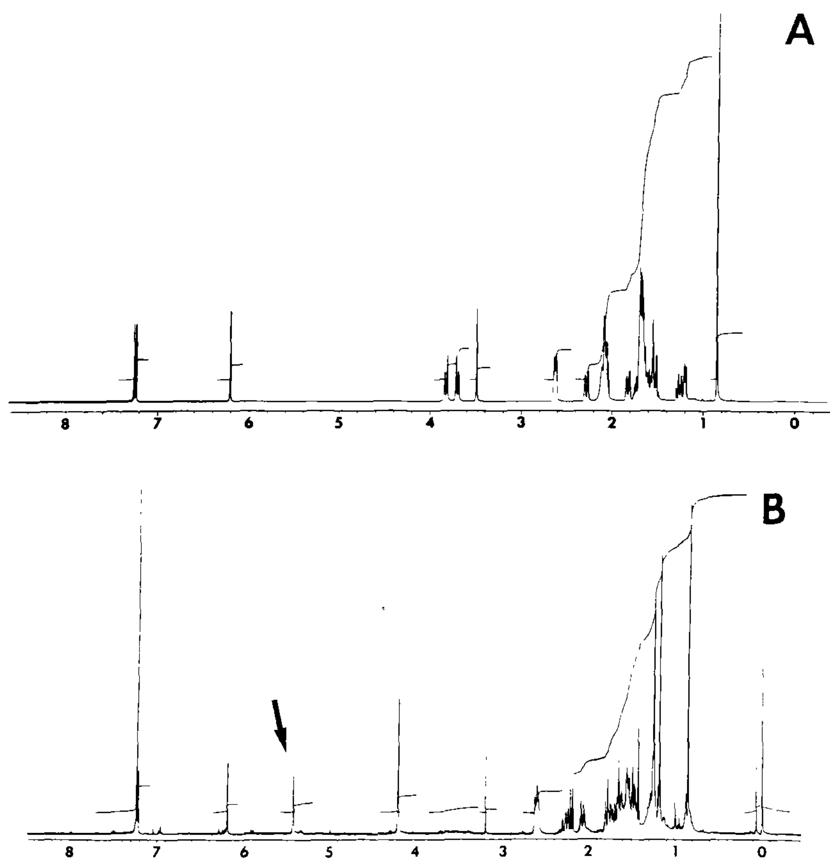

Fig. 4 AB. ^{1}H-NMR spectra of **A** Cafestol, **B** Dehydrocafestol
400 MHz; 10 mg cafestol, ca. 1 mg dehydrocafestol in $CDCl_3$

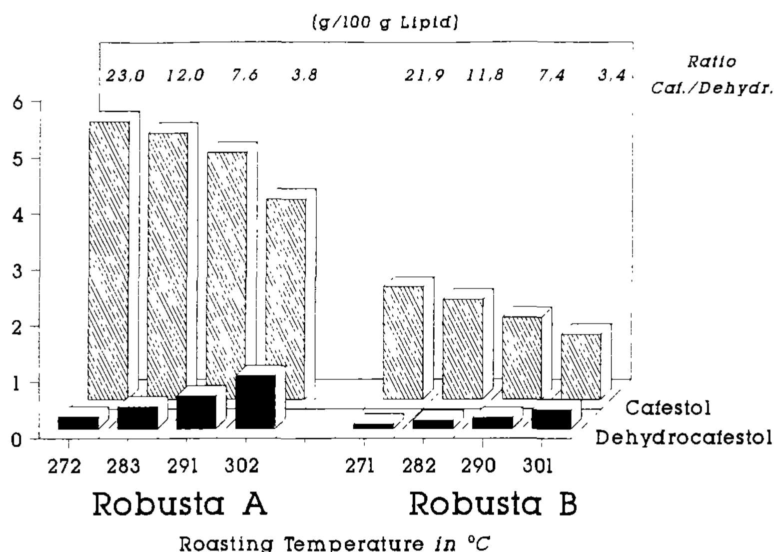

Fig. 5. Contents of Cafestol and Dehydrocafestol in coffee roasted at different temperatures

Acknowledgement
We have to thank Dr. V. Sinnwell for NMR analysis.

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Summary

In roasted coffee, dehydrocafestol and dehydrokahweol have been identified as degradation products of the diterpenes cafestol and kahweol. The dehydrocafestol content increases as the roasting temperature rises.

Zusammenfassung

In Röstkaffee wurden Dehydrocafestol und Dehydrokahweol als Abbauprodukte der Diterpene Cafestol und Kahweol identifiziert. Der Gehalt an Dehydrocafestol nimmt mit steigender Rösttemperatur zu.

PROGRESS IN THE ANALYSIS OF PROANTHOCYANIDINS IN FRESHLY PREPARED COFFEE PULP

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INTRODUCTION

Coffee pulp is a byproduct of the wet processing of coffee cherries, much of which is dumped in water courses causing serious pollution. For economic and environmental reasons attempts have been made to utilize coffee pulp as animal feed but when used at levels in excess of 10% of normal rations feed utilisation and growth rate are impaired (Bressani & Braham, 1985). Various components have been blamed for these effects but recently it has been shown that the caffeine and phenolic acids contents are too low and the tannins are now generally considered to be responsible (Clifford & Ramírez-Martínez, 1990; Ramírez-Martínez & Clifford, 1990).

The tannins of coffee pulp have been analysed using various procedures. Zuluaga-Vasco and Tabacchi (1980) reported that sun dried arabica pulp contained 0·41% hydrolysable tannins and 1·64% formaldehyde-precipitatable condensed tannins, whereas García *et al.* (1985) reported 2·56% alkali-soluble condensed tannins using a method detecting flavan-3-ols which may also be present in the range 0·19-0·86% (Ramírez-Martínez, 1988). It has since been shown that the reports of hydrolysable tannins probably arose from a misclassification of phenolic acids and that condensed tannins dominate this fraction (Clifford & Ramírez-Martínez, 1991).

Using the condensed tannin-specific Porter's reagent it was shown that sun dried arabica coffee pulp contained 1·40-1·47% db tannins but it was subsequently observed that the tannin content of the dried pulp apparently declined to 0·16-0·19% db after a years storage and it was recognised that the values originally obtained might have been underestimates of the tannin content of fresh pulp. An improved procedures for extracting condensed tannins from sun dried coffee pulp led to values for crude condensed tannins of 3·82 $\pm$ 0·43% db (as oligomers) or 6·72 $\pm$ 0·72% db (as dimers) (Clifford *et al.* 1991). This paper provides data for the analysis of coffee pulp immediately after removal from freshly harvested coffee cherries, and for the same material after drying. In addition, the extracts of crude tannins have been partially purified and fractionated into ethyl acetate-soluble dimer-rich and ethyl acetate-insoluble oligomer-rich fractions.

MATERIALS

Recently harvested ripe coffee cherries (*C. arabica* var. Red Bourbon, Red Catuai and Yellow Catuai) were supplied by the Estación Experimental Agrícola de Bramón, Venezuela. All reagents were standard analytical grade items from reputable commercial sources.

METHODS

Detection of proanthocyanidins in fresh coffee pulp by direct addition to Porter's reagents.
The method previously described (Clifford and Ramírez-Martínez, 1991) was applied to 200 mg of freshly prepared whole pulp free from pigmented exocarp.

Proanthocyanidins extraction from freshly prepared undried coffee pulp.
Ripe coffee cherries were depulped by hand and 5 g of pulp were immediately blended with 20 ml of aqueous 70% acetone in a Sorvall omnimixer for 10 min. The resulting mixture was centrifuged in a Sorvall refrigerated centrifuge at 10,000 rpm for 10 min and the supernatant was filtered through a Whatman No. 1 filter paper. The pellet was extracted in aqueous 70% acetone until the supernatant gave a negative response to the Folin-Denis reagent (AOAC, 1984). The filtered aqueous acetone extracts were pooled and concentrated by low pressure evaporation at $< 30°C$.

The concentrated aqueous extract was lyophilized and the brownish lyophilisate extracted sequentially (1:20 m/v) with petroleum ether ($\times 3$) to remove pigments and lipids, with ethyl acetate ($\times 7$) to extract simple phenols, flavonoids and proanthocyanidin dimers and with dichloromethane ($\times 3$) to eliminate caffeine. The resulting ethyl acetate-insoluble fraction contained the proanthocyanidin oligomers. The ethyl acetate extracts containing the proanthocyanidin dimers were pooled and lyophilized. The three lyophilisates were analysed as described below.

Proanthocyanidin extraction from freshly prepared coffee pulp after drying.
Coffee pulp was air-dried for 14 days, left overnight in an oven at 30°C, ground and sieved through a 10 mesh sieve. Aliquots (5 g) were suspended in 20 ml of aqueous 50% acetone and shaken at room temperature for 30 min, but otherwise fractionated identically to the undried pulp.

Quantification of condensed tannins using Porter's reagents.
Aliquots of the lyophilisates from fresh and dried pulp were prepared in triplicate as previously described (Clifford and Ramírez-Martínez, 1991; Clifford *et al.* 1991). Two samples were autoxidised and the third served as an unheated control. The control corrected E_{550} values were interpreted by reference as appropriate to the $E^{1\%}_{550}$ values recommended by Porter *et al.* (1986) and Porter (1989) for quantification of dimeric and oligomeric 4→8 linked proanthocyanidins.

RESULTS AND DISCUSSION

The immediate testing of freshly prepared coffee pulp, free from pigmented exocarp, by direct addition to Porter's reagents yielded a red colour indicating the presence of condensed tannins, but the large moisture content of the pulp ($\approx 80\%$) disturbed the reagent composition and interfered in colour development and it was not possible to interpret the result quantitatively. This is the first unequivocal observation of condensed tannins in freshly prepared coffee pulp and in direct contrast to the results of Zuluaga-Vasco and Tabacchi (1989) who believed that the tannins were formed only on sun drying.

The proanthocyanidins contents of fractions isolated from the fresh and dried coffee pulp from three cultivars of arabica coffee are presented in Table 1. The factor for procyanidin oligomers (470) was used for the crude and ethyl acetate-insoluble fractions, whereas the factor for dimers (270) was used for the ethyl acetate-soluble fraction.

The condensed tannins contents for the crude lyophilisate from freshly prepared pulp (0·60—0·91% db before drying and 0·88-1·19% db after drying) are similar to the 1·40—1·47% db and 3·82-6·77% db previously obtained (Clifford and Ramírez-Martínez, 1991; Clifford *et al.* 1991). Such small values for the freshly harvested pulp were somewhat unexpected since it has been noted that the apparent condensed tannin content declined during post harvest storage of the pulp.

It is interesting to note that not only does there seem to be a net production of tannin on drying, but also that while fresh pulp from all three cultivars contains more dimers than oligomers, this relationship is reversed on drying, strikingly so in the case of pulp from Red Catuai. Further studies are in progress, and more detailed papers are in preparation and will be published elsewhere.

Table 1. The proanthocyanidins contents[a] of freshly harvested and dried coffee pulp from three cultivars interpreted as procyanidin equivalents (% db) using $E^{1\%}{}_{550}$ according to Porter[b].

Cultivar and method of pulp preparation	Crude lyophilisate	Proanthocyanidin dimer-rich ethyl acetate-soluble fraction	Proanthocyanidin oligomer-rich ethyl acetate-insoluble fraction
Red Bourbon (Fresh)	0·73	0·72	0·27
Red Bourbon (Dried)	0·88	0·15	0·33
Yellow Catuai (Fresh)	0·91	0·82	0·15
Yellow Catuai (Dried)	1·19	0·11	0·35
Red Catuai (Fresh)	0·60	0·58	0·11
Red Catuai (Dried)	0·89	0·11	0·75

[a] mean $\pm$ s.d., N $\geq$ 6

[b] $E^{1\%}{}_{550}$ = 270 for dimers; $E^{1\%}{}_{550}$ = 470 for oligomers (Porter *et al.*, 1986; Porter, 1989)

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SUMMARY

This communication reports the extraction, quantification and fractionation of condensed tannins from freshly prepared coffee pulp and the effects of oven drying. Tannin content, which was 0·60—0·91% db before drying, rose to 0·88-1·19% db after drying, and was accompanied by the apparent conversion of proanthocyanidin dimers to oligomers.

RÉSUMÉ

Ce manuscrit rend compte de l'extraction, de la quantification et de la fractionation pour les tannines dans la pulpe fraiche de café, et les effets de sécher. La quantité de tannines, qui était 0·60-0·91% ms avant de sécher, après s'agrandit à 0·88-1·19% ms. Aussi, il aperçu que les dimers étaient transformés aux oligomers.

SEPARATION OF METAL CHELATING COMPOUNDS IN INSTANT COFFEE

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The effect of coffee brew on mineral nutrition has recenttly been noticed. Iron deficiency anemia is prevalent for malnourished persons in area such as Costa Rica where coffee is commonly consumed, and an inhibitory effect on non-heme iron absorption in human subject has been reported[1,2]. Roasting of green bean of coffee is essential to produce aroma, taste and color compounds in coffee brew. Polyphenolic compounds such as chlorogenic acid and tannin have been known to be are active in metal chelation, which would be involved with antioxidative activity and biological availability of metal ions in food system. Phenolic compounds as well as sugars and amino acids are degraded and reacted each other by roasting to form more active compounds in metal chelation than the coumpounds originally present in the green bean. There have been few studies stating that they have been separated as metal chelating compounds[3].

The purpose of this study is to separate iron(II) chelating compounds from instant coffee.

Materials and Methods

Sample.

Instant coffee of lyophilized type, Nestle Gold Blend was offered by Nestele Co. Ltd., Japan and was stored at 4C.

Preparation of Fe(II)-coffee complex

Coffee(1kg) was added with 3 l of 10mM $FeSO_4$ dissolved in 10 mM hexamine buffer(pH 5.0) containing 10mM KCl and stirred. The solution was allowed to stand overnight, and centrifuged. The precipitates formed was washed with the pH 5.0 hexamine

buffer and centrifuged. This washing procedure was repeated three times in order to remove unreacted ferrous ion. The washed precipitates was dissolved in 1 l of 1% ammonia solution, and the residual material was again dissolved in the ammonia solution. The Fe(II)-coffee complex soluble in the ammonia solution was combined, and then lyophilized this being designated as Sample A. As a marker of the Fe-complex, iron in the solutions during the preparation was determined by atomic absorption spectrophotometry(Shimadzu AA-670) without ashing

Separation of Sample A by ion-exchange column chromatography.

Sample A(14g) was dissolved in water, and loaded into a column (4.0 x 40 cm) of Amberlite IRA-410(OH^-). After the column had been washed with 3 column volumes of water, it was eluted with 1N HCl. The eluate was collected in fractions of 200 ml, and measured for iron concentration by atomic absorption spectrophotometry without ashing procedure.

The fractions with high iron content was loaded into a column(4 x 40 cm) of Amberlite IR-120B(NH_4^+). The column was eluted with 3 column volumes of water and 1N ammonia. The eluate was also collected into fractions this being measuring for the iron concentration. The fractions with high iron concentration was acidified with conc. HCl and the formed precipitate was collected by centrigugation. The precipitate was dissolved in a diluted ammonia solution, and the soluble portion was lyophilized this being designated as Sample Ap.

Separation of the Fe-coffee complex by cellulose column chromatography.

Sample Ap(6.0g) was dissolved in about 30 ml of 1% ammonia, and dispersed into 10g of Celite(Hyflo super cel). The resulting gruel-like material was lyophilized, and pulverized in a motor and pestle. A cellulose column(6 x 60 cm) was prepared with Cellulose mikrokristallin(Merck) which had been washed with a mixed solution of n-propanol and water(5:2 v/v). The lyophilized material was suspended in the starting eluent and put on the top of the cellulose column. The eluents were prepared by step-wise change in mixing ratio(v/v) of n-propanol and 1% ammonia solution from 5:2, 3:2 and 1:1. Each eluent was 3 L, and the eluate, being collected into fractions of 20 ml, was monitored by the absorbance density at 470nm and iron concentration according to atomic absorption spectrophtometry. The eluted peak components were concentrated *in vacuo*, and their yield and iron content were determined. Iron was determined by atomic absorption spectrophotometry after the dried samples were dissolved in a 1% ammonia solution.

Estimation of molecular weight of Sample Ap component

Molecular weight of Sample Ap components was estimated by using HPLC with a column of TSK-gel G-3000PW(Tosoh, Japan: 7.5 mm x 60 cm) connected with a precolumn of TSK PWH(Tosoh, Japan :7.5 x 75 mm). The eluent was pH 6.8 phosphate buffer(20mM) containing 0.1M KCl, and molecular markers used were proteins, which were cytochrome C(12,400), carbonic anhydrase(29,000), albumin(66,000) and alcohol dehydrogenase(150,000) obtained from Sigma. The elution was detected by the absorbance at

Dissociation constant.

Dissociation constant was measured by dialysis-equilibrium method in pH 4.0 acetate buffer(0.01M).

Results and Discussion

Preparation of Fe-Coffee complex.

The method to prapare and separate the Fe-coffee complex leads to select the property of the complex. The Fe-coffee complex obtained here is soluble in an alkaline solution, and insoluble in a weak-acidic solution. Since ferrous sulfate is not soluble in alkali, the presence of iron in this alkali-soluble complex is indicative of the formation of iron (II) chelating compound(s). The change in iron concentration of the solutions during the preparation of the complex is shown in Table 1, where total quantity of soluble iron is larger in an alkaline condition than in an acidic condition after the complex formation. In this study

The Fe-coffee complex (Sample A) is black-colored materials, and the iron content is 3.60 mg/g. The yield is 1.58% from the coffee.

Separation of Sample A by ion-exchange column chromatography.

Asakura et al. revealed that zinc chelating substance in instant coffee was acidic by electrophoresis[??]. As shown in Table 1, most Fe-coffee complex were retained with neither anion- nor cation-exchange columns, and almost no iron was dissociated from the ligands from the coffee regardless of the passage of the Fe-complex through a strong cation exchanger. It is indicative of strong chelating activity of the Fe-coffee complex separated.

The Fe-coffee complex was recovered as precipitate by acidification of the solution. The yield of the Fe-coffee complex(Sample Ap) purified by ion-exchange chromatographies was 0.9% of the instant coffee.

Separation of the Fe-coffee complex by cellulose column chromatography.

Cellulose column chromatogram of Sample Ap is shown in Fig.1. Sample Ap was separated into 4 fractions from Ap I to Ap IV by raising the mixing ratio of 1% ammonia in the eluent. Table 2 shows the yield and the iron content of each Ap component separated by the cellulose column chromatography. Ap I and II eluted with the starting eluent hardly contain iron, while Ap III and IV contain iron being regarded as Fe-coffee complex. In this study iron in the complex had been checked as the marker for chelating substances, and the separation procedures cause to release iron from the ligands with a low stability constant resulting in the potent indicator of iron-chelating activity in the advanced process of separation.

Molecular weight estimation of Sample Ap component

The molecular weight of the Sample Ap components prepared by the cellulose column chromatography is shown in Table 2. The molecular weight of Sample Ap-I and -II were found to be less

than 12,400 (about 9,000 by extrapolation of the calibration curve) and 15,000, respectively. Sample Ap-III and -IV were found to be 36,000 and 50,000, respectively, which were larger than those for Sample Ap-I and -II. It is of a great interest that the elution order of the Sample Ap components eluted by raising the polarity of the eluent on the cellulose column chromatography is correlated with iron content and molecular weight.

The yield of the Sample Ap I-III components from the coffee sample is 0.06%, and that of the Ap-IV is one order less than that of Ap-III. Although the major Fe-coffee complex is the Sample Ap III in this study, the yield of the Sample Ap-III and -IV seems to be variable to some extent according to our repeated works. It suggests that the conditions for the separation process used may give effects to the stability of the Fe-complex formed.

Dissociation constant

Apparent dissociation constant of Ap-III for Fe(II) was found to be 5.56×10^{-6} by dialysis-equilibrium method. The value seems to be larger than supposed. Since the iron content of Ap-III is found to be 4.6mg/g, the metal binding sites with strong chelation are supposed to be occupied with iron, which would not be released by ion-exchanges and acidic conditions used during the separation prcess. The determined value is supposed to be for the sites for weak chelation.

This study revealed that the instant coffee contains more than 0.06% of iron chelatig compounds. The properties of iron(II) chelation such as the estimation of stability constant and its polymerization due to metal chelation are in the progress.

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DETERMINATION OF TRIGONELLINE AND NICOTINIC ACID BY HPLC

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Abstract

A new method for the determination of trigonelline and nicotinic acid, a thermal degradation product, has been developed. Sample preparation includes a filtration step over C-18 extraction cartriges. Recovery is nearly complete. The separation is acieved by an ion exclusion HPLC system.
The effects of the roasting parameters (temperature, duration, roast colour) on the thermal decomposition of trigonelline are demonstrated in this paper.
Quantitative data are presented in a variety of coffee samples, e.g. different green coffees, R & G, SC.

RAPPORT DE SYNTHÈSE / SUMMARY REPORT

Rapport de synthèse

Biotechnologie

La session Biotechnologie s'est fixé pour objectif d'établir le bilan des apports récents de la biotechnologie, de la biochimie et de la génétique à l'amélioration des caféiers et à la production de cafés de qualité. Cette session a permis de présenter deux exposés magistraux, seize communications et douze affiches, tous d'un grand intérêt scientifique. Le premier exposé magistral du Dr W. Powell (SCRI), présenté par K. Chalmers, a permis de faire connaître les techniques moléculaires et leurs premières applications aux caféiers. Le second, du Dr V. Pétiard (Francereco), nous a donné une vue d'ensemble des différentes biotechnologies basées sur la maîtrise de la culture *in vitro* des caféiers. L'ensemble des présentations de cette session peut être synthétisé sous trois thèmes.

1. Les marqueurs moléculaires et biochimiques

Les premières applications aux caféiers des techniques de marquage de l'ADN (RFLP, RAPD, PCR) par le SCRI (exposé K. Chalmers), Francereco (exposé M. Paillard) et l'ORSTOM (exposé J. Cros) ont été développées pour caractériser et identifier le matériel végétal, établir des phylogénies, détecter des introgressions et, à terme, établir une carte génétique des caféiers.

La taille du génome des caféiers et la variation de la quantité d'ADN ont été précisées par les chercheurs de l'ORSTOM (S. Hamon). Enfin, le polymorphisme biochimique des caféiers africains et malgaches étudiés par J. J. Rakotomalala (FOFIFA) conduit à renouveler la classification taxinomique des caféiers.

Une première utilisation de ces marqueurs est dès maintenant possible pour l'identification des caféiers ; à moyen terme, des corrélations avec les caractères agronomiques et technologiques assureront la relation avec les sélectionneurs. Des coopérations entre les laboratoires qui maîtrisent les techniques moléculaires (SCRI, Francereco, ORSTOM) et les équipes d'amélioration et de sélection des caféiers permettront d'optimiser l'utilisation de cette nouvelle approche.

2. Les différentes biotechnologies basées sur la maîtrise de la culture *in vitro* des caféiers

Ces techniques ont été expérimentées dans les années 80 et ont évolué avec l'avancée des connaissances. Elles débouchent sur différents développements importants pour les caféiers.

a) La conservation du matériel végétal et des variétés améliorées dans des collections en champ ainsi que leur échange peuvent être abordés autrement. Pour le stockage de vitroplants, F. Engelmann (ORSTOM) a mis en évidence un marqueur précoce des altérations physiologiques en cours de conservation à différentes températures. La conservation à long terme dans l'azote liquide, appelée cryoconservation, a largement progressé. La déshydratation et la congélation d'embryons zygotiques et d'embryons somatiques ont été présentées par B. Florin (Francereco) et par A. Abdelnour (CATIE). La première expérience d'encapsulation-déshydratation des méristèmes a été réussie par l'équipe ORSTOM (S. Mari).

b) La multiplication *in vitro* des caféiers est développée par microbouturage et microgreffage au CATIE (B. Bertrand), par induction de rejets multiples sur hypocotyle au SCRI (N. E. Nyange) et par différentes voies de régénération au CIFC (M. E. Carneiro) et au CATIE (C. Brocherieux).

Les progrès les plus notables concernent l'embryogenèse somatique grâce au développement de la culture en milieu liquide à Cenicafé (M. E. Aponte) et l'utilisation de bioréacteurs par Francereco (J. P. Ducos) et par DNAP (C. Noriega), bien que les effets génétiques étudiés à l'IAC (J. C. S. Ramos) restent prépondérants. Les taux de multiplication atteints sont remarquables et ouvrent des perspectives d'applications variées. En outre, les conditions de croissance des vitroplants sont améliorées au CIRAD (D. Bieysse) par modification des facteurs environnementaux.

c) La fixation de lignées et la création de variétés de *C. arabica* par **haploïdisation** progressent lentement. La formation de colonies cellulaires haploïdes à partir de microspores a été obtenue au CATIE (B. Neuenschwander). L'embryogenèse somatique sur microspores et anthères obtenue au CIFC (M. E. Carneiro) mérite d'être contrôlée au niveau cytologique.

d) La création de variations somaclonales chez les caféiers issus d'embryogenèse somatique est en cours d'étude à DNAP (M. Sondhal) et Francereco (V. Pétiard). L'amélioration des cultivars par la sélection *in vitro* de cals est actuellement tentée au SCRI (N. E. Nyange) en présence de filtrats de cultures de *Colletotrichum coffeanum,* agent du CBD.

e) La transformation génétique des caféiers progresse. Elle a été réussie par *Agrobacterium rhizogenes* à Francereco (J. Spiral) sur des embryons somatiques d'*Arabica,* de *Canephora* et d'*Arabusta.* De l'ADN étranger est aussi introduit dans les tissus de caféiers grâce au canon à particules au CIRAD (J. Van Boxtel). Enfin, la régénération de protoplastes est étudiée à l'Université de Compiègne (J. Grèzes) en relation avec la transformation génétique et la production de métabolites secondaires. Les perspectives ouvertes par l'introduction de gènes étrangers dépendent du progrès des connaissances sur les bases biochimiques et moléculaires des caractères agronomiques et biochimiques.

3. Biosynthèse des composés biochimiques des caféiers

Les recherches présentées portent essentiellement sur la synthèse des précurseurs des alcaloïdes dérivés des purines. Les différentes voies de production et l'accumulation de ces alcaloïdes aux différents stades de développement des tissus sont étudiées aux Etats-Unis (G. R. Waller) et au Japon (H. Ashihara). De même, G. M.

Nazario (Université de Californie) a montré que chaque alcaloïde est le produit d'une voie de biosynthèse spécifique à partir des purines endogènes.

Un nouveau métabolite a été mis en évidence dans les cultures cellulaires de caféiers à l'Université de Zurich (B. H. Schulthess). Le Dr T. Baumann de cette même université a étudié l'interdépendance de la caféine et de l'acide chlorogénique et la régulation de leur synthèse.

A. Charrier
Professeur à l'ENSA M

Agronomie

Je dois tout d'abord remercier mes anciens collaborateurs et amis qui m'ont aidé à faire cette synthèse des journées d'Agronomie, MM. Avelino, Charmetant, Decazy et Eskes.

Les sessions d'Agronomie ont débuté par un exposé général que j'ai eu moi-même l'honneur de présenter, sur le thème : « Quelles caféicultures pour demain ? » Cet exposé fait ressortir la distance existant entre le possible, une culture du caféier très performante, appliquant les données de la recherche, et le réel, une caféiculture trop souvent de type primitif ne bénéficiant en rien des connaissances acquises, avec une tendance marquée vers des pratiques extensives proches de la cueillette, du fait de la crise actuelle.

Des propositions pour faire mieux passer le message technique en milieu rural, et pour orienter les recherches dans le sens d'une réduction des coûts de production servent de conclusion, avec le souhait qu'une entente pour des prix convenables soit rapidement trouvée, permettant aux producteurs d'avoir une juste rémunération de leur travail et une possibilité d'accéder aux progrès techniques.

On a ensuite examiné les avancées récentes en matière de **génétique** et de **sélection.** Plusieurs grands thèmes ont été abordés.

En ce qui concerne les ressources génétiques, une méthodologie a été présentée par M. Noirot pour l'échantillonnage de la variabilité. Elle permettra de constituer une « core » collection, qui servira de base de travail pour les différents programmes d'amélioration génétique.

Les relations entre les espèces ont été évoquées sous deux aspects : la fertilité des hybrides, par J. Louarn, et la compatibilité au greffage, par E. Couturon. Ces deux approches mettent en évidence la distance génétique existant entre les caféiers de l'Est et de l'Ouest de l'Afrique.

Selon M. Bayetta Bellachew, l'évaluation de lignées éthiopiennes en collection confirme leur adaptation préférentielle aux conditions de leur région d'origine.

En ce qui concerne l'hybride interspécifique Arabusta, Y. Adu-Ampomah, pour le Ghana, et D. Le Pierrès, pour la Côte-d'Ivoire, ont fait état des études qui portent sur le système de reproduction des hybrides F1, l'évaluation des générations F2 et F3 et sur les rétrocroisements sur l'Arabica. Une légère amélioration de la fertilité en F3 a été notée.

Pour *Coffea canephora,* T. Leroy a rendu compte des paramètres génétiques estimés grâce au schéma de Sélection Récurrente Réciproque, qui a abouti à la sélection de sept hybrides et de soixante-dix clones. Au Ghana, la sélection clonale conduite par Y. Adu-Ampomah a produit des clones améliorateurs. P. Lashermes a exposé la méthode d'exploitation des haploïdes doublés en Côte-d'Ivoire : des hybrides, aussi performants que certains clones, ont été obtenus.

La transmission de la résistance à la rouille orangée liée au facteur SH3 a été étudiée par T. D. Kukhang dans le cadre d'une coopération CIRAD-Papouasie-Nouvelle-Guinée. Ce facteur semble complexe, ce qui peut expliquer sa durabilité relative en Inde et en Indonésie.

L'état d'avancement des programmes d'amélioration dans ces deux pays a été présenté respectivement par M. S. Sreenivasan et S. Mawardi, puis, pour la Papouasie-Nouvelle-Guinée, par P. Charmetant.

Enfin, des résultats prometteurs ont été présentés pour une stratégie d'amélioration des Arabica basée sur l'exploitation d'hybrides F1. Une hétérosis importante a été observée au Cameroun pour la production, par M. Boccara, et en Ethiopie, par M. Bayetta Bellachew, pour la vigueur dans le jeune âge.

C. O. Agwanda a évoqué les problèmes posés par la production de semences hybrides au Kenya.

Finalement, M. Cadena, de Colombie, a proposé la méthode de M. d. P. Moncada B., qui permet de réduire le temps nécessaire à l'évaluation de la productivité. On peut ainsi envisager une réduction importante de la durée des cycles de sélection.

En **phytopathologie,** sept communications et six affiches ont été présentées, soit huit études sur la rouille orangée et cinq autres sur le CBD (anthracnose des baies du caféier).

En ce qui concerne le CBD, dû à *Colletotrichum coffeanum sensu* Hindorf, le travail de C. Lambot au Burundi a montré l'effet d'applications de zinc sur l'incidence du CBD. V. Aubin et ses collaborateurs ont mesuré le temps nécessaire à la chute des baies après infection au Cameroun et ont montré que les fongicides systémiques agiraient en ralentissant le développement du champignon dans les baies. Pour l'obtention de tests précoces de sélection de *Coffea arabica* contre le champignon, M. Rigouzzo et ses collègues soulignent l'importance de la mise en corrélation précise des niveaux de sensibilité au CBD observés au champ et des réactions obtenues en laboratoire. La biotechnologie semble devoir jouer un grand rôle au Cameroun dans ce domaine. C'est aussi le cas en Tanzanie où N. E. Nyange développe ses recherches. Enfin, C. J. Rodrigues nous a fait part des derniers travaux du CIFC au Portugal, au sujet de la pathogénécité de différents isolats de CBD et de la possibilité de trouver quelques génotypes résistants.

En ce qui concerne la rouille orangée, le CIFC a mis en évidence l'existence de nouvelles races infectant les Catimor, ce qui pourrait représenter une sérieuse menace pour les variétés issues de l'Hybride de Timor.

F. Holguin, dans sa présentation, a montré qu'il existe de nouvelles races de rouille orangée, non seulement sur Catimor mais aussi sur *C. canephora.* Les races qui attaquent les Catimor sont plus virulentes et plus agressives que celles qui attaquent *C. canephora.*

Dans le même sens, C. Montagnon suggère l'existence de différentes races de rouille orangée infectant le *C. canephora* en Côte-d'Ivoire, mais la variabilité génétique de ce caféier permet la mise en place d'une stratégie d'amélioration à la fois pour la résistance à cette maladie, au scolyte des branchettes et à la sécheresse. M. C. Silva et ses collaborateurs, du CIFC au Portugal, se sont intéressés aux relations hôtes-parasite entre la rouille orangée et différentes plantes hôtes et non-hôtes. Dans le cas des relations incompatibles, la croissance du champignon est arrêtée le plus souvent au stade de la formation des haustoria.

M. E. M. Guedes et ses collaborateurs ont mis en évidence, au Portugal, l'existence de composés chimiques, type stéroïdes, dans les urédospores de *Hemileia vastatrix* et pensent qu'il y a là de nouvelles possibilités de lutte. Finalement, trois études épidémiologiques sur la rouille orangée ont été présentées. J. H. Whan, de Papouasie-Nouvelle-Guinée, a souligné la liaison inversement proportionnelle entre l'altitude ou la température

et l'incidence de la maladie. J. Avelino a démontré, au Guatemala, l'importance de la charge en fruits du caféier sur le développement de l'épidémie. La maladie provoque des dégâts en basse altitude, ce qui justifie l'emploi de la lutte chimique. La fertilisation devrait être un moyen de réduire l'incidence de la maladie. Il faut enfin mentionner le travail de l'équipe de l'ORSTOM de Nouvelle-Calédonie qui est parvenue à l'élaboration d'un modèle permettant des estimations du développement des épidémies, à partir de données pathologiques, agronomiques, pédologiques et climatiques.

Les travaux portant sur les **ravageurs du caféier** ont fait l'objet de cinq communications orales et de huit affiches.

Les thèmes de recherche abordés s'inscrivent tous dans un concept de gestion intégrée des ravageurs. Plusieurs stratégies de lutte ont été évoquées. Certes, la lutte chimique fait toujours l'objet d'expérimentations en laboratoire et au champ : quatre insecticides sont évalués par B. Padi pour leur efficacité contre les Epicampoptères, chenilles défoliatrices.

L'évaluation du comportement du matériel végétal vis-à-vis des ravageurs a mis en évidence la possibilité de sélectionner des lignées ou des génotypes résistants à certains aléas, ainsi que les mécanismes de cette résistance. Pour cela des tests précoces d'évaluation ont été mis au point.

Dans le cadre d'une coopération CIRAD/Guatemala, F. Anzueto a étudié le comportement de diverses lignées de caféiers éthiopiens ainsi que celui de certains Robusta vis-à-vis de deux populations de *Meloidogyne* spp. A. Garcia a décrit la sensibilité de diverses espèces de *Coffea* aux attaques de la cochenille *Dysmicoccus cryptus.* C. Montagnon a montré en Côte-d'Ivoire la possibilité de sélectionner des caféiers pour leur résistance au scolyte des branchettes. O. Guerreiro (CIRAD/Brésil) a présenté un travail faisant état de méthodes originales d'évaluation de toxines de *Bacillus thuringiensis,* contre *Perileucoptera coffeella,* dans le but de les utiliser pour la création d'un caféier génétiquement transformé et résistant à ce ravageur.

Deux affiches, l'une présentée par L. Villain *et al.*, l'autre par D. Remond *et al.*, définissent la répartition spatiale et la distribution des populations du nématode *Pratylenchus* sp. et du scolyte des baies, *Hypothenemus hampei,* ce qui permet l'élaboration de méthodes d'échantillonnage pour l'évaluation du niveau des populations de ces ravageurs, utilisables dans un programme de lutte. B. Dufour a identifié les principales espèces de cochenilles du caféier ; il a étudié leur répartition géographique et fait un premier inventaire de leurs ennemis naturels en Amérique centrale. Les méthodes de contrôle envisagées porteront sur la correction du déséquilibre biologique.

Dans le cadre de l'amélioration des méthodes de lutte contre le scolyte des baies, *Hypothenemus hampei,* par une meilleure connaissance des médiateurs chimiques, deux affiches élaborées par A. Lopez présentent, l'une, une méthode rapide et performante d'analyse de la distribution des femelles vierges et fécondées se développant dans les fruits, l'autre, la morphologie et la distribution des sensilles des antennes des mâles et des femelles. F. Mathieu a exposé une affiche montrant la dynamique de sortie des femelles du fruit d'origine en présence de cerises vertes ; les stimuli qui interviennent dans la sortie des cerises sont analysés.

Parallèlement à ces travaux, relatifs à la gestion intégrée des principaux ravageurs, une étude présentée par B. Bouyjou porte sur l'analyse des facteurs d'origines entomologique et bactérienne responsables du goût de pomme de terre et sur les actions propres à corriger ce défaut.

En matière d'**Agrotechnie**, un certain nombre d'affiches ont traité, avec Y. Edjamo et ses collègues, de la densité optimale des plantations en fonction de la dimension des arbustes, selon l'altitude, et de l'influence de différentes densités d'ombrage sur les rendements.

Des recherches sur la fumure organique en Indonésie ont été présentées par M. Winaryo et ses collègues, tandis que les avantages et les inconvénients de divers types de couvertures destinés à remplacer le paillage ont été discutés par D. Snoeck et ses collaborateurs du Burundi, montrant ainsi un souci marqué pour tenter d'obtenir une production endogène d'azote et une protection du sol.

La définition des caractéristiques des sols les plus favorables au caféier Arabica témoigne du souci de M. Admasu Shiferaw de ne pas installer la culture dans des conditions défavorables ; on peut rattacher à ce souci le travail effectué au Nigeria par A. R. Owaiye, qui décrit les sols aptes à la culture de l'Arabica.

P. E. Harding a étudié, en Papouasie-Nouvelle-Guinée, les fluctuations du contenu des feuilles en éléments minéraux, selon les exigences saisonnières de la plante, ce qui permet de mieux utiliser l'analyse foliaire pour le choix de la fumure.

L'importance de la fréquence des cueillettes sur la quantité et la qualité du café récolté a été mise en relief par M. Osei-Bonsu et ses collaborateurs.

Au niveau de la récolte, M. Woelore a exposé les conditions optimales de la fermentation en fonction de l'altitude. L'étude de la microflore des pulpes de café, conduite par S. Denis et ses collègues de l'ORSTOM, fait ressortir l'existence de souches de champignons filamenteux capables de dégrader la caféine, et apporte un premier élément de connaissance sur leur physiologie.

Le rapport de M. Castillo relatif à la mise en place, au Mexique, d'un digesteur anaérobie pour la dépollution des eaux résiduaires à la sortie d'une station de dépulpage, avec production de gaz, mérite une mention particulière par le fait qu'il s'agit d'une réalisation concrète en milieu paysan, par les paysans, dans le cadre d'une coopération franco-mexicaine.

Enfin, la présentation faite par P. Charmetant de la caféiculture australienne nous projette dans un certain futur. Le « tout mécanique », dicté par des nécessités socio-économiques, qui s'épanouit en Australie, ne sera sûrement pas un modèle universellement valable, mais les travaux menés pour sa mise au point sont remarquables, et l'on doit surtout souligner la réflexion qui a conduit à la conception d'une telle technologie adaptée à des conditions très spéciales.

On ne saurait terminer ce tour d'horizon rapide sans mentionner la communication de J. J. Osorto, Directeur de l'IICA-PROMECAFÉ. Sous une forme résumée, il a en effet brossé le tableau des réalisations de son organisation depuis treize ans. Il s'agit là d'un véritable modèle de coopération régionale, puisque PROMECAFÉ a su fédérer les activités de ses huit Etats membres dans le domaine de la recherche et dans celui du développement. C'est aussi un modèle de coopération internationale, puisque les résultats obtenus sont le fruit de l'activité conjointe des chercheurs centraméricains, du CIFC au Portugal et surtout de la France avec une forte contribution du ministère français des Affaires Etrangères (crédits, bourses pour des formations doctorales) et du CIRAD (six chercheurs permanents). Nous ne saurions faire mieux que de souhaiter voir ce modèle adopté dans d'autres régions.

R. A. Muller
ex-Directeur scientifique de l'IRCC

Effets physiologiques

Au cours de cette journée « Café et Santé », présidée par le Professeur G. Debry et le Docteur R. Viani, on a pu suivre cinq exposés et neuf communications qui rendent compte de l'évolution des connaissances concernant les relations entre la consommation de café et la santé.

Comme le souligne S. Heyden, au cours de ces dix dernières années les relations entre la consommation de café et la santé ne sont plus seulement envisagées en termes de nocivité éventuelle, mais aussi en tant qu'effets bienfaisants. Il s'agit là d'un changement récent des préoccupations des scientifiques à l'égard du café.

Beaucoup d'études épidémiologiques ou expérimentales réalisées jusqu'alors n'ont pas la signification qui leur a été donnée en raison d'une part des doses utilisées et d'autre part des incertitudes concernant le recueil des données et l'interprétation des résultats.

En effet, comme le rapporte P. Ducimetière, il n'est pas aisé de connaître les consommations exactes de café, notamment durant une longue période. M. J. Arnaud démontre bien d'ailleurs que pour établir une corrélation entre la consommation de caféine et ses effets physiologiques, il est nécessaire, du fait des imprécisions de l'enquête alimentaire et de la grande variabilité du métabolisme et de la clairance de la caféine, de vérifier les données par la mesure des concentrations plasmiques et salivaires en caféine.

P. Ducimetière insiste également sur les difficultés de la prise en compte, lors des études épidémiologiques, des très nombreux facteurs qui peuvent ausi être impliqués dans le déterminisme des maladies imputées à la consommation de café. Parmi les facteurs qui créent la confusion, ce sont essentiellement les consommations de tabac et d'alcool, car elles sont en très forte corrélation positive avec celle de café. D'autres facteurs agissent vraisemblablement, comme par exemple le style de vie, qui peut être une cause majeure de l'excès de consommation de café. Pour toutes ces raisons et parce que, trop souvent, l'existence d'une corrélation positive est abusivement transformée en relations de cause à effet, un certain nombre de maladies ont été imputées à tort au café.

Les effets physiologiques de la consommation de café font l'objet de plusieurs communications :

Le café n'a pas les effets nocifs cardiovasculaires qu'on lui attribue. Le café décaféiné accroît la fréquence cardiaque (I. Höfer) par rapport au café. Il n'a pas été observé de relation dose-effet entre la consommation de café et la fréquence cardiaque (M. Hasenfratz). En revanche, la consommation de café prévient l'hypotention post-prandiale des personnes âgées et les crises asthmatiformes chez les adolescents (S. Heyden).

Par rapport à celle de café décaféiné, la consommation de café augmente l'activité locomotrice et diminue les sensations migraineuses ; les durées des effets physiologiques qui sont secondaires à la consommation de café ou à l'abstinence sont différentes (I. Höfer).

Comparée à un placebo, la caféine accroît la vigilance (I. Höfer, M. Hasenfratz) et améliore l'humeur (A. Smith). Les performances mentales sont augmentées (A. Smith, M. Hasenfratz), mais de manière variable selon le type de travail à réaliser (A. Smith). Toutefois, il existe une relation négative entre la dose de caféine et les performances mentales, à l'inverse de la vigilance (M. Hasenfratz).

Ces différents effets pourraient être expliqués par l'action de la caféine et du café sur le métabolisme énergétique et le débit sanguin cérébral.

Les augmentations de l'utilisation cérébrale du glucose induites par la caféine sont bien corrélées avec l'activité locomotrice et le cycle veille-sommeil chez le rat. De plus, le métabolisme cérébral ne semble pas développer de tolérance aux effets stimulants de la caféine. L'adénosine est un des facteurs intervenant dans le couplage dynamique entre le débit sanguin et le métabolisme du cerveau (A. Nehlig).

L'existence d'une dépendance à la caféine ne peut être affirmée. La notion de dépendance à une drogue est pharmacologiquement bien définie ; or, les troubles qui sont secondaires à l'abstinence de la caféine ne répondent pas à ces critères. A l'inverse des toxicomanies, la consommation de caféine n'entraîne pas le besoin d'augmenter les doses, aussi bien chez les animaux que chez les humains. Les céphalées secondaires à l'abstinence de caféine sont de courtes durées et n'accroissent pas le désir de reprendre la consommation de caféine (K. Bättig).

La thermogenèse, l'oxydation des glucides et des graisses sont augmentées par la caféine, mais plus faiblement chez les sujets obèses que chez ceux dont le poids est normal pour la taille (Y. Schutz). Enfin, il est à nouveau prouvé que l'action hypercholestérolémiante du café est due aux lipides contenus dans l'huile de café. Elle ne concerne donc que le café non filtré (A. Huggett, C. Scaccini) et en particulier le café bouilli.

Les études expérimentales sur les relations éventuelles entre la consommation de café et la cancérogenèse illustrent les difficultés importantes rencontrées au cours des expériences.

La présence d'ochratoxine A, produite par diverses espèces de champignons, a été constatée dans des essais préliminaires à confirmer. La torréfaction ne détruit pas cette toxine (C. Schlatter), mais les doses consommées sont très nettement inférieures à celles qui, expérimentalement, favorisent l'induction de tumeurs rénales cancéreuses chez l'animal.

Le café contient des substances anti-oxydantes qui ont des actions favorables ou défavorables constatées *in vitro* et dépendantes de la dose. Les effets favorables à la santé semblent pouvoir être attribués aux polyphénols contenus dans le café. De faibles quantités de café suppriment, *in vitro,* l'effet mutagène de certains oxydants, inhibent la peroxydation lipidique et la formation de malondialdéhyde. Toutefois, l'extrapolation à l'homme de ces résultats obtenus *in vitro* implique une très grande prudence (R. Turesky).

Il a aussi été démontré que les grains de café vert et deux fractions de leurs extraits prévenaient le cancer de la cavité buccale chez le hamster. Cette action anticarcinogène n'est pas détruite par la torréfaction (E. G. Miller).

Ces nouvelles données confirment la nécessité de poursuivre les études expérimentales et épidémiologiques, car dans l'état actuel des connaissances il n'existe pas de preuves permettant d'affirmer qu'aux doses de la consommation humaine le café puisse avoir une part de responsabilité dans la survenue des cancers. Les interprétations des résultats des études expérimentales doivent toujours tenir compte des doses utilisées et de la durée de l'exposition au café, tandis que celles des données épidémiologiques doivent être attentives à l'exactitude du recueil des données et à la prise en compte des facteurs de confusion, notamment les consommations d'alcool et de tabac.

Pr G. Debry
texte présenté par le Dr R. Viani
Vice-Président de l'ASIC

Technologie

Les communications de la session Technologie portaient essentiellement sur les aspects de la qualité du café torréfié/moulu et soluble, avec en outre une présentation sur les caractères physico-chimiques des méthylxanthines.

Dans le premier exposé, K. Cale a décrit la récupération des composés aromatiques bénéfiques, ayant des

caractères vineux, fruité et à goût de beurre, par l'adsorption sélective des sorties de l'hydrolysat thermique des procédés de fabrication du café soluble, avec des adsorbants non polaires.

H. Cammenga a examiné les solubilités des méthylxanthines et a montré que le taux de caféine dans les procédés simples pouvait être déterminé en mesurant la densité de la solution aqueuse de caféine.

D. Hinman a étudié les isothermes de sorption pour le gaz carbonique sur café torréfié et moulu ; une équation permettant de calculer la pression finale dans les emballages de café en mesurant le volume du gaz carbonique total a été présentée.

Dans une présentation vidéo spectaculaire, M. Petracco a développé un modèle pour expliquer la « dynamique d'infusion » compliquée dans une cafetière espresso. Des équations différentielles de l'hydrodynamique ont été mises au point par l'interprétation des données expérimentales classiques, qui peuvent être remplacées par des « règles locales ». Dans l'avenir, ceci pourrait conduire à une étude de ce procédé en utilisant la géométrie « complexe » et « fractale ».

L. E. Small a décrit un modèle physico-chimique de la dégradation de l'acide chlorogénique et l'évolution aiguë du gaz carbonique — facteurs clefs dans le gonflement des grains. Un procédé technologique permettant de produire un meilleur café à haut rendement avec une meilleure homogénéité de torréfaction a été examiné.

D. Bassoli et A. de Castro ont présenté un procédé de séchage par atomisation à basse température qui donne un café soluble avec moins de notes aromatiques à caractère caramel ou brûlé. Ce procédé assurerait une meilleure conservation de l'arôme, même en comparaison avec la lyophilisation.

Chimie

La session Chimie a également reflété le haut niveau atteint par les chercheurs travaillant sur le café. Un matériel moderne très sophistiqué a été utilisé afin d'obtenir une meilleure compréhension du sujet qui est la qualité du café.

Dans la première communication plénière, C. T. Ho a décrit la réaction entre glucides et protéines/peptides — appelée réaction de Maillard — pendant la torréfaction du café. Il a souligné le rôle de la réaction de désamination des composés protéiques et a décrit des voies qui conduisent à la formation des constituants de l'arôme du café pendant la torréfaction. Les réactions qui mènent à la formation de dix catégories de composés volatils du café ont été examinées.

G. Cohen a fait un rapport sur les avantages de l'utilisation d'un détecteur à chimioluminescence du soufre pour l'analyse de l'espace de tête du café. Une amélioration très importante de la sélectivité, de la linéarité et de la sensibilité aux composés sulfurés de ce détecteur, comparé aux détecteurs classiques, a été démontrée.

H. Bade-Wegner *et al.* ont déterminé le 2-méthylisobornéol dans des cafés torréfiés et ont constaté que les taux dans le Robusta étaient toujours plus élevés que ceux de l'Arabica.

W. Grosch *et al.* ont appliqué un test basé sur la dilution d'un isotope stable pour caractériser et quantifier les différences distinctes dans certains composés à impact aromatique entre les cafés Robusta et Arabica.

J. Boosfeld *et al.* ont associé différentes techniques chromatographiques et spectroscopiques pour caractériser deux isomères de la 2,4-décadiène dans le café vert.

L'influence de la température et de la disponibilité d'oxygène sur la libération des composés volatils et du gaz carbonique, ainsi que sur l'oxydation des lipides dans le café torréfié a été examinée par M. C. Nicoli *et al.*

Dans la deuxième communication plénière, H. G. Maier a fait le bilan des recherches sur les composés non volatils du café et a donné des exemples des applications pratiques qui avaient été élaborées au sein de son institut de recherche.

Une étude sur la détermination quantitative des sucres libres dans le café a été décrite par R. M. Noyes. Les équilibres matériels mesurés pendant la torréfaction et l'extraction ont été mis en corrélation à l'aide d'un modèle mathématique, ce qui permet de prévoir les profils des sucres.

Le profil des acides gras individuels libres et liés dans les cafés Arabica et Robusta a été analysé par K. Speer et ses collaborateurs.

Différentes méthodes chromatographiques ont été utilisées par H. Steinhart pour séparer et caractériser les mélanoïdines dans les extraits de café ; la distribution des poids moléculaires a été déterminée dans plusieurs fractions.

H. Stahl et T. Parliment ont trouvé que le furfuryle mercaptan est le principal composé du goût produit dans les systèmes modèles cystéine/pentose. Ils ont examiné la cinétique de sa formation en fonction du temps, de la température et du pH.

C. Severini *et al.* ont étudié la cinétique d'extraction des composés non volatils du café, ainsi que leur comportement pendant le stockage des boissons de café.

Dans une communication plénière sur le principe et l'application des « nez électroniques », P. N. Bartlett a décrit l'imitation du système olfactif humain en remplaçant des neurones sensoriels par des détecteurs de gaz non spécifiques, qui fonctionnement soit avec des oxydes métalliques soit avec des résistors chimiques à polymère conducteur. Des exemples concernant les arômes du café et de la bière, où les différences entre les origines ont pu être caractérisées, ont été donnés.

B. Guyot a décrit une méthode rapide pour la détermination de la caféine et de l'humidité dans les cafés Robusta verts et torréfiés, ainsi que dans tous les mélanges d'Arabica et de Robusta. En utilisant la spectroscopie proche infrarouge, seule une purification simple est nécessaire. Avec une bonne courbe d'étalonnage, l'écart-type s'est révélé petit.

Les fractions des acides phénoliques après dérivation ont été analysées dans différents cafés sauvages en utilisant LC/MS et GC/MS, par J. J. R. Rakotomalala *et al.*

La formation de radicaux stables libres pendant la torréfaction du café est connue grâce à la spectroscopie de résonnance électronique. B. Goodman et ses co-auteurs ont montré la capacité des différentes variétés de café de désactiver les radicaux d'oxygène qui pourraient jouer un rôle dans le processus biologique de vieillissement.

En utilisant des techniques basées sur la spectroscopie à plasma à couplage inductif, les taux des métaux présents à l'état de traces dans une large sélection de grains de cafés verts ont été étudiés par R. Macrae et ses coauteurs. Les résultats ont été reliés à la qualité sensorielle et à l'origine géographique des grains.

L'évolution physique pendant la torréfaction des cafés verts Arabica et Robusta a été suivie par C. Gutierrez *et al.* en utilisant la microscopie électronique à balayage. Des valeurs de la porosité et de la distribution de la taille des pores ont été obtenues par analyse d'images.

M. Ortola *et al.* ont étudié l'adsorption et la diffusion de l'eau dans le café torréfié. Des isothermes d'adsorption pour l'eau ont été utilisés afin de caractériser la structure des pores dans du café torréfié. La diffusion était très lente, ce qui a été attribué à l'interconnexion des pores extrêmement fins.

L. Moy a décrit un détecteur à barrettes à l'oxyde d'étain qui peut distinguer des échantillons de café torréfiés à différents degrés.

Affiches

La session affiches comportait également des contributions remarquables.

Par l'application de la chromatographie sur échangeur anionique et d'exclusion, les glucides extractibles des cafés Arabica et Robusta ont été caractérisés par V. Leloup *et al.* ; l'influence de la torréfaction et de la température d'extraction a été étudiée.

La stabilité thermale du 2-méthylisobornéol (MIB), un composé révélateur des cafés Robusta, a été étudiée par F. Rouge et ses co-auteurs. Ils ont constaté une réduction considérable du MIB dans les Robusta après traitement à la vapeur et torréfaction. Ils ont signalé, également, des taux de MIB dans certains Arabica plus élevés que ceux cités dans des références bibliographiques antérieures. Cette contradiction a été examinée après la présentation de Bade-Wegner, mais n'a pas pu être résolue. Des études supplémentaires sur la fonction du MIB dans les cafés verts après stockage seront nécessaires.

Les taux des lipides dans des cafés préparés de façons différentes ont été présentés par K. Speer. Le même auteur a identifié par HPLC et MS deux produits inconnus issus de la déshydratation des terpènes.

Les fractions d'arôme des boissons de café entraînables à la vapeur ont été étudiées par R. Silwar, avec une considération particulière donnée aux relations polarité/extractibilité.

S. Homma et M. Murata ont réussi l'isolation et la caractérisation des composés bruns de café capables de chélater le fer.

J. Zapp a montré une méthode nouvelle de détermination de la trigonelline et de l'acide nicotinique dans le café par HPLC à exclusion d'ions.

Dr O. Vitzthum
Secrétaire Scientifique de l'ASIC
(traduction)

Summary report

Biotechnology

The objective of the Biotechnology session was to review the recent contributions of biotechnology, biochemistry and genetics to the improvement of coffee plants and to the production of quality coffees. This session enabled two lectures, sixteen papers and twelve poster displays to be presented, each of considerable scientific interest. The first lecture, by Dr. W. Powell (SCRI), given by K. Chalmers, introduced molecular techniques and their leading applications to coffee plants. The second, by Dr. V. Pétiard (Francereco) gave an overall view of the various biotechnologies based on expertise in the *in vitro* culture of coffee plants.

The presentations given in this session may be summarised under three headings.

1. Biochemical and molecular markers

The first applications to coffee plants of DNA marking techniques (RFLP, RAPD, PCR) by SCRI (presentation K. Chalmers), Francereco (presentation M. Paillard) and ORSTOM (presentation J. Cros) were developed to characterise and identify plant material, to establish phylogenies, to detect introgressions and, in the long term, to draw up a genetic map of coffee plants.

The size of different coffee genomes and variations in the quantity of DNA were described by researchers from ORSTOM (S. Hamon). Finally, the biochemical polymorphism in African and Malagasy coffee plants studied by J. J. Rakotomalala (FOFIFA) leads to a revision of the taxonomic classification of coffee plants.

A first use of these markers to identify coffee plants is now possible ; in the medium term, correlations with agronomic and technological characteristics will open links with the breeders. Cooperation between the laboratories possessing expertise in molecular techniques (SCRI, Francereco, ORSTOM) and the research teams concerned with the breeding and improvement of coffee plants will enable this new approach to be perfected.

2. The different biotechnologies based on expertise in the *in vitro* culture of coffee plants

These techniques were tested during the 1980's and have evolved with the advancement of knowledge. They have resulted in various important developments concerning coffee plants.

a) The conservation of plant material and improved varieties in field collections and their exchange can now be approached differently. For the storage of vitroplants, F. Engelmann (ORSTOM) has revealed an early marker of physiologic change occurring during storage at different temperatures. Long-term storage in liquid nitrogen (cryoconservation) has progressed enormously. The dehydration and freezing of zygotic and somatic embryos were presented by B. Florin (Francereco) and by A. Abdelnour (CATIE). The team from ORSTOM (S. Mari) has successfully performed the first encapsulation-dehydration of meristems.

b) The *in vitro* propagation of coffee plants is being developed using micro-cuttings and micro-grafts at CATIE (B. Bertrand), through induction of multiple shoots on hypocotyls at SCRI (N. E. Nyange) and by various means of regeneration at CIFC (M. F. Carneiro) and at CATIE (C. Brocherieux).

The most notable progress concerns somatic embryogenesis thanks to the development of culture in liquid medium at Cenicafé (M. E. Aponte) and the use of bioreactors by Francereco (J. P. Ducos) and by DNAP (C. Noriega), whilst the genetic effects studied at IAC (J. C. S. Ramos) are of utmost importance. The levels of multiplication achieved are remarkable and open up various prospects for application. In addition, the conditions of vitroplant growth are being improved at CIRAD (D. Bieysse) through the modification of environmental factors.

c) The fixation of strains and the creation of varieties of *C. arabica* by **haploidisation** are progressing slowly. The formation of colonies of haploid cells starting from microspores has been achieved at CATIE (B. Neuenschwander). The somatic embryogenesis of microspores and anthers obtained at CIFC (M. F. Carneiro) deserves to be investigated at cellular level.

d) The creation of somaclonal variations in coffee plants obtained from somatic embryogenesis is currently being studied at DNAP (M. Sondhal) and at Francereco (V. Pétiard). The improvement of cultivars by *in vitro* selection of calluses is being tried out at SCRI (N. E. Nyange) in the presence of filtrates from cultures of *Colletotrichum coffeanum* the agent responsible for CBD.

e) The genetic transformation of coffee plants is progressing. It has been achieved using *Agrobacterium rhizogenes* at Francereco (J. Spiral) on somatic embryos of Arabica, Canephora and Arabusta. Foreign DNA has also been introduced to coffee plant tissues using a particle cannon at CIRAD (J. van Boxtel). Finally, protoplast regeneration is being studied at the University of Compiègne (J. Grèzes) in relation to genetic transformation and the production of secondary metabolites. The prospects opened up by the introduction of foreign genes depend on the progress of knowledge concerning the biochemical and molecular bases of agronomic and biochemical characteristics.

3. Biosynthesis of the biochemical components of coffee plants

The research presented concerns essentially the synthesis of the precursors of purine alkaloids. The different biosynthetic pathways and the accumulation of these alkaloids at different stages of tissue development are being studied in the USA (G. R. Waller) and in Japan (H. Ashihara). In a similar vein, G. M. Nazario (University of California) has demonstrated that each alkaloid is the product of a specific biosynthetic pathway starting with endogenous purines.

A new metabolite has been revealed in coffee plant cell cultures at the University of Zurich (B. H. Schulthess). Dr. T. Baumann from the same university has studied the interdependence of caffeine and chlorogenic acid and the regulation of their synthesis.

A. Charrier
Professor at ENSA M
(translation)

Agronomy

I must first thank my long-time colleagues and friends who have helped me prepare this summary of the Agronomy sessions, Mssrs. Avelino, Charmetant, Decazy and Eskes.

The Agronomy sessions began with a general talk, which I myself had the honour of presenting, on the theme : « Which coffee cultures for tomorrow ? » This talk pointed out the distance existing between the possible — coffee culture that is very successful, applying the information gained by research — and the actual — coffee culture too often primitive, taking no benefit from knowledge acquired and, because of the present crisis, having a marked tendency towards extensive practices near harvesting.

The talk concluded with some proposals for better passing the technical message in the rural environment and for directing research towards reducing production costs, with the wish that agreement on acceptable prices be quickly found and so allows the producers fair remuneration for their work along with the chance to have access to technical progress.

We next discussed recent advances in **genetics and selection.** Several broad themes were covered.

Concerning genetic resources, a method was presented by M. Noirot for the sampling of variability. It will enable a core collection to be built up, which will serve as a workbase for the different genetic improvement programmes.

Two aspects of the relationships between coffee species were treated : hybrid fertility by J. Louarn, and grafting compatibility by E. Couturon. These two approaches demonstrated the genetic distance existing between coffees from East and West Africa.

According to M. Bayetta Bellachew, the evaluation of Ethiopian strains in collection confirms their preferential adaptation to their area of origin.

Regarding the interspecific hybrid Arabusta, Y. Adu-Ampomah, for Ghana, and D. Le Pierrès, for Côte-d'Ivoire, gave an account of some work on the reproductive system of F1 hybrids, on the evaluation of F2 and F3 generations and on Arabica backcrossings. A slight improvement in fertility in F3 was noted.

For *Coffea canephora,* T. Leroy reported on the genetic parameters, estimated according to the Reciprocal Reccurrent Selection Scheme, which led to the selection of seven hybrids and seventy clones. In Ghana, clonal selection carried out by Y. Adu-Ampomah has produced high-yielding clones. P. Lashermes presented the method of exploitation of double haploids used in Côte-d'Ivoire : some hybrids as productive as certain clones have been obtained.

The transmission of resistance to orange rust linked to factor SH3 has been studied by T. D. Kukhang under a CIRAD- Papua New Guinea cooperation programme. This factor seems complex, which may explain its relative durability in India and Indonesia.

Accounts of the progress made in improvement programmes in these two countries were given by M. S. Sreenivasan and S. Mawardi respectively, followed by P. Charmetant for Papua New Guinea.

At last, promising results were presented for an Arabica improvement strategy based on the use of F1 hybrids. A large heterosis was observed in Cameroon by M. Boccara for production and in Ethiopia by M. Bayetta Bellachew for vigour at a young age.

C. O. Agawanda described the problems of hybrid seed production in Kenya.

Finally, M. Cadena from Colombia proposed the M. d. P. Moncada B. method, which enables the time necessary to evaluate productivity to be reduced. A considerable reduction in the duration of selection cycles should thus be possible.

— In **phytopathology,** seven papers and six poster displays were presented, describing eight studies on orange rust and five on Coffee Berry Disease (CBD).

Concerning CBD, caused by *Colletotrichum coffeanum sensu* Hindorf, the work of C. Lambot in Burundi has demonstrated the effect of applications of zinc on the incidence of CBD. V. Aubin and his co-workers in Cameroon have measured the time necessary from infection to berry fall and have shown that the systemic fungicides act by slowing the development of the fungus in the berries. In developing early selection tests for *Coffea arabica* resistance to the fungus, M. Rigouzzo and colleagues stress the importance of precisely correlating the levels of susceptibility to CBD observed in the field with the reactions obtained in the laboratory. Biotechnology seems destined to play a major role in this field in Cameroon. This is also the case in Tanzania where N. E. Nyange is carrying out his research. Finally, C. J. Rodrigues described the latest research at CIFC in Portugal concerning the pathogenicity of different CBD isolates and the possibility of finding resistant genotypes.

Concerning orange rust, CIFC has revealed the existence of new strains infecting Catimor, which may represent a serious threat to the varieties produced from the Timor Hybrid.

F. Holguin in his presentation showed that new strains of orange rust exist not only on Catimor but also on *C. canephora.* The strains attacking Catimor are more virulent than those attacking *C. canephora.*

On the same subject, C. Montagnon suggests the existence of different strains of orange rust infecting *C. canephora* in Côte-d'Ivoire. However, the genetic variability of this coffee plant enables an improvement strategy to be adopted which deals at the same time with resistance to this disease, to twig-borer and to drought. M. C. Silva

and colleagues at CIFC in Portugal have studied host-parasite relationships between orange rust and various host and non-host plants. In cases of incompatibility, the fungus growth is arrested most often at the stage of haustorium formation.

M. E. M. Guedes and colleagues in Portugal have demonstrated the existence of steroidal compounds in the uredospores of *Hemileia vastatrix* and think that there may be new control possibilities here. Finally, three epidemiological studies on orange rust were presented. J. H. Whan of Papua New Guinea pointed out the inversely proportional relationship between altitude or temperature and the incidence of the disease. J. Avelino in Guatemala has demonstrated the importance of the loading of fruits on the coffee plant to the development of the disease. The disease causes damage at low altitude, justifying the use of chemical control. Fertilisation should also be a means to reduce the incidence of the disease. The work of the ORSTOM team in New Caledonia must be mentioned. They have succeeded in developing a model that enables estimations of epidemic development to be made using pathologic, agronomic, pedologic and climatic data.

Work concerning the **pests of coffee plants** was the subject of five talks and eight poster displays.

All the research themes considered concerned integrated pest management. Several control strategies were evoked. Of course, chemical control still remains the subject of laboratory and field experimentation : four insecticides were evaluated by B. Padi for their effectiveness against the *Epicampoptera*, the defoliating caterpillars.

The evaluation of the reaction of plant material to pests has revealed the possibility of selecting strains or genotypes resistant to certain hazards, as well as the mechanisms of this resistance. To achieve this, early-evaluation tests were developed.

In the framework of a CIRAD/Guatemala cooperation programme, F. Anzueto has studied the behaviour of various strains of Ethiopian coffee plants, as well as some Robusta, to two populations of *Meloidogyne* spp.

A. Garcia described the susceptibility of various *Coffea* species to the root mealy bug *Dysmicoccus cryptus.* C. Montagnon in Côte-d'Ivoire has shown the possibility of selecting coffee plants for resistance to twig borer. O. Guerreiro (CIRAD/Brazil) presented work showing original methods for the evaluation of the use of *Bacillus thuringiensis* toxins against *Perileucoptera coffeella,* with the aim of using them in the creation of a genetically-transformed coffee plant resistant to this pest.

Two posters, one presented by L. Villain *et al.*, the other by D. Remond *et al.*, defined the spacial repartition and population distribution of the nematode *Pratylenchus* sp. and the coffee berry borer, *Hypothenemus hampei,* which allow the elaboration of sampling methods for the evaluation of population levels in these pests, of use in control programmes. B. Dufour identified the principal species of mealy bugs found on coffee ; he has studied their geographic distribution and has made a first inventory of their natural enemies in Central America. The control measures envisaged concern the correction of the biological imbalance.

A. Lopez presented two posters on the subject of improvements in the control of coffee berry borer, *Hypothenemus hampei,* through better knowledge of chemical mediators. The first, a rapid and effective method of analysing the distribution of virgin and fertilized females developing in the fruits. The second, showing the morphology and distribution of the receptors on male and female antennae. F. Mathieu displayed a poster showing the dynamics of female emergence from the fruit of origin in the presence of green cherries ; the stimuli associated with emergence from the cherries were analysed.

In parallel to this work on integrated management of the principal pests, a study presented by B. Bouyjou concerned the analysis of factors of entomological and bacterial origins responsible for potato off-flavour, and specific actions for correcting this defect.

Concerning **Agronomic techniques,** posters by Y. Edjamo and colleagues referred to the optimum density of plantations as a function of plant size and altitude, and to the influence on the yield of different degrees of shading.

Research on organic manuring in Indonesia was described by M. Winaryo and colleagues, whilst the advantages and disadvantages of various types of coverings designed to replace mulching were discussed by D. Snoeck and colleagues from Burundi, showing by this a marked concern for trying to obtain a local production of nitrogen and a protection of the soil.

The definition by M. Admasu Shiferaw of soil characteristics most favourable to Arabica coffee plants bears witness to his concern that planting in unfavourable conditions be avoided ; with the same concern, work carried out in Nigeria by A. R. Owaiye describes soils suitable for the culture of Arabica.

P. E. Harding in Papua New Guinea studied the fluctuations in the mineral contents of the leaves with the seasonal demands of the plant, thus enabling better use of foliar analysis in the choice of manure.

The importance of picking frequency on the quantity and quality of coffee harvested was stressed by M. Osei-Bonsu and co-workers.

Concerning post-harvest treatment, M. Woelore described the optimum conditions for fermentation as a function of altitude. The study of the microflora of coffee pulp, carried out by S. Denis and colleagues from ORSTOM, revealed the existence of strains of filamentous fungi capable of metabolising caffeine, and provided a first indication of their physiology.

The report by M. Castillo on the installation in Mexico of an anaerobic digester for the clean-up of residual water at the outflow of a pulping station, with production of gas, merits special mention since it involves a concrete accomplishment in a farming environment by the farmers themselves, within a French-Mexican cooperation programme.

Lastly, the presentation given by P. Charmetant on Australian coffee growing provided a glimpse of a possible future. The « Fully Mechanised », which has blossomed in Australia, dictated by socio-economic necessities, will surely not become a universally-applicable model, but the work carried out in its development is remarkable and one must stress the thought that went into the conception of such a technology adapted as it is to very special conditions.

It would not be possible to end this rapid overview without making mention of the paper by J. J. Osorto, Director of IICA-PROMECAFÉ. In the form of a summary, he sketched the achievements of his organisation over thirteen years. They constitute a veritable model of regional cooperation since PROMECAFÉ was able to associate the activities of the eight member states in the fields of research and development. It is also a model of international cooperation since the results obtained are the fruit of joint activities between researchers from Central America, CIFC Portugal and particularly France, with a large contribution from the French Ministry of Foreign Affairs (credits, grants for doctoral studies) and CIRAD (six permanent researchers).

We could not wish for more than to see this model adopted in other regions.

R. A. Muller
ex-Scientific Director of IRCC
(translation)

Physiology

During the day devoted to « Coffee and Health » five papers and nine statements reported on the progress in the state of knowledge on the relation between coffee consumption and health.

As S. Heyden stresses, during the last ten years the relations between coffee consumption and health are not only considered in terms of negative effects but also in terms of beneficial effects. This reflects a recent shift in the scientists' way of considering coffee.

Many epidemiological and experimental studies carried out up to now are not as meaningful as they were said to be because, on the one hand, the amounts used and, on the other, because of the uncertain manner in which data were collected and the results were analysed.

Actually, as P. Ducimetière points out, it is not easy to make exact measurements of coffee intake, especially over an extended period of time. M. J. Arnaud, moreover, shows that in order to make a correlation between caffeine consumption and its physiological effects, because of imprecise dietary intake studies, and the great individual variability in caffeine metabolism and clearance, data have to be checked by measuring plasma and saliva caffeine levels.

According to P. Ducimetière, in epidemiological studies, it is difficult to cover all the many factors that may also be involved in determining the causes of diseases that are blamed on coffee consumption. Smoking and alcohol consumption are the main factors that create confusion because there is a strong positive correlation with coffee intake. There are probably other factors also, such as lifestyle, which can be major causes for drinking too much coffee. For these reasons and because all too often the existence of a positive correlation is erroneously transformed into a cause and effect relationship, coffee has wrongly been blamed for certain diseases.

Many papers delt on the physiological effects of coffee consumption.

Coffee does not have the detrimental cardiovascular effects for which it was once blamed ; decaffeinated coffee causes a higher heart rate than caffeinated coffee does (I. Höfer). No dose-effect relationship was observed between coffee consumption and heart rate (M. Hasenfratz). On the other hand, coffee consumption can prevent postprandial hypotension in elderly patients and asthma attacks in adolescents.

If compared with decaffeinated coffee, the consumption of caffeine-containing coffee increases motor activity and decreases headaches. Time courses of the physiological effects that are secondary to coffee consumption or abstinence are different (I. Höfer).

Compared to placebo, caffeine increases wakefulness (I. Höfer, M. Hasenfratz) and improves the mood (A. Smith). Mental performance is enhanced (A. Smith, M. Hasenfratz), but in varying manners, depending on the nature of the task to be performed (A. Smith). This said, there is a negative relationship between the caffeine dose and the mental performance, which is the opposite for alertness (M. Hasenfratz).

These different effects could be explained by the effects of caffeine and coffee on the energy metabolism and the blood flow to the brain. The increase in the cerebral glucose utilization, which is induced by caffeine, can be correlated with the motor activity and the sleep-wake cycle in rats. Furthermore, the cerebral energy metabolism does not seem to develop tolerance to the stimulating effects of caffeine. Adenosine is one of the main factors in the dynamic coupling of blood flow and brain metabolism (A. Nehlig).

The existence of caffeine dependency cannot be ascertained. The notion of dependence on a drug is « pharmacologically » well defined, but disorders that can be taken as secondary effects to caffeine abstinence do not meet the criteria. Unlike drug addiction, caffeine consumption does not stimulate a need to increase doses, either in animals or in people. The transient headaches that are secondary effects to caffeine abstinence are ephemeral and do not increase the desire to consume caffeine again (K. Bättig).

Thermogenesis, and carbohydrate and lipid oxidation are increased by caffeine, more so in obese people than in people whose weight is normal for their size (Y. Schutz). Last, more evidence showed that the effect of coffee on serum cholesterol is due to the fats in the coffee oils, and thus only applies to unfiltered coffee (A. Huggett, C. Scaccini), to boiled coffee in particular.

Experiments on the possible relation between coffee consumption and cancer illustrate manifold experiment-related difficulties. Ochratoxin A, that is produced by various types of fungal species, was found in preliminary tests which need confirmation. Roasting does not destroy this toxin (C. Schlatter), but human intake levels are far lower than the doses given in the experiment that induced cancerous kidney tumors in animals.

Coffee contains anti-oxidants which, depending on the dose, have beneficial or detrimental effects, as was seen *in vitro*. The beneficial effects for health seem to be traced to the polyphenols in coffee. In small doses, coffee can suppress the *in vitro* mutagenicity of certain oxidants, inhibit the lipid peroxidation and malondialdehyde formation. Extreme caution must be exercised before extrapolating these effects, obtained *in vitro,* to man (R. Turesky).

Green coffee beans and two fractions of coffee bean extracts have been shown to inhibit cancer of the buccal pouch in hamsters. The cancer prevention activity is not destroyed by roasting (E. G. Miller).

These new data confirm the need to continue the experimental epidemiological studies because the present state of the art does not provide proof that substantiates the thesis that human intake levels of coffee can have an effect on the onset of cancer. Any analysis of results from experimental studies must specify the doses used and the duration of coffee consumption, while epidemiological data should insist on the accuracy of the data collected and to proper adjustment for factors of confounding in particular smoking and alcohol consumption.

Pr. G. Debry
summary presented by Dr. R. Viani
Vice-President of ASIC
(translation)

Technology

The papers in the Technology session concentrated on the quality aspects of roast and ground, and instant coffee with one additional paper on the physico-chemical properties of the methylxanthines.

In the first communication, K. Cale described the recovery of beneficial aroma compounds with buttery, fruity and winey characteristics by selective adsorption of the thermal hydrolyzate streams from instant coffee processes using non-polar adsorbents.

H. Cammenga investigated the solubilities of the methylxanthines and demonstrated that the concentration of caffeine in single process streams could be determined by the measurement of the density of the aqueous caffeine solution.

D. Hinman studied the sorption isotherms for carbon dioxide on ground roast coffee ; an equation to calculate terminal pressure in coffee packages by measurement of the total carbon dioxide volume was presented.

In a spectacular video presentation M. Petracco developed a model explaining the complicated « Brewing Dynamics » in an Espresso coffee machine. Differential equations of hydrodynamics were developed by interpretation of classical experimental data which may be repla-

ced by « local rules ». In the future this could lead to an investigation of this process using « complex » and « fractal » geometry.

L. E. Small described a physico-chemical model of the degradation of chlorogenic acid and the sharp evolution of carbon dioxide key factors in bean puffing. Technology was discussed that produces improved high yield coffee having better roast uniformity.

D. Bassoli and A. de Castro presented a low temperature spray drying process which yielded an instant coffee product with less caramel and burnt/overcooked flavor notes. Better aroma retention even when compared to freeze drying is claimed for this process.

Chemistry

The Chemistry session again reflects the high standards achieved by coffee researchers. Modern highly sophisticated equipment was used to obtain a better understanding of the issue of coffee quality.

In the first plenary paper in Chemistry, C. T. Ho reported on the reaction of carbohydrates and proteins/peptides — the so-called Maillard reaction — during the roasting process of coffee. He stressed the role of the deamination reaction of protein materials and described pathways leading to the formation of coffee aroma compounds during roasting. The reactions leading to the formation of ten classes of volatile coffee compounds were discussed.

G. Cohen reported on the benefits of using the sulfur chemiluminescent detector for analysis of the coffee headspace. A dramatic improvement in the selectivity, linearity and sensitivity to sulfur compounds of this detector compared with conventional detectors was demonstrated.

H. Bade-Wegner *et al.* quantified 2-methylisoborneol in roasted coffees and found the levels in Robusta to be consistently higher than in Arabica.

W. Grosch *et al.* applied a stable isotope dilution assay to characterize and quantitate the distinctive differences in certain aroma impact compounds between Robusta and Arabica coffees.

J. Boosfeld *et al.* combined various chromatographic and spectroscopic techniques for the characterization of two 2,4-decadiene isomers in green coffee.

The influence of temperature and availability of oxygen on the release of volatiles and carbon dioxide as well as lipid oxidation in roast coffee were discussed by M. C. Nicoli *et al.*

In the second plenary paper, H. G. Maier reviewed the status of research on non-volatile coffee compounds and gave examples of practical applications that were elaborated by his research institute.

A study on quantification of free sugars in coffee was reported by R. M. Noyes. Material balances measured during roasting and extraction were correlated by a mathematical model, allowing the prediction of sugar profiles.

The pattern of the individual free and bound fatty acids in Arabica and Robusta coffees was analysed by K. Speer and co-workers.

Different chromatographic methods were used by H. Steinhart for the separation and characterization of melanoidins from coffee extracts ; the molecular weight distribution was determined in several fractions.

H. Stahl and T. Parliment found that furfuryl mercaptan is the major flavor compound formed in cysteine/pentose model systems. They discussed the kinetics for its formation in relation to time, temperature and pH.

C. Severini *et al.* investigated the extraction kinetics of non-volatile coffee compounds as well as their behavior during storage of coffee brews.

In a plenary paper on the principle and application of « electronic noses », P. N. Bartlett described the mimicking of the human olfactory system by substitution of the sensory neurones with non-specific gas sensitive devices. Sensors could be metal oxides or conducting polymer chemiresistors. Examples were given for coffee and beer odors, where differences between origins could be characterized.

B. Guyot described a fast method for the determination of caffeine and moisture in green and roasted Robusta as well as for all blends of Arabica and Robusta. Using Near Infrared Spectroscopy only a simple clean-up procedure is necessary. With a good calibration curve, the standard deviation was found to be small.

The phenolic-acid containing fractions after derivatization have been analysed in a selection of wild coffees using LC/MS and GC/MS by J. J. R. Rakotomalala *et al.*

The formation of stable free radicals during roasting of coffee is known from Electron Resonance Spectroscopy measurements. B. Goodman and co-authors demonstrated the abilities of different types of coffee to deactivate oxygen radicals, which may play a role in the biological ageing process.

Using techniques based on Inductively Coupled Plasma Spectroscopy, the amount of trace metals in a wide range of green coffee beans were investigated by R. Macrae and co-authors. The results were related to the sensory quality and to the geographical origin of the beans.

The physical changes occurring during the roasting of Arabica and Robusta green coffee were monitored by C. Gutierrez *et al.* using scanning electron microscopy. Porosity values and pore size distribution were obtained by image analysis.

M. Ortola *et al.* studied the adsorption and diffusion of water into roasted coffee : adsorption isotherms for water were used to characterize the pore structure of the roasted coffee. The diffusion was very slow, and this was attributed to the interconnection of extremely fine pores.

L. Moy described a tin oxide sensor array system able to discriminate between coffee samples subjected to varying degrees of roast.

Posters

In the poster session also there were some remarkable contributions.

Applying anion-exchange and size-exclusion chromatography, the extractable carbohydrates from Arabica and Robusta coffee were characterized by V. Leloup ; the influence of roasting and extraction temperature was investigated.

The thermal stability of 2-methylisoborneol (MIB), an indicator substance of Robusta coffees, was investigated by R. Liardon and co-authors. They found a considerable reduction in MIB in Robusta after steaming and roasting. They also reported amounts of MIB in certain Arabica higher than those reported in previous references. This discrepancy was discussed at the end of the Bade-Wegner paper and could not be resolved. Further investigation of the function of MIB in green Arabica after storage will be necessary.

The lipid contents of differently prepared coffee brews were reported by K. Speer.

The same author identified two unknown dehydration products from diterpenes by means of HPLC and MS.

The steam volatile aroma fraction of coffee beverages with special respect to polarity/extractability relationships was investigated by R. Silwar.

S. Homma and M. Murata succeeded in the isolation and characterization of iron-chelating brown-coloured coffee compounds.

J. Zapp showed a new method for determination of trigonelline and nicotinic acid in coffee by ion-exclusion HPLC.

Dr. O. Vitzthum
Scientific Secretary of ASIC

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