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Food Chemistry 82 (2003) 397–402

Food Chemistry

www.elsevier.com/locate/foodchem

Profile and levels of bioactive amines in green and roasted coffee

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Received 1 October 2002; accepted 18 November 2002

Abstract

The profile and levels of bioactive amines in green and roasted coffee were investigated. *Coffea arabica* L., variety 'catuaí vermelho', was harvested in São Sebastião do Paraíso, state of Minas Gerais, Brazil. The green coffee was roasted at two degrees—American and French (300 °C for 6 and 12 min, respectively). The samples were analysed for bioactive amines. Roasted samples were also analysed for moisture content, water activity and *L*a*b** colour characteristics. Total amine levels in green coffee ranged from 3.03 to 4.44 mg/100 g. The predominant amines in green coffee were serotonin and putrescine, followed by spermidine and spermine. The degree of roasting did not affect moisture content or water activity of the coffee bean. Hue angle and saturation were higher for American than for French coffee. The profile and levels of bioactive amines in roasted coffee differed significantly from green coffee. The prevailing amine in roasted coffee was serotonin, followed by spermidine. Putrescine and spermine were not detected in roasted coffee. The presence of agmatine was detected in French roasted coffee. American roasted coffee had lower amine levels than French, which indicated that, the stronger the degree of roasting, the higher were the total levels of amines.

Keywords: Bioactive amines; Coffee; Roasting; Serotonin; Agmatine

1. Introduction

Bioactive amines are organic bases of low molecular weight which participate in normal metabolic processes in living tissues (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Lima & Glória, 1999). They can be classified as polyamines and biogenic amines. Polyamines such as putrescine, spermidine and spermine, have been found in all higher plants. They are formed by de novo synthesis (Fig. 1) and are involved in important physiological processes, such as fruit growth and development (Esti, Volpe, Massignan, Compagnone, La Notte, & Palleschi, 1998). Enhanced polyamine biosynthesis often occurs concurrently with growth. In view of the universal distribution and ability to interact with nucleic acids, they are considered to be of fundamental importance in growth processes (Smith, 1981). Polyamines may also have functions in cell differentiation and membrane stabilization (Tabor & Tabor, 1976). They can bind to membranes, prevent lipid peroxidation and scavenge free radicals involved in the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene (Krebsky, Geuns, & De Proft, 1999). In bacteria and plants, putrescine is also apparently involved in mechanisms for cell pH control (Smith, 1981).

The biogenic amines (histamine, putrescine, tyramine, tryptamine, 2-phenylethylamine and cadaverine) can be naturally present in some plant tissues. The distribution of these compounds has been a useful tool in chemotaxonomic studies. The metabolic function of these amines in plants is not well established, though they are the precursors of a series of alkaloids. Like the uncommon amino acids, the biogenic amines may benefit the plant which produces them by acting as deterrents to insect predators and foraging animals and, for this reason, they may be of agricultural significance (Smith, 1977). The levels of biogenic amines may vary due to

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0308-8146/03/$ - see front matter
doi:10.1016/S0308-8146(02)00560-5

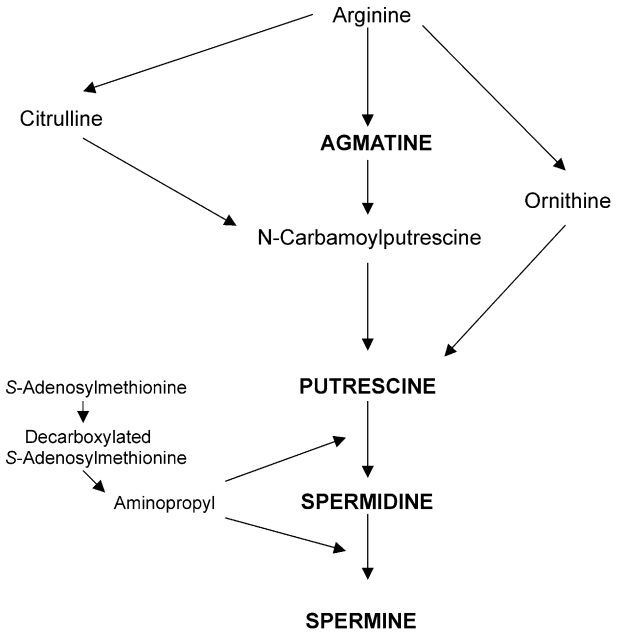

Fig. 1. Biosynthesis of polyamines (Lima & Glória, 1999).

many factors, including degree of ripening, storage conditions, variety of plant and conditions of growth (Udenfriend, Lovenberg, & Sjoerdsma, 1959). Biogenic amines can also be formed during storage or processing of the products by thermal or bacterial enzymatic decarboxylation of free amino acids (Fig. 2) (Halász et al., 1994; Lima & Glória, 1999; Udenfriend et al., 1959).

Few papers have reported the bioactive amines content of fruits and vegetables (including coffee) or the influence of processing on bioactive amine levels. Brazil is the largest worldwide coffee producer. Several studies have been undertaken to understand and improve coffee quality. The influence of roasting on the chemical composition of coffee has been studied. Some amino acids (arginine, cystein, lysine, serine) and carbohydrates (sucrose, arabinogalactan), volatile, nonvolatile and phenolic acids are known to decrease during roasting (Feldman, Ryder, & Kung, 1969). Amorin, Basso, Crocomo, and Teixeira (1977) investigated the levels of polyamines in green and roasted coffee. According to their work, three polyamines were detected in green coffee: putrescine, spermine and spermidine. The biogenic amines were not investigated. After roasting the beans at 240 °C for 12 min, the only polyamine detected by these investigators was putrescine. However, putrescine is degraded during boiling and commercial canning (Glória, Daeschel, Craven, & Hilderbrand, 1999; Yen, 1992), which are mild heat treatments compared to roasting.

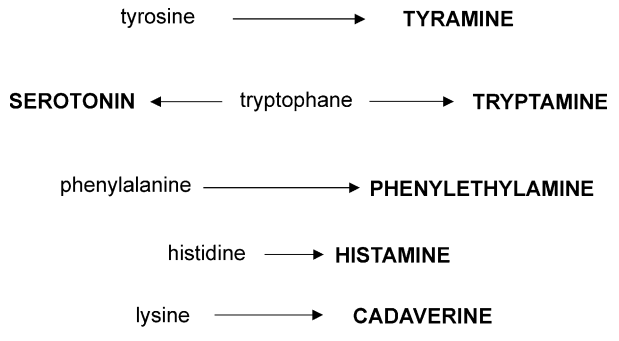

Fig. 2. Synthesis of biogenic amines (Halász et al., 1994).

The object of the present work was to investigate the profile and levels of bioactive amines—polyamines and biogenic amines—in green coffee and to evaluate the influence of two degrees of roasting—American and French—on bioactive amines levels and profile.

2. Materials and methods

2.1. Materials

Coffee samples of *Coffea arabica* L., variety 'catuaí vermelho' cultivated in the experimental farm of Empresa Brasileira de Pesquisa Agropecuária de Minas Gerais—EPAMIG São Sebastião do Paraíso, state of Minas Gerais, Brazil, were used. Coffee was harvested, sun-dried, dehulled and taken to the laboratory. Some of the samples were submitted to two different degrees of roasting (Probat roaster, Germany): 300 °C for 6 min (American roasting) and 300 °C for 12 min (French roasting). The experiment was performed in triplicate.

Both green and roasted samples were analysed for bioactive amines. Roasted samples were also analysed for moisture content, water activity and CIE $L^*a^*b^*$ colour characteristics. Each analysis was performed in duplicate.

Bioactive amine standards were purchased from Sigma Chemical Co. (St. Louis, MO, EUA). They included putrescine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, agmatine sulphate, cadaverine dihydrochloride, serotonin hydrochloride, histamine dihydrochloride, tyramine, tryptamine and 2-phenylethylamine dihydrochloride.

All reagents were of analytical grade, but HPLC reagents were LC grade. Ultrapure water was obtained from a Milli-Q System (Millipore Corp., Milford, MA, USA). The mobile phases were filtered in HAWP and HVWP membranes, used, respectively, for aqueous and organic solvents (47 mm diameter and 0.45-μm pore size, Millipore Corp., Milford, MA, USA).

2.2. Methods of analysis

2.2.1. Determination of bioactive amines

Amines were extracted from samples (5 g) with 7 ml 5% trichloroacetic acid (TCA). After agitation for 5 min in a vortex mixer, the slurry was centrifuged at 10,000×*g* at 4 °C, and the supernatant was collected.

The solid residue was extracted twice more with volumes of 7 and 6 ml of TCA. Supernatants were combined and filtered through 0.45 μm membranes. The amines were separated by ion-pair reverse phase HPLC and quantified fluorimetrically after post-column derivatization with *o*-phthalaldehyde (OPA), as described by Vale and Glória (1997).

Liquid chromatography was performed using a LC-10AD system connected to a RF-551 spectrofluorimetric detector at 340 and 445 nm of excitation and emission, respectively, and to a CBM-10AD controller (Shimadzu, Kyoto, Japan). A reversed-phase μBondapak C18 column, 300×3.9 mm i.d., 10 μm, was used with a μBondapak C18 guard-pak insert (Waters, Milford, MA). The mobile phases were: A, solution of 0.2 M sodium acetate and 15 mM 1-octanesulfonic acid sodium salt, adjusted to pH 4.9 with acetic acid, and B, acetonitrile. The flow rate was set at 0.6 ml/min and the gradient was: 13 min at 11% B, 19 min at 29% B, 24 min at 11% B, and 55 min at 11% B. The post-column derivatization reagent was delivered at 0.4 ml/min. It consisted of 1.5 ml Brij-35, 1.5 ml mercaptoethanol and 0.2 g OPA dissolved in a 500 ml solution of 25 g boric acid and 22 g KOH (pH adjusted to 10.5 with 3% KOH). The column and the post-column reaction apparatus were at room temperature (23±1 °C). The identification of amines was performed by comparison of retention time of amines in samples to standard solutions and also by addition of the suspected amine to the sample. Amine levels were calculated by direct interpolation in a calibration curve.

2.2.2. Moisture content

Moisture content was measured according to the Karl Fisher method (IAL, 1985).

2.2.3. Water activity

Water activity was determined using the water activity analyser TESTO—650-Testo Gmbh & Co. (Lenzkirch, Germany).

2.2.4. CIE L*a*b* colour characteristics

Colour characteristics were determined using a Colortec PCM (Clinton, USA) according to the methodology described by Stark, Fawcet, Tucker, and Weatherall (1996). The illuminant used was D65 (day light). Determinations of L^* (luminosity), a^* (intensity of green to red) and b^* (intensity of yellow to blue) were performed in triplicate. From these data, saturation [$C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$] and hue angle [$h = \tan^{-1} b^*/a^*$] were calculated.

2.3. Statistical analysis

To investigate the influence of roasting on the moisture content, water activity, colour characteristics and bioactive amine levels, the data was submitted to analysis of variance and the means were compared by the Duncan test at 5% probability.

3. Results and discussion

3.1. Types and levels of bioactive amines in green coffee

Among the amines investigated, putrescine, spermidine, spermine and serotonin were detected in green coffee. The presence of spermine, spermidine and putrescine was also detected in green coffee beans (*Coffea arabica* L. var. Mundo Novo) by Amorim et al. (1977). The presence of spermine and spermidine in coffee is expected as they are widely distributed in plants and play important roles in cellular division and growth (Bardocz, 1995; Halász et al., 1994). The presence of putrescine is also expected since it is a precursor of spermidine and spermine (Lima & Glória, 1999).

Serotonin, which was also detected in coffee, can be found in many vegetable species, such as banana, tomato, avocado, eggplant, plum (Adão, 1998; Smith, 1977; Starling, 1998; Udenfriend et al., 1959), where it plays a role in plant protection. The presence of serotonin (5-hydroxytryptamine) in coffee wax was described by Kele and Ohmacht (1996) and in Astra coffee by Stranc (1993). According to Kele and Ohmacht (1996), serotonin plays an important role in cerebral metabolism and it is a potential drug with different activities, for example, vasoconstrictive, antihypertonic, antiallergic, antipsychotic, antitabac. Stranc (1993) reported on a Polish coffee, called Astra, which contained 20–40 mg serotonin/100 g and was entitled to be sold as 'low irritant' coffee in Germany and Switzerland.

Total amine levels ranged from 3.03 to 4.44 mg/100 g (Table 1). Such values are lower than those found by Amorim et al. (1977) for 'Mundo Novo' coffee, which varied from 6.00 to 8.40 mg/100 g. However, they are similar to those found by Starling (1998) for different vegetables.

Serotonin and putrescine were the prevailing amines in green coffee, followed by spermidine and spermine, as shown in Fig. 3. The prevalence of spermidine over spermine is in accordance with an observation by Starling

Table 1
Types and levels of bioactive amines in green coffee

Value	Amine levels (mg/100 g)				
	Putrescine	Spermidine	Spermine	Serotonin	Total
Minimum	0.91	0.51	0.42	0.96	3.03
Maximum	1.63	0.68	0.73	1.63	4.44
Mean	1.03	0.60	0.44	1.13	3.21
CV (%)	10	11	19	13	4

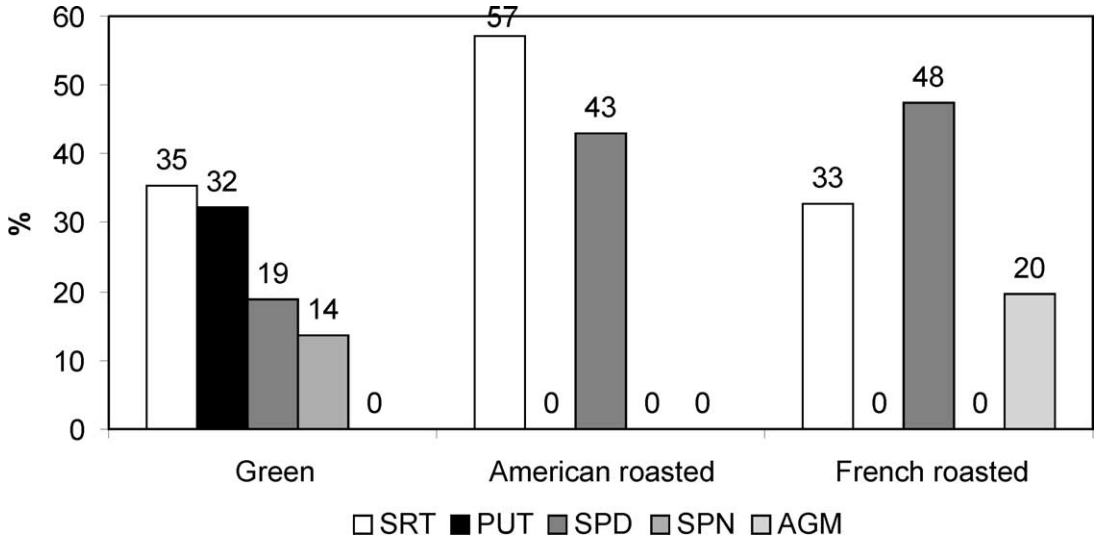

Fig. 3. Contribution of bioactive amines to total levels in green, American and French roasted coffee (SRT = serotonin, PUT = putrescine, SPD = spermidine, SPN = spermine, AGM = agmatine).

(1998) for different vegetables, in which spermidine is usually more predominant than spermine. Among the vegetables analyzed by Starling (1998), the prevalence of putrescine over spermidine was only observed for eggplant.

Comparing results from this study with those reported by Amorim et al. (1977), large differences in amine levels were observed. Such differences could be related to coffee variety (Mundo Novo×Catuaí) and also to cultivation conditions, such as, soil type, mineral deficiency, stress, and physiological stage. Potassium deficiency caused putrescine accumulation in coffee beans (Cirilo, 2002), and also in leaves of barley, radish, pea, bean and spinach (Basso & Smith, 1974). Magnesium deficiency was observed to cause putrescine accumulation in barley, pea and bean (Basso & Smith, 1974). Total polyamine levels increased during ageing of wheat embryos (Cecilia, Bagni, & Floris, 1974). Polyamine levels varied during the development and ripening of eggplant (Rodriguez, López, & Chaves, 1999) and banana (Adão, 1998).

3.2. Influence of the degree of roasting on bioactive amines in coffee

The results regarding the influence of the degree of roasting on the physico-chemical quality of coffee are described on Tables 2 and 3. The different degrees of roasting did not significantly affect moisture content or water activity, which varied from 3.70 to 3.76 g/100 g and from 0.37 to 0.40, respectively. According to França, Oliveira, and Vitorino (2002), roasting of coffee is characterized by two phases—drying and pyrolysis. In the first one, which lasts a few minutes, there is a significant decrease of moisture content with time. However, during pyrolysis, there are negligible differences on moisture levels with time. Based on these results, at 6 and 12 min of roasting, the samples had undergone dehydration and were in the pyrolysis phase.

The degree of roasting, however, significantly affected colour characteristics. American roasting produced

Table 2
Physico-chemical characteristics of American and French roasted coffee

Characteristics	Roasting	
	American	French
Water content (g/100 g)	3.70±0.47	3.76±0.38
Water activity	0.40±0.04	0.37±0.04
Color		
*L**	32.65±2.99	20.67±1.32b
*a**	11.98±1.46	7.12±1.41b
*b**	18.16±2.85	6.97±2.00b
*C*ab* (saturation)	22.08±2.53	9.90±1.69b
*h*ab* (hue angle)	56.46±6.98a	44.27±9.19a

Mean values with the same letter in the same line do not differ significantly by the Duncan test at 5% probability.

Table 3
Levels of bioactive amines in green and roasted coffee

Bioactive amine	Levels (mg/100 g) in coffee		
	Green	American roasted	French roasted
Putrescine	1.03±0.09	nd	nd
Spermine	0.44±0.08	nd	nd
Spermidine	0.60±0.07a	0.12±0.02c	0.20±0.01b
Serotonin	1.13±0.15a	0.16±0.05c	0.29±0.22b
Agmatine	nd	nd	0.12±0.05
Total	3.21±0.13a	0.28±0.06c	0.61±0.13b

Mean values with the same letter in the same line do not differ significantly by the Duncan test at 5% probability.

lighter grains (> L^*), with higher intensities of a^* and b^*, than French. Significantly higher saturations and hue angles were observed for samples submitted to American roasting than to French.

During roasting, there was a significant change in amine profile and levels. There was a total loss of putrescine and spermine. This result suggests that putrescine and spermine are sensitive to the temperature/time used during roasting. The loss of putrescine (Glória et al., 1999; Nout, Ruikes, Bouwmeester, & Beijaars, 1993; Shalaby, 2000; Yen, 1992) and spermine (Glória et al., 1999; Shalaby, 2000) during heat treatment has been described. According to Yen (1992), there were 30 and 90% losses of putrescine in straw mushroom during boiling and commercial canning, respectively. During home preparation of tempe, Nout et al. (1993) also observed that stewing (100 °C/10 min) caused some reduction of putrescine, whereas frying (170 °C/3–5 min) had stronger reducing effects on putrescine. During canning of albacore tuna at 117 °C/80 min (Glória et al., 1999), there were significant losses in the levels of spermine (62%) and putrescine (55%). During cooking of germinated legume seeds, there was total loss of putrescine and spermine (Shalaby, 2000). Considering the higher temperatures associated with roasting, a more deleterious effect from these amines would be expected.

There was a significant decrease in spermidine and serotonin levels during roasting. In the first 6 min of roasting, spermidine levels decreased to 22% of the original levels. However, at 12 min roasting, the levels had increased to 36%. Similar behaviour was also observed for serotonin: in the first 6 min of roasting, levels dropped to 15% of the initial level but increased to 28% at 12 min roasting. The loss of serotonin due to heat treatment was described by Garcia and Mariné (1983) during the frying of tomatoes. However, Nagatsu (1991) reported that serotonin could be formed by thermal decarboxylation of 5-hydroxytryptophan. The loss of spermidine during boiling of germinated legume seeds was observed by Shalaby (2000). However, Glória et al. (1999) did not find any significant difference in spermidine levels during canning of albacore tuna. These results suggest that these amines are sensitive to heat treatment. However, on prolonged exposure, there was formation of these amines, probably due to thermal decarboxylation of the precursor amino acids.

During roasting for 12 min (French style), there was formation of agmatine. Agmatine can be formed by decarboxylation of arginine, as indicated in Fig. 1 (Maretzki, Thom, & Nickell, 1969). The presence of this amino acid has been reported in green coffee at levels varying from 2.28 to 4.72 g/100 g (Feldman et al., 1969). These investigators also observed that arginine was lost during roasting, which could corroborate the findings in this study.

The profile of bioactive amines in roasted coffee is indicated in Fig. 3. In American roasted coffee, serotonin was the prevailing amine, followed by spermidine. In French roasted coffee, spermidine was the prevailing amine followed by serotonin and agmatine. Total amine levels also differed significantly, with higher levels found in green coffee, followed by French roasted and by American roasted, as indicated in Table 3.

4. Conclusion

Green coffee was characterized by the presence of four bioactive amines, including putrescine, spermidine, spermine and serotonin. Serotonin and putrescine were the prevailing amines followed by spermidine and spermine. During roasting, there was a total loss of putrescine and spermine. There was also a decrease of spermidine and serotonin. However, samples submitted to 12 min roasting—French roasting—contained significantly higher levels of spermidine and serotonin compared with 6 min—American roasting. Extensive roasting also caused agmatine to be formed. French and American roasted coffee differed from each other with respect to colour characteristics and the levels of spermidine, serotonin and agmatine.

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