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Volume I

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Volume I

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OSHIMA K. — F.M.I Corp., 2-10-10, Hammaatsu-cho, Minato-ku, Tokyo
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SAWAMURA I. — Ajinomoto General Foods Inc., 9-67, Kitaitami, Itami-shi, Hyogo
SEKINE E. — Sekine Coffee Co., Ltd., 1-23-14, Shinmachi, Nishi-ku, Osaka
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SHINA H. — Oyama Coffee Systems Co., Ltd., 1208-4, Dei-Isonomiyaura, Oyama-shi, Tochigi
SHIMOHARA T. — Nestlé Japan Ltd., 1700, Terakudo, Hosojima aza, Shimada-shi
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SHINKAWA S. — Coca Cola (Japan) Company, Limited, Shibuya P.O. Box 10 Tokyo
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TSUGE Y. — Hamaya Co., Ltd., 2-5-20, Kawaraya-cho, Chyuo-ku, Osaka
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ASIC'95
THE 16TH INTERNATIONAL CONFERENCE ON COFFEE SCIENCE APRIL 9-14 1995
16e COLLOQUE SCIENTIFIQUE INTERNATIONALE DU CAFE du 9 au 14 avril 1995
第16回 国際コーヒー科学会議 1995年 4月 9日~14日
RAMSA
RAMSA

OPENING SPEECH
by Tatsushi Ueshima, president of ASIC

Ladies and Gentlemen,

Before proceeding with my opening speech, I would like to ask all of you to join me in expressing sincere condolences for those who perished in the January 17th Hanshin-Awaji earthquake. I would also kike to express my sincere hope for a quick recovery to the victims of the quake and especially to those members of the coffee industry who suffered therefrom. It was most grateful for the expression of sympathy and encouragement from all of our overseas friends of the coffee industry immediately following the quake.

The disaster was widely reported, even overseas. I wish to share with all of you the pleasure I feel that against overwhelming odds the 16th ASIC conference is taking place here in Kyoto as scheduled.

As the current president of ASIC, the host, as chairman of the organizing committee and as president of the All Japan Coffee Association, the conference supporters, I now proclaim the 16th international conference on coffee science officially open.

Today we are especially fortunate to hear from our honored guests Mr. Ohkawara, the Minister of Agriculture, Forestry and Fisheries, Mr. Armaki, the Governor of Kyoto Prefecture, Mr. Tanabe, the Mayor of Kyoto City, and our special overseas guest, Mr. Boecklin, President of the U.S. National Coffee Association.

I greatly regret that our Honorary President, Mr. René Coste, who led the first fifteen ASIC conferences, spanning a period of over thirty years, is unable to be with us this year due to advanced age. I would like to express my gratitude to Dr. Ernesto Illy, the First Vice President, who worked with Mr. Coste on ASIC conferences since the beginning, Dr. Rinantonio Viani, the ASIC Administrative Secretary, Dr Otto Vitzthum, the Asic Scientific Secretary and the Board members for their guidance and cooperation in organizing this conference. I am also very pleased with the active participation in this conference of over 450 people from 32 countries, which has made such a splendid ASIC'95 possible. I would like to emphasize that this is the first ASIC conference ever to be held in Asia.

Asia for many years has been a major coffee producing region, and more recently the number of producer countries in the region has increased. The rapid growth of coffee consumption in Asia is expected to continue, thus drawing attention to the importance of the region for both coffee production and consumption.

Looking back at the history of coffee imports to Japan, we see that the first 18 tons of green coffee were imported to Japan in 1877, nearly 120 years ago. Import volumes grew rapidly after that time, totaling some 100 tons in 1900 and an impressive 3000 tons in 1940. Imports were stopped during the unhappy war years, but since the end of world war II, growth has been so rapid that the 1994 import total, which included everything from green beans to finished products, was over 380,000 tons or over 6.3 million 60-kilo bags. The coffee market has grown so quickly that consumption is now 120 times greater than at the turn of the century . According to ICO figures, Japan is now the world's third largest net coffee consuming nation, following the U.S. and Germany.

After the war the Japan coffee industry grew as instant coffee became popular in the home, and later, growth continued thanks to increased consumption of ground coffee. The style of consumption of ground coffee has shifted in recent years, moving from the coffee houses to the home and has expanded total volume. In 1969 my father developed ready-to-drink canned coffee, a product that has fueled volume growth. Ready-to-drink canned coffee has been a success story unique to Japan, where approximately one-fourth of green coffee imports are used in this category. The growth and development of the Japan coffee industry has benefited from the country's economic growth. Although the Japanese food culture is becoming more modern, traditional foods are not being displaced ; they are being retained, while foods such as coffee are being incorporated into the native food culture. We in the Japanese coffee trade have a strong desire to promote coffee as a popular daily drink, and will make every effort to increase the demand for coffee so that we can live up to the expectations of the coffee-growing countries.

Next I would like, briefly, to talk about the contents of this conference. Because this year's conference is taking place in Asia, it is natural that about two-thirds of the participants come from the Asia region. When looking at the various fields of presentation, and not counting the 12 keynote speeches, there are 138 presentations ; overseas attendees will make 95 and Japanese attendees will make 43. Looking at the categories, we see that 63 presentations will be devoted to agronomics ; they will be given mainly by participants from the producing countries. On the other hand, there are only five technical papers on physiology. This is because coffee is already recognized as a healty beverage. This figure is fewer by one than the number of keynote speeches on the subject of coffee physiology.

Now I would like to talk a litte about the host city of Kyoto. It is one of the oldest cities in Japan and has a rich tradition. Last year it celebrated the 1200th anniversary of the transfer of the capital from Nara which had been the capital city prior to 794. Kyoto was the capital of Japan until about 120 years ago, a period of over 1000 years. Kyoto in April is a city of flowers where the national flower, the cherry blossom, blooms gloriously. We are delighted to be holding this conference, that brings together so many participants from abroad, during the best season of the year, in a city that has been so fondly protected and nurtured by its loving citizens. I hope that you will all enjoy the splendors of Kyoto during your short stay here.

Last but not least, on behalf of the organizers, I wish to take this opportunity to express my appreciation to ASIC Board members and to the members of the organizing committee for their many creative ideas and their energetic contributions to ensuring the success of the conference, to the Kyodo-Senden Agency, to ISS the Inter-language Service System and to the Meiko Travel Service for their cooperation in realizing our plans, and to the management and staff of the Kyoto Grand Hotel for providing us with such lovely meeting facilities.

We hope that this conference, through its contribution to coffee science and production, will play a role in furthering the world's coffee industry and will be a great success. Let me end by fervently requesting your cooperation in this endeavor.

Thank you very much.

THE EFFECT OF COFFEE AROMA ON THE BRAIN FUNCTION : RESULTS FROM THE STUDIES OF REGIONAL CEREBRAL BLOOD FLOW THROUGH POSITRON EMISSION TOMOGRAPHY AND EVENT-RELATED POTENTIAL (ERP)

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In the last two decades, the study of the effect of coffee on the human brain function has yielded a lot of insights that may elucidate the physiological bases of psychological phenomena associated with drinking coffee[4)7)]. However, few attempts have so far been made at the change of the brain function induced by coffee aroma which may alter mental activity. As well as other odorants, the effect of coffee aroma has been mainly measured by psychological tests or subjective ratings which depend on the individual subjective feeling[1)].

In our laboratory, event-related potential(ERP) has already been employed to study the effect of odorants on the mental activity from psychophysiological aspect. ERP is thought to be electrophysiological representation of information processing in the human brain. Unlike previous psychophysiological methods such as electrodermal activty, heart rate and pupillary light reflex, ERP is able to demonstrate the processing of a sensory input in the brain in terms of electrical activity. We have already investigated the effect of odors of some essential oils by using ERP as a tool and showed that the ability to process a visual input increased under the favorite odor. Although positron emission tomography(PET) provides reliable data on regional cerebral blood flow(rCBF), it has scarcely utilized for the research of odor[2)]. With the cooperation of the reseachers of Research Institute for Brain and Blood Vessels, Akita, we have measured the change of rCBF under the odor of whiskey and alcohol. The results showed that rCBF of ten healthy males under the odor of whiskey increased in the right hemispheric areas, in particular, in the right nucleus accumbens septi[23)]. The P300, one of the ERP components, elicited from the same subjects showed increase in amplitude with decrease in latency under the odor of whiskey, suggesting that the ability of processing of an auditory input was activated in parallel with the increase in cerebral blood flow.

In our preliminary study, coffee aroma increased the amplitude and decreased the latency of

the P300 elicited by the auditory oddball task. However, no significant statistical differences were obtained. Thus we assumed that the effect of coffee aroma could be more firmly proved by examining it from another physiological aspect. Therefore, we introduced PET to examine the effect of coffee aroma from the aspect of cerebral blood flow. Our second concern was to investigate the effect of coffee aroma on the sensory input of another modality. Thus, we recorded ERP of normal subjects by using the visual oddball task giving them coffee aroma. Among several ERP components, the P300 was recorded by the visual oddball task. The P300 amplitude is thought to reflect the amount of the information processing resources allocated to the process of stimulus evaluation[3)8)9)10)12)22)25)-28)].

METHOD

Experiment 1. RCBF

Subjects

Subjects comprised of nine healthy right-handed males with a mean age of 25.6 ± 2.5 years. They were all right-handed and four of them were habitual smokers. Each was given a through explanation of the experiment and appropriate remuneration was provided to them.

Procedure

Samples

Roasted coffee(20 g, ground) and lavender oil(2 ml) were employed. Distilled water was used as a control. Each sample was put into a small glass bottle, and placed 5 cm from the subject's nostrils as the scanning was performed.

Measurement of rCBF

The PET scanner here used was the HEADTOME IV, and 14 slices were obtained at the intervals of 6.5 mm parallel to the anterior commissure/posterior commissure(AC-PC) line. $H_2{}^{15}O$ was used as the nuclide which was administered intravenously 15 seconds prior to the scanning. Each scanning lasted 90 seconds. Aforementioned three samples were presented twice, i.e., each subject was scanned six times. During the scanning, each of the subjects was instructed to perform the auditory oddball task as mentioned below.

Data thus obtained were standardized to a total cerebral blood flow of 50 ml/100 g/min., and two measurement values for each sample for individual subjects were averaged. The regions of interest(ROIs) were determined as following areas of both hemispheres: piriform cortex, hippocampus, nucleus accumbens septi, orbitofrontal cortex, hypothalamus, thalamus, transverse gyrus of Heschl, anterior part of superior temporal gyrus, posterior part of superior temporal gyrus, anterior part of middle temporal gyrus, posterior part of middle temporal gyrus and angular gyrus. These 24 ROIs were localized with MRI and scaled to the dimensions of the PET images for each subject.

The stimuli used for the auditory oddball task

The stimuli consisted of two tone bursts of different frequencies. The rare stimulus(p=0.2) was

a 2000 Hz tone and the frequent stimulus(p=0.8) was a tone of 1000 Hz. They were binaurally presented via earphone for 100 msec(10 msec rise and fall times) at 80 dB SPL in random order. The number of stimuli presented in each scanning was 150 times in all. During the scanning, the subject was instructed to breathe through his nose and to count the number of the rare stimuli in silence.

Experiment 2:ERP

The visual P300 was elicited during color discrimination task with ambient odors.

Subjects
Fourty right-handed students(20 males, 20 females) with a mean age of 21.7 ± 0.8 years participated in this experiment as paid volunteers. They had no significant illness including otolaryngeal diseases and five of them were habitual smokers. Informed consent to take part in the experiment was obtained from all the subjects.

Procedure
Samples
Roasted coffee, lemon oil, lavender oil and isovaleric acid were employed. We used distilled water again as a control. Each sample was put on a laboratory dish and set in the desicator with a piece of cloth(30 cm × 40 cm) for twenty-four hours in order that the odor of the sample might be absorbed into the cloth sufficiently. Twenty grams of coffee beans were ground just before they were set in the desicator. The quantity of another samples was 2 ml each. After the absorption, and just before the start of each experimental block, the cloth was took out from the desicator and put on the subject's breast.

The stimuli used for the visual oddball task and the instruction to the subject
The experiment was done in a sound attenuated and electrically shielded chamber. The subject was indicated to gaze at the circle(r= 4 cm) displayed constantly on a CRT screen placed in front of him at a 180 cm distance. Two rectangles(6 cm × 1.5 cm) of different colors(red and blue) were presented for 100 msec within the circle in random order. The red one was the rare stimulus(p=0.2) while the blue was the frequent(p=0.8). The stimuli delivered to each subject 150 times in total for each experimental block. The subject was instructed to perform mental counting of the red one with the cloth on his breast as mentioned above. For each subject, five experimental blocks in which we used different five odors were performed in random order.

EEG recording and analysis
EEG was recorded(bandpass down 3 dB at 0.2 and 120 Hz) with Ag/AgCl electrodes at Cz, Pz, P3 and P4 referred to the linked ears. EEG was AD converted at a rate of 500 Hz and stored on an optical disk. Then EEG was analyzed off-line. EEG was averaged for a 1024 msec period from 100 msec prior to stimulation. Artifact-free twenty epochs for rare stimuli were selected by sight and averaged. The baseline was the mean value of the voltage preceding 100 msec of stimulation.

RESULTS

1. RCBF

No significant differences were found in the value of rCBF among the samples in any ROI. However, the rCBF value under coffee aroma was the highest in the eight ROIs to twelve of the right hemisphere: piriform cortex, hippocampus, nucleus accumbens septi, orbitofrontal cortex, hypothalamus, thalamus, transverse gyrus of Heschl and anterior part of superior temporal gyrus of the right hemisphere. On the contrary, in case of the left hemisphere, it was the highest only in the three ROIs(transverse gyrus of Heschl, posterior part of superior temporal gyrus and angular gyrus) and the lowest in the seven ROIs(hippocampus, orbitofrontal cortex, hypothalamus, thalamus, anterior part of superior temporal gyrus, anterior and posterior parts of middle temporal gyrus). Under the lavender oil odor, the rCBF value was the highest in the three ROIs of the right hemisphere and in the four ROIs of the left. There was a significant difference in the rank of the rCBF value among the samples in the right hemisphere($\chi^2=10.520$; $p<0.05$), and in the left($\chi^2=13.121$; $p<0.05$)(Fig.1).

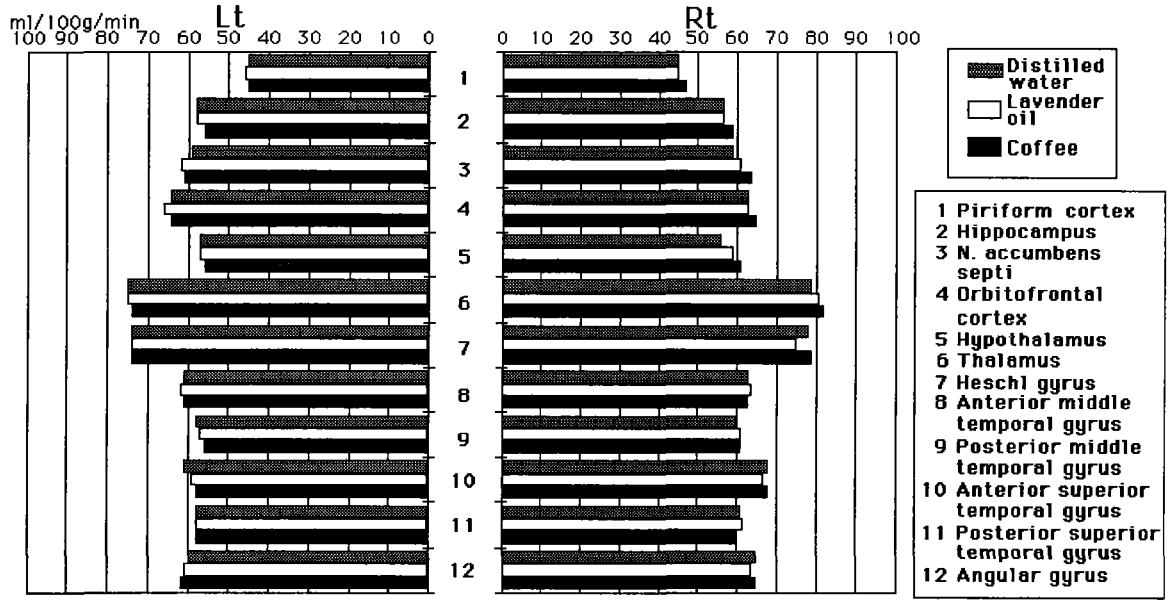

Fig. 1 Comparison of rCBF value among the three samples

Furthermore, the rCBF value under coffee aroma was higher in the right hemisphere than that in the left hemisphere at any pair of the twelve areas. On the other hand, in cases of the other two samples, the rCBF value in the four ROIs(piriform cortex, hippocampus, nucleus accumbens septi, orbitofrontal cortex) was higher in the left side compared to that in the right side. In other eight areas, it was higher in the right side than in the left.

2. P300

1)P300 peak latency

A sample × sex × electrode site analysis of variance was performed on the peak latency value.

The analysis revealed no significant differences among the samples, although a significant interaction was found between sample and sex($F=3.5407$; $p<0.01$). The P300 latency under coffee aroma was the shortest among the samples in the most sites for both male and female subjects(Fig.2,3).

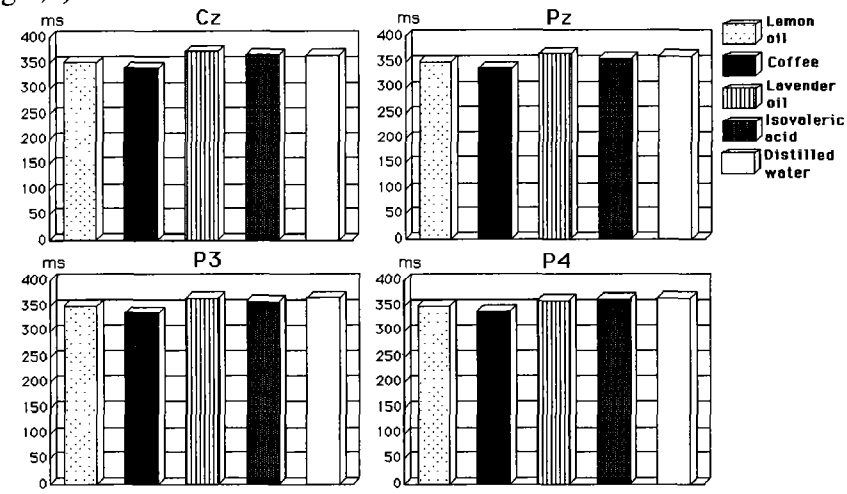

Fig. 2 Mean P300 Latency(male)

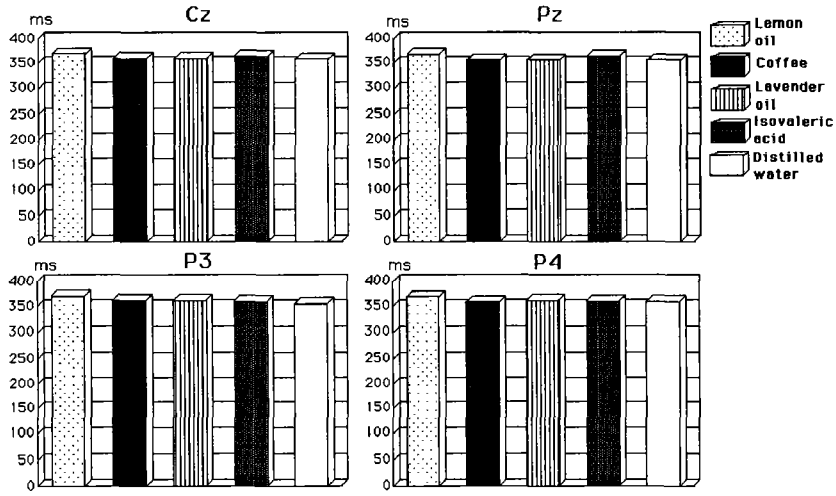

Fig. 3 Mean P300 Latency(female)

2)P300 amplitude

A sample x sex x electrode site analysis of variance for peak amplitude showed significant F value for sample($F=5.9379$; $p<0.01$), sex($F=6.8616$; $p<0.01$), and interaction between sample and sex($F=2.7505$; $p<0.05$). In the male subjects, isovaleric acid showed the smallest P300 amplitude among the five samples at the most electrode sites. The result included significant sample differences in 10 of the 16 sets(Fig.4). While more differences were found among the samples in case of the females. The P300 amplitudes under the lemon oil odor was the largest in all the sites. The P300 amplitudes under coffee aroma were significantly larger than those under isovaleric acid odor in all sites. Moreover, the P300 amplitudes under coffee aroma were significantly larger than under lavender oil odor and distilled water at the most sites(Fig.5).

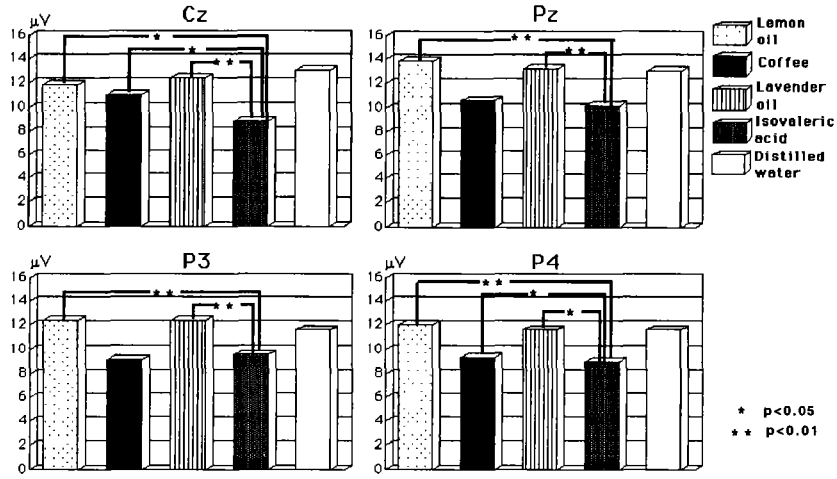

Fig. 4 Mean P300 Amplitude(male)

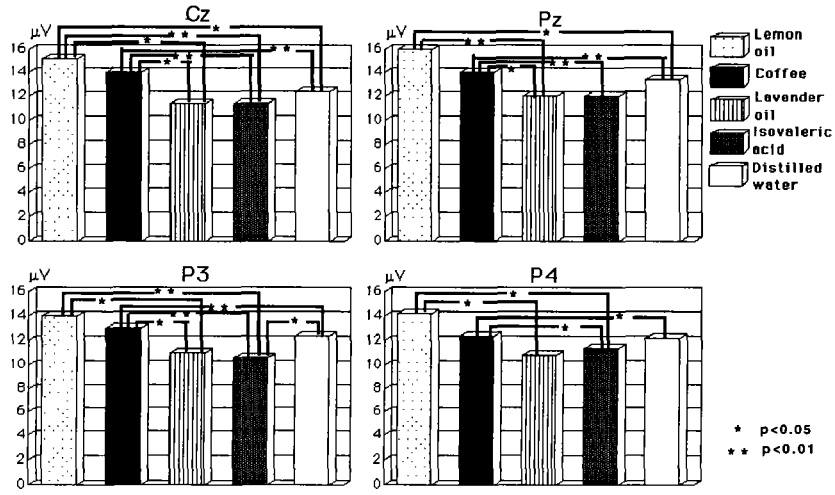

Fig. 5 Mean P300 Amplitude(female)

With respect to the sex differences, the P300 amplitudes of the odorous samples except lavender oil were larger in the females compared to the males in all sites, although statistical significance was obtained only in the case of coffee aroma.i.e, the P300 amplitude at P3 of the females was significantly larger than that of the males($t = 2.320$; $p<0.05$).

As concerns hemispheric differences, no significant differences were obtained between the P300 amplitude at P3 and at P4 in any sample with both male and female subjects.

DISCUSSION

There have been reported by many researchers that coffee has a variety of effects on cognition and behavior based on the brain function. Among numerous components in coffee, most studies were conducted on caffeine[11)19)20)21)24)]. It has been already clarified that caffeine acts as a stimulant on the central nervous system. It elevates the excitability of hippocampus in rat and increases the frequency of alpha activity in human. With respect to the effect of caffeine on

rCBF in human[14)-18)], reduction in rCBF in whole areas without interregional differences was reported by Cameron et al. in 1990[2)]. Behaviorally, it increases the rate of information processing and reduces reaction time. As concerns the mental activity, caffeine increases the anxiety of normals and patients with some psychiatric illnesses such as anxiety disorder, depression, and psychosomatic diseases. However, all of these effects including undesirable effects are brought by oral or intravenous administration of considerably high or moderate doses of caffeine.

As only the odor of each sample was presented to the subject in this experiment, however, the amount of caffeine absorbed through breathing was as little as negligible. Therefore, the findings obtained here about coffee are thought to be produced by coffee aroma itsself.

The results of the rCBF measurement show that coffee aroma increases the rCBF exclusively in the right hemispheric areas especially in the piriform cortex, the hippocampus, the nucleus accumbens septi and the orbitofrontal cortex which are closely related to the processing of an olfactory input. On the contrary, it seems that coffee aroma, different from the other two samples, has an inhibitory effect on the rCBF of these four areas in the left hemisphere. It is generally considered that the right hemisphere is associated with emotional function[5)13)]. In particular, the four areas mentioned above including some parts of the limbic systems are thought to play a significant role to express emotion. For example, the nucleus accumbens septi is thought as the center of pleasant feeling. In addition to those areas, the rCBF value under coffee aroma in the right hypothalamus was the highest among the three samples. The hypothalamus is an important relay center in the pathways cocerned with emotional expression[6)].

With respect to the other areas, the rCBF value was higher in the right hemisphere compared with the left in all the three samples. It may because that the tone burst was used as a stimulus. As tone burst is processed dominantly in the right hemisphere, it seems reasonable that rCBF is higher in the right side than in the left. Thus the unique effect of coffee aroma on the brain function can be found by measuring the rCBF.

The differences of the P300 amplitudes among the five samples show that each sample acts on the visual information processing in a different way. The small P300 amplitude under isovaleric acid suggests that the unpleasant odor decreases the amount of the information processing resources allocated to the process of stimulus evaluation. The difference of the P300 amplitude between the male and the female subjects was especially distinct in case of coffee aroma compared with that of the other four samples. The result above indicates that coffee aroma can increase the ability of visual discrimination particularly in the females.

The results obtained from the rCBF and the ERP experiments demonstrate that coffee aroma has a variety of effects on human cognitive function and behavior. Aromatherapy, the method to improve mental and physical activity by using the odors of essential oils, has become popular in many countries including Japan. The oils employed in this study are quite often used in aromatherapy. As mentioned above, coffee aroma has different effects from those of the two essential oils on the brain function, it can be a good candidate of aromatherapy for the people who do not show remarkable responses to the oils, especially for those who like coffee but show physically adverse responses to caffeine.

Moreover, coffee aroma can be used to improve the unpleasant feeling in some psychiatric

illness without any side effect because it acts on the areas associated with emotion. As this study employed the normal subjects, further investigation should be performed on the patients with moderate emotional disorders in order to clarify whether coffee aroma is effective for the mentally pathological state or not.

SUMMARY

The effect of coffee aroma on the brain function was compared with the other odors by measuring rCBF and ERP. RCBF was measured in 24 ROIs in both hemispheres by using PET scanner. The P300, one of the ERP components, was recorded during the visual oddball task.

The rCBF value under coffee aroma was highest among the three samples(coffee aroma, lavender oil, distilled water) in the most ROIs of the right hemisphere particularly in the four areas(piriform cortex, hippocampus, nucleus accumbens septi, orbitofrontal cortex) closely related to emotional expression, and processing of an olfactory input. Compared with the rCBF value under coffee aroma of the left hemisphere, the value of the right hemisphere was higher in any pair of ROIs. On the other hand, rCBF value of the aforementioned four areas including some parts of the limbic systems was higher in the left hemisphere compared with in the right hemisphere under the other two samples. In the P300 experiment, the effect of coffee aroma was compared with that of lemon oil oder, lavender oil odor, isovaleic acid odor and distilled water. Different from the result of the male subjects, the P300 amplitudes of the female subjects under coffee aroma were significantly larger than those under isovaleric acid odor, and under lavender oil odor. Moreover, the amplitudes of the females was significantly larger than those of the males but lavender.

The hemispheric differences of rCBF caused by coffee aroma show the physiological evidence of the unique effect of coffee on human brain function through its aroma. Furthermore, it is suggested from the result of ERP that coffee aroma affects males and females in a different way.

ACKNOWLEDGEMENTS

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PSYCHOPHYSIOLOGY OF COFFEE IN SENSORY DOMAIN

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1. Introduction

Coffee was regarded as medicine from its very beginning. This prejudice was often reversed by the motives that were not always related to health. Today the controversy about the effects of coffee on health is still not resolved. When we turn our attention to the effects of coffee on mental activities, the beneficial activity is that it offers stimulation without subsequent depression.

Since the extraction of caffeine from coffee beans in 1820, the effects of coffee have been largely ascribed to the pharmacological activities of xanthine derivative. Many systematic studies of the effects of caffeine on mental activities and moods have been carried out through 1970-80's. However, coffee is unique in its aroma and aroma of coffee reaches our sense first. In fact, all the taste of coffee starts from aroma.

In this present study, effects of coffee on psychophysiological functions were studied from the view point of its effects on sensory domain.

2. Methods

Subjects:

Subjects were young female students who participated in experiments as paid

volunteers. All of the subjects were neurologically and psychiatrically normal. Numbers of subjects ranged form 16 to 10 according to sessions. Only instruction they had was to sleep well the night before the experiment.

Experimental sessions:

Three experiments were designed for coffee aroma, taste and drink, and caffeine. Each subject participated in only one of these three sessions. Each experiment started around 1:00 pm and lasted about 4 hours. Experimental session consisted of control and experimental sessions in both of which physical, psychological and psychophysiological measurements were included.

Procedure:

Before the subjects underwent to the control session, they were asked about her night sleep with sleep check list. and their physical conditions were also checked by questionnaire. Control session started with the measurement of heart rate and blood pressure, and, then, moved on Critical Flicker Fusion Test(CFFT) and Choice Reaction time Test(CRT). These were followed by psychophysiological recording in which Contingent Negative Variation(CNV) was recorded. A series of these procedures were ended by Feeling Profile evaluation. In the experimental session, psychophysiological recording preceded to the other measurements and tests.

In the following sections, the results for physical measurements(heart rate and blood pressure) and mental functions (CFFT and CRT) were excluded, as these measurements and tests did not show any changes between the control and experimental condition of each experimental session.

Psychological evaluation:

In addition to the CFFT and CRT, six categories of psychological states were rated on the analogue scale ranging from 0 to 100 %. These categories were "elated", "depressed", "favored", "ill-favored", "relaxed", "irritated". These were thought to be constituents of pleasant and unpleasant feelings and schematic presentation of these rated is useful to know the characteristics of feeling induced in the experimental session.

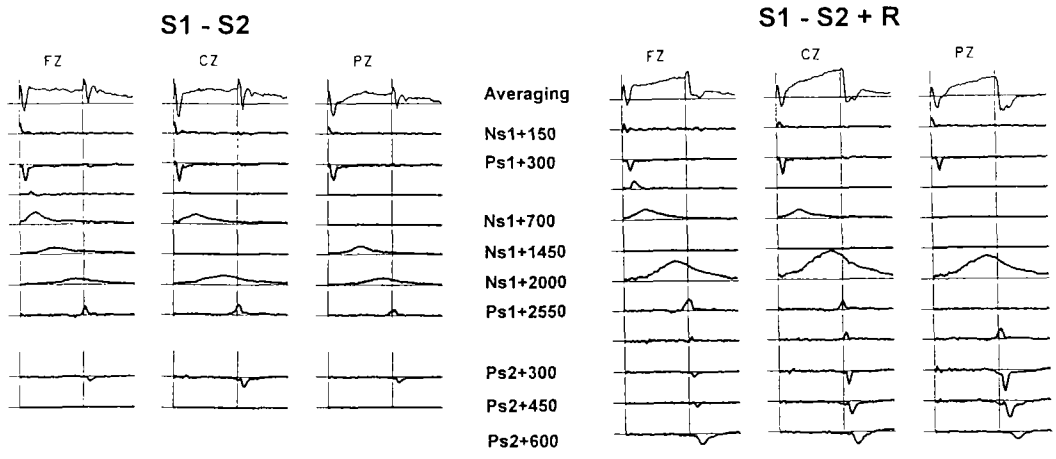
Psychophysiological recording:

Electroencephalogram(EEG) was recorded from the 19 electrode sites placed on the scalp according to the 10/20 Electrode System with time constant of 5.0 sec. Event-related potentials were recorded by S1(click)-S2(white noise) and S1-S2+R paradigms in which S1-S2 interval was 2.5sec. In the latter paradigm, "R" was to interrupt the S2 as soon as possible when

the S2 was presented. The psychophysiological recording and its data processing method were described in detail elsewhere(Saito and Yamamoto, 1986).

Figure-1 shows an example of the results obtained by our method of analysis. Among the number of CNV basis waves, three main basis waves(Ns1+700, Ns1+2000 and Ps2+300) underwent to further analysis. Ns1+700 is called in the following context as early CNV basis wave, and Ns1+2000 as late CNV basis wave and Ps2+300 as late positive slow basis wave respectively.

Figure-1. Basis Wave Constructure of CNV



3. Results

1) Experiment of coffee aroma

Sixteen young females participated in coffee aroma experiment. Twelve out of the sixteen successfully completed the experiment. In this coffee aroma experiment, after the control session, roasted coffee beans were ground for each subject in the shielded room and then glass balloon used for providing the room with coffee aroma. When all the coffee filtered down into the lower bowl, the subject allowed to come into the room and CNV was recorded in scented environment. It took about 20 min to record CNV. Feeling profile was assessed soon after CNV recording.

Feeling profile in the scented condition is illustrated on the upper left in Figure-2. Subjective evaluation of their feelings shows rather relaxed and favored type with some elated score. It is also interesting to note that there were some ill-favored and irritated scores at the same time. As a whole, this profile indicates that their feeling profile was a pleasant one.

Mean magnitude of the CNV basis waves and their increase ratios to the paradigm shift are shown in Table-1 and, in Figure-3, the ratios are illustrated schematically. In the control

condition, the increase ratio of the early CNV basis wave was 1.75 and this ratio decreased to 1.13 in the experiment condition scented by coffee aroma, although the mean magnitude of this basis wave was increased in the scented condition.

Figure-2. Feeling Profiles of Four Experimental Conditions

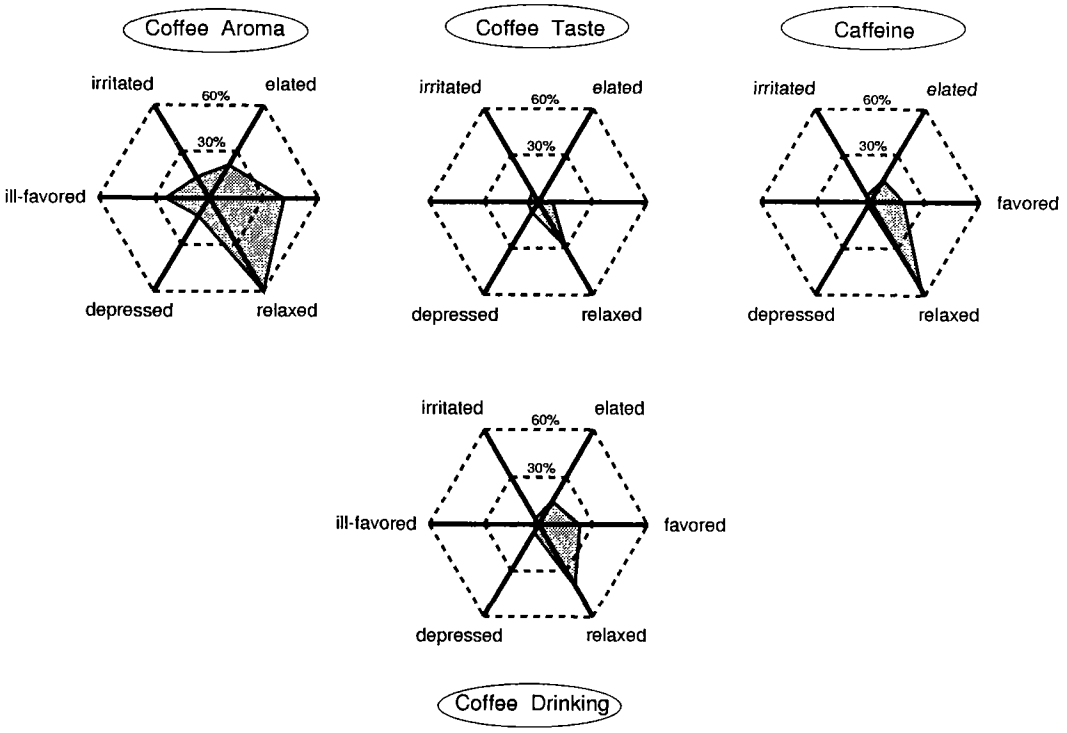


Table-1. Basis Wave Measurements

	Early CNV Basis Wave			
	Control		*Scented*	
	Lt		Rt	
	R(-)	R(+)	R(-)	R(+)
magnitude	70.3	120.7	111.9	127.3
(x50μvms)	37.2	33.8	41.9	51.8
Increase Ratio		1.75		1.13

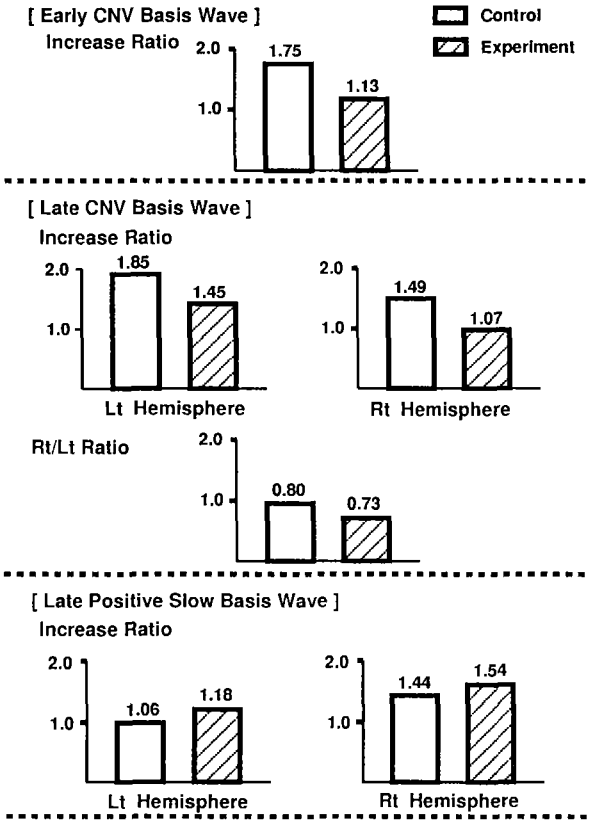
	Latee CNV Basis Wave							
	Control				Experiment			
	Lt		Rt		Lt		Rt	
	R(-)	R(+)	R(-)	R(+)	R(-)	R(+)	R(-)	R(+)
magnitude	145.2	209.2	170.7	255.6	207.7	301.4	235.6	254.4
(x50μvms)	36.9	82.4	38 0	87.3	52.1	93.8	52.0	111.3
Increase R		1.85		1.49		1.45		1.07
Rt / Lt Ratio				0.80				0.73

	Rate Positive Slow Basis Wave							
	Control				Experiment			
	Lt		Rt		Lt		Rt	
	R(-)	R(+)	R(-)	R(+)	R(-)	R(+)	R(-)	R(+)
magnitude	12.6	13.4	10.2	14.8	17.7	21.0	10.8	16 6
(x50μvms)	6.6	6.8	5.7	10.7	5.8	13.9	7.4	15.6
Increase R		1.06		1.44		1.18		1.54

The same kind of results were obtained about the late CNV basis wave, showing decreased ratios on the both hemispheres in the scented condition. The Rt/Lt ratio was also decreased in the scented.

Late positive slow basis wave were increased its magnitude in the S1-S2+R paradigm on the both hemispheres and the increase ratio of this basis wave was larger in the scented condition than in the control condition.

Figure-3. CNV Profile of Coffee Aroma



2) Feeling profile in the four experimental conditions

Feeling profiles of four experimental conditions are illustrated in Figure-2. The upper three profiles from the left to the right show the profile for the coffee aroma, taste and caffeine and the bottom one is the feeling profile after coffee drink. None of the upper three profiles can explain by itself the bottom one. As far as the feeling profile concerns, the feeling induced by coffee seems to be compound.

3) Early CNV basis wave in the four experimental conditions

Increase ratios of the early CNV basis wave are illustrated in Figures-4 and -5. The increase ratio of the early CNV basis wave decreased in the coffee aroma condition, while it increased in the coffee taste and caffeine conditions. The increase of this ratio in the coffee drink

condition seems to be ascribable to the sensation induced by coffee taste or the pharmacological effects of caffeine, or to the both.

Figure-4. Increase Ratios of Early CNV Basis Wave

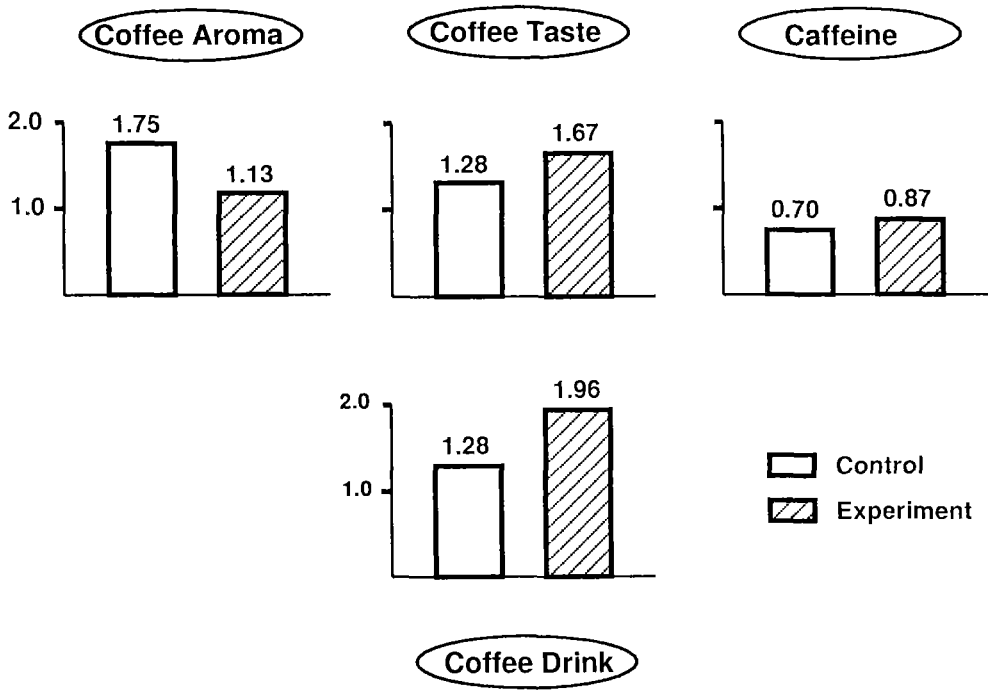
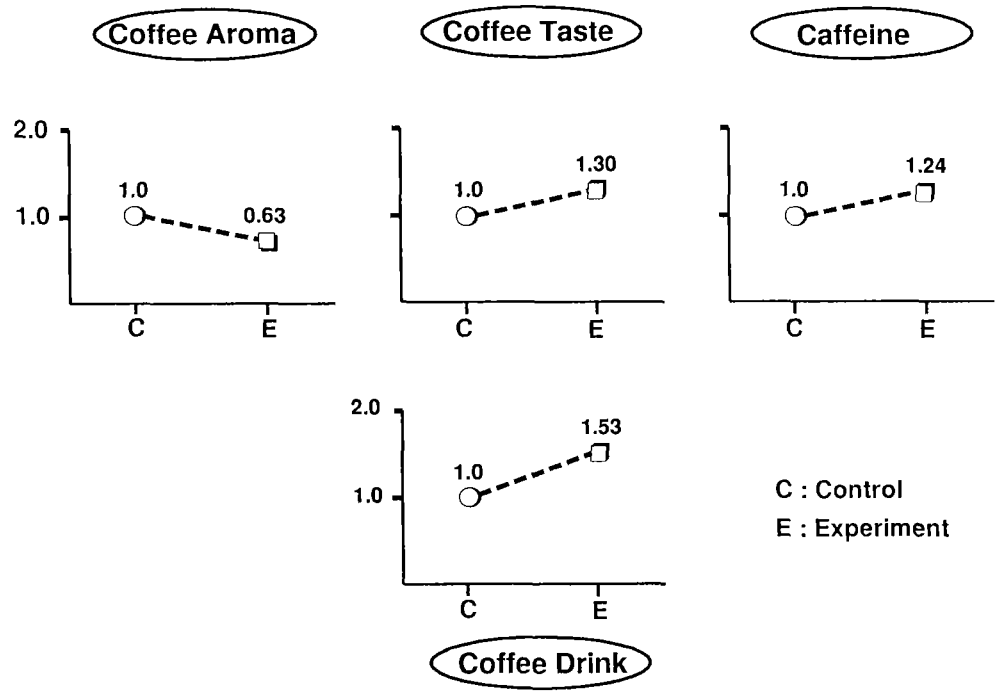


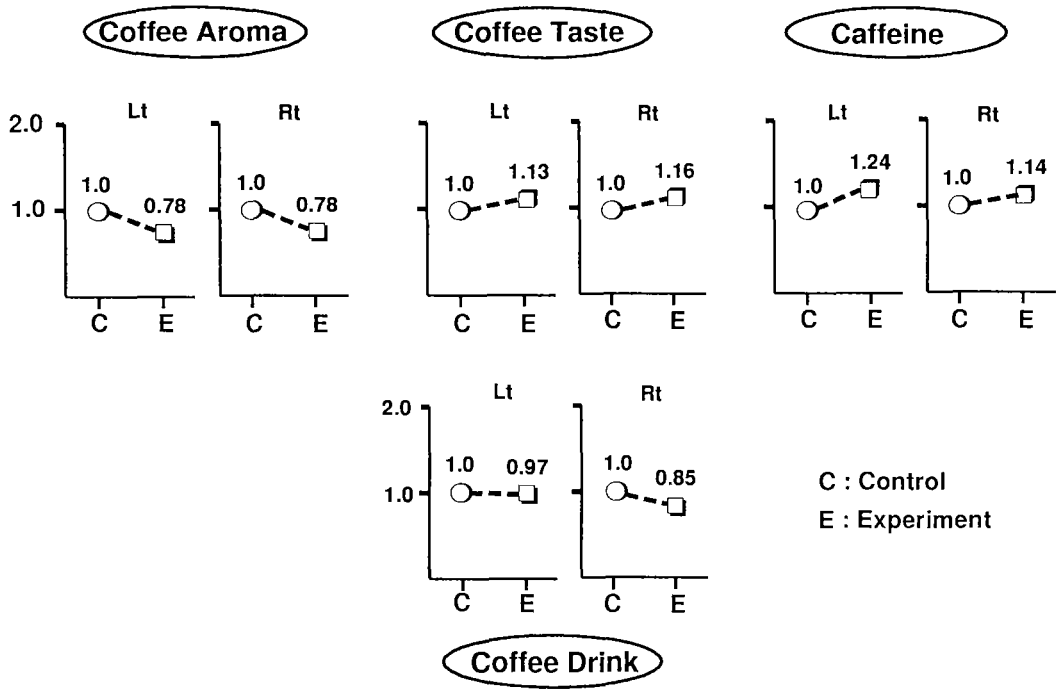
Figure-5. Relative Ratios of Early CNV Increase Ratio



4) Late CNV basis wave in the four experimental conditions

The hemispheric increase ratio of late CNV basis wave became smaller in the coffee aroma condition than in the control condition, while it became larger in both the taste and caffeine conditions. The results in the coffee drink condition, especially on the right hemisphere, were quite similar to that of the coffee aroma condition showing a decreased ratio in the experimental condition. This was also reflected in the Rt/Lt. ratio. These results of the late CNV basis wave in the coffee drink condition corresponded well to the feeling profile induced after drinking coffee (Figure-6)

Figure-6. Relative Ratios of Late CNV Increase Ratio



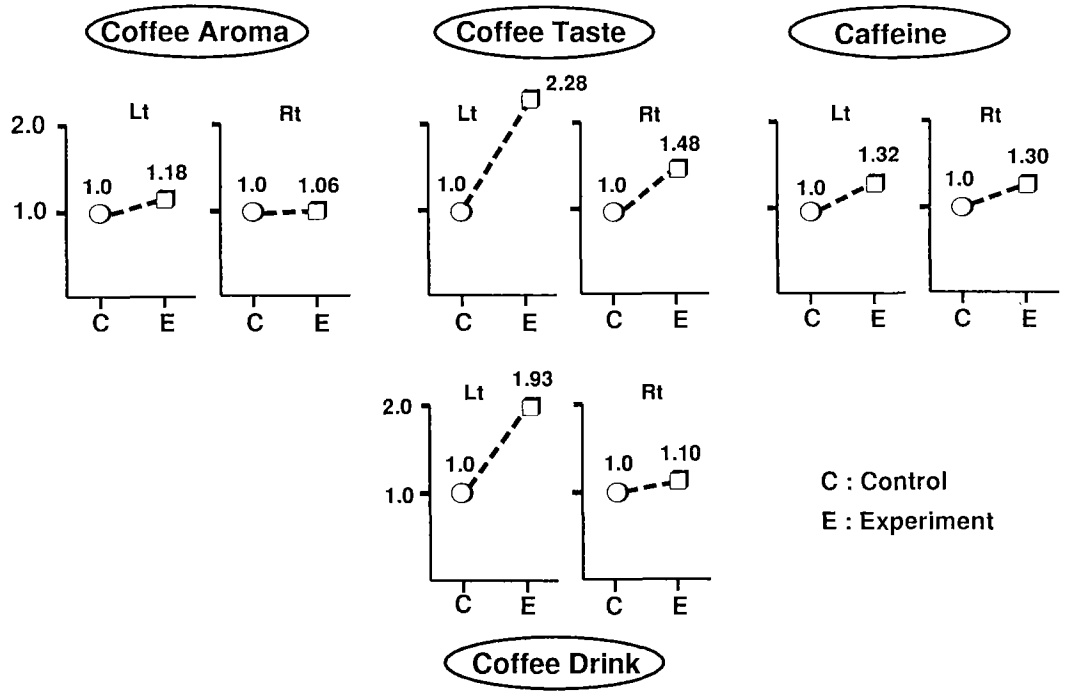
5) Late positive slow basis wave in the four experimental conditions

Late positive slow basis wave increased its magnitude in all four experimental conditions showing more increased ratios. This was especially dominant on the left hemisphere in the taste and coffee drink conditions. The results in the coffee drink condition might be ascribable to the effects of sensation induced by coffee taste (Figure-7).

4. Discussion

As have been already told in the legends of coffee origin, the beneficial activities of coffee is that it offers stimulation without subsequent depression. In 1820, caffeine extracted

Figure-7. Relative Ratios of Increase Ratio of Late Positive Slow Basis Wave



from coffee beans by Runge and this xanthine derivative was also extracted from tea leaves. Many effects of coffee have been attributed to the pharmacological effects of caffeine.

In a number of studies, it has been reported caffeine has such positive effects on mental activities as reduction of reaction time(Smith et al, 1977), increased alertness and improvement in maintaining attention. On the other hand, a number of studies suggested that caffeine actions are most pronounced under conditions of mental fatigue, whereas in well-rested subjects it appears difficult to demonstrate effects of caffeine(Weiss & Latis, 1962; Lieberman et al, 1986).

However, coffee has many attributes related to various sensory domains. Coffee aroma reaches our sense first and this olfactory stimulus may possible to raise some sensation and, then, feeling. Feelings induced by odor change psychophysiological cerebral functions related to arousal and cognition (Yamamoto, 1993). Coffee aroma induced changes in the CNV basis waves clearly indicate that some aspects of psychophysiological effects of coffee might be attributable to the aroma of coffee.

Effects of coffee on taste modality is also another point of interest. the fact that sensation of taste did not affected feeling might be attributable to the character of this modality which, anatomically, involves three cranial nerves; facial, glossopharyngeal and vagus nerves. In addition to this, it is also known that general sensation of oral cavity is conveyed by the trigeminal nerve. Although a detail of the relationship between these cranial nerves are not

elucidated, functional convergence will be supposed. As far as present data concern, sensation of coffee taste seems to affect together with caffeine cognitive and orienting function.

5. Summary

Psychophysiological effects of coffee were studied by feeling profile and CNV measurement in the experiments coffee aroma, taste and caffeine.

It appeared that both of coffee aroma and taste were important factors to elucidate the effects of coffee on psychophysiological brain functions. Effects of coffee on feeling were attributed mainly to coffee aroma, while coffee taste may affect information processing mode on the left hemisphere.

Both of coffee taste and caffeine seem to affect the cerebral orienting response. Neither Coffee aroma nor caffeine alone is attributable to causative factor of the effects of coffee on psychophysiological functions. Effects of coffee on psychophysiological bran functions are of compound and complex ways.

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CAFFEINE AND VISUAL PERCEPTION

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INTRODUCTION

The considerable amount of caffeine containing substances in the diet of millions of people, together with a strong emphasis on cognitive work and mental efficiency in daily life, justifies research trying to answer the question what the effects of caffeine are on human information processing activities. Caffeine is generally classified as a 'stimulant'. It has been claimed to have facilitating actions on information processing through actions on energetical resources. Measures of cortical brain activity might serve as an indication of these energetical effects of caffeine. With increasing energy, the electroencephalogram (EEG) shows more activation and changes towards faster frequency and lower-amplitude activity. In accordance with previous EEG studies on caffeine effects, a reduction in alpha and delta power was found after caffeine, interpreted as reflecting elevated levels of energy (Bruce, Lader, and Marks, 1986; Etevenon et al., 1989; Kenemans and Lorist, in press; Newman, Stein, Trettau, Coppola, and Uhde, 1992; Saletu, Anderer, Kinsperger, and Grünberger, 1987). Because studies of the EEG have revealed some task-specific patterning in this activity (Gale and Edwards, 1983), the reported results might imply that caffeine has an effect on cognitive processing, although EEG measures seem less suitable to uncover more detailed effects of caffeine on cognitive processing.

The principal aim of this paper is to evaluate the specific effects of a single amount of caffeine, similar to a dose found in two average cups of coffee, on processes involved in the processing of visual information. Regarding the literature on the effects of caffeine on task related activities, virtually every conceivable result has been obtained, indicating that caffeine may be facilitating, detrimental, or ineffective with regard to its effects on information processing. However, a central problem of studies investigating the effects of caffeine on human performance is that of most of the cognitive tasks used, the underlying structure is unclear. At best, these studies give only a global insight in the types of tasks that are most sensitive to caffeine effects. An interpretation of

results in terms of effects on different cognitive processes is often not possible, and for this reason generalisations of caffeine effects to other task situations are difficult to make.

VISUAL PERCEPTION

Where (spatial) and what (non spatial) characteristics of visual information

Complex activities such as the perception and identification of visual information encompass several levels of analyses. Among the theories, describing how the information processing system handles visual information, Treisman's feature-integration theory (1988, 1993) is currently one of the most influential. A basic assumption of this theory is that different stimulus characteristics or features are analysed in specialised modules. Physiological evidence indeed indicates that information about where a stimulus resides in our visual environment is analysed in the primary visual area and the parietal lobes in the upper middle of the brain, while the recognition of particular visual patterns (e.g., colour and form) concerns brain areas that go from the primary visual area in the back of the brain out into the temporal lobes in the lower middle of the brain (see Posner and Raichle, 1994; Wijers, Lange, and Mulder, in press). In Treisman's theory three different levels of analyses of visual information are distinguished. In an early stage of processing spatial features are analysed. The outcome of this analysis shows where all feature boundaries are located, but not which features are located where. At the second stage, non spatial features of visual stimuli such as colour (hue, brightness, and saturation) and form (frequency, orientation, shape, and size) are analysed. The different spatial and non spatial features are subsequently conjoined, to form a coherent representation of the visual information. These conjunctions will generally characterise a more complex and structured representation of visual information than the stimulus information picked up early in the analysis of visual information. Notice that location is regarded as a distinct feature in vision (see also Duncan, 1993; Schneider, 1993), that is, conjunctions are only correctly identified when they are also correctly localised.

The role of attention in visual perception

Attention is required to locate and conjoin features. Without attention different features are either not conjoined or conjoined incorrectly. A second function of attentional control in visual perception concerns the selective processing of specific features or conjunction of features, in order to prevent an overload of the capacity limited information processing system. For example, when spatial information is available in advance (e.g., a spatial cue), the attentional window might restrict the number of features used to identify environmental information. Attention has often been regarded as some sort of spotlight that can be directed to different locations in the visual field. Detection of global shape can be done with a broad setting of the attentional window. Accurate localisation and conjoining of features for individual objects require a more narrowly focusing of the attentional spotlight.

CAFFEINE AND SPATIAL FEATURES

In a study reported by Lorist, Snel, and Kok (1994), processes involved in the perception and identification of visual information were manipulated by degrading the quality of visual stimuli (see Figure 1.). More precisely, in

the degraded stimulus condition the stimulus' distinctiveness relative to background was worse as compared with the intact condition. The

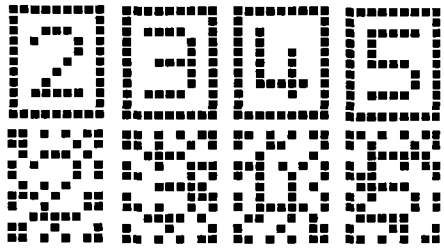

Figure 1. Stimuli used by Lorist, Snel, and Kok (1994).

effects of caffeine were found to be more pronounced when the quality of visual information was bad, that is, in a situation in which a greater demand was placed upon perceptual processes. These results indicated that processes involved in the perception of visual stimuli are affected by caffeine. The implication of these findings are twofold. First, an effect of caffeine on the analyses of spatial stimulus features can be assumed. In that case, the greater influence of caffeine in the degraded stimulus condition might be explained by a better perception of feature boundaries. Second, caffeine might have an effect on selective attention mechanisms.

CAFFEINE AND NON SPATIAL FEATURES

To verify whether caffeine has an effect on the analysis of non spatial stimulus characteristics, an experiment was designed in which subjects had to select visual stimuli on the base of a combination of these non spatial features (Kenemans and Lorist, in press). The stimuli used in this experiment were presented at a fixed position, but differed with regard to spatial frequency and orientation (see Figure 2). The results of this experiment indicated that caffeine improved the speed and efficiency of information processing independent of stimulus characteristics, as reflected in faster reactions and an increase in correct responses. However, patterns of brain activity, found to be differentially sensitive to spatial frequency and orientation of visual stimuli (Harter
and Previc, 1978; Kenemans, Kok, and Smulders, 1993; Kenemans, Smulders, and Kok, 1995), were not affected by the administration of caffeine. It seems that the processing of specific non spatial features is not influenced by caffeine

The second possibility, suggested above as a feasible mechanism through which caffeine may act, was by an influence on selective attention mechanisms. In this experiment (Kenemans and Lorist, in press), selectivity of processing was indexed by the difference between brain potentials elicited in stimuli possessing the combination of relevant feature dimensions and stimuli having irrelevant feature dimensions. The results indicated that caffeine had no effect on selective attention mechanisms involved in the processing of these non spatial features (e.g., orientation, spatial frequency). Putting the results together, it seems that caffeine affects perceptual processing of visual stimuli in a specific way. The processing of spatial features is affected to a greater extent by caffeine than the processing of non spatial features, and caffeine does not affect the selection process based on these non spatial features.

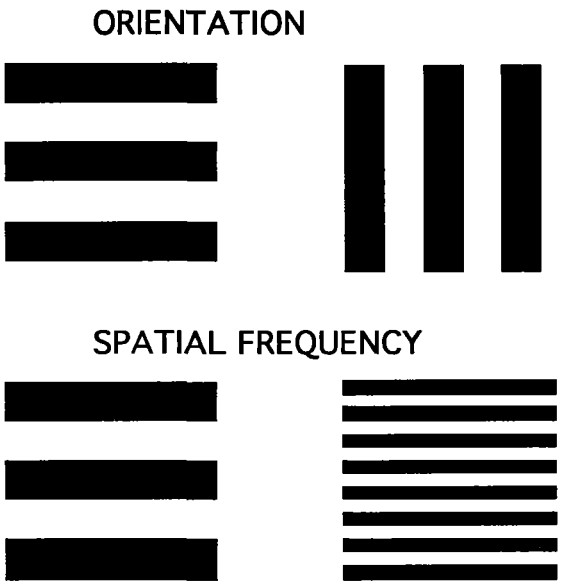

Figure 2. Stimuli used by Kenemans and Lorist (in press).

CAFFEINE AND ATTENTION

The next step in localising the effects of caffeine on human information processing system is to take a closer look on the effects of caffeine (200 mg/kg or 3 mg/kg) attention. More precisely, the effects of caffeine were evaluated in a situation in which subjects had to direct their attention to a *restricted* number of display *positions* on which stimulus items could be presented (focussed attention condition: Lorist, Snel, Mulder, & Kok, in press; Lorist, Snel, Kok, & Mulder, 1994; Lorist, Snel, Kok, & Mulder, submitted), and in a situation in which they had to attend to all items presented on the display, independent of the specific location (divided attention condition: see Lorist, Snel, Kok, & Mulder, submitted). Thus in the focussed attention condition subjects had to make a selection between relevant and irrelevant stimulus features, a process which did not occur in the divided attention task conditions. The difference in brain activity associated with selection on the base of spatial frequency and orientation (Kenemans and Lorist, in press), and the activity pattern elicited by diagonal as a stimulus-selection criterion (Lorist, Snel, Mulder, & Kok, in press; Lorist, Snel, Kok, & Mulder, 1994; Lorist, Snel, Kok, & Mulder, submitted) indicate that both types of selection are based on distinctive attention mechanisms, requiring different brain processes (Harter and Previc, 1978; Kenemans, Kok, and Smulders, 1993; Wijers, 1989). The selective attention mechanism involved in the second case, relies on a feature unspecific attention mechanism. This selection mechanism was found to be influenced by caffeine. The amount of caffeine comparable to two cups of regular coffee appears to make people less easily distracted by irrelevant information, and improves the sensory perceptual processing of stimuli presented at attended locations. In terms of the feature-integration theory (Treisman, 1988, 1993) this effect can be interpreted as reflecting a more active suppression of the processing of information presented on irrelevant locations.

CONCLUSION

In summary, it can be concluded that a moderate dose of caffeine affects visual information processing by affecting the analysis of spatial stimulus features (where characteristics of information). The perception of non spatial features (what characteristics) is not influenced. Moreover, caffeine appears to improve the selective processing of specific points in visual space.

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ABSTRACT

The human information processing system is a complex system. For example, the perception of visual information encompasses a set of functionally different cognitive processes, needed to analyse different visual stimulus characteristics. The effects of caffeine on the perception of these different characteristics of visual information, were examined in more detail in two studies. The results indicate that caffeine affects processes involved in the analysis of information about where a stimulus resides in our visual environment. The analysis of non spatial stimulus characteristics seems to be unaffected by caffeine. In addition, caffeine appears to make people less easily distracted by irrelevant information.

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THE CHOLESTEROL-ELEVATING FACTOR FROM COFFEE BEANS

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The effect of coffee consumption on the heart and the vascular system has been controversial for a long time. In 1963, Paul et al. reported that coffee consumption could be a risk factor for cardiovascular diseases (1). In other epidemiological investigations this association was not confirmed, mainly because of confounding by smoking habits: people consuming large amounts of coffee are likely to smoke more than those drinking no or moderate amounts of coffee. This hampered the elucidation of the effect of coffee use on the risk of developing cardiovascular disease. The suspected higher risk was clearly not mediated through effects of coffee drinking on blood pressure, as the impact of chronic consumption of coffee or of caffeine on blood pressure is small.

The relationship between coffee and serum levels of cholesterol was doubted by many researchers, but in 1983 Thelle and coworkers (2) reported an undeniable positive association of coffee drinking with serum cholesterol levels in Norwegian men. This group also found in an experiment that withdrawal of coffee reduced serum cholesterol levels by 0.55 mmol/L (3). Studies in other populations, however, did not reveal an elevating effect of coffee consumption on serum cholesterol. The method of coffee brewing appeared to be crucial. In Scandinavian countries, coffee was prepared traditionally by boiling coarsely ground coffee beans with water and decanting the brew into the cup without the use of a filter. Ahola and coworkers in Helsinki (4) showed that this specific type of "boiled" coffee increased the levels of serum cholesterol, whereas filtered coffee did not show any effect. A similar response in serum cholesterol was

obtained by Bak and Grobbee (5) with unfiltered coffee prepared by infusing coffee grounds with water of 93°C. Interestingly, not the heating or boiling procedure itself was the crucial factor for the cholesterol-raising capacity, but it was the presence or absence of a paper filter that made the difference. This was in line with data from epidemiological studies: only people consuming boiled and unfiltered coffee had higher levels of serum cholesterol.

Zock and coworkers in Wageningen proposed that the cholesterol-elevating compound was carried with coffee bean particles that are present in unfiltered coffee brews. After centrifugation of boiled coffee a sediment of bean particles was present at the tube bottom, but there was also a small layer of fatty substance floating atop. Boiled coffee was found to contain 1-2 g of lipid material per liter. Boiled coffee prepared in an identical way but decanted through a paper filter before consumption hardly contained any lipids. Thus, the lipid material present in boiled coffee was retained by this paper filter. A daily dose of 1.3 g of this coffee lipid substance, corresponding to about 7 cups of boiled coffee, elevated serum cholesterol by 23% in ten healthy volunteers (6). In the same subjects, serum levels of triglycerides were elevated by 55%. The cholesterol-elevating factor from coffee beans thus appeared to be a lipid.

Coffee beans contain 10-15% (w/w) of oil (7). The main representatives are triglycerides, which consist of fatty acids attached to a glycerol molecule. Consumption of gram amounts of fatty acids hardly affect blood lipoprotein profile, but coffee oil also contains 6-11% unsaponifiable lipids, among which fatty acid esters of diterpene alcohols and several sterols, tyramine-compounds and small amounts of hydrocarbons. One of these non-triglyceride lipids could be the cholesterol-raising factor.

Our research for the identity of the factor started with an experiment in which volunteers consumed oil that was pressed from coffee beans. This oil raised serum cholesterol, and the laborious process of preparing and centrifuging large amounts of boiled coffee could further be avoided. We tried to find an animal model to ease the study of the underlying mechanisms, but none of the selected animals -- rats, gerbils, hamsters, rabbits, and Cebus or Rhesus monkeys -- responded to coffee oil, irrespective of the administered amount (8,9). The response of serum lipids to the coffee factor seemed to be specific for humans, and the remaining part of our research thus concentrated on volunteers. In a trial with 63 volunteers lasting six weeks, we showed that the cholesterol-elevating factor in fact was one of the unsaponifiable organic compounds present in coffee oil (10). We then tested coffee oil that was stripped of diterpene alcohols, which form the major part of the unsaponifiable substance of coffee oil. In an experiment of 5 weeks with 43 volunteers we showed that such oil had no longer cholesterol-raising capacity (10) (**figure 1**). We then analyzed activities of several common liver function enzymes in the sera of these volunteers. These enzymes are indicative of liver functioning. We found that consumption of 2 g of coffee oil per day raised the activity of alanine aminotransferase (ALAT; SGPT), though not pathologically. After withdrawal of the treatment, alanine aminotransferase levels returned to normal. The course of γ-glutamyltransferase (γ-GT) was even more remarkable: its activity declined during coffee oil consumption and showed a sharp rebound rise after withdrawal of the treatment (10) (**figure 1**).

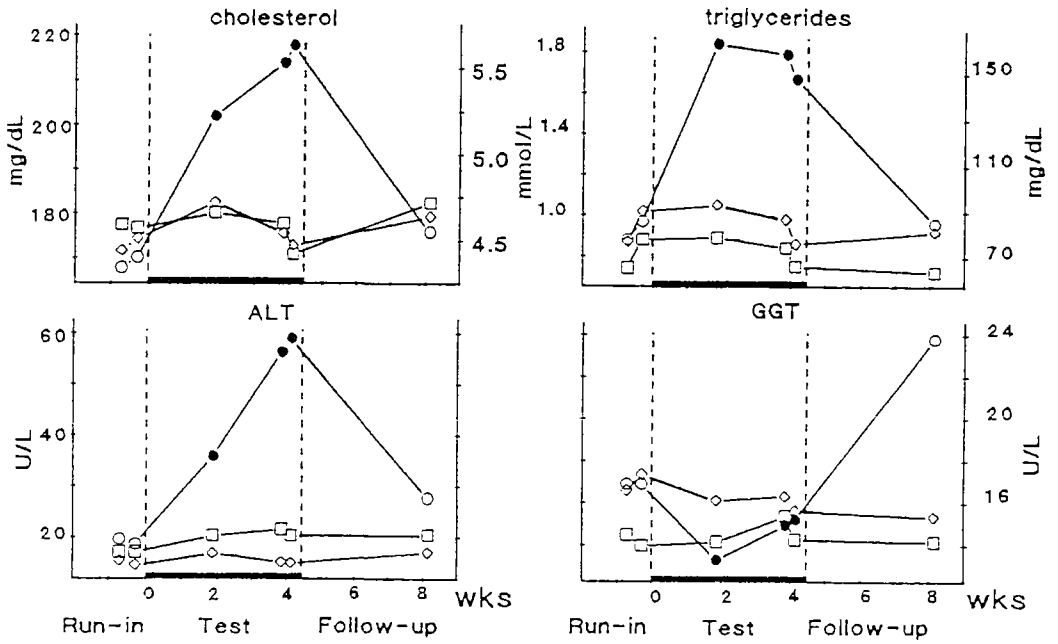

Figure 1 The effect on serum levels of total cholesterol, triglycerides, alanine aminotransferase and γ-glutamyltransferase of consumption of 2 g of placebo oil (□), coffee oil (●) or coffee oil without cafestol and kahweol (◇) per day in healthy volunteers (n=12-16 per group). The test period is indicated by a horizontal black bar. During the run-in period the volunteers swallowed 2 g of placebo oil per day. No treatment was given during the follow-up period (10).

The main diterpenes from coffee beans are cafestol and kahweol (**figure 2**). Robusta beans are almost devoid of kahweol but contain a minor third diterpene -- 16-O-methylcafestol -- which is absent in Arabica beans. In our next experiment, we tested the efficacy of cafestol and kahweol, that had been purified from coffee oil. We gave a mixture of these diterpenes dissolved in placebo oil to three volunteers for six weeks. Cafestol and kahweol sharply elevated serum cholesterol. Similar responses were also obtained in the other parameters that are sensitive to consumption of coffee oil, such as a rise of triglycerides, a reduction of HDL and creatinine, a rise of alanine aminotransferase activity, and a reduction of γ-glutamyltransferase activity followed by a rebound rise after withdrawal of treatment (10). This almost completed the identification: cafestol, possibly together with kahweol, is the cholesterol-elevating factor from coffee beans. Activity of 16-O-methylcafestol is unknown, but intake levels of this diterpene are very low.

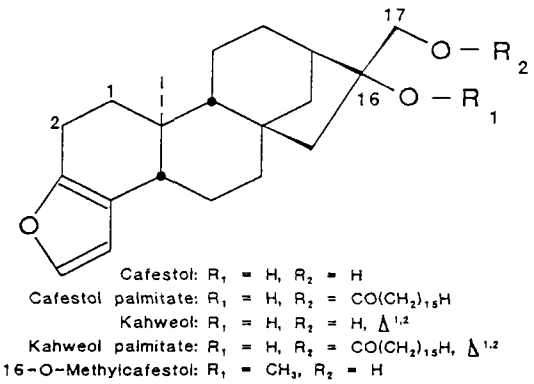

Figure 2 Structure of diterpenes from coffee. Kahweol has an additional double bond between carbon atoms 1 and 2. During the roasting processes small amounts of decomposition products of cafestol and kahweol are formed.

We wondered whether the effects on the liver site as seen with consumption of coffee oil and purified diterpenes would also be present with consumption of regular boiled coffee. Our colleagues from Helsinki provided us with sera from an experiment with consumers of boiled versus filtered coffee, in which we found that boiled coffee also raises alanine aminotransferase activity, and decreases that of γ-glutamyltransferase (10). We organized a case-control study with our colleagues in Norway to investigate the effect of chronic consumption of boiled coffee. Serum levels of cholesterol were higher and γ-glutamyltransferase activities were lower in 150 boiled-coffee drinkers compared to 159 matched filtered-coffee drinkers, but the difference in alanine aminotransferase was very small; the mildly hepatocellular effect of cafestol and kahweol as suggested by the elevations of alanine aminotransferase activity could thus be transient (10). The time course of the physiological effects of coffee diterpenes with prolonged exposure to unfiltered coffee will be subject to further research.

The identification of the factor enabled us to estimate the cholesterol-elevating capacity of various coffee brews, by measuring their content of the hypercholesterolemic compounds. The Scandinavian type of boiled coffee was the main type of coffee consumed in experimental and epidemiological studies showing higher cholesterol levels upon coffee drinking. We found that a 150 mL-cup of Scandinavian boiled coffee provides on average 3 mg of cafestol and 4 mg of kahweol. Similar diterpene concentrations were found in French press coffee (also called cafetiere-coffee or plunger-coffee, **figure 3**) -- prepared by pouring boiling water onto coarsely ground coffee in a glass jug after which the liquid is separated from the sediment by pushing down a screen strainer.

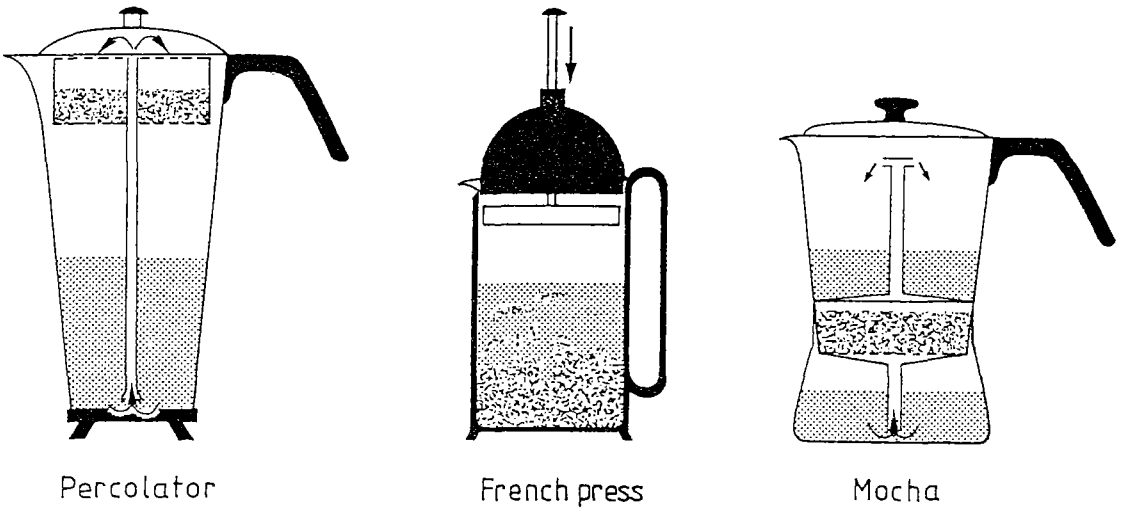

Figure 3 Brewing principles for percolated, French press (cafetiere or plunger) and mocha coffee.

We estimated that daily consumption of five cups of Scandinavian boiled or French press coffee on average will result in a rise of serum cholesterol of 0.2-0.3 mmol/L (8-12 mg/dL) (**figure 4**). Turkish or Greek coffee -- prepared by boiling finely ground or powdery coffee with water and decanting the brew into the cup without the use of a filter -- also provides considerable amounts of cafestol, mainly because of a high amount of floating coffee bean particles decanted and consumed with the liquid. Up to 90% of the diterpenes present in a cup of Turkish/Greek coffee may be carried by floating coffee bean particles (11), and levels of cafestol and kahweol in Turkish/Greek coffee vary largely with the amount of particles decanted with the brew. We showed that diterpenes from these bean particles are well absorbed and raise serum cholesterol and serum activity of alanine aminotransferase similar to those administered with coffee oil (11). The procedure of brewing espresso coffee is also a very efficient way to extract diterpenes from coffee grounds, but intake levels of cafestol and kahweol are lower, as this type of coffee is usually served in very small quantities. We estimated that the amount of diterpenes ingested daily with five cups of espresso coffee will elevate serum cholesterol on average by 0.10 mmol/L (4 mg/dL). Effects of Neapolitan mocha coffee are even lower (**figures 3 and 4**).

Surprisingly, percolated coffee appeared to contain very low levels of cafestol and kahweol. In the percolator pot (**figure 3**), coffee is constantly recirculated through a bed of coarsely ground coffee, which seems to function as a filter cake retaining the diterpenes from the brew. Instant or soluble coffees contain only negligible amounts of cafestol and kahweol, for both decaffeinated and regular brands. Finally, chemical analyses confirmed our findings from experiments with volunteers that filtered coffee has no cholesterol-elevating capacity (**figure 4**).

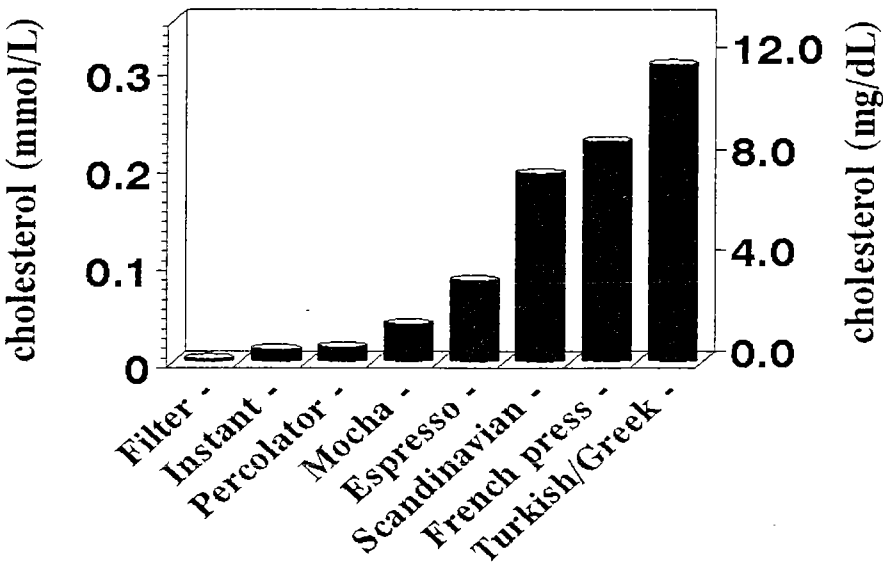

Figure 4 The estimated effect of daily consumption of five cups of coffee per day on serum levels of cholesterol (12). Estimations are based on the observation of Weusten-van der Wouw et al (10) that every 10 mg of cafestol consumed per day raises serum cholesterol by 5 mg/dL (0.13 mmol/L).

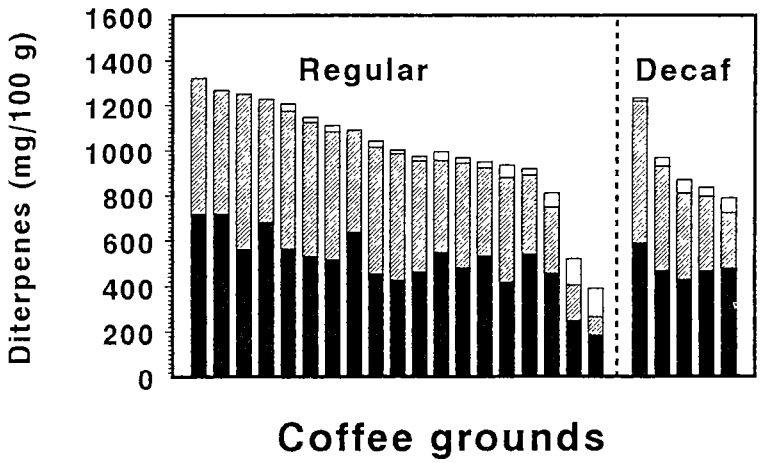

Figure 5 Levels of cafestol (■), kahweol (▨) and 16-O-methylcafestol (□) in commercial roast and ground coffees. Values are given in mg of free alcohols per 100 g of grounds. 'Regular' refers to caffeine-containing products, 'Decaf' refers to decaffeinated products (12).

Finally, we determined the diterpene content in various commercial roast and ground coffees (**figure 5**). We found that cafestol content varies little. Coffee grounds with lower levels of cafestol are blends from Arabica and Robusta beans, as is indicated by concurrent lower levels of kahweol and higher levels of 16-O-methylcafestol. Amounts of diterpenes in decaffeinated beans, or in coffee brews prepared from decaffeinated coffee beans, do not substantially differ from their caffeine-containing counterparts (12).

In conclusion, coffee beans and some types of coffee brew -- but not the regular type of coffee that has passed a paper filter -- contain compounds that in milligram quantities per day actively raise serum cholesterol levels, and that appear to affect liver function. Elucidation of the underlying mechanisms may produce new insights into the metabolism of lipoprotein metabolism. Our further investigations will therefore also concentrate on the mechanism of action of these powerful cholesterol-elevating substances.

SUMMARY

Suspicions regarding a possible link between coffee consumption and cardiovascular diseases go back a long way, but associations seen in epidemiological studies were often due to the fact that coffee drinkers smoke more. The effects of habitual coffee or caffeine intake on blood pressure are small. Caffeine also does not affect serum cholesterol levels. However, in the early 1980s Thelle and coworkers in Norway discovered that the unfiltered coffee as consumed in that country did raise cholesterol. It was later found that this effect disappeared if the coffee was filtered through a paper filter. In collaboration with the European coffee industry we have recently identified the substances responsible for this effect. They are the diterpenes cafestol and kahweol, which are present in small amounts in the fat in coffee beans. They are not removed by decaffeination. They are extracted into the brew in techniques where no filters are used such as Scandinavian boiled coffee, French press (Melior or cafetiere) coffee, Turkish/Greek coffee, and to a lesser extent also espresso and mocha coffee. Coffee diterpenes raise LDL cholesterol and triglycerides, and appear to slightly lower HDL cholesterol. The high intakes of boiled coffee in Scandinavia have probably contributed to the high rates of coronary heart disease in these countries. Not much is known about underlying mechanisms. Possibly, cafestol and kahweol exert their effects by affecting liver function: they increase serum activity of alanine aminotransferase and decrease that of γ-glutamyltransferase.

Patients at increased risk of heart disease who drink large amounts of coffee should be advised to select brews low in diterpenes. As for paper-filtered coffee, there is no compelling evidence for any type of adverse effect on the heart or blood vessels.

We would like to acknowledge coworkers and colleagues in the Netherlands and abroad for their attributions to this research, and the Netherland Heart Foundation.

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EFFECT OF FILTERED COFFEE INTAKE ON LIPID METABOLISM

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Introduction

Along with the advances in the scientific technology, many investigations have been done to clarify the mechanism of atherosclerosis. In the pathogenesis of atherosclerosis, low density lipoprotein (LDL) and high density lipoprotein (HDL) both play an important role. Modified LDL has a strong capacity to form atherosclerotic lesions, while HDL protects against the development of such lesions through the reverse cholesterol transport system.

Previous investigations have shown that coffee, especially boiled coffee, increases the serum cholesterol and LDL cholesterol levels[1-5]. On the other hand, some investigators have reported that consumption of filtered coffee increases both LDL and HDL cholesterol[6]. These conflicting findings suggest that the kind of coffee and the method of brewing may be important determinants of the influence on lipid metabolism.

In the present study, we tried to clarify the effect of filtered coffee on lipid metabolism and atherosclerosis by investigating the

serum lipid and apolipoprotein levels, the oxidative susceptibility of LDL, and the reverse cholesterol transport system.

Subjects and Methods

Subjects

The subjects were 64 healthy normolipidemic young men and women, who were students of our medical college. Before envollment, informed consent was obtained from all the subjects and their daily coffee or tea drinking habits were ascertained. They all normally consumed one to two cups of coffee or tea per day.

The subjects were randomly divided into five groups. Group A (8 men) consumed 5 glasses of water per day and group B (15 men) consumed 5 cups of coffee per day. Group C (16 women) consumed 5 cups of cups of coffee daily, while group D (16 men) and group E (11 women) both consumed 5 cups of tea daily.

Study Protocol

All subjects abstained from beverages containing caffeine for at least 4 weeks before the start of the study period. Then each group consumed water, coffee, or tea as specified above for 4 weeks. Otherwise, they maintained a normal daily routine.

Before the study and at weekly intervals during the study period, body weight was recorded and a venous blood sample was collected after an overnight fast.

Analytical Methods

Plasma lipoproteins were separated by preparative ultracentrifugation[6] before the study and at the end of the 4-week study period. Cholesteryl ester transfer protein (CETP) activity was determined by the method of Albers et al.[7]. The oxidative susceptibility of LDL was measured by the method of Esterbauer et al.[8]. Serum lipids, cholesterol, triglycerides, and

phospholipids were measured by enzymatic methods.
Apolipoproteins were detected by immunoturbidometry. The concentration of LpAI, which contains only ApoA-I, was determined by rocket immuno-electrophoresis.
The significance of differences was determined by Student's t-test and ANOVA.

Results

Serum lipid and apolipoprotein levels.

Changes of the serum cholesterol concentration during experimental period are presented in Table 1. The initial cholesterol level did not differ significantly among the 5 groups. A statistically significant increase was found at the end of the study in the women who consumed coffee, while a slight decrease of cholesterol was found in the men who drank tea. Changes of the HDL cholesterol level are also presented in Table 1. After the consumption of 5 cups of coffee daily, a marked increase of HDL cholesterol was found in the female subjects despite the lack of a significant change in the male subjects. Changes of apolipoprotein A-I are also presented in Table 1. As with HDL cholesterol, marked elevation of ApoA-I was only found in the female subjects who drank coffee. Despite the marked elevation of ApoA-I in female subjects, the elevation of ApoA-II with coffee consumption was minimal.

Oxidative susceptibility of LDL.

The composition of LDL lipids is presented in Table 2. LDL cholesterol levels were significantly decreased by coffee consumption in female subjects, but there were no significant changes of other LDL lipids.

Changes of the oxidative susceptibility of LDL, which is one of the key parameters in the development of premature atherosclerosis, are also shown in Table 2. We found no significant

changes after the consumption of water. However, in the male subjects, both coffee and tea consumption caused a marked reduction of the oxidative susceptibility of LDL. In the female subjects, the decrease was not statistically significant, probably due to the small number of women investigated.

Reverse cholesterol transport system.

The HDL2 cholesterol level is known to have a negative correlation with coronary heart disease. HDL2 cholesterol, triglyceride, and phospholipid levels were significantly increased by coffee intake in the in the female subject, but there was no significant change of HDL3 lipids.

CETP is a major component of the reverse cholesterol transport system and has an important influence on HDL cholesterol level. We found no significant changes of CETP in present study. LpAI is an important anti-atherogenic HDL, as reported previously. We found a marked elevation of LpAI with coffee intake in the female subjects, despite no significant change of LpAI/AII concentration.

Discussion

After the intake of coffee, female subjects showed a significant increase of LpAI, ApoA-I, HDL2, and HDL cholesterol, all of which are known to be anti-atherogenic lipoproteins. Althogh the mechanism of this increase of anti-atherogenic lipoproteins needs further investigation, the influence of female hormones may explain the sex difference in the response of HDL to coffee consumption. Female hormones, especially estradiol, have an anti-atherogenic effect. Administration of estradiol was found to increase the synthesis of ApoA-I in some experimental studies. Thus, combined action of female hormones and a substance contained in coffee may

influence the synthesis of ApoA-I and increase these anti-atherogenic lipoproteins.

On the other hand, we found a marked reduction of the oxidative susceptibility of LDL after the consumption of coffee and tea. Coffee and tea are known to contain some antioxidant substances and these may have reduced the oxidation of LDL.

In conclusion, moderate filtered coffee intake may have a potential protective effect on atherosclerosis by increasing of HDL-C and reducing the oxidative susceptibility of LDL.

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Abstract

In the pathogenesis of atherosclerosis, high density lipoprotein (HDL) plays an important protective role, while oxidized low density lipoprotein (LDL) promotes atherogenesis. To clarify the relationship of coffee intake to atherogenesis, we investigated the effect of filtered coffee intake on serum lipid levels (especially HDL-C) and the oxidative susceptibility of LDL in normolipidemic healthy young subjects. The subjects abstained from caffeine-containing beverage for four weeks, and were divided into three groups (a water group, a coffee group, and a tea group). The coffee group consumed five cups of coffee per day for 4 weeks, while the water group similarly consumed five glasses of water and the tea group consumed 5 cups of tea daily. There were no significant changes of serum lipid levels (total cholesterol, triglycerides, free cholesterol, and phospholipids) in the water and tea groups. In the coffee group, HDL-C levels did not change significantly in male subjects, but increased significantly in female subjects. In addition, the oxidative susceptibility of LDL was significantly reduced in the coffee and tea groups compared with the water group.

In conclusion, moderate filtered coffee intake may have a potential protective effect against atherosclerosis by increasing HDL-C and reducing the oxidative susceptibility of LDL.

KEY WORDS: filtered coffee/ HDL/ oxidative susceptibility of LDL/ LpAI/ CETP

Table 1 Changes of lipid and apolipoprotein concentrations

mg/dl		water	coffee		tea	
		men	men	women	men	women
TC	0w	198 ± 30	171 ± 24	177 ± 29	180 ± 25	162 ± 44
	1w	193 ± 24	164 ± 25	178 ± 27	180 ± 24	158 ± 37
	2w	181 ± 30	162 ± 29	184 ± 29	173 ± 26	164 ± 38
	3w	179 ± 25	169 ± 29	181 ± 27	173 ± 23	160 ± 39
	4w	183 ± 25	167 ± 30	187 ± 30*	173 ± 24	166 ± 42
HDL-C	0w	69 ± 16	60 ± 14	72 ± 19	64 ± 13	59 ± 14
	1w	64 ± 13	57 ± 10	73 ± 27	65 ± 13	62 ± 12
	2w	68 ± 15	57 ± 12	77 ± 15	63 ± 14	65 ± 14*
	3w	67 ± 16	59 ± 13	81 ± 16**	63 ± 13	63 ± 13
	4w	70 ± 17	59 ± 11	84 ± 17**	64 ± 12	64 ± 12
ApoA-I	0w	166 ± 17	145 ± 21	153 ± 20	150 ± 24	139 ± 26
	1w	155 ± 13	131 ± 15*	152 ± 18	143 ± 21	140 ± 20
	2w	161 ± 13	137 ± 22	165 ± 15**	146 ± 20	140 ± 28
	3w	159 ± 15	140 ± 22	162 ± 18*	143 ± 22	142 ± 24
	4w	168 ± 27	141 ± 20	170 ± 19**	145 ± 20	149 ± 29*
ApoA-II	0w	40 ± 4	35 ± 5	31 ± 4	37 ± 6	30 ± 5
	1w	39 ± 3	32 ± 4	30 ± 3	35 ± 5	29 ± 5
	2w	38 ± 3	33 ± 6	32 ± 4	35 ± 5	30 ± 5*
	3w	37 ± 3	34 ± 6	31 ± 3	35 ± 5	29 ± 5
	4w	40 ± 5	34 ± 6	33 ± 5**	36 ± 6	30 ± 6

* $p < 0.05$, ** $p < 0.01$ (mean ± SD)

Table 2 Changes of the oxidative susceptibility of LDL

		water	coffee		tea	
		men	men	women	men	women
LDL-C	0w	95 ± 35	81 ± 21	84 ± 25	86 ± 18	90 ± 34
(mg/dl)	4w	85 ± 28	81 ± 25	73 ± 29*	81 ± 23	90 ± 30
LDL-TG	0w	25 ± 9	18 ± 6	27 ± 10	18 ± 5	25 ± 14
(mg/dl)	4w	26 ± 12	17 ± 5	21 ± 7	16 ± 2	23 ± 8
LDL-PL	0w	73 ± 21	71 ± 13	67 ± 18	75 ± 17	59 ± 25
(mg/dl)	4w	67 ± 18	71 ± 22	58 ± 19	69 ± 21	57 ± 22
Lag Time	0w	61 ± 7	56 ± 11	54 ± 6	54 ± 11	52 ± 4
(min)	4w	64 ± 6	63 ± 8**	57 ± 5	62 ± 11**	56 ± 6
Propagation	0w	12 ± 2	13 ± 2	13 ± 2	13 ± 2	10 ± 2
(/min)	4w	12 ± 2	15 ± 2	13 ± 2	14 ± 2	11 ± 3

* $p < 0.05$, ** $p < 0.01$ (mean ± SD)

Table 3 Changes of the reverse cholesterol transport system

		water	coffee		tea	
		men	men	women	men	women
HDL2-C	0w	38 ± 16	36 ± 16	41 ± 18	38 ± 13	34 ± 15
(mg/dl)	4w	37 ± 16	38 ± 15	49 ± 18**	43 ± 11	37 ± 12
HDL2-TG	0w	7 ± 2	7 ± 3	9 ± 3	7 ± 4	6 ± 7
(mg/dl)	4w	8 ± 4	8 ± 7	12 ± 3**	6 ± 3	5 ± 6
HDL2-PL	0w	75 ± 26	78 ± 31	81 ± 33	84 ± 29	91 ± 29
(mg/dl)	4w	79 ± 34	84 ± 33	97 ± 34**	91 ± 19	63 ± 19
HDL3-C	0w	20 ± 4	20 ± 5	16 ± 3	19 ± 7	21 ± 6
(mg/dl)	4w	21 ± 3	18 ± 4	16 ± 5*	19 ± 4	22 ± 3
HDL3-TG	0w	7 ± 2	7 ± 3	6 ± 2	5 ± 3	5 ± 3
(mg/dl)	4w	7 ± 2	6 ± 3	7 ± 2	5 ± 2	6 ± 4
HDL3-PL	0w	74 ± 8	81 ± 14	62 ± 8	81 ± 15	55 ± 10
(mg/dl)	4w	79 ± 8	74 ± 12	64 ± 7	75 ± 11	60 ± 10
LpAI	0w	63 ± 16	71 ± 26	58 ± 17	59 ± 18	58 ± 16
(mg/dl)	4w	67 ± 27	62 ± 19	77 ± 16**	60 ± 7	63 ± 11
LpAI/AII	0w	96 ± 10	79 ± 16	96 ± 11	94 ± 19	94 ± 27
(mg/dl)	4w	99 ± 16	80 ± 13	95 ± 15	81 ± 14	98 ± 28
CETP	0w	26 ± 3	22 ± 5	27 ± 5	24 ± 5	26 ± 6
(%)	4w	23 ± 9	22 ± 5	28 ± 6	24 ± 5	24 ± 5

* $p < 0.05$, ** $p < 0.01$ (mean ± SD)

CHEMOPROTECTIVE EFFECTS OF COFFEE AND ITS COMPONENTS CAFESTOL AND KAHWEOL : EFFECTS ON XENOBIOTIC METABOLISING ENZYMES

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Introduction

The literature on coffee and human cancer is complicated by an association between tobacco usage and coffee consumption (1). Nevertheless there is increasing evidence to suggest that ingestion of coffee may offer some protection against colorectal cancer (2-7). For example in a review in 1991, the International Agency for Research on Cancer (IARC) reported that 11 out of 12 epidemiological studies have indicated an inverse relationship between coffee consumption and the risk from colorectal cancer and it was concluded that "the collective evidence is compatible with a protective effect" (8). Several epidemiology studies performed since 1991 have provided additional evidence to indicate that coffee can significantly lower the risk for colorectal cancer. For example, a recent study performed in Sweden, a country with high coffee consumption, clearly indicated a dose-response pattern of findings compatible with a protective effect of coffee against colon cancer (7). Interestingly in this study, tea appeared to have no effect on colon cancer suggesting that the effect is not related to caffeine consumption per se. The data concerning the inverse relationship between coffee consumption and the incidence of colorectal cancer is not entirely consistent. This may be due to several reasons such as confounding effects of diet or other lifestyle factors, or the method of brewing. The latter parameter has not been critically investigated.

Animal studies have provided additional support for the chemoprotective effects of coffee and have led to the identification of some of the components that may be responsible for these effects. For example, chronic exposure of rodents to instant coffee resulted in a decreased incidence of spontaneous tumours which could not be solely accounted for by a calorific restriction effect of caffeine (9,10). Several studies have demonstrated that green coffee inhibits the development of 7,12-dimethylbenz[a]anthracene (DMBA)-induced carcinogenesis in different experimental animal cancer models (11,12). Subsequent investigations led to the identification of the lipidic diterpenes cafestol and kahweol as two potentially chemoprotective agents in green coffee beans (13,14). Roasted coffee beans were also found to protect against DMBA-induced cancer (15). Although cafestol and kahweol may have contributed to this protective effect, there was evidence to suggest that chemoprotective agents formed during roasting play a more important role. A further indication that cafestol and kahweol cannot account for all of the protective effects of coffee on colorectal cancer is that these compounds are extracted to different extents during brewing (16). While certain coffees such as Scandinavian-type boiled coffee contain relatively large amounts of these components, some of the more commonly consumed brews such as filtered coffee and instant coffee contain very little cafestol and kahweol. Thus it is probable that the putative anti-cancer effects of coffee are due to a combination of different coffee components.

The mechanisms responsible for the chemoprotective effects of coffee or of its diterpene constituents have not been elucidated although two hypotheses have been put forward. Firstly, it has been postulated that coffee might inhibit the excretion of bile acids (17,18) which are believed to be promoters of colon cancer (19). There is some circumstantial evidence to suggest that cafestol and kahweol may act through this mechanism. These components have been found to increase serum cholesterol levels in man (20) and experimental animals (21), a potential consequence of decreased bile secretion. Furthermore we have recently obtained experimental evidence to demonstrate that this effect on cholesterol is preceded by an alteration in biliary clearance (A. Huggett - personal communication). An alternative hypothesis is that coffee constituents act as blocking agents by producing a co-ordinated enhancement of multiple enzymes which detoxify carcinogens (22). This latter hypothesis is supported by the finding that cafestol and kahweol produce a marked enhancement of glutathione S-transferase (GST) activity in the liver and small bowel in experimental animals (13,23). This family of enzymes, which are involved in the detoxification of many xenobiotics including putative dietary carcinogens such as the heterocyclic amines, are considered to be an important target for chemoprotective dietary agents.

The purpose of the present study was to investigate which particular glutathione S-transferase isoforms are the targets for cafestol and kahweol and to determine whether these coffee components may also have blocking effects on carcinogen activation via alternative mechanisms such as a modulation of cytochrome P450 activity, thus directing carcinogen metabolism along non-genotoxic pathways (Figure 1). For this initial study the effects of cafestol and kahweol on the activity of these enzymes in liver was examined since this organ is the major site of carcinogen metabolism.

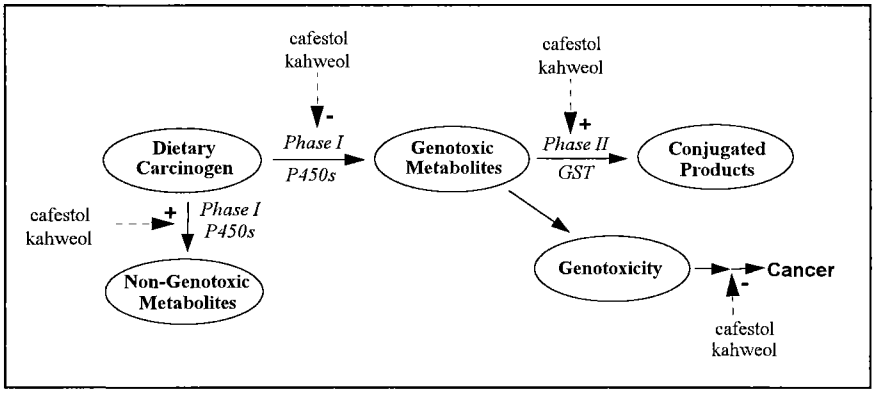

Figure 1. Potential mechanisms responsible for the chemoprotective effects of cafestol and kahweol.

Materials and Methods

Male Sprague-Dawley rats (3 weeks of age) were obtained from Iffa Credo S.A. (L'Arbresle, France). The animals were housed individually and fed certified laboratory chow (Nafag 890, Nähr und Futtermittel A.G, Gossau, Switzerland) as basal diet. Following 11 days on basal diet the animals were randomly allocated to five groups (A-E) comprising 10 animals each. A mixture of cafestol and kahweol palmitates (C+K) in a proportion of 52.5% and 47.5 % respectively was solubilized at different concentrations in a 50:50 mixture of corn and palm oils employed as a vehicle. Group A received basal diet containing 2.5% of the vehicle. Groups B-E received basal diet with 2.5% of the vehicle containing increasing amounts of C+K to give final concentrations of C+K in the diet of: B - 92 ppm; C - 420 ppm; D - 2300 ppm; E - 6200 ppm.

After 28 days on the test diets, which were provided ad libitum, the animals were fasted for 20 hours, anaesthetised and blood was collected from the abdominal aorta for analysis of plasma total cholesterol (24). The livers were excised and used as outlined in Figure 2 for RNA isolation, and for preparation of microsome and cytosolic fractions according to standard methodologies.

Northern analyses to examine the effect of C+K on the expression of messenger RNA (mRNA) for GST isoenzyme subunits and selected cytochrome P450s were performed as described previously using specific oligonucleotide probes for hybridisation (25,26). Enzymatic assays using "semi-specific" substrates were used to investigate the effect of C+K on the activity of GST isoenzymes in the liver cytosol fraction (27) and on the activity of microsomal cytochrome P450s (28,29).

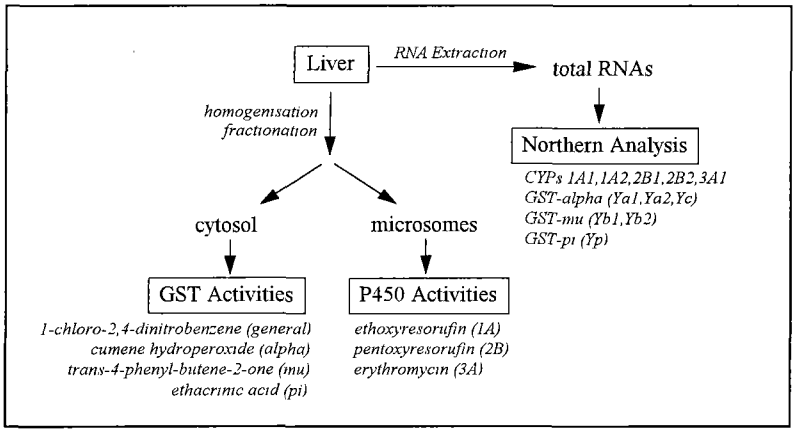

Figure 2. Analyses performed on liver samples from animals treated with cafestol and kahweol.

Results

The test diets produced no abnormal behaviour and the experimental animals remained in good health throughout the study. Cafestol and kahweol produced a dose-dependent increase in plasma total cholesterol levels (Figure 3) and this reached statistical significance ($P < 0.01$) for groups D and E compared to control animals (group A).

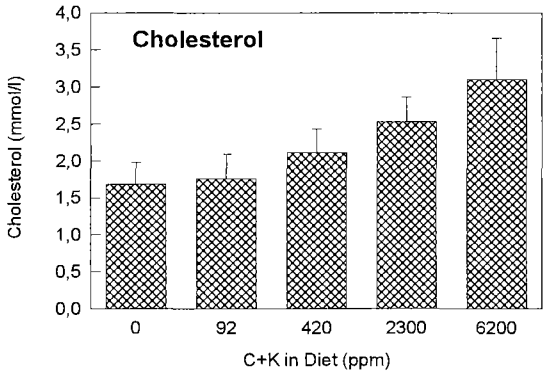

Figure 3. Effect of cafestol and kahweol palmitates (C+K) on plasma cholesterol levels in Sprague Dawley rats (mean±S.D.).

The total activity of liver glutathione S-transferases was measured using 1-chloro-2,4-dinitrobenzene as a substrate. Cafestol and kahweol produced a dose-dependent increase in activity as shown in Figure 4.

Northern analysis, performed to identify which particular glutathione S-transferase isoenzymes were induced by cafestol and kahweol, demonstrated differential effects on different isoenzymes and their particular subunits (Figure 6). The expression of mRNA for the Ya2 and Yc subunits of glutathione S-transferase alpha were increased by C+K while mRNA for the Ya1 subunit was unaffected. However these alterations in mRNA expression had no effect on the enzymatic activity of the cytosolic glutathione S-transferase alpha isoenzyme which was unaffected by C+K (data not shown). Similarly C+K produced an increase in the expression of mRNA for only the Yb1 subunit of the mu form of glutathione S-transferase while it had no effect on the mRNA expression of the Yb2 subunit, resulting in no overall effects on the enzymatic activity of this GST isoenzyme (data not shown). In the case of the pi form of glutathione-S-transferase, a large increase in both mRNA expression and enzymatic activity were observed at dietary concentrations of C+K of 2300 and 6200 ppm (Figure 5).

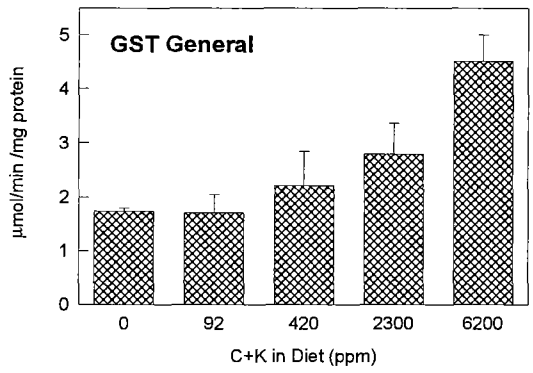

Figure 4. Effect of cafestol and kahweol palmitates (C+K) on total liver glutathione S-transferase activity in Sprague Dawley rats (mean±S.D.).

Northern analysis, performed to identify which particular glutathione S-transferase isoenzymes were induced by cafestol and kahweol, demonstrated differential effects on different isoenzymes and their particular subunits (Figure 6). The expression of mRNA for the Ya2 and Yc subunits of glutathione S-transferase alpha were increased by C+K while mRNA for the Ya1 subunit was unaffected. However these alterations in mRNA expression had no effect on the enzymatic activity of the cytosolic glutathione S-transferase alpha isoenzyme which was unaffected by C+K (data not shown). Similarly C+K produced an increase in the expression of mRNA for only the Yb1 subunit of the mu form of glutathione S-transferase while it had no effect on the mRNA expression of the Yb2 subunit, resulting in no overall effects on the enzymatic activity of this GST isoenzyme (data not shown). In the case of the pi form of glutathione-S-transferase, a large increase in both mRNA expression and enzymatic activity were observed at dietary concentrations of C+K of 2300 and 6200 ppm (Figure 5).

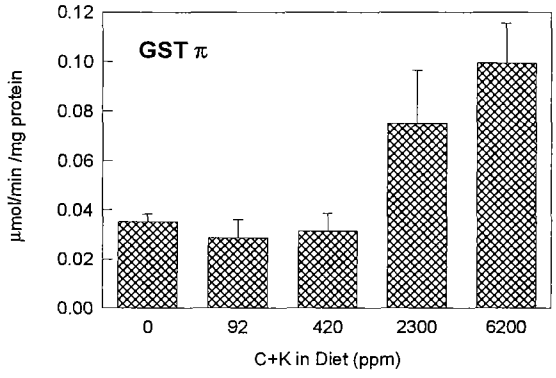

Figure 5. Effect of cafestol and kahweol palmitates (C+K) on liver glutathione S-transferase pi activity in Sprague Dawley rats (mean±S.D.).

Cafestol and kahweol also showed differential effects on the expression of mRNA for some specific cytochrome P450 isoenzymes. Although no effect was observed on the expression of CYP 1A1 (data not shown), C+K at 6200 ppm in the diet cause a marked increase in the expression of CYP 2B1, while an important reduction in the expression of mRNA for CYP 3A1 was observed at 2300 ppm C+K (Figure 5). These effects were confirmed at the enzymic level using semi-specific substrates (data not shown).

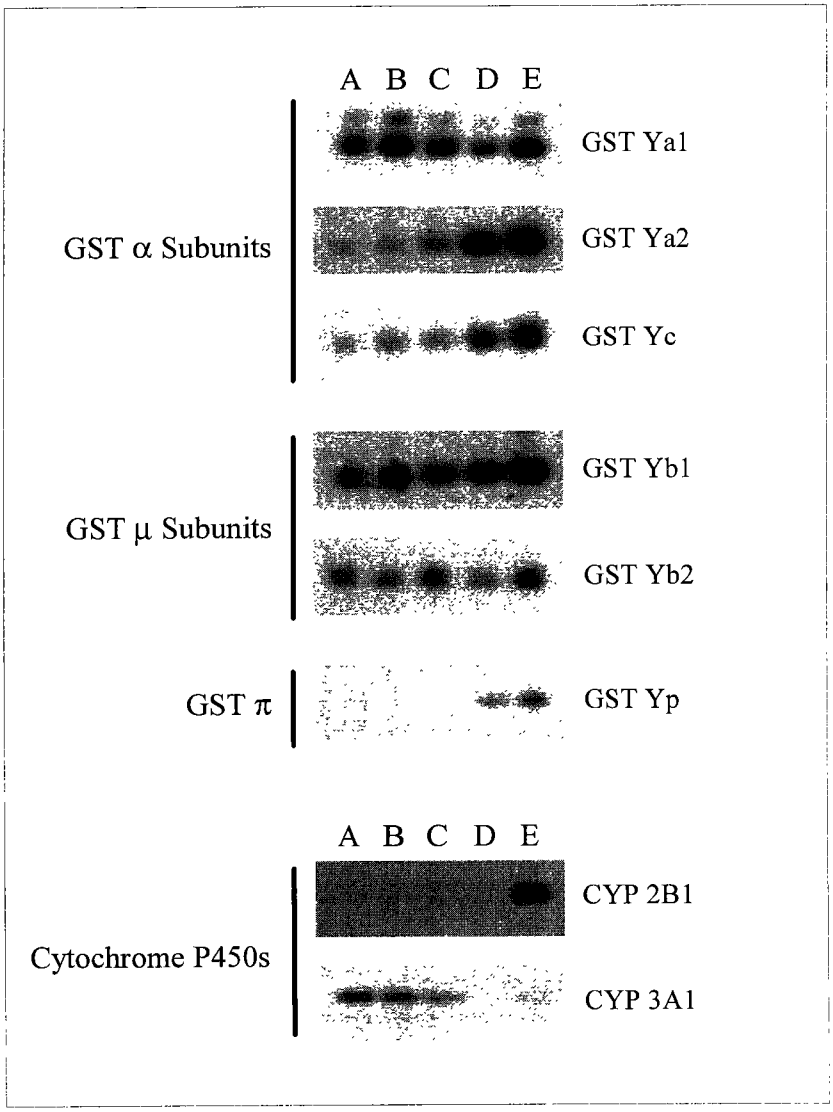

Figure 6. Effect of cafestol and kahweol palmitates on the expression of mRNAs for glutathione S-transferase subunits and cytochrome P450 isoenzymes.

Discussion

Increasing attention is currently being paid to the possibility that natural dietary constituents may provide a protective effect against human cancers. However, coffee, which contains over 1000 different individual natural components, has received little attention in this respect despite the fact that the human epidemiological evidence indicating a protective effect against colorectal cancer is relatively strong. One of the key mechanisms by which dietary agents are believed to protect against cancer is by the enhancement of host detoxification systems, thus preventing cancer causing agents from reaching or reacting with critical target sites. Since they represent a major detoxifying system for carcinogens, the glutathione S-transferases have been the subject of several studies aimed at demonstrating the potential protective effects of natural food constituents. As originally reported by Wattenberg and his co-workers (13), in the present study we have confirmed that cafestol and kahweol are inducers of the total activity of glutathione S-transferases. Interestingly the amount of the diterpenes required to produce a significant increase in this parameter was essentially the same as that which produced an effect on plasma cholesterol levels.

Glutathione S-transferases are a family of isoenzymes with different substrate specificities and regulatory controls. There have been very few studies performed to investigate which of the different glutathione S-transferases is the target for cafestol and kahweol. Di Simplicio et al. (23) reported that cafestol palmitate produced an induction of the activity of GSTμ in mouse liver. This is in contrast to the findings in our study in rats in which the activities of GSTα and GSTμ were essentially unaffected in animals fed a mixture of cafestol and kahweol palmitates. The reason for this discrepancy is unknown but may highlight a species selectivity in the response of GSTs to coffee diterpenes. Interestingly, despite a lack of effect at the enzymatic level, we found that the expression of messenger RNAs for certain of the subunits of GSTα and GSTμ were elevated in rats treated with C+K suggesting a differential regulation of the different subunits and also a limiting effect of particular subunits. The major specific effect of C+K was a marked dose-dependent increase in the activity of GSTπ. This was paralleled by an increase in the expression of mRNA for the Yp subunit. It should be noted that the effect of C+K on GSTπ was completely reversible (data not shown) and immunohistochemical studies demonstrated that no focal lesions were present livers of animals treated with C+K (I. Perrin - personal communication).

The present study is the first to address the possibility that cafestol and kahweol alter the expression of Phase I xenobiotic metabolising enzymes. Although C+K had no effect on the expression of messenger RNA for cytochrome P450 1A1, they produced a marked elevation in mRNA for cytochrome P450 2B1. However since this latter finding was only detected at the highest dose of C+K it may represent a secondary non-specific effect of the coffee diterpenes. In animals treated with C+K there was a dose-dependent decrease in messenger RNA for cytochrome P450 3A1. This effect was already noticeable at the threshold dose for effects on GST-π and cholesterol indicating it to be a primary effect. Interestingly the cytochrome P450 3A family is involved in steroid metabolism and is tempting to postulate that this induction may be related to the effects of C+K on cholesterol regulation.

The results we have obtained are consistent with a chemoprotective effect of cafestol and kahweol. However the mechanism for this effect may be more complicated than a simple induction of glutathione S-transferases. Cafestol and kahweol may also alter carcinogen activation by the modulation of Phase I xenobiotic metabolising enzymes, and their effects on cholesterol metabolism and biliary clearance leave open the possibility that they may act on later events during the multi-step process of cancer. Although our results indicate that the consumption of certain specialised coffee brews (boiled coffee, French Press coffee, Turkish coffee), which contain significant amounts of cafestol and kahweol, may confer a protective effect against dietary carcinogens via the induction of glutathione S-transferases, the likely negative health aspects of these coffee components related to their effects on cholesterol metabolism must also be considered (20). Therefore, at the present time cafestol and kahweol cannot be regarded as potential prophylactic dietary supplements.

There is increasing evidence to suggest that components formed during the coffee roasting process are in fact the major chemoprotective agents in the more commonly consumed brewed coffees. We have preliminary data to demonstrate that brewed roast and ground coffee also induces liver glutathione S-transferase activity in Sprague Dawley rats and that this effect is unrelated to cafestol and kahweol. Our current work is focused on the investigation of the effects of commonly consumed coffee brews on carcinogen detoxification systems in both hepatic and non-hepatic tissues including the colon, and the extension of these studies to the examination of human tissue and cell lines.

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Summary

Human epidemiology studies have demonstrated that consumption of moderate amounts of coffee results in a lower risk of colon cancer. In addition long-term feeding studies in animals have shown that coffee increases lifetime with a concomitant decrease in the incidence of spontaneous tumours. The mechanisms or components responsible for these chemoprotective effects of coffee are unknown. One of the potential targets for this beneficial activity is the inhibition of activation or stimulation of detoxification of carcinogenic components of the diet. We have investigated the effects of roast and ground coffee brew and also of the coffee-specific diterpenes cafestol and kahweol (C+K) on the activity of carcinogen metabolising enzymes (phase I and phase II xenobiotic metabolising enzymes) in rodents.

In the first instance our analyses focused on liver samples from rats fed a mixture of C+K. These components produced a dose-dependent increase in the activity of certain glutathione S-transferase isoenzymes in the liver. These enzymes are generally considered to be important carcinogen detoxifying enzymes. In addition C+K produced changes in the activity of several different specific P450 isoenzymes which are involved in carcinogen activation and detoxification. Roast and ground coffee produced some similar alterations in the activities of these phase I and phase II xenobiotic metabolising enzymes also consistent with a likely net detoxification of dietary carcinogens. These effects may be responsible for some of the chemoprotective activities of coffee.

Des études épidémiologiques humaines ont démontré qu'une consommation modérée de café était liée à un risque diminué de cancer du colon. Chez le rat, l ' incorporation de café dans la diète allonge la durée de vie des animaux tout en diminuant l'incidence des cancers spontanés. Les mécanismes d'action ainsi que les constituants responsables de cet effet chimioprotecteur sont inconnus. Une des cibles potentielles de cette action bénéfique est l'inhibition de l'activation ou la stimulation de la détoxication de substances cancérigènes présentes dans la nourriture. Nous avons étudié, chez le rongeur, les effets du café et de diterpènes spécifiques du café, le cafestol et le kahwéol (C+K), sur l' expression d'enzymes impliquées dans la biotransformation de substances cancérigènes.

Dans un premier temps, l'analyse s'est concentrée sur des échantillons de foie de rats nourris par une diète contenant un mélange de C+K. Ces composés ont produit une augmentation dose-dépendante de l' activité de certaines isoenzymes de la glutathion S-transférase. Ces enzymes sont généralement considérées comme étant d'importance dans la détoxication de carcinogènes. Il a également été observé que le C+K modifiait significativement l'activité de plusieurs cytochromes P450, enzymes impliquées dans les processus d' activation/détoxication de substances cancérigènes. Le café semble produire des effets similaires qui pourraient être responsables de son action chimioprotectrice.

SOME NOTES ON COFFEE, CAFFEINE AND HEALTH

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1 - Introduction

Coffee is a beverage containing hundreds of chemical species, the best known being caffeine, a mild stimulant. Because of its extensive use coffee is one of the most widely studied food components, although much remains to be learned about its effects which may be detrimental to health. Some recent knowledge on coffee has been summarized in a book (1).

2 - Notes on consumption and composition of coffee

It is hard to assess coffee's effects because coffee is not a single entity and is influenced by a number of factors. For instance the two commercial species Arabica and Robusta coffee have different composition; Arabica contains more lipids and trigonelline than Robusta (respectively 17 and 11% and 1.0 and 0.7% in medium-roasted coffee beans, dry weight) while Robusta contains more caffeine and chlorogenic acids (2.4 and 1.3% and 3.1 and 2.7% respectively). (2)

The composition of coffee beans changes considerably in relation to roasting at different temperatures. The roasting loss ranges between 13% in the northern countries to 20% in Mediterranean areas. The formation of polymeric brown pigments, known as melanoidins, may consequently differ qualitatively and quantitatively (2).

The brewing techniques too make up different beverages: boiled coffee, espresso, filter coffee, Greek/Turkish "mud" coffee, liquid coffee, Mocha, percolated coffee, soluble coffee and decaffeinated coffee are some of the most popular preparations. The amount of ground coffee, the size of the cup, the caffeine extraction

efficiency and therefore the amount of caffeine and fluid taken per day have been described (3), showing marked differences in the various countries. Europe is the continent with the largest consumption (4.6 kg of green coffee per person per year) followed by North and Central America (3.6 kg) while in Asia it is only 0.3 kg. However within Europe consumption varies widely: for instance in Finland coffee consumption is about five times more than in Spain.

Considering the composition of coffee, attention has focussed on the purine alkaloids, the best characterized component of coffee on account of the presence of caffeine (1,3,7 trimethylxanthine). Besides caffeine there are three dimethylxanthines: theophylline (1,3 dimethylxanthine) theobromine (3,7 dimethylxanthine) and paraxanthine (1,7 dimethylxanthine). These dimethylxanthines too are present in higher concentrations in Robusta than in Arabica coffee (2). Other caffeine analogs found in minor amounts in coffee are theacrine and linerine, but very little is known about their pharmacology.

Several 5-hydroxytryptamides (5-hydroxytryptamine esterified with several C20-C26 fatty acids) are part of the complex indicated as "irritating substances" because 5-hydroxytryptamine released in the intestine may exert an effect on the contraction and permeability of the digestive tract in sensitive people (2). The presence of several chlorogenic acids confer antioxidant and oxygen-scavenger activity on the drink, and may be responsible for some of its anticancer activity (4) as also discussed in this meeting (T. Tanaka and H. Mori; V.C. Nguyen et al.). Attentions is now tending towards the unsaporifiable lipid fraction of coffee. Among the diterpenes, cafestol and kahweol are typical of Robusta (5-7) and Arabica respectively (8-9). These diterpenes have hypercholesteremic effect (M.B. Katan, this meeting) which may explain the hyperlipemic effect observed with boiled but not filtered coffee (10-12). Cafestol and kahweol, in addition, show cancer- protective activity in animal experiments (13-14). These effects are probably related to an increase of some isoenzymes such glutathione transferase and cytochrome P450 which are involved in carcinogen activation and detoxification (A.C. Huggett et al., this meeting).

All these studies illustrate the importance of non-caffeine components in coffee although it may be premature to draw any conclusion before the quantitative aspects are worked out.

3 - Notes about caffeine

The kinetics and metabolism of caffeine are now well known in several animal species including man (15). Caffeine is sufficiently lipophilic to reach all tissues including the brain. Its effects, both central and peripheral, are reinforced by the formation of active metabolites such as the dimethylxanthines. Paraxanthine is the major metabolite in man (16-17) and theophylline in monkeys (18), while in rats the three dimethylxanthines are present in similar amounts (19-20).

Several factors affect the metabolism of caffeine: it is stimulated by smoking (21-23), high repeated intake of coffee (24-25), cruciferous vegetables (26), polybrominated biphenyls (27) and inhibited by the last trimester of pregnancy (28-29), liver diseases (30-33), exercise (34-35), aging (36-37), and obesity (38).

The rate of demethylation and oxidation of xanthines derived from caffeine differs depending on the animal species. In humans less than 6% are trimethyl derivatives while in rats these metabolites account for about 40% (15). Several uracil derivatives are formed from caffeine, paraxanthine and theobramine (39-40) and are excreted in urine, while an acetylated uracil derivative (AFMU) has been detected only in man (41). No pharmacological effects have been demonstrated for the uracil metabolites.

All the data on kinetics and metabolism of caffeine point to the difficulty of relating its disposition in man with that of any single animal species, making it difficult to extrapolate data from animal studies to man.

Though we now have a clear understanding of caffeine's metabolism today same cannot be said for its mode of action. A recent review by Daly (42) reports the pleiotropic interactions of coffee with norepinephrine, dopamine, serotonin, acetylcholine, GABA and glutamate systems in the brain. In addition caffeine blocks the two classes of adenosine-receptors which are linked to inhibition (A1) (43) and activation (A2) (44-45) of adenylate cyclase *via* G proteins. Another way to affect the level of cyclic AMP is blockade of phosphodiesterase, an effect observed with caffeine and other methylxanthines (46-47). However, all these effects occur at high doses, so they may be relevant for explaining the pharmacological activities of caffeine but are unlikely to play any role in the stimulant effect of coffee. Therefore much remains to be done to ascertain the mechanism by which coffee affects the CNS or other physiological functions.

4 - Coffee and cardiovascular epidemiology

It is impossible to summarize all the studies which have investigated the possible detrimental effects of coffee on the cardiovascular system. Epidemiological studies on coffee, being retrospective or prospective, are very difficult to interpret because of the many confounding factors which include smoking, alcohol and dietary habits. In addition, systematic bias may be introduced by the fact that cardiovascular patients have often been forbidden - particularly in the past - to drink coffee. Only a few studies will be mentioned in this review.

Heyden (48-49) reported a five year community-based trial, know as HDFP (Hypertension Detection and Follow-up Program) which recruited 10,064 hypertensive patients. This study could not find any difference in all-cause mortality when comparing non drinkers and three classes (low, medium, high) of coffee drinkers. The same applied when mortality was splith between cerebrovascular and all other cardiovascular-related mortality. There was a strong association between smoking and coffee and, as expected, smokers had significantly higher mortality than non-smokers.

Another investigation, known as the CARDIA study (50), recruited 5,115 young men and women to establish whether there is any association between caffeine-containing beverages and blood pressure or lipoproteins. The results have been inconsistent and no relation could be proved.

A much larger study was organised by Klatsky et al. (51) who followed 128,934 persons for ten years, with 4501 deaths. Except for a slightly increased risk of acute myocardial infarction among heavier coffee users (more than four cups a day), there was no increase in the risk of mortality for all deaths including those for hypertension, chronic coronary disease, cardiomyopathy, stroke, respiratory diseases, cirrhosis and suicides.

Sander Greenland (52) has reviewed eight case-control and 14 cohort studies. The results were considered "ambiguous" regarding the existence and the size of the coffee effect although the danger of drinking ten cups of coffee or more per day could not be ruled out concerning myocardial and coronary death. Different conclusions were reached by Myers and Basinski (53), who reviewed the same studies.

Franceschi (54), in a critical review, considered eight case-control and eleven prospective studies concerning coffee and myocardial infarction. She concluded that epidemiological studies cannot rule out a risk factor for heavy coffee drinkers although she recognizes the problem of confounding factors such as smoking.

A recent editorial in the New England Journal of Medicine (55) recommends that clinicians should not advise patients to change their life-style habits "unless the risk is large". Therefore, persons at high risk of myocardial infarction should consider limiting their coffee intake to fewer than four cups per day (56) until firmer results become available.

5 - Coffee and digestive cancer epidemiology

Another topic that has attracted much attention in recent year is the possible association between coffee and digestive cancers. La Vecchia has made extensive studies and published a comprehensive review (57). In this field once again it is essential to stress the difficulty arising from confounding factors, particularly smoking and alcohol. The data today seem to exclude any relation between coffee use and cancer of the liver, upper digestive tract and stomach. As regards colorectal cancer, nine case-control studies from different countries consistently report a risk below unity (0.5 - 0.8) for coffee drinkers in relation to non-drinkers (58-67). Other smaller studies showed no association (68-69) or an increased risk (70).

A biological explanation of the apparent protection of coffee against colorectal cancer may be based on the reduction of bile acid and neutral sterol secretion by substances present in coffee, considering (65) that bile acids are potent promoters of carcinogenesis in animals (71-72). An additional factor is the protective effect of cafestol and kahweol on the metabolism of cancerogenic agents, as well as the antioxidant properties of chlorogenic acids.

However it must also be considered that the current tendency to advise people with digestive diseases not to drink coffee may introduce a bias in its favour.

Controversial results have been obtained on the association between coffee consumption and pancreatic cancer. In 1981 a positive association was reported by MacMahon et al. (73) but this was not confirmed by 19 subsequent studies summarized by La Vecchia (57). The positive finding was probably related to the residual confounding with cigarette smoking, the single recognized risk factor for pancreatic cancer (74). Therefore the majority of studies today is largely reassuring about any strong association between coffee consumption and pancreatic cancer.

6 - Concluding remarks

These notes summarize the efforts of a large number of scientific institutions to detect any possible associations between coffee (and caffeine) consumption and different kinds of diseases. Taken together, the information available today does not refute the safety of coffee, particularly if it is drunk in moderation. In addition some potential advantages come to light on the basis of recent investigations concerning coffee components other than xanthines. It is in this area that future investigations should concentrate. The possible dangers of excessive coffee consumption must however continue to be explored, especially to detect subgroups of the population who might be particularly at risk.

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PREVENTION OF EXPERIMENTAL TUMORIGENESIS IN COLON, LIVER, AND ORAL CAVITY BY DIETARY CHLOROGENIC AND CAFFEIC ACIDS

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1- Introduction

Dietary factors play an important role in human health and in the development of certain chronic diseases including cancer (Doll and Peto, 1981). Recent research has focused on the presence of a number of non-nutrients that possess antimutagenic and anticarcinogenic properties. These are now frequently referred to as chemopreventive agents (chemopreventers). Currently, cancer chemoprevention (a strategy to block or reverse carcinogenesis processes before the development of invasive cancer) is frequently described as a new scientific field of oncology.

The frequent consumption of fresh fruit and vegetables is associated with a low cancer incidence (Block et al., 1992), although it is not known with certainty which components in fruit and vegetables contribute to inhibiting tumor development in humans. Almost all fresh fruit and vegetables contain rich amounts of naturally occurring phenolic compounds in addition to several vitamins. The mode of action of most chemopreventers are still unknown, although it appears that many of them are

antioxidants (Stavric, 1994; Tanaka, 1992; Tanaka, 1994; Tanaka et al., 1995), and as such, they may scavenge free radicals formed in the body. The phenolic acids chlorogenic acid (CGA) and caffeic acid (CA) present in coffee beans are hydroxycinnamic acids found in many fruits and vegetables in their free state and in a variety of derivatives (Sondheimer, 1958).

We present here our recent work on the suppressing effects of CGA and CA on chemically-induced colon, liver, and oral carcinogenesis initiated with methylazoxymethanol (MAM) acetate or 4-nitroquinoline 1-oxide (4-NQO). Also, the modulatory effects of two extracts (K-401 and K-409) from coffee beans on the development of aberrant crypt foci (ACF), which are known to be precursor lesions for colon cancer, were examined in rats treated with a colon carcinogen azoxymethane (AOM).

2- Materials, methods, and results

<Experiment I> Inhibitory Effects of CGA on MAM Acetate-induced Colon and Liver Carcinogenesis in Hamsters: Syrian golden hamsters of both sexes were divided in 4 experimental. Animals in groups 1 and 2 were initiated with a single i.v. injection of MAM acetate (20 mg/kg body weight) to induce colon neoplasms. Starting 1 week after the initiation, group 2 was fed the diet containing 250 ppm CGA for 23 weeks. Group 3 was fed the CGA diet alone for 24 weeks. Group 4 was fed the basal diet (CE-2) alone. At the end of the study (24 weeks), complete necropsy was performed on all animals. After fixation in 10% buffered formalin, all tissues including large intestine and liver were processed for histological diagnosis. Large intestinal tumors (adenoma and adenocarcinoma) developed in the cecum and the upper-third of the colon of hamsters in groups 1 and 2, while no such tumors were found in groups 3 and 4 (Tables 1 and 2). The combined incidence of adenoma and adenocarcinoma in males or both sexes in group 2 were significantly lower than that of group 1 ($P<0.05$). The incidence of adenocarcinomas in group 1 was 40% in each sex, while no adenocarcinomas were present in animals of group 2.

Table 1. Incidence of large intestinal tumors in hamsters fed diets with or without chlorogenic acid (CGA)

	Group 1	Group 2	Group 3	Group 4
Treatment	MAM acetate alone	MAM acetate + CGA	CGA alone	No treatment
Incidence of large intestinal tumors				
1) Adenoma + carcinoma				
♂ + ♀	10/20 (50%)	3/24 (13%)[a]	0/20 (0%)	0/30 (0%)
♂	6/10 (60%)	2/12 (17%)[b]	0/10 (0%)	0/15 (0%)
♀	4/10 (40%)	1/12 (8%)	0/10 (0%)	0/15 (0%)
2) Adenoma				
♂ + ♀	6/20 (30%)	3/24 (13%)	0/20 (0%)	0/30 (0%)
♂	4/10 (40%)	2/12 (17%)	0/10 (0%)	0/15 (0%)
♀	2/10 (20%)	1/12 (8%)	0/10 (0%)	0/15 (0%)
3) Carcinoma				
♂ + ♀	8/20 (40%)	0/24 (0%)[c]	0/20 (0%)	0/30 (0%)
♂	4/10 (40%)	0/12 (0%)[b]	0/10 (0%)	0/15 (0%)
♀	4/10 (40%)	0/12 (0%)[b]	0/10 (0%)	0/15 (0%)

[a-c]Significantly different from group 1 by Fisher's exact probability test ([a]$P<0.01$, [b]$P<0.05$, and [c]$P<0.001$).

Table 2. Incidences of liver cell foci and liver tumors in hamsters fed diets with or without chlorogenic acid (CGA)

	Group 1	Group 2	Group 3	Group 4
Treatment	MAM acetate alone	MAM acetate + CGA	CGA alone	No treatment
Liver cell adenoma				
♂	0/10 (0%)	0/12 (0%)	0/10 (0%)	0/15 (0%)
♀	1/10 (10%)	0/12 (0%)	0/10 (0%)	0/15 (0%)
Bile duct adenoma				
♂	2/10 (20%)	0/12 (0%)	0/10 (0%)	0/15 (0%)
♀	2/10 (20%)	3/12 (25%)	0/10 (0%)	0/15 (0%)
Hemangioma				
♂	1/10 (10%)	0/12 (0%)	0/10 (0%)	0/15 (0%)
♀	1/10 (10%)	0/12 (0%)	0/10 (0%)	0/15 (0%)
Liver cell foci (/cm²)				
♂ + ♀	10.8 ± 2.7[a]	6.0 ± 2.7[b]	0	0
♂	10.0 ± 3.8	5.3 ± 2.3[b]	0	0
♀	11.2 ± 4.7	6.4 ± 3.0[b]	0	0

[a]Mean ± SD.
[b]Significantly different from group 1 by Student's *t*-test ($P<0.01$).

As for liver cell foci that are precursor lesions for liver cell cancer, the density (/cm^2) of group 2 was significantly smaller than that of group 1 ($P<0.01$), as indicated in Table 2.

<Experiment II> Inhibitory Effects of CGA and CA on 4-NQO-induced Oral Carcinogenesis in Rats: Male F344 rats were divided into 6 groups and groups 1-3 were initiated with 4-NQO (20 ppm) in the drinking water for 5 weeks. Groups 2 and 3 were respectively fed the diets mixed with 250 ppm CGA and 500 ppm CA for 7 weeks of the initiation phase. Groups 4 and 5 were fed CGA and CA containing diets alone, respectively throughout the study. Group 6 was fed the basal diet alone. All animals were sacrificed 32 weeks after the start. After complete necropsy, all organs including tongue were processed for histology. Also, morphometric analysis of number and area of silver-stained nucleolar organizer regions (AgNORs) protein, which is cell proliferation biomarker, was done. The incidences of tongue neoplasms in groups 2 and 3 were significantly smaller than those of group 1 ($P<0.01$) (Table 3). No tongue carcinomas were found in groups 2 and 3.

Table 3. Incidence of tongue neoplasms in rats fed diets with or without chlorogenic acid (CGA) and caffeic acid (CA)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Treatment	4-NQO alone	4-NQO + CGA	4-NQO + CA	CGA alone	CA alone	No treatment
Body weight at sacrifice	370 ± 17[a]	360 ± 50	334 ± 38[b]	351 ± 45	333 ± 29	334 ± 18
Liver weight at sacrifice	14 ± 2	16 ± 3	13 ± 2	14 ± 2	12 ± 3	12 ± 1
Rats with tongue neoplasms	9/15 (60%)	2/15[c] (13%)	0/15[d] (0%)	0/8 (0%)	0/8 (0%)	0/12 (0%)
Rats with tongue papilloma	1/15 (7%)	1/15 (7%)	0/15 (0%)	0/8 (0%)	0/8 (0%)	0/12 (0%)
Rats with tongue carcinoma	9/15 (60%)	1/15[c] (7%)	0/15[d] (0%)	0/8 (0%)	0/8 (0%)	0/12 (0%)

[a]Mean ± SD.
[b]Significantly different from group 1 by Student's *t*-test ($P<0.01$).
[c, d]Significantly different from group 1 by Fisher's exact probability test ([c]$P<0.01$ and [d]$P<0.001$).

Similarly, the frequencies of preneoplastic tongue lesions (hyperplasia and dysplasia) in groups 2 and 3 were significantly lower than group 1 ($P<0.05$) (Table 4). Mean number of AgNORs/nucleus and average area of AgNORs/nucleus of the tongue

squamous epithelium in groups 2 and 3 were significantly smaller than those of group 1 ($P<0.05$) (Table 5).

Table 4. Incidence of tongue preneoplastic lesions in rats fed diets with or without chlorogenic acid (CGA) and caffeic acid (CA)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Treatment	4-NQO alone	4-NQO + CGA	4-NQO + CA	CGA alone	CA alone	No treatment
Hyperplasia	15/15 (100%)	9/15[a] (60%)	10/15[b] (67%)	0/8 (0%)	0/8 (0%)	0/12 (0%)
Dysplasia	14/15 (93%)	10/15 (67%)	11/15 (73%)	0/8 (0%)	0/8 (0%)	0/12 (0%)
Mild dysplasia	6/15 (40%)	2/15 (13%)	2/15 (13%)	0/8 (0%)	0/8 (0%)	0/12 (0%)
Moderate dysplasia	9/15 (60%)	3/15[b] (20%)	3/15[b] (20%)	0/8 (0%)	0/8 (0%)	0/12 (0%)
Severe dysplasia	14/15 (93%)	5/15[c] (33%)	6/15[a] (40%)	0/8 (0%)	0/8 (0%)	0/12 (0%)

[a-c]Significantly different from group 1 by Fisher's exact probability test ([a]$P<0.01$, [b]$P<0.05$, and [c]$P<0.001$).

Table 5. Morphometric analysis of AgNORs in the non-lesional tongue squamous epithellum

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Treatment	4-NQO alone	4-NQO + CGA	4-NQO + CA	CGA alone	CA alone	No treatment
Number of AgNORs/nucleus	2.5±1.0[a]	1.8±1.7[b]	1.7±1.4[b]	1.4±1.1	1.5±0.8	1.5±0.9
Total area of AgNORs (μm^2)	3.1±1.9	2.8±1.4[b]	2.7±1.0[b]	2.4±1.0	2.5±0.8	2.5±0.8

[a]Mean ± SD.
[b]Significantly different from group 1 by Student's t-test ($P<0.05$).

<Experiment III> Inhibitory Effects of Extracts from Coffee Beans on the Development of AOM-induced Colonic ACF in Rats: Male F344 rats were divided into 6 groups. Groups 1-3 were initiated with AOM by weekly subcutaneous injections

(15 mg/kg body weight) to induce ACF in the colon. Groups 2 and 3 were respectively fed the diets containing 1,000 ppm K-401 containing 36.9% CGA and 0.6% caffeine and 1,000 ppm K-409 21.9% CGA and 6.5% caffeine) for 5 week of the initiation phase. Groups 4 and 5 were given the test chemicals alone. Group 6 was served as an untreated control. The experiment was terminated 5 weeks after the start and the frequency of ACF was determined. Ornithine decarboxylase (ODC) activity in the colonic mucosa was also assayed. Feeding of both extracts significantly reduced the occurrence of ACF ($P<0.05$) (Table 6). ODC activities in rats treated with AOM together with K-401 or K-409 were smaller than that of rats given AOM alone.

Table 6. Incidence of aberrant crypt foci (ACF) and colonic ODC activity in rats treated with AOM and coffee beans exatracts K-401 and K-409

Group no.	Treatment	No. of rats examined	No. of ACF/colon	No. of aberrant crypts/focus	ODC activity (pmol $^{14}CO_2$/h/mg protein
1	AOM alone	8	102.2 ± 17.3	1.91 ± 0.16	99.7 ± 28.8
2	AOM + K-401*	8	78.0 ± 10.3[a]	1.76 ± 0.04[b]	76.0 ± 16.4
3	AOM + K-409*	8	85.0 ± 11.8[b]	1.84 ± 0.16	126.7 ± 50.3
4	K-401	4	0	0	75 6 ± 24 7
5	K-409	4	0	0	91 6 ± 16 4
6	No treatment	4	0	0	83 7 ± 23 7

*K-401 containing 36.9% chlorogenic acid and 0.6% caffeine and K-409 containing 21.9% chlorogenic acid and 6.5% caffeine were mixed with a basal diet at a concentration of 1,000 ppm.
[a,b]Significantly different from group 1 ([a]$P<0.01$ and [b]$P<0.05$).

3- Discussion

The results described here clearly indicate that dietary administration of CGA and CA inhibits chemically-induced large intestinal, liver, and oral carcinogenesis in rodents. Moreover, feeding of CGA and CA did not cause any toxicity under each experimental condition. The findings are in close agreement with epidemiological data showing a negative association between coffee drinking and certain cancers and several experimental observations (Stavric, 1992). Previously, the attention of the epidemiologic studies was focused on the possible association between coffee drinking and the development of cancer at different sites. However, a recent meeting of IARC (the International Agency for Research on Cancer) to evaluate the carcinogenic risk to humans of coffee, tea, caffeine and other methylxanthines to humans concluded that only for the urinary bladder was there some evidence that

coffee drinking may produce or possess certain carcinogenic risk for humans (IARC Working Group, 1991). There was no evidence that coffee may cause cancer to other organs. Furthermore, Rosenberg et al. suggested that heavy coffee consumption may reduce the risk of colon cancer (Rosenberg et al., 1989). The results in Experiment I and III might support their findings. CA esters, recently, were reported to suppress the occurrence of ACF (Rao et al., 1993). Also, coffee or caffeine inhibited pancreas, mammary gland, oral carcinogenesis (Stavric, 1992). As for other constituents of coffee beans, two Diterpenoids kahweol and cafestol present in coffee beans have antineoplastic activity (Tanaka, 1994; Tanaka, 1995).

The potential anticarcinogenenic mechanisms of phenolic compounds including CGA and CA when given during the initiation phase (Experiments II and III) involves (a) the induction of detoxification enzymes including glutathione *S*-transferase (Steinmetz and Potter, 1991); (b) the prevention of the formation of principle carcinogens from precursor compounds; and (c) the block of the reaction of carcinogens with critical cellular macromolecules in the target organ (Wattenberg, 1992). The inhibitory effects of feeding of CGA during postinitiation phase (Experiment I) might be due to alteration of cyclooxygenase and/or lipoxygenase activity in the target organs (Reddy et al., 1992; Tanaka et al., 1993b). Cell proliferation plays an important role in multistage carcinogenesis with multiple genetic changes (Cohen and Ellwin, 1990; Preston-Martin et al., 1990; Hunter and Pines, 1994; Hartwell and Kastan, 1994). Lowering of polyamine levels through the inhibition of their synthetic enzyme activities such as ODC might prevent the proliferative activity of neoplastic cells (Pegg, 1988; Tanaka et al., 1993a). Therefore, CGA and CA might also suppress oral carcinogenesis by lowering cell proliferation through alteration of arachidonic pathways.

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5- Summary

Possible inhibitory effects of CGA, CA, and coffee beans extracts on chemically-induced tumorigenesis were examined using colon, liver, and oral carcinogenesis models. dietary feeding of CGA and CA during the initiation or postinitiation phase of carcinogenesis effectively suppressed the occurrence of neoplasms and preneoplastic lesions of these organs. The findings may also suggest that such inhibition might be related to lowering the cell proliferation activity.

ON THE DESMUTAGENICITY MECHANISM OF COFFEE

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1-Introduction

Coffee is a popular beverage which is consumed daily world-wide. The safety of coffee concerning cancer was intensively investigated by many authors. Some dicarbonyl compounds such as glyoxal, diacetyl and methylglyoxal, were identified in coffee extracts (Kasai et al., 1982). Methylglyoxal was reported to be mutagenic towards *Salmonella typhimurium* TA 100 without S-9 mix. Diacetyl, glyoxal are mutagenic towards TA 100 with S-9 mix (Bjeldanes et al., 1979). Fung et al., (1988) reported that chlorogenic acid and caffeic acid were mutagenic in the mouse lymphoma assay but negative in the *Salmonella* assay. Hydrogen peroxide is also reported to be a major contributor to coffee mutagenicity and genotoxicity in vitro (Nagao et al., 1986; Rinkus et al., 1990). Recently, Kato et al. (1994) reported on the possible occurrence of new mutagens with the DNA breaking activity in coffee other than hydrogen peroxide. On the contrary, Yamaguchi et al., (1986) reported that coffee extracts inhibited the mutagenicity of various mutagen, especially heterocyclic amines, and they implied that the decrease of mutagenicity by coffee extracts was due to inhibiting the metabolic activation but S-9 (P-450). Obana et al., (1987) also reported that coffee suppressed the induction of SOS responses not only by activated Trp-P-1 but also by UV or AF-2. Thus, coffee shows both mutagenicity and desmutagenicity. Significantly, it is important to clarify that which one is the main specific activity of coffee.

The aim of this study is to clarify the desmutagenic mechanism of coffee against heterocyclic amine.

2- Materials and Methods

Materials. Coffee beans from Tanzania were kindly supplied by Atto Coffee Co., Ltd (Japan). The beans were roasted to 3 different degree: mild, medium and deep, then extracted (10g/100 ml) with distilled water,

methanol or diethylether by refluxing for 30 min. Then, the extracts were lyophilized to powder and were used as sample. Caffeine, Chlorogenic acid (Wako Pure Chemicals Co., Ltd) and Trigonelline (Tokyo Kasei Co., Ltd) were heated at 180℃ for 20 min, then were used as sample.

Preparation of High and Low molecular weight fraction from Coffee extract. Coffee extract was dialyzed against distilled water for 3 days through cellulose tubing (Visking), the nondialyzable and dialyzable fractions were lyophilized and used as high and low molecular weight fraction respectively.

Mutation assay. Mutagenicity was assayed by the preincubation method of Ames'test, using *Salmonella* TA 100 in the absence of S-9 mix. Desmutagenicity against Trp-P-1 was also assayed by the preincubation method, using *Salmonella* TA 98 in the presence of S-9 mix.

Preparation of activated Trp-P-1. Activated Trp-P-1 was prepared by the method of Arimoto et al.(1980). An aqueous solution (700 μ l) of Trp-P-1 (5 μ g) was incubated with 3.5 ml of S-9 mix (100 μ l of S-9) at 37 ℃ for 20 min with shaking. Then acetone was added, the mixture was stood on ice for 15 min, before being centrifuged at 3000 rpm for 10 min at 0℃. The precipitate containing activated Trp-P-1 was collected, acetone was eliminated by evaporation at room temperature under vacuo. Activated Trp-P-1 was dissolved in 1.5 ml of distilled water and used as sample.

Measurement of hydroxyl radical and superoxide anion. The scavenging activity of Coffee extract against hydroxyl radical or superoxide anion was carried out by the using of ESR (JEOL JES-RE1X ESR spectrometer X band). Hydroxyl radical was generated by Fenton reaction with the using of hypoxanthine (Sigma Chemical, 2 mM) 50 μ l, xanthine oxidase (Boehringer Mannheim, 0.4U/ml)750 μ l, Diethylenetriamine-N,N,N",N"'-pentaacetic acid (Wako Pure Chemical, 1mM) 50 μ l and DMPO (8.8 mM) 20 μ l used as trapping reagent, Coffee extract was also added at a volume of 100 μ l.

The operation conditions for the ESR spectrometer were as follows: microwave power, 8 mM; modulation frequency, 100 KHz ; modulation amplitude, 0.1 mT ; time constant, 0.1 sec ; scanning time, 2 min ; scanning field, 335.0 ± 5 mT ; microwave frequency, 9.41 GHz. A solution of 1.0 μ M of Tempol was used as a primary standard for the ESR spectra. The sample was measured in an aqueous quartz flat cell with an effective sample volume of 130 μ l.

3- Results and Discussion

Mutagenicity and Desmutagenicity of Coffee Extract.

Mutagenicity and desmutagenicity of extracts of Coffee beans heated at different conditions are shown in Table I. Mutagenicity assayed by *Salmonella typhimurium* TA 100 in the absence of S-9. Hitherto, some papers reported that Coffee extract showed mutagenicity towards *Salmonella* TA 100 without S-9 mix (Bjeldanes et al., 1979) or TA 98 with S-9 mix (Shibamoto et al., 1984). Although this mutagenicity was very weak. The concentration of Coffee used in these reports might be higher than that of this study. On the contrary, desmutagenicity of extracts from mild, medium and deeply heated Coffee was observed. The desmutagenic intensity increased with the higher degree of heating i. e., desmutagenic compounds generated by the heating process.

In order to find out compounds responsible for the desmutagenic activity, Coffee extract was dialyzed and divided into high and low molecular weight fraction. The results are shown in Table II. Low molecular weight

Table I . Mutagenicity and Desmutagenicity of Extracts of Coffee Beans Heated at Different Conditions

Sample		Mutagenicity		Desmutagenicity		
		sample mg/plate	TA-100 -S-9 Revertants	sample mg/plate	Trp-P-1 μg/plate	TA-98 +S-9 Revertants (inhibition %)
Water extract	Raw	4	68±5	2	0.1	801±24
	Mild	4	78±14	2	0.1	502±56(43)
	Medium	4	84±2	2	0.1	179±2 (79)*
	Deep	4	88±13	2	0.1	159±40 (82)*
control		0	61±3	0	0.1	887±51
Methanol Extract	Raw	4	58±6	1	0.075	378±32 (45)*
	Mild	4	30±0	1	0.075	161±20 (76)*
	Medium	4	52±7	1	0.075	131±4 (81)*
	Deep	4	49±4	1	0.075	74±13 (89)*
control		0	47±0	0	0.075	686±6
Diethyl ether Extract	Raw	4	75±8	2	0.1	620±85(34)***
	Mild	4	81±6	2	0.1	471±37(49)**
	Deep	4	78±5	2	0.1	220±16 (76)***
control		0	73±8	0	0.1	941±135

Raw,mild,medium or deeply heated coffee beans were extracted with water,methanol or diethylether. Mutagenicity and Desmutagenicity were measured by Ames'test using Salmonella typhimurium TA-100 or TA-98.

*p<0.001 statistically significant against control.
**p<0.01 statistically significant against control.
***p<0.1 statistically significant against control.

fraction as well as high molecular weight fraction showed strong desmutagenicity against Trp-P-1. Low molecular weight fraction decreased with higher degree of heating, while high molecular weight fraction increased with the higher degree of heating. High molecular weight product of amino carbonyl reaction, the so called Melanoidins were reported to have strong desmutagenicity against Trp-P-1 (Lee et al., 1994), these melanoidins may exist in high molecular weight fraction of Coffee extracts. Since Coffee contains a large amount of amino compounds and carbonyls. Besides, the yield of low molecular weight fraction is much higher than that of high molecular weight fraction (Table Ⅲ). Therefore, it is estimated that low molecular weight fraction takes a main role in the desmutagenicity of Coffee.

Low molecular weight products of Coffee extract contain characteristic components such as caffeine, chlorogenic acid and trigonelline. These compounds were heated at 180 ℃ for 20 min, then their mutagenicity and desmutagenicity were measured by Ames' test. The results are shown in Table IV. No mutagenicity was observed, on the contrary, desmutagenicity against Trp-P-1 was observed for caffeine, chlorogenic acid and trigonelline. The desmutagenicity of caffeine was very weak, but that of chlorogenic acid and trigonelline showed strong desmutagenic activity, and this activity increased when chlorogenic acid and trigonelline were heated, especially trigonelline i. e., some desmutagenic compounds were generated by the heating process. In this study, the desmutagenicity of some generated compounds were measured, 3-ethylpyridine, 4-ethylpyridine, 4,4-dipyridyl showed strong desmutagenic activity against Trp-P-1.

Table Ⅱ. Mutagenicity and Desmutagenicity of High Molecular Weight and Low Molecular Weight Fraction of Coffee Extract

Sample (water extract)		Mutagenicity sample mg/plate	Mutagenicity TA-100 -S-9 Revertants	Desmutagenicity sample mg/plate	Desmutagenicity Trp-P-1 μg/plate	Desmutagenicity TA-98 +S-9 Revertants (inhibition %)
Raw	High	4	38±4	2	0.075	627±46
	Low	4	42±2	2	0.075	279±32(44)**
Deep	High	4	46±1	2	0.075	79±12 (84)*
	Low	4	49±16	2	0.075	66±22 (87)*
control		0	35±3	0	0.075	502±58

Coffee extract was dialyzed against water at 4℃ for 48h in cellophane tube.
Mutagenicity and Desmutagenicity was measured by Ames'test using Salmonella typhimurium TA-100 or TA-98.
*p<0.001 statistically significant against control.
**p<0.01 statistically significant against control.

Table Ⅲ. High Molecular Weight Fraction and Low Molecular Weight Fraction obtained by the Dialysis of 1g of Coffee Extract

Sample		High molecular (%)	Low molecular (%)
Coffee	Raw	169.7 (18.3)	756.4 (81.7)
	Mild	210.3 (23.0)	703.3 (77.0)
	Medium	233.1 (26.5)	646.5 (73.5)
	Deep	298.3 (30.0)	693.0 (70.0)

(unit : mg)

Table Ⅳ. Mutagenicity and Desmutagenicity of Raw and Heated Ingredients of Coffee Beans

Sample		Mutagenicity sample mg/plate	Mutagenicity TA-100 -S-9 Revertants	Desmutagenicity TA-98 +S-9 Revertants (inhibition %)
Raw	Caffeine	2	59±11	868±40(16.7)*
	Chlorogenic acid	2	67±7	542±9((47.9)**
	Chlorogenic acid	4	66±1	461±29(55.7)**
	Trigonelline	2	76±4	1192±127
	Trigonelline	4	102±8	1033±128
Control		0	70±4	1041±55
Heated	Caffeine	2	67±7	683±50(25.6)
	Chlorogenic acid	1	65±1	93±61(89.2)***
	Trigonelline	2	71±12	375±41(56.3)**
	Trigonelline	4	96±7	156±11(81.8)**
Control		0	70±4	858±65

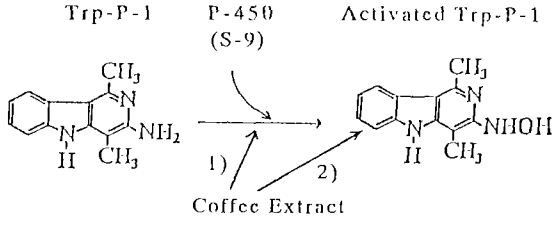
Coffee ingredients were heated at 180℃ for 20 min.
Mutagenicity and Desmutagenicity were measured by Ames'test using Salmonella typhimurium TA-100 or TA-98.
*p<0.05 statistically significant against control.
**p<0.01 statistically significant against control.
***p<0.001 statistically significant against control.

Desmutagenic Mechanism of Coffee Extract.

Coffee extracts were reported to exhibit desmutagenic activity against Trp-P-1, however its mechanism was not know. In this study, we investigated wether the desmutagenic activity of Coffee extract against Trp-P-1 was due to the inhibitory effect of Coffee extract on S-9 mix or on activated Trp-P-1 or both (Fig. 1). Table V shows the enzymatic activity of S-9 treated with or without Coffee extract by measuring the mutagenicity of Trp-P-1. The results show that there is no difference in the enzymatic activity between S-9, before and after Coffee extract treatment i.e., Coffee extract did not inhibit the enzymatic activity of S-9.

The inhibitory of Coffee extract against activated Trp-P-1 was also investigated (Table VI). Coffee extract strongly inhibited the mutagenicity of activated Trp-P-1, and this inhibition is dose dependent. From these results, it can be concluded that Coffee extract inhibits the mutagenicity of activated Trp-P-1, but not S-9, to exhibit its desmutagenicity.

Besides, the scavenging activity of Coffee extracts against hydrogen peroxide, hydroxyl radical and superoxide anion was investigated by the using of ESR. At a concentration of 40 ppm, Coffee extract completely scavenged 88mM hydrogen peroxide. At a concentration of 250 ppm, Coffee extract scavenged 90% of generated hydroxyl radical. Moreover, at a level of 700 ppm, Coffee extract scavenged 90% of supeoxide anion.



Possibility : 1) Coffee extract blocks the activity of P-450
2) Coffee extract blocks the acivity of Activated Trp-P-1

Fig.1. Speculated Desmutagenic Mechanism of Coffee Extract against Trp-P-1

Table V. Effect of Coffee Extract against P-450 (S-9) Activity

Sample (water Extract)	Trp-P-1 mg/plate	TA-98 +S-9 Revertants (inhibition %)
S-9 + coffee	1	1120±118
S-9 control	1	1131±165

S-9 (100 μ l) and deeply heated coffee extract(10mg)was incubated at 37 ℃ for 1h in 0.25M phosphate buffer (pH7.4 ,2ml).

Table VI. Effect of Coffee Extract against Activated Trp-P-1 at various concentration

Sample	Desmutagenicity		
	sample mg/plate	Trp-P-1 μg/plate	TA-98 +S-9 Revertants (inhibition %)
water extract	2	0.1	60(91%)
control	0	0.1	667
water extract	2	0.15	113(86%)
control	0	0.15	813
water extract	2	0.2	223(78%)
control	0	0.2	1012

Heated coffee beans were extracted by water.
Activated Trp-P-1 was prepared by the incubation with S-9 mix at 37℃ for 1h.

4- Summary

Desmutagenic mechanism of Coffee extract against Trp-P-1 was investigated by the using of *Salmonella typhimurium* TA 98 under the presence of S-9 mix, then the followings were made clear: 1) Low molecular weight fraction of Coffee extract showed strong desmutagenicity. 2) Desmutagenicity of roasted chlorogenic acid and trigonelline was very strong, while that of caffeine was very weak. 3) Some degradation products of trigonelline such as 4-ethylpyridine, dipyridyl showed strong desmutagenicity. 4) Coffee extract reacted with activated Trp-P-1 rather than S-9 to exhibit its desmutagenicity. 5) Coffee extract showed strong scavenging activity against hydroxyl radical and superoxide anion.

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COFFEE, AN OVERVIEW FROM THE SITE OF ASIAN TRADITIONAL MEDICINE

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INTRODUCTION

The coffee intake in Asian countries has widely increased in recent years due to the westernization of the life style although tea has been the traditional beverage for most of those nations since ancient times. According to the legendary, coffee was formerly taken as food or medicine instead of the present use as beverage. This seems to be associated with the thought of Asian traditional medicine that considers a common origin for food and medicine. The three great traditional medicines are Chinese, *Ayurveda* (Indian Medicine) and *Unani* (Arabic Medicine). These systems are practiced to present date and have developed throughout the Asian countries before the introduction of Western medicine. They have in common the holistic fundamental thought, *i.e.*, the human anatomy and physiology are intimately bound to other physical systems. This reports a bibliographic survey carried out in order to investigate the position of coffee in Asian traditional system of medicine. On the other hand, some tests on the biological activities of coffee extracts were performed with the aim to find a relationship between coffee intake and health, by measuring their effects on myocardial beating, scavenging active oxygens and suppressing hepatitis B virus surface antigen.

I. Medicinal uses of coffee in Asia

Japan - Coffee was introduced in Japan for the first time in the end of 18th century or at the beginning of 19th century, in the late era of *Yedo*, through overseas trading with Netherlands. One of the earliest reports on the experiences of coffee intake were done by Shokusanjin Ota (大田蜀山人), a writer and the first Japanese to drink coffee, and scholars as C. P. Thunberg and Siebold. During this period various western culture flowed in Japan and coffee was one of the subjects introduced through translations of Dutch textbooks. Physicians who were in charge of the translations of such references have described the pharmacological effects of coffee in "*Komo Honzou*(紅毛本草)" (1783) by Yamamoto (Fig. 1), and by Kai Hirokawa(廣川 獬) in "*Kosei Shimpen* (厚生新編)" (1811) and "*Nagasaki Bunkenroku* (長崎聞見録)" (1795) (Fig. 2). The explanations of coffee were influenced in part by the knowledge of the physicians on Chinese traditional medicine. The effectiveness of coffee

under the view of *Kampo* medicine, which had developed in Japan (originally from China), can be briefly interpreted as follows, taking the observations of Kai Hirokawa as an example:

Fig. 1. Description of the Pharmacological Effect of Coffee in "*Komo Honzo* (紅毛本草)," (Translated from "*Huishoudelijk Woordenboek*" in Japan. 1783)

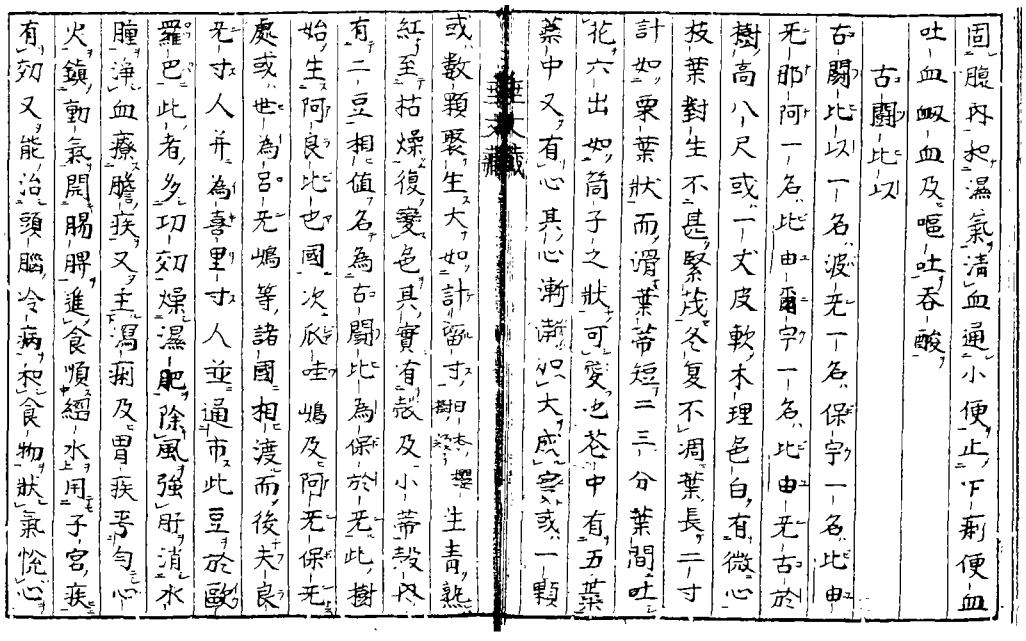
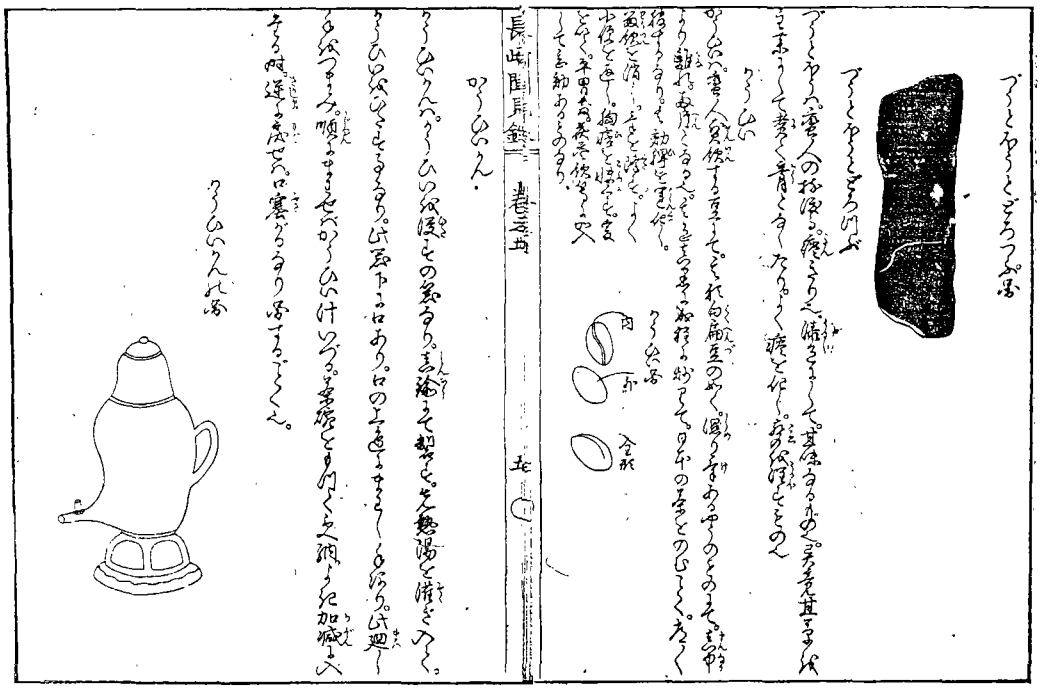
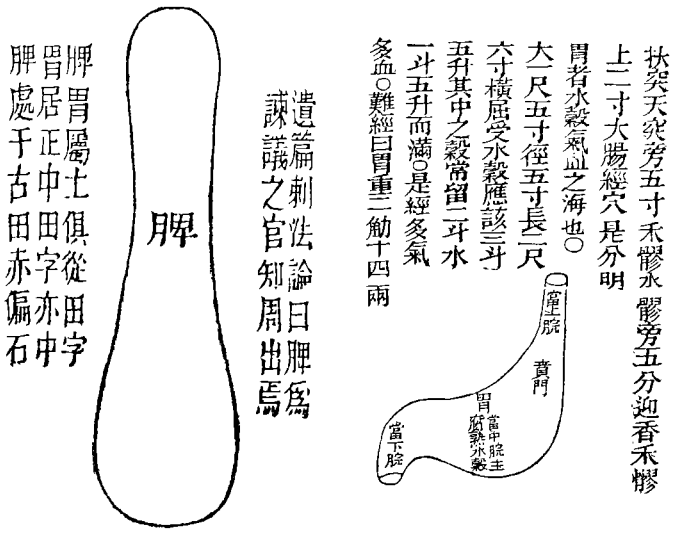


Fig. 2. Description of the Pharmacological Effect of Coffee in "*Nagasaki bunkenroku* (長崎聞見録)," ("The Report of Experience in Nagasaki," 1795)



1. Tonifies spleen (spleen(脾; *hi*) is the organ associated to stomach(胃; *i*). It helps the digestion and distribute the energy throughout the body).(Fig. 3)

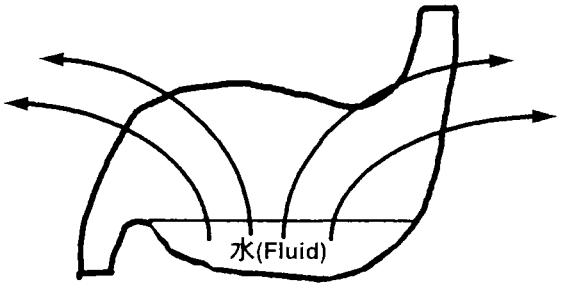
Fig. 3 The Spleen "脾" and the Stomach "胃" in Kampo Medicine



2. Removes stasis of body fluids in the epigastric region (eliminates the harmful effects of accumulated fluid in the body and does not mean exactly the diuretic action); (= Concept of "Removal of *Inai-teisui* [胃内停水].") (Fig. 4)

Fig. 4 Concept of Removal stasis of body fluids in the epigastric region

溜飲を消す（胃内停水を去る）



3. Regulates the excess of "Ki (Qi; 気[the elemental energy])" flow.
4. Enhances production of urine（利尿作用）.
5. Effective if added to "Hei-i-san(平胃散)" or "Bukuryo-in(茯苓飲)" (*Kampo* prescriptions having the above properties).

China - A renowned and great traditional system of medicine was developed in China. This had already established its maturity at the time coffee was taken to Europe, therefore the use of coffee as one of the herbal drug was not promptly incorporated in China. Moreover, the tea intake had been established in the nation with a tradition over a thousand years. A bibliographical survey was carried out to seek for any evidence of the use of

coffee in Chinese traditional medicine. However the only description of coffee in the pharmaceutical field was found in "Xinhua bencao gangyao(新华本草纲要)," where coffee is described on its morphology, natural features and pharmacological effects, properties attributed mostly to caffeine. On the other hand, people of minor tribe, called "Uygur," in northwest China has been used coffee under the influence of Islam and it is described in "Pharmacography of Uygur(维吾尔药志)."(Fig. 5)

Fig. 5. "*Pharmacography of Uygur* (维吾尔药志)."

71. 咖 啡

(قەھۋە) كافى 咖 啡

Semen Caffeae

【植物来源】 茜草科（Rubiaceae）咖啡属多种植物。其中以阿拉伯咖啡（Coffea arabica L.）分布最广。本植物为灌木或小乔木，高5～8米，基部通常多分枝，老枝灰白色，节膨大，幼枝无毛，呈压扁状。叶薄革质，卵状披针形或披针形，长6～14厘米，宽3.5～5厘米，顶端渐尖，基部楔形或略钝，边缘或呈浅波状，两面无毛，下面脉腋内有小孔或无、中脉在叶两面均凸起，侧脉每边7～13条，叶柄长8～15毫米，托叶阔三角形，长3～6毫米。聚伞花序数个簇生于叶腋，每1花序通常有花2～5朵，总花梗无或极短，花芳香，花梗长0.5～1.5毫米，苞片4枚，异形，2枚阔三角形，另2披针形，萼管状，长2.5～3毫米，顶端5齿裂，花冠白色，一般长10～18毫米，顶部通常5裂，雄化5，药伸出冠外，子房2室，花柱长12～14毫米，柱头2裂。浆果成熟时阔椭圆形，红色，长12～16毫米，直径10～12毫米，外果皮硬膜质，中果皮肉质，有甜味，种子2枚，长8～10毫米，直径5～7毫米。花期3～4月。（图71—1）

原产埃塞俄比亚和阿拉伯半岛，现广植于全世界热带地区。我国海南岛有栽培。

【药材性状】 除去种皮的咖啡，呈椭圆形或卵形，长8～10毫米，宽5～7毫米，中间部厚为3～4毫米，背面隆起，腹面平坦，有稍弯曲的纵沟及纸样种皮痕迹，呈黄色至暗棕色。生品无芳香，炒后而具芳香气味。

【功能主治】 开窍，利尿，健胃，止泻。用于精神不振，小便不利，腹泻、痢疾和食欲不佳等症。

【附　　注】 咖啡有时可混入伪品，伪者有以淀粉豆壳粉混合用模型冲压后，烘烤而成；亦测得粉末咖啡，通常用豆类或橡子的种仁掺伪，需加注意，通常以显微鉴别。前者有淀粉粒，后者可观察到豆类的栅状细胞。

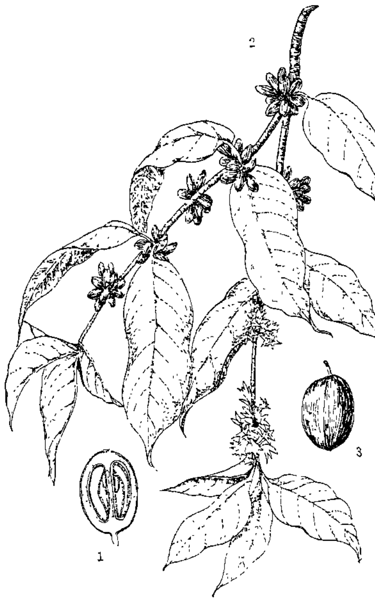


图71—1 阿拉伯咖啡 Coffea arabica L.
1.果纵剖面 2.花果枝 3.果

India and Sri Lanka - The traditional medicine practiced in these countries is mainly ***Ayurveda***. Coffee seems to have given few influences in this traditional medicine, since it is described briefly in the modern literature only. Here, like in China, tea has been the predominant beverage.

Arabic Countries - A Greek medicine developed among Islamic people to a system of medicine called ***Unani***. This is practiced today in Pakistan and few other South Asian countries. It is known that the use and the name of coffee started in the sphere of Islamic culture. The use of coffee as a medicine has been confirmed in an investigation on the crude drug market of Turkey and Syria by Baser *et al.*[1] and Honda *et al.*[2]. In Yemen[3], a decoction of green beans and dried fruits has been used more often then roasted beans.

II. The biological activities of the coffee extract

Materials and Methods

1. Reagents and media

Eagle's minimal essential medium (Eagle's MEM) and Dulbecco's modified minimal essential medium (Dulbecco's MEM) were purchased from Nissui Laboratories (Tokyo, Japan); fetal calf serum (FCS) from ICN Biomedicals Japan Co., Ltd. (Osaka, Japan); xanthine oxidase (XOD, from butter milk), caffeine, xanthine and nitroblue tetrazolium (NBT) from Wako Pure Chemicals (Osaka, Japan); caffeic acid and chlorogenic acid from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

2. Analytical apparatus and chromatography

UV spectrometer model UV-2200 (Shimadzu Corporation, Kyoto, Japan). The analytical conditions of HPLC for caffeine were as follows: column, Develosil-ODS-T-5 (Nomura Chemicals Co., Ltd.); eluent, $CH_3CN:H_2O$

(65:35); flow rate, 0.5 ml/min; detector UV (270 nm). The same condition was applied to the analysis of chlorogenic acid and caffeic acid except for the eluent that was CH_3OH:H_2O:H_3PO_4 (40:60:0.05) and detector UV (325 nm).

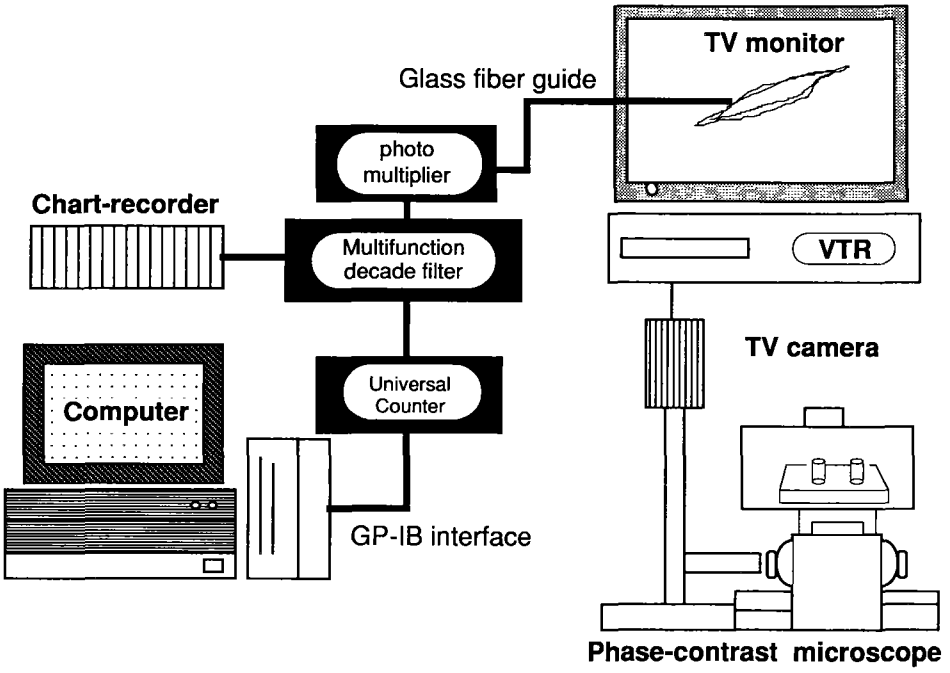
3. Extraction and quantitative analysis

One hundred grams each of green beans and roasted beans of coffee from Brazil, Colombia, Ethiopia, Indonesia (Sumatra Mandhelin), and Tanzania were extracted with hot water using a home coffee maker. The water extract was partitioned with AcOEt to remove fatty acids and other water-insoluble substances. The remaining water layer was freeze-dried to give coffee extracts that were used for the quantitative analysis of chemical constituents and in the experiments for biological activity.

4. Effect on the spontaneous myocardial cell beating

Preparation of myocardial cell sheets: Myocardial cell culture was prepared by a modified method of Goshima[4]. Briefly, cells were isolated from hearts of 14-16 day mice embryos and seeded (2-5 x 10^6 cells/ml) onto a glass cover strip coated with fibronectin in a 35 mm plastic Petri dish containing Eagle's MEM. It was incubated at 37°C under an atmosphere of 5% CO_2 for 48h. The cell-attached glass strip was mounted upside down on a small chamber in a stainless plate (1mm thickness). The chamber was filled with medium and the cell beating was analyzed by an instrument described by Namba *et al*[5].(Fig. 6)

Fig. 6. Block diagram of apparatus



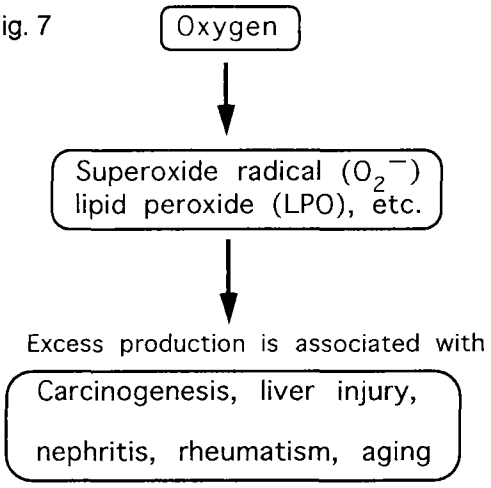
Myocardial cell beating analysis: The spontaneous beating of the cell sheet was observed under a phase-contrasted microscope at 37°C. The control cell beating was measured and the effects of the coffee extracts were assessed by exchanging the medium to the same medium supplemented with coffee extracts. The same point of a cell sheet was observed in the course of a measurement.

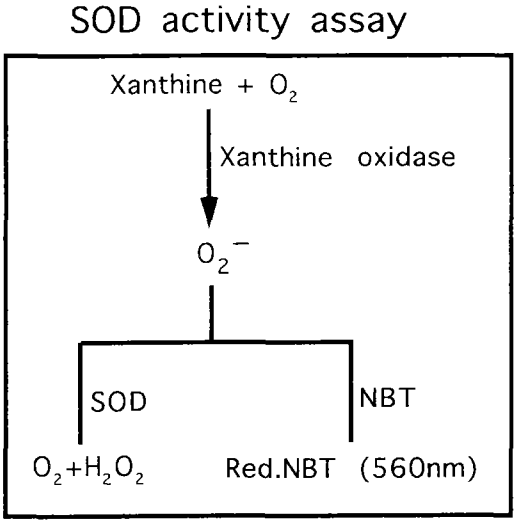
5. Free radical scavenging effect (Fig. 7)

Determination of SOD-like activity : The production of superoxide anions in xanthine-XOD system was calculated following the methods of Imanari *et al.*[6]. Briefly, in a reaction mixture containing xanthine, NBT, and the test sample, XOD was added and the absorbance of reduced NBT was measured at 560 nm.

Lipid peroxidation assay: The reaction mixture (1.0ml) for lipid peroxidation was composed of 0.2 mM ascorbate, 0.01 mM $FeSO_4$, various concentrations of coffee extracts and the microsomal suspension (2 mg protein/ml). After incubation at 37°C for 20 min, lipid peroxide in the reaction mixture was measured by the method of Ohkawa *et al.*[7].

Fig. 7

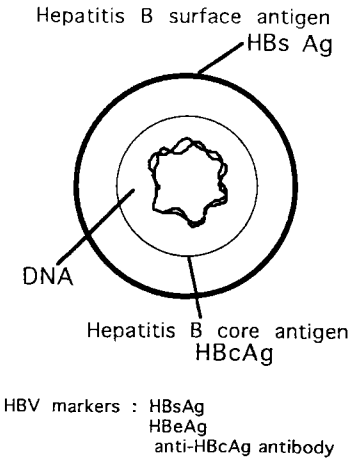


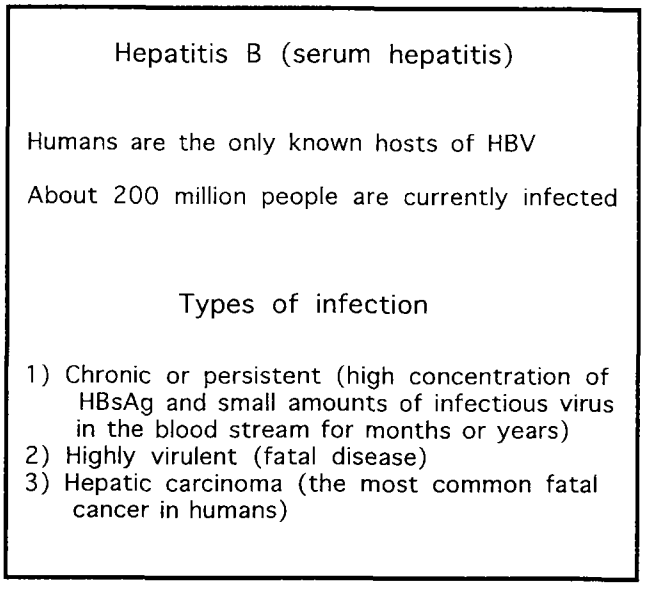


6. Anti-HBV activity (Fig. 8)

Cell culture: Human hepatoma cell line (PLC/PRF/5) (Macnab *et al.*[8]) were grown in Dulbecco's MEM supplemented with 10% heat-inactivated FCS, 2 mmol of glutamine, 0.1mg streptomycin and 50 units of penicillin/ml. The tests were carried out in 24-well plastic tissue culture plates, seeded with $2x10^4$ cells per well and incubated at 37°C under 5% CO_2.

Fig. 8 Schematic diagram of hepatitis B virus (HBV)





Tests for the antiviral effects: For the assessment of antiviral effect the coffee extracts were added to the confluent cell (7-day culture) at the concentrations of 0.5, 1.0 and 2.0 mg/ml. A pretreatment of 2 h was followed by a culture of 24 h. The 24 h culture supernatant was harvested for the quantitation of HBsAg by the method of ELISA, using a monoclonal antibody to HBsAg (MBL, Nagoya, Japan). The cytotoxicity was monitored by the trypan blue exclusion method.

Results and Discussion

I. Quantitative analysis of the constituents of coffee extracts:

The extraction of coffee beans with hot water yielded 11.2 - 21.2 % of extract (w/w) for green or roasted coffee, without any notable differences in samples from various origins and intensity of roast. Caffeine content ranged from 0.5 to 0.9 % (w/w) in green and roasted coffee beans while chlorogenic acid content varied according to the roast. Generally its concentration was highest (2.5-3.22%) in green coffee decreasing to 0.7 - 1.4% in light roasted and about 0.1% in dark roasted coffee. Caffeic acid content was very low compared to above two constituents.

II. Effects on the spontaneous cell beating

The cultured myocardial cells from the early stages of embryonic hearts are known to have spontaneous moving activity and in monolayer sheets they have a pacemaking mechanism similar to intact hearts. Since they are free from neutral and systemic influences, direct calcium antagonistic effects can be observed in this system. In this experiment, the spontaneous beating rate of myocardial cell was slightly increased in the presence of the extract of coffee from Colombia, however this was not observed in any other sample. Caffeine, caffeic acid or chlorogenic acid did not have significant effect in this experimental conditions. This result shows that the effects of coffee in cardiac system is probably a consequence of stimulation of central nervous system. (Fig. 9, 10)

Fig. 9 Effect of the coffee extracts on myocardial cell beating rate

	Relative beating rate (%)		
	5min	10 min	20 min
Control	102.3 ± 0.9	101.5 ± 1.0	101.5 ± 1.4
Green beans (0.1mg/ml)	100.9 ± 1.1	103.1 ± 1.6	102.9 ± 2.9
Light roast beans (0.1mg/ml)	105.6 ± 1.9	108.5 ± 2.9	102.9 ± 2.9
Dark roast beans (0.1mg/ml)	104.9 ± 4.5	107.8 ± 6.0	109.1 ± 6.1
Norepinephrine (0.01mM)	122.0 ± 2.2*	124.2 ± 1.6*	123.4 ± 0.8*
Caffeine (0.01mM)	98.4 ± 6.6	95.2 ± 2.9	107.4 ± 2.9

Values are expressed as mean ± S.E. of four independent experiments, significanly different from control, *$p<0.001$. The origin of coffee beans was Brazil.

Fig. 10 Effect of the coffee extracts on myocardial cell beating amplitude

	Relative beating amplitude (%)		
	5min	10 min	20 min
Control	103.0 ± 2.4	102.5 ± 2.7	102.6 ± 2.4
Green beans (0.1mg/ml)	101.2 ± 2.1	101.1 ± 3.6	102.1 ± 4.3
Light roast beans (0.1mg/ml)	99.7 ± 2.5	98.5 ± 3.2	98.1 ± 4.0
Dark roast beans (0.1mg/ml)	102.2 ± 3.5	101.4 ± 3.4	100.9 ± 5.3

Values are expressed as mean ± S.E. of four independent experiments.
The origin of coffee beans was Brazil.

III. Free radical scavenging effect

The excessive production of active oxygen is associated with several affections such as cancer, rheumatism, nephritis and process of aging. With the aim of studying the medical properties of coffee in preventing such diseases or precocious aging, the coffee extracts were tested *in vitro* for active oxygen scavenging effects. The 50% active oxygen scavenging concentration (IC_{50}) ranged from 2.4 to 10 μg/ml. In general green coffee showed to be stronger than the roasted beans in scavenging superoxide anions. The IC_{50} of caffeic acid, a known antioxidant, was 1.0 μg/ml and of chlorogenic acid was 7.8 μg/ml. A similar pattern was observed for lipid peroxidation, but the values of IC_{50} ranged from 15 to 32 μg/ml. (Fig. 11-12)

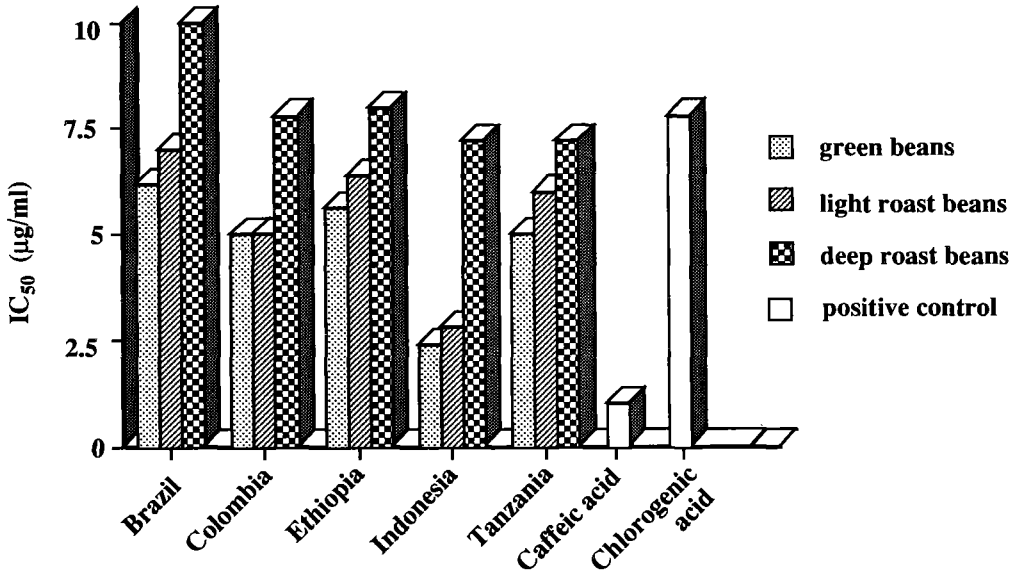


Fig. 11. The 50% inhibitory concentration (IC_{50}) of coffee extracts on the formation of superoxide anion in the reaction of xanthine/xanthine oxidase.

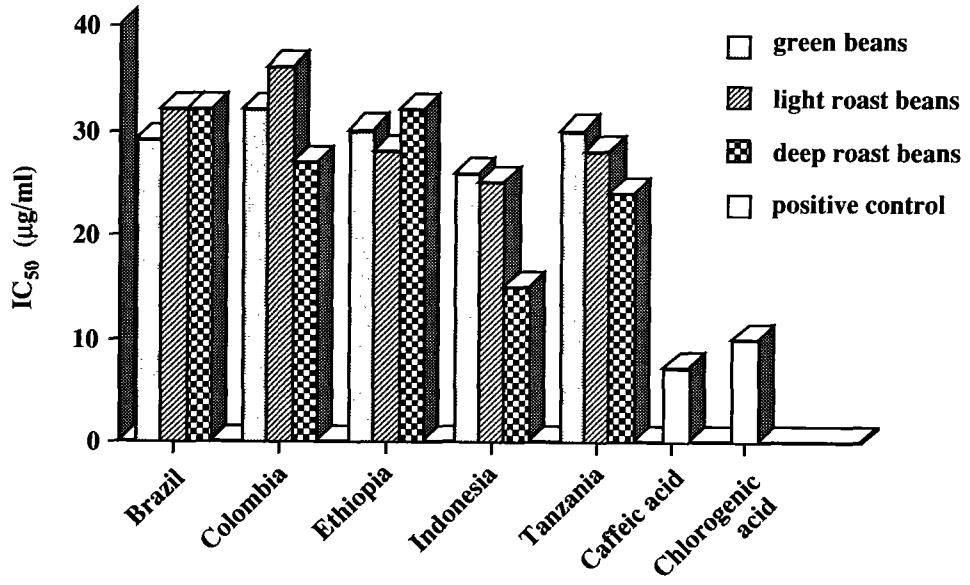


Fig. 12. The 50% inhibitory concentration of coffee extracts on lipid peroxidation in rat liver microsomes

IV. Anti-HBV activity

Since no great differences were observed on the chemical constituents and above biological activity tests among coffee of different origins, coffee from Brazil was randomly selected to test for the anti viral activity against hepatitis B virus (HBV).

Green coffee suppressed the secretion of surface antigen of HBV (HBsAg) in the cell culture medium by 30% while light and dark roasted coffee extracts suppressed by 50% at the concentration of 0.5 mg/ml. As shown in Fig. 15, green beans extracts gave strongest suppressive effects in a dose dependent manner if compared with other samples. Cytotoxicity was not significant in any of the samples tested. Two constituents of coffee extracts, chlorogenic acid and caffeic acid showed a dose dependent suppression (100% suppression at 0.5 mg/ml) while caffeine had no influence on HBsAg secretion. (Fig. 13)

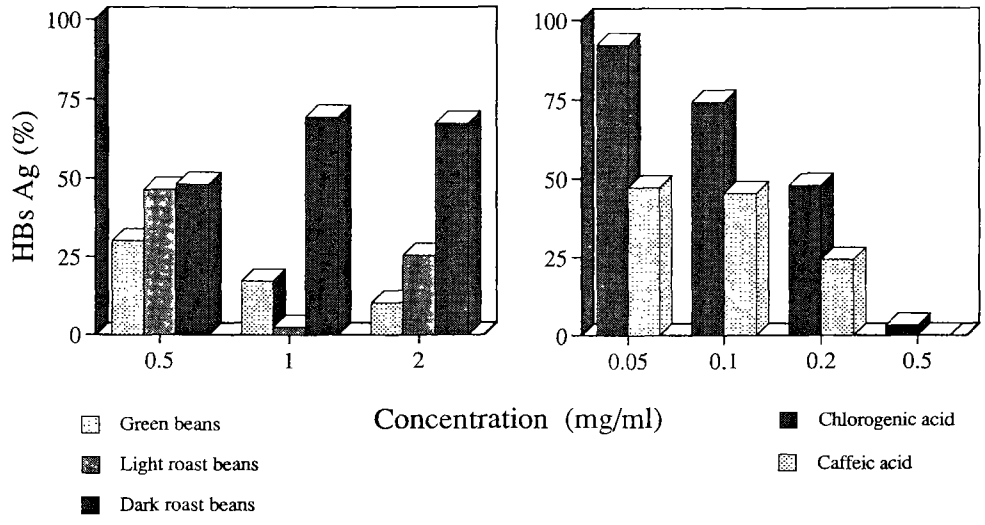


Fig. 13 Effects of coffee extracts, chlorogenic acid and caffeic acid on the secretion of hepatitis B surface antigen (HBsAg)

Conclusion

From the literature survey on Chinese and Arabic traditional system of medicine, the uses of coffee were based on its diuretic and central nervous system stimulant properties, attributed in general to caffeine. On the various biological tests the water extract of coffee did not show any notable effect on myocardial cell beating, however, showed superoxide anion scavenging effects, inhibitory activity of lipid peroxidation and suppression of HBsAg. These biological activities are closely related to the presence of caffeic acid derivatives, specially chlorogenic acid. These findings suggest that coffee has properties to prevent deleterious actions of free radicals and viral infections besides its stimulant effect.

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Summary

The medicinal properties of coffee such as diuretic and stimulant, due to the effect of caffeine, have been known since ancient times and nowadays it has been a popular beverage around the world. This study dealt with the uses of coffee in traditional medicines of Asian countries and some biological activities related to aging, infectious diseases and cardioprotective effects.

Materials and Methods **:** The water extracts of coffee from different sources: Colombia, Brazil, Ethiopia, Indonesia, and Tanzania were tested for the biological activities in the following methods. a) Inhibitory effects on the lipid peroxidation; b) suppression of hepatitis B virus surface antigen (HBsAg); c) effects on the cultured myocardial cells.

Results **:** From the literature survey on Chinese and Arabic traditional systems of medicine, the uses of coffee were based on its diuretic and central nervous system stimulant properties, attributed in general to caffeine. On the various biological tests the water extract of coffee showed inhibitory activities for the lipid peroxidation and for the formation of HBsAg. A slight increase of beating rate on the myocardial cells was observed in the presence of the extract of coffee from Colombia, however this was not observed in any other sample. Caffeine, caffeic acid or chlorogenic acid did not have significant effect in this experimental conditions. This result shows that the effects of coffee in cardiac system is probably a consequence of stimulation of central nervous system.

ON THE SCAVENGING ACTIVITY OF COFFEE EXTRACT AGAINST REACTIVE OXYGEN SPECIES

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1-Introduction

Reactive oxygen species, such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen have been suggested as major causes of cancer, heart disease and aging. These oxygen species appear to be toxic in vitro and vivo because they initiate the chain reaction of lipid peroxidation.

Thus, the control of these reactive oxygen species in vivo is an important problem. Recently, relationship between coffee and reactive oxygen species was elucidated. Hydrogen peroxide was reported to be generated from coffee by various authors (Nagao et al., 1986; Rinkus et al., 1990; Stadler et al., 1994). However, Shi et al., (1991) reported that caffeine scavenged hydrogen radical. Thus, it is necessary to make clear the relationship between coffee extract and reactive oxygen species.

Therefore, the aims of this study are : (1)To investigate the generation and scavenging activity of coffee extract. (2)To compare the radical scavenging activity of various kind of coffee beans and some brand of commercial instant coffee.

2-Materials and Methods

Materials.

Six kinds of coffee beans : Colombia, El Salvador, Tanzania, Sumatra **Mandarin** , Brazil and Java Robusta were kindly supplied by Atto Coffee Co., Ltd. Instant coffee were purchased from local market. Coffee beans were roasted and milled at ordinary conditions and concentration of 2mg/10ml. Extracts from instant coffee were prepared at the same conditions.

Hydrogen peroxide measurement.

Hydrogen peroxide was measured by the Chemiluminescence HPLC method.This analytical system consisted of normal phase HPLC (JASCO PU-980) and a chemiluminescence detector.The conditions were as follow : column, JASCO Finepak SIL(5 μm, 250 $\times$ 4.6mm) ; column oven (JASCO 860-CO, at 30℃) ; solvent, chloroform:methanol:1-propanol:water (10:90:40:1 v/v) ; flow rate, 1.0ml/min ; detector, JASCO 825-CL. Chemiluminescence reagent was prepared by dissolving 10μg/ml of cytochrome C (from horse heart, type VI, Sigma Chemical, Tokyo) in 50mM borate buffer (pH10.0). The flow rate of the chemiluminescence reagent was 1.1ml/min (JASCO-980 PU pump)(Miyazawa et al., 1987).

Measurement of hydroxyl radical and superoxide anion.

The scavenging activity of Coffee extract against hydroxyl radical or superoxide anion was carried out by the using of ESR (JEOL JES-RE1X ESR Spectrometer-X band). Hydroxyl radical was generated by Fenton reaction using hydrogen peroxide(1mM) 50 μl, $FeSO_4$ (0.01mM) 50 μl and DMPO (Labotec Co., Ltd., 8.8 mM) 20 μl as trapping reagent, then Coffee extract was added at a volume of 100 μl. Superoxide anion was generated by using of hypoxanthine (Sigma Chemical, 2mM) 50 μl, xanthine oxidase (Boehringer Mannheim, 0.4U/ml) 50 μl, Diethyl-enetriamine-N,N,N',N"-pentaacetic acid(Wako Chemical, 1mM) 50 μ l and DMPO (8.8mM) 20 μ l as trapping reagent, Coffee extract was also added at a volume of 100 μ l.

The operation conditions for the ESR Spectrometer were as follows : microwave power, 8mM ; modulation frequency 100KHz, modulation amplitude,0.1mT ; time constant, 0.1sec ; scannig time, 2min ; scanning field, 335.0 $\pm$ 5mT, microwave frequency 9.41GHz. A solution of 1.0 μ M of Tempol was used as a primary standard for the ESR spectra. The sample was measured in an aqueous quartz flat cell with an effective sample volume of 130 μ l.

3-Results and Discussion.

Generation capacity of reactive oxygen species of Coffee extract.

Rinkus et al. (1990) reported that from 12 freshly prepared brewed and instant coffee preparations, six of them contained an amount of 3$\sim$29 μ M hydrogen peroxide, while in other six none could be detected.They also estimated that adventitious formation of hydrogen peroxide is a confounding factor in the analytical and in vitro genotoxicological testing of Coffee. Recently, Stadler et al. (1994) reported that hydrogen peroxide in freshly prepared coffee was formed at a level of 400$\sim$450 μ M after 1hr of incubation. The report also estimated that the generation of hydrogen peroxide is highly dependent on the quantities of polyphenolics, transition metals, oxygen, and the biological chemical endpoints used for measurement.

In this study, we measured the quantity of hydrogen peroxide in freshly prepared coffee extract in a diluted solution (80ppm) by Chemiluminescence HPLC and ESR, and found that hydrogen peroxide was generated at a negligible amount. While Coffee extract at a concentration of 30ppm scavenged more than 95% of 0.6ppm hydrogen peroxide(Table I). From this result, it can be estimated that the scavenging activity is higher than the generating activity of hydrogen peroxide of Coffee extract. However, this dual property may depent on the concentration of Coffee extract, and it must be made clear in the future.

We also measured the generation of radicals by ESR from Coffee extract at a range of 5ppm～5% concentration. The results showed that the generating radical quantity was negligible at this condition, even when iron was added at a concentration of 4 μM or 40 μM.

Table Ⅰ.

Scavenging Activity of Coffee extract against Hydrogen Peroxide

Coffee concentration	Scavenging(%)
7.5ppm	59
15.0ppm	64
30.0ppm	96/100*
60.0ppm	95
120.0ppm	97/98*

Chemiluminescence measurement conditions: Cytochrome c(10 μg/ml) and luminol (1 μg/ml) mixture in Borate Buffer (0.2M, pH10.0) were added to 0.6ppm hydrogen peroxide. ＊Chemiluminescence was measured after incubated at 37℃ for 30 min.

Scavenging activity of Hydroxyl radical by Coffee extract.

Scavenging activities of extracts from various kinds of Coffee beans against hydroxyl radicals are shown in Table Ⅱ. The hydroxyl radical was generated by Fenton reaction(2.27mM Fe^{2+} with 227mM H_2O_2), DMPO was used as trapping reagent, Coffee also was added at a concentration of 29ppm. From this result, coffee beans from Colombia and El Salvador showed strong scavenging activity, while coffee beans from Brazil and Java Robusta showed rather weak activity.

The scavenging activities of 4 kinds of instant coffee against hydroxyl radical are shown in Table Ⅲ. When Coffee extracts were used at a concentration of 4.5～45.5ppm, the scavenging activities were observed at a range of 35～80%. There are differences on the scavenging capacity among these 4 kinds. The differences on the composition of these coffee may be the main cause.

Table Ⅱ.

Inhibitory effect of Coffee bean extract on the formation of DMPO-OH radical

Coffee (29ppm)	Spin concentration(μM)	Inhibitory effect(%)
Colombia	5.72	79.2
El Salvador	11.00	60.0
Tanzania	17.11	37.8
Sumatra Mandarin	18.07	34.3
Brazil	19.66	28.5
Java Robusta	19.80	28.0
Control	27.50	-

The hydroxyl radical was produced by Fenton reaction. 2.27mM Fe^{2+} with 227mM H_2O_2 containing of DMPO (880mM) used as trapping reagent.
ESR conditions : Modulation amplitude; 0.05mT(100kHz), recording range; 5mT, recording time 2min, time constant; 0.1s, microwave power; 8mW(9.414GHz).

Table Ⅲ.
Inhibitory effect of Instant Coffee extract on the formation of DMPO-OH radical

Coffee (ppm)	Spin concentration(μ M)	Inhibitory effect(%)
A (4.5)	13.83	49.7
(45.5)	11.85	56.9
B (4.5)	15.13	45.0
(45.5)	6.27	77.2
C (4.5)	10.95	60.2
(45.5)	7.21	73.8
D (4.5)	17.63	35.9
(45.5)	5.80	78.9

The hydroxyl radical was produced by Fenton reaction. 2.27mM Fe^{2+} with 227mM H_2O_2 containing of DMPO (880mM) used as trapping reagent.
ESR conditions : Modulation amplitude; 0.05mT(100kHz), recording range; 5mT, recording time 2min, time constant; 0.1s, microwave power; 8mW(9.414GHz).

Scavenging activity of Superoxide anion by Coffee extract.

Scavenging activities of extracts from various kinds of Coffee beans against superoxide anion are shown in Table Ⅳ. At a concentration of 75ppm, the scavenging activity was from 3.0% to 34%. From this result it can be said that, the scavenging activity of hydroxyl radical is stronger than the scavenging activity against superoxide anion in Coffee. Scavenging activity of instant coffee against superoxide anion shows the same tendency (Table Ⅴ).

Since reactive oxygen species induces various kind of diseases in biological systems, the scavenging activity of Coffee against these oxygen species is a problem of interest.

Table Ⅳ.
Inhibitory effect of Roasted Coffee extract on the formation of DMPO-O_2^- radical

Coffee (75ppm)	Spin concentration(μ M)	Inhibitory effect(%)
Colombia	2.22	3.0
El Salvador	2.14	6.6
Tanzania	2.02	12.0
Sumatra Mandarin	2.50	-
Brazil	1.50	34.3
Java Robusta	1.56	31.9
Control	2.29	-

The superoxide anion radical was produced by HPX-XOD reaction. 40mM HPX with 0.8unit ml^{-1} XOD containing of DMPO(660M) used as trapping reagent.
ESR conditions : Modulation amplitude; 0.05mT(100kHz), recording range; 5mT, recording time 2min, time constant; 0.1s, microwave power; 8mW(9.414GHz).

Table V.

Inhibitory effect of Instant Coffee extract on the formation of DMPO-O_2^- radical

Coffee (37.5ppm)	Spin concentration(μ M)	Inhibitory effect(%)
A	1.02	55.6
B	0.99	56.6
C	1.41	38.6
D	2.05	31.3
Control	2.29	-

The superoxide anion radical was produced by NADPH-PMS reaction. 55 μM NADPH with 5.5 μM PMS containing of DMPO(660M) used as trapping reagent.
ESR conditions : Modulation amplitude; 0.05mT(100kHz), recording range; 5mT, recording time 2min, time constant; 0.1s, microwave power; 8mW(9.414GHz).

4-Summary

The generating and scavenging activity of Coffee extracts of various kinds of Coffee beans and instant coffee on reactive oxygen species were investigated and the followings were made clear : (1)At low concentration level of about 80ppm, the scavenging activity was stronger than the generating activity of hydrogen peroxide. (2)Coffee bean extracts exhibited strong scavenging activity against hydroxyl radical and superoxide anion. These scavenging activities were observed dose-dependent. (3)The scavenging capacities of different Coffee bean extract against hydroxyl radical were as follows : Colombia>El Salvador>Tanzania>Sumatra Mandarin >Brazil>Java Robusta. (4)The scavenging capacities of different Coffee bean extracts against superoxide anion were as follows : Java Robusta>Brazil>Tanzania>El Salvador>Colombia>Sumatra Mandarin (5)The scavenging capacities of 4 kinds of instant Coffee against both hydroxyl radical and superoxide anion were observed. These capacities were stronger than that of Coffee beans.

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PROTECTIVE EFFECT OF CAFFEINE APOPTOSIS INDUCED BY ANTHRACYCLINES

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Introduction

Apoptosis (cell death) plays an important role in tumor growth, embryonic development metamorphosis, and hormone-dependent atrophy, as a physiological event regulating the cell number or eliminating damaged cells (Gerschenson and Rotello, 1992). Recent data have demonstrated that apoptosis may be involved in cell death induced by chemotherapeutic agents such as anthracyclines (Ling *et al.*, 1993), cisplatin (Barry *et al.*, 1990), topoisomerase I inhibitors such as camptothecin (Kaufman, 1989) and topoisomerase II inhibitors such as etoposide (Sun *et al.*, 1994). The molecular mechanism of apoptosis remains unknown.

Caffeine (CAF), the xanthine derivative, has been shown to confer cytoprotection against such chemotherapeutic agents *in vitro*(Traganos *et al.*, 1993)The mechanism of protection action by CAF against the pharmacological activity of DNA targeting drugs is unclear. It has also been shown that the treatment of CAF can potentiated cellular toxicity of anticancer drug (Tomita and Tsuchiya *et al.*, 1989). Nevertheless, there does not appear to be a correlation between apoptosis and cytoprotection.

In the present study, we demonstrated that CAF markedly reduced the toxicological action of the anthracyclines.

Materials and Methods

Mouse P388 leukemia cells were kindly supplied by Japanese Cancer Research Resource Bank, Tokyo, Japan. The cells were maintained in suspension culture in RPMI 1640 medium supplemented with 10 % FBS, 5 X 10^{-5} M 2-mercaptoethanol, penicillin (100 units /ml) and streptomycin (100 μg/ml). They were grown in an atmosphere of 5 % CO_2 and 95 % air at 37 °C in a humidified incubator. Females DBA/2 and DBA/2 X C57BL/6 (BDF_1) mice were obtained from Japan SLC Inc., Hamamatsu, Japan. Cytotoxic activity of a drug was assessed by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma)-based colorimetric assay. The content of anthracycline was then determined fluorimetrically (Bachur, 1970) in an Hitachi spectrofluorometer (650-10S type). The incorporation into acid-insoluble material was determined after 1 h incubation of P388 cells (10^6 cells/ml) with [methyl-^{3}H]thymidine([^{3}H]Thd, 1 μCi, Amersham) or [5-^{3}H]uridine ([^{3}H]Urd, 1 μCi, NEN Products). Values for the increase in life span (ILS) % to evaluate the

antitumor effects were calculated from the mean survival time (MST) of treated animals relative to that of control animals.

Results and Discussions

The effect of CAF on anthracycline-induced growth inhibition in P388 cells lines was examined. Various doses of anthracyclines (0.001-0.5 μg/ml) were added to the growth medium in the presence or absence of CAF and exposed for 1 h and then incubated in drug-free medium for 48 h. As shown in Fig.1, in this cell

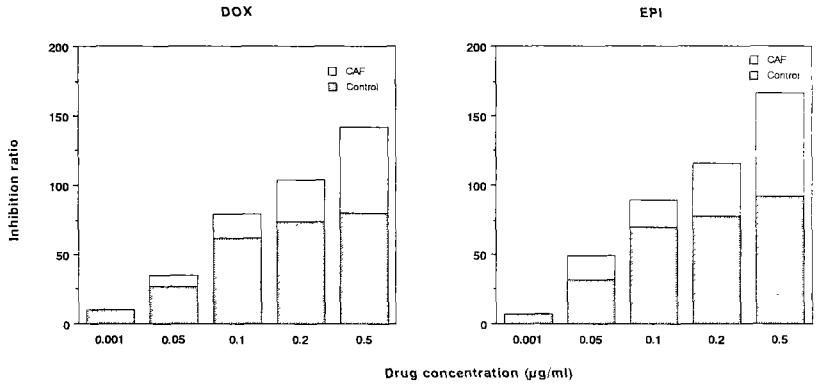

Fig. 1. Abrogation of anthracycline cytotoxicity *in vitro* by caffeine. P388 cells were exposed to anthracyclines in the presence or absence of CAF for 1 h. Then the cells were washed and reincubated in drug-free medium for 48 h. Cytotoxicity was determined using MTT assay.

line, cytoprotection was observed after CAF exposure. The influence of CAF on cellular accumulations of anthracyclines is shown in Fig. 2. P388 cells were incubated with anthracyclines (1 μg/ml) in the presence or

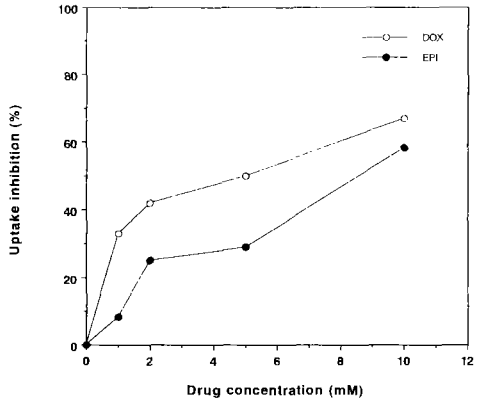

Fig. 2. Inhibition of anthracycline uptake by CAF. P388 cells were exposed to EPI (1 μg/ml) in the presence or absence of CAF. Anthracycline content of cells were measured fluorometrically.

absence of CAF for 1 h. An increase in the amount of CAF caused the inhibition of anthracycline uptake. The incorporation rate of [^{3}H] TdR into the DNA of the cells decreased significantly for an exposure time of 1 h of 1 μg/ml anthracyclines (Fig. 3). The inhibition of the incorporation of [^{3}H] uridine into the RNA by same drug was also observed. A decrease of incorporations of radiolabeled TdR and uridine induced by anthracyclines was markedly inhibited by CAF. We studied the effect of CAF on G_2 phase arrest by 0.1-.5 μg/ml of EPI using flow cytometry. As shown in Table 1, EPI caused G_2/M phase accumulation of P388 cells. This accumulation by EPI was diminished by pretreatment of 10 mM of CAF.

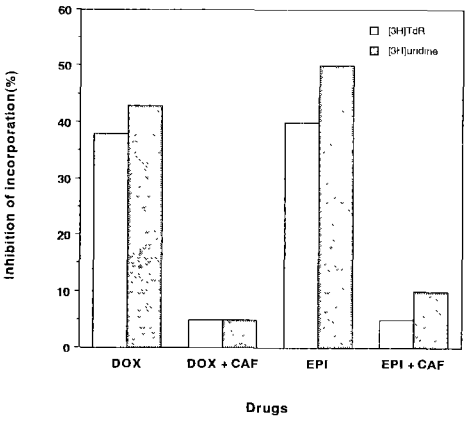

Fig. 3. Effects of anthracyclines on the incorporation of labelled precursors into DNA and RNA and protective effect of CAF. P388 cells (1 x 10^5 cells/well) were incubated with anthracylines in the presence or absence of 10 mM CAF for 1 h and then washed free of drug with fresh warm medium. Incorporation of [^{3}H]TdR (1 μCi/ml) and [^{3}H]uridine (1 μCi/ml) into TCA-insoluble material was determined.

Table 1 Percentage of cells in each DNA compartment following EPI treatment.

Time post-treatment		Phase	EPI (μg/ml)			
			0	0.1	0.2	0.5
6 h	Control	G_1	48.3	25.7	1.2	1.4
		S	46.2	65.2	69.8	74.2
		G_2 + M	5.5	8.9	28.9	24.4
	10 mM CAF	G_1	44.9	38.7	36.5	29.2
		S	50.0	54.5	52.8	53.4
		G2 + M	5.1	6.8	10.7	17.4
24 h	Control	G_1	45.7	21.7	3.0	4.4
		S	46.9	36.7	61.1	35.9
		G_2 + M	7.4	41.6	35.8	59.8
	10 mM CAF	G_1	51.1	57.0	57.7	35.6
		S	45.6	35.1	32.0	37.2
		G_2 + M	3.3	7.9	9.7	27.2
48 h	Control	G_1	51.0	57.0	22.5	23.1
		S	44.3	13.4	47.4	73.5
		G_2 + M	4.8	29.6	30.1	3.5
	10 mM CAF	G_1	47.9	51.8	48.8	12.0
		S	45.4	41.7	44.2	50.0
		G_2 + M	6.7	6.5	7.1	37.9

Cells were treated with EPI for 1 h at 37 °C, washed, and incubated in drug-free medium. The cells were harvested, fixed, and stained .

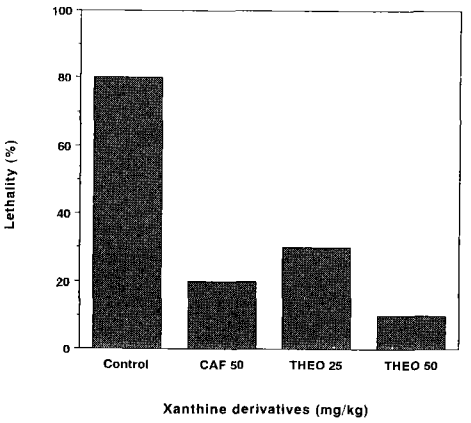

Fig. 4. Protective effects of xanthine derivatives on lethal dose of EPI in mice. CAF or theophylline (THEO) was given inltraperitoneally 10 min before the administration of EPI. Animals were observed for 15 days after the administrations of EPI.

A single dose of 20 mg/kg of EPI (i.p.) significantly reduced body weight in mice 3 days after injection (data not shown). The treatment with CAF at a dose of 50 mg/kg (i.p.) significantly protected the decrease in body weigh induced by EPI. The protective effects of CAF and theophylline on EPI-induced lethality in mice are presented in Fig. 4. The death of mice injected with EPI (20 mg/kg) was markedly diminished by the administration of CAF or theophylline. Next, we examined whether CAF protects cells against the antitumor effect of EPI. However, survival rate of EPI (1-10 mg/kg, i.p) in the combination with CAF (25-100 mg/kg, i.p.) was almost the same compared to that of EPI alone group (Table 2).

Table 2 Effect of CAF on the antitumor activity of EPI in P388 leukemia bearing mice

Treatment[a] (Dose, mg/kg)	MST ± S.E.[b]	ILS[c]
(A) Control	10.5 ± 0.40	
EPI 5	16.5 ± 0.42	57
+ CAF 25	17.0 ± 0.71	62
+ CAF 50	19.7 ± 0.71	73
+ CAF 100	17.8 ± 0.79	70
EPI 10	20.0 ± 0.70	90
+ CAF 25	19.8 ± 1.31	89
+ CAF 50	19.0 ± 0.86	81
+ CAF 100	19.5 ± 0.20	86
(B) Control	11.3 ± 0.33	
CAF 50	11.2 ± 0.31	-1
EPI 1	17.7 ± 0.68	57
+ CAF 50	17.8 ± 0.85	58
EPI 5	22.8 ± 1.16	102
+ CAF 50	22.0 ± 0.87	95

(A): [a]P388 cells (10^6 cells) were implanted i.p. into BDF_1 mice on Day 0 and then drugs were given i.p. on Day 1. (B) [a] P388 cells (10^6 cells) were implanted i.p. in to BDF_1 mice on Day 0 and then drugs were given i.p. on Days 3, 6, and 9. [b] Mean survival time. Each value represents the mean and standard error of 6-10 mice. [c] Increases in life-span. Values calculated on the basis of the MST of the treated vs. control.

In the present investigation, CAF prevented cell death induced by anthracyclines. A significant cytoprotection

was observed when cells were exposed to CAF prior to anthracyclines. The data presented here provide indirect evidence that initiation of anthracyclines-induced apoptosis may need to be coupled to a cell cycle-mediated event. In a cell-cycle study, we indicated that the G_2-phase arrest by anthracycline was inhibited by treatment with CAF, suggesting that cell cycle arrest was inhibited by CAF may be important for protection. Furthermore, the protective effect of CAF was also observed in EPI-treated mice. Administration of CAF prior to EPI treatment appears to prevent death induced by EPI. This suggests that CAF will be useful for reducing the toxicity of anthracycline.

Summary

We studied the effects of the xanthine derivative, caffeine (CAF), on cell killing by anthracycline antibiotics *in vitro* and *in vivo*. CAF reduced the cytotoxicity of epirubicin (EPI) in cultured P388 leukemia cells. The treatment with CAF diminished G_2 blocking effects induced by anthracyclines in cells. CAF also weakened the inhibition of anthracycline-induced nucleic acid synthesis in cells. When CAF was added to the growth medium before EPI exposure, a modification in intracellular EPI accumulation was observed. The treatment with CAF significantly diminished the lethality of anthracyclines in mice. Thus, CAF inhibited cell death induced by EPI *in vitro* and *in vivo*. CAF, however, did not affect the antitumor activity of EPI of mice bearing P388 leukemia. These results suggest the antagonistic effect of CAF on the toxicological actions of anthracyclines in cells and mice. From these data, this protection by CAF may be important in reducing side effects of the antitumor drug.

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EFFECTS OF COFFEE DRINKING ON THE ACTIVITY OF AUTONOMIC NERVOUS SYSTEM IN HUMAN : HIGH RESOLUTION BY A POWER SPECTRAL ANALYSIS

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1. Introduction

Coffee beans contain some physiological and pharmacological active components. Especially, caffeine is known as a stimulant of central nervous system and contraction of the blood vessels. Not so many studies in detail have been done so far noninvasively on its effect on the activity of autonomic nervous system. Power spectral analysis of heart rate variability is a useful mathematical non invasive method for analyzing cardiac autonomic nerve activity [1-4]. It is a kind of method to quantify heartbeats' fluctuation, and offers us a curve or drawing which shows a relation ship between the periodically fluctuating components included in heart rate variability and its power. It has been developed and applied to various pathological diagnosis such as diadetes [5,6]. In this study, we have attempted to develop a computer-implented power spectral analysis of heart rate variability which is a useful noninvasive method, and to determine the possibility of its application to the field of physiological functions of food.

2. Materials and Methods

Subjects

Eight healty subjects were recruited from our laboratory with their informed consent, five males and three females, aged 21-41 years old (mean 25.6 years). Some of them regularly drank caffeinated coffee.

Equipment and data analysis

The bear-to-beat instantaneous heart rate was measured using ear sensor. Power spectral analysis (R-R analysis) was then performed by means of Fast Fourier Transform to obtain the drawing of power spectra. In power spectrum, low frequency component (0.03-0.15 Hz; LO component) is considered to reflect both sympathetic and parasympathetic nerve activities and high frequency component (0.15-0.8 Hz; HI component) to reflect only parasympathetic nerve activity. It was compared integrated values of each component (LO, HI and TOTAL: LO + HI) to evaluate the variations in autonomic nerve activity as time passes.

Experimental procedures

Before and after drinking all subjects were in the sitting position on a comfortable chair under constant room temperature. Subjects fasted from foods and drinks for at least 2 hours before measurements. After subjects resting on a chair for more than 15 minutes, instantaneous heart rate was measured before and after taking caffeine-containing drink or coffee drink.

2. Results and discussion

Each of the integrated values of power spectral analysis (LO, HI and TOTAL) changed after taking caffeine-containing drink and coffee. Figure 1 and 4 represent a typical set of TOTAL component of autonomic nerve activity as time passes. After taking of caffeine-containing drink, total autonomic nerve activities were increased and reached a maximum level at 25 min as shown in Fig.1. Figure 2 shows a typical set of raw R-R interval data and corresponding spectra obtained from the subjects before and after taking caffeine-containing drink or caffeine's drink (control) at 25 min. In these spectrum, low frequency component (0.03-0.15 Hz; LO component) is considered to reflect both sympathetic and parasympathetic nerve activities (black out parts) and high frequency component (0.15-0.8 Hz; HI component) to reflect only parasympathetic nerve activity. These data suggest that caffeine-containing drink evokes total autonomic nerve activity, especially sympathetic nerve activity. In the same time, the effect of caffeine-containing drink on the subject sleepiness was evaluated by oneself. As shown in Fig 3, caffeine-containing drink significantly shake off drowsiness and sleepiness.

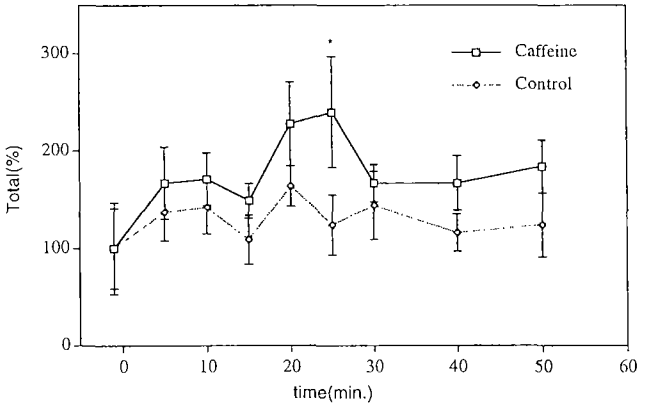

Fig.1 Effect of taking caffeine-cotaining drink on the activity of autonomic nervous system. Each value is shown as relative mean of TOTAL integrated value ± SEM compared to one before taking the drink (n=5-7). *P <0.05 vs Control

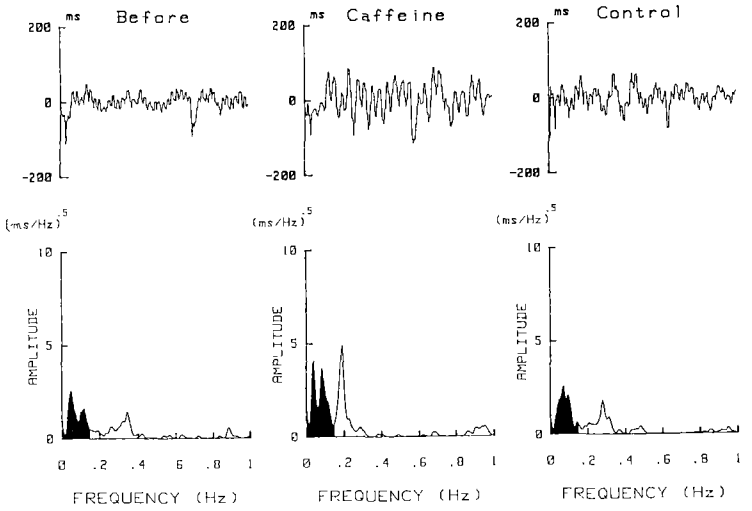

Fig.2 A typical set of raw R-R interval data and corresponding spectra obtained from before, after taking caffeine-containing drink. t=25(min.)

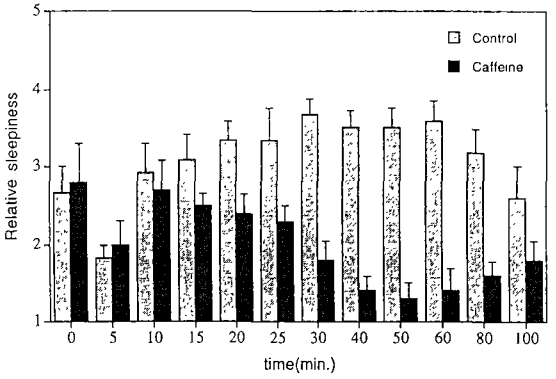

Fig.3 Effect of caffeine on human sleepiness (subjective value). Each value is shown as mean±SEM(n=5-6). A bigger number shows that the subject was more sleepy: Max.level=5(very sleepy), Min.level=1(very vigil).

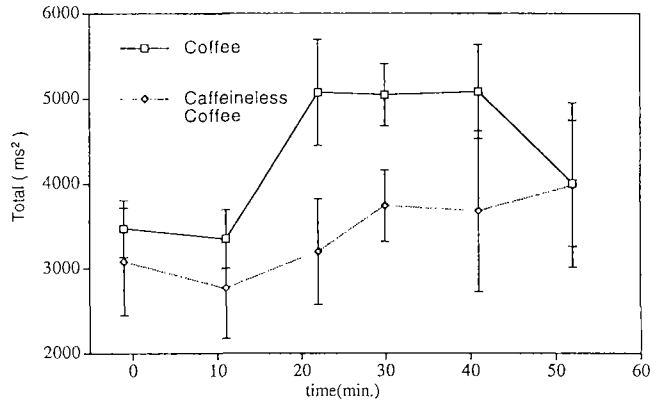

Fig.4 Effect of coffee drinking on the activity of autonomic nervous system. Each value is shown as mean of TOTAL integrated value ± SEM (n=5).

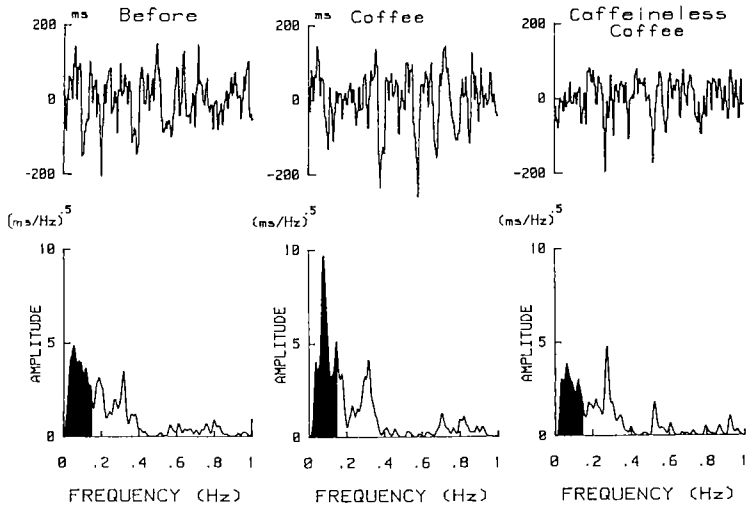

Fig.5 A typical set of raw R-R interval data and corresponding spectra obtained from coffee drink or caffeineless coffee drink. t=41(min.)

Figure 4 shows the TOTAL component of power spectral analysis before and after taking coffee or caffeinless coffee. Both drinks, especially coffee drinking, increase the TOTAL autonomic nerve activity and reached a maximum level at 22 min. and then maintained this level to 41min. Figure 5 shows a typical set of raw R-R interval data and corresponding spectra obtained from the subjects before and after taking coffee drink or caffeineless coffee at 41 min. These data suggest that coffee drinking evokes total

autonomic nerve activity, both sympathetic and parasympathetic nerve activity. On the other hand, caffeinless-coffee drink slightly stimulate LOW component in compared with coffee drink. As shown in Fig. 2, caffeine-containing drink stimulates sympathetic nerve activity. These our results might suggest that coffee and caffeine-containing drink evoke total autonomic nerve activity, especially sympathetic one.

3. Summary

Coffee beans contain some physiological and pharmacological active components. Especially, caffeine is known as a stimulant of central nervous system and contraction of the blood vessels. Not so many studies in detail have been done so far noninvasively on its effect on the activity of autonomic nervous system (ANS). In this study, we have attempted to develop a computer-implanted power spectral analysis of heart rate variability which is a useful non invasive method, and to determine the possibility of its application to the field of physiological functions of food. The subjects who volunteered for this study were 8 healthy people. After drinking of coffee, it was observed by a power spectral analysis that the activity of ANS was augmented. Drinking of caffeinated coffee showed a greater effect on the activity of ANS than that of decaffeinated one. Our present results suggest the possibility of the application of a power spectral analysis to the field of physiological functions of food.

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ANTIOXIDANT ACTIVITIES OF CHLOROGENIC ACIDS

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1. Introduction

Coffee bean phenolics have been of interest to a number of workers since Payen bestowed chlorogenic acids on them in 1846(1). Chlorogenic acids include about ten closely related compounds which are esters of hydroxy-cinnamic acid and/or its derivative with quinic acid. These compounds constitute about 8% of green coffee beans and 4% of roasted coffee beans(2).

Recently, naturally occurring plant phenolics have attracted much attention in relation to their physiological potential. Depending upon the conditions chlorogenic acids can be either beneficial or detrimental to biological processes. Investigators have reported an inhibitory effect of chlorogenic acid on lipoxygenase activity in prostaglandin metabolism(3), inhibition of oxidation of vitamin A(4), protection against oxidation of epinephrine (5), inhibition of retinoic acid 5,6-epoxidation(6), and antiviral activity (7), while others showed that chlorogenic acid is a potent cocarcinogenic agent (8) and an inducer of DNA damage (9). In the present report antioxidant activities of chlorogenic acids were investigated by using 1,1-diphenyl-2-picrylhydrazyl(DPPH) radical scavenging system and superoxide anion mediated linoleic acid peroxidation system *in vitro*. A mechanism of linoleic acid peroxidation inhibition by chlorogenic acid was proposed.

2. Materials and methods

Materials. Linoleic acid (99.9 %), xanthine oxidase (XOD), 5-O-caffeoylquinic acid(5-CQA), caffeic acid, *dl*-α-tocopherol were purchased from Sigma. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was from Wako. 3,5-O-Dicaffeoylquinic acid(3,5-DCQA) was isolated from coffee beans. All other chemicals used were commercial products of the highest grade.

Radical scavenging activity on DPPH radical. Radical scavenging activity was assayed according to the method of Blois(10). The reaction mixture contained, in a total volume of 3.5 ml, 3 ml of 0.1 mM DPPH (in 95 % EtOH) and 0.5 ml of the test compound (in 95 % EtOH). After allowing the mixture to stand at room temperature for 20 min, the absorbance of the remaining DPPH was determined colorimetrically at 517 nm. The scavenging activity was measured as the decrease in absorbance of the DPPH expressed as a percentage of the absorbance of a control DPPH solution without test substances. The mean value was obtained from triplicate determinations.

Antioxidant activity of linoleic acid peroxidation. Linoleic acid peroxidation was assayed according to the method of Tien et al (11).The reaction mixture contained, in a total volume of 5.02 ml, 4 ml of 1.25 mM linoleic acid micelles, 0.5 ml of 1 mM EDTA-Fe^{3+} (1.1 mM EDTA, 1 mM $FeCl_3$), 0.5 ml of 0.3 M acetaldehyde, 0.01 ml of 18.5 U ml^{-1} XOD and 0.01 ml of the test compound, in 30 mM NaCl (pH 7.0). Reactions were initiated by adding XOD. The absorbance of conjugated diene formation was determined colorimetrically at 234 nm. The inhibitory activity was expressed by the decrease in conjugated diene formation after 10 min.

3. Results

Scavenging activity on DPPH radicals

At a concentration of 10 μM,5-CQA, 3,5-DCQA, caffeic and ferulic acids had scavenging activities of 37, 50, 28 and 17 %, respectively (Table 1). Both *dl*-α-tocopherol and ascorbic acid had 19 % scavenging activity. DPPH radical scavenging activities of these compounds increased dose-dependently at concentrations ranging from 1 to 50 μM (Fig. 1). *p*-Coumaric acid showed no reaction with the DPPH radical. Comparing the scavenging activities to that of cysteine, 1 mol of 5-CQA reacted with ca 4 mol of radical, and 1 mol of 3,5-DCQA with ca 6 mol of radical(12).

Table 1. Radical scavenging activities of chlorogenic acids and related compounds on DPPH

Addition (10μM)	Scavenging activity (%)
5-0-Caffeoylquinic acid	37
3,5-0-Dicaffeoylquinic acid	50
Caffeic acid	28
Ferulic acid	17
p-Coumaric acid	0
dl-α-Tocopherol	19
Ascorbic acid	19
Cysteine	7

The scavenging activity was measured as the decrease in absorbance of the DPPH radical expressed as a percentage of the absorbance of a control DPPH radical solution without test substances.

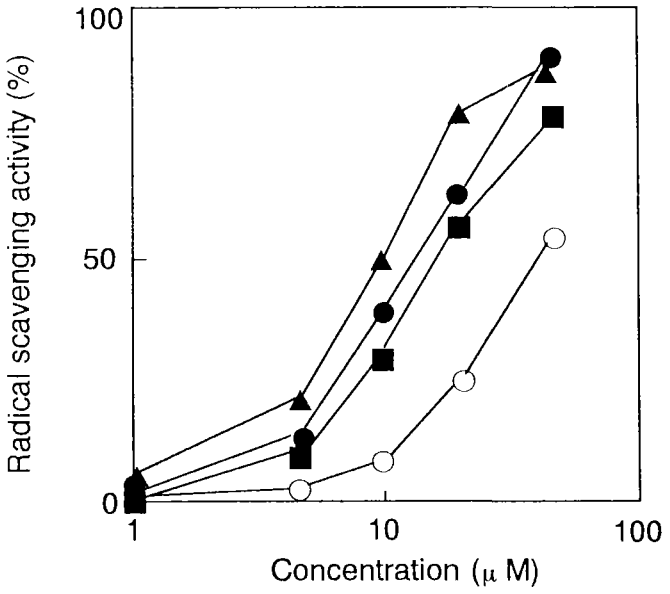

Fig.1. Radical scavenging activities of chlorogenic acids and ralated compounds on DPPH : 5-0-Caffeoylquinic acid(●); 3,5-0-Dicaffeoylquinic acid(▲); Caffeic acid(■); Ferulic acid(○).

Antioxidant activity on linoleic acid peroxidation

Exposure of linoleic acid micelles to superoxide resulted in an increase in absorbance at 234 nm (Fig. 2 curve a). Both the initial rate of conjugated diene formation and the amount of diene formed after 10 min, decreased dose-dependently on addition of 5-CQA(Fig. 2 curves b-d). At a concentration of 20μM, 5-CQA, 3,5-DCQA, caffeic and ferulic acids inhibited the formation of conjugated diene by 55, 74, 56 and 40 % respectively (Table 2). *dl*-α-Tocopherol and ascorbic acid inhibited by 84 and 48 %, respectively. Mannitol (5 mM) showed 82 % inhibition.

Table 2. Inhibitory activities of chlorogenic acids and related compounds on conjugated diene formation.

Addition (20μM)	Inhibitory activity (%)
5-O-Caffeoylquinic acid	55
3,5-O-Dicaffeoylquinic acid	74
Caffeic acid	56
Ferulic acid	40
p-Coumaric acid	2
dl-α-Tocopherol	84
Ascorbic acid	48
Mannitol (5mM)	82

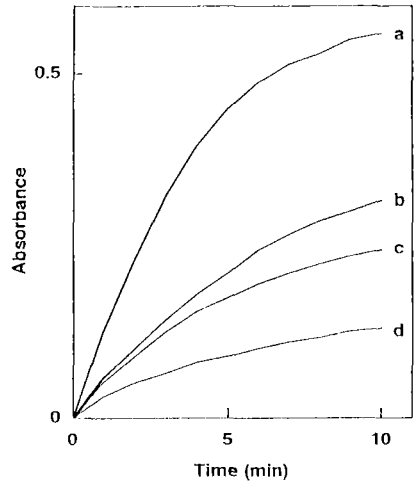

Fig.2. Inhibitory activity of 5-O-Caffeoylquinic acid on conjugated diene formation. (a)No addition;(b)10μM 5-CQA;(c)20μM 5-CQA;(d)50μM 5-CQA.

4. Discussion

5-CQA, 3,5-DCQA and caffeic acid exhibited scavenging activities on the DPPH radical. Radical scavenging activities of these compounds were stronger than those of *dl*-α-tocopherol or ascorbic acid at 10 μM. It is interesting that the coffee bean phenolics were so much more effective than ascorbic acid, assuming that the reactions may proceed as suggested by Blois (10).

5-CQA, 3,5-DCQA and caffeic acid inhibited the formation of conjugated diene in the early stage of linoleic acid peroxidation. The inhibitory activity of 3,5-DCQA was stronger than those of 5-CQA, caffeic and ascorbic acids, whereas *dl*-α-tocopherol was the most inhibitory among others tested. The greater inhibitory power of 3,5-DCQA compared to 5-CQA or caffeic acid is to be expected as it contains two dihydroxy functions per molecule.

5-CQA and 3,5-DCQA were much more inhibitory to conjugated diene formation than mannitol. For the same inhibition, the concentration of mannitol, a selective hydroxy radical scavenger, required to be ca 200-fold greater than that of 3,5-DCQA. This suggests that 5-CQA and 3,5-DCQA are not only selective hydroxy radical scavengers at the concentrations employed, but also peroxy radical scavengers. Therefore, antioxidant activities of 5-CQA and 3,5-DCQA may not only result from inhibition of the initiation of lipid peroxidation but also from inhibition of the subsequent step. During the formation of conjugated diene, a change in the retention time of these compounds was observed by HPLC because of the formation of new compounds with different retention times as the scheme suggests.

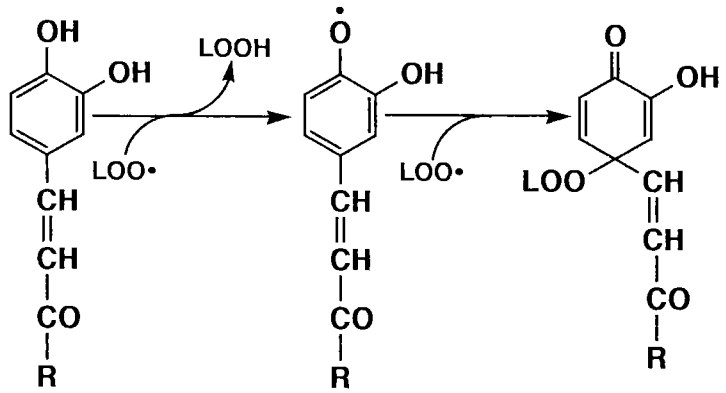

Fig.3. A proposed mechanism of antioxidant activity by chlorogenic acid.

We propose a mechanism of antioxidant activity of chlorogenic acids as follows. First, chlorogenic acid may act as a H-atom donor to the peroxy radicals (LOO·), and may react with another peroxy radical converting it into much less active products (Fig.3). Inhibitory activities of 5-CQA and 3,5-DCQA on the autoxidation of fatty acids may thus be because they terminate the chain radical reaction. 5-CQA and 3,5-DCQA, belong to a class of small molecules that react rapidly with peroxy radicals and, hence, are potentially important biological antioxidants.

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6. Summary

Antioxidant activities of chlorogenic acids and related compounds were investigated by both the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging system and the superoxide anion-mediated linoleic acid peroxidation system. At 10 μM, 5-CQA 3,5-DCQA and caffeic acid showed more scavenging activities on DPPH than *dl*-α-tocopherol or ascorbic acid. DPPH radical scavenging activities of these compounds increased dose-dependently at concentrations ranging from 1 to 50 μM. 5-CQA, 3,5-DCQA and caffeic acid inhibited the formation of conjugated diene from linoleic acid. The inhibitory activity of 3,5-DCQA was stronger than that of 5-CQA or caffeic acid.

PROTECTION BY « COFFEE CHERRY » OF DEVELOPMENT AND GROWTH OF SPONTANEOUS MAMMARY TUMOURS IN MICE

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1-Introduction

Today, numerous chemically synthesized medicines have been most extensively used. While there is no doubt of their contribution to human health, they are sometimes harmful and generally the greater the effects, the more toxic they are. For this reason, the significance of herbal medicines and pharmaceutical effects of natural products have recently been recognized in Japan as well as in the world. Coffee cherry (CC), residue after removal of coffee beans, is mostly discarded or used as manure, however, a little of them is used locally as a source of healthy drink in some producing districts.

A protective role of a hot water extract of CC was found in the development and growth of spontaneous mouse mammary tumours, a representative animal model for human breast cancer, and is reported in this communication.

2- Materials and methods

Dry matter of CC donated by PT TOARCO JAYA, Jakarta, Indonesia, was extracted repeatedly with hot water and the supernatants were pooled and dried *in vacuo*. The extract was dissolved with tap water (0.5 %) and served as the sample in both Experiments I and II. A high mammary tumour strain of SHN mice (Nagasawa et al. , 1976; Staats, 1985) were used.

In Experiment I, the control and the experimental mice were given the tap water and the sample, respectively, beginning 2 months of age and all mice were checked for palpable mammary tumours every 7 days and tumourous mice were killed within a week by decapitation under the light ether anaesthesia after recording the total number of tumours. Blood was collected from the trunk and serum was stored at -70 °C for assay of prolactin by radioimmunoassay (Yamamoto and Kikuyama, 1982) and free fatty acid by acyl Co A oxidase method. ^{1}H-NMR spectra (Fujii et al. , 1993) and blood glucose level (Cauley et al. , 1959) were determined in non-tumourous mice at 2 and 5 months and at 8 months of treatment, respectively.

In Experiment II, Mice bearing mammary tumours at size of 5-7 mm were divided into two groups. The control and the experimental groups received the tap water and the sample, respectively, for 10 days and the change of mammary tumour size was recorded.

In both Experiments, mice were kept in teflon cages with wood shavings (4-5 each), maintained in an air- and light- controlled windowless animal room and provided with commercial pellets (Nihon Nosan Kogyo KK, Yokohama, Japan) and water *ad libitum*.

At autopsy, the bilateral third thoracic mammary glands were prepared for the wholemount evaluation and examined under the 10-fold magnification for both normal and preneoplastic mammary gland growth.

The activities in normal and neoplastic mammary tissue of thymidylate synthetase (TS) and thymidine kinase (TK); i. e. , DNA synthesizing enzymes in *de novo* and salvage pathways of pyrimidine, respectively, were also determined (Sakamoto et al. , 1987).

3-Results and discussion

Experiment I : As shown in Figure 1, mammary tumours appeared from 4 months of treatment (6 months of age) in the control, however, it was 2 months later in the experimental group given CC. Furthermore, the cumulative incidence at each month was always lower in the experimental group and the difference in mammary tumourigenesis between groups was statistically significant. Total number of tumours per mouse differed little between groups.

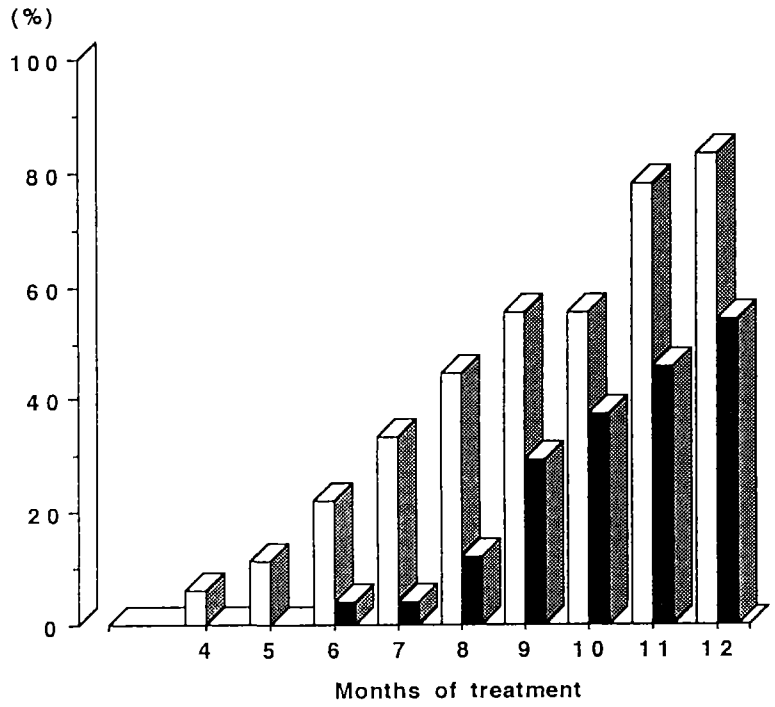

Figure 1. Cumulative incidence of mammary tumours in each group [□ Control (n=18), ■ Experimental (n=24)]. Experimental group received CC beginning 2 months of age. The difference between groups in mammary tumourigenesis considering simultaneously the incidence and the onset age of tumours was statistically significant by multiple classification method of analysis of variance (Snedecor and Cochran, 1980).

Table 1 Normal and preneoplastic mammary gland growth in each group. (Mean ± SEM).

Group	Normal glands		Hyperplastic alveolar nodules (HAN)	
	Rating [1]	Area [2] (mm^2)	Number [3]	Area [4] (mm^2)
Control	2.5 ± 0.3 (13)	291 ± 15 (13)	11.8 ± 2.4 (11)	1.77 ± 0.21 (144)
Exp.	1.8 ± 0.2 (13)	237 * ± 21 (13)	12.9 ± 2.3 (13)	1.29 * ± 0.11 (172)

[1] The degree of formation of end-buds or lobulo-alveoli was rated from 1 to 7 in increments of 1 (Nagasawa et al., 1980). The value represents the mean of the bilateral third thoraicic glands.
[2] Area binding the tops of ducts by straight lines was measured by a computerized digitizer (Model LA-535, PIAS, Tokyo, Japan) as an index of mammary duct extention; Mean of the bilateral glands.
[3] Sum of the bilateral glands.
[4] Area measured by a computerized digitizer.
Number of estimates is in the parentheses. * Significantly different at $P < 0.05$.

Areas of both normal mammary glands and HAN were significantly lower in the experimental group than in the control (Table 1). Moreover, mammary rating was also apparently lower in the experimental mice than in the control. These indicate that the growth of both normal and preneoplastic mammary glands were inhibited by CC, although it was not reflected by the dynamic aspect of the mammary glands, DNA synthesizing enzyme activities, which were different little between the control and the experimental groups (data not shown).

Most of the urinary component levels were higher (Figure 2) and serum free fatty acid level was lower in the experimental group than in the control [1.51 ± 0.14 μEq/ml (n=5) and 0.82 ± 0.16 μEq/ml (n=6) in the control and the experimental groups, respectively]. There was little difference between groups in any of serum levels of prolactin and glucose, weights of anterior pituitary, adrenals and ovaries and the pattern of oestrous cycles (data not shown).

Experiment II : As shown in Figure 3, the mammary tumour growth was significantly declined compared to the control by the free access of CC. On the other hand, the treatment affected little the growth of normal and preneoplastic mammary glands and endocrine organ weights.

Present results show that hot water extract of CC protected spontaneous mammary tumourigenesis. The mechanism of the effects is not clear at present, however, modulation or acceleration by CC of metabolism, which was estimated by the increased excretion of urinary components and decreased free fatty acid level in the circulation, may contribute at least partly to the mammary tumour protection. CC alternation of endocrine system, which has a major role in the induction of mammary tumours, may be minor, since no endocrine parameters were affected by the CC treatment.

Finally, CC may be promising as a chemoprophylatic agent of breast and other types of cancers.

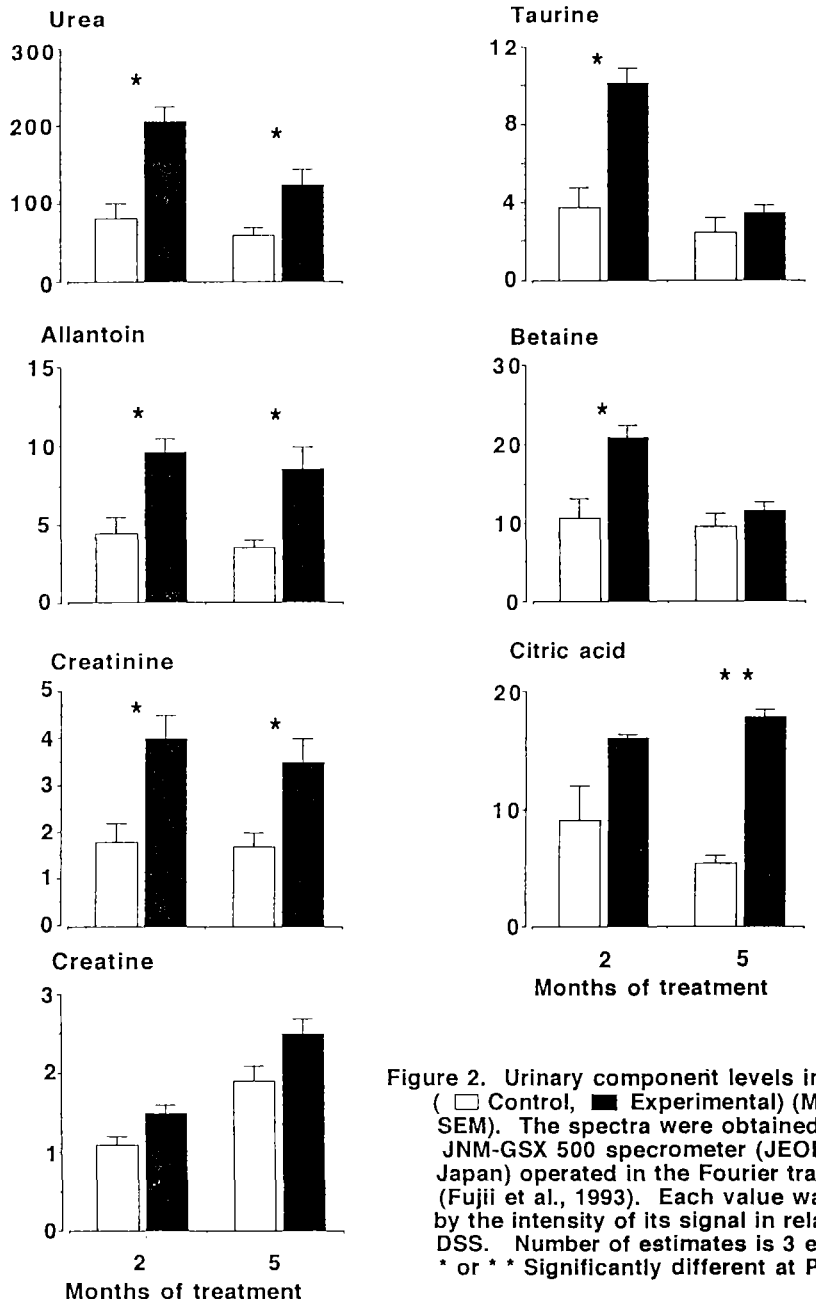

Figure 2. Urinary component levels in each group (□ Control, ■ Experimental) (Mean ± SEM). The spectra were obtained by JEOL JNM-GSX 500 specrometer (JEOL, Tokyo, Japan) operated in the Fourier transform mode (Fujii et al., 1993). Each value was estimated by the intensity of its signal in relation to that DSS. Number of estimates is 3 each.
* or * * Significantly different at $P < 0.05$ or 0.01.

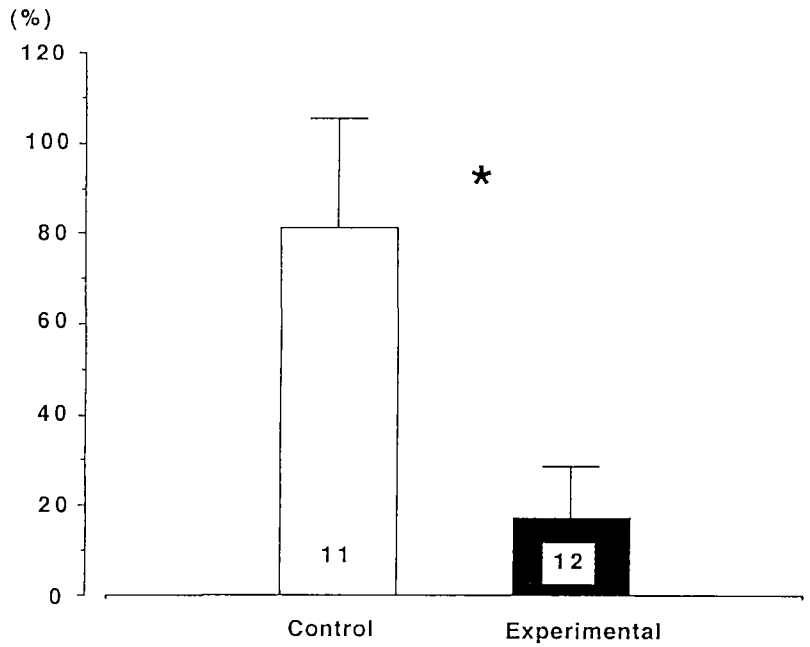

Figure 3. Growth rate (percent change) of mammary tumour size in each group (Mean ± SEM). CC was given to mice bearing mammary tumours (5-7 mm) for 10 days. Number of estimates is in the column. * Significantly different at $P < 0.05$.

4-Summary

Chronic ingestion of extract of coffee cherry (CC), residue after removal of coffee beans, induced a mark suppression of the development and growth of spontaneous mammary tumours in a high mammary tumour strain of SHN virgin mice. Growth of normal and preneoplastic mammary glands were also apparently inhibited by the long-term treatment with CC. The excretion of urinary components such as urea, allantoin and creatinine was increased, and serum free fatty acid level was decreased by CC. Little modulation of the endocrine system was noted by the treatment. Finally, the chemoprophylatic role of CC in tumours is promising.

Acknowledgements

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OBESE WOMEN WITH REDUCED THERMOGENIC RESPONSE TO CAFFEINE HAVE DIFFICULTIES IN LOSING BODY WEIGHT

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1 - Introduction

Various studies have suggested that a reduced basal metabolic rate (BMR) may precede obesity (1-3). However, it is unclear whether obese subjects with a reduced BMR have difficulties in losing body weight with treatment. Caffeine, which is a constituent of many beverages, such as coffee, tea and some cola drinks, stimulates energy expenditure in rodents (4,5) and humans (6-8), although its use as a slimming agent has never been justified by any clinical documentation of weight-reducing properties. Brown adipose tissue (BAT) is a heat-producing organ of diet-induced thermogenesis (9) and non-shivering thermogenesis (10). A deficient or reduced BAT function plays an important role in the development of obesity in experimentally obese rodents (9,11). However, it is unclear whether obesity is due to a thermogenic mechanism because we cannot measure BAT function directly in humans. Therefore, in this study, we conducted the following two investigations to clarify how obese subjects with reduced BMR and/or reduced thermogenic response to caffeine via reduced BAT thermogenesis lose body weight. In the first study, we examined in mice the effect of caffeine on BAT thermogenesis by measuring guanosine-5'-diphosphate (GDP) binding (which is a thermogenic indicator of BAT), oxygen consumption in BAT mitochondria, BAT temperature, and resting metabolic rate (RMR). In the second study, we measured BMR and thermogenic responses to caffeine in the 136 obese women who were treated with a combined low calorie diet and exercise regimen for two months.

2 - Effects of caffeine on BAT thermogenesis and metabolic rate in mice

Under anesthesia by intraperitoneal injection of urethane , mice were placed in a box kept at 33±3°C. Small thermistors (IT-21, Sensortex, N.J., USA) were inserted under the intracapsular BAT (IBAT) pad and 3 cm into the rectum of 15 female CRJ-ICR mice (age 12 weeks, weighing about 30g, Charles River Japan, Tokyo) as described elsewhere (12). After a stable baseline had been achieved (about 30 min), caffeine (1,3,7-trimethylxanthine)(Wako Chemical Industries, Osaka) in saline solution, was injected intraperitoneally (i.p.) at a dose of 40 or 60mg/kg. Saline only was injected as a control. BAT (T_{BAT}) and core (T_{core}) temperatures were monitored for 60 min after administration. RMR was estimated using a closed-circuit metabolic system (Environics, USA). RMR was measured for 60 min after the injection in 6 female ICR mice. BAT thermogenesis was assessed by measuring GDP binding and oxygen consumption in BAT mitochondria. Thirty minutes after the injection of caffeine (40 and 60 mg/kg) or saline, 8 female ICR mice in each groups were killed by cervical dislocation at 0900h. IBAT was rapidly removed and dissected from connective tissue. IBAT samples were weighed and homogenized in an ice-cold medium containing 250 mM sucrose and 5mM TES (pH 7.2). The mitochondria of IBAT were isolated by differential centrifugation and then the mitochondria protein content was measured (13). GDP binding and oxygen consumption in IBAT mitochondria was determined as described elsewhere (14). IBAT samples from two mice were combined for one measurement of GDP binding and oxygen consumption. Statistical analysis was done using Bonferroni t-test, following ANOVA.

Table 1. Effect of caffeine (40 and 60 mg/kg) on IBAT weight, IBAT mitochondrial protein content, GDP binding, and oxygen consumption in IBAT mitochondria, and RMR.

		Control	Caffeine (40 mg/kg)	Caffeine (60 mg/kg)
IBAT weight (mg)	(n=4)	104.7±0.8	98.7±1.0	101.1±0.9
Mitochondrial protein content in IBAT (mg)	(n=4)	1.05±0.14	1.10±0.08	1.10±0.11
GDP binding (pmol/mg·mitochondrial protein)	(n=4)	143.9±5.9	152.3±7.1	169.2±4.2*
Oxygen consumption (nmol/min·mg·mitochondrial protein)	(n=4)	22.6±0.6	24.5±2.0	31.3±1.3**
RMR (ml/min·$BW^{0.75}$)	(n=6)	21.0±1.1	23.1±1.0	30.8±1.6*

Values are mean±SE. BW, body weight (kg). Significant difference between control and caffeine: * $p<0.05$; ** $p<0.01$.

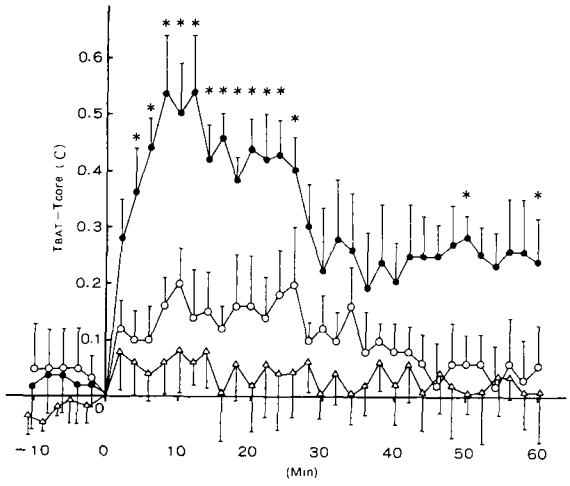

Fig. 1. Effect of intraperitoneal injection of caffeine and saline on changes in BAT temperature relative to core temperature ($T_{BAT}-T_{core}$). ●, caffeine (60 mg/kg) ($n=5$); ○, caffeine (40 mg/kg) ($n=5$); △, saline ($n=5$); $^*p<0.05$ (*vs.* saline); Values are mean±SE for 5 mice.

As shown in Figure 1, the mean changes in TBAT relative Tcore (TBAT-Tcore) after the intaraperitoneal injection of 40 mg/kg caffeine was slightly higher than that after the injection of saline. After the injection of 60 mg/kg caffeine, TBAT elevated from 37.1±0.1°C to 38.1±0.2°C, but Tcore elevated only from 37.1±0.1°C to 38.1±0.2°C. No significant differences in IBAT weight and mitochondrial protein content in IBAT were observed among control and caffeine (40 and 60 mg/kg) groups. GDP binding and oxygen consumption in IBAT mitochondria, and RMR in 60 mg/kg caffeine-injected group were significantly increased compared with those in the control group (Table 1).

3 - Effects of caffeine on RMR in healthy adult female

To select the optimal dose of caffeine, we measured the BMR and the RMR in ten non-obese healthy adult females (mean age; 25.5±1.2 years; mean body weight; 49.5±2.7 kg; mean body mass index (BMI); 19.8±0.9 kg/m^2; mean % body fat: 20.4±1.1 %; mean lean body mass (LBM): 39.4±2.1 kg). We used a closed-circuit indirect calorimeter that analyses oxygen alone (Sanborn-Wedge type spirometer; metabograph, Model SS-80; Fukuda Medical Laboratory Co., Tokyo, Japan) with a mouth piece, and the measurements were made in a temperature-controlled room (22 - 24 °C). The BMR was measured when the subjects were in the supine position, 30 min after they awoke in the morning. The subjects spent the night before each test at the university hospital, and the BMR values were obtained during a 10 min measurement period. After measurement of BMR, the ten subjects received physiological saline orally, and RMR was measured 30 and 60 min later in the same way as for BMR. On the second day of the experiment, the subjects received 4 mg caffeine/kg ideal body weight per os, dissolved in

physiological saline, and the RMR was measured again. One week later, a caffeine-loading test (8mg/kg ideal body weight) was performed per os.

As shown in Figure 2, in the ten healthy females the BMR was 3.65±0.27 kJ/min (3.31-40.02 kJ/min). The BMR/LBM was 0.093±0.009 kJ/min/kg (0.083-0.107 kJ/min/kg), and the RMR at 30 min after 4 mg/kg caffeine loading was significantly increased to 4.22±0.44 kJ/min (3.72-4.91 kJ/min) and to 4.29±0.41 kJ/min (3.68-5.08 kJ/min) 30 min after 8 mg/kg, compared with the baseline measurements of the control group given only physiological saline. The RMR at 60 min after caffeine loading was 4.06±0.47 kJ/min (3.61-4.88 kJ/min) and 4.45±0.48 kJ/min (3.60-5.46 kJ/min) for 4 mg/kg and 8 mg/kg caffeine loading, respectively. The within-subject coefficient of variation on the days that BMR was measured was 2.08±1.96 %. The between-day coefficient of variation was 6.49±0.63%. The thermogenic response to caffeine, measured at 30 min after caffeine loading at a dose of 4 mg/kg ideal body weight was 15.5±5.2% (8.4-23.4%), calculated by the following formula: $[(RMR_{30min\ after} - BMR)/BMR] \times 100\%$. Because the RMR at 30 min after caffeine loading at a dose of 4 mg/kg ideal body weight increased significantly and as the values at a dose of 4 mg/kg ideal body weight did not differ from those of 8 mg/kg ideal body weight, the caffeine loading test at 4 mg/kg ideal body weight was chosen as the optimal dose of caffeine and was measured in the subsequent experiment.

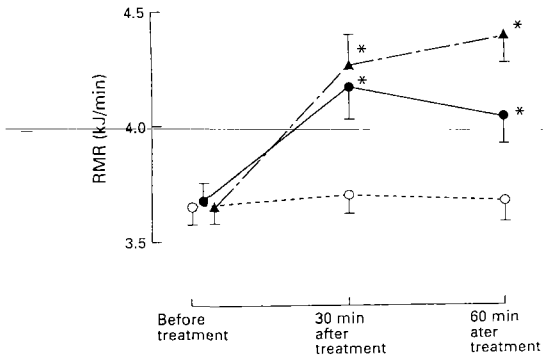

Figure 2 Effects of caffeine on resting metabolic rate (RMR) in ten lean control subjects: (▲--▲) caffeine (8 mg/kg ideal body weight, *per os*), (●—●) caffeine (4 mg/kg ideal body weight, *per os*), (O—O) control (physiological saline, *per os*). * $P < 0.05$ *vs.* control and before treatment.

4 - BMR, thermogenic response to caffeine and body weight loss following a combined low calorie diet and exercise treatment

We studied 136 women, assessed as obese (mean age: 42.6±1.4 years; mean duration of obesity: 4.6±1.7 years; mean body weight: 73.2±3.8 kg; mean BMI; 30.9±1.5 kg/m^2) who came to the out-patient clinic of our university hospital for treatment of their overweight condition. We also examined ten age-matched lean controls (mean age: 43.1±1.5 years; mean body weight: 50.4±2.6 kg; mean BMI; 21.5± 0.9 kg/m^2) (Table 2). Any obese or lean subjects who had complications such as diabetes mellitus and hypertension were excluded from this study. These women were all habitual drinkers of coffee or Japanese tea with a mild to moderate consumption (50-200 mg/day caffeine). However because heavy caffeine intake induces tolerance to at least some of the effects of caffeine (15), we excluded from the study those subjects who had a habitual intake of more than 300 mg caffeine per day (corresponding to three cups of coffee). The caffeine intake was assessed by the answers given to a questionnaire on the consumption of coffee, tea, Japanese tea, and cola beverages. None of the participants took any medications during the study. All subjects abstained from coffee and Japanese tea for 24 h before the initiation of each test. The BMR was measured in all subjects in a supine position at 30 min after they awoke in the morning. Then, caffeine (4 mg/kg ideal body weight) was given per os, and the RMR was measured 30 min after caffeine loading. The obese patients were asked to follow a daily 4184-5020 kJ diet (carbohydrate 60%, protein 23%, fat 17%)(104.6 kJ(25kcal)/kg ideal body weight) (16) and an exercise regimen (mean 10,000 steps/day: mean 1255 kJ (300 kcal/day) (16). They were asked to record their food intake and exercise, were seen weekly or every other week, and body weight was measured at each consultation. Skin fold thicknesses at the biceps, triceps, subscapular and supura-iliac regions were measured using calipers. The proportion of body fat (% body fat) was estimated from skinfold measurements, and lean body mass (LBM) was calculated by subtracting body fat from body weight. All data are given as means±s.d. Data were analyzed by one-way or two-way ANOVA. After justification by ANOVA, the Bonferroni t-test was performed. Single correlations were also calculated between the different variables.

In the 136 obese females, the BMR was 3.57 ± 0.46 kJ/min (2.61-4.27 kJ/min) and the BMR/LBM was 0.077 ± 0.011 kJ/min /kg LBM (0.054 - 0.097 kJ/min/kg LBM). The thermogenic response to caffeine was significantly increased to 4.06 ± 0.51 kJ/min (2.75 - 5.30 kJ/min) in terms of measured values and to 13.8 ± 6.2% (0.7 - 24.8%) in terms percentage values. However, these values for BMR, BMR/LBM and the thermogenic response to caffeine did not differ significantly from the values of the age-matched lean controls, which were 3.32 ± 0.11 kJ/min (3.18 - 3.59 kJ/min), 0.083 ± 0.009 kJ/min /kg LBM (0.071 - 0.094 kJ/min/kg LBM) and 15.2 ± 5.0% (9.6 - 23.0%), respectively, although these indices were widely spread among individual subjects. We analyzed the records of the subjects' food intake and exercise during

the two months of diet and exercise treatment. We found that they ate 5188 ± 172 kJ/day (1240 ± 41 kcal/day) and walked 8386 ± 170 steps/day. After the two-month treatment, body weight had significantly (P<0.001) decreased from 73.2 ± 3.8 kg to 69.4 ± 2.3 kg, and the BMI had decreased from 30.9 ± 1.5 kg/m^2 to 29.3 ± 1.0 kg/m^2 (P<0.001) , with percentage body fat also decreasing.

Table 2 Physical characteristics, BMR and thermogenic response to caffeine

	Age (years)	*Body weight (kg)*	*BMI (kg/m^2)*	*Ideal body weight (%)*	*% Body fat (%)*	*LBM (kg)*	*Duration of obesity (years)*	*BMR (kJ/min)*	*BMR/LBM (kJ/min/ kg LBM)*	*Thermogenic response to caffeine (%)*
Lean controls (n = 10)	43.1 ± 1.5	50.4 ± 2.6	21.5 ± 0.9	97.7 ± 1.0	20.7 ± 1.1	40.0 ± 1.6		3.32 ± 0.11	0.083 ± 0.009	15.2 ± 5.0
Obese subjects (n = 136)	42.6 ± 1.4	73.2 ± 3.8[a]	30.9 ± 1.5[a]	140.5 ± 3.5[a]	36.7 ± 1.4[a]	46.3 ± 1.4[a]	4.6 ± 1.7	3.57 ± 0.46	0.077 ± 0.011	13.8 ± 6.2
Obese group with BMR < 3.10kJ/min (n = 30)	42.9 ± 1.5	72.9 ± 3.8	30.8 ± 1.5	140.0 ± 3.9	36.7 ± 1.3	46.2 ± 1.5	4.8 ± 1.7	2.93 ± 0.16	0.065 ± 0.006	11.8 ± 5.6
Obese group with BMR ≥ 3.10kJ/min (n = 106)	42.5 ± 1.4	73.3 ± 3.8	30.9 ± 1.5	140.4 ± 4.5	36.7 ± 1.5	46.4 ± 1.4	4.6 ± 1.7	3.78 ± 0.30[c]	0.082 ± 0.086[c]	14.5 ± 6.3[b]
Obese group with thermogenic response to caffeine < 5.2% (n = 24)	42.8 ± 1.6	73.6 ± 2.9	31.0 ± 1.0	141.0 ± 3.6	36.8 ± 1.1	46.5 ± 1.3	4.8 ± 0.9	3.23 ± 0.33	0.069 ± 0.007	4.2 ± 1.1
Obese group with thermogenic response to caffeine ≥ 5.2% (n = 112)	42.6 ± 1.4	73.1 ± 3.9	30.8 ± 1.6	140.0 ± 5.7	36.7 ± 1.5	46.3 ± 1.5	4.6 ± 1.8	3.64 ± 0.45[d]	0.079 ± 0.011[d]	15.9 ± 4.8[d]

All data are presented as means ± s d.
[a] *P < 0.01 (lean controls vs. obese subjects);*
[b] *P < 0.05 (obese with BMR < 3.10 vs. obese with BMR ≥ 3.10);*
[c] *P < 0.01 (obese with BMR < 3.10 vs. obese with BMR ≥ 3.10);*
[d] *P < 0.01 (obese with thermogenic response < 5.2% vs. obese with thermogenic response ≥ 5.2%).*

Table 3 and Figure 3 show single correlations between body weight loss after two months and the different initial characteristics. The BMR, the BMR/LBM and the thermogenic response to caffeine were significantly correlated with reduction in body weight (r = 0.3621; r = 0.3196; r = 0.6943, respectively, P<0.001) (Figure 3). Furthermore, there was a significant correlation (r = 0.3156, P<0.001) between BMR and the thermogenic response to caffeine. However, there was no correlation between body weight loss and initial body weight , BMI, percentage body fat, LBM or duration of obesity. The variable that showed the best correlation coefficient with body weight loss was the thermogenic response to caffeine. When the criterion of a BMR less than 3.10 kJ/min (less than two standard deviations below the mean of the age-matched lean controls) was used to define an obese group with reduced BMR, there were 30 obese women in this category, and their body weights were significantly (P<0.05) decreased, from 72.8 ± 3.8 kg to 70.3 ± 3.5 kg, after two months of treatment. When the criterion of less than 5.2% of the thermogenic response to caffeine (less than two standard deviations below the mean of the age-matched lean control) was used to define an obese group with reduced thermogenic response to caffeine, there were 24 obese subjects in this

group. Their body weights were not reduced after treatment (73.6 ± 2.9 kg *vs.* 72.3 ± 2.4 kg). As shown in Table 2, there were no significant pre-treatment differences in age, duration of obesity, body weight, BMI, percentage body fat or LBM in these obese groups.

Table 3 Simple correlations between body weight loss after treatment and initial characteristics of obese subjects

Independent variable	*Correlation coefficient (r)*	*Statistical significance (P <)*
Body weight	0.1467	n.s.
BMI	0.1478	n.s.
Percentage body fat	0.1392	n.s.
LBM	0.1290	n.s.
Duration of obesity	0.0520	n.s.
BMR	0.3621	0.001
BMR/LBM	0.3196	0.001
Thermogenic response to caffeine	0.6943	0.001

n.s. = not significant (P > 0.05).

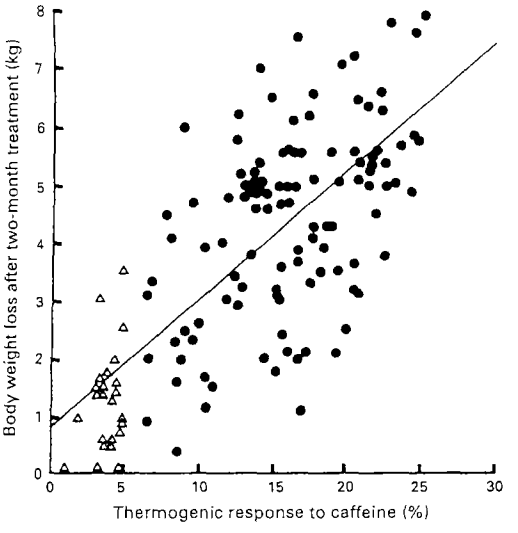

Figure 3 Correlation between thermogenic response to caffeine and body weight loss after treatment (n = 136): r = 0.6943 (P < 0.001), y = 0.2134 x +0.8460.
(Δ) *Obese group with reduced thermogenic response to caffeine* (< 5.2%, i.e. less than two standard deviations below the mean of age-matched lean controls). (●) Obese group with thermogenic response ≥ 5.2%.

Discussion

It is reported (17) that there is BAT in the perirenal and axillar regions even in humans as well as in rodents. However, because in humans it is difficult to examine BAT function directly, there is no report on obesity in patients caused by reduced BAT theremogenesis. Therefore, we first investigated in mice, whether caffeine could promote RMR via the activation of BAT function. After this hypothesis was verified (18), we measured RMR response to caffeine in obese women. Our results indicate that there were significant correlations in obese subjects between body weight loss and BMR, between body weight loss and BMR/LBM, and between body weight loss and the thermogenic response to caffeine. There was no correlation between body weight loss and duration of obesity, initial body weight, percentage body fat or LBM. However, we found that body weight loss was significant in an obese group with reduced BMR (30 obese subjects), while body weight loss did not occur in an obese group with reduced thermogenic response to caffeine (24 obese subjects), although initial body weight, percentage body fat and LBM did not differ between these two groups as

shown in Table 2. Furthermore, the coefficient of correlation (r = 0.6943) between body weight loss and thermogenic response to caffeine was much stronger than that between weight loss and BMR (r = 0.3621), indicating that the thermogenic response to caffeine would be a more appropriate index than BMR to predict body weight loss after treatment in obese women. This result also indicates that obese subjects who have a reduced thermogenic response to caffeine will find it difficult to reduce body weight with conventional diet and exercise treatment. Therefore, such obese subjects should be distinguished as having obesity with reduced thermogenesis as opposed to obese subjects with normal thermogenesis (19). Subjects with reduced thermogenesis may require treatment consisting of a very low calorie diet (20,21) or they may need a metabolic activator, such as ß3-adrenoceptor agonist (22,23,24,25) to activate BAT thermogenesis and stimulate lipolysis of white adipose tissue. Ravussin et al. (1), by measuring 24 h energy expenditure, found that a low rate of energy expenditure may contribute to obesity, and that the metabolic rate during rest in subjects who gained > 10 kg was significantly less than in subjects who did not gain weight. It seems that our present results coincide with their findings (1), although they believed that reduced metabolic rate was a cause of body weight gain, whereas we think of it as a cause of difficulty in losing body weight. The BMR and RMR values in Japanese women with reduced metabolic rates were much lower than those of the American Indians measured by Ravussin et al.(1)

The mechanism by which caffeine activates resting metabolism in rodents and humans (8,26), involves promotion of the release of catecholamines from the adrenal medulla (23,24) and inhibition of the action of cyclic AMP phosphodiesterase (29), thus increasing cellular cyclic AMP, which is a link between the activation of BAT cellular receptors and the oxidsation of fatty acids within BAT (30,31). It has also been reported that caffeine increased BAT cellularity and protein content (4), although the first study revealed that caffeine activates BAT thermogenesis. Furthermore, it has been reported that lactate and triglyceride production and increased vascular smooth muscle tone may be responsible for a major part of the thermogenic effect of caffeine (26). Although, in this experiment, we used caffeine to assess the rise in RMR in obese women, substances such as nicotine (32,33) and ß3-adrenoceptor agonists also activate resting metabolism. Therefore, to determine whether our findings for obese subjects are specific for caffeine, it would be necessary to carry out overload tests of these substances. It is also important to determine which organs and tissue, e.g. muscle, liver, digestive organs and BAT, are most responsible for causing reduced thermogenesis in these subjects, a topic which is still unclear.

In conclusion, this study showed that there were considerable individual variations in BMR and the thermogenic responses to caffeine in obese women, and that there were significant correlations between body weight loss and BMR, and between body weight loss and thermogenic response to caffeine. In the light of these findings, we suggest that a reduced response to caffeine would be a more appropriate index than BMR to predict body weight loss. Additionally, obese subjects with reduced thermogenesis in response to caffeine could find it

difficult to lose body weight with conventional treatment, and could thus require a very low calorie diet, possibly controlled by treatment with a metabolic activator.

Acknowledgment

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Abstract

Obese women with reduced thermogenic response to caffeine have difficulties in losing body weight

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Reduced function of brown adipose tissue (BAT), which is a main effector organ of diet-induced thermogenesis as well as of non-shivering thermogenesis, is regarded with keen interest as a cause of obesity in various experimentally obese animals. However, it is unclear whether there is obesity due to reduced thermogenesis in humans because we cannot measure BAT function directly in humans. To clarify this point, we examined 136 obese women (mean age: 42.6±1.4 years; mean duration of obesity: 4.6±1.7 years; mean body weight: 73.2±3.8 kg; mean BMI; 30.9±1.5 kg/m^2) by measuring the resting metabolic rate (RMR) before and 30 min after a caffeine [which activates BAT function and RMR in mice] overloading test at a dosage of 4mg/kg. The women were divided into a group with normal thermogenesis (106 patients, 77.9%) and a group with reduced thermogenesis (36 patients, 22.1%). Two months after a diet and exercise therapy, body weight, BMI and fat percentage were significantly reduced in the group with the normal thermogenesis, but almost unchanged in the group with the reduced thermogenesis. The thermogenic response to caffeine was markedly correlated with loss of body weight ($r = 0.6943$, $P<0.001$). These results suggest that there may be obesity due to reduced thermogenesis even in humans, and it should therefore be treatable with drugs (e.g. ß-3 adrenoceptor agonist) which activate thermogenesis, in addition to a diet and exercise therapy.

ANALYSIS OF COFFEE'S ACTION ON NEPHRITIS AND TUMOR THROUGH *IN VITRO* TESTING

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1. Introduction

The accumulation of extracellular matrix in glomeruli is the pathological feature of kidney disease. Transforming growth factor-beta (TGF-ß) has been reported to be a factor that is involved in the matrix accumulation; the accumulation of pathological matrix in experimental glomerulonephritis is attributed to overexpression of TGF-ß (Okuda *et al.*, 1990). Glomerular matrix production and matrix accumulation in the injured glomeruli are suppressed by administration of an antibody raised against TGF-ß to glomerulonephritic rats (Border *et al.*, 1990). One of the matrix components, the proteoglycan decorin, can bind TGF-ß, neutralize its biological activity (Yamaguchi *et al.*, 1990), inhibit the increased production of extracellular matrix, and attenuate manifestations of disease (Border *et al.*, 1992). These findings suggest that the inhibition of TGF-ß action may block further worsening of nephritis.

Tumor cells have two biological characteristics, that is, endless proliferation and metastasis. Some food components have been found to suppress both tumor growth (Yagasaki *et al.*, 1994) and metastasis (Taniguchi *et al.*, 1992) in animals with transplanted tumors.

Coffee contains caffeine, tannin, aroma constituents and so forth. It is therefore interesting to learn whether or not coffee can interfere with TGF-ß activity and suppress the proliferation and/or metastasis of tumor cells. In this monograph, we describe coffee's potentiality to modify these biological events.

2. Methods of investigating coffee's action

As a strategy to investigate coffee's action, we employed *in vitro* assay systems using cultured cells prior to *in vivo* study. This strategy may contribute to development of *in vitro* assay

systems for both replacement of experimantal animals and assessment of physiologically functional substances in foods and natural sources.

Inhibitory action of coffee on TGF-ß was evaluated by using mink-derived Mv1Lu cells (ATCC, CCL-64), their proliferation being inhibited by TGF-ß (Coimbra *et al.*, 1991). Briefly, Mv1Lu cells were maintained in high glucose DMEM with 10% fetal calf serum. The cells were seeded at $4X10^3$/0.1 mL in each well of a 96-well plate. After 1 hr, recombinant human TGF-ß1 (2 ng/mL, Takara Shuzo Co., Kyoto) alone or in combination with test substances were added, and cells were cultured for 22 hr. Maximum inhibition of Mv1Lu proliferation commenced to appear at a concentration of 2 ng/mL of TGF-ß (data not shown). Cells were then pulsed with 14.8 kBq/well of [methyl-^{3}H] thymidine (740 GBq/mmol, NEN Research Product, Boston, USA) for 2 hr. The cells were fixed with methanol-acetic acid (3:1), washed three times with 80% methanol, and lysed with 2 mM SDS/0.2N NaOH. Radioactivity in the cell lysate was counted, and TGF-ß activity was calculated.

Antitumor effect of coffee was evaluated by using an ascites hepatoma line of AH109A cells. The coffee's effect on AH109A proliferation was estimated by the method described previously (Yagasaki *et al.*, 1992) with a slight modification. Briefly, AH109A cells maintained in DM-160 with 10% newborn bovine serum were subcultured at $1X10^5$/0.6 mL in each well of a 24-well plate. They were cultured for 44 hr in the absence or presence of test substances. The cells were then given MTT reagent, and cultured for another 4 hr. After extracting formazan formed in AH109A cells with 0.04 N HCl/isopropanol, the difference between absorbance at 570 nm and 630 nm was measured. Control (no test substances) value was expressed as 100%. The coffee's effect on invasion, an important step of metastasis, was measured by co-culture system of AH109A with mesentery-derived cells (Akedo *et al.*, 1986). Briefly primary cultured cells (M cells) from mesentery of Donryu rats were seeded at $1.5X10^5$/3 mL in a 60 mm dish and cultured for 7~10 days to attain confluent state. AH109A cells ($2.4X10^5$) were then seeded on the monolayers and cultured without or with test substances for 48 hr. Invaded AH109A cells and colonies underneath M cell layers were measured by a phase-contrast microscope. Results were expressed as number of invaded cells and colonies/cm^2.

3. Results and discussion

Effect of coffee extract on human TGF-ß-induced inhibition of Mv1Lu proliferation is illustrated in Figure 1. Hot water extract of coffee (extracted from 10 g coffee with 150 mL boiled water) was sterilized by membrane filtration (0.2 μm), and added to experimental media at concentrations of 0~1%. TGF-ß stongly suppressed the proliferation of Mv1Lu in the absence of coffee extract. The TGF-ß-induced inhibition was partly but significantly canceled by coffee extract at concentrations of 0.1% and 0.3%, while the same concentrations of coffee extract exerted no significant influence on the proliferation of Mv1Lu cells without TGF-ß treatment (Figure 1, left). Relative changes from control (no coffee extract) assay are also shown (Figure 1, right). From these results, TGF-ß action is suggested to be interrupted in part by some component(s) contained in coffee extract.

Effect of coffee extract on the proliferation and invasion of AH109A cells is illustrated in Figure 2. In this case, coffee extract was concentrated to 10 times original concentration, and added to experimental media at concentrations of 0~1%. Coffee extract dose-dependently suppressed the invasion of AH109A cells underneath M cell layers (Figure 2, right), whereas it had no effect on the proliferation of AH109A cells (Figure 2, left). It is important to know whether or not effective component(s) would be absorbed from the gastrointestinal tract. Rats were given oral intubation of concentrated coffee extract (1 mL/100g body weight), blood was withdrawn 0, 0.5, 1, 2 and 3 hr

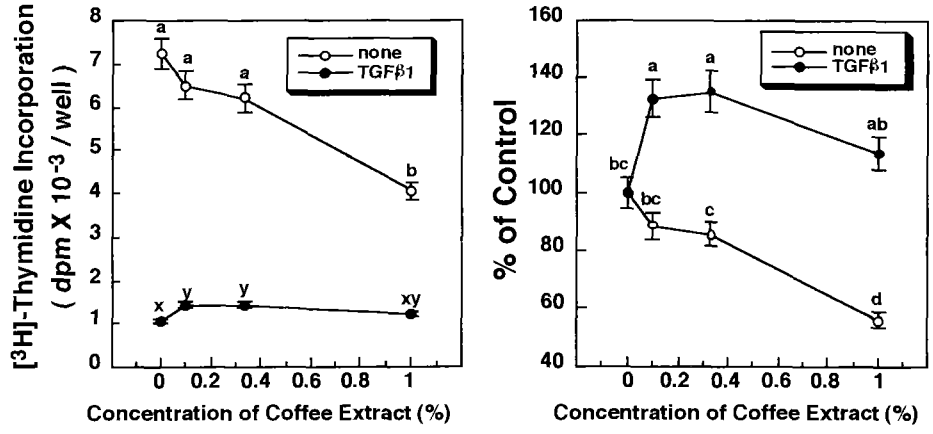

Figure 1. Effect of Coffee Extract on hTGF-β1-induced Inhibition of Mv1Lu Proliferation

Each value represents the mean ± SEM for 4 assays. Values not sharing a common letter are significantly different at $P < 0.05$ by Duncan's multiple-range test.

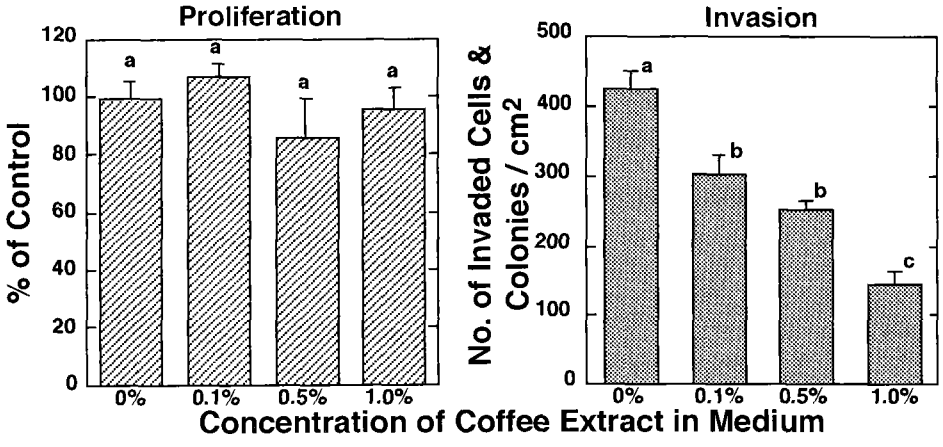

Figure 2. Effect of Coffee Extract on Proliferation and Invasion of AH109A.

Each value represents the mean ± SEM for 3 (proliferation) or 10 (invasion) assays. Values not sharing a common letter are significantly different at $P < 0.05$ by Duncan's multiple-range test.

after the intubation, and the effect of coffee-loaded rat serum on the proliferation and invasion of AH109A was tested *in vitro*. Coffee-loaded rat serum time-dependently suppressed the invasion of AH109A, while the same serum had no effect on the proliferation of AH109A (data not shown). Coffee contains caffeine, so we next tried to examine the action of caffeine, using concentrations equivalent to the amounts of the component contained in the experimental media shown in Figure 2. Caffeine had no effect on the proliferation but rather stimulated the invasion of AH109A (data not shown). These results suggest that caffeine may not be responsible for the coffee's inhibitory action on the invasion of AH109A cells.

From these *in vitro* results, coffee is suggested to possess potentiality to interfere with the action of TGF- ß and to suppress the invasion of hepatoma cells. Coffee is then expected to reduce glomerulonephritis and to suppress metastasis of tumor cells. *In vivo* studies should be done to clarify these aspects using animal models such as nephritic (Okuda *et al.*, 1990; Coimbra *et al.*, 1991; Fujisawa *et al*, 1995) and tumor-bearing (Taniguchi et *al.*, 1992; Yagasaki *et al.*, 1994; Miura *et al.*, 1995) animals.

4. Summary

Transforming growth factor-beta (TGF-ß) has been established to be a factor that aggravate glomerulonephritis by stimulating extracellular matrix production and its accumulation in the kidney. This suggests that the inhibition of TGF-ß action may suppress further worsening of nephritis. Tumor cells have two biological characteristics, *i.e.*, endless proliferation and metastasis. It is interesting to know whether or not coffee can interfere with TGF-ß activity and suppress the proliferation and/or metastasis of tumor cells. We have examined these points using *in vitro* assay systems. Inhibitory action of coffee on TGF-ß activity was evaluated by using mink-derived Mv1Lu cells, their proliferation being inhibited by TGF-ß. Antitumor effect of coffee was evaluated by using rat ascites hepatoma line of AH109A cells. The effect on AH109A proliferation was measured with the MTT method, while that on invasion, an important step of metastasis, was measured by co-culture of AH109A with mesentery-derived cells (M cells). Hot water extract of coffee was sterilized by membrane filtration and added to experimental media. Coffee was found to weakly interfere with TGF-ß action and to suppress the invasion of AH109A underneath M cell layers. Effective component(s) might be absorbed across the gut, since serum from coffee extract-loaded rats also inhibited the invasion. Of components present in coffee extract, caffeine seems not to be responsible for the inhibitory action of coffee on invasion. From these *in vitro* results, coffee is expected to reduce nephritis and to suppress tumor metastasis. *In vivo* studies using animal models such as nephritic and tumor-bearing animals will clarify the anticipation.

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INSTRUMENTAL AND SENSORY ANALYSIS OF COFFEE VOLATILES

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1 Introduction

Studies on the volatile components causing the unique flavor of roasted coffee have been carried out since more than one hundred years. According to my knowledge, Bernheimer (1880) was the first who reported the identification of some coffee volatiles, e.g. methylamine, pyrrole.

However, the first exhaustive research on the aroma of coffee was performed by Reichstein and Staudinger in the years 1921-30. They isolated a yellow-colored oil from large quantities of roasted and ground coffee and identified in the oil over 70 substances by the preparation of crystalline derivatives, and by chemical analysis (Reichstein and Staudinger, 1955). The authors maintained that not a single one of the substances identified causes the coffee flavor. However, they emphasized that a highly diluted aqueous solution of 2-furfurylthiol "exhales a pleasant note indicative of coffee".

It is well-known that the introduction of capillary gas chromatography (GC) and mass spectrometry (MS) has stimulated flavor research very strongly. Numerous papers on the composition of coffee volatiles have been published in which the identification of more than 800 compounds has been reported (for overviews see Flament, 1989, 1991; Maarse and Visscher, 1992). At this time, however, the question, which of these volatiles stimulate the characteristic flavor of roasted coffee, was not answered.

Calculation of the ratio of the concentration of a volatile compound to its odor threshold (Rothe and Thomas, 1963) was the first approach to show the importance of a compound to the flavor of a particular food. The result is denoted aroma value, odor unit or odor activity value (OAV) (for overview see Grosch, 1993).

On the basis of this odor unit concept, Tressl (1989) confirmed 2-furfurylthiol as key aroma compound of roasted coffee and suggested, in addition, that 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-ethyl-3,5-dimethylpyrazine, 2-/3-methylbutanal, diacetyl, guaiacol, 4-vinylguaiacol and some other volatiles were important contributors to the coffee flavor.

Subsequently, based on the odor unit concept of Rothe and Thomas (1963), a procedure has been developed for the analysis of food flavors (Grosch, 1993; Grosch, 1994; Guth and Grosch, 1994). The procedure consists of the following steps:

- Localization of the potent odorants in capillary gas chromatograms of the volatile fraction by CHARM analysis (Acree et al., 1984) or by aroma extract dilution analysis (AEDA; Ullrich and Grosch, 1987).
- Identification of these potent odorants.
- Quantification and calculation of the OAV.
- Sensory studies of aroma models.

It is shown in the following that this procedure reveals the character impact odorants of coffee flavors.

2 Screening for potent odorants

Potent odorants of coffee were screened by AEDA and gas chromatography/olfactometry of headspace samples (GCO-H).

AEDA of the roasted powder from Arabica coffee was performed by Holscher et al. (1990, 1991) and by Blank et al. (1992). The results of both screening experiments are in agreement with regard to the odorants which have to be taken into account as important contributors to the aroma of coffee. In particular, the high flavor dilution (FD)-factors reported by the authors for 3-mercapto-3-methylbutylformate, 2-furfurylthiol, methional, 2-ethyl-3,5-dimethylpyrazine, 3-isobutyl-2-methoxypyrazine, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone and ß-damascenone suggest that these odorants belong to the key components of the coffee flavor.

AEDA is limited to odorants boiling higher than the solvent used for the extraction and dilution steps. Furthermore, odorants boiling in the same range as the extraction solvent might be partially lost during the concentration of the extract by distilling off the solvent. To overcome

these limitations, AEDA was completed by GCO-H (Holscher and Steinhart, 1992; Semmelroch and Grosch, in press).

The results obtained by GCO-H of the powders and the brews prepared from roasted Arabica (*Coffea arabica*) and Robusta coffees (*Coffea canephora var. robusta*) are summarized in Table 1.

On the basis of their high FD-factors, 2,3-butanedione, 2,3-pentanedione, 3-methyl-2-buten-1-thiol, methional, 2-furfurylthiol and 3-mercapto-3-methylbutylformate were the key highly volatile odorants of both powders, and might be responsible for the top notes of the coffee aroma. The FD-factors of these and of the odorants **1** to **4**, **6**, **7**, **21**, **22** and **30** were identical in both coffee samples, as they differed, at the most, by only one dilution step, which is within the limit of error of the GCO-H.

In contrast, clear differences in the odor potencies were found for 2-methyl-3-furanthiol, 2,3-diethyl-5-methylpyrazine and the unknown **23**, which belong to the key odorants of only the Robusta coffee powder (Table 1).

The results listed in Table 1 indicate that the powders and the brews agreed with regard to compounds **5** and **8** as major odorants. The well-known, strong difference between the overall odours of the powders and the brews was mainly due to a decrease of the FD-factors of the thiols **2**, **9**, **11**, **13** and **20** as well as to an increase in the FD-factors of acetaldehyde, propanal, methylpropanal, 3-methylbutanal and dimethyltrisulphide in the brews. Furthermore, the guaiacols **28** and **29** were detectable in the brew of Robusta coffee, and **29** also in that of Arabica coffee.

3 Quantitative data, odor activity values

AEDA and GCO-H are only screening procedures, because the results are not corrected for the losses of the odorants during both, the isolation procedure and the capillary gas chromatography. Furthermore, in the dilution experiments the odorants are completely volatilized and then evaluated by sniffing, whereas the volatility of the compounds in coffee depends on interactions with water and with the non-volatile components. For these reasons, quantification of odorants and calculation of OAVs are the required, next steps to correct the simplifications implicit in the dilution experiments.

The odorants were quantified by using the corresponding odorants labelled with a stable isotope (deuterium or carbon-13) as internal standards. This technique, which is denoted "stable isotope dilution assay", provides the most accurate data since, apart from small isotope effects, the labelled standards have chemical and physical properties identical to those of the odorants to be quantified (Grosch, 1993).

Table 1. GCO-H of roasted coffee powders and brews[a]

No.	Compound[b]	FD-factor			
		Powder[c]		Brew[d]	
		Arabica	Robusta	Arabica	Robusta
1	Acetaldehyde	25	25	125	62.5
2	Methanethiol	5	12.5	1	1
3	Propanal	5	5	25	25
4	Methylpropanal	5	5	25	25
5	2,3-Butanedione	62.5	125	125	125
6	3-Methylbutanal	12.5	25	62.5	62.5
7	2-Methylbutanal	5	12.5	25	25
8	2,3-Pentanedione	125	125	125	62.5
9	3-Methyl-2-buten-1-thiol	62.5	62.5	5	5
10	Unknown	n.d.	5	n.d.	n.d.
11	2-Methyl-3-furanthiol	25	125	5	5
12	Methional	62.5	62.5	25	25
13	2-Furfurylthiol	62.5	125	12.5	12.5
14	Unknown	n.d.	5	n.d.	n.d.
15	Dimethyltrisulphide	n.d.	1	12.5	25
16	1-Octen-3-one	n.d.	n.d.	5	1
17	Unknown	1	25	n.d.	1
18	Unknown	n.d.	n.d.	5	5
19	Unknown	n.d.	n.d.	5	5
20	3-Mercapto-3-methylbutylformate	62.5	62.5	1	1
21	2-Ethyl-3,5-dimethylpyrazine	25	62.5	25	62.5
22	Guaiacol	12.5	25	25	12.5
23	Unknown	25	125	25	25
24	2,3-Diethyl-5-methylpyrazine	25	125	25	125
25	Unknown	12.5	62.5	1	25
26	2-Isobutyl-3-methoxypyrazine	25	1	125	1
27	Unknown	1	5	1	5
28	4-Ethylguaiacol	n.d.	n.d.	n.d.	5
29	4-Vinylguaiacol	n.d.	n.d.	5	25
30	(E)-ß-Damascenone	5	5	n.d.	n.d.

[a] Source: Semmelroch and Grosch (in press).

[b] The compounds which appeared in one of the coffee samples with an FD-factor of at least five are compared.

[c] The coffee powder (100 mg) in a closed vessel of 250 mL was analysed at room temperature.

[d] Hot water (1.1 L) was filtered through the coffee powder (54 g); the coffee brew (10 mL, 70°C) in the closed vessel (250 mL) was analysed by GCO-H.

n.d.: not detectable in the highest headspace volume of 25 mL which corresponds to an FD-factor of one.

Table 2. Concentrations (mg/kg) and odor activity values (OAVs) of the odorants in roasted powders of Arabica and Robusta coffees[a]

No.	Odorant	Arabica[b]		Robusta[b]	
		Conc.	OAV[c]	Conc.	OAV[c]
1	2-Furfurylthiol	1.08	$1.1x10^5$	1.73	$1.7x10^5$
2	Methional	0.24	$1.2x10^3$	0.095	$4.8x10^2$
3	3-Mercapto-3-methylbutylformate	0.13	$3.7x10^4$	0.115	$3.3x10^4$
4	3-Methyl-2-buten-1-thiol	0.0082	$2.7x10^4$	0.0083	$2.8x10^4$
5	Guaiacol	4.2	$1.7x10^3$	28.2	$1.1x10^4$
6	4-Ethylguaiacol	1.63	$3.0x10^1$	18.1	$3.6x10^2$
7	4-Vinylguaiacol	64.8	$3.2x10^3$	177.7	$8.9x10^3$
8	Vanillin	4.8	$1.9x10^2$	16.1	$6.5x10^2$
9	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	109.0	$1.1x10^4$	57.0	$5.7x10^3$
10	5-Ethyl-4-hydroxy-2-methyl-3(2H)-furanone	17.3	$1.5x10^4$	14.3	$1.2x10^4$
11	3-Hydroxy-4,5-dimethyl-2(5H)-furanone	1.47	$7.5x10^1$	0.63	$3.0x10^1$
12	5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone	0.16	$2.0x10^1$	0.085	$1.0x10^1$
13	2-Ethyl-3,5-dimethylpyrazine	0.33	$1.7x10^2$	0.94	$4.7x10^2$
14	2,3-Diethyl-5-methylpyrazine	0.095	$1.0x10^2$	0.31	$3.0x10^2$
15	2-Isobutyl-3-methoxypyrazine	0.083	$1.7x10^4$	0.012	$2.4x10^3$
16	2-Isopropyl-3-methoxypyrazine	0.0033	$8.3x10^2$	0.0014	$3.5x10^2$
17	(E)-ß-Damascenone	0.195	$2.6x10^5$	0.205	$2.7x10^5$
18	2,3-Butanedione	50.8	$3.4x10^3$	47.8	$3.2x10^3$
19	2,3-Pentanedione	39.6	$1.3x10^3$	19.8	$6.6x10^2$

[a] Source: Semmelroch et al (1995), Semmelroch and Grosch (in preparation).

[b] The Arabica coffee was from Colombia and the Robusta coffee from Indonesia.

[c] The OAV was obtained by dividing the concentration of the compound by the odor threshold (Table 3).

The amounts of 19 odorants found in powders of roasted Arabica and Robusta coffees are listed in Table 2. The Robusta coffee contained more 2-furfurylthiol with 1.73 mg/kg than the Arabica with 1.08 mg/kg. Analysis of two other samples (Grosch et al., 1994) showed the concentrations of 1.96 mg/kg (Robusta) and 1.71 mg/kg (Arabica).

The sample of Arabica (Table 2) was much higher in methional (no. **2**), the furanones **9**, **11** and **12**, as well as in 2-isobutyl-3-methoxypyrazine (**15**)

Table 3. Odor threshold of coffee volatiles in water

No.	Compound	Threshold (μg/L)
1	2-Furfurylthiol	0.01
2	Methional	0.2
3	3-Mercapto-3-methylbutylformate	0.0035
4	3-Methyl-2-buten-1-thiol	0.0003
5	Guaiacol	2.5
6	4-Ethylguaiacol	50
7	4-Vinylguaiacol	30
8	Vanillin	25
9	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	10
10	5-Ethyl-4-hydroxy-2-methyl-3(2H)-furanone	1.15
11	3-Hydroxy-4,5-dimethyl-2(5H)-furanone	20
12	5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone	7.5
13	2-Ethyl-3,5-dimethylpyrazine	2.0
14	2,3-Diethyl-5-methylpyrazine	1.0
15	2-Isobutyl-3-methoxypyrazine	0.005
16	2-Isopropyl-3-methoxypyrazine	0.004
17	(E)-ß-Damascenone	0.00075
18	2,3-Butanedione	15
19	2,3-Pentanedione	30
20	Propanal	10
21	Methylpropanal	0.7
22	2-Methylbutanal	1.3
23	3-Methylbutanal	0.35
24	Methanethiol	0.2

Source: Semmelroch et al. (1995); Semmelroch and Grosch (in preparation).

and 2,3-pentanedione (**19**). On the other hand, the phenolic odorants **5** to **7** and the trialkylated pyrazines **13** and **14** predominated in the Robusta sample. Earlier published quantitative data (Tressl, 1989; Heinrich and Baltes, 1987), obtained with conventional methods, agreed in so far as higher levels of phenolic compounds and of the furanones **9** and **10** in Robusta and Arabica samples, respectively, had been reported. However, most of the concentration levels found by means of conventional methods are lower than those reported here. Furthermore, it is very difficult to quantify accurately with conventional methods the odorants occurring in very low concentration levels in coffee and the labile sulfur compounds, e.g. **1** to **4** in Table 2.

Table 4. Concentrations (μg/L) and odor activity values (OAVs) of eight most potent odorants in brews prepared from Arabica and Robusta coffees[a]

No.	Odorant	Arabica[b]		Robusta	
		Conc.[b]	OAV[c]	Conc.[b]	OAV[c]
1	2-Furfurylthiol	19.1	1910	39.0	3900
2	(E)-ß-Damascenone	1.3	1730	1.5	2000
3	3-Mercapto-3-methylbutylformate	5.5	1570	4.3	1230
4	3-Methylbutanal	550	1570	925	2640
5	Methylpropanal	800	1140	1380	1970
6	Methanethiol	210	1050	600	3000
7	5-Ethyl-4-hydroxy-2-methyl-3(2H)-furanone	840	730	670	580
8	2-Methylbutanal	650	500	1300	1000

Source: Semmelroch and Grosch (in preparation).

[a] The brews (1 L) were prepared by filtration of hot water (1.1 L, ca. 95°C) through the coffee powder (54 g).

[b] The concentration was determined by stable isotope dilution assays.

[c] The OAV was calculated by dividing the concentration of the compound by the odor threshold (Table 3).

Under the assumption that it belongs to the typical odorants of Robusta coffee (Vitzthum et al., 1990), 2-methylisoborneol (MIB) was determined by a stable isotope dilution assay in various Robusta and Arabica coffee samples (Bade-Wegner et al., 1994). MIB levels in the Robusta coffees (0.12-0.43 μg/kg) were consistently higher than in the Arabicas (<0.02 μg/kg). However, sensory studies with brews from Arabica coffee, to which MIB had been added, did not confirm the significance of MIB for the coffee flavor (Rouge et al., 1994).

OAVs were calculated to get a first insight into the importance of the odorants quantified for the aroma of the coffee samples. The calculation of the OAVs is based on the odor thresholds listed in Table 3. A comparison of the data indicates that the thiols **1**, **3**, **4**, ß-damascenone (**17**) and the pyrazines **15** and **16** have the lowest threshold values. Therefore, it is not surprising that most of these compounds (**1**, **3**, **4**, **15** and **17**) belong to the odorants showing the highest OAVs at least in one of the coffee samples (Table 2). In addition, guaiacol (**5**) in particular in the Robusta coffee, as well as furanone **9** in the Arabica coffee and furanone **10** in both coffee samples are very potent odorants on the basis of their high OAVs.

Table 5. Odor profiles of Robusta coffee brew and its model[a]

Attribute	Robusta coffee	
	brew	model R
Roasty/sulfury	2.7	2.3
Earthy/musty	2.4	1.7
Sweetish/caramel	1.2	1.3
Buttery	0.7	1.5
Green/peasy	1.0	0.7
Smoky	2.6	2.7

Source: Semmelroch and Grosch (in preparation).

[a] The intensity of the odor notes was scored at room temperature on the scale 0 (absent) to 3 (strong). The results obtained by six panelists were averaged.

Brews were prepared from the Arabica and Robusta coffees, cooled to room temperature, then spiked with the labelled internal standards and finally extracted with a solvent. The odorants were quantified in the extracts by mass chromatography.

The most potent odorants of the brews on the basis of OAVs are listed in Table 4. The thiols **1** and **3**, ß-damascenone (**2**) as well as the *Strecker* aldehydes **4** and **5** showed the highest OAVs in both brews. However, the ranking of the potent odorants was different. The sequence in the Arabica coffee brew was **1** followed by **2** to **6**, whereas in the Robusta coffee brew **1** was followed by **6**, **4**, **2**, **5** and **3**.

4 Sensory study

To verify whether the odorants quantified are indeed the key odorants of the Robusta coffee brew, a corresponding model was prepared. In the concentration levels found in the Robusta coffee brew a total number of 24 odorants was dissolved in water. After adjustment of pH 6.0 the odor profile of this model was compared at room temperature with that of the original brew.

The overall odor of the model was described by the panelists as clearly coffee-like. Nevertheless, there were some differences to the odor profile of the Robusta coffee brew (Table 5). In particular, the earthy/musty note was weaker and the buttery note was more intensive in the model.

However, two unknown earthy odorants (nos. **23** and **25** in Table 2) were perceived with high FD-factors during GCO-H of the coffee powders and brews. Possibly, these odorants, which have been also detected by AEDA (Blank et al., 1992), are necessary to intensify the earthy/musty odor note in the model. Furthermore, their presence might reduce the intensity of the buttery note in the odor profile of the model.

5 Conclusion

The progress in aroma research has led to the identification of most of the key odorants of roasted Arabica and Robusta coffee. These compounds are suitable as indicators to objectify flavor differences caused by e.g. the raw materials, green coffee processing, roasting, grinding and storage. Methods for an accurate quantification of the indicators are available.

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Summary

The potent odorants of roasted Arabica (*coffea arabica*) and Robusta coffees (*Coffea canephora, var. robusta*) were screened by aroma extract dilution analysis and then quantified in powders and brews by stable isotope dilution assays. An aqueous solution of the odorants at the same concentration levels, which had been found in the brew of Robusta coffee, were judged to meet very well the overall odor of the brew.

MATERIAL BALANCE IN ROAST AND GROUND AND BREWED COFFEE

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Introduction

Roast and ground coffee is a complicated matrix of cellular remnants and oil droplets left behind by the combination of pyrolysis and combustion that is coffee roasting. There has been considerable effort to maximize the retention of the volatile aromas of coffee through the technology of roasting, grinding and packing, and the design of the equipment used in the plant for these processes. Of some interest in these schemes is the efficiency of the retention, that is, what is the effect on the overall retention of a change in process design. Headspace aroma measurements are relatively simply performed, and give an accurate measure of the delivery of aroma when the package is opened, but quantitation of the retention of aroma has required more difficult analyses. This work will address the measurement of the distribution of aroma between headspace and bulk in roast and ground coffee, and between the brew and the cup headspace, and will present a simple headspace method for the quantitation of total light volatiles. The samples considered are commercial blends in current use in the US market, and some experimental samples prepared with different levels of retained aroma.

Methods and Experimental

Some measurements of the total aroma in roast and ground coffee can be found in the literature (1,2), done by standards addition to coffee brews. This method is relatively arduous. Other experimental examples of the quantitation of aroma volatiles have been performed using exhaustive distillation and recovery (3,4); these methods often lead to artifacts, and also do not directly address the question of distribution. One or other of these more stringent method is necessary for full recovery of some of the more tightly bound aroma components. This work will focus on the lighter volatiles known to be of importance in the consumer perception of coffee quality (2,5).

Dry R&G Headspace The most direct headspace method of quantitation of total aroma in coffee would be the exhaustive stripping of the aroma through a gas sweep, and indeed this procedure was attempted.

However, the process is very slow, and substantial amounts of water are recovered as well. In the absence of a means of strict separation of the ubiquitous water from the swept material, quantification of the aroma in the collected sweep gas is impossible. In order to avoid these difficulties, and in addition to measure the distribution of individual species, we have used an extrapolative technique, based on the assumption of Henry's-Law behavior in the sorbed aromas. Samples of 0.5, 5, 10, 15 and 35g were placed in 120cc vials sealed with septa, and a gas chromatogram of the headspace taken. It was found that plots of weight/headspace concentration *vs* weight were quite linear over the whole range, as shown in Figure 1. As the weight in the vial approaches zero, and the fraction of aroma in the headspace approaches 1, we can calculate the initial aroma concentration in the solid from the parameters of the straight line. The procedure follows from the assumption of Henry's Law. For Henry's Law sorption of an aroma species, the amount of aroma sorbed in the solid is linear in the partial pressure

1... $m_{s,i} = m_c K_i c_i$

where $m_{s,i}$ is the mass of aroma component i sorbed, in μg
m_c is the mass of coffee, g
K_i is the Henry's Law constant for the i^{th} component, in $cm^3.g^{-1}$
and c_i is the concentration of the i^{th} component in the headspace, in $\mu g.cm^{-3}$

The amount of the i^{th} component in the headspace is

2... $m_{hs,i} = c_i V$

where $m_{hs,i}$ is the amount of the i^{th} component in the headspace, in μg
and V is the headspace volume

The total amount of the i^{th} component is that brought into the vial with the solid, given by the concentration in the solid, which is what we wish to know:

3... $m_{total,i} = m_c X_i$

where X_i is the concentration of the i^{th} component in the solid originally.

Since the total amount of the aroma component is equal to the sum of the amount in the headspace and that sorbed in the solid at equilibrium, the amount present when packed can be determined from

4... $m_c X_i = m_c K_i c_i + c_i V$

which can be rearranged to give

5... $$\frac{m_c}{c_i} = m_c\left[\frac{K_i}{X_i}\right] + \frac{V}{X_i}$$

Now, when the headspace concentration data from gas chromatography are plotted as m_c/c_i *vs* m_c, as seen in Figure 1, the parameters K_i and X_i can be determined from the slope and intercept.

Once the amount of aroma contained in the solid is known, the ratio of headspace aroma to aroma held in the solid can be calculated. A typical package (vacuum bag) in the US market contains 369g in a total package volume of about 850cc. Scaled to the vials used, this is equivalent to 54g/120cc in our experiment. Using the

equations above, the ratio of aroma in the headspace to that in the solid in the package is

$$6... \quad \frac{c_i V}{m_c X_i} = \frac{V}{m_c K_i + V}$$

Brew Headspace The distribution of aroma between the brew and the headspace above it was done by the method of standards addition. Solutions of eight important headspace compounds were made in acetone, and dosed into a brew sample at two different levels. Brew solutions were prepared by adding zero, one or two 1-mL doses of the standard solution to enough 80°C water to make a final volume of 50mL, then adding this solution and a THF internal standard to 2.5g of R&G and sealing it in a 120cc vial thermostatted at 80°C. After 5 minutes, the solution headspace was sampled into a gas chromatograph. The results were all normalized to the internal standard concentration, and a normalized increment per mL of added standard solution calculated according to the formula

$$7... \quad NI_{mL,1} = \left[\frac{A_{i,j}}{A_{IS,j}} - \frac{A_{i,ctl}}{A_{IS,ctl}} \right] \cdot \frac{1}{V_j}$$

where i refers to the i^{th} compound
j is the j^{th} standard addition (added volume amount)
ctl refers to the control, *e.g.*, no addition
and V_j is the volume added in the j^{th} addition, *e.g.*, 1 or 2mL

Absolute solution concentrations could then be determined by considering that the amount of solute in the solution added is equal to

$$8... \quad NI_{\mu,i} = NI_{mL,i} c_i$$

The concentration of that solute in the brew solution is then

$$9... \quad c_{i,brew} = NI_{\mu,i} / V_{brew}$$

Results

Headspace Aroma -- Table 1 shows the bulk concentrations and their standard errors, and the sorption parameters for the compounds studied. The samples studied are two standard US domestic products from

Table 1

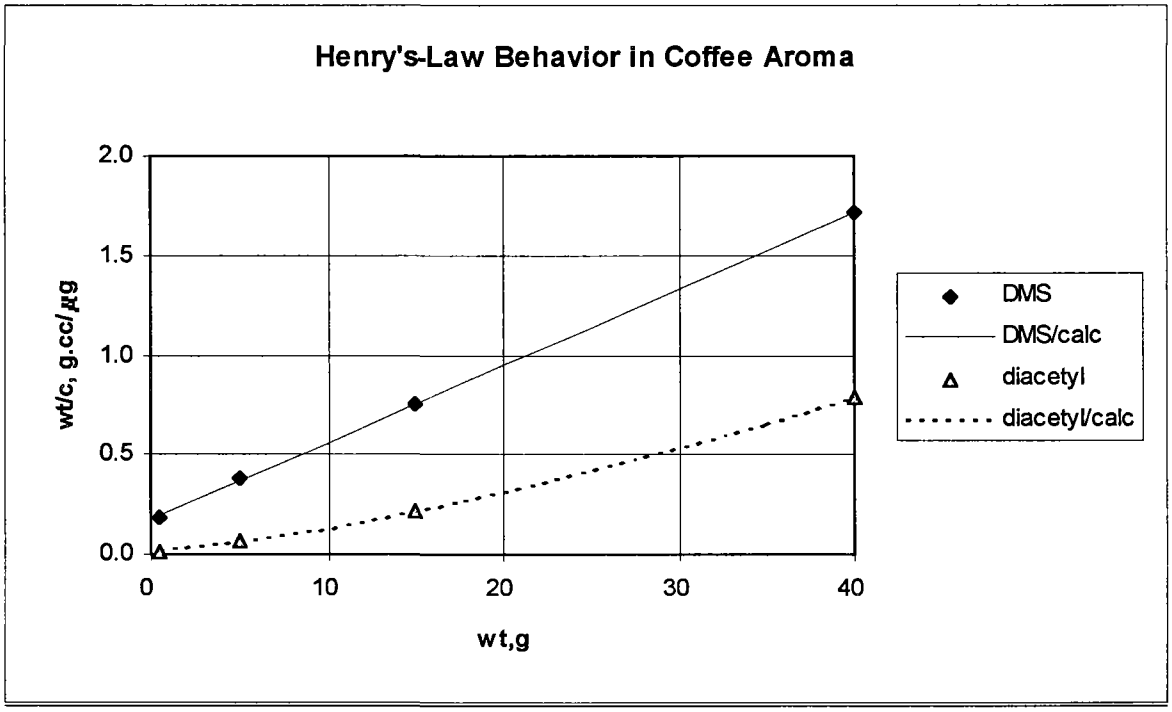
	US-A			US-B			Expt-Typical			Expt-Gentle		
	X, µg/g	s(X)	K, cm³/g	X, µg/g	s(X)	K, cm³/g	X, µg/g	s(X)	K, cm³/g	X, µg/g	s(X)	K, cm³/g
DMS	0.8	0.3	45	0.5	0.4	40	0.7	0.1	26	1.3	0.4	22
Me-Ac	4.5	1.5	113	3.4	1.9	79	0.3	0.1	30	0.9	0.7	42
IBA	13.8	2.9	54	10.8	4.5	56	18.0	2.5	87	26.1	9.4	86
Diacetyl	20.4	20.7	549	15.3	12.9	244	-4.0	8.5	-79	23.1	29.0	235
2Bu-one	7.3	1.9	292	5.1	3.8	200	-1.8	6.6	-189	2.7	5.3	146
2MeFuran	15.0	1.9	58	16.4	6.1	62	37.3	36.2	140	36.3	20.4	89
IVA	10.2	1.6	87	9.2	3.7	100	1000	87000	10700	23.9	26.8	176
2,3Pe-dione	7.9	2.8	234	8.7	3.9	209	-3.0	5.5	-86	47.0	327.0	867

two different manufacturers, labeled US-A and US-B; there are in addition to experimental samples, one produced under normal plant conditions of hold-up and temperature, and the other produced under gentle-handling conditions.

The two US domestic R&G samples agree quite well in their aroma content, and it can be seen that the change in process effected in the experimental samples was successful in increasing the retention of aroma.

The table shows as well (through negative or extremely high values of X) that certain of the headspace compounds are not well-fit by the linear Henry's-Law model, notably diacetyl, 2-butanone and 2,3-pentane dione. Figure 1 illustrates the difference between the behavior of the compounds which follow Henry's Law and those which don't; the curvature is essentially the same in plots of all of the three compounds (diacetyl, 2-butanone, and 2,3-pentane dione) which fail to follow Henry's Law, and the typical result is a negative calculated value of the intercept.

Figure 1



Aroma Concentrations from Standards Additions -- The measurements of aroma concentration *via* standards addition yielded results reasonably similar to those derived from the R&G headspace alone. Table 2 shows the results, and Figure 2 shows the ratios between the values calculated according to the two methods.

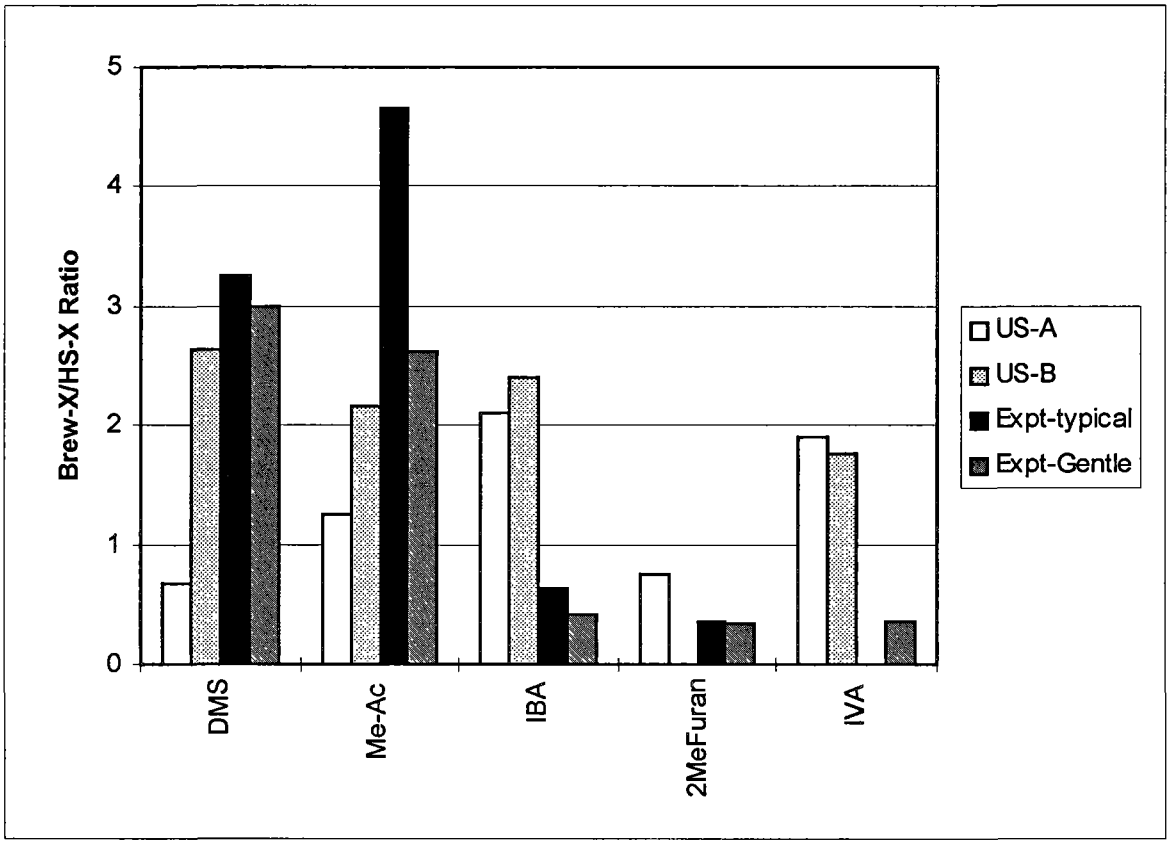
Table 2

	US-A			US-B			Expt-Typical			Expt-Gentle		
Cmpd	X(hs), µg/g	X(brew), µg/g	X(brew)/ X(hs)	X(hs), µg/g	X(brew), µg/g	X(brew)/ X(hs)	X(hs), µg/g	X(brew), µg/g	X(brew)/ X(hs)	X(hs), µg/g	X(brew), µg/g	X(brew)/ X(hs)
DMS	0.8	0.5	0.7	0.5	1.4	2.6	1.4	4.4	3.2	2.7	7.9	3.0
Me-Ac	4.5	5.7	1.3	3.4	7.3	2.2	0.7	3.2	4.7	1.7	4.4	2.6
IBA	13.8	28.9	2.1	10.8	25.8	2.4	35.9	22.8	0.6	52.1	21.9	0.4
Diacetyl	20.4	18.6	0.9	15.3	35.1	2.3	-7.9	23.8	-3.0	46.2	25.2	0.5
2Bu-one	7.3	6.5	0.9	5.1	7.7	1.5	-3.6	6.7	-1.9	5.3	7.3	1.4
2MeFuran	15.0	11.4	0.8	16.4	-508	-31.0	74.5	26.7	0.4	72.7	25.0	0.3
IVA	10.2	19.6	1.9	9.2	16.1	1.8	2000	19.2	0.0	47.7	17.4	0.4
2,3Pe-dione	7.9	17.2	2.2	8.7	27.6	3.2	-5.9	22.1	-3.7	94.0	20.6	0.2

The agreement between the aroma content calculated from the headspace and that from standards additions to the brew is reasonable, considering the very different nature of the expression of the aroma into headspace and brew; most of the values are within a factor of 2 of each other. In most cases the lighter volatiles seem

better expressed into the brew than into the headspace. Here too, it can be seen that the behavior of the species which don't obey Henry's Law is much more regular.

Figure 2



Discussion

The results seen here suggest that it is only a few of the lightest aroma compounds which show a Henry's-Law type of sorption in the coffee. For this small number of the important aroma compounds studies here, a simple headspace technique can determine the total retention of these compounds in the coffee. With a few exceptions, the standards additions to the brew give results consistent with others seen in the literature, and must remain a preferred method. Although the headspace analysis fails to give results useful for calculating the total amount of several of these volatiles, the behavior of the curves does give a little more information about the nature of the sorption for these species. The curvature of the plots of wt/c *vs* wt in all of the plots which are non-linear is directed upwards; this implies that the curvature of the sorption isotherms for these compounds in coffee is likewise positive. This behavior does not derive from simple solution behavior, and illustrates that the compounds are present at far less than saturation.

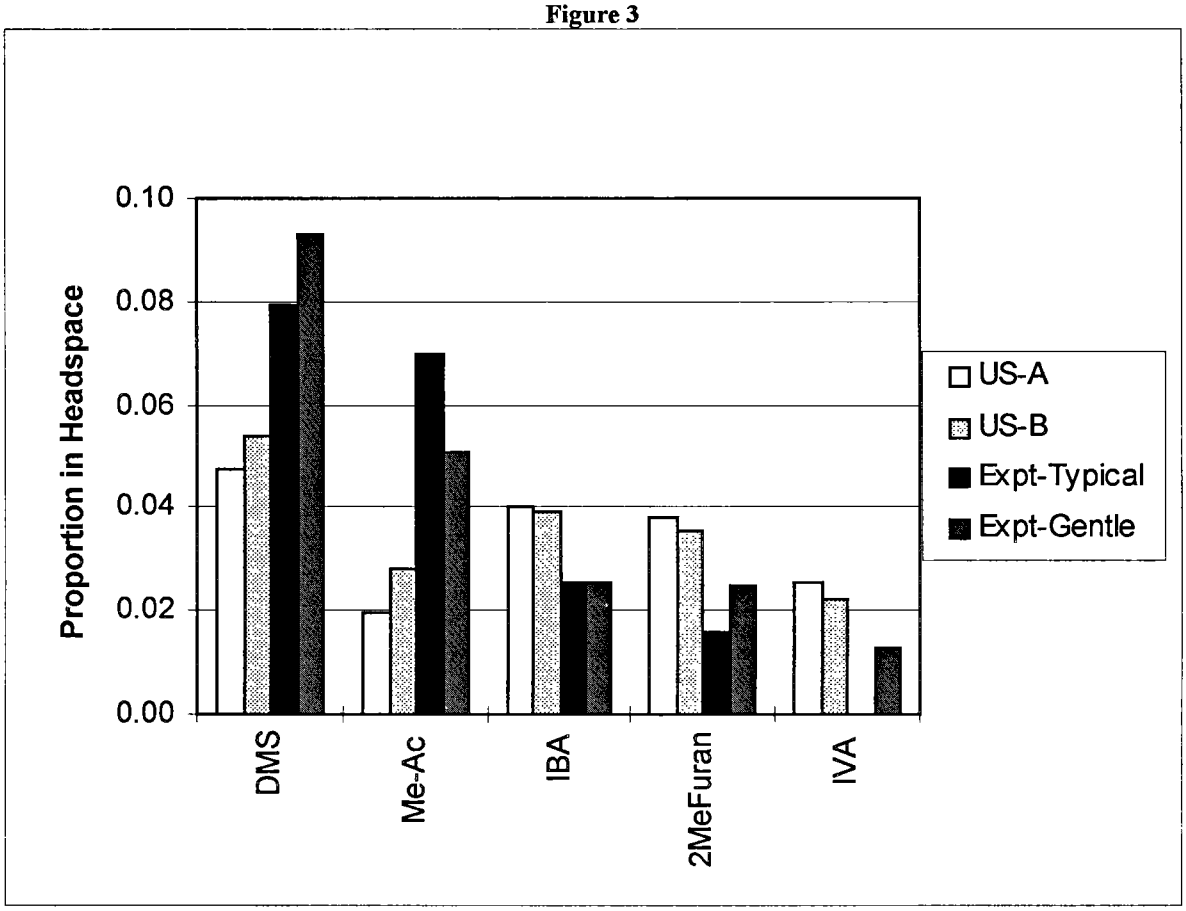
The data can also be used to illustrate the relative volatility of the compounds in the presence of coffee. This can be done calculating the distribution between the headspace and the bulk using the bulk concentrations from either of the methods used to determine it, and the headspace concentrations from the GC measurements. Though the plots are non-linear for some of the compounds, the straight-line plots of wt/c *vs*

wt are accurate enough to calculate the headspace concentration at the density found in a package. The calculation is made by rearranging Equation 5 above,

$$10.. \quad \frac{cV}{mX} = \frac{V}{mK+V}$$

The package weight and volume can be inserted along with the X and K parameters. Figure 3 shows this result for the samples studied here. As expected, the lighter volatiles are distributed more toward the headspace.

Figure 3



Conclusions

The data show that a method using only GC headspace of dry R&G can determine the total volatile composition of roast and ground coffee, but that the assumptions are only valid for the lightest of the aroma volatiles. The measurements agree reasonably well with an established method. The utility of determining the total volatile composition is in its applicability to process design, and other aspects of coffee conditioning requiring a consideration of aroma loss or material balance. The data show that for those compounds in the study which do not obey Henry's Law, the sorption isotherms are concave upward.

Acknowledgments

The assistance of Ms S Miesner and Mr E Civinskas in performing the gas chromatographic measurements and in providing technical assistance is gratefully acknowledged.

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Summary

The relationship between headspace and bulk concentrations of light aroma volatiles in coffee is shown. The model allows the calculation of the amount of sorbed volatiles from GC headspace measurements. Levels of the order of 10µg/g were found. The results were compared with those generated from standards addition.

Résumé

Le rapport entre l'espace de tête et les concentrations d'arômes volatils légers absorbés dans le café est démontré. Le modèle permet le calcul de la quantité de volatils absorbés à partir de mesures d'espace de tête en chromatographie gazeuse. Des niveaux de l'ordre de 10µg/g ont été détectés. Les résultats ont été comparés à ceux obtenus par l'addition de standards.

THE EFFECT OF CREAMER/MILK ADDITION TO COFFEE AROMA RETENTION IN COFFEE SOLUTION

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1. Introduction

Creamer/milk addition to black coffee is the most common practice of the household consumers, restaurants and institutional users in preparing coffee beverages. The purposes of the creamer/milk addition are: to develop a desirable color change, to impart a body to the coffee solution, to reduce bitter and sour tastes and to reduce astringency of the coffee. The ingredients of creamer/milk are: fat, protein and carbohydrate which can affect the properties of the creamer/milk products and their performance as a creamer/milk. Also, aroma interactions with fats, proteins and carbohydrates affect the retention of volatiles within the coffee solution and thereby, the levels of aroma in the gaseous phase. Consequently, these aroma interactions affect the amount of headspace aroma above the coffee solution.

The objectives of this study were: (1) to observe the effect of different kinds of creamer/milk addition on the total headspace aroma of the coffee solution; (2) to observe the influence of the creamer/milk addition on the aroma retention of individual compounds in the coffee solution; (3) to observe the difference of selectivity between the Frima containing vegetable oil and milk containing milk fat for the individual compounds.

2. Materials and Method

2.1 Materials

Reduced Fat Non-Dairy Creamer (RFNDC)

Reduced fat non-dairy creamer is a non-dairy coffee creamer which was produced in DSF pilot plant. The ingredients are corn syrup, sodium caseinate, dipotassium phosphate, emulsifier and hydrogenated vegetable oil 15%

Frima

Frima is a commercial non-dairy coffee creamer which is produced by DSF. The ingredients are corn syrup, sodium caseinate, dipotassium phosphate, emulsifier and hydrogenated vegetable oil 35%.

Milk

Milk is a commercial whole milk product which is produced by DSF. The ingredients are milk fat 3.5%, protein 3.2%, lactose 4.7%, ash 0.6% and soluble solids content 12%.

Non Fat Milk Solid (NFMS); Skimmed Milk Powder

Non fat milk solid is a commercial skimmed milk powder which is produced by the Seoul Milk Company. The ingredients are protein 35%, milk fat 1.0%, lactose 52.5%, ash 8.5% and others 3%.

Milk Fat

Milk fat was prepared by centrifuging fresh cream (30% fat). The cream separates into three phases: milk protein separates to the bottom, whey protein separates to the middle and milk fat separates to the top. The fat was taken from the top of the centrifuge tube. The centrifuge conditions were: 1 hour centrifuging, 5℃ constant temperature and 5000 rpm. The ingredients are fat 70% (purity ≥ 97%) with a moisture of 30%.

Commercial Coffee Products

Four kinds of korean commercial coffee products (two different freeze dried coffee products and two different spray dried coffee products) were used.

Table 1. Composition of 4.00 g of creamer/milk in 100 g of solution

unit : g

		RFNDC	Frima	Milk	NFMS	Milk Fat
Fats and oils	Milk fat			1.17	0.04	4.00
	Vegetable oil	0.60	1.40			
Carbohydrates	Corn syrup	3.04	2.24			
	Lactose			1.57	2.10	
Protein		0.20	0.20	1.07	1.40	
Others		0.16	0.16	0.19	0.46	
Total		4.00	4.00	4.00	4.00	4.00

2.2 Method

Sample Preparation

1.5 g of soluble coffee were weighed into a 250 ml headspace vial. 4.0 g (solid base) of creamer/milk was then added to the coffee powder. Distilled water was then added to make the solution up to mark 100 g total weight. A 25 μg internal standard, Tetrahydrofuran (THF-Aldrich Chemical Co., Inc.) was then added. The headspace vial was sealed with a septum and then secured by a cap. The headspace vial was stirred and heated at 80℃ for 30 minutes in a water bath. 5 ml of the headspace aroma was then injected into a gas chromatograph (GC) with a purge & trap system and flame ionization detector (FID). The results are expressed as the peak concentration of the 11 compounds calibrated by the internal standard.

Instrument Conditions

An Hewlett Packard 5880A gas chromatograph with a purge and trap system and FID was used. The column used was a Chrompack CP-Sil 5 CB (30 m x 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 20 ml/min. The temperature program was: 0℃ for 2 min, then raised 5℃/min until 180℃ was reached. These conditions were held for 5 minutes.

Statistical Analysis

The mean values of each compound are averages of five measurements. The standard deviation (S.D) of each sample and compound has also been calculated. The mean values have been plotted on the graph below. The t-test has been applied to determine whether the creamer/milk addition has changed the 11 compounds composition. The test gives a confidence value of whether the two series of measurements have the same mean value. A very low value indicates differing mean values, while a high percentage means that the two measurements are likely to be the same result. The last column summarizes whether the measured compounds have changed due to the creamer/milk addition. Here, 'same' means no change in the compound concentration and 'different' indicates a concentration difference between the two samples.

3. Results and Discussion

Aroma Pattern of Black Soluble Coffee

Figure 1 shows the headspace chromatogram of black soluble coffee. Several peaks between 2 and 17 minutes retention time are present. The eleven most significant peaks were selected for data analysis. Also, the aroma pattern of reduced fat non-dairy creamer, Frima, milk, non fat milk solid and milk fat without addition of any other components was measured by gas chromatography under the same conditions. All samples did not show any relevant aroma compounds which occurred in the black coffee. 2, 3-Butanedione and 2, 3-Pentanedione were present but at a concentration below accurate measurement.

The Effect of Creamer/Milk Addition on Total Headspace Aroma Retention

Figure 2 shows that the total headspace aroma content of all samples was decreased after the creamer/milk was added. The total headspace aroma was found to be in the order of black coffee > coffee with reduced fat non-dairy creamer > coffee with milk > coffee with Frima > coffee with non fat milk solid > coffee with milk fat. Figure 3 shows that the higher the fat content of the creamer, the higher the retention of the aroma compounds in the solutions containing non-dairy coffee creamer. Figure 4 shows that in the solutions containing milk, the protein as well as the fat increases the aroma retention.

The Effect of Creamer/Milk Addition to Individual Compounds

Figures 3 and 4 show that 2-Methylpropanal, 3-Methylbutanal, 2-Methylbutanal and 2-Methylfuran were decreased by the creamer/milk addition. 2, 3-Butanedione, 2-Butanone and 2, 3-Pentanedione were not decreased as much by the creamer/milk addition.

The Difference of Selectivity between the Frima and the Milk

Figure 5 shows that there was a difference of selectivity between the Frima containing vegetable oil and the milk containing milk fat for the eleven individual compounds. The Frima has more selectivity to the acetaldehyde and acetone than the milk.

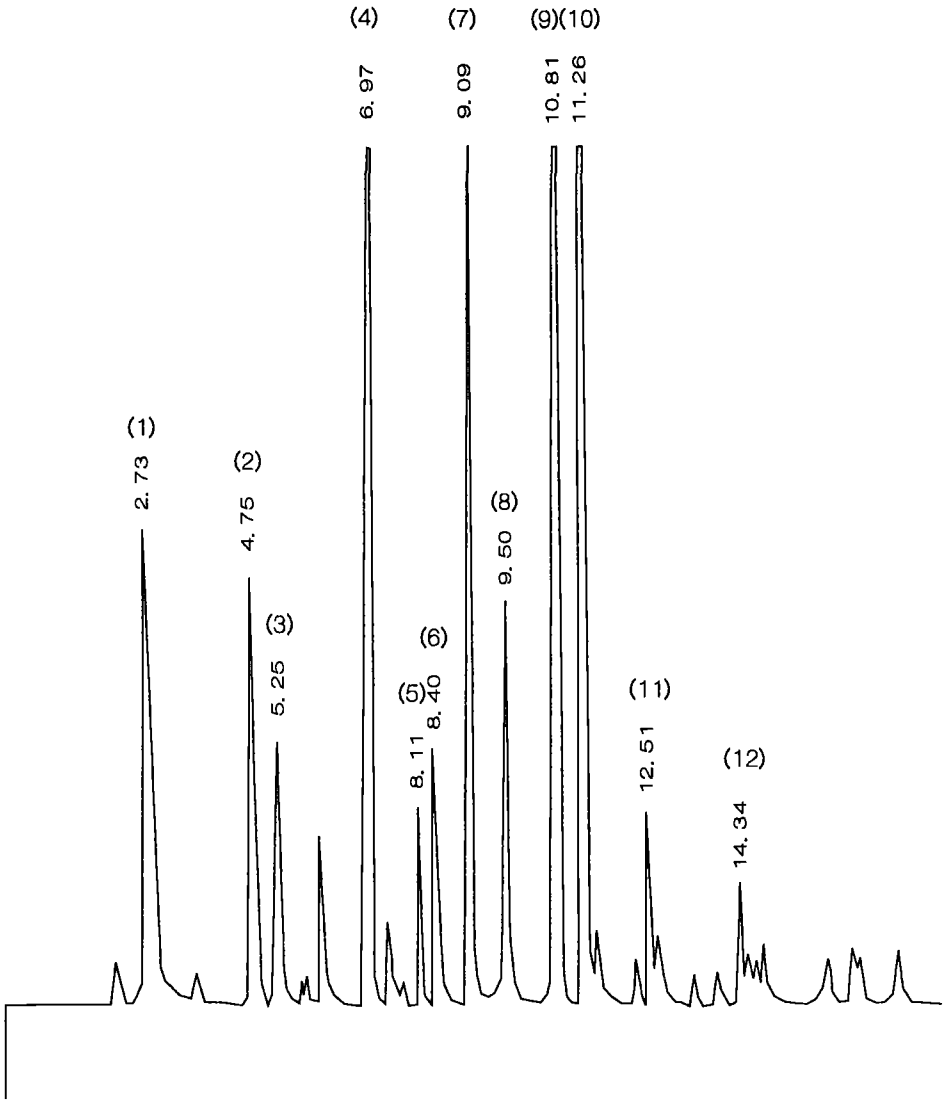


Fig. 1. Chromatogram of Black Coffee. Peaks: (1) Acetaldehyde: (2) Acetone: (3) Furan: (4) 2-Methyl propanal (IBA): (5) 2,3-Butanedione: (6) Butanone (MEK): (7) 2-Methyl furan: (8) Tetrahydrofuran (Internal Standard): (9) 3-Methyl butanal: (10) 2-Methyl butanal: (11) 2,3-Pentanedione: (12) Pyrazine.

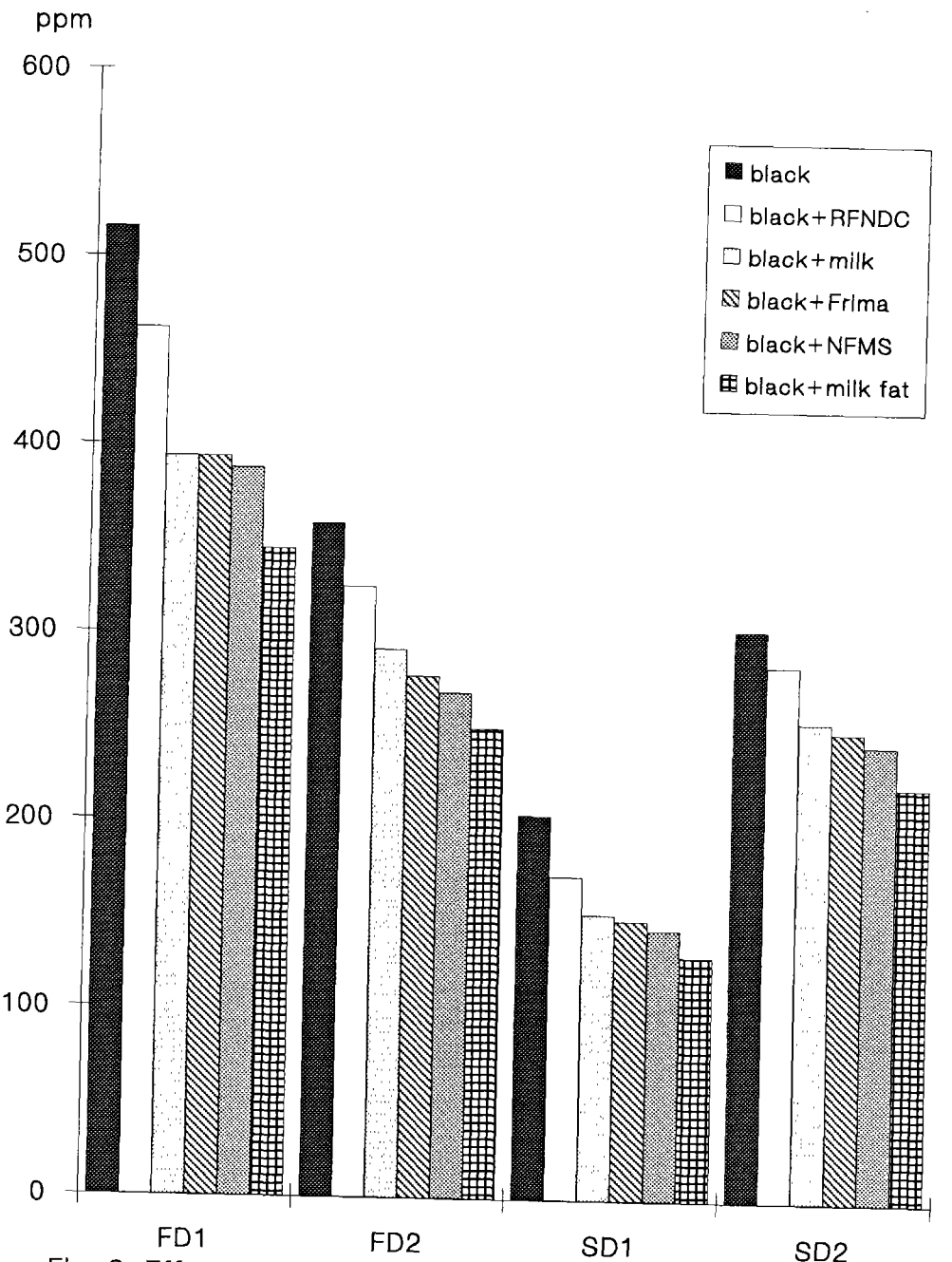


Fig. 2. Effect of creamer/milk addition on aroma retention in coffee solutions

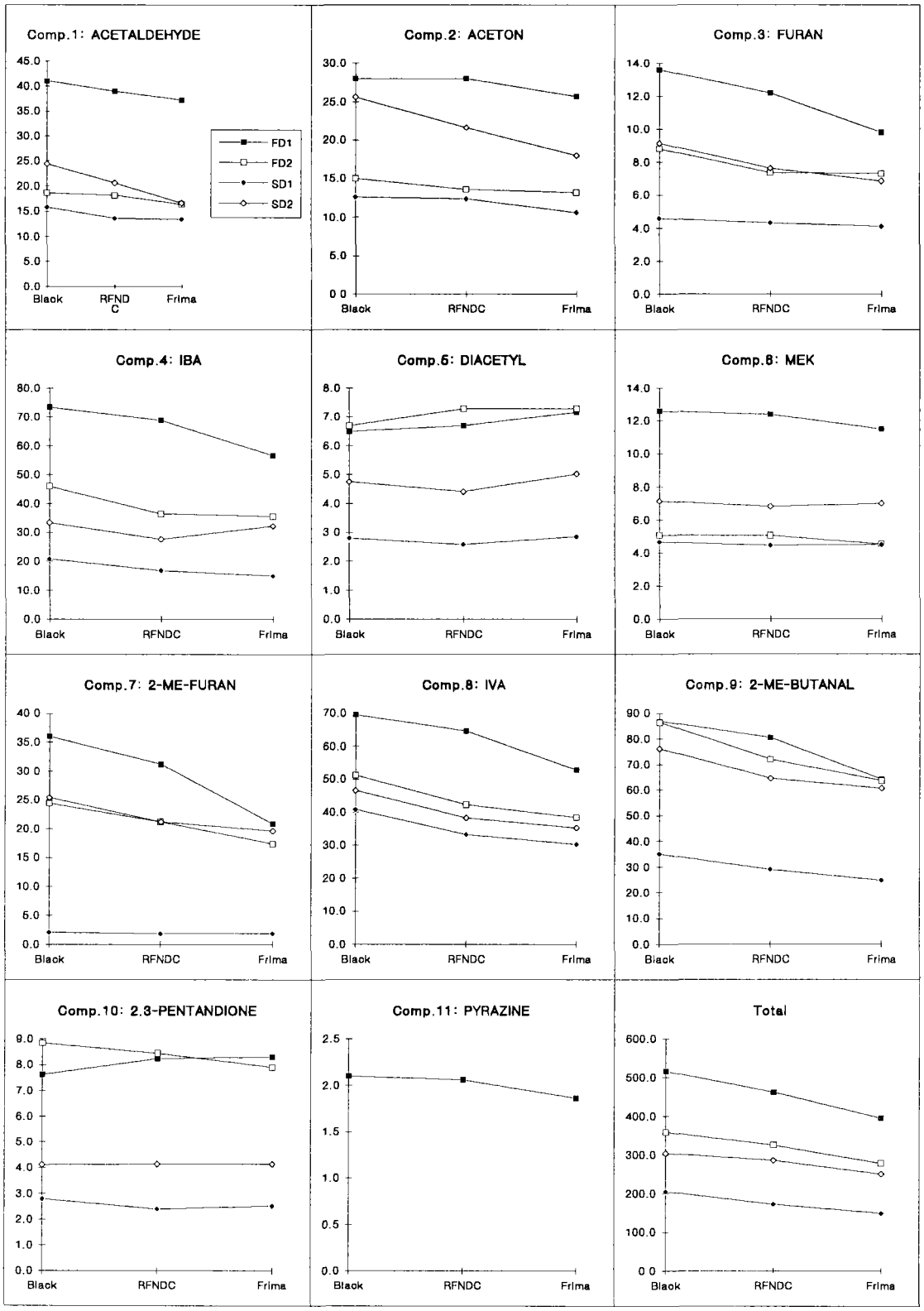


Fig.3. Effect of creamer products addition on the aroma retention of compound 1 to 11 and total aroma in coffee solution

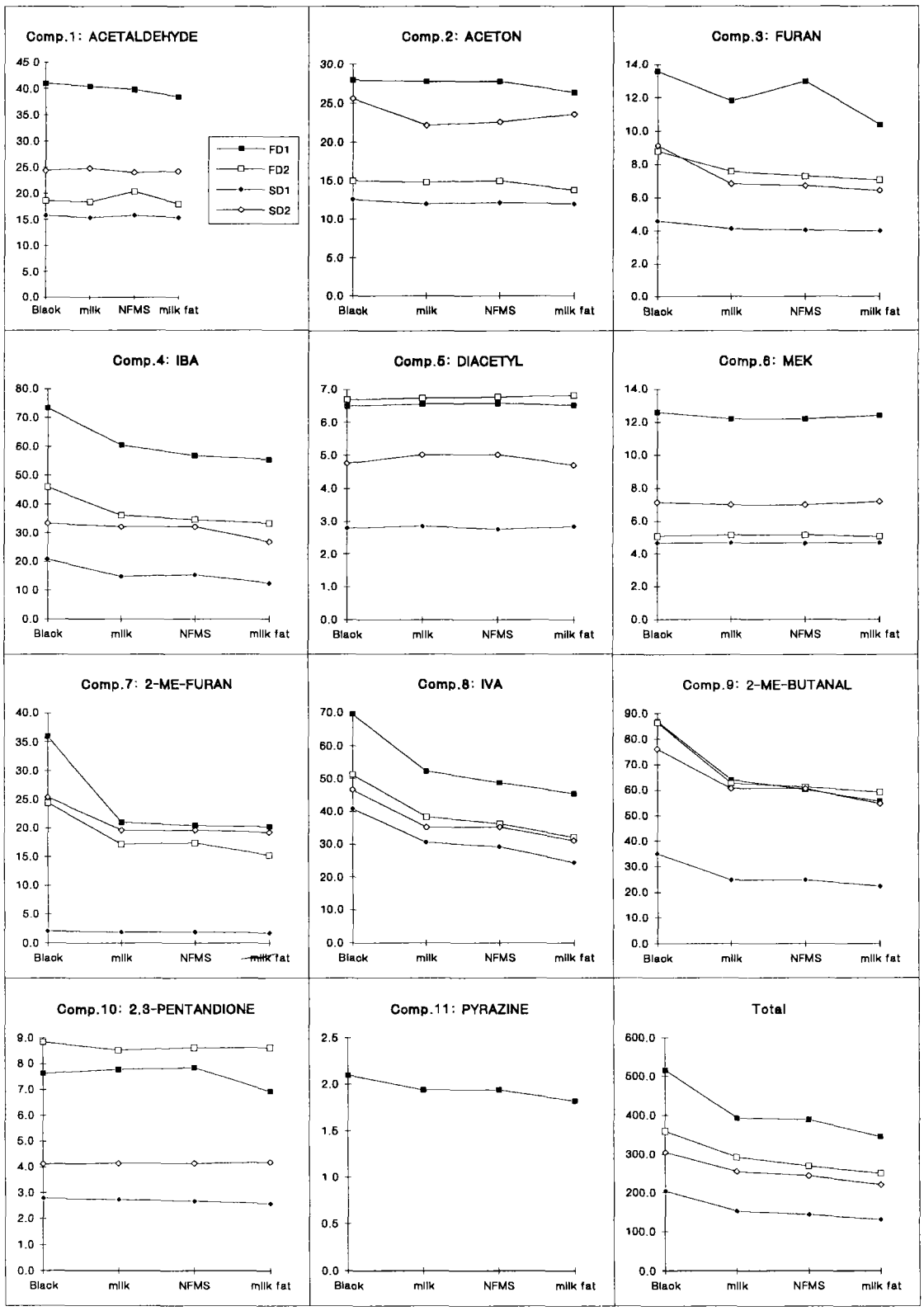
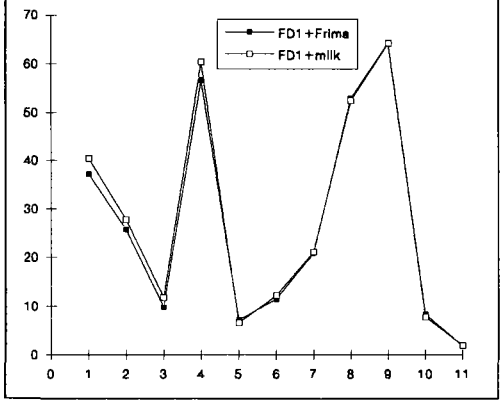


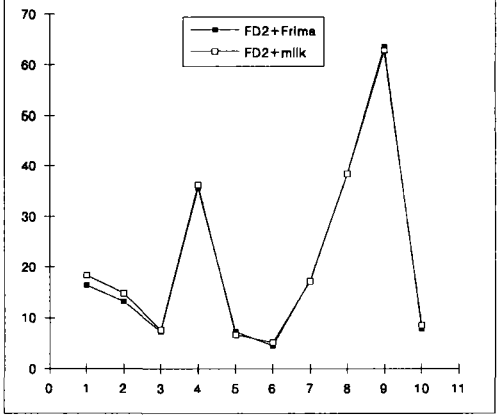
Fig.4. Effect of milk products addition on the aroma retention of compound 1 to 11 and total aroma in coffee solution

	FD1+Frima		FD1+milk			
	average	Std. Dev.	average	Std. Dev.	%t	5% limit
1	37.2	1.643	40.4	1.140	0.77	different
2	25.7	1.095	27.8	1.643	4.63	different
3	9.8	0.837	11.8	0.837	0.56	different
4	56.6	2.302	60.4	1.140	1.11	different
5	7.2	0.792	6.6	0.152	14.90	same
6	11.5	0.158	12.2	0.837	10.25	same
7	20.8	1.304	21.0	1.225	80.91	same
8	52.8	1.789	52.4	1.140	68.44	same
9	64.2	1.304	64.2	1.304	100.00	same
10	8.3	0.274	7.8	0.277	1.81	different
11	1.9	0.055	1.9	0.089	13.33	same
total	394.2	20.462	393.6	5.413	95.15	same



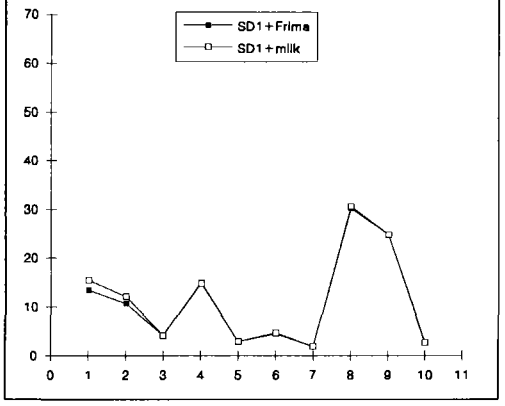
a

	FD2+Frima		FD2+milk			
	average	Std. Dev.	average	Std. Dev.	%t	5% limit
1	16.4	1.140	18.4	1.342	3.81	different
2	13.2	0.707	14.8	1.304	4.46	different
3	7.3	0.354	7.6	0.295	21.28	same
4	35.6	2.408	36.2	1.304	63.81	same
5	7.3	0.192	6.7	0.305	1.10	different
6	4.5	0.239	5.1	0.297	0.70	different
7	17.4	1.140	17.2	1.095	81.11	same
8	38.4	1.140	38.4	1.517	100.00	same
9	63.6	2.302	62.8	1.789	55.75	same
10	7.9	0.251	8.5	0.455	3.16	different
total	277.8	12.538	292.2	8.075	6.65	same



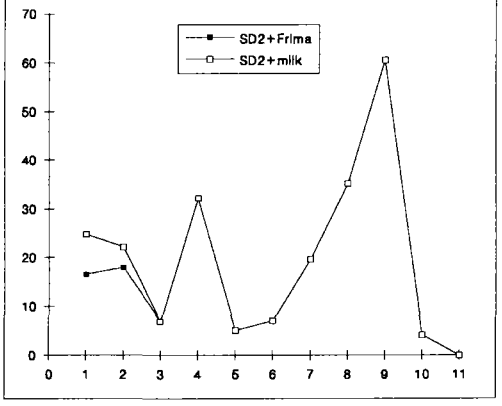
b

	SD1+Frima		SD1+milk			
	average	Std. Dev.	average	Std. Dev.	%t	5% limit
1	13.4	0.548	15.4	1.140	0.81	different
2	10.6	0.894	12.0	1.000	4.86	different
3	4.1	0.164	4.1	0.192	100.00	same
4	15.0	1.225	14.8	0.837	77.08	same
5	2.9	0.134	2.9	0.134	100.00	same
6	4.5	0.255	4.7	0.219	32.01	same
7	1.9	0.055	1.9	0.084	66.74	same
8	30.2	0.837	30.6	1.140	54.63	same
9	24.8	1.643	24.8	1.643	100.00	same
10	2.5	0.130	2.7	0.167	4.93	different
total	148.6	5.030	152.2	5.450	31.05	same



c

	SD2+Frima		SD2+milk			
	average	Std. Dev.	average	Std. Dev.	%t	5% limit
1	16.6	0.548	24.8	1.304	0.00	different
2	18.0	1.000	22.2	2.168	0.46	different
3	6.8	0.134	6.8	0.134	100.00	same
4	32.2	0.837	32.2	0.837	100.00	same
5	5.0	0.192	5.0	0.192	100.00	same
6	7.0	0.100	7.0	0.100	100.00	same
7	19.6	0.548	19.6	0.548	100.00	same
8	35.2	1.304	35.2	1.304	100.00	same
9	60.6	0.548	60.6	0.548	100.00	same
10	4.1	0.195	4.1	0.195	100.00	same
total	249.8	5.975	254.6	5.505	22.63	same



d

Fig. 5. Difference of selectivity between the Frima and the Milk: a. FD1: b. FD2: c. SD1: d. SD2

4. Conclusion

In conclusion, the experimental results show that the headspace GC data measured above all coffee solutions containing creamer/milk are different from the headspace GC data measured above black coffee. This difference in GC data between the black coffee samples and the coffee solutions containing creamer/milk, can be described as a "Milk Penetration" effect. Ingredients of the creamer/milk, (i.e. fat, protein, carbohydrate) can affect the the retention of aroma in the coffee solution. The total headspace aroma was found to be in the order: black coffee > coffee with reduced fat non-dairy creamer > coffee with milk > coffee with Frima > coffee with non fat milk solid > coffee with milk fat. The higher the fat content of the creamer, the higher the retention of aroma compounds in the samples containing non-dairy coffee creamer. In the samples containing milk, the protein as well as the fat increases the aroma retention. Among the eleven compounds, 2-Methylpropanal, 3-Methylbutanal, 2-Methylbutanal and 2-Methylfuran were decreased by creamer/milk addition. 2, 3-Butanedione, 2-Butanone, 2, 3-Pentanedione were not as much decreased by creamer/milk addition. There was a difference of selectivity between the Frima containing vegetable oil and the milk containing milk fat for the eleven individual compounds. The Frima has more selectivity to the acetaldehyde and acetone than the milk.

5. Acknowledgment

We thank Dr. O. G. Vitzthum and Dr. A. Hässelbarth for assistance in the screening test.

6. References

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7. Summary

To investigate the effect of creamer/milk addition to aroma retention in coffee solution, four kinds of korean commercial coffee products were selected as the test coffees. Two different creamers, commercial whole milk, non fat milk solid and milk fat were used as the creamer/milk. The headspace GC data measured above all coffee solutions containing creamer/milk are different from the headspace GC data measured above black coffee. This difference in GC data between the black coffee sample and the coffee solution containing creamer/milk, is a "Milk Penetration" effect. The total headspace aroma content of all samples was decreased after the creamer/milk was added. The total headspace aroma was found to be in the order ; black coffee > coffee with reduced fat non-dairy creamer > coffee with milk > coffee with Frima > coffee with non fat milk solid > coffee with milk fat. The higher the fat content of the creamer, the higher the retention of aroma compounds in the non-dairy coffee creamer. In the milk products, the protein as well as the fat increases the aroma retention.

OFF-FLAVOR ELUCIDATION IN CERTAIN BATCHES OF KENYAN COFFEE

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1 INTRODUCTION

Raw materials are main cost factors in coffee manufacturing. Costs are primarily driven by the worldwide green coffee price level which is a function of sufficient supply of high quality coffee. The quality of coffee is still - and probably has always been - one of the major driver for consumer preference. Among the worldwide segment of high quality beans green coffee from Kenya is well recognized among experts and consumers for the *fine acidity* and the *full, fruity, flowery body* in the cup. However, amongst the most critical threats to high coffee quality are off flavors and taints that may occur sporadically in green coffee processing as well as in roast coffee manufacturing. In autumn 1992, several lots of Kenyan coffee appeared on the market that exhibited a strong *hardish* off-flavor that had not been observed in coffees from that area previously. More and more it became clear that there might arise a serious quality problem for Kenyan coffees. The objective of the present investigation was to elucidate the off-flavor causing substance and to explain its sporadic occurrence in Kenyan coffees.

2 EXPERIMENTAL

2.1 Materials

Green coffees were from five different lots from Kenya and were classified as *hardish*. The absence of the *hardish* note in a control Kenyan coffee was confirmed by trained coffee experts. Roasting was performed on a benchtop scale fluidized bed roaster for 3 min at 265°C. Beverages were prepared from 50 gms roasted coffee per liter water. Due to the limited supply of *hardish* Kenyans, *rioy* Brazil coffee was used for quantitative method development. Pure 2,4,6- and 2,3,6-trichloroanisole (TCA) as well as 2,4,6-trichlorophenol (2,4,6-TCP) reference chemicals were purchased from Aldrich (Steinheim, Germany). Pro-

chloraz manganese chloride complex (prochloraz-Mn) was obtained in form of a 50% powder formulation under the registered trade mark Octave® from Schering AG (Berlin, Germany).

2.2 Isolation of Volatiles

The volatiles were isolated by simultaneous distillation/extraction (SDE) for 3 h [1,2]. Green coffees were ground with a Condux® mill (average particle size 0.5-1 mm) and 100 gms were placed in a 2 L round bottom flask together with 1 L of distilled water. For quantitative measurements the internal standard 2,3,6-TCA (1.2 μg in 1 mL tert. butylmethylether) was added. 50 mL of a mixture of n-pentane/diethylether (1+1, v/v) was used as solvent. The raw extract was dried over anhydrous sodium sulfate, filtered off into a 50 mL finger flask and concentrated to about 1.5 mL by means of a Vigreux column (250 x 10 mm) at a water bath temperature of 45°C, subsequently.

2.3 Gas Chromatography (GC), GC-Olfactometry and Mass Spectroscopy (MS)

GC separations were performed on Hewlett Packard® gas chromatographs type 5890 series II equiped with a DB-5 capillary column (60 m x 0.25 mm, film thickness 0.25 μm; J&W Scientific®). Alternatively, DB-1701 columns (60 m x 0.25 mm, film thickness 0.25 μm) were used. Injection volumes were 2 μl applied by a programmable temperature variable injector system (Gerstel®, Mülheim, Germany). The injector temperature profile ranged from 60°C to 200°C at a heating rate of 12°C/min. The oven temperature profile was 35°C held for 2 min then 5°C/min to 220°C, held for 10 min.

GC-olfactometry was conducted on a Carlo Erba® gas chromatograph type 4200 using the same capillary columns mentioned above but with larger inner diameter (0.32 mm). The GC-effluent was split 1:1 by means of a glass-cap-cross splitter [3] for flame ionization detection as well as for simultaneous olfactometric evaluation by the human nose. Sniffing was performed by 3 trained testers.

GC-MS was performed on a Finnigan MAT 95Q® mass spectrometer (EI mode, ionization energy 70 eV, emission current 1 mA, source temp. 220°C). The components of interest were quantified by GC-MS, using the internal standard method. For a higher selectivity the mass tracks m/z 195, 197, 210 and 212 were evaluated for a higher selectivity.

2.4 Parametric Studies

The optimal SDE time was checked by running 4 experiments with *rioy* Brazil coffee according to 2.2 and keeping the extraction time for 1, 2, 3 and 4 h, respectively. Reproducibility was checked by analyzing 5 samples of the same *rioy* lot according to 2.2. The influence of pH during extraction was determined by performing the SDE under controlled pH conditions at pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 9.0 after adjusting the pH with hydrochloric acid and sodium hydroxide, respectively, and addition of commercially available buffer systems.

2.5 Extraction of 2,4,6-TCA During Filter Brew Preparation

Filter brews were prepared from each 100 gs of a roasted and ground *rioy* Brazil coffee (300-500 μm average particle size) by careful extraction with boiling tap water. Exactly 2 L of coffee beverage were obtained. The beverages as well as the wet coffee spent grounds were transferred into a 4 and 2 L round bottom flasks, respectively, together with the internal standard 2,3,4-TCA. 1 L of distilled water was added in case of the wet coffee spent grounds. All samples were extracted according to 2.2.

2.6 In Vitro Stability of Prochloraz-Mn in Hydrolytic and Oxidative Media

Each 0.5 gms of the 50% powder formulation prochloraz-Mn were suspended in 50 mL citrate buffer pH 2.0, 50 mL of 10% aqueous sodium carbonate (pH 11.35) as well as in 50

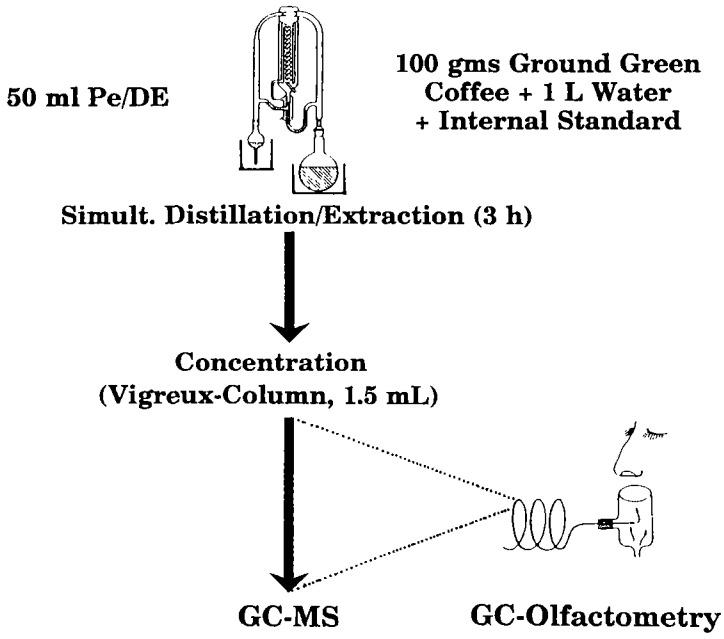
mL distilled water (pH 7.28) as a control. The suspensions were heated under reflux for 30 min. After filtering off the undissolvable particles, the hydrolysates were neutralized (pH 6.0) and an aliquot of each filtrate of 25 mL was mixed with 3 mL of concentrated hydrogen peroxide (30%). This oxidative medium was heated under reflux for another 30 min. Each hydrolysate was extracted two times with 25 mL n-pentane/diethylether (1+1, v/v) directly after hydrolysis and neutralization as well as after the oxidation step. The organic phases were dried over sodium sulfate and concentrated to about 1 mL. In a separate experiment 0.5 gms of the pesticide formulation was extracted with 3 mL hexane to check the presence of low molecular weight breakdown products. Each 1 μL of the organic phases was injected for GC-olfactometry and GC-MS.

3 RESULTS AND DISCUSSION

3.1 Background

During more than 70 years of coffee aroma research a wide range of off-flavor causing compounds have been identified in coffee. In 1988, a characteristic *peasy* off-flavor note in Rwandian coffee (goût de pommes de terres) was traced back to significantly elevated levels of 2-methoxy-3-isopropyl pyrazine [4]. 2-Methylisoborneol was detected in Robusta coffees at substantially higher levels compared with Arabicas and contributes to the characteristic *earthy, musty* flavor [5]. Robustas are usually grown in lower and hence more humid altitudes. Quite recently, Grob et al., 1992, published a paper that deals with the contamination of coffee by jute or sisal bag batching oils which might be related to so called *sack* off flavors [6]. The contamination was monitored by the indicator compounds pristane and phytane. Furthermore, it is intriguing to note that even halogenated compounds are present in coffee. Spadone et al., 1990, found that 2,4,6-trichloroanisole (2,4,6-TCA) is the major contributor to the *hardish, phenolic, medicinal, musty rioy* flavor that is pertinent for about 20 % of Brazilian coffees. Although characteristic for that specific coffee type, the *rioy* flavor is unac-

Figure 1: Scheme of the experimental approach.



ceptable in high quality coffee blends. However, the occurrence of off-flavors and taints in Kenyan coffee has been extremely unusual. Nevertheless, tasting of several roasted lots from the 1991/92 crop by trained tasters indicated the presence of a *hardish, musty, phenolic, medicinal* off-note that was quite well known from *rioy* Brazilian coffees.

3.2 Analytical Procedure

Figure I gives an idea of the analytical methodology for the elucidation of the *hardish* Kenyan off-flavor which was derived from a procedure that had been developed to detect and quantify the *rioy* flavor in Brazilian coffees [2]. Off-flavors caused by the chemical class of halogenated compounds are usually not formed during roasting but are present already in the green state. Therefore, it was possible to conduct the analyses with green coffee to facilitate the procedure dramatically because of a much lower number of volatile compounds compared with the corresponding roasted sample. The chosen extraction time of 3 h is a compromise between the requirements of good recovery and the limitation of time consumption because slightly higher 2,4,6-TCA levels were found even after 4 h hours of extraction compared with the 3 h value (Table I).

Table I: Relationship between quantitative data of 2,4,6-TCA and extraction time.

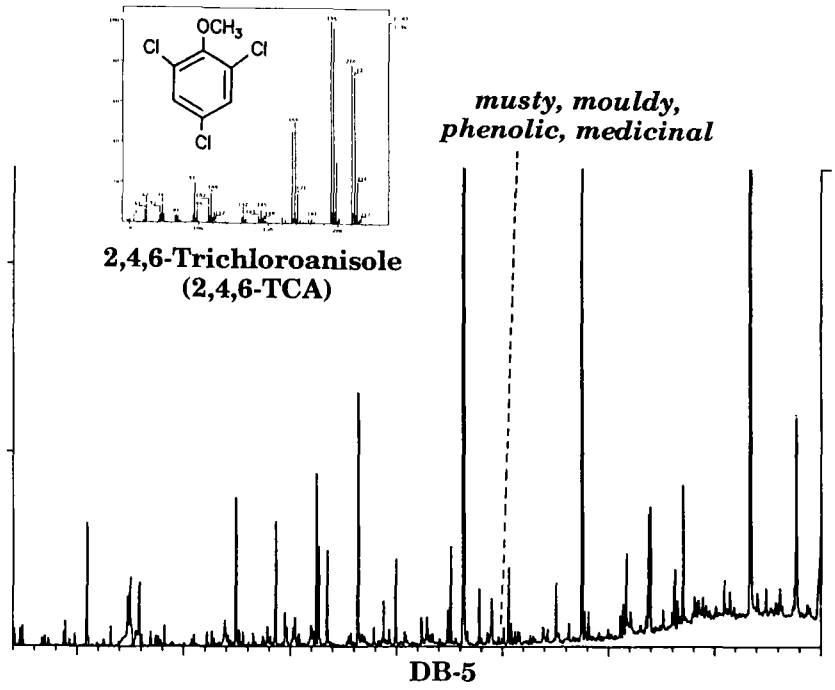
	Extraction Time			
	1h	**2h**	**3h**	**4h**
µg/kg 2,4,6-TCA	13.4	14.1	15.9	18.5

The influence of pH on the recovery is shown in Table II. The quantitative data of 2,4,6-TCA are scarcely affected in the pH range from 2.0 to 9.0. However, a steep loss of recovery was observed between pH 5.0 and 6.0 in case of 2,4,6-TCP. However, pH adjustment prior to extraction was abandoned because qualitative detection of 2,4,6-TCP is still possible at this specific pH range which represents the natural pH of an aqueous ground green coffee suspension. To improve accuracy of the method 2,3,6-TCA was used for internal standardization. There was no evidence, either from the literature or according to our experience, that this compound occurs in coffee naturally. The reproducibility of the methodology is expressed by a relative standard deviation of 2.8% in the parts per billion level and was therefore fully acceptable. The lower detection limit accomplished by mass selective GC-MS detection was 100 pg/L for coffee beverages and 5-10 ng/kg in case of green coffees. The use of deuterated 2,4,6-TCA as internal standard for even more precise quantification was abandoned due to coelution under the chosen GC conditions and the occurrence of a large number of isotope peaks usually present in halogenated compounds.

Table II: Influence of pH during SDE on quantitative data of 2,4,6-TCA and 2,4,6-TCP (n.d.: not detectable).

pH	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**	**9.0**
µg/kg 2,4,6-TCA	10.6	9.8	11.4	12.0	10.4	9.8	10.2
µg/kg 2,4,6-TCP	5.4	4.8	5.4	5.5	2.6	0.6	n.d.

Figure 2: GC-olfactometry of an aroma extract of *hardish* Kenyan coffee (experimental conditions see text).



3.3 Chemical Identification of the Off-Flavor

GC-olfactometry was applied as a powerful tool to detect positive key aroma compounds as well as off-flavors and taints in complex mixtures such as coffee aroma. The concentrated volatiles isolated from spoiled Kenyan coffee was evaluated by the human noses of highly experienced testers. Besides numerous odor notes typically present in green coffee aroma extracts [7], one intense *musty, phenolic* odor note was perceived at the sniffing port, although that note was not recognized as a distinctive peak by flame ionization detection (Figure 2). Sniffing was conducted over the whole chromatogram and performed with two capillary columns of different polarity but no further *musty* off notes were perceivable that might contribute to the *hardish* note as well. The *musty* note was not present in *non-hardish* control samples. The off note was identified as 2,4,6-TCA by comparison of GC retention and mass spectral data with authentic reference chemicals (Figure 2,Table III). 2,4,6-TCP was identified qualitatively but did not give a *musty* odor during elution at the sniffing port because of much higher sensory threshold values [8].

Table III: GC retention data of 2,4,6-TCA, 2,3,6-TCA and 2,4,6-TCP calculated according to [9], (column parameter: 60m x 0.32 mm, 0.25 μm film thickness).

Compound	DB-5	DB-1701
2,4,6-TCA	1319	1398
2,3,6-TCA	1380	1463
2,4,6-TCP	1363	1529

3.4 Quantification and Sensory Relevance

2,4,6-TCA is one of the most powerful odorants known so far and a notorious off-flavor compound in food. An extensive review about the occurrence of 2,4,6-TCA is given by reference [10]. In water, 2,4,6-TCA shows one of the lowest odor thresholds known that goes down to 0.03 ng/L (ppt). In coffee 2,4,6-TCA was identified earlier as a major contributor to the *hardish, phenolic* or *medicinal rioy* flavor in Brazil coffees [2]. The authors recognized odor thresholds down to 8 ng/L for direct odor perception and 1-2 ng/L for taste perception in coffee beverages.

The results of quantification of 2,4,6-TCA by means of mass selective GC-MS are shown in Table IV. The extraction rate during filter brew preparation was determined using roasted *rioy* Brazil coffee. It was found to be about 20%. A theoretical level of 0.4 μg 2,4,6-TCA/L coffee brew can be calculated based on the assumption of 20% extraction rate and a brew strength of 50 gms coffee powder per liter. Actually 0.6 μg 2,4,6-TCA/L were found. This is quite close to the theoretical value and therefore about five hundred times the taste threshold value in a coffee beverage. On the assumption that approximately 1000 green beans correspond to 1 kg, then about 10-25 *rioy* beans in 1 kg could affect the overall taste in a negative way. This could be the reason why the *rioy* flavor is difficult to conceal by blending with neutral beans.

As Table IV indicates, contents of 2,4,6-TCA in *hardish* Kenyans were in the low parts per billion level ranging from 4.3 to 7.9 μg/kg. This is lower than the levels found in typical *rioy* Brazilians which are usually larger than 10 μg/kg. However, the amounts detected in Kenyans are still high enough to cause an off-note. 2,4,6-TCA could not be detected in the *non-hardish* control.

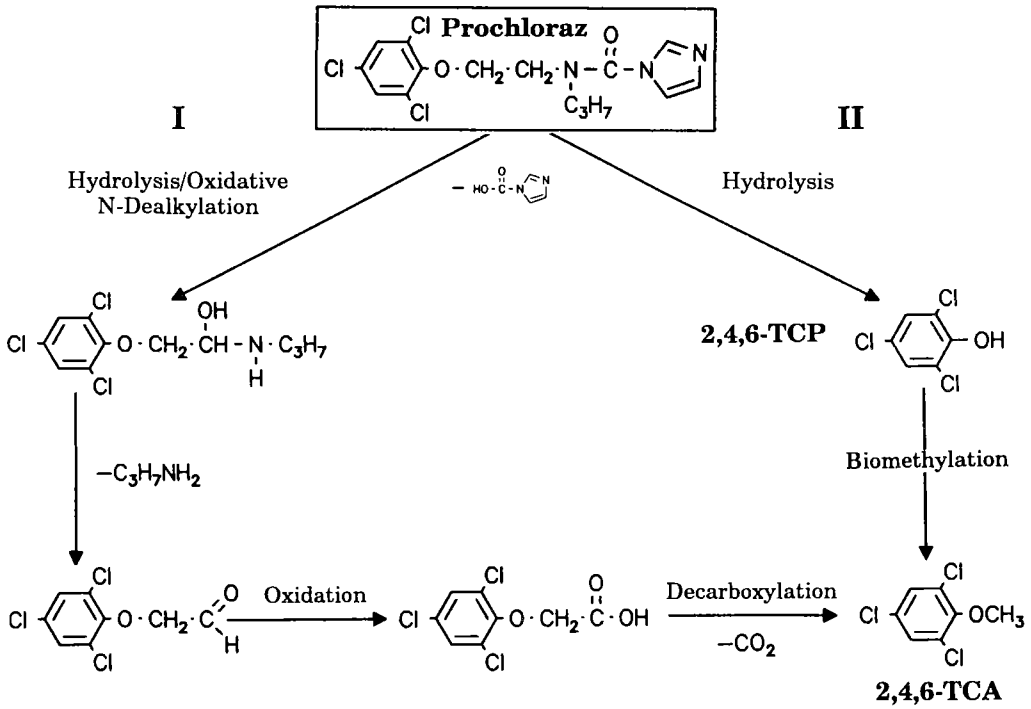
Table IV: Contents of 2,4,6-TCA in various coffee samples (n.d.: not detectable, *: μg/L beverage).

Coffee Sample	μg/kg 2,4,6-TCA
Rioy Brazilian - roasted	36.1
Rioy Brazilian - spent grounds	28.2
Rioy Brazilian - filter brew	0.6*
Hardish Kenyan I	7.9
Hardish Kenyan II	7.9
Hardish Kenyan III	5.4
Hardish Kenyan IV	4.3
Hardish Kenyan V	5.4
Non-hardish Kenyan VI (control)	n.d.

3.5 Hypothetical Formation Pathways of 2,4,6-TCA in Kenyan Coffees

Having been elucidated and quantified, the question is raised as to the explanation for the presence of 2,4,6-TCA in certain batches of Kenyan coffee. With regard to the *rioy* flavor in Brazilian coffees a natural generation of 2,4,6-TCA by microorganisms was discussed [11]. Biochlorination of green coffee phenols by molds and/or bacteria may yield the intermediate product 2,4,6-TCP. The corresponding phenol was actually identified in *rioy* Brazils and may undergo biomethylation to generate 2,4,6-trichloroanisole [2]. On the other hand, the same authors discussed an external contamination by polychlorophenols as one explanation for the

Figure 3: Hypothetical degradation pathway of prochloraz (according to [12] with modifications suggested by the present work).



rioy flavor. Recently, Kenyan authors considered this issue and suggested that the sporadic occurrence of the new type of *hardish* off-flavor could be related to the use of the fungicide prochloraz-Mn [12]. Prochloraz-Mn (1-N-propyl-N-[2(2,4,6-trichlorophenoxy)ethyl] carbamoyl imidazole) was recommended and applied to control brown eye spot and coffee berry disease [13]. Figure 3 obviously shows that the basic chemical element of 2,4,6-TCA is present already within the prochloraz molecular frame. 2,4,6-TCA and/or 2,4,6-TCP, respectively,

Table V: Generation of 2,4,6-TCP and 2,4,6-TCA after in vitro treatment of prochloraz-Mn (+: detectable by GC-olfactometry and MS; n.d.: not detectable).

Kind of Chemical Treatment		2,4,6-TCP	2,4,6-TCA
Hydrolyis	Dist. Water	+	n.d.
Oxidation	H_2O_2	+	+
Hydrolyis	pH 2.0	+	n.d.
Oxidation	H_2O_2	+	+
Hydrolyis	pH 11.35	+	n.d.
Oxidation	H_2O_2	+	+
Hexane Extraction		+	n.d.

may be formed via a special hypothetical degradation pathway under hydrolytic and oxidative conditions depicted in Figure 3. Subpathway (I) includes hydrolysis, oxidative dealkylation, oxidation and decarboxylation and yields 2,4,6-TCA. Pathway (II) may lead to the formation of 2,4,6-TCP by hydrolysis that may undergo biomethylation to yield 2,4,6-TCA finally.

To answer the question whether the formation of 2,4,6-TCP and 2,4,6-TCA from prochloraz-Mn may in principle be possible, several simple experiments were conducted to check its stability in hydrolytic and oxidative media. The results of these trials are summarized in Table V. 2,4,6-TCP could be clearly detected after hydrolysis in alkaline and moderately acidic media as well as in a neutral aqueous environment. It should be noted that traces of 2,4,6-TCP were already present in the pure fungicide without any chemical pretreatment. 2,4,6-TCA could not be detected after hydrolysis. However, oxidation with hydrogen peroxide after hydrolysis generated clearly detectable levels of 2,4,6-TCA in all three experiments. The present results reveal that the generation of 2,4,6-TCA from prochloraz-Mn seems to be likely at least as a form of side stream metabolism that may be favored by wet weather and a damp environment. It is probably important to note that the application of prochloraz-Mn has since been deliberatly abandoned by the manufacturer and that the *hardish* off-flavor has not been observed in Kenyan coffee from recent crops.

4 SUMMARY AND CONCLUSIONS

2,4,6-TCA was identified qualitatively and quantitatively as the key off-flavor causing substance in coffees from Kenya that exhibited a characteristic *hardish* off-flavor. Quantitative data reveal low 2,4,6-TCA extraction rates during filter brew preparation; however, the levels detected were much above the threshold values. A natural generation of 2,4,6-TCA by microrganisms is generally accepted; on the other hand, chemical contamination via previous application of the pesticide prochloraz-Mn cannot be fully excluded in the present case. The *hardish* off-flavor was not observed during recent crops after suspending procloraz-Mn from application on coffee. The present findings urgently reveal that green coffee producers and coffee manufacturers must be aware of the serious threat that off-flavor compounds such as 2,4,6-TCA can mean to high quality coffee standards.

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SUMMARY. *In 1992, several batches of Kenyan coffee appeared on the market that exhibited a hardish, phenolic or musty off-flavor that had not been known before in coffees from that area. This paper describes the chemical elucidation of the off-flavor causing substance. Aroma concentrates from green hardish Kenyan coffee were investigated using GC-olfactometry in combination with GC-MS. The hardish note was identified as 2,4,6-trichloroanisole, a compound that is well known to cause the rioy note in Brazilian coffees. Quantification gave values in the low parts per billion level. These amounts are much above the odor threshold of 2,4,6-trichloroanisole in coffee beverages. Finally, possible explanations for the sporadic occurrence of this off-flavor are given.*

RESUME. *En 1992, il y a eu quelques lots de café verts en provenance du Kenya, ayant des traces d'arômes défectueux, qui furent reconnus comme phénoliques, moissis ou durs, ce qui jusqu'à ce jour n'étaient pas encore connu dans les cafés verts de cette région. Le rapport ci-joint essaie d'éclaircir les responsables chimiques de ces composants arômatiques défectueux. Les concentrats aromatiques des cafés verts du Kenya, furent analysés par moyen de GC olfactométriques en combination avec des GC-MS. Il fut reconnu comme étant responsable des arômes défectueux, l'association 2,4,6-trichloroanisole, une substance déjà connue comme étant la responsable pour l'odeur riotée des cafés brésiliens. Des mesures quantitatives ont donné dans les parties inférieures des valeurs de l'ordre du domaine du billion. Ces quantités sont nettement supérieures au seuil de perception du 2,4,6-trichloroanisole dans des boissons de café. En conclusion, il est discuté des explications possibles, auxquelles seraient du ces apparitions sporadiques de ces arômes défectueux.*

CHARACTERIZATION OF METAL-CHELATING COMPOUNDS IN INSTANT COFFEE

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The effect of brewed coffee on mineral nutrition has recently been noticed. Iron-deficient anemia is prevalent in malnourished individuals in such countries as Costa Rica, where coffee is commonly consumed.[1] In addition, studies on non-heme iron absorption in humans[2] and rats[3] have been reported. The roasting process for green coffee beans is essential to produce the aroma, taste and color compounds in brewed coffee. Polyphenolic compounds such as chlorogenic acid and tannin are known to be active in metal chelation, which is involved with the antioxidative activity in foods and the availability of trace elements in biological systems. Roasting the green coffee bean can also degrade and activate phenolics, sugars and amino acids to form additional active compounds involved in metal chelation.

There have been few studies on separating these metal-chelating compounds in roasted coffee. Therefore, the purpose of this study is to separate and characterize zinc(II)- and iron(II)-chelating compounds from instant coffee of the lyophilized type.

I. Preliminary methods.

Firstly, an Fe(II) coffee complex was detected by gel-permeation column chromatography, using a pH 4 acetate buffer (0.01M) containing ferrous sulfate as the eluent. Coffee samples were charged into the gel-permeation column for HPLC. Eluates were collected in fractions and measured for Fe concentration by atomic absorption spectrophotometry and by their optical density values in the UV and visible light range. As shown in Fig. 1, the zones of troughs or peaks in the Fe concentration curve correspond to ferrous ion bound with the coffee constituents.

Instant coffee was separated into its fractions by ion-exchange column chromatography, solubility in mixed solvents, gel permeation column chromatography and cellulose column chromatography. We finally isolated

aspartic acid as an Fe(II)-chelating compound in instant coffee as an enantiomorphic mixture.[4] Free aspartic acid and total amino acids were determined in regular coffee and instant coffee. The ratio of the free aspartic acid content to total amino acids was found to be more than two times larger in instant coffee than in regular coffee.

We next used tetramethylmurexide (TMM), a chelating titration reagent for Zn(II), in order to assay the zinc ion bound with the coffee constituents. The assay system used a pH 5 hexamine buffer (10 mM) with 50 μM $ZnCl_2$, 0.05% of a sample, and 10 mM KCl. After adding 0.2 ml of 1.6 mM TMM (the ammonium salt) to 4 ml of the assay mixture, the optical density values at 460 nm and 530 nm were measured. A calibration curve for free Zn(II) concentration was prepared by assuming linearity between the ratio of absorbance (460 nm/530 nm) and free Zn(II) concentration (Fig. 2).

Instant coffee was fractionated by ion-exchange column chromatography, solubility in mixed solvents, and reversed-phase HPLC. For every fractionation step, samples were determined for their zinc-chelating ability by the TMM method. Citric acid was finally isolated as a ligand in instant coffee for Zn(II).[5] A Scatchard plot for the Zn(II) citric acid complex was prepared by using the TMM method, and it gave 3.50×10^{-5} [M] for the dissociation constant (Kd).

The first and second methods led to the detection of the low molecular-weight compounds, aspartic acid and citric acid. The reason why these low molecular weight compounds were isolated as ligands is due to the fact that the Zn(II) or Fe(II) binding ability of a test sample is shown as μmole of Zn(II) per gram of sample charged. It would have been very laborious to determine in the usual way apparent Kd values for the coffee fractions at every separation step.

We were, however, forced to change methods to detect the Zn(II)-coffee complex. We prepared a Zn(II)-coffee complex, purification being followed by measuring the zinc content in each purified sample as a marker of the Zn(II)-coffee complex at every separation step.

2-1. Test preparation of an insoluble metal-coffee complex.

Coffee (1.50 g) was dissolved in water, and adjusted to pH 4, 5, 6, 7 and 8 with N-HCl or N NaOH. Each coffee solution was mixed with a salt solution and again adjusted to the respective pH values, making up to 150 ml with a final concentration of 1 mM of the metal ion. The salts added was $FeSO_4$, $CuSO_4$, and $ZnSO_4$, and after adding one of these salt solutions to a coffee solution, a rapid color change was observed. This apparent color change was indicative of chelation of coffee brew in respect of the metal ions.

The optical density at 400 nm was increased in each coffee solution with the addition of a salt, being greatest with ferrous sulfate, and next with cupric sulfate. Zinc sulfate only increased the optical density a little.

Aliquots (10 ml) of each mixed solution were put into test tubes and allowed to stand at 37℃ for 6 hr. The insoluble material was filtered off, and the filtrate was subjected to colorimetry at 645 nm as an index of the quantity of organic compounds after adding potassium dichromate dissolved in diluted sulfuric acid.

The addition of a salt to the coffee solution produced a significant amount of an insoluble complex, high pH favoring the production of an

insoluble complex with Fe(Ⅱ) and Cu(Ⅱ). The amount of the insoluble Zn(Ⅱ)-coffee complex was found to be greatest at pH 5, and it is suggested that the higher the pH, the more soluble the zinc-complex becomes.

2 2. Purification of the Zn(II)-coffee complex.

1) Preparation of the complex.

Coffee (1 kg) was added to 3 of a pH 5 hexamine buffer (10 mM) containing 10 mM $ZnCl_2$ and 10 mM KCl. The precipitate formed was collected and washed with the hexamine buffer containing 10 mM KCl. The precipitate was then dissolved in 1% ammonia and lyophilized (sample A, 19.96g). Zinc present in the solution during preparation was determined by atomic absorption spectrophotometry without ashing.

The preparation and separation procedures for the Zn(Ⅱ) coffee complex were utilized to select required complex properties. The Zn(Ⅱ) coffee complex obtained was soluble in a basic solution and insoluble under acidic conditions. Since zinc chloride is not soluble under alkaline conditions, the alkali-soluble complex is indicative of the formation of Zn(Ⅱ) chelating compound(s). The Zn(Ⅱ)-coffee complex (sample A) was black in color, its zinc concentration was 1.01 mg/g and the yield from coffee was 2.00%.

2) Separation of sample A by ion-exchange column chromatography.

Sample A (20 g) was dissolved in water and loaded into an Amberlite IRA 410 (OH^-) column. After the column had been washed with water, it was eluted with 1 N HCl. The eluate was collected in fractions, and measured for Zn concentration by atomic absorption spectrophotometry without ashing

The fractions with high Zn content were loaded into an Amberlite IR 120 ($NH4^+$) column. The column was washed with water, eluted with 1 N ammonia, and the fractions measured for zinc concentration. The fractions with high zinc concentration were acidified with concentrated HCl, and the resulting precipitate was collected by centrifugation. The precipitate was dissolved in a dilute ammonia solution, and the soluble portion was lyophilized (sample Ap, 13.45 g).

We revealed that the Zn(Ⅱ) chelating substances in instant coffee were acidic by electrophoresis,[6] the Zn(Ⅱ)-coffee complex not being retained in either anion or cation exchange columns. Almost no zinc dissociated from the ligands present in the coffee after passage of the Zn (Ⅱ) coffee complex through a strong cation exchanger, indicating that the separated Zn(Ⅱ)-coffee complex was a strong chelator.

The Zn(Ⅱ) coffee complex was again recovered as a precipitate by acidifiying the solution. The yield of the Zn(Ⅱ) coffee complex (sample Ap) after purification by ion-exchange chromtotography was 1.34 %, and its zinc content was 4.36 mg/g.

3) Separation of the Zn(Ⅱ) coffee complex by cellulose column chromatography.

Sample Ap (12.42 g) was dissolved in 1% ammonia and dispersed in Celite, the resulting gruel like material then being lyophilized and pulverised. A cellulose column (6 x 60 cm) was prepared with Microkristallin Cellulose (Merck) which had been washed with a mixed solution of n propanol and water (5:2 v/v). The lyophilized and pulverized material was suspended in the starting eluent and poured into the cellulose column.

The eluents were prepared by step-wise changes in the mixing ratio (v/v) of n-propnanol and 1% ammonia from 5:3, 3:3 to 1:1. Each eluent was 3 the resulting eluate being collected in fractions and monitored for optical density at 470 nm. The zinc content was measured by atomic absorption spectrophotometry. The eluted peak components were concentrated *in vacuo* and their yields determined.

The cellulose column chromatogram of sample Ap is shown in Fig. 3. Sample Ap was separated into four major fractions, names Ap I-IV, by increasing the proportion of 1% ammonia in the eluent. Ap I and II hardly contained any zinc, while Ap III and IV contained the zinc(II)-coffee complex. In this study, zinc was used as the marker for chelating substances. The separation procedure released zinc from the ligands with low stability constants, which indicates weak activity for zinc chelation.

The yield of the Sample Ap I and II components from the coffee sample was 0.343%, and those of samples Ap III and IV were 0.129% and 0.012%, respectively. Although the major Zn(II) coffee complex was sample Ap III in this study, the yields of samples Ap III and IV varied. This suggests that the conditions used for the separation process used may have affected the stability of the Zn(II)-coffee complex.

2-3. Molecular weight and dissociation constant for sample Ap-III of the Zn(II)-coffee complex.

The molecular weights of the sample Ap components were evaluated by gel-permeation HPLC, being 700-1,100 for samples Ap I and II. Sample III and IV had molecular weights of 20,500 and 32,000 with small amounts of other component(s) in the 5:2 eluate. Sample Ap IV also had another minor component with m.w. larger than 32,000 to exceed the range of the calibration curve. The elution order for the sample Ap components, which was achieved by raising the eluent polarity for cellulose column chromatography is correlated with the zinc content and molecular weight.

The dissociation constant was measured by preparing a Scatchard plot, using the TMM method. The total zinc content of sample Ap-III was determined by atomic absorption spectrophotometry. The apparent dissociation constants of sample Ap III for Zn(II) were 3.33×10^{-8} and 2.67×10^{-7} [M], and the numbers of binding sites were 1.6 and 4.0, respectively.

2-4. Effect of exposing samples Ap III and IV to Zn(II) and EDTA by cellulose column chromatography.

Since samples Ap III and IV were the major Zn(II)-coffee complexes and their yields seemed to be variable, these samples were exposed to zinc chloride or EDTA, and their chromatographic behavior in a cellulose column was observed. Samples Ap III and IV were dissolved in diluted ammonia, and $ZnCl_2$ or EDTA was added before the solution was dispersed on Celite, lyophilized and chromatographed. Elution was monitored by detecting optical density in visible light range for the color peaks and by atomic absorption spectrophotometry for zinc.

Further exposure of sample Ap III to zinc chloride gave a component that was eluted with a 1:1 (v/v) mixture of n-propanol and 1% ammonia. This component coincided with a zinc peak, suggesting the formation of another Zn(II)-coffee complex with similar molecular size to that of sample Ap IV.

The exposure of sample III to EDTA resulted in no shift of the major peak, while producing a small peak in the 5:2 eluate, but all the zinc was

eluted in the 5:2 eluate. Therefore, sample Ap III seems to have varied in molecular size and polarity depending on the Zn(II) concentration as shown in Fig. 4.

Rechromatography of sample Ap IV in the cellulose column seems to have dissociated Ap IV to produce peaks in the 5:2, 3:2 and 1:1 eluates. Further exposure of sample Ap IV to Zn(II) gave peaks in these same three eluates. The exposure of sample Ap IV to EDTA gave peaks in the 5:2, 3:2 and 1:1 eluates with all the zinc appearing in the 5:2 eluate. The behavior of sample Ap IV during cellulose column chromatography was thus more variable than that of Ap III.

2 5. Analyses and chemical characteristics of Sample Ap III.

1) Analyses.

If phytate is involved in the formation of polymers during roasting of green coffee beans, a strong contribution from the phosphoric group to chelation would be expected.[7] However, phosphorus was scarcely detected by the modified Bartlett method.

Phenolics were determined by colorimetry with the Folin-Denis method, using pyrocatechol and chlorogenic acid as calibration standards, resulting in a phenolic content of 9.5% and 30%, respectively.

Ap III underwent alkali fusion, the yield of the ethereal extract being 46%. This ethereal extract was analyzed for phenolics by three dimensional HPLC, and pyrogallol, protocatechuic acid, pyrocatechol and p hydroxycinnamic acid were determined. The total amount of these phenolics approximately accounts for the value determined by the Folin-Denis method. Hydrogen consumed in the catalytic reduction was 65% of the theoretical amount of unsaturated bonds.

2) Characteristics.

Ap III had a black appearance, was a 20,000 Dalton polymer, and was not soluble under acidic conditions. Zinc(II) was not dissociated with strong anion and cation exchangers.

The Zn(II) content affected the polarity and molecular size of Ap III. -Log Kd for Ap of the Zn(II)-complex was 8.74, and its quantity in instant coffee was 0.1%.

3-1. Purification of the Fe(II)-coffee complex.

Iron (II) chelating compounds were separated from instant coffee by the same procedures as those used for the zinc-coffee complex.

Cellulose column chromatography of sample Ap gave four components, Ap I-IV. Ap I and II hardly contained any iron. The total yields of Ap III and IV were 1.10 g and 0.05 g from 1 kg of instant coffee, the iron content being 4.62 mg/g and 7.30 mg/g, respectively.

The molecular weights of Ap I-IV were evaluated to be 9,000, 15,000, 36,000 and 50,000 Daltons, respectively, by gel-permeation HPLC.

3 2. Dissociation constant for sample Ap-III of the Fe(II)-coffee complex.

The dissociation constant of Ap III for iron (II), which was determined by the dialysis-equilibrium method, was 5.56×10^{-6} [M]. Ap III was dialyzed against EDTA for two days, and 60% of Fe was released. Elemental, IR and NMR analyses of Ap III showed the presence of double bonds, hydroxyl groups, and amines or imines.

3 3. Effect of exposing sample Ap-III to Fe(II) and chelators by cellulose column chromatography.

Further exposure of Ap III from the Fe(II) coffee complex to Fe(II) resulted in Ap migrating to the position of Ap IV by cellulose column chromatography. Gel permeation HPLC supported the fact that Ap III was converted to Ap IV. Several types of Fe(II) chelators such as EDTA, o phenanthroline and bipyridyl were used to release Fe(II) from Ap III. EDTA was found to be involved in the formation of complexes with Ap III without dissociating Fe(II), while o-phenanthroline and bipyridyl were involved in the formation of Ap IV without dissociating Fe(II).

3 4. Preparation of the Fe(II)-model coffee complex.

Reagent mixtures of model coffee were roasted to investigate which materials in green coffee beans were involved in the formation of ferrous-chelating compounds. A solution of $FeSO_4$ was added to an aqueous solution of a roasted mixture to form the Fe(II) model coffee complex. The complexes were purified by dissolving in 1% ammonia and precipitating in 2 N HCl.

The essential compounds for forming the Fe(II)-coffee complex were sucrose, chlorogenic acid and amino acids, formation of the complex being enhanced by the addition of lysine and valine.

Conclusion:

Some compounds in brewed coffee could form a complex with trace elements such as zinc and copper. The quantity of the complex formed from the coffee brew being at the 0.1 % level, with Kd less than or equal to that of citric acid ($-\log Kd \fallingdotseq 8$). The nutritional effect of these coffee chelators on the bioavailability of trace elements would be very small.

An Fe(II)-coffee complex was also prepared from instant coffee and purified by the same procedure as that used for the zinc-coffee complex. Some compounds in the coffee brew could form a very strong complex with Fe(II). Although the dissociation constant (Kd) of this complex is still unknown, the Kd value for Ap of the Fe(II)-coffee complex seems to be less than that of the Zn(II)-coffee complex, the quantity in the coffee brew also being 0.1% level.

As long as a well-nourished person does not exceed a moderate consumption of brewed coffee, there would be no cause of iron defficiency. Controlling the formation of these strong chelating compounds during processing and tests on animals can be expected.

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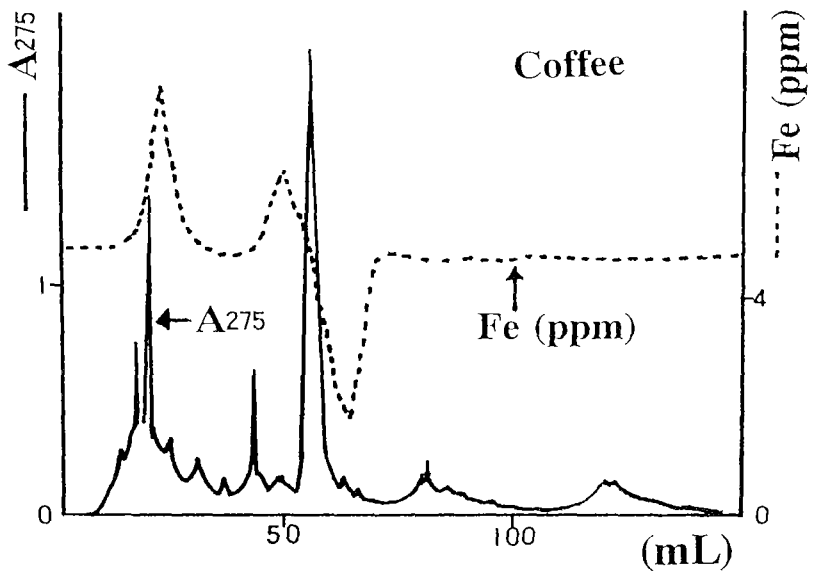

Fig. 1. Gel permeation chromatography equilibrated with 0.1 mM $FeSO_4$ in pH 4.0 acetate buffer (10 mM).

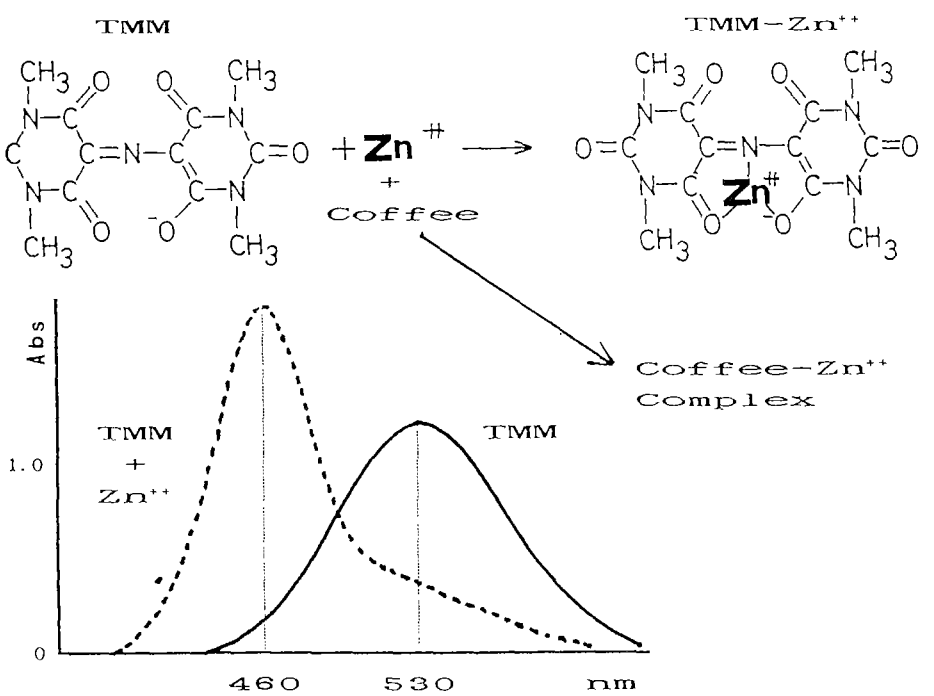

Fig. 2. Colorimetry of the zinc ion with tetramethylmurexide.

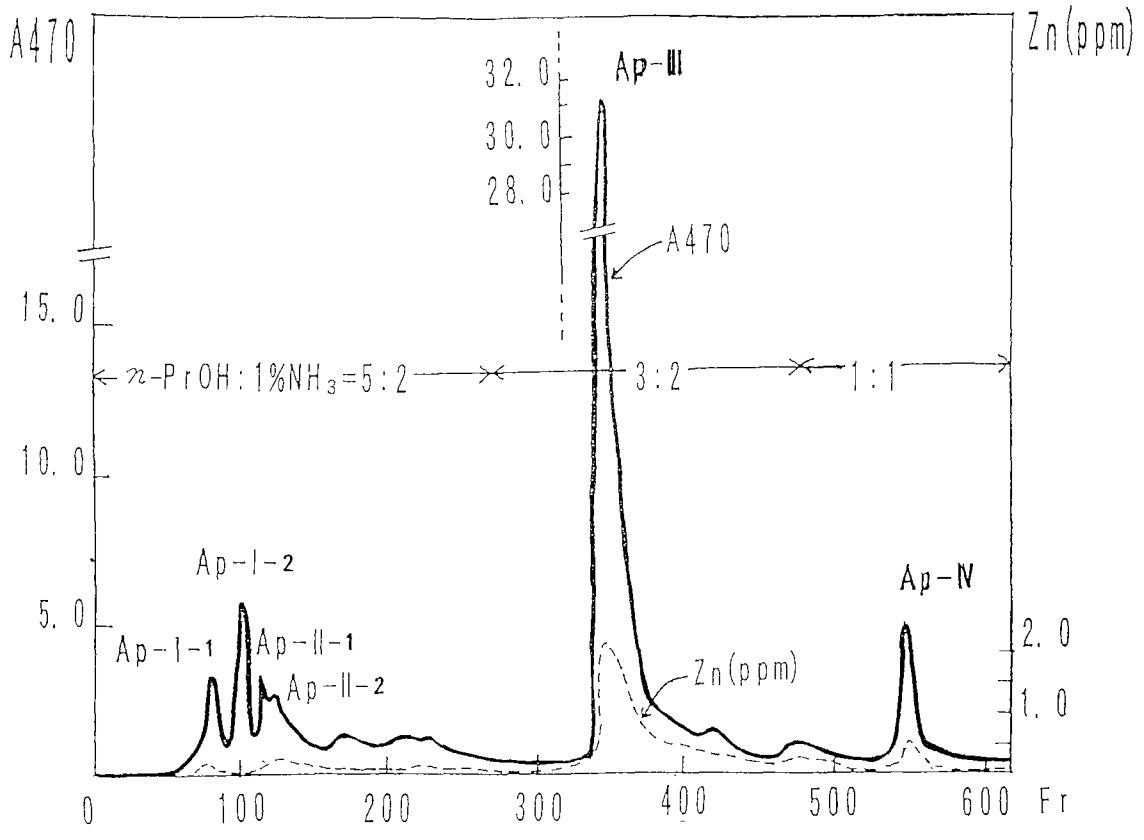

Fig. 3. Cellulose column chromatography for sample Ap Ⅲ of the zinc(Ⅱ)−coffee complex.

Column: Mikrokristallin cellulose (Merck), 6 x 60 cm

Eluent: mixture of 5:2, 3:2 and 1:1 (v/v) of n−propanol and 1% ammonia.

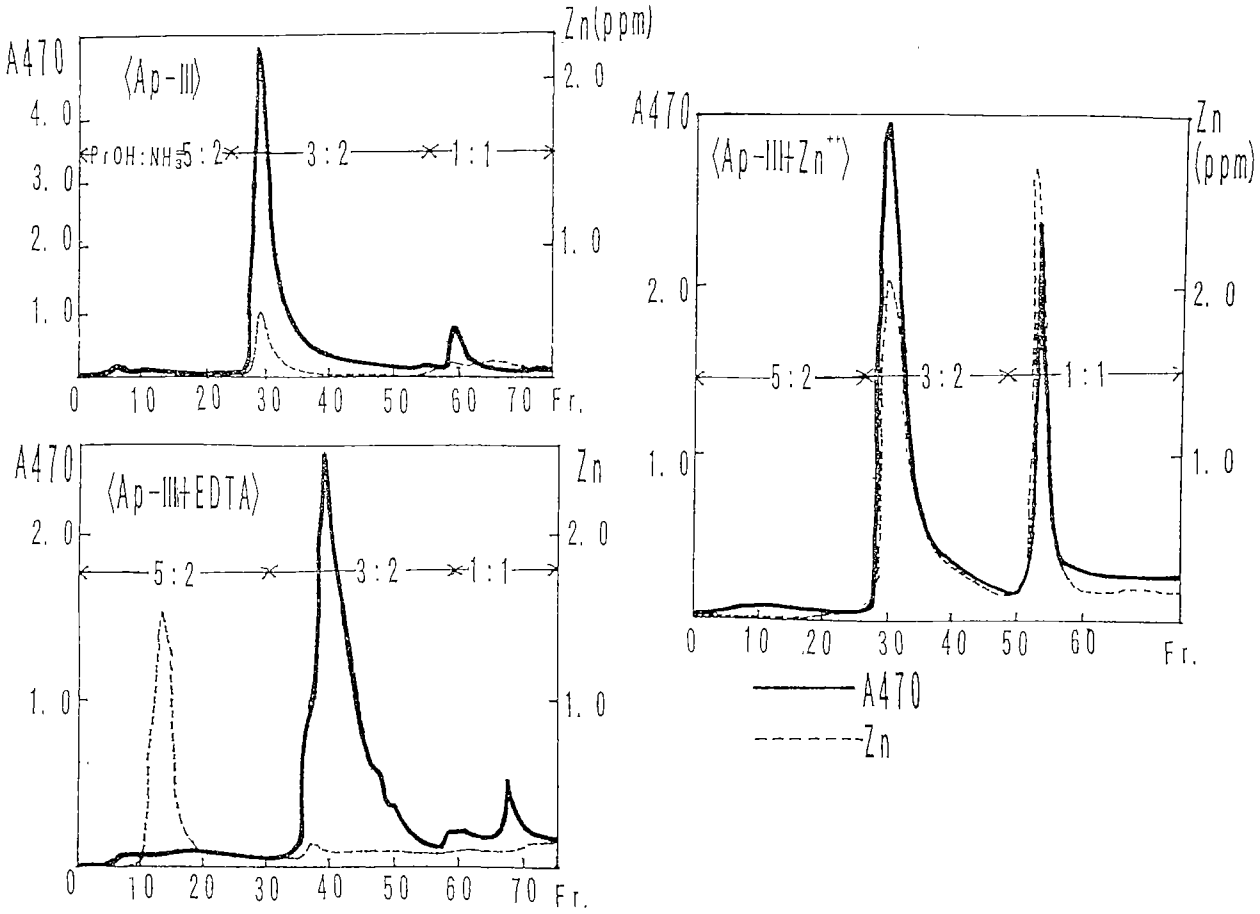

Fig. 4. Cellulose column chromatography for sample Ap Ⅲ of the Zinc(Ⅱ)−coffee complex further exposed to zinc(Ⅱ) and EDTA.

DEGRADATION MECHANISMS OF CHLOROGENIC ACIDS DURING ROASTING

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Chlorogenic acids (CGA) are ubiquitous in plant kingdom, in which they participate to a wide number of plant metabolisms. They control germination and cell growth, participate to defense mechanisms or act as lignin precursor[1].

CGA are particularly abundant in green coffee[2], but esters of quinic (QA) and caffeic (CA) acids are predominant, compared to those of quinic and ferulic (FA). CGA is degraded upon roasting. Many complex chemical pathways have been suggested, among which are hydrolysis, polymerisation, association[1] or volatile formation[3]. This work proposes possible chemical pathways of CGA degradation obtained in following CGA becoming during a roasting kinetics.

MATERIAL & METHODS

A green Colombian coffee is roasted at 240°C over 600s. Samples are regularly withdrawn along the roasting kinetics and finely ground.

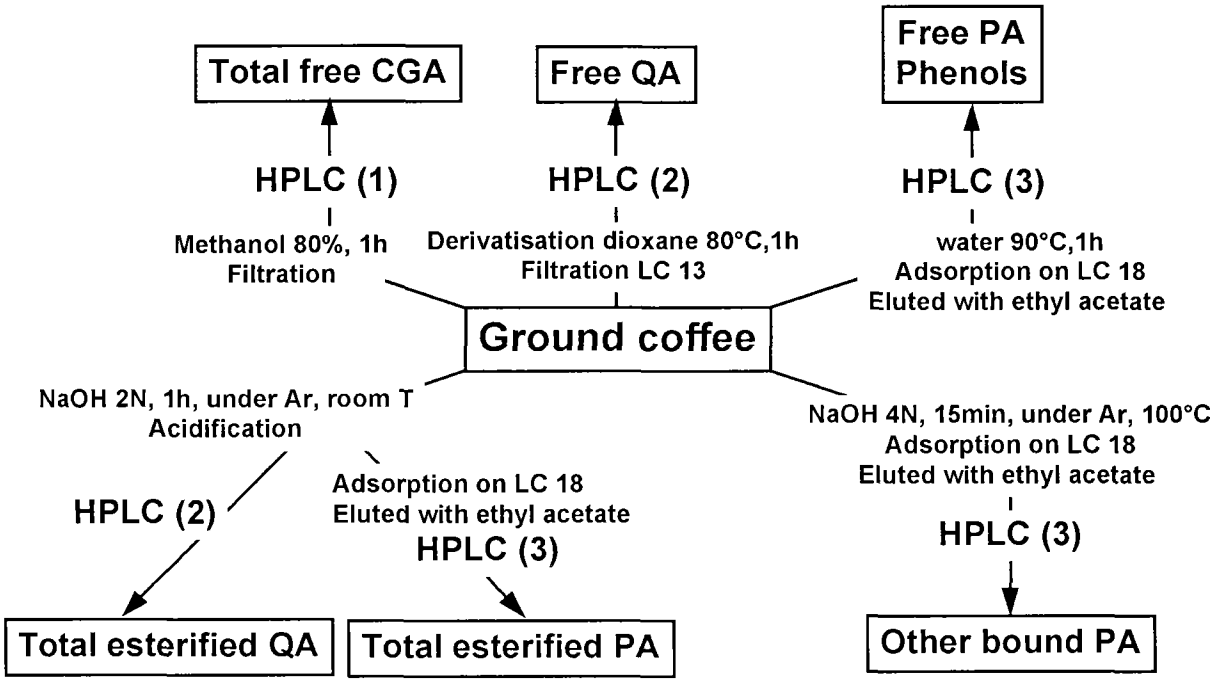
A combination of chromatographic methods is used to quantify CGA, QA, CA, FA and some phenol components (**Figure 1**).

HPLC 1 CGA are analysed on a Spherisorb S5 ODS1 column (250x4.5mm) eluted at 1mL/min room temperature in a H_3PO_4/CH_3CN gradient (92/8 to 50/50). Detection is performed at 320nm.

HPLC 2 QA is analysed on a Nucleosil 100-5 C18 column (250x4mm) (Marcherey-Nagel) eluted at 1mL/min 20°C in a CH_3CN/H_2O gradient (25/75 to 100/0). Detection is performed at 265nm.

HPLC 3 CA, FA and phenol components are analysed on an Aminex HPX-87H column (300x7.8mm) (Biorad) eluted at 0.6mL/min 65°C in a H_2SO_4 0.01N/CH_3CN 90/10. Detection is performed at 290nm.

Figure 1 Analytical approach for the quantification of CGA, QA, CA, FA and phenol components.



RESULTS & DISCUSSION

CGA rapidly disappear during the roasting process, but all CGA isomers are not similarly sensitive to thermal treatment as shown in **Figure 2**. 5-caffeoylquinic acid (5CQA) and dicaffeoylquinic acid (diCQA) are those decreasing the more rapidly. 4CQA and 3CQA isomers show peculiar behaviour. A temporary increase in their content is observed at the early stage of roasting suggesting that diCQA are partially hydrolysed leading to CQA and a caffeic moiety. After 2 minutes roasting all CGA are equally disappearing.

The phenolic moieties are never freed (free phenolic < 0.01%) during the roasting process (**Figures 3-4**). New esterified forms are transitorily produced in the early stage of roasting up to a half of initial content. On longer time the esterified phenolic acids

decrease up to one-third of initial content through degradation process. Phenol components such as catechol, 4-ethylcatechol, guaiacol and 4-ethylguaiacol were detected in trace amounts (<0.1%) explaining part of the two-third degraded phenolic acids. No bound species other than esterified were detected.

Figure 2 Degradation kinetics of CGA isomers during a roasting performed at 240°C (expressed in g/100g green coffee dry basis).

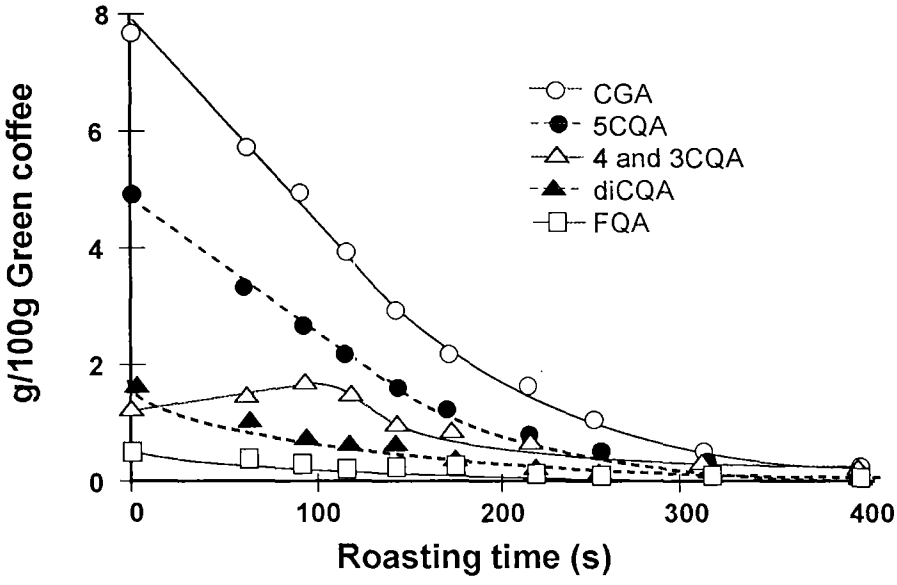


Figure 3 Behaviour of caffeic moieties during roasting (expressed in g/100g green coffee dry basis).

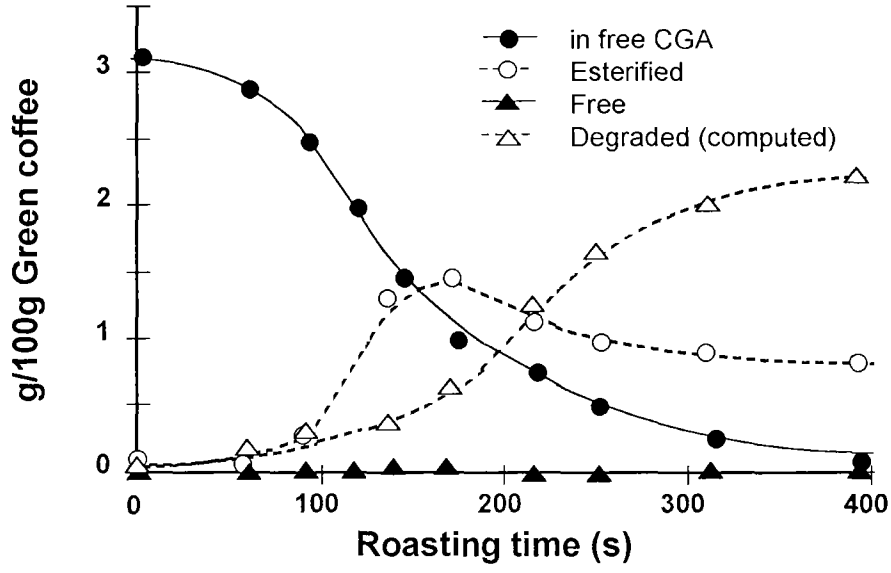


Figure 4 Behaviour of ferulic moieties during roasting (expressed in g/100g green coffee dry basis).

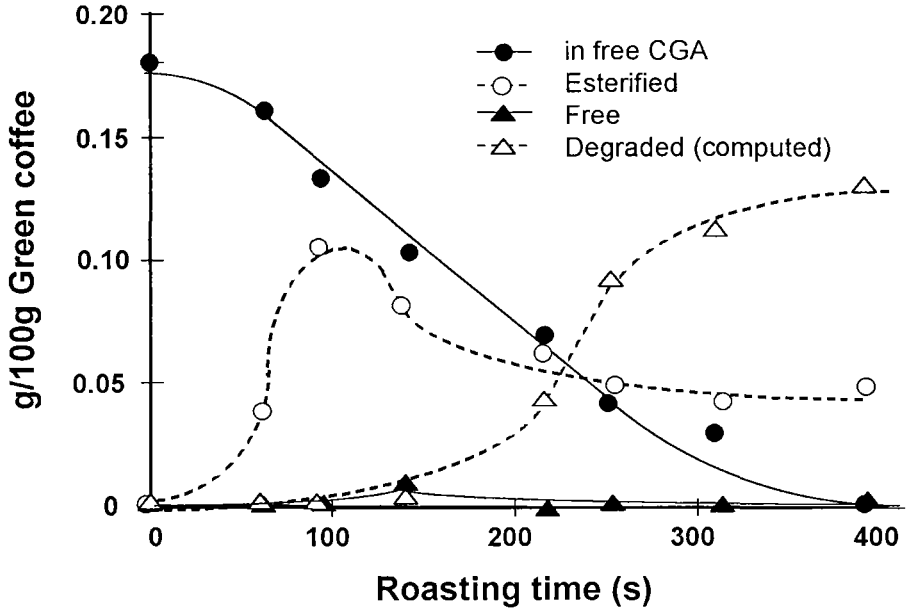
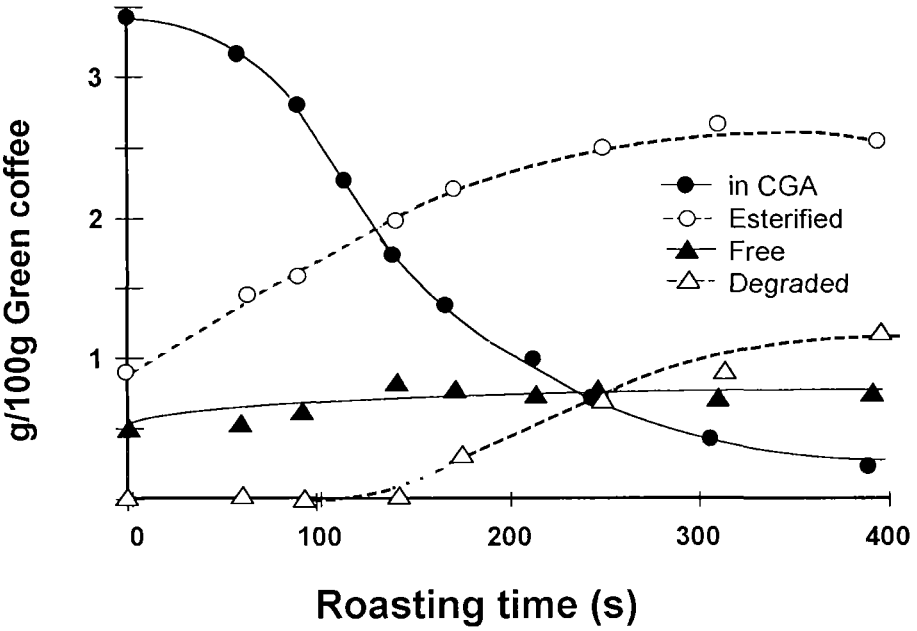


Figure 5 Behaviour of quinic moieties during roasting (expressed in g/100g green coffee dry basis).



Free quinic acid is already present in green coffee (**Figure 5**) and is slightly increased in the early stage of roasting. However the quinic moiety of CGA mostly undergoes esterification and values a half of quinic acid initial content. Degradation process is only observed in later stage of the roasting process. Degradation finally concerns one fourth of total quinic acid. Degradation products such as catechol, phenol, pyrogallol and hydroquinone were detected as traces.

Comparing the amounts of newly esterified phenolic and quinic moieties (**Figure 6**), it was observed that their amounts were similar in the early stage of roasting. On longer time the phenolic moiety was more quickly released than the quinic one. It suggests that part of CGA could undergo esterification through the free carboxylic group of the quinic acid and an hydroxyl group of e.g. carbohydrate or protein. 5CQA could be more reactive than 4CQA or 3CQA, the latter being more sterically hindered for the esterification reaction to take place.

A scheme of the possible routes for CGA degradation is proposed in **Figure 7**. At low roasting time, the coffee contains low amount of water which can be used for hydrolysis reactions. DiCQA are hydrolysed producing CQA and caffeic acid moiety. CQA and particularly 5CQA, probably the most reactive, may enter esterification reactions, leading to bound CGA. At the same time caffeic acid is degraded into catechol, 4-ethylcatechol. At high roasting time, the phenolic moiety is first released and immediatly degraded, whereas the quinic one is more difficult to free. A slight degradation is finally observed.

Figure 6 Comparison of esterified quinic and phenolic moieties along roasting (expressed in g/100g green coffee dry basis).

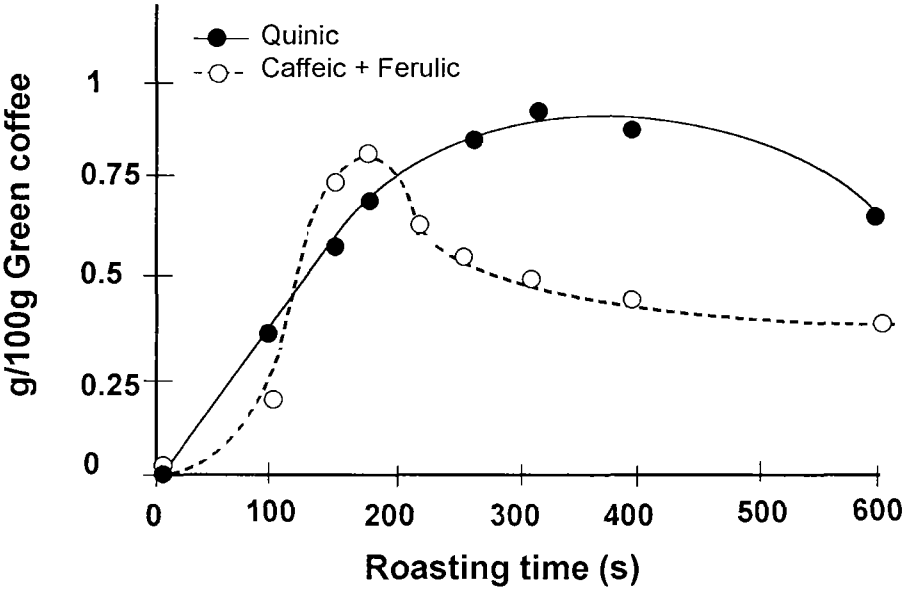
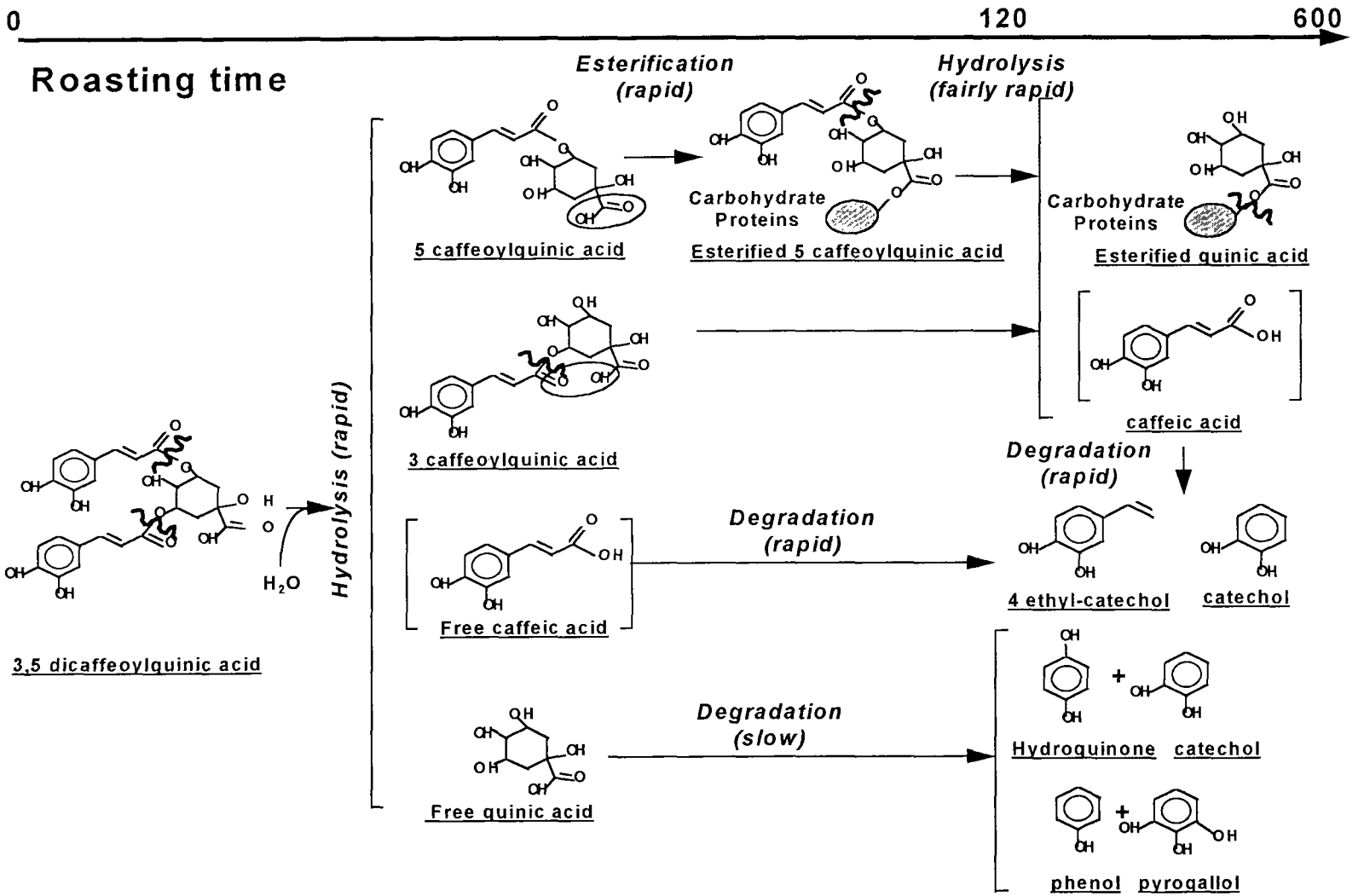


Figure 7 Suggested degradation mechanism of 3,5diCQA during roasting kinetics



SUMMARY

The fate of chlorogenic acids (CGA) during a medium slow roasting process (240°C) was kinetically investigated (0-600s) on a Colombian Arabica coffee. Chromatographic methods to analyse chlorogenic acid isomers, as well as their phenolic (caffeic CA, ferulic FA) and quinic moieties are presented. At low roasting time (<120s), diCQA are hydrolysed into CQA and CA. 5CQA isomers rapidly undergo esterification, producing bound chlorogenic acids. On longer roasting time, phenolic and quinic moieties whatever their occurrence form, start degrading generating diverses phenols components.

RESUME

Les modifications chimiques subies par les acides chlorogeniques (CGA) au cours d'un procédé de torréfaction moyenne lente (240°C, 0-600sec) ont été suivies sur un café Arabica Colombien. Des méthodes chromatographiques permettant la caractérisation qualitative et quantitative des isomères d'acides chlorogéniques (diCQA, CQA, FQA) et de leurs précurseurs, acides phénolique (CA, FA) et quinique (QA), sont présentées. Au début de la torréfaction (<120s), les diCQA sont hydrolysés en CQA et CA. Concomittamment les 5CQA sont esterifiés conduisant à la formation de CGA liés. Au delà de 120s, les CA et QA quelles que soient leur forme (CGA, estérifiés), commencent à se dégrader en composés phénoliques divers.

CGA	Chlorogenic acids
diCQA	Dicaffeoylquinic acid
CQA	Caffeoylquinic acid
FQA	Feruloylquinic acid
CA	Caffeic acid
FA	Ferulic acid
QA	Quinic acid

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A NOVEL HYDROXYCINNAMIC ACID DERIVATIVE OF COFFEE BEAN

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1. Introduction

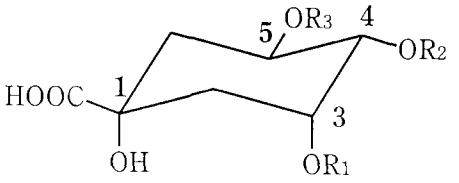
Coffee bean contains many kinds of hydroxycinnamic acid derivatives or chlorogenic acids (Fig. 1).[1] Chlorogenic acids are family of mono- and di-acyl quinic acids. The commoner acylating residues are caffeic acid, ferulic acid, and p-coumaric acid. The main compound among them is 5-caffeoylquinic acid, so-called chlorogenic acid.[2] These compounds are considered to be precursors of brown pigments of coffee[3,4] and shows astringency.[5] During the study on metal-chelating compounds from roasted coffee[6-8] and on the change in phenolic compounds by roasting coffee, we found an unidentified peak on three dimensional high-performance liquid chromatography (HPLC). In this report, we described the identification of hydroxycinnamic acid derivatives of coffee bean on HPLC with photodiode-array detector and the isolation and the identification of a novel compound, p-coumaroyl-(L)-tryptophan (13).

2. Materials and Methods

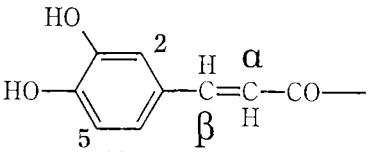
General. Melting point (mp) was determined by Yanagimoto micromelting point measuring instrument. Spectroscopic measurements were carried out using the following instruments: Hitachi 200-20 (UV), JEOL JMX-102/JMA-DA6000 (FABMS), and JEOL JNM-GX 270 (NMR). CD_3OD was used as a solvent and TMS as an internal standard for NMR. 3-Nitrobenzylalcohol was used as a matrix and NaI was added to a sample for FABMS.

Analysis of phenols of raw coffee bean. Raw coffee bean

(Coffea canephora var. robusta) was obtained from UCC Uejima Coffee Ltd. (Tokyo, Japan). Several grams of raw coffee bean was freezed with liquid nitrogen and pulverized by coffee mill. Ground coffee bean (0.5 g) was boiled in 20 ml of 70% methanol for 15 min. The filtrate and residue were separated by filtration. The residue was further extracted by the same procedure 3 times. The combined filtrate were analyzed by HPLC.
Analytical HPLC was performed under the following condition with photodiode-array detector; column, YMC-pak R-ODS-10 (Yamamura, Kyoto, Japan); pump, Hitachi L-6320 (Tokyo, Japan); photodiode-array detector, Hitachi L-4500 (Tokyo); eluent, CH_3CN and 5% acetic acid (2:98, v/v) for 10 min, the linear gradient from CH_3CN and 5% acetic acid (2:98, v/v) to (30:70, v/v) for 40 min, and CH_3CN and 5% acetic acid (30:70, v/v) for 10 min; flow rate, 1.0 ml/min.

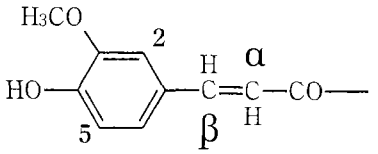


quinic acid

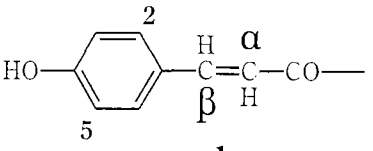


caffeoyl

	R1	R2	R3
3-CQA (1)	caffeoyl	H	H
4-CQA (3)	H	caffeoyl	H
5-CQA (2)	H	H	caffeoyl
5-CoQA (4)	H	H	coumaroyl
5-FQA (5)	H	H	feruloyl
3,4-diCQA (6)	caffeoyl	caffeoyl	H
3,5-diCQA (7)	caffeoyl	H	caffeoyl
4,5-diCQA (8)	H	caffeoyl	caffeoyl
3,4-FCQA (9)	caffeoyl (feruloyl)	feruloyl (caffeoyl)	H
3,5-FCQA (10)	caffeoyl (feruloyl)	H	feruloyl (caffeoyl)
4,5-FCQA (11)	H	caffeoyl (feruloyl)	feruloyl (caffeoyl)



feruloyl



coumaroyl

Figure 1. Structures of Hydroxycinnamic Acid Derivatives (Compounds **1-11**).

Transesterification of 5-chlorogenic acid. 3- and 4-chlorogenic acids were prepared by transesterification of 5-chlorogenic acid using tetramethylammonium hydroxide.[91] Twenty microliter of 10% tetramethylammonium hydroxide in MeOH (Tokyo Kasei Organic Chemicals, Tokyo, Japan) was added with immediate mixing to a 100 µl of 0.36% 5-chlorogenic acid (Nacalai Tesque,

Kyoto, Japan) in MeOH . After 5 min at room temperature, the reaction was terminated by adding a 20 µl of 2.5 M acetic acid. After dried up, the mixture of chlorogenic acids was applied to HPLC.

Isolation. About hundred grams of raw coffee bean was pulverized by coffee mill and phenolic compounds were three times extracted by 70% aqueous methanol. The combined extracts were concentrated *in vacuo*, and cinnamic acids derivatives were extracted by ethyl acetate. The ethyl acetate layer was dried by Na_2SO_4 and concentrated *in vacuo*. This crude paste was loaded into a reversed-phase open column (SS1020T, Senshu Kagaku, Tokyo, Japan; 15ϕ x 200 mm). It was successively developed with a mixture of CH_3CN and 5% acetic acid (5:95, 10:90, 15:85, 20:80, 25:75, and 30:70, v/v), each fraction being monitored by analytical HPLC. Fractions containing respective hydoxycinnamic derivatives were collected and concentrated *in vacuo*. The obtained fractions were then loaded into a preparative HPLC (column, YMC-pack D-ODS-10, 20ϕ x 250 mm (Yamamura, Kyoto); pump, Hitachi L-6000 (Tokyo); detector, Hitachi L-4200 UV-VIS type (Tokyo); eluent, a mixture of CH_3CN and 5% acetic acid; flow rate, 9.99 ml/min. Compound **5** (peak 5 on analytical HPLC) was obtained from the eluent of CH_3CN and 5% acetic acid (10:90, v/v) and compounds **6** (peak 6), **7** (peak 7), and **8** (peak 8) were from the eluent of CH_3CN and 5% acetic acid (15:75, v/v). Compounds **9** (peak 9), **10** (peak 10), **11** (peak 11), and **12** (peak 12) were obtained from the eluent of CH_3CN and 5% acetic acid (20:80, v/v), and compounds **13** (peak 13) were from the eluent of CH_3CN and 5% acetic acid (25:75, v/v).

Determination of absolute configuration of tryptophan. Isolated compound **13** was hydrolysed in 1 N NaOH at 70°C for 1 hr. Tryptophan in the hydrolyzate was analyzed by a chiral solvent-generated phase of HPLC according to the method of Wernicke[10] with some modifications. The HPLC condition was performed under the following condition; column, YMC-pak R-ODS-10 (Yamamura); pump, Hitachi L-6320; photodiode-array detector, Hitachi L-4500; eluent, MeOH and the solution of 1 mM copper(II) acetate and 2 mM L-phenylalanine (pH 4.5) (35:65, v/v); flow rate, 0.4 ml/min.

3. Results and Discussion

Identification of cinnamic acid derivatives by HPLC.

To prevent confusion, we point out that this paper use the preferred IUPAC numbering system for chlorogenic acids. Figure 2 showed typical chromatogram of raw coffee bean, which was shown at 320 nm. Morishita *et al.*[11] and Clifford and Javis[12] analyzed hydroxycinnamic acid derivatives by HPLC and used MeOH/phosphoric acid/water or CH_3CN/formic acid/water as an eluent. Here we adopted CH_3CN/acetic acid/water, because it showed better separation on the ODS column used here. Peaks 1-13 were numbered on the chromatogram. Caffeine having absorption maximum at 270 nm and being not detected at 320 nm was eluted about 2 min earlier than

peak 5. UV spectra of peaks 1-13, being directly measured by three dimensional HPLC with photodiode-array detector, were categorized into four patterns (A, B, C, and D, Fig. 3). Pattern A (peaks 1-3 and 5-11) showed spectra with absorption maximum at 295 and 320 nm, being almost the same as that of caffeic acid. The spectrum of peak 4 (pattern B) showed absorption maximum at 305 nm, corresponding with that of coumaric acid. The spectrum of peak 12 (pattern C) showed absorption maximum at 290 and 320 nm, and the spectrum of peak 13 (pattern D) showed absorption maximum at 290 nm and shoulder at 305 nm.

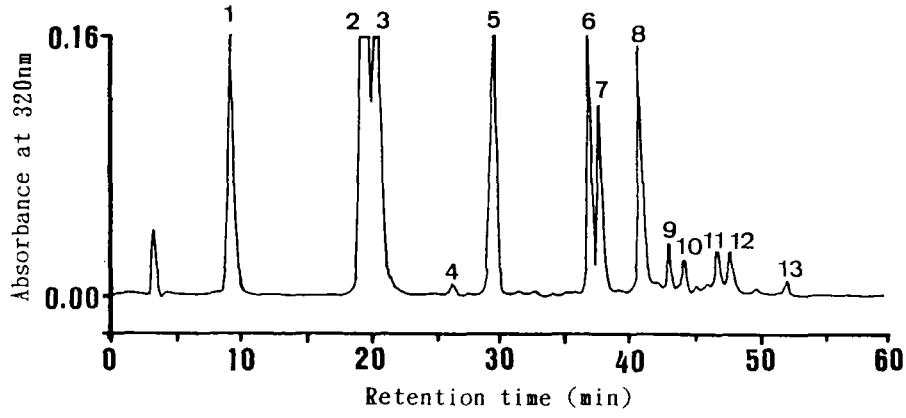


Figure 2. Chromatographic Pattern of Extracts of Raw Coffee Bean on HPLC.
Column, YMC-pak R-ODS-10; eluent, CH_3CN and 5% acetic acid (2:98, v/v) for 10 min, the linear gradient from CH_3CN and 5% acetic acid (2:98, v/v) to (30:70, v/v) for 40 min, and CH_3CN and 5% acetic acid (30:70, v/v) for 10 min; flow rate, 1.0 ml/min; Wavelength for detection, 320 nm.

Peaks 1-4 were identified by the retention time, their UV spectra on HPLC with photodiode-array detector and the comparison with standards or data of literatures.[11, 13] 3-chlorogenic acid and 4-chlorogenic acid were prepared by transesterification of 5-chlorogenic acid using tetramethyl ammonium hydroxide.[9] As a result, peaks 1, 2, 3, 4, and 5 were identified as 3-chlorogenic acid (**1**), 5-chlorogenic acid (**2**), the major and so-called chlorogenic acid in coffee bean, 4-chlorogenic acid (**3**), and p-coumaroylquinic acid (**4**), respectively.

As peaks 5-13 were not identified only by the retention time, their UV spectra on HPLC and the data of literatures, they were isolated and analyzed by NMR. Phenolics were extracted by 70% methanol from pulverized raw coffee bean. After concentration, phenolics were transferred to ethyl acetate layer. This fraction was separated by reversed-phase open column chromatography, and each compound was finally isolated by preparative HPLC.

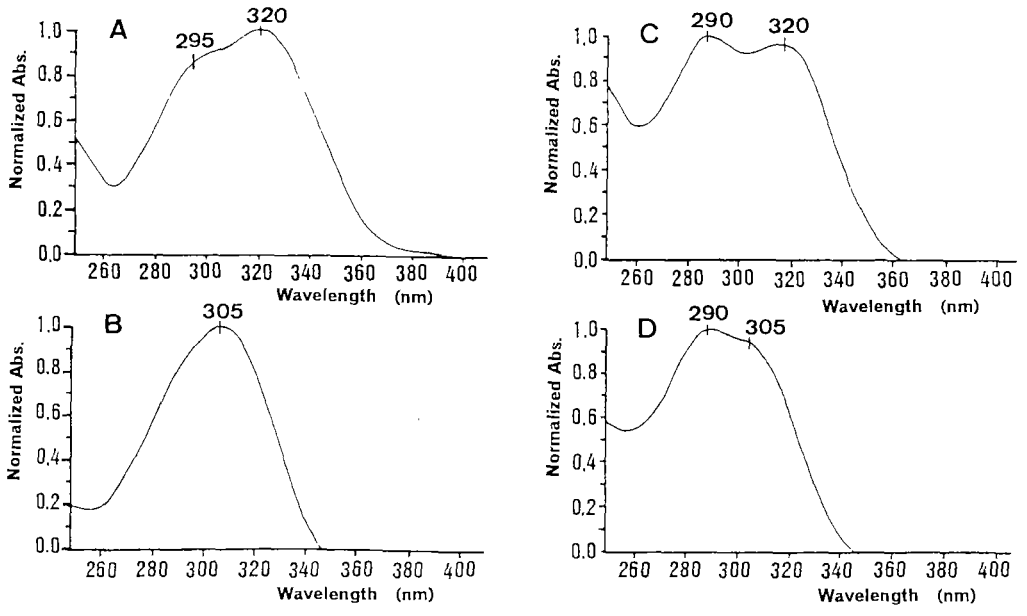


Figure 3. UV Spectra Pattern of Peaks 1-14 on HPLC.
UV spectra were recorded by photodiode-array detector. A, peaks 1-3, 5-11; B, peak 4; C, peak 12; D, peak 13

Peak 5, its NMR spectrum being almost the same as that of 5-CQA except that it had a signal at δ4.1 ppm of $-OCH_3$, was identified as 5-feruloylquinic acid (5-FQA, **5**).

Peaks 6-8 were di-caffeoyl quinic acids.[14] These compounds contained 2 molecules of caffeic acids and 1 molecule of quinic acid, and were identified as 3, 4-dicaffeoylquinic acid (3, 4-diCQA, **6**), 3, 5-diCQA (**7**), and 4, 5-diCQA (**8**), respectively, by their NMR data and UV-absorption spectra (Fig. 3). The signals of protons at position 3 (multiplet), position 4 (doublet doublet), and position 5 (triplet doublet) of the residue of quinic acid on their NMR spectra were respectively assigned from the proton-proton coupling pattern of quinic acid. The position of quinic acid to which caffeic acid was connected by ester bond was determined by the change of the chemical shift from quinic acid, because the signal at the position to which caffeic acid was connected shifted 1.1 to 1.7 ppm to down field.

The ^{1}H-NMR data and the UV-absorption spectra (Fig. 3) of peaks 9, 10, and 11 showed that they contained caffeic acid, ferulic acid and quinic acid, and they were identified as 3-caffeoyl-4-feruloylquinic acid or 4-caffeoyl-3-feruloylquinic acid (**9**), 3-caffeoyl-5-feruloylquinic acid or 5-caffeoyl-3-feruloylquinic acid (**10**), and 4-caffeoyl-5-feruloylquinic acid or 5-caffeoyl-4-feruloylquinic acid (**11**), respectively.[15, 16] The

positions of quinic acid to which caffeoyl and feruloyl groups were connected were determined by the same discussion as already described. However, it was not determined from the chemical shifts that either caffeoyl or feruloyl group was connected to either of two connected positions. This was also described by Clifford et al.[15]

Peak 12 was identified as caffeoyl-tryptophan (**12**, Fig. 4)[17] from the ^{1}H-NMR data and the UV-absorption spectrum (Fig. 3). The UV-absorption spectrum (absorption maximum at 290 and 320 nm) was similar to that of the mixture of tryptophan (absorption maximum at 290 nm) and caffeic acid (absorption maximum at 320 and 285 nm). The NMR signals were assigned by the comparison with those of tryptophan and caffeic acid.

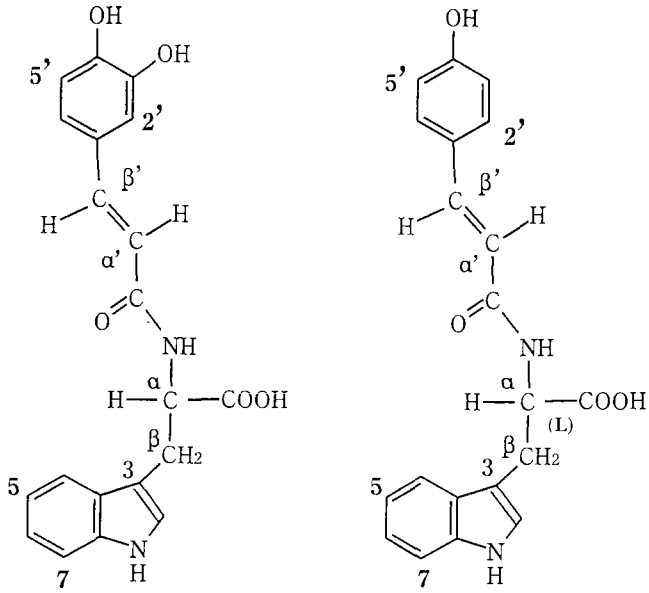


Figure 4. Structures of Caffeoyltryptophan (**12**) and Coumaroyl-(L)-Tryptophan (**13**).

Identification of compound **13**.

As peak 13 (compound **13**) was not identified from the data of literatures on HPLC analysis, we identified it by instrumental analyses. The yield was about 7 mg from 100 g coffee bean. Compound **13** was obtained as amorphous white powder, mp >208°C (decomp). It has molecular weigh 350 (FABMS m/z: 373 $(M+Na)^+$) and

molecular formula $C_{20}H_{18}N_2O_4$ (HR-FABMS m/z: calcd for $C_{20}H_{18}N_2O_4Na$, 373.1164; found, 373.1149). The UV spectrum (λmax 290 and 305 nm) were different from chlorogenic acids (λmax 295 and 320 nm) and similar to a mixture of tryptophane (λmax 290 nm) and p-coumaric acid (λmax 305 nm). Its ^{1}H- and ^{13}C-NMR spectral data are summarized in Table I, showing the existence of moieties of tryptophan and p-coumaric acid. When the data of caffeoyltryptophan and compound **13** were compared, the signals originated of 4-substituted ortho-diphenol in caffeoyl group and those of para-substituted phenol in coumaroyl group were only different. The existence of two trans vinyl protons of coumaroyl group and a tryptophanyl residue was clearly shown by ^{1}H-NMR. The existence of coumaroyl group was also supported by the fragmentation peak (base peak) of FABMS at m/z 147. After hydrolyzing compound **13**, the produced tryptophan was then analyzed by reversed-phase HPLC with chiral eluent.[10] In this analysis, tryptophan formed a complex with L-phenylalanine and Cu(II), and D-tryptophan and L-tryptophan were eluted with retention times of 39.7 min and 62.9 min, respectively. As a result, it was shown that **13** contained L-tryptophan. Compound **13** was thus identified as p-coumaroyl-(L)-tryptophan (Fig. 4), which was a novel

Table I. NMR Data of Compound **13**.

Position	δ_H (ppm)	δ_C (ppm)
2	7.37 (1H, s)	125.2
3		109.6
4	7.85 (1H, d, J=7.8)	120.1
5	7.32 (1H, t, J=7.8)	122.7
6	7.25 (1H, t, J=7.8)	119.4
7	7.57 (1H, d, J=7.8)	112.1
8		138.4
9		128.5
α	5.10 (1H, t, J=7.6)	59.7
β	3.50 (1H, dd, J=14.5, 7.6)	28.5
	3.65 (1H, dd, J=14.5, 7.6)	
COOH		174.4
1'		127.2
2'	7.65 (1H, d, J=8.5)	131.1
3'	7.04 (1H, d, J=8.5)	116.7
4'		161.0
5'	7.04 (1H, d, J=8.5)	116.7
6'	7.65 (1H, d, J=8.5)	131.1
α'	6.72 (1H, d, J=15.8)	115.5
β'	7.69 (1H, d, J=15.8)	146.6
CO		171.0

hydroxycinnamic or amino acid derivative, while caffeoyl-

tryptophan[17] and caffeoyl-tyrosine[18] have been reported as components of coffee bean.

The contents of caffeoyltryptophan and p-coumaroyl-(L)-tryptophan in raw coffee bean used here were 110 and 30 mg/100 g, respectively, which were determined by analytical HPLC.

Here, Coffea canephora var. robusta was only used for isolation of compound **13**. It is reported that C. robusta contained more chlorogenic acids than Coffea arabica, being another commercially important coffee bean. We are interested in the distribution of this new compound in various coffee beans, its change by roasting, and the relationship between the compound and the quality of coffee.

Acknowledgements. We are grateful to UCC Uejima Coffee Ltd. and Japan Coffee Association for supplying coffee bean.

Summery

Hydroxycinnamic acid derivatives of coffee bean were analyzed by three dimensional high-performance liquid chromatography. Thirteen peaks were detected at 320 nm using a reversed-phase column and were categorized into 4 groups by their UV-absorption spectra. Eleven chlorogenic acids and caffeoyltryptophan were identified and a novel hydroxycinnamic acid derivative, p-coumaroyl-(L)-tryptophan, was isolated from coffee bean and identified by instrumental analyses.

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SUPEROXIDE ANION SCAVENGING ACTIVITY AND METAL CONTENTS OF COFFEE

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INTRODUCTION

Superoxide anion radical, which is an active form of molecular dioxygen, has both beneficial and harmful effects to organisms. Especially, superoxide anion is proposed to cause oxidative injury to cells and tissues due to the successive formations of more reactive oxygen spiecies such as hydrogen peroxide and hydroxyl radicals (1-4). Coffee which contains both polyphenols like tannins and metalloproteins like superoxide dismutases (5) is highly expected to scavenge the active oxygen species such as superoxide anion. We studied the beneficial effect of coffee in terms of both superoxide anion scavenging activity (SSA) and metal contents of coffee which we take every day, by evaluating with ESR (electron spin reasonance)-spin trapping (6,7), neutron activation analysis and flameless atomic absorption methods.

EXPERIMENTAL

Materials

Coffee beans such as Bluemountain, Colombia, Jamaica, Kilimanjaro, Mandarin, Mocha and Santos, and instant coffees like Kilimanjaro and Mocha were commercially available.

Rooibos-tea and Gyokuro-tea (Japanese green tea) were also commercially available products. Xanthine oxidase (XOD, grade 1, butter-milk) and superoxide dismutase (SOD, bovine erythrocytes) were obtained from Sigma Chemical, Co., St. Louis, USA. Hypoxanthine (HPX), 60% nitric acid, 30% hydrogen peroxide, 60% perchloric acid and standard solutions (1000ppm) of copper, iron, manganese and zinc were purchased from Wako Pure Chemicals, Co., Osaka, Japan. Diethylenetriaminepentacetic acid (DTPA) was obtained from Dojin do, Co., Kumamoto, JAPAN. 5,5-Dimethyl-1-pyrroline-1-oxide (DMPO) was from Labotec, Co., Tokyo, JAPAN.

Methods

ESR Measurement

ESR spectra were recorded on a JEOL JES-RE1X spectrometer using an aqueous quartz flat cell (JEOL LC-12 ESR cuvette) with an inner size of 60mm × 10mm × 0.31mm and an effective volume of 160 μl. Instrumental conditions were as follows : Field modulation frequency 100KHz, modulation amplitude 0.1mT and output power 5mW. All experiments were carried out at room temperature (22℃).

Preparation of Sample

A 24g of coffee powder made by a coffee mill (Toshiba, HCD580M) was passed by 480ml of hot water (81℃) for 8min. For the extracted solution, superoxide anion scavenging activity (SSA) was estimated by ESR-spin trapping method using DMPO . Superoxide anion radicals were generated with a hypoxanthine-xanthine oxidase (HPX-XOD) reaction system. The sampling procedure was as follows : A 100mM (1M =1 mol dm^{-3}) sodium phosphate buffer solution (pH7.4) was used for a solvent. Solutions of 2.0mM HPX (a), 1.0mM DTPA (b), various concentrations of extracted sample solution (c) and 0.5unit XOD/ml (d) were prepared before use. XOD solution was stored in an ice bath to prevent the inactivation during the experiment. A 50 μl of (a), 30 μl of (b), 50 μl of (c) or the vehicle and 10 μl of 25% DMPO (diluted with water) were put into a test tube. To the solution, 50 μl of (d) was added. After quick stirring for 5 sec, 200 μl of the solution was taken into a flat cell. The test solution thus contains the final concentrations of 0.42mM HPX, 1.38mM DTPA, 93.4mM DMPO, 0.105mM XOD and various concentrations of the extracted sample solution.

SSA Measurement

A quantitative analysis of DMPO-OO(H) adduct by ESR spectrometry was performed under the following conditions for obtaining a high reproducibility of the spin adduct yields. Recording of the ESR spectrum started 40sec after the addition of XOD. The recording rate was 5mT min^{-1}. The signal intensity of the lowest field peak in the spectrum was normalized as a relative signal height against the standard signal intensity due to the manganese (II) marker doped in MgO. The scavenging activity of the sample is expressed as the IC_{50} value, the 50% inhibition concentration of the superoxide anion radicals generated by the HPX-XOD system (8) .

Flameless Atomic Absorption Spectrometry (FAA)

Copper, iron, manganese and zinc contents in the samples were measured by FAA for wet digested samples as follows : Wet digestion of coffee beans or extracted coffee solutions for FAA measurement were performed in 50ml beakers. A100mg of coffee beens or 5ml of extracted coffee solutions were treated with repeated addition of 60% nitric acid, 30% hydrogen peroxide and 60% perchloric acid at 200℃ on a hotplate in a draft chamber. When the digestion was completed, the white crystals were dissolved in 6% nitric acid to be a total volume of 5ml. For this wet digested solution, the metal contents (copper, iron, manganese and zinc) were determined by FAA using a Shimazu AA-6500 spectrometer. Calibration curves for these 4 metal ions were obtained with the corresponding standard metal solutions.

Neutron Activation Analysis (NAA)

Other metal contents were quantitated by NAA as follows : Coffee powder samples and standards for sodium analysis on filter papers, were sealed in double-layered chlorine-free, polyethylene bags, and subjected to NAA. The sealed samples were packed in a pneumatic tube and activated by irradiation at 5000kW for 30sec or 60min in the Research Reactor of Kyoto University. The thermal, epithermal and fast neutron fluxes were 2.34×10^{13}, 8.40×10^{11} and 4.80×10^{12} neutrons $cm^{-2}s^{-1}$, respectively. Measurements of gamma-rays were performed with a PGE Ge(Li)detector (active volume, 90ml) equipped with a Northan (4096 channels) pulse height analyser. Subsequent data reduction was achieved with a series of Fortran computer program written by the staff of the Research Reactor Institute of Kyoto University.

RESULTS

Superoxide Anion Scavenging Activity (SSA) of Coffee as Evaluated by ESR-Spin Trapping Method

ESR-spin trapping was performed by using DMPO to examine superoxide anion scavenging activity (SSA) of extracted coffee solutions. Signal due to superoxide anions ($\cdot O_2^-$) detected by ESR-spin trapping technique was typically depicted in Fig. 1. The spectrum was identified as DMPO-OO(H) spin adduct by the coupling constants a^N = 1.41mT, a_β^H = 1.12mT and a_γ^H = 0.138mT by comparing its simulated hyperfine coupling constants (Fig. 1(a)).

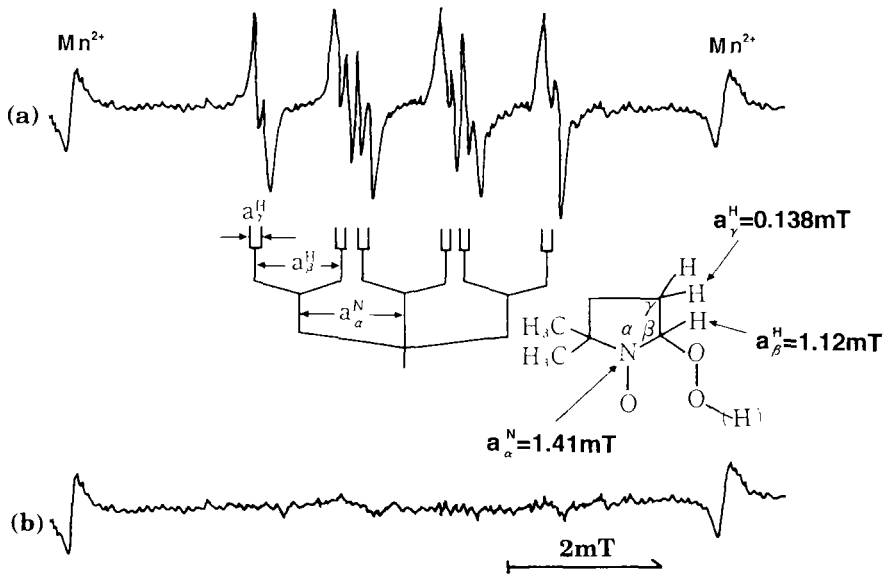

Fig. 1 ESR-spin trapping of $\cdot O_2^-$ generated by HPX-XOD system (a) and that (b) in the presence of extracted Colombia coffee (5mg/ml)

These signals due to $\cdot O_2^-$ disappeared by adding coffee solutions (Fig. 1(b)) depending on their concentrations as shown in Fig. 2. SSA was evaluated by IC_{50} value which is the concentration of coffee extent causing 50% scavenging of superoxide anions generated by the HPX-XOD system . The IC_{50} values of coffees imported from different countries were thus estimated to be about 260～750 μ g/ml (Table 1).

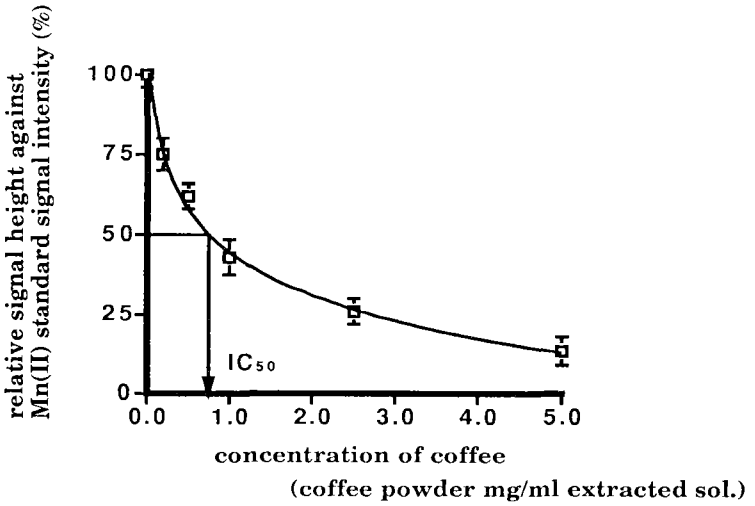

Fig. 2 Evaluation method of SSA of coffee

Table 1. Superoxide anion scavenging and SOD-like activities of coffees

Type of Coffee	Brand	IC_{50}±S.D. (μg/ml)	SOD-like activity±S.D. (SOD unit/ml)
Powder coffee	Kilimanjaro	746±67	473±56
	Mandarin	685±110	511±82
	Santos	575±97	612±103
	Bluemountain	472±116	745±184
	Mocha	352±122	997±348
	Jamaica	298±91	1186±359
	Colombia	259±33	1361±166
Instant coffee	Kilimanjaro	217±21	464±46
	Mocha	168±7	601±25
Others	Rooibos tea	833±201	21±5
	Gyokuro	154±42	7340±2058

Values are means±S.D.s for three experiments.

Elements in Coffee Powder as Determined by Neutron Activation Analysis (NAA) Method

NAA was performed to determined the elements in coffee powder. Fig. 3 shows the gamma-ray spectrogram of a coffee (Mocha) powder after neutron irradiation. For example, ^{51}Cr (half life, 27.8d) was detectable by the gamma-ray peak at 320.0 KeV. Thus, the 320.0KeV peak was used to calculate the Cr concentration in the coffee. Likewise, ^{82}Br, ^{134}Cs, ^{46}Sc, ^{27}Mg, ^{86}Rb, ^{65}Zn, ^{60}Co, ^{24}Na, ^{52}V, ^{42}K, ^{140}La, ^{82}Al, ^{56}Mn, ^{38}Cl and ^{49}Ca were

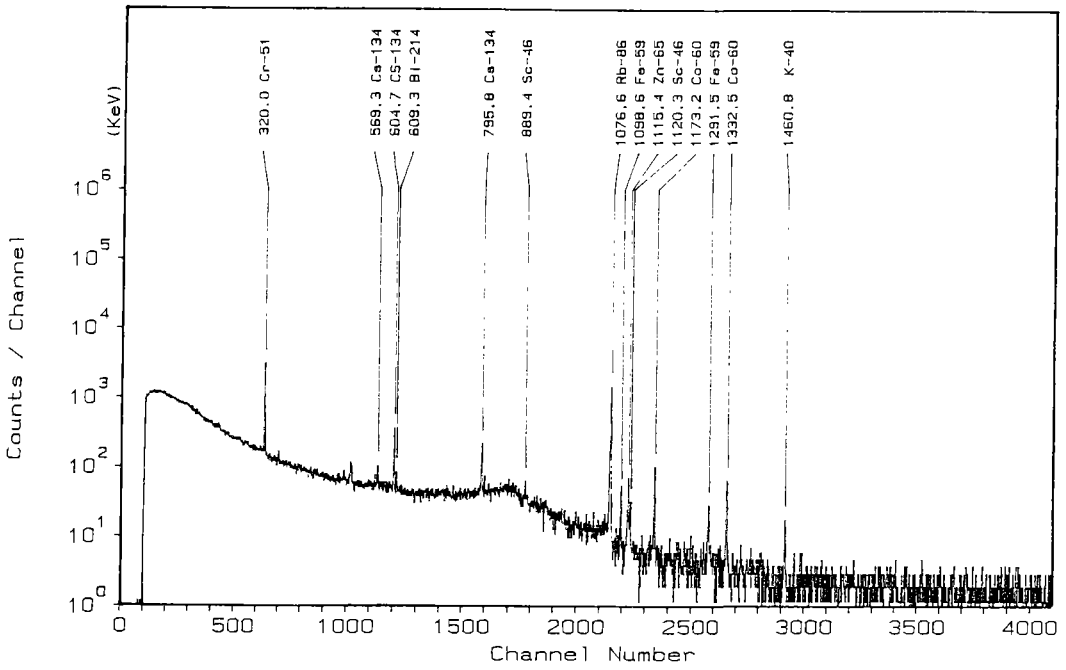

Fig. 3 Gamma-ray spectrogram of a coffee powder (Mocha)

determined by the gamma-ray peaks at 554.0, 889.3, 1014.4, 1077.9, 1115.5, 1173.2, 1368.5, 1434.1, 1524.6, 1596.5, 1778.7, 1810.6, 2167.6 and 3084.4 KeV, respectively. The concentrations of these elements in coffee powders were summarized in Table 2. The average levels of the elements in coffees were found in the following order, K (4.5mg/ 100mg powder) > Mg (480 μ g) > Co (430 μ g) > Ca (290 μ g) . Concentrations of iron(Fe),

Table 2. Concentrations of the elements in coffees, as determined by NAA method

Type of Coffee	Brand	Na	K	Rb	Cs	Mg	Ca	Al	Cl	Br
Instant coffee	KILIMANJARO	894	109000	115	0.26	8280	3450	83.7	1510	4.22
		38.9	2788	1.35	0.01	341	86.1	3.10	42.6	0.1
	MOCHA	676	102000	93.6	0.26	7450	3710	83.4	1420	5.62
		29.4	2609	1.10	0.01	307	92.6	3.09	40.1	0.07
Powder coffee	COLOMBIA	19.4	41400	21.5	0.09	4330	3120	64.2	347	20.2
		0.84	1059	0.25	0.002	178	77.8	2.38	9.79	0.25
	JAMAICA	45.6	47600	18	n.d.	5430	4200	53.5	662	0.89
		1.98	1217	0.21	n.d.	223	105	1.98	18.7	0.01
	KILIMANJARO	18.1	41200	65	0.04	4810	2300	52.5	276	8.98
		0.79	1054	0.76	0.001	198	57.4	1.95	7.79	0.11
	MANDARIN	17.5	43800	69.1	0.14	4310	2120	110	285	22
		0.76	1120	0.81	0.003	177	52.90	4.08	8.04	0.28
	MOCHA	21.1	44700	29.2	n.d.	4610	2660	167	324	6.29
		0.92	1143	0.34	n.d.	190	66.4	6.19	9.14	0.08
	SANTOS	14.5	52500	35.8	0.06	5240	2830	75.5	556	12.1
		0.63	1343	0.42	0.001	216	70.6	2.80	15.7	0.15
Others	ROOIBOS TEA	2300	2600	5.5	0.041	2200	1700	140	2700	27

copper(Cu), zinc(Zn) and manganese(Mn) were 0.34 μ g, 0.42 μ g, 0.50 μ g and 6.49 μ g / 100mg coffee powder, respectively, giving the following order, Mn > Zn > Cu > Fe.

Four Metal Ions (Fe, Cu, Zn and Mn) in Extracted Coffee Solution as Determined by Flameless Atomic Absorption (FAA) Spectrometry

FAA spectrometry was performed to examine the 4 metal ions (Fe, Cu, Zn and Mn) in extracted coffee solutions. These 4 metal ions were involved in the active site of SOD to scavenge $\cdot O_2^-$. Thus, the occurrence of these 4 metal ions may be expected for SOD-like activities. The concentrations of 4 metal ions involved in coffee solutions are shown in Table 3. The average concentrations of the 4 metal ions (Fe, Cu, Zn and Mn) in extracted coffee solutions were found to be 0.27 μ g, 0.08 μ g, 0.13 μ g and 0.58 μ g / 100mg coffee powder, respectively, being in the following order, Mn > Fe > Zn > Cu.

DISCUSSION

We measured superoxide anion scavenging activity (SSA) of coffees imported from different countries. SSA of 7 coffees were found to be in the order of Colombia > Jamaica > Mocha > Blue-mountain > Santos > Mandarin > Kilimanjaro. The SSA activity of Colombia was approximately three times higher than that of Kilimanjaro, as evaluated by IC_{50} values. Coffees examined were found to have SSA in the range of 470~1360 SOD

Brand	Sc	V	Cr	Mn	Fe	Co	Cu	Zn	La	Ce
KILIMANJARO	< 0.01	n.d.	22	75.1	410	8290	36.8	4.39	n.d.	n.d.
(INSTANT)	< 0.001	n.d.	0.42	1.37	7.34	140.67	0.58	0.07	n.d.	n.d.
MOCHA	92.7	0.31	19.5	83.4	372	5380	32.2	3.36	n.d.	n.d.
(INSTANT)	2.06	0.006	0.38	1.52	6.66	91.3	0.51	0.05	n.d.	n.d.
COLOMBIA	74	n.d.	17.9	73	355	5510	488	6.67	n.d.	n.d.
	1.65	n.d.	0.34	1.33	6.36	93.5	7.68	0.10	n.d.	n.d.
JAMAICA	29.9	n.d.	13.3	74.5	351	4680	539	5.15	n.d.	n.d.
	0.7	n.d.	0.26	1.36	6.29	79.4	8.48	0.08	n.d.	n.d.
KILIMANJARO	n.d.	n.d.	21.2	84.3	339	5060	355	3.96	n.d.	n.d.
	n.d.	n.d.	0.41	1.53	6.07	85.9	5.59	0.06	n.d.	n.d.
MANDARIN	< 0.01	n.d.	18.7	45.8	327	2250	352	4.47	n.d.	n.d.
	< 0.001	n.d.	0.36	0.83	5.86	38.2	5.54	0.07	n.d.	n.d.
MOCHA	133	n.d.	17.8	42.7	325	n.d.	245	4.39	0.08	n.d.
	2.96	n.d.	0.34	0.78	5.82	n.d.	3.86	0.07	0.001	n.d.
SANTOS	72.2	n.d.	23.8	68.9	320	4000	548	5.2	0.73	0.86
	1.61	n.d.	0.46	1.25	5.73	67.9	8.62	0.08	0.005	0.01
ROOIBOS TEA	0.022	0.22	0.22	59	110	0.088	< 6.8	5.8	0.31	0.58

n.d. = not detected, upper value : μ g/g coffee powder, lower value : μ mol/g coffee powder

Table 3. Concentrations of Fe, Cu, Zn and Mn in the extracted coffee solutions

Brand	Fe	Cu	Zn	Mn
BLUEMOUNTAIN	2.76	0.56	0.61	5.94
	0.049	0.009	0.009	0.108
COLOMBIA	2.18	0.68	1.52	6.14
	0.039	0.011	0.023	0.112
JAMAICA	2.92	0.56	0.68	6.30
	0.052	0.009	0.010	0.115
KILIMANJARO	3.02	0.78	1.70	5.64
	0.054	0.012	0.026	0.103
MANDARIN	2.52	0.73	1.49	5.04
	0.045	0.012	0.023	0.092
MOCHA	2.38	1.06	1.73	5.08
	0.043	0.017	0.026	0.092
SANTOS	2.78	1.23	1.14	6.28
	0.050	0.019	0.017	0.114

upper value : μg/g coffee powder, lower value : μ mol/g coffee powder

unit / ml of coffee extracted, indicating that coffees have relatively good SSA, compared with that of Rooibos tea, but less active than Japanese green tea, Gyokuro.

We also determined the element contents in coffees and detected the following elements, Na, K, Rb, Cs, Mg, Ca, Al, Cl, Br, Sc, V, Cr, Mn, Fe, Co, Cu, Zn, La and Ce. Almost coffee beans have been found to accumulate Co, Fe, Cr and Sc remarkably, compared with their concentrations in almost plants (9) . In Mocha Cs and Co were not detected, and in Jamaica Cs was not detected. In Mocha and Santos La was detected, and Ce was detectable in Santos.

On the other hand, the 4 metal ions (Fe, Cu, Zn and Mn) in extracted coffee solutions were estimated. These 4 metal ions are involved in the active site of SOD to scavenge $\cdot O_2^-$. Thus, the occurrence of these 4 metal ions may relate to the SOD-like activity of the coffee.

We tried to find correlations between the SSA and metal contents or their ratios in coffee. As shown in Fig. 4, the correlation between the SSA and the ratio of Mn/Fe was found, in which the correlation coefficient of the linear regression was 0.762 for a total of 7 points. This result suggests that the SSA of coffees depends partially on the metal ratios in coffee. In fact, the occurrences of Cu, Zn-, Fe- and Mn-SOD in coffee have been reported (5). Thus, every time when we drink a cup of coffee (130ml), SSA which corresponds to 61～177 kilounit SOD will be taken. Investigations on this line is continued.

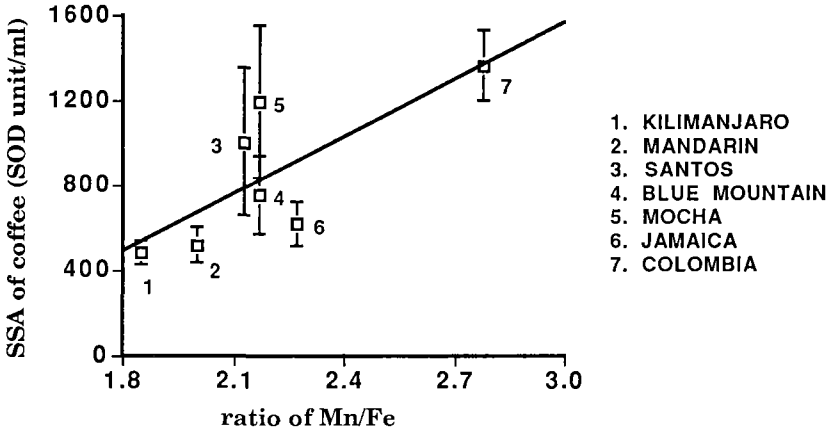

Fig. 4 The correlation between the SSA and the ratio of Mn/Fe

Linear correlation was found to be y = 904X-1144 (r = 0.762).

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ABSTRACT

SUPEROXIDE ANION SCAVENGING ACTIVITY AND METAL CONTENTS OF COFFEE

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Superoxide anion radical which is an active form of molecular dioxygen has both beneficial and harmful effects to organisms. Especially, superoxide anion is proposed to cause oxidative injury to cells and tissues due to the successive formations of more reactive oxygen spiecies such as hydrogen peroxide and hydroxyl radicals. Coffee which contains both polyphenols like tannins and metalloproteins like superoxide dismutases is highly expected to scavenge the active oxygen species such as superoxide anion. We studied the beneficial effect of coffee in terms of both superoxide anion scavenging activity (SSA) and metal contents of coffee which we take every day, by evaluating with ESR (electron spin reasonance)-spin trapping, neutron activation analysis (NAA) and flameless atomic absorption (FAA) methods. Coffees imported from different countries were found to have SSA in the range of 470～1360 SOD unit/ml of coffee extract, indicating that almost coffees examined have relatively good SSA. Nineteen sorts of elements were detected by both NAA and FAA methods, depending on the kind of coffee. A correlation between SSA and the metal ratio Mn/Fe was found, indicating the occurrence of SOD-like activity of coffee. Thus, every time when we drinke a cup of coffee (130ml), SSA which corresponds to 61～177 kilounit SOD was indicated to be taken.

ANALYSIS OF ACIDS IN COFFEE BY CAPILLARY ELECTROPHORESIS

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Introduction

The acid fraction in coffee is an important factor in the sensory quality of the beverage. Although the chlorogenic acids are usually analyzed using HPLC(1), there are various types of procedure available for the estimation of the other major acid type, the aliphatic acids. These procedures typically use HPLC (2), GC (3) or ion-exchange chromatography (4), none of which offers the combination of rapid analysis with base-line separation of all the important coffee aliphatic acids.
A recently developed technique, capillary electrophoresis (CE), offers an additional means of determining aliphatic acids. CE has a better separative ability than chromatography-based methods and analysis is rapid. CE has been shown to be effective for the analysis of organic acids in various types of foods and beverages (5) and preliminary work has indicated that it is suitable for the analysis of acids in coffee extracts (4,6). For organic acids, a uv-absorbing buffer system is set at a pH at which the acids are essentially fully ionized. Most successful separations of the anions have been obtained by the addition of a cationic surfactant to reverse the electroosmotic flow (EOF), together with injection at the cathode. The anions are monitored shortly before the anode by indirect detection in the uv. A CE procedure which incorporates these approaches for the analysis of aliphatic acids in coffee is described in this publication.

Experimental

Sample Preparation

Green Colombian Arabica and Indonesian Robusta beans were roasted batch-wise (40g) in a Henzler fluidized-bed jet-zone roaster for 3 min at a series of temperatures from 210 to 290°C.
Green and roasted beans were extracted (0.8g/100 ml) at 40°C in an ultrasonic bath for 10 min.

Capillary Electrophoresis

A Hewlett Packard 3D CE capillary electrophoresis system was used. Applied voltage was set at -20 kV and the temperature at 25°C. An 80.5 cm (72 cm to detector) fused silica capillary was used with an I.D. of 50 μm. For quantitation, the injection mode was hydrostatic (50 mbar for 10 sec, gave injected volume of 10 nL) rather than electrokinetic (see below). The buffer used was 25 mM phthalate at pH 5.5, containing CTAB (cetyltrimethylammonium bromide, 0.2 mM) to reverse the EOF. The acids were monitored as their anions by indirect detection at 240 nm.

Results and Discussion

Method Optimization

The pH of the phthalate buffer selected was 5.5. This was because of the strong buffering action of phthalate at this pH, and, based on pK values (7), the coffee acids were essentially present in the ionized form. An electropherogram of the organic acids in a roast coffee extract obtained using electrokinetic injection is shown in Figure 1. Peak identification was by comparison to standards. Although separation was excellent, calibration experiments showed that the peak area response for the individual acids was not linear. This was attributed to a bias towards faster moving ions, a known problem with this injection technique. Consequently, for the purposes of quantitation, hydrodynamic injection, which results in a repeatable defined injection volume, was used.

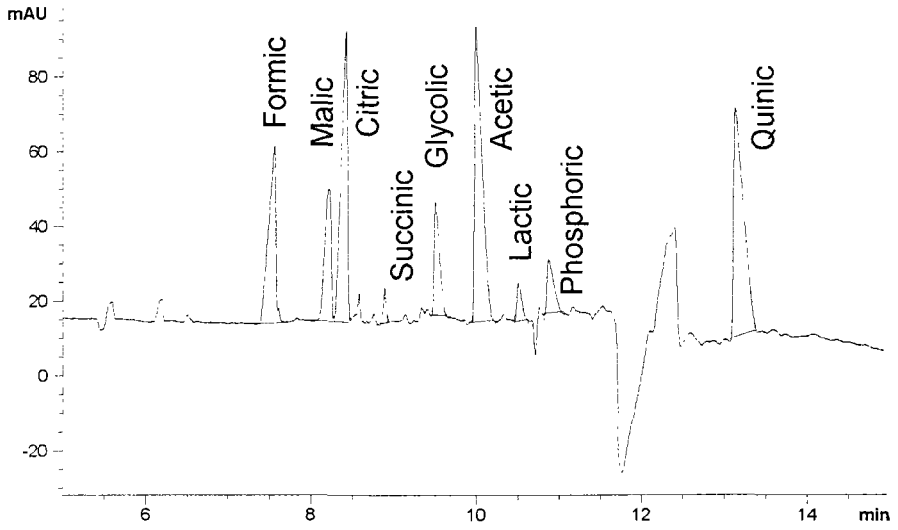


Fig. 1: Electropherogram of roast coffee extract; **electrokinetic** injection

An electropherogram, obtained from a hydrodynamically injected sample, is shown in Figure 2. Under these conditions, a large negative peak was obtained. The peak is probaly not attributable to the EOF, because the negatively charged quinide ion, which should migrate faster than the EOF, appears later. The negative peak was not considered to be a problem, because it did not obscure any of the peaks associated with the coffee acids. A possible exception was pyroglutamic acid, which Maier and his group (8) have found in roasted coffee extracts. Injection of a solution of a standard sample failed to give a peak.

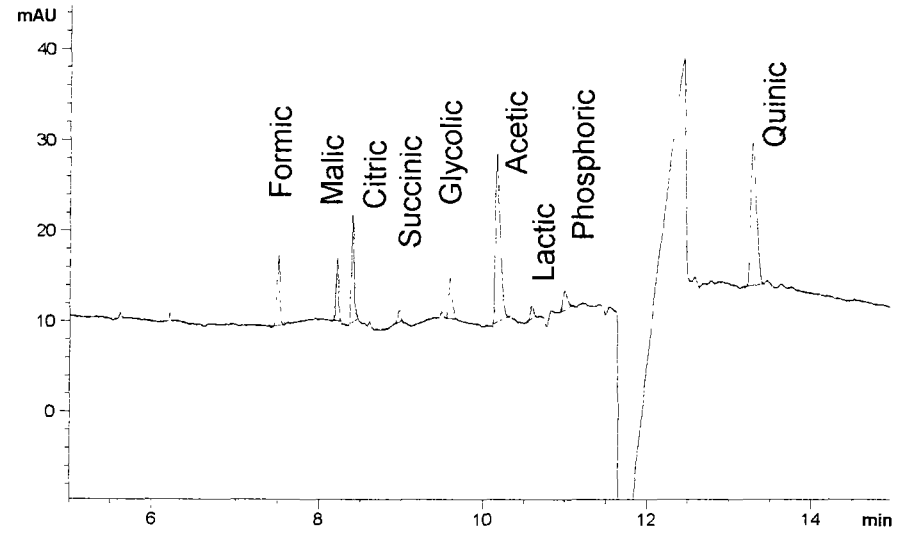


Fig. 2 Electropherogram of roast coffee extract; **hydrodynamic** injection

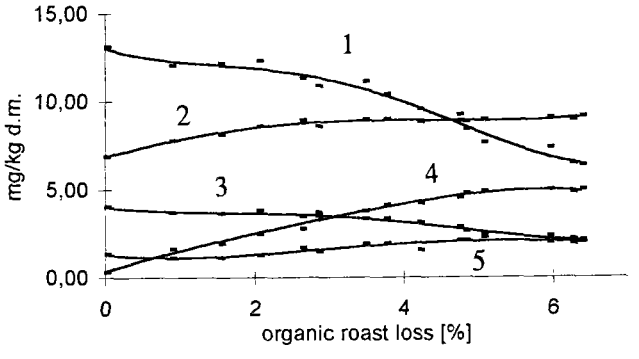
The method was calibrated, using standard substances, for each of the major acids identified in the electropherogram. Migration time inconsistency can be a problem with CE, this was prevented by passing 0.1N NaOH solution and then buffer through the capillary between each run (Table 1). Response for the individual acids was linear over a concentration range from 5 to 250 ppm in injected sample (Table 1). In addition to the aliphatic acids, phosphoric acid can also be monitored. Chlorogenic acids appeared later in the electropherogram, the isomers were not separatable. As these can be analyzed conveniently using a HPLC-based procedure (1), no further attempt was made to analyze them by CE.

Table 1: Migration times, response factors and associated standard deviation for coffee acids. Response factor = peak area / weight

Acid	Mt [min]	RSD [%]	Response Factor	RSD [%]
Formic	7.49	0.26	0.94	1.84
Malic	8.20	0.24	0.72	3.98
Citric	8.39	0.25	0.41	2.25
Succinic	8.94	0.26	1.11	1.19
Glycolic	9.58	0.24	0.85	1.68
Acetic	10.14	0.24	2.04	4.57
Lactic	10.58	0.24	0.43	1.25
Phosphoric	10.98	0.23	0.49	3.30
Quinic	13.26	0.24	1.11	1.90

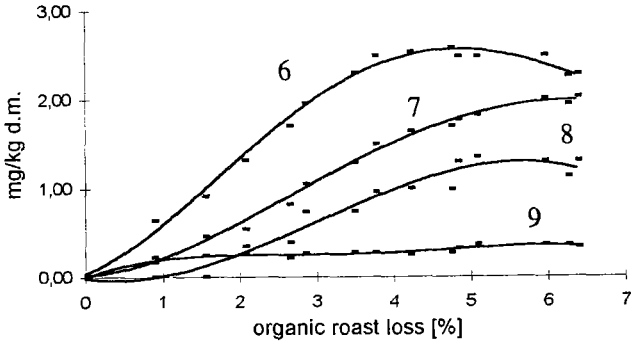
Acids in Coffee - Effect of Roast

Figures 3 and 4 illustrate the contents of the major aliphatic (and phosphoric) acids in Colombian Arabica beans as a function of roast (expressed as organic roast loss). The sample range was from green to roasted at the limit of organoleptic acceptability. The principal acids in the green beans were malic, citric and quinic (chlorogenic acids were also present but were not monitored). As roasting progesses, the content of the first two decreased whereas those of quinic and the other aliphatic acids increased. Yields of quinic acid increased with roasting due to the cleavage of chlorogenic acid (8). At higher roasts, the yields of formic and acetic acid began to fall off. The yield of the former, because of its higher volatility, was initiated at a lower organic roast loss. Yields of the other aliphatic acids, which were less volatile and are presumably breakdown products of the polymeric carbohydrate fraction, tended to level off at the higher roasts. At even higher roasts (not studied here) the yield of volatile acids continued to fall off, whereas that of the less volatile acids was steadier (8).



1: Citric 2: Quinic 3: Malic 4: Acetic 5: Phosphoric

Fig. 3 Acids vs. organic roast loss for Colombian Arabica Beans



6: Formic 7: Glycolic 8: Lactic 9: Succinic

Fig. 4 Acids vs. organic roast loss for Colombian Arabica beans

Comparison of acid yields as a function of roast loss for the Colombian Arabicas and Indonesian Robustas (Tables 2 and 3) revealed some interesting differences. The content of phosphoric acid was significantly higher in Robusta beans. Yield of phosphoric acid after roasting also seemed to indicate a higher yield of phytic acid (produces phosphoric acid on roasting) in Robustas. As only one sample of each was measured, a generalization cannot be made; however, Franz and Maier (9) found that four Robusta samples all had higher phytic acid and total phosphorous content than four Arabica samples. Quinic acid yields on roasting were higher in Robustas, reflecting their higher chlorogenic acid content (10). The yields of the other aliphatic acids in roasted samples tended to be higher in the Colombian beans.

Table 2: Acid yield [g/kg d.m.] as a function of organic roast loss for **Colombian Arabicas**

Roast Loss [%]	Formic	Malic	Citric	Succinic	Glycolic	Acetic	Lactic	Phosphoric	Quinic
0.00	n.d.	4.02	13.11	n.d.	n.d.	0.29	n.d.	1.45	6.87
3.31	2.29	3.29	11.10	0.28	1.28	3.76	0.73	1.88	8.96
4.20	2.53	3.11	9.53	0.25	1.64	4.18	1.00	1.69	8.81
5.06	2.47	2.49	7.66	0.36	1.81	4.86	1.35	2.30	8.94
6.37	2.28	2.00	6.34	0.33	2.02	4.98	1.30	2.18	9.12

Table 3: Acid yield [g/kg d.m.] as a function of organic roast loss for **Indonesian Robustas**

Roast Loss [%]	Formic	Malic	Citric	Succinic	Glycolic	Acetic	Lactic	Phosphoric	Quinic
0.00	0.18	2.47	13.50	0.13	n.d.	0.15	n.d.	2.79	4.70
3.25	0.86	2.40	13.38	0.23	0.47	1.98	n.d.	4.05	10.01
4.15	1.31	2.20	12.26	0.37	0.86	2.73	0.51	5.47	10.82
4.99	1.48	1.93	10.79	0.43	1.04	3.00	0.71	6.35	12.32
6.47	1.61	1.45	8.38	0.45	1.30	3.25	0.77	5.95	13.19

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Summary

A capillary electrophoresis-based method for the determination of the aliphatic acids and phosphoric acid in coffee extracts has been developed. The procedure has several advantages: analysis is rapid, resolution is excellent, data is reproducible and the instrument is convenient to use. Sensitivity is at least as good as chromatography-based methods. The method was used to monitor the yields of the acids as a function of organic roast loss for samples of Colombian Arabica and Indonesian Robusta beans.

Résumé

Une methode a base de capillaire électrophorèse a été développé pour la détermination de l'acide aliphatique et l'acide phosphorique dans les extraits de café. Le procédé a plusieurs avantages: l'analyse est rapide, la résolution est excellente, les données sont reproductible et les instruments sont faciles à l'utilisation. La sensitivité est au moins aussi bonne que la méthode a base chromatographique. La méthode a été utilisé pour surveiller la concentration d'acide comme fonction organique torréfié perdue a l'aide de grains Robusta de provenance colombienne, et indonesienne.

FATTY ACID ESTERS OF THE 16-O-METHYLCAFESTOL

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Introduction

Up to now, the concentration of the new diterpene 16-O-methylcafestol (16-OMC) in coffee mixtures was always determined after saponification of the coffee oil. Hence, it was impossible to give information on the bound components. So far, they were of no interest because we intended to estimate the Robusta concentration in Arabica coffee mixtures by analysing the total 16-OMC content. By this method introduced in 1989, it was possible to detect Robusta concentrations in mixtures definitely from 2 % onward and higher [1,2].

Now, investigations revealed that there are only minor 16-O-methylcafestol concentrations in the uncombined state in the lipid fraction of coffee (1,0 - 3,4 % of the total percentage) [3]. Consequently, the greater part has to be bound. Concerning the two other diterpenes kahweol and cafestol detected in coffee, it is known that these are present mainly esterified with fatty acids. For cafestol and kahweol, up to 8 different fatty acid esters were identified [4-6].

We already mentioned on the EURO FOOD CHEM 1991 and the last ASIC that the diterpene 16-OMC, too, is esterified with different fatty acids [7,8].

Isolation of the 16-O-methylcafestol fatty acid esters

In order to identify the various 16-OMC esters, at first a pure fraction has to be isolated containing only the 16-OMC esters. Therefore, it is necessary to separate the other compounds of coffee oil such as sterol esters, sterols, phosphatides, free fatty acids, free diterpenes and mainly the triglycerides (Fig. 1).

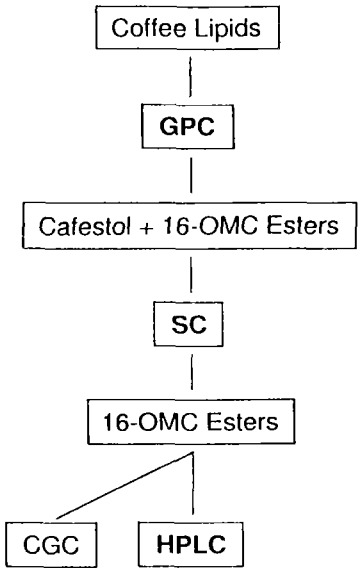

Fig. 1. Scheme of isolation of the 16-OMC esters

These accompanying substances can be separated by different chromatographic methods. Using gel permeation chromatography (Bio Beads S-X3) and the eluent ethyl acetate/cyclohexane (1/1), so far, the 16-OMC esters and triglycerides are separated with success. However, the 16-OMC esters are still present in the same fraction together with sterol esters and cafestol esters. By a subsequent solid phase extraction on silica cartridges, finally, a fraction is achieved which only includes the 16-OMC esters.

Identification of the 16-O-methylcafestol esters

- Capillary gas chromatography

At first, it was tried to separate the 16-OMC esters by means of capillary gas chromatography. Using an on-column injector, a short non-polar column and an extreme temperature programme, a splitting into several signals is achieved. However, owing to high molecular weights, retention times are very long (Fig. 2).
But these conditions could not be transferred to our CGC/MS system - no on-column injector was at disposal and it was impossible to thermostat the transfer line to 300 °C.

Consequently, the components were separated only insufficiently, but because of the acquired mass spectra it was possible to assign a few of the signals to definite esters. Nevertheless, we found it more promising to separate the individual esters by semipreparative HPLC and subsequently, to identify by solid-probe mass spectrometry.

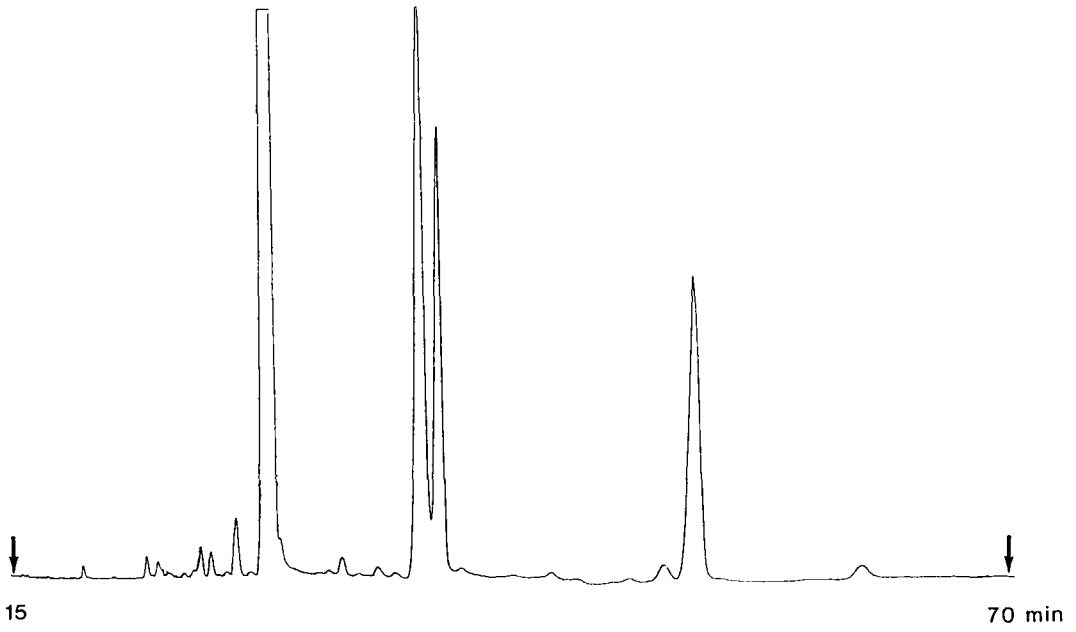

Fig. 2. Gas chromatogram of 16-OMC fatty acid esters
column: DB-5, 15 m x 0,32 mm id.; temperature progr. 100°C, 30°C/min to 300°C, isotherm 65 min; detection: FID; injection: 1 µl cold-on-column

- Semipreparative HPLC and solid-probe mass spectrometry

In 1987, PETTITT [9] tried to separate some kahweol and cafestol fatty acid esters by reversed-phase-HPLC using an acetonitrile/water gradient. However, the result was extremely inadequate because many substances co-elute under the mentioned conditions.

Therefore, we tested a method published by SWACZYNA and MONTAG [10,11]. They succeeded in appropriately separating 15 cholesteryl esters - these are compounds of a structure similar to that of diterpene esters - using an RP-18 phase and the solvent mixture of acetonitrile/iso-propanol (50/50). Changing the mixture to 70 % of acetonitrile and 30 % of iso-propanol, adequate separation of the 16-OMC esters was achieved (Fig. 3). In order to be able to separate greater quantities, we used a semipreparative column of a diameter of 10 mm.

The isolated esters were identified by solid-probe mass spectrometry. Besides the 16-OMC palmitate already assumed by PETTITT, esters of 16-OMC with fatty acids such as C_{18}, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, C_{20}, C_{22} and C_{24} were identified, in addition, the 16-OMC esters with the fatty acid $C_{20:1}$ and some odd-numbered fatty acids such as C_{17} (Fig. 7), C_{21} (Fig. 8) and C_{23}. The occurrence of these minor components is not surprising because various odd-numbered fatty acids were already identified in the triglycerides of coffee oil. We reported on this on the last ASIC [8].

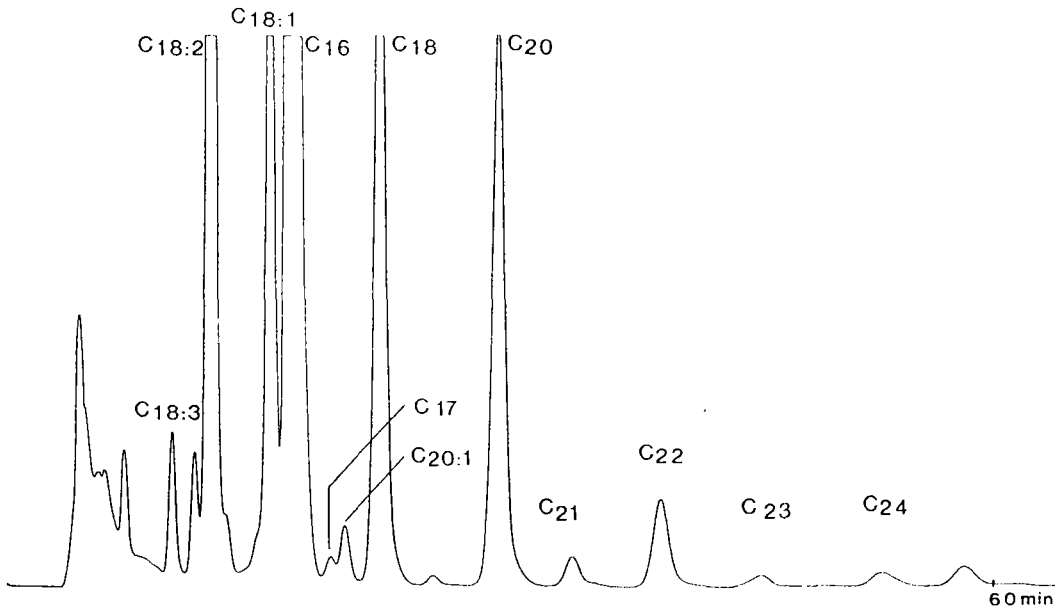

Fig. 3. HPLC chromatogram of 16-OMC fatty acid esters
column: LiChrosorb RP-18, 7 µm, 250x10 mm; eluent: acetonitril/iso-propanol 70/30 (v:v); flow rate: 4,0 ml/min; detection: 220 nm

Synthesis of the 16-OMC fatty acid esters

In order to be able to investigate in greater detail the most important 16-OMC fatty acid esters, it was intended to make them by synthesis. Often, acylation of alcohols is performed using carboxylic acid, however, for sterically hindered alcohols and hence, for 16-OMC, as well, acylation goes on considerably easier and at higher yields with acid chloride.

16-OMC + fatty acid chloride

reaction

16-OMC + fatty acid + 16-OMC ester

Na_2CO_3-solution

16-OMC + 16-OMC ester

SC

16-OMC ester

Fig. 4. Scheme of synthesis

In the following, the principle of the synthesis is described in short (Fig. 4):
Free 16-OMC reacts with the fatty acid chloride at room temperature. After the reaction, besides the formed diterpene fatty acid ester, additionally, the free fatty acid and the not converted 16-OMC are present in the reaction vessel. Adding sodium carbonate solution to precipitate the free fatty acid and applying chromatographic methods, finally, the pure synthesized ester is obtained which can be tested with respect to purity by means of HPLC and diode array UV detection.

HPLC analysis of 16-OMC esters

For HPLC analysis, however, we do not use the semipreparative RP column but an analytical column of a very small pore's diameter (3 µm). With KÖLLING [12], this column stood the test for difficult separation problems in nucleotide/nucleoside analytics. The 16-OMC esters are separated very satisfactorily and quantified under the conditions mentioned beneath (Fig. 5).

Though the UV spectra of saturated and unsaturated fatty acid esters of 16-OMC are different, all esters can be detected at a wavelength of 220 nm.

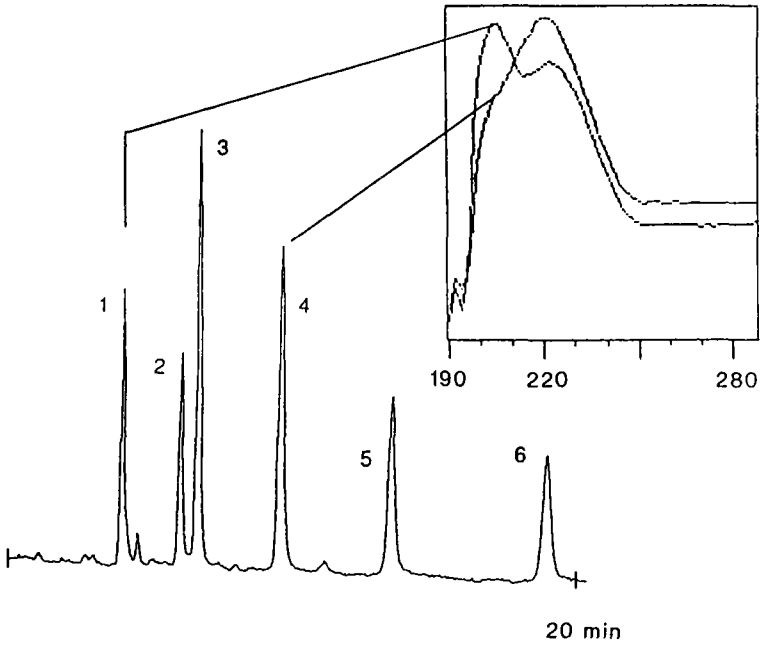

Fig. 5. HPLC chromatogram of a mixed standard solution of 16-OMC fatty acid esters
column: Nucleosil 120-3 C_{18}, 250x8x4; eluent: acetonitril/iso-propanol 65/35 (v:v); flow rate: 0,9 ml/min; detection: 220 nm
1 linoleate, **2** oleate, **3** palmitate, **4** stearate, **5** arachidate, **6** behenate

Stability behaviour of some of the 16-OMC esters during roasting

We found in previous investigations that the total concentration of 16-OMC does not decrease during roasting [13]. Now SEHAT et al. [14] tested to what extent the individual fatty acid esters of 16-OMC are diminished by roasting. Therefore, he analysed a Madagascar coffee roasted at four different temperatures for 2.5 minutes (Fig. 6).

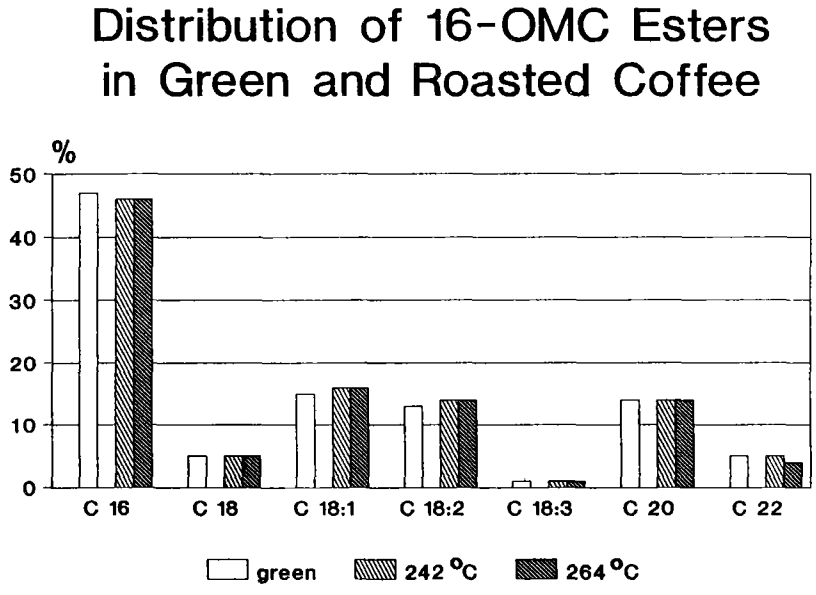

Fig. 6.

As it is seen, in spite of different roasting temperatures, the proportional distribution for the diterpene esters is almost the same. Obviously esters are stable during roasting.

Conclusions

The diterpene 16-OMC is present in coffee oil esterified with at least 12 fatty acids. By this analysis and synthesis method described above the behaviour of some of the 16-OMC fatty acid esters can be investigated directly, for example, during roasting process, too.
These investigations will be continued with regard to the fatty acid esters of kahweol and cafestol as well.

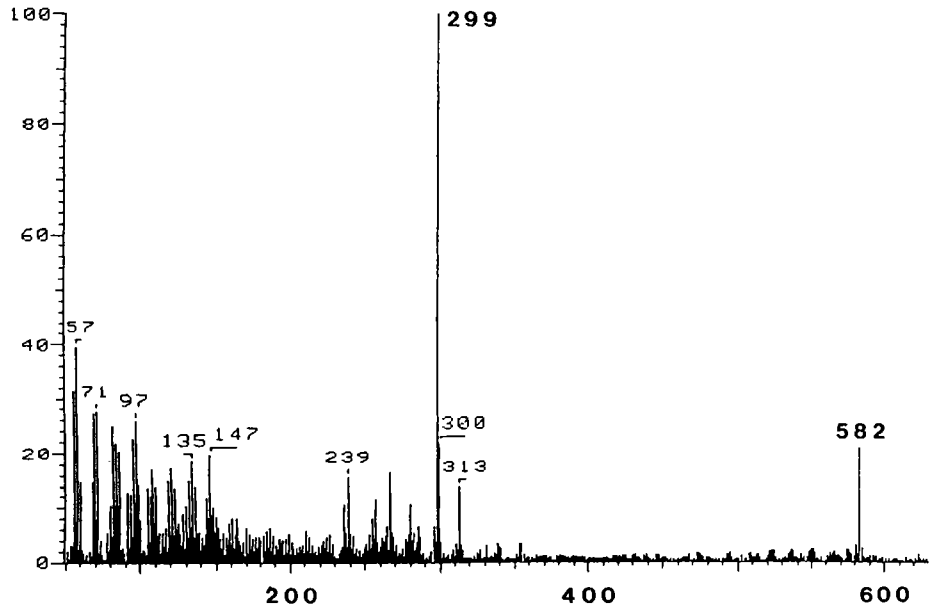

Fig. 7. Solid-probe mass spectrum of 16-OMC heptadecanoate

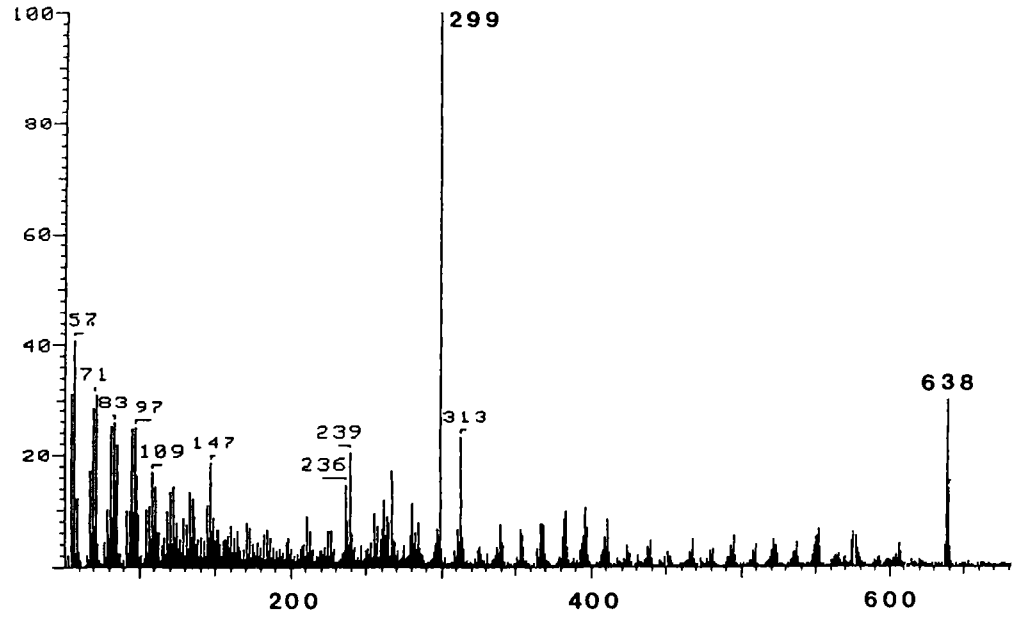

Fig. 8. Solid-probe mass spectrum of 16-OMC heneicosanoate

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Summary

The diterpene 16-OMC occurs in Robusta coffees. There are only small concentrations in free form, the greatest part is esterified with fatty acids - as it is already known for the two other diterpenes kahweol and cafestol occurring in coffee.
Using gel permeation chromatography equipped with a Bio Beads S-X3 column and subsequent chromatography on silica cartridges, it is possible to separate various 16-OMC fatty acid esters from Robusta coffee oil. 12 fatty acid esters were isolated by semipreparative RP-HPLC and identified by solid-probe mass spectrometry.
The 16-OMC esters detected in coffee oil can be won as standard matters by synthesis and separated on a Nucleosil-C_{18} analytical column. Thus, for the first time, the behaviour of individual esters could be investigated directly. In a coffee roasted at various temperatures it was proved that the proportional ester distribution is not changed by roasting.

Zusammenfassung

Das Diterpen 16-O-Methylcafestol kommt in Robusta-Kaffees vor. Nur geringe Anteile liegen in freier Form vor, der größte Teil ist hingegen - wie schon für die beiden anderen im Kaffee vorkommenden Diterpene Kahweol und Cafestol bekannt ist - mit Fettsäuren verestert.
Durch Gelpermeationschromatographie an Bio Beads S-X3 und anschließender Säulenchromatographie an Kieselgel Einmaltrennsäulen gelingt es, aus Robusta-Kaffeeöl verschiedene Fettsäureester des 16-O-Methylcafestols zu isolieren. Eine Trennung der einzelnen Ester erreicht man mit HPLC an RP-18-Säulen. Durch halbpräparative HPLC konnten 12 Fettsäureester isoliert und ihre Identität über Schubstangen-Massenspektrometrie abgesichert werden.
Durch Synthese lassen sich die im Kaffeeöl nachgewiesenen 16-O-Methylcafestolester als Standardsubstanzen gewinnen. Damit war es möglich, das Verhalten der einzelnen Ester erstmals direkt zu verfolgen. In einem bei unterschiedlichen Temperaturen gerösteten Kaffee konnte nachgewiesen werden, daß die prozentuale Esterverteilung durch die Röstung keine Veränderung erfährt.

THE FREE RADICAL SCAVENGING ACTIVITY OF CAFFEINE IN COFFEE EXPOSED TO FENTON-TYPE OXIDATION

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INTRODUCTION

Recently, strong evidence has accumulated showing that coffee may exert antimutagenic and anticarcinogenic effects both *in vitro* (Obana *et al.*, 1986; Stadler *et al.*, 1994) and *in vivo* (Stalder *et al.*, 1990; Abraham, 1989). These beneficial antioxidant properties are apparently linked to polyphenolic constituents (Stich *et al.*, 1991), and also to products that arise during the roasting process (Macku and Shibamoto, 1991). Recently caffeine, the major alkaloidal constituent of coffee, was reported to be an efficacious scavenger of the highly reactive hydroxyl radical, but without mention of the specific reaction products (Shi *et al.*, 1991). Studies addressing the free radical scavenging activities of coffee have demonstrated that this beverage can exert potent antioxidative effects (Morrice *et al.*, 1993; Stadler *et al.*, 1994), analogous to those already observed for green and black tea and their catechin and tannin-type polyphenolics. On the other hand, the presence of stable organic free radicals in roasted coffee beans and in coffee solutions has been demonstrated by electron paramagnetic resonance (EPR) techniques (Santanilla *et al.*, 1981; Troup *et al.*, 1988). These free radicals are generally attributed to organic compounds and are relatively stable and unreactive, and their formation may be associated with the charring of polysaccharide coffee components during roasting (Morrice *et al.*, 1993).

In this study we demonstrate that caffeine, present as a natural constituent in coffee, is an efficient scavenger of the deleterious hydroxyl radical *in situ*, forming the major reaction product 8-oxocaffeine (1,3,7-trimethyluric acid), which can be monitored in coffee by analytical HPLC with electrochemical detection (ECD) or LC-MS/MS techniques.

EXPERIMENTAL

Coffee preparation. Coffee solutions (Instant and roast and ground, all European shelf brands) were acidified (1 M HCl) and fractionated on C-18 Chromabond columns. After penetration of the coffee solution, the columns were eluted with 2 mL of a solvent mixture A + B (7 + 3), comprised of A = 95 % water, 5 % MeOH, 2 mM acetic acid; B = 95 % MeOH, 5% water, 2 mM acetic acid. The effluent was concentrated *in vacuo*, filtered, and analysed by HPLC.

HPLC-ECD. Reversed-phase HPLC employed a Supelcosil LC-18 DB column (5 µm, 25 cm x 4.6 mm), with solvents A = 50 mM citric acid/NaOH at pH 5.25 and B = 100 % MeOH. Flow initially isocratic at 10 % B for 40 minutes, then going to 80 % B over 10 minutes and returning to 10 % B over 10 min, eluting 8-oxocaffeine at t_R 37.2 minutes. ECD was with a glassy carbon electrode operated at 31°C, potential set at + 0.85 versus the AgCl/KCl reference electrode. Absorbance (UV) was monitored simultaneously at 290 nm.

LC-MS/MS. The experiments were carried out using a Finnigan TSQ 700 mass spectrometer connected *via* a thermospray interface to a Waters 600-MS pump and a Waters 717 autosampler. Detection was achieved by tandem mass spectrometry after collision induced dissociation of the protonated molecular ion of 8-oxocaffeine (m/z 211). The daughter ion $[M+H-CH_3]^+$ was selectively recorded.

Isolation of 8-oxocaffeine. 8-Oxocaffeine was isolated from Instant coffee powder by solid phase extraction on C-18 cartridges and subsequent TLC in solvent systems $CHCl_3$:MeOH:NH_4OH (140:60:1.5, v/v) and $CHCl_3$:Me_2CO:*n*-BuOH:NH_4OH (3:3:4:1, v/v). The crude 8-oxocaffeine fraction was further purified by HPLC, employing a Supelcosil LC-18-DB column with a ammonium acetate/MeOH gradient.

Exposure of caffeine to Fenton's reagent. Caffeine (2.6 mM) was incubated (1 - 6 h) at 37°C in a KPO_4 buffer (80 mM) at different pH levels in the presence of EDTA (0.5 mM), $FeCl_3$ (5.6 ppm), ascorbic acid (1 mM), and H_2O_2 (500 µM). The reaction was terminated by addition of catalase (40 units) and EtOH (final conc. 2.3 M). An internal standard, β-hydroxyethyltheophylline, was added (10 µg) to each of the terminated incubation mixtures which were stored at -20°C until analysis. Products were identified and quantified by HPLC with UV (288 nm) detection.

Oxidation experiments with instant coffee. Coffees were prepared at a concentration of 16.7 mg/mL. Typical reaction mixtures (final conc.) were comprised of EDTA (0.5 mM), $FeCl_3$ (5.6 ppm), and H_2O_2 (4.4 mM), and incubated at 37 °C for 1 h. Analysis for 8-oxocaffeine was by HPLC with ECD using the conditions as described above.

RESULTS

Oxidation of Caffeine. Exposure of caffeine to Fenton's reagent in KPO_4 buffered solution resulted in the formation of one major product, namely 8-oxocaffeine, reaching up to 6.1 % turnover of the total caffeine after 1 h and close to 7.2 % turnover after 6 h at pH 6.8 (Table 1). Omission of vitamin C in the presence of H_2O_2 and Fe^{3+} did not result in 8-oxocaffeine production (Control A). Caffeine alone in the presence of vitamin C (Control B) also showed the formation of the C-8 hydroxylated purine, probably attributable to spurious amounts of transition metals in the incubation mix leading to the generation of active oxygen species *via* reduction of O_2.

Table 1. Fenton Catalysed Formation of 8-Oxocaffeine (Expressed as %age of 8-Oxocaffeine Formed) from Caffeine at Different pH Levels Over Time[a].

Incubation Conditions	Incubation Time (h) at 37°C		
	1	3	6
Control A (without vitamin C)	0	0	0
Control B (without H_2O_2/Fe)	0.2	1.0	1.3
pH 5.5	5.0	5.3	5.8
pH 6.0	5.5	6.3	6.7
pH 6.8	6.1	6.6	7.2
pH 7.4	5.2	5.8	6.2
pH 8.2	5.6	4.9	5.1

[a]All incubations containing EDTA, Fe^{3+}, and H_2O_2. Entries represent averages of duplicate determinations with β-hydroxyethyltheophylline as internal standard.

Quantification of 8-oxocaffeine in coffee. In order to quantify 8-oxocaffeine, the coffee solutions were spiked with different known amounts of standard compound (Figure 1) and the amount in the non-spiked sample extrapolated from a linear regression equation. In all cases, there was a good linear relationship between 8-oxocaffeine concentration in the coffee and peak area (HPLC-ECD or LC-MS/MS), giving correlation coefficients (r^2) of≥ 0.996. The detection limits of 8-oxocaffeine were 0.5 - 1.0 ppm in coffee (Table 2), and gave a linear response range from 2.22 ng to 100 ng of spiked 8-oxocaffeine in the coffee solution.

As depicted in Figure 2A, the characteristic fragmentation pattern of 8-oxocaffeine enabled detection (daughter ion at m/z 196) of levels of as low as 1-1.5 ppm in a coffee extract, revealing a chromatogram almost completely free of interferences from other coffee constituents (Figure 2B). Extraction of six different instant coffees showed that 8-oxocaffeine was present in all the brands that were analysed (Table 2), ranging from 4 to 31 ppm (µg/g coffee powder).

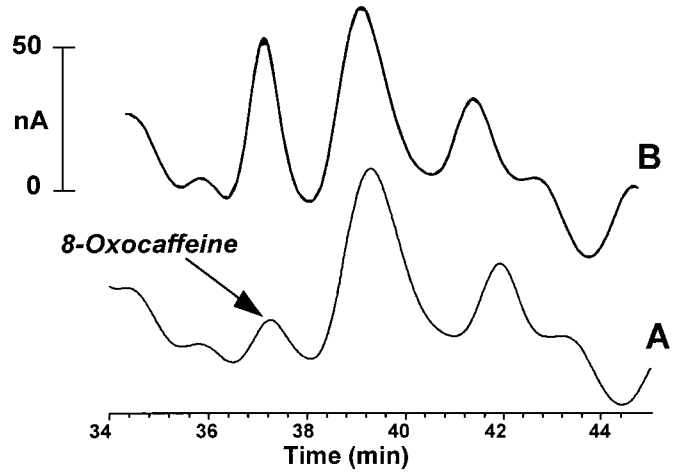


Figure 1. Excerpt of an HPLC Profile (ECD) of Solid-Phase Treated Coffee. A, non-spiked; B, spiked with 35.5 ng 8-Oxocaffeine Standard.

Also, there was a good correlation of the values of coffee samples analysed by LC-MS/MS and the identical samples analysed using HPLC with ECD. There was no significant difference in the 8-oxocaffeine content in the light roasted brand compared to the medium and dark roasted coffees, which suggests that the degree of roasting does not necessarily reflect 8-oxocaffeine production. This was further corroborated by the strong variation in the 8-oxocaffeine content within the medium roast brands. The levels of 8-oxocaffeine in a freshly prepared cup of coffee (instant or R & G) prepared with Millipore grade water and incubated at 37°C did not increase over a period of one hour, which demonstrates that hydroxyl radical production under these conditions is negligible. Comparison of the levels of caffeine (2.8 to 3.4 % of coffee powder) in the individual coffee brands, revealed absolutely no correlation of 8-oxocaffeine content and the concentration of caffeine. The levels of 8-oxocaffeine in the R & G brands were lower than compared to the instant coffees and are calculated as per total coffee grounds and not as per soluble filtered solids. As exemplified by R & G brand A, a slight increase in the 8-oxocaffeine level was observed after keeping the brew on a hot plate at 75 °C for 1 h (2.9 to 3.9 ppm), having taken into account the loss of water during this time.

Table 2. Quantification[a] of 8-Oxocaffeine (ppm) in Instant, Roast and Ground (R & G), and Green Coffee by HPLC-ECD and LC-MS/MS Techniques.

Coffee Brand[b]	LC-MS/MS	HPLC-ECD
Instant A - light roast	13.1	15.7
Instant B - medium roast	11.2	13.3
Instant C - medium roast	4.6	4.8
Instant D - medium roast	21.6	31.6
Instant E - dark roast	10.7	17.1
Instant F - decaffeinated	n.d	15.5
R & G Brand A	n.d.	2.9
R & G Brand B	n.d.	6.5
Green Coffee beans	< 0.5	< 0.5

[a] Each coffee sample (2.5 g/150 mL water) was spiked with three different concentrations of 8-oxocaffeine, linear regression calculated.
[b] European shelf brands
n.d. = not determined.

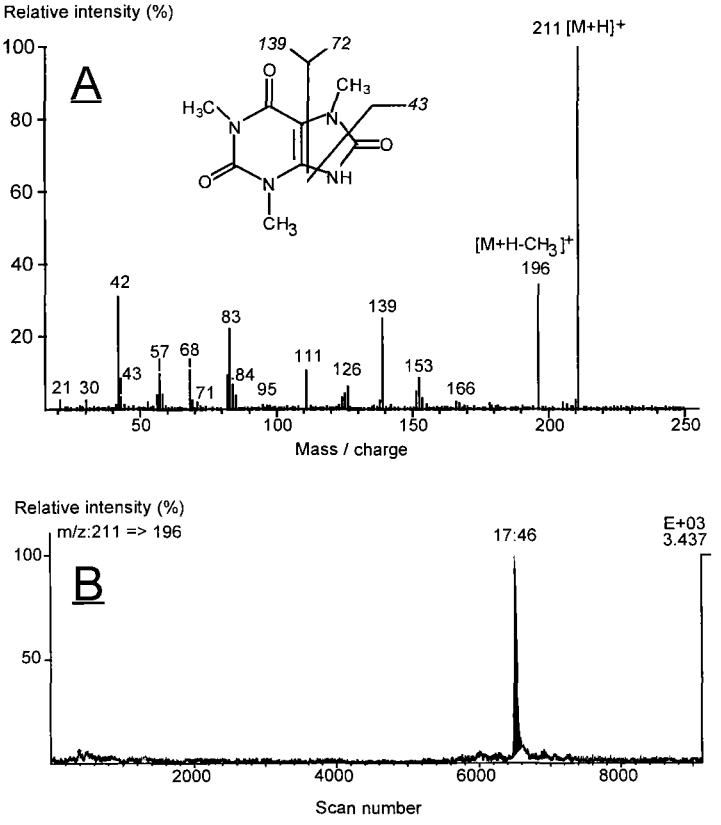


Figure 2. Daughter Mass Spectrum of the Protonated Molecular Ion (m/z 211) of 8-Oxocaffeine (A); and (B) LC-MS/MS Detection of 8-Oxocaffeine in a Coffee Extract.

Influence of pH, oxygen, hydrogen peroxide and transition metals. In order to demonstrate the free radical scavenging activity of caffeine *in situ*, coffees were fortified with transition metals and EDTA, and the formation of 8-oxocaffeine monitored by HPLC-ECD. The dependency of C-8 oxidation of caffeine on pH is portrayed in Figure 3. The relative formation of 8-oxocaffeine in coffee treated with iron/EDTA was evident at all pH values tested, and reached a maximum at pH 6.8. This result shows that Fenton chemistry takes place under acidic (pH 5.5) conditions, and is even potentiated at neutral and slightly alkaline pH values, probably due to more facile autoxidation of polyphenolics and subsequent ease of reduction of Fe^{3+} to the active Fe^{2+} state.

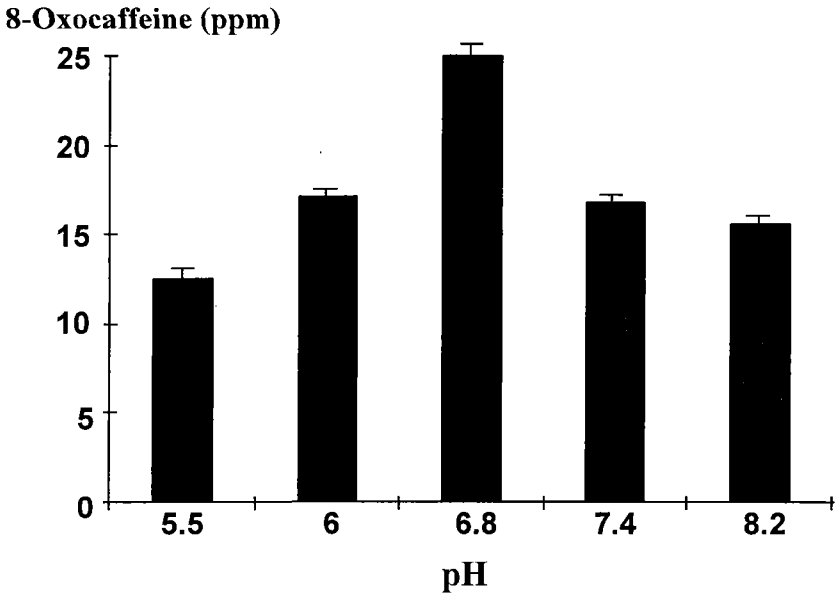


Figure 3. Influence of pH on the Formation of 8-Oxocaffeine in Coffee. Values are Averages (n =3)± s.d.

The importance of O_2 in the production of 8-oxocaffeine was demonstrated by purging an aqueous green bean slurry with O_2 for 16 h at room temperature. This showed a significant increase in 8-oxocaffeine (<0.5 to 1.9 ppm) which was not detected in the same beans that were stirred for the same period of time (16 h) but without oxygenation, substantiating that this enhancement was not due to gradual extraction of possible endogenous 8-oxocaffeine. Analogously, instant coffee that was purged with oxygen at ambient temperatures also displayed a prominent increase (factor 2) in the level of 8-oxocaffeine. This result again reflects the importance of O_2 and H_2O_2 in free radical mediated oxidation reactions.

The effect of addition of transition metals on 8-oxocaffeine formation in instant coffee is portrayed in Figure 4. As anticipated, the greatest increase in the level of 8-oxocaffeine was observed when the brew was fortified with ferric iron (Fe^{3+}). Even low levels of Fe^{3+} (5.5 ppm relative to the total reaction volume) increased 8-oxocaffeine 6 to 7-fold after an incubation time of 1 h. Addition of H_2O_2 simultaneously with Fe^{3+} revealed up to 18 times more 8-oxocaffeine than detected in the control brew after the same incubation time.

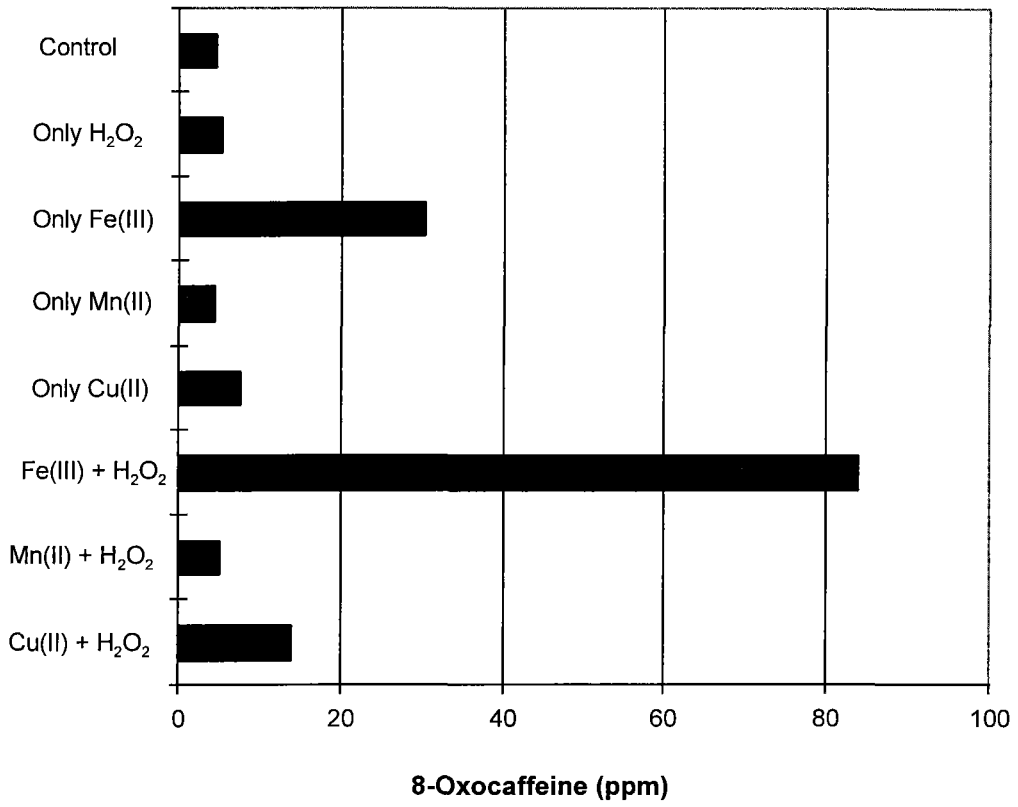
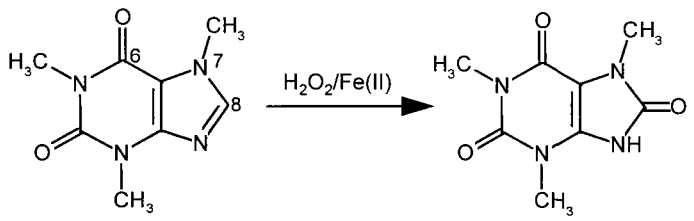


Figure 4. Influence of Transition Metals on 8-Oxocaffeine Formation in Coffee.

However, adding equivalent amounts of Cu^{2+} increased the level of 8-oxocaffeine only by a factor close to 2, and Mn^{2+} fortification of the brew had practically no effect at all, in accord with the inability of Mn to participate in Fenton chemistry (Gutteridge and Bannister, 1986). Moreover, in model systems, Cu^{2+}-EDTA complexes have been reported to be less effective Fenton catalysts as compared to Fe^{3+}-EDTA complexes (McCord and Day, 1978), also observed here by the lower concentration of 8-oxocaffeine after treatment with Cu^{2+} *vs* Fe^{3+}.

DISCUSSION

Only a few uric acids have been detected and quantified in green coffee beans, namely 1,3,7,9-tetramethyluric acid (theacrine), O^2,1,9-trimethyluric acid (liberine) and O^2,1,7,9-tetramethyluric acid (methylliberine), the levels of which range between 7 and 110 ppm (dry wt) for liberine, and ca. 11 ppm for theacrine (Kappeler and Baumann, 1985; Suzuki*et al.*, 1992). The uric acid analogue 8-oxocaffeine is not a product of purine metabolism in the coffee plant, since it was absent in fresh green coffee beans. The detection of 8-oxocaffeine in roasted coffees suggests that it is generated during the roasting process, and one can envisage its formation as an addition of the hydroxyl radical at the carbon-8 position of the methylxanthine nucleus (Scheme 1).



Caffeine **8-Oxocaffeine**

Scheme 1. Formation of 8-Oxocaffeine From Caffeine in the Presence of Hydrogen Peroxide and Fe^{2+}.

8-Oxocaffeine is a normal by-product of caffeine metabolism in the human liver, and is excreted in the urine of man and rodents after the injestion of caffeine (Yesair *et al.*, 1984). Interestingly, 8-oxocaffeine has also been classified as a good radical scavenger and potent antioxidant in model systems (Nishida, 1991). In addition, 8-oxocaffeine may be useful as a chemical marker of oxidation in quality assessment and shelf-life determination of coffee and caffeine-rich foods and beverages. As EPR techniques have already shown, free radical production can to a certain extent reflect the degree of decomposition of coffee beans (Ikeya *et al.*, 1989). Further studies are now underway to ascertain the significance of related methylxanthines as radical scavengers and uric acid derivatives as markers of free radical generation in foods and beverages, and their potential application also *in vivo*.

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Summary

Caffeine subjected to a Fenton-type reaction with Fe^{3+}/EDTA/vitamin C results in the formation of one major product, namely 8-oxocaffeine (1,3,7-trimethyluric acid). This C-8 hydroxylated purine base was detected in roast and grounds and in instant coffee using HPLC with electrochemical detection (ECD) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques. Levels of 8-oxocaffeine in coffee ranged between 4 and 35 ppm. Exposure of coffee brew to oxidation by addition of Fe^{3+} and hydrogen peroxide resulted in a significant increase in the 8-oxocaffeine level (18-fold vs the control). Results show that the formation of this compound reflects the free radical scavenging activity of caffeine *in situ*, and its formation is dependent on oxygen tension, hydrogen peroxide and transition metal availability.

Résumé

La caféine réagissant avec le mélange Fe^{3+}/EDTA/vitamine C selon une réaction de type Fenton conduit majoritairement à la formation de 8-oxocafféine (acide 1,3,7-triméthylurique). Cette purine hydroxylée sur la position 8 a été détectée dans le café moulu rôti et dans le café instantané par HPLC avec détection électrochimique (ECD), et par chromatographie liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS). Les niveaux de 8-oxocafféine dans le café se situent entre 4 et 35 ppm. L'exposition de l'infusion de café à l'oxidation par addition de Fe^{3+} et de peroxyde d'hydrogène conduit à une augmentation significative des niveaux de 8-oxocafféine (18 fois le contrôle). Ces résultats montrent que la formation de ce composé traduit la capacité de la caféine à piéger *in situ* les radicaux libres et que la formation de 8-oxocafféine est dépendante de la disponibilité de l'oxygène, du peroxyde d'hydrogène et des métaux de transition.

ANTIOXIDANT SUBSTANCES IN COFFEE EXTRACTS

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1-Introduction

There is a record that a coffee bean has been used by human being as medicine in the first encounter of coffee bean. Since then, we had learned to drink coffee after roasting coffee bean and now, the aroma and taste are loved and drunken by peoples of all over the world.

Coffee bean contains about 7～8% chlorogenic acid. This concentration of chlorogenic acid is very high. And we can not find any other plants which contain high concentration of chlorogenic acid like coffee. We are very interested in why coffee bean contains high concentration like this. Chlorogenic acid in coffee bean has been considered as a "very bad" constituent which has "the worst influence" on the coffee aroma and taste under the condition that a coffee is used as just drinking.

Recently in Japan, the research of constituent which has physiological activity function in a plant has become very popular, many reports of these researchs have been described , in which many reports in terms of these researchs accomplishment focus on chlorogenic acid and it's constituent, coffee acid, which have physiological function in plant. In the report of these researchs, especially, chlorogenic acid has been found to have the function which inhibits 5-Lipoxgnase enzyme activity, and there is another report that it has antiallergic disease , asthma, inflammation, arteriosclerosis preventive action by the application of it's function. Moreover, it has found and reported that Chlorogenic acid has pharmacological action such as carcinogenesis inhibitory action, antiinfluenza virus and suggest the possibility of developing the new way of using coffee bean.

In this experience, we report the result of application research in terms of the development of new product and make-up material by applying particularly chlorogenic acid ant-oxidation function to the hold-back agent of the freshness for the sea foods.

2. Constituent in coffee bean (Description by Table-1)

CONSTITUENT	GREEN BEAN	ROAST BEAN	CONSTITUENT	GREEN BEAN	ROAST BEAN
PROTEIN	11.6	3.1	HEMICELLULOSE	23.0	24.0
CAFFEINE	1.2	1.3	CELLULOSE	12.7	13.2
TRIGONELLINE	1.1	0.7	CHLOROGENIC ACID	7.6	3.5
LIPID	11.4	11.9	LIGNIN	5.6	5.8
SUCROSE	7.3	0.3	NOT DEFINED	14.0	31.7
REDUCING SUGAR	0.7	0.5			

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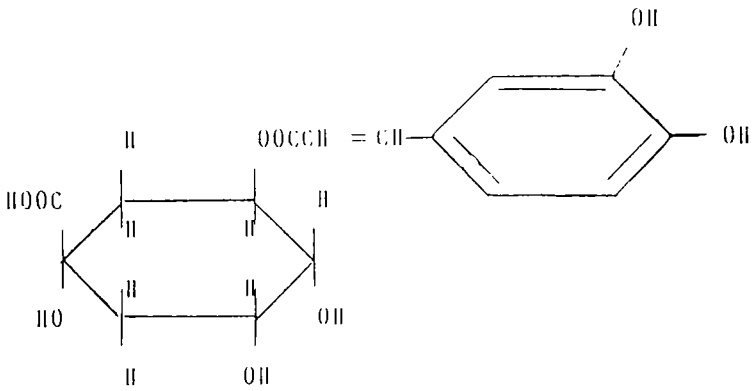
[Table-1 The explanation of point]
The content of Chlorogenic acid is very high, which is exception to other plants. According to the result of analysis done by presenter, the content of Chlorogenic acid has various value ranging from 7.7% to 11.8% depending on origine, species, quality.

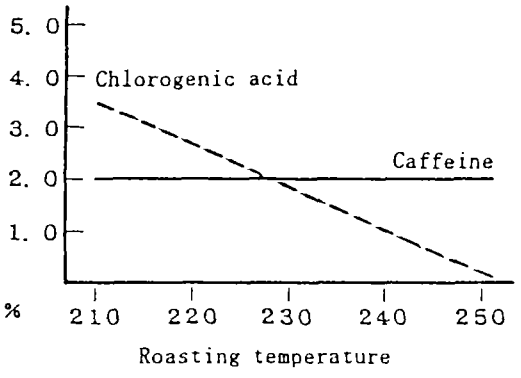
3. Chemical structure of Chlorogenic acid (Explanation by Fig-2)

Chlorogenic Acid
1,3,4,5-Tetrahydroxycyclohexanecarboxlic Acid
3-(3,4-Dihydroxycinnamate)



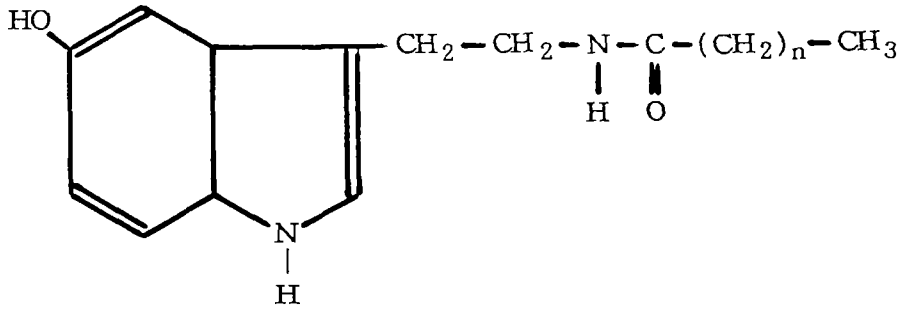
[Fig-2 Explanation of the point]
Chlorogenic acid is a compound which Quinic acid combined to Coffee acid.
Morishita et al. reported in Japan that 11 kinds of Chlorogenic acid isomer exist in a coffee bean.

4. Heat stability of Chlorogenic acid (Explanation by Fig-3)



[Explanation of the point Fig-3]
Explanation of the relation between roast temperature and heat degradation of chlorogenic acid.
1) 50% Chlorogenic acid is degraded by light roast at roast temperature 210℃.
2) 50% Chlorogenic acid is degraded by medium roast at roast temperature 230℃.
3) 100% Chlorogenic acid is degraded by fullcity roast at roast temperature 250℃.
4) Caffeine is stable at heat and not degraded by even hard roast condition.

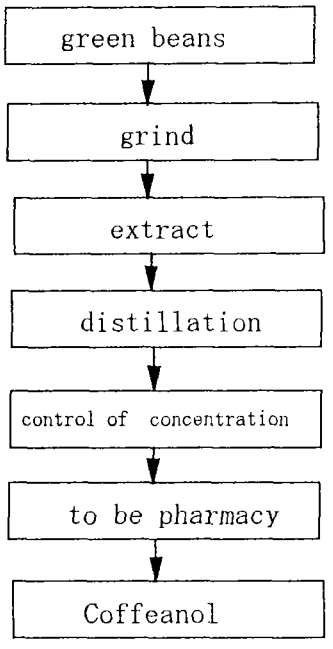
5. Other constituent Fatty acid amide of Serotonin having anti-oxidation action



[Fig-4 Explanation of the point]
0.5%~0.1% Fatty acid amide of Serotonin exists in coffee bean.
This compound has a very strong anti-oxidation being equal to BHA, BHT.

6. Application as food additives(anti-oxidation)

The process of production for green bean extract and it's constituent (explanation by Fig-5 & Table-2).



extraction by 40% Ethanol

recovering Ethanol

arranging for the blend of Glycine, Citric acid

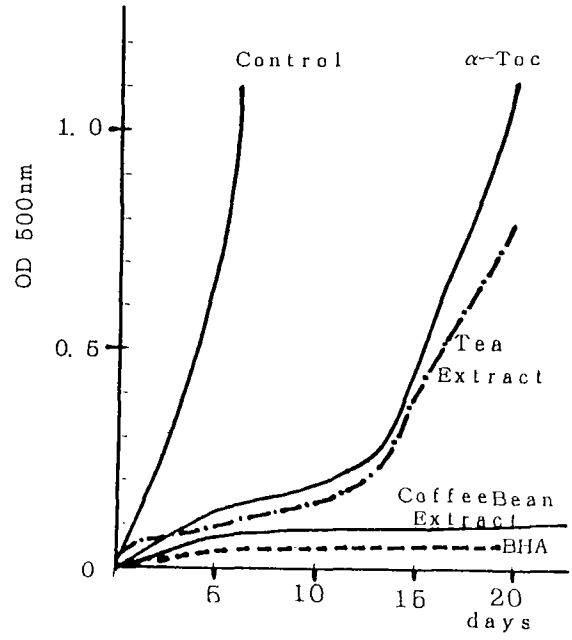
COMPOSITION

EXTRACTION OF COFFEE GREEN BEAN	10%
GLYCINE	3%
CITRIC ACID	2%
ETHANOL	20%
FOOD MATERIAL	65%

[Fig-5 & Table-2 Explanation of the point]

1) The example of success being able to have made coffee extraction commercialized for the porpoise of anti-oxidation as food additives is not found in not only Japan but also any other countries except UCC. UCC has succeeded in this for the first time.
2) Serotonin fatty acid amide which has strong anti-oxidation is extracted by using Ethanol. and we applied for patent of processing as well as the result.
3) We found that the anti-oxidation of the coffee extraction is multiplied by blending with Glycine.

7. Anti-oxidation of Coffee green bean extraction (coffeanol-A) (Explanation of Fig-6)



[Fig & Table-6 Explanation of the point]

These samples were settled in the incubater at 50℃ and the changing of their collor were measured. After 50 days, Coffeanol A didn't change so much in it's collor and indicated the same effect as food additives(anti-oxidant), BHA. It was considered that -Tocophenol didn't have an effect on coffee green bean extraction because of it's insolube character to water.

8. The effect of holding the freshness for sea foods (Explanation by Picture-1)

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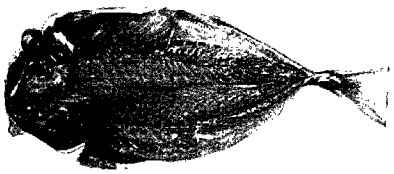
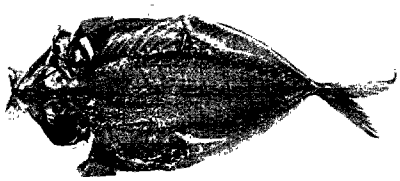
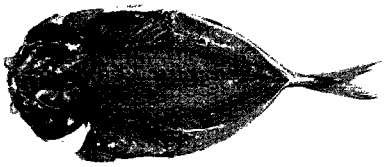


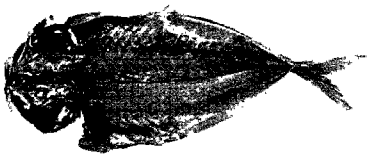
5% NaCl



Coffeanol 0.5%



Catechin 0.5%



Tocopherol

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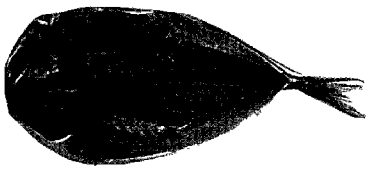
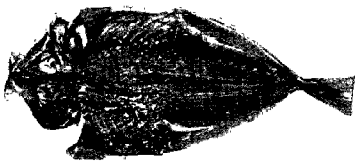
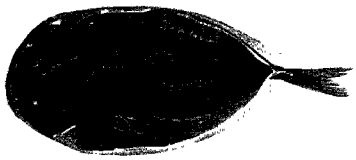


5% NaCl



Coffeanol 0.5%



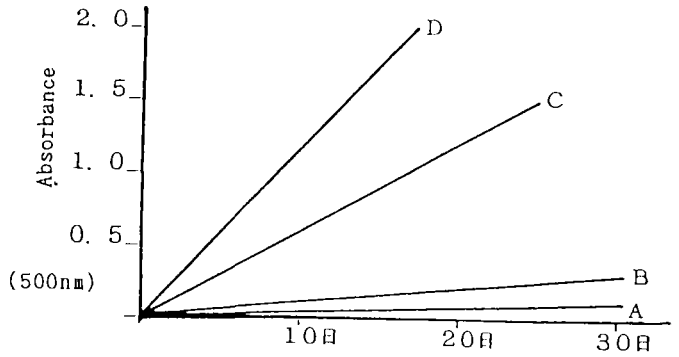
Catechin 0.5%

Tocopherol

[Explanation of the point by Picture-1]

- Browning was admitted in the cace of using 5% NaCl and tea Catechin.
- Not so greate changing of color was admitted in the cace of using Coffeanol, and that it is easy to handle becase of it's water-soluble chracter.
- Not so greate changing of color was admitted in the cace of using Tocopherol, but it is not easy to handle without emulsifying.

9. Other characters (Explanation of Picture-2)



A : Coffeanol(0.05% solution)
B : Coffeanol(iron added to 0.05% solution)
C : Tea extract(0.05% solution)
D : Tea extract(iron aded to 0.05% solution)

[Explanation of the point by the picture-2]

Catechin in tea extract and Chlorogenic acid in a coffee bean extract as well as a Polyphenol which abundantly exists in a plant can be a substrate in the process of enzymatic browning, oxidizing and browning easily by the action of polyphenol oxidase. Generally, The Browning reaction is recognized as deterioration of quality and unfavorable phenomenon.

The writer stored coffee bean and tea extract at 50℃, and measured the change of browning reaction with the time passing . The result is described in Table-5. The change of coffee bean extract browning reaction is very moderate compared with tea extract, particularly, the tea extract was colored greatly in the presence of iron ion. We observed that the coffee extract were not influenced by iron ion.

In the case of using tea extract for sea food, meat as anti-oxidant, the conspicuous browning reaction phenomenon of Catechin for the above iron ion is that hemoglobin iron in blood which is contained in a meat reacts with Catechin and the color of blood changed into blackish. It is not good phenomenon for the processed food product which appearance is very important such as sea food, meat and has been problem for use.

On the other hand, since the change of coffee bean extract browning reaction is very moderate and is not influenced by iron ion, we can conclude that the application of Coffeanol in the case of using for meat and processed food product as hold-back agent of the freshness very high.

10. The holding the freshness and the quality function of Coffeanol

The food constituents of sea food, meat, vegetable and other processed food products are changed by the function of bacteria, enzyme, ultraviolet rays, and their freshness, quality become not good. Coffeanol has anti-oxidation action, absorption ultraviolet rays action, inhibitory action for Tyrosinase, and that in addition to these functions, Coffeanol is a water-soluble, so , particularly, it has grate holding freshness action to processed sea food products.

We examined the effect of holding freshness function in terms of Coffeanol, tea extract, Tocopherol by using the opened horse mackerel. The example of this experience is described as follows.

[Method]

Each of the horse mackerel which back was opened was dipped into the prepared liquid
1～4 group as follows for 30 minuets, after removed the water, sample was put into the tray, rapped and stored in the refrigerator at 5 . The appearance of these sample changed by the time passing was examined.

[group]

Dipping liquid was prepared by adding 0.5% each addition agent, 1～4 as follows , to 5% saline solution.

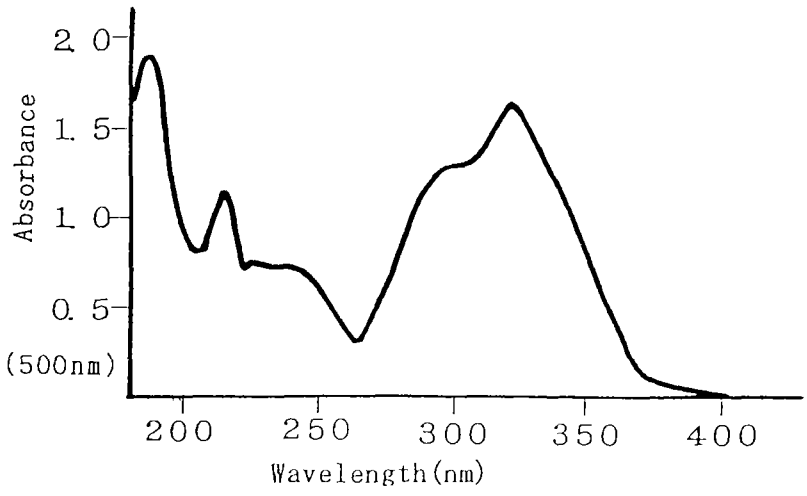
1. control (non addition)
2. 5% addition of coffeanol solvent
3. 0.5% addition of tea extract
4. 0.5% addition of tocopherol emulsion

[Result]

Days of preservation / experiment group	2 days	5 days	10 days
1	△	×	×
2	◎	◎	○
3	○	△	×
4	○	△	×

◎ : While the sample were dipping into prepared liquid, the color and luster had been kept what they had and their smell didn't change
○ : While the sample were dipping into prepared liquid, the color had been kept what they had, but the luster had become weak a little.
△ : Fading the color and luster of the sample meat are observed.
× : Greate fading the color and luster of the sample meat are observed.
×× : Greate fading the color and luster of the sample meat are observed and it had a acidification odor.

11. The application as makeup material
Absorption function of ultraviolet ray



[Explanation by the point Fig & Table-7]

The action of UV to organism is different, so the range of UV is classified into three ranges, less than 290nm UV-C resign, 290～320nm UV-B range, 320～400nm UV-A range.

The resign of Coffeanol UV absorption covers UV-B and UV-A, and strong absorption band is observed in 280nm～350nm range (Table-4).

UV ray of UV-A, UV-B resign is profitable for synthesizing vitamin D, sterilizing function and so on, on the contrary, they have some harmful function that promote acute inflammation, discoloration , aging and carcinogenicity of skin. UV also has the functions which promote deterioration of freshness and quality such as discoloration and oxidation of lipid for foods stuffs. Therefore, the protection for UV is needed. In the case of applying for the foods stuffs, the absorption function of Coffeanol UV can be greatly expected to have the effect on protecting of discoloration and promoting of stability for unoxidation in the preservation for freshness and quality.

12. Last word

In the recent research in relation to a coffee, the accomplishment of the research in terms of physiological activity function which Chlorogenic acid has will be very attractive suggestion on considering of developing coffee industry.

We, UCC Coffee Group will make a further research with the hope of that the accomplishment of our research will be a new foundation for developing world coffee industry.

THE MAILLARD REACTION AND COFFEE FLAVOR

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St. Paul, MN 55108, USA

In recent years there have been numerous reviews published on the role of the Maillard reaction in producing food flavors (Mottram, 1994; Tressl et. al., 1994; Baltes, 1990; Ledl, 1990) and even specifically the contribution of the Maillard reaction to coffee flavor (Flament, 1989; Ho et. al., 1993; Shibamoto, 1991; Tressl et. al., 1993; Holscher and Steinhart, 1994; Silwar and Lullmann, 1993) to mention a few. These reviews have very adequately detailed the numerous chemicals which have been found to result from the Maillard reaction, proposed mechanisms of formation and tabulated sensory properties of a large percentage of these compounds. Thus it would suit little purpose here to present long lists of chemicals and mechanistic pathways for the Maillard reaction in general. I prefer instead to discuss some of the most recent research on the Maillard reaction and relate this to what we know of coffee aroma.

The most recent tabulation of aroma compounds identified in coffee is the 1989 TNO-CIVO list. While there are recent supplements to this list, coffee has not been updated. You will note in Table I that nearly 800 compounds have been identified in coffee as of 1989. The present number is likely to be little above this since it becomes increasingly difficult to identify additional compounds. As you might expect, the largest general grouping consist of the heterocyclic compounds accounting for 80-85% of the total number of volatiles identified (Silwar and Lullmann, 1993). In quantitative terms, the total amount of steam distillable volatiles (excluding acids) in Arabica coffees was determined to be from 700 to 800 ppm (Silwar et. al., 1987). Since Silwar et. al., (1987) estimated that ca. 170 compounds are present at concentrations ranging from 1-150 ppm, one can appreciate that the vast majority of the volatiles identified in coffee are present at extremely low concentrations.

Table I. Volatile compounds identified in coffee (TNO-CIVO, 1989).

Hydrocarbons	74	Alcohols	20
Carbonyls, aldehydes	102	Acids	25
Esters	31	Lactones	3
Bases	216	Sulfur compounds	97
Acetals	1	Ethers	2
Nitriles and amides	4	Phenols	48
Furans	126	Epoxides, pyrans,	
Oxazol(in)es	35	Coumarins	7

An analytical chemist might be content to leave this tabulation as it is or go on to even expand the list of volatiles identified. However, if we wish to gain something useful from all this work our task must be to simplify this list and be able to focus on determining the aroma chemicals actually responsible for coffee aroma. Holscher and Steinhart (1994) suggested that the actual number of volatiles contributing to coffee aroma may range from 60 to 80. Professor Grosch and coworkers in previous publications (Semmelroch et. al., 1995; Blank et. al., 1992; Sen et. al., 1991; Blank et. al., 1991) as well as a part of this meeting have narrowed this list even further to 29 compounds. In a recent publication, they reported on the odor activity values of 14 compounds in this list (Table II).

Table II. Odor activity values of volatiles in the aroma of roast and ground Arabica coffee (Semmelroch et. al., 1995).

Compound	Odor Activity Value	Formation Mechanism*
(E)-β-Damascenone	2.7×10^5	Carotene degrad.
2-Furfurylthiol	1.7×10^5	Maillard reaction
3-Mercapto-3-methylbutylformate	3.7×10^4	Maillard reaction
5-Ethyl-4-hydroxy-2-methyl-3(2H)-furanone	1.5×10^4	Maillard reaction
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	1.1×10^4	Maillard reaction
Guaicol	1.7×10^3	Phenol degrad.
4-Vinylguaicol	1.1×10^3	Phenol degrad.
Methional	1.2×10^3	Maillard reaction
2-Ethyl-3-dimethylpyrazine	165	Maillard reaction
2,3-Diethyl-5-methylpyrazine	95	Maillard reaction
3-Hydroxy-4,5-dimethyl-2(5H)-furanone	74	Maillard reaction
Vanillin	48	Phenol degrad.
4-Ethylguaicol	32	Phenol degrad.
5-Ethyl-3-hydroxy-4- methyl-2(5H)-furanone	21	Maillard reaction

* Holscher and Steinhart (1994)

If one goes through this list of volatiles and considers the chemical mechanism responsible for the formation of each volatile compound, the Maillard reaction is well represented. One notes that 5 of the most « potent » 8 compounds are the result of the Maillard reaction. Thus one can readily appreciate why the Maillard reaction is given the credit it is for contributing to coffee aroma. One can not overlook the fact, however, that other reactions do yield potent aroma compounds and these contribute to coffee aroma. In that vein, it is of interest that β-damascenone has the highest odor activity valve of the volatiles listed in Table II. While that does not necessarily mean it is the most important volatile contributing to coffee aroma, it strongly suggests that it plays an important role in coffee aroma (Grosch, 1994). Damascenone comes from the thermal degradation of the carotenoids. While we have recognized the role of carotenoids in the development of tea flavor, they have largely been overlooked as being major contributors to coffee flavor. Another reaction whose prominence in contributing to coffee aroma is noted by the data in Table II is the degradation of phenolic compounds. The data available to date suggests that phenol degradations are second in importance (to the Maillard reaction) in contributing to coffee aroma.

Recent Advances in Maillard Chemistry

Recognizing the importance of the Maillard reaction to coffee aroma, it seems worthwhile to review what has happened in the area of Maillard research (limited to aroma compounds) in the last few years. Due to the large number of publications in this area, only some articles and authors can be selected for mention.

Chemical Mechanisms – The most basic work on the Maillard reaction has focused on determining the detailed chemical pathways leading to the formation of aroma chemicals (see review by Ledl, 1990). It has been a habit (albeit a bad one) amongst flavor chemists upon the identification of a new flavor compound to speculate on the mechanism of its formation. While this speculation has likely been based on sound theoretical chemistry, there has seldom been any experimental data to support the proposed chemical mechanism and thus mechanisms have often remained little more than speculation. However, substantial work has recently been published by Tressl and

coworkers in this regard (Tressl et. al., 1994; Rewicki et. al., 1994; Rewicki et. al., 1993; Tressl et. al.; 1993). They have put an emphasis on the use of stable isotope labeled precursor materials and determination of label incorporation into aroma compounds.

One of the benefits of this research has been a much better understanding of the interconversions of the initial reactants in the Maillard reaction (e.g. glucose and fructose) (Rewicki et. al., 1994). While different sugars lead to the formation of some specific aroma compounds, the commonality of Maillard reaction products irrespective of the starting carbohydrate had not been understood.

The majority of other research has focused on elucidating the mechanisms of formation of specific aroma compounds. To that end, substantial knowledge has been gained in how furans, furanones, pyranones, pyridines, pyrazines (see also the work of Weenan and Tjan, 1994), pyrroles, pyrrolines, thiophenes and thiophenones are formed during the Maillard reaction. An interesting side benefit of this work has been that this knowledge has lead to improved synthesis mechanisms of some compounds including those of the « kahweofuran » type and mono and bicyclic α-enamino-ketones (Rewicki et. al., 1993). This mechanistic work has led to the observation that some of the Maillard intermediates are extremely reactive and can form flavors at room temperature that typically are formed only at high temperatures. A example is the observation of Wedzicha and Edwards (1991) that 3-deoxyglucosone will react with cysteine at room temperature to produce a cooked meat flavor. Our traditional view of Maillard flavors is that we must use relatively high temperatures to accomplish the formation of some specific flavor chemicals. This view is supported later in this presentation when I will discuss research on reaction kinetics. However, it appears that high temperatures are not required if the proper precursors are present. An example of this is the formation of alkyl pyrazines in Swiss cheese. We know that alkyl pyrazines contribute roasted nutty notes to foods and it is widely believed that they can only be formed by high temperature roasting. Swiss cheese is not roasted so how does this product have pyrazines? The unique precursors of the alkyl pyrazines most commonly result from Strecker degradations (Maillard reactions). However, alkyl pyrazine precursors can also result via microbial metabolism. The metabolic intermediates of Swiss cheese starter cultures react during aging of cheese to yield a nutty flavor without heating.

Flavor Precursors - A large proportion of the research conducted on the Maillard reaction related to aroma generation has involved the use of model systems. Model systems comprised of various combinations of amino acids and sugars have been heated at selected conditions and the resulting flavor volatiles isolated and identified. The results of these studies have been the identification of volatile Maillard products, their required precursors and the conditions for their formation.

Professor Ho and coworkers, Rizzi (1989) and Bohneusteingel and Baltes (1992) have expanded the study of Maillard aroma precursors to consider the contribution of peptides and intact proteins. We have known for many years that the ε-amino group of lysine in proteins will participate in the Maillard reaction leading to the formation of a host of volatile compounds. It is also reasonable to expect that the deamidation of asparagine and glutamine will provide free ammonia as a participant in the Maillard reaction. Ho et al. (1993) and Bohneusteingel and Bates (1992) have presented substantial data that demonstrates that the deamidation of glutamine readily occurs and contributes more importantly to pyrazine formation than the α-amino group. Ho et al. (1993) also presented data that demonstrates significant amounts of pyrazines are formed from dipeptides. It is of particular interest that the dipeptide alanine-aspartic acid produced a relatively large amount of 2,5-dimethyl- 3-ethyl pyrazine, a compound considered to be very important to coffee aroma (Tressl 1989).

Ho et al. (1992) also has studied the contribution of peptides to Maillard generated aromas by investigating the aroma compounds formed by heating glycine and glutathione based peptides (1-4 units) and the corresponding free amino acid constituents. The glycine-based peptide studies found the peptides readily produced volatile Maillard products upon heating. Similar results were observed for glycine and triglycine and for diglycine and tetraglycine. They also found that alkyl 2-(1H)-pyrazinones are peptide specific products. Their further work with glutathione and the individual glutathione free amino acids demonstrated that different and unique volatile profiles result from the heating of peptides verses component free amino acids.

Izzo et al. (1993) conducted more studies with peptides and intact proteins. They chose to study the ability of gluten protein to yield volatile compounds when heated with glucose. It is of interest that the native gluten formed less pyrazines than gluten which had been partially deaminated. One would have expected the native gluten, having an abundance of amide groups, to give up a substantial amount of ammonia which is a good precursor for pyrazines. Izzo et al. (1993) explained this contradiction by noting that free ammonia can, under the proper conditions, contribute to color development as opposed to aroma development.

Ho has summarized his work in this area in the following way. The size, sequence and chemical conformation of a peptide will influence the aromas formed during heating and may control how much of each compound is formed. Ho has also noted that proteins contribute to the Maillard reaction by liberating ammonia.

These studies and observations undoubtedly contribute some understanding of coffee aroma formation during roasting, but they also complicate it. We find that the protein content of coffee decreases from about 12% in the green bean to about 3% in the roasted product. The proteins comprised in the green bean are likely to participate in the formation of coffee aroma either as intact proteins, hydrolyzed products or a free amino acids. Thus we cannot only consider the reaction of free amino acids, but we must also consider polymer forms of the amino acids. When we better define the true determinants of roasted coffee aroma, it will be interesting to determine their precursors in the coffee bean.

Lipid Participation in the Maillard Reaction – There has been considerable interest in studying the how the presence of lipids will influence the Maillard reaction (Whitfield, 1992; Farmer and Mottram, 1994). Coffee contains about 11.6% lipids in the green bean and thus the Maillard reaction occurring during roasting occurs in the presence of lipids. There is little question that Maillard reaction products will influence the degradation of lipids during heating and also that lipid degradation products will participate in the Mallard reaction. I will take a few minutes to review the current status of this research.

Substantial work has been done reacting amino acids and sugars in the presence and absence of lipids. The volatiles formed in each of these systems have been isolated and quantified permitting qualitative and quantitative comparisons to be made. Qualitative changes have been clearly demonstrated by numerous researchers (Ohinishi and Shibamoto, 1984; Huang et.al., 1987; Farmer et.al., 1991; Farmer and Whitfield, 1993). Compounds containing long carbon chains can only originate from fatty acid precursors. These compounds are formed as the result of thermally induced oxidation of lipids subsequently participating in the Maillard reaction. Other researchers have chosen to evaluate the participation of individual lipid oxidation products (e.g. saturated or unsaturated aldehydes) in model amino acid/sugar reactions (Hwang et.al., 1986; Kawai and Ishida, 1987; Chiu et.al., 1990; Zhang and Ho, 1989). Again numerous reaction products were formed which are unique to the presence of lipid. It is of interest (and certainly not unexpected) that the volatile compounds formed during the Maillard reaction depend on the lipid which is used in the system. Farmer and Mottram (1992) have done extensive studies in this area and some of their data are presented in Table III. As anticipated, when there were no lipids present in the model system, no long chain derivatives of flavor compounds were found in the system. However, when lipids, particularly egg derived phosphatidyl choline (PC) were included in the system, a different aroma profile resulted. The phospholipids used in this study contained from 22 to 34% polyunsaturated fatty acids which readily thermally decompose to reactive aldehydes.

Table III. Influence of lipid on the relative formation of lipid specific volatile sulfur compounds in Maillard systems.

	Cysteine:Ribose with				
Compound	alone	+Bovine triglyceride	+Bovine phospholipid	+Phosphatidyl-choline	+Phosphatidyl-ethanolamine
2-Pentylpyridine	0	0.005	0.054	1	0.082
2-Hexylthiophene	0	0	0.15	1	0.36
2-Pentylthiapyran	0	0.0001	0.091	1	0.36
1-Heptanethiol	0	0	0	1	0.25
1-Octanethiol	0	0	0	1	0.30

Farmer and Mottram, 1990.

Mottram and Farmer (1990) have also provided data to demonstrate that the presence of lipids will change the basic course of volatile formation during the Maillard reaction (Table IV). It is particularly interesting that the sulfur-containing volatiles resulting from cysteine/ribose reaction were reduced in the presence of lipids. The neutral triglyceride had the least effect while the beef phospholipid and phosphatidyl-ethanolamine had the greatest effect. Farmer and Mottram (1990) noted that the beef phospholipid and the phosphatidyl-ethanolamine (egg PE) both contain an ethanolamine group. This ethanolamine group may compete for the any reactive carbonyls and thus reduce the extent of the Maillard reaction. Farmer and Mottram (1994) also noted that the two

lipid are also very high in unsaturated fatty acids. The thermal degradation of these lipids would generate aldehydes which also would readily compete with sugars for NH_3 and H_2S.

Table IV. Influence of lipid on the relative formation of volatile sulfur compounds in Maillard model systems.

Compound	Cysteine:Ribose with alone	+Bovine triglyceride	+Bovine phospholipid	+Phosphatidyl-choline	+Phosphatidyl-ethanolamine
2-Mercapto-3-butanone	1	0.97	0.45	0.51	0.48
2-Mercapto-3-pentanone	1	0.72	0.49	0.53	0.53
2-Methyl- 3-furanthiol	1	0.42	0.15	0.27	0.24
2-Thiophenethiol	1	0.32	0.15	0.46	0.14
2-Methyl- 3-thiophenethiol	1	0.076	0.0025	0.19	0.028

Farmer and Mottram, 1990.

If we return to the Maillard reaction and coffee aroma, it does not appear that unique lipid/Maillard products make a significant contribution. Of the more than 800 volatiles identified in coffee, one does not find long chain derivatives in this list. Coffee glycerides appear to be reasonably heat stable and do not degrade into Maillard reactive components. This does not mean that coffee lipids (more correctly coffee glycerides) do not have any influence on coffee aroma, however, it appears that any effect is minor and indirect.

Reaction Kinetics Studies – In the more than 80 years which have passed since Louis Maillard (1912) first studied the reaction of glycine and glucose, a large amount of work has been published on the loss of nutrients, formation and fate of intermediates (many of these are flavor compounds) and formation of color. Labuza (1994) has recently lamented that little research has been directed at '... developing mathematical equations based on sound chemical principles that could be used to make valid predictions of color, flavor etc. under various formulations, processing and storage conditions...' relating to the Maillard reaction. Labuza is a colleague of mine and I have very often heard him use the phrase 'cook and look research'. This is how he describes research where one chooses a model system and heats it for some arbitrary time and temperature (often unrealistic) then looks to see what has happened. Few studies have been methodically planned, all variables controlled (e.g. pH), products adequately quantified and a sufficient range in times and temperatures of heating employed to permit the development of mathematical relationships useful in kinetic predictions. However, substantial kinetic work has been done on the loss of reactants related to nutrition or color formation (change in physical appearance).

The Maillard reaction follows first order kinetics for amine loss (until the amine starts recycling), first order kinetics for the loss of reducing sugars and pseudo first order kinetics for the formation of brown pigments (Labuza, 1994). Flavor compounds are intermediates in these reactions and are transient in nature. Consequently the kinetics of flavor compound formation tend to be more complex. Despite this, kinetic data on a limited number of flavor compounds has recently appeared in the literature. Some of the earliest work is that of Leahy and Reineccius (1989 a,b) reporting on the kinetics of pyrazine formation in simple amino acid:sugar model systems (glucose, fructose or ribose with lysine or asparagine). The authors found that pyrazine formation followed pseudo zero order reaction kinetics with an activation energy ranging from 27-45 Kcal/mole. The lower E_a was for pyrazine while the higher E_a's were for more highly substituted pyrazines. A high E_a value is characteristic of reactions that proceed only under the higher temperatures associated with roasting. Since pyrazines give characteristic roasted, toasted notes, the high E_a s for pyrazine formation are reasonable. Parliament and Stahl (1994) studied the kinetics of the generation of furfuryl mercaptan in a cysteine:ribose model system. They found that this compound also has a high E_a (ca. 50 Kcal/mole). This suggests that high roasting temperatures are also required for the formation of this compound. Furfuryl mercaptan also followed zero order reaction kinetics and its' formation was favored at low pH. Furfuryl mercaptan exhibits a maximum concentration and then decreases with heating time. Thus furfuryl mercaptan serves as a precursor to the formation of other products with continued heating.

A tabulation of other kinetic studies is presented in Table V. Only a portion of these data will be commented on. One point of interest is the low E_a's required of the Strecker aldehydes methional, isovaleraldehyde and

phenylacetaldehyde. These compounds are readily formed at low temperatures and may serve as indicators of product degradation upon storage due to Maillard browning. The broad range of E_as reported for sugar fragmentation products (e.g. furfural, 2-acetylfuran, 5-methyl furfural and pyranone) demonstrates the strong system dependence for the formation of these compounds. Schirle-Keller and Reineccius (1992) observed high E_as for these products when cysteine was the only amino acid source. Chang and Reineccius (1994) used a much more reactive amino acids and greatly enhanced the production of these same compounds (presumably lower E_a's were required). Another point of interest is that few of the volatiles studied followed the pH relationship (associated with Maillard browning) of a greater loss of reactants and higher degree of color formation at higher pH values. The only volatiles that followed this relationship were the pyrazines. All of the other compounds showed different relationships to pH. This demonstrates a pH sensitivity to either their formation or stability in the system.

Table V. Summary of kinetic information on flavor compounds.

Compound	Activation energies KCal/Mole	
Pyrazines	27 TO 45a	
Furfural	35.2b	17.5 to 25e
2-Acetyfuran	36.2b	17.7e
5-Methylfurfural	37b	
DI(H)-Di(OH)-6-Methyl pyranone	30.7b	16d
Diethylsulfide	28.1c	
Methional	19 to 27c	
2-Acetylthiophene	30 to 32c	
2-Furfurylthiol	48 to 50e	
Strecker aldehydes	14.4 to 23.1d	

a Leahy and Reineccius, 1989a, b
b Schirle-Keller and Reineccius, 1992
c Chan and Reineccius, 1994a
d Chan and Reineccius, 1994b
e Parliment and Stahl, 1994

Maillard Reaction and the Glass Transition Theory - In recent years we have heard a great deal about glass-rubber transition theory and extremely detailed reviews have been published (Slade and Lavine, 1991). Simply put, this theory proposes that a polymeric material, when in the solid state, may exist as either a rubbery or glassy state. The rubbery state has a large free volume which readily permits diffusion to occur, while the glassy state is very rigid and will not permit diffusion. Researchers have tended to equate the glassy state to product stability, for example in encapsulated flavors. Factors that determine whether a material is in the rubbery or glassy state include; the inherent properties of the food itself, water activity and temperature. To provide you with an appreciation for some typical glass transition values (T_g); a 10 DE maltodextrin has a T_g of ca. 90°C, a 20 DE maltodextrin a T_g of 80°C, and a 36.5 corn syrup solids a T_g of 45°C. These T_g values are all at a water activity (a_w) of 0.20. We would observe a decrease in T_g with an increase in a_w.

Initially, the glass transition theory was offered as an explanation for nearly all physical and chemical reactions in dry foods. However, the validity of this theory has been brought to question by numerous researchers. For example, one would expect electrical conductivity to be influenced by T_g. Yet Noel et al. (1994) have shown that there is no inflection in electrical conductivity of carbohydrates in glassy states at T_g. In addition, LeMesta et al. (1991) have found that glass transition theory does not explain the mobility of small molecules in polymeric solids. As research continues, it appears that the glass transition theory explains only a small part of the chemical phenomena that occur in dry food products during storage. There is mounting evidence that the Maillard reaction may be influenced by glass transition in some systems (Buera and Karel, 1994). The data presented in Figure 1 are for a lactose/CMC/threalose/xylose/lysine model system. Karmas et al. (1992) have suggested that the change in kinetic behavior occurs at T_g. They found a significantly slower Maillard reaction rate when the system was in the

glassy state. As you will note, there is only one data point presented below T_g and thus the conclusions based on this data is less convincing than they could be.

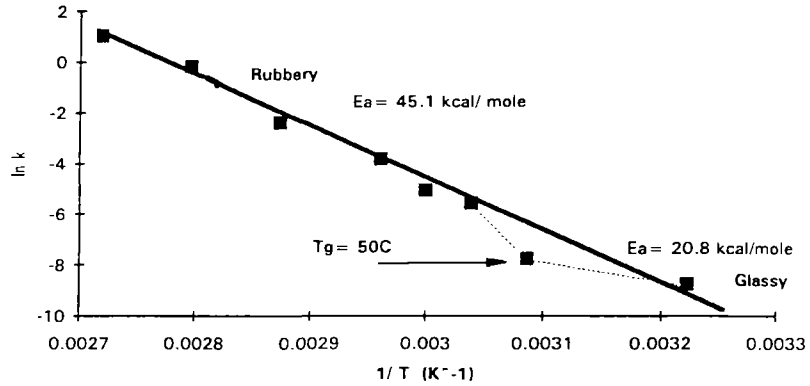

Figure 1. Browing in lactose/CMC/threalose/xylose/lysine model systems (Karmas et al. 1992).

Summary

I believe we can unquestionably say that the Maillard reaction plays a key role in the formation of coffee aroma. Recent studies carried out on the Maillard reaction have made general advancements in our fundamental knowledge of this reaction. The recent well planned studies involving complex precursors, rigorous kinetic studies and consideration of the physical state of a food will prove much more useful than past « cook and look » analytical studies. With respect to coffee aroma, works such as these will provide an understanding of the precursors that lead to producing a good coffee aroma. A few years ago only free amino acids were considered as aroma precursors, while peptides and proteins are now considered to play an important role in flavor formation. Rigorous mechanistic work is defining how individual aroma compounds are being formed and kinetic studies are defining the reaction conditions necessary for their formation. However, studies on glass transition theory related to the Maillard reaction have thus far failed to support the global application of this theory in predicting the reactions in dry food products.

In my opinion, our next step in studying the importance of the Maillard reaction on coffee aroma should be to step away for a moment and allow sensory, chemical and instrumental studies to merge. We could allow this non-targeted approach in the kinetic and mechanistic studies to continue; with a lot of time and laboratory resources wasted. We must have, before we continue chemically and instrumentally, a clear definition coffee aroma. Only then can we efficiently and effectively direct our Maillard research to focus on these key aroma compounds, their precursors and the processing conditions needed to generate these important aroma components. It is futile to attempt to study the formation of the more than 800 volatile compound thus far identified in coffee. Sensory, chemical and instrumental scientist must narrow this list to ca. 25 most sensorially significant compounds. Then, research efforts to define mechanistic and kinetic parameters of the formation of this limited number of coffee aroma components would be feasible and translate directly into practical guidelines for producing and controlling the formation of good coffee aroma

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Brief Summary

The Maillard reaction plays an important role in the development of coffee aroma. Recent studies carried out on the Maillard reaction have made general advancements in our fundamental knowledge of this reaction. However, I believe that sensory, chemical and instrumental studies must be completed on coffee aroma in order to permit Maillard research to focus on the aroma constituents of greatest importance. We must have a clear definition coffee aroma. Only then can we efficiently and effectively direct our Maillard research to focus on these key aroma compounds, their precursors and the processing conditions needed to generate these important aroma components. It is futile to attempt to study the formation of the more than 800 volatile compound thus far identified in coffee. Sensory, chemical and instrumental scientist must narrow this list to ca. 25 most sensorially significant compounds. Then, research efforts to define mechanistic and kinetic parameters of the formation of this limited number of coffee aroma components would be feasible and translate directly into practical guidelines for producing and controlling the formation of good coffee aroma.

COFFEE ADULTERATION AND A MULTIVARIATE APPROACH TO QUALITY CONTROL

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1. Introduction

Coffee is one of the most widely traded commodities in the world and a beverage enjoyed by millions. Coffee authenticity and quality issues are complicated by the fact that coffee is processed, prepared, and consumed in a variety of ways according to local practices. There are "French" coffees that contain chicory, and "Viennese" coffees containing fig. There are light roasts, dark roasts, coarse grind, fine grind, percolated, drip, boiled, expresso, and Turkish coffees. There are green coffee quality issues having to do with geographical origin, the level of defects, bean maturity, whether wet or dry processed. Most commercial coffees are blends of two species *(Coffea arabica and C. canephora)* that differ widely in quality and are priced differently on the world market. Thus, there is a continuum of quality based on blend alone. Soluble coffees are subjected to an assortment of extraction, concentration, and drying processes. All of these variables result in "coffees" with a variety of chemistries, making it difficult for anyone to agree as to what constitutes the real thing.

Coffee is defined[1] as a, "General term for the fruits and seeds of plants of the genus *Coffea*, generally the cultivated species, as well as products from these fruits and seeds in different stages of processing and use, intended for consumption." It is further noted that, "This applies to products such as cherry coffee, husk coffee, parchment coffee, green coffee, monsooned coffee, polished coffee, decaffeinated coffee, roasted coffee as beans or ground, coffee extract, instant coffee and coffee brew." So technically, a product could contain any of the above at any level and be considered coffee. From a quality standpoint, consumers and producers alike would agree that coffee beans must be roasted before brewing. Coffee cannot contain high levels of husk, parchment, or other defects, and still deliver the flavor and aroma associated with the coffee beverage.[2] So there are boundaries within which the coffee processor must operate to ensure a quality beverage.

An attempt to define coffee adulteration[3] reads, "to debase by mixing with something inferior or spurious. Tapioca chips, date seeds, coffee husk, tamarind seeds or shells, roasted peas and cereals are added to coffee as adulterants." More generally, adulteration can be viewed as the deliberate addition of a less expensive filler for economic benefit. Whether it be chicory, cereals, maltodextrins, or even *robusta* coffee in a product declared to be *arabica*, any deliberate misrepresentation should be regarded as adulteration. Authentic, on the other hand, implies that something *is* what it is purported to be.

Foods and beverages in the United States are required to be labeled in a manner which is neither false nor misleading. Thus, a product labeled as coffee should contain coffee and nothing else. Otherwise, the name should be modified to reflect the presence of added ingredients. For example, *coffee beverage* might be

appropriate and the product should be accompanied by an Ingredient Statement indicating what else it contains. In this context, this paper will highlight some useful tools for detecting some common adulterants and controlling coffee quality.

2. Methods

2.1. Insoluble and Soluble Matter

The simplest form of adulteration is the addition of filler to extend a product. Soluble coffee is produced by hot-water, high-pressure extraction of roast, ground coffee. Extraction yields typically range from 40-55%, leaving nearly half of the coffee to be discarded. The presence of insoluble matter could be evidence of add-back of spent grounds or some other filler to extend the product. Reynolds et al.[4] illustrate results from gravimetric analysis of instant coffee spiked with grounds over the range 0.2-0.6 g/kg. Although the experimental variability was significant below about 0.5 g/kg, results were acceptable at 1 g/kg, well above the "normal" level of insoluble matter in instant coffee. Thus, filtering and weighing is a suitable first step for detecting the addition of insoluble extenders to soluble coffee.

Screening roast and ground coffee calls for a counter approach, where water-*soluble* extractives are measured.[5-6] Although somewhat dependent on type of coffee, degree of roast, and grind, a simple extraction of similar roast, ground coffees give remarkably reproducible numbers (*%RSD* < 1). Pure coffee will typically yield 29-30% (db) soluble solids. On the other hand, cereals, and coffee substitutes such as chicory contain more free sugars after roasting and yield 68-70%. This makes it possible to detect the co-blending of coffee with cereals, barley, corn, chicory, and other such substances.

2.2. Caffeine and Chlorogenic Acids

Two soluble compounds that are determined fairly easily are caffeine and the chlorogenic acids (CQA). In our laboratory, both are determined in a single run by reversed-phase HPLC with UV detection (280 and 330 nm). Caffeine is typically between 0.9-1.2% in arabica coffee and 1.6-2.4% in robusta. CQA are typically 1-2%, slightly higher in robustas than arabicas. Because they are readily soluble in hot water, there is an inverse relationship between extraction yield and percent by weight in the extract. Thus, soluble coffee contains these compounds in expected ranges that depend on extraction yield and to a lesser extent, blend. A caffeine result of 2.2% (**Figure 1**) for a coffee/chicory mix might also be achieved with a low caffeine (0.9%) arabica coffee and an extraction yield of over 40%. However, arabica beans give lower yield than robustas and most soluble producers would opt for the latter. A robusta at 1.6% caffeine and 55% yield would exhibit 2.9% caffeine in the finished product. The point is that unusually low caffeine and/or CQA levels could be an indication of the presence of a non-coffee substance. However, it is conceded that natural fluctuations and processing effects can lead to wide variations.

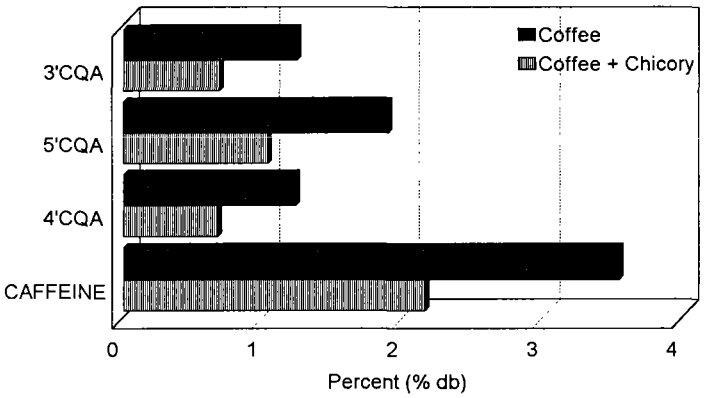

Figure 1. Percent (% db) of caffeine and chlorogenic acids (CQA) in soluble coffee, and in a coffee/chicory mixture.

Regarding green coffee quality, it should be mentioned here that CQA profile can be a useful indication of bean maturity.[7] The diCQA/CQA ratio is higher for discolored green coffee, a characteristic of immature fruit. It is noteworthy that sucrose, essential for flavor development, is less than 10% of that in healthy green coffee.

2.3. Sugars

Sugar composition is another example where expected values can be used to check coffee authenticity. Newman[8] illustrates this for soluble coffee containing chicory and glucose solids. His calculation assumes that

pure soluble coffee contains about 3% caffeine, 5% aldose, and negligible ketose sugars. Based on typical values in chicory and glucose solids, the composition of the unknown was calculated as follows:

TYPICAL VALUES	% CAFFEINE	% KETOSE SUGAR	% ALDOSE SUGAR
Extracted Coffee Solids	3.00	0	5.00
Extracted Chicory Solids	0	57.50	10.00
Glucose Solids	0	0	90.70
Unknown Sample	0.16	15.80	63.40

In the unknown sample:

% coffee = 0.16/3.00 = 0.052 or 5.2%

% chicory = 15.8/57.5 = 0.28 or 28%

% glucose solids = [63.4 - 0.052(5.0) - 0.28(10.0)]/90.7 = 0.67 or 67%

The analysis of sugars was facilitated by the development of High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD).[9-10] An interlaboratory study led by J. Prodolliet[11] in conjunction with the European Association of Soluble Coffee Manufacturers (AFCASOLE) found this method to be superior to more commonly used methods for sugars analysis.[12-13] Chromatograms of a standard sugar mixture and a soluble coffee are shown in **Figure 2.**

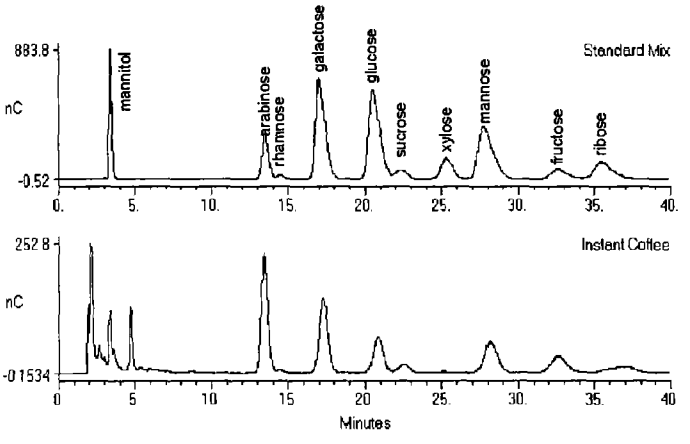

Figure 2. HPAE-PAD chromatograms of a standard mixture of monosaccharides, and typical instant coffee.

Results are given in **Figure 3** for soluble coffee and a coffee/chicory mixture. Note the high level of fructose from the fructan, inulin, in chicory. Chicory also contains higher levels of free glucose and sucrose. Pure coffee contains higher levels of arabinose and galactose. Maltodextrins, starches, or other glucose solids may be added to coffee extracts to aid spray-drying, to encapsulate flavor and aroma volatiles, or simply to extend the product. These are characterized by abnormally high glucose levels, with somewhat diluted levels of the other sugars **(see Figure 4).**

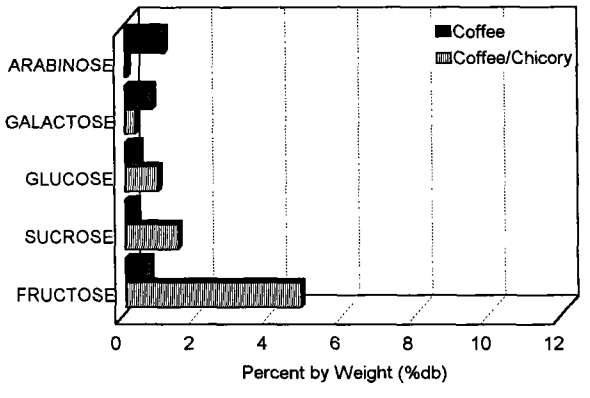

Figure 3. Percent (% db) of some free sugars in soluble coffee and a soluble coffee/chicory mixture.

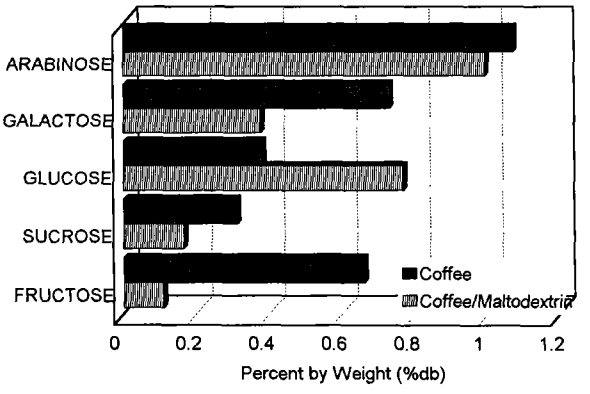

Figure 4. Percent (%db) of some free sugars in soluble coffee and a soluble coffee/maltodextrin mixture.

Green coffee defects such as parchment or hulls deterioriate the coffee beverage when co-extracted.[2] This woody material contains a xylan polysaccharide, which upon hydrolysis yields xylose. Very little xylose is found in the coffee bean. Although differences in xylose content with geographical origin have been noted[14], the determination of xylose in hydrolyzed coffee can be a useful quality measure. **Figure 5** contains chromatograms of a typical hydrolyzed soluble coffee, and hydrolyzed coffee hulls.

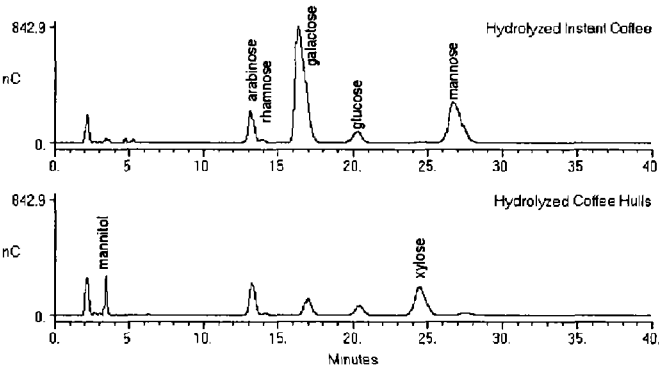

Figure 5. HPAE-PAD chromatograms of hydrolyzed instant coffee, and hydrolyzed coffee hulls.

2.4. Coffee Blend

Speer[15] reported the isolation and identification of 16-O-methylcafestol, a diterpene alcohol found only the oil of robusta coffee and not in arabica. **Figure 6** contains HPLC chromatograms of 50% and 2% robusta/arabica blends obtained in our laboratory. Neglecting differences in content among robustas of different countries, the method allows the quantitation of as little as 2% robusta in a roast and ground coffee blend. We have confirmed Speer's findings in that we have not found the compound in any arabica coffees to date.

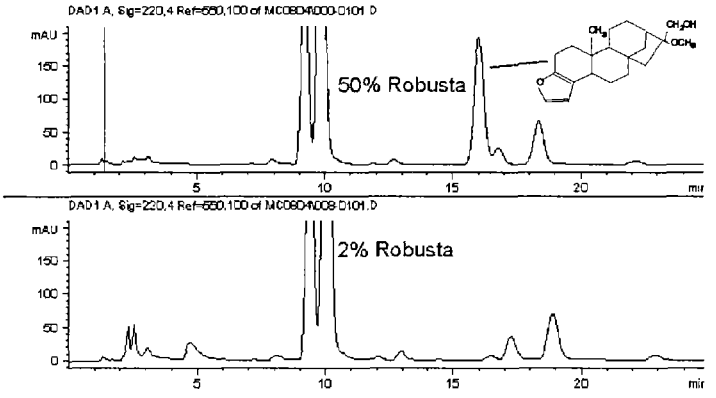

Figure 6. HPLC chromatograms of coffee blends showing 16-*O* methylcafestol: (top) a 50% robusta/arabica blend, (bottom) a 2% robusta/arabica blend.

2.5. Multivariate Methods

In addition to those compounds already mentioned, coffee contains over 800 volatiles that have been identified. These include furans, aldehydes, ketones, pyrazines, pyridines, pyrroles, thiols, and phenols, to name a few. It is estimated that perhaps 170 or so compounds are responsible for coffee's characteristic aroma.[16] This is too many compounds to monitor individually. In our laboratory, we use a combination of headspace gas chromatography and HPLC methods. Multivariate exploration and classification methods (PCA, HCA) are applied to make optimum use of these data.

Figure 7 is a dendrogram from Heirarchical Cluster Analysis (HCA) of GC and HPLC data on several commercial soluble coffees. Sample similarity or difference is based on a Euclidean distance calculated from all variables (*n*). In this dataset, many samples are similar, however there are some apparent outliers.

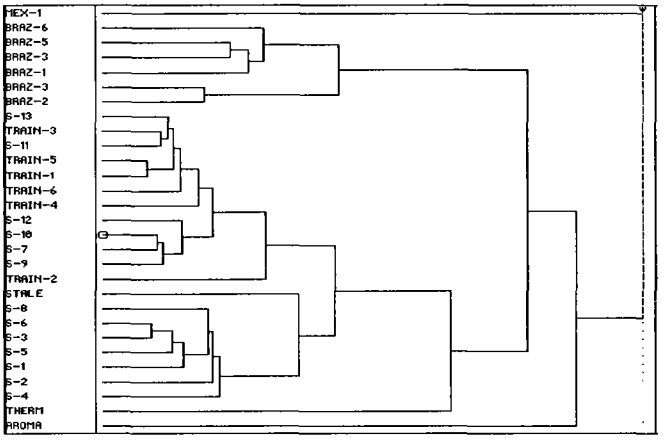

Figure 7. Heirarchical Cluster Analysis (HCA) of GC and HPLC data from analysis of some commercial soluble coffees.

To visualize the interrelationship between samples we applied Principal Components Analysis or PCA (**Figure 8**). In PCA, the original *n* variables are linearly combined to construct 2 or 3 orthogonal axes that contain the majority of variance in the dataset. Any remaining variance is ascribed to noise. Briefly, the original data matrix is multiplied by its transpose to construct a correlation matrix, which is then decomposed into two matrices, a *loadings* matrix and a *scores* matrix . The loadings matrix is the relationship of the original variables to the new axes (PC's), while the scores matrix contains the samples' new coordinates. The data are reconstructed by taking the scores matrix *times* the loadings matrix--*transposed.* Any difference between the original and the calculated data is the *residuals* matrix. The sum of the residuals-*squared* for each sample across all variables yields a Squared Prediction Error (SPE)[17], which will be discussed in more detail later.

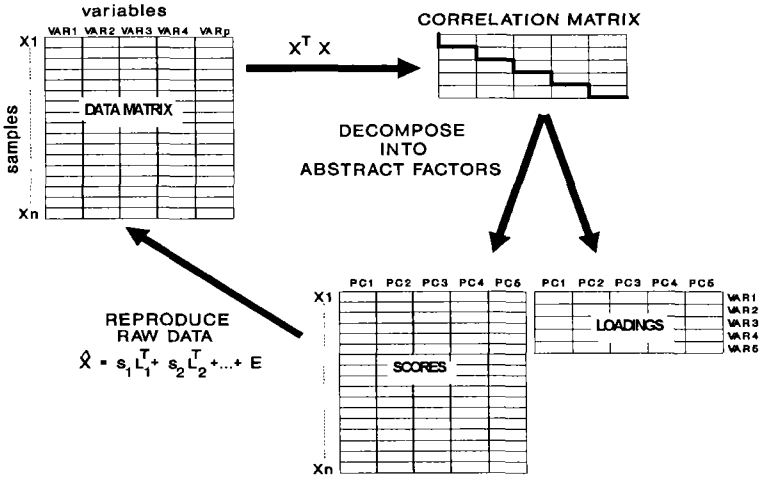

Figure 8. Basic steps in Principal Components Analysis (PCA).

The Loadings plot **(Figure 9)** illustrates the relationship of the original variables to the new axes. Note that the majority of volatile compounds load high on PC1, or to the right of the plot. Certain non-volatiles, in particular total sugars (after hydrolyis of sample) load to the left. Free sugars are high on PC 2, while some furans lie tend toward the bottom. Note that similar classes of volatile compounds, such as pyrazines, ketones, diones, aldehydes tend to load together within the volatiles cluster.

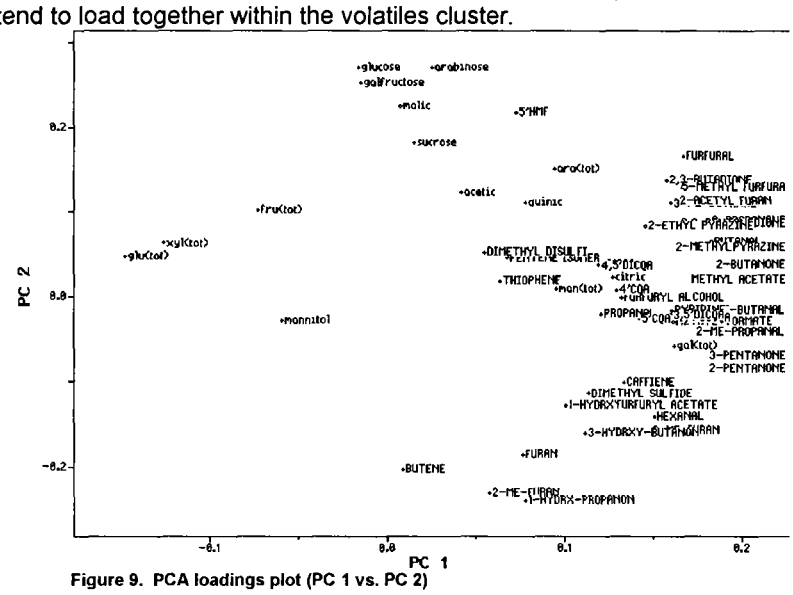

Figure 9. PCA loadings plot (PC 1 vs. PC 2)

Figure 10 is the scores map of the same samples illustrated in Figure 7. For the sake of discussion, we will assume that the training set (labeled TRAIN) is normal product. We can now readily identify the outlying samples. Recalling the loadings, the sample to the left of the plot (MEX) is relatively low in volatiles, yet higher in total glucose, fructose, and xylose than other samples. This is indication that the sample contains filler material, most likely glucose solids and possibly chicory. The sample may also have been co-extracted with a high level of coffee hulls. The sample toward the bottom (THERM) contains low levels of free sugars, yet higher levels of some furans. Furans are generated at the expense of sugars upon heating, so it is likely that this sample was thermally abused. The sample on the right (AROMA) contains a higher level of volatiles, perhaps

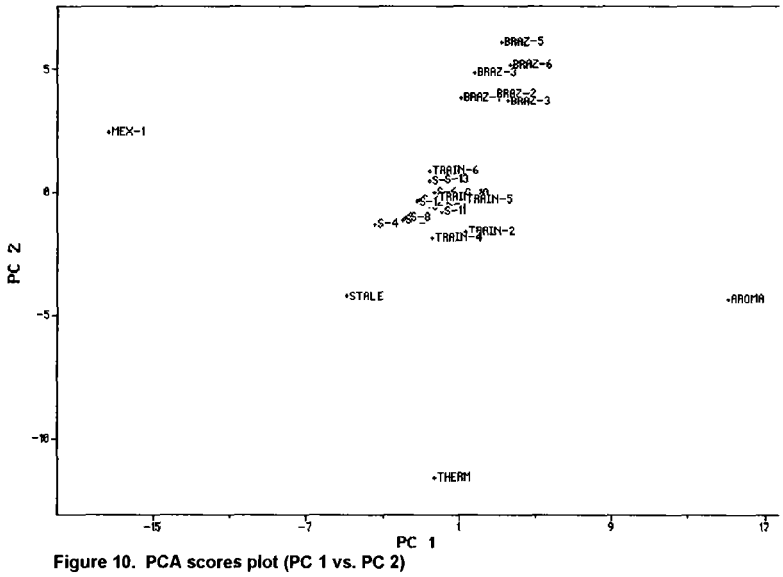

Figure 10. PCA scores plot (PC 1 vs. PC 2)

due to "aromatization" with volatiles from another source, or freeze-drying, a process that retains more volatiles. The cluster of samples at the top of the plot (BRAZ) is characterized by higher levels of all free sugars, as well as 5-HMF. This could be an indication of different extraction conditions, and not necessarily adulteration. The sample labeled STALE was judged so by flavor experts and is clearly outside of the cluster of normal samples.

The *residuals* matrix is the difference between the original data and that calculated from PCA scores and loadings. The SPE is the sum of the residuals-squared for each sample, and is a useful number for describing how well, or how poorly, a sample fits the model. If a suitable PCA model is developed for the "normal" coffees, then a sample that does not "fit" the model will have a high SPE. **Figure 11** is a plot of SPE vs. sample number. The outlying samples are readily identified. It is possible to calculate statistical limits at a user specified confidence level, above which a sample is considered to be a statistical outlier. Plots of this sort are useful for control charting. The advantage of the multivariate procedure over the classical Shewart control chart is that rather than monitoring a single variable with time, we are in essence monitoring several variables, the variance of which is contained in 2 or 3 principal components.

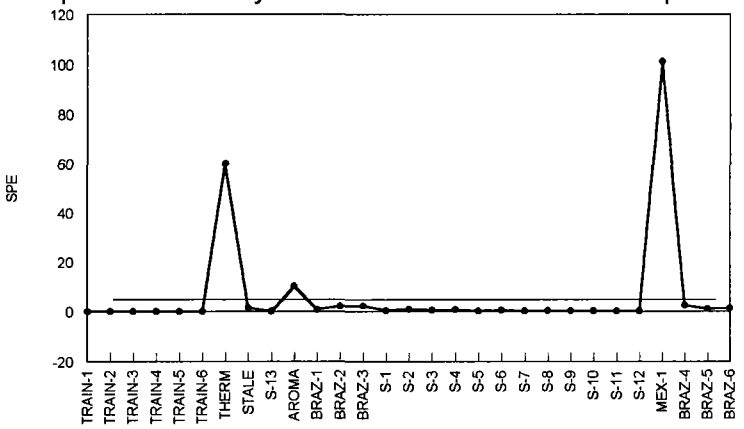

Figure 11. A multivariate statistical control chart: squared-prediction error (SPE) vs. sample number.

3. Conclusions

Coffee is a very complex beverage enjoyed by millions in a variety of ways. There is wide latitude for agreement between buyer and seller as to standards of coffee quality. This paper has reviewed some of the more commonly used methods used to check for authenticity, detect deliberate misrepresentation, and make quality assessments. These methods include physical tests, chemical analysis, and comparison with expected results. Although it is possible in some cases to identify unusual samples from these tests, coffee as a natural product is susceptible to wide variations. Multivariate statistical techniques (HCA, PCA) when applied to GC-headspace and HPLC data show promise as additional tools for classification and quality control of coffee products.

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ABSTRACT

Coffee adulteration issues are complicated by the fact that most commercial coffees are blends of *Coffea arabica* (arabica) and *C. canephora* (robusta). *Arabicas* command a higher price and are considered to be of better quality than *robustas*. Furthermore, coffees are processed, prepared, and consumed in a variety of ways according to local practices. Examples are "French" coffees that contain added chicory, or "Viennese" coffees containing fig. Green coffee quality, processing, the level of defects, roasting, and grinding all influence the final product. Soluble (Instant) coffees are further subjected to an assortment of extraction, concentration, and drying processes. Methods for detecting adulteration have focused on extraneous sugars from undeclared chicory, cereals, or other fillers. Quality measures such as the percentage of robusta in a blend, or the levels of defects (coffee parchment, husks, discolored beans) have also received attention. Multivariate statistical techniques show promise as tools for classification and quality assurance of soluble coffee. Heirarchical Cluster Analysis (HCA) and Principal Components Analysis (PCA) applied to GC-headspace and HPLC data permit grouping of samples according to origin and/or processing conditions. A method of quality control of soluble coffee that utilizes PCA compression of multivariate data and examination of residuals is illustrated. Examples of outlier detection and identification of unusual or adulterated samples is given.

INFLUENCE DU MODE DE PRÉPARATION DU CAFÉ VERT ROBUSTA SUR SA COMPOSITION CHIMIQUE ET SES QUALITÉS ORGANOLEPTIQUES

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INTRODUCTION

La qualité d'un café dépend de plusieurs facteurs : les génotypes, les zones de culture, les pratiques culturales, la maturité des cerises, les traitements post récolte, la torréfaction et le conditionnement.

Quelques études sur la qualité du café ont déjà permis de mettre en évidence l'influence de certains facteurs sans toutefois déterminer le rôle et l'importance de chacun avec précision.

Cramer (1), Teixera (2) et Leroy (3) ont montré l'importance du facteur génétique et l'existence d'une variabilité clonale pour la qualité. Leroy et al. (4) a également mis en évidence des différences concernant les qualités technologiques et organoleptiques de génotypes de caféiers issus de deux populations en sélection, Guinéens et Congolais. Guyot et al. (5, 6) ont étudié l'évolution des propriétés physiques, chimiques et organoleptiques d'un clone de café Robusta en fonction de la maturité. Vincent et al.(7) puis Texeira (2)ont également mis en évidence un effet préparation qui fait apparaître comme meilleurs les cafés préparés par voie humide. L'utilisation d'enzymes pour la préparation du café a déjà été effectuée par Arunga (8), Amorim (9) et plus récemment par Tchana (10) sans préjudice pour la qualité.

De ces études il ressort que le facteur génétique et le facteur mode de préparation sont parmi les plus importants, sans pour autant négliger les autres facteurs; ceux-ci ne pourront d'ailleurs être étudiés que si l'influence de ces deux facteurs est parfaitement connue.

L'étude que nous avons entreprise consiste en une expérimentation testant 3 modes de préparation : voie sèche (VS), voie humide (VH), et voie humide avec enzyme (VHE), sur 3 clones de café ROBUSTA (J21, C6, M5) présentant des propriétés technologiques, chimiques et organoleptiques très différentes. Les différentes analyses physiques, chimiques sur cafés verts et organoleptiques sur cafés torréfiés ont permis de :

- mettre en évidence les modifications dues aux modes de préparation.

- de classer les différents échantillons par des analyses statistiques multivariées grâce aux 6 répétitions effectuées au niveau des modes de préparation des échantillons.

MATERIEL ET METHODES

PROTOCOLE DE PREPARATION DES ECHANTILLONS DE CAFE VERT

Les échantillons ont été préparés sur la plantation industrielle de la CFSO à ABONG MBANG au CAMEROUN.

Trois modes de préparation voie sèche,voie humide, et voie humide avec enzyme ont été testés sur trois clones de café ROBUSTA J21,C6 et M5 présentant des propriétés physiques, chimiques et organoleptiques différentes.

- J21 : origine JAVA, bonne production, bon rendement à l'usinage (3,5), bonne granulométrie.
- C6 : origine COTE D'IVOIRE, bonne production, petits grains en majorité.
- M5 : origine MADAGASCAR, rendement correct à l'usinage (4,3), très forte granulométrie.

Pour tous les clones, les cerises sont cueillies mûres, au moment du pic de récolte (11); elles proviennent d'une même parcelle et les lots ainsi obtenus sont nettoyés et débarrassés des cerises flottantes par flottaison. Ce traitement a permis d'éliminer en grande partie les cerises scolytées. 15 kg de cerises ainsi obtenus sont traités par répétition pour chaque clone et 6 répétitions sont effectuées pour chaque traitement.

- TRAITEMENT PAR VOIE SECHE **(VS)**

  5 kg de cerises sont mises à sècher au soleil en couche mince et sont remués 3 à 4 fois par jour. La durée du séchage a été de l'ordre de 3 semaines environ pour une teneur finale en eau de 12% environ. Après séchage les cerises sont décortiquées et le café obtenu est nettoyé et trié pour donner l'échantillon de café vert à traiter.

- TRAITEMENT PAR VOIE HUMIDE **(VH)**

  5 kg de cerises sont dépulpées sous eau avec un dépulpeur à tambour. Les pulpes folles sont éliminées et le café dépulpé est mis à fermenter dans un seau plastique percé de trous pendant 18 heures. Après fermentation le café parche est lavé soigneusement jusqu'à disparition complète du mucilage restant.

- TRAITEMENT PAR VOIE HUMIDE AVEC ENZYME **(VHE)**

  5 kg de cerises sont dépulpées sous eau avec un dépulpeur à tambour. Les pulpes folles sont éliminées et le café dépulpé est mis à fermenter dans un seau plastique sous eau en présence d'enzyme (*).(5 ml de solution enzymatique pour 5 l de café dépulpé). La durée de fermentation est de 2 heures environ. Après fermentation le café parche est lavé soigneusement jusqu'à disparition complète du mucilage restant.

** L'enzyme utilisé est de la RAPIDASE 9211 (fabriqué par GIST BROCADES) qui a une forte activité pectinolytique.*

Pour les traitements voie humide, le café parche est mis à sécher au soleil en couche mince, après égouttage,et remué 3 à 4 fois par jour. La durée du séchage a été de l'ordre de 2 semaines environ pour une teneur finale en eau de 11,5% environ. Après séchage le café parche est déparché et le café obtenu est nettoyé et trié pour donner l'échantillon de café vert à traiter.

Avant analyse les échantillons de cafés verts sont tamisés (tamis à ouverture de $13/64^{ème}$ de pouce) pour éliminer les brisures et les fèves mal formées ou piquées.

CARACTERISATION DES MODES DE TRAITEMENT

Les différences induites par les trois modes de traitement ont été caractérisées par des :

- analyses chimiques sur cafés verts.
- tests organoleptiques sur cafés torréfiés.

Une étude statistique multivariable (ACP et AFD) est ensuite effectuée à partir des données obtenues.

Analyses chimiques des cafes verts.

Les principaux constituants chimiques des cafés verts ont été analysés d'après les méthodes de dosage développées et appliquées au café :

- analyses chimiques : pH, acidité totale (11), caféine (12), principaux isomères de l'acide chlorogénique (13), saccharose, fructose(11).

Analyses organoleptiques des cafes torréfiés.

TORREFACTION : Les échantillons (150 g) sont torréfiés avec un torréfacteur de laboratoire PROBAT dans des conditions opératoires strictement identiques. (température et durée de torréfaction). Le contrôle de la torréfaction est effectué au niveau du bilan de torréfaction : perte et augmentation de volume des grains et au niveau de la couleur mesurée par un Chromamètre MINOLTA 100S et caractérisée par les coordonnées L,a,b des échantillons après torréfaction. Les échantillons ont été torréfiés clone par clone soit 18 échantillons et conservés à - 20°C.

TESTS ORGANOLEPTIQUES : Le café boisson est préparé par décoction à raison de 7g de poudre de café pour 100 ml d'eau de VOLVIC. Le mélange est ensuite filtré et la solution ainsi obtenue peut ensuite être dégustée.

Le jury est composé de 8 dégustateurs expérimentés. Le dispositif expérimental est celui utilisé par MOSCHETTO (14). Les échantillons de cafés correspondant aux 3 modes de traitement VS, VH, VHE, sont dégustés ensemble pour chaque répétition et classés par rapport à un témoin constant et connu. Ce témoin est composé d'un mélange en égale partie de 3 échantillons correspondant aux 3 modes de traitement du même clone à tester. Il est dégusté avant chacun des 3 cafés testés et est utilisé pour les 6 répétitions concernant le même clone. Les caractéristiques notées par les dégustateurs sont les suivantes : intensité aromatique, corps et force, acidité, amertume, astringence, synthèse (échelle de notation de 1 à 5).

RESULTATS ET DISCUSSIONS

ANALYSES CHIMIQUES

Les résultats des analyses chimiques pour chaque clone et chaque mode de préparation (moyennes des 6 répétitions) sont résumés Tableaux 1 et 2.

Les différences observées sont en fonction du mode de préparation ; les principales conclusions sont les suivantes :

- La teneur en caféine diminue de 3% environ avec l'effet du trempage dansle cas de la préparation par voie humide ; dans ce cas il y aurait exosmose de la caféine.
- Les teneurs en composés phénoliques (isomères de l'acide chlorogénique (C5Q)) et en saccharose sont plus importantes lors du traitement par voie humide enzymatique que pour les autres traitements voie sèche (7 à 15 % pour les acides chlorogéniques totaux, 20 à 38 % pour le saccharose)et

Tableau 1 : Analyses chimiques des clones J21, C6 et M5

REF	CAFEINE %	SACCHAROSE %	pH	ACIDITE(ml)	FRUCTOSE %
J21					
VS	2.30	5.38	5.85	16.88	0.19
VH	2.22	5.69	5.80	18.96	0.28
VHE	2.26	6.48	5.74	19.62	0.19
C6					
VS	2.52	3.99	5.87	16.40	0.17
VH	2.46	5.27	5.79	17.37	0.28
VHE	2.46	5.50	5.77	17.50	0.19
M5					
VS	2.94	4.76	5.87	18.60	0.15
VH	2.82	5.42	5.80	19.47	0.26
VHE	2.81	6.23	5.77	19.58	0.19

Tableau 2 : Teneur (%) en composés phénoliques des clones J21, C6 et M5 (Moyenne générale)

REF	C3Q	C5Q	C4Q	F5Q	C34Q	C35Q	TOTAL
J21							
VS	0.41	4.14	0.40	0.48	0.53	0.55	6.50
VH	0.45	4.53	0.31	0.59	0.55	0.63	7.09
VHE	0.47	4.74	0.29	0.68	0.60	0.72	7.48
C6							
VS	0.41	4.84	0.58	0.55	0.51	0.58	7.47
VH	0.40	4.81	0.66	0.54	0.49	0.74	7.67
VHE	0.39	4.91	0.72	0.56	0.54	0.86	7.97
M5							
VS	0.57	5.25	0.42	0.81	0.64	0.77	8.45
VH	0.60	5.70	0.43	0.85	0.72	0.91	9.22
VHE	0.62	6.02	0.36	0.97	0.73	0.98	9.67

Tableau 3 : Résultats des tests organoleptiques des clones J21, C6 et M5 (Moyenne générale)

REF	I.AROMATIQUE	C.FORCE	ACIDITE	AMERTUME	ASTRINGENCE	SYNTHESE
J21						
VS	2.836	3.565	2.815	3.628	3.272	2.628
VH	3.380	2.730	3.712	2.460	2.898	3.690
VHE	3.378	2.658	3.667	2.565	2.938	3.710
C6						
VS	2.857	3.607	2.232	3.878	3.168	2.378
VH	3.502	2.585	3.793	2.460	3.128	3.858
VHE	3.503	2.668	3.753	2.400	3.043	3.732
M5						
VS	2.752	3.607	2.670	3.897	2.459	2.313
VH	3.377	2.658	3.658	2.502	3.045	3.628
VHE	3.377	2.690	3.710	2.545	3.275	3.815

voie humide (4 à 6 % pour les acides chlorogéniques totaux, 4 à 14 % pour le saccharose). Ces différences proviennent de la dégradation de ces composés, oxydation des acides chlorogéniques en chlorogénoquinones d'une part et décomposition du saccharose en glucose et fructose d'autre part, au cours du séchage pour la voie sèche ; la durée du séchage des cerises est plus longue que celle correspondant au café parche (1 semaine environ) à conditions atmosphériques égales.
Le traitement par voie humide enzymatique étant de courte durée (2 heures), il diminue la dégradation de ces composés, ceci étant très important pour le saccharose qui fait partie des précurseurs d'arôme dans les réactions de MAILLARD.
La teneur en fructose est variable suivant le mode de traitement et dépend surtout de la teneur en saccharose et des conditions de dégradation.
- Le traitement par voie humide augmente l'acidité caractérisée par le pH et l'acidité totale (exprimée en ml de NaOH 0,1N pour 10g de café vert). Cette augmentation est variable suivant les clones mais on constate que l'utilisation d'enzyme renforce l'acidité totale.

ANALYSES ORGANOLEPTIQUES

Les résultats des tests organoleptiques (moyenne des 6 répétitions et des 8 dégustateurs pour chaque répétition) sont résumés Tableau 3.
Ces résultats confirment les variations des analyses chimiques observées sur café vert :

- grande différence entre la voie sèche et les deux voies humides, traitement avec ou sans enzyme ; cette observation est valable pour les 3 clônes, cependant une comparaison entre clone n'est pas possible car chaque clône a été dégusté par rapport à son propre témoin. Le traitement par voie humide permet l'augmentation de l'intensité aromatique, de l'acidité, et de la qualité (note de synthèse) et la diminution du corps et de l'amertume.
L'astringence est la seule caractéristique qui varie différemment en fonction des clônes étudiés.
- comportement pratiquement identique entre les deux voies humides, traitement avec ou sans enzyme. Les caractéristiques des cafés boissons sont semblables.

ANALYSES STATISTIQUES DES RESULTATS.

La réalisation des analyses statistiques a été effectuée par le logiciel STATITCF.
Les données concernant les différents échantillons de cafés verts et les tests organoleptiques correspondant aux cafés torréfiés ont d'abord été traitées séparément puis ensembles après fusion des 2 fichiers.
Deux méthodes d'analyses multivariées ont été utilisées :
- analyse en composantes principales (ACP)
- analyse factorielle discriminante (AFD)

Matrice de corrélations

Une matrice de corrélations a été établie entre les variables chimiques caractérisant les cafés verts et les caractéristiques organoleptiques des cafés torréfiés correspondant. (Tableau 4).

Cette matrice met en évidence des corrélations :
- positives entre les caractéristiques organoleptiques suivantes : intensité aromatique, acidité, synthèse et les variables chimiques : saccharose, fructose et acidité totale.
- négatives entre les caractéristiques organoleptiques suivantes : amertume, corps et force et les variables chimiques : saccharose, fructose et acidité totale.

Tableau 4 : Matrice de corrélation entre variables chimiques et caractéristiques organoleptiques.

	I.ARO	C.FOR	AC	AMERT	ASTRI	SYNTH	CAF	SACCH	pH	ACT	FRUCT	C3Q	C5Q	C4Q	F5Q	C34Q	C35Q	TAC
I.ARO	1.000																	
C.FOR	-0.771	1.000																
AC	0.862	-0.836	1.000															
AMERT	-0.845	0.917	-0.906	1.000														
ASTRI	-0.374	0.372	-0.350	0.460	1.000													
SYNTH	0.893	-0.838	0.935	-0.919	-0.465	1.000												
CAF	-0.186	0.148	-0.149	0.207	0.367	-0.199	1.000											
SACCH	0.552	-0.657	0.739	-0.690	-0.257	0.703	-0.239	1.000										
pH	-0.726	0.796	-0.795	0.817	0.303	-0.801	0.285	-0.808	1.000									
ACT	0.363	-0.508	0.544	-0.498	-0.119	0.468	0.277	0.696	-0.541	1.000								
FRUCT	0.560	-0.529	0.566	-0.588	-0.329	0.577	-0.267	0.225	-0.277	0.183	1.000							
C3Q	-0.026	-0.107	0.111	0.055	0.178	0.056	0.767	0.241	-0.043	0.711	-0.110	1.000						
C5Q	0.222	-0.285	0.244	-0.249	0.139	0.244	0.820	0.150	-0.186	0.575	-0.059	0.838	1.000					
C4Q	0.146	-0.024	-0.018	-0.033	-0.009	0.028	0.044	-0.474	0.093	-0.639	0.008	-0.490	-0.081	1.000				
F4Q	0.095	-0.208	0.200	-0.172	0.102	0.180	0.739	0.310	-0.185	0.740	-0.148	0.939	0.890	-0.427	1.000			
C34Q	0.113	-0.216	0.237	-0.190	0.059	0.194	0.656	0.379	-0.196	0.766	-0.065	0.899	0.805	-0.490	0.892	1.000		
C35Q	0.437	-0.529	0.531	-0.486	0.022	0.491	0.633	0.365	-0.435	0.577	0.017	0.619	0.833	0.040	0.733	0.715	1.000	
TAC	0.247	-0.317	0.268	-0.277	0.121	0.274	0.828	0.178	-0.213	0.575	-0.064	0.828	0.990	-0.040	0.892	0.817	0.864	1.000

- faibles entre l'astringence et les acides chlorogéniques totaux (0.12). Cette observation est en contradiction avec les travaux de M.Naish et al.(15). Ce fait peut s'expliquer par la torréfaction qui détruit entre 80 et 90% des acides chlorogéniques.

On note également de fortes corrélations entre certaines caractéristiques organoleptiques : l'intensité aromatique est corrélée avec l'acidité (0.86), la synthèse (0.89), l'amertume (-0.85) et corps et force (-0.77). Dans le cadre des analyses chimiques la caféine est fortement corrélée aux acides chlorogéniques totaux (0.83). Ces résultats ont déjà été observés lors de tests organoleptiques (6).

Ces résultats montrent que la composition chimique d'un café vert est en relation directe avec sa qualité organoleptique. Les critères de qualité sont les suivants:

- forte teneur en sucres et bonne acidité.
- faible teneur en caféine et en acides chlorogéniques.

Les conditions de modes préparation qui favoriseront et developperont ces critères sont donc à utiliser dans le cas de cafés verts ROBUSTA.

Influence du mode de préparation.

L'analyse en composante principale (ACP) a permis, à partir des analyses chimiques effectuées sur cafés verts, de classer les échantillons d'après leur mode de préparation. (Figure 1)
Le tableau 5 résume le descriptif des variables dans l'ACP.

Tableau 5 : Desciptif des variables dans l'ACP

	INERTIE	VARIABLES (corrélation)
AXE 1	55.4 %	CAFEINE 72.9 % ACIDITE 79.9 % TAC 93.1 %
AXE 2	21.2 %	SACCHAROSE 84.8 % pH - 77.2 %

Ces résultats montrent que le classement des échantillons se fait sur l'axe 1 d'après les teneurs en acides chlorogéniques et en acidité totale, sur l'axe 2 d'après la teneur en saccharose et le pH. L'ACP effectuée sur les analyses chimiques met donc en évidence pour chaque clone un effet traitement important.

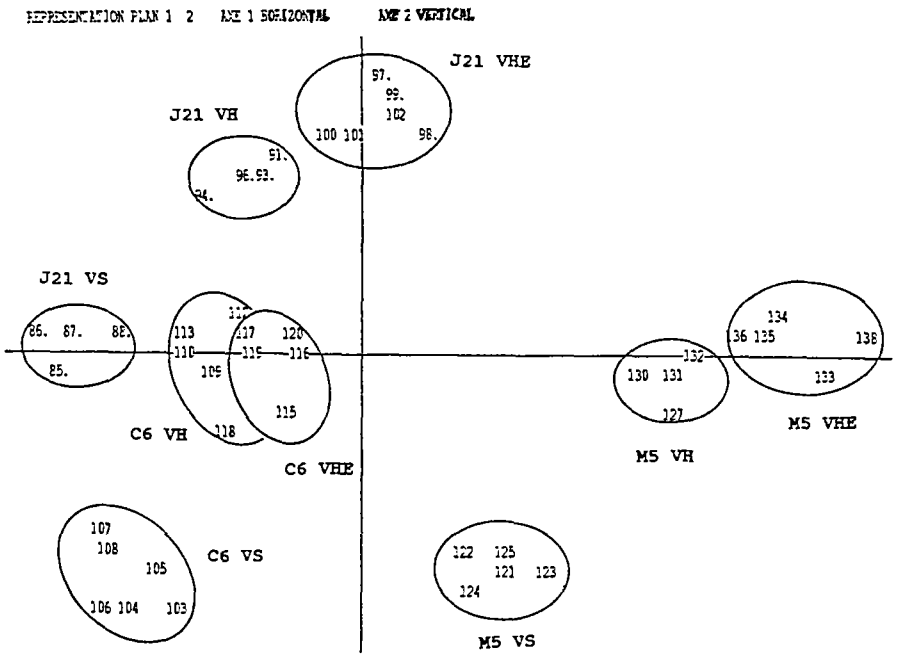

Figure 1 : ACP effectuée à partir des analyses chimiques.

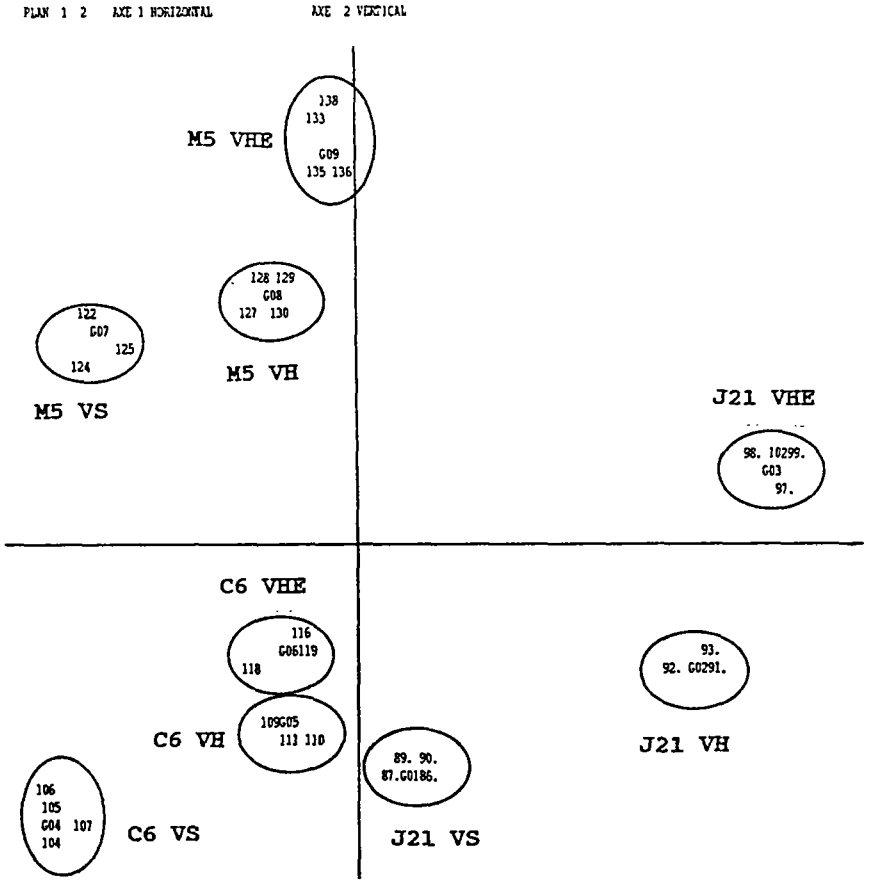

Figure 2 : AFD effectuée sur l'ensemble des données. (Critère de groupe mode de préparation et clone).

Une analyse factorielle discriminante (AFD) effectuée en prenant comme critère de groupe les clones et les modes de préparation (9 groupes) donne 100% d'échantillons bien classés. Les groupes correspondant aux modes de préparation VH et VHE sont très proches en particulier pour les clones J21 et C6.

L'effet traitement peut être évalué à l'effet clone en comparant les distances de MAHALANOBIS de chaque groupe lors d'AFD efféctuées en prenant comme critère de groupe soit les modes de préparation soit les clones. (Tableaux 6 et 7).

Tableau 6 : Distances de MAHALANOBIS entre groupes correspondant aux modes de préparation.

GROUPE N°	1	2	3
1	0.0000		
2	2.3485	0.0000	
3	2.4085	2.3134	0.0000

Groupe 1 : VS Groupe 2 : VH Groupe 3 : VHE

Tableau 7 : Distances de MAHALANOBIS entre groupes correspondant aux clones.

GROUPE N°	1	2	3
1	0.0000		
2	2.3988	0.0000	
3	2.4398	2.4079	0.0000

Groupe 1 : J21 Groupe 2 : C6 Groupe 3 : M5

D' après les résultats de ces deux tableaux les distances de MAHALANOBIS entre groupes correspondant aux modes de préparation et aux clones sont équivalentes ; ceci confirme que l'effet mode de préparation est aussi important que l'effet génétique pour ces trois clones analysés.

Les tests organoleptiques confirment ce comportement identique des 3 clones. Une AFD faite sur les caractéristiques organoleptiques en prenant comme critère de groupe les modes de préparation met en évidence les mêmes propriétés pour chaque clone. (Tableau 8).

Tableau 8 : Caractéristiques fournies par AFD sur les clones à partir des données organoleptiques.

CLONES	% ECHANTILLONS BIEN CLASSES	INERTIE AXE 1	INERTIE AXE 2
J21	67.4	99.5	0.5
C6	72.9	99.5	0.5
M5	66.0	99.1	0.9
GLOBAl	74.1	99.9	0.1

L'axe 1 représente toutes les caractéristiques organoleptiques (acidité et int.aromatique d'un côté, amertume et corps de l'autre) et les échantillons sont séparés uniquement suivant cet axe. Les groupes VH et VHE sont situés de part et d'autre de l'axe 1 (côté acidité) et en opposition du groupe VS situé sur l'axe 1.(côté amertume).

Un traitement global des 3 clones par AFD permet d'obtenir les mêmes résultats. Tous les échantillons VS sont bien classés et seuls les échantillons VH et VHE sont confondus en partie (7 sur 18 pour chaque groupe).

Le tableau 9 représente les distances de MAHALANOBIS entre les différents groupes.

Tableau 9 : Distance de MAHALANOBIS entre groupes correspondant aux modes de préparation.

GROUPE N°	1	2	3
1	0.0000		
2	2.0912	0.0000	
3	2.0711	0.4052	0.0000

Groupe 1 : VS Groupe 2 : VH Groupe 3 : VHE

Les résultats de cette AFD montrent que les analyses organoleptiques permettent de distinguer les échantillons VS d'une part et VH et VHE d'autre part, par contre les échantillons VH et VHE ne peuvent être discriminés.

Une ACP a été effectuée en fusionnant les fichiers de données chimiques et organoleptiques après avoir fait la moyenne par rapport aux dégustateurs pour les données organoleptiques.

L'inertie suivant les axes 1,2 et 3 est respectivement de 44.8, 29.7 et de 10.3 %. L'axe 1 correspond essentiellement à la qualité (acidité, intensité aromatique, synthèse, amertume) et à certaines analyses chimiques (saccharose, acidité totale, acides chlorogéniques), l'axe 2 à l'instringence et aux teneurs en caféine et C3Q, et l'axe 3 aux teneurs en C4Q et en saccharose.

Suivant les axes 1 et2 on observe un regroupement des clones J21 et C6 suivant les modes de préparation. Les échantillons VS sont séparés des échantillons VH et VHE comme décrit précédemment. Cette analyse met en évidence le comportement différent du clone M5 par rapport aux clones J21 et C6. Cependant le comportement au niveau préparation reste identique.

Dans le plan 1-3 la séparation se fait au niveau de l'axe 3 pour les clones et au niveau de l'axe 1 pour les modes de préparation.

L'AFD effectuée sur l'ensemble des données et en prenant comme critère de groupe les clones et les modes de préparation montre que 100 % des échantillons sont bien classés dans chaque groupe et que la discrimination est possible même au niveau des modes de préparation VH et VHE. (Figure 2).

CONCLUSIONS

Les résultats de cette étude concernant le café ROBUSTA ont mis en évidence deux caractéristiques principales obtenues par les modes de préparation VH et VHE par rapport au mode de préparation VS lors des tests organoleptiques :

- augmentation de l'acidité et de l'intensité aromatique.
- diminution de l'amertume et du corps.

Les traitements VH et VHE améliorent donc la qualité d'un café ROBUSTA et ce quel que soit le clone. Ces résultats avaient déjà été observés par VINCENT (7) sur des cafés ROBUSTA et ARABUSTA. Les différences de qualité mises en évidence lors des tests organoleptiques et correspondant à chaque traitement sont comparables pour chaque clone et l'effet mode de préparation est aussi important que l'effet génétique.

Au niveau de la composition chimique du café vert les variations que l'on peut observer sont dues principalement aux effets de deux opérations technologiques :

- le trempage qui provoque une légère diminution de la teneur en caféine et une augmentation de l'acidité. Cette acidité peut être due à une flore bactérienne favorisant la formation d'acides organiques. On observe également une diminution de la teneur en saccharose en fonction de la durée de fermentation (comparaison VH et VHE).
- le séchage avec dégradation de certains composés comme les acides chlorogéniques et le saccharose. Cette dégradation est fonction des conditions de séchage en particulier de la durée du séchage (comparaison VS et VH). En effet lors du traitement VS la durée de séchage est plus

longue que pour un traitement voie humide. (environ une semaine à conditions climatiques égales). Pendant cette étape, la flore microbienne peut dégrader les constituants chimiques et cette action sera proportionnelle à la durée du séchage.

D'après l'ensemble de ces résultats, le traitement par voie humide (VH) tel qu'il a été réalisé lors de ces essais présente le meilleur compromis sur les possibilités de caractérisation et d'évaluation des propriétés spécifiques, en particulier le potentiel aromatique, d'un clone de café ROBUSTA.

RESUME : La qualité d'un café dépend, outre de son patrimoine génétique, de son mode de préparation. Une expérimentation, testant 3 modes de préparation : voie sèche, voie humide et voie humide avec enzyme, effectuée au Cameroun sur 3 clones de café ROBUSTA J21, C6, M5 présentant des propriétés technologiques, chimiques, et organoleptiques différentes a permis de mettre en évidence les modifications de qualité dûes aux conditions de préparation.

L'amélioration de la qualité par voie humide (avec ou sans enzyme) correspond d'une part à une augmentation de l'acidité et de l'arôme et d'autre part à une diminution du corps et de l'amertume ; les transformations chimiques pendant les opérations de fermentation et de séchage étant responsables des modifications des caractéristiques organoleptiques.

Les analyses statistiques multivariées montrent que l'effet mode de préparation est aussi important que l'effet génétique et que l'utilisation d'enzyme ne diminue pas la qualité d'un café lors de sa préparation par voie humide.

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AMINO ACID PATTERN OF STEAM TREATED COFFEE

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INTRODUCTION

Since the thirties, when Lendrich developed the steam-treatment of raw coffee beans prior to the roasting process, industry has been producing so-called „health coffees" for people with a sensitive stomach. Several studies have proven the better acceptability of those pretreated coffees, but nobody has found a chemical reason for this. Initially, Lendrich maintained that these coffees contain less chlorogenic acids, but this has been proven wrong. There is therefore no suitable analytical process to distinguish between untreated and treated coffees. Up to now, this has only been possible when the raw coffee can also be analysed.

Because of this, STEINHART et al. (1990) began investigation of the melanoidins, a group of brown pigments of high molecular mass, that is formed out of amino acids, carbohydrates and chlorogenic acids during the roasting process by means of a Maillard reaction. The melanoidins were separated on a gel column into five fractions, the first one with a molecular mass over 100.000 Dalton being significantly reduced in pretreated coffees. STEINHART and PACKERT (1993) confirmed this and reported that this fraction had 50% less mannose in its molecular structure. Therefore, a significant difference in the quantity as well as in the structure of those high molecular melanoidins seems likely. This observation might lead to a successful analytical procedure.

To understand more about the reasons for this difference between treated and untreated coffee, we analysed the amino acid, carbohydrate and chlorogenic acid contents of treated and untreated, raw and roasted coffees. Subject of this paper are our results on the changes in the amino acid pattern during the steam treatment of coffee in relation to the duration and the pressure of the treatment.

EXPERIMENTAL

Coffee Samples

Table 1 shows the analysed coffee samples; both the industrially treated coffees and their raw untreated counterparts were a gift from CR3-Kaffeeveredelung, Bremen.

Table 1: Coffee samples studied

Origin	Treatment	Organic Roasting Losses
Arabica Mixture	untreated	raw, 6.1%, 10.4%, 13.5%
	industrially treated 1bar 1h	raw, 6.1%, 10.3%, 13.4%
	laboratory treated 0.8bar 1h	raw, 6.5%
	laboratory treated 0.8bar 2h	raw, 8.7%
	laboratory treated 0.8bar 4h	raw, 6.2%
Arabica Columbia	untreated	raw, 9.0%
	industrially treated 1bar 1h	raw, 8.6%
Arabica Tanzania	untreated	raw, 8.5%
	laboratory treated 0.8bar 1.5h	raw, 8.4%
Robusta Indonesia	untreated	raw, 5.6%, 10.6%, 12.9%
	industrially treated 1.5bar 40min	raw, 6.6%, 10.3%, 14.0%
	industrially treated 3bar 40min	raw, 6.2%, 10.9%, 14.2%
	laboratory treated 0.8bar 1h	raw, 6.9%
	laboratory treated 0.8bar 2h	raw, 7.6%
	laboratory treated 0.8bar 4h	raw, 5.9%
	laboratory treated 0.4bar 4h	raw, 6.3%
Robusta Cameroun	untreated	raw, 8.5%
	laboratory treated 0.8bar 1.5h	raw, 9.5%
Robusta Thailand	untreated	raw, 8.4%
	laboratory treated 0.8bar 1.5h	raw, 7.9%
Robusta Sri Lanka	untreated	raw, 5.7%
	laboratory treated 0.8bar 1.5h	raw, 7.7%

Laboratory treatment was performed in a Fissler pressure cooker; the roasting process took place in a Probst laboratory roaster.

Analytical Procedure

Analysis of Free Amino Acids

5 g of finely ground coffee powder were extracted four times with 3% sulphosalicylic acid in 0.01 N hydrochloric acid. The filtered, combined extracts were brought to a defined volume. An aliquot was adjusted to pH

2.2 with a sodiumcitrate buffer and applied to a cation exchange resin. Elution of the amino acids was carried out with 4 N ammonia solution; the elute was desiccated in a vacuum centrifuge. To derivatise the amino acids, a redrying solution (dist.water:ethanol:triethylamine, 2:2:1, v:v:v) was added. After desiccation, the coupling solution (dist.water:ethanol:triethylamine:phenylisothiocyanate, 1:7:1:1, v:v:v:v) was added and the mixture was allowed to stand for 20 min at room temperature. After desiccation, the residue was dissolved in 1 ml of the starting eluent.

Analysis of Protein Bound Amino Acids

40 mg of the coffee powder were weighed into a 20 ml Pyrex vial and placed in a beaker. The bottom of the beaker was covered with 6 N hydrochloric acid and tryptamine. The beaker was put into a flat flange reaction beaker and evaporated. Hydrolysis took place in a drying cabinet for 20 hrs at 110°C. The dry residue was dissolved in 0.1 N hydrochloric acid and brought to a defined volume. An aliquot was then adjusted to pH 2.2 with a sodiumcitrate buffer. Clean-up by cation exchange and derivatisation were carried out as described above.

High Performance Liquid Chromatography

Chromatography was performed on a Superspher 100 RP18 endcapped (4 µm, 250 x 4 mm, Merck, Germany) using a gradient program. Flow rate was 1.0 ml/min. Detection took place at 245 nm. Identity of the peaks was confirmed with a diode array detector (Waters).

RESULTS AND DISCUSSION

Amino Acids in Raw Coffee

Free amino acids in raw coffees have been analysed extensively by TRAUTWEIN and ERBERSDOBLER (1989). They observed significant differences between Arabicas and Robustas, but they did not analyse any steam treated coffees. In general, our data are in accordance with theirs. Figure 1 shows as an example the decrease of every single free amino acid in the Arabica Mixture during a steam treatment at 0.8 bar, 100% being the data from the untreated coffee.

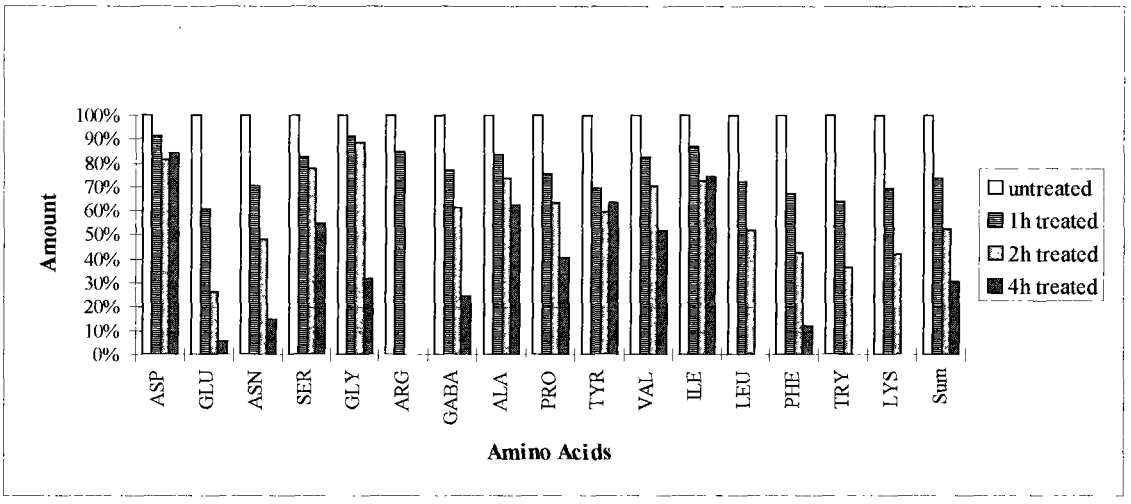

Figure 1: Decrease of the free amino acids during the steam treatment in the Arabica Mixture

The other coffees were to show nearly the same behaviour of every amino acid, but there were major differences in the extent of the decreases. This is displayed in figure 2 for the sum of the free amino acids.

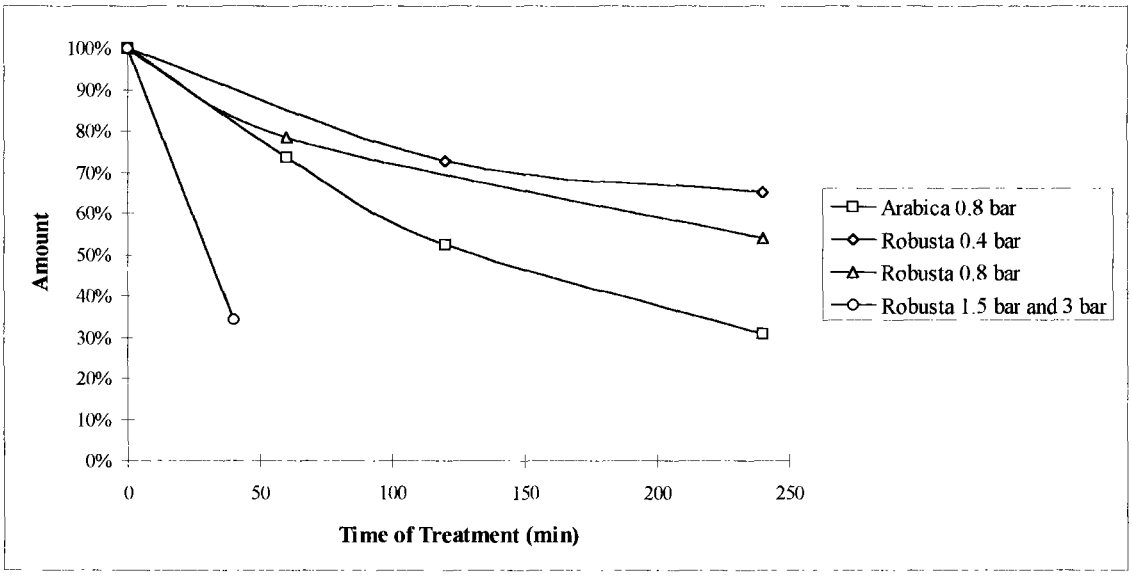

Figure 2: Decrease of the sum of the free amino acids in relation to duration and pressure of treatment

This holds good for every Arabica or Robusta studied. As can be seen, the free amino acids decrease significantly during the treatment. This happens in the form of a curve. This curve becomes steeper when a higher pressure is used, but there is a limit to this: the Robusta treated at 1.5 bar loses the same amount of amino acids as the Robusta treated with 3 bar. The curve is steeper for Arabicas than for Robustas if they are treated at the same pressure. Therefore, it can be concluded that Arabicas react more sensitively to the treatment than do Robustas. Generally, industrially pretreated coffees enter the roasting process with only half of their original amount of free amino acids.

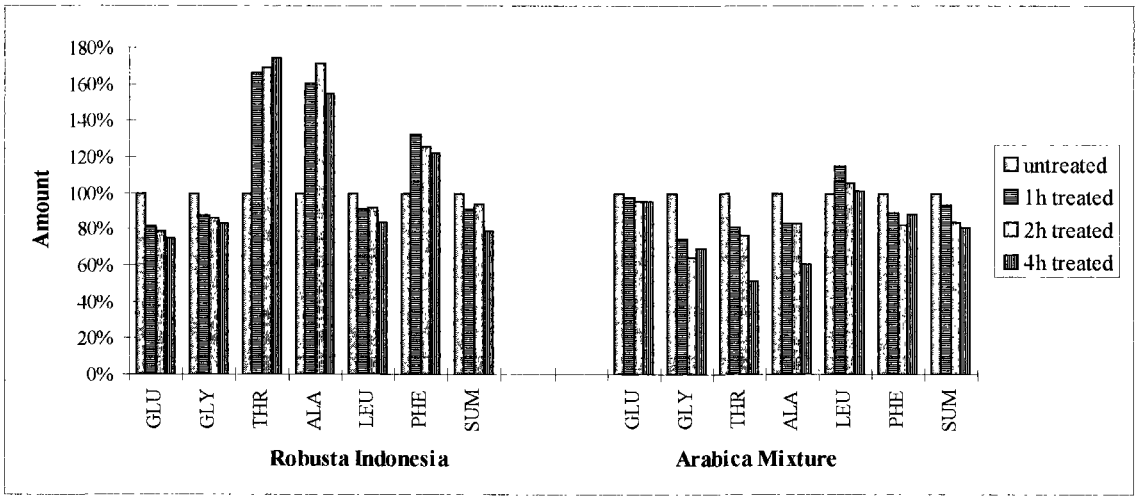

Figure 3: Changes in the amount of selected protein bound amino acids related to the duration of treatment in the raw Arabica Mixture and the raw Robusta Indonesia

Figure 3 displays the changes in a few selected protein bound amino acids during the steam treatment. Contrary to the results of NEHRING (1991), the protein bound amino acids of the coffees studied decreased during the treatment. This decrease was observable in all Arabicas and Robustas studied, except for the Robusta Indonesia treated with 3 bar. This is shown in figure 4.

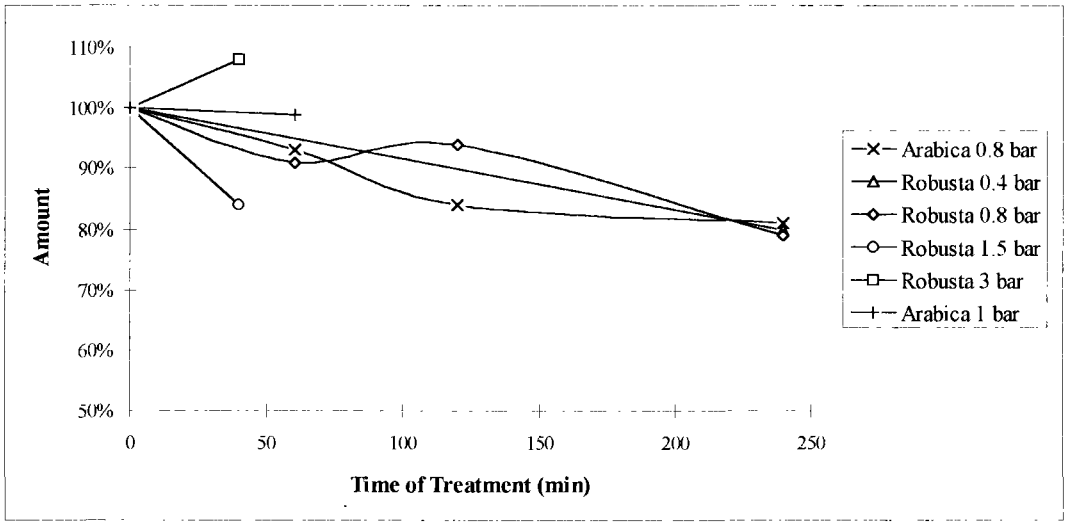

Figure 4: Changes in the sum of the bound amino acids during the steam treatment

There was a general decrease in the sum of the protein bound amino acids during the treatment. It seemed to be independent of the pressure applied, but to be related to the duration of the treatment. After 60 minutes of steam treatment, the sum amounted to 95% of the original value; after 4 hrs only 80-85% were left. An exception to this rule was the Robusta treated at 1.5 bar. Here the decrease was more rapid. The Robusta treated at 3 bar even showed an increase in the amount of the bound amino acids. This might result from a fragmentation of structural protein, making it accessible for the acidic hydrolysis.

Therefore, an industrially treated coffee enters the roasting process with about 90% of the protein bound amino acids and only 50% of the free amino acids of the untreated coffee.

Amino Acids in Roasted Coffee

Free amino acids were not detectable in the roasted coffee samples.

Figure 5 shows the behaviour of the protein bound amino acids during the roasting process in the untreated and treated Arabica Mixture and Robusta Indonesia. The tendency is the same in every coffee sample studied: first a remarkable loss of bound amino acids to 35-60% of the original amount at an organic roasting loss of 6%, then a slight increase to 45-60% at an organic roasting loss of 13.5%.

This could be caused by the fragmentation of structural protein, as described before for the bound amino acids in raw coffee samples. The untreated Arabica, its structural protein untouched by any pretreatment, at first shows a significant loss, then, when its protein breaks up, a remarkable increase in the hydrolysable bound amino acids. The pretreated Arabica shows only the decrease, with hardly any increase during the roasting process.

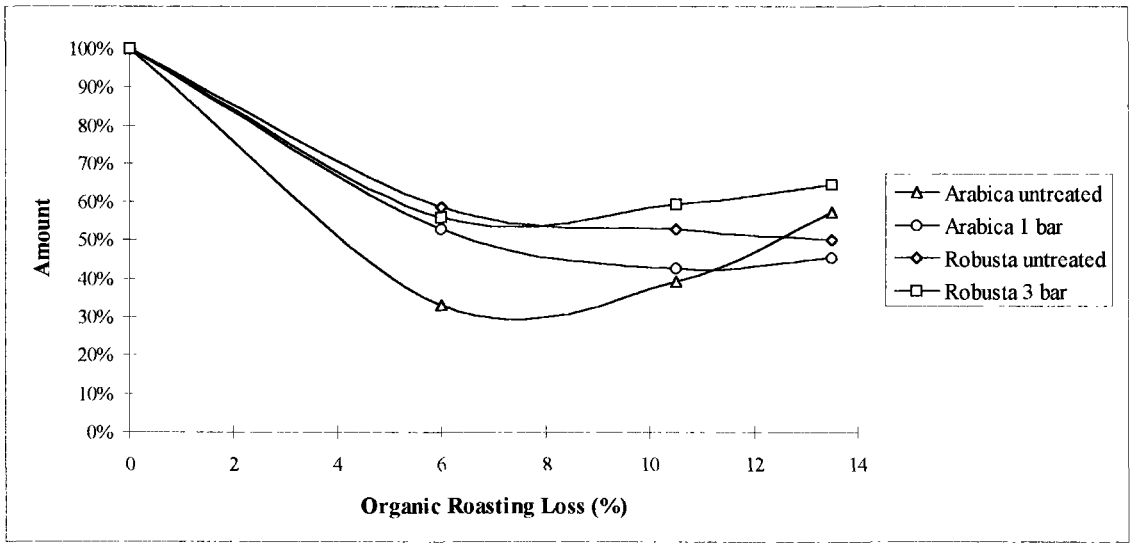

Figure 5: Changes in the amount of bound amino acids during the roasting process

The Robusta that had been pretreated at 3 bar, but only for 40 min, displayed an increase in the bound amino acids in the raw coffee, as shown in figure 4. These broken structural proteins seem to be destroyed in the first part of the roasting process, resulting in the large decrease visible in figure 6. Later on in the roasting process, more structural protein is set free, so that the amount of the hydrolysable bound amino acids again increases, but only slightly.

To compare treated and untreated coffees, figure 6 displays the sum of the protein bound amino acids in the Arabica Mixture and the Robusta Indonesia, roasted to an organic roasting loss between 6 % and 7 %, in relation to the time and pressure of the treatment.

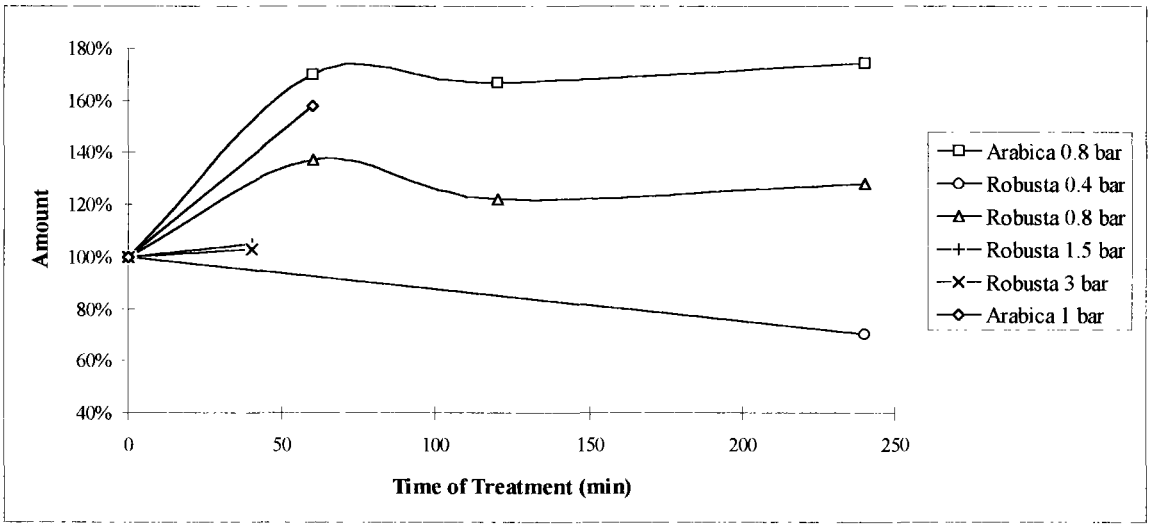

Figure 6: Changes in the amount of bound amino acids related to duration and pressure of treatment

As can be seen, the Arabica displays a higher amount (about 165%) of amino acids in the treated coffee than in the untreated. Time of treatment and pressure seem to be of less importance, since the amount does not increase more with a longer duration or a higher pressure of the treatment. The other Arabicas studied show about the same behaviour, to a greater or lesser extent. The amount of protein bound amino acids after the roasting process in pretreated Arabicas can therefore be stated to be about 150% of that of the untreated counterpart.

In Robustas, the difference between treated and untreated coffees is less obvious. As indicated in figure 6, the Robusta treated at 0.8 bar contained about 125% of the bound amino acids of the untreated coffee, whereas those Robustas treated at 1.5 bar and those treated at 3 bar remain at the same level as the untreated coffee. Of the other Robustas studied here, some show a slight increase, some a slight decrease in the amino acids compared to their untreated counterparts. Thus there is no significant difference remaining between steam treated and untreated Robusta coffees after the roasting process.

CONCLUSIONS

From our data, the following conclusions can be drawn:

- Free amino acids in raw coffees degrade significantly during the steam treatment, more so in Arabicas than in Robustas; the higher the applied pressure, the larger the decrease.
- Protein bound amino acids in raw coffees degrade independently of specie as well as of the pressure applied during the treatment.
- During the roasting process, the amount of bound amino acids decreases to about 50% of the amount in the raw coffee, then increases again to 60%.
- The pretreated roasted Arabicas comprise about 150% of the protein bound amino acids of the untreated coffee. In Robustas, no significant difference between treated and untreated coffee after the roasting process is observable.

SUMMARY

3 Arabica and 4 Robusta coffees of different origin, steam treated under various conditions and untreated, raw and roasted to different degrees, were analysed for their free and protein bound amino acid content. It can be stated that the steam pretreatment of coffee has a considerable effect upon its amino acid content. Free amino acids degrade significantly, more in Arabicas and less in Robustas, during the treatment, this being related to duration and pressure of the treatment. The same applies to the protein bound amino acids, but this decrease is independent of the pressure of the treatment. After the roasting process, Arabicas show significantly more protein bound amino acids in the pretreated than in the untreated coffee. In Robustas, there is no such difference. Free amino acids were not detectable in the roasted coffees.

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CARBOHYDRATE PROFILES IN BEVERAGES LIKE COFFEE : METHODS AND OBJECTS OF INVESTIGATIONS

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Introduction

Some beverages of plant origin, like manna or coconut milk, can be used straight from the plant, others require preparatory action. This may entail expelling, like squeezing an orange for juice, or pressing, like for a piece of sugar cane, or infusion of the dried or roasted parts of the plant to yield a pot of herbal tea or coffee. Preferences are location specific and change with time.

Beverages for infusion are the subject of investigation to be presented here. Amongst these, in Germany, there are roasted cereals, marketed as „Kornkaffee mit Zichorie", grain coffee with chicory, which in the days before the European coffee directive came into effect was sold as „Ersatzkaffee", coffee substitute. It has no caffeine, but a taste of roast, and was esteemed because of its price, salutarity, and, for a number of years, to support national autarchy.

Travelling eastward, to the Arab origin of coffee, Yemen, on the other hand, dried coffee husks are sold in the markets to make the common brew „Qishr" which contains caffeine but tastes like tea, if I remember correctly, unless it is too highly sweetened... and it usually is.

Further on in Asia, ginseng dried roots are consumed as brewed beverages, and, nowadays commercialised as Instant Powder. In Germany it is not drunk as beverage, but used as a medicine.

Going further east, to ASIC'95, here in Kyoto, for coffee people such as we, the better known instant products are Instant Coffee or Tea, or even instant coffee substitutes, the preparative action for the final consumer reduced to: mix with cold or hot water.

In vegetable matter, major constituents are the carbohydrates produced by the plant through photosynthesis, to form the mono, di- and so on, up to poly-saccharides, and other glycoside containing macromolecules. Composition depends on the plant itself, the botanical and local origin, and the state of maturity, and is influenced by the following steps to lead to product consumption.

For industrial instant manufacturing, there are requirements on the reproducibility of the conditions in which raw materials and processes are to be used. Consequently, a certain degree of standardisation is introduced into the subject, and the peculiarities of home brewing are counterbalanced.

Considering this, carbohydrate analysis is widely used for characterisation of natural variability and processing of vegetable instant products like soluble coffee.

The common analytical scheme entails two procedures: direct identification of the individual free mono-, di- or trisaccharides of the given or prepared solution, and, - starting prior to completion of the identification process, - analytical acid hydrolysis of the sample in order to determine the constitutive monosaccharides as the total content of individual sugars. Inpassing, I might say that in this scheme, the disaccharides are analysed twice, first in their initial state and then after hydrolysis, as contributors to the total quantity of glucose and fructose respectively.

Objects

As objects of our investigation, with a special interest in instant coffee, we had looked on the related beverages as well, namely,

- extracts of roasted seeds like coffee or cereals,
- roasted roots: chicory,
- dried leaves: tea,
- other dried & roasted plant material: qishr, and
- dried roots: ginseng.

In the following diagram carbohydrate profiles for the different infusion solubles are given, expressed as dry matter content of mono- and disaccharide sugars in free and total carbohydrate analytical preparations.

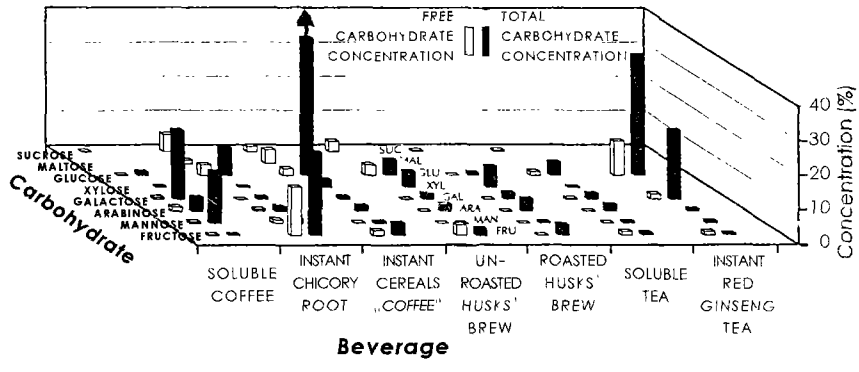

Fig.1 Carbohydrate patterns of soluble coffee and some coffee like beverages (dry matter basis, average values)

In soluble coffee, the most prominent carbohydrate is the galactose in the totals, followed by mannose and arabinose, in a concentration range of roughly between 15 : 15 : 5 and 20 : 10 : 5 percent, with a marked variation depending on origin. These sugars originate from the polymeric structures of parent galactomannans and arabinogalactans, which have been discussed in various ASIC presentations during the last decades[1], focused on the polysaccharides in the differently soluble fractions of coffee. Other sugars are only to be found in very limited amounts.

As locally competing beverages from the coffee plant there are infusions of „qishr", hulls, skins, husks and parchment surrounding the coffee seed. The Figure gives the profiles of lab brews of Yemenite qishr, both unroasted and roasted. Its cellular structure is clearly different from the bean, since the pattern is contrary to soluble coffee characteristics: it shows only small quantities of

arabinose and galactose, and is marked by sucrose, glucose and fructose in the brew of the unroasted, by total fructose after roasting, and, as a non bean characteristic, by a dominant content of total xylose, the wood sugar of xylans.

Like instant coffee, chicory extracts are prepared through roasting and extraction of the root. As this mainly consists of the polysaccharide inuline, the solubles' carbohydrate profiles, in consequence, are dominated by fructose and glucose moieties, both in free and total profiles.

Roasted cereal seeds, with starch as the principal component, processed into an instant product in a way similar to coffee, show, in the case of malt-coffee, maltose, and in any case, glucose as the main characterising sugar after hydrolysis; this is not surprising. The profiles demonstrate as well the presence of some xylose, from the xylans of grain hulls, which evidently cannot be totally removed in the preparatory steps.

Tea as an infusion of green or fermented leaves - in this Figure we have a black tea extract - shows a balanced content of free glucose and fructose, and some total galactose and arabinose in the carbohydrates' profile. If the tea is of a quality that includes parts of twigs, xylose can also be found, not given in this figure.

The ginseng extract we look at last, is the soluble powder of dried roots. According to our analysis, they seem to consist of galactoglucan polysaccarides, with a marked content of free glucose, maybe originally from the root or added during production. This lies outside our scope of study.

The data demonstrate that carbohydrate analysis may well serve as a tool for distinguishing the nature and even the origin of instant products, to reflect processing and mixing they underwent.

Methods

Since the development of chromatographic separation techniques, the analysis of carbohydrates may be efficiently performed through gas chromatography with pre-column esterification for the volatilisation of the polyhydroxy compounds, or via high pressure liquid chromatography with post column derivatisation for sensitivity enhancement to detect such low concentrations as found here for most of the sugars. In 1988, for free and total carbohydrate determination in instant coffee, a combination of GC and a special HPLC procedure had been proposed in two ISO draft standards[2]. In ASIC 1991, this was the method of the presentation by Noyes[3]. An overall method for both free and total carbohydrates was given by Blanc in 1989[4]. It entails HPLC on amino modified silica phases and spectrometric detection after tetrazolium blue reaction, restrained on reducing sugars.

Since then, ISO has approved an overall HPLC anion exchange method (HPAE), using special HPLC equipment with amperometric detection[5], presented to ASIC in 1991 by Prodolliet[6]. This is also useful for the sugar alcohol mannitol, another characteristic component of coffee hulls, which is not destroyed by roasting.

As we wanted to use the HPLC standard equipment available in our laboratory for both free and total carbohydrates, including the non-reducing ones, we chose the 1988 ISO draft HPLC method for total carbohydrates mentioned above, which is based on the work of Femia and Weinberger of 1987[7], and modified it to serve both purposes. The principle of the analytics unchanged, - aqueous isocratic separation on the lead-loaded cation exchange column, post-column reaction with p-aminobenzoylhydrazide after acid catalysed conversion of the non reducing sugars, and spectrophotometric detection at 410 nm -, we perform a direct HPLC of the purified aqueous sample solution for free carbohydrates on the same column as for the totals of the hydrolysed sample. The signal-to-noise ratio is to be kept under control by low pulsation solvent delivery. As in a wood

pulp study[8], we could enhance the chromatographic resolution between coeluting mannose and fructose and of xylose/glucose by use of two analytical columns in series. The temperature was adjusted to 45°C, all other conditions as described. The long-term precision data conform to those reported by Noyes.

A comparison with the new ISO method was performed through out-of-competition participation in two collaborative studies for HPAE[9], and gave results of sufficient consistency.

To give a visual idea, the chromatogram of pure instant malt-coffee is presented in Fig. 2, together with chromatograms of model carbohydrate solutions of soluble coffee concentration widths. The superposition underlines once again the message of Fig. 1: Signals characteristic for malt-coffee carbohydrates, namely maltose, glucose and xylose, are easily to recognise in the coffee substitute, whilst the triple of sugars characterising soluble coffee -total galactose, mannose, and arabinose- is nearly absent; the former ones would serve, if detected in a soluble coffee sample amongst the latters, to indicate an admixture or adulteration.

To show the difference, an HPAE chromatogram of malt-coffee is given in Fig. 3. It was run on the same substance in a commercial laboratory under „ISO 1994" conditions.

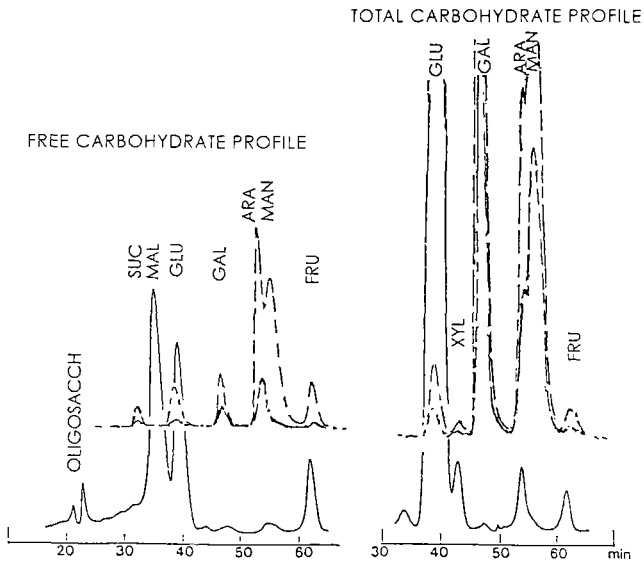

Fig. 2 Instant malt coffee carbohydrate profiles, run with cation exchange HPLC
dotted: range of soluble coffees' carbohydrates

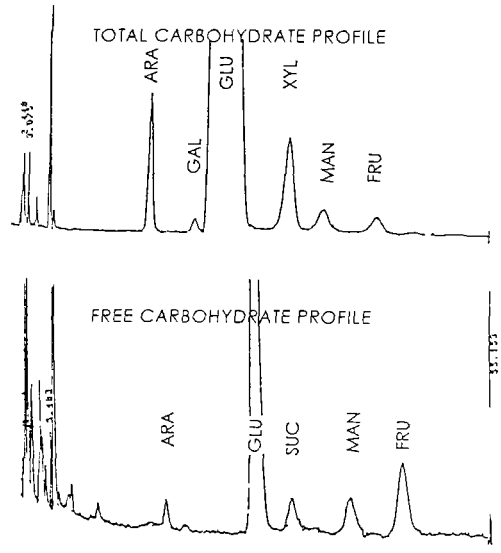

Fig. 3 Instant malt coffee of Fig. 2, HPAE carbohydrate profiles
courtesy of Lab.Dr.Scheutwinkel, Berlin

HPAE gives a dramatically better base line resolution which is easier to integrate; mannitol, if present, might have been determined. The data for higher concentrations, like in this special case for total glucose, can be achieved within the linearity range of the same run. The cation exchange method on the other hand gives additional information on the oligosaccharides, even not resolved, and further maltose can be determined (which in HPAE is not looked at due to very long retention time).

Even though there are differences in appearance, the results in the main values conform, and the analytical conclusions on the products can be achieved by both methods.

Analytical Results

Currently, we use the method described here in quality control against accidental and fraudulent addition of non bean material to soluble coffee, analysing soluble coffees and coffee substitutes,

the data of Fig. 1 being averages of the analysis. With the pool of data for soluble coffee obtained from samples from known production sites, and presumed to be pure instant coffee, we focused on the limited amount of certain sugars which can serve to distinguish coffee, coffee substitutes, and coffee husks, that means on free and total fructose and glucose and on total xylose.

The variety of natural contents, blends and processing yields a distribution presented in the histograms of Fig. 4, where agglomerations of preferred ranges and maximum values can be deduced from the sets of concentration values. A statistical treatment of such data is demonstrated by White in this colloque[10].

In a detailed look at xylose, Fig. 5, most of the soluble coffee analysed in our runs show concentrations of around 0.25%, with 90% of the results below 0.4%. Values of samples exceeding this amount of xylose may reflect a content of husks and branches, as we analysed in qishr, in tea containing twigs, and in instant cereals coffee.

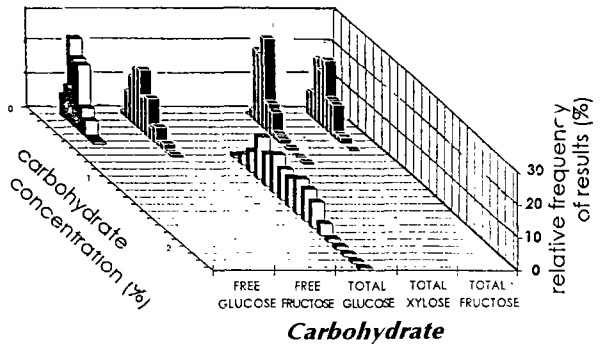

Fig. 4 Histograms of free and total glucose, fructose and of total xylose concentrations in some 700 soluble coffees of various production sites

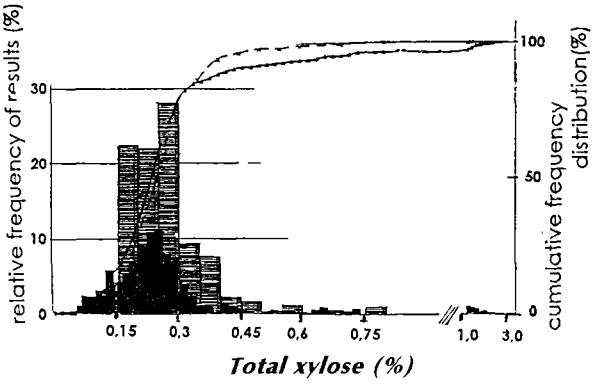

Fig. 5 Total xylose distribution in soluble coffees: cation exchange HPLC data of Fig. 4, compared with HPAE data* *Ref. 11

Within the same figure, the data are compared to those found by Prodolliet[11] in 700 soluble coffees samples from the international market, orders of magnitude equal to those we analysed. The distributions of both investigations are corresponding, with a smoother envelope in HPAE because of its somewhat higher resolution, results outside the preferred range differring due to different origins of the two sets of samples.

Conclusion

The method of sugar analysis reported here, run on standard HPLC equipment, is suitable to measure the carbohydrate pattern of instant coffee and instant coffee substitutes for daily quality assurance of product authenticity. The results are compatible to those determined according to the HPAE method just approved by ISO standard 11292. They show an equivalent distribution for the minor components to serve as distinctive marks in carbohydrate profiles.

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Summary

Carbohydrate profiles of vegetable matter infusions show characteristic patterns, to be recognised by HPLC analytics. Chromatograms of beverages of different origin, similar to soluble coffee, indicate some minor constituent sugars to serve as distinctive marks within this group. A suitable routine analytic by cation exchange HPLC, to be run with standard equipment, is presented and compared to the HPAE-method just introduced as ISO standard for soluble coffee.

Résumée

Les profiles des carbohydrates dans des breuvages d'origine des plantes montrent des distributions typiques, lesquelles sont à analyser avec des différentes techniques HPLC. Études des produits similaires au café soluble, soit instantanés soit infusés marquent quelques glucides simples comme indicatifs pour un mélange. L'analyse des carbohydrates est effectuée par séparation sur une résine échangeuse des cations et détection spectrophotométrique après derivatisation avec un appareillage standard HPLC. La méthode est trouvé compatible avec une nouvelle norme internationale ISO pour le café soluble.

THE TASTE SENSOR

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1. Introduction

Taste is constructed from five basic taste qualities. The first is sourness produced by hydrogen ions. The second is saltiness of NaCl and KCl. The third is bitterness. Quinine and caffeine produce bitterness. The fourth is sweetness, produced by sucrose, glucose, and so on. The last is umami, which implies "deliciousness" in Japanese term. Monosodium glutamate (MSG), disodium inosinate (IMP) and disodium guanylate (GMP) show umami [1-4].

Taste substances are received by the biological membrane of gustatory cells in taste buds on tongue (Figure 1). Recently, we have developed a multichannel taste sensor whose transducer is composed of lipid membranes [5-13]. This sensor can detect tastes in a manner similar to human gustatory sensation. The output of the sensor is not the amount of taste substances but the taste quality (and also magnitude); different output patterns were obtained for different taste groups such as sourness and saltiness, whereas similar patterns were obtained for taste substances in the same group such as MSG, IMP and GMP, which have an umami taste, and NaCl, KCl and KBr for saltiness.

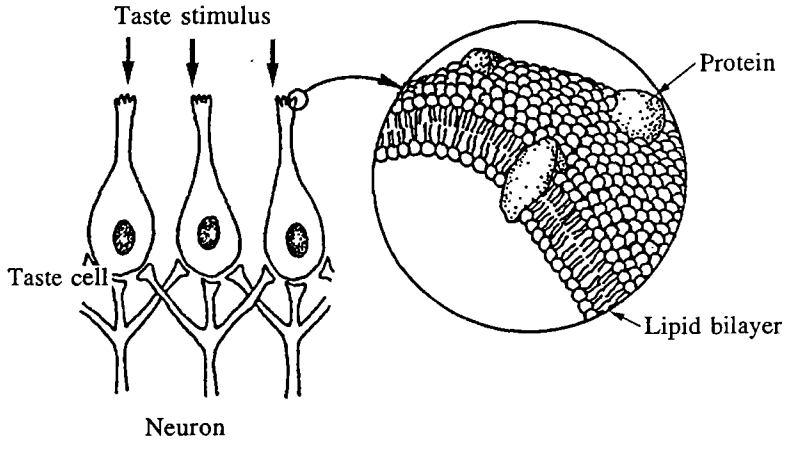

Figure 1. Taste reception in biological systems

This sensor was developed on a concept very different from that of conventional chemical sensors, which selectively detect specific chemical substances. However, taste cannot be measured in those terms even if all the chemical substances contained in foodstuffs are measured. Humans do not distinguish each chemical substance, but express the taste in itself. Nevertheless, the relationship between chemical substances and taste is not clear. It is also not practical to arrange so many chemical sensors with respect to the number of chemical substances, because one kind of foodstuff is composed of over 1000 chemical substances. Moreover, humans experience interactions between taste substances, such as the suppression effect or the synergistic effect. A taste sensor should measure these effects; the aim is not to measure the amount of each chemical substance but to measure the taste itself, and to express it quantitatively. The recently developed sensor satisfies this request.

The principle of the taste sensor and the application to coffee are mentioned here. Quantification of the taste of several origins of coffee became possible using the taste sensor.

2. Multichannel taste sensor

Previous studies [14-18] showed that lipid membranes had the ability to sense the taste by responding to taste substances; i.e., lipid membranes are useful materials to transform the taste to electric signals. A recently developed multichannel taste sensor, where transducers were composed of lipid membranes immobilized with a polymer, has higher sensitivity and better reproducibility than those of humans [5-13].

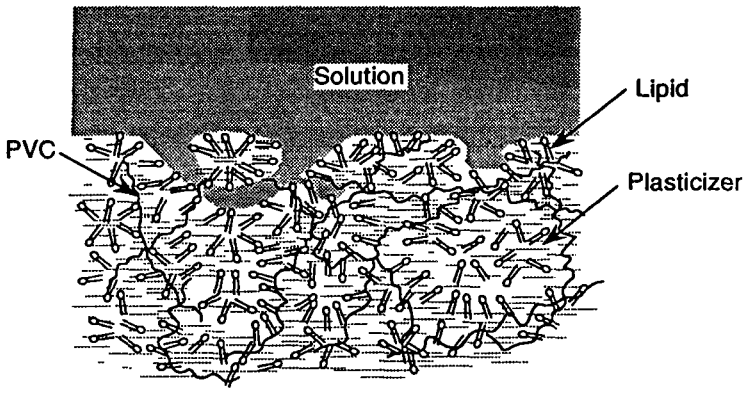

Figure 2. Surface structure of a lipid-PVC membrane

Eight kinds of lipid analogues were used for preparing the membranes [5-7]. Depending upon the object to be measured, we prepared different lipid materials [8-13]. For example, the mixed membranes composed of dioctyl phosphate and trioctyl methyl ammonium chloride were used for measurements of amino acids [12]. The lipid membrane was a transparent, colorless and soft film with ca. 200 μm thickness. Figure 2 shows a schematic illustration of the surface structure of a lipid-PVC membrane.

Lipid membranes were fitted on a multichannel electrode. Figure 3 shows a front view and a cross section of the electrode. The electrode was made from Ag/AgCl wires, which were 1.5 mm in diameter, in 100mM KCl solution in the cone-shaped hole of a basal acrylic board.

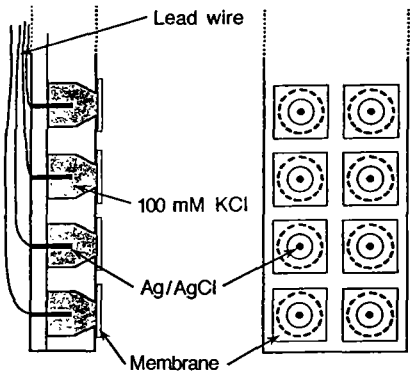

Figure 3. Multichannel electrode

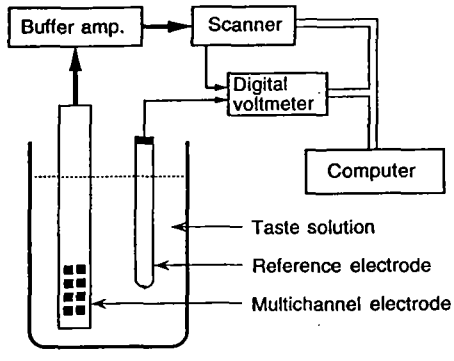

Figure 4. Measurement system

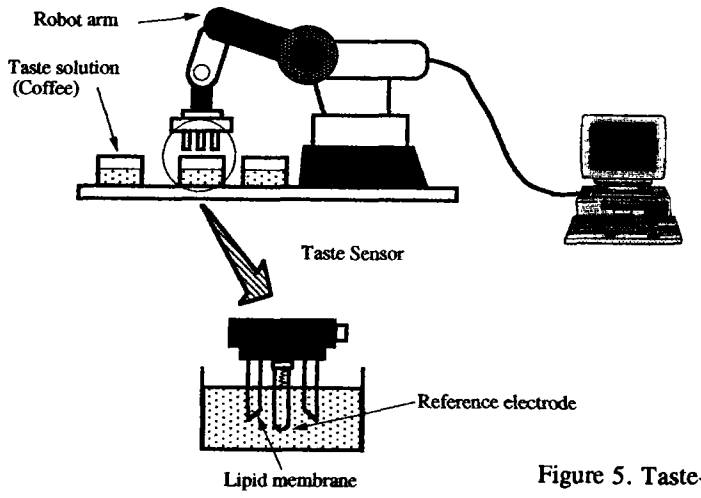

Figure 5. Taste-sensing system (SA401)

The multichannel electrode was connected to an 8-channel scanner through high-input impedance amplifiers (Fig. 4). The selected electric signal from the sensor was converted to a digital code by a digital voltmeter and was fed to a computer. Then, a voltage difference between the lipid membrane electrode and a reference electrode was measured.

The commercial taste-sensing system (SA401) of Anritsu Corp. has the mechanism essentially similar to the above. The detecting sensor part is made of seven electrodes of lipid membranes, and is controlled mechanically by a robot arm, as illustrated in Fig. 5. Measurements of the taste of coffee were made using SA401. The membranes were No.SB01AAC1 for channels 1-3, No.SB01AAF1 for ch. 4 and 5, and No.SB01AAJ1 for ch. 6 and 7, which were immersed in the standard coffee, Saldvador used here, for at least a month. The response electric potentials were taken using this coffee as the origin of electric potential pattern.

Typical five primary taste substances, HCl (sour), NaCl (salty), quinine (bitter), sucrose (sweet) and MSG (umami) were studied [5]. Figure 6 shows the electric potential pattern from 8 channels for five kinds of taste qualities of sour, salty, bitter, sweet and umami. The pattern of each taste substance is different, and hence each taste substance can be easily discriminated. The reproducibility is very high, because the standard deviations are smaller than 1% or so. The taste sensor shows similar response patterns to the same group of taste. Sour substances such as HCl, citric acid and acetic acid show similar response patterns. Bitter substances, quinine, $MgSO_4$ and phenylthiourea, show similar patterns.

Therefore, we can conclude that this taste sensor can respond to the taste in itself. This fact is very important, because we must measure the taste (not chemical substances). The sensor had detection errors (in the unit of logarithmic concentration) 0.73% for saltiness, 0.65% for sourness and 3.4% for bitterness in the mixed aqueous solution [19]. Humans usually cannot distinguish two tastes with a concentration difference below 20% [3]. Here, 20% means the error of 7.9% (=log 1.2). Therefore, ability of detection of the sensor is superior to that of humans.

Taste of amino acids was studied using the taste sensor [12]. Taste of amino acids has had the large attention so far because each of them elicits complicated mixed taste itself, as can be understood from the example where L-valine produces sweet and bitter tastes at the same time.

Figure 7(a) shows the response patterns to typical amino acids [12], each of which elicits different taste quality in humans [20]. Each channel responded to them in different ways depending on their tastes. L-Alanine, glycine and L-threonine taste mainly sweet. Only for these amino acids, the potentials of channels 1 and 2 decreased. L-Glutamic acid and L-histidine monohydrochloride, which taste mainly sour, increased each of the potentials of channels 1-5 to almost the same degree.

On the other hand, L-tryptophan, which elicits almost pure bitter taste, increased the potentials of channels 1, 2 and 3 greatly. This tendency was also observed for other amino acids which mainly exhibit bitter taste: L-phenylalanine and L-isoleucine. L-Valine and L-methionine, which taste mainly bitter and slightly sweet, decreased the potential of channel 5; the responses of channels 1 and 2 were small. For monosodium L-aspartate eliciting mainly umami taste in humans, the response pattern was different from those of the other amino acids.

Figure 7(b) shows the data points plotted in the scattering diagram on PC 1 and PC 2 by the principal component analysis. The first principal axis (PC 1) reflects bitterness and sweetness. The second principal axis (PC 2) reflects sourness and umami. Amino acids are classified clearly into five groups by the taste sensor.

The response of the sensor to amino acids was compared with the results of panel tests [20]. The response potentials from the eight membranes were transformed into five basic tastes by multiple linear regression. This expression of five basic tastes reproduced human taste sensation very well.

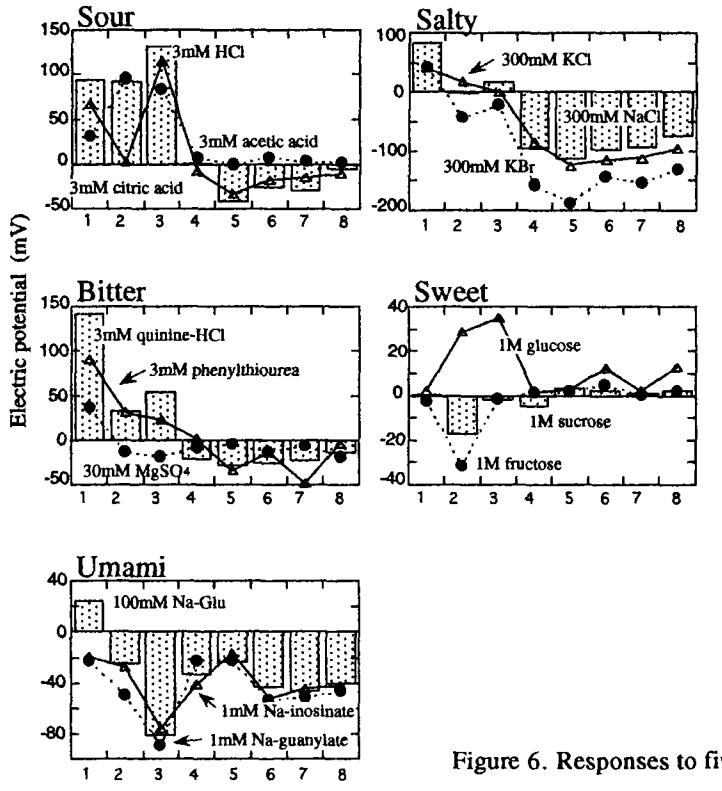

Figure 6. Responses to five basic taste substances

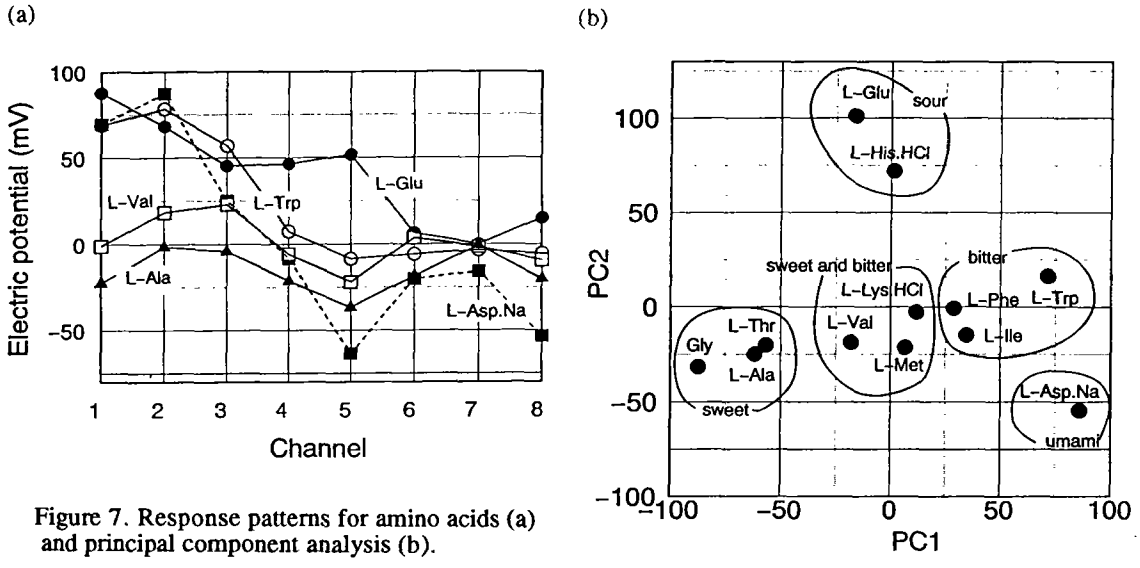

Figure 7. Response patterns for amino acids (a) and principal component analysis (b).

3. Expression of taste by four basic tastes

We tried to make an artificial taste solution, which shows a similar taste to some commercial aqueous drinks, by combination of basic taste substances by comparing electric potential patterns of the taste sensor [11]. Four different concentrations were prepared for each of basic taste substances: 1, 3, 10, 30 mM for HCl; 30, 100, 300, 1000 mM for NaCl and sucrose; 0.03, 0.1, 0.3, 1 mM for quinine. We prepared 4^4(=256) mixed solutions with different compositions by combination of these four types of basic solutions.

The above-mentioned 256 mixed solutions were measured with the taste sensor. Therefore, data on the output electric potential pattern were taken for the 256 solutions. While the data on each channel output were dispersed discretely in the four-dimensional space constructed from four different concentrations, we approximated them by a quadratic function of the concentrations. The data can be expressed by a set of the eight quadratic functions (corresponding to 8 channels) of concentrations of four taste substances.

Two commercial drinks of different brands were chosen as test aqueous drinks. Figure 8 shows the electric potential patterns of these two drinks. The discrimination is very easy because of small standard deviations. We attempted to fit the above patterns constructed from eight functions to the output electric potential pattern of one of commercial drinks (let us call "drink A" for convenience) by minimizing L :

$$L = \sum_{i=1}^{8} (V_{mi} - V_i)^2 , \qquad (1)$$

where V_{mi} and V_i are the output electric potentials for the above mixed solutions (expressed by the quadratic function) and drink A, respectively, with i denoting the output from channel i. As a result, we obtained one mixed solution whose output pattern was nearest to that of drink A.

The best combination of the concentrations for basic taste substances was obtained: 2 mM HCl, 50 mM NaCl, 0.2 mM quinine and 100 mM sucrose. Figure 8 shows that the pattern for the mixed solution is surely closer to that of the drink A compared to another drink. The sensory evaluation by humans was also made; i.e., the above-mentioned two commercial drinks and the mixed solution were tasted, and their tastes were compared to one another. The sensory tests by humans showed that this mixed solution produced almost the same taste as drink A.

The τ scale is effective to express the taste strength [1, 2]. The concentration of each taste substance can be transformed into the taste strength. If we consider that HCl has the strength two times as large as tartaric acid, the above mixed solution is composed of 4.04 sourness, 2.03 saltiness, 5.01 bitterness and 2.24 sweetness in terms of the τ scale.

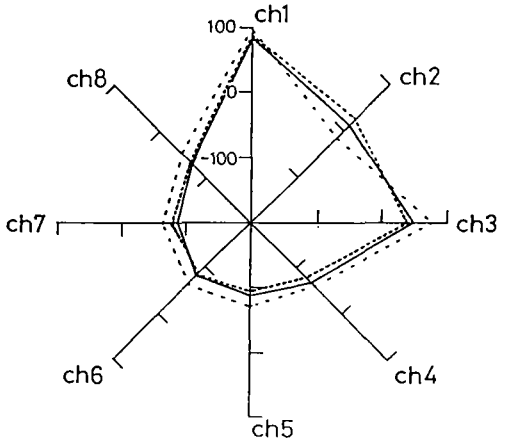

Figure 8. Aqueous drinks and mixed solution. ——, drink A; – – – –, another drink; ·····, mixed solution whose taste is similar to drink A.

4. Discrimination of taste of coffee

The taste-sensing system SA401 of Anritsu Corp. was applied to measure the taste of several origins of coffee and also commercial canned coffees.

Figure 9 shows the response electric potentials for 10 origins of coffee, Brazil (Santos No.2), Guatemala (SHB), Jamaica (Blue Mountain No.2), Hawaii Kona (Extra Fancy), Kenya (AA), Tanzania (AA), Colombia (Excelso), Indonesia Mandheling (Grade1), Indonesia WIB 1 and Indonesia AP 1, relatively measured form the electric potential for Salvador (CS), which was used here as the standard coffee (i.e., the zero level of electric potential pattern). We can see that Kenya shows the largest pattern, while AP 1 shows the smallest pattern. Since the standard deviations of the measuring system were ± 0.3mV, these origins of coffee could be easily discriminated.

Figure 10 shows the result of principal component analysis applied to the electric potential patterns in Fig. 9. Each origin of coffee is found to be dispersed on the two-dimensional plane of the first (PC1) and second (PC2) principal components. The contribution rates to PC1 and PC2 are 82.2% and 13.8%, respectively; hence we can satisfactorily discuss the taste of coffee on the two-dimensional plane. In other words, the taste of coffee can be quantified by at least two independent expressions.

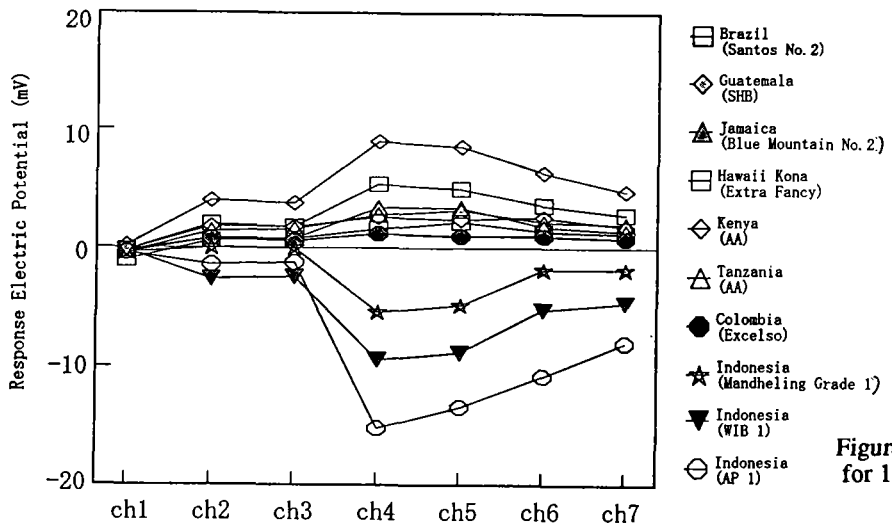

Figure 9. Response patterns for 10 origins of coffee.

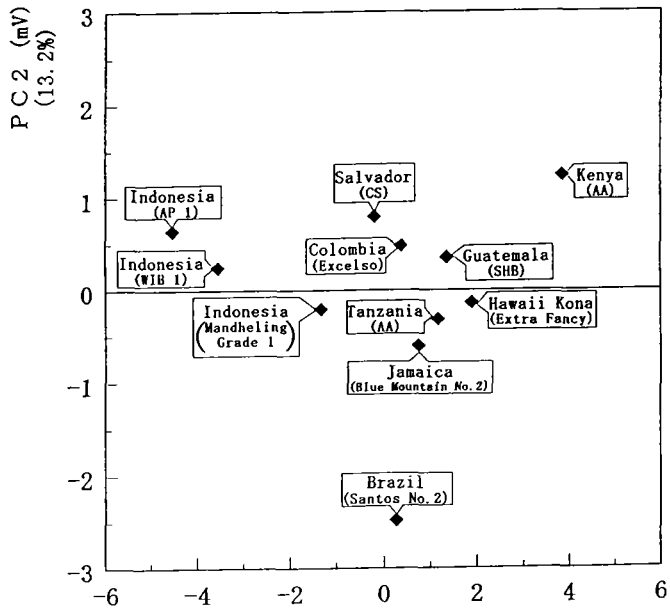

Figure 10. Principal component analysis applied to the response patterns for 10 origins of coffee.

As is well known, the basic words characteristic of the taste of coffee are "acidity" and " bitterness". We hereafter use the term "acidity" instead of "sourness", because "acidity" is always used to express a basic, desirable sharp and pleasing taste produced by organic acids contained in coffee [21]. Figure 11 shows the comparisons between the response electric potential and the sensory panel test for acidity (a) and bitterness (b). The correlation coefficients were 0.98 for acidity using channel 2 and 0.94 for bitterness using channel 5. This result implies that channels 2 and 5 can be utilized to quantify acidity and bitterness, respectively. For example, Kenya has strong acidity while Mandheling shows strong bitterness; this fact can be expressed by using the quantified scale obtained from the sensor output.

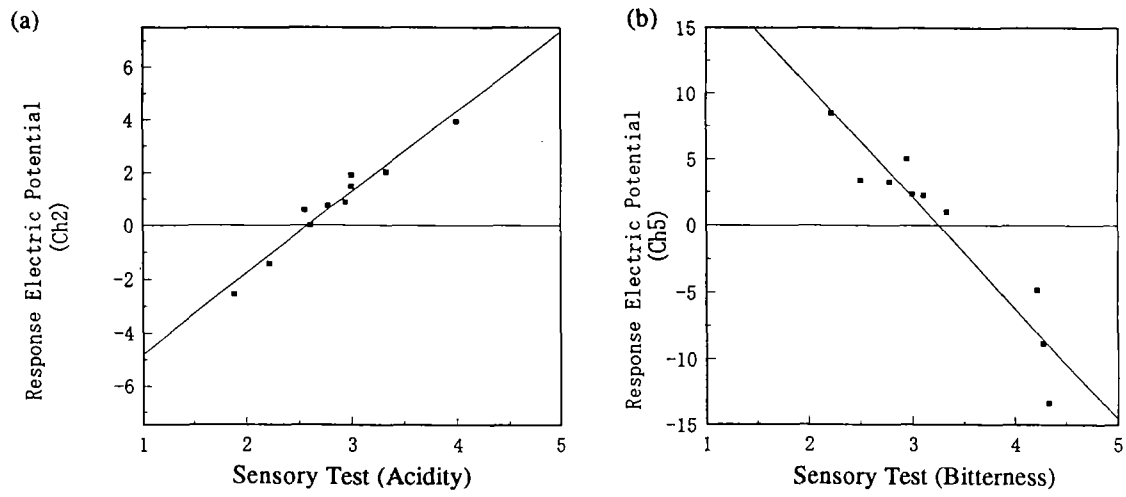

Figure 11. Comparisons between the sensor output and the sensory panel test.

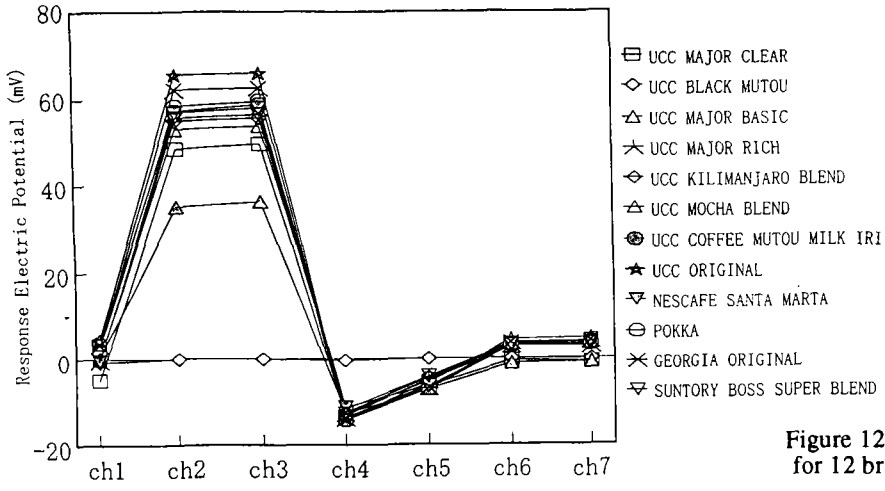

Figure 12. Response patterns for 12 brands of canned coffee.

Let us next mention application of the taste-sensing system to commercial canned coffees. Figure 12 shows the response electric potentials for 12 brands of canned coffee, measured from the origin of electric potential for UCC BLACK MUTOU, which is sugarless canned coffee. We can see that the response potentials are different from each other and much larger than the case of brewed coffee in Fig. 9. Channels 2 and 3 responded largely to coffees with milky taste. We can discuss the taste of canned coffee using this result.

Figure 13 is the result of principal component analysis applied to Fig. 12. From the comparison with sensory tests, PC1 seems to reflect "milky taste" and PC2 may reflect "body taste". It should be noted that the taste sensor can always output this result reliably. In addition, the sensitivity is as high as ± 0.3mV, and hence the minute difference of taste of different brands (and also the same brand with different production date) of coffees can be easily detected.

5. Summary

The taste sensor showed a similar pattern in the same group of taste, while quite different patterns were obtained for different taste groups. In fact, the similar outputs were obtained for, e.g., NaCl, KCl and KBr eliciting saltiness, whereas their patterns differed clearly from those for other basic taste qualities. The taste of amino acids was classified into several groups in accordance with human taste sense. These facts imply that taste is measured with a fashion similar to the human gustatory sensation.

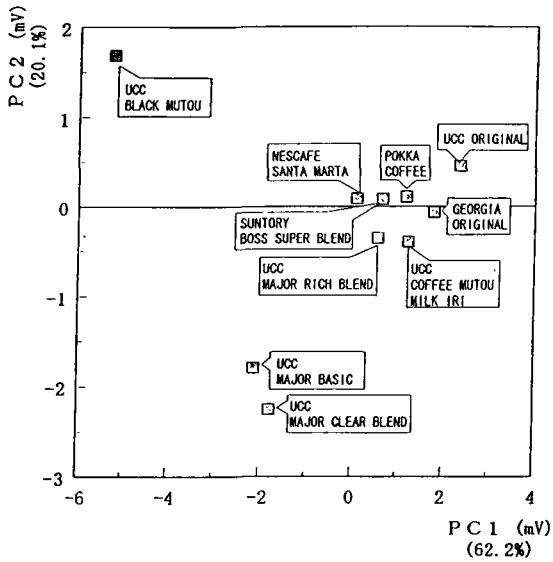

Figure 13. Principal component analysis applied to the response patterns for 12 brands of canned coffee.

Different origins of coffees were measured using the taste sensor. As a result, they were easily discriminated; very high correlations were found between the output of the taste sensor and the sensory panel tests by humans for acidity and bitterness. Commercial canned coffees were also measured, and the two-dimensional taste map was obtained. The taste sensor has the sensitivity, reproducibility and durability superior to those of humans. If we regard the ordinate in Fig. 11, i.e., the response electric potential, as the measure of acidity (and bitterness), then the taste of coffee can be quantified by this quantified, objective measure.

The taste sensor will be applicable for quality control of coffee and also for the automatic production. The sense of taste is vague and largely depends on subjective factors of human feelings. If we compare the standard index measured using the taste sensor with the sensory evaluation, we will be able to assess the taste objectively. Moreover, the mechanism of information processing of taste in the brain as well as the reception at taste cells will be clarified by developing a taste sensor which has output similar to that of the biological gustatory system.

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MEASUREMENT OF COFFEE TASTE USING LIPID MEMBRANE TASTE SENSORS

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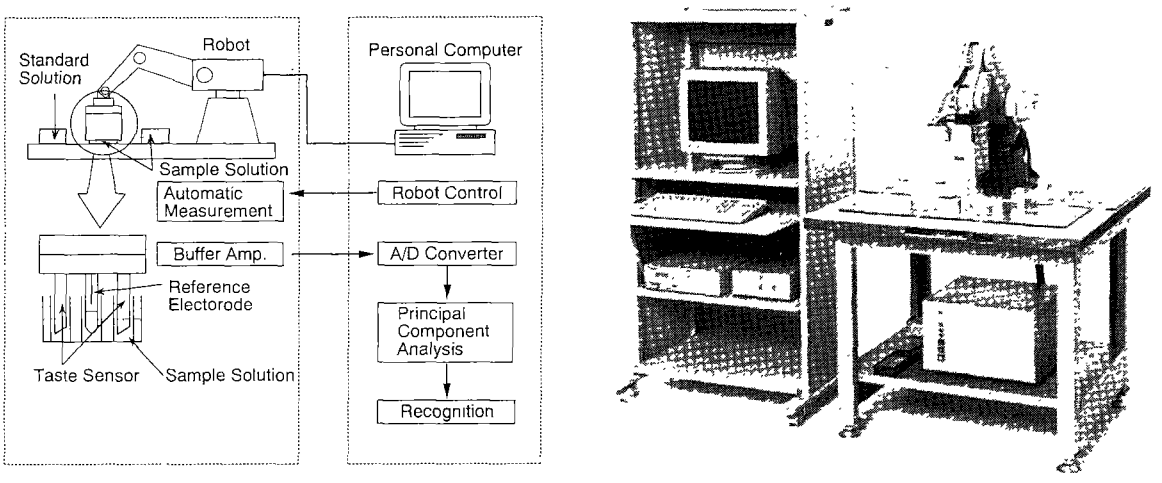
1. Introduction

Various types of sensors have been developed by the present. Many quality control processes have been automated and the accuracy has remarkably improved by these sensors. However, taste sensing relies on the sensory evaluation. Sensory evaluations are influenced by the physical and mental conditions of panels. To automate the quality control of taste, the development of the taste sensor that resemble to human sense of taste is necessary. There are two reasons why development of a taste sensor is delayed. The first reason is that foods and drinks contain a large number of taste substances. When conventional chemical sensors, which has selectivity, is employed, it is difficult to prepare sensors corresponding to every taste substance. The second reason is that the taste is changed by interactions of taste substances. For example, suppression effect between sweetness and bitterness, and synergistic effect of *umami* are reported [1-3]. Even if taste substances contained in food were analyzed precisely, it is difficult to express the taste while including the influence of interactions.

Multichannel taste sensor which is entirely different from the concept of conventional chemical sensor has been developed. It is modeled upon taste receptors of living organism. The taste sensor is made with artificial lipid membrane which responds to taste substances [4-7]. It does not detect taste substances one by one, but rather detects whole taste in a general manner like human sense of taste. Taste sensing system SA401 employing the taste sensor is able to measure beverage in a few minutes per sample. It is possible to distinguish brand of beverages such as beer or Japanese *sake*.

In this study, instant coffee and the substances in coffee were measured by the taste sensing system, and the effects of a preliminary treatment of coffee solution was investigated. In the measurement of instant coffee, brands were discriminated sufficiently. But an analysis of the measurements showed high correlation of sensor



(a) (b)

Figure 1. Outline of taste sensing system SA401 (a) and appearance (b)

responses with acidity. The discrimination of delicate taste other than sourness was not sufficient. Therefore, the preliminary treatment is used in attempt to coffee to increase information from sensors. As a result, response to taste other than sourness was increased and the discrimination capability was improved.

2. Taste sensing system SA401

Taste sensing system SA401 discriminates taste by using lipid membrane taste sensors. SA401 is composed of seven taste sensors, robot and a personal computer (Figure 1). The personal computer analyzes the response patterns from the sensors and control the robot which carries out measurement automatically.

The mechanism of taste in living organism has not been clarified completely yet. The following is thought as a model of it. There are various taste receptors which have different response properties. Lipids in biomembrane of the receptors play an important role [8,9]. Signal pattern from them are processed in a neural network and are recognized as some kinds of taste. Taste sensor is modeled upon these taste receptors of a living organism (Figure 2). The taste sensor uses a artificial lipid membrane. The response property of a artificial lipid membrane to a taste substance varies with lipid. Response pattern of those sensors is processed by a personal computer and is discriminated.

The measurement method of this system is relative value measurement. Difference between standard solution and sample solution are measured. Standard solution is the same kind of drink as the objects to be measured. The optimum combination of seven sensors differs by the objects. The sensor set is preconditioned with standard solution and become specialized for the object. Measurement accuracy is improved by this method. An automatic measurement by an industrial robot is intended for labor-saving and regularization of measurement condition. The limit of difference in taste concentration that average human is able to discriminate is 20% [10,11]. As for SA401, the detection limit of difference in concentration is 1-2% in the case

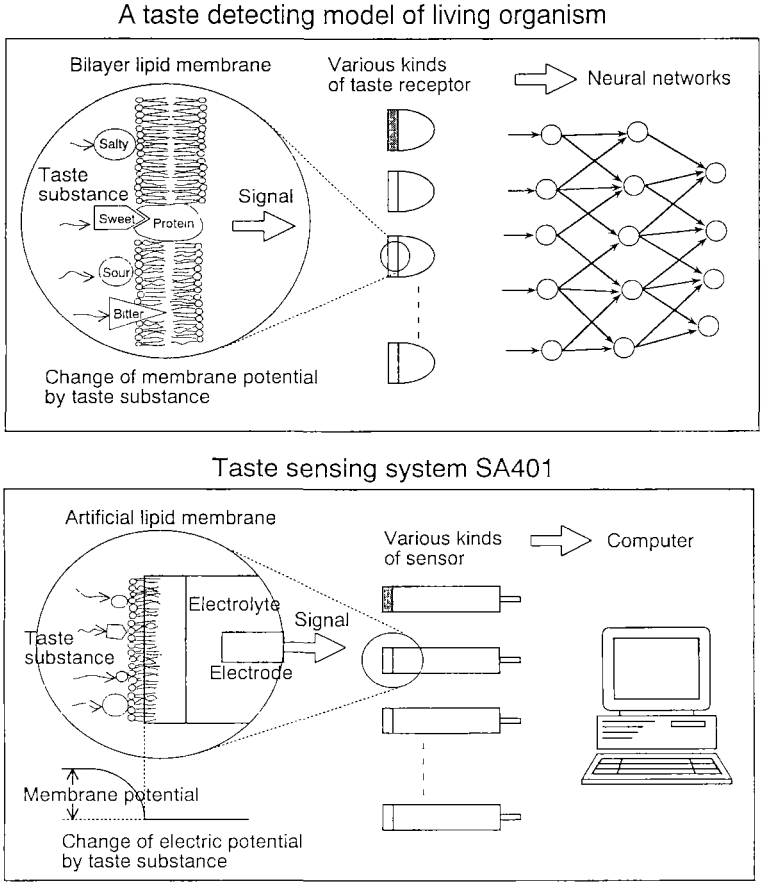


Figure 2 Model of taste sensing system

of salt and acid [12].

The personal computer analyzes response patterns from the taste sensors with principal component analysis. A neural network compares the processed data with learned data which was measured beforehand and classifies them. Two-dimensional representation of the taste distribution is displayed on the computer screen.

3. Measurement of coffee

Nine brands of instant coffee and eleven substances contained in coffee were measure with the taste sensing system. Table 1 shows the combination of sensors that was used in the present study. The procedure of this measurement is as follows. A standard solution is measured several times until the sensors become stable. The electric potentials of stabilized sensors is used as the reference value. Then a sample solution is measured. The measurement value of the sample is the difference in electric potential of the sensor between sample solution and the reference solution. This procedure was repeated according to the number of samples. Each sample was measured five times.

Table 1. *Combination of sensors*

channel	Lipid
1	dioctyl phosphate
2	cholesterol
3	oleic acid
4	decyl alcohol
5	oleylamine
6	lecithin
7	phosphatidyl serine

Table 2. Correlation coefficients between sensors and pH (instant coffee)

	ch1	ch2	ch3	ch4	ch5	ch6	ch7	pH
ch1	+1.00	+0.90	+0.93	+0.94	+0.87	+0.91	+0.89	-0.73
ch2		+1.00	+0.99	+0.99	+0.94	+1.00	+1.00	-0.85
ch3			+1.00	+0.99	+0.91	+0.99	+0.99	-0.80
ch4				+1.00	+0.91	+0.99	+0.99	-0.80
ch5					+1.00	+0.93	+0.92	-0.96
ch6						+1.00	+1.00	-0.84
ch7							+1.00	-0.83
pH								+1.00

3. 1 Measurement of instant coffee

Nine brands of instant coffee were measured. Sample solutions were made with 2g of coffee powder and 140g of pure water by following the instruction on the labels.

Figure 3 shows the response patterns of the sensors. The mean value of five measurements of each sample is plotted in a radar chart. Coffee I is used as standard solution, so the measurements of coffee I are zero level. Figure 4 is the result of principal component analysis. The scatter in each sample includes error of five measurements and change of sample itself with elapse of time. The difference of these coffees are large enough to be discriminated in comparison with measurement errors. A contribution rates of first (PC1) and second principal component (PC2) are 95.8% and 2.2% respectively. Table 2 shows the correlation coefficients between each channel of sensors and pH in this measurement. All channel have high correlation coefficients to each other, and negative correlation with pH. Therefore, PC1 is equivalent to sourness.

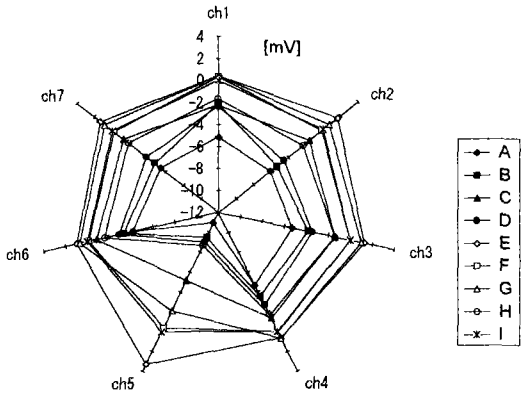


Figure 3. Response patterns for instant coffee

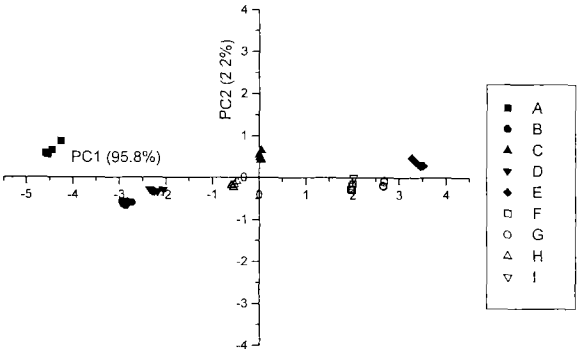


Figure 4. Principal component analysis applied to the response patterns for instant coffee

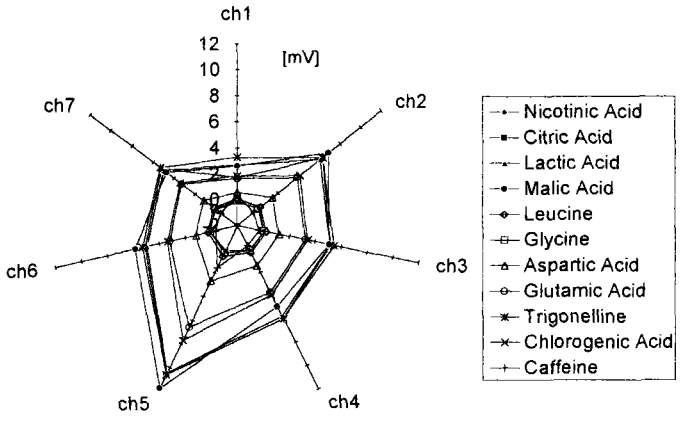


Figure 5. Response patterns for eleven substances in coffee

Table 3. Substances added to coffee and their quantities

substance	quantity (mg / 100g)	pH
Nicotinic Acid	0.3	5.42
Citric Acid	20.0	5.07
Lactic Acid	23.0	5.13
Malic Acid	20.0	5.06
Leucine	4.0	5.45
Glycine	5.0	5.42
Aspartic Acid	6.0	5.38
Glutamic Acid	21.0	5.22
Trigonelline	30.0	5.16
Chrogenic Acid	50.0	5.22
Caffeine	96.0	5.44

3. 2 Measurement of components of coffee

Components of coffee were measured to investigate delicate taste differences. Instant coffee I is used as the standard solution. Eleven substances were selected from main components of coffee and added to coffee I. Table 3 shows the substances and their quantities. The quantity of substance is approximately equal to what contained in 100g of coffee generally [13], except malic acid, torigonelline and chlorogenic acid. That is, the concentration of the substances approximately doubled.

Figure 5 shows the response patterns. The response potentials became small in comparison with Figure 3. This shows that the difference of taste is smaller than that of instant coffees. Figure 6 shows the result of principal component analysis. The contribution rate of PC1 and PC2 are 99.6% and 0.2% respectively. Organic acids such as citric acid, malic acid and lactic acid changed greatly from the standard solution. Amino acids such as leucine and glycine changed slightly. From the coefficient of correlation (Table 3), PC1 is equivalent to pH. There is almost one-dimensional information from the sensors. Therefore, it is easy to discriminate the strength of sourness, but is difficult to distinguish differences of delicate taste other than sourness.

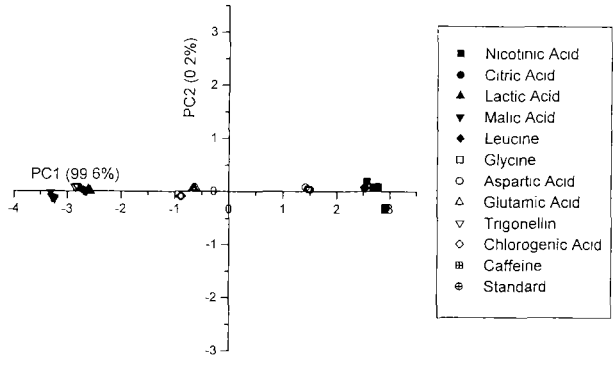


Figure 6 Principal component analysis applied to the response patterns for 11 substances in coffee

3. 3 Preliminary treatment to coffee

A preliminary treatment to coffee was attempted to improve the discrimination capability of delicate taste other than sourness. When milk is added to coffee, it is experienced that the taste becomes mild. It feels that sourness abates and other delicate tastes appears. Thereupon, milk and skim milk were added to coffee. Nine brands of instant coffee with milk and that with skim milk were measured. The quantities of milk and skim milk are 10ml and 1.2g per 140ml respectively.

Figure 7 shows response patterns of nine brands of coffee with milk and with skim milk, and Table 4 shows correlation coefficients of each channel and pH. They are almost the same as ones of black coffee (Table 2) except for ch3. The correlation between ch3 and other channels are low. The response patterns of ch3 changed by adding milk or a skim milk. From the correlation coefficient, seven sensors are divided into three groups. They are ch3, ch5 and others. Figure 8 shows the result of principal component analysis of measurements of three channels of sensors (ch3, ch5 and ch7). Figure 8 (a) shows the result of black coffees (same as Figure 4), but the measurements are reduced to three channels. In the case of adding milk, contribution rates of PC1 and PC2 are 76.8% and 22.2% respectively. PC2 is increased by 5.8 times. Coffee F and Coffee I are discriminated clearly. In the case of skim milk, contribution rates of PC1 and PC2 are 89.8% and 8.6% respectively. Resolution is improved by adding milk or skim milk.

Additions of milk and skim milk to coffees added with eleven substances were also attempted. The standard solution is the same, coffee I of black.

Figure 9 shows response patterns. Table 5 shows coefficient of correlation of each channel and pH. The correlation between ch3 and other channels are decreased, in the case that skim milk was added. Figure 10 shows the result of principal component analysis of measurements of three channels (ch3, ch5 and ch7). Figure 10 (a) shows the result of black coffees (same as Figure 4), but the measurements are reduced to three channels. The contribution rate of PC2 is 0.5%. The change of contribution rate by decreasing channels is small, because of high correlation of all channels. In the case that added milk, contribution rate of PC1 and PC2 is 98.6% and 1.2% respectively. PC2 is doubled. In the case of skim milk, PC1 is 90.1% and PC2 became 9.7%. PC2 is increased significantly and two-dimensional information is obtained by addition of skim milk. Citric acid and lactic acid, which are not clearly separated in Figure 10 (a), are spread in PC2 direction and sufficient discrimination is possible.

3. 4 Discussion

In the case of principal component analysis applied to response patterns for nine brands of instant coffee without milk, contribution rate of PC2 is 3.3%. Because of that instant coffees are blended, their difference are small in comparison with differences in origins. In the result of measurement of eleven substances in coffee which is attempted to discriminate delicate taste difference, nothing but strength of sourness was detected. Many commonest are contained in coffee, differences of coffee taste are not explained by these eleven substances. Other substances also affect tastes of coffee. In comparison with discriminations of brands of instant coffee, the improvement of discriminating resolution of eleven substance by addition of milk is small. This is interpreted that milk has greater effect on substances other than the eleven substances.

The response patterns of ch3 were change by addition of milk and skim milk, and the discriminating

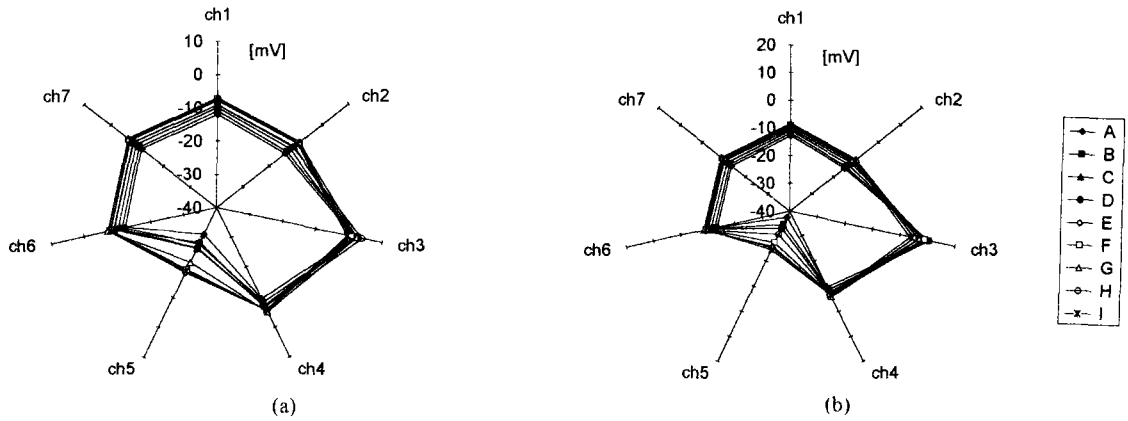


Figure 7. Response patterns for nine brands of instant coffee with milk (a) and with skim milk (b)

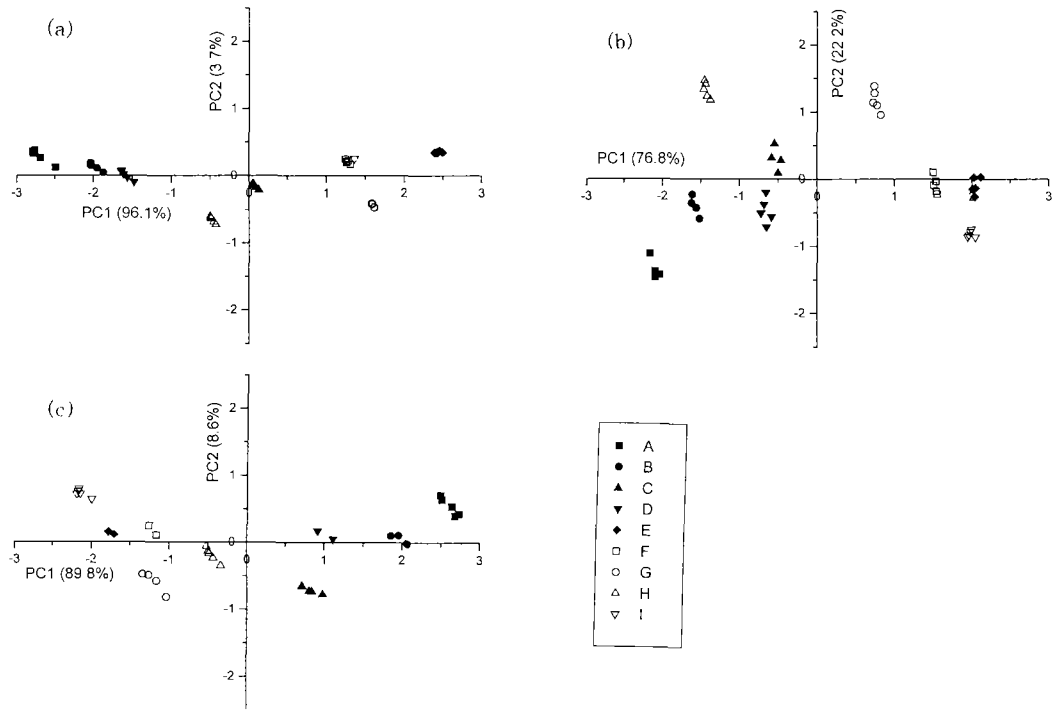


Figure 8. Principal component analysis applied to the response patterns of ch3, ch5 and ch7 for instant coffee of black(a), with milk(b) and with skim milk(c)

Table 4. Correlation coefficients between sensors and pH in the case of instant coffee with milk (a) and with skim milk (b)

(a)

	ch1	ch2	ch3	ch4	ch5	ch6	ch7	pH
ch1	+1 00	+0 99	-0.41	+0 99	+0 90	+0.99	+0 99	-0 87
ch2		+1.00	-0.42	+0.98	+0 90	+0.99	+1 00	-0 87
ch3			+1.00	-0 93	-0.73	-0.34	-0.37	+0 77
ch4				+1.00	+0.89	+0 98	+0 98	-0.84
ch5					+1.00	+0.86	+0 87	-0 99
ch6						+1.00	+1 00	-0.82
ch7							+1.00	-0 84
pH								+1.00

(b)

	ch1	ch2	ch3	ch4	ch5	ch6	ch7	pH
ch1	+1.00	+1 00	-0 74	+0 99	+0 93	+0.99	+0.99	-0.77
ch2		+1 00	-0.76	+0.98	+0 93	+0.99	+1 00	-0.77
ch3			+1.00	-0 69	-0.84	-0.72	-0 74	+0.73
ch4				+1.00	+0.91	+0.97	+0.97	-0 76
ch5					+1 00	+0 89	+0.91	-0 93
ch6						+1 00	+1.00	-0 70
ch7							+1.00	-0 74
pH								+1 00

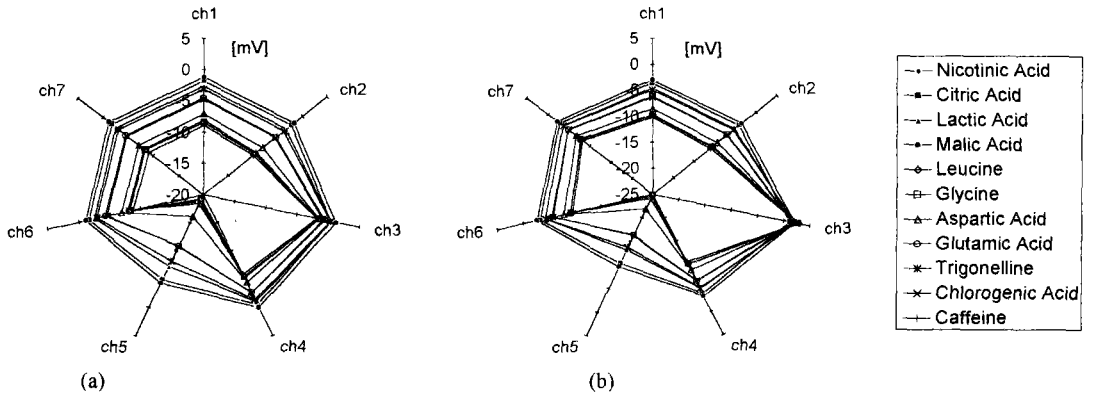


Figure 9. Response patterns for eleven substances in coffee with milk (a) and with skim milk (b)

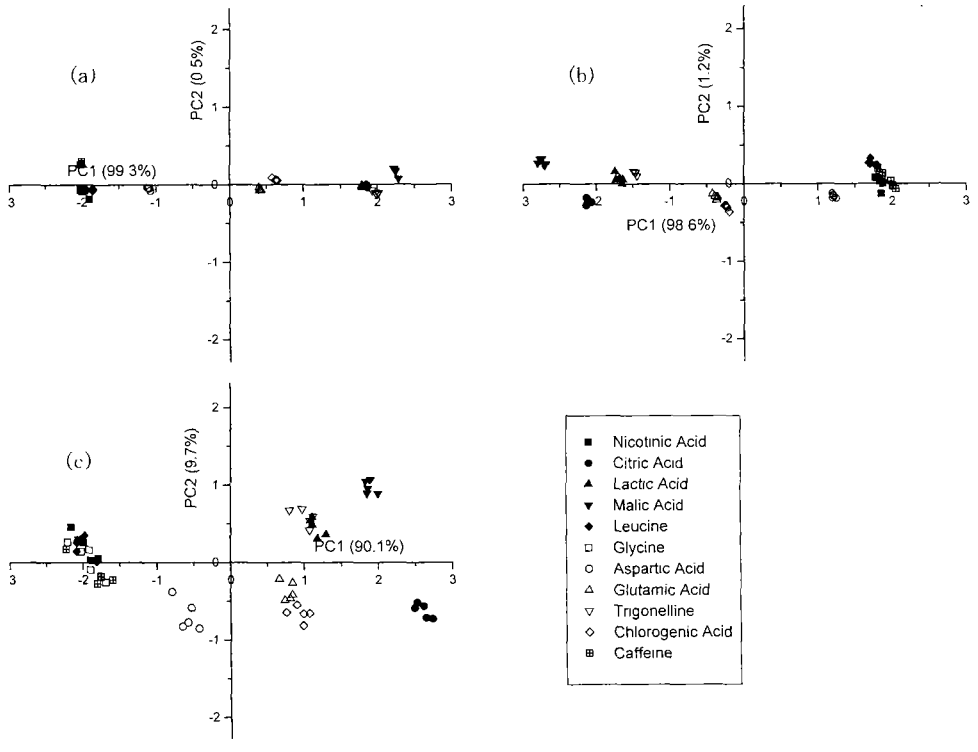


Figure 10. Principal component analysis applied to the response patterns of ch3, ch5 and ch7 for eleven substances in coffee of black(a), with milk(b) and with skim milk(c)

Table 5. Correlation coefficients between sensors and pH in the case of eleven substances with milk (a) and with skim milk (b)

(a)

	ch1	ch2	ch3	ch4	ch5	ch6	ch7	pH
ch1	+1.00	+1 00	+092	+0.99	+0.99	+1 00	+1 00	-0 96
ch2		+1.00	-0 76	+0.99	+1.00	+1.00	+1.00	-0 97
ch3			+1.00	+0.95	+0.89	+0 92	+0.92	-0.79
ch4				+1.00	+0.98	+0.99	+0.99	-0 92
ch5					+1 00	+0.99	+0.99	-0 98
ch6						+1.00	+1 00	-0.96
ch7							+1 00	-0 96
pH								+1 00

(b)

	ch1	ch2	ch3	ch4	ch5	ch6	ch7	pH
ch1	+1.00	+1.00	-0.79	+1.00	+1 00	+1.00	+1.00	-1.00
ch2		+1.00	-0.79	+1.00	+0.90	+1.00	+1 00	-1.00
ch3			+1.00	-0.79	-0 82	-0.79	-0.79	+0 82
ch4				+1.00	+1.00	+1.00	+1.00	-0 99
ch5					+1 00	+1 00	+1 00	-1.00
ch6						+1 00	+1.00	-0.99
ch7							+1.00	-0 99
pH								+1 00

resolution were improved. Addition of commercial creaming powder had almost the same result, but its effect was inferior to that of milk. The principle is not elucidated as yet.

4. Summary

Instant coffee and the components of coffee were measured by the taste sensing system which used lipid membrane taste sensors. The brands of instant coffee were discriminated easily and the difference of strength of sourness appeared clearly. In the case of measurement of components of coffee, only one-dimensional information was obtained, because all the response patterns are related to pH. Therefore it was difficult to detect a difference of delicate taste other than sourness. Thereupon, the preliminary treatment to coffee was tried to increase the information. The response of ch3 changed by adding milk or skim milk to coffee. Different output patterns from other channels were obtained. In case of discrimination of brands of instant coffee contribution rate of PC2 improved by 5.8 times and the discriminating resolution was improved. The difference of delicate taste other than sourness was detected as PC2. The preliminary treatment to coffee increases the information and has the effect of improving discriminating resolution.

An improvement of the resolution by the preliminary treatment to coffee will greatly contribute to quality control of coffee and development of new products. Improvements of taste sensors and studies of connection between response of sensor and sensory evaluation are being carried out.

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RELATIVE EXTRACTION YIELDS OF GREEN COFFEE

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INTRODUCTION

In the manufacturing of instant coffee, the yield that is obtained from the starting green coffee beans is critical to determining the economics of the instant coffee process. Within the overall yield calculation, the extraction yield that is obtained is a major factor. A great deal of work has been done by many individuals to optimize the extraction yields obtained in the instant coffee process. This work has covered such areas as extraction column design as well as optimizing the extraction parameters such as the extraction temperature profile and the draw off factor. This work examines the effect that the type of coffee can have on the extraction yield. Not only are the differences between Arabica coffees and Robustas coffees examined, but a study of the origin country and the level of defects in the coffee was done in order to quantify what effect those variables have on the extraction yield.

On an overall basis, the green yield for an instant coffee product is defined as the pounds of soluble coffee that are obtained for a given amount of green coffee, that is, the pounds soluble divided by the pounds of green coffee. For example, a green yield of 40% would mean that 40 pounds of soluble coffee were obtained from 100 pounds of green coffee beans. However, the instant coffee process involves a number of different processing steps and for each of these processing steps a yield can be determined. Typically, the overall green yield for instant coffee is broken down into the following main components. 1) The green cleaning yield which measures the losses which occur in cleaning the incoming green coffee. 2) The roasting yield which measures the losses which occur during the roasting process. 3) The extraction yield which measures the amount of soluble solids extracted per unit of roasted coffee. This yield may be calculated in

different ways. In this work, the dry extraction yield refers to the amount of dry solids in the liquid extract divided by the amount of dry roasted coffee. That is, the roasted coffee weight is adjusted to a moisture free basis. 4) A process yield which measures the amount of solids lost between the liquid extract and the final packaging. The product of these four yields gives the overall green yield as shown in this equation:

Green Yield = Cleaning Yield x Roasting Yield x Extraction Yield x Process Yield.

The importance of the extraction yield in this calculation cannot be overlooked. Let's look at an example of the sensitivity of the extraction yield. Assuming a cleaning yield of 99%, a roasting yield of 85%, an extraction yield of 45%, and a process yield of 95%, the green yield calculates as 36%. But if the extraction yield were to improve to 47.5% (and all other yields remained the same), then the green yield would improve to 38%. If the green coffee blend used in this example cost $1.50 per pound, then the instant coffee would cost $4.17 per pound with the extraction yield of 45% and $3.95 per pound with an extraction yield of 47.5%. That represents a 5% reduction in the price of the soluble coffee based solely on the raw material cost.

CURRENT WORK

This work examined the effect that the type of green coffee can have on the extraction yield. A total of 77 green coffee samples were extracted using an autoclave extractor in which we were able to hold constant extraction conditions. Of these 77 coffees, 51 were Arabica coffees and 26 were Robusta coffees. The geographic origin of these coffees was varied in order to quantify the effect that source country had on the extraction yield.

In order to maintain the required constant extraction conditions, a laboratory autoclave (Parr Instrument Company, Model Number 4522M, 2000 ml) was used to extract roasted coffee in a two stage extraction process. In the first stage, 35 grams of ground roasted coffee were placed into the autoclave. 700 milliliters of distilled water were added to the autoclave, the vessel was sealed, and the temperature was brought up to 90 degrees centigrade while the water/coffee slurry was being agitated. The slurry was maintained at 90 degrees for 20 minutes at which time the slurry was cooled to a temperature that was safe enough to handle. The slurry was decanted and the fresh extract was saved. The partially extracted grounds were placed back into the autoclave and an additional 700 milliliters of water were added. This mixture was then brought up to 190 degrees centigrade and that temperature was maintained for 20 minutes in order to hydrolyze the coffee. At the conclusion of the extraction cycle, the slurry was cooled and the extract was decanted from the grounds and saved as the hydrolysis extract.

For each autoclave run, the concentration of dissolved solids was determined on both the fresh extract and the hydrolysis extract by refractive index. Both a fresh yield and a hydrolysis yield were determined. These two yields were added together to determine the total extraction yield.

A reproducibility study was conducted in order to quantify the amount of variation in total extraction yield that one would attribute to technique and measurement variability. For the reproducibility study, a blend of Arabica coffee and Robusta coffee was extracted six times. The results of the reproducibility study are shown in Table 1.

Table 1

REPRODUCIBILITY STUDY
Blend of Arabicas and Robustas

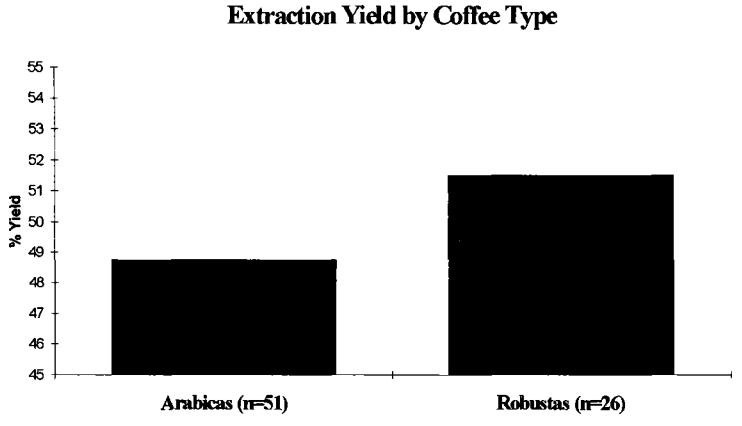
Run		Total Dry Extraction Yield
1		57.37%
2		54.76%
3		57.13%
4		56.85%
5		55.20%
6		54.89%
	Mean	56.03%
	Sigma	1.21

Although the sigma for this reproducibility study may appear somewhat high, it is reasonable when one considers that a difference in the extract concentration of only 0.1% represents a difference in the yield calculation of 2% due to the 20:1 water to coffee ratio that is used. And this type of variation from run to run is typical of what we have seen in other studies of this nature. For the purposes of determining whether or not the differences seen in extraction yield between one type of coffee and another is meaningful, we will simplify the matter by using a difference of 2 sigma, or 2.4%, as a meaningful difference in extraction yield.

RESULTS AND DISCUSSION

The data from the 77 autoclave extraction runs can be stratified in a number of ways. Graph 1 shows the yield obtained from the 51 Arabica samples compared to the 26 Robusta samples.

Graph 1

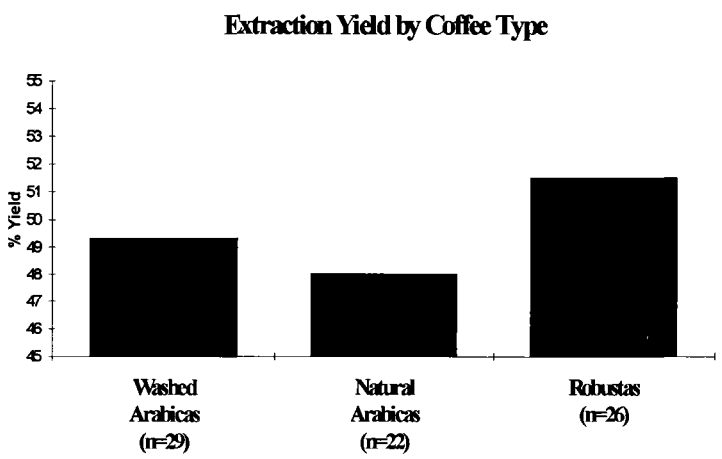


The average yield for the Arabicas was 48.76% and the average yield for the Robustas was 51.50%. On the average, the yield difference between these two types of coffees just meet the

criteria of being a meaningful difference. However, the yield difference between the Arabicas and the Robustas appears to be low. In other work that has been conducted in our laboratories, we have found that the yield difference between an Arabica coffee and a Robusta coffee can be as much as 10%. I would speculate here that the extraction conditions being used for the autoclave may be over extracting the coffee from what is seen in an industrial extractor and, thus, minimizing the differences in yield.

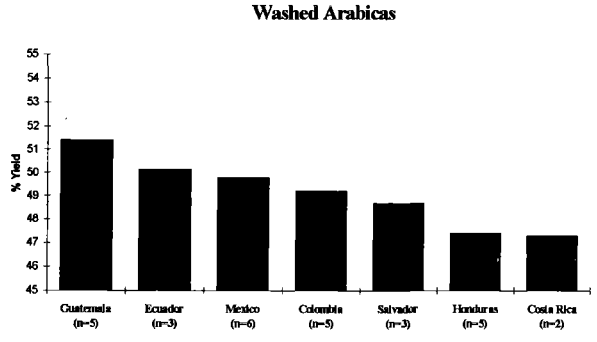
The data can be further stratified by looking at the two types of Arabica coffees - washed Arabicas and natural Arabicas - separately as shown in Graph 2.

Graph 2

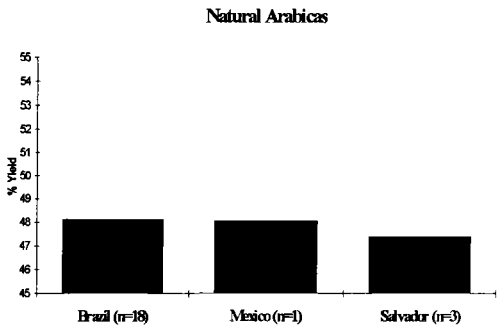


The washed Arabicas averaged 49.32% while the natural Arabicas averaged 48.02%. Based on the variation in the methodology, the difference in yields between washed Arabicas and natural Arabicas does not appear to be meaningful. The washed Arabicas and the natural Arabicas can be further stratified by the country of origin as shown in Graphs 3 and 4.

Graph 3



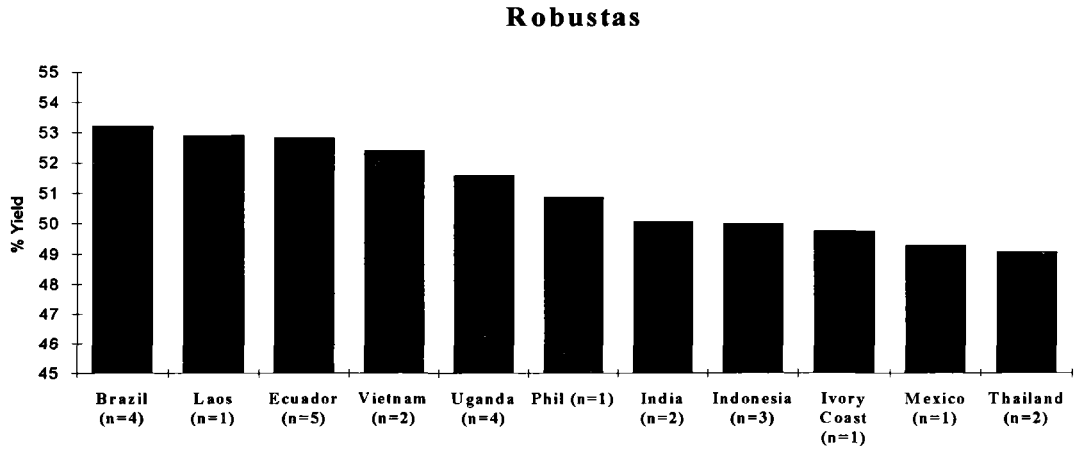
Graph 4



For the washed Arabicas, the highest yield was seen for the coffees from Guatemala averaging 51.41% with a sample size of 5. The lowest yield was seen on the Costa Rica samples (n=2) which averaged 47.32%. The difference in yield between the various washed Arabicas appears to me important and needs to be taken into consideration when sourcing this type of coffee for an instant process. For the natural Arabicas, only three countries were studied. Brazil Arabicas had the highest yield with an average of 48.12% followed by Mexico at 48.05% and Salvador at 47.39%. These differences are judged to not be meaningful.

The Robusta samples can be broken out by country as shown in Graph 5.

Graph 5

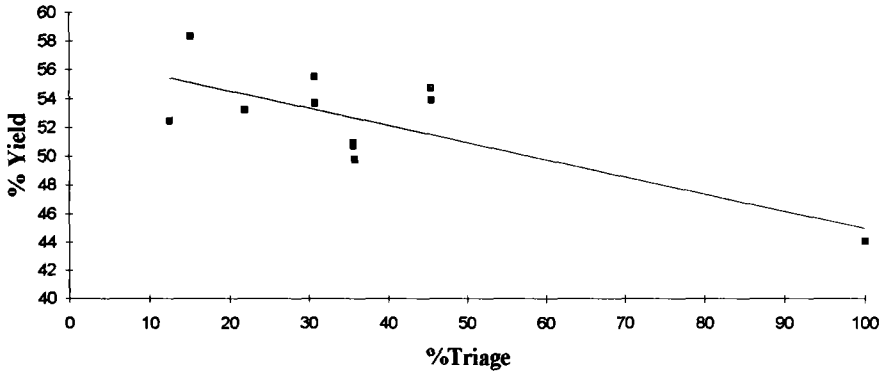


The Conilon Robusta from Brazil gave the highest yield at 53.22%. In other studies that we have conducted, we have found that the Conilon Robusta consistently gives one of the highest yields of all of the Robustas. Robustas from Laos, Ecuador, and Vietnam gave yields which averaged above 52%. On the low end, the Robustas from Indonesia, Ivory Coast, Mexico, and Thailand gave yields which averaged below 50%.

In order to better understand some of the reasons why the various Robusta coffees would give different yields, a study was conducted on Indonesian Robusta coffee where the level of triage, or defects, was varied from 12.5% by weight up to 45%. Additionally, a sample of pure triage that represented 100% coffee defects was roasted and extracted. The resulting extraction yield was plotted versus the level of triage and a linear regression was performed on the data. The results of this study are shown in Graph 6.

Graph 6

Total Dry Extraction Yield vs % Triage - Indonesian Robusta



The equation for the linear regression is: Yield = (-0.1190)*(% Triage) + 56.91. The regression fit is .57 indicating that 57% of the yield variation observed in the data can be explained by the variation in the level of triage. The negative slope for this regression analysis indicates that for every 10% increase in the level of defects in a green coffee sample, one could expect to see a yield decrease of approximately 1%.

The relationship between the extraction yield and the level of triage in the green coffee is important when doing an economic evaluation of a coffee for use in an instant coffee process. For.example, in deciding between two coffees to purchase, the cheaper of the two coffees may in fact result in a more expensive instant coffee if the overall green yield for that coffee is, on a percentage basis, poorer than the price difference. A coffee which results in a 5% lower green yield would need to be priced 5% lower than the coffee it is being compared to. The following expression can be used to make this comparison:

if (P2/Y2) < (P1/Y1), then coffee 2 is a better overall value

where P is the price of the coffee (1 or 2)
and Y is the yield of the coffee (1 or 2)

SUMMARY

The extraction yield obtained in an instant coffee operation can be influenced by a number of variables. Of those variables, the type of green coffee used, the country from which it is sourced, and the level of triage in the coffee can all have an impact on the extraction yield that will be obtained. By no means are the numbers stated in this report definitive. Depending on the type of extraction system being used, and the operating conditions for that system, the effect of the green coffee variables on extraction yield may be significant or negligible. The important point to keep in mind is that the type of green coffee being used is another variable that must be considered in the economics of an instant coffee process.

RÉSUMÉ

Le taux d'extraction obtenu lors de la fabrication de café instantané peut être influencé par plusieurs facteurs. Le genre de café vert, le pays d'origine et le niveau de triage peuvent tous avoir un effet sur le taux d'extraction obtenu. (Les chiffres sur ce rapport ne sont pas définitifs.)

Selon le système et les conditions d'extraction utilisés, l'effet de ces différents facteurs peut être important ou négligeable. Ce qu'il faut retenir, c'est le fait que le café vert utilisé est l'un des facteurs qui peut avoir un effet sur les coûts de production du café instantané.

CHLOROGENIC ACIDS AND CAFFEINE CONTENTS OF MONSOONED INDIAN ARABICA AND ROBUSTA COFFEES COMPARED WITH WET AND DRY PROCESSED COFFEES FROM THE SAME GEOGRAPHIC AREA

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INTRODUCTION

In India, the two commercial varieties of coffee viz; Arabica coffee from the species ***Coffea arabica*** Linnaeus and Robusta coffee from ***Coffea canephora*** Pierre ex Froehner, are mainly grown in the southern states of India i.e. Karnataka, Kerala and Tamilnadu. Arabica and Robusta coffees may be prepared by either of two methods, namely **wet process** and **dry process**. In the wet process, the cherries undergo pulping, fermentation, washing and drying at estate level, and further drying, peeling, polishing and grading for size at the curing works. The graded beans are then subjected to garbling/sorting and cleaning to achieve the Indian standard specification. Arabica parchment coffee is designated as Plantation coffee and Robusta washed coffee is termed Robusta parchment coffee.

In dry processing the berries are harvested, sun dried till the outer skin, the parchment cover and the pulp between dry up to form a thick husk leaving the beans loose inside. The dried cherry is then decorticated by passing through a huller, graded and sorted according to prescribed standard. The final product of this process is known as cherry/unwashed/dry-processed/natural coffee. The parchment and cherry coffees are graded for trade at curing works as per the size of beans such as A, B, AB, C and so on (*Anon.*, 1983).

A specialty of Indian coffee is the monsooned coffee which is in good demand in Scandinavian countries. This coffee is specially prepared to cater to the special needs of buyers. Monsooned coffee is prepared in west coast of India during monsoon season. This process uses only A grades of whole-crop cherry or AB grades of Arabica and Robusta coffees which have been exposed to a humid atmosphere, causing them to absorb moisture up to 15-16 %. This results in swelling to one and half times the normal size of cherry beans and a change in colour to pale white or golden/light brown. These swollen coffees are then polished through hullers, graded and garbled by sorter. Through monsooning the dry processed coffee acquires a special neutral mellow flavour. The grades which are specially sought after in the International market are Monsooned Arabica/Monsooned Malabar AA and Monsooned Robusta AA In the International market there

is a high demand for Indian plantation coffee, washed and unwashed Robusta coffees for use in the best blends.

In the study reported here, the contents of caffeine and individual chlorogenic acids have been determined in the three major types of processed arabicas and robustas from the same growing region in India.

MATERIALS AND METHODS

The raw/green coffee samples of commercial grades (flat beans) of wet, dry and monsoon processed Indian Arabica and Robusta were kindly supplied by Consolidated Coffee Limited, Pollibetta, Kodagu, Karnataka, India. These were:

Arabica coffee

1) Wet-Processed/washed Arabica: Plantation-A (fig. 1a).
2) Dry-processed/ unwashed Arabica: Arabica Cherry-AB (fig. 1b).
3) Monsooned Arabica: Monsooned Malabar-AA (fig. 1c).

Robusta coffee

1) Wet-processed/washed Robusta: Robusta Parchment-AB (fig. 2 a).
2) Dry-processed/unwashed Robusta: Robusta Cherry-AB (fig. 2b).
3) Monsooned Robusta-AA (fig. 2c).

Standard 5-CQA (Caffeoylquinic acid), caffeine, caffeic acid and triflouroacetic acid (TFA) were obtained from Sigma Chemical Company Ltd. Poole, Dorset, U.K. Methanol and acetonitrile of HPLC grade were obtained from Fisons Ltd. Loughborough, U.K. Water means distilled water unless otherwise specified. All other reagents were standard items from reputable commercial sources.

Carrez reagent A was prepared by dissolving 21.9 g zinc acetate dihydrate in water containing 3.0 g of glacial acetic acid and diluting to 100 ml with water. Carrez reagent B was prepared by dissolving 10.6 g of potassium ferrocyanide trihydrate in 100 ml water. These reagents were stored at -4°C.

Calibration standards of caffeine and 5-CQA were prepared as necessary in 70% v/v aqueous methanol

EXTRACTION: Extracts of each green coffee sample were prepared in triplicate by refluxing 0.5g ground bean in 70% (v/v) methanol (4 x 25 ml, 25 min each) using a Tecator Soxtec HT-1043 continuous extraction unit as described in authors' earlier research paper *(Balyaya & Clifford,* 1995).

HPLC analysis: A Spectra physics P-4000 gradient pump coupled to a Spectra physics AS-3000 auto sampler and a Spectrafocus forward optical scanning detector were used in conjunction with a 250 × 4·6 mm id column packed with Spherisorb 5ODS 2 (fully capped with 5 μm reverse phase packing material having a very low level of residual silanol groups) supplied by Hichrom (Theale, Berkshire, UK). Chromatographic and spectral data were collected and integrated using Spectra focus software on IBM PS/2 computer. Sample/ standard 5-CQA and caffeine (aqueous methanolic 70 % v/v) 20 μl each was analysed using a linear gradient from 100 % solvent A (aqueous 0·5% triflouroacetic acid) to 100 % solvent B (45 % acetonitrile in 0·5 % aqueous TFA) in 56 minutes at a flow rate of 1 ml/min as this gradient provides good resolution of peaks. Peaks were detected at 280 nm for caffeine and 315 nm for chlorogenic acids. As required, UV spectra were recorded between 200 and 360 nm. A specimen chromatogram of 70 % methanolic extract of Indian Monsooned Arabica and Robusta green coffee AA grade are shown in Fig.3 and 4 , respectively.

Fig.1 a **Plantation A**

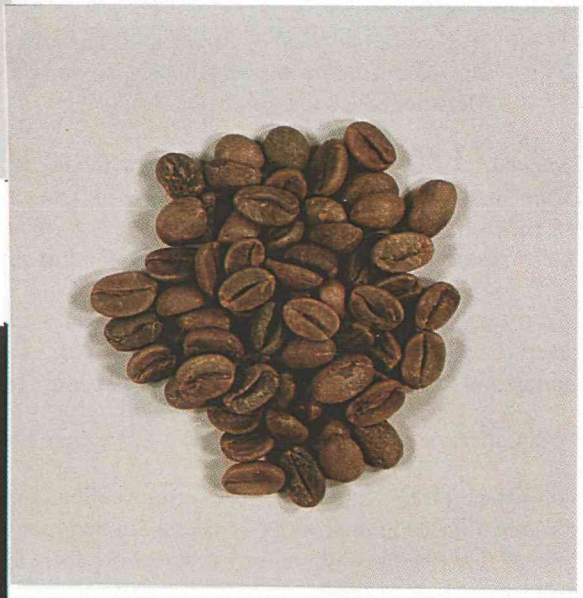

Fig. 1 b **Arabica Cherry AB**

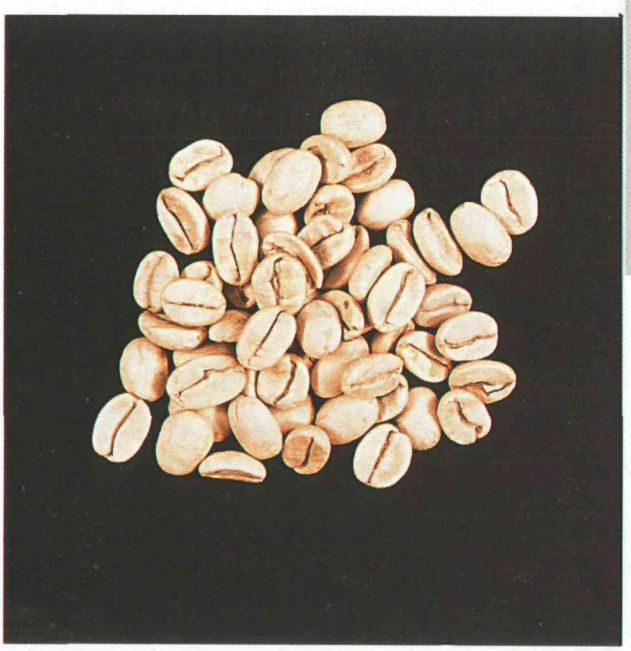

Fig. 1 c **Monsooned Malabar AA**

Fig. 2 a **Robusta Parchment AB**

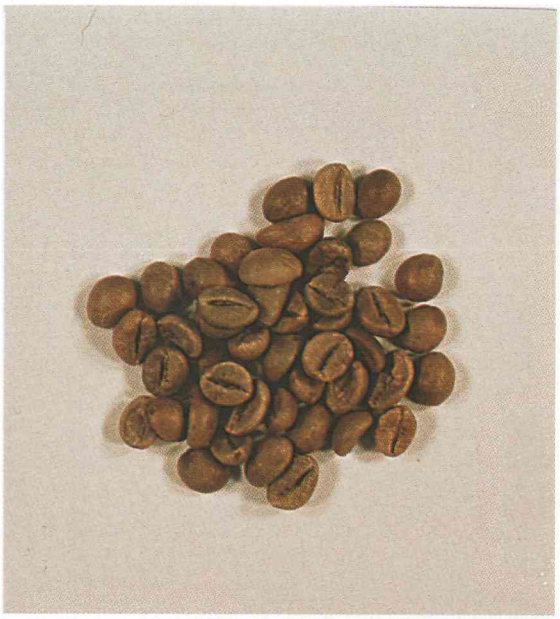

Fig. 2 b **Robusta Cherry AB**

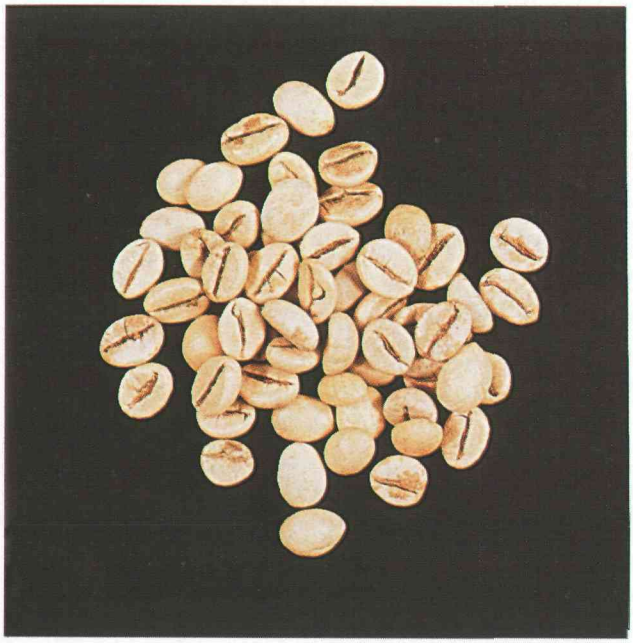

Fig. 2 c **Monsooned Robusta AA**

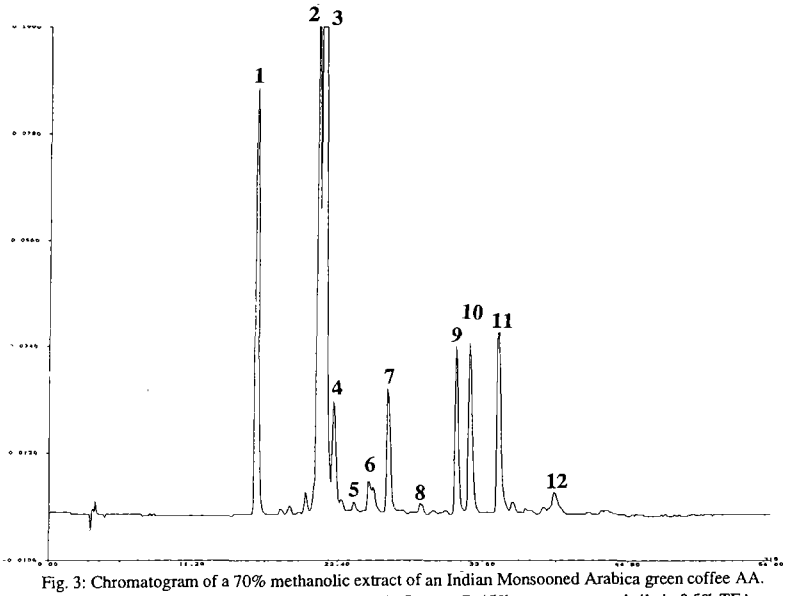

Fig. 3: Chromatogram of a 70% methanolic extract of an Indian Monsooned Arabica green coffee AA. Chromatograhic conditions: Solvent A 0·5% TFA; Solvent B 45% aqueous acetonitrile in 0·5% TFA; gradient 100% A to 100% B linearly in 56minutes; column 25 cm × 4.6 mm packed with Spherisorb ODS2 5µ; 20µl injection; detection at 315 nm. Peaks: 1 = 3-CQA; 2 = 4-CQA; 3 = 5-CQA; 4 = Caffeic acid; 5 = 5-*p*-CoQA; 6 = 4-FQA; 7 = 5-FQA; 8= unknown 1; 9 = 3,5-diCQA; 10 = 3,5-diCQA; 11 = 4,5-diCQA; 12 = unknown 2.

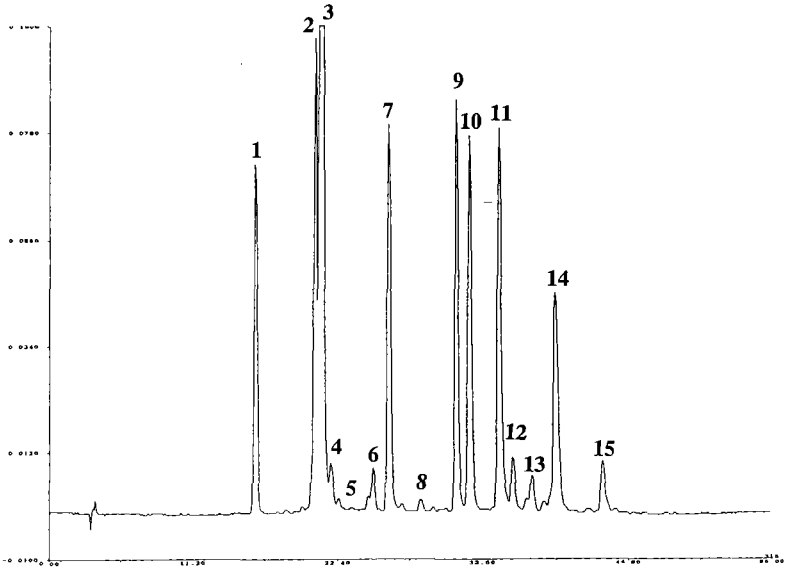

Fig. 4: Chromatogram of a 70% methanolic extract of an Indian Monsoonesd Robusta green coffee AA; detection at 315 nm. Peaks: 1 = 3-CQA; 2 = 4-CQA; 3= 5-CQA; 4= Caffeic acid; 5 = 5-*p*-CoQA; 6 = 4-FQA; 7 = 5-FQA; 8 = unknown 1; 9 = 3,5- diCQA; 10 = 3,5- diCQA; 11 = 4,5 -diCQA; 12 = 3C,4FQA + 3F,4CQA; 13 = 3c,5FQA + 3F,5CQA; 14 = Caffeoyl-tryptpphan with 4C,5FQA + 4F,5CQA on leading edge; 15 = unknown 2.

Quantification of individual chlorogenic acids and caffeine was by peak areas comparison with standard 5-CQA and caffeine calibrations respectively. The simple regression equation was used for the CGA without correction for individual response factors, as described by *Balyaya & Clifford* (1995).

Moisture content: Duplicate 0·5 g samples of each ground sample of green beans were dried to constant weight at 105 °C in vacuum oven.

RESULTS AND DISCUSSION

Moisture contents

The mean moisture contents (% wt/wt) of three processed commercial grades of Indian Arabica and Robusta green coffees are given below:

Indian Arabica coffee

Washed (wet-processed): Plantation A = 7·80 %

Dry-processed (unwashed): Cherry AB = 6·50 %

Monsooned Malabar AA = 7·27 %

Indian Robusta coffee

Wet-processed (washed): Parchment AB = 7·80 %

Dry-processed (unwashed): Cherry AB = 7·50 %

Monsooned AA = 7·37 %

The moisture content of these green coffees are commensurate with good commercial practice (Clarke, 1985).

Chlorogenic Acids and Caffeine Contents

Comparative data for the contents of individual CGA and caffeine (% dry basis) of wet, dry and monsoon processed Arabica green coffees and similar processed Robusta green coffees are shown in Tables 1 and 2 respectively.

In the authors' earlier study, between-replicate variation was quantified at not more than 6 % of the mean for the quantitatively major CGA and not more than 30 % for the minor CGA.

The washed Arabica coffee showed lower level of total chlorogenic acids than dry processed Arabica coffee. The higher level of total CQA, FQA and diCQA contents in Arabica Cherry AB may be due to the presence of beans from ripe, unripe and over ripe cherries whereas only mature cherries are harvested for wet processing. There were no significant differences in caffeine content between wet and dry processed Arabica coffee samples.

TABLE 1: THE CONTENT OF INDIVIDUAL CHLOROGENIC ACIDS AND CAFFEINE (%DRY BASIS) IN INDIAN WASHED, *UNWASHED AND MONSOONED ARABICA GREEN COFFEES OF COMMERCIAL GRADE*

Compound Quantified	**Wet- Processed Arabica coffee (Plantation A) Mean (n=3)**	**SD**	**Dry-processed Arabica coffee (Arabica Cherry AB) Mean (n=3)**	**SD**	**Monsooned Arabica coffee (Monsooned Malabar AA) Mean (n=3)**	**SD**
3- CQA	0·34	0·01	0·40	0·004	0·63	0·003
4-CQA	0·44	0·01	0·50	0·004	0·71	0·01
5-CQA	3·64	0·01	4·23	0·01	4·19	0·01
CQA Subtotal	**4·41**	**0·03**	**5·12**	**0·01**	**5·54**	**0·02**
Caffeic acid	**0·06**	**0·01**	**0·08**	**0·01**	**0·19**	**0·01**
5-*p* CoQA	0·05	0·004	0·07	0·01	0·05	0·002
4-FQA	0·06	0·003	0·05	0·004	0·05	0·001
5-FQA	0·33	0·004	0·35	0·004	0·26	0·002
FQA Subtotal	**0·39**	**0·01**	**0·40**	**0·000**	**0·31**	**0·004**
Unknown 1	0·04	0·001	0·04	0·004	0·02	0·001
3,4-diCQA	0·15	0·01	0·18	0·001	0·24	0·001
3,5-diCQA	0·41	0.01	0·37	0·004	0·30	0·01
4,5diCQA	0·21	0·01	0·27	0·001	0·34	0·002
DiCQA Subtotal	0·78	**0·02**	**0·82**	**0·004**	**0·87**	**0·01**
3C,4FQA+3F,4CQA						
3C,5FQA+3F,5CQA						
CFQA+FCQA Subtotal						
Caffeoyl-Tryptophan						
Unknown 2	0.08	0.003	0.07	0·00	0·07	0·001
Total CGA	***5·81***	***0·05***	***6·60***	***0·03***	***7·05***	***0·04***
Caffeine	**1·27**	**0·014**	**1·51**	**0·014**	**1·52**	**0·05**

TABLE 2: THE CONTENT OF INDIVIDUAL CHLOROGENIC ACIDS AND CAFFEINE (% DRY BASIS) IN INDIAN WASHED, UNWASHED AND MONSOONED ROBUSTA GREEN COFFEES OF COMMERCIAL GRADE

Compound Quantified	Wet-Processed Robusta coffee (Robusta Parchment AB) Mean (n=3)	SD	Dry-Processed Robusta coffee (Robusta Cherry AB) Mean (n=3)	SD	Monsooned Robusta coffee (Monsooned Robusta AA) Mean (n=3)	SD
3-CQA	0·45	0·01	0·35	0·01	0·64	0·004
4-CQA	0·59	0·01	0·47	0·03	0·74	0·01
5-CQA	4·64	0.03	3·29	0·07	4·50	0·010
CQA Subtotal	**5·68**	**0·03**	**4·11**	**0·08**	**5·88**	**0·02**
Caffeic acid	**0·08**	**0·01**	**0·06**	**0·01**	**0·12**	**0·02**
5-*p* CoQA	0·05	0·001	0·04	0·003	0·02	0·002
4-FQA	0·10	0·01	0·10	0·002	0·09	0·004
5-FQA	0·90	0·02	0·85	0·03	0·69	0·004
FQA Subtotal	**1·00**	**0·02**	**0·95**	**0·02**	**0·78**	**0·004**
Unknown 1	0·08	0·01	0·09	0·01	0·02	0·01
3,4-diCQA	0·52	0·003	0·41	0·01	0·71	0·01
3,5-diCQA	0·76	0·02	0·60	0·01	0·71	0·01
4,5-diCQA	0·50	0·01	0·45	0·01	0·84	0·01
DiCQA Subtotal	**1·79**	**0·03**	**1·45**	**0·02**	**2·26**	**0·01**
3C,4FQA+3F,4CQA	0·13	0·004	0·15	0·01	0·13	0·004
3C, 5FQA+3F,5CQA	0·13	0·006	0·12	0·004	0·08	0·01
CFQA+FCQA Subtotal	**0·27**	**0·01**	**0·26**	**0·01**	**0·21**	**0·01**
Caffeoyl-Tryptophan	**0·55**	**0·004**	**0·45**	**0·01**	**0·57**	**0·003**
Unknown 2	0·18	0·001	0·14	0·002	0·12	0·001
Total CGA	***9·68***	***0·05***	***7·55***	***0·09***	***9·99***	***0·06***
Caffeine	**2·99**	**0·03**	**2·90**	**0·001**	**2·52**	**0·06**

Monsooned Arabica coffee showed higher contents of total CQA and diCQA, and thus total CGA compared with unwashed and washed Arabica coffees. However, from table 1, it is clear that there was a lower content of FQA in monsooned coffee than in wet or dry processed Arabica coffees. It is also clear from table 1 that Monsooned Arabica coffee had a higher content of **caffeic acid** than wet or dry processed Arabica coffees. This seems to be the most significant compositional difference characterising the monsooned coffee.

Previous studies of *Clifford and Kazi* (1987) have shown that there is an increase in the CQA : diCQA ratio which seems to be a general feature of the final 5-6 weeks of seed maturation. *De Menezes* (1994) reported similarly that there was a statistically significant association between the CQA : diCQA ratio and the level of maturity in samples of *Coffea arabica* cv vermelho prepared by both dry and wet processing methods.

It is generally accepted that the quality of Indian Arabica coffee is rated as: Monsooned arabica coffee > Plantation coffee > Arabica cherry coffee.

It is possible that the higher levels of CQA, diCQA and thus total CGA, and the greater content of caffeic acid in monsooned Arabica coffee may be due in some way to the method of preparation and contribute directly or indirectly to the quality of the beverage, in particular the neutral mellow flavour.

Mellow A balanced coffee whose basic organoleptic characteristics are just at the right level, with none particularly apparent, giving an impression of roundness, and reflecting a harmonious balance in the strength/body - not too acid, not too bitter but dense and rich flavour as defined by *Lingle* (1988).

However there were no significance difference in caffeine contents between dry, monsooned and wet processed Arabica coffees.

The equivalent data for wet, dry and monsoon processed green robusta coffees are presented in table 2. The wet processed (AB) grade of Robusta coffee had a higher level of 5-CQA and 3,4-diCQA (both $p < .0{\cdot}05$) total CQA, 3,5-diCQA and total CGA ($p < 0{\cdot}01$) than the dry processed Robusta cherry AB coffee beans. There were no significant difference in total FQA and caffeine contents between these two samples of coffee. As was seen for Monsooned Arabica cherry coffee there is a lower level of total FQA content found in Monsooned Robusta cherry coffees than in wet or dry processed Robustas ($P > 0{\cdot}05$). There were also significant differences in contents of total CQA, caffeic acid, total diCQA and total CGA ($p < 0{\cdot}01$) in Monsooned Robusta coffees compared with Robusta Cherry AB coffee. The Monsoon Robusta AA coffees had greater content of 3-CQA, caffeic acid, ($P > 0{\cdot}01$) and lesser total FQA ($p < 0{\cdot}05$) than Robusta Parchment AB coffee. There were no statistically significant differences in the contents of caffeine, total CFQA or Caffeoyl-tryptophan between the three Robusta coffee samples even at the 5 % level. Comparatively, Monsooned Robusta AA coffee had higher level of total CQA, Caffeic acid, total diCQA and greater total CGA content despite a lower FQA content compared with Robusta Parchment and Cherry AB coffees.

Conclusion

Monsooned arabica and robusta coffees differ significantly in composition compared with dry and wet processed coffees produced in the same region.

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DETERMINATION OF THE PARTITION COEFFICIENTS OF COFFEE VOLATILES USING STATIC HEADSPACE

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Summary

Air/water " K_v (w) " , air/coffee oil " K_v (o) " and oil/water " K_p (o/w) " partition coefficients of volatiles were determined using static headspace analysis. Three different methods are described, their advantages and limitations discussed. Examples are given for standard solutions or more complex coffee aroma condensates.

Résumé

Les coefficients de partage de composés volatils entre l'air et l'eau " K_v (w) ", l'air et l'huile " K_v (o) ", et l'huile et l'eau " K_p (o/w) " ont été déterminés par espace de tête statique. Trois approches différentes sont décrites, leurs avantages et limites sont discutés. L'application à des solutions standards ou à des mélanges plus complexes, tels des condensats d'arômes de café est présentée.

1. Introduction

The aroma perceived from coffee samples depends on partition coefficients of volatiles between the matrix and air. Very little data have been published for components present in coffee aroma. *R.G. Buttery et al. (1969) and G. Mazza (1980)* have used the combination of static headspace and liquid injection to calculate partition coefficients in air/water systems. The application of static headspace using external calibration has been described by *P.E. Nelson (1968), B. Kolb (1992)) and J.Bakker et al. (1994).*

Recently, *D. Stuart (1993)* reported a way to obtain partition coefficients without calibration. Our purpose was, like for Stuart, to find simple and reliable methods to determine K_v or K_p without calibration. The partition coefficients could be estimated then independently of the initial concentration of volatiles in the matrix of interest.

2. Theory

Henry's low gives the relation between the molar fraction of a solute x_i and its partial pressure above the solution P_i : $\mathbf{P_i = H_i \times x_i}$ with $\mathbf{H_i = P_0 \times \gamma_i}$, P_0 is defined as the vapour pressure of the pure compound and γ_i as its activity coefficient. The Henry's equation can be rewritten for concentrations instead of pressure: $\mathbf{C_g = K_v \times C_l}$ ("g"for gas and "l"for liquid, K_v proportional to H_i). C_g is correlated with peak area measured by headspace.

In this publication, we are going to consider different **partition coefficients K** :

system air (g) / water (w)	$\mathbf{K_v (w) = C_g / C_w}$
system air (g) / coffee oil (o)	$\mathbf{K_v (o) = C_g / C_o}$
system coffee oil (o) / water (w)	$\mathbf{K_p (o/w) = C_o / C_w = K_v(w) / K_v(o)}$

Two different methods are proposed to determine K_v, and one method is described to calculate K_p when K_v are not available.

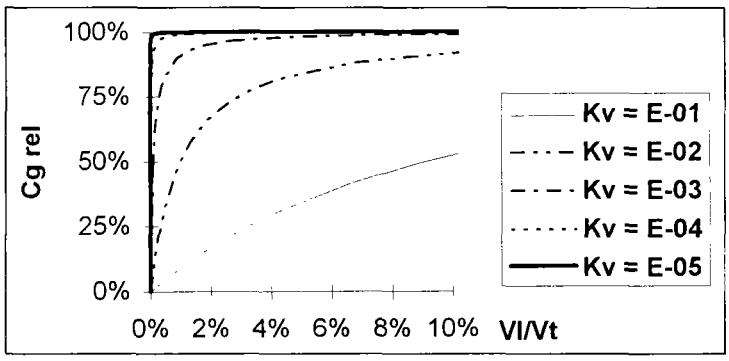
2.1 Variable filling ratio method (a)

C_g is measured above an **increasing amount V_l** of solution having a **constant concentration C_t**. The total volume is kept constant $V_t = V_g+V_l$ but the mass of compound is variable $M_t = (C_g \times V_g) + (C_l \times V_l)$. C_g can be expressed by the equation:

$C_g = M_t / \{V_g + (V_l / K_v)\}$ then $1/C_g$ is a linear equation

$\mathbf{1/C_g = a + (b/V_l)}$ where $\mathbf{a = \{1/(K_v \times C_t)\} - 1/C_t}$ and $\mathbf{b = V_t/C_t}$

This method allows to use linear regression to calculate K_v . For low K_v, it requires small V_l/V_t ratio, meaning a precise determination of V_t , and a precise addition of small liquid volume V_l in the container. The dependence of C_g with filling ratio is shown in Figure 1.



The variable filling ratio method developed in our lab in 1990 (unpublished results) is very similar to the method described by *D. Stuart (1993)*. Lower filling ratios considered here allowed us to determine low volatility coefficients.

Figure 1: Gas concentration (C_g rel) as a function of filling ratio (V_l / V_t)

2.2 Dilution method (b)

C_g is measured for the **same mass of compounds at different dilution**. The global mass M_t and the total volume V_t are kept constant, but the liquid volume is variable.

$$K_v = \frac{\{(C_{g1} \times V_{l1}) - (C_{g2} \times V_{l2})\}}{\{(C_{g2} \times V_{g2}) - (C_{g1} \times V_{g1})\}}$$

The dilution factor should be at least of 1 to 5. Low K_v requires dilution of 1 to 100 as it can be seen in Figure 2.

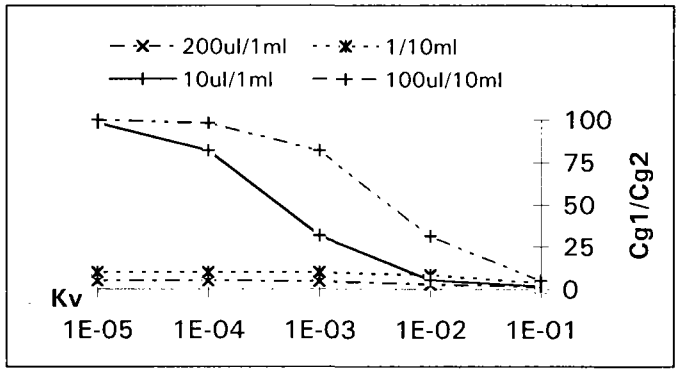


Figure 2: Variation of C_{g1}/C_{g2} ratio as a function of K_v and dilution factor.

2.3 Two solvents method (c)

C_g is measured above a **set of three solutions** of **well defined volumes V_l**, the **global mass of compound M_t being constant** in the three solutions.

Solvent A + solute: $V_{A1} = V_{A2} = V_{A3}$

Solvent B (free of solute) $V_{B1} = 0$ $V_{B2} = 0.5 \times V_{A1}$ $V_{B3} = V_{A1}$

The concentration in the gas phase for solution 1 and 2 or 3, can be expressed as:

$$C_{g1} = M_t / \{V_{g1} + (V_{A1}/K_{vA})\}$$

$$C_{gi} = M_t / \{V_{gi} + (V_{Ai}/(K_{p(B/A)} \times K_{vB})) + (V_{Bi}/K_{vB})\} \quad \text{with } \mathbf{i = 2 \text{ or } 3}$$

Assuming the activity is constant and $M_g << M_l$, the equations above can be simplified and partition coefficient derived:

$$K_{p(B/A)} = 2 \times (C_{g1}-C_{g2})/C_{g2} \quad \text{or} \quad K_{p(B/A)} = (C_{g1}-C_{g3})/C_{g3}$$

As shown in Figure 3, the higher the volatility coefficients K_{vA} and K_{vB}, the higher the error on K_p.

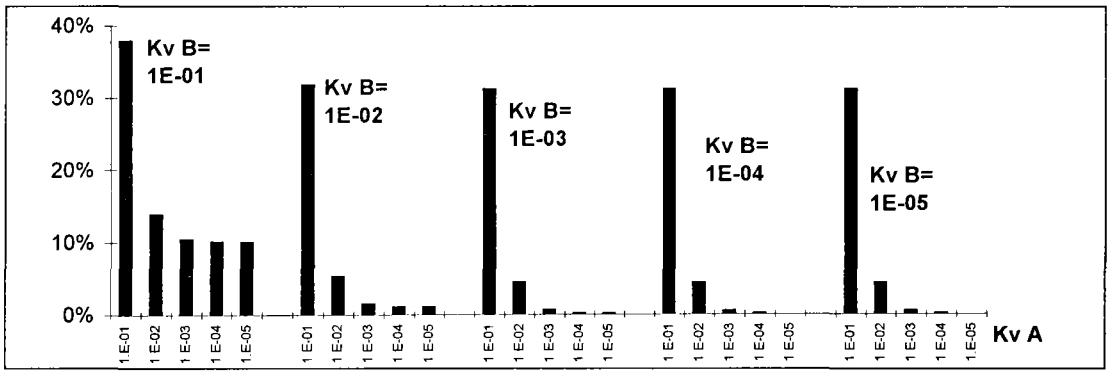


Figure 3 : Error on K_p in percent depending on K_vA and K_vB magnitudes

3. Experimental

The concentrations of standard solutions ranged between 10 and 2000 ppm in water and up to 2% in coffee oil. Samples were conditioned in silanised 22 ml vials (Teflon coated septa) at 30, 40, 60 or 80°C for an equilibration of 2 hours. The automated headspace injections (15-30µl gas injected) were performed either with a Perkin Elmer HS100 (splitless injection) or a Hewlett-Packard 193195A (split injection). Volatiles were eluted on DBWAX column and detected with a flame ionisation detector.

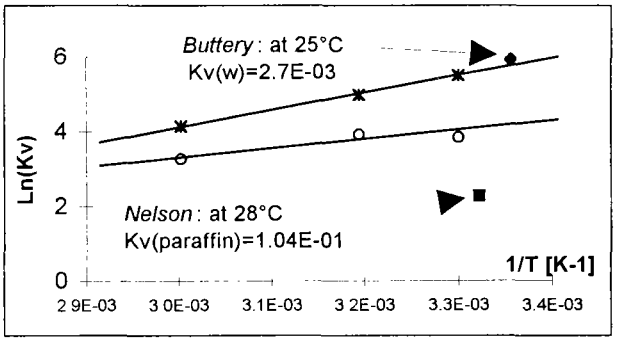
4. Applications

4.1 Acetaldehyde in water or coffee oil

Partition coefficients obtained using variable filling ratio method (a) or dilution method (b) are summarised in the Table 1 and compared with published data in Figure 4:

Table 1 : Partition coefficients for acetaldehyde between 30°C and 60°C for air/water, air/oil and oil/water (method used is in brackets)

Acetaldehyde	T = 30°C (a)	T = 40°C (b)	T = 60°C (b)
K_v (w)	4.17 E-03	7.0 E-03	1.6 E-02
K_v (o)	2.19 E-02	2.0 E-02	3.8 E-02
K_p (o/w)	0.19	0.35	0.42



The expression of $\ln(K_v)$ as a function of 1/T showed a good correlation between our data and those reported by *Buttery et al. (1969).*

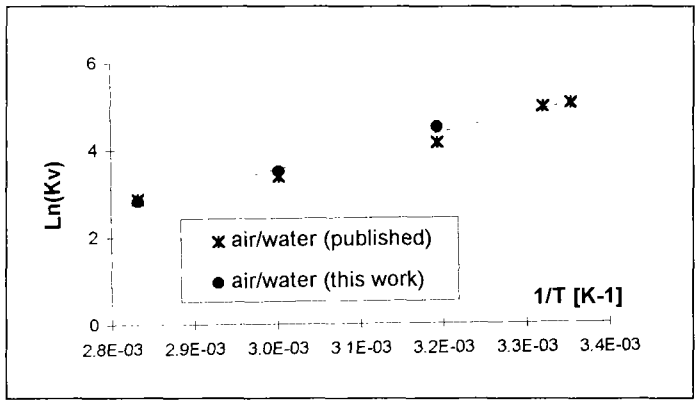
Figure 4 : Relation between $\ln(K_v)$ and 1/T for acetaldehyde in water (*) or in coffee oil (o).

4.2 Ethylacetate in water

C_g was determined using dilution method (b) above four different solutions (0.1ml/1000ppm[1], 1ml/100ppm[2], 5ml/ 20ppm[3] and 10ml/10ppm[4]). K_v was calculated from the pairs 1-2, 1-3, 1-4, 2-3 and 2-4. The precision of the method is reported in Table 2.

Table 2 : Partition coefficient for ethylacetate in air/water system (method b)

Ethylacetate	T = 40°C	T = 60°C	T = 80°C
K_v (w)	1.11 E-02	2.91 E-02	6.00 E-02
+/-	0.36 E-02	0.26 E-02	1.5 E-02



We observed a good fit between our data and those published by Voilley (1993) and Kolb (1992), when reporting $\ln(K_v)$ as a function of 1/T (see Figure 5).

Figure 5 : Dependence of $\ln(K_v)$ with temperature for ethylacetate in water

4.3 Isobutanal in water or coffee oil

The partition of isobutanal between oil and water was compared using dilution method (b) and two solvent method (c), and a good agreement was found. Coefficient values calculated from standard solutions or coffee aroma condensates were close as it is shown in Table 3.

Table 3 : Partition coefficients at 40°C for isobutanal in water, oil and emulsion (method used in brackets)

Isobutanal	K_v (w)	K_v (o)	K_p (o/w)
Standard	2.47 E-02 (b)	6.81 E-03 (b)	3.63 (b) 3.10 (c)
Coffee Aroma	2.20 E-02 (b)	9.10 E-03 (b)	2.42 (b)

4.4 Temperature dependence of K_v for high volatiles

The volatility measured at different temperatures is reported in Table 4. For both aldehydes, K_v increased more in water than in oil. The situation was reversed for the disulphide, suggesting different solubility properties.

Table 4 : K_v values as a function of temperature and solvent

Compound	Solvent	T = 40°C	T = 60°C	T = 80°C
Isobutanal	water	2.34 E-02	7.96 E-02	1.07 E-01
Isobutanal	coffee oil	7.96 E-03	1.64 E-02	2.37 E-02
3-methylbutanal	water	2.67 E-02	6.56 E-02	1.26 E-01
3-methylbutanal	coffee oil	4.10 E-03	7.54 E-03	1.04 E-02
dimethyldisulfide	water	8.89 E-02	1.52 E-01	2.31 E-01
dimethyldisulfide	coffee oil	7.47 E-04	1.79 E-03	5.75 E-03

4.5 Partition coefficients at 60°C for medium volatiles

Some values obtained for important components of coffee aroma are reported in Table 5. The medium volatility of pyrazines could be evidenced in air/water and air/oil systems.

Table 5 : Partition coefficients in water and oil for some important coffee volatiles

Compound	Solvent	K_v (std mixture)	K_v (coffee aroma)	K_p (o/w)
2-methylpyrazine	coffee oil	1.17 E-03 (a)	2.50 E-03 (b)	0.98 (c)
2-methylpyrazine	water		2.80 E-03 (b)	1.12 (b)
2-ethylpyrazine	coffee oil	8.65 E-04 (a)	9.30 E-04 (b)	1.60-2.14 (c)
2-ethylpyrazine	water		5.20 E-03 (b)	5.59 (b)
2,6-dimethylpyrazine	coffee oil	7.92 E-04 (a)		1.19 (c)
trimethylpyrazine	coffee oil	6.05 E-04 (a)		1.92-2.29 (c)
diacetyle	coffee oil	1.03 E-02 (a)	1.20 E-02 (b)	0.55-0.75 (c)
diacetyle	water	7.71 E-03 (a)	6.55 E-03 (b)	
2,3-pentanedione	coffee oil	4.06 E-03 (a)	6.20 E-03 (b)	2.7-3.7 (c)
2,3-pentanedione	water	1.33 E-02 (a)	1.00 E.-02 (b)	
furfural	coffee oil	1.22 E-03 (ab)		1.7-2.8 (c)
furfural	water	1.13 E-03 (ab)		
ethanethiol (<1% O_2)	coffee oil	2.13 E-02 (a)		11.7

5. Conclusion

Three headspace methods have been described allowing the determination of partition coefficients K_v and K_p between volatiles and matrix. The methods proposed did not require gas concentration calibration, but needed multiple analysis. Precision range lied between 10 and 30% and a good linearity was observed between $\ln(K_v)$ and 1/T. Good agreements were found with published data. Very low partition coefficients could not be assessed in this work due to the small volume of headspace injected.

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STUDIES ON VOLATILE COMPOUNDS IN A SERIES OF ROASTED COFFEE BEANS. CHANGES OF THE AMOUNTS OF VOLATILE COMPOUNDS AND TOTAL SULFUR

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1. Introduction

More than 800 volatile components have been identified in roasted coffee beans[1]. Almost all these components are presumed to be formed in roasting process. The changes of volatiles in roasting process were partially described in several papers[2-5].However,there are still many unknown parts in the formation of volatiles in roasting process.In this paper, the changes of volatile components in a series of nine roast steps(Luminosity [L-value]: 38 to 16.8) of coffee beans were investigated using five varieties of Arabica and Robusta coffee beans. The semi-quantitative gas chromatographic(GC) data were obtained from the water brew solvent extraction method and were analyzed by the method of principal component analysis (PCA). Then the results were compared with sensory evaluations of coffee brews.

For the first time as an analytical attempt,the changes of the amounts of total sulfur were examined in roasted coffees by inductively coupled plasma-atomic emission spectrometer (ICP-AES). Generally it is known that 96 identified sulfur components[1],play an important role because of its low threshold in coffee flavor[2,6]. ICP-AES method is usually used in quantitative analysis of heavy metals in water solution. However,there are no papers in which ICP-AES method are applied to analyze sulfur in coffee beans[7].

2. Experimental

Material

All samples of roasted coffee beans were supplied by TAKASAGO COFFEE Co.Ltd.(Tokyo). Four kinds of Arabica (Brazil Santos No.4/5(BR),Colombia Excelso(CO),Guatemala SHB(GU),Mocha Jimma(MO)) and a kind of Robusta(Indonesia AP-1(RO)) were used for analysis. Each coffee was roasted at nine different degrees (L-value: 38.0 , 35.5 , 29.4 , 27.0 , 26.6 , 24.6 , 21.4 , 18.4 , 16.8 ; with an error of about 0.5). All samples were ground (maximum size 1000 μ m) and stored at -30℃ before use.

Extraction

a) The coffee sample (10 g) was extracted with 60 ml of water at room temperature in under stirring for 30 min and filtered.The coffee residue was washed with water (30 ml x 2) .The combined filtrate was extracted with n-pentane/ether=2/1(v/v) (150ml x 2). The organic layer was dried over anhydrous sodium sulfate, concentrated to ca. 30mg by Kuderna-Danish apparatus,and analyzed by GC and GC/MS. Identifications were made by the original library searches based on the comparison with retention times and mass fragments to authentic standards.

b) The coffee sample (2.5 g) was extracted with 10 ml of absolute ethanol at room temperature in stirring for 5 hours. The supernatant was filtered by membrane filter(0.45um), diluted to 5 times with ethanol, and analyzed by ICP-AES.The operating conditions were shown in Table 1 and dimethyl sulfoxide solutions were used as standard samples of sulfur(0 to 20 ppm as S). Here,since non-volatile sulfer compounds could be extracted by absolute ethanol, volatile compounds were removed from the extracts by the molecular distllation and the resulted residues were used as blank value.

3. Results and Discussion

Total Volatile Components

The recovered total amounts of volatile components were showed in Figure 1. The total amount of volatile components reached to maximum (ca. 2500 ppm) at L-value 20 to 18(French to Italian roast) and it seemed there were no relation with the varieties of coffee beans. However, the changes of the amounts of individual components were different as described later. In strong roast,volatile components were decreased based on higher evaporation than formation.

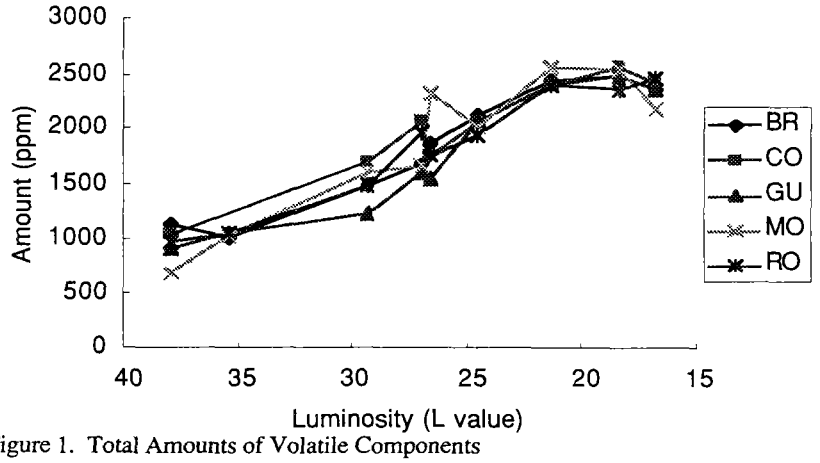

Figure 1. Total Amounts of Volatile Components

Furans

Furans were major volatile components (ca. 40%) in roasted coffee. The total furanaldehydes and furans(excluded aldehydes) were analyzed,and their change patterns were different as shown in Figure 2 and 3. The former reached to maximum (210 to 280 ppm) in high roast (about L 25), while the latter was maximal (810 to 970 ppm) in french roast(about L 20) in Arabica coffees. It was assumed the difference is related to high reactivity of furanaldehydes. Both volatile amounts and change of its were small in every roasting degree in Robusta compared with Arabica. This might be explained based on not only amounts of precursors but also the difference in the way of thermal decomposition of precursors owing to the physical factor of the tissue of beans[8]. These results support that Arabica possess generally more sweet flavor than Robusta.

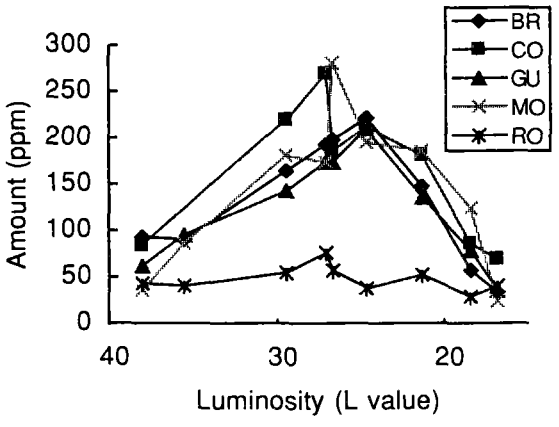

Figure 2. Total Furanaldehydes

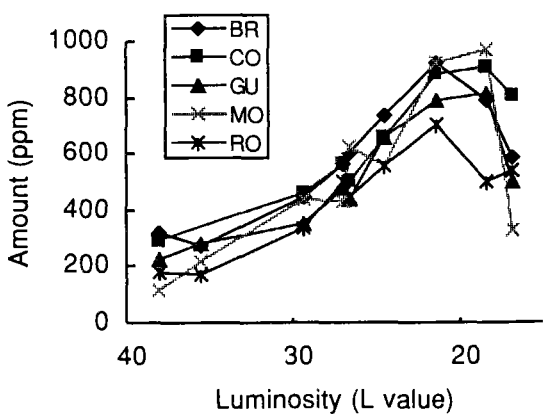

Figure 3. Total Furans(excluded Furanaldehydes)

Acids

The change of volatile amount of acids (Figure 4) was similar to that of furanaldehydes. The change of acetic acid (Figure 5) in the total acids is pronounced in light roasted coffee. It should be resulted by the difference of precursors.

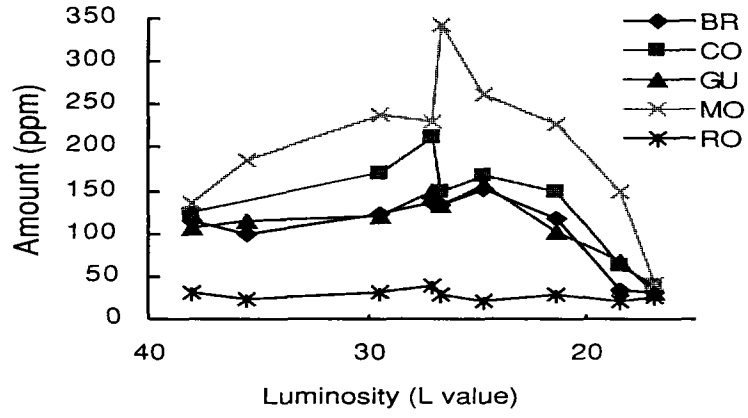

Figure 4. Total Acids

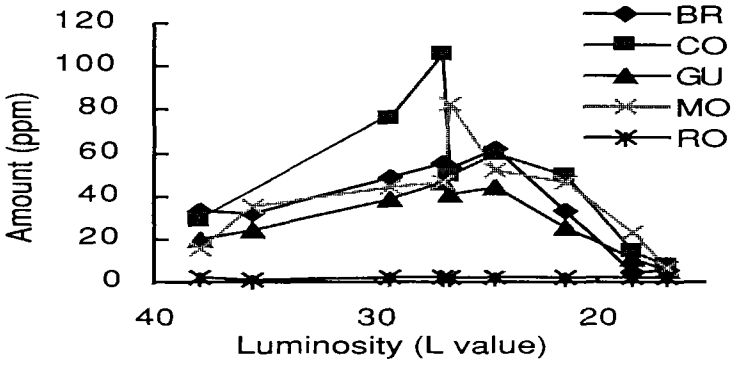

Figure 5. Acetic acid

Pyrazines

It was appeared that pyrazines increased according to the roasting level as shown in Figure 6. However, the changes of amounts of pyrazines was generally smaller than that of other components, and the large amounts of them tend to decrease at less than about L 22. It is known that pyrazines possess roasted nutty,green,cereal and earthy aroma qualities[2]. Then it is said that pyrazines are the body note of roasted coffee. Pyrazines compared with other components are relatively high amounts in light roast ,so pyrazines characters are conspicuous. In opposite, they are relatively low in French roast,so other components characters (i.e. pyrroles and phenols) are conspicuous.

Pyrroles and Pyridines

In Arabica coffees, pyrroles and pyridines increased according to the roasting level (Figure 7) and the increase of pyridine was remarkable in french roast (Figure 8). On the other hand,pyridine decreased in french roast (the maximum amount is ca. 70 ppm) in Robusta. The difference is explained that trigonelline which was precursor of pyridine [9] was contained less in green Robusta than Arabica[3]. As shown in Figure 9, 2-acetylpyrrole was maximum at L 22 (ca. 47 ppm) in Robusta and at L 18 (ca. 50 ppm) in Arabica.

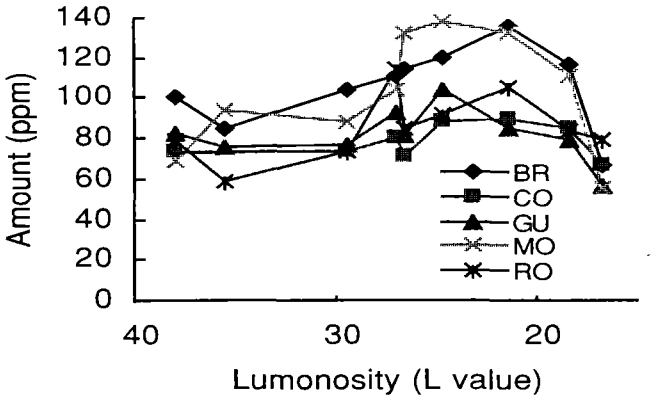

Figure 6. Total Pyrazines

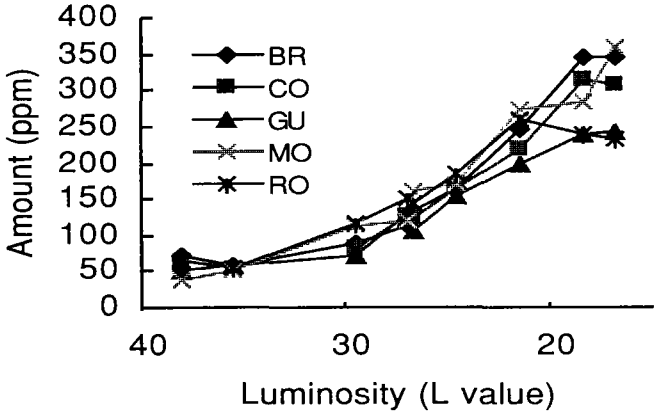

Figure 7. Total Pyrroles(Pyridines)

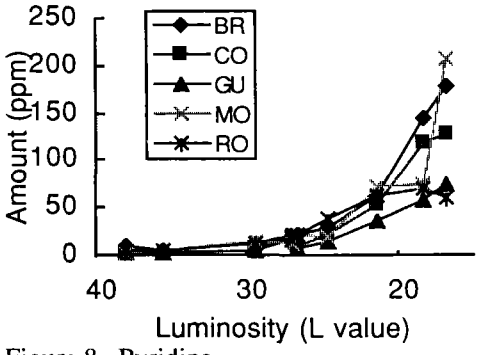

Figure 8. Pyridine

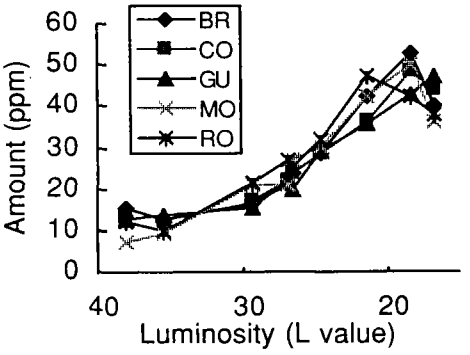

Figure 9. 2-Acetylpyrrole

Phenols

Phenols increased remarkably in french roast(L18) in Arabica,while they increased in high roast(L26) in Robusta(Figure 10). The phenol/guaiacol ratios,on the basis of the sensory characters, increased according to the roasting level in Arabica. These ratios stayed at constant level in Robusta (Figure 11). It was considered that these results were occurred from the difference of compositions of chlorogenic acid which is generally known as precursors of phenol and guaiacol[3].

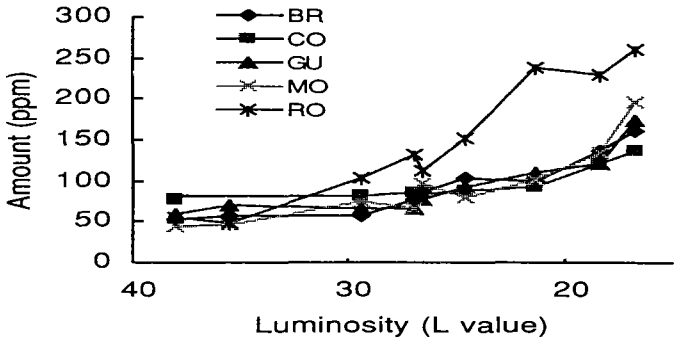

Figure 10. Total Phenols

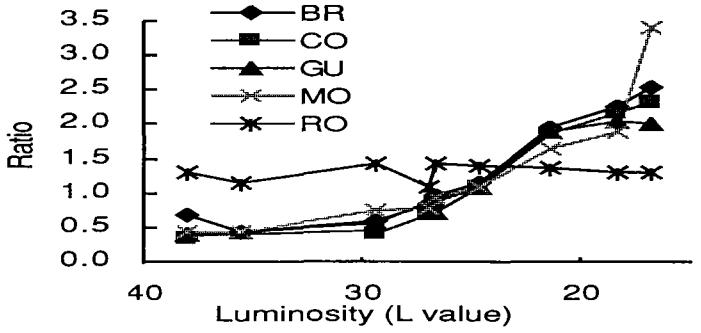

Figure 11. Phenol/Guaiacol Ratio

The Relations between Volatile Components and Sensory Evaluations

As mentioned above, volatile components were divided into seven groups (furanaldehydes,other furan derivatives,acids,pyrroles including pyridines,phenols and others) by the structure. The roast degree was taking alternate roasting (five) steps for simplification and PCA was carried out to observe the relations between components and roasting level. Furanaldehydes(I-axis) and pyrroles (II-axis) were selected. Then, the result was modified to see the relations of the amount between furanaldehydes (X -axis) and pyrroles (Y-axis) as Figure 12. Except Robusta, the amount of furanaldehydes was proportional to that of pyrroles from L 38 to L 26 and the relation was negative from L 26 to L 16. These results indicated that the way of roasting reaction which occurred in the beans was common in each coffee and only the amounts of components (precursors) were different. After sensory evaluations in each brewed coffee, the scores analyzed by PCA and the result showed in Figure 13. I(Y)-axis which indicated the roasting degrees and II(X)-axis which indicated the character of coffee (i.e. Mild,Sourness) were selected. The shape of Figure 13 are nearly agreeable with that of Figure 12. It was suggested that these results might be able to presume the sensory evaluation results by the relations between furanaldehydes and pyrroles in roasted coffee.

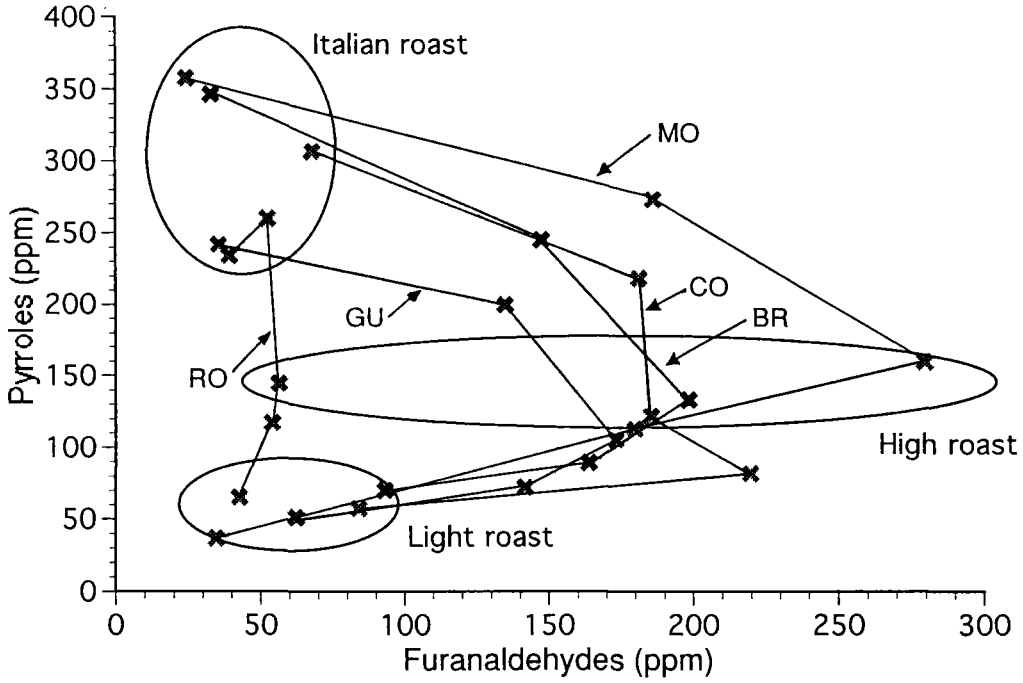

Figure 12. The Relations of the Amounts between Furanaldehydes and Pyrroles

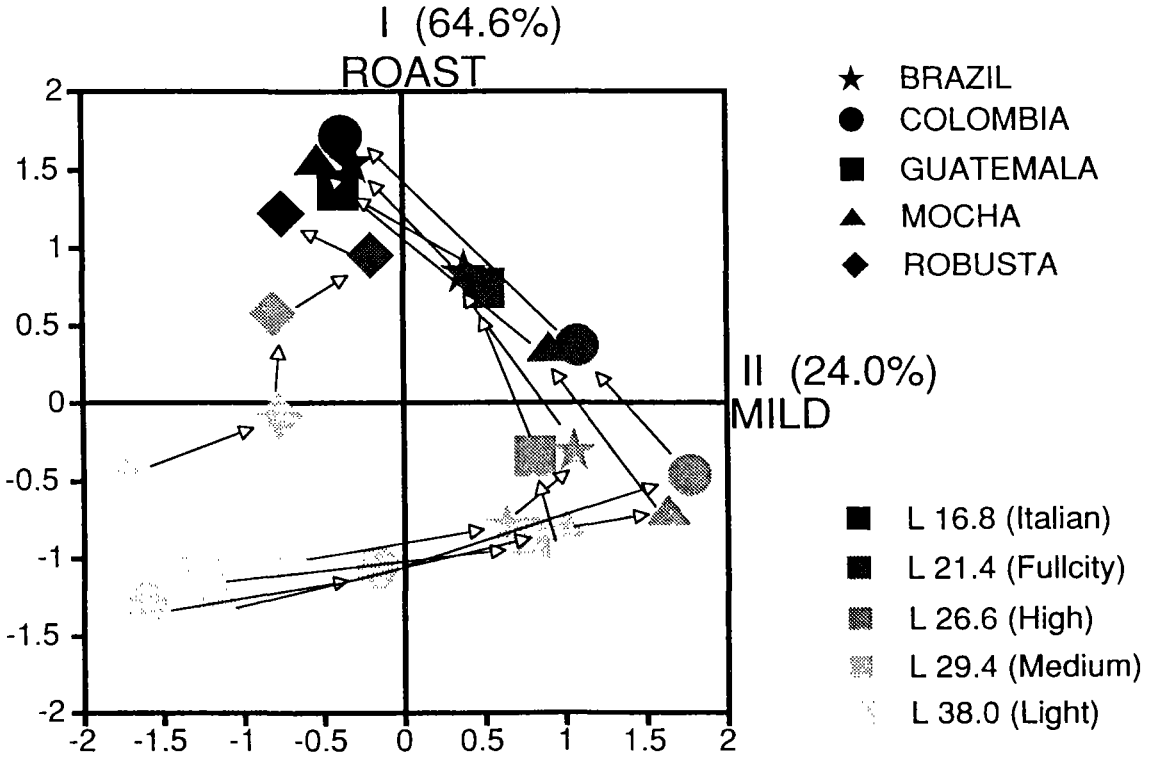

Figure 13. Pattern of Sensory Data, Analyzed by PCA

Total Sulfur

The total amount of sulfur increased according to the roasting(Figure 14). The highest amount(65 ppm at L 16.8) was detected in Robusta and the lowest(25 ppm at L 18.4) in Colombia . The amounts were larger in Robusta than Arabica and its difference was getting larger when the roasted degrees become stronger. It is known that Green Robusta possess more sulfur containing amino acids[10] than Arabica,which are decomposed by roasting. Our experimental results were not conflicting the literatures.

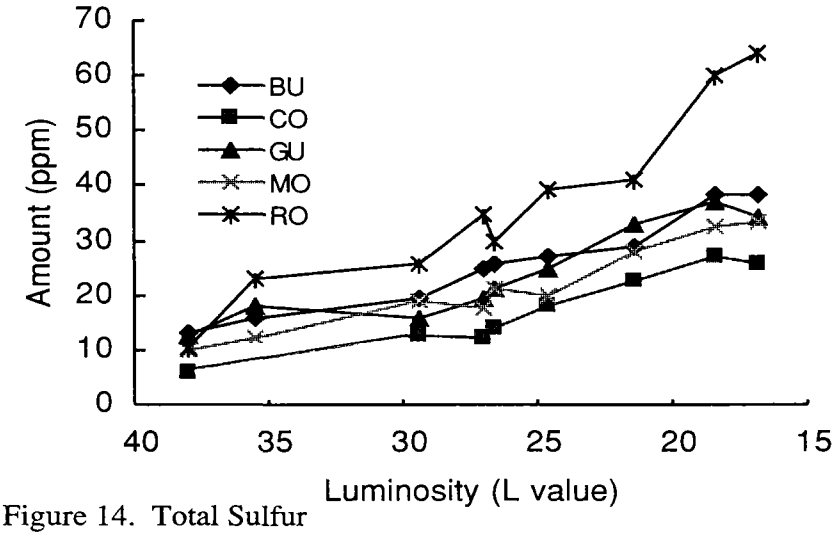

Figure 14. Total Sulfur

Table 1. ICP operating conditions

Instrument	: Seiko Instruments Inc. SPS1500 VR plasma spectrometer
Forward RF power	: 1.76 kW
Analytical line	: 182.02 nm
Integration time	: 5 sec.
Source gas	: argon
Gas flows	: cooling gas 18 l/min, plasma gas 1.5 l/min sample transport gas 0.2 l/min
Standard sample	: dimethyl sulfoxide absolute ethanol solutions (0 to 20 ppm as S)

4. Summary

The changes of volatile compounds in a series of nine roast steps(L-value:38 to 16) of coffee beans were investigated using five varieties of Arabica and Robusta coffee beans. The semi-quantitative GC data were made by water brew solvent extraction method. The total amount of volatile compounds reached to maximum (ca. 2500 ppm) at L-value 20 to 18(French to Italian roast) and, depending upon the kinds of coffee beans, some characteristic differences in volatile compounds were found. For example, the big differences were observed in the amounts of acids and furanaldehydes between Arabica and Robusta . In Arabica coffees, it was recognized that the correlation of the amounts of furanaldehydes and pyrroles in high roast showed the characters of coffee beans (i.e. Mocha,Colombia, Brazil etc.). And this result agreed with that of PCA of sensory evaluations.

For the first time the changes of the total amounts of sulfur were studied in roasted coffees by ICP-AES. The results indicated that total sulfur amounts increased in strong roast and were larger in Robusta compared with Arabica, and its difference was getting larger in strong roast.

Acknowledgment

We are grateful to TAKASAGO COFFEE CO.,LTD.(Tokyo,Japan) for material support and advice.

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HEADSPACE GAS CHROMATOGRAPHIC ANALYSIS AND SENSORY EVALUATION OF VARIOUS DOMESTIC AND FOREIGN-MADE COMMERCIAL ROASTED AND GROUND COFFEES

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1. Introduction

Generally consumed coffee in market may show different taste depends on the roasted and ground (R & G) brands. In this study, the chemical characteristics of the coffee which seemed to determine the taste of coffee, such as pH, titratable acidity (TA), roasting color (RC) and aroma were analyzed and sensory evaluation was conducted.

2. Materials and Methods

1) Materials

Twenty different brands of roasted and ground coffee were used (Table 1).

2) Methods

(1) pH & Titratable Acidity

pH was measured using pH-Meter (Metrohm 636, Titroprocessor, SWISS) and titrant was calculated from the equation, N/10-NaOH, with the end point of 6.28.

(2) Roasting color

Roasting color was determined by using the Photovolt colorimeter (Model 670) with color filter for the R & G coffee (Triamber).

(3) Sensory evaluation

Seven professional panelists were selected. The standard sample was blended with Brazil coffee, Columbian coffee and Robusta coffee at 1:1:1 ratio and medium roasted. The samples were prepared by pouring 100 ml of 95℃ (after leaving boiling water at room temperature for 2 minutes) distilled water into the 130 ml glass container with 5 g of R & G coffee. As soon as the coffee powder was precipitated, the aliquot part was used

as analysis. The evaluation was conducted at the sensory evaluation room at 10:00 am and 2:00 pm. The numbered samples were randomly ordered and distilled water was used for goggling between samples. The evaluation was conducted as described in Takayuki (1981).

(4) Headspace gas chromatograph (HSGC) method

① Ten g of coffee was placed in the 250 ml Erlenmeyer flask and 200 ml of 95℃ distilled water was added. Coffee was brewed for 20 seconds.
② Fifteen ml of the extract was placed in the 25 Headspace vial and 3 % 1-butanol was added as Internal standard. The vials were sealed with septum.
③ Sample was placed in the 65℃ water bath for 30 minutes.
④ Two ml of headspace aroma was taken using gas tight syringe and injected into GC.
⑤ Each sample was analyzed by multiplying the internal factor.

(5) Instrument conditions

The Hewlett Packard 5880 gas chromatography was equipped with flame ionization detector. The Carbopack glass column (6 ft x 1/4 inch O.D.) packed with 0.2% cabowax 1500 on 80/100 mesh was used. Helium was used as carrier gas at a flow rate of 30 ml/min. The temperature program was; 60℃ for 2 min, then raised 5℃ /min until 150℃ was reached. These conditions were hold for 5 minutes. Injector temperature was 150℃ and detector temperature was 230℃ .

3. Results and Discussion

1) pH, TA, Roasting color

As shown in Table 2, with the higher roasting color, lower pH and the higher TA were observed. The average roasting color of Korean brand R & G coffees was 47, compared with the average roasting color of imported R & G coffee was 54.

2) Sensory evaluation

Table 3 shows the results of sensory evaluation. The sourness scale indicates as 0 for the standard (medium sourness), 3 for the strongest sourness and -3 for the weakest sourness. The most sour R & C coffee showed average RC of 54.7, pH of 5.02 and TA of 3.98. The average values of the weakest sour R & C coffee were RC of 34.6, pH of 5.40 and TA of 2.26. The bitterness showed the opposite results compared with the sourness. The bitterness showed higher as the RC was higher. With strong Undesirable bunt notes (UBN, 1 ~ 3), RC was 40 while with weaker UBN (-1 ~ -3), the RC was 56.8. Overall flavor quality (OFQ) indicates the qualified aroma as acale of 1 to 3 and disqualified aroma as scale of -1 thru -3. Those R & G coffee with OFQ of 1 ~ 3 showed the average RC of 54 and OFQ of -1 ~ -3 showed the average RC of 33.7.

3) The characteristics of aroma

The typical head space aroma chromatogram is shown in Figure 1. Tabel 4 is the list of retention time and the molecular structure of the each compound. The compositions of aroma pattern and the chromatogrms of six compounds with high concentration in the R & G coffees are shown in Figure 2-1 thru Figure 2-7. Among the headspace aroma compounds, 2-methyl furan showed the correlation with RC. 2-methyl furan is formed from the pyrolytic separation of coffee components when the coffee bean was roasted. It has a distinct aroma as ether and also related to the harshness. Therefore, the multiple regression analysis was conducted for the relationship between the 2-methyl furan and roasting color;

R square = 0.712

Analysis of variance;

Source	DF	SS	V	F	PROB F (0.01)
Regression	1	7.615	7.615	44.53	8.29
Error	18	3.081	0.171		
Total	20	10.696			

Regeression coefficient; $a_0 = 4.45$, $a_1 = -0.06$

The F value of the regression analysis was 44.53 as shown above so that 2-methyl furan and roasting color was correlated at the confidence level of 99%.

4. Conclusion

The relationship between the chemical characteristics of roasted & ground coffee and the taste and aroma was investigated using gas chromatography and the sensory evaluation. According to the sensory evaluation, R & G coffees with the strong sourness showed the average pH of 5.02, TA of 3.98, roasting color of 54.7. Those with the weak sourness showed the average pH of 5.40, TA of 2.26 and the average roasting color of 34.6. These results showed that less roasted coffee had more sourness. The average RC of R & G coffees with strong UBN was 40 and those of R & G coffees with weak UBN was 56.8. Therefore, the more undesirable burnt note was observed as coffee was roasted more. The OFQ (overall flavor quality) also showed the higher value with higher RC. The average RC of R & G coffees with higher OFQ was 54 while the overall average RC of R & G coffee was 33.7. One of the aroma components found was 2-methyl furan which was related to the harshness of coffee. The amount of 2-methyl furan was also increased with higher RC.

5. References

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Table 1. R & G coffee samples used in the experiment

Sample No.	Manufacturing country	Buying place	Sample No.	Manufacturing country	Buying Place
1	Korea	Seoul	11	Japan	Tokyo
2	Korea	Seoul	12	Japan	Tokyo
3	Korea	Seoul	13	Japan	Tokyo
4	Korea	Seoul	14	Japan	Tokyo
5	Korea	Seoul	15	Japan	Tokyo
6	Korea	Seoul	16	Japan	Tokyo
7	Korea	Seoul	17	Japan	Tokyo
8	Korea	Seoul	18	Japan	Tokyo
9	Korea	Seoul	19	U.S.A.	Seoul
10	Japan	Tokyo	20	U.S.A.	Seoul

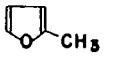
Table 2. Comparisons of pH, titratable acidity(TA) and roasting color of samples.

Sample No.	pH	TA	Roasting color
1	5.302	3.529	49
2	5.358	2.880	38
3	5.182	3.630	35
4	5.474	2.154	32
5	5.096	4.692	60
6	5.240	2.402	44
7	4.896	5.894	53
8	5.051	3.874	58
9	4.954	3.837	60
10	4.968	6.151	60
11	5.018	4.004	48
12	4.967	4.088	60
13	5.086	3.032	60
14	4.985	3.472	52
15	5.055	3.376	56
16	4.953	4.345	57
17	5.577	1.171	30
18	5.418	1.512	38
19	4.919	2.823	48
20	5.036	2.614	46

Table 3. Average data of sensory evaluation results

Sample No.	St.R.A.	U.B.A.	A.S.T.	O.A.Q.	Bitter	Sour	O.F.St.	O.F.Q.
1	-1	0	1	1	1	1	1	1
2	0	2	0	-1	3	-1	1	0
3	-2	2	0	-1	2	-2	0	-1
4	-3	3	-2	-3	3	-3	2	-2
5	2	-2	2	2	-2	2	1	1
6	1	0	0	0	1	0	1	1
7	2	-3	2	2	-2	3	1	2
8	2	-2	2	2	-2	2	0	1
9	3	-3	2	2	-3	3	1	2
10	2	-3	2	2	-2	2	2	2
11	3	-3	2	2	-3	2	2	2
12	1	-1	0	1	-2	2	0	0
13	1	1	1	1	-1	2	1	1
14	1	0	1	1	-1	1	1	1
15	1	-2	2	2	-2	2	0	2
16	2	-3	2	2	-3	2	-1	2
17	-2	3	-1	-2	3	-2	2	-2
18	-2	2	1	-2	2	-2	2	-1
19	0	2	-1	0	0	1	0	0
20	1	0	1	0	-1	2	1	1

Table 4. Peak identification of headspace gas chromatogram of R & G coffee by comparison of relative retention time

Peak Number	Retention Time	Compound	Molecular Structure
1	0.35	Light hydrocarbon (methane, ethane and ethylene)	CH_4 C_2H_6 C_2H_2
2	0.61	Acetaldehyde	CH_3CHO
3	0.81	Methylformate	HCO_2CH_3
4	1.72	Acetone Carbondisulfide	CH_3COCH_3 CS_2
5	2.45	Methylacetate	CH_3COOCH_3
6	3.47	Isobutyraldehyde	$(CH_3)_2CHCHO$
7	5.70	Diacetyl	$(CH_3CO)_2$
8	6.55	2-Methylfuran	
9	8.53	2-Methylbutanal	$C_2H_5CH(CH_3)CH_2OH$
10	8.97	Isovaleraldehyde	$(CH_3)_2CHCH_2CHO$
11	9.57	Internal standard (1-Butanol)	$CH_3CH_2CH_2CH_2OH$

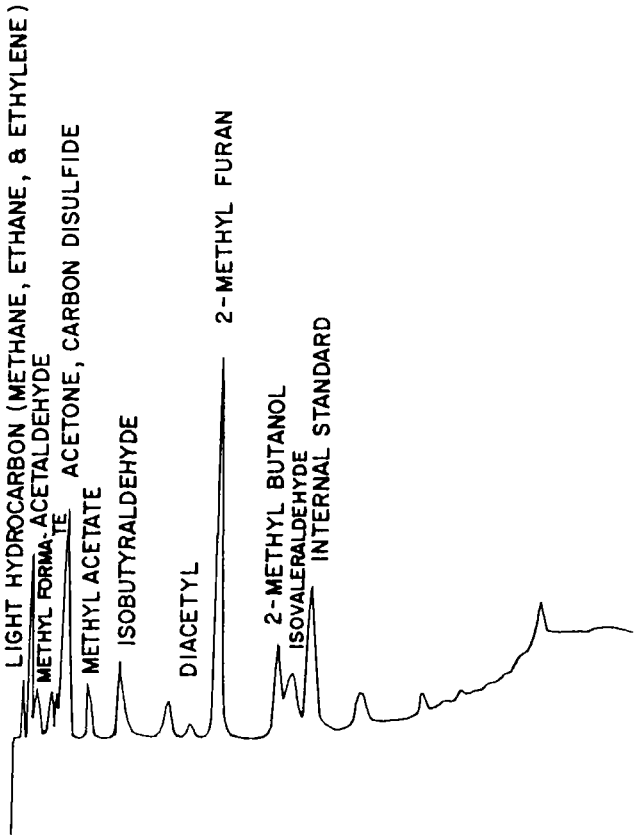


Fig.1. Headspace gas chromatogram of roasted and ground coffee

Hewlett Packard 5880 GC equipped with FID, column: 0.2% Carbowax 1500 on 80/100 mesh Carbopack C, 6 ft glass, Carrier : N_2 (30ml / min)

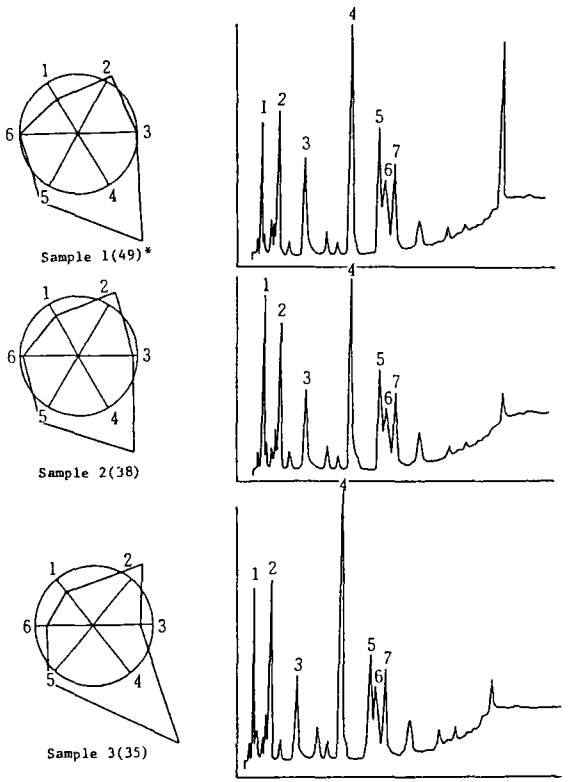


Fig. 2-1 Composition of aroma pattern and gas chromatogram of domestic R. & G. coffee

* ( ) Roasting Color.
1. Acetaldehyde
2. Acetone carbon disulfide
3. Isobutylaldehyde
4. 2-Methyl furan
5. 2-Methyl butanol
6. Isovaleraldehyde
7. Internal standard

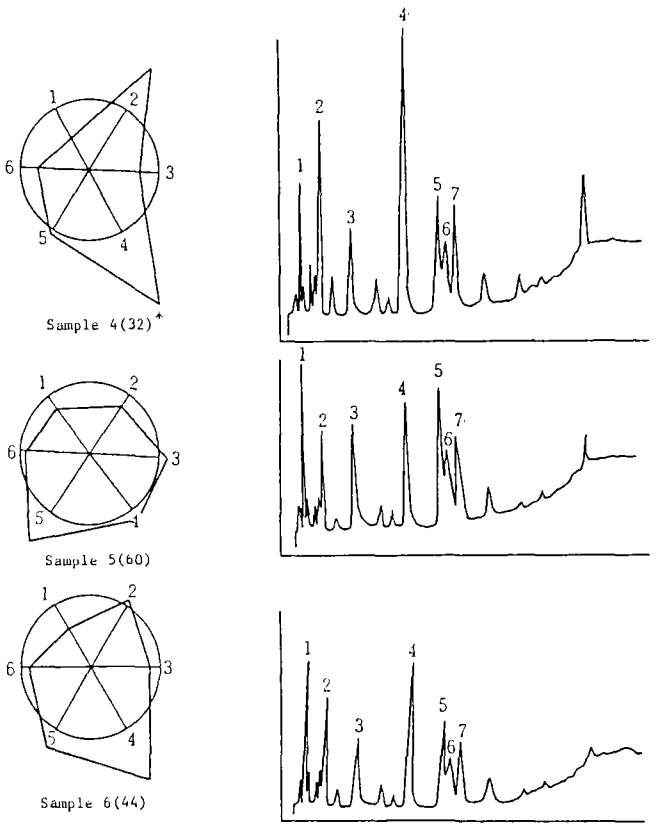


Fig.2 -2 Composition of aroma pattern and gas chromatogram of domestic R. & G. coffee

* ( ) Roasting Color.
1. Acetaldehyde
2. Acetone carbon disulfide
3. Isobutylaldehyde
4. 2-Methyl furan
5. 2-Methyl butanol
6. Isovaleraldehyde
7. Internal standard

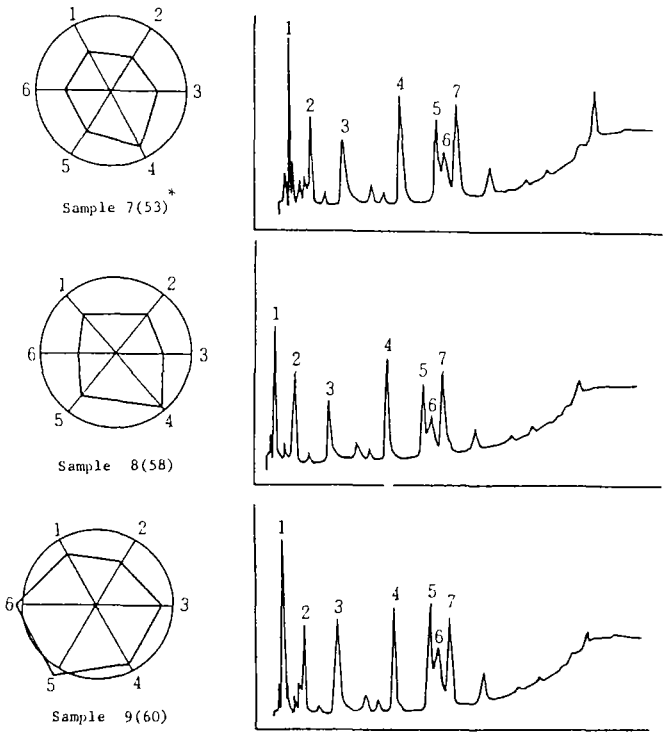


Fig.2 -3 Composition of aroma pattern and gas chromatogram of domestic R. & G. coffee

* ( ) Roasting Color.
1. Acetaldehyde
2. Acetone carbon disulfide
3. Isobutylaldehyde
4. 2-Methyl furan
5. 2-Methyl butanol
6. Isovaleraldehyde
7. Internal standard

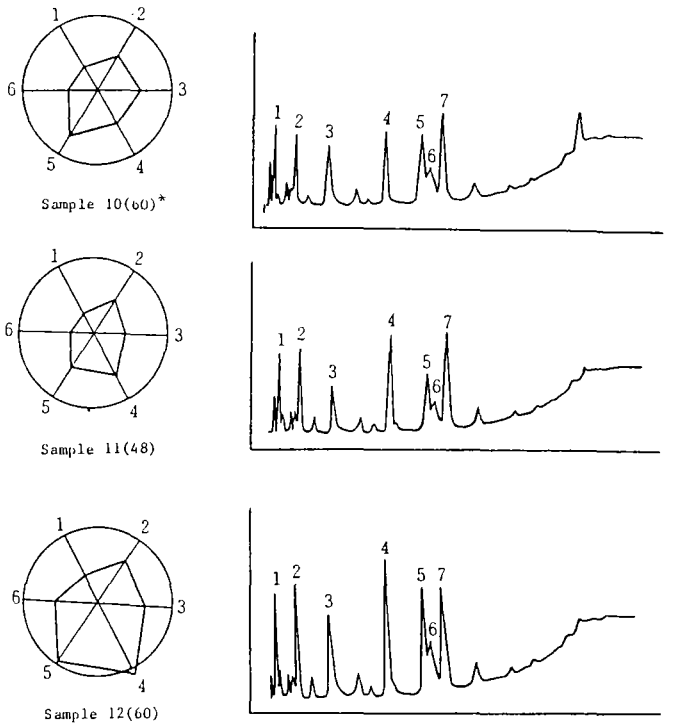


Fig.2 -4 Composition of aroma pattern and gas chromatogram of foreign R. & G. coffee (Japan)

* ( ) Roasting Color.
1. Acetaldehyde
2. Acetone carbon disulfide
3. Isobutylaldehyde
4. 2-Methyl furan
5. 2-Methyl butanol
6. Isovaleraldehyde
7. Internal standard

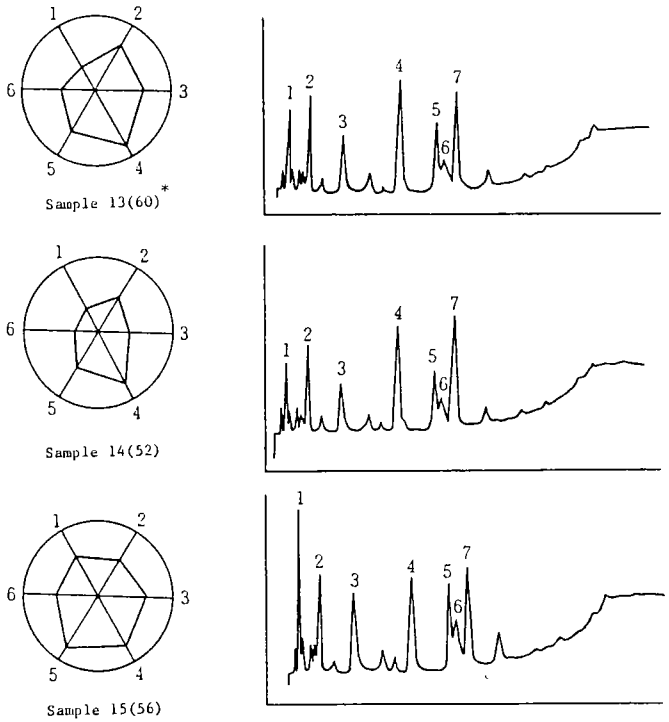


Fig. 2-5 Composition of aroma pattern and gas chromatogram of foreign R, & G. coffee (Japan)

* ( ) Roasting Color.
1. Acetaldehyde
2. Acetone carbon disulfide
3. Isobutylaldehyde
4. 2-Methyl furan
5. 2-Methyl butanol
6. Isovaleraldehyde
7. Internal standard

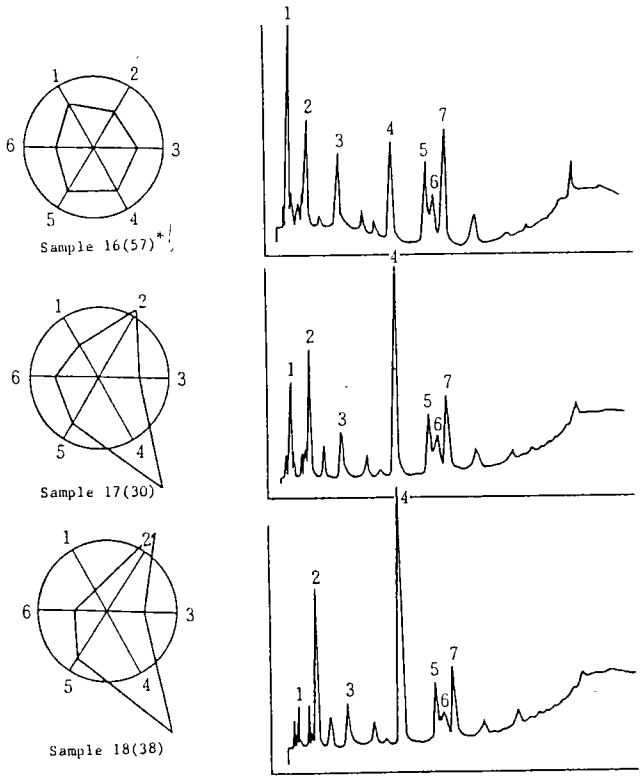


Fig. 2-6 Composition of aroma pattern and gas chromatogram of foreign R. & G. coffee (Japan)

* ( ) Roasting Color.
1. Acetaldehyde
2. Acetone carbon disulfide
3. Isobutylaldehyde
4. 2-Methyl furan
5. 2-Methyl butanol
6. Isovaleraldehyde
7. Internal standard

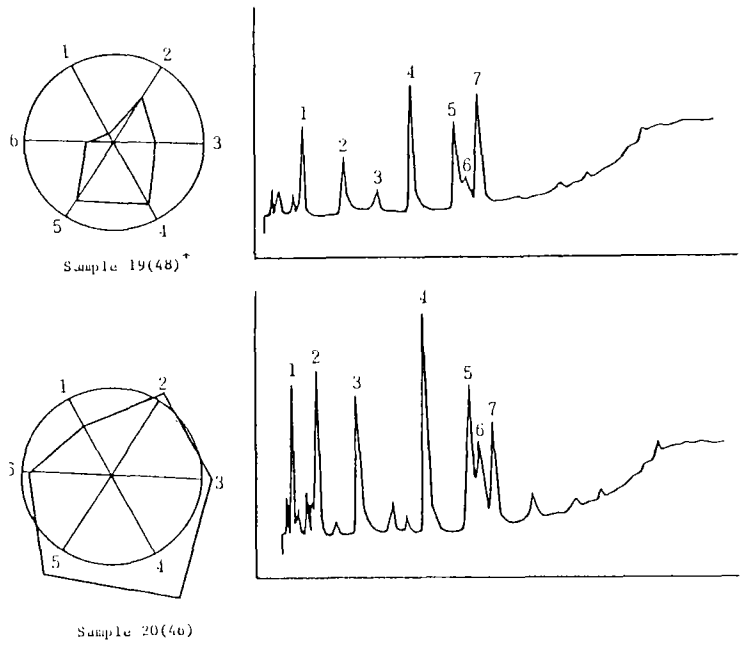


Fig. 2 - 7 Composition of aroma pattern and gas chromatogram of foreign R. & G. coffee (U.S.A)

* ( ) Roasting Color.
1. Acetaldehyde
2. Acetone carbon disulfide
3. Isobutylaldehyde
4. 2-Methyl furan
5. 2-Methyl butanol
6. Isovaleraldehyde
7. Internal standard

6. Summary

Twenty different kinds of roasted and ground coffees, 9 domestic and 11 foreign coffee products, were analyzed using a headspace gas chromatographic technique and sensory evaluation. Among many aroma compounds, acetaldehyde, methylformate, diacetyl, 2-methylfuran were detected from 10 samples analyzed. Overroasted coffees showed increased amount of 2-methylfuran which may give a harshness to the dark-roasted coffee. For sourness, pH and total acidity were measured and there was a close relationship between roasting degree and sourness, i.e., high sourness samples had low pH and high total acidity. By using a Photovolt colorimeter, a relatively good range of roasting color for the panelists was about 54. When this color number became higher, less desirable burnt note was recognized. The average roasting color of the foreign coffee was 54 and that of the domestic coffee was 47. This indicates the domestic coffees was stronger in roasting degree.

DETERMINATION OF ACID CONTENT IN COFFEE BEANS AND COFFEE

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1-Introduction

Green coffee bean contains various acids, such as chlorogenic, citric and malic acids. Slight change in their content readily leads to differences in the aroma and quality of the coffee bean, and content may change easily by processing, roasting and extraction. Acid assay of coffee beans and coffee is thus important for quality control. Common methods involve pH measurement and acid-base titration (Firestone, 1989). However, insufficient accuracy and error due to inadequate pH jump are attendant problems owing to variation in acid pKa. Carboxylic acids in coffee beans or coffee are determined by gas chromatography (GC)(Englhardt and Maier, 1985a; Englhardt and Maier, 1985b; Wada et al., 1987; Markelov and Guzowski, 1993), isotachophoresis (Englhardt and Maier, 1985b) and high-performance liquid chromatography (HPLC) with UV detection (Busling et al., 1982; Pfeiffer and Radler, 1985; Badoud and Pratz, 1986). But these methods have limited application : GC may be used only for volatile components, and HPLC with UV detection for chromophores. The authors thus undertook to develop a sufficiently reliable and accurate method for acid determination. A method for determining free fatty acid in oils was previously developed based on reduction prepeak current of 2-methyl-1,4-naphthoquinone (VK_3). Electrochemical detection appears simple and adequate for any acid determination without complicated acid derivation and thus was used in the present study.

2-Experimental

Reagents

Chlorogenic acid (3-caffenoylquinic acid)(96%), caffeic acid (98%), quinic acid (98%) (Table I) were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). All other acids used (Table I) were of reagent

grade and used without further purification. Ethanol solution containing 3 mM VK_3 and 38 mM $LiClO_4$ served as the supporting electrolyte solution for voltammetry and the carrier solution for flow injection analysis (FIA).

Apparatus

A potentiostat equipped with a wave generator (Model HAB-151, Hokuto Denko, Ltd., Tokyo, Japan), electrochemical cell and recorder were used to conduct the voltammetry. A glassy carbon disk (diameter, 6 mm, Tokai carbon Co., Tokyo, Japan), Ag/AgCl and platinum served as the working, reference and counter electrodes, respectively. The flow injection system was comprised of a pump (Model DMX-2200-T, SNK. Ind. Co., Ltd., Tokyo, Japan), sample injector valve (Model 7125, Rheodyne Inc., Cotati, CA, U.S.A.) with a 5 μl loop, walljet type electrochemical cell, potentiostat (Model 312, Fuso Seisakusyo Co., Kawasaki, Japan) and recorder (Model 807-IT, Jasco Co., Tokyo, Japan). The walljet type electrochemical cell was constructed of a glassy carbon working electrode, Ag/AgCl reference electrode, stainless steal counter electrode and trifluorochloroethylene resin cell body. Air dissolved in the supporting or carrier solution was removed by bubbling pure nitrogen gas.

Sample preparation

Coffee bean oil was extracted from roasted coffee bean powder with ether using a soxhlet extractor. For coffee extraction, 250 ml hot water were poured onto 20 g roasted coffee bean powder on filter paper.

Table I. Acids used

formic acid : $HCOOH$

acetic acid : CH_3COOH

fumaric acid : $HOOCCHCHCOOH$

malic acid : $HOOCCHOHCH_2COOH$

citric acid : $HOOCCH_2C(OH)(COOH)CH_2COOH$

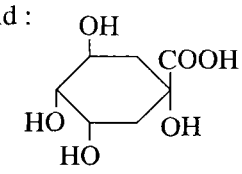
palmitic acid : $C_{15}H_{29}COOH$

stearic acid : $C_{17}H_{35}COOH$

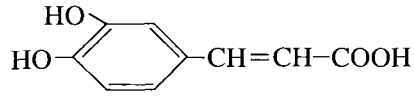
oleic acid : $C_{17}H_{33}COOH$

linoleic acid : $C_{17}H_{31}COOH$

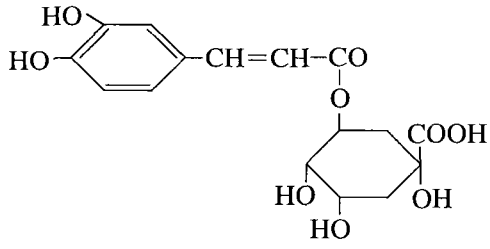
quinic acid :



caffeic acid :



chlorogenic acid :



3-Results and Discussion

Voltammetry of Vitamin K3 in the Presence of Acid

The presence of a trace acid component in solution caused a prewave prior to the reduction wave of quinone. The height of this prewave was proportional to acid concentration. The effects of proton donors on the polarographic behavior of quinones in aqueous and non-aqueous solution have been studied by Takamura and Hayakawa (Takamura and Hayakawa, 1971). In recent studies (Takamura et al., 1993, Fuse et al., 1995), free fatty acids in fats and oils gave rise to a prepeak on the voltammogram of VK3 at a potential more positive than that of the VK3 reduction peak in ethanol solution containing 38 mM $LiClO_4$ and 3 mM VK3. This proton donor effect was the basis for the developed new method for acid value determination of fats and oils (Kusu et al., 1994). VK3 gave a reduction peak at -0.80 V vs. Ag/AgCl (Fig. 1,a, peak I). Following the addition of formic acid to the solution, a prepeak appeared on the voltammogram at -0.45 V.(Fig. 1,b, peak II). Prepeaks of VK3 in the presence of acetic, citric, malic, chlorogenic, quinic and caffeic acids each present at 0.20 mM are shown in Fig. 2. Half-peak potentials, $E_{p/2}$, for acetic, citric, malic, chlorogenic, quinic and caffeic acids were -0.40, -0.32, -0.32,-0.28, -0.29, and -0.39 V vs. Ag/AgCl, respectively. The concentration of each acid was the same but current height differed for each prepeak.

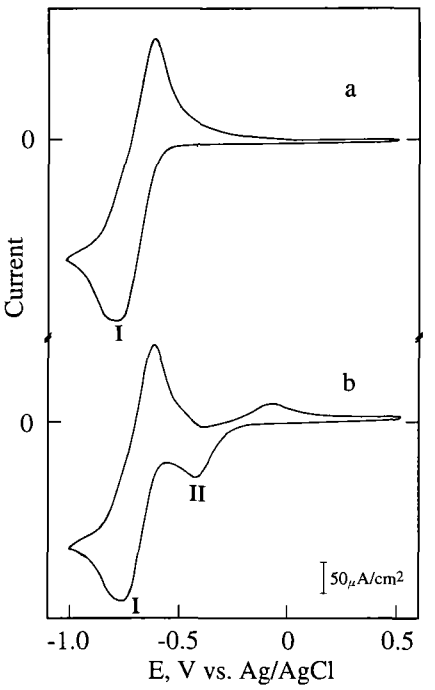


Figure 1. Voltammograms of 3 mM VK3 (a) without and (b) with 1.5 mM formic acid in 38 mM $LiClO_4$ ethanol solution. Potential sweep rate : 5 mV/s.

The electrode reaction is given by:

$$HA \rightleftharpoons H^+ + A^- \qquad [1]$$

$$Ka = [H^+][A^-]/[HA] \qquad [2]$$

$$VK3 + H^+ \rightleftharpoons VK3 \cdot H^+ \qquad [3]$$

$$VK3 \cdot H^+ + H^+ + 2e \rightleftharpoons VK3 \cdot H_2 \qquad [4]$$

where HA denotes the acid (Takamura and Hayakawa, 1971). As long as the dissociation of HA (equation 1) is in equilibrium at the electrode surface, and the protonated species, $VK3 \cdot H^+$, is produced so rapidly that VK3 is reduced as $VK3 \cdot H^+$ at the electrode surface, $E_{p/2}$ of the prepeak of the reduction reaction of equation 4 can be derived as follow :

$$E_{p/2} = (RT/2F)\ln Ka + \text{const.} \qquad [5]$$

$$= -0.030\ pKa + \text{const.} \quad (25^oC)$$

In Fig. 3, plots $E_{p/2}$ against pKa, where the first dissociation constant of di- or tri-carboxylic acid is adopted as Ka. The plots demonstrated a linear relation with a slope of -0.04 V/pKa. The electrode reaction would thus appear explainable by equation 4. Peak current heights, i_p, of prepeaks for mono-, di-, and tri-carboxylic acids increased in this order (Fig. 5). The relation between i_p for mono-carboxylic acids and

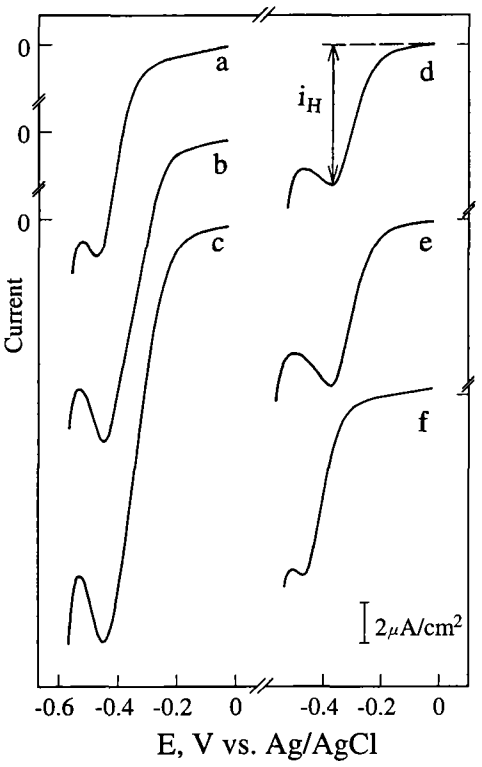


Figure 2. Prepeaks of VK3 with (a) acetic acid, (b) malic acid, (c) citric acid, (d) chlorogenic acid, (e) quinic acid, and (f) caffeic acid.
Acid concentration : 0.20 mM. Potential sweep rate : 5 mV/s.

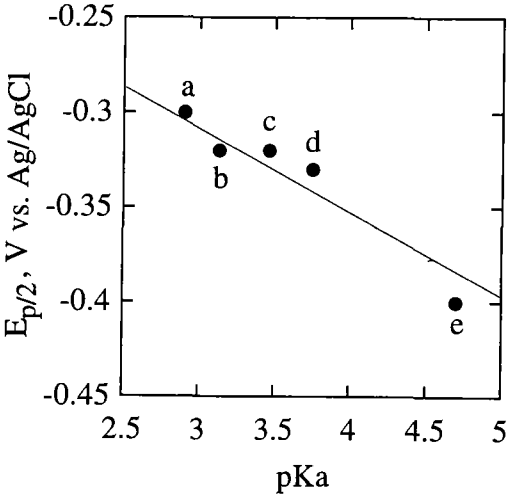


Figure 3. Relation between half-peak potential and pKa of (a) bromoacetic acid, (b) citric acid, (c) malic acid, (d) formic acid, (e) acetic acid.

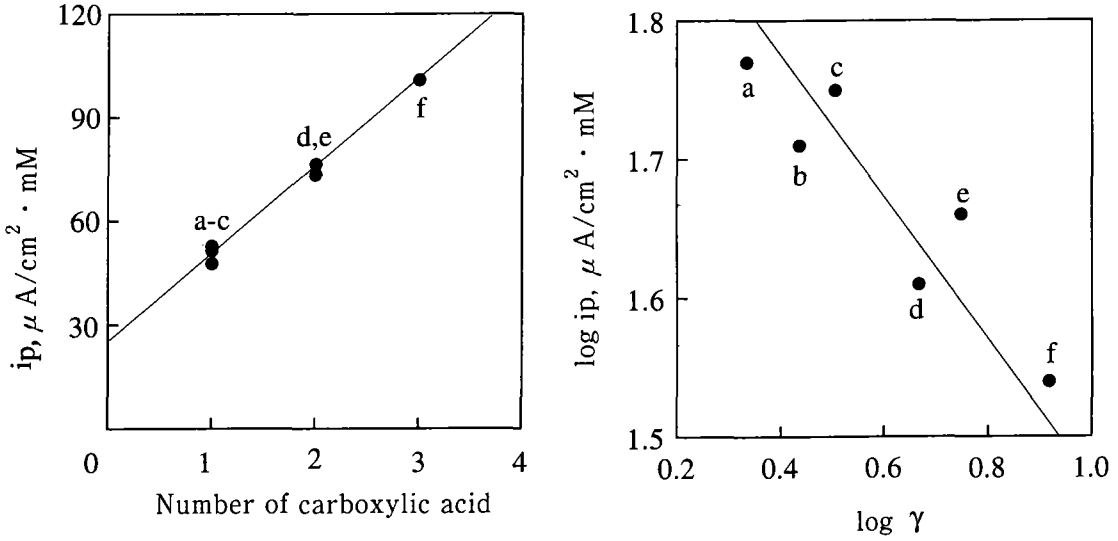


Figure 4. Effect of number of carboxylic acid in a molecule on prewave height. Acids : (a) acetic acid, (b) caproic acid, (c) palmitic acid, (d) malic acid, (e) fumaric acid, (f) citric acid.

Figure 5. Effect of molecular radius of acid on prewave height. Acid concentration : 0.20 mM. Acids : (a) formic acid, (b) bromoacetic acid, (c) acetic acid, (d) quinic acid, (e) caffeic acid, (f) chlorogenic acid.

molecular radius, r, is shown in Fig. 5. When an electrode reaction was controlled by undissociated acid species diffusion, the current peak was proportional to the square root of the radius of the acid molecular. Logarithm of i_p vs. logarithm of r gave straight line with a slope of -1/2. The current of the prepeak would thus appear to be controlled by the diffusion of an undissociated acid species. Acid content in a sample solution of coffee (Brazil) was determined using the calibration curve of caffeic acid by voltammetry, was 8.4 mM. Determination of the content for the same sample by conventional acid-base titration indicated a value of 7.2 mM.

Determination of higher fatty acids in coffee bean oil

Prepeaks for various concentrations of chlorogenic acid are shown in Fig. 6. i_p was linearly related to the concentration of each acid used. However, slope differed according to molecular structure. However, the determination of higher fatty acids in the oil of coffee beans may be possible by voltammetry, since current density at the concentration and peak potential of each prepeak for different free higher fatty acids in oils was essentially the same. The voltammogram for an oil sample extracted from coffee bean is shown in Fig. 7.

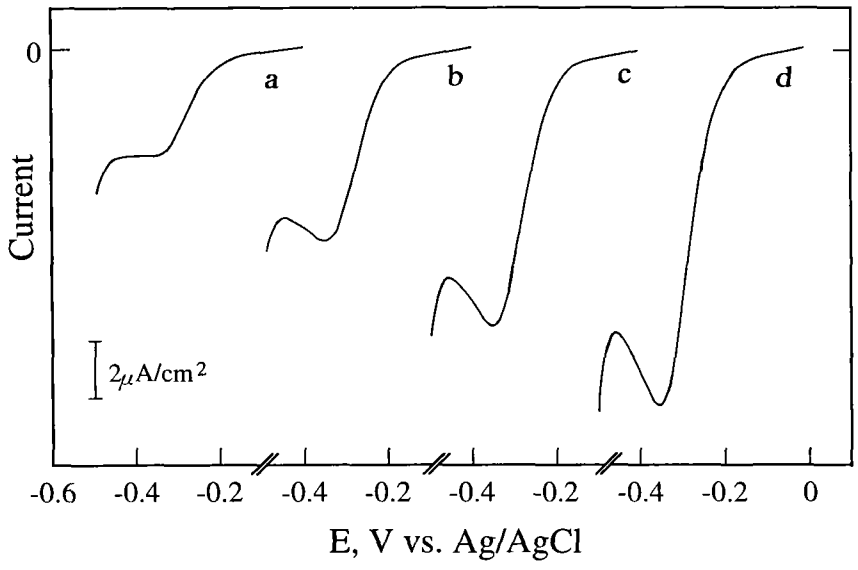


Figure 6. Prepeaks of VK3 with various concentration of chlorogenic acid. Concentrations : (a) 0.098, (b) 0.19, (c) 0.28, (d) 0.38 mM.

Table II Content of higher fatty acid in coffee bean oil

Sample	Oil content in coffee bean (%)	Acid content in oil (mmol/g)
Colombia	8.86	0.083
Brazil	10.32	0.12

The prepeak appeared at -0.39 V, suggesting the absence or only a trace amount of chlorogenic acid whose prepeak potential was less negative. Oil content values of coffee bean and content of higher fatty acids as determined are shown in Table II.

Flow Injection Analysis

Voltammetry was applied to electrochemical detection of acids in flow injection analysis (FIA). The sample solution (5 μl) was injected into the carrier solution at a flow rate of 0.6 ml/min. All acids examined were monitored at - 240mV vs. Ag/AgCl. FIA signals of chlorogenic acid are shown in Fig.8. These signals along with acid concentrations ranging from 0.01 - 0.3 mM (50 - 1500 pmol/test) are shown in Fig.9. Relative standard deviation was at 0.102 mM acetic acid of 1.4 % (n=10). For the determination of only one acid, FIA is simple and rapid for the acid determination. But in the case of several acids, their separation is requisite for maximally accurate determination. High-performance liquid chromatography (HPLC) should be conducted for such cases.

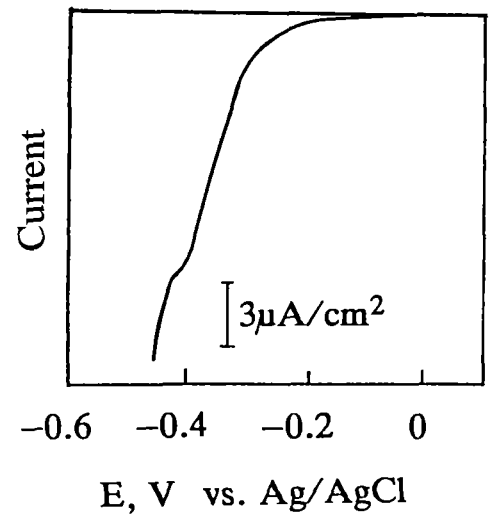


Figure 7. Prepeaks of VK3 with 1.12 g coffee bean oil in 40 ml 38 mM $LiClO_4$ ethanol solution.

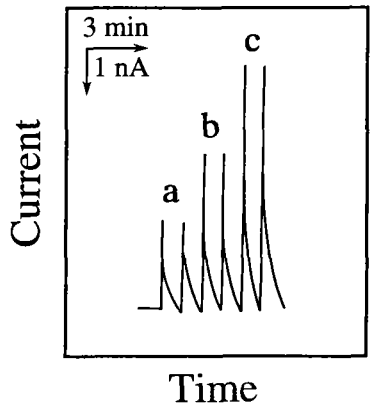


Figure 8. FIA signals for (a) 45, (b) 90 (c) 135 ng/test of caffeic acid. Applied potential : -240 mV vs. Ag/AgCl. Injection volume of sample solution : 5 μl. Flow rate : 0.6 ml/min.

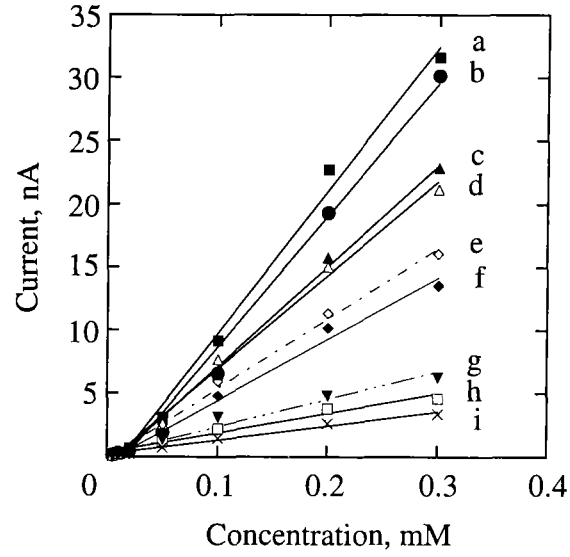


Figure 9. Calibration curves for (a) citric acid, (b) chlorogenic acid, (c) quinic acid, (d) malic acid, (e) fumaric acid, (f) formic acid, (g) acetic acid, (h) caffeic acid, and (i) palmitic acid by FIA.

In a preliminary experiment, HPLC using an ODS column with the electrochemical detector was carried out for a sample containing several fatty acids (Fig. 10). Provided a column that gives adequate separation is used, the present electrochemical method should prove sufficient for quality control of coffee beans and coffee.

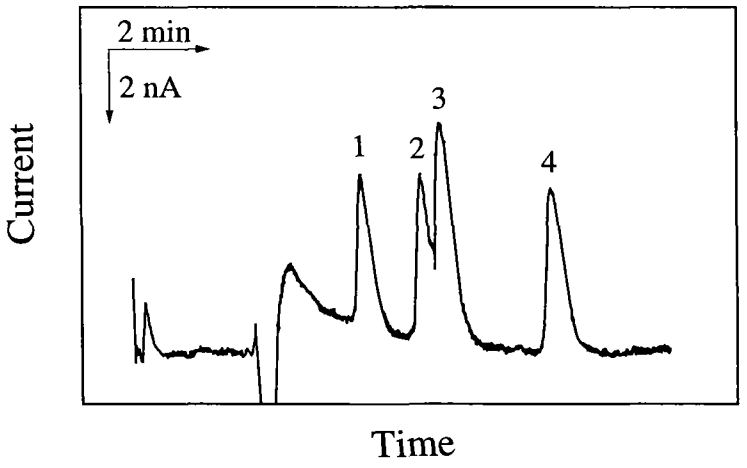


Figure 10. Chromatogram of fatty acids
1 : linoleic acid, 2 : oleic acid, 3 : palmitic acid, 4 : stearic acid
Acid concentration : 0.10 mM.
Column : LiCrospher 100 RP-18, 250 x 4 mm i.d., 5 µm.

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Summary

The present study was conducted to establish a reliable accurate method for determining acid concentration. When acids such as chlorogenic, caffeic, quinic, acetic and citric acids are present in ethanol solution containing 2-methyl-1,4-naphthoquinone (VK3), the voltammetric reduction peak of VK3 is accompanied by a prepeak, whose peak height is proportional to acid concentration. Flow injection analysis (FIA) using an electrochemical detector with a glassy carbon electrode and carrier solution of ethanol containing 38 mM $LiClO_4$ and 3 mM VK3 is presently used for acid determination. FIA signals and acid concentration were found in this study to be linearly related between 0.01 - 0.3 mM.

CONTRIBUTION TO COMPOSITION AND REACTIVITY OF COFFEE PROTEIN

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1.The recent knowledge on coffee protein

Basic work on coffee protein was done between 1960 and 1980, frequently in the whole ground coffee without protein isolation. Later on coffee protein has been investigated scarcely. Untill now our knowledge on the molecular properties of coffee proteins and its changes caused by the technological process of roasting is rather small.

Informations on the amino acid composition are given e.g. by THALER et al. (1962,1963), on the electrophoretic behaviour by BADE and STEGEMANN (1982), and on the thiol- and disulfide-groups by MEICHELBECK and ZAHN (1968).

2.The objectives of this investigation

One aim of our interest in the coffee protein research is to improve the knowledge on the soluble coffee protein fraction, the main share of it is considered as the storage proteinof the coffee bean On the base of that there is a second point of view. Proteins are reactive biopolymers in virtue of the composing amino acids. During coffee roasting the amino acid side chains could react in different manner (dependent on the temperature and time of heat influence) and the proteins could be modified in different ways too. According to that abilities any contribution of the proteins to coffee aroma should not be excluded.

Unfortunately it is extremely difficult to recognize changes of the amino acids and/or the coffee proteins in its original environment inside the coffee bean.Therefore we prepared a pure water soluble coffee protein isolate.The protein isolate was used to study some molecular data and further to find out whether anything at all has happened after heating the protein 6 minutes up to about 200°C (473 K) simulating coffee roasting.

3. Results: Coffee protein isolate

Overall composition of the isolate

Protein according to Barnstein (Nx6,25)	89,0%
Ash (thermograv. procedure)	4,0%
Plant Phenols (complex formation with Fe-ion, calc. as chlorogenic acid)	0,3%
Water (thermograv. procedure)	6,0%
Carbohydrate (hydrolysis, TLC): Gal, Ara, Rha, Glc	
Coffein (HPLC)	0,04%

Protein type

Solubility experiments as well as fractionated precipitation revealed the presence of proteins of the albumin and of the globulin type.The relation is about 15% albumins to 85% globulins.

The electrophoresis pattern demonstrates the complex composition of the coffee protein isolate. This impression is confirmed by the number of different N-terminal aminoacids.

Amino acid composition

The values of several amino acids in this investigation deviate from the results of THALER. The reason for it is assumed in the different composition of the samples.

Amino acid	Isolate	Heated product	watersoluble share	insoluble share
		all data in mol%		
	n = 10	n = 12	n = 12	n = 11
Asx	7,8	9,1	4,9	8,0
Glx	28,2	22,8	25,9	18,7
Ser	5,8	4,0	4,2	4,0
Thr	4,2	3,2	2,7	3,4
Gly	8,8	10,3	12,5	11,3
Ala	9,4	7,2	8,1	7,3
Arg	3,6	5,4	5,3	5,3
Pro	5,1	6,5	9,4	6,5
Val	5,1	5,9	5,5	6,7
Met	1,8	not detectable	not det.	not det.
Ile	3,2	3,8	3,6	4,8
Leu	6,3	9,5	9,4	10,1
Trp	<0,4	not detectable	not det.	not det.
Phe	3,6	5,2	4,1	5,6
Lys	3,2	4,1	2,9	4,8
His	2,2	1,2	0,8	1,6
Tyr	1,7	1,8	2,2	2,9

Thiol- and disulfide-groups

SH:

<0,1 nmol / mg pure protein (photometrical method acc. to TANNHAUSER et al./1984/)

SS:

95 - 99 nmol S^- / mg pure protein (method acc. to TANNHAUSER et al./1984/)

N-terminal amino acids

Main compounds	Minor compounds (<5%)
Gly 36%	Leu, Arg, Asn, Gln, His,
Glu 15%	Ile, Lys, Met, Phe, Pro,
Asp 10%	Ser, Thr, Val

Data are given in % of the total amount of the N-terminal amino acids.

Electrophoretic behaviour

PAGE

Figure indicates a SDS-electrophoresis pattern. The molecular masses of the reduced protein monomers are in the range between 20 - 30 kD (major bands) and 30 - 45 kD (minor bands)

Isoelectrofocusing

The main share of bands is found between pH 5,0 and pH 7,0, but single ones could be seen up to about pH 8,5.

If some protein bands occur below pH 3,5, artefacts are assumed because polyphenols can be detected as well.

4. Results: "roasted" coffee protein isolate

Temperature fields with endothermic changes

Differential scanning calorimetrie reveals two fields of endothermic reactions. The first one is at about 150°C (423 K), the second one at 215°C (488 K). The last modification is accompanied with the beginning of a loss in mass as thermogravimetric measurement indicates.

Solubility of the heated coffee protein isolate

After the model-heating nearly half of the protein (45 - 46%) dissolves in dist. water at

room temperature. A share of the peptide bonds is cleaved and formation of peptides starts. The remaining product is unsoluble in all common polar solvents.

Amino acid composition

The amino acid compositions of the heated isolate as well as the values of the soluble and the insoluble protein fractions are given in the table.

5. Discussion

-The protein isolate investigated mainly consists of the storage globulins of coffee bean. It is colourless, tasteless, and does not smell.

-Glx is the main amino acid. Compared with other plant storage globulins only a smaller Asx content was found. About a quarter of the amino acids have reactive side chains.

-The amount of SH-groups is very low in spite of a careful exclusion of oxygen during handling. But the presence of thiol-groups is recognized electrophoretically, using the method of TAKAHASHI et al. (1990). The result agrees with MEICHELBECK et al. Approximately a third of the SS-groups reacts quick, the remaining share seems to be buried.

-The protein isolate contains small amounts of carbohydrate. According to the reaction with Concanavalin A subsequent to electrophoretic separation three of the protein spots are identified as glycoproteins.

-The molecular weight distribution determined by SDS-PAGE of the reduced protein agrees in the main bands with the data of BADE et al. Some bands in a higher molecular range are detected additionally.

-The pattern of isoelectrofocusing reveals the complex composition of the coffee albumins and globulins once more. Focusing in a gradient from pH 2 to pH 9 yields about 35 individual proteins, but with narrow gradients the number of bands increases further. The isoelectric points of the major bands range from pH 5 to pH 7

-Differential scanning calorimetric and thermogravimetric studies of the protein isolate indicate two endothermic molecular reactions. The last one is near the popular roast temperature of about 200°C (473 K). A remarkable loss in weight happens when temperature rises to about 220°C (493 K).

-After heating the protein isolate has a yellowish to light brownish colour and a light roast-like taste and smell.

- After heating procedure a surprisingly low change of amino acid composition has confirmed, but a large part of the reaction product is soluble in cold dist. water. An explanation for this behaviour is given amongst others by electrophoresis, indicating the existence of peptides with a molecular mass smaller than 10 kD. The temperature level of 200°C is near at a critical point: Amino acid decomposition is not yet very strong whereas peptide formation perceptibly occurs.

6. Conclusion

Protein isolate has been shown as a reactive constituent part of coffee under conditions similar to the roasting process. Only few amino acids with polar side chains are lost at 200°C, but stronger changes are expected at a temperature of 220°C and more. Especially with regard to the formation of peptides the possible role of coffee protein as an aroma precursor should be taken in consideration. Peptides formed from coffee protein isolate are precursors of coffee aroma as well as amino acids (LUDWIG and ARNOLD,unpublished results).

7. Material and Methods

a) Material

Coffee samples were commodities of the JACOBS - SUCHARD GmbH. The protein was prepared from mature green Columbia C. arabica beans harvested in 1992.The total protein comes to 14,6 - 0,1% d.m. (total nitrogen minus non protein nitrogen).

b) Methods

Protein preparation

Coffe samples were carefully ground at low temperature in the absence of oxygen. The removal of lipids was achieved by treatment with cold aceton, the removal of polyphenols by succeeding extraction with an aceton-water mixture (80:20, v:v). After that proteins were extracted with an aqueous coffein-solution (1%, m:v). Treatment with polyamide powder, membranfiltration and protein precipitation by means of ammonium sulfate addition delivers a precipitate and after dialysis and lyophilization a white protein isolate. Nearly 45% of the total coffee protein was obtained.

Amino acid composition
Protein was hydrolyzed with 6N hydrochloric acid at 106°C (379 K). The relation was 1: 5 000 (m:v).
The amino acids were treated with DABS-Cl (4-dimethylamino-azobenzene-4'-sulfonylchloride), separated by HPLC (C-18-column, a gradient of 25 mM KH_2PO_4 pH 6,8 and acetonitril / 2-propanol 80.20 (v:v)) and detected at 436 nm.

N-terminal amino acids
Protein was treated with DABITC (4-dimethylamino-azobenzene-4'-isothiocyanate). Amino acids were separated by HPLC (DB-C-18 column, a gradient of 55 mM CH_3COONa pH 5,1 and acetonitril) and detected at 436 nm.

Electrophoresis
PAGE is carried out according to MAURER (1968).
SDS-PAGE is carried out according to LAEMMLI (1970) but without stacking gel.
Electrofocussing is carried out according to GÖRG et al. (1978)

Heat procedure
A 10% water containing dry protein sample was heated in a closed glas tube in the presence of nitrogen 6 min at 200°C (473 K) dipping the vessel in a fluid bath. The end temperature was reached within about half a min. Rapid cooling to 20°C (293 K) stopped reactions.

Differential scanning calorimetry
A METTLER DSC-30 device was used. Temperature rate 10°C/min; Nitrogen atmosphere.

Thermogravimetry
A METTLER TG-50 device was used. Temperature rate 10°C/min; air.

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9.Acknowledgement

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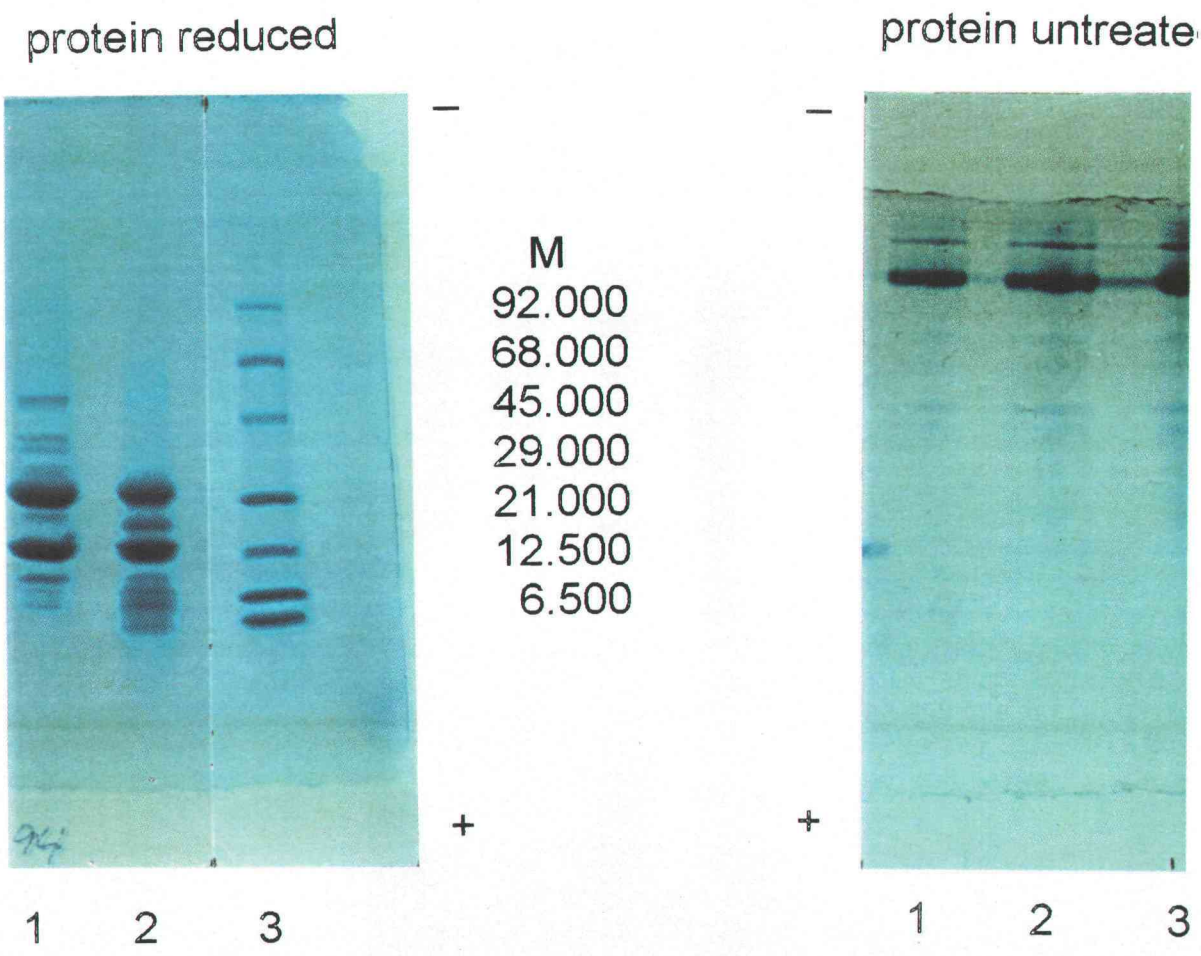
Mol.-weight-estimation by SDS-PAGE

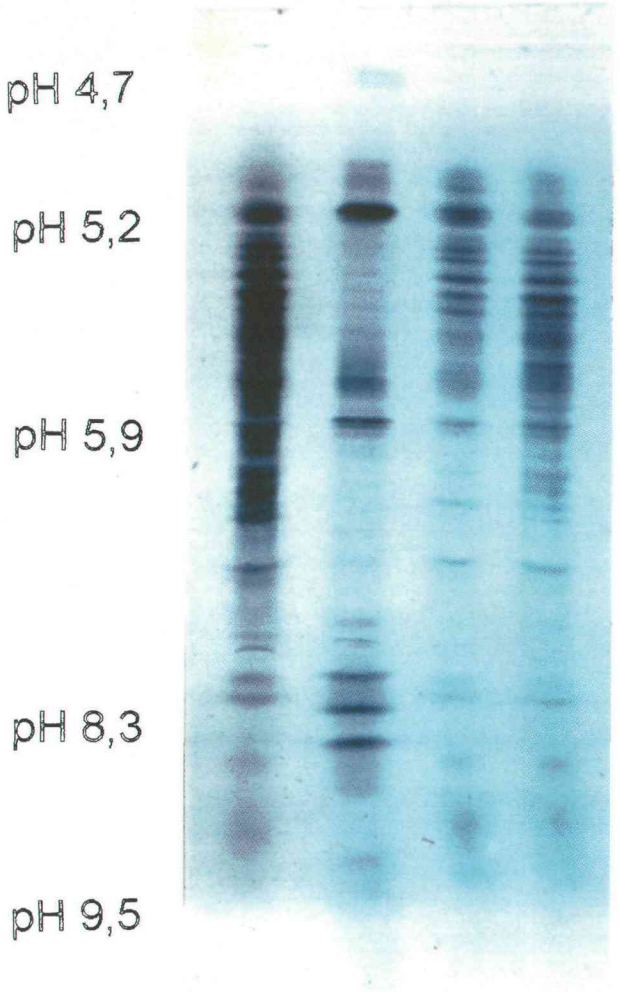


1: coffee globulin (reduced)
2: protein isolate
3: marker

protein isolate (untreated

Isoelectric focusing pattern of protein isolate

pH-gradient 3-11

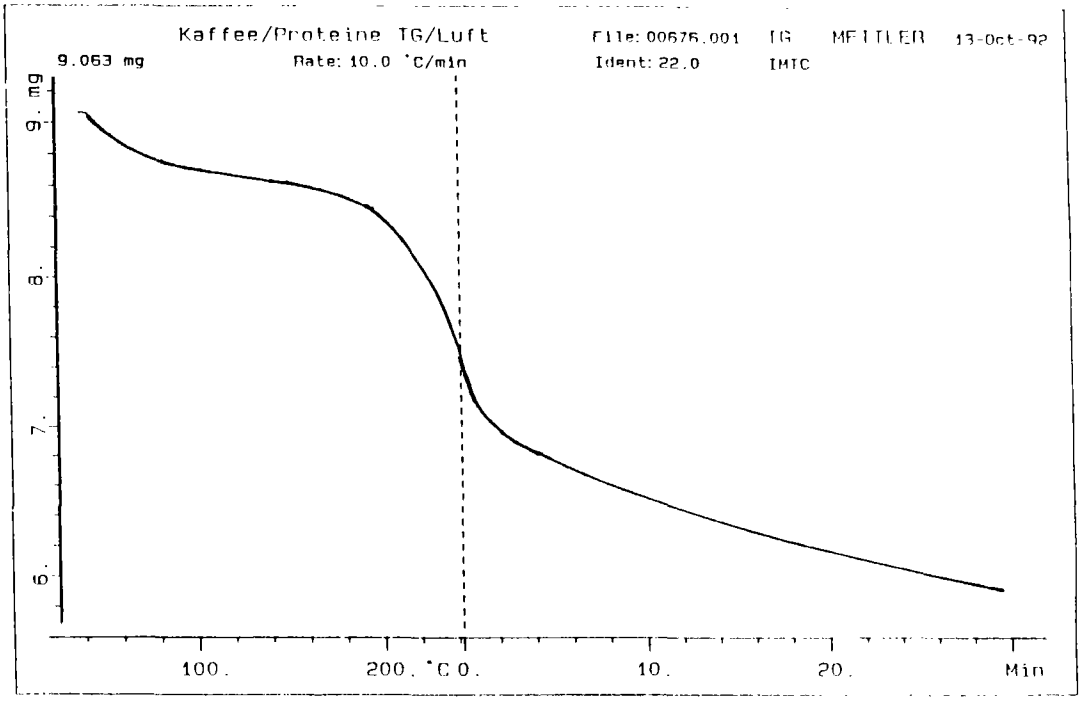


1 2 3 4

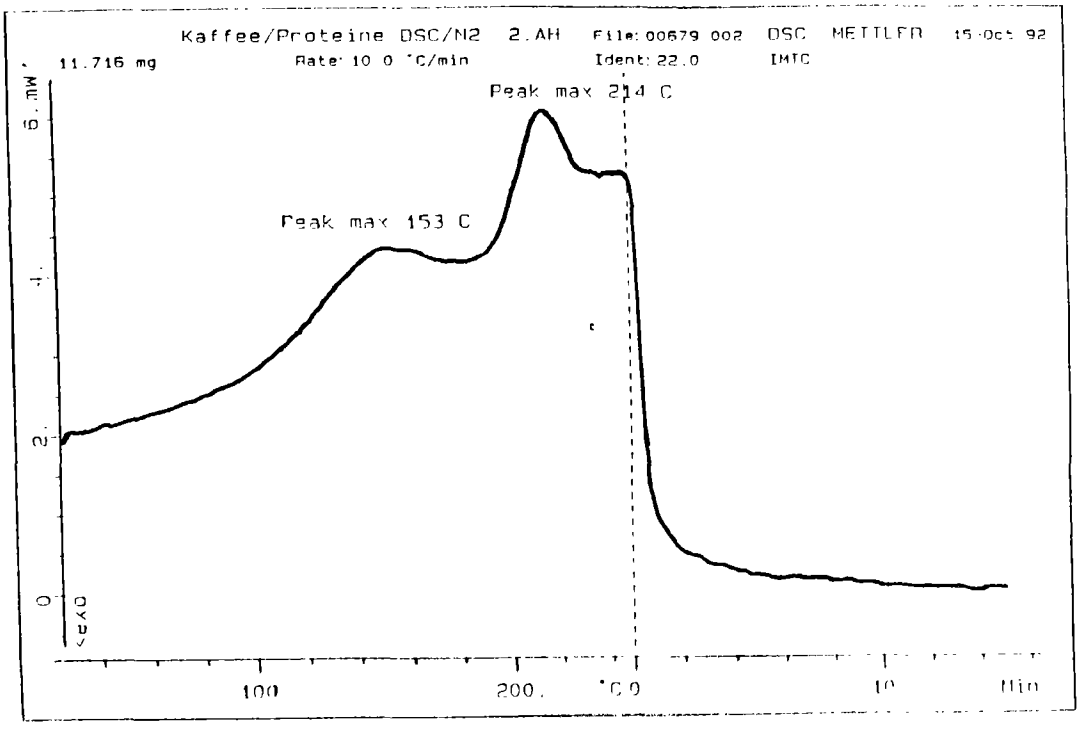
1,3,4 protein isolate (diff. conc.)

2 marker

Thermogravimetric diagram



Differential scanning calorimetric diagramm



CARBOHYDRATES IN STEAM TREATED COFFEE

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INTRODUCTION

As previously described (STEINHART and LUGER, 1995), we analysed the amino acid, carbohydrate and chlorogenic acid contents of treated and untreated coffees to learn more about the reasons for the proven difference in the melanoidin fraction with the highest molecular mass. This fraction has been stated to be significantly diminished in pretreated coffees (STEINHART et al., 1990) and to contain about 50% less mannose (STEINHART and PACKERT, 1993). Subject of this paper are our results on the changes in the carbohydrate contents during the steam treatment of the raw coffee beans in relation to duration and pressure of the treatment.

EXPERIMENTAL

Coffee Samples

Table 1 displays the studied coffee samples. The industrially treated coffees and their untreated counterparts were a gift from the CR3-Kaffeeveredelung M. Hermsen, Bremen.

Table 1: Coffee samples studied

Type	Origin	Treatment
Arabica	Mixture	untreated industrially treated 1bar 1h laboratory treated 0.8bar 1h laboratory treated 0.8bar 2h laboratory treated 0.8bar 4h
Arabica	Columbia	untreated industrially treated 1bar 1h
Arabica	Tanzania	untreated laboratory treated 0.8bar 1,5h
Arabica	Ethiopia	untreated laboratory treated 0.8bar 1,5h
Arabica	Columbia	untreated laboratory treated 0.8bar 1,5h
Robusta	Indonesia	untreated industrially treated 1.5bar 40min industrially treated 3bar 40min laboratory treated 0.8bar 1h laboratory treated 0.8bar 2h laboratory treated 0.8bar 4h laboratory treated 0.4bar 4h
Robusta	Cameroun	untreated laboratory treated 0.8bar 1.5h
Robusta	Indonesia	untreated laboratory treated 0.8bar 1.5h
Robusta	Thailand	untreated laboratory treated 0.8bar 1.5h
Robusta	Tanzania	untreated laboratory treated 0.8bar 1.5h
Robusta	Sri Lanka	untreated laboratory treated 0.8bar 1.5h

Laboratory treatment was performed in a Fissler pressure cooker.

Analytical Procedure

5 g of finely ground coffee powder were mixed with 10 g sea sand and 1 g calciumcarbonate and extracted in a Soxhlet apparatus with hexane for 2 hrs. Mono- and oligosaccharides were then extracted with 80% ethanol for 4 hrs. The polysaccharides remained in the residue. An aliquot of the remainder was hydrolysed with 2 M trifluoroacetic acid for 2 hrs at 121°C in a drying cabinet. The residue was diluted with distilled water, filtered and brought to a defined volume. An aliquot was desiccated in a vacuum centrifuge, thiolysed with propanethiol:trifluoroacetic acid 4:1 (v:v) for 30 min at room temperature and silylated with 5 % trimethylchlorosilane in bis(trimethylsilyl)trifluoroacetamide for 20 min at 75°C.

The extract was cleared according to Carrez, brought to a defined volume and filtered. An aliquot was desiccated in a vacuum centrifuge, dissolved in distilled water and diluted with the fourteenfold amount of acetonitrile. An aliquot of this solution was then applied to a silica-gel column. Monosaccharides were eluted with acetonitrile:dist.water 97:3 (v:v), oligosaccharides were eluted with acetonitrile:dist.water 90:10 (v:v). The monosaccharide fraction was desiccated, thiolysation and silylation took place as described above.

The oligosaccharide fraction was brought to a defined volume. An aliquot was then desiccated in a vacuum centrifuge and silylated as described above.

Gas Chromatography

Gas chromatography was performed on a HP-5 column (30 m, 0.32 mm i.d., 0.25 µm thickness, Hewlett Packard, Germany). Carrier gas was helium with 1 ml/min, split 1:10, injection volume 1 µl. Detection took place by means of a flame ionisation detector. Time program was: 180°C, no halt, with 10°C/min to 220°C, 5 min halt, with 2°C/min to 225°C, no halt, with 7°C/min to 270°C, 15 min halt.

RESULTS AND DISCUSSION

Monosaccharides

Reducing sugars in steam treated raw coffee have been analysed by Maier et al.; unfortunately they did not publish their results. MAIER (1989) only reported that pretreated coffee had a higher amount of reducing sugars. Our results confirm this. Figure 1 shows the changes in the monosaccharides during the steam treatment in the Robusta Indonesia. The coffees were laboratory treated at 0.8 bar.

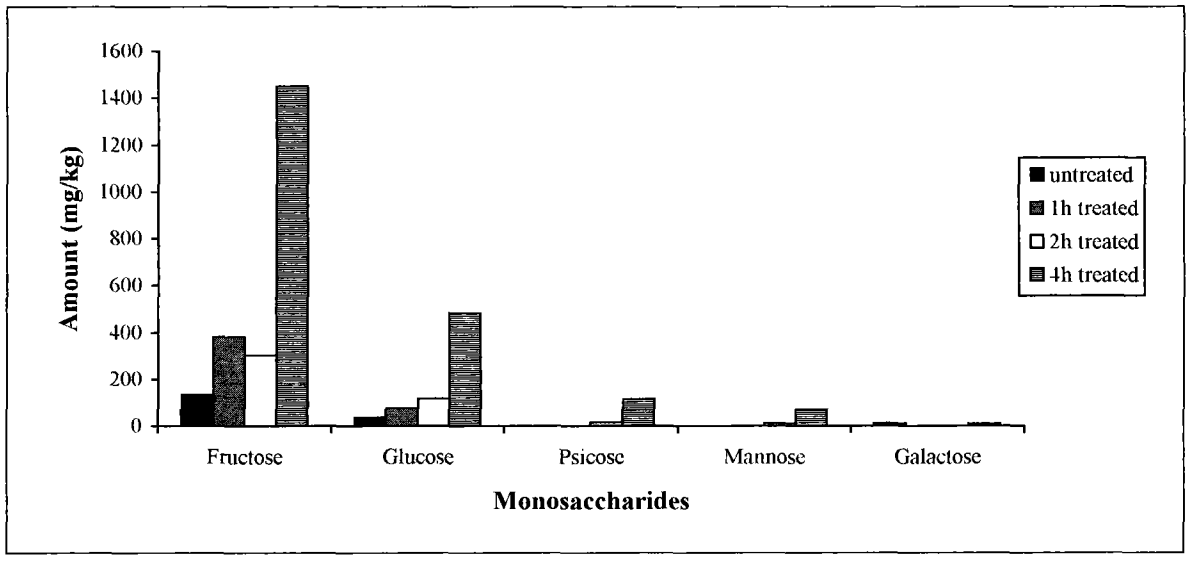

Figure 1: Changes in the monosaccharides of the Robusta Indonesia during the steam treatment

Ribose, xylose, arabinose and rhamnose were also detectable, but to lesser amounts than those carbohydrates displayed above. As indicated in figure 1, the amount of every monosaccharide except galactose increased remarkably during the treatment. Galactose seemed to be destroyed as soon as it is set free from oligo- or polysaccharides. Striking is the development of psicose, a carbohydrate that was detected by BLANC and PARCHET (1989) in soluble coffees. This behaviour was the same for every coffee analysed, whether an Arabica or a Robusta. The amount of the increase did though vary with the applied pressure. This is shown in figure 2.

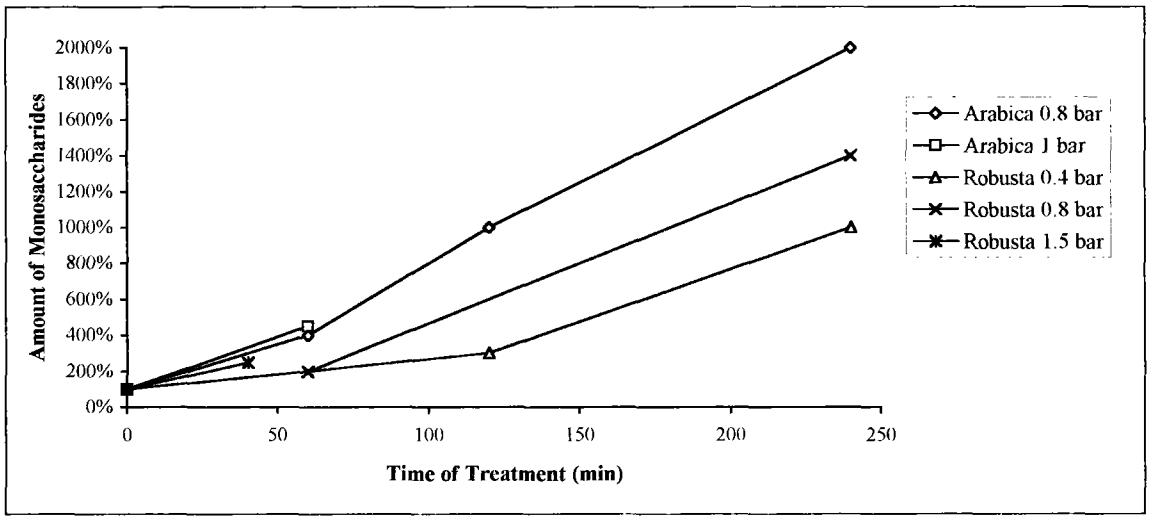

Figure 2: Changes in the sum of the monosaccharides during the treatment

These curves are valid for every Arabica and Robusta studied. It was observed that Arabicas showed a faster increase of their monosaccharide contents than did Robustas. In addition the increase steepened with the applied

pressure, as indicated in the data for those Robustas treated at 0.4bar, 0.8bar and 1.5bar. The Robusta treated at 3bar (not displayed in figure 2) reached the curve of the Arabica treated at 0.8bar.

Oligosaccharides

The main oligosaccharide in raw coffee is saccharose with an amount of 1.8-7.7% dry matter basis, raffinose and stachyose are also detectable (MAIER, 1981). In the Arabicas and Robustas studied here we detected saccharose amounts of about 5% in the untreated coffees without any significant difference between the species. During the steam treatment, saccharose decreased notably. This is displayed in figure 3.

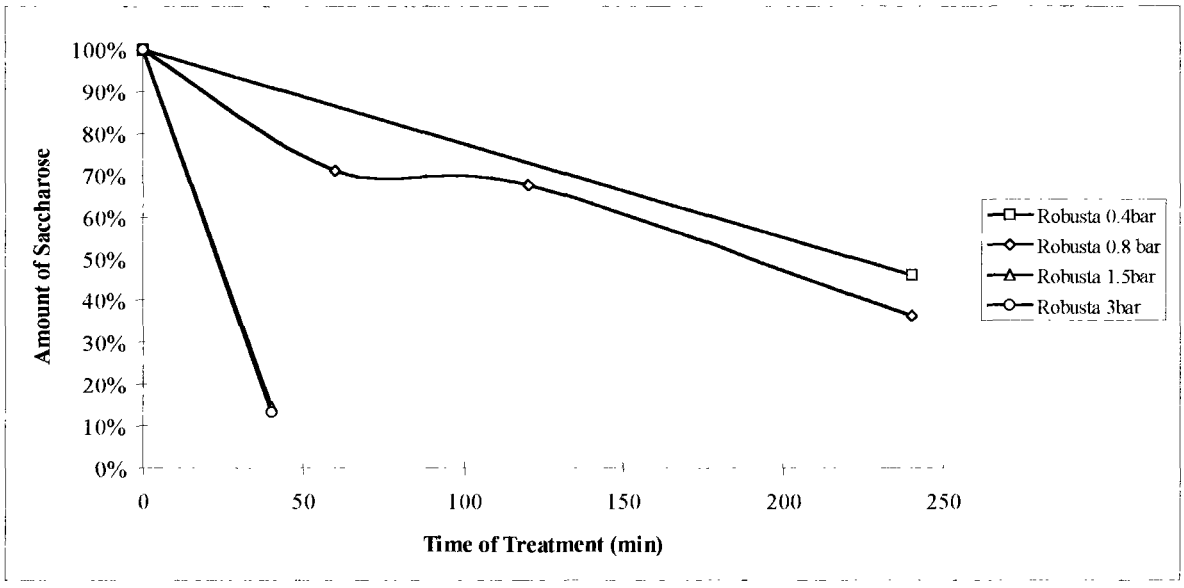

Figure 3: Decrease in the saccharose content during the steam treatment of the Robusta Indonesia with different pressures

As indicated in figure 3, the higher the applied pressure was, the greater was the decrease in the amount of saccharose. However, there was an end to this: the Robusta treated with 3bar showed no greater decrease than the Robusta treated with 1.5bar. There was no difference observable either between the Arabicas and the Robustas or between the different origins.

Polysaccharides

Polysaccharides form the group of substances with the greatest amount in raw coffee. Arabicas are reported to comprise about 53% on a dry matter basis, Robustas about 44% (STREULI, 1973). Monomers of the polysaccharides are mainly mannose, galactose and arabinose and to a lesser extent xylose and rhamnose (MAIER, 1981). Our data confirmed this. Between pretreated and untreated coffees, no difference was observed.

SUMMARY

5 Arabicas and 6 Robustas of different origin and pretreatment were analysed for their carbohydrate contents. During the steam treatment, fructose and glucose increased remarkably in their amounts; mannose, xylose, rhamnose, arabinose and, most striking, psicose were detectable after the treatment. These increases were observed to be connected with the applied pressure as well as with the coffee specie. Saccharose decreased during the treatment, also in connection with the applied pressure, but not with the specie. Polysaccharides showed no significant changes during the treatment.

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PROTECTIVE EFFECTS OF CAFFEIC ACID ESTERS AGAINST H_2O_2-INDUCED CELL DAMAGES

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1. Introduction

Dietary plants contain various kinds of polyphenols, which we take in milligram quantities from food sources every day. Extensive investigations of the biological effects of polyphenols have revealed their contradictory functions: induction of genotoxic activities such as DNA lesions, mutations and chromosome aberrations *vs.* suppression of these genotoxic activities in carcinogenic compounds; carcinogenicity *vs.* anticarcinogenicity at every stage of *in vivo* experiments.

Caffeic acid is a polyphenol, bearing a catechol-like aromatic ring with two hydroxyl groups. Chlorogenic acid, which consists of quinic acid esterified to the carboxyl acid group of caffeic acid, are widely distributed among numerous plant species including coffee bean. Caffeic acid phenethyl ester (CAPE) was isolated from propolis (a product of honeybee hives) that has been used in folk medicine as a potent antiinflammatory agent. Caffeic acid and these esters have shown contradictory functions *in vitro* and *in vivo* experiments as described below. Caffeic acid induced forestomach squamous cell carcinoma in rats. It is, however, reported that CAPE and caffeic acid inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA) -induced tumor promotion in mouse skin and azoxymethane (AOM) -induced aberrant crypt foci formation in rat colon . Caffeic acid was clastogenic in Chinese hamster ovary cells.

On the other hand, CAPE and caffeic acid methyl ester (CAME) significantly inhibited 3,2'-dimethyl-4-aminobiphenyl (DMAB) -induced mutagencicity in *Salmonella typhimurium* strains TA98 and TA100. In the course of these studies, it was found that caffeic acid and its esters inhibited many enzymes *in vitro* and *in vivo*, *e.g.*, ornithine decarboxylase in mouse skin and adenocarcinoma cells and lipoxygenase in colonic preneoplastic lesions and cloned mastocytoma cells.

Some researchers ascribed clastogenic activity of caffeic acid to H_2O_2 formed in its autoxidation process, because catalase abolished the clastogenic activity (1). Catecholic compounds have been known to autoxidize and produce H_2O_2 especially in the presence of transition metal ions such as Fe^{3+}, Cu^{2+}, and Mn^{2+}. In the presence of these transition metal ions, caffeic acid enhanced hydroxyl radical (·OH) formation (2) and caused oxidative damage to cellular and isolated DNA (3,4,5). Addition of the transition metals enhanced the clastogenic, mutagenic, and convertogenic activity in caffeic acid (6) and chlorogenic acid (7). In these cases, H_2O_2 formed during the autoxidation process of caffeic acid seems to be critical species for the damages. In contrast, we recently found from a colony-formation assay that certain polyphenols including CAME protected Chinese hamster V79 cells against the cytotocity of H_2O_2 (8). This result suggests that caffeic acid would have the opposite effect on the cytotoxicity of H_2O_2. We also found that caffeic acid inhibited H_2O_2-induced DNA strand breakage in the presence of cytochrome *c* instead of "free" ferric ion in a model reaction system(4). These results prompted us to investigate the effects of caffeic acid or its esters on H_2O_2-induced DNA strand breakage in mammalian cells without addition of the transition metal ions to the medium. In the present study, we investigated the effects of caffeic acid ethyl ester (CAEE) on H_2O_2-induced cytotoxicity, DNA damage in the cells, and lipid peroxidation of liposome in the presence of cytochrome *c*.

2. Methods

Reagents Ethyl esters of caffeic acid and ferulic acid were prepared by esterification, purified by recrystallization from ethyl acetate, and identified by mass spectroscopy (Figure 1).

Colony formation assay Protective effects of the polyphenols, namely caffeic acid, ferulic acid, caffeic acid ethyl ester (CAEE) and ferulic acid ethyl ester (FAEE), against H_2O_2-induced cytotoxicity were assessed by a colony-formation assay. Chinese hamster lung fibroblast V79 cells were seeded in 60-mm petri dishes (200 cells/dish) and incubated in 5 ml of minimum essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) in a humidified atomosphere of 5% CO_2 in air at 37°C.

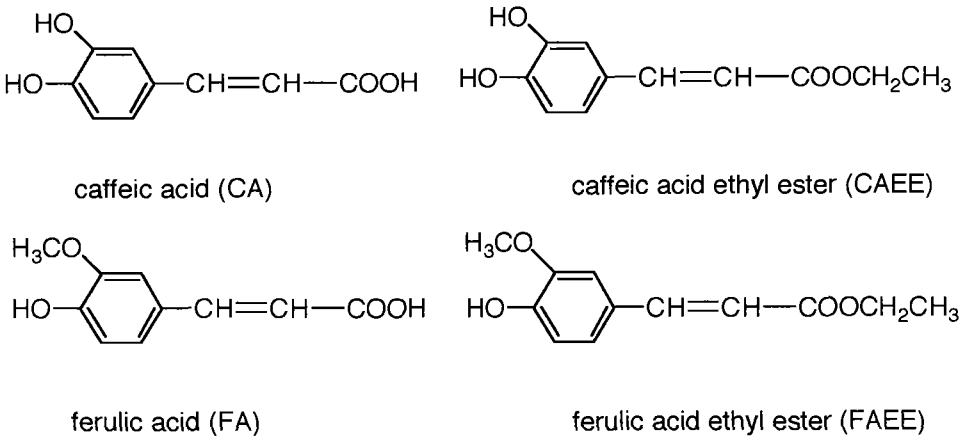

Figure 1 Structures of caffeic acid, ferulic acid and their ethyl esters.

After changing the medium to 5 ml of MEM free of FBS, an ethanolic solution of each polyphenol (up to 40 μl) was added to the medium, and the cells were incubated for 4 hours. After being washed with HEPES-buffered saline (HBS) (pH 7.3), the cells were treated with H_2O_2 (60 μM) in 5 ml of HBS for 30 min.

After culture in MEM supplemented with 10% FBS for 5 days, the number of colonies was counted. The survival (% of control) was calculated by the number of colonies of the cells treated with the polyphenols and/or H_2O_2 by the number of colonies of the untreated control cells. The results are expressed as the means and standard deviations (SD) of four separately treated cultures.

DNA precipitation assay V79 cells (1.0 x 10^6 cells / ϕ=10 mm dish) were incubated in MEM (10% FBS) for 4 hours. Then, 10 μCi [methyl-^{3}H]-thymidine was added to the medium, and the cells were incubated for 24 hours. After the cells were trypsinized and seeded into 24-multi wells (1.0 x 10^5 cells/well), the cells were allowed to grow for 15 hours in MEM (10% FBS). After being washed with phosphate buffered saline (PBS), the cells were treated with the indicated amount of H_2O_2 in 1 ml of PBS for 30 min at 37°C. After the cells were treated with 300 μl of lysis buffer (10 mM Tris, 10 mM EDTA, 0.05 M NaOH, 2% SDS, pH 12.4) for 1 min, 300 μl of 120 mM KCl were gently added to the well. The wells were incubated at 65°C for 10 min in a water bath, after which 400 μl of the lysate was gently transferred into Eppendorf tubes and kept for 5 min in an ice bath. A DNA-protein K-SDS complex formed in this process precipitated. Low-molecular single-strand DNA released from the bulk of the DNA was separated from the precipitate by centrifugation at 3500 rpm for 10 min at 10°C. The supernatant was transferred to a glass microfiber filter (Whatman, GF/C, ϕ=2.5 cm). These were serially washed in 5% TCA and methanol, and their radio

activity counted in a scintillation counter. The percentage of precipitated DNA was calculated for each sample and normalized to the value of precipitated DNA in the untreated controls, taken as 100%. In all plots the points represent the mean of 4 independent determinations and the vertical bars represent the standard deviation. The data were analyzed with Student's *t*-test, comparing the control (the cells treated only with H_2O_2) and the groups treated with a polyphenol and H_2O_2.

Measurement of thiobarbituric acid reacting substances (TBARS) formed during lipid peroxidation of liposome in the presence of H_2O_2 and cytochrome *c*. Small unilamellar vesicles (SUV) were prepared from egg lecithin. SUV solution (10 mg liposome/ml) was diluted with 10 mM sodium phosphate buffer (pH 7.4) to produce the final concentration of liposome (1 mg/ml). Indicated amounts of H_2O_2, cytochrome *c*, diethylenetriamine pentaacetic acid (DTPA), and each polyphenol were added to the diluted SUV solution. After incubation for 4 hours at 37°C, 1 ml of the reaction mixture, 0.1 ml of 10% methanolic solution of 10% butylated hydroxy toluene (BHT), and 2 ml of TBA solution (15% w/v TCA, 0.375% w/v TBA and 0.25 N HCl) were mixed, heated for 15 min in a boiling water bath, and measured its absobance at 535nm.

3. Results and Discussion

Since no polyphenols used in our experiments were toxic at 100 μM, we compared the protective effects of the polyphenols against H_2O_2-induced cytotoxicity by changing their concentrations up to 75 μM. As shown in figure 2, the survivals of the cells treated with 60 μM of H_2O_2 in the absence of the polyphenols were less than 5%, and only CAEE showed protective effects against the cytotoxicity of H_2O_2. In addition, the polyphenols other than CAEE did not show any protective effects even at the higher concentrations. This suggests that the difference of the protective effects between CAEE and the other polyphenols is not quantitative but qualitative. It is well known that carboxylic acid esters like fluorescein diacetate and quin-2 ester diffuse across the plasma membrane and are hydrolyzed by cytosolic esterases to yield the respective impermeable carboxylic acid and alcohol. We suppose that CAEE is permeable to the plasma membrane and cleaved by cellular esterases to yield caffeic acid and ethanol. Considering the diffusion factors described above, we compared effects of CAEE on H_2O_2-induced DNA single-strand breaks in the cells with those of FAEE by DNA precipitation assay. We first determined the dose dependence of the ratio of precipitated DNA with H_2O_2 concentration.

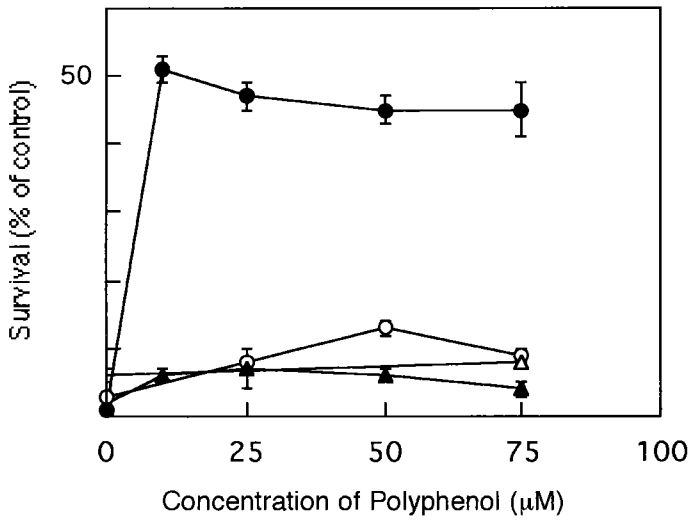

Figure 2 Dose effects of the polyphenols against the cytotoxicity of H_2O_2. V79 cells were treated with each polyphenol at the indicated concentrations in MEM (-FBS) at 37°C for 4 hours, and subsequently, with H_2O_2 (60 μM) in HBS for 30 min. After culture in MEM (+FBS) for 5 days, the number of colonies was counted. (●)CAEE (○)caffeic acid (▲) FAEE (△) ferulic acid.

Figure 3 shows that the effect of H_2O_2 was linear up to 30 μM, above which it tends to level off. When the cells were pretreated with CAEE, the ratio of precipitated DNA increased with the concentration of CAEE (Figure 4). This result indicates that CAEE inhibited H_2O_2-induced DNA single-strand breaks. On the other hand, the ratio of precipitated DNA of the cells pretreated with FAEE was not essentially changed, indicating that FAEE had no protective effects against H_2O_2-induced DNA single-strand breaks. The protective effects of CAEE both on H_2O_2-induced cytotoxicity and H_2O_2-induced DNA single-strand breaks, and no effects of FAEE on these damages suggest that both H_2O_2-induced damages might be ascribed to common mechanisms. In the presence of the transition metal ions such as Fe^{3+}, Cu^{2+} and Mn^{2+}, caffeic acid caused oxidative damage to cellular and isolated DNA (3,4,5).

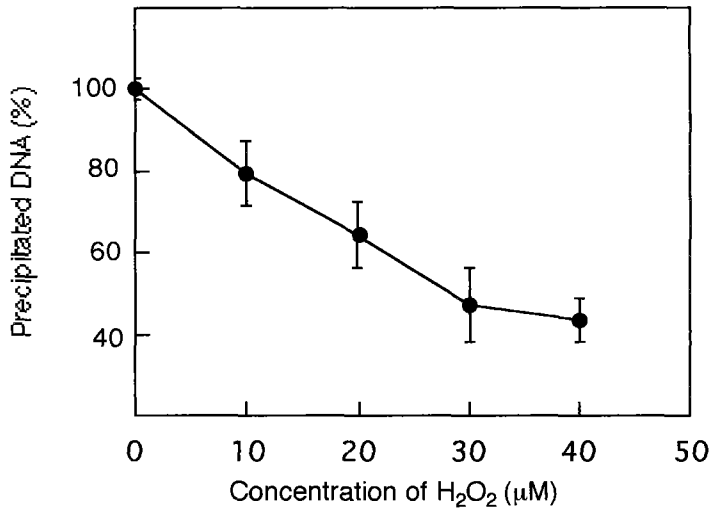

Figure 3 DNA precipitation assay to determine single-strand breaks in cells exposed to H_2O_2.

If these metal ions were sufficiently present in cells and catalyzed H_2O_2-induced reactions, CAEE would enhance H_2O_2-induced cell damages. Inhibitory effects of CAEE in the present study, therefore, indicates that the transition metals have no critical roles in the H_2O_2-induced cell damages. It is proposed that H_2O_2-induced and cytochrome *c*- catalyzed lipid peroxidation in mitochondrial membrane triggers oxidative cell damages. We found that TBARS increased with inrease in H_2O_2 concentration, indicating that H_2O_2 induced lipid peroxidation in liposomal membrane in the presence of cytochrome *c* (data not shown). Since sufficient DTPA was present in the reaction mixture, the lipid perxoxidation should not be catalyzed by free iron ions but cytochrome *c*. Under these conditions, we compared the effects of caffeic acid and CAEE on the lipid peroxidation with those of ferulic acid and FAEE and found that caffeic acid and CAEE inhibited the lipid peroxidation. On the other hand, the inhibitory effects of ferulic acid and FAEE were not so evident (Table 1). Consequently, CAEE showed inhibitory effects in all assays in the present study, and FAEE did not. These common contrasts in our present results would be useful to clarify the antioxidative effects of polyphenols against H_2O_2-induced cell damages.

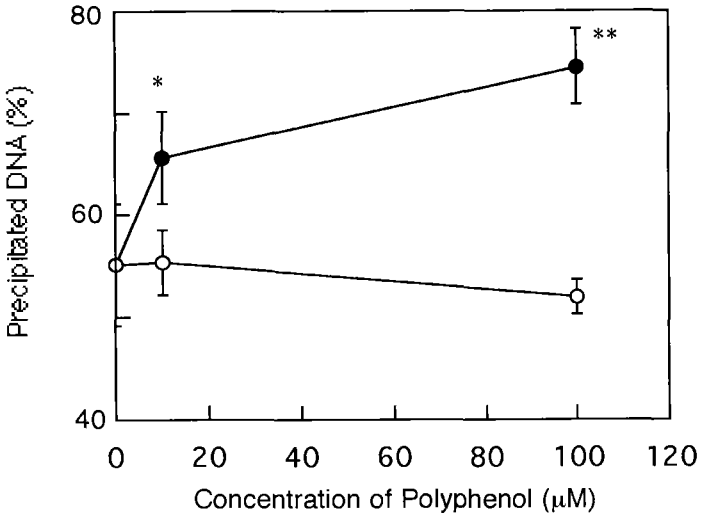

Figure 4 Dose effects of preincubation of V79 cells with CAEE or FAEE on the production of single-strand breaks in the cells exposed to H_2O_2. At the time that the cells were plated into wells, the media were supplemented with the indicated amount of CAEE(●) or FAEE(○). After 15 hours, cells were treated with H_2O_2 (40 μM) for 30 min. The data were analyzed with Student's *t*-test, comparing the group treated with H_2O_2 and the group treated with H_2O_2 and the indicated polyphenol. *P<0.05, **P<0.01.

Table 1.
Effects of the polyphenols on liposome lipid peroxidation induced by H_2O_2 in the presence of cytochrome *c*.

Polyphenol	TBARS (nmol MDA eq / mg lipid)
Control	0.80 ± 0
+ caffeic acid	0.12 ± 0.01
+ CAEE	0.21 ± 0
+ ferulic acid	0.72 ± 0.01
+ FAEE	0.63 ± 0.04

H_2O_2 (200 μM), cytochrome *c* (10 μM), DTPA (20 μM), and each polyphenol (50 μM) were added to the liposome solution (1 mg/ml). Reaction mixtures were incubated for 4 hours at 37°C. Data are the means of duplicate samples.

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Summary

Oxidative cell damages of caffeic acid have been ascribed to H_2O_2 formed in its autoxidation process. In the presence of transition metal ion such as Fe^{3+}, Cu^{2+}, and Mn^{2+}, caffeic acid enhanced hydroxyl radical (·OH) formation and caused oxidative damages such as chromosomal aberration, mutagenicity, and single strand breaks of cellular and isolated DNA.

We found that caffeic acid esters showed protective effects against H_2O_2-induced cell damages in the absence of these metal ions. Cytotoxicity and DNA single strand breaks caused by H_2O_2 were assessed by a colony formation assay and a DNA precipitation assay, respectively. In both assay, caffeic acid ethyl ester showed protective effects. Also in model systems in the presence of cytochrome *c* instead of free Fe^{3+}, caffeic acid and its esters inhibited H_2O_2-induced DNA single strand breaks and H_2O_2-induced lipid peroxidation. These results suggest that caffeic acid could act as antioxidant *in vivo*, where these transition metal ions are scarcely found as their free forms.

PHYSICAL AND CHEMICAL CHANGES DURING HOT STORAGE OF THE COFFEE BEVERAGE

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Introduction

The deterioration of the coffee beverage during hot storage is a problem known to both the catering service and the consumer. To handle demand during peak periods, coffee is brewed in large quantities in advance and is kept hot until needed. Thus, the quality of the product is reduced in the service area. The ability to take countermeasures requires knowledge of quality impact parameters.

The sensory character of the coffee beverage is composed of visual attributes, of odor and taste. Visual features can be divided into color and turbidity.

Study of color and turbidity

PANGBORN [1982] observed visually a strong increase in intensity of color and turbidity in the first hour of keeping hot at 95°C. At 60°C and 80°C the observed changes were rather small. ROTHFOS and OESTREICH-JANZEN [1991] found, with colorimetric methods at storage temperatures between 20°C and 80°C, a decrease in the brightness value L* and the red-green value a* according to the L*a*b*-system of CIE terminology fixed in DIN 6174. This system is based on a color space defined by three space coordinates. The L*a*b* color space is shown in fig. 1. Red and green terminate the horizontal a*-axis; yellow and blue terminate the horizontal b*-axis. The vertical L*-axis represents brightness (black: $L^* = 0$, white: $L^* = 100$). The measuring geometry was 45°/0° (fig. 2). To study turbidity, we used nephelometry. The principle of the instrument used is presented in fig 3. The result is given as % of reflected light referred to the incident light.

Fig. 1: Color space of the CIE-L*a*b*-system

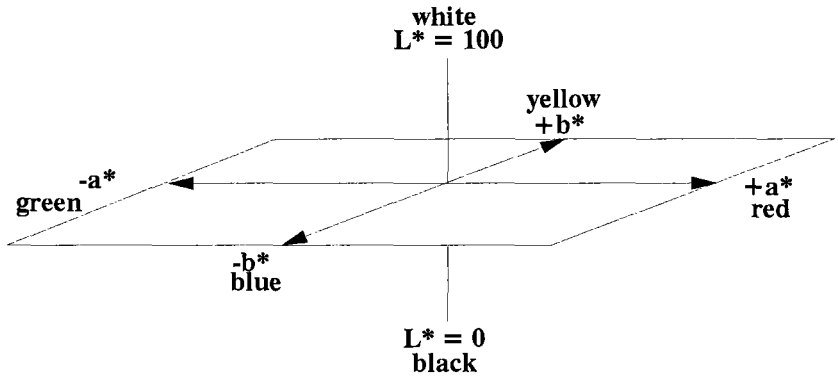


Fig 2: Equipment for measuring the transflectance of liquors (45°/0°)

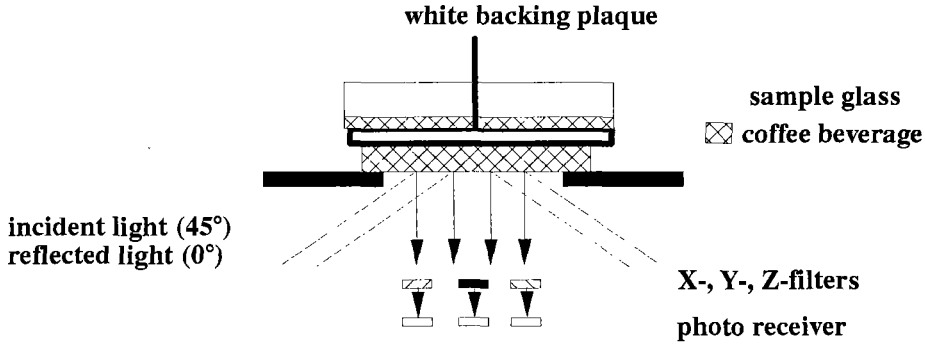
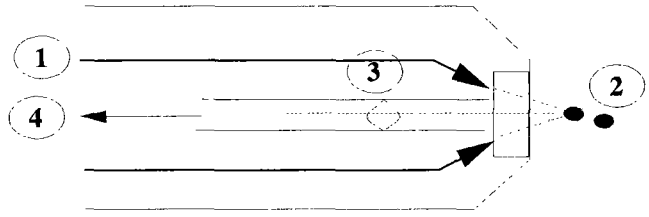


Fig. 3: Equipment for measuring the turbidity by nephelometry



(1) Incidence of IR light
(2) Reflection, due to particles in liquor
(3) Focus of reflected light
(4) Measurement by the receiver optics

A beverage of arabica, provenance Columbia, middle roasted, was brewed in a coffee machine with 1,5% dry substance. The samples were kept hot at 80°C in a water bath. The headspace in one half of the samples was approx. 10 ml; in the other half approx. 400 ml. For measurements one sample was removed from the water bath, whilst the rest remained closed in order to in order to preserve a "closed system" with no further access of air. Color and turbidity were measured at ambient temperature. Additionally, the change in turbidity was measured at 80°C in the samples with approx. 400 ml headspace.

Fig. 4 shows only a little difference in color during the hot storage with a headspace of 10 ml. A headspace of 400 ml led, however, to a constant decrease of L*. The values for a* and particularly for b* showed a similar tendency. The turbidity measured at 80°C increased with time of storage, as can be seen in fig. 5.

Fig. 4: Changes of color in a coffee beverage, stored in a water bath (80°C), headspace HS 10 ml/400 ml (transflectance measurement 45°/0°)

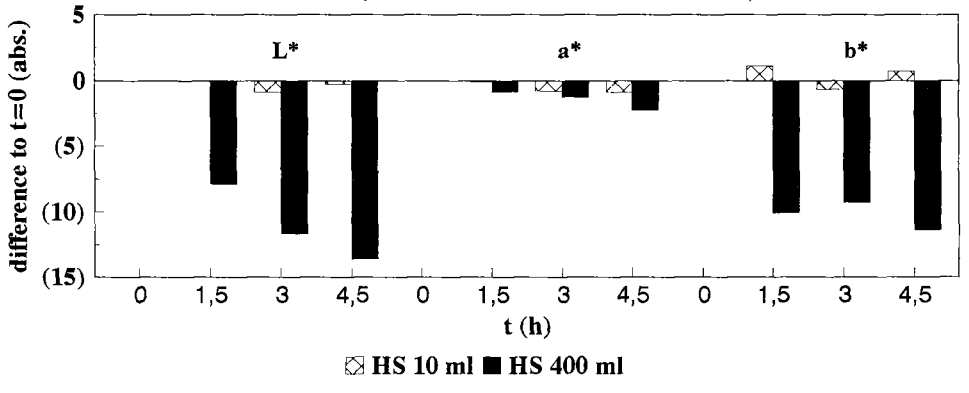


Fig. 5: Intensity of the turbidity (It) during hot storage of the coffee beverage, headspace HS 400 ml (water bath 80°C; measurement 80°C)

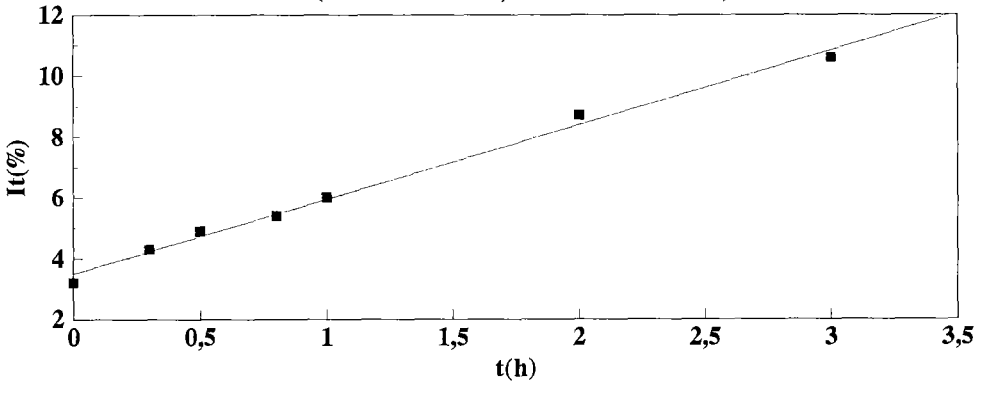
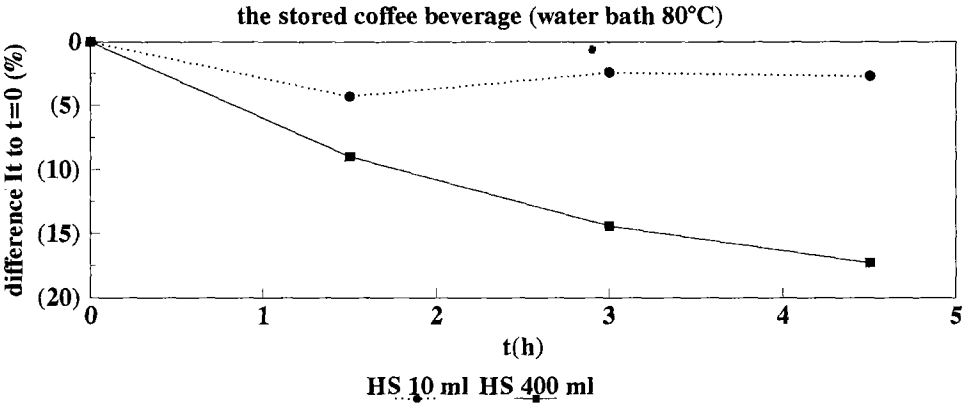


Fig. 6, however, shows a decreasing turbidity with a 400 ml headspace volume measured at 20°C. Little change occurred in the samples kept hot with a headspace of 10 ml.

Fig. 6: Influence of the headspace HS (10 ml/ 400 ml) on the intensity of turbidity (It) in the stored coffee beverage (water bath 80°C)



Oxygen suggested itself as the reason for this difference and it was proved to be so by passing oxygen through the system. The color of the beverage was markedly deepened (fig. 7). The turbidity highly increased in the beverage measured at 80°C and decreased in the cooled beverage (fig. 8). In the oxygen treated beverage floc particles appeared.

Fig. 7: Influence of passing oxygen through the coffee beverage on the changes of the color (water bath 80°C/0,5h; transflectance 45°/0°)

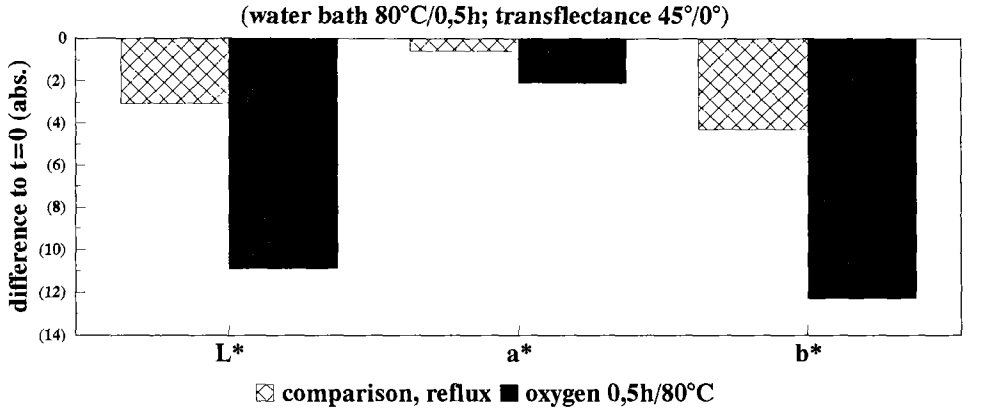
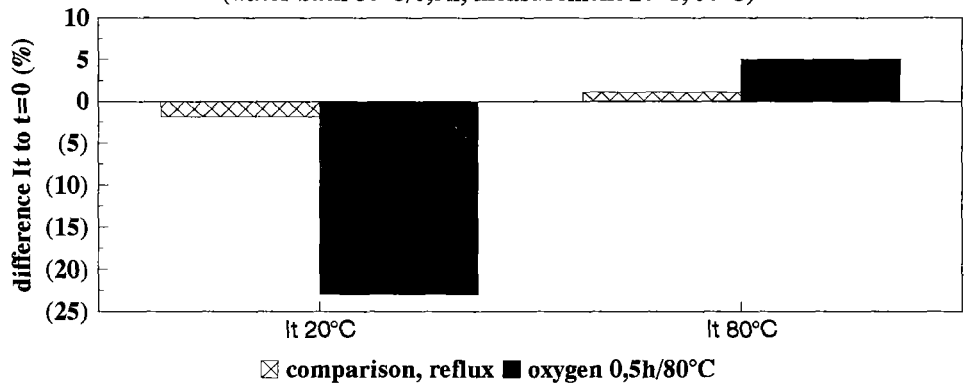


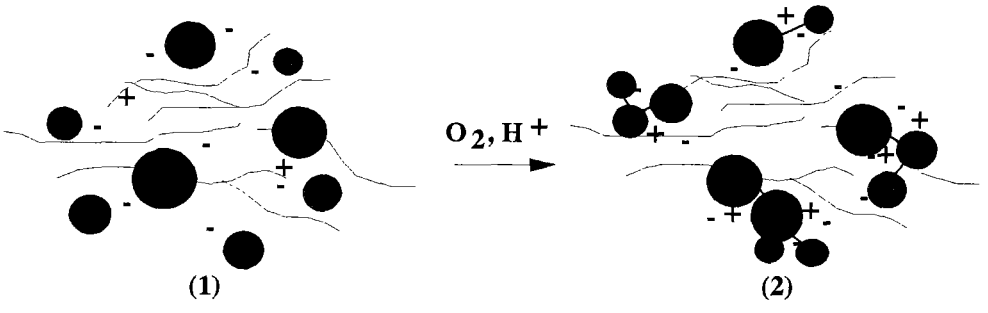
Fig. 8: Influence of passing oxygen through the coffee beverage on the intensity of turbidity It (water bath 80°C/0,5h; measurement 20°C, 80°C)



It may be concluded from these results that oxygen induces reactions between the melanoidins which are responsible for the dark color of coffee. The components are of complex structure due to the roasting process. As is known from the literature on the subject, the components contain phenolic groups [HEINRICH and BALTES, 1987 a-b] so that phenolic oxidation reactions are quite likely.

The model shown in fig. 9 was deduced. In the hot beverage, micelles of melanoidins are thermally unfolded. Phenolic groups are easily accessible. In reacting with oxygen, smaller complexes of melanoidins are coupled to larger aggregates with a growing tendency to becoming insoluble. Oxidative aggregation is accompanied by rising electrostatic interactions resulting from hydrolytic reactions [MAIER et al., 1984]. This hydrolysis may lead to a reduction in size of the submicelles.

Fig. 9: Model of the aggregation of melanoidin micelles

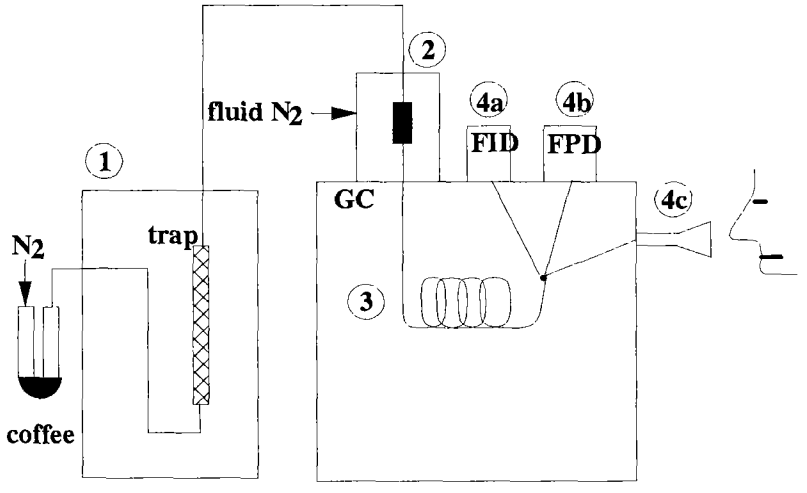


(1) Thermally induced unfolded melanoidin micelles
(2) Aggregation of micelles, due to oxidation of phenolic groups and electrostatic interactions

Study of volatiles

The volatiles were studied by dynamic headspace gas chromatography. A scheme of the equipment is given in fig. 10. The volatiles were purged out of the sample with nitrogen and were trapped on a column of Tenax GC-charcoal-silica gel. After thermal desorbtion, the volatiles were concentrated in the cryo module at -120°C. The compounds were thermally desorbed, separated on a capillary column, and detected by a flame ionization detector (FID), a flame photometry detector selective for sulfur compounds (FPD) and a sniffing device. The identification of compounds was according to retention times given by other authors [HOLSCHER und STEINHART, 1992 a-b; BLANK et al., 1992] and supplementing the beverage with these substances. The reproducibility of peak areas was high, due to the automatization of the process. A HRG-chromatogram with FID and FPD as detectors is shown in fig. 11. Of particular interest were those compounds representing oxidative reactions.

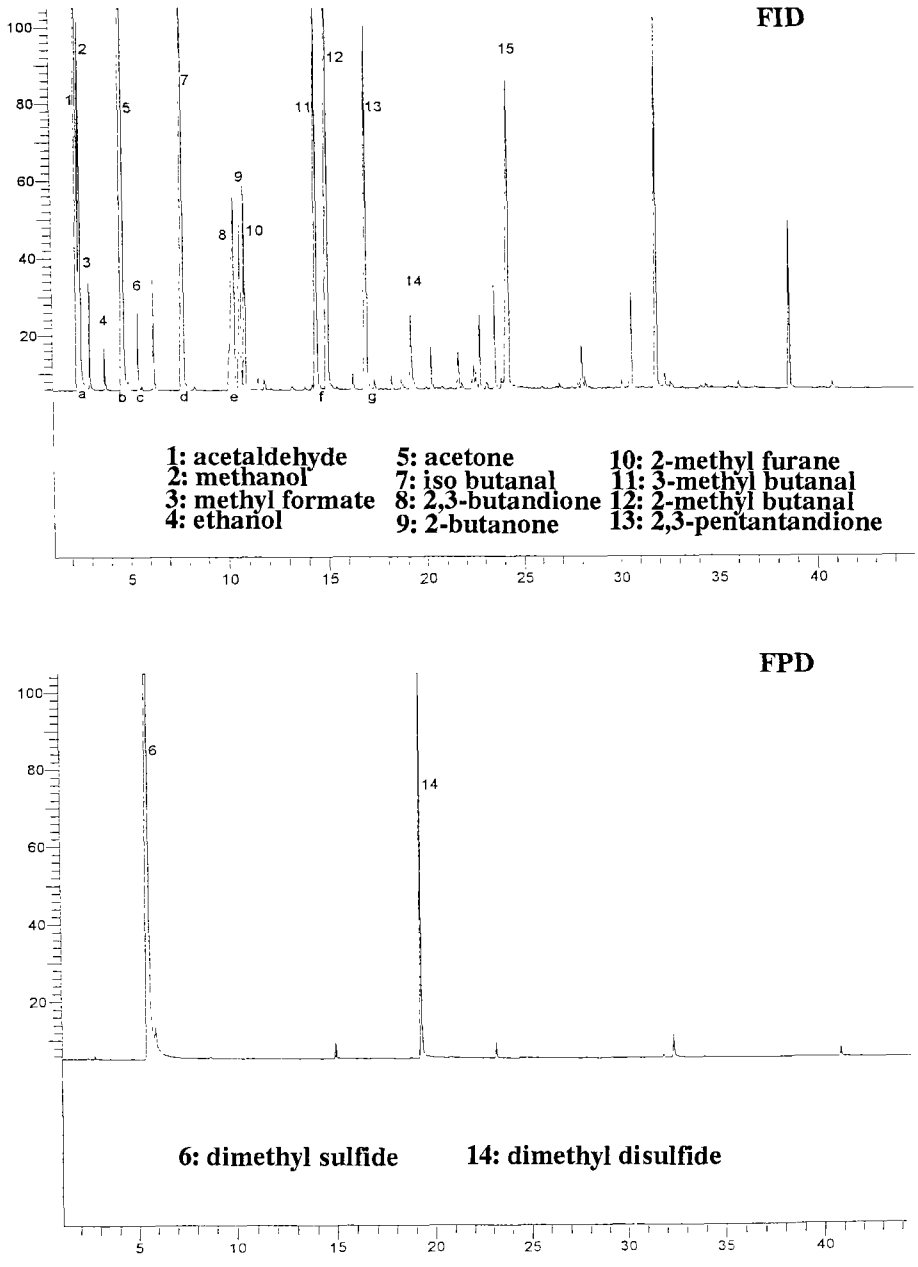
Fig. 10: Equipment for dynamic headspace gas chromatography



(1) Isolation by purge & trap
(2) Concentration by fluid nitrogen (-120°C)
(3) Separation of the compounds via capillary
(4) Detection by FID (4a), FPD (4b) and a sniffing device (4c)

Common indicators of ageing of roasted coffee are M/M (methanol/2-methyl furane) and M/B (methanol/2-butanone). According to several authors, an increase of M/M and a decrease of M/B depending on the storage conditions has been observed [REYMOND et al., 1962; ARACKAL and LEHMANN, 1979; KWASNY and WERKHOFF, 1979; SPADONE and LIARDON, 1989]. As sulfur compounds are of high importance for the freshness of roasted coffee [HOLSCHER and STEINHART, 1992 a-b], dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) were included in the calculations, and changes of DMS/B and DMDS/B were observed during hot storage.

Fig. 11: Purge & Trap - HRGC; chromatograms of the coffee beverage, detection at FID and FPD (trap Tenax GC/active charcoal/silicagel; capillary MB 54/25 m/0,25 mm i.d./1 μm)



The influence of oxygen was identified by storing the samples under the same conditions as in the experiments on changes of color and turbidity (water bath 80°C, headspace approx. 10 ml/400 ml). The changes in indices M/M, M/B, DMS, and DMDS/B are shown in fig. 12 over a storage time of 4.5 hours. With respect to M/M and M/B, these indices show the same tendency as in roasted coffee. Because of the changes in the beverage stored under a headspace volume of 400 ml, oxygen is taken to be the reason for this difference. A high sensitivity for oxidative reactions is also observed, with DMS/B and DMDS/B showing a marked decrease. From the decrease of methyl formate independent of the headspace volume (fig. 13), the occurrence of the hydrolytic reaction type was concluded.

Fig. 12: P&T-HRGC; changes of the indices M/M, M/B, DMS/B, DMDS/B during hot storage and with a headspace HS 10 ml/400 ml (water bath 80°C/4,5h)

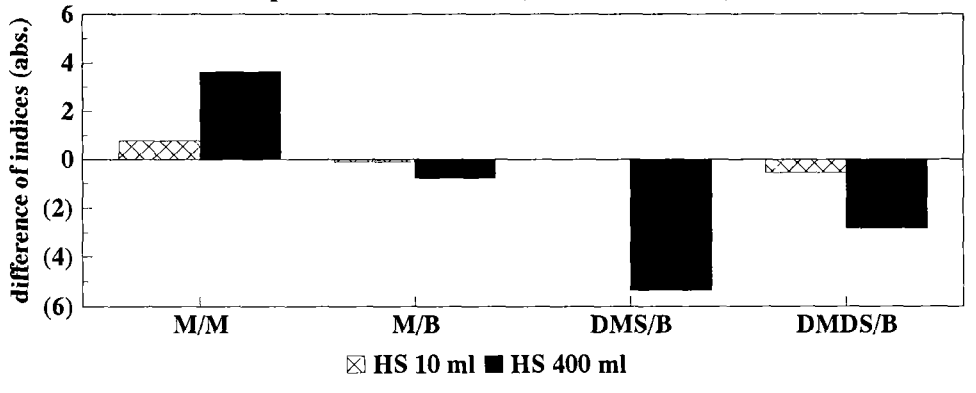
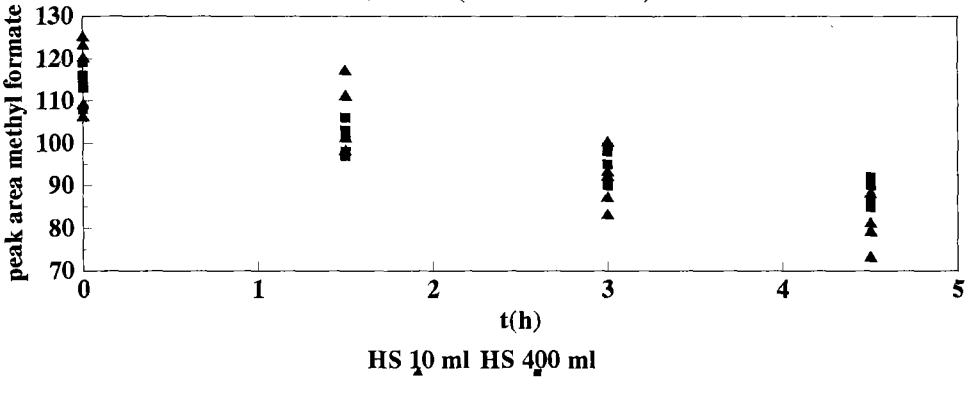


Fig. 13: P&T-HRGC; peak area of methyl formate during hot storage and with a headspace HS 10 ml/400 ml (water bath 80°C)



Summary

By investigating color, turbidity, and volatiles, oxygen was shown to be an important factor in the deterioration of the coffee beverage during hot storage. Among the non-volatiles, oxidative reactions of melanoidins were identified by a marked deepening of color with increasing accessibility of oxygen. Oxygen led to a higher turbidity measured in the hot beverage but led to a lower turbidity in the cooled beverage. A structural model including oxidative and hydrolytic reactions was developed. The volatiles were studied by dynamic headspace gas chromatography. Indices from ageing of roasted coffee were found to be transferable to the ageing of the coffee beverage. Two new indices containing sulfur compounds were calculated and showed high sensitivity for oxidative reactions.

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PIPECOLIC ACID FROM THE DEVELOPING FRUITS (PERICARP AND SEEDS) OF *COFFEA ARABICA* AND *CAMELLIA SINENSIS*

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INTRODUCTION

In tea plants, γ-glutamylmethylamide (γ-GM) is an effective precursor of the biosynthesis of caffeine (Konishi *et al.*, 1972); this unusual amino acid is metabolized as a one-carbon compound via methylamine and HCHO (Suzuki, 1973; Fig. 1). Methylamine may also serve as a precursor of caffeine in coffee plants (Preusser, 1967). However, little is known about the occurence of γ-GM and its participation in caffeine biosynthesis in coffee plants.

In contrast to γ-GM in tea plants, a proline analogue, pipecolic acid (Fig. 2), is fairly widespread in plants generally (Fowden, 1970; Bell, 1980) and we previously reported the level of this imino acid in mulberry (*Morus alba*) (Suzuki and Kohno, 1983, 1987). Recently, pipecolic acid, along with γ-GM, has also been detected by two-dimensional thin-layer chromatography (2D-TLC) of the amino acids in green tea (Suzuki and Higuchi, unpublished results).

In the present study, as part of our extensive efforts to clarify various aspects of the metabolism of caffeine and related compounds and their role in different organs and species (see Suzuki *et al.*, 1992 and Terrasaki *et al.*, 1994), we first examined the occurence of γ-GM in unripe and ripe fruits (pericarps and seeds) of *Coffea* spp. and tea plants. This paper also describes (i) the detection of pipecolic acid in unripe fruits of these plants and (ii) the inhibition of root growth of germinating seeds of *M. alba* and *Astragalus sinicus* exposed to 5-20mM pipecolic acid.

MATERIALS AND METHODS

Plant materials. The leaves and immature fruits of *Coffea* spp. (*C. arabica, C. canephora, C. liberica, C. bengalensis*) were harvested 29 September 1994 in a greenhouse of the Faculty of Agriculture, Kobe University, Kobe, Japan and stored in a deep freezer until required for extraction. Fruits of tea (*Camellia sinensis* cv. Komakage) were collected in Takeda Herbal Garden, Kyoto, Japan at 3-4 week intervals from 9 September to 31 October 1994 and kept frozen in a deep freezer until extraction.

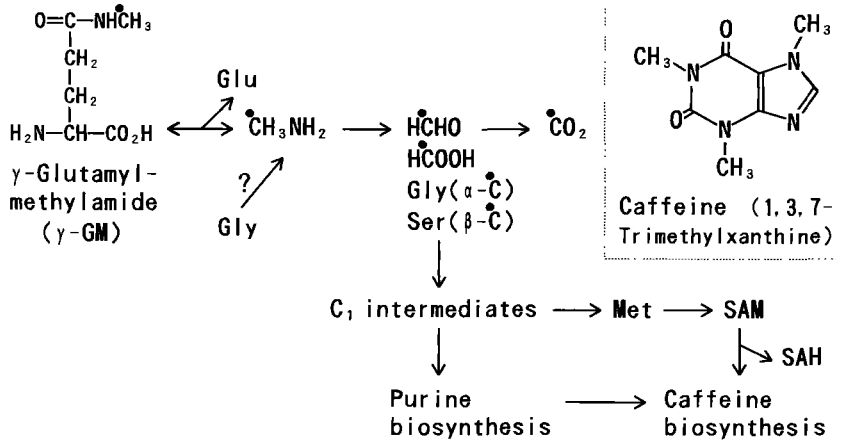

Figure 1. One-carbon metabolism and caffeine biosynthesis in tea plants (Suzuki and Takahashi, 1977)

SAM:S-adenosyl-L-methionine SAH:S-adenosyl-L-homocysteine

Extraction and analysis of free amino acids. Before analysis of the samples, the fruits of *C. arabica* and *C. bengalensis* were separated into pericarps and seeds (Suzuki and Waller,1985), whereas the fruits of tea were separated into pericarps, seed coats and cotyledons (Terrasaki *et al.*,). Samples were extracted as described elsewhere (Suzuki, 1982; Suzuki and Kohno, 1983). In brief, each sample was ground with 80% EtOH and washed sea sand in a mortar. After centrifugation, the residue was washed twice with 80% EtOH. The supernatant and washings were passed through a column of Amberlite IR-120 (H^+ form) and the regin was washed with water. The amino acids were then eluted with 3M-NH_4OH and the eluates were dried *in vacuo* at a temperature below 40° C. The dried samples were dissolved in water and analyzed by 2D-TLC on silica gel 60 plates (Merck, Germany). The solvents used were (a) PhOH-H_2O (3:1, w/w) and (b) n-BuOH-AcOH-H_2O (4:1:1, by vol.). Amino acids were revealed by their reaction with ninhydrin sprays. Cyclic imino acids (Fig. 2) were detected by the isatin-coloration (Piez *et al.*, 1956; Kondo, 1957).

Identification of the cyclic imino acids. Identification was based on R*f* and color reactions on TLC (Tab. 1). Ninhydrin reacts with the cyclic imino acids on the plate to give distinctive colors. Prolin appears yellow, fading to brown. Hydroxyproline is brick-red, while pipecolic acid (Wako Pure Chemical Ind., Ltd., Osaka, Japan) appears blue. All of these cyclic imino acids also give a blue to greenish-blue color with isatin.

Germination experiments. To determine the phytotoxic effect of pipecolic acid, aqueous solutions of the authentic compound in a series of concen-

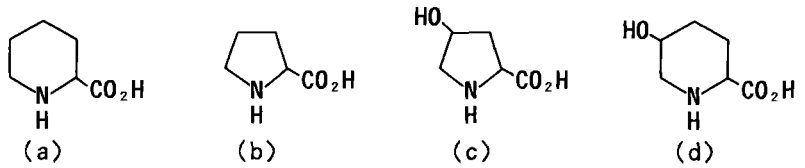

Figure 2. Some naturally occurring cyclic imino acids in plants

(a) Pipecolic cid (b) Proline (c) Hydroxyproline
(d) 5-Hydroxypipecolic acid

trations (1-30mM) were bioassayed with Chinese milk vetch (*A. sinicus*), mulberry (*M. alba* cv. Shin-ichinose), and weeping mulberry (*M. alba* var. *pendulous* DIPPEL) as test materials. Seeds were initially soaked in 70% EtOH for 3 min, surface-sterilized in a saturated solution of chlorinated lime for 30 min, washed in running water and rinsed twice with distilled water. They were then placed on two layers of Advantec filter paper (No. 2) in petrei dishes and allowed to germinate at 25±1°C. Distilled water served as a control and exogenous caffeine (5mM), a known phytotoxic compound (Chou and Waller, 1980; Rizvi *et al.*), was also similarly tested as reference.

RESULTS AND DISCUSSION

Cyclic imino acids in immature fruits of tea and coffee

From the R*f* values in two-dimensional paper chromatography in solvents (a) and (b), and color reactions produced by ninhydrin sprays, pipecolic acid and 5-hydroxypipecolic acid present in *M. alba* were detected previously (Suzuki, 1982; Suzuki and Kohno, 1983). These were further identified by the color reaction with isatin and absorption spectra after reaction with ninhydrin in glacious AcOH (Suzuki, unpublished results; see also Piez *et al.*, 1956 and Kondo, 1957). In the present study, two ninhydrin- and isatin-positive substances on 2D-TLC of the free amino acids of the immature fruits (pericarps, seed coats and cotyledons) of tea, developed with solvents (a) and (b), successively, were coincident with cyclic imino acids, hydroxyproline and pipecolic acid (Fig. 3; Tabs 1 and 2).

Among the free amino acids which have been isolated from *Coffea* spp., proline, hydroxyproline and pipecolic acid were also detected by 2D-TLC in the immature fruits (pericarps and seeds) of *C. arabica* (Tabs 1 and 3). Pipecolic acid also occurred in young leaves of *C. arabica*, but were not detected in the fruit of *C. bengalensis* (Tab. 3).

Pipecolic acid and γ-glutamylmethylamide in tea fruits

In leaves of tea seedlings, the methyl group of γ-GM is an effective precursor of the biosynthesis of caffeine (Konishi *et al.*, 1972); this non-protein

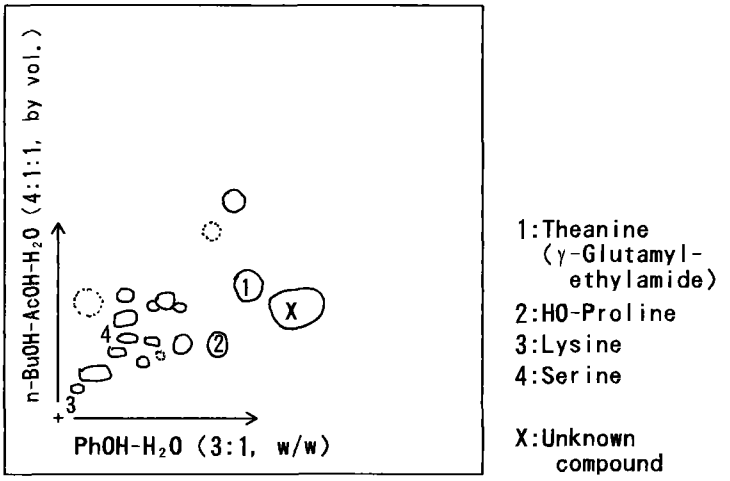

Figure 3. Two-dimensional thin-layer chromatographic profile of amino acids of the immature seed (cotyledon) of tea harvested on September 9

Table 1. Characterization of imino acids in the developing fruits of tea and coffee plants

	Rf values*		Nihydrin color	Isatin color
	(1)PhOH-H_2O (3:1, w/w)	(2)BuOH-AcOH-H_2O (4:1:1, by vol.)		
Proline	0.54	0.20	yellow	blue
HO-Proline	0.40	0.21	red-brown	green-blue
Pipecolic acid	0.58	0.30	blue	green-blue
Unknown X	0.58	0.30	blue	green-blue

*Silica gel 60 (Merck)

Table 2. The relative composition* of selected amino acids of immature and mature fruits of tea and made tea

Plant part or souce	Date of sampling	Theanine	γ-GM	Pipecolic acid	Lysine
Pericarp	Sep 9	++	—	+++	—
	Oct 11	++	—	++	+
	Oct 31	+++	—	+++	—
Seed coat	Sep 9	±	—	+	—
	Oct 11	—	—	—	—
	Oct 31	+	—	—	—
Seed	Sep 9	++	—	+++	+
(cotyledon)	Oct 11	++	—	++	++
	Oct 31	+++	—	—	++
Green tea (1) sencha		+++	±	+	+
Green tea (2) bancha		+	—	±	—
Black tea		++	—	+	+

*Based on reaction with ninhydrin on silica gel 60 plates.

amino acid is metabolized as a one-carbon compound via methylamine and HCHO (Suzuki, 1973; Suzuki and Takahashi, 1977; Fig. 1). The biosynthetic capacity for the synthesis of caffeine, using [8-^{14}C]adenine, has also shown to be high in developing flower buds, pericarps and seed coats of tea (Fujimori and Ashihara, 1990; Terrasaki *et al.*, 1994). In the present study, however, γ-GM was not detected in these tissues, in particular harvested in September (Fig. 3; Tab. 2). Judging from the spot-size and the intensity of the ninhydrin coloration, serine was an abundant amino acid (data not shown). This indicates that the β-carbon atom of serine may be an important methyl group donor for caffeine biosynthesis in developing flower buds, pericarps and seed coats of tea (Fig. 1). Active synthesis of theobromine and caffeine from [3-^{14}C]serine in shoot tips of tea has been described (Suzuki and Takahashi, 1976).

In contrast to γ-GM, in pericarps and cotyledons of the immature fruits of tea harvested in September, pipecolic acid was the major amino acid, although theanine (γ-glutamylethylamide) occurred as an abundant amino acid (Tab. 2), as is in the leaves (data not shown). It should be noted, however, that in the mature dried fruit (31 October) pipecolic acid disappeared drastically in the cotyledon, while it was still the abundant amino acid in the discarded pericarp (Tab. 2). Alternatively, lysine increased in the matured cotyledons. These results suggest the close relation of the metabolism between lysine and pipecolic

acid, as described in other organisms (Rodwell, 1969; Mazelis, 1980). Thus lysine but not pipecolic acid dose serve as a nitrogen storage compound in tea seeds. Since pipecolic acid was also found in made tea (green tea and black tea) (Table 2), further study on the level and role of pipecolic acid in tea fruits during development and seed formation as well as in the flush shoots and young leaves sholud be needed.

Pipecolic acid in coffee plants

In leaves, pericarps and seeds of *Coffea* spp., γ-GM was not thoroughly detected (Tab. 3), indicating that γ-GM may not be the precursor of caffeine in *Coffea* plants. Thus methylamine may be directly oxidized to HCHO and then metabolized as a one-carbon compound in coffee plants (Fig. 1).

In contrast to this, with the exception of the fruit of *C. bengalensis*, pipecolic acid occurred in young leaves and unripe fruits of *Coffea* spp. analyzed, although lysine was also present in these tissues (Tab. 3). Like mature tea seeds (cotyledons), pipecolic acid almost disappeared in the mature seeds of *C. arabica*. Thus, although pipecolic acid was first found in seeds of *Phaseolus* spp. and other Leguminosae plants (except for *Vigna* spp.) (Zacharius *et al.*, 1954; Bell, 1971), its occurence in seeds of *Coffea* spp. (except for *C. begalensis*) is closely associated with fruit development and seed formation, similar to tea seeds.

Inhibitory effects of aqueous solutions of pipecolic acid

Little is known about the role of pipecolic acid in the developing fruits of tea and coffee plants (Tabs 2 and 3). The accumulation of pipecolic acid under water stress or saline conditions or in response to Mg- or Cl-deficiencies in higher plants has been described (Stewart and Larher, 1980). Some non-protein amino acids, in particular present in fruits and seeds, exhibit toxicity against various predators and thus function as natural protectants of such tissues (Rosenthal, 1982; Harborne and Turner, 1984; D'Mello, 1995). Some of these compounds may also have phytotoxic activity, i.e., inhibit germination or growth of

Table 3. The relative composition* of selected amino acids of leaves and fruits (pericarps and seeds) of coffee plants and roasted coffee**

	Plant part	Date of sampling	γ-GM	Pipecolic acid	Lys	Pro	HO-Pro
C. arabica	Young leaf	Sep 29	—	+	+	+	+
	Pericarp	Sep 29 (a)	—	++	+	++	+
		Dec 5 (b)	—	±	+	+	+
		Jan 17 (c)	—	±	+	+	±
	Seed	Sep 29	—	++	+	++	+
		Dec 5	—	+	+	+	++
		Jan 17	—	±	+	+	+
C. bengalensis	Young leaf	Sep 29	—	±	+	+	—
	Pericarp	Sep 29 (a)	—	—	+	±	+
	Seed	Sep 29	—	—	+	±	+
C. canephora	Young leaf	Sep 29	—	+	+	++	+
C. liberica	Young leaf	Sep 29	—	+	+	+	++

*Based on reaction with ninhydrin on silica gel 60 plates.
**No free amino acids could be detected in roasted coffee.
(a)~(c)Colors of fruit epidermis: (a) green, (b) yellow-red, (c) red-brown.

Table 4. Inhibitory effects of pipecolic acid in aq. soln. on growth of seedlings of Chinese milk vetch, weeping mulberry, and mulberry (cv. Shin-ichinose) after 5 or 10 days at 25°C*

		Concentration (mM)				
		1	5	10	20	30
Chinese milk vetch	Pipecolic acid					
	Radicle	n.d.**	n.d.	78±9	47±8	33±7
	Hypocotyl	n.d.	n.d.	105±13	100±11	94±8
	Caffeine					
	Radicle	n.d.	15±4	n.d.	n.d.	n.d.
	Hypocotyl	n.d.	28±5	n.d.	n.d.	n.d.
Weeping mulberry	Pipecolic acid					
	Radicle	102±14	28±6	25±5	n.d.	n.d.
	Hypocotyl	99±10	95±8	83±9	n.d.	n.d.
	Caffeine					
	Radicle	n.d.	18±5	n.d.	n.d.	n.d.
	Hypocotyl	n.d.	20±6	n.d.	n.d.	n.d.
Mulberry (cv. Shin-ichinose)	Pipecolic acid					
	Radicle	n.d.	n.d.	72±10	42±8	29±7
	Hypocotyl	n.d.	n.d.	110±15	104±12	55±8

*Data presented as percentage of growth (length) of the control (distilled water), average of 30 seedlings (±SD). Chinese milk vetch, 5 d; weeping mulberry, 10 d; mulberry (cv. Shin-ichinose), 10 d.

**n.d. =Not determined.

various species, but may also adversely affect their own species (Friedman and Waller, 1983). In the seeds of Chinese milk vetch, mulberry, and weeping mulberry tested in the present study, pipecolic acid has been detected (Higuchi and Suzuki, unpublished results). The tested compounds (DL- and L-pipecolic acid) exhibited phytotoxicity of the radicle growth of germinating seeds of these plants at concentrations of 5-20mM (Tab. 4), although the compound had little effect on percent germination (data not shown).

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SUMMARY

The methyl groups of methylamine and γ-glutamylmethylamide (γ-GM) are precursors of the biosynthesis of caffeine in leaveas of tea seedlings and mature plants. Methylamine is also a precursor of caffeine biosynthesis in leaves of *Coffea arabica*; however, γ-GM was not detected on two-dimensional thin-layer chromatography (2D-TLC; silica gel 60, Merck) of the free amino acids of the young developing leaves and unripe fruit of *C. arabica*.

In contrast to this, two nihydrin- and isatin-positive substances on 2D-TLC of the free amino acids of the developing immature fruits (pericarps and seeds) of tea were coincident with cyclic imino acids, pipecolic acid and hydroxyproline. In the maturing seeds, pipecolic acid almost disappeared, but lysine was detected. Pipicolic acid together with proline and hydroxyproline was also detected in the developing leaves and unripe fruits (pericarps and seeds) of *C. arabica*.

The inhibition of growth of Chinese milk vetch (*Astragalus sinicus*) and weeping mulberry (*M. alba* var. *pendula* Dippel) exposed to 20 and 5mM pipexcolic acid, respectively, was found to occur in the radicle of germinating seeds.

EFFECTS OF FAR INFRARED HEATING ON COFFEE BEANS ROASTING

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INTRODUCTION

Coffee beans are generally roasted with flames of liquid or gas fuels or a hot air generating therefrom. In recent years, large-sized roasters of the hot air type are mainly employed for commercial purposes. It may be said that the "convection heat transfer" is the major heat transfer process. In Japan, on the other hand, far infrared heaters of the radiation heat transfer type have become popular and effectively employed in drying coatings, molding plastics, heating and drying foods, etc. Infrared radiation is classified into near infrared radiation, mean infrared radiation and far infrared radiation. Although the wavelength region for each has never been clearly defined, a wavelength region exceeding 3~4 μm is generally regarded as the far infrared region. According to IEC 50 (841)(1983)[International Electrotechnical Vocabulary, Chapter 841 : Electroheat] and Japan Electro-Heat Association, the near infrared, mean infrared and far infrared regions correspond respectively to 0.78~2 μm, 2~4 μm and 4~1000 μm. According to JIRA (Japan Far Infrared Rays Association), the near infrared and far infrared regions correspond respectively to 0.78~3 μm and 3~1000 μm.

To establish a new method for roasting coffee beans, we have developed a coffee roaster with the use of a ceramic far infrared heater and put it into practical use.[1] Although the results of sensory test indicated that the coffee prepared by this method differed from those prepared by the existing methods, the difference has not been clarified from chemical and physical viewpoints. Accordingly, we have made attempts from every point of view so as to evaluate the method.

Based on the results of these experiments, it is estimated that the coffee beans roasted by the far infrared heating method show a rapid increase in the internal temperature but suffer from little damage on the surface and uniformly and quickly lose the moisture. Thus they are uniformly heated even at the core. Regarding the taste, it is assumed that the coffee is rich in the aroma components and has a light acidity, thus differing from those prepared by the existing methods. These results agree with the sensory evaluation.

SAMPLES AND EXPERIMENTAL PROCEDURES

1. Experiment on uniformity of heating

1-1. Measurement of increase in temperature of coffee beans during heating process

A thin thermocouple was inserted into the center of green coffeebeans (Jamaica PW, 18 mesh) and the increase in the temperature was measured in a system for comparing far infrared heating with hot air heating (NGK INSULATORS, LTD.; H7GS-71173). This system made it possible to heat coffee beans separately with a far infrared heater (NGK INSULATORS, LTD.: INFRASTEIN) and by convection heating with a hot air. In this experiment, the temperature in the oven was set to 200℃. The outputs of the far infrared heater and the hot air generator were both 1 Kw. The green coffee beans were made static at the center of the oven while supporting with the cord of the thermocouple inserted therein so as to avoid the contact of the coffee beans with the wall of the oven, etc.

1-2. Measurement of moisture content loss of coffee beans during heating process

By using a small-sized far infrared coffee roaster (NGK INSULATORS, LTD; IRT-003, H7GS-71169), 200g of green coffee beans (Indonesia AP-1) were heated with far infrared heaters alone or a hot air alone in a roasting drum regulated to 200 ℃. During this heating process, changes in the weight of the coffee beans were monitored with the passage of time. This roaster had an oven made of a heat insulating material. In this oven, a cylindrical rotary drum having a perforated side wall and mixing blades could rotate horizontally and a number of ceramic far infrared heaters (NGK INSULATORS, LTD; INFRASTEIN) were provided around the drum. In addition to these far infrared heaters, this device was also equipped with a hot air generator which could be used either alone or together with the far infrared heaters.

1-3. Evaluation of roasted beans by Electron Spin Resonance (ESR)

Green coffee beans (Tanzania AA) of a single lot were roasted with a hot air roaster and a far infrared roaster to the same roasting level (L value). The L values of the beans roasted by the hot air method and the far infrared method were respectively 25.25 and 25.31.

As the far infrared roaster, the above-mentioned small-sized far infrared coffee roaster (NGK INSULATORS, LTD.;IRT-003, H7GS-71169) was used. As the hot air roaster, "JETROAST"(ARAKAWA ENGINEERING WORKS., LTD.) was used. In the case of this hot air roaster, green coffee beans were fed into a heat-resistant glass cylinder which had been closed at both ends and fixed vertically. Then a hot air was jetted from a pipe, which was protruded from the center of the upper face of the cylinder nearly to the center of the lower face thereof, and the coffee beans were roasted under stirring. After the completion of the roasting, the bottom of the cylinder was opened and then the coffee beans fell into a cooling tank.

After paring the surface off with a cutter, each of the several hundred coffee beans was divided into the surfacepart (about 1/3) and the core (about 2/3). The silver skin at the core was eliminated. Then they were each powdered followed by the comparison of the amounts of radicals by ESR.

1-4. Evaluation of roasted coffee beans by Nuclear Magnetic Resonance(NMR)

Green coffee beans (Cuba Crystal Mountain) of a single lot were roasted with a hot air roaster and a far infrared roaster to thereby give half-roasted samples at the early stage of roasting and light-roasted ones. The structures of the roasters were the same as those described in 1-2 and 1-3.

In the half-roasted state, the moisture contents of the samples obtained by the hot air heating and the far infrared heating were respectively 5.7% and 5.0%. In the light-roasted state, the moisture contents of the samples obtained by the hot air heating and the far infrared heating were respectively 3.0% and 2.8%.

For each sample, the ratio of relativly free water (W-1) to relativly bonded water (W-2) was measured with the use of NMR (manufactured by JEOL, MU25 Pulse NMR System). Also, the moisture distribution was observed by Magnetic Resonans Imaging(MRI).

1-5. Comparison of the brown colored compounds content in the surface of coffee beans to that in the core thereof

Green coffee beans (Brazil No. 2) were roasted by far infrared heating. The structure of the roaster was the same as the one described in 1-2. For comparison, marketed coffee beans (100 % Brazil Arabica) were purchased.

After paring the surface off with a cutter, each of the several hundred coffee beans was divided into the surface part (about 1/3) and the core (about 2/3). The silver skin at the core was eliminated. Then they were each powdered.

Then the obtained powders were each extracted with hot water and filtered under definite conditions. The soluble solid contained in these extracts were adjusted to a constant level and then the absorbances at 400 nm were compared.

2. Experiment of effects on taste through component analysis

2-1. Analysis of organic acids in extract by High Performans Liquid Chromatography(HPLC)

Green coffee beans (Brazil No. 2) of single lot were roasted with a hot air roaster and a far infrared roaster. The structures of the roasters were the same as those described in 1-2 and 1-3. Then citric acid, malic acid, formic acid and chlorogenic acid were determined by HPLC.

HPLC conditions (Instrument: SHIMADZU, LC-6A)

Citric acid, malic acid and formic acid:

Column: SCR-102 (H) Mobile phase: 5 mM $KClO_4$
Flow rate: 0.8 ml/min Temp.: 42℃ Detector: IC

Chlorogenic acid:
Column: Shim-pack CLC-ODS (M) Mobile phase: 4% Acetate/Methanol = 85/15
Flow rate: 1.0 ml/min. Temp.: 50℃
Detector: UV (270 nm)

2-2. Analysis of aroma component composition of roasted coffee beans by Gas Chromatography(GC) and Gas Chromatography-Mas Spectrometry(GC-MS)

Green coffee beans (Tanzania AA) of a single lot were roasted by hot air heating, "direct heating" and far infrared heating to the same roasting level (L value). The L values thus achieved were respectively 23.68, 23.40 and 23.41. The structures of the hot air roaster and the far infrared roaster were the same as those described in 1-2 and 1-3. The "direct heating roaster" had an oven made of a heat insulating material and provided with a cylindrical rotary drum almost at the center therein. The rotary drum was provided with stirring blades therein and supported in such a manner as to allow horizontal rotation. Under this drum, a gas burner was located for heating.

From these roasted beans thus obtained, aroma components were collected by steam-distillation under definite conditions to thereby give samples.

20 μl of each sample was introduced into a 100 ml bubbling trap. Then an activecarbon filter was fitted into one side while Tenax TA was fitted into another side. The aroma components were adsorbed by sucking from the Tenax TA side with a small-sized pump at room temperature for 10 minutes.

Thermal Desorption Cold Trap Injection Conditions:

<GC>

Column : TC-WAX 60 m x 0.25 mm I.D. 0.25 μm
Detector : FID (250℃)
Column Temp. : 50 ~ 210℃ (50℃, 5 min. hold 3℃/min.) Carrier Gas : He 0.3 bar
Instrument : GL Sciences Inc. GC-380, Sic Labchart 80 Injector : TCT
Desorption Time : 10 min. Desorption Temp. : 200℃
Injection Time : 5 min. Desorption Flow Rate : 10 ml/min.
Cold Trap Temp. : -130℃ Injection Temp. : 250 ℃

<GC-MS>
Column : TC-Wax 60 m x 0.25 mm I.D. 0.25 μm
Column Temp. : 50 ~210 ℃ (50℃, 5 min. hold 3℃/min.)
Carrier Gas : He 0.3 bar Interface Temp. : 210℃
Ionsource Temp. : 200 ℃ Vacuum (torr) : 4 x 10^{-7}
Ion Accelerate : 3.0 Kv Ionization Voltage : 70 eV
Instrument : HITACHI M-80B, M-0101 CPU Injector : TCT
Desorption Time : 10 min. Desorption Temp.: 200℃
Ionization : 5min. Injection Flow Rate : 10ml/min.
Cold Trap Temp. : -130℃ Injection Temp. : 250 ℃

2-3. Determination of odor intensity of extract with odor sensor

Green coffee beans (Tanzania AA) of a single lot were roasted by hot air heating and far infrared heating to the same roasting level (L value). The structures of the hot air roaster and the far infrared roaster were the same as those described in I-2 and I-3. The L values thus achieved by the hot air heating and the far infrared heating were respectively 25.25 and 25.31. The

roasted beans were extracted with hot water under definite conditions to thereby give samples.

Each extract was covered with a box and the intensity of the odor in the box was measured by using a portable odor sensor (NEW COSMOS ELECTRIC CO., LTD.: Model XP-329).[2] To carry out the measurement under the same conditions, the time from the extraction to the measurement and the temperature of the extracts were set constant.

2-4.Evaluation of extract by Taste Sensing System

Green coffee beans (Tanzania AA and Brazil No. 2, each a single lot) were roasted to the same roasting level(L value) by hot air heating, "direct heating" and far infrared heating (in the case of Tanzania AA) and by "direct heating" and far infrared heating (in the case of Brazil No. 2). Regarding Tanzania AA, the L values achieved by the hot air heating, "direct heating" and far infrared heating were respectively 23.68, 23.40 and 23.41. Regarding Brazil No. 2, the L values achieved by the "direct heating" and far infrared heating were respectively 24.31 and 24.34. These roasted beans were extracted with hot water under the same conditions to thereby give samples.

By using Taste Sensing System (ANRITSU CORPORATION, SA401) with the use of multichannel tastesensors using electric potentialchanges in lipid membranes, "Principal componet analysis" were applied to the output signals obtained from each sensor. Thus differences in taste among these samples were evaluated.[3,4,5,6]

RESULTS AND DISCUSSION

1. Experiment on uniformity of heating

1-1. Measurement of increase in temperature of coffee beans during heating process

Fig. 1 shows the results. The temperature of the green coffee beans increased faster in the far infrared heating oven than in the convection heating oven. Namely, it took about 9 minutes that the green coffee beans were heated to the oven temperature, i.e., 200℃ in the case of the convection heating, while it took about 4 minutes in the case of the far infrared heating.

In the case of the far infrared heating, the temperature of the coffee beans exceeded the oven temperature, which indicates that the increase in the temperature of coffee beans in the far infrared heating depends not on the oven temperature but on the heater temperature.

When an aluminum foil barrier was provided in the space between the far infrared heater and the green coffee beans in the far infrared heating, the temperature increased very slowly. This fact also proves the effect of radiation heat transfer.

1-2. Measurement of moisture content loss of coffee beans during heating process

Fig.2 shows the results. After 10 minutes, about 7% of the moisture was lost in the case of the hot air heating, while about 9% of the moisture was lost in the case of the far infrared heating. Thus it can be estimated that the moisture is reduced faster in the far infrared heating.

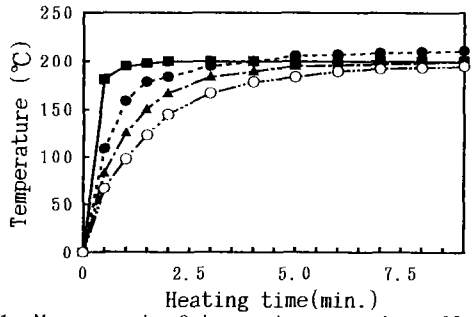

Fig.1 Measurment of temp. increase in coffee beans during heating

■—■ Atmospheric temp.
●···● Far infrared heating
▲---▲ Hot air heating
○-·-○ Far infrared heating of coffee beans covered with aluminum foil barrier

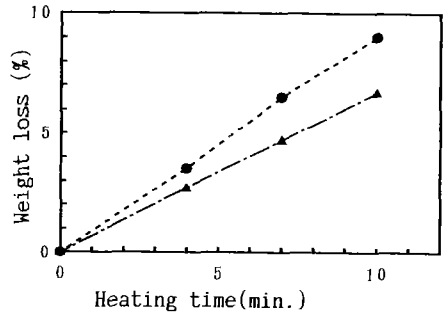

Fig.2 Measurment of moisture loss in coffee beans during heating

●···● Far infrared heating

▲---▲ Hot air heating

1-3. Evaluation of roasted coffee beans by ESR

In a compound, electrons are maintained stable since they always exist in the form of a pair. However unpaired electrons are sometimes formed due to light, heat or oxygen. In recent years, measuremens of radicals has been attempted to estimate the production date of potato chips[7], to estimate the age of corals[8], to date tooth or bones excavated from remains[9] and to estimate the time after bleeding of bloodstain.[10] Also, a geen tea of a lower grade contains radicals in the larger amount, which is seemingly due to the progress of decomposition. Thus it is said that the flavor reversely correlates to the radical amount.

From this point of view, we attempted to evaluate the roasting conditions of coffee beans. When the amounts of radicals in green beans, light-roasted beans, medium-roasted beans and dark-roasted beans were determined, the radicals increased as roasting proceeded, as a matter of course. This tendency was common to the hot air heating and the far infrared heating.

When the radicals contained in the surface parts and the core parts of green beans and medium-roasted beans (hot air heating and far infrared heating) were measured, the hot air heated beans showed a higher increase ratio of the surface radicals, as shown in Table 1. Thus it is assumed that the far infrared heated beans suffer from less damage at the surface part.

1-4. Evaluation of roasted coffee beans by NMR

Table 2. shows the results. Further, the moisture distributions in half-roasted coffee beans (at the early stage of roasting) were observed by MRI. Fig. 3 shows the results. The whiter part indicates the higher moisture content. Compared with the far infrared heated sample (right), the moisture was concentrated toward the core in the hot air heated one (left).

These results suggest the following phenomena. In the hot air heating, moisture is rapidly vaporized from the surface of the beans exposed to the hot air and thus clustered at the core. In the case of the far infrared heating, in contrast, the beans are uniformly heated and thus the moisture is uniformly distributed. When the hot air heating is further continued, even the bonded water(W-2) on the surface begins to evaporate. Thus the hot air heated beans undergo overheating at the surface.

Table.1 Evaluation of roasted coffee beans by NMR

	A	B	C
Radicals on surface / Radicals at core	1.06	1.10	1.25

A : Green beans
B : Far infrared
C : Hot air

Table.2 Evaluation of roasted coffee beans by NMR

		A	B
Harf-roasted	Total moistur(%)	5.7	5.0
	W - 1 (%)	65.3	61.0
	W - 2 (%)	34.7	39.0
light-roasted	Total moistur(%)	3.0	2.8
	W - 1 (%)	93.1	80.0
	W - 2 (%)	6.9	20.0

A : Hot air heating
B : Far infrared heating

1-5. Comparison of contents of brown colored compounds on the surface and at the core of beans

Fig. 4 shows the results. A higher absorbance shows the larger content of brown colored compounds. The hot air roasted beans contained the brown colored compounds in a larger amount on the surface than at the core. In contrast, the content of the brown colored compounds on the surface of the far infrared roasted beans was almost the same as that at the core.

Accordingly, it is estimated that the hot air roasted beans undergo strong browning due to overheating on the surface while the far infrared roasted beans undergo uniform browning.

Fig.3 Evaluation of roasted coffee beans by MRI

Right : Far infrared heating
Left : Hot air heating

2. Experiment on effects of taste by component analysis

2-1. Analysis of organic acids in extract by HPLC

Figs.5 and 6 show the results. As roasting proceeded, citric acid, malic acid and formic acid in the far infrared roasted beans were reduced. Chlorogenic acid showed little difference.

With the progress of roasting, acids in coffee increase due to the thermal decomposition of sucrose. As roasting further proceeds, the acids per se are thermally decomposed and reduced. It is assumed that the decrease in acids in the far infrared roasted beans begins at the early stage since the temperature of the coffee beans is uniformly elevated.

2-2. Analysis of aroma components by GC and GC-MS

Fig. 7 shows the results. The content of the aroma components in the far infrared roasted beans was larger by about 10% and by about 25% respectively than those of the hot air roasted coffee beans and the "direct heating roasted ones".

Depending on the characteristics, the aroma components of coffee can be classified into carbonyls, N-compounds, furans, phenols, miscellaneous and others. Fig. 8 shows the results of the analysis of the coffee beans roasted by hot air heating, far infrared heating and "direct heating".

The far infrared roasted sample was almost comparable to the hot air roasted sample in the qualities of the aroma but different therefrom in the content of the aroma components.

2-3. Measurement of odor intensity of extract by odor sensor

Fig. 9 shows the results. Although the measurement data differ from lot to lot, the far infrared roasted coffee always showed a higher odor intensity.

Thus it is assumed that the extract of the far infrared roasted coffee beans is superior in aroma.

2-4. Evaluation of extract by Taste Sensing System

The recent development in sensing techniques has made it possible to develop a system whereby the taste of a sample can be evaluated in a manner closely similar to human tasting characteristics. It depends on the output response patterns of lipid membrane sensors simulating those in the cells located on the surface of human tongue. There have been reported analytical data on amino acids[11], tomato juice[12] and beer[13] obtained by using such a system.

Fig. 10 shows the results of the evaluation of coffee extract by Taste Sensing System. The Tanzania samples roasted by the hot air heating and the "direct heating" are located at the same position, while the one roasted by the far infrared heating is apart therefrom. The Brazil sample roasted by the "direct heating" is apart from the one roasted by the far infrared heating. Thus it is assumed that a coffee roasted by the far infrared heating differs in taste from those roasted by the existing methods. Based on the results of sensory evaluation, the "first principal component" seemingly affects the intensity of acidity. Namely, the acidity is reduced as the spot shifts toward the right. The Brazil coffee is weaker in acidity than the Tanzania coffee and the far infrared roasted sample is weaker in acidity in each case. It is assumed that the "second principal component" relates to bitterness. These samples show little difference in the location of the spots of the second principal component, which might be caused by the same roasting level.

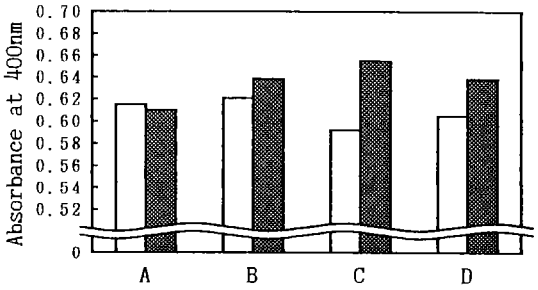

Fig.4 Comparison of brown colored compounds on the surface and at the core of roasting coffee

A : Far infrared roasting
B ~ D : Marketed coffee beans
□ : Core
▒ : Surface

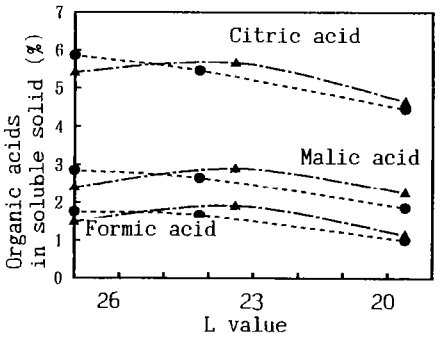

Fig.5 Determination of citric acid, malic acid and formic acid by HPLC

●---● Far infrared heating
▲---▲ Hot air heating

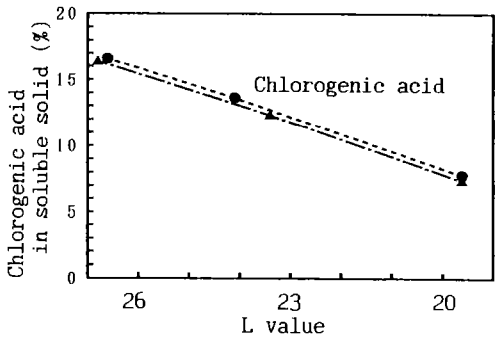

Fig.6 Determination of chlorogenic acid by HPLC

●---● Far infrared heating
▲---▲ Hot air heating

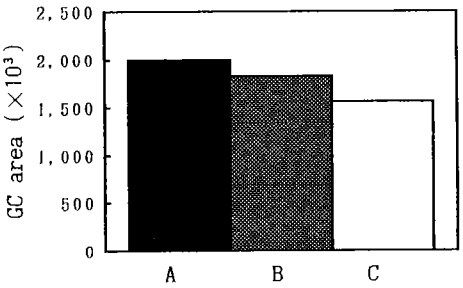

Fig.7 Analysis of aroma component content in roasted coffee beans

■ Far infrared heating
▒ Hot air heating
□ "Direct heating"

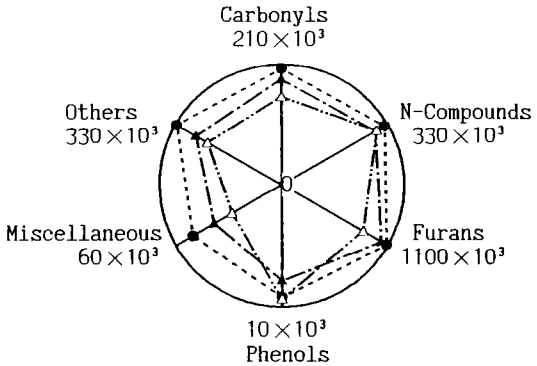

Fig.8 Analysis of aroma component composition of roasted coffee beans

●---● Far infrared heating
▲---▲ Hot air heating
△---△ "Direct heating"

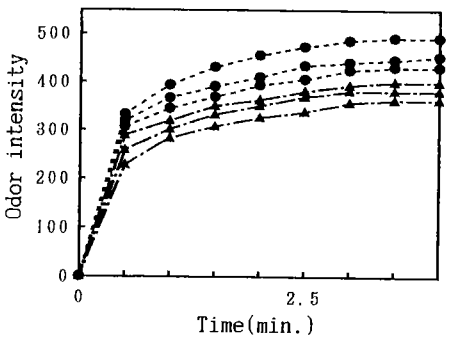

Fig.9 Measurement of odor intensity of extract with odor sensor

●---● Far infrared heating
▲---▲ Hot air heating

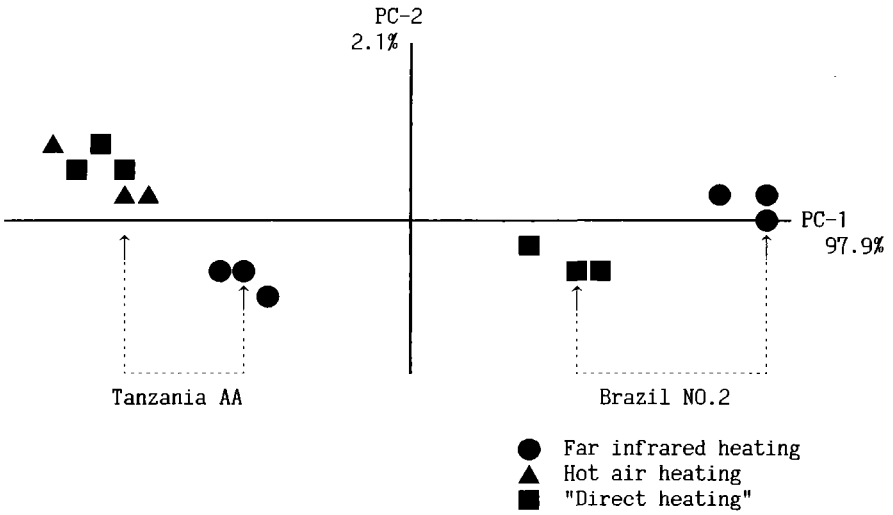

Fig.10 Evaluation of extract with taste sensing system

SUMMARY

We have conducted various experiments in order to clarify how coffee beans roasted with the use of a far infrared heater as a heatsource differ from those roasted by the existing methods, i.e., hot air heating and "direct heating". The results are as follows.

1) When the temperature in the oven was regulated to 200℃, coffee beans roasted with a far infrared heater showed quicker increase in temperature than those roasted by hot air.

2) When the temperature in a roasting drum was regulated to 200℃, far infrared roasted coffee beans showed quicker decrease in moisture content than hot air roasted ones.

3) Analysis by ESR indicated that far infrared roasted coffee beans showed less increase in radicals in the surface thereof than hot air roasted ones.

4) Analysis by NMR indicated that far infrared roasted coffee beans showed more uniform distribution of moisture and a higher rate of bonded water than hot air roasted ones, thus suggesting that the former samples suffered from uniform decrease in moisture.

5) Compared with hot air roasted coffee beans, far infrared roasted ones showed uniform formation of brown colored compounds.

6) Changes in the contents of citric acid, malic acid and formicacid were monitored with the progress of roasting. As a result, far infrared roasted coffee beans showed changes from an earlier stage than hot air roasted coffee beans did. As the roasting proceeded, the far infrared roasted coffee beans contained these acids in a smaller amount (when compared at the same roasting level). No difference was observed in the change of chlorogenic acid content.

7) When coffee beans of the same variety were roasted to the same level, the content of aroma components of far infrared roasted ones was larger by about 10% and by about 25% respectively than those of the hot air roasted coffee beans and the "direct heating roasted ones". The far infrared roasted coffee beans showed no large difference in the composition of aroma components from the hot air roasted ones.

8) When coffee beans of the same variety were roasted to the same level, the odor intensity of the extract of far infrared roasted coffee beans exceeded that of hot air roasted one.

9) When coffee beans of the same variety were roasted to the same level, the taste (evaluated by Taste Sensing System) of the extract of hot air roasted coffee beans was comparable to that of " direct heating roasted ones", while the extract of far infrared roasted coffee beans was different from them (i.e., weaker acidity).

Based on these experimental results, it is assumed that far infrared roasted coffee beans show a quick increase in the internal temperature but suffer from less damage on the surface, show a uniform and quick decrease in moisture and thus undergo uniform heating even at the core. From the viewpoint of taste, the extract of the far infrared roasted coffee beans is distinguishable from the existing ones, since it is rich in aroma components and shows a light acidity.

In the present study, coffee bean samples of the same variety were roasted to almost the same roasting level with the use of small-sized roasters of almost the same scale. Further work is needed from the viewpoints of the structure of roaster and standardizing the energy level.

ACKNOWLEDGEMENT

We gratefully acknowledge the invaluable assistance of NGK INSULATORS, LTD., JEOL DATUM, ANRITSU CORPORATION and NAGAOKA PERFUMERYCO., LTD.

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REDUCTION OF GASEOUS ROASTER AND COOLER EMISSIONS IN THE RFB SYSTEM

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The "RFB" batch roaster, manufactured by NEUHAUS NEOTEC, is used by major roasting companies throughout the world. Roasting and cooling is performed in two separate but geometrically identically shaped "RFB" chambers. (see diagram I)

In these RFB chambers the coffee is intensively mixed by the roasting respective cooling air and the shape of the chamber leads to what is described as rotating fluidized bed. The coffee beans in the batch are lifted and bow-like conveyed to the slope side where they slide back to the bottom plate, thus rotating around an imaginary axis. Discharging is effected by use of gravity by opening the gate at the lowest point of the bottom plate.

The hot air is recirculated in a closed bypass-loop during feed and discharge. During the entire process, the overpressure caused by the excess gases of the roasting process and burner affluent is directed via an auxiliary burner through a catalyzer system.

The today's problem in many areas, especially with dense population, is the smell and emission caused by the uncleaned cooling process in most systems:

We successfully introduced a patented system in the RFB which uses a common catalyzing system for both roaster and cooler affluent. (see diagram II) A few facts make this simple and effective process possible: As soon as the set point and coffee temperature of the beans is reached the coffee batch is discharged within only two seconds or less by gravity into the cooling chamber. At the same time a high pressure jet system quenches the beans over the entire length of the chamber while the cooling air is reduced to "boiling bed conditions". The intensiveness and eveness of the water spray into the boiling bed enables to reach the moisture set-point within 10 to 15 seconds, depending on the target. During that period the steam containing high concentration of odour and emission is guided via a bypass to the common catalyzer whose temperature has increased for approx. 10 C waiting the moisture steam injection. At the same time the cooler main atmospheric outlet is simply closed. After quenching is terminated the cooling system with its bypass is purched for another 10 seconds by cooling air only.

Measurement (see diagram III) showed that during these intensively quenching and cooling periods the coffee bean temperature is rapidly decreased according to the curve and reaches about 80 C already after approx. 1 minute.

After the quenching and purching period the bypass is closed and the cooling air is led to the atmosphere again.

The advantage of this system is not only a simple solution of reducing roaster and cooler affluent with its containing odour, this system also insures that cooling and final moisture targets could be reached with high consistency.

Usually this catalyzing system works with Honeycomb structure metal or caramic catalyst platin/palladium coated. The following datas were achieved at a catalyzer temperature of 450°C.

The measurement results (see diagram IV) showed that both, the roaster and the cooler exhaust concentration, is below today's regulation set by the local authorities.

Depending on the catalyzer temperature a total carbon concentration of 12 mgC/m^3 respective 19 mgC/m^3 in the roaster exhaust could be reached.

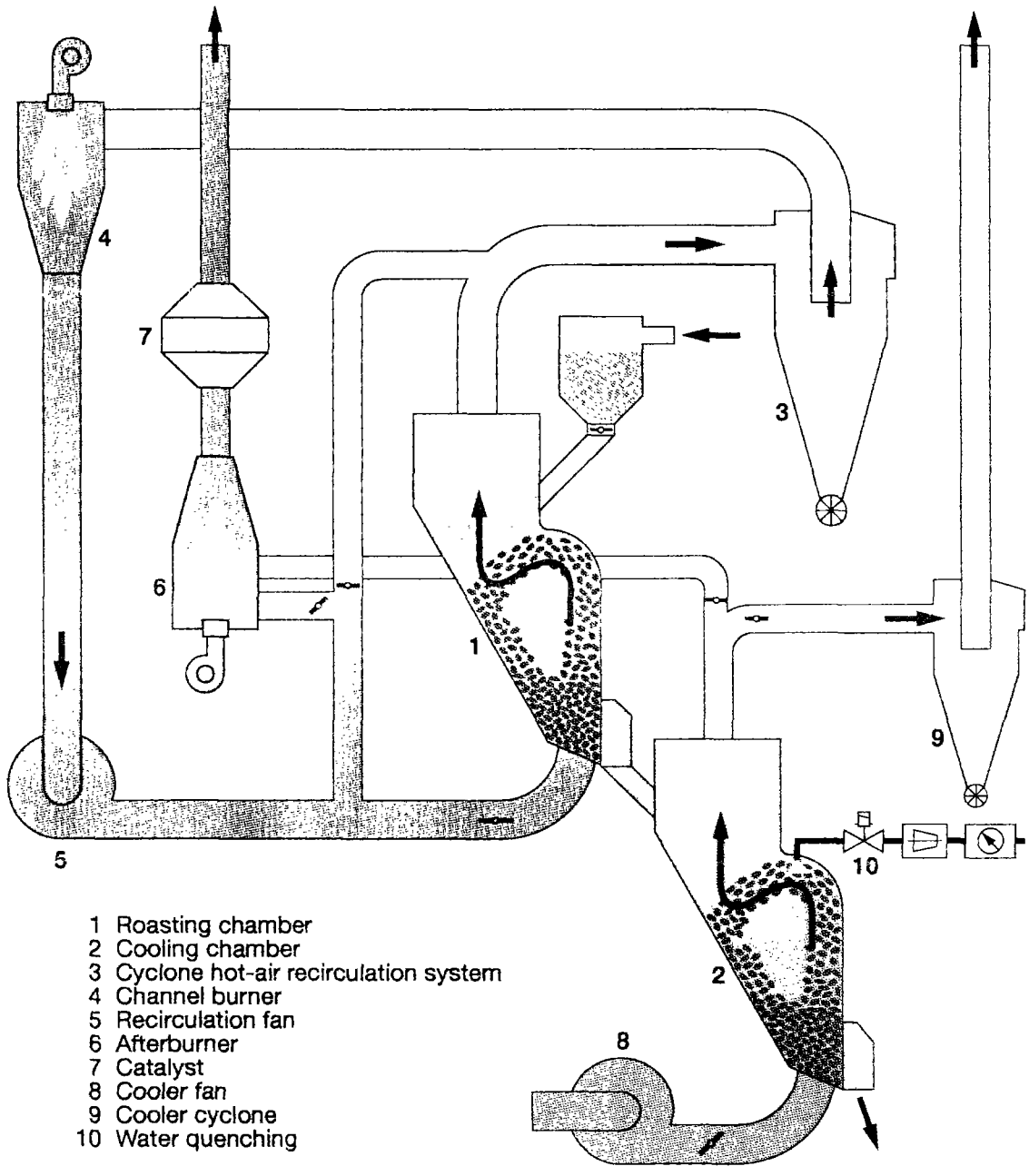
The NOx value was measured between 21 mg/m^3 and 48 mg/m^3. The variation is depending on roast degree of the coffee and is increasing with higher catalyzer temperature. (see table V)

Olfactometric measurements were also executed with the result of 5.000 - 7.000 OU/m^3 roaster exhaust and 8.000 - 10.000 OU/m^3 cooler exhaust.

In case of using a biological washer a further odour reduction of up to 95 % would be possible which means that a odour concentration of 1.000 - 3.000 OU/m^3 can be reached.

RFB Rotating Fluidized Bed Roaster
Air Cooling Phase

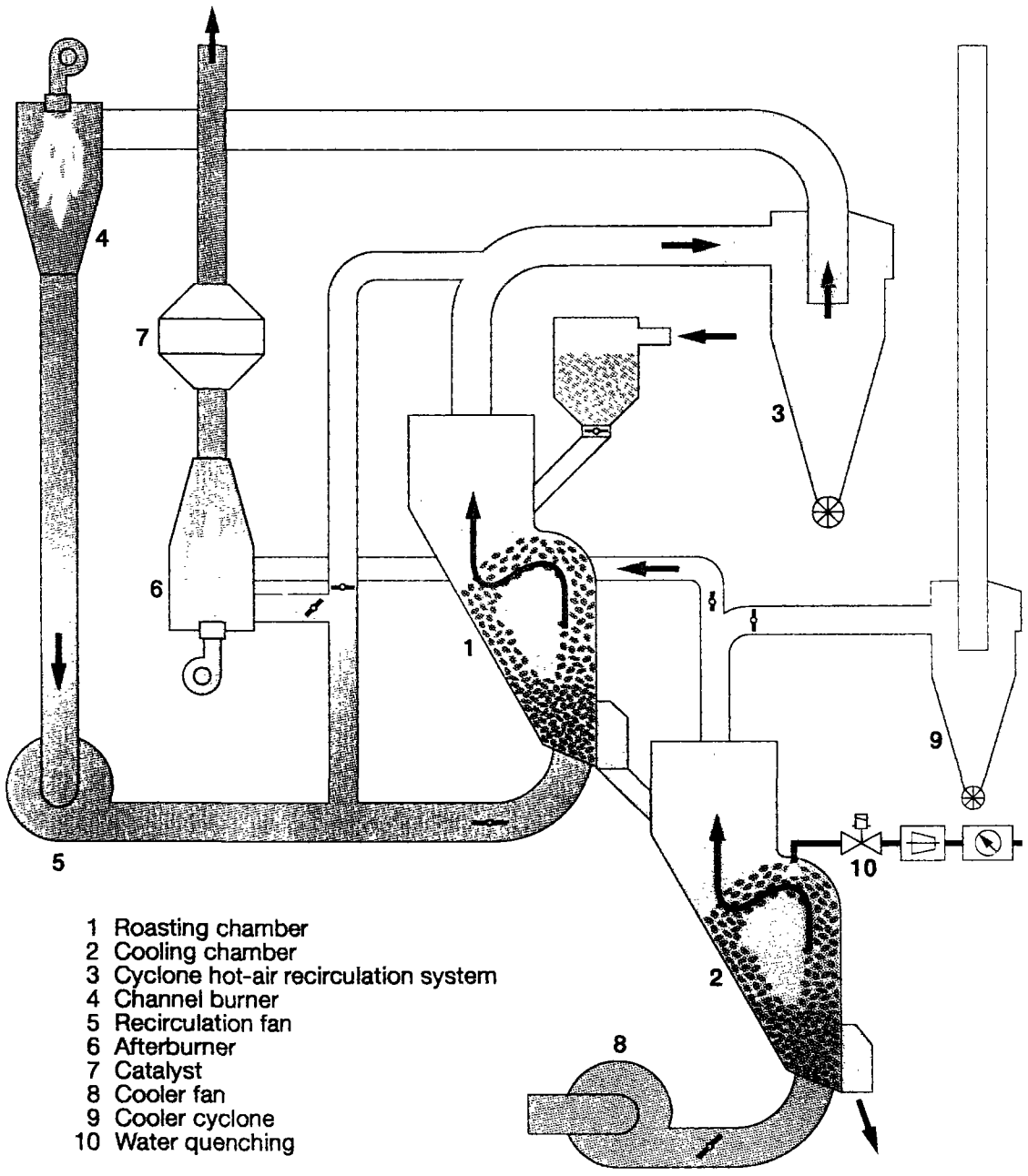
I



1 Roasting chamber
2 Cooling chamber
3 Cyclone hot-air recirculation system
4 Channel burner
5 Recirculation fan
6 Afterburner
7 Catalyst
8 Cooler fan
9 Cooler cyclone
10 Water quenching

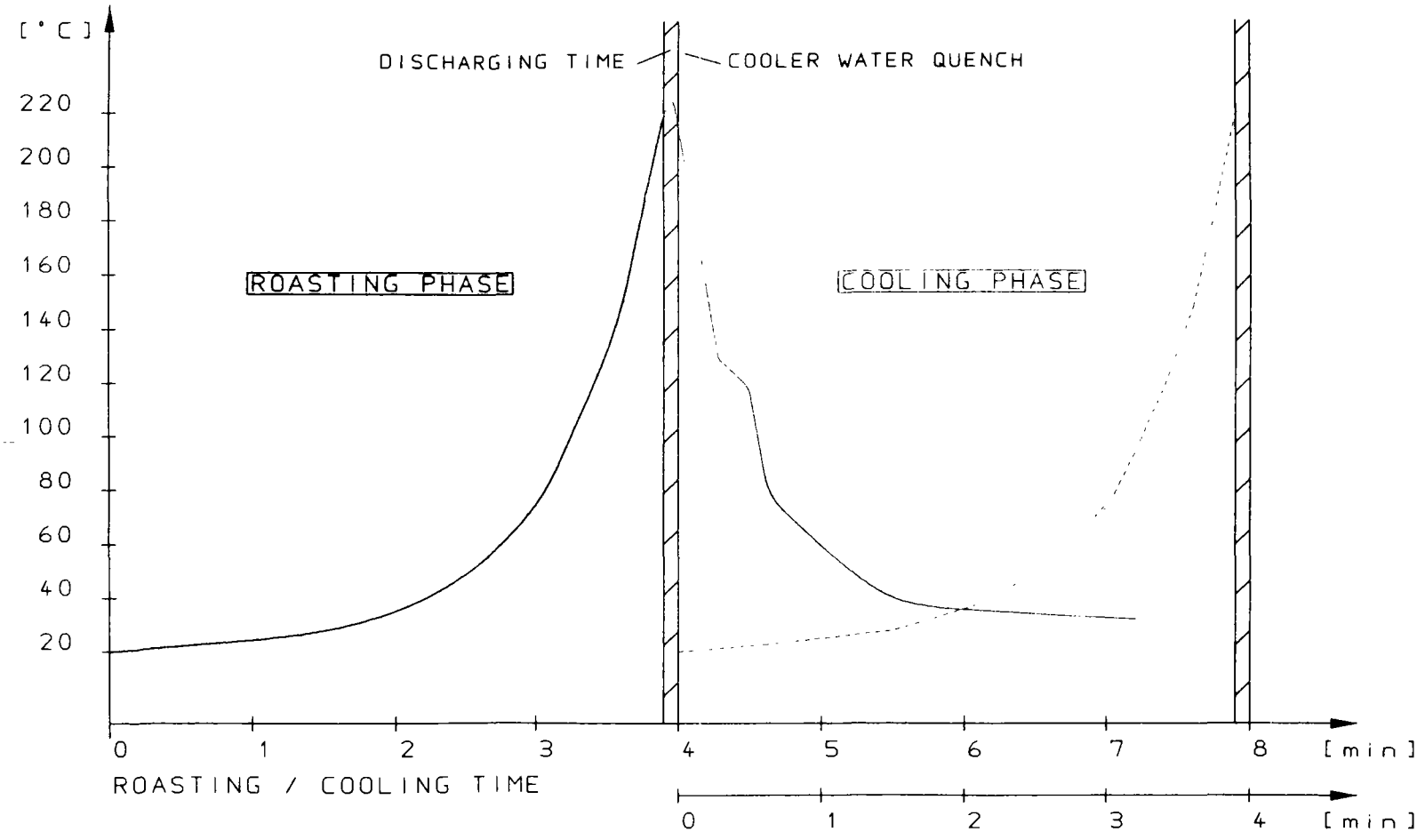
RFB Rotating Fluidized Bed Roaster
Cooler Quench Cycle Phase

II



1 Roasting chamber
2 Cooling chamber
3 Cyclone hot-air recirculation system
4 Channel burner
5 Recirculation fan
6 Afterburner
7 Catalyst
8 Cooler fan
9 Cooler cyclone
10 Water quenching

TEMPERATURE DEVELOPMENT OF THE COFFEE BEAN DURING ROASTING AND COOLING



IV ROASTER AND COOLER EMISSION MEASUREMENTS

NOx :

approx. 210 - 480 mg NOx/m_N^3

limiting conditions:

- a catalyst temperature of 420°C
- a medium roast

OLFACTOMETRIC :

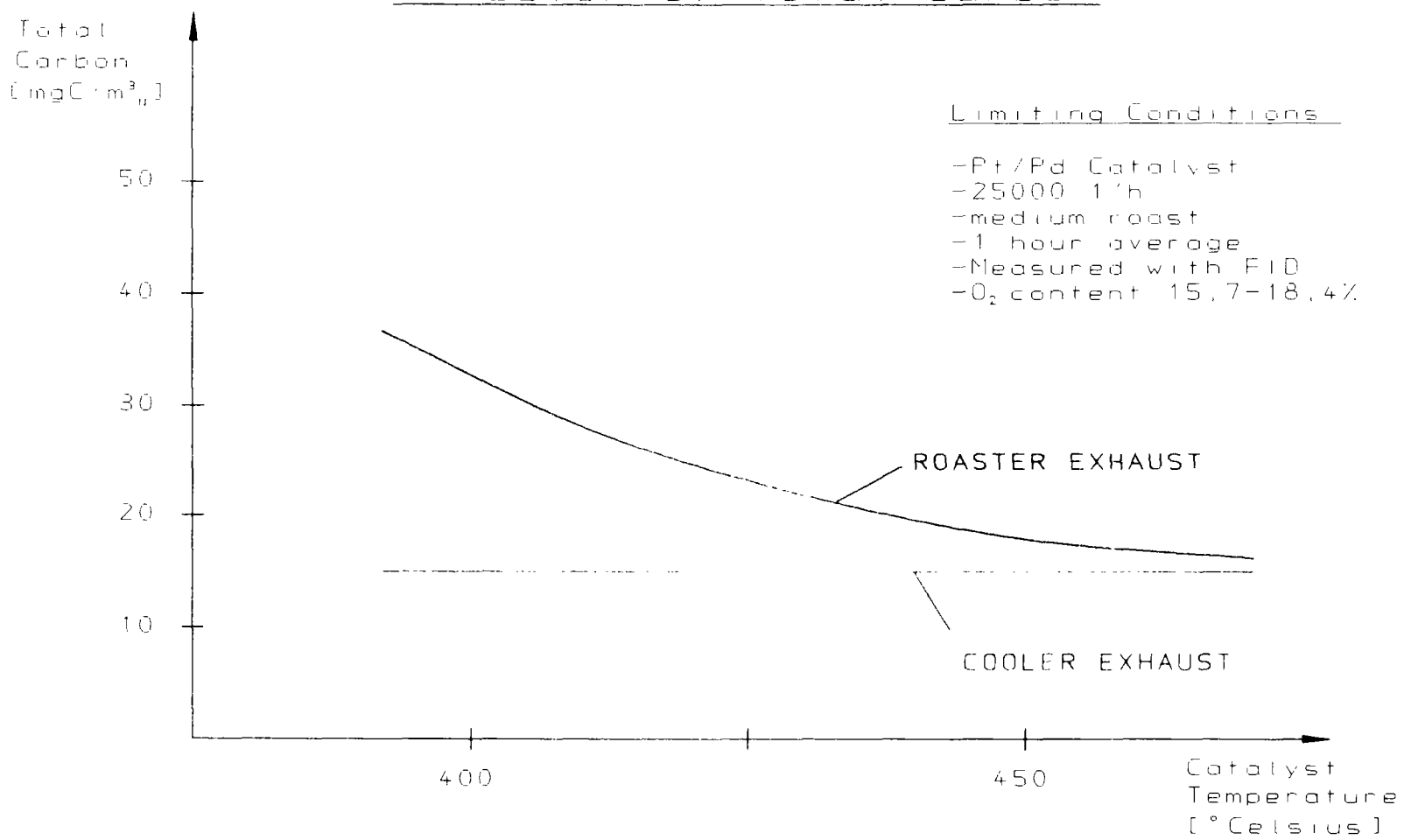
a) Catalytic reactor

roaster exhaust approx. 5.000 - 7.000 OU/m_N^3
cooler exhaust approx. 8.000 - 10.000 OU/m_N^3

b) Biological Washer

reduction of odor emissions up to 95 %
common roaster/cooler exhaust approx. 1.000 - 3.000 OU/m_N^3

V Emission of Total Carbon



EFFECT OF WATER AND COFFEE ACIDITY ON EXTRACTION. DYNAMICS OF COFFEE BED COMPACTION IN ESPRESSO TYPE EXTRACTION

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1 — Introduction

A common concern for every one in charge of preparing coffee extract from the householder, to the bar waiter or factory operator, is to carry out a reproducible extraction operation that will insure high and consistent coffee brew quality. These goals will be more easily achieved if we can arrive at a better understanding of coffee extraction.
Coffee extraction is a complex and dynamic process in which coffee particles and water react with each other through a multitude of phenomena. Many relationships have yet to be introduced and understood. Our present discussion will focus on the influence of water and coffee origins on coffee bed compaction. We will investigate how a chemical reaction, displacement of the bicarbonate equilibium caused by coffee acid solubilization results in a physical modification of coffee bed structure.
We have been concerned with espresso coffee type extraction which is probably the most sensitive to this phenomenon.

2 - Materiel & Methods

A Turmix espresso machine with a pump delivering a flow up to a pressure of 15 bar was used. Except for experiments with pure origins, the roasted coffee used was a blend of Arabicas and Robustas R&G coffee dose was transfered in a specific filtering pan applying the same procedure. Coffee bed was slightly pressed, under same conditions, with a piston.
Roasted coffee beans were ground using a Probat grinder UW 45 L with 2 sets of rollers. The coffee particle size distribution was measured in a solvent phase by means of laser type analyser Malvern 2600. The analysis range was from 10 to 1000 microns. Average particle size distribution was around 300 microns.

For the investigation of water effect, only one batch of R&G coffee was used.
In the case of the coffee origin study, the different samples were ground so that average particle

size distribution was in the range of 300 microns ± 10 microns.
Raw tap water had the following content : Calcium 100mg/l, Magnesium 20mg/l, Sodium 8mg/l, Potassium 2mg/, Bicarbonate 380mg/l, Chlorure 15mg/l, Nitrate 15mg/l, Sulfate 15mg/l.

3 - Results & Discussion

In Espresso extraction, or more generally in any initial phase of extraction, two significant steps, wetting (water fills in coffee particle voids. Inter and intra particle gas is removed out of the coffee bed) and percolation (mass transfer between coffee particles and water) are occuring. We will focus on them as they are the most complex, dynamic and important phases which determine overall extraction conditions .

Many independent variables can be imposed on the extraction process (SIVETZ, 1979) :

- geometry of percolation equipment
- green coffee blend, roast and grind
- water quality
- water temperature
- extract / roast coffee ratio.

The resulting product characteristics, referred to here as dependent variables are mainly :

- organoleptic characteristics of the brew (taste, aroma, appearance)
- yield of solubles from R&G coffee
- concentration of solubles in the extract
- resistance to flow in the R&G coffee bed.

Our work will focus on the relationship between independent variables sch as green coffee origin, roasting conditions, and water quality on resulting resistance to flow in the R&G coffee bed, extraction flow rate and extraction time.

Extraction Initial Transient Phase

In the case of Espresso extraction, flow rate varies in a large extent during the initial extraction phase when coffee bed compaction is occurring (resistance to flow in the R&G coffee bed is increasing).
Dynamics of extraction pressure and flow rate can be studied on Figure 1.

Espresso Extraction consists in two successive phases :

- *transient phase* : wetting (water fills in coffee particle voids. Inter and intra particle gas is removed out of the coffee bed) and percolation (mass transfer between coffee particles and water) occurs simultaneously
- *extraction steady state phase* : resulting from the dynamics of the first one.

The transient phase can itself be divided in two sub phases :

- *phase 1* : flow rate and pressure increase, in accordance with Darcy's law.
- *phase 2* : the most spectacular . According to the pump characteristics, pressure increases while flow rate decreases. Coffee bed compacts. This sequence occurs until an equilibrium is reached ;pressure and flow rate are thus stabilized. Steady state phase (third phase) remains until the end of the extraction.

Phase 1 lasts about 1 second, phase 2 around 4-5 seconds. Phase 3 is the longest : from 15 seconds for an usual espresso extraction time to 45 seconds or more if problems occur.

The transient phase has already been introduced by other authors (Illy 1963, Petracco 1993). Mineral content of extraction water can induce significant variations of the transient phase. As a consequence, steady state pressure achieved at end and the extraction time by its dependence on the steady state flow rate are both influenced by the water.

Influence of Extraction Water

Water is composed of many anions and cations. According to the nature of soil in which water percolates, the Calcium (Ca 2+) and Magnesium (Mg 2+) content can vary. The high proportion of these cations in water (measured as hardness) can cause problems of pipe scale cup as they produce insoluble salts (mainly carbonates, but also sulfates and silicates) when heated up. To avoid this problem, water is treated either by softening or demineralization (figure 2).

Softening treatment, carried out by processing water through a brine column (resin with Nacl), exchanges Calcium (Ca2+) from the water and replaces it by Sodium (Na+). The treated water is enriched in Solium carbonate (NaHCO3).
Demineralization is performed in 2 steps: water first passes through a cationic type resin column retaining Ca++ and Mg++ cations and replacing them with H+. It passes second through an anionic type resin column capturing Cl-, HCO3-, SO4-- anions and replacing them with hydroxide OH-. Treated Water contains all salts of initial strong acids and some dissolved CO2 in proportion of initial bicarbonates of the raw water.

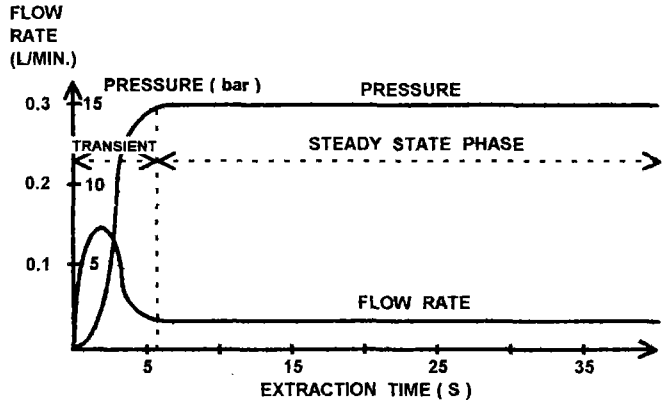

Fig. 1 : Dynamics of espresso extraction

WATER	TIME (S)	EXTRACTION PRESSURE (BAR)
TAP	27.5	6.4
DEMINERALIZED	30.4	8.5
SOFT	42.0	12.8

Table 1 : Effect of water on extraction condition

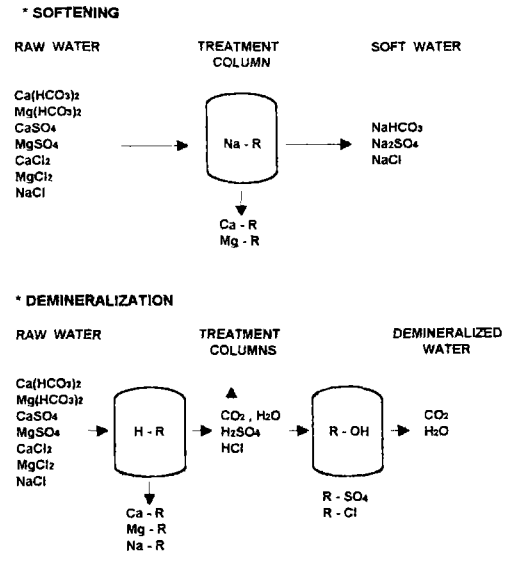

Fig. 2 : Water treatments

Table 1 shows the effect of extraction water on extraction conditions with all other parameters unchanged. Extraction was performed with a draw off ratio of 8.0 (5g R&G coffee, 40 g extract).
Soft water increases extraction time by 50% and extraction pressure by 100% compared to raw tap water. Soft water induces higher compaction of the coffee bed and demineralized water has a medium effect. Any mixture of 2 of these 3 kinds of water gives intermediate results.

Effect of Bicarbonate Ions

To understand influence of water ions an extraction, we modified the content of water. We investigated among different salts the influencing ones.
As reported in Figure 3, the extraction time and pressure increase significantly when the bicarbonate ion concentration (HCO3-) is raised. Demineralized water with a sodium carbonate concentration around 0.15 g/l displays an effect similar to the soft water.

As bicarbonate ion HCO3- has a significant pH buffer effect, the influence of the initial pH of the water on extraction conditions was investigated. According to Figure 4, initial pH of the extraction water influences extraction conditions : lower pH induces milder extraction conditions with shorter extraction time and lower pressure drop. Soft water with an initial pH of 5.3, close to the final extract pH (5.3 to 5.0), recovers an effect similar to raw tap water.
At this stage, it is important to mention that whatever the initial extraction water pH, the final extract pH remains the same. Coffee extract with a high proportion of acids has a strong pH buffer effect. This means that the phenomenon under discussion here is induced by the coffee extract solubilization and emphasized by the water mineral content. The effect of pH change is more significant when pH decreases (especially below pH 7.0) rather than increases.

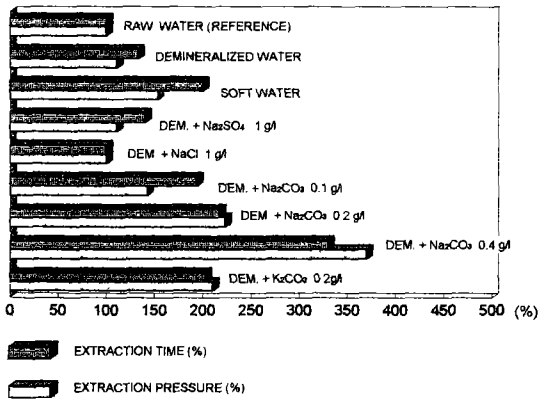

Fig. 3 : Influence of water ions on extraction

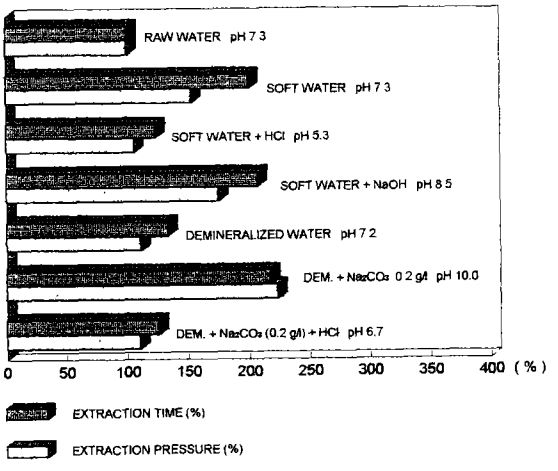

Fig. 4 : Effect of inital water pH

Bicarbonate - Carbonic Acid Equilibrium

From this point, we can hypothesize that the compaction effect taking place in the transient phase is related to the bicarbonate ions and the displacement of its equilibrium according to the pH. According to pH of a salt, every "acid" form is in equilibrium with its corresponding "base".
In the case of calco-carbonic equilibrium including carbonate ions, bicarbonate ions and dissolved CO2 the situation is as follows (Table 2)

ACID	<=======>	BASE	
HCO3-	<=======>	CO3-- + H+	pK1 = 10.2
H2O + CO2	<=======>	HCO3- + H+	pK2 = 6.4

Table 2 : Carbonate - Bicarbonate and Carbonic Acid Equilibrium

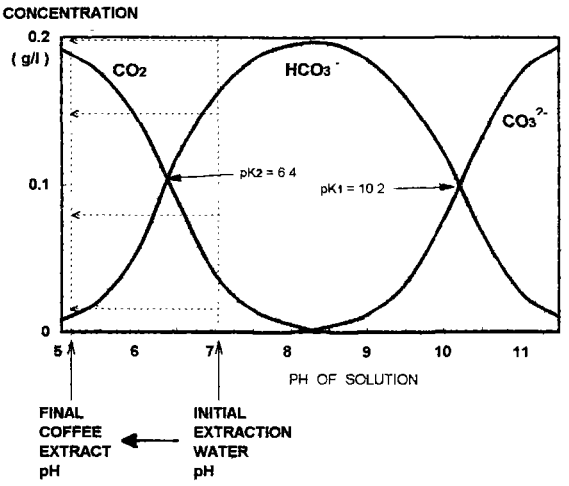

Fig. 5 : Effect of pH on bicarbonate equilibrium

When pH drops by addition of ions H+, equilibrium is displaced toward the acid side, resulting in a production of bicarbonates HCO3- from carbonate CO3-- or production of dissolved CO2 from bicarbonates HCO3-. We can specify the proportions of these different components according to the pH (Figure 5).

Compaction process : Influence of Bicarbonate Equilibrium displacement

During extraction, pH of water drops from around 7.0 - 7.5 to 5.5 - 5.0, the final pH of the extract. This means that the calco-carbonic equilibrium is deeply modified with the majority of ions switching from bicarbonate HCO3- to dissolved carbonic acid CO2. Carbonic acid CO2 solubility in hot water is low and further decreases when the pressure of the liquid is reduced, according to the Henry's law. This situation occurs throughout the coffee bed, as we can decompose flow across the bed through a multitude of thin layers where outlet pressure is supposed to be lower than inlet pressure, the difference being the pressure drop through this layer.

The excess CO2 in excess is released as gas. As CO2 gas creates turbulences and moreover as CO2 gas requires much more volume than its corresponding liquid form, the local velocity of extraction fluid increases significantly (the free section for flow is reduced). Pressure drop increases as the square of flow velocity.

During the first part of extraction, coffee bed is not stabilized and is susceptible to modification (Illy 1963, Petracco 1993, Sivetz 1979). The pressure drop increase introduces more energy with the liquid. This flow energy favors displacement of coffee bed particles and a gradual compaction of coffee bed as a reaction. Coffee bed porosity decreases, inducing again itself as a reaction, a higher pressure drop.
Coffee bed particle swelling due to the wetting process of extraction water is also responsible for a reduction of coffee bed porosity and an increase in the pressure drop. We can imagine that coffee bed compaction is induced layer after layer, the layer closer to the pump being, at the same instant, more compacted than the next one.

All these chain reactions accumulate, amplifying the compaction process. The compaction continues until an equilibrium pressure drop is reached according to the energy released by the pump. The coffee bed is then finally compacted and extraction pressure and flow are stabilized at values compatible with the energy released by the pump. During this stationary phase, as extraction is still going on, the CO2 equilibrium is still displaced toward CO2 production. Neverthless, no further compaction occurs since an equilibrium has been reached between the "energy supplier", the pump, and the "energy consumer", extract flow through the compacted coffee bed. CO2 degassing, occuring all along extraction, contributes to foam and emulsion generation which is such a nice feature of Espresso coffees.

The importance of this initial transient phase is great for two reasons. First, the coffee bed is not stabilized and is susceptible to remodeling. Second, the physico-chemical reaction induced is very intense during the first phase of extraction. As proposed by Zanoni (1991) and Voiley (1984), extraction proceeds by a double mechanism ie, direct almost instantaneous dissolution of free solubles at the particle surface followed by diffusion of free solubles from the porous interior. This assumption, even if denied by Spiro (1993), is nevertheless more appropriate when using a fine grind (250 microns average particle size) as for espresso type extraction.
In an espresso type extraction lasting around 30 seconds overall, we found that more than 50% of the total solids are extracted within the first extract drops (draw off ratio 0.7) in a few seconds. The remaining 40 - 45% solubles are extracted during the stationary state phase with a draw off ratio from 5.0 to 10.0 and lasting about 20 seconds.

Some authors (Sivetz 1979, Illy & Viani 1994) reported increase of percolation time when using alkaline waters or very soft or demineralized water with a hardness below 8 French degrees. Illy & Viani (1994) wondered if pH could affect percolation time in association with hardness. Petracco (1993), in a detailed description of the transient phase explaining partly how coffee bed compacts through the formation of a bottom layer densely packed with fine particles, was questionning if this phase could not be related to chemical reactions of extraction, triggered by high temperatures in the vicinity of the boiling point of water (100oC). The preceding authors introduced the compaction process and explained partly how it occurs. Our work supports this and earlier explanations by focusing on the physico-chemical changes occuring during percolation.

Influence of coffee origins on compaction process

In the preceding part of our work, we explained how the coffee solubles influence the carbonic equilibrium and induce compaction of the coffee bed. We are now going to study the influence of coffee origin on the release of these coffee solubles and on coffee compaction. We introduced our assumption that coffee acidity was responsible for displacement of CO2 equilibrium. By relating coffee acidity to coffee origin, we can explain how coffee origin affects extraction conditions.
Coffee titrable acidity is the amount of caustic soda necessary to neutralize the pH of coffee extract

It is expressed in Meq/kg at pH 6.6 (some authors raise it at pH 8.0).

During this investigation, different coffees were roasted under similar conditions, ground to the same particle size and espresso type extractions were conducted with regular raw tap water. Results are reported in Table 3. Extraction time between Togo (Robusta) and Colombia is extended by more than 100%, Santos coffee induces a 60% increase. The pH of Colombia extract is the lowest (5.14) and its acidity the highest (14.5 Meq/kg at pH6.6) among the 3 tested coffees. The acidity of the extract is related to its buffer effect and to its pH. Colombia coffee induces higher pressure and longer extraction pressure because the displacement of the carbonic equilibrium leading to CO2 gas release is extended as compared with Santos or Togo coffees. Results related to a short draw off ratio (0.7) are reported in Table 4.

Coffee origin	EXTRACTION		EXTRACT	
	Time (S)	Pressure (bar)	pH	ACIDITY (Meq/kg at pH 6.6)
COLOMBIA	27.1	11.6	5.14	14.5
SANTOS	20.4	11.3	5.39	11.0
TOGO	12.5	10.6	5.62	8.6

Table 3 : Extraction of different coffee origins and influence of coffee acidity. Draw off ratio 8.0

Coffee origin	EXTRACTION		EXTRACT	
	Time (S)	Pressure (bar)	pH	ACIDITY (Meq/kg at pH 6.6)
COLOMBIA	5.0	11.6	5.05	68.0
SANTOS	4.0	11.3	5.30	51.0
TOGO	3.0	10.6	5.40	40.0

Table 4 : Extraction of different coffee origins and influence of coffee acidity during the transient phase. Draw off ratio 0.7

Acidity increases about 4.6 times over along draw off ratio. This emphasizes once more the extreme importance of the intense solubilization and the consecutive reactions occuring during the transient phase. In their study of chemical variation of coffee extract during extraction, Severini et al (1993) quantified the pH and acidity of the different fractions of the extract. The trend and absolute values are very similar to our findings.

On the average during the transient phase, Colombia extract pH is 5.05 and the acidity stands at 68 Meq/kg at pH 6.6. Togo coffee, by comparison, shows a pH of 5.4 while the acidity rises at 40 Meq/kg. Maier (1987), in his study about coffee acids, reported higher titrable acidity for Arabica coffees than for Robusta ones. Sivetz (1979) also mentioned that different types of green coffees percolate differently.

4-Conclusion

The ultimate goal of any experimental work on coffee extraction is to improve understanding of the fundamental extraction dynamics that have a bearing on operation, quality, cost, control and consistency decisions. With this work, we contributed to a better understanding of the coffee bed compaction, especially in the case of espresso extraction.

We explained how a chemical reaction, related to the coffee origin or water content, enhances a physical modification of the coffee bed.
Preparing a good espresso coffee requires proper equipment, fine coffee, quality water and ample of experience. We should be alert to new ideas and findings contributed from many disciplines.
Though extraction seems to be fairly responsive to variations in water or coffee origins, practical adjustments of the operation is limited. However,this work, if integrated with other recent findings, technologies or modelization programs, could contribute to an improved understanding of the extraction process.

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SUMMARY

Espresso extraction is characterized by a dynamic compaction of the coffee bed. This compaction is influenced both by the nature of extraction water and the coffee origin. The physical reaction of compaction is emphasized by a chemical reaction ie, displacement of the bicarbonate equilibrium induced by the acidity (pH drop) extracted from coffee. The excess dissolved CO2 is released as gas. It creates flow turbulences and increases the pressure drop, resulting in a coffee bed compaction. The extent of this chemical reaction is influenced both by the nature of the ions, the pH of the extraction water, and by the acidity of coffee. Extraction time and pressure varie within a range of -50% ltingto +100% from standard conditions. Soft water, water with a higher initial pH or acid coffees such as Arabicas, induce higher compaction while raw tap water , acid water or Robusta coffees have the opposite effect.

RESUME

INFLUENCE DE LA NATURE DE L`EAU ET DE L`ACIDITE DU CAFE SUR L`EXTRACTION.
DYNAMIQUE DU COMPACTAGE DU LIT DE CAFE DANS LE CAS DE LA PREPARATION D`UN CAFE ESPRESSO

L`extraction Espresso est caractérisée par un compactage dynamique du lit de café. Cette réaction physique est amplifiée par une réaction chimique, déplacement de l`équilibre des ions bicarbonates causée par l`acidité du café. Le CO2 dissous produit en excés est relargué sous forme gazeuse. Ce gaz entraine des turbulences dans le flux liquide et une augmentation de la perte de charge. Le lit de café se compacte ainsi. L`intensité de cette réaction chimique est influencée par la nature des ions, le pH de l`eau d`extraction, et par l`acidité du café. Les variations engendrées à travers la durée et la pression d`extraction se situent dans une fourchette de -50% a +100% par rapport au standard. L`eau adoucie, une eau d`extraction á pH initial élevé ou des cafés Arabicas développant une acidité titrable elevée, induisent une forte compaction à l`opposé d`une eau de source ou de nappe, d`une eau acide, ou des cafés Robustas.

RECENT TRENDS IN SOLUBLE COFFEE

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From the first appearance of instant coffee the readily soluble coffee powder has had a distinct taste and flavour - different from coffee brewed in the traditional way from the same blend of coffee beans.

In the early process the coffee was stewed for a long time in the extraction process, and then a low concentration extract was subjected to high temprature during the drying stage. This resulted in a product with little flavour, a stuwed taste and only a slight coffee aroma.

For many years the development work within the instant coffee field was mainly directed towards improving the economy - higher yields and lowr production cost were the main goals because most of the consumers of instant coffee had not been traditional coffee drinkers and did not know how a freshly brewed good coffee could taste. This has changed!

Today most consumers demand a high quality product, meaning that the convenience of instant coffee should be coupled with a taste and aroma that is close to a cup of brewed coffee.

In order to achieve this, it has been necessary to look into the three main process steps involved in the manufacture of instant coffee:

1. extraction including preparation of the roast coffee before the extraction starts
2. concentration
3. drying

As to the extraction there are two important factors in the extraction process that influence the quality namely that extraction of the aroma solids and extraction and hydrolyzation of the remaining part of the solids should be kept separate and the extraction time for both extract streams should be kept at a minimum. This has recently been achieved in a new process which is explained in the following:

The extraction really starts with the preparation of the coffee before the actual extraction itself takes place. In order to ensure an effective extraction, where all coffee particles come into contact with the extraction water, the ground coffee is prewetted by hot water in a mixer under conditions ensuring a uniform wetting of the roast and ground coffee. The prewetting, in fact, has two purposes i.e. avoiding channelling of the extraction water and assisting in the degassing of the roast and ground coffee. The degassing is an important feature because gas entrapped in the coffee particles will oppress the extraction and lead to foam formation hampering the flow of water through the roast and ground coffee.

The prewetted coffee is filled into the extractors and to ensure correct packing of the coffee, air is removed from the extractor at the same time. After filling of the coffee into the extractor a high vacuum is drawn in order to remove all remaining gas in the coffee.

The actual extraction is a high-speed double extraction. The double extraction means that extraction of the aroma compounds at temperatures below 120°C takes place separately and as fast as 10–15 minutes. The resulting aroma extract is of an extremely high quality, and despite the short extraction time the efficiency is very good due to the prewetting, degassing and turbulent flow.

The aroma extraction is followed by extraction of the hydrolysed compounds in the coffee. Also this part of the extraction is done at water velocities up to 2.5 times higher than in conventional extraction, resulting in a much shorter extraction time and a turbulent flow. For this part of the extraction, too, the shorter extraction time results in a better extract quality, and the turbulent flow increases the extraction efficiency.

The higher water velocity during extraction combined with the prewetting and degassing results in an increase of the average overall yield of the extraction of 4–5% and still with a much improved quality of the extract.

Higher liquid velocity and shorter extraction time have been made possible by the design of the extractors and the pre-preparation of the roast and ground coffee. The overall extraction time has been decreased from the conventional 200–240 minutes to approx. 90 minutes.

The new extractor design which has small thermal masses (no heavy flanges) incorporates filter designs that ensure even liquid velocity over the entire volume of the extractor. Due to the short cycle time, the coffee volume per time unit decreased to less than half the conventional volume, resulting in smaller extraction vessels. These are arranged in a circle in order to facilitate filing and to ensure the shortest possible interconnecting piping.

Due to the short cycle time, accurate and optimal operation of the valve manifold is essential, and therefore the operation is automated by actuators on valves and a simple time relay based control panel. Experience has shown that this is a great advantage in producing a continuous quantity of high quality extract.

Extract Handling

The two streams of extract are treated completely separately.

The aroma extract is normally filtered and cooled to approx. 5°C in order not to lose or degrade any of the precious aroma substances.

The hydrolysed extract is filtered or centrifuged, depending on the market requirements.

Concentration

The aroma extract, which contains all the desirable aroma and flavour, should be treated as leniently as possible. The best result is obtained if this extract is freeze concentrated. In the freeze concentration the water is removed from the extract in the form of ice crystals which in fact is pure water. Therefore, the aroma loss and thermal degradation will be zero. The aroma extract is ideally suited for freeze concentration because it contains no hydrolysed particles which could cause a viscosity problem.

A cheaper but less perfect way to treat this extract is to strip off the aroma compounds and then concentrate the rest of the extract together with the hydrolysed extract. The aroma stripping should preferably be an integrated part of the extraction in order to avoid oxidation and thermal degradation of the aroma compounds.

The hydrolysed part of the extract, possibly together with the stripped aroma part, can economically concentrated in a falling-film evaporator which will remove some undesirable flavour compounds from the extract. The falling-film evaporator will concentrate the extract up to approx. 55 wt% which is ideal for a subsequent drying with minimum aroma loss.

Mixing

The two extract streams are mixed before further processing. Mixing can be done in different proportions in order to suit different customer requirements.

Drying

The development of the multistage Fluidized Spray Dryer was initiated by NIRO A/S about 10 years ago, because of customer demand for granular products with good redispersion characteristics.

The dryer concept was developed on basis of various NIRO drying processes including: spray drying, two-stage drying and fluid bed spray agglomeration.

The basic elements of the FSD process are:

- Introduction of liquid and hot air for the spray zone at the top of the drying chamber. This generates a zone of co-current down flow of drying air and particles, making the dryer suitable for heat-sensitive materials.

- Introduction of warm air to the fluid bed integrated into the bottom of the chamber causes fluidization and entrainment of fines into the upper section of the chamber to the spray zone causing agglomeration.

- Extraction of spent drying gases and fines from the top of the drying chamber promoting turbulence.

- Recirculation of fines from the fines separator(s).

- Particles large enough to remain in the fluid bed and strong enough to withstand attrition in the bed are discharged as non-dusty product from the integrated fluid bed.

- The product from the integrated fluid bed is discharged into a separate fluid bed for further agglomeration, drying to final moisture and cooling.

Each product particle is built-up from a number of primary spray particles with a fairly small particle size which ensures low drying temperature and good flavour retention. Typical average particle size of the product leaving the integrated fluid bed is in the range of 300–500 microns. When this powder is dried and cooled in the fluid bed for post drying it can be bulk packed and shipped as high class spray dried powder.

Since the final product agglomerates are consisting of a number of small particles, the dimensions of the spray drying chamber are significantly smaller than for a classical spray tower for coffee powder, where particles originate from basically one droplet. Due to this fact, significant savings in building height are achieved.

STABILITY IMPROVEMENT OF ROASTED AND GROUND COFFEE BY OXYGEN ABSORBENT

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Introduction

It is well known that aroma compounds in roasted and ground coffee (R&G) are very reactive with oxygen and that the staling of coffee and the development of stale flavors is mainly caused by the oxidation of aroma compounds[1]. For this reason, vacuum packaging in high oxygen barrier materials such as steel or aluminum are generally used for the production of R&G. When using high barrier packaging materials, additional reduction of the oxygen content present in the package after closure can further contribute to obtaining excellent storage stability in R&G coffee[2]. This is accomplished through the use of oxygen absorbents, the benefits of which were examined in this study.

The void volume in a package of R&G is the source of the oxygen which promotes product degradation. In the most common package types for vacuum packed R&G coffee; rigid steel cans and multilayer flexible bags, the can tends to have a larger void volume than the bag. Also, due to the mechanical differences of the two packing systems and the desire to minimize the risk of cans denting or imploding, the initial vacuum level in cans tends to be limited to -700 mmHg whereas higher vacuums can be applied to bags. This generally results in poorer storage stability for canned products than bagged ones as shown in Figure1.

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[1] Sivetz, M., Desrosier, N., Coffee Technology 565-566 (1979), AVI Publishing Company

[2] Hinman, D. C., 14th ASIC Colloquium, 1991, 165-174

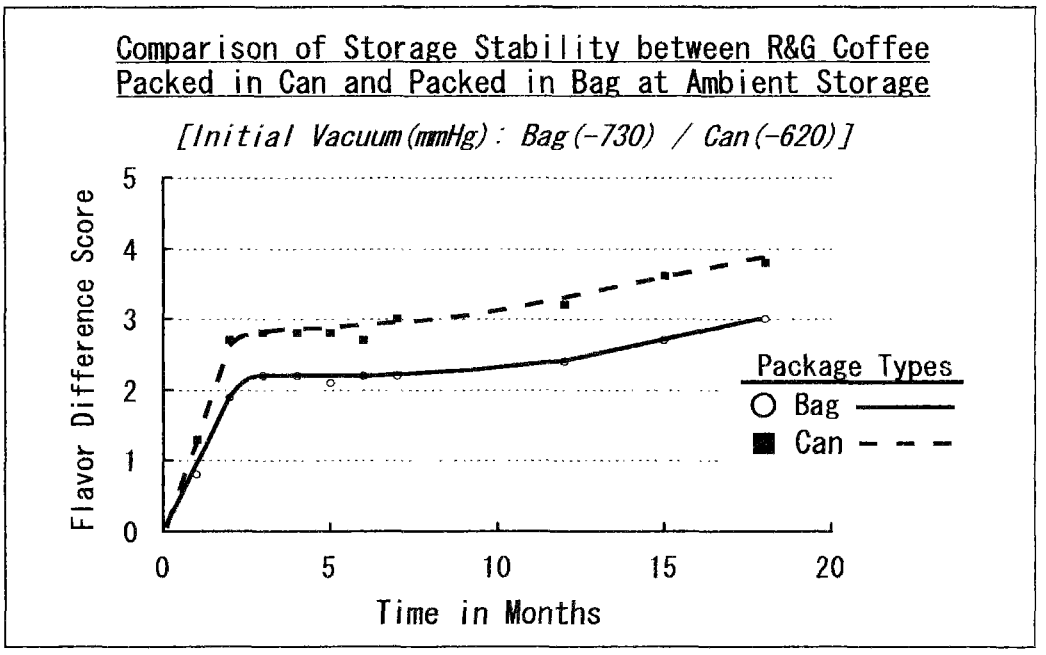

Figure-1

An effective method for removing the residual oxygen present in the void space of vacuum packed coffee is to use an oxygen absorbent. Oxigen absorbents mainly consist of iron compounds and the method of oxygen reduction is achieved via the oxidation of iron compounds as follows:[3]

$$2\,Fe + 3/2\,O_2 + 3\,H_2O \rightarrow 2\,Fe(OH)_3$$
$$2\,FeO + 1/2\,O_2 + 3\,H_2O \rightarrow 2\,Fe(OH)_3$$
$$2\,Fe(OH)_2 + 1/2\,O_2 + H_2O \rightarrow 2\,Fe(OH)_3$$

Some types of oxygen absorbents for R&G coffee can also absorb carbon dioxide, because carbon dioxide competes with oxygen for iron oxide as shown below:[4]

$$FeO + CO_2 \rightarrow FeCO_3$$

Some absorbents contain compounds for the specific absorption of carbon dioxide. This is usually achieved by the reaction between carbon dioxide and alkaline compounds like calcium hydroxide as follows:[4]

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O$$

[3] Nakamura, H., Hoshino, J., Shyokuhin-no-Eiseikanri 543-581 (1985), Sanshyu-Shoboh Publishing Company

[4] Nawata, T., Komatsu, T., Ohtsuka, M., Japanese Patent Dis. Shyou 56-133027 (1981)

Water (H_2O), which is the by-product of this reaction, is available for promoting the oxidation of iron powder and thus can theoretically improve oxygen removal. Further, the inclusion of carbon dioxide absorbents is also beneficial in vacuum packed R&G coffee products because it helps to maintain sub-atmospheric pressure in the package and prevents package distortion as CO_2 evolves naturally from the coffee.

Materials and Methods

Roasted and ground coffees for the study were prepared in a commercial process. Different varieties of Arabica green coffees were blended and roasted to a 'medium-to-light' roast color. The roasted whole beans were then stored overnight at room temperature, and ground the following day to a typical filter coffee size distribution (MPS = 680μ). The R&G coffee was immediately vacuum packed into steel cans or in the case of vacuum bagging, allowed to rest for two hours at room temperature for degassing prior to packing.

Absorbent packets that absorb only oxygen or both oxygen and carbon dioxide (Mitsubishi Gas Chemical Company) were examined for their ability to extend the storage life of vacuum packed roasted and ground coffee. Four types were selected for study, as shown in Table 1. These absorbents were selected based on their ability and capacity to absorb the calculated initial oxygen volume in the voidage of a can[5]. One packet of each absorbent was packed separately into a can, both with and without 200g of R&G coffee, and sealed under -620 mmHg vacuum. Cans without absorbent were also prepared as controls. Vacuum bag samples without absorbent were sealed under -730 mmHg and used as a reference standards for storage stability testing. The experimental design is set out in Table 2.

All experimental samples (except for reference standards for flavor evaluation stored at -20°C) were stored at ambient temperature for about two years. Brew flavor was evaluated periodically to assess the effect of oxygen absorbents on storage stability of the vacuum canned R&G coffee. Brew flavor evaluations were conducted by expert panels who determined the differences between samples and vacuum bagged reference standards. The flavor difference scale is presented in Table 3.

Measurements of the oxygen content and the pressure in the can were made during the first 160 hours of storage. Oxygen content was measured using an Oxygen Analyzer Model RO-101 (IJIMA Products Mfg. Co. Ltd.) and vacuum was measured using a vacuum gauge. After measurement, the values were converted into free oxygen volumes in the cans as follows:

[5] The calculated initial oxygen volumes in the cans assuming atmospheric conditions and room temperature were: 24.6 cc (packed without R&G coffee) and 17.6 cc (packed with R&G coffee).

$$V(O_2) = \frac{(760\ mmHg - Vs)}{760\ mmHg} \times \frac{P(O_2)}{100} \times \text{Void Volume}$$

where:

$V(O_2)$ = free oxygen content in can at atmospheric pressure (cc @ 760 mmHg)

Vs = vacuum in can (mmHg)

$P(O_2)$ = oxygen (O_2) content, (%)

Void volume = empty can volume (cc) - [(g coffee)/(absolute coffee density in g/cc)]

Table-1

Specifications of Oxygen Absorbents

Code	Type of Absorbent	Designed Minimum Capacity for absorption (cc / packet)
A	Oxygen Only	20
B	Oxygen Only	50
C	Oxygen & Carbon Dioxide	25 (O_2) / 250 (CO_2)
D	Oxygen & Carbon Dioxide	50 (O_2) / 500 (CO_2)

Table-2

Experimental Design for Sample Preparation

Package Type	Initial Vacuum (mmHg)	R&G Coffee Weight(g)	Degas	Absorbent
Can	-620	200	-None-	-None-
Can	-620	-None-	-None-	Yes
Can	-620	200	-None-	Yes
Bag	-730	200	2hrs	-None-

Table-3

Definition of Flavor Difference Score

Flavor Difference Score	Definition
0	Identical to flavor standard
1	Very slight difference
2	Slight difference (within normal initial variation range)
3	Moderate difference
4	Moderate - to - large difference
5	Large difference

Results and Discussion

1. Comparison of Oxygen Absorption Rates

Changes in the free oxygen volume in partially evacuated cans (without R&G coffee present) is shown in Figure 2. When R&G coffee was not present, the absorbent B(50/0) absorbed oxygen fastest, and the absorbent A(20/0) was second fastest. The absorbent C(25/250) which also had the ability to absorb carbon dioxide, surprisingly absorbed oxygen more slowly than the absorbent A(20/0) whose designed oxygen absorption capacity was lower than absorbent C. This phenomenon may have been caused by the difference in the effective surface area for chemical reaction of oxygen amongst these absorbents. In the case of absorbent C(25/250), which contains reagents both for oxygen and carbon dioxide absorption, the effective surface area of the reagent for oxygen absorption is probably smaller than that of the absorbent A(20/0) which contains only the reagent for oxygen absorption.

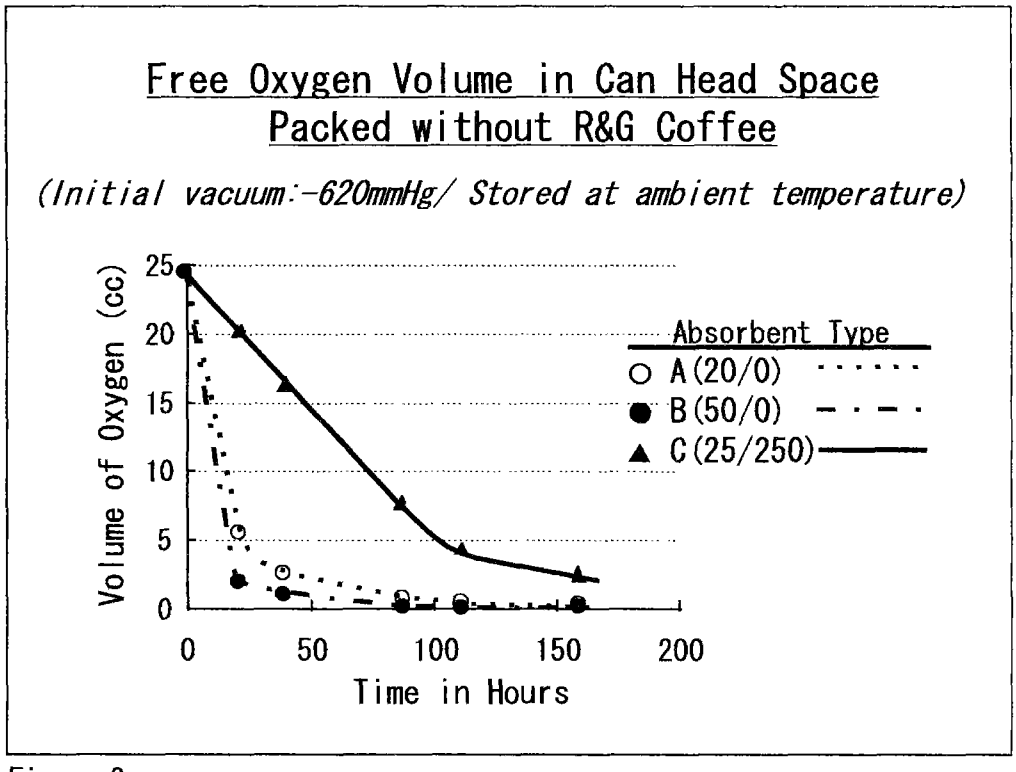

Figure-2

Changes in the free oxygen in the void volumes of cans containing both absorbent and R&G coffee is shown in Figure 3. All samples containing any kind of oxygen absorbent absorbed oxygen faster than the control sample containing R&G coffee alone. But the absorption rate of every absorbent was slower in the presence of coffee than in an empty can. Particularly, the samples containing absorbent A(20/0) and B(50/0) which did not have any specific ability to absorb carbon dioxide, absorbed oxygen much more slowly in the presence of R&G coffee. This is believed to be due to the competition for iron oxide between oxygen and carbon dioxide evolved from the coffee as mentioned in the introduction. However, the fact that even the absorbent C(25/250) which had a carbon dioxide absorbing capacity absorbed oxygen slower when packed with R&G coffee suggest a possibility that coffee aroma compounds react with the chemical reagents, thereby reducing their oxygen scavenging effectiveness.

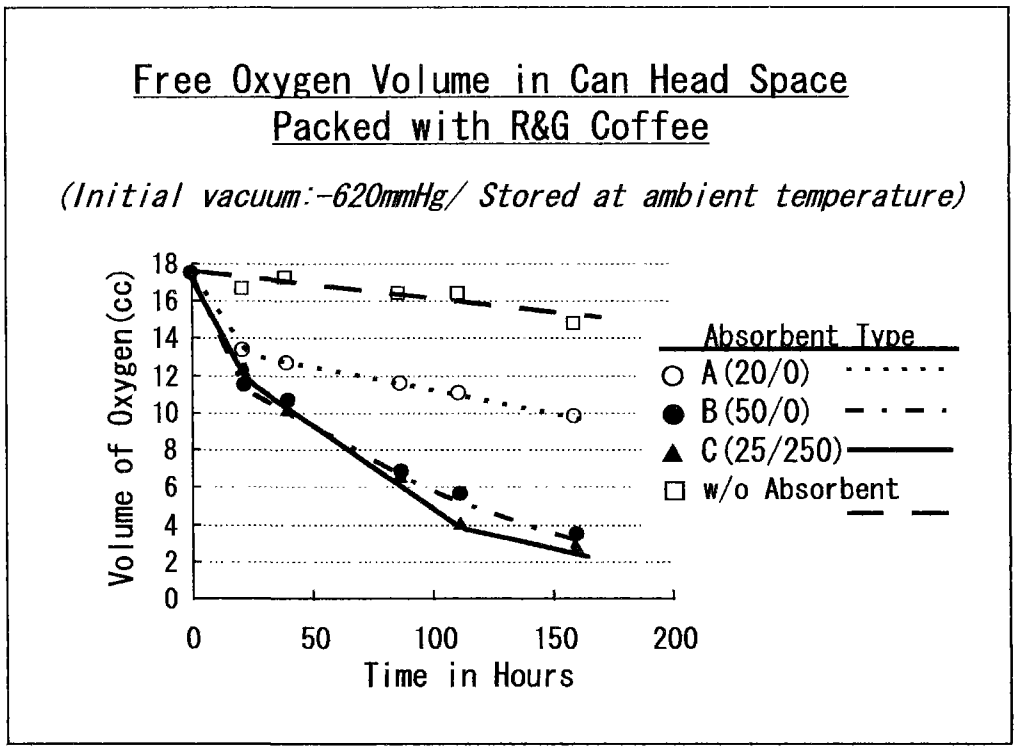

Figure-3

2. Effect of Oxygen Absorbents on the Storage Stability of Vacuum Canned R&G Coffee

Changes in the flavor difference scores with time for each canned R&G coffee (containing different kinds of oxygen absorbents) is shown in Figure 4. The sample containing the absorbent C(25/250) showed the best storage stability amongst all the canned samples, and closest to the bag type reference. Samples containing the absorbent B(50/0) or D(50/500) for which the designed oxygen absorption capacities were the highest, showed the poorest storage stability. A chemical flavor note was detected in these samples, and was especially strong in the sample containing absorbent D, which showed off-flavors in the earliest stages of storage. Off-notes were not detected in the samples containing A(20/0) or C(25/250), suggesting that excessively high oxygen capacity in the absorbents may be detrimental to flavor quality. The generation of a chemical flavor note seems to be related to reactions between coffee aroma compounds and chemical reagents in the absorbents. That is, absorbents which have a large absorption capacity for oxygen such as absorbent B(50/0) and D(50/500) must have reacted with coffee aroma compounds and formed many compounds which contribute to a chemical flavor. These reactions must also have occurred in the samples containing low capacity absorbents A(20/0) or C(25/250), but the quantity of the compounds which cause to chemical flavor note must have been small enough not to be detected by organoleptic evaluations. This hypothesis needs to be confirmed by chemical analyses of the aroma compounds using gas chromatography.

There seem to be two main reasons why the samples which contained absorbent C(25/250) showed the best storage stability. One is that the designed absorption capacity of this absorbent for oxygen must have been properly matched to the load and thus a chemical flavor note was not generated. The other is that an appropriate absorption rate for oxygen must have been maintained by incorporating the function of carbon dioxide absorption and thus the oxidation of R&G coffee was efficiently prevented. The fact that the samples which contained absorbent D(50/500) exhibited a stronger chemical flavor note than those which contain absorbent B(50/0) and further, that the samples which contained absorbent C(25/250) did not exhibit a chemical flavor note suggests a possibility that absorption capacity for carbon dioxide may be related to the generation of a chemical flavor note. This will be confirmed in a further study which will examine samples packed with absorbents which absorb only carbon dioxide.

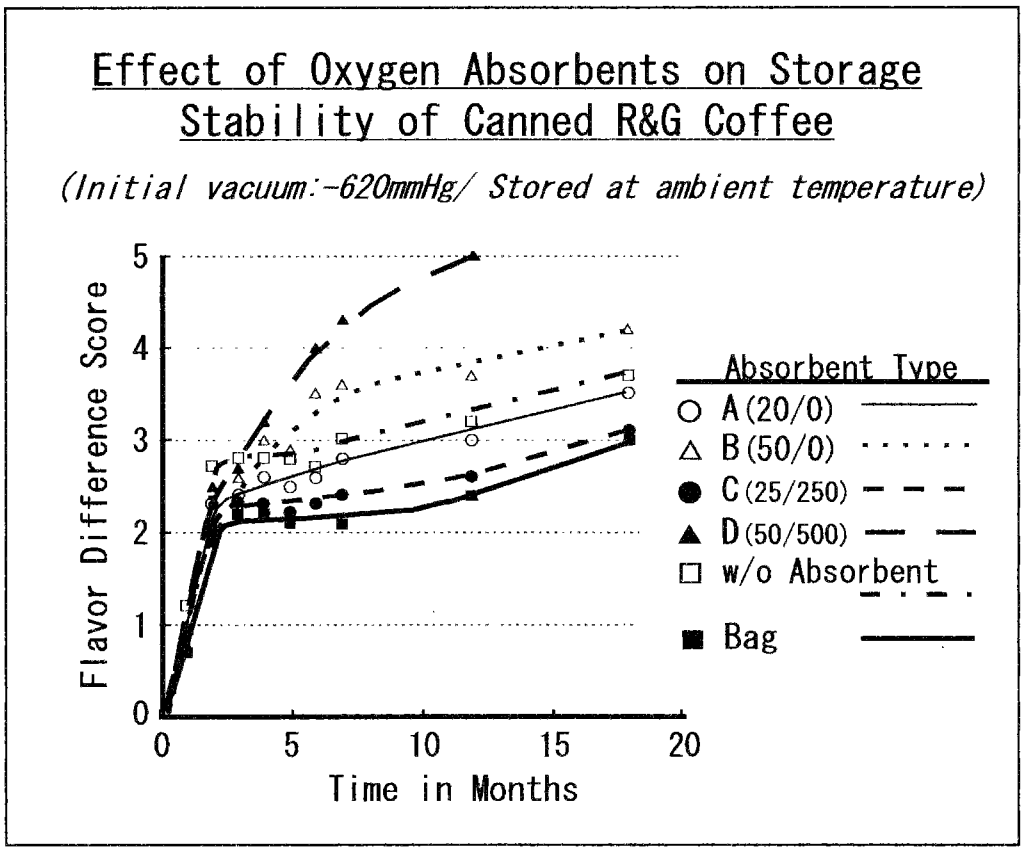

Figure-4

Conclusions

1. An improvement in the storage stability of vacuum canned R&G coffee has been demonstrated by the application of oxygen absorbents. The absorbent C(25/250) which can absorb both oxygen and carbon dioxide gave the best storage stability among examined absorbents and its stability was very close to vacuum bagged R&G coffee.

2. Key factors which should be considered when oxygen absorbents are applied to R&G coffee packing are absorption capacity for oxygen and function for absorbing carbon dioxide. An oxygen absorbent which has proper absorption capacity against initial oxygen volume in a package can absorb oxygen without generating a chemical flavor note. The absorption of carbon dioxide is essential to keep absorption speed of oxygen high enough to eliminate the oxidation of coffee aroma compounds.

Summary

The void volume in a package of R&G is the source of the oxygen which promotes product degradation. In the most common package types for vacuum packed R&G coffee; rigid steel cans and multilayer flexible bags, the can tends to have a larger void volume than the bag and also, due to the mechanical differences of the two packing systems and the desire to minimize the risk of cans denting or imploding, the initial vacuum level in cans tends to be made lower than the values applied to bags. This generally results in poorer storage stability for canned products than bagged ones.

An effective method for removing the residual oxygen present in the void space of vacuum canned coffee using an oxygen absorbent was examined to improve storage stability. Four kind of oxygen absorbents which can absorb oxygen only or can absorb both oxygen and carbon dioxide and also had a different level of oxygen absorption capacity were selected and a storage test with samples containing one of packet of these absorbents were conducted.

An improvement in the storage stability of vacuum canned R&G coffee was demonstrated by the application of oxygen absorbents. Key factors which should be considered when oxygen absorbents are applied to R&G coffee packing are absorption capacity for oxygen and function for absorbing carbon dioxide. An oxygen absorbent which has proper absorption capacity against initial oxygen volume in a package can absorb oxygen without generating a chemical flavor note. The absorption of carbon dioxide is essential to keep absorption speed of oxygen high enough to eliminate the oxidation of coffee aroma compounds.

THE INCORPORATION OF COFFEE PULP IN ANIMAL FEED. REDUCTION OF GROWTH IMPAIRMENT BY FERMENTATION

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INTRODUCTION

Coffee pulp, which is basically a waste product, represents about 30% of the coffee fruit on a weight basis, and in countries where wet processing of coffee is applied, it frequently causes serious environmental problems, since it is either left to ferment in open spaces or dumped in water courses (Braham & Bressani, 1980)

Levels above 10% have been shown to impair growth when incorporated into animal feed, probably due to the caffeine content (Clifford & Ramirez-Martinez, 1991a; Braham & Bressani, 1980) or to the presence of low molecular weight phenols and tannins. Since tannins complex with proteins and both soluble and insoluble condensed tannins are present in coffee pulp (Clifford & Ramirez-Martinez, 1991b), such a complexation could account for the negative nitrogen balance which results (Mehansu et alii, 1987).

The incorporation of coffee pulp into animal feed would be of both economic and environmental interest if this inhibitive effect could be overcome. Previous studies have shown the ability of *Lactobacillus plantarum* to reduce the tannin content of fresh coffee pulp (Menezes et alii, 1993) and this paper reports on the chemical composition of the fermented pulp and on feeding trials with rats to determine the efficiency of this process in reducing those factors in the coffee pulp which impair the growth of rats.

MATERIALS

Fresh coffee pulp was provided by the Coffee Experimental Station in Campinas and the culture of *Lactobacillus plantarum* was obtained from the Tropical Culture Collection of the Fundação Tropical de Pesquisas e Tecnologia "André Tosello" in Campinas.

METHODS

Fermentation by L.plantarum: Batches of 1Kg were homogenized in a domestic blender, inoculated with 1% *Lactobacillus plantarum* (24 hour culture in MRS medium) and 1% sacarose (Menezes *et alii*, 1993) and held at room temperature in closed plastic bags for 3 days, this as previously determined, being the period necessary for the pH to lower to pH 3.6. After verification of the pH, the fermented batches were either frozen or used directly.
Fibre content: This was determined according to Goering & Van Soest (1970).
Acidity, pH, fat, humidity, protein and ash determinations: All were determined according to AOAC (1984).
Carbohydrate content: This was determined by the anthrone method (Dreywood, 1946)
Extraction of acetone soluble tannins: The method of Clifford & Ramirez-Martinez (1991b) was used without the addition of formic acid.
Proanthocyanidin content: This was determined using the method of Porter *et alii* (1986), using grape-seed tannins as the reference standard.
Feeding trials: Growth tests were carried out using groups of 08 21-day old Wistar rats and the PER methodology of Pellet & Young (1980) and Pike & Brown (1975). Two control diets were used, one based on casein and the other a non-protein diet. Substitution with fermented coffee pulp was carried out in the test diets at levels of 10% and 20%. The composition of the control casein diet was according to AOAC (1984) with the exception of the vitamin and mineral supplements, which were according to the American Institute of Nutrition (Reeves et alii, 1993). Thus the control diet was composed of 10% protein (casein - 80% protein), 8% fat (soybean oil), 1% vitamin supplement, 3.5% mineral supplement and 5% fibre (cellulose), the remainder being 25% sugar and 75% corn starch. In the test diets, adjustments were made for the protein and fibre contents of the fermented pulp.

RESULTS AND DISCUSSION

Chemical composition

Table 1 shows the values obtained for the chemical composition of both the fermented and non-fermented coffee pulps.

Table 1 - Chemical Composition

Analysis	*Fermented pulp*	*Non-fermented pulp*
pH	3.8	4.8
acidity-mls 0.1N NaOH/100g	112.9	32.5
% fat	1.1	1.0
% humidity	77.9	72.4
% protein	5.1	3.0
% fibre	8.3	8.4
% ash	1.6	1.6
% tannins (Porter)	0.0	0.8
% carbohydrate	4.8	6.0

In general these results were as expected, based on the previous experiments, and it is interesting to note that the porter-reactive tannins were completely degraded under the conditions used, which did not occur in earlier assays with aqueous slurries.

Diet formulation

Based on the composition of the fermented pulp, and making allowances for the protein and fibre contents, which were sufficient to upset the balance, the test diets were formulated. The composition of these diets is shown in Table 2 together with those of the two control diets.

Table 2 - The composition of the test & control diets (100g portions)

Nutrient - grms	*Control diets*		*Test diets*	
	Proteic	*Non-proteic*	*10%*	*20%*
Casein (80% protein)	12.5	0.0	11.9	11.2
Soybean oil	8.0	8.0	8.0	8.0
Vitamin supplement	1.0	1.0	1.0	1.0
Mineral supplement	3.5	3.5	3.5	3.5
Cellulose (fibre)	5.0	5.0	4.2	3.4
Coffee pulp	0.0	0.0	10.0	20.0
Sugar (sucrose)	17.5	20.6	15.4	13.2
Corn starch	52.5	61.9	46.1	39.6

Feeding trials

In each group the rats were offered 15g of feed per day and weighed after 0 and 15 days. The residue weight was subtracted from the amount offered to each rat in order to calculate the weight ingested. The protein content of each diet was also determined in order to calculate the weight of protein ingested.

The PER was calculated using the following formula:

$$\text{PER} = \frac{\text{Weight gain + non-proteic loss in weight}}{\text{Protein consumption}}$$

The PER was then corrected according to the standard PER value for casein of 2.5, average values being calculated for each group. In this way a PER value of 2.58 was calculated for the 10% coffee feed and a value of 3.65 for the 20% coffee feed. These values confirm that the fermentation process with *Lactobacillus plantarum* eliminated the effect of those factors in the coffee pulp responsible for impairing growth in rats, since the 10% substituted feed gave practically the same value as the casein control diet and the 20% substituted feed gave a considerably higher value, presumably due to other factors in the fermented pulp, which actually stimulated growth.

CONCLUSIONS

It was concluded that the fermentation of coffee (Coffea arabica) pulp with *Lactobacillus plantarum* in closed containers at room temperature with the addition of 1% sucrose, produced a pulp which could be substituted into animal feed at a level of 20%. The fermented pulp showed a pH of 3.8, low enough to be stable, and since the substituted feed actually stimulated growth when compaired to the standard casein diet, it is to be supposed that substitutuion could be carried out at higher levels.

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RESUMÉ

La réduction dans le contenu des tanins de la pulpe de café frais dûe à une fermentation avec *Lactobacillus plantarum* a été déjà demontrée.

Cet article informe sur la composition chimique de la pulpe fermentée et sur des essais alimentaires avec des souris pour déterminer l'efficacité de ce procéde en réduire les facteurs de la pulpe de café qui préjudiquent la croissance.

SUMMARY

The reduction in the tannin content of fresh coffee pulp by fermentation with *Lactobacillus plantarum* has already been demonstrated.

This paper reports on the chemical composition of the fermented pulp and on feeding trials with rats to determine the efficiency of this process in reducing those factors in the coffee pulp which impair the growth of rats.

COFFEE WASTES : QUALITY OF EFFLUENT WATER FROM THE PASTEURIZING OF COFFEE PULP

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1. Introduction.

During the period 1993-94, Mexico produced approximately 4,150 000 sacks of coffee (60 kg each), which places it amongst the principal coffee producers of the world. This production process generates by-products, these being coffee pulp, husk and mucilage, which are generally not used and whose quantities amount to 72.21, 29.8 and 9.96 million tons respectively (SARH, 1991). The pulp is a particular problem because it is customary to throw it all directly into the rivers. A promising and viable alternative for this by-product is the cultivation of edible mushrooms. The success of this technology can be seen in the development, within only the last 10 years, of various production units throughout the country run by small and micro businesses producing from 1-100 kg of mushroom per day (Villegas 1994)

The cultivation of mushrooms upon coffee pulp fits well within the ideology of organic agriculture and the criteria of sustainable cultivation. The technology has been developed along lines ensuring its suitability to local conditions and has many advantages such as the recycling of the pulp, the rapid and easy production of a food rich in protein and low in fat and which finally creates an end-product that can be used as an organic fertiliser or feed for cattle or other ruminants.

Taking all factors into account, the cultivation of edible mushrooms can be seen as an ecologically sound activity as it facilitates the recycling of waste products, the process itself however generates waste products, such as plastic and waste water. Until now, due attention has not been paid to the study of the effluent produced, mainly because in the small scale production units involved quantities are low and contamination as yet not serious. However considering the rapid proliferation of similar production units, this problematic element of the process must be tackled as soon as possible.

This study was carried out to evaluate environmental impact by means of determination of the physico-biochemical properties of the waste water produced by the rehydration and pasteurization of *Pleurotus ostreatus* cultivated upon coffee pulp. The result obtained will be the base upon which to carry out studies towards the development of an effective treatment for this waste water and ensure a truly beneficial impact of edible mushroom production units upon the economy and ecology of the region.

2. Background

2.1 Coffee pulp as substrate for the production of edible mushrooms.

After the pioneering work of De Leon et al 1983 , Martinez 1984 and Martinez *et al* 1984, who discovered that it was technically possible to produce *Pleurotus ostreatus,* upon coffee pulp, various lines of research have been set up to test the use of this waste product alone (fresh or dehydrated) fermented or mixed with other by products for the production of edible mushrooms. Other waste products that have been used in the preparation of these mixtures with coffee pulp, have included: citronella pulp; sugar cane pulp; straw; coca husks and coconut fibre (De León et al 1983, Martinez-Carrera *et al* 1985, 1986, Calvo y Sánchez 1993, González *et al.* 1993, Méndez-Carreto *et al.* 1994). The results obtained with coffee pulp, in terms of biological efficiency are amongst the highest recorded to date for *Pleurotus* (approximately 1.6 kg of fresh mushrooms for Kg of dry pulp). A mixture of wood shavings and coffee pulp has been used for the cultivation of *Lentinus boryanus* and *Lentinus edodes* (Mata y Gaitán-Hernández 1991).

The mushrooms that have so far been cultivated upon coffee pulp belong to different genera:

Pleurotus: P. djamor, P. ostreatus, P. flabellatus, P. ostreatus var. *floridae*
Lentinus: L. edodes, L. boryanus, L. tigrinus
Auricularia: A. fuscosuccinea, A. polytricha
Volvariella: Volvariella sp.
Agaricus: Agaricus bisporus

Although various genera of mushroom have been cultivated experimentally using coffee pulp, only in the case of *Pleurotus ostreatus* has production achieved commercial proportions (Guzmán 1993, Martínez-carrera 1986)

2.2. Production process for *Pleurotus* sp.

The process for the cultivation of this mushroom was established by Martinez-Carrera and Guzman in 1985 and includes the following stages: preparation of the inoculum, which can be achieved using a solid medium, using cereal grains sterilised for 30 minutes at 121°C and then inoculated with a fragment of the chosen strain (Martinez-Carrera et al 1988); or by the use of a liquid inoculum for which the fungus is grown submerged, and regularly shaken then inoculated directly into the substrate. This technique is used for other mushrooms (Stamets and Chilton 1983, Ohsaki 1993), but in areas where coffee pulp is used as a substrate, liquid inoculum is not at commercial level.

Preparation of coffee pulp: This can be fermented aerobically (Martinez-Carrera et al 1985, 1988), or rehydrated if previously dried (Soto et al 1987) The pulp is then pasteurised by submerging it in hot water at roughly 85°C for 30-40 minutes. Finally, the pulp is left to drain and cool ready for inoculation. The inoculated substrate is incubated to encourage mycelial development and ultimately the development of fruiting bodies . (Zadrazil and Kurtzman 1982, Guzmán *et al* 1993).

3. Materials and Methods

For this study, a small unit producing *Pleurotus ostreatus*, was chosen operating at 500 metres above sea level in the municipality of Cacahoatan, Chiapas, Mexico and with a capacity of 15 kg. of fresh fungus daily.

The determination of levels of nitrogen, sulphur, phosphorous and potassium were carried out in accordance with the methodology proposed by AOAC (William 1984). The determination of the chemical oxygen demand (COD) using the micromethod proposed by the American Public Health Association (1979), that of the volatile fatty acids according to Wasser 1990 and the solid analysis using the Mexican official norm DG-A-AA-B-1973.

The water under analysis came from the two phases involved in the preparation of the coffee pulp, *i.e.* those of rehydration and pasteurization which are carried out separately in metal drums with a capacity of 200 litres, into which an average of 117± 16.9 and 173± 11.4 litres of water are introduced respectively. In this quantity of water 20.7± 1.98 Kg of coffee pulp are submerged with an existing humidity of 8.2± 2.98 %. the analysis were carried out on the water flowing into the production unit and on the contaminated water after one or two rehydration and pasteurization operations.

4. Results and Discussion

4.1 Water consumption in the mushroom production unit.

Water is required for the preparation of the raw material that serves as a vehicle for the inoculum (seeds or grain), and for the pasteurization and rehydration of the vegetable residue that will serve as the substrate for mushroom growth (Coffee pulp). Yet more water is required for watering and for the maintenance of humidity in the fruiting chamber and for the general cleaning of the production unit. Table 1 shows a summary of the quantities of water required for each activity.

The amount of water necessary to maintain adequate humidity in the fruiting chambers depends upon the location of the unit and upon the season. During the rainy season in the typical tropical conditions found in coffee producing countries it is probable that no watering will be necessary, whereas in the dry season 3 or 4 waterings may be necessary per day.

Table 1 Water consumption during the production of *Pleurotus ostreatus* from coffee pulp

Activity	Water consumption
Cleaning and hydration of the grain	3 ± 0.5 lt/ kg. grain
Rehydration of coffee pulp	5.68 ± 0.8 lt./kg pulp
Pasteurization of coffee pulp	8.36 ± 0.8 lt./kg pulp
Watering in the fruiting chamber	Variable
Cleaning	-

4.2 Characteristics of waste water from Rehydration and Pasteurization in the cultivation of *Pleurotus ostreatus*

Table 2 presents the solids content of the waste water from rehydration and pasteurization comparing it to the composition of the water that enters the production unit. The total solids increases from 206 ppm in the water entering the unit to 18 842 ppm after rehydration and increasing further to 22, 991 after pasteurization. The passing of these solids into the water gives it its highly contaminating character. Statistical analysis showed no difference between both types of pasteurization and rehydation effluentes produced. The differences observed at a level of significance of 5% merely confirm that water used twice for the same operation are more contaminated than those used only once. On the other hand it can be seen that the majority of these solids form part of the dissolved solids content, which renders inoperable most possible physical treatment process. When comparing these data with those obtained by Bello *et al.* in 1993 concerning waste water from the processing of coffee, it can be seen that the waste water in the present study displays a much higher solids content, thereby leaving the production unit more highly contaminated as a result of an extended period of contact between pulp and water (and with the water at higher temperature in the case of the pasteurization process) than that observed in the processing of coffee.

Table 3 shows the principal chemical characteristics of the waste under consideration. The inflowing water shows the normal range of characteristics of water that has not been contaminated by organic matter, it is notable however the presence of potassium and SO_4 in this source of water, which is due to the characteristics of the soil in this region (Evergenyi, 1990). In general, both the pasteurization and rehydration process provoke a slight acidification of the water changing the pH from an initial value of 6.3 to a value of 5.83 after two cycles of use. This acidification is due to the presence of pectin, chlorogenic acids etc. in the pulp, but in real term the acidification is slight and statistical analysis did not show any significant differences at 5% level between the different types of water studied.

In terms of what is referred to as the COD, it can be seen that from an initial value of 5.5 ppm, The COD rises to 28, 507 ppm after being used to rehydrate two lots of pulp and rises further to 56, 693 after the second pasteurization. these values are very high in comparison to other waste waters and indicate a high level of contamination (Bello *et al* 1993). Statistical analysis shows a significant difference between the waste water from the different stages of the process, indicating at 5% confidence level that the 2nd pasteurization is the most contaminated. This can be explained by the high temperatures employed in this operation which facilities the diffusion of the components into the water. The second most contaminated stage, according to the same statistical

analysis, is the second rehydration, and the third group includes waste water from the first rehydration and first pasteurization, all different and more contaminated by organic matter than the inflowing water.

Table 2. Some physical characteristics of waste from the production of *Pleurotus ostreatus* upon coffee pulp.

Parameter	Inflow	1st Rehydration	2nd Rehydration	1st Pasteurization	2nd pasteurization	C.V. (%)
Temperature	28 b	26.6 b	25.26 b	90 a	89 a	5.33
Total solids (ppm)	206.6 c	5810 b	18842 a	7477.3 b	22991.3 a	26.31
Total dissolved solids (ppm)	200 d	5200 c	18770 b	7225.13 c	22991 a	0.49
Floating material	0	0	0	0	0	
Sediment	0	0	0	0	0	

Values in the same raw with the same letter are not statistically different at the level of 5%

Table 3. Some chemical characteristics of the waste water from pasteurization and rehydration during the Process of producing *P. ostreatus* using coffee pulp.

Parameters	Inflow	1st soaking	2nd soaking	1st Pasteurization	2nd Pasteurization	C.V. (%)
pH	6.3 a	6 a	5.83 a	6.26 a	5.83 a	6.32
Total Alkalinity (ppm)	87 c	100 a	8500 a	1275 b	7900 a	6.24
COD (ppm)	5.5 d	5604.64 c	28507.4 b	7728.3 c	56693 a	17.0
NH_3-N (ppm)	0 c	6.608 b	12.618 a	5.67 b	12.45 a	17.7
Phosphorous (ppm)	0	Traces	Traces	Traces	Traces	
VFA (meq/l)	0 c	0.61 b	3.33 a	0.66 b	2.61 a	10.8
SO_4 (ppm)	11.95 c	151.5 cd	1143.5 b	237.06 c	2240.5 a	43.2
Caffeine (%)	0	0.01	0.02	0.036	0.02	0.9
Potassium (ppm)	2.404 c	1160.7 b	4453.7 a	1081.6 b	3761.9 a	33.1

Values in the same raw with the same letter are not statistically different at the level of 5%

After the second cycle of pasteurization and rehydration, levels of 12.6 and 12.45 ppm of ammoniacal nitrogen were obtained respectively. The water coming into the production unit, the inflow, shows no trace of nitrogen, however, as a result of contact with the coffee pulp it becomes enriched with this component. Statistical analysis does not show differences between the process of pasteurization and that of rehydration, for which reference is made to the diffusion of ammoniacal nitrogen which indicates that the temperature is not the decisive factor in the diffusion of this component. However, a significant difference is observed in the case of frequency of use of the water. With repeated use of the water the greater is the amount of nitrogen transmitted. this enrichment is important when considering the possibility of using these waste waters as a cultivation broth, as the nitrogen present could serve as a substrate for the development of a chosen microorganism.

The observations made on ammoniacal nitrogen apply also to the case of volatile fatty acids. The inflowing water lacks fatty acids of low molecular weight, but due to contact with the cultivation medium, such substances

are passed into the water reaching concentrations of 2.61 and 3.33 ppm for waste water from pasteurization and rehydration respectively. There is no significant difference at 5% level between the VFA content of the two categories of waste water.

The incorporation of volatile acids into the effluent is an important aspect as it is known that these acids are the principal substrate for methanogenic microorganisms. This implies that the waste water under study could be employed for anaerobic digestion processes and the production of biogas (NAS 1977).

The incorporation of $SO_4^=$, potassium and caffeine into the water used in the process contributes to their further enrichment as a culture medium. Caffeine for example at low concentrations has been proven to stimulate the growth of various fungi (Martínez-Carrera 1988, Calvo y Sánchez 1994) If the waste water is deficient in anything, it is Phosphorous, which is only present in traces. This factor is important in that it could detain the growth process of microorganisms as has already been reported in the case of the anaerobic digestion of coffee pulp (Sánchez and Martin 1993)

In terms of microbiological characteristics of the three phases of water under study, the inflowing water contained cells of *Klebsiella* sp. which indicates that the water is not drinkable. This situation can be explained by the fact that the inflow water is drawn off from a nearly river, carrying with it soil microorganisms. *Klebsiella* was found once again in the waste water from rehydration, which also carried populations of Enterobacteria and yeasts. This further contamination can be explained by the contact between the water and the raw material during the rehydration of the latter. Coffee pulp, upon leaving the processing unit (beneficios) contains a diversity of flora according to the methods used. The waste water from pasteurization did not present a single contaminating organism during the testing, however, during processing occasional contamination by *Coprinus* spp. has been observed. *Coprinus* is considered as a thermoresistant fungi and its spores on occasion resist pasteurization treatment (Stamets and Chilton 1983).

Waste water from the washing and depulping of coffee has been characterised in presentations of previous studies. These studies have been of major importance in the determination of treatment systems for these effluents. Given that during the production of edible mushrooms the substrate used (coffee pulp) remains in closer contact with the water used than is the case for the water used in coffee processing (beneficios), the level of contamination is higher. When comparing the results obtained by Bello *et al* 1993, it can be seen that although the acid content is similar (pH 5.31± 0.76) there is a marked difference in organic matter content. In waste water from coffee processing plants low COD values are reported (2480.31 ppm), but on evaluation of microbiological quality the same authors found pathogenic bacteria present such as *Salmonella, Klebsiella*, and *Enterobacter* among others. In 1989, Morales reported on the physico-chemical and bacteriological characteristics of the coffee beneficio at Chocoman in Veracruz, Mexico. This author presented results similar to those of Bello *et a .op. cit.* but in Cali, Colombia however results showed a higher COD at 15, 450 ppm (Arias 1987) caused by the reduced volumes of water used in the region. This shows that depending upon the volume of water used for dispulping and fermenting, the waste water from coffee beneficios could reach contamination loads similar to those reported here for pasteurization.

5. Perspectives in the Treatment and use of Waste Water.

The production of *P. ostreatus* generates waste water characteristic of the technology used. Although rehydration of the substrate is advisable (Guzmán *et al.* 1993) this is a stage of the process that could be eliminated, which if widely accepted could reduce the amount of water used in the process.

The production units here exemplified are small businesses with scant resources and do not have the means to install more efficient forms of pasteurization/sterilization such as pressure or vapor, these too would reduce water consumption but are more profitable for large scale production.

The treatment of waste water depends upon the characteristics of the water concerned, the level of purification required and the characteristics of the site. It is clear that to obtain water free of contamination it is necessary to implement various treatments methods in sequence. Given the characteristics of the waste water that is produced as a result of mushroom cultivation, in which dissolved solids predominate, treatment must focus on the dissolved organic matter. One of the best ways to achieve this is the use of microorganisms within which various possibilities have been tested including the production of biogas.

The production of fungi using these waste water is feasible and various species from the genera *Aspergillus*, *Penicillium* and *Trichoderma* which produce interesting metabolites are candidates for this method. In the case of the waste water produced as a result of the pasteurization process, the high temperature of the outflow (>80°C) facilitates the elimination of microorganisms that might otherwise compete with the organism being cultivated. It is also possible to think in terms of the cultivation of the same fungus *Pleurotus*, by submerged

fermentation, which generates the provision of inoculum for the production unit of the edible fruiting bodies (Sánchez and Calvo 1994). It should be noted however that although the fungi grow within this substrate of waste water, reducing the dissolved organic material content, there is no guarantee of total purification.

The treatment that eliminates the greatest quantity of organic matter is that of anaerobic digestion, coupled with a third treatment similar to the process proposed for the waste water from the depulping of coffee (Bello and Castillo 1994). Studies carried out in our laboratories have shown that with the use of an anaerobic filter packed with pumice-stone it is possible to eliminate 70% of the COD using a continuous process with a retention time of 36 hours. This technology should be improved still further.

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ABSTRACT

Using coffee pulp for the cultivation of different kinds of edible mushrooms has been found to be suitable in coffee producing countries. The present work was carried out with the main objective being to evaluate the quality of the waste water generated by a small production plant of *Pleurotus ostreatus* from coffee pulp. Samples were taken from the waste water from the rehydration and pasteurization of the coffee pulp in order to evaluate the water consumption and pollution levels of the effluent. Using standard methods for examination of water and waste water, the effluents from rehydration and pasteurization were found to have high polluting conditions above all from the pasteurization process where the thermal treatment extracts more constituents from coffee pulp and presents the majority of the solid component in dissolved form. The possibility of utilization of such a waste water for the cultivation of other mushrooms and for anaerobic digestion is discussed.

SPRAY-DRYING INSTANT COFFEE PRODUCT AT LOW TEMPERATURE

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1.INTRODUCTION

Instant coffee is made by processing coffee beans through the following steps: roasting, grinding, extraction, concentration, drying and packing. Of all characteristics that coffee possesses, perhaps its most appealing attribute is the rich coffee aroma. Evaporation that takes place during the production of instant coffee significantly dissipates this rich aroma.
Freeze drying and spray drying are the most frequently used methods for producing instant coffee. Many manufacturers use a freeze drying process because it is thought that such a process reduces the changes in aroma characteristics normally caused by heat and oxidation. However, the long vacuum time required for this process leads to a relatively substantial loss of aroma. In addition, milling and sifting must occur at low temperatures and drying requires several hours.
As these processes require a considerable investment in equipment and energy, the finished product becomes rather expensive.

The spray drying process, on the other hand, can be accomplished with relatively simple equipment. This process permits large scale production with lower costs and results in a product with low density and good flowability. However, usual spray drying temperatures of 200

-300℃ cause aroma loss and impart a caramel flavor to the product.
Low temperature spray drying was attempted using a single nozzle system which permits considerable control over the temperature and volume of hot air entering the drying chamber. With this system it was possible to increase the initial drying rate for sprayed droplets that passed through the single nozzle and to cool down the dried particles in the bottom of the drying chamber. As a result, aroma retention was about twice that of the high temperature spray drying process. Also, aroma retention was greater than that of the freeze drying process. These results were published in ASIC(1).

Further, it has been reported from experimentation with the use of maltodextrine that greater aroma retention can be achieved by a 40% increase in solids concentration (2-7). A common method to increase the level of solids prior to drying is vacuum thermal concentration, which causes loss of aroma(8).
In recent years, attempts have been made to use a freeze concentration method.
Compared with vacuum thermal concentration, this method allows an increase in solids to be achieved with barely any aroma loss. Because of its advantages, the freeze concentration method is being used more frequently as a part of the process to produce instant coffee. However, with the increase in solids there is also an increase in viscosity. Therefore it becomes difficult to separate ice particles from the concentrate and the extent to which solids concentration can be achieved becomes limited(9). This paper investigates the changes in aroma retention and density of instant coffee produced by low temperature extraction, freeze concentration and low temperture spray drying.

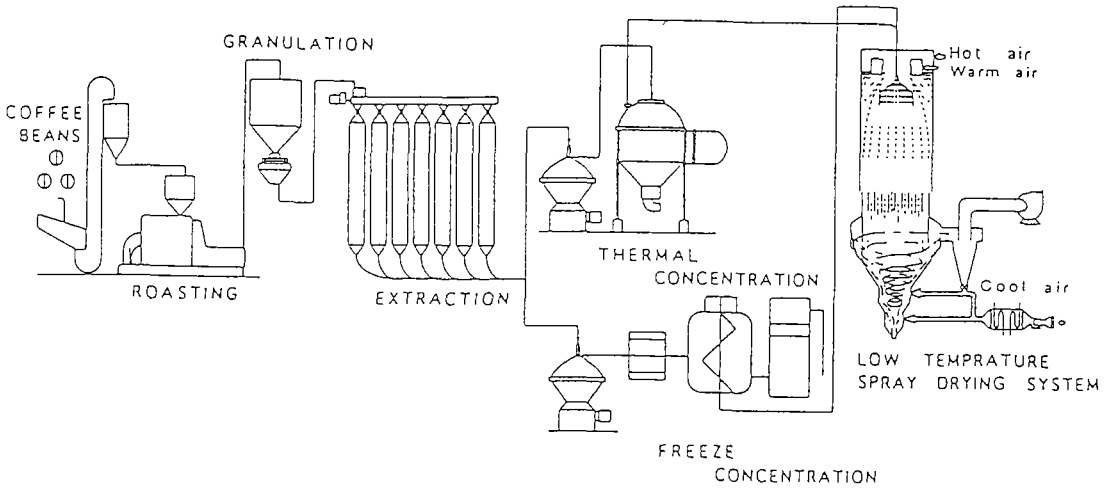
2. Method and Material

2.1 Materials

Figure 1 shows a flow-diagram of instant coffee production. A blend of Arabica (70%) and Robusta (30%) was roasted, ground and extracted according to current industrial standards. The resulting extract was divided into two parts. One part was freeze concentrated (T.S. 30, 43 %) and spray dried at low temperature (120,140,160,or 18

0℃). The other part was thermally concentrated (T.S. 43%)

Fig-1 Flow-diagram of Instant Coffee Production



2.2 Analytical Methods

A. Analysis of aroma

Coffee extract or powder was analyzed for volatile aroma, either by adsorption onto TENAX or by purge and trap techniques, using gas chromatography oven conditions of 35℃ initially, increasing at 3℃/min to 220℃.

B. Analysis of Density

Density was measured by the a tapping method (precisely 200ml of powder was measured by filling a stainless tub ediameter 5.5cm, length 8.4cm -with powder through a funnel, tapping the tube 120 times then removing any excess powder with a leveling device.)

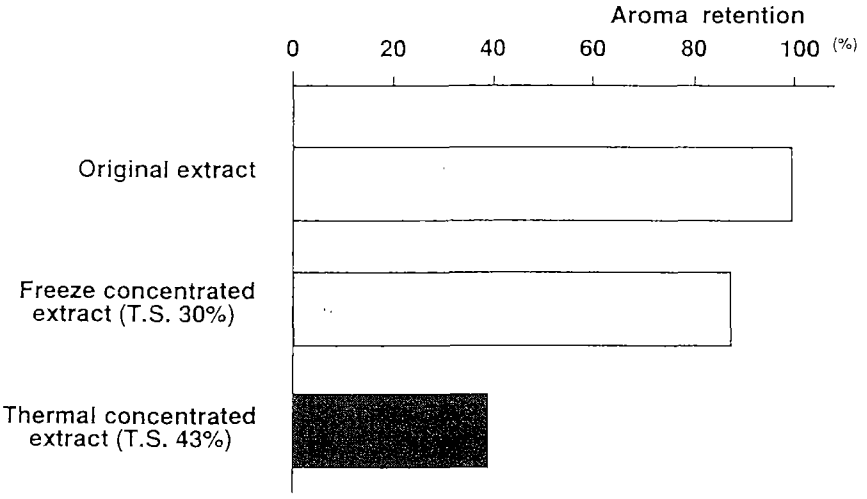
3. Results and Discussion

3.1.Comparison of the Effect of Concentration Method on Aroma Retention

A comparison of the aroma retention after thermal concentration (T.S. 43%) and freeze concentration (T.S. 30%) was conducted.

With freeze concentration, viscosity rose as the level of solids increased and the separability of ice particles and extract decreased. As a result, the extent to which solids could be concentrated was limited. Two types of concentrate were examined by gas chromatography with TENAX to compare the retention of aroma in each type of concentrate. Figure 2 shows the retention of aroma using the extract as an index (equal to 100%). Using the thermal concentration method the aroma retained was only 39%. That is, about 60% of the aroma was lost in this process. With freeze concentration, in contrast, 86% of the aroma was retained. The results indicate that freeze concentration is an effective means to reduce moisture content prior to the drying process.

Fig-2 Comparison of aroma retention at different concentration method



However, with the freeze concentration method , loss of aroma during the drying process is likely to occur because of the low solids content. Therefore methods aimed to increase the solids concentration were investigated. Figure 3 shows the relationship between solids concentration and viscosity. Using conventional extraction methods viscosity rises rapidly when the solids concentration reaches about 30%. Here, extraction was conducted at low temperature to suppress hydrolysis which causes the rise in viscosity during extraction. Under the conditions employed the viscosity rose slowly and a concentration of 43% could be achieved.

Fig-3 Relationship between viscosity and concentration in extraction method

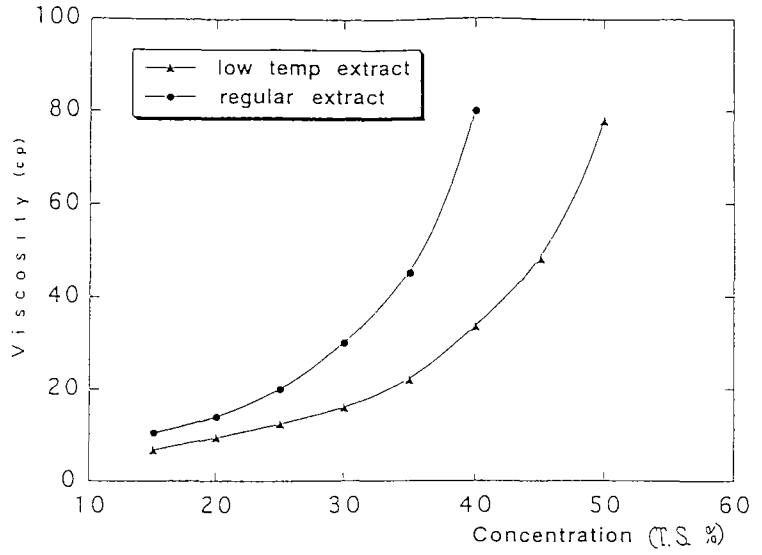
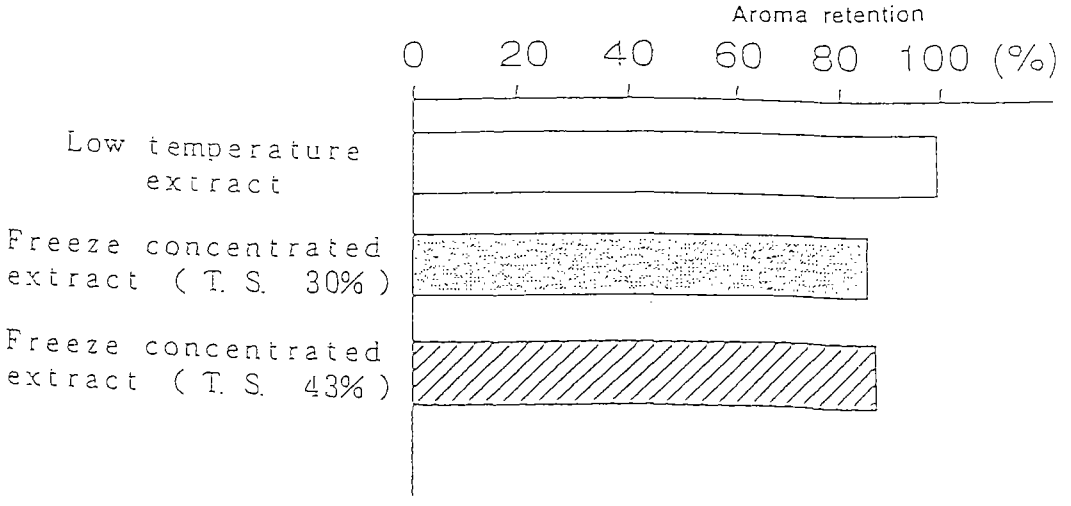


Figure 4 shows the aroma retention achieved when the total solids was 43%. There was no significant difference in aroma retention between the low level concentrate of T.S. 30% and the concentrate of T.S. 43% produced by freeze concentration.

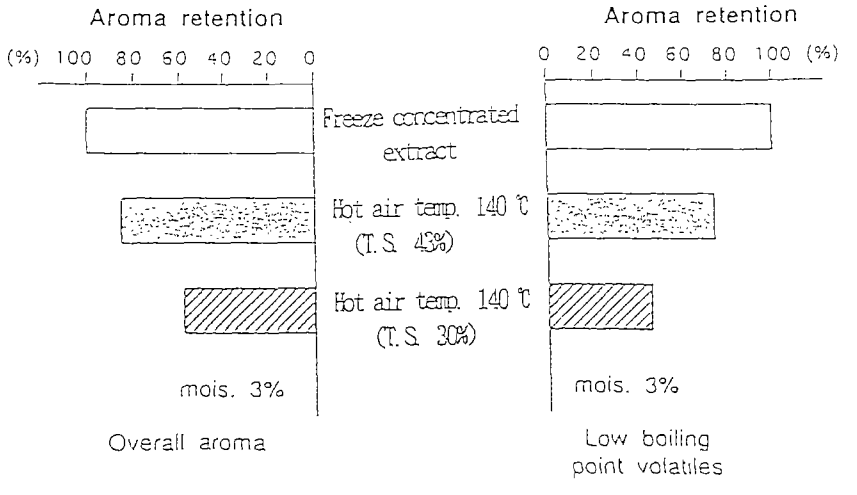
Fig-4 Aroma retention at different freeze concentration



3.2.Comparison of Aroma Retention at Different Levels of Concentration after Low Temperature Spray Drying

A comparison was made of the aroma retention of two freeze concentrates (T.S. 30% and T.S. 43%) produced by low temperature spray drying (hot air temperature 140℃). These results are shown in Figure 5. The overall retention of aroma (total sum of peak areas) is shown on the left. It is evident that the retention of low boiling point aroma increases as the solids concentration increases. The reason for this is that as the solids concentration decreases, the powder particles become smaller and more particles are brought into the cyclones causing aroma loss. The loss of aroma, however, is counteracted by the more rapid formation of a dry skin around the droplets of a higher concentration.

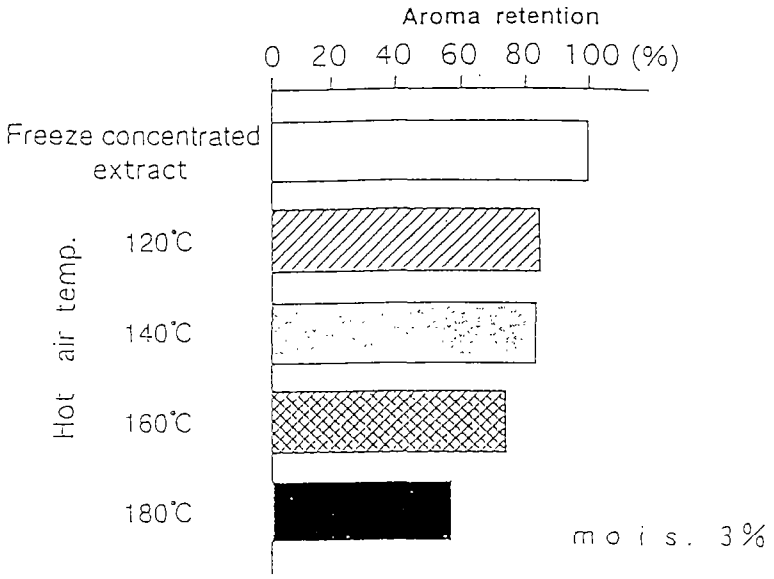
Fig-5 Comparison of aroma retention at different levels of concentration after low temperatire spray drying.



3.3.Comparison of Aroma Retention at Different Hot Air Temperatures

A comparison was made of aroma retention after spray drying of a freeze concentrated extract (T.S. 43%) at temperatures of 120,140,160 and 180℃. The retention of aroma is shown in Figure 6. An index of 100 is used to represent the freeze concentrated extract. It is evident that aroma retention decreased as the temperature increased.

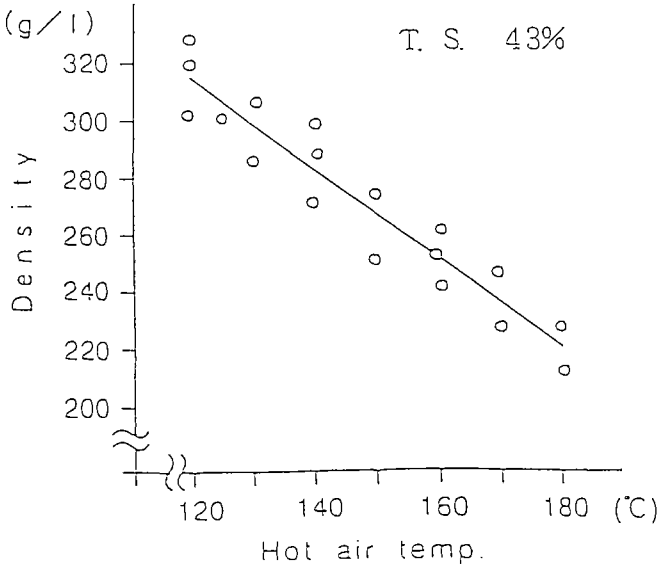
Fig-6 Effect of hot air temperature on aroma retention



3.4.Controllability of Density in the Low Temperature Spray Drying Method

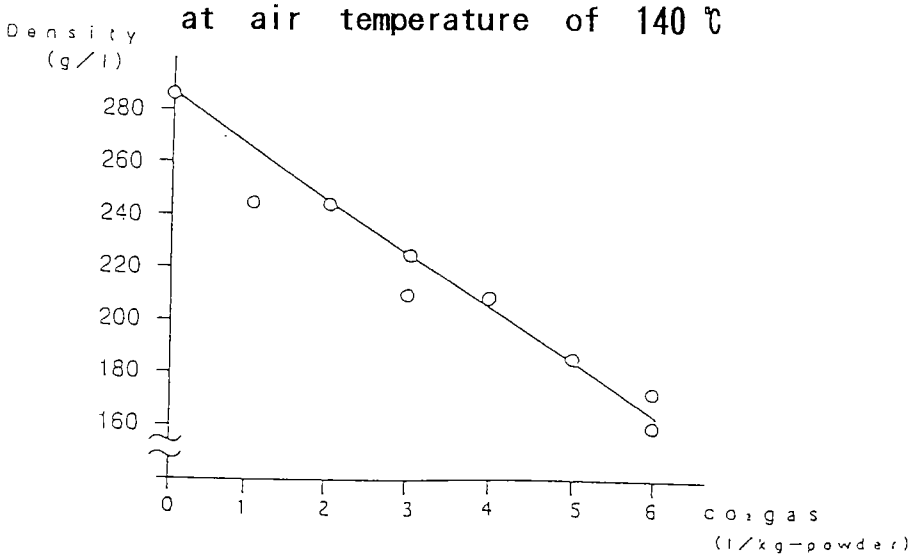
The controllability of density, a critical characteristic of instant coffee, was investigated. Figure 7 shows the change in density under varying temperature conditions using the low temperature spray drying method.

Fig-7 Relationship between density and hot air temperature in spray drying



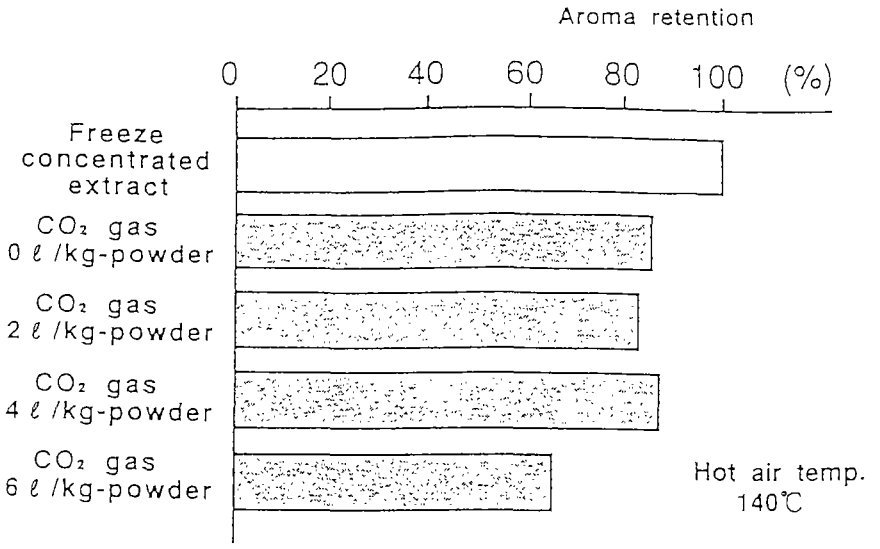
Results indicate that the lower the air temperature, the higher the density became, so it is thought that air temperature has a significant impact on the density. Generally it is believed that when the diameter of the droplets is small and the hot air temperature is high the density of the powder will decrease. This was reported by Greenwood and King (10) in their report which would indicate that when the diameter of the droplets is small,the rise in temperature of the droplets is great, thereby promoting droplet expansion.
By lowering the air temperature and increasing the air flow, drying time for the low temperature drying method can be shortened in order to obtain better aroma retention. When this is done the spray particle is dried before full expansion occurs. However, as previously stated a higher air temperature affects aroma retention. Figure 8 shows the results of spray drying coffee extract in which CO_2 has been dissolved. The results indicate that during low temperature spray drying, even when droplets have not fully expanded, density can be controlled by increasing the amount of CO_2 added to the extract.

Fig-8 Relationship between density and CO_2 gas at air temperature of 140 ℃



The effect of addition of CO_2 on aroma retention is shown in Figure 9. While density was maintained at a normal level, the addition of CO_2 gas had no significant impact on aroma retention.

Fig-9 Influence of the addition of CO_2 on aroma retention



4.Conclusions

1) When extraction of coffee beans is done at low temperature, with further processing by freeze concentration and low temperature spray drying, the resulting coffee powder closely resembles the original extract.

2) Powder obtained by spray drying at a higher concentration of solids shows better aroma retention.

3) It is possible to control the density of coffee powder by the addition of CO_2 to the extract prior to low temperature spray drying. The use of CO_2 has no significant influence on aroma retention of the coffee.

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SUMMARY

The effect of spraying at higher solids concentration on aroma retention in powder is reported.
An advantage of freeze concentration is that little loss of volatile components occurs, but compared with evaporation, the extent of concentration achieved is limited because of viscosity. In order to achieve a higher concentration of solids with the freeze concentration method, low temperature extraction was applied. The powder obtained by spray drying at higher concentration of solids showed better aroma retention.
Also, we have investigated means to control the density of powder produced using this low temperature hot air spray drying system. As the hot air temperature was decreased, the density increased. The powder density could be controlled by injection of CO_2 gas into the concentrated extract. This treatment did not adversely affect aroma retention.

AROMA AND ENZYME RETENTION DURING DRYING

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1 Introduction

Many desirable and undesirable changes occur during drying of food. For example, aroma is the most important quality in coffee. So, it is essential to prevent the aroma loss during drying of coffee liquids. Changes of heat-sensitive materials such as proteins and enzymes also affect the food quality very much.

To predict the retention of aroma and enzyme during drying of liquid foods, mathematical models that consider the simultaneous heat and mass transfer and the degradation reaction are needed. Furthermore, many physical and phenomenological parameter values must be obtained for the model calculation. This is not an easy task.

Among those parameters the diffusion coefficient D which depends strongly on water concentration W is the key parameter, as the aroma is entrapped during drying due to the "selective diffusion mechanism" which is based on the concentration dependent D [1, 2, 3, 5, 6].

The enzyme retention can be predicted on the basis of the above-mentioned model with the inactivation rate constants k which also depend on W as well as temperature T.

In this paper, I will present a short review on aroma and enzyme retention during drying based on the experimental results with a single droplet of liquids containing sugars [7, 8, 9].

2 Experimental

A schematical drawing of the experimental setup for the drying of a single droplet is shown in **Fig.1** [8]. A solution was suspended from a glass filament by a microsyringe. The droplet(4 to 8 μL) was subjected to a constant temperature and flat-flow-velocity air. Periodically, the air was stopped and the weight of

the drop was determined from the deflection of the glass beam balance. The drop temperature T_d was recorded continuously from the output of a thermocouple inserted into the drop. In order to measure the remaining activity of the enzyme or the ethanol concentration in the drop during drying, the drop was suspended from a glass filament which was removable from the drying apparatus. At different lengths of drying time, the drop was removed and dissolved in an appropriate buffer solution and the enzyme activity or the ethanol concentration was assayed. The enzyme used is β-galactosidase from *Aspergillus oryzae* (Amano Seiyaku,Japan) and its activity was measured according to the standard method with o-nitrophenylgalactoside as the substrate. The ethanol concentration was measured by the enzymatic analysis with alcohol dehydrogenase.

Sugars employed in this study are monosaccharide (glucose, galactose, mannose), disaccharides(sucrose, maltose and lactose), oligosaccharide [maltodextrin(MD) of dextrose equivalents(DE), DE=11 and DE=40, Matsutani Kagaku Kogyo, Japan] and high-molecular weight polysaccharides[Dextran T-10 and T-70, Pharmacia, Sweden]. A model food sample [an aqueous solution (20 wt%) of lactose (11wt%), casein (5wt%), bovine serum albumin (2wt%), sodium chloride (2wt%)] was also used as a sample.

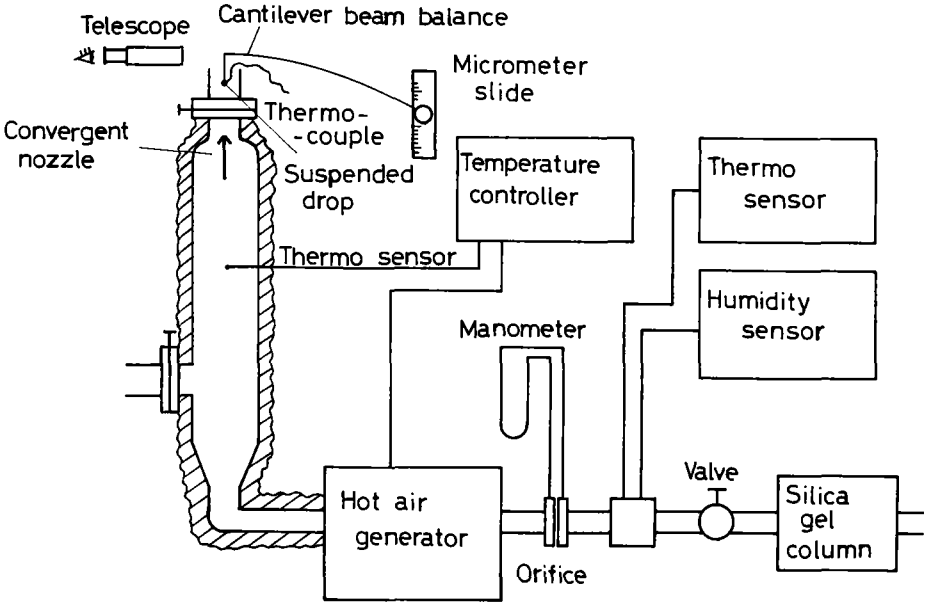

Figure 1: Set-up for the drying experiment of a single suspended droplet[8]

3 Drying behavior

Figure 2 shows typical experimental results for the drying of a single droplet with mono- and disaccharides as a sample solution. The drop temperature T_d remains constant just after the start of the drying, which is almost equal to the wet-bulb temperature. After this constant-rate period, T_d rises to the air temperature T_A gradually and the drying rate($-d\bar{u}/dt$) decreases. The deviation of the experimental results from the computer simulated ones with the experimental D and A_W data is possibly due to the internal circulation of liquid at the early stage of the drying. The observation by means of a video camera equipped with a magnifying lens showed that the internal circulation of liquid occurred at the beginning of the drying and terminated at the time when T_d started to rise from the wet-bulb- to the air temperature. When a small amount of agar-agar(0.07%) was added to a glucose solution, the experimental results approached the computer simulation(data not shown).

In the case of lactose drops, the formation of a solid phase was observed at the end of the constant rate

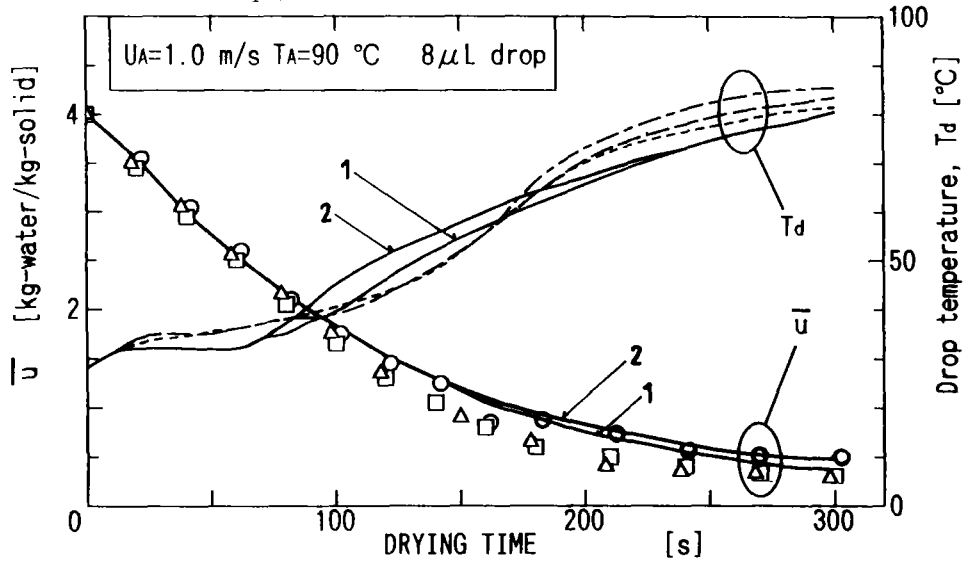

Figure 2: Average moisture content $\bar{u}$ and drop temperature T_d as a function of drying time during drying of a single droplet of various mono- and di-saccharide solutions[8].
Key:○ and - - - for lactose, □ and — — — for sucrose, △ and — - — for glucose, 1 for glucose(model calculation),2 for sucrose(model calculation).

period, and then the drying rate was lower than that for sucrose. Probably, the surface crust prevented water from evaporating from the surface of the drop.

In **Fig.3,** the drying histories of high-molecular weight sugars are shown. Clearly, the constant rate periods are much shorter than those in Fig.2 due to the low D values of high molecular weight sugars. Although the convection was not remarkable for these sugars, the experimental results for maltodextrin (MD) of DE=11 deviated from the computer simulated ones with the progress of the drying. Moreover, the drying rates of Dextran T70, the molecular weight of which is higher than MD(DE=11), are equal to or higher than that of MD(DE=11) at the end of the drying. A most possible reason for these phenomena is the deformation of the droplets with the progress of the drying. The droplets shrunk irregularly with a number of wrinkles, which are responsible for the increase of the drying rate.

4 Aroma retention

Ethanol was lost very rapidly at the beginning of the drying, and then retained constant(**Fig.4**). When we compare the ethanol retention in Fig.4 and the temperature histories in Figs. 2 and 3, it is clearly seen that the most of the aroma loss takes place in the constant rate period. Therefore, the final aroma retention increased with increasing molecular weight of sugars. These results are understandable in terms of the selective diffusion mechanism [1, 2, 3, 4, 5, 6]. No appreciable difference was found among the ethanol retention in monosaccharides (glucose, galactose, and mannose) and also in disaccharides (sucrose, maltose and lactose) (data not shown).

5 Enzyme retention

In contrast to the aroma loss which mainly occurs in the constant rate period, the enzyme inactivation takes place in the falling rate period characterized as the "*regular regime period*" [1, 5, 7]. Most enzymes

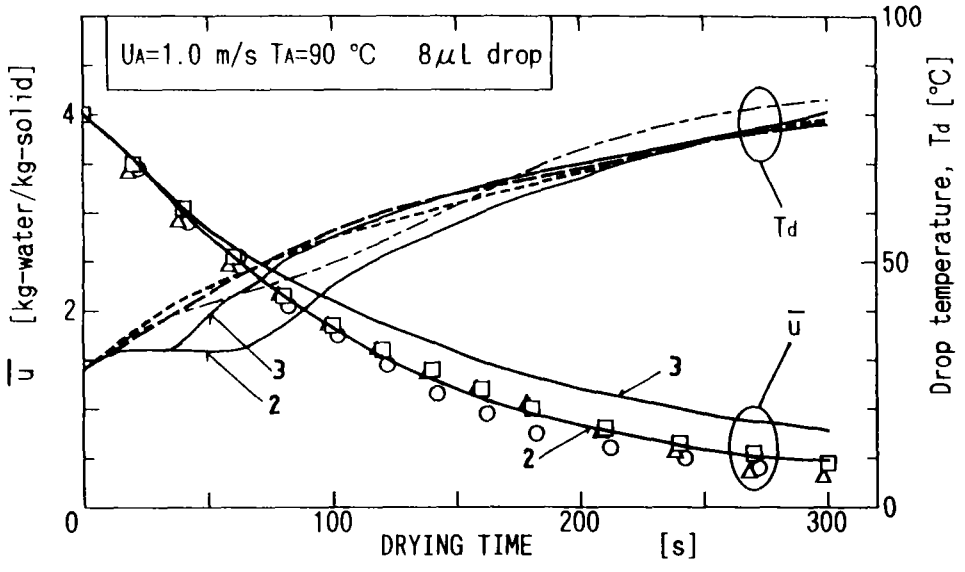

Figure 3: Average moisture content $\bar{u}$ and drop temperature T_d as a function of drying time during drying of a single droplet of various oligo- and poly-saccharides[8].
Key:○ and — - — for maltodextrin (DE=40), □ and — — — for maltodextrin (DE=11), △ and - - - - for Dextran T-70, 2 for sucrose(model calculation), 3 for maltodextrin of DE=11 (model calculation).

become highly stable at low water concentrations [1, 5, 7]. Therefore, a high enzyme retention is obtained when the water content of a drying specimen falls to very low levels before the temperature of the sample becomes high. Based on this consideration, we can expect from Figs. 2 and 3 that the enzyme retention becomes lower with an increase of molecular weight. The experimental results in Fig.4 support this expectation. However, the enzyme retention of very high molecular weight sugars was as high as that for smaller ones (data not shown). This high enzyme retention is due to the acceleration of the drying caused by the deformation mentioned above, since at the final stage of the drying both the temperature and water content histories of the drop were quite similar to those for small sugars. The initial enzyme activity was retained almost completely in the case of lactose, a substrate of β-galactosidase while the activity at t=300s for maltose drops was lower than that of sucrose and lactose. As the protecting power is different from solute to solute [7], it is still difficult to predict how a particular solute can stabilize a given protein. A sugar that has a high glass transition temperature and a very weak interaction with proteins may be a good stabilizer. Further research should be done toward this direction as hot air convective drying as a method for biopreservation will become more important in pharmaceutical as well as food industries.

6 Discussion

The drying behavior and the enzyme and the aroma retention behavior observed in this study can be schematically summarized in **Fig.5** [8]. Numerical calculation for predicting the aroma loss during drying requires multicomponent diffusion equations. Such diffusion coefficients are not readily obtained experimentally. Numerical calculation itself is also a hard task. We are now developing simple, still accurate methods for predicting the drying rate, and the aroma and the enzyme retention based on *short-cut calculation procedures*.

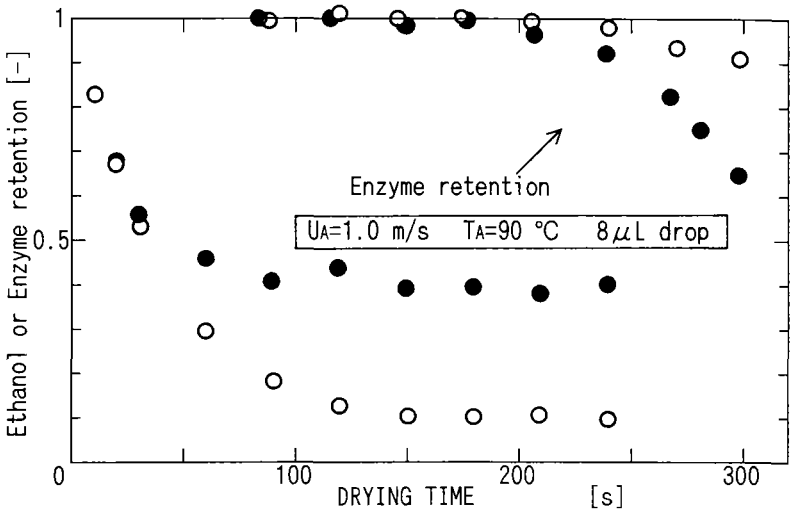

Figure 4: Ethanol and enzyme retention[8].The drying conditions are the same as in Figs. 2 and 3. The droplets of 20 wt% carbohydrate solutions (○=sucrose, ●=maltodextrin of DE=11) contained 1000 ppm ethanol for the ethanol retention measurement and 0.07 wt% β-galactosidase for the enzyme retention measurement.

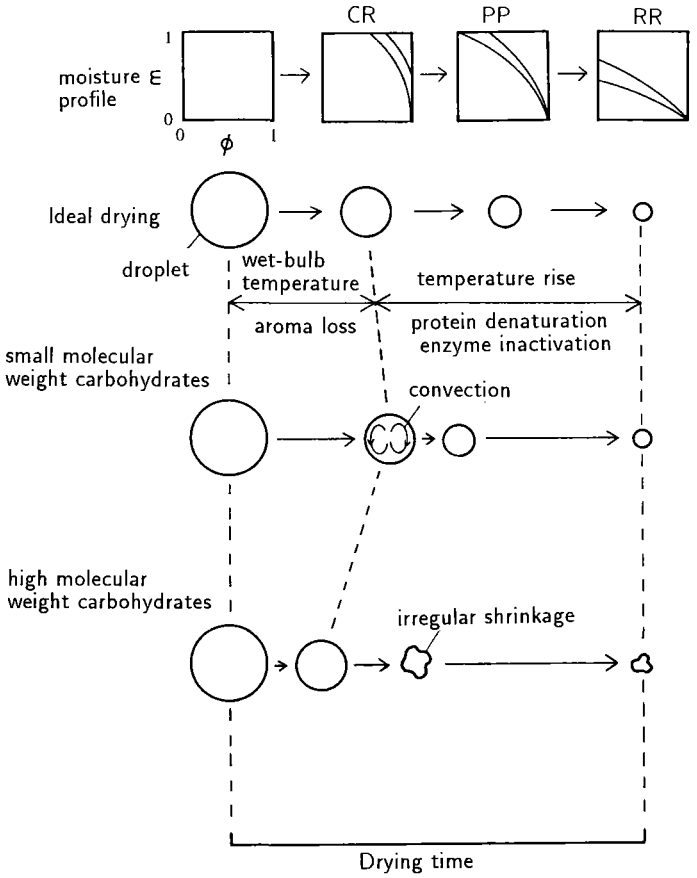

Figure 5: Schematical drawing of the drying behavior of a single droplet[8].
m=dimensionless moisture content, ϕ=dimensionless space coordinate. ϕ=1 means the droplet surface. CR=constant-rate, PP=penetration period, RR=regular regime [5, 1]

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Summary

Many physical and bio-chemical changes occur during drying of liquid foods such as coffee. Among them, aroma loss and enzyme inactivation(or protein denaturation) are most important subjects to be considered in food industries.

Our study showed that the aroma is lost at the beginning of the drying (the constant-drying rate period). After this period the enzyme is inactivated sharply. We also found that the aroma retention increases with an increase in the molecular weight of the dissolved solid, which is understandable by the selective diffusion theory, and that the protective power of the dissolved solid to the enzyme retention is different from solid to solid, which is not easily systematized.

The computer simulation program was developed to predict the aroma and the enzyme retention during drying. However, as the determination of the transport properties such as diffusivity needed in the simulation is difficult and time consuming, simple approximate prediction methods are required. In addition to the losses predicted by this model, there may be some additional losses in the actual drying processes, which must be considered carefully.

NEW DEVELOPMENT IN FOAMING AND FREEZING OF EXTRACT FOR FREEZE-DRIED COFFEE

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1. Introduction

Freeze-drying of coffee has proved to be a very successful method of achieving a high preservation of flavour, improve shelf stability and prepare the product in a way that gives the end user a product which is very convenient to use.

Over the years the customer demand for freeze-dried coffee of high quality with almost no variation in e.g. taste and colour has proved to be of growing importance.

For many years much attention has been paid to obtaining a colour (appearance) of the freeze-dried product which is identical with roasted and ground coffee. The work done has mainly been concentrated on single level freezing methods for the coffee extract and on freeze-drying of frozen granules. Many of the methods described in the literature are based on processes which are difficult to transfer to industrial production of freeze-dried coffee.

Control of colour can be obtained with melt-back of the coffee granules during the freeze-drying process. The melt-back of the frozen granules is established by a relatively high pressure in the drying chamber above the eutectic point for the product. This results in a darker appearance of the freeze-dried coffee. The method is rarely used in industrial production of freeze-dried coffee due to some loss of aroma. Nevertheless, the drying pressure can to some extent be used to adjust the colour of the product without the occurrence of partial melting.

It is known that the freezing process for the extract has a great influence on the colour of the freeze-dried product. Therefore, much attention has been paid to the freezing system, and it has been found that a slow freezing rate for the coffee extract results in a darker colour than a quick freezing rate. Freezing methods resulting in a darker colour of the freeze-dried product are given in Ref. 1 and 2. It is described that a dark colour is primarily related to a slow freezing of the coffee extract. Due to the slow freezing, the water in the coffee extract creates a pattern with large crystal structure, the orientation of the crystals is random. The crystal structure in a fast frozen coffee extract is highly ordered with parallel pores with a shape like needles. The random crystal structure results in the dark colour of the freeze-dried product whereas the parallel pores give a light appearance of the dried product. To obtain the long freezing time, a practical method is freezing on relatively long belts, this method is widely recog-

nized in the industry. The belt is normally divided into various zones where the temperature or the amount of cooling media and the speed of the belt can be adjusted. Working with these parameters it is possible to adjust the freezing time for the product and thereby influence the colour. Due to the relation between the freezing time and the capacity, this method has some limitations in practice - the freezing time must be kept within reasonable limits leading to a restriction in the obtainable darkness of the product.

With the described limitations in mind, a study of parameters which affect the colour of the freeze-dried product has been carried out to see whether it is possible to obtain a better control of the colour compared to the known processes described above. The impact on the colour in each of the processes freezing, foaming, and freeze-drying has been studied.

2. The freezing characteristics of coffee extract

Due to the composition of coffee extract, formation of ice crystals in the water takes place at a relatively large temperature interval. Formation of ice crystals depends on the temperature and the contents of solids in the coffee extract.

A coffee extract with 38% dry matter will not contain any ice crystals at -3°C, but at -5°C 20% of the water in the extract is transformed into ice crystals and at -8.5°C 50% of the water is ice crystals. A freezing curve is shown in figure No. 1.

It must be kept in mind that to some extent the shape of the ice crystals in the coffee extract will depend on the freezing time and the freezing method.

The viscosity of the coffee extract increases as the temperature drops and the amount of ice crystals is growing. The extract is liquid and pumpable until approximately 40% of the water has been transformed into ice crystals. This behaviour gives the possibility of applying different freezing methods at different freezing levels, i.e. multi level freezing instead of single level freezing.

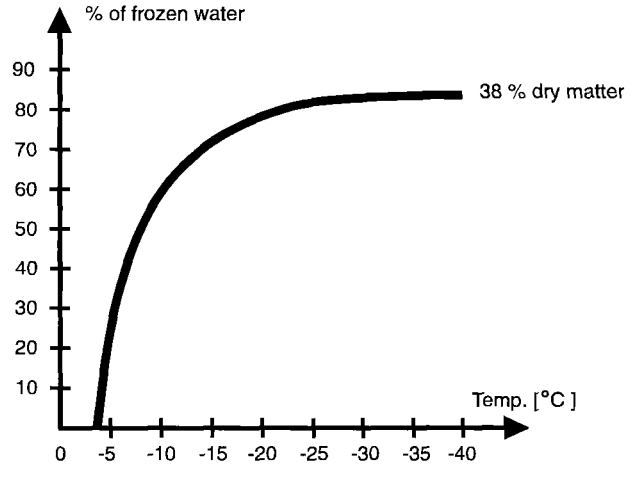

Figure No. 1 Freezing curve for coffee extract with 38% dry matter

3. The process

The freezing characteristics of coffee extract have been the main inspiration for the development of the process for freezing and foaming of coffee extract. The different levels of the process as they have been during the tests are described below.

Fresh coffee extract is supplied to the process. The coffee extract is cooled to a temperature level where formation of ice crystals in the water will begin. At this point foaming of the product takes place. The distribution of the foaming gas in the coffee extract is very uniform because no ice is present in the product at this stage. Due to the cooling of the coffee extract, the viscosity of the product has increased. The high viscosity gives a stable product where the injected gas is captured.

After the foaming process the coffee extract is frozen in two processes. In the first freezing process (prefreezing) the product is in the liquid phase, in the second freezing process the product transforms from the liquid into the solid phase.

In the liquid phase the product is cooled to a temperature where approximately 30% of the water in the product is frozen. The cooling and freezing of the coffee extract in the liquid phase take place in scraped surface heat exchangers, with this freezing method it is possible to keep the product under agitation during the freezing process.

The product is then pumped to a freezing belt where the transformation into the solid phase takes place. To avoid instant cooling of the product causing a light colour on the slab surface, the temperature of the freezing belt in the loading area is adjusted to the same level as the temperature of the coffee extract. Transfer of cooling energy is achieved with cold air throughout the belt freezing.

After granulation and sieving the granules are loaded on trays and freeze-dried. The pressure during the freeze-drying is kept at a constant level without fluctuations where no partial melting of the granules occurs.

4. Materials and methods

The tests were carried out at Atlas Industries A/S' laboratory using the equipment described below.

Coffee extract

All the tests were performed with industrially produced Colombian coffee extract, Arabica blend with a dry matter content of 38%.

Cooling and prefreezing

To cool and prefreeze the coffee extract, a scraped surface heat exchanger of the type Gerstenberg & Agger was used.

Foaming

Foaming of the coffee extract was performed in a foaming device developed by Atlas Industries A/S. The foaming device injects the foaming gas directly into the coffee extract through nozzles which control the bubble size of the foaming gas in the coffee extract. Nitrogen (N_2) was used as foaming gas.

Freezing

The freezing was performed in a pilot belt freezing system with air as cooling media. The layer thickness of the slab was 6 mm. A typical freezing profile for the blast freezing system is shown in figure No. 2.

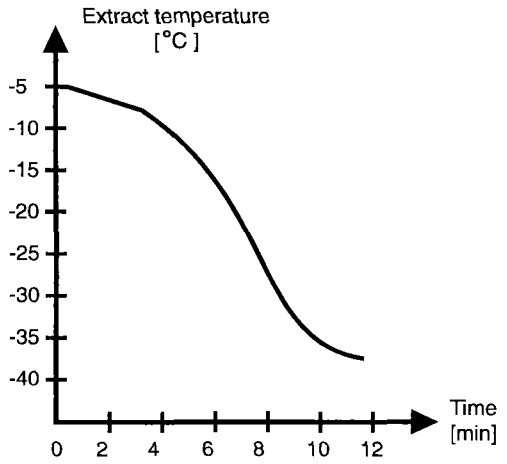

Figure No. 2: Typical freezing profile for freezing of coffee extract with 38 % dry matter.

Granulation and sieving

The frozen slabs were granulated in an Atlas Industries A/S laboratory granulator type H-300 equipped with a 4 mm screen. The granules were sieved on a 2.5 / 0.84 mm sieve.

Freeze-drying

The frozen granules were freeze-dried in an Atlas Industries A/S pilot freeze-drier type RAY 1.

The layer thickness was 17 mm, the drying pressure 0.4 mbara. The drying time was 6.7 hours and the maximum product temperature was 60°C.

Analysis of bulk density

The dried product was analysed for bulk density in accordance with ISO 8460.

Analysis of colour

The colour of the dried product was measured on a Dr. Lange Reflection meter of the type LK 100.

5. Discussion

The influence on the colour in the freezing, foaming and drying processes used for producing freeze-dried coffee has been evaluated. It has been found that the colour of the dried product can be described in the function:

$$\text{Colour} = f(FT_{lp}, A_{lp}, Fo, FT_{sp}, MT, P_{FD})$$

where

FT_{lp}: Freezing temperature in the liquid phase
A_{lp}: Agitation of the coffee extract in the liquid phase
Fo: Foaming degree
FT_{sp}: Freezing time in the solid phase
MT: Maturing time of the prefrozen coffee extract (liquid phase)
P_{FD}: Freeze-drying pressure

The parameter having the most significant impact on the colour is the combination of the freezing temperature and the agitation when the coffee extract is in the liquid phase. Compared to the other parameters the effect is tremendous. A change of the freezing temperature in the liquid phase of only 0.16°C will lead to the same change in colour of the freeze-dried product as if the freezing time in the solid phase is altered 4 minutes. Maturing of the prefrozen extract for 1 hour results in the same darkening of the freeze-dried product as a temperature rise of 0.16°C. The agitation of the product has the advantage of increased transfer of energy per cooling area and a more uniform temperature throughout the product - supercooling along the cooling surface is avoided and thereby the formation of many small ice crystals and small gas bubbles. With correct agitation the ice crystals can be relatively large leading to a dark product.

The degree of foaming also has a certain but not as significant impact on the colour of the coffee extract. Further, this parameter is limited due to the demand for a fixed bulk density of the freeze-dried coffee.

The influence of the freeze-drying pressure on the colour is limited by two factors, melt-back of the frozen granules which will result in loss of the aroma and a practical obtainable minimum pressure. The closer the pressure is to the maximum pressure, the darker the product will get. In practice, the pressure is kept below the maximum pressure due to the risk of melt-back. This gives a very limited area of variation for the pressure, 0.2 to 0.3 mbar absolute pressure. Compared to the temperature/agitation in the liquid phase a change of the pressure of 0.05 mbara will give the same change in colour as the earlier mentioned 0.16°C. It is important to remember that the temperature can be adjusted within 2-3 degrees Celsius.

As colour and bulk density must be regarded as two of the most important parameters for the freeze-dried coffee, and as the parameters are linked together via the level of foaming, a study has been made on the total area of variations possible for the two parameters. As can be seen in figure No. 3 it is possible to obtain a relatively larger variation of the colour at a low bulk density compared to the high bulk density. The figure shows that the process gives wide possibilities of colour and bulk density.

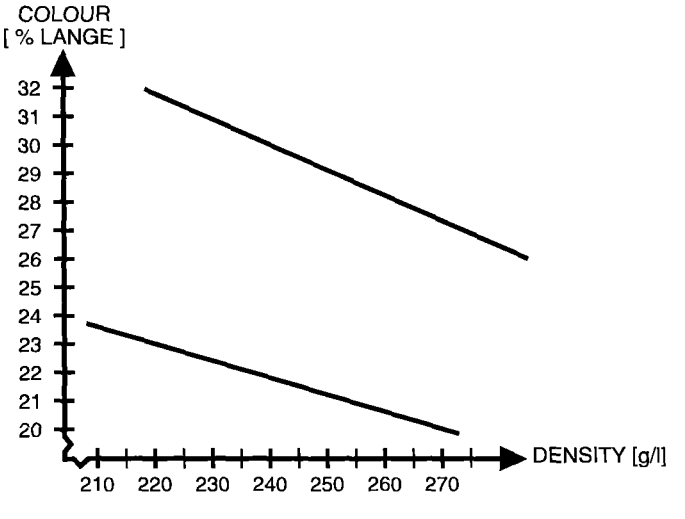

Figure No. 3: Area of variation for colour and bulk density, freeze-dried coffee with 38% dry matter.

6. Conclusion

Freeze-dried coffee has to fulfil a range of quality parameters. Colour and bulk density are some of the vital ones. The study has been concentrated on design of a foaming and freezing process where it is possible to obtain a high span in the colour and the bulk density of the freeze-dried product.

Splitting up the freezing process in two processes, one process for the liquid phase (prefreezing) and a different process for the final freezing, where the coffee extract is frozen to the solid phase, gives a wide range for control of the colour.

The relation between the freezing temperature and the agitation in the liquid phase has been found to have the most significant impact on the colour of the freeze-dried product.

The combination of freezing and agitation in the liquid freezing process contributes to a uniform temperature distribution in the product during the process. Supercooling of the product along the cooling surface is avoided and thereby the formation of many small ice crystals. The ice crystals created are relatively large resulting in a dark product.

The freeze-drying pressure, maturing time for the prefrozen extract and freezing time in the solid phase have been found to be of minor importance in order to control the colour of the freeze-dried product.

Other types of extract than the one used during the tests have shown the same relations regarding colour and bulk density.

7.1. Summary

The importance of techniques which give a colour (appearance) of the freeze-dried coffee almost identical with roasted and ground coffee has been increased as the customer demand for high quality freeze-dried coffee has been growing.

With the basis in the freezing characteristics of coffee extract a process for freezing and foaming of coffee extract has been developed which, compared to methods used hitherto, gives a bigger area of variation for colour and bulk density of the freeze-dried product.

The freezing process described consists of two levels. In the first level the coffee extract is frozen in the liquid phase, in the second level the product is transformed from the liquid into the solid phase.

The relation between the freezing temperature and the agitation during the freezing in the liquid phase has been found to have the most significant impact on the colour of the freeze-dried product.

7.2 Résumé

L'importance des techniques donnant une couleur (apparence) au café lyophilisé presque identique à celle du café torréfié et moulu s'est augmentée avec la demande croissante des clients pour du café lyophilisé de haute qualité.

Un nouveau processus, par lequel du nitrogène est ajouté à l'extrait de café qui est ensuite congelé, a été développé sur la base des caractéristiques de congélation de l'extrait de café. Par comparaison avec les méthodes antérieures, le nouveau processus offre une gamme plus étendue de variations de couleurs et de masses volumiques du produit lyophilisé.

Le processus de congélation décrit s'effectue dans deux étapes. Premièrement, l'extrait de café est congelé dans la phase liquide, ensuite le produit est transformé de liquide en solide.

Il a été constaté que le rapport entre la température de congélation et l'agitation pendant le temps de congélation dans la phase liquide a l'influence la plus significative sur la couleur du produit lyophilisé.

8. List of references

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EFFECTS OF EXTRACT PRETREATMENT VIA MICROFILTRATION ON THE CONCENTRATION OF COFFEE EXTRACT VIA REVERSE OSMOSIS

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Introduction

Concentration is a technique which is widely used in food industry to increase the stability of liquid food products. In the instant coffee process, concentration is used not only to reduce water activity and increase stability but also to improve volatile retention across the subsequent drying process.

There are several commercial techniques used for concentration. Concentration by heat, freeze concentration and membrane concentration are well known. Membranes have been mostly applied in the dairy industry because temperature can be kept low and energy cost can be minimized [1,2,3,4,5,6]. When reverse osmosis membranes are applied to coffee concentration, a problem in operation has been observed. The flux of the permeate is reduced after a short period of time.(Fig. 1)

Similar problems have been reported for other applications of membranes such as UF (Ultra Filtration) and RO (Reverse Osmosis) to food and beverage products. The primary cause is reported to be the accumulation of large molecules on the surface of the membranes [7,8,9,10]. Therefore, it is assumed that the problem is similar for coffee; that is, a gel layer of large molecules of the soluble solids in the coffee extract deposits on the RO membrane. This gel layer would be difficult to remove by common cleaning methods and would cause fouling.

In this study, reduction of the levels of high molecular weight material in coffee extract using MF (Micro Filtration) or UF prior to RO membrane concentration was investigated as a mean of improving the efficiency of RO concentration. The characteristics of concentrating MF-pretreated coffee extract by RO was also analyzed with a concentration polarization model.

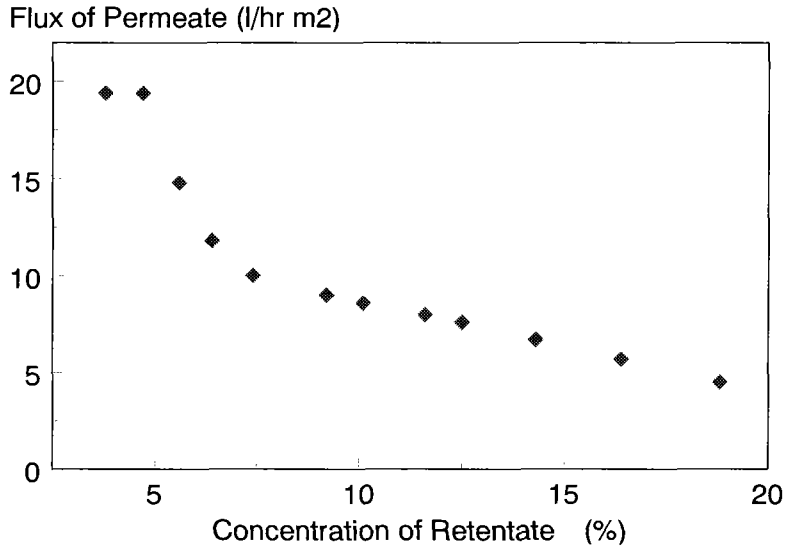

Fig.1 Change of Flux of Permeate in RO Concentration of Non-Pretreated Coffee Extract

Materials and Methods

1. Coffee extract

Coffee extract having approx. 4% soluble solids concentration (as is basis) extracted from roasted Arabica coffee beans was fed to RO concentration system.

2. Measurement of molecular weight distribution of coffee solids

Distribution of the molecular weight of soluble solids was measured by a High Speed Gel Permeation Chromatograph (GPC). The column was TSK gel GMPW, 7.8 mm ID x 300mm L and the eluent was water with 0.6ml/min. flow rate. A refractive index (RI) detector was used. The coffee extract was diluted with water to 2% concentration and 40μl was injected onto the GPC column.

3. Pretreatment of coffee extract

Ultrafilter (UF) having 20,000 MW cut-off and Ceramic Micorfilter (MF) membranes having 0.8 and 1.8 μm pore sizes and 0.2 m^2 surface area (manufactured by Ceraver) were used to pre-treat coffee extract under 4kg/cm^2 pressure. The observed soluble solids rejection (Robs) was calculated by the following equation with measured concentrations of the feed and the permeate.

$$Robs=(Cb-Cp)/Cbx100 \qquad (1)$$

Where Cb: soluble solids concentration of feed extract (%) and Cp: soluble solids concentration of permeate (%)

4. Reverse osmosis experiments

A plate and frame type RO membrane type HR98 manufactured by DDS having 0.325m^2 surface area was used for the concentration of coffee extracts using 25 kg/cm^2 pressure.

Results and Discussions

1. Change of molecular weight distribution across RO concentration

Figure 2 shows a chromatogram of GPC analysis for the RO feed extract. A 5,000 dalton standard, consisting of α-1,6 polymers of maltotriose (Showa-Denko), was used to calibrate the GPC. A retention time of 30 minutes was observed for the standard. Large molecule carbohydrates in coffee solids are different from the standard, consisting of polymers of mannose, glucose, galactose and arabinose. Therefore, the exact molecular weight of the carbohydrates in coffee solids could not be determined by

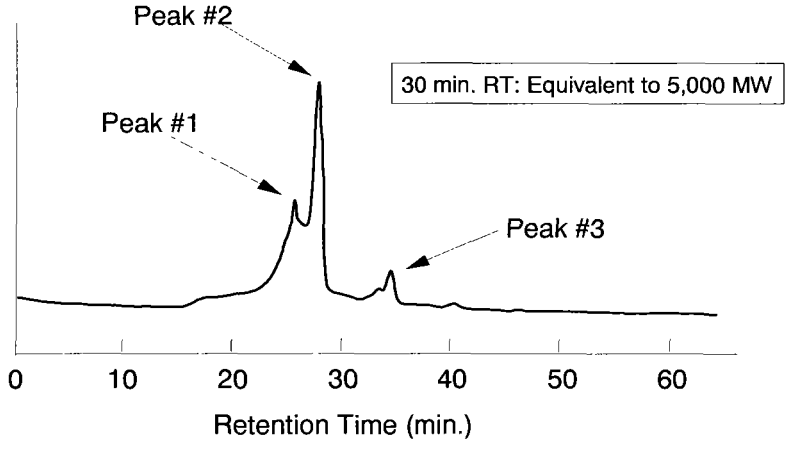

Fig. 2 Gel Permeation Chromatograph for RO Feed Coffee Extract (4% Conc.)

the retention times of the peaks on the chromatograms. However, a change in the distribution was observed across the RO concentration process, since larger molecules elute faster than smaller molecules based on the principle of GPC.

For the RO feed extract, the ratio of the heights of the first peak (#1) and the last peak (#3) on the chromatogram was 3.13; This ratio fell to 2.29 for the RO retentate, as shown in Table 1. Although the compounds corresponding to the peaks were not identified, it was obvious that the proportion of larger

molecules in the coffee solids was reduced across the RO concentration process. The proportion of heights of the second (#2) and the last peak (#3) also changed across the process. The concentration of coffee solids in the permeate was zero (0) % and the rejection of solids was 100%.

Table 1 Ratio of Peak Heights of High MW and Low MW

Peaks for Calculation	RO Feed	RO Retentate
#1/#3	3.13	2.29
#2/#3	6.82	5.11

It was therefore assumed that there was an accumulation and precipitation of larger molecules on the surface of the membrane. This increases the resistance of permeation and causes the decrease of flux in RO concentration.

2. Pretreatment of coffee extract by MF and UF membranes

The result of the GPC analysis suggested that reduction or removal of large molecules in coffee extracts should be effective for increasing the flux.

Based on bench-top screening of MF for pre-treatment of coffee extracts prior to RO concentration, a Ceramic MF having 0.8 μm pore size and UF having 20,000 MW cut-off were evaluated for the

Table 2 Solid Rejection and Flavor Change across MF and UF for Pretreatment of Coffee Extract

	Ceramic Microfilter (MF)	Ultra Filter (UF)
	Pore Size (μm)	Rejection MW
	0.8	20,000
Concentration (%)		
Feed	3.9	4.3
Permeate	3.2	2.8
Solid Rejection(%)	19.0	36.0
Average Flux (l/hr m2)	40	11
Change of Flavor	Similar to Feed	Weak in Cup Strength

pretreatment in a pilot plant scale. Expert flavor panelists organoleptically evaluated the permeates of those membranes. The permeate of the MF showed little difference from the feed extract, while the permeate of the UF showed weaker cup strength and weaker bitterness. (Table 2)

For the permeate of the MF membrane having 0.8 μm pore size, the ratio of the heights of the first (#1) and the last (#3) peaks of the GPC chromatograms was 2.06, as shown in Table 3. The ratio was smaller than that of non-treated coffee extract fed to RO (3.13) and was similar to that of RO retentate (2.29) with non-treated extract. This indicated that the large molecules in coffee extract, which were assumed to accumulate in the RO system, could be reduced by the MF membrane pre-treatment. This suggested that the pretreated extract by the MF could improve the efficiency of RO concentration. The solids rejection with the MF membrane having 0.8 μm pore size was 19%, which was much less than that of the UF membrane having 20,000 MW cut-off (36%), shown in Table 2. Solids rejection represents yield loss for this treatment and it would be preferable to minimize the solids loss and yet reject as much high molecular weight material as possible for the subsequent RO concentration process. From those evaluation, the ceramic MF membrane having 0.8 μm pore size was satisfactory for pretreatment in the RO concentration process for improving its productivity.

Table 3 Ratio of Peak Heights of Peak #1 and Peak #3 with GPC for MF Pretreated Coffee

	RO Feed		Permeate of Ceramic MF (0.8μm)		RO Retentate
#1/#3	3.13	>	2.06	≈	2.29

3. RO concentration of the permeate from ceramic MF

As described in the last section, the ceramic MF membrane having 0.8 μm pore size could reduce the level of large molecules and did not make any change in coffee flavor. The characteristics of RO concentration of the permeate from the MF membrane was therefore investigated. As a reference, non-treated coffee extract (feed for MF) was diluted with water to prepare the same concentration extract for RO studies as the MF permeate.

a) Change of the flux

The relationship between the concentration of retentates and the flux of permeate in RO concentration is shown in Fig.3. The initial fluxes for the MF permeate and the non-treated extract (reference) were almost identical. However, for the non-treated extract, the flux rapidly decreased as the concentration of the retentate increased. When the concentration of retentate was more than 10%, the flux for the non-treated extract leveled off. The flux declined again at retentate concentrations exceeding 15%. On the other hand, much higher fluxes were observed for the MF permeate, although the flux decreased as the concentration increased.

The flux of permeate, Jv, is described by the following equation (2) when the concentration polarization model is considered to apply[11,12,13,14]:

$$Jv=kln\{(Cm-Cp)/(Cb-Cp)\} \quad (2)$$

where Jv: flux (l/m^2 hr), Cm:concentration of solids on the surface of membrane (% solid), Cb:concentration of solids in retentate (% solid), Cp:concentration of solids in permeate (% solid), k:permeation factor (l/m^2 hr).

The solids rejection across the RO system was 100%, since the solids concentration in the permeates was zero (0). That is, Cp =0 in Eq.(2) and thus Eq.(2) can be transformed to the following Eq.(3):

$$Jv=kln(Cm/Cb) \quad (3)$$

As shown in Fig.3, the flux of RO permeate for the MF treated extract decreased linearly as the log of the concentration of retentate increased. The flux for the non-treated extract fell sharply to approximately 5% concentration of retentate. The following regression equation described the relationship between the concentration of the retentate and the flux of the permeate for the MF treated extract:

$$Jv=17.3\ ln(24.5/Cb) \quad (4)$$

The equation is of the form of the concentration polarization model, Eq.(3), and correlates well with the decrease of flux observed for the concentration of MF treated extract. When the equation is extrapolated to Jv=0, Cb=24.5% is obtained. This means that the concentration of the polarization layer on the surface of the membrane was 24.5% and the concentration represented the theoretical maximum concentration with the operating conditions employed.

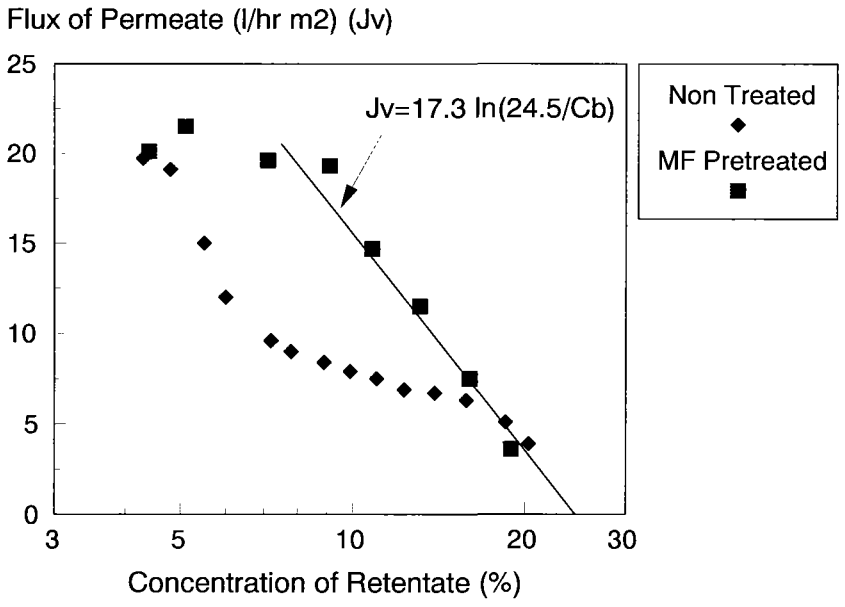

Fig. 3 Change of Flux in RO Concentration for MF Pretreated Extract

b) Rate of concentration increase

Figure 4 shows the relationship between processing time and solids concentration in the retentate for both non-treated coffee extract and MF treated extract. It took only 120 minutes to concentrate MF treated extract (initial concentration: 4%) to 15% concentration, while it took 180 minutes for non-treated coffee extract to reach the same concentration. It can therefore be concluded that the productivity of RO concentration of coffee extracts can be improved by 50% when MF membranes are applied to the pretreatment of the coffee extract.

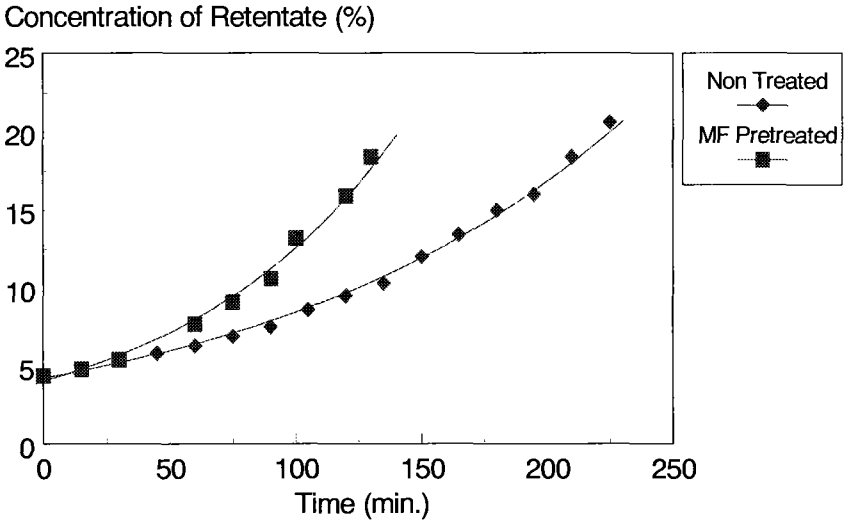

Fig. 4 Increase of Concentration of RO Retentate for Non-Pretreated and MF Pretreated Coffee

Summary

When a Reverse Osmosis (RO) is applied to concentration of coffee extracts, a sharp decrease of flux of the permeate is observed. This results in taking too much time to concentrate the extract and obstructs application of RO to concentration of coffee extracts. Molecular weight distributions of feed and concentrated coffee extracts via RO were measured with Gel Permeation Chromatograph (GPC), and the analysis indicated that the decrease of the flux was attributed to an accumulation of large molecules on the RO membrane. Filtration of coffee extract through a Ceramic Microfilter (MF) having 0.8 μm pore size did not affect flavor characteristics of the coffee extarct. This pretreatment reduced as much large molecules in coffee extarct as accumulating on the RO membrane with non-treated extract. As a result, the pretreatment avoided the sharp decrease of the flux of permeate in RO concentration. The change of the flux was well described by a concentration polarization model and this suggested little gel layer was formed on the RO membrane when the pretreatment was applied. The pretreatment

of coffee extract via MF, thus, significantly improved the efficiency in the concentration of coffee extarcts via RO.

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APPLICATION OF FLAVOR SENSOR TO COFFEE

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1. Introduction

It is said that the technical innovations in future depend on the development of novel sensors. In these days, even house-hold electrical appliances involve high performance sensors which have been employed exclusively in specialized fields.

On the other hand, it is required to create a pleasant space for the amenity of life and sensors for various sensitivities have been studies for this purpose.

Although sensors for sensitivities should be equivalent for physical functions, it is highly difficult to objectively evaluate, in particular, "odors". Thus studies in this field falls behind others at present.

A number of workers have proposed methods for classifying odors for a long time and, in its turn, it is finally required to establish fundamental criteria for evaluating odors, i.e., those similar to the three primary colors and five primary tastes. Although Henning, Amoore et al. suggested primary odors, these criteria are not applicable to anyone and the number of the standards and the expressions vary over a wide range.

Anyway, there are a number of fundamental odors and countless combinations of them, which makes the development of odor sensors difficult.

Under these circumstances, we have developed a system for analyzing an odor (a flavor sensor) equivalent for physical functions with the use of metal oxide semiconductors. Man recognizes an odor based on its qualities and strength. This system aims at visualizing and numerically indicating this recognition. Thus it has been proved that an odor can be objectively judged in "pleasantness/unpleasantness". Then attempt is made to apply this system to the analysis of coffee having a highly complicated flavor constitution.

There have been reported a number of findings on the flavor of coffee with the progress in analytical instruments. It is considered that flavor components of coffee amount 600 and it is also said that a number of components still remain unidentified. However no component which can be regarded as "the essence of coffee" has been found out from among these components, which

suggests the complicate constitution of the coffee aroma.

It is an object of this study to objectively judge the coffee aroma by analyzing coffee having a complicated flavor by the flavor sensor and examining the qualitative and quantitative tendency of the coffee aroma based on the information obtained from the outputs of the sensor. It is another object of this study to discuss the availability of this system as a new indication for the evaluation of coffee qualities.

2. Materials and Methods

2.1 *Metal oxide semiconductor sensors*

Gaseous components of odors include oxidizing gases and reducing gases. In the daily life under common conditions, reducing gases exist in general.

The principle of a semiconductor sensor resides in that when a reducing odor molecule comes in contact with a N-type sensor, the electron density of the semiconductor is elevated and its electrical resistance is thus lowered. Examples of the N-type semiconductors employed at present include ZnO, SnO_2, Fe_2O_3 and TiO_2. These sensors are frequently used in a heated state (about 300℃) and therefore scarcely affected by humidity, different from those which are operated at room temperature. Semiconductor sensors are classified into sintered type sensors and thin-film type sensors prepared by, for example, metallizing. Thus it is possible to establish the sensitivity characteristics for various odors by altering the surface structure of a sensor. Namely, a sintered sensor has a porous structure and an extremely large surface area. Thus geometrically small-sized molecules and volatile molecules with a low boiling point penetrate into the inside of the sensor to thereby cause changes in the electrical characteristics of the sensor at a high sensitivity. On the other hand, large-sized molecules cannot enter the inside of the sensor and thus scarcely cause any change in the electrical resistance. In contrast, the adsorption efficiency of a thin-film type sensor is not affected by the molecular size. From the viewpoint of proportion, it captures larger molecules than a sintered type sensor does and, therefore, is usable as a sensor having different gas characteristics. That is to say, a sintered type sensor is highly sensitive to light odors, while a thin-film type sensor is highly sensitive to heavy ones. Accordingly, it is possible to separately capture various odors with the use of these sensors (Fig. 1).

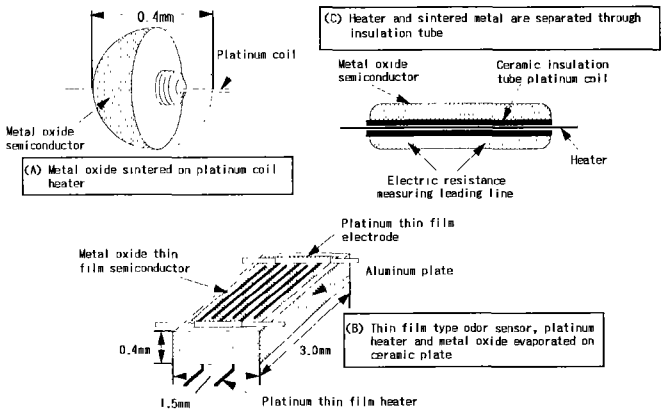

Fig. 1 Various types of metal oxide semiconductor odor sensors

In this system, 6 metal oxides differing in gas characteristics from each other are used as sensors. This system is completed by forming sintered SnO_2 and ZnO and thin-film type sensors by taking advantage of the above-described characteristics thereof and integrally uniting 6 sensors which are sensitive to, in particular, hydrogen sulfide, ammonia, aromatic compounds, hydrocarbons, alcohols and general odors.

2.2 Summary of flavor sensor system and measurement procedure

This system is summarized in Fig. 2. The measurement was per formed in the following manner. Before the measurement, it was confirmed that each sensor had been stabilized at 3 ± 1 mV. Then a sample was inserted into a chamber. The aroma components vaporized in the chamber were captured by 6 sensors located above.

Then the electrical signal of each sensor was amplified by an amplifier and transmitted to a computer via a data collector. In the computer, the signal was indicated on CRT and the diffusion of the aroma components in the chamber was numerically confirmed by real-time processing (the abscissa: time) (Fig. 3). By dividing the time-serial data with time axis, the output signal from each sensor at a definite measurement time can be observed in the form of a radarchart (a hexagon graph). Also, the area of this radar chart (integrated value: S) can be calculated. S is used as a parameter of the flavor strength. All of these data can be printed out by a plotter.

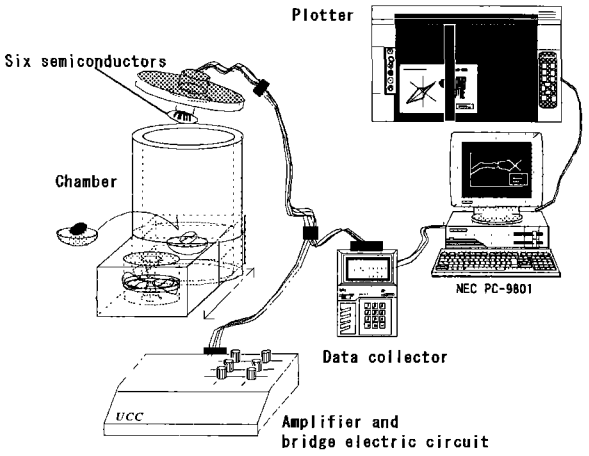

Fig. 2 Diagram of the sensor system

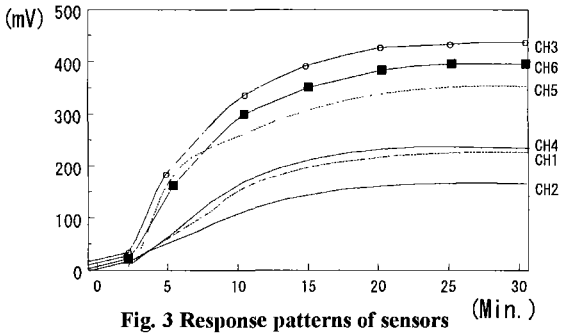

Fig. 3 Response patterns of sensors

2.3 Preparation of sample

The coffee sample was prepared in the following manner. Green coffee beans were roasted with a test roaster (manufactured by Probat). Immediately after the completion of the roasting, the beans were ground (finely grinding: Bomac Coffee Cutter BM570) to thereby give the sample.

2.4 Instrumental analysis

For using as the indicator of the qualities of the sample and comparing with the responses from the sensors, the roasting level of the test sample was examined and headspace analysis was performed by gas chromatography.

If necessary, the test sample was also subjected to GC-MS (gas chromatography coupled to mass spectrometry).

The roasting level was determined as a color meter value with the use of a color meter COROLLETE 3 (manufactured by Probat).

In the headspace analysis by GC, GC-14A (detector: FID), HSS-2B (manufactured by Shimadzu) was used.

As the column, a capillary column (DB-WAX, 30 m x 0.25 mm, film thickness, manufactured by J&W Scientific) was used. The column oven was maintained at 40℃ for 5 minutes and then the temperature was elevated to 200℃ at a rate of 5℃/min.

The GC-MS analysis was effected by using a GC-MS system manufactured by Perkin Elmer. The column and the heating conditions of the oven were the same as those employed in the above case of GC.

3. Results and Discussion

3.1 Confirmation of reproducibility in the measurement

To confirm the reproducibility of the sensor output, a known substance was employed as a sample and analyzed repeatedly. That is to say, an artificial coffee flavor was employed as the sample, 50 μ l of the sample was injected into a glass vial and analyzed by the flavor sensor as such. The measurement was effected in a clean booth controlled at a temperature of 25±2℃ under a relative humidity of 25±5%. The results are given in Table 1 As Table 1 clearly shows, almost the same result was obtained every time, which indicates that this system has a high reproducibility.

Table 1 Basic statistics of sensor's outputs

	Ch.1(mV)	Ch.2(mV)	Ch.3(mV)	Ch.4(mV)	Ch.5(mV)	Ch.6(mV)
Rep.1	161.5	66.0	237.8	65.0	166.3	265.1
Rep.2	152.3	70.0	236.0	70.0	167.0	275.8
Rep.3	178.5	73.0	265.3	79.8	172.5	286.5
Rep.4	182.5	79.0	270.6	78.5	182.5	300.2
Rep.5	163.1	83.2	280.6	71.5	165.2	290.0
Rep.6	156.3	79.5	243.5	70.9	189.4	252.0
Mean (mV)	165.7	75.1	255.6	72.6	173.8	278.3
S.D.*	11.1	6.0	17.3	5.1	9.1	16.1
R.S.D.(%)**	6.7	7.9	6.8	7.0	5.2	5.8

* Standard deviations

** Relative standard deviations

3.2 Responses of sensor to various samples

When various odors were analyze, the sensor showed a particular tendency. Fig. 5 shows a part of these results wherein the sensor outputs are expressed in radar charts. The odors of ammonia, iso- valeric acid and *Natto* are regarded as unpleasant odors, while the odors of lavender, lemon and rose are regarded as pleasant ones. Then it has been confirmed that each of the pleasant odors shows a pattern like a fountain pen tip with extension of the output along the channels 3 and 6. On the other hand, the unpleasant odors show each a largely deformed pattern. Although the feeling of pleasantness/unpleasantness widely varies from person to person and cannot be easily generalized, the same tendency was observed in the analyses of other samples. It is therefore expected that the degree of pleasantness may be determined by regarding the pen tip-like pattern of the chart of a pleasant odor as a standard and numerically expressing the deviation therefrom.

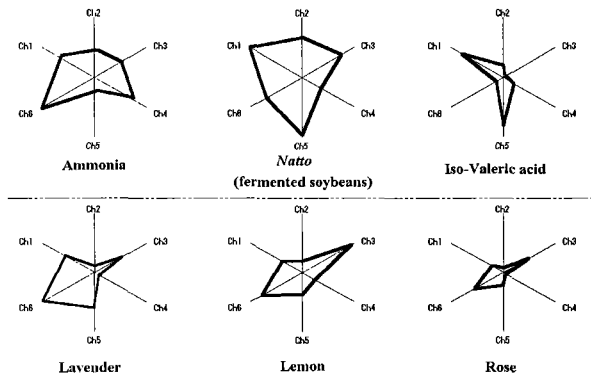

Fig. 4 Response patterns for various samples

3.3 Sensor responses to coffee

To study the differences depending on the origin in the sensor responses and sensor outputs of coffee, fresh-roasted samples of various origins were ground and analyzed by the sensor. The roasting levels of these samples were adjusted to 100 to 110 (measured by the color meter), i.e., from 20.9 to 22.4 in terms of L value. The results are given in Fig. 5. As Fig. 5 clearly shows, coffee of every origin shows a radar chart of pen-tip shape. Based on the results described above, it is concluded that the odors of these coffee samples are regarded as "pleasant" by the sensor. Regarding the difference in sensor outputs depending on the origins, the difference from Arabica to Robusta can be understood to a certain extent from the radar charts. However samples of the same species [for example, Brazil Santos (No. 2) and El Salvador (CS)] were similar to each other in the sensor response and hardly distinguishable based on the radar charts. Thus these output data were statistically analyzed.

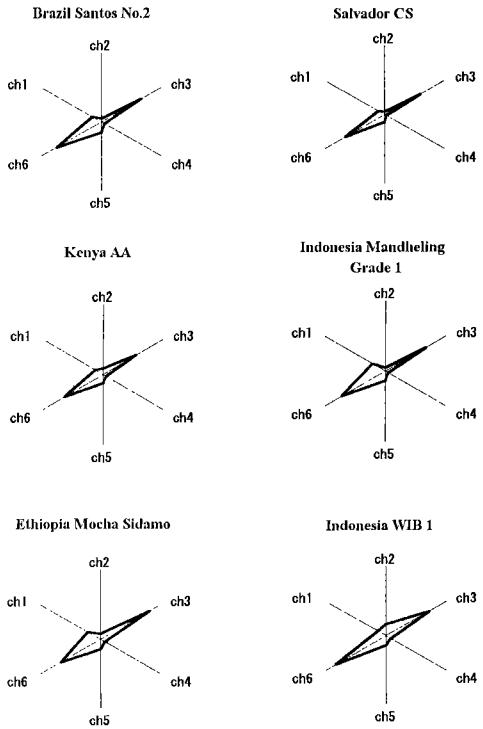

Fig. 5 Response patterns for coffees

Based on the experimental data obtained above, the principal components were analyzed. The term "principal component analysis" means a method of multiple linear regression by which data of higher orders are reduced into those of lower orders while minimizing the loss of information. That is to say, the data are converted into a group of variables called "principal components" which do not correlate to each other but correspond to linear linkages of original variables. The data groups are taken out in the order of importance as, for example, the principal component 1 (PC1), the principal component 2 (PC2) and so on and thus the original variables can be mostly illustrated by a smaller number of variables.

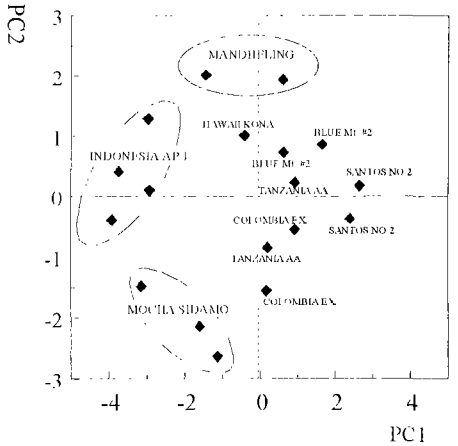

Fig. 6 The principal component analysis of sensor's outputs to coffees

Figs. 6 show the results in graphs. To avoid the influence of the absolute amount, the ratio of the output of each channel to the sum of the outputs of all sensors was subjected to the analysis as the original variable. The contribution ratios of the inherent values of the principal component 1 (PC1), the principal component 2 (PC2) and the principal component 3 (PC3) were respectively 57.6%, 31.9% and 8.1%. The principal components 1 to 3 showed relatively large values, which indicates that the flavor sensor outputs provides

three-dimensional information of coffee. It is also suggested that the flavor sensor can distinguish coffee flavors, though it cannot be concluded in general since variation in qualities in coffees of the same origin would affect largely.

Next, the sensor outputs were subjected to cluster analysis. The term "cluster analysis" means a method of multiple linear regression by which the distance between samples is calculated from the variables and thus samples are classified into some groups. Fig. 7 shows the results. First, large clusters were formed by Arabica and Robusta species. From the cluster of the Arabica species, Mandheling separated out followed by Colombia Excelso and Mocha Sidamo. It is considered that the formation of these clusters agree with the sensory evaluation by man to a certain extent. That is to say, it seems that this sensor can express the qualities of coffee flavor.

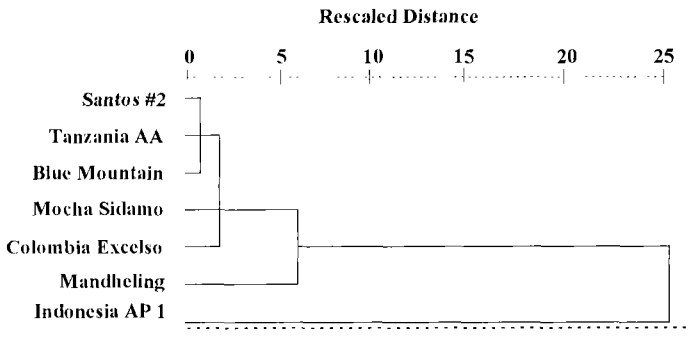

Fig. 7 Results of cluster analysis of sensor's outputs for coffees

3.4 Comparison of instrumental analysis with sensor responses

To develop a sensor, it is important to understand the direction characteristics of the sensor. Thus the sensor outputs were compared with the result of instrumental analysis and discussed. Simultaneously with the analysis by the flavor sensor, samples were subjected to headspace GC and the measurement with the color meter and the results were compared with each other. As the sensor output, the PC1 obtained by the principal component analysis and the radar chart area (integrated value) were employed as indications.

Fig. 8 shows the result of the comparison between the sensor output and the roasting level measured by the color meter. As Fig. 8 clearly shows, the sensor output highly correlated to the color meter data ($R^2 = 0.8026762$). This fact indicates that this sensor found out the characteristic flavor corresponding to each roasting level and expressed it in a fuzzy manner. On the contrary, the sensor response largely depended on the roasting level. It is therefore considered that the roasting level should be strictly regulated in order to examine the flavor tendency depending on the origin.

Subsequently, Fig. 9 shows the result of the comparison between the sensor output (integrated value) and the headspace GC output (total peak area). As Fig. 9 clearly shows, the sensor output highly correlated to the GC output ($R^2 = 0.91219$).

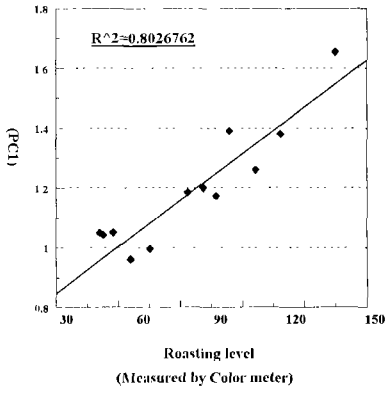

Fig. 8 Comparison between the sensor outputs and the roasting level

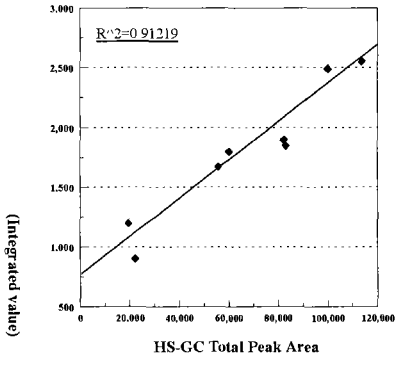

Fig. 9 Comparison between the sensor outputs and the GC outputs

Based on this fact, it is considered that this sensor recognized aroma components over a wide range and could express a flavor qualitatively too.

3.5 Sensor responses to samples with offensive taste and smell

The most serious problem in the importation of green coffee beans resides in the quality control. In the field of the quality control, sensory tests play an important role similar to physicochemical analysis. In particular, it is frequently troublesome to detect substances having delicately offensive taste or smell by physicochemical analysis. Thus the detection of these substances frequently depends on sensory tests with those skilled in the art. However it is difficult to completely eliminate subjective factors from these sensory tests. Also, a decrease in the reproducibility and deterioration in sensitivity due to, for example, physical conditions are unavoidable in such a case. It is therefore expected that the objectivity, reproducibility and sensitivity can be improved by combining these sensory tests with the analysis by the sensor.

To research the responses of this sensor to a substance with offensive taste and smell, therefore, a sample having a medicine-like odor was analyzed by this sensor. As a control, a sample, the odor of which was evaluated as normal by the sensory test, of the same importation lot was employed. The roasting levels of these samples were adjusted to 107 (L value = ca. 22). To compare the output results, these samples were also analyzed by GC-MS.

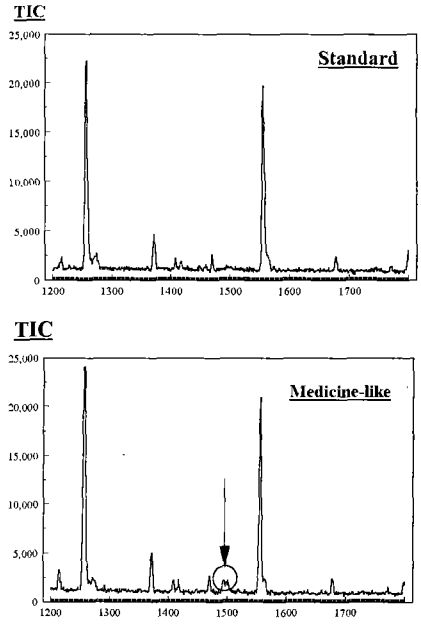

Fig. 13 Total ion chromatogram of cofee with medicine like smell

Fig. 10 show the results of the total ion chromatography charts obtained by the analysis by GC-MS. Although the sample having the medicine-like smell shows several peaks which are not observed in the control sample, these peaks are weak in strength. Thus these data cannot be retrieved from a library by a software and, therefore, it is required to effect troublesome operations such as extraction or concentration.

Fig. 10 shows the sensor output patterns of these samples obtained by using the flavor sensor. As Fig. 10 clearly shows, the control shows a pattern like a pen-tip, while the sample with the medicine-like smell shows a deformed pattern. It is highly risky to consider that this difference is simply caused by the medicine-like smell. However, it can be concluded at least that this sensor can distinguish these samples from each other without requiring any complicated process as the one of GC and has a high sensitivity to a substance with an offensive taste or smell.

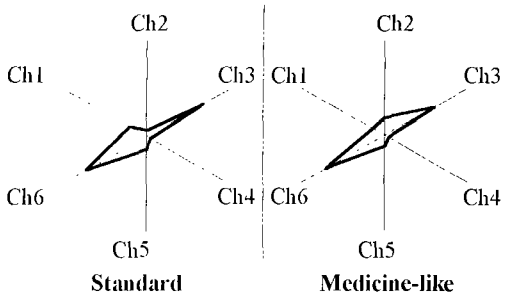

Fig. 10 Response patterns of the sensor outputs for coffee with medicine-like smell

4. Problems in Future

In this study, the measurement and the analysis were carried out while controlling temperature and humidity and ascertaining the reproducibility, which

somewhat damaged the convenience of the process, i.e., one of the benefits to be achieved. Further studies are needed to correct the variation in an open system. Although no influence of humidity has been reported in this paper, it is an important factor in the measurement in an open system. There have been published a number of reports on the measurement of odors with the use of semiconductors which include one wherein a carrier gas is used in a closed system, one wherein an odor is fed into a chamber with a pump and measured, etc.

Also, the recognition of flavors and humidity widely varies. Attempts have been made to avoid the influence of humidity by controlling the humidity at a constant level with an instrument or developing a sensor suffering from no influence of humidity per se. When we feel an odor, however, it is considered that we recognize the odor including the influence of humidity. It frequently happens in our daily life and, therefore, should be discussed in the course of the development of flavor sensors equivalent for the physical functions and sensitivities.

5. Conclusion

The beginning of the research of this sensor system resided in the idea to economically develop a convenient measuring method to be used in an atmospheric system such as a pH meter used in the aqueous system. It is therefore desirable that this system is a simple one to be used in an open system and the sensor is a semiconductor which costs low and is excellent in durability.

We have developed this system based on this idea. Furthermore, this sensor has attracted attention of workers in various fields from the viewpoint of sensitive measurement, in particular, the relation between flavor and physical functions. Under these circumstances, we analyzed various flavors by the flavor sensor equivalent for the physical functions developed recently.

As a result, it was suggested that this sensor system would express odors both quantitatively and qualitatively. Then coffee, which is considered to have the most complicated aroma constitution, was tested by this sensor and the sensor responses were analyzed and discussed.

As a result, it is suggested that this sensor system would recognize the flavor of coffee both quantitatively and qualitatively.

6. Summary

To carry out a pattern analysis of odor, an odor analysis system with a human sense of smell was developed by using unique metal oxide semiconductor sensors (four kinds of SnO_2 and two kinds of ZnO). Elements of an odor to human include six substance of nitrogen, sulfur, hydrocarbon, hydrogen, oxygen, and aromatic compounds. They can be sensed by six types of semiconductor respectively. This newly developed system can detect odor quality by the shape of hexagon graphs which are formed by depending on responses of six sensors. Measurement of many types of odor indicated that pleasant odors to human have a hexagon graph like a fountain pen tip. The system was applied to the discrimination of roasted coffees,. Hexagon graphs of coffees looked like a fountain pen tip, but identification of coffees with hexagon graphs was difficult due to complexity and the resemblance of their aromas. The principal component analysis of sensor's outputs can characterize several origins of coffee. The sensor's output's had a high correlation to roasting level and to outputs of gas chromatography.

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STUDIES ON ROASTING OF LOW GRADE INDIAN COFFEE BEANS (BLACK, BROWN, BITS) USING SPOUTED BED ROASTER

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Introduction.

Roasting plays a vital role in processing of green coffee. Flavour and Aroma characteristics of the product depend on the temperature and duration of roasting. In conventional drum roasters, the beans are subjected to drastic temperature conditions for prolong time. This poses many problems like uneven roasting, loss of quality and fire hazard. Due to variations in particle size and mixed variety of beans, processing of low grade Indian coffee (Black, Brown, Bits) in commercial roasters is not viable. In India about 1,80,000 tonnes of green coffee is produced, out of which the low grade (Black, Brown, Bits) forms 6 - 8% amounting to 14,000 tonnes /annum. Due to non availability of suitable roasters, this material is not utilised in commercial level for consumption (Sunderarajan,1989).

This has suggested the use of spouted bed technology for upgrading the low grade (Black, Brown, Bits) coffee beans. Laboratory scale roasters of capacity 1kg/batch and 5kg/batch have been developed and roasting process was carried out for these low grade coffee beans also different blends of low grade with superior varieties.

Materials and Methods:

The spouted Bed Roasters consists of a cylindrical spouting chamber (pyrex glass) with a conical stainless steel bottom having central spout connected to hot air supply unit. Digital temperature recording unit with thermocouple sensors help in accurate measurement of the air and product temperature during roasting. Material procured from curing works are used in 1kg and 5kg in batch roasters. (Fig.4.Roaster)

For the production of instant coffee, roasted beans were ground to an average diameter of 1.95 mm (0.077") mesh size using a hammer mill. Instantisation was done using standard procedure (Sylla, 1989.), but with gravity extraction.

Analysis of ground and instantised powders were done as per the AOAC official method.

Results and Discussions:

Fig.1. Shows the roasting times for various air temperatures for Black, Brown, Bits in 1kg and 5kg/batch units along with that of peaberry beans. It can be seen that similar trends are exhibited in both cases and at high temperatures the roasting time between the two are not much varied.

As Sivetz (1991) suggested, roasting of coffee beans at temperature of 260°C with roasting time of 4 to 10 MINUTES produces excellent taste of coffee. The present data on roasting of Black, Brown, Bits is in good agreement with the reported values.

Fig.2. Shows the final product temperature for 1kg and 5kg/ batch roasters. It can be seen that the product temperature are same in both the cases and they are within the range of 220°C. As given by Sivetz (1991) the product temperature is less than the optimal range of 232°C at which maximum aroma and flavour develops.

Table.1. Shows the product characteristics of roasted and ground coffee for Black, Brown, Bits and blends with superior variety. It can be seen that the data on Black, Brown, Bits using Spouted Bed compare well with the commercial sample.

Table.2. Shows the characteristics of Instantised coffee powder from Black, Brown, Bits and other varieties like Arabica, Robusta and Peabery are compare well with the commercial sample. Hence the Spouted Bed can be used for upgrading Black, Brown, Bits with superior varieties.

Conclusions.

Spouted Bed roasting can be adopted for processing of Black, Brown, Bits at temperature of 260°C at the roasting time of 8 to 10 minutes. The Black, Brown, Bits coffee beans can be upgraded by blending with superior grade to obtain products similar to the superior varieties. The characteristics of roasted and ground as well as instant coffee compare well with the market sample.

Acknowledgment

The authors wish to express their thanks to Sri. A.Ramesh, Head, Food Engg and Dr.M.L.Shankaranarayana , Head, Plantation Products for their valuable guidance and encouragement, and also wish to thank to Mr.C.T.Murthy, Scientist for his suggestions.

Summary:

This paper gives in brief the work carried out on the process for upgrading the low grade Indian green coffee (Black, Brown, Bits) which are considered to be rejects. Black,Brown and Bits form a by-product in coffee curing works. In India there is major constraint in using these grades of raw materials for ground and instant coffee production due to non availability of advanced technological process for roasting. The present study examines the hot air roasting of these varieties for the production of roasted ground coffee as well as instant coffee.

The study reports the data on Spouted Bed Roasting of this low grade green coffee at temperatures in the range of 200 to 260°C. The product analysis of ground and instant coffee is presented. It is observed that the final product from these grades compare well with the the commercial samples of superior grades .Blends of these with superior grades can also be roasted to meet the consumers needs for their specific requirements.

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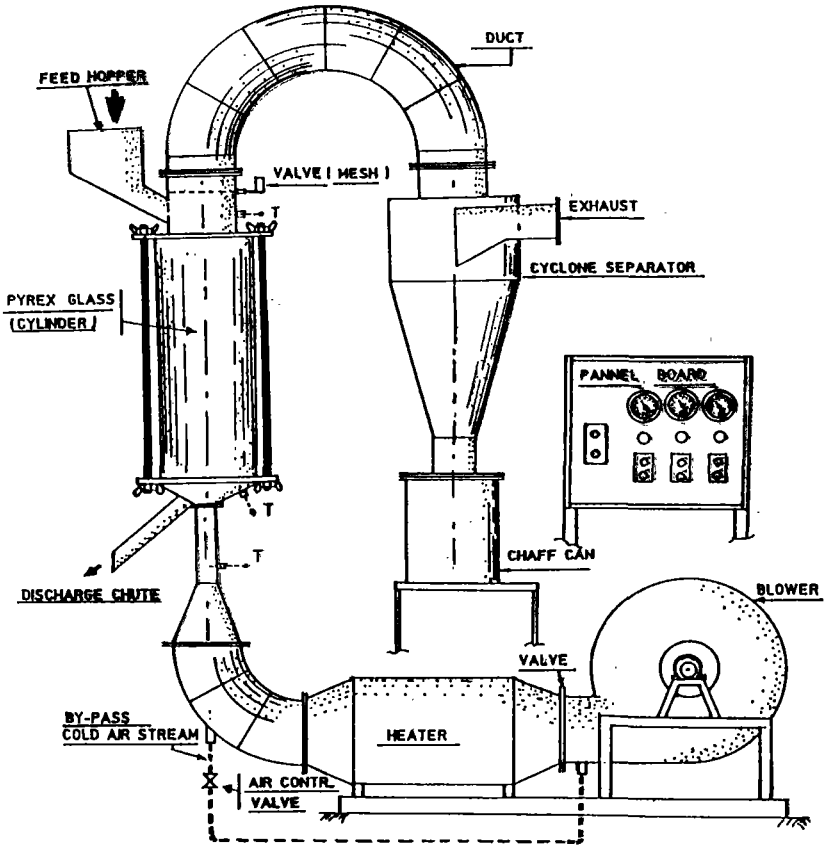

Fig. 4. **SPOUTED BED COFFEE ROASTER**

UNLESS OTHERWISE MENTIONED ALL DIMENSIONS ARE IN MILLIMETRES

SPOUTED BED COFFEE ROASTER (CAP. 5 Kg./BATCH)				
SCALE	1 : 10	DRAWN	K.Padmanabha	20-3-95
DRG. NO.	2-287-42	TRACED	—	
REV. NO	00	CHECKED		
		APPROVED		
CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE MYSORE - 570013 INDIA				cftri MYSORE

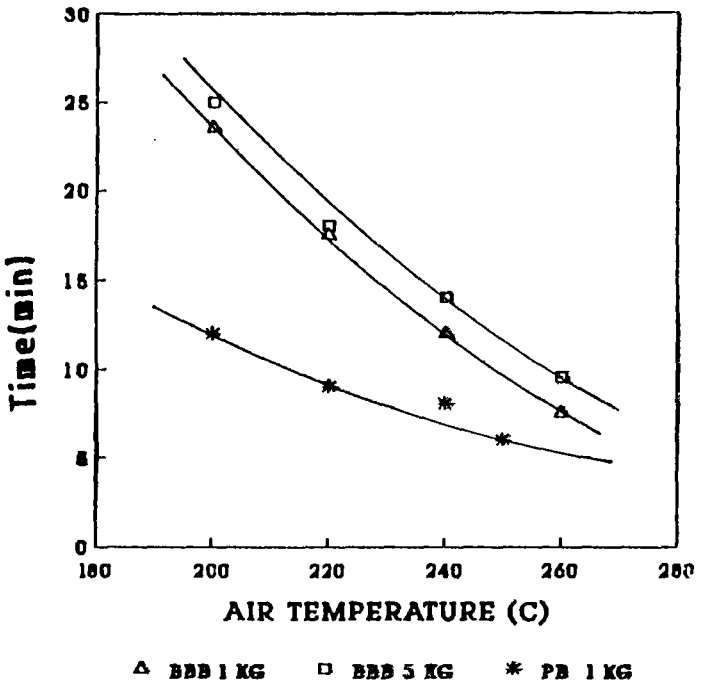

FIG. 1, ROASTING TIME Vs AIR TEMPERATURE

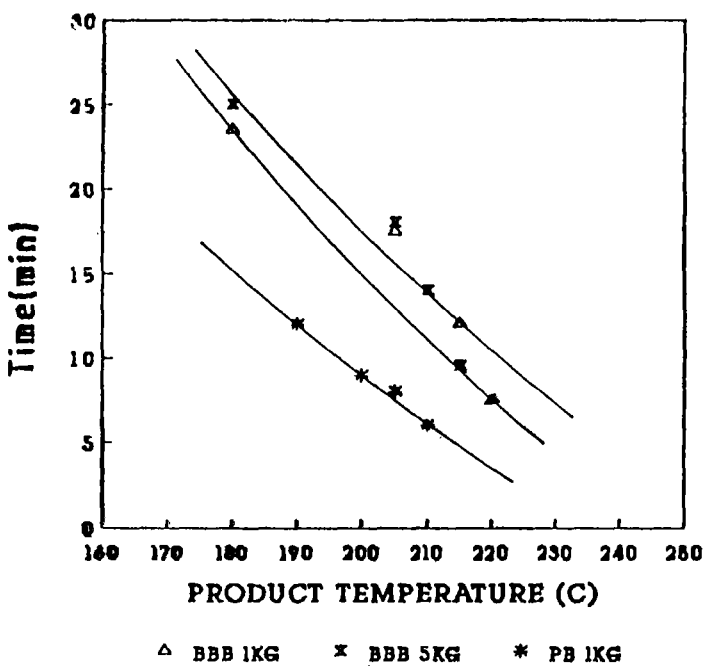

FIG.2, TIME Vs PRODUCT TEMPERATURE

Table.1. PRODUCT CHARACTERISTICS ON ROASTED AND GROUND POWDER
(Sl.No. 1 to 4 BBB 1kg/batch and 5 to 8 BBB 5kg/batch)

SL. No.	Inlet temp(°C)	Product temp(°C)	Roast time	Bulk[a] Density	Moisture cont(%)	T.S.S[b] (%)	Caffeine cont(%)	Chl.genic acid(%)	Colour
1.	200	180	23.5	0.45	1.36	27.22	2.2	5.33	13.1
2.	220	205	17.5	0.38	1.18	29.17	2.31	3.22	9.8
3.	240	215	12.0	0.38	1.67	31.47	2.36	3.95	9.8
4.	260	220	7.5	0.33	1.12	33.52	2.63	3.96	9.9
5.	200	180	25.0	0.50	1.04	27.06	1.76	6.65	16.0
6.	220	200	18.0	0.45	1.24	27.34	1.96	5.68	13.9
7 .	240	210	14.0	0.43	1.19	29.37	1.75	5.58	13.4
8.	260	215	9.5	0.41	1.36	29.78	1.89	5.83	13.0
Blends of superior variety and BBB									
9.	240	200	17.0	--	1.55	29.09	2.07	3.25	9.4
10.	240	200	10.0	--	2.33	29.69	2.03	4.74	11.2
Roasted and ground commercial sample:					1.12	29.55	1.88	5.04	10.4

a. Bulk density in gram/cc, b. T.S.S-Total soluble solids.

Table.2. PRODUCT CHARACTERISTICS OF INSTANT POWDER

Sl. No.	Variety	Moisture content(%)	Caffeine content(%)	Chlorogenic acid(%)	Colour
1.	BBB	3.57	3.97	10.55	12.6
2.	Peabery	4.44	2.86	9.56	10.2
3.	Robusta	1.2	5.5	11.68	10.7
4.	Arabica	4.99	3.05	9.15	15.3
Commercial sample :		3.93	3.65	11.59	18.0

DEGRADATION OF COFFEE POLYSACCHARIDES BY *TRICHODERMA VIRIDE* No. 8 ISOLATED FROM SOIL

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INTRODUCTION

Coffee beans, which are seeds of coffee trees belonging to the genus *Coffea* of the *Rubiaceae* family, are regarded as a food material containing nutrient components similar to other nuts such as almond, walnuts, peanuts, etc. In fact, it is said that coffee beans were taken as such or processed into a preserve food by grinding and mixing with oil in old days[1),2)].

Today, however, the almost only one use of coffee beans comprises roasting coffee beans, grinding the roasted coffee, and extracting the ground coffee with hot water to there by give a coffee extract for drinking.

On the other hand, green coffee beans contain polysaccharides such as cellulose, mannan, arabinogalactan, etc. in an amount of 40 to 50% in terms of dry matters. The high toughness of coffee beans is mainly caused by these polysaccharides (fibers)[3),4),5)]. This property established by the polysaccharides hinders the application of coffee beans as a food material. In addition, these polysaccharides are the main component of the grounds coffee formed in the present application method.

To improve the processing suitability and the utilization rate of coffee, therefore, degradation of the coffee polysaccharides is highly important and it is urgently required to establish techniques therefore[6),7)].

We isolated a strain No. 8 from a soil, which was identified as *Trichoderma viride* and it can degrade coffee grounds [8)]. In this study, we incubated *T. viride* No. 8 in media containing grounds coffees, green coffee and roasted coffee and examined the degradation of coffee beans during the cultivation and the coffee-degrading rates and the coffee-degrading products observed after the cultivation.

MATERIALS AND METHOD

1. Organism

T. viride No.8 was isolated from a soil sample obtained from the Osaka at 1985, and degraded grounds

coffee which was added into a liquid medium[8].

2. Material and pretreatments

Green coffee of two origins were prepared : Uganda Robusta and Colonbia Arabica. Roasted coffee was prepareed by roasting to a medium degree of each green coffee industrially. Grounds coffee was prepared by extracting of roasted coffee (Arabica : Robusta 50 : 50 blends) industrially. Green coffee, roasted coffee and grounds coffee were grinded to particle size 3 mm or less, and were boiling with 1%-NaOH for 3, 5, 7 hours, then washing two times with disitilled water and drying in an oven at 100℃. The dryed coffee ground to particle size 1 mm or less, 1 to 2 mm, 2 mm over.

3. Culturale conditions

T. viride No.8 strain was preincubated by reciprocal shaking (120 strokes/min, 5 cm span) at 28℃ for 5 days in 100 ml of YM-broth (Difco). The basal medium containing 4.0 g of NH_4NO_3, 0.8 g of $MgSO_4 \cdot 7H_2O$, 5.6 g of KH_2PO_4, 3.6 g of yeast extract in 1000ml of distilled water was prepared at pH 6.2 . Each 500ml Erlenmeyer flask contained 55ml of the basal medium was added in 4.5g of coffee samples, and sterilized autoclaving at 120℃ for 20 min. A portion of 1ml of pre-culture (YM-broth) was inoculated medium, and then incubated at 28℃ with reciprocal shaking (120 strokes/min, 5 cm span).

4. Measurment of degrating rate and reducing sugar

After cultivation, the amount of dry matter remaining coffee beens was determined by washed the insoluble material two times with disitilled water on the mesh (opening of 0.85 mm) and dryed in an oven at 100℃. Degrading rate (DR : %) was calculated by the following equation : $DR=W_A/W_O \times 100$ (W_O : coffee of before cultivation, 4.5g. W_A : remaining coffee of after cultivation). In order to measure the reducing sugar in the medium supernatant which were obtained by centrifugation at room temperature (3000 × g for 10 min), measured by Somogyi-Nelson method.

5. TLC of monosaccharides and oligosaccharides

The supernatant was concentrated with evaporator. The concentrates was passed through colomns of Dowex 1×2 and Dowex 50 with deionized water successively. Sugar in the medium concentrates were assayed by thin layer chromatography (TLC) using a silica gel plate (Kisel Gel 60, Merck) and 2-propanol : aceten : 0.1M-lactic acid (4 : 4 : 2, V/V) as a solvent system. Sugar on the plate were detected by spraying dipenylamine-aniline-phosphoric acid reagent, followed by heating at 100℃ for 10 to 20 min.

RESULTS AND DISCUSSION

1. Pretreatments

Before the degradation of coffee beans by *T. viride* No.8, the degree of grinding and the conditions for the NaOH treatment were discussed. Coffee grounds adjusted to a particle size of 3 mm or less were treated with NaOH for 0 (i.e., an untreated lot), 3, 5 and 7 hours. Compared with the untreated sample, the samples treated with the 1%-NaOH for 3 hours or longer were degraded to an extremely high extent. The coffee-degrading rate of the sample treated for 5 hours was almost 4.2 times higher than that of the

untreated sample. The sample treated for 7 hours showed a further elevated coffee-degrading rate, i.e., about 4.3 times higher than that of the untreated sample (Table1).

Table 1 Degrading rate of grounds coffee in each NaOH-treatments by *T. viride* No.8

sample	Degrading rate (%) Incubation time (days)			
	1	3	5	7
none	0.5	2.7	9.3	18.9
3h	1.3	10.5	21.3	48.7
5h	1.8	13.9	48.2	79.8
7h	2.1	23.4	64.8	81.6

Table 2 shows the coffee-degrading rates of grounds coffee, which had been ground to particle sizes in 3 degrees, on the days 1, 3, 5 and 7 of the cultivation. The sample of a particle size of 1 mm or less showed the highest coffee-degrading rates on the days 3, 5 and 7. Compared with the sample of a particle size of 1 mm or less, the sample of a particle size of 1 to 2 mm showed a somewhat low coffee-degrading rate on the day 3, a coffee-degrading rate of about 80% on the day 5 and that of about 89% on the day 7 (i.e., almost comparable to the sample of a particle size of 1 mm or less). The coffee-degrading rate of the sample of a particle size of 2 mm or more showed no increase but remained at about 13.6% evenon the day 7.

Table 2 Degrading rate of grounds coffee in each particle size by *T. viride* No.8

sample	Degrading rate (%) Incubation time (days)			
	1	3	5	7
< 1mm	3.3	51.6	82.7	89.8
1mm-2mm	2.0	16.4	80.0	88.7
2mm >	0.7	5.3	11.1	13.6

Wolfrom et al.[3] and Bradbury et al.[4] reported that coffee polysaccharides mainly comprise aabinogalactan, cellulose and mannan. Similar to the cases of other nuts, these coffee polysaccharides are bonded to each other via hydrogen bondings or attached to lignin, proteins and polyphenol and thus maintaina high strength. To degrade coffee beans by *T. viride* No. 8, it is therefore necessary to reduce the crystallinity of cellulose contained in the coffee beans and to remove lignin though a treatment with analkali. It is also important to grind the sample (substrate) to thereby enlarge the surface area of the substrate coming into contact with the enzyme. In this study, the samples were boilked with 1%- NaOH for 5 hours and thecoffee beans were ground to a particle size of 1 to 2 mm by taking the efficiency of the degradation of the coffee beans, the loss of the substrate during the processing and the coast of the materials into consideration.

2. Degradation of coffee by *T. viride* No. 8

The *T. viride* No. 8 was incubated for 7 days in the media containing the pretreated grounds coffee, the green coffees and the roasted coffees. When observed with the naked eye, it was confirmed that the

grounds coffee, the green Uganda coffee and the roasted Uganda coffee in the media had been almost completely degraded by this strain and converted into viscous liquids. In contrast, the green Colombia coffee and the roasted Colombia coffee had not been degraded but remained in the media in a large amount. Compared with other 3 samples, the medium containing the Colombia coffee showed little pellet-like mycelia and suspending conidia of *T. viride* No. 8.

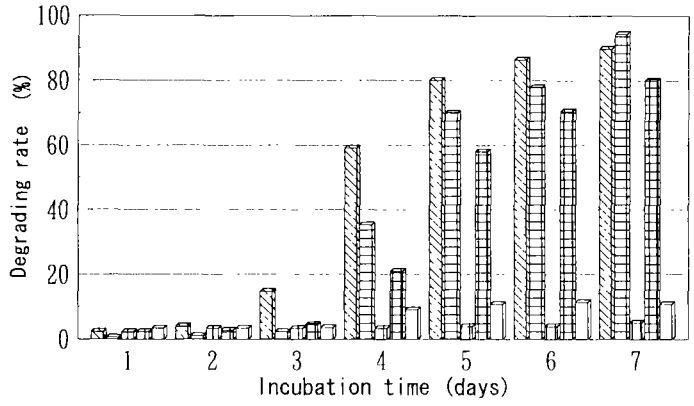

Fig. 1 Degrading rate of coffee by *T. viride* No. 8

Grounds coffee Green Uganda Green Colombia
Roasted Uganda Roasted Colombia

Fig. 1 shows the coffee-degrading rate of each sample with the passage of time. The degradation of the grounds coffee started on the day 3 of the cultivation and the coffee-degrading rate amounted to about 60% and about 90% respectively on the days 4 and 7.

The degradation of the green Uganda coffee started on the day 4 of the cultivation and the coffee-degrading rate amounted to 94% on the day 7. In contrast, the green Colombia coffee was hardly degraded even on the day 7 of the cultivation (coffee-degrading rate: 5%).

Similar to the green Uganda coffee, the degradation of the roasted Uganda coffee started on the day 4 of the cultivation and the coffee-degrading rate amounted to 80% on the day 7. On the other hand, the roasted Colombia coffee showed a coffee-degrading rate of 11% on the day 7 of the cultivation.

It is known that *T. viride* has a high productivity of lignocellulose hydrolase[9]. Supposing that the degradation of coffee with this strain is caused by the degradation of coffee polysaccharides, it is assumed that the degrading products comprise monosaccharidesand oligosaccharides and thus a cultural medium shows an increase in reducing sugars. Thus the reducing sugars in the cultural media were determined with the passage of time.

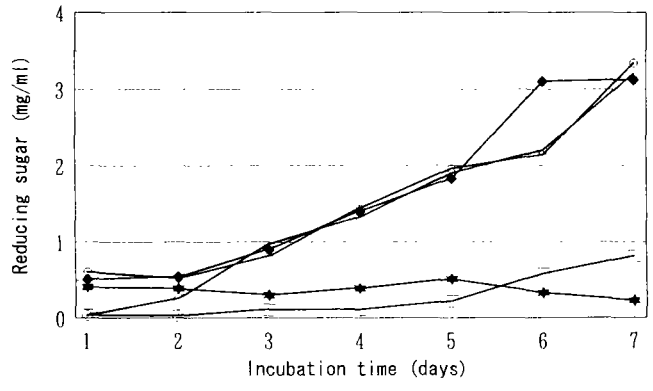

Fig. 2 Production of reducind sugar in the medium by *T. viride* No. 8

Grounds coffee Green Uganda Green Colombia
Roasted Uganda Roasted Colombia

Reducing sugars in the cultural media containing the grounds coffee, the green Uganda coffee and the roasted Uganda coffee showed each an increase in reducing sugars from the day 3 of the cultivation. On the other hand, the media containing the Colombia coffee, which showed low coffee-degrading rates, scarcely showed any increasein reducing sugars. Namely, the reducing sugar concentrations of the samples of Colombia coffee on the day 7 corresponded to 1/4

to 1/10 of hose of other 3 samples. The coffee grounds showed the highest concentration of reducing sugars (3.3 mg/ml) followed by the green Uganda coffee (3.2 mg/ml) and the roasted Uganda coffee (3.12 mg/ml) (Fig. 2). These results indicate that the degradation of coffee by *T. viride* No. 8 would be mainly caused by the degradation of polysaccharides.

On the other hand , the major reason why the Colombia was hardiy degraded by *T. viride* No.8. It is considered that the degradation is inhibited by some facter contained in the Colombia coffee which suppress the growth of *T. viride* No.8 or enzyme activity.

3. Production of saccharides by cultivation

The monosaccharides and oligosaccharide produced through the degradation of the coffee polysaccharides were analyzed by TLC (Fig. 3). As a result, major 3 components were detected from the degrading products of grounds coffee, green Uganda coffee and roasted Uganda coffee respectivily.

By comparing the Rf values of these products with the Rf values of standard saccharides, these products were identified as glucose, mannose and cellobiose. These results indication that *T. viride* No.8 degraded coffee polysccharides, and produced such succharides as mannose, glucose and cellobiose.

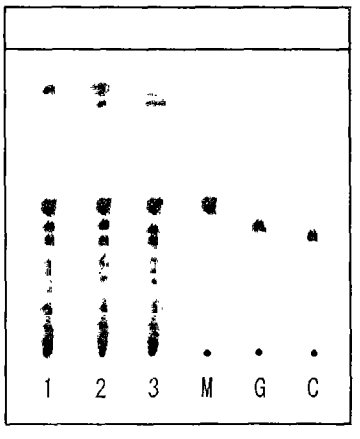

Fig.3 TLC of production from coffee sample by *T. viride* No. 8

1:Grounds coffee 2:Green Uganda
3:Roasted Uganda M:Mannose
G:Glucose C:Cellobiose

CONCLUSIONS

1. In this study, coffee samples boiling with 1%-NaOH for 5 hours and ground to a particle size of 1 to 2 mm by taking efficiency of the degradation of the coffee.
2. During 7 days cultivation, each coffee-degrading rate of the grounds coffee, the green Uganda coffee and the roasted Uganda coffee was about 90%. On the other hand, the green Colombia coffee and the roasted Colombia coffee was hardly degraded.
3. Reducing sugar in the cultural media containing the grounds coffee, the green Uganda coffee and the roasted Uganda coffee showed each an increase.
4. *T. viride* No.8 degraded coffee polysaccharides, and produced such as mannose, glucose and cellobiose.

SMUMMARY

The soluble solid in roasted coffee which is extracted by hot water consists of about 30 to 40%, and the insoluble consisits of about 50% polysccharides, mostly cellulose, mannan and arabinogalactan.

We have already isolated *Trichoderma viride* No.8 from soil, which degraded grounds coffee. In this study, we reported the cultural conditions for degrading of the green coffee, the roasted coffee and the grounds coffee by *T. viride* No.8, and coffee-degrading products by cultivation.

Green coffee, roasted coffee and grounds coffee were boiling with 1%-NaOH for 5 hours, and ground to particle size 1 to 2 mm respectively. Cultural conditions, 4.5g of this treated coffee added into 55ml of a liquid medium, and then cultivated at 28℃ with reciprocal shaking (120 strokes/min,5 cm span). After

cultivation for sevral days, coffee-degating rate was calculated on mesuring each weight of coffee (on the opening of 0.85 mm mesh)at dry condition, and reducing sugar in medium was measured by Somogyi-Nelson method. During 7 days cultivation, each coffee-degrading rate of the green coffee (origin : Uganda), the roasted coffee (origin : Uganda) and the grounds coffee was about 90%. Concentration of reducing sugar in medium was increased, and this succharides consists of almost mannose, glucose cellobiose.

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USE OF HIGH PRESSURE TREATMENT FOR STABILIZING COFFEE BREW DURING STORAGE

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Introduction

It is well known that a series of chemical and physico-chemical modifications take place during the storage of coffee brew. In particular as storage temperature increases, the pH of the brew decreases; moreover, the extract becomes pregressively turbid. These modifications, as well as changes in aroma and taste, become more pronounced when the product is sterilized by high temperature treatment. Over the last few years high pressure treatments have received considerable attention for their applications in food preservation as an alternative to sterilization processes.

Materials and methods

Arabica coffee, "Italian style" roasted and ground, was extracted by percolation using a water:powder ratio of 10:1. 50 cc of coffee brew was packed in a flexible film and pressed with an hydrostatic press QFP6 Asca Brown Boveri. The accelerated ageing of the samples was carried out using an air circulating oven at 40°C for 15 days. The pH of the brew was tested by a Crison micro pH 2001 pH meter ; the optical density was measured by a Perkin Elmer 550 SE UV/VIS spectrophotometer at 420 and 700 nm.

Results and Discussion

From the kinetic constant values (table 1) it is evident that the behaviour of the pH decrease was similar for all the samples. Figure 1 shows that the pasteurized sample had a higher pH starting value, but the kinetic decrease was the same as in the other samples. The substitution of the pastorization with the pressure treatment did not help the chemical stability of the product.
Regarding the optical properties of the pressurized and pasteurized coffee brew, in figure 2 the absorbance values at 420 nm versus the storage time are shown. The behaviour of the optical density of the samples was quite regular for brews pressurized at 3000 and 7000 bar for 10' and for pasteurized brew, but the sample treated at 5000 bar showed anomalous values during storage. In fact this level of pressure, 5000 bar, is considered a critical point for microbiological activity and for protein structure changes; we could hypothize that the coffee brew, in which polyphenolic pigments and pseudo-colloids of proteinic nature are present, was destabilized by this particular pressure treatment. This hypothesis is confirmed by data obtained from the shorter treatments (1' and 5') and from the optical density data at 700 nm for the same samples. These results are being elaborated.

Table 1 - Kinetic constants and statistical data of the pH of the coffee brews treated at pressures of 3000, 5000 and 7000 bar for 1', 5' and 10' and pasteurized at 65° for 25',obtained by fitting the regression lines. The data concern the pH decrease versus the storage time at 40°C.

Pressure (bar)	Treatment time (min)	Statistical data			
		r	p	SEM	K
3000	1'	- 0.966	<0.01	0.087	- 0.037
	5'	- 0.962	<0.01	0.090	- 0.038
	10'	- 0.948	<0.01	0.087	- 0.036
5000	1'	- 0.985	<0.01	0.082	- 0.035
	5'	- 0.968	<0.01	0.092	- 0.039
	10'	- 0.971	<0.01	0.083	- 0.035
7000	1'	- 0.963	<0.01	0.085	- 0.036
	5'	- 0.972	<0.01	0.079	- 0.034
	10'	- 0.967	<0.01	0.081	- 0.034
Pasteurized		- 0.890	<0.05	0.084	- 0.033

r = correlation coefficient
p = significance
SEM = Standard Error of Mean
K = Kinetic constants ($days^{-1}$)

Summary

A coffee brew, obtained by using the percolation method of extraction was subjected to three different pressure levels (3000, 5000 and 7000 bar) and to pasteurization (65°C for 25'). After the processes, the coffee brew was stored at 40°C for the "accelerated ageing". The quality of the products was then checked by following some indicators such as pH and optical density (420 and 700 nm) during storage. Biological stability was reached in all cases, but the pressure treatment did not increase shelf-life with respect to the chemico-physical characteristics, if the brew was not stored at low temperatures. The pressure of 5000 bar was a critical pressure for the stability of the colloidal fraction of the coffee.

Résumé

Du café sous forme de boisson, obtenu avec la métode d'extraction de la percolation, a été traité avec trois differentes pressions (3000, 5000 et 7000 bar) et pasteurizé à une température de 65°C pour 25 minutes. Aprés le processus, le café a été conservé à une temperature de 40°C constants pour le vieillissement accéléré. La qualité du produit a été côntrolée ensuite en suivant les variations de pH et de l'absorbance à 420 et à 700 nm. La stabilité biologique a été obtenue dans tous les cas, mais le traitement avec la pression élévée n'a pas porté à une "shelf-life" plus longue pour ce qui concerne les caracteristiques physico-chimiques. La pression de 5000 bar a été une pression critique pour la stabilité de la fraction colloïdale du café.

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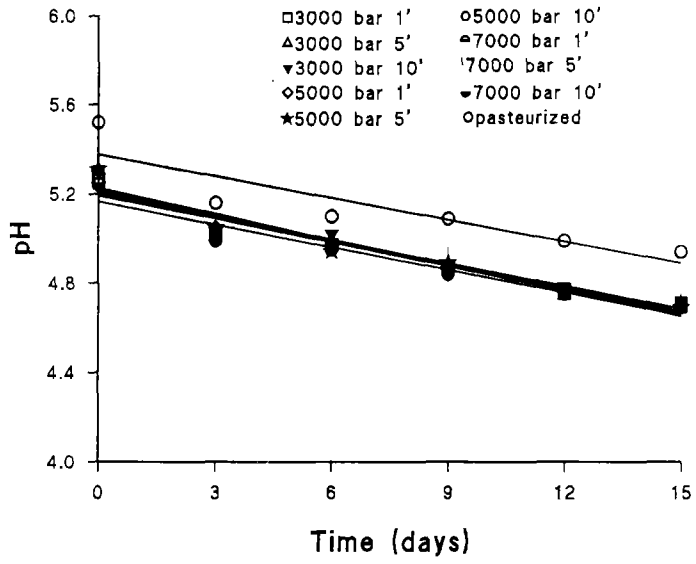

Figure 1 -- pH values of coffee brews treated at three pressure levels (3000, 5000 and 7000 bar) for three different times (1', 5' and 10') and pasteurized (65°C for 25') versus storage time at 40°C.

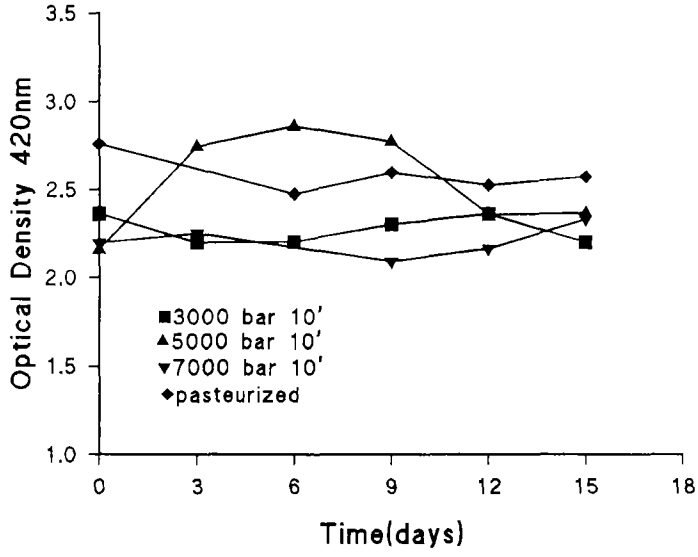

Figure 2 -- Optical density at 420 nm of coffee brews treated at 3000, 5000 and 7000 bar for 10 minutes and pasteurized (65°C for 25') versus storage time at 40°C.

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