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Original Article

Profile and levels of bioactive amines in instant coffee

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Abstract

The levels of ten free bioactive amines, as well as pH and color characteristics, were determined in different types and brands of instant coffee. The amines were extracted with trichloroacetic acid and quantified by ion-pair HPLC, post-column derivatization with *o*-phthalaldehyde and fluorimetric detection. Overall, nine amines were detected: serotonin, cadaverine, tyramine, spermidine, putrescine, histamine, agmatine, phenyletylamine and spermine. Tryptamine was not detected in any sample. Tyramine was present in every sample, followed by cadaverine and serotonin. Total amine levels in the dry instant coffee varied from 0.28 to 2.76 mg/100 g. Overall, serotonin was present at higher levels followed by cadaverine, tyramine and spermidine. Significantly higher tyramine levels were found in decaffeinated coffee and higher cadaverine levels were detected in decaffeinated and organic coffee. The levels of amines varied among lots of the same brand and among brands. The color characteristics varied among types of instant coffee. The pH of the beverage varied from 4.86 to 5.15, with higher levels in decaffeinated coffee. There was significant positive correlation between pH and the levels of tyramine and agmatine. Investigations are needed to ascertain the impact of these amines on coffee flavor and on human health, and to determine the factors which affect amine formation and accumulation in instant coffee.

Keywords: Instant coffee; Bioactive amines; pH; Color; Decaffeinated; Organic

1. Introduction

Coffee is one of the most popular and widely consumed beverages throughout the world due to its pleasant taste and aroma and stimulant effect. Furthermore, a number of recent studies have found beneficial health properties attributed to coffee (Yen et al., 2005; Farah et al., 2006b). Even though instant coffee was invented in 1901, it was commercially available only in 1938. Its popularity and use has increased significantly because of the ease and speed of preparation and long shelf life (Trugo and Macrae, 1984; Nogueira and Trugo, 2003).

In recent years the number of studies on the composition of coffee has increased significantly, mainly concerning phenolic acids and nitrogenous compounds such as caffeine and trigonelline. However, few studies have been undertaken on bioactive amines, which play important roles in plant development and also in human health. In plants, the amines are required for growth, they control intracellular pH, response to stress, and defense responses to pathogens, insects and predators (Eliassen et al.; 2002; Glória, 2005; Kalac and Krausová, 2005). The profile and levels of bioactive amines in a product can be used as a quality index, reflecting the quality of the raw materials or the hygienic conditions prevalent during processing (Lima and Glória, 1999). With regard to human health, some amines are required for normal development and growth, response to stress, inhibition of lipid peroxidation, stabilization of membranes, maturation of the gastrointestinal tract, whereas others are vasoactive or psychoactive (Glória, 2005; Kalac and Krausová, 2005).

Amorim et al. (1977) were the first to study the levels of free amines in coffee. However, only 25 years later, further studies were undertaken (Casal et al., 2002, 2004; Cirilo et al., 2003; Oliveira et al., 2005; Vasconcelos et al., 2007). According to these studies, green coffee was reported to contain mainly putrescine, spermidine and spermine.

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Nomenclature

AGM	agmatine
CAD	cadaverine
HIM	histamine
PHE	phenylethylamine
PUT	putrescine
SPD	spermidine
SPM	spermine
SRT	serotonin
TRM	tryptamine
TYM	tyramine

Putrescine was the prevalent amine followed by spermidine and spermine. The presence of other amines were also found in green coffee, among them serotonin (Cirilo et al., 2003; Casal et al., 2005; Vasconcelos et al., 2007), tyramine, histamine and cadaverine (Casal et al., 2002, 2004). Serotonin was found at high levels whereas tyramine, histamine and cadaverine were present at low levels. When comparing amines in *Coffea arabica* L. and *Coffea canephora* var. robusta, the levels of putrescine were lower and of tyramine were higher in the latter (Casal et al., 2002, 2004). Besides the differences on amine levels among coffee varieties, there were also significant differences on the levels of amines on samples from different origins (Casal et al., 2002), cultivation practices (Cirilo et al., 2003), sensory quality of the beverage (Amorim et al., 1977; Oliveira et al., 2005), coffee bean quality (Vasconcelos et al., 2007) and also the analytical methodology used in the extraction of the amines (Casal et al., 2004, 2005).

Studies undertaken by Oliveira et al. (2005) indicated that coffee beverages of low sensory quality (Rio) contained significantly higher levels of putrescine compared to high sensory quality (Soft). Furthermore, histamine and tryptamine were also detected in coffee beverages of low sensory quality. According to Vasconcelos et al. (2007), some amines which are not commonly detected in coffee can be found in defective coffee beans: histamine was detected in black, immature and sour beans; tryptamine was found in immature and sour beans; and cadaverine was detected in black beans. Based on the fact that coffee beverages of low sensory quality can contain a higher percentage of defective beans, they can also contain the amines which are characteristic of defective beans. Therefore, uncommon amines in green coffee could indicate the presence of defective beans and, consequently, a beverage of low sensory quality.

During roasting of coffee, there is a significant decrease on the levels of amines. The higher the temperature or the time used during roasting the lower the levels of amines, up to a point in which no amines are detected (Amorim et al., 1977; Cirilo et al., 2003; Casal et al., 2004, 2005). However, coffee beans submitted to a light roast contain low levels of serotonin (Cirilo et al., 2003; Casal et al., 2004, 2005; Vasconcelos et al., 2007). Furthermore, during roasting of coffee for 12 min at 300 °C, Cirilo et al. (2003) detected the formation of agmatine. It was suggested that the formation of this amine was possible by decarboxylation of arginine or by the release of this amine from conjugated forms (Cirilo et al., 2003; Casal et al., 2005).

No information was found on the levels of bioactive amines in instant coffee. During production of instant coffee, the grains are roasted, submitted to extraction under pressure at high temperatures (180 °C), and the extract is dehydrated by spray or freeze drying (Nogueira and Trugo, 2003). Therefore, due to the scarcity of data on the levels of bioactive amines in instant coffee and the relevance of these compounds to food quality and human health, the objective of this study was to investigate the profile and levels of bioactive amines in instant coffee. The color characteristics of the dried instant coffee and the pH of the beverage were also investigated in order to obtain further information on the quality of instant coffee.

2. Material and methods

2.1. Material

Sixty-eight samples of instant coffee were purchased at grocery stores in Belo Horizonte, MG, Brazil, from July 2002 until December 2003. The samples comprised glass jars of at least 100 g of three types (regular, decaffeinated and regular organic), 12 brands and five different lots from each brand. The dried instant coffee samples were analyzed for the profile and levels of bioactive amines and color characteristics. The beverages were prepared according to the instructions provided by the manufacturers, e.g. 1.5 and 3.0 g per 50 mL of water for freeze dried and spray dried samples, respectively. The prepared beverages were analyzed for pH.

The standards of bioactive amines were purchased from Sigma Chemical Co. (St. Louis, MO, USA). They included spermine (SPM) tetrahydrochloride, spermidine (SPD) trihydrochloride, putrescine (PUT) dihydrochloride, agmatine (AGM) sulfate, cadaverine (CAD) dihydrochloride, serotonin (SRT) creatinine sulfate, histamine (HIM) dihydrochloride, tyramine (TYM) hydrochloride, tryptamine (TRM) and 2-phenylethylamine (PHE) hydrochloride.

The reagents were of analytical grade, except those used in HPLC analysis which were chromatographic grade. Water was purified in Milli-Q (Millipore Corp., Milford, MA, USA). The mobile phases were filtered in HAWP and HVWP membranes (0.45 μm pore size, Millipore Corp.) for aqueous and organic solvents, respectively, and the extracts were filtered in HAWP membranes.

2.2. Analytical methods

2.1.1. Determination of free bioactive amines

The free amines were extracted from the instant coffee samples (5 g) with 20 mL of 5% trichloroacetic acid (TCA) and filtered through 0.45 μm pore diameter HAWP membrane. The amines were separated by ion pair-HPLC using reversed phase μBondapak C18 column (300 × 3.9 mm i.d., 10 mm) (Waters, Milford, USA), and quantified after post column derivatization with *o*-phthalaldehyde and fluorimetric detection at 340 nm of excitation and 445 nm of emission as described by Cirilo et al. (2003).

The amines were identified by comparison of the retention time of peaks in the sample in relation to standards and confirmed by the addition of the suspect amine to the sample. The concentrations of the amines were determined by direct interpolation in individual standard curves with $R^2 \geqslant 0.9926$. The limits of determination were 0.02 mg/100 g for spermidine, spermine, agmatine, putrescine, cadaverine, histamine, tyramine and phenylethylamine and 0.04 mg/100 g for serotonin and tryptamine. Previous studies indicated lack of interference from other N containing compounds such as aminoacids (Vale and Glória, 1997; Cirilo et al., 2003). Standard solution containing 4 μg/mL of each of the ten amines was analyzed in between every four samples as a quality control parameter.

2.2.2. Determination of the pH

The pH was determined in the beverage prepared with the instant coffee according to instructions by the manufacturers. A digital pH meter (Digimed DM20, DIGICROM Analítica Ltda, Santo Amaro, SP, Brazil) was used after calibration with appropriate buffers (IAL, 1985).

2.2.3. Color characteristics

The color characteristics of the dry instant coffee were determined by means of the CIE $L^*a^*b^*$ color system using a ColorTec PCM (Accuracy Microsensor, Pittsford, USA). Luminosity (L^*) values were taken directly and chroma and hue were calculated using the respective formulas: $c^* = (a^{*2} + b^{*2})^{1/2}$ and $h_{ab} = \tan^{-1} b^*/a^*$ (Borges et al., 2002).

2.3. Statistical analysis

All of the analyses were performed in duplicate. The data were submitted to analysis of variance (ANOVA) and the means were compared by the Duncan test at 5% probability using SIGMA STAT 2.0 (Systat Software Inc, Richmond, CA, USA). The existence of significant correlation between amines and physicochemical characteristics was determined by Pearson correlation (Sampaio, 1998).

3. Results and discussion

3.1. Profile of free bioactive amines in instant coffee

Nine of the ten amines investigated were detected in the samples. Although tryptamine was monitored, it was not detected in any sample. Tyramine was the prevalent amine, being detected in every one of the 68 samples analyzed. It was followed by cadaverine (88%), serotonin (72%), spermidine (65%), phenylethylamine (57%), putrescine and histamine (54%). Agmatine and spermine were only detected in 29 and 18% of the samples.

The presence of spermidine in coffee was expected since this amine is widely distributed in plants, where it plays important roles in cell division and growth (Flores et al., 1989; Bardócz, 1995; Cirilo et al., 2003). The presence of putrescine was also expected, as it is an obligate intermediate in the synthesis of spermidine (Lima and Glória, 1999; Walters, 2003). The prevalence of spermidine over spermine is in accordance with findings by Valero et al. (2002) and Kalac et al. (2002) for different vegetables.

Several of the amines detected in instant coffee were previously identified in green or roasted coffee, except for phenylethylamine which was detected in coffee samples for the first time. The presence of most of these amines was described in green coffee beans, among them, putrescine, spermidine, spermine and serotonin (Amorim et al., 1977; Cirilo et al., 2003; Casal et al., 2004; Vasconcelos et al., 2007). Tyramine and cadaverine were found at low concentrations ($\leqslant 0.31$ and $\leqslant 0.04$ mg/100 g, respectively) in green robusta coffee (Casal et al., 2004). Agmatine was detected in samples submitted to high roasting temperatures (Cirilo et al., 2003). Furthermore, histamine, tryptamine and cadaverine were detected in coffees of low cup quality and in defective coffee beans: black, immature and sour (Oliveira et al., 2005; Vasconcelos et al., 2007).

No information was found in the literature on bioactive amines in instant coffee. However, since it is the extract from roasted coffee, it should contain the same types of amines found in roasted coffee. Most of the free amines present in green coffee are degraded during the roasting process. However, roasted coffee contained mainly serotonin, which seems to be the most resistant amine to the roasting process. Some other amines like putrescine, spermidine and spermine could be detected in light roasts, whereas when submitted to a dark roast, agmatine could be formed (Amorim et al., 1977; Cirilo et al., 2003; Casal et al., 2004). The presence of different types of amines in instant coffee could indicate that they were introduced during instant coffee processing: during extraction, concentration or dehydration processes. Furthermore, they could have been present in the roasted coffee at the conjugated form which were hydrolyzed during processing. Studies are needed to identify the source of these amines.

The presence of serotonin has been reported in many fruits and vegetables, such as, banana, avocado, tomato, eggplant, and plum (Flores et al., 1989; Adão and Glória, 2005).

Table 1
Levels of free bioactive amines in samples of dry instant coffee

Values	Levels of amines (mg/100 g)									
	SPD	SPM	PUT	AGM	SRT	CAD	TYM	HIM	PHE	Total
$n+$	44	12	37	20	49	60	68	37	39	68
Minimum	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.28
Maximum	0.77	0.41	0.53	0.53	1.80	0.81	0.55	0.14	0.75	2.76
Median	0.06	0.00	0.04	0.00	0.36	0.27	0.11	0.04	0.04	1.01
Mean	0.11	0.03	0.08	0.04	0.39	0.27	0.14	0.04	0.05	1.15
CV (%)	169	293	142	216	98	79	74	110	197	56

$N = 68$; $n+$ = number of samples containing the respective amine; CV = coefficient of variation; SPD = spermidine; SPM = spermine; PUT = putrescine, AGM = agmatine; SRT = serotonin; CAD = cadaverine; TYM = tyramine; HIM = histamine; PHE = phenylethylamine.

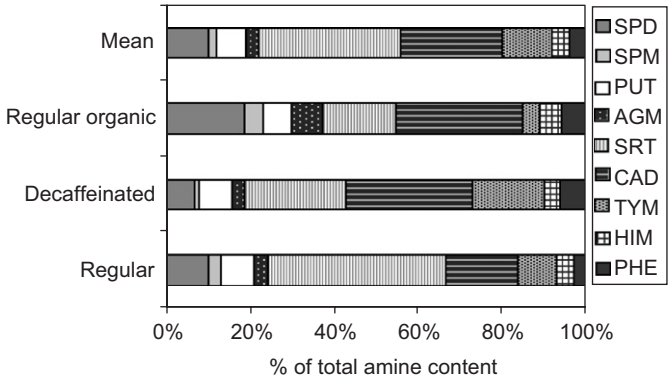

Fig. 1. Contribution of each amine to total mean amine levels in different types of instant coffee: regular, decaffeinated and regular organic. SPD = spermidine; SPM = spermine; PUT = putrescine, AGM = agmatine; SRT = serotonin; CAD = cadaverine; TYM = tyramine; HIM = histamine; PHE = phenylethylamine.

According to Flores et al. (1989) and Glória (2003), serotonin plays important role in plant protection. The presence of serotonin in coffee is interesting since it has physiological action in humans, as a neurological mediator (Casal et al., 2004).

3.2. Levels of free bioactive amines in instant coffee

The levels of amines detected in the samples analyzed are indicated in Table 1 Total amine levels varied widely, from 0.28 to 2.76 mg/100 g. Higher mean levels of amines were observed for serotonin, followed by cadaverine, tyramine and spermidine.

The levels of amines varied widely among samples, as indicated by the coefficients of variation. Higher variation was observed for spermine, followed by agmatine and phenylethylamine. However, lower variations were observed for tyramine, followed by cadaverine and serotonin, which were the amines present at higher amounts.

No information was found in the literature on the levels of amines in instant coffee. The levels of serotonin, spermidine, and putrescine were similar to those found in roasted coffee as reported by Amorim et al. (1977), Cirilo et al. (2003), Casal et al. (2004) and Vasconcelos et al. (2007). However cadaverine, tyramine, histamine and phenylethylamine, which were not present in roasted coffee, were found in some samples of instant coffee at significant levels. Studies are necessary to ascertain the factors affecting the formation and accumulation of these amines in instant coffee.

3.3. Profile and levels of free amines in different types of instant coffee

The samples of instant coffee could be grouped according to the type into regular, decaffeinated and regular organic (Fig. 1). The same nine amines were detected in each group, however, the contribution of each amine to total levels varied significantly. In regular coffee, serotonin contributed most to total levels, followed by cadaverine, spermidine and tyramine. In decaffeinated coffee, cadaverine contributed the most to total levels, followed by serotonin and tyramine. In regular organic coffee cadaverine was also the amine which contributed the most to total levels, followed by spermidine and serotonin.

The mean levels of amines detected in each type of instant coffee are indicated in Fig. 2. The amines found at higher levels were serotonin, cadaverine, tyramine and spermidine. Significant difference among the types was observed for cadaverine and tyramine. Higher cadaverine levels were detected in decaffeinated and regular organic instant coffee, whereas higher tyramine levels were detected in decaffeinated samples.

According to Nogueira and Trugo (2003), the composition of instant coffee can be affected during processing and also by the species and varieties of coffee used in the blends. Robusta green coffee was observed to contain higher tyramine levels; therefore, the use of this coffee variety at a higher proportion in the blend could provide a coffee with higher tyramine levels (Casal et al., 2004). Since regular organic instant coffee differed significantly from regular instant coffee with respect to cadaverine levels, it is probable that organic cultivation practices can favor cadaverine formation and accumulation in coffee.

3.4. Profile and levels of free amines in different brands of instant coffee

The total levels of amines in regular, decaffeinated, and organic instant coffee grouped according to the respective brands are indicated in Fig. 3. Overall, among the 12 brands included in this study, ten produced regular instant, six decaffeinated and only two regular organic instant coffees. Total amine levels varied from 0.30 to 2.86 mg/100 g (mean of 1.07 mg/100 g) for regular instant coffee; 0.38 to 2.51 mg/100 g (mean of 1.29 mg/100 g) for decaffeinated; and 0.41 to 2.53 mg/100 g (mean of 1.41 mg/100 g) for regular organic instant coffee. Even

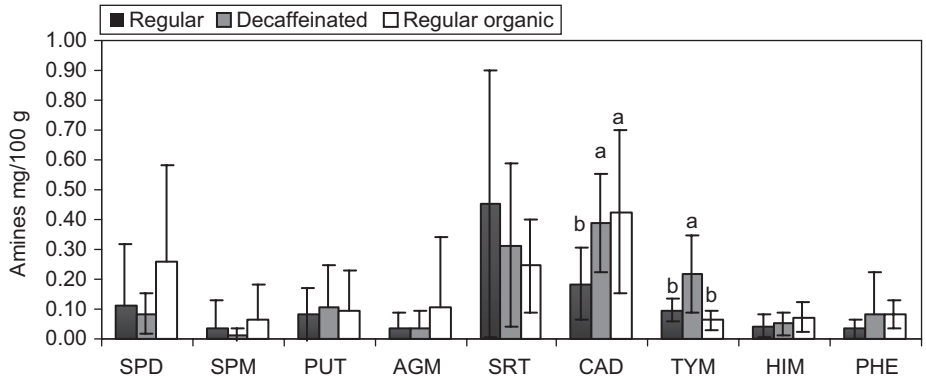

Fig. 2. Levels of free bioactive amines in different types of instant coffee: regular, decaffeinated and regular organic. SPD = spermidine; SPM = spermine; PUT = putrescine, AGM = agmatine; SRT = serotonin; CAD = cadaverine; TYM = tyramine; HIM = histamine; PHE = phenylethylamine. Mean values ± standard deviations for each amine with different letters are significantly different (Duncan test, $P \leqslant 0.05$).

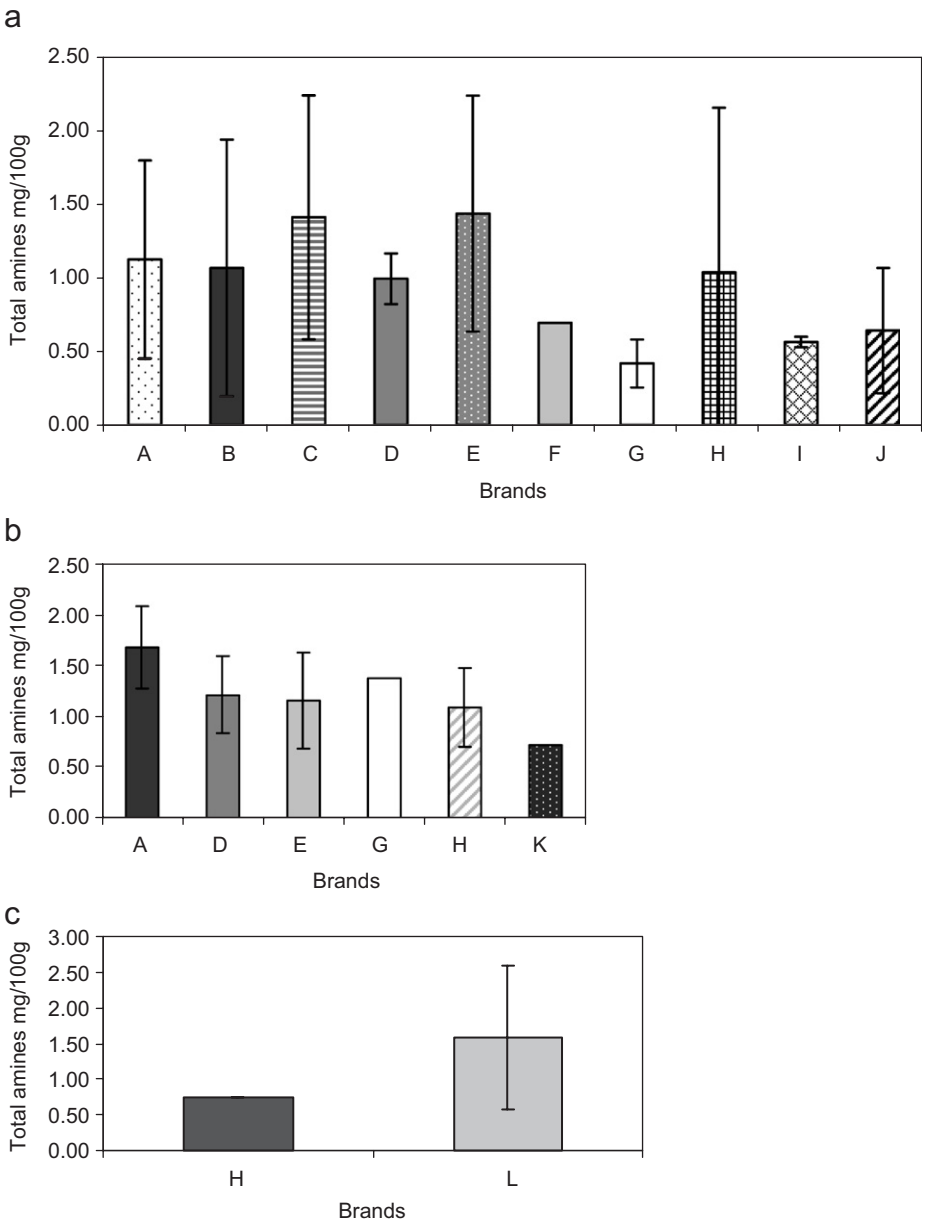

Fig. 3. Total levels of free bioactive amines in different brands of instant coffee: (a) regular; (b) decaffeinated; and (c) regular organic coffees. There is no significant difference on total amine levels among brands, Duncan test, $P > 0.05$.

though mean levels varied among brands, the difference was not significant. This is probably due to the large variation on amines levels among samples from different lots of the same brand.

One brand (H) had the three different types of instant coffee, whereas three (A, D and E) had regular and decaffeinated coffees in the market. However, no significant difference was observed on total amine levels among them.

3.5. Profile and levels of free amines in different brands of the instant coffee beverage

The levels of amines calculated for 50 mL of the instant coffee beverage from the different brands, prepared according to the manufacturers' recommendation varied widely (Table 2). The levels of serotonin varied from not detected to 7.8 μg; tyramine from 0.3 at 4.9 μg and cadaverine from 0.2 to 6.0 μg. The significance of the levels of these amines in the coffee beverage is not well established. Very few sensory data on amines are available. Wang et al. (1975) reported that free polyamines have an unpleasant, almost putrid, ammoniacal odor. However, the pH of the coffee as well as the other components present can play a role on the amines threshold. This represents an area that deserves further attention.

Table 2
Calculated levels of serotonin, tyramine, cadaverine and total free bioactive amines in a 50 mL cup of different types and brands of the instant coffee beverage

Instant coffee	Levels of amines (μg) in 50 mL beverage			
Types/Brands	Serotonin	Tyramine	Cadaverine	Total amines
Regular				
A	4.3	1.2	2.0	11.2
B	3.7	0.6	2.6	10.6
C	7.8	0.9	3.8	18.3
D	6.5	1.0	0.6	9.9
E	3.4	2.0	5.0	28.6
F	nd	2.3	1.7	6.9
G	nd	2.2	2.2	5.5
H	3.0	0.3	0.2	5.1
I	4.0	0.9	1.3	5.6
J	2.7	1.0	0.9	6.4
Decaffeinated				
A	5.5	1.9	6.0	16.9
D	3.9	1.4	3.7	12.2
E	2.6	2.0	3.3	23.0
G	1.2	4.9	0.4	18.0
H	0.5	3.7	2.4	5.5
K	nd	1.3	4.4	7.2
Regular organic				
H	1.7	0.3	0.9	4.0
L	2.2	0.6	4.8	15.8

Levels were calculated from mean values ($n = 5$) of individual amines in the respective dry instant coffee; nd–not detected (determination limit = 0.4 μg/50 mL for serotonin).

According to Lima (2006), four to five 50 mL cups of coffee should be consumed daily in order to benefit from coffee's functional properties. Considering an intake of 5 cups of the beverage with the highest levels of tyramine and serotonin, the ingestion of these amines would be, respectively, 24.5 and 39.0 μg daily. According to Halász et al. (1994) and Glória (2005), high levels of tyramine (10 mg/100 g of food) can cause hypertensive responses in normal individuals. Therefore, the levels of tyramine in the coffee samples would not represent any risk to consumers. No information was found regarding serotonin, however, the presence of this amine in coffee could be interesting since it has physiological roles in humans as a neurological mediator (Casal et al., 2004).

3.6. pH and color characteristics of instant coffee

The pH and the color characteristics of the samples are indicated on Table 3. The pH varied among the different types of instant coffee, with significantly lower values found in regular organic coffee, compared to regular which was lower than the decaffeinated coffee. There was also significant difference on the color characteristics among the different types of samples. Overall, higher values were observed for the regular organic instant coffee, followed by decaffeinated, which was followed by regular coffee. The luminosity of roasted coffee correlates inversely with the degree of roasting (Borges et al., 2002; Farah et al., 2006a). However, no correlation has been established for instant coffee.

Correlation studies of the levels of amines with the pH and the color characteristics indicated significant positive correlation of tyramine and agmatine with the pH ($R = 0.434$, $P = 0.003$ and $R = 0.293$, $P = 0.048$, respectively). These results suggest that the higher the pH, the higher the levels of tyramine and agmatine. There was no

Table 3
pH values and CIE $L^*a^*b^*$ color characteristics of the different types of instant coffee

Characteristics	Mean value ± standard deviation/type of instant coffee		
	Regular	Decaffeinated	Organic
pH*	5.05 ± 0.07 b	5.15 ± 0.14 a	4.86 ± 0.04 c
Color characteristics**[a]			
L^*	14.61 ± 2.20 c	20.61 ± 4.69 b	33.48 ± 3.02 a
a^*	6.61 ± 2.03 b	7.91 ± 3.63 b	11.53 ± 4.01 a
b^*	3.73 ± 2.33 c	12.56 ± 8.03 b	33.51 ± 6.73 a
c^*	9.01 ± 5.15 c	15.35 ± 8.00 b	36.24 ± 3.99 a
H^o	32.89 ± 17.47 b	54.03 ± 15.02 a	67.15 ± 9.28 a

*Determined on the beverage; ** determined on the dry instant coffee. Mean values with different letters in the same line are significantly different (Duncan test, $P \leq 0.05$). L^* = luminosity; c^* = chroma and h = hue (as measured by $c^* = (a^{*2} + b^{*2})^{1/2}$ and $h_{ab} = \tan^{-1} b^*/a^*$ [Borges et al., 2002]).

[a]The color characteristics of the dry instant coffee were determined by means of the CIE $L^*a^*b^*$ color system using a ColorTec PCM (Accuracy Microsensor, Pittsford, USA).

significant correlation between color characteristics and the levels of amines.

4. Conclusion

The profile and levels of bioactive amines in instant coffee was investigated for the first time. Nine of ten amines investigated, among them, serotonin, cadaverine, tyramine, spermidine, putrescine, histamine, agmatine, phenyletylamine and spermine were detected in the samples. Although monitored, tryptamine was not detected in any sample. Tyramine was found in every sample analyzed, followed by cadaverine (88%) and serotonin (72%) whereas agmatine and spermine were the least frequent amines, detected in only 29 and 18% of the samples, respectively. Overall, the amine present at higher levels was serotonin, followed by cadaverine, tyramine and spermidine. Significantly higher tyramine levels were found in decaffeinated coffee and significantly higher cadaverine levels were detected in decaffeinated and regular organic coffee. The levels of amines varied among lots of the same brand and among brands. There was significant positive correlation between tyramine or agmatine and the pH. The levels of amines in the coffee beverage were calculated. However, further studies are needed in order to determine the impact of these amines in coffee flavor and on humans health. Furthermore, the factors affecting amine formation and accumulation in instant coffee must be determined.

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