JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Flavoring Components of Raw Monsooned Arabica Coffee and Their Changes during Radiation Processing

PRASAD S. VARIYAR,*,† RASHEED AHMAD,§ RAJEEV BHAT,§ ZAREENA NIYAS,† AND ARUN SHARMA†

Food Technology Division, BARC, Trombay, Mumbai 400 085, India, and Department of Botany, Mangalore University, Mangalagangotri, India

Volatile aroma principles, nonvolatile taste constituents (caffeine and chlorogenic and caffeic acids), and glycosidically bound aroma compounds of monsooned and nonmonsooned raw arabica coffee were analyzed using gas chromatography−mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC). Among the most potent odor active constituents known to contribute to the aroma of the green beans, 3-isopropyl-2-methoxypyrazine, 3-isobutyl-2-methoxypyrazine, 4-vinylguaiacol, β-damascenone, (*E*)-2-nonenal, *trans*,*trans*-2,4-decadienal, phenylacetaldehyde, and 3-methylbutyric acid were detected by GC-MS in both samples. A decrease in content of methoxypyrazines and an increase in 4-vinylguaiacol and isoeugenol resulted in a dominant spicy note of monsooned coffee. These phenolic compounds exist partly as their glycosides, and their release from the bound precursors during monsooning accounted for their higher content in monsooned coffee. A considerable decrease in astringent chlorogenic acid as a consequence of hydrolysis to bitter caffeic acid was noted in monsooned coffee. Radiation processing of nonmonsooned beans at a dose of 5 kGy resulted in an increased rate of monsooning. At this dose a quantitative increase in most of the aroma active components could be observed in all samples studied. Hydrolysis of chlorogenic acid to caffeic acid was noted in radiation-processed monsooned coffee beans irrespective of whether the treatment was carried out before or after monsooning. These changes were, however, not observed in irradiated, nonmonsooned coffee beans, suggesting an enzymatic rather than a radiolytic cleavage of chlorogenic acid. A rationale behind the mechanism of monsooning and radiation-induced enhancement of the monsooning process is discussed.

KEYWORDS: Monsooned coffee; γ-radiation; raw coffee beans; volatiles; caffeine; chlorogenic acid

INTRODUCTION

Monsooned coffee, a speciality coffee of India, is known for its characteristic taste and aroma (*1*). This coffee commands a higher price, compared to the other Indian coffees, on the international market (*1*). Monsooning is a natural process of curing wherein dry raw coffee beans belonging to Arabica (*Coffea arabica* L.) and Robusta (*Coffea canephora* Pierce ex Froehner) varieties are exposed to moist monsoon winds prevailing in the coastal regions of Mangalore and Tellicherry of Karnataka and Kerala states (India), respectively. During this process the moisture content of coffee beans increases from an initial 10−12 to 18−22%, and they double in size (*1*). The original green color is lost, and they have a bleached appearance. The final product has a lower bulk density and possesses a unique mellow flavor (*1*). Although there are several reports in the literature (*2*−*5*) on the flavoring constituents of conventional (green as well as roasted) nonmonsooned coffee beans, no information exists to date on the nature of these constituents in monsooned coffee.

Due to their higher moisture content, monsooned coffee beans are prone to insect infestation (*Aracerus fasciculatus*) and microbial contamination, thereby reducing their shelf life. With the likely ban on the use of chemical fumigants in food-processing in the near future, it is imperative to find an alternative and effective method to control fungal contamination and insect infestation in monsooned beans. In recent years, irradiation of foods by exposing them to ionizing radiation such as γ-rays and electron beams has emerged as an effective tool for shelf-life extension, hygienization, and overcoming quarantine barriers (*6*). Food irradiation has gained importance as it is not only safe but leaves no toxic residues and is also economically viable (*7*, *8*). A radiation dose in the range of 0.6−0.9 kGy was shown (*9*, *10*) to result in a 100% mortality of the coffee bean weevil (*Araecerus fasciculatus*) and thus could be employed for insect disinfestation of coffee beans. Diaz et al. (*11*) have noted that the flavor or aroma of roasted coffee beans was unaffected by radiation treatment up to a dose of 0.5

* Author to whom correspondence should be addressed (telephone 022-25590560; fax +91-22-25505151; e-mail pvariyar@rediffmail.com).
† BARC.
§ Mangalore University.

10.1021/jf030408q CCC: $25.00
Published on Web 12/06/2003

kGy. However, no report exists so far on the efficacy of using γ-radiation as a means of disinfestation/decontamination of raw monsooned coffee.

The present work therefore attempts to determine the nature of volatile aroma principles, nonvolatile taste constituents (caffeine and chlorogenic and caffeic acids), and glycosidically bound aroma compounds (aroma glycosides) of monsooned coffee and ascertains the impact of radiation processing on these constituents in this well-known variety of speciality coffee.

MATERIALS AND METHODS

Chemicals. All solvents used were of analytical grade, purchased from Merck (Mumbai, India), and distilled before use. Diethyl ether was made peroxide free before use. 2-Ethylhexanol, 3-isopropyl-2-methoxypyrazine, 3-isobutyl-2-methoxypyrazine, 4-vinylguaiacol, β-damascenone, (*E*)-2-nonenal, *trans*,*trans*-2,4-decadienal, phenylacetaldehyde, and 3-methylbutyric acid were procured from Aldrich Chemical Co. (Milwaukee, WI), whereas isoeugenol was obtained from Merck (Schuchardt, Germany). Chlorogenic (3-caffeoylquinic acid) and caffeic acids were purchased from Sigma Chemical Co. (St. Louis, MO), and caffeine was obtained from HiMedia Laboratories Ltd. (Mumbai, India).

Coffee Beans. Freshly hulled commercial green coffee beans (Arabica variety) were collected from the local coffee-curing works (Aspinwall and Co.) situated at Mangalore. The samples were divided into three equal lots. One lot served as nonmonsooned control samples and was designated as Arabica nonmonsooned (ANM). The second lot was subjected to natural monsooning as described below and termed as Arabica monsooned (AM), and the third lot was exposed to an irradiation dose of 5 kGy prior to the monsooning process and labeled as Arabica irradiated and monsooned (AIM). The nonmonsooned (ANM) and naturally monsooned (AM) beans were also subjected to γ-irradiation at a dose of 5 kGy and designated ANMI and AMI, respectively.

Monsooning Process. Dry green coffee been samples (ANM and ANMI, 10−12% moisture) were spread on the floor (to a height of 8−10 cm) of well-ventilated godowns located at the coffee-curing works, Mangalore, India, during the monsoon period (July−September). The beans were raked at regular intervals to avoid mold growth and to facilitate uniform moisture uptake. Once the monsooning process was over, as determined by the increased size and moisture levels (18−22%), the beans were packed in airtight polyethylene bags. The samples, namely, AM and AIM, thus obtained were stored in a deep freezer (−30 °C) until further analysis.

Irradiation. Irradiation of coffee beans (ANM and AM) to an average absorbed dose of 5 kGy was carried out in a package irradiator (AECL, Ottawa, Canada) having a dose rate of 34 Gy/min at the Food Technology Division, FIPLY, BARC.

Isolation of Volatile Compounds. Coarse coffee bean powder (150 g) was mixed with 300 mL of distilled water and subjected to steam distillation using a Likens−Nickerson simultaneous distillation−extraction apparatus (SDE) (*12*). Ether was used as an extracting solvent, and the distillation was continued for 2 h. The solvent was then removed from the extract under a slow stream of nitrogen gas to obtain the volatile oils. Samples were stored in a deep freezer (−30 °C) until further analysis by gas chromatography−mass spectrometry (GC-MS).

GC-MS Analysis. The aroma concentrates obtained above were subjected to GC-MS analysis using a Shimadzu QP-5050A series GC-MS instrument. The instrument was equipped with a GC-17A gas chromatograph and provided with a DB-1 (dimethyl polysiloxane, J&W Scientific) capillary column (length = 30 m, i.d. = 0.25 mm, and film thickness = 0.25 μm). The operating conditions were as follows: column temperature, programmed from 60 to 200 °C at the rate of 4 °C/min, held at initial temperature and at 200 °C for 5 min and programmed further to 280 °C at the rate of 10 °C/min, held at final temperature for 20 min; injector and interface temperatures, maintained at 210 and 230 °C, respectively; carrier gas, helium, at a linear flow rate of 0.9 mL/min; ionization voltage, 70 eV; electron multiplier voltage, 1 kV. Peaks were identified by comparing the mass fragmentation pattern with that of standard spectra available in the spectral library (Flavor and Fragrance and Wiley/NIST libraries) of the instrument, with retention time of standards as well as their retention indices (RIs). RIs of the analytes on DB-1 column were determined on the basis of retention times of *n*-alkanes C_6-C_{16} prior to injection of sample. Peaks were quantified using isoeugenol as external standard injected (in the range of 10−100 ng) into the GC-MS equipment under identical conditions as above.

Isolation of Aroma Glycosides. Finely powdered coffee bean powder (ANM and AM, 50 g each) was extracted with 80% aqueous methanol (3 × 50 mL). The extract was concentrated under vacuum in a Buchi flash evaporator (Buchi Rotavapor R-114, Flawil, Switzerland) to obtain an aqueous solution. The solution thus obtained was diluted to 3 times its volume with distilled water and then passed through an Amberlite XAD-2 column according to the procedure reported by Gunata et al. (*13*). After removal of sugars and free terpenes by elution with water and diethyl ether, respectively, aroma glycosides were eluted with methanol. The methanol eluate was evaporated to dryness, dissolved in distilled water, and then washed three times with an equal volume of diethyl ether to remove traces of free volatiles and other impurities. The aqueous solution containing the aroma glycosides was concentrated and then subjected to acid hydrolysis (1 N HCl, 1 h, 100 °C). The hydrolysate containing free terpenes was extracted into diethyl ether. The organic layer was washed free of acid and dried over sodium sulfate, and the solvent was removed under a slow stream of nitrogen. The isolate was subjected to GC-MS analysis under the same conditions as above. The remaining aqueous solution was freeze-dried and the residue subjected to acetylation using pyridine/acetic anhydride (1:1) overnight at room temperature. Solvent was removed under vacuum, and the residue was dissolved in chloroform. GC-MS analysis of this solution under identical conditions as above showed the presence of glucose and fructose as the major sugar residue. Except for minor amounts of galactose, no other constituents could be detected.

Extraction of Caffeine and Chlorogenic Acids. Coffee beans (50 g) were finely powdered in a Moulinex coffee grinder (model 684) and soaked in 80% methanol (100 mL) for 2 h. The slurry obtained was extracted in a homogenizing blender at high speed for 5 min and filtered under suction. The residue was then re-extracted with 80% aqueous methanol (2 × 100 mL) as mentioned above until the filtrate was colorless. The filtrate obtained was pooled and then concentrated as above. The aqueous solution (~45 mL) obtained after the removal of methanol was washed with diethyl ether (3 × 10 mL) in a separating funnel to remove unwanted lipids. The compounds of interest were then extracted with chloroform (3 × 10 mL). The chloroform layer was washed and evaporated to dryness under vacuum. The residue thus obtained was made up to 1% solution in chloroform. The chloroform solution was stored in a deep freezer (−30 °C) until further analysis.

High-Performance Liquid Chromatography (HPLC) Analysis. HPLC analysis was carried out on a Pharmacia LKB-LCC 2252 HPLC instrument provided with a UVW monitor 2141. Samples (5 μL) were analyzed on a C-18 column (250 × 4.6 mm, particle size = 10 μm, Shandum Scientific Ltd.) using a linear gradient from 100% solvent A (water/acetic acid, 95:5) to 100% solvent B (methanol/acetic acid, 95:5) over a period of 65 min at a flow rate of 1 mL/min (*14*). The peaks were detected at 280 nm. Compounds of interest were identified by comparing their retention times (t_R) with those of standard compounds under the same HPLC conditions.

Quantification of Caffeine, Chlorogenic Acid, and Caffeic Acid. Estimation of caffeine, chlorogenic acid, and caffeic acid content in various samples was carried out by external standard method using authentic standards. In the case of chlorogenic acid and caffeic acid, aliquots of each of the standard sample ranging in concentration from 1 to 10 μg were injected into the HPLC column under the same experimental conditions as above. Concentrations of caffeine injected, however, ranged from 10 to 100 μg. A plot of concentration (μg) versus peak area (mm^2) was used to obtain a standard curve. The contents of the above compounds in various samples were calculated from the standard curve and expressed as grams per 100 grams of green berries.

Data Analysis. All data presented are the mean of three independent determinations. Statistical analysis was done using a paired *t* test and ANOVA (Micrococal Origin 4.1 software).

Table 1. Concentration of Volatile Compounds Identified in Different Samples of Coffee Beans by GC-MS[a]

Sl no.	retention index	compound identified	concn of volatiles (μg/kg of green beans) nonmonsooned (ANM)	nonmonsooned, irradiated (ANMI)	monsooned (AM)	monsooned, irradiated (AMI)	irradiated, monsooned (AIM)
1	930	3-methylbutanoic acid	22.38 ± 0.12	3.12 ± 0.09			
2	1018	phenylacetaldehyde	16.76 ± 0.10	18.12* ± 0.14	4.18 ± 0.11	8.57 ± 0.09	9.64 ± 0.10
3	1075	(*E*)-2-nonenal	9.57 ± 0.09	9.39* ± 0.12	9.51* ± 0.08	10.25 ± 0.11	13.43 ± 0.07
4	1089	3-isopropyl-2-methoxypyrazine	3.75 ± 0.16	3.73* ± 0.13	2.98 ± 0.13	2.97 ± 0.11	2.93 ± 0.08
5	1176	3-isobutyl-2-methoxy pyrazine	<0.5*	<0.5*	<0.5*	<0.5*	<0.5*
6	1270	*trans*,*trans*-2,4-decadienal	4.21 ± 0.09	4.28* ± 0.12	3.91* ± 0.11	3.83 ± 0.12	3.80 ± 0.08
7	1311	4-vinylguaiacol	30.76 ± 0.32	31.72* ± 0.29	76.20 ± 0.33	78.72 ± 0.38	114.30 ± 0.43
8	1335	isoeugenol			8.57 ± 0.15	8.54 ± 0.12	8.56 ± 0.16
9	1363	β-damascenone	3.92 ± 0.13	3.91* ± 0.07	3.83* ± 0.11	2.92 ± 0.09	3.93 ± 0.11

[a] Mean value ± standard error, n = three independent determinations. An asterisk (*) indicates the mean is not significantly different from nonmonsooned sample at 0.05 level ($p < 0.05$).

RESULTS AND DISCUSSION

An increased rate of reaction occurring during monsooning was observed when green coffee beans were subjected to γ-radiation prior to the monsooning process. The rate of monsooning increased with radiation dose with optima at 5 kGy. At this dose the berries could be kept free of insect infestation and microbial contamination without producing any perceivable changes in their sensory properties either immediately after irradiation or during storage. Hence, 5 kGy was used as the radiation dose in the present study.

Volatile Essential Oils. Green coffee beans are generally devoid of any appealing aroma or flavor. However, they possess a large number of volatiles that undergo changes during roasting, resulting in pleasant aroma characteristics. Monsooning of green coffee results in a speciality coffee having a spicy aroma (*15*).

Steam distillation of different green coffee bean samples yielded a pale yellow oil with a characteristic green, earthy, and peasy note. The oil yields (w/w) obtained from ANM, AM, AIM, ANMI, and AMI were found to be 0.013 ± 0.004, 0.009 ± 0.0005, 0.043 ± 0.013, 0.01 ± 0.0007, and 0.164 ± 0.012%, respectively. The lower yield of oil in monsooned coffee could be explained by its higher moisture content (22 ± 1.2%) and hence a lower bulk density compared to nonmonsooned coffee (10−12%). Irradiation had no effect on the oil yield of nonmonsooned coffee (means not significant at $p < 0.05$ and 0.01). The higher oil yield obtained in AIM and AMI samples could be attributed to the greater extractability of essential oil constituents as a result of increased breakdown of polysaccharides and starch in high moisture containing monsooned coffee when subjected to radiation processing.

It is known that SDE when carried out at normal atmospheric pressure can give rise to artifacts. However, most of the potent odorants known to contribute to the aroma of green coffee beans (*2*) such as 3-isopropyl-2-methoxypyrazine, 3-isobutyl-2-methoxypyrazine, 4-vinylguaiacol, β-damascenone, (*E*)-2-nonenal, *trans*,*trans*-2,4-decadienal, phenylacetaldehyde, and 3-methylbutyric acid were identified in all of the samples analyzed in the present study. **Table 1** lists the major aroma active constituents identified by GC-MS. The content of most of these compounds also matched literature values wherein a milder extraction technique such as vacuum distillation was used (*2*).

Methoxypyrazines are widespread in the plant kingdom and are known to possess the characteristic note of nonmonsooned green coffee beans. 3-Isobutyl-2-methoxypyrazine, the character impact compound of bell peppers, has a low threshold in water of 1 part in 10 (*12*) and hence is a powerful odorant effective even in low amounts (*16*). By aroma extract dilution analysis (AEDA) and odor activity value (OAV) measurements Czerny and Grosch (*2*) have shown that the aroma of raw coffee (nonmonsooned) is primarily caused by 3-isobutyl-2-methoxypyrazine. Vizthum et al. (*4*), on the basis of high-resolution gas chromatography−olfactometry, attributed the characteristic green coffee smell to 3-isobutyl-2-methoxypyrazine and its isopropyl derivative. Thus, the peasy and earthy notes exhibited by the volatile oils isolated from different samples in the present study could be ascribed to the presence of the above two pyrazines. However, as noted in **Table 1** the oil was characterized by a very low content of 3-isobutyl-2-methoxypyrazine. This could possibly be attributed to the characteristic of Indian green coffee. Lee and Shibamoto (*17*) have also noted a similar trend in oils from Hawaiian green coffee beans that were devoid of heterocyclic compounds other than 3-isopropyl-2-methoxypyrazine. Besides these two major aroma active constituents, other components are also known to play a role in the overall aroma of nonmonsooned green coffee. (*E*)-2-Nonenal at low concentrations imparts a fresh-brewed woody character and tends to reduce the harsh acidic and astringent notes of coffee (*18*, *19*). This aldehyde, along with *trans*,*trans*-2,4-decadienal, another odor active constituent with a peanuty flavor (*20*), is formed by oxidative degradation of unsaturated fatty acids occurring in nonmonsooned coffee. 4-Vinylguaiacol (spicy odor), β-damascenone (honey-like fruity odor), phenylacetaldehyde (honey-like odor), and 3-methylbutyric acid with a sweaty odor are also reported (*2*) as important odor active compounds of green nonmonsooned coffee and identified in this study. Although the content of 4-vinylguaiacol was found to be lower than literature values (*2*), that of β-damascenone was far higher than reported (*2*). These quantitative differences could possibly be due to varietal variation as explained above. Guyot et al. (*5*) have shown the strong fruity bynote in raw coffee to be contributed by ethyl 2-methylbutyrate and isoamyl acetate. These compounds were, however, not detected in the present study. Green nonmonsooned coffee beans are reported (*21*, *22*) to contain appreciable amounts of hydrocarbons, mainly lower alkanes and alkenes, volatile esters, and terpenes. Many of these components were also detected in the volatile oils under study. However, these constituents were present in minute amounts and hence not included in **Table 1**.

Significant quantitative differences could be noted in the identified constituents (**Table 1**) between the various oils under study. Considerable decreases in the contents of 3-methylbutanoic acid and phenylacetaldehyde were clearly discernible (**Table 1**) in monsooned coffee compared to the nonmonsooned sample. Monsooned coffee samples were characterized by the absence of 3-methylbutanoic acid. 3-Isopropyl-2-methoxypyrazine also showed a decreasing trend. The contents of (*E*)-2-

Table 2. Volatile Compounds in Glycosidically Bound Fraction from Arabica Nonmonsooned and Arabica Monsooned Coffee

compound	retention index	relative area % ANM	relative area % AM
3-methylbutanoic acid	930	1.33	
2-ethylhexanol	1020	1.38	
4-vinylguaiacol	1311	0.97	
isoeugenol	1335	0.44	
caffeic acid	1478	11.08	9.21
2-methylisoborneol[b]	1489	0.50	

[a] Data are the average of three independent determinations. [b] Identified by comparing mass fragmentation pattern with that of standard spectra available in the spectral library; standard not available.

nonenal, *trans*,*trans*-2,4-decadienal, and β-damascenone were, however, not affected during the monsooning process. Interestingly, a considerable increase in phenolic compounds such as 4-vinylguaiacol and isoeugenol could be observed in the monsooned beans. In fact, isoeugenol could be hardly detected in ANM samples. Czerny and Grosch (*2*) have reported a masking of peasy odor of 3-isobutyl-2-methoxypyrazine by other odor active components formed during the roasting process, thus imparting a pleasant odor note to roasted coffee. Furthermore, a strong link between raw material composition and the final flavor profile of roasted material was noted by Holscher (*23*). Thus, the lowering in content of methoxypyrazines and a concomitant increase in phenolic compounds with high flavor dilution values may result in a masking of the peasy note, resulting in the dominance of a pleasant spicy aroma that characterizes commercial roasted monsooned coffee. Radiation treatment increased the content of individual volatile principles in AIM and AMI samples compared to AM samples, in most cases as a result of the greater extractability of essential oil constituents for reasons explained earlier.

Aroma Glycosides. Aroma glycosides are odorless nonvolatile flavor precursors ubiquitously distributed in the plant kingdom (*24*). During maturation, processing, or storage these compounds are able to release free aroma compounds by enzymatic or acid hydrolysis (*24*). The free aroma compounds thus released can enhance/modify the overall flavor profile. Galactosidase enzymes from green coffee beans are known to hydrolyze a broad spectrum of glycosidic substrate (*25*). Due to the higher moisture content in AM than in ANM samples, an increased enzymatic activity including galactosidase activity is expected in AM beans. It was therefore of interest to determine the nature of aroma glycosides in ANM and AM coffee. **Table 2** lists the volatile compounds identified in the glycosidically bound fraction of the above samples. 3-Methylbutanoic acid, 2-ethylhexanol, 4-vinylguaiacol, isoeugenol, and 2-methylisoborneol are the major volatile constituents identified in ANM samples. Interestingly, none of these compounds could be detected in AM beans, suggesting their release from glycosidic precursors during the monsooning process. The increase in content of isoeugenol and 4-vinylguaiacol during monsooning as noted above could thus be explained. 3-Methylbutanoic acid, 2-ethylhexanol, and 2-methylisoborneol were, however, not detected in free volatiles of AM beans. Weckerle et al. (*26*) have earlier reported the presence of 3-methylbutanoyl-6-*O*-α-D-glucopyranosyl-β-D-fructofuranoside in Arabica green coffee beans. Linalool and 3-methylbut-2-enyl disaccharides have also been isolated and identified in the green beans (*26, 27*). However, the latter two compounds have not been detected in the present study. 2-Methylisoborneol, which imparts a key earthy note to Robusta coffee, is often used as a marker to distinguish Arabica and Robusta coffees (*28*). It is therefore surprising to note the occurrence of the glycosidic precursor of this compound in Arabica coffee. Interestingly, caffeic acid was identified as a major component in the bound fraction of both ANM and AM samples. The presence of glycosidic precursors of cinnamic acids has earlier been reported in several fruits (*29*). A high content of fatty acids accounting for almost 80% of the chromatogram could be noted in this fraction in the present study (data not shown in **Table 2**). Palmitic acid (48%) and methyllinolelaidate (25%) were the major acids identified. This could possibly be due to incomplete removal of these compounds during the extraction process. Fatty acids have, however, been reported in the glycosidic fraction of many fruits.

Table 3. Total Content of Caffeine, Chlorogenic Acid, and Caffeic Acid (Grams per 100 g) in Different Samples of Arabica Coffee Beans[a]

sample	caffeine	chlorogenic acid	caffeic acid
ANM	1.21 ± 0.05	0.28 ± 0.02	0.0
AM	1.85 ± 0.06	0.027 ± 0.002	0.014 ± 0.004
AIM	1.93 ± 0.07	0.019 ± 0.003	0.027 ± 0.005
ANMI	1.80 ± 0.06	0.092 ± 0.003	0.0
AMI	1.95 ± 0.07	0.033 ± 0.004	0.016 ± 0.003

[a] Mean value $\pm$ standard error, n = three independent determinations. Mean significantly different at $p < 0.05$ between different samples (within columns).

Nonvolatile Constituents. The HPLC profile of the chloroform extract of different samples of green coffee beans is shown in **Figure 1**, and the distribution (g/100 g) of caffeine, chlorogenic acid, and caffeic acid contents is summarized in **Table 3**. The caffeine content of dry green Arabica coffee beans has been reported to be in the range of 0.58−1.7% with an average value of ~1.16% (*19*). Caffeic acid, chlorogenic acid, and caffeine contents of green coffee beans were recently estimated by Sakakibara et al. (*30*) and found to be 166, 698, and 4032 μmol/100 g, respectively, on a fresh weight basis. The results obtained here are thus in agreement with the reported literature values.

Increases in caffeine content by 53 and 60% were recorded in AM and AIM samples, respectively, compared to ANM beans. Radiation treatment of nonmonsooned samples (ANMI) also resulted in a 50% increase in caffeine content. The increase observed could possibly be due to the higher extractability of this compound as a result of enhanced water uptake in AM samples as well as increased breakdown of polysaccharides and starch in radiation-treated samples. An increase in caffeine content by only 6.5% was, however, noted in AMI beans compared to the AM samples in the present study. Caffeine has a distinct bitter taste but is known to account for only ~10% of the perceived bitterness in coffee (*31*). Changes in this compound may not therefore significantly alter the bitterness of monsooned coffee.

Chlorogenic acid is a major phenolic compound responsible for the astringency of green coffee. This compound, an ester of caffeic acid, is also known as caffoylquinic acid with the aglycon portion made up of caffeic acid. 3-Caffeoylquinic acid is the major chlorogenic acid present in coffee. This acid accounts for 60.4% of the total chlorogenic acids in coffee (*32*). 4-Caffeoylquinic acid (6.04%), 5-caffeoylquinic acid (30.2%), 3,4-dicaffeoylquinic acid (0.03%), 3,5-dicaffeoylquinic acid (2.72%), and 4,5-dicaffeoylquinic acid (0.302%) are the other chlorogenic acids reported to be present in coffee (*32*). Caffeic acid, a bitter-tasting compound, is normally present only in trace amounts in wet processed Arabica coffee (*33*). This was also

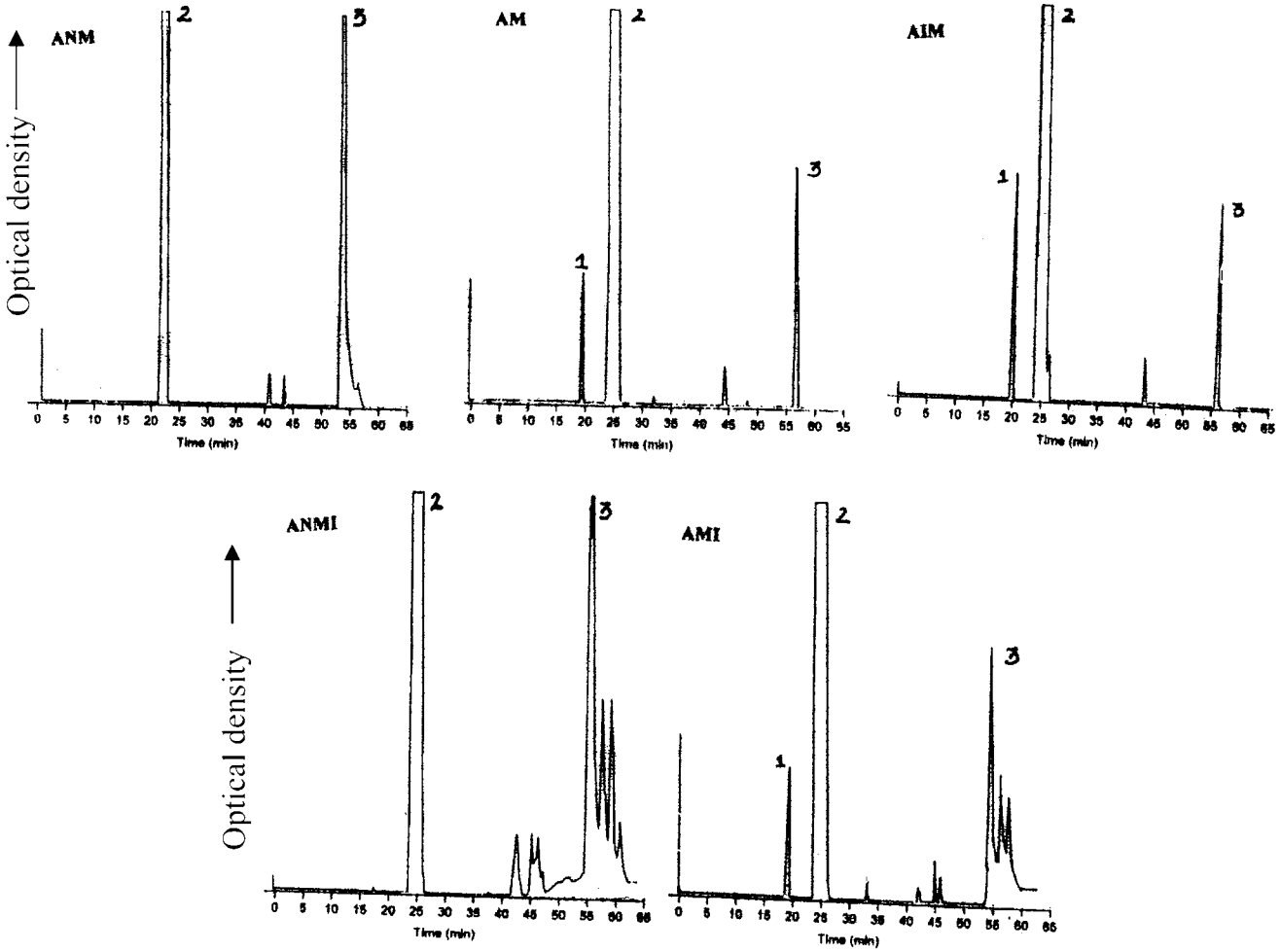

Figure 1. HPLC profile of chloroform extract of different coffee bean samples: 1, caffeic acid; 2, caffeine; 3, chlorogenic acid (abbreviations as given in the text).

noted in the present study, where nonmonsooned green beans were found to be devoid of caffeic acid (**Figure 1**).

A substantial decrease in chlorogenic acid and a corresponding increase in the caffeic acid content were recorded in AM and AIM beans in the present study. Hydrolysis of chlorogenic acid into caffeic acid during monsooning could thus be inferred. This trend was also noted in AMI samples. Degradation of chlorogenic acid during steaming of green beans as a consequence of increased water uptake was earlier reported by Maier (*34*). Liberation of free phenolic groups glycosidically bound as esters or ethers during sun-drying of coffee beans as a result of hydrolysis have also been reported (*32*). Interestingly, caffeic acid was not detected in radiation-processed nonmonsooned samples despite a substantial decrease in chlorogenic acid content in this sample (**Figure 1**). This suggests that radiation processing degrades chlorogenic acid rather than hydrolyzing it to produce caffeic acid. Deshpande and Aquilar (*35*) have, however, observed that radiation treatment of coffee beans does not affect the chlorogenic content. Thus, under high moisture conditions as prevalent in AM beans, degradation of polysaccharides during radiation treatment may possibly effect closer interaction of enzymes with the substrate, resulting in increased hydrolysis of chlorogenic acid to caffeic acid. Hydrolysis of chlorogenic acid to caffeic acid on standing for some hours on a hot plate and a consequent increase in bitterness in coffee brew was reported earlier (*20*). An appreciable decrease in astringent chlorogenic acid and an increase in caffeic acid may thus result in increased bitterness of monsooned coffee.

Thus, the whole process of monsooning may be attributed to the enzymatic activities taking place in the coffee bean during the monsooning. Increased bitterness in monsooned coffee could be associated with the enhanced hydrolysis of chlorogenic acid to caffeic acid, whereas the characteristic spicy aroma of the processed product can be attributed to release of isoeugenol and 4-vinylguaiacol from its glycosidic precursors. Irradiation treatment might be playing a significant role by breaking down complex sugars or polysaccharides, facilitating an increase in moisture uptake and increased enzymatic activity. A closer interaction of the enzymes with the substrates may thus result in the release of aglycons such as caffeic acid and isoeugenol that impart an exotic flavor to monsooned coffee. Further work relating to the role of enzymes in the monsooning process as well as correlation of chemical changes in monsooned coffee with sensory parameters needs to be investigated.

ACKNOWLEDGMENT

We thank Shri V. N. Sawant for technical help rendered during the course of this work.

LITERATURE CITED

(1) Ahmad, R. Trivia and Science of Monsooned Coffee. *Indian Coffee LXIV* **2000**, *4*, 3−8.

(2) Czerny, M.; Grosch, W. Potent odorants of raw Arabica coffee. Their changes during roasting. *J. Agric. Food Chem.* **2000**, *48*, 868−872.

(3) Semmerlroch, P.; Grosch W. Studies on character impact odorants of coffee brews. *J. Agric. Food Chem.* **1996**, *44*, 537−543.
(4) Vitzthum, O. G.; Werkhoff, P.; Ablanque, E. Fluechtige Inhaltsstoffe des Rohkaffees. In *7th International Conference on Coffee*, Hamburg, 1975; ASIC: Paris, France, 1976; pp 115−123.
(5) Guyot, B.; Cros, E.; Vincent, J.-C. Characterization and identification of the compounds of the volatile fraction of a sound green Arabica coffee and malodorous Arabica coffee. In *10th International Conference on Coffee*, Salvador, 1982; ASIC: Paris, France, 1983; pp 253−259 (in French).
(6) Sharma, A. Scientific Aspects of Radiation Processing of Food Products. In *Seminar on Radiation Processing of Food Products*; Indian National Academy of Engineering and Department of Atomic Energy, BARC: Mumbai, India, 2000.
(7) Diehl, J. F. *Safety of Irradiated Foods*; Dekker: New York, 1990; p 4.
(8) Wilkinson, V. M.; Gould, G. W. *Food Irradiation, A Reference Guide*; Butterworth-Heinemann: Oxford, U.K., 1996; pp 46−48.
(9) Manoto, E. C.; Blanco, L. R.; Mendoza, A. B.; Resilva, S. S. Disinfestation of copra, desiccated coconut and coffee beans using gamma radiation. In *Insect Disinfestation of Food and Agricultural Products by Irradiation*, proceedings of the final research coordination meeting, Beijing, China, 1987; IAEA: Vienna, Austria, 1991; pp 105−125.
(10) Soemartaputra, M. H.; Purwanto, Z. I.; Chosdu, R.; Haryadi, R. S.; Rahayu, A. Radiation disinfestation of dry leaf tobacco and coffee beans. In *Insect Disinfestation of Food and Agricultural Products by Irradiation*, proceedings of the final research coordination meeting, Beijing, China, 1987; IAEA: Vienna, Austria, 1991; pp 153−168.
(11) Diaz, M.; Loaharanu, S.; Vokal, L. Changes in flavour and taste of irradiated coffee beans. In *Proceedings of an IAEA/FAO/WHO Symposium*, Wageningen, The Netherlands, 1997; IAEA: Vienna, Austria, 1978; pp 539−543.
(12) Nickerson, G. B.; Likens, S. T. Gas chromatographic evidence for the occurrence of hop oil components in beer. *J. Chromatogr.* **1966**, *21*, 1−5.
(13) Gunata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J. Chromatogr.* **1985**, *331*, 83−90.
(14) Seo, A.; Morr, C. V. Improved high performance liquid chromatographic analysis of phenolic acids and isoflavonoids from soybean protein products. *J. Agric. Food Chem.* **1984**, *32*, 530−533.
(15) http:/www.ebelcoffee.cz/guide.htm; Cyber Smiths Interactive Media Publishing: Czech Republic, 2001.
(16) Murray, K. E.; Whitefield, F. B. The occurrence of 3-alkyl-2-methoxy pyrazines in raw vegetables. *J. Sci. Food Agric.* **1975**, *26*, 973−986.
(17) Lee, K. G.; Shibamoto, T. Analysis of volatile components isolated from Hawaiian green coffee beans (*Coffea arabica* L.). *Flavour Fragrance J.* **2002**, *17*, 349−351.
(18) Grosch, W. Flavour of coffee. *Nahrung* **1998**, *42*, 344−350.
(19) Parliament, T. H.; Clinton, W.; Scarpellino, R. *trans*-2-Nonenal: Coffee compounds with novel organoleptic properties. *J. Agric. Food Chem.* **1973**, *21*, 485−487.
(20) Vitzthum, O. G. Thirty years of coffee chemistry research. In *Flavor Chemistry, Thirty Years of Progress*; Teranishi, R., Wick, E. L., Hornstein, I., Eds.; Kluwer Academic: New York, 1999; pp 117−133.
(21) Merritt, C.; Robertson, D. H., Jr.; McAdoo, D. J. The relationship of volatile compounds in roasted coffee beans to their precursors. In *Procceedings of the 4th International Scientific Colloquim On Coffee*; ASIC: Amsterdam, The Netherlands, 1969; pp 144−148.
(22) Gutmann, W.; Werkhoff, P.; Barthels, M.; Vitzthum, O. G. Vergleich der Head space-Aroma profile von Arabusta-Kaffe mit Arabica- und Robusta-Sorten. In *Procceedings of the 8th International Scientific Colloquim On Coffee*; ASIC: Abidjan, 1977; pp 153−161.
(23) Holscher, W. Comparison of some aroma impact compounds in roasted coffee and coffee surrogates. In *Flavour Science, Recent Developments*; Taylor, A. J., Mottram, D. S., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1996; pp 239−244.
(24) Crouzet, J.; Chassagne, D. Glycosidically bound volatiles in plants. In *Naturally Occurring Glycosides*; Ikan, R., Ed.; Wiley: Chichester, U.K., 1999; pp 225−274.
(25) Yagi, F.; Eckhardt, A. E.; Goldstein, I. J. Glycosidases of Ehrlich ascites tumor cells and ascitic fluid-purification and substrate specificity of α-*N*-acetylgalactosaminidase and α-galactosidases: comparison with coffee beans. *Arch. Biochem. Biophys.* **1990**, *280*, 61−67.
(26) Weckerle, B.; Gati, T.; Tohl, D.; Schreier, P. 3-Methyl butanoyl and 3-methyl-but-2-enyl disaccharides from green coffee beans (*Coffea arabica*). *Phytochemistry* **2002**, *60*, 409−414.
(27) Weckerle, B.; Toth, G.; Schreier, P. Linalool disaccharides as flavour precursors from green coffee beans (*Coffea arabica*). *Eur. Food Res. Technol.* **2003**, *216*, 6−10.
(28) Blank, I.; Grosch, W. On the role of (−)-2-Methylisoborneol for the aroma of Robusta coffee. *J. Agric Food Chem.* **2002**, *50*, 4653−4656.
(29) Latza, S.; Gansser, D.; Berger, R. G. Carbohydrate esters of cinnamic acid from fruits of *Physalis peruviana, Psidium guajava* and *Vaccinium vitis-idaea*. *Phytochemistry* **1996**, *43*, 481−485.
(30) Sakakibara, H.; Honda, Y.; Nakagawa, S.; Ashida, H.; Kanazawa, K. Simultaneous determination of all polyphenols in vegetables, fruits, and teas. *J. Agric Food Chem.* **2003**, *51*, 571−581.
(31) Voilley, A.; Sauvageot, F.; Durand, F. Influence sur I' amartume d' café boisson, de quelques parametres d' extraction. In *Procceedings of the 8th International Scientific Colloquim On Coffee*; ASIC: Abidjan, 1977.
(32) Spiller, M. A. The chemical components of coffee. In *Caffeine*; Spiller, G. A., Ed.; CRC Press: New York, 1998; pp 97−161.
(33) Feldman, R. S.; Ryder, W. S.; Kung, J. T. Importance of nonvolatile compounds to the flavor of coffee. *J. Agric. Food Chem.* **1969**, *17*, 733−739.
(34) Maier, H. G. Status of research in the field of non-volatile coffee components. In *Internationalen Wissenschaftlichen Kolloquim ueber Kaffee*, Montpellier, 1993; ASIC: Paris, France, 1994; pp 567−576.
(35) Deshpande, S. N.; Aquilar, A. A. Effects of roasting temperatures and γ-irradiation on the content of chlorogenic acid, caffeic acid and soluble carbohydrates of coffee. *Int. J. Appl. Radiat. Isot.* **1975**, *26*, 656−661.

Received for review June 2, 2003. Revised manuscript received October 4, 2003. Accepted October 5, 2003. We thank the Board of Research in Nuclear Science (BRNS), Department of Atomic Energy, Government of India, for financial support (Section 2000/35/1 BRNS 1-6-2000) provided during the course of this study.

JF030408Q