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Original article

Effect of roasting on chlorogenic acids, caffeine and polycyclic aromatic hydrocarbons levels in two *Coffea* cultivars: *Coffea arabica* cv. Catuaí Amarelo IAC-62 and *Coffea canephora* cv. Apoatã IAC-2258

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Summary During coffee roasting process, several substances may be formed or eliminated. The influence of roasting on polycyclic aromatic hydrocarbons (PAHs), caffeoylquinic acids (CQAs) and caffeine levels was studied in *Coffea arabica* cv. Catuaí Amarelo IAC-62 and *Coffea canephora* cv. Apoatã IAC-2258, roasted in three roasting degrees. CQAs and caffeine were determined simultaneously by HPLC-DAD, and PAHs by HPLC-FLD. Caffeine levels were higher in canephora (1486–1884 mg per 100 g) than in arabica (1110–1255 mg per 100 g) and increased up to 21% at darker roasts. Summed CQA levels were higher in green coffee (4661 and 4946 mg per 100 g) and decreased at darker roasts (234 and 377 mg per 100 g), showing no difference between the coffee cultivars studied. PAH summed levels varied from 0.052 to 0.814 μg kg^{-1} (arabica) and 0.108 to 0.392 μg kg^{-1} (canephora). No correlation was observed between roasting degree, coffee cultivar and PAH levels. Results were also analysed using hierarchical cluster analysis and principal component analysis.

Keywords Caffeine, chlorogenic acids, *Coffea arabica*, *Coffea canephora*, coffee, polycyclic aromatic hydrocarbons, roasting.

Introduction

Over the years, polycyclic aromatic hydrocarbons (PAHs) have attracted attention because of their carcinogenic potential. PAHs are formed during incomplete combustion or pyrolysis of organic matter. Humans can be exposed to PAHs through different routes, and for the general population, food is one of the major routes of exposure. Their presence in food originates mainly from processing and cooking (drying, smoking, roasting, frying, grilling), although some food groups can also be contaminated by environmental PAHs that are present in the air (by deposition), soil (by transfer) and water (by deposition and transfer) (WHO, 1998, 2005, EFSA, 2008).

Polycyclic aromatic hydrocarbons can occur as contaminants in different types of food such as vegetable oils, tea, coffee, seafood, alcoholic drinks, vegetables, fruits, guarana powder, smoked and grilled meat, and the contamination levels are presented in wide range of variation, which may vary from not detected to 112.0 μg kg^{-1} (in soybean oil) (Camargo & Toledo, 2003; Bishnoi *et al*., 2005; Camargo *et al*., 2006, 2011; Tfouni *et al*., 2007; Rey-Salgueiro *et al*., 2009; Visciano *et al*., 2009; Farhadian *et al*., 2010).

The International Agency for Research on Cancer (IARC) has classified benzo(a)pyrene, the most-known and most-studied PAH, in the group 1 as carcinogenic to humans (IARC, 2010). The Joint FAO/WHO Expert Committee on Food Additives evaluated thirty three PAHs during its 64th meeting and concluded that thirteen of them were clearly carcinogenic and genotoxic, including the four compounds selected for this study (WHO, 2005).

Coffee is one of the most important Brazilian crops, and in the last 2009 season, the country produced 2.37 million tons of green coffee; where 73.1% of the production was of the arabica species and 26.8% was canephora (robusta). Among ground roasted coffee commercially available in the market, some are produced exclusively with *Coffea arabica* and others with a blend of *C. arabica* and *Coffea canephora*. Brazil is the world's largest coffee exporter; in 2008, almost 1.57 million tons of green coffee was exported to different

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countries such as Germany, USA, Italy, Belgium and Japan (ABIC, 2010; CONAB, 2010).

Coffee roasting process is responsible for the products characteristics and final quality. In this process, several substances such as chlorogenic acid (CGA) and caffeine are formed or eliminated, providing flavour, acidity and body (Melo, 2004). On the other hand, undesirable compounds may also be formed, which is the case of PAHs (Kruijf *et al*., 1987).

When studying PAHs, both substances (CGA and caffeine) are of interest. Caffeine is known to form a complex with PAHs, which may be related to PAH transference to the coffee brew (Kolarovic & Traitler, 1982; Navarro *et al*., 2009), and CGA may be a precursor of PAH formation (Sharma & Hajaligol, 2003). The main groups of CGA found in green coffee beans include caffeoylquinic acids, dicaffeoylquinic acids and feruloylquinic acids, with at least three isomers for each group. Of the nine main chlorogenic acids isomers present in green coffee, caffeoylquinic acids (CQAs) represent from 76 to 82% (Farah & Donangelo, 2006).

Different studies have shown that coffee composition varies according to species and cultivar, i.e. differences in amino acids, caffeine and chlorogenic acids levels were described for different coffee species, cultivars and roasting degrees (Martin *et al*., 1998; Ky *et al*., 2001; Campa *et al*., 2005; Farah *et al*., 2005; Murkovic & Derler, 2006; Perrone *et al*., 2008). Mendonça *et al*. (2007) evaluated sugars, ethereal extract, polyphenols and caffeine levels from sixteen different cultivars of *C. arabica*, and the results showed a significant variation among them. In a study conducted by Aguiar *et al*. (2005) with six cultivars of *C. canephora,* differences in the levels of soluble solids, lipids, chlorogenic acids and caffeine were found.

There are few data regarding PAH content in ground roasted coffee, and the studies hardly ever inform the coffee species or cultivar studied. As there are many possible PAH precursors and the composition of coffee beans vary among species and cultivars, the formation, composition and concentration of these carcinogenic compounds might depend on these parameters and likewise on the roasting conditions.

Multivariate analytical techniques have been widely used for food quality control to assess authenticity of food products and also their classification according to variety and/or geographical origin. It has been applied for products such as wine, olive oil, honey, vegetables, coffee, juice and rice (Kallithraka *et al*., 2001; Tzouros & Arvanitoyannis, 2001; Arvanitoyannis *et al*., 2005, 2008; Arvanitoyannis & Vlachos, 2007, 2008). Hierarchical cluster analysis (HCA) and principal component analysis (PCA) are very popular multivariate statistical methods, which are used mainly to examine data structure and interpret patterns of influence (Ferreira *et al*., 2000).

Thus, the objective of the present study was to evaluate the possible influence of the roasting process on arabica and canephora coffees from specific cultivars, on the levels of PAHs, chlorogenic acids and caffeine, in ground roasted coffee. For this purpose, two green coffee samples were collected (*C. arabica* cv. Catuaí Amarelo IAC-62 and *C. canephora* cv. Apoatã IAC-2258) and roasted in three different roasting degrees, and the levels of four carcinogenic PAHs (benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene), three caffeoylquinic acids (3-CQA, 4-CQA and 5-CQA) and caffeine were determined. Authors adopted the IUPAC numbering system for chlorogenic acids. To better understand the relationships between the analysed compounds, the roasting process and the cultivars studied, the experimental results were analysed using multivariate methods HCA and PCA.

Materials and methods

Materials

Samples and roasting

In September 2007, samples of *C. arabica* cv. Catuaí Amarelo IAC-62 and *C. canephora* cv. Apoatã IAC-2258 cultivated in the region of Campinas-SP, Brazil, and developed by the Agronomic Institute of Campinas (IAC) were collected. Both cultivars are widely cultivated in the country as they enabled the use of new and specific areas to improve coffee production.

The green coffee beans were obtained by the dry method, in which coffee cherries were harvested and dried under the sun until they reached 12% moisture content and the dried outer parts were mechanically removed. Subsequently, beans were roasted in a Probat roaster (Probatino model; Leogap, Curitiba, PR, Brazil) with temperature set at 200 °C and roasting time of 7 min (for light roast), 10 min (medium roast) and 12 min (dark roast). Each roasting batch contained 1 kg of green coffee beans. Roasted beans were ground in a fine grind using a La Cimbali Special grinder (Cimbali, Milano, Italy) with ring nut number 4 (providing an average particle size of 400 μm or less) stored in glass flasks with screw caps and kept frozen (−18 °C) until analysis.

Samples were roasted to obtain three roasting degrees: light, medium and dark, which were determined, in three replicates, by the Agtron/SCAA Roast Color Classification System, using an E10-CP Agtron Coffee Roast Analyser (Agtron, Reno, NV, USA). Numeric results were correlated with the discs and also with the roasting degree as follows: no. 25–45: dark, no. 55–65: medium and no. 75–95: light.

Samples of ground roasted coffee were analysed in duplicate for the presence of caffeine, caffeoylquinic acids (3-CQA, 4-CQA and 5-CQA) and PAHs [benz(a)anthracene (BaA), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF) and benzo(a)pyrene (BaP)].

Standards and reagents

BaA and BaP standards were purchased from Supelco Inc. (Bellefonte, PA, USA), while BbF and BkF were purchased from Aldrich Chemical Co. (Steinheim, Germany). Caffeine and 5-CQA standards were acquired from Sigma-Aldrich (St Louis, MO, USA). A mixture of 3-CQA, 4-CQA and 5-CQA was prepared from 5-CQA using the isomerisation method described by Trugo & Macrae (1984).

Hexane, cyclohexane, methanol and N,N-dimethylformamide (HPLC grade) were purchased from Tedia Company Inc. (Fairfield, OH, USA). HPLC-grade acetonitrile and reagent-grade anhydrous sodium sulphate, glacial acetic acid, phosphoric acid and hydrochloric acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). Silica gel (70–230 mesh, ASTM) was from Merck AS Chemical Industries (Darmstadt, Germany). Reagent-grade crystallised zinc acetate and potassium hexacyanoferrate (II) were from Synth (Diadema, SP, Brazil). Water was obtained from a Millipore (Milford, MA, USA) Milli-Q water purification system and Millex HV 0.45-μm filter from Millipore.

Carrez solution I was prepared by dissolving 21.9 g of crystallised zinc acetate and 3 mL of glacial acetic acid in distilled water and diluting to 100 mL. Carrez solution II was prepared with 10.6 g of potassium hexacyanoferrate (II) in 100 mL of distilled water.

Methods

Moisture (dry matter) analysis

Moisture content of the samples was determined by loss on drying. In ground roasted coffee, the determination was performed according to AOAC Official Method 968.11 (Horwitz, 2005). In green coffee beans, the analysis was carried out according to the Brazilian Ministry of Agriculture Livestock and Food Supply guidelines (MAPA, 1992).

Caffeine and caffeoylquinic acids analysis

Sample preparation. Caffeine and caffeoylquinic acids (3-CQA, 4-CQA and 5-CQA) were determined simultaneously using a method of extraction and clean-up based on the one described by Trugo & Macrae (1984).

Coffee samples were homogenised, 0.5 g was weighted in a 100-mL volumetric flask, 20 mL of methanol/water (20:80, v/v) was added, and the flask was placed in an ultrasonic bath for 5 min. Carrez solution I (2 mL) and Carrez solution II (2 mL) were added, and the solution was diluted to 100 mL with methanol/water (20:80, v/v). Flask was agitated and left to stand for 10 min, and the final solution was filtered through a 0.45-μm filter and analysed by HPLC with a diode array detector.

HPLC. The analyses were carried out using a Shimadzu (Kyoto, Japan) HPLC apparatus equipped with an LC-20AT quaternary pump, an in-line degasser, a Rheodyne® 7725i injector (Rohnert Park, CA, USA) (20 μL sample loop) and a diode array detector SPD-M20A (detection at 272 nm for caffeine and 324 nm for CQAs). Data were acquired and processed with LC solution software. A C18 column (Lichrosphere 100, 250 × 4 mm, 5 μm particle size; Merck, Darmstadt, Germany) was used, and the mobile phase, at a flow rate of 1 mL min^{-1}, consisted of a gradient of A: acetonitrile and B: water (adjusted to pH 2.7 with phosphoric acid) + 1 g L^{-1} NaH_2PO_4. The gradient was programmed as follows: from 0 to 25 min 8% of A, 25–30 min increase to 80% of A, 30–35 min 80% of A, 35–40 min decrease to 8% of A, 40–45 min 8% of A. The peaks of caffeine and CQAs in the samples were identified by comparing the retention time with that of the standards and by their UV spectrum (200–400 nm), which were also used to verify the purity of the peaks.

Quantification and method validation. The three isomers peaks (3-CQA, 4-CQA and 5-CQA) were identified by comparing chromatograms, retention times and elution order with the ones reported by the HPLC column manufacturer, under the same chromatographic conditions.

The external standard plot method was used for quantification of caffeine, 5-CQA and the sum of the 3 CQAs isomers, with the latter been done by adding up the areas of the three CQAs peaks. Duplicate HPLC injections of caffeine and CQA standard solutions in water were used to construct linear regressions lines (peak area ratios vs. concentration). For this purpose, six concentration levels ranging from 0.004 to 0.1 mg mL^{-1} for caffeine and 5-CQA and from 0.008 to 0.5 mg mL^{-1} for the isomers mix standard were used. Results are expressed in dry matter basis.

To determine the accuracy and repeatability of the method, two ground roasted coffees were acquired from the market: a decaffeinated and a dark roasted sample. Recovery studies were carried out by spiking the decaffeinated sample with two concentrations of caffeine (500 and 1000 mg per 100 g, in five replicates) and the dark roasted sample with two concentrations of 5-CQA and the CQA isomers (1000 and 2000 mg per 100 g, in five replicates). Recoveries were calculated from the differences in amounts between the spiked samples and unspiked controls.

Repeatability of the method was evaluated through the relative standard deviation (RSD) associated with

measurements of the compounds performed during recovery analyses.

Polycyclic aromatic hydrocarbons analysis

Extraction and clean-up. Polycyclic aromatic hydrocarbons extraction and clean-up procedures were based on the methods described by Badolato *et al*. (2006) and Tfouni & Toledo (2007).

Homogenised samples (5 g) were extracted using a Soxhlet extractor with 150 mL of hexane. After 6 h, the hexane was transferred to a separating funnel and extracted with three aliquots of N,N-dimethylformamide-water (9:1, v/v) (50, 25 and 25 mL). Then, 100 mL of a 1% sodium sulphate solution was added to the combined extract and re-extracted with 50, 35 and 35 mL aliquots of cyclohexane. The final solution was dried with anhydrous sodium sulphate and concentrated on a rotary evaporator to 5 mL at 40 °C.

The concentrated extract (5 mL) was purified by column chromatography on silica gel. A glass column (200 × 10 mm i.d.) was packed with 5 g of deactivated silica gel (15% water) and 1 g of anhydrous sodium sulphate on the top. 5 mL of extract was applied to the top of the column and eluted with 85 mL of cyclohexane. The first 10 mL was discarded, and the 10–85 mL fraction was concentrated to about 1 mL on a rotary evaporator at 40 °C and dried under a flow of nitrogen. Finally, the residue was dissolved in 2 mL of acetonitrile, filtered through a 0.45-μm filter and analysed by HPLC with fluorescence detection.

HPLC. The analyses were carried out using a Shimadzu (Kyoto, Japan) HPLC apparatus equipped with an LC-20AT pump, an SIL-20AT autosampler (injection volume of 20 μL), a CTO-20A column oven and an RF-10A xl fluorescence detector (excitation wavelength 290 nm and emission wavelength 430 nm). Data were acquired and processed with LC solution software. A C18 column (Vydac 201 TP54, 250 × 4.6 mm, 5 μm particle size; Vydac, Hesperia, CA, USA) at 30 °C was used, and the mobile phase consisted of 75% acetonitrile and 25% water, at a flow rate of 1 mL min^{-1}.

Quantification and method validation. The external standard plot method was used for quantification. Duplicate HPLC injections of PAH standard solutions in acetonitrile were used to construct linear regressions lines (peak area ratios vs. PAH concentration). For this purpose, six concentration levels ranging from 0.1 to 2.0 ng mL^{-1} were used. The limit of detection (LOD) for each PAH was defined as the concentration of the analyte that produced a signal-to-noise ratio of three. Peaks were identified by comparing the retention time with that of the standards and by re-injecting the extract in the chromatograph with the addition of standard solution. Results are expressed in dry matter basis.

Accuracy and repeatability data were obtained through recovery studies carried out by spiking a roasted ground coffee sample with three different concentrations of PAH standard solutions at levels of 1.0, 2.0 and 3.0 μg kg^{-1}. The spiked samples and the unspiked controls were analysed in five replicates. Recoveries were calculated from the differences in total amounts of each PAH between the spiked and unspiked samples.

Repeatability of the method was evaluated through the RSD associated with measurements of the PAHs performed during recovery analyses.

To maintain analytical quality control, for each batch of samples analysed, a spiked sample (similar to the ones used in the recovery study) was analysed simultaneously.

Statistical analysis

Data were processed using the software Statistica (Statistica 5.5; Stat Soft Inc. Tulsa, OK, USA) by one-way ANOVA with comparison of means (Tukey test) with 95% confidence, multivariate chemometric techniques, HCA and PCA.

Results and discussion

Each coffee sample was roasted to obtain three different roasting degrees: light, medium and dark. The repeatability of the process was evaluated by performing the roasting process at least twice for each degree of roast. For the *C. arabica* cv. Catuaí Amarelo, the following roasted samples were obtained: two light, four medium and three dark, while for *C. canephora* cv. Apoatã, four light, two medium and three dark roasted coffees were obtained and analysed.

Moisture analysis

The results obtained for moisture analysis were 8.5% and 9% for *C. canephora* cv. Apoatã and *C. arabica* cv. Catuaí Amarelo, respectively. For ground roasted coffee, *C. arabica* presented levels from 1% to 2.2%, while moisture content in *C. canephora* ranged from 0.8% to 2.3%.

Caffeine and caffeoylquinic acids analysis

Mean recovery and RSD for caffeine and CQAs are summarised in Table 1. Recoveries ranged from 95.3% to 96.0% for caffeine, with RSD ranging from 0% to 1.2%. For CQAs, recoveries varied from 83.0% to 93.4% and RSD from 0.6% to 7.0%. These results are considered satisfactory for determinations at mg g^{-1} levels (Horwitz *et al*., 1980).

Table 2 presents the caffeine and caffeoylquinic acid content determined in the coffee samples analysed from different degrees of roast.

Table 1 Recovery (R) and relative standard deviation (RSD) for the analysis of caffeine, 5-CQA and the sum of CQA isomers in ground roasted coffee

Spike level (mg per 100 g)	R (%)[a]	RSD (%)
Caffeine		
500	96.0	0
1000	95.3	1.2
5-CQA		
1000	85.0	0.6
2000	83.0	6.8
Σ CQA isomers		
1000	93.4	7.0
2000	83.2	3.5

[a]$n = 5$.

5-CQA, 5-caffeoylquinic acid; Σ CQA isomers, sum of 3-caffeoylquinic acid, 4-caffeoylquinic acid and 5-caffeoylquinic acid.

Table 2 Mean levels of caffeine 5-CQA and the sum of CQA isomers in coffee samples with different roasting degrees, results expressed in dry matter basis

	Mean level (mg per 100 g) (*n*)[a]			
	Green[b]	Light[b]	Medium[b]	Dark[b]
Caffeine				
arabica	1110 a (1)	1192 b (2)	1255 c (4)	1222 bc (3)
canephora	1486 a (1)	1698 b (4)	1884 c (2)	1848 c (3)
5-CQA				
arabica	2901 a (1)	1172 b (2)	306 c (4)	88 d (3)
canephora	3454 a (1)	978 b (4)	172 c (2)	142 c (3)
Σ CQA isomers				
arabica	4661 a (1)	2702 b (2)	786 c (4)	234 d (3)
canephora	4946 a (1)	2317 b (4)	463 c (2)	377 c (3)

[a]Mean of *n* samples, in duplicate.

[b]Roasting degree.

Values in the same row with the same letter are not statistically different ($P < 0.05$).

As can be observed, caffeine levels are higher in the *C. canephora* cv. Apoatã than in the *C. arabica* cv. Catuaí Amarelo. These results are in accordance with the ones previously reported by other authors, where *C. canephora* samples showed higher caffeine levels than *C. arabica* (Macrae, 1985; Casal *et al*., 2000; Ky *et al*., 2001; Rodrigues *et al*., 2007). Considering the roasting degree, there was a difference in the caffeine content, with *C. arabica* cv. Catuaí Amarelo medium roasted presenting 12% more caffeine than the green coffee and *C. canephora* cv. Apoatã samples (medium and dark roast) showing an increase of 21% during process. Nevertheless, Casal *et al.* (2000) reported a slight decrease in caffeine content for both *C. canephora* (cv. Robusta) and *C. arabica* under strong roasting conditions. However, when temperatures of roast were between 140 and 160/200 °C, authors reported an increase in caffeine content in comparison with green coffee. Similar results were obtained in the present study. One could expect a reduction in caffeine levels as a result of sublimation. However, the increase in caffeine levels during processing could be due to the fact that during roasting, the weight of the green beans can be reduced by 20% or more (10% water, 10% dry matter) (Macrae, 1985), and as results are reported in dry matter basis, they are only corrected for the loss of water. The roasted bean may also contain small but significant weights of entrapped carbon dioxide, which is released during roasting (Clarke, 1985).

The highest CQA contents were found in green coffee, and differently from caffeine, the levels decreased in coffee samples as the roast was darker (Table 2). A reduction of 42% and 53% was found in *C. arabica* cv. Catuaí Amarelo and *C. canephora* cv. Apoatã light roasted coffee, respectively. The dark roasted samples presented the lowest CQAs content in comparison to the original green coffee (mean reductions of 94% for Catuaí Amarelo and 92% for Apoatã). There was no statistical difference ($P < 0.05$) in CQA levels between the cultivars studied within the same roasting degree. According to Perrone *et al.* (2008), the reduction in CQA levels during processing is a consequence of thermal breakage of carbon–carbon covalent bonds, resulting in isomerisation in the initial roasting stages and epimerisation, lactonisation and degradation in the latter stages.

Farah *et al.* (2005) reported similar results in a study performed with other cultivars of *C. arabica* and *C. canephora*, which also presented decreasing CQAs levels under stronger roasting conditions. Nevertheless, the *C. canephora* cv. Robusta sample presented levels of CQAs 27% higher than the *C. arabica* ones (cv. Bourbon and Longberry). Perrone *et al.* (2008) also reported CQAs content 27% higher for *C. canephora* cv. Conillon samples than for *C. arabica* (cv. Mundo Novo and Catuaí Vermelho).

The 5-CQA isomer represents, in green coffee, 62% and 70% of the sum of isomers for *C. arabica* cv. Catuaí Amarelo and *C. canephora* cv. Apoatã, respectively (Table 2). In roasted coffee, this proportion is lower, with 5-CQA representing 33–37% of the summed CQA isomers for Catuaí Amarelo and from 29 to 42% for Apoatã. Farah *et al.* (2005) also reported, in *C. arabica*, cv. Bourbon and Longberry, and *C. canephora* cv. Robusta, higher decrease in 5-CQA levels during roasting when compared to 3 and 4 isomers of caffeoylquinic acids.

Polycyclic aromatic hydrocarbons analysis

Mean recovery, RSD and LOD for BaA, BbF, BkF and BaP are presented in Table 3. Recoveries obtained for

Table 3 Limits of detection (LOD), recovery (R) and relative standard deviation (RSD) for PAH analysis in ground roasted coffee

PAH	LOD (μg kg^{-1})	Spike level (μg kg^{-1})	R (%)[a]	RSD (%)
		1.0	83	12
BaA	0.04	2.0	66	17
		3.0	66	22
		1.0	82	10
BbF	0.04	2.0	67	18
		3.0	66	18
		1.0	74	12
BkF	0.02	2.0	67	16
		3.0	67	15
		1.0	87	12
BaP	0.02	2.0	70	17
		3.0	72	22

[a]$n = 5$.

BaA, benz(a)anthracene; BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene; BaP, benzo(a)pyrene.

different PAH ranged from 66% to 87%, and the RSD ranged from 10% to 22%. LODs were from 0.02 to 0.04 μg kg^{-1}. The calibration curves obtained for the PAHs studied were linear with correlation coefficients between 0.995 and 1.000. These results may be considered satisfactory for determinations at μg kg^{-1} levels and fit the performance criteria for methods of BaP analysis proposed by the European Union (Horwitz *et al.*, 1980; CEC, 2007), showing that the analytical method used is suitable for the analysis of the four PAHs in ground roasted coffee. Tables 4 and 5 present the PAH levels determined in the coffee samples from different roasting degrees.

In all samples analysed, at least one PAH was detected. The most representative ones were BbF and BaA, detected in 94% and 89% of the samples, respectively, while BkF and BaP were detected in only five and three of the evaluated samples. Considering that BaA and BbF were more frequently determined than BaP, the use of BaP as a marker for PAH presence may not be suitable, as stated by EFSA (2008).

In green coffee samples, the summed PAH content were 0.086 and 0.108 μg kg^{-1} for *C. arabica* cv. Catuaí Amarelo and *C. canephora* Apoatã, respectively. Roasted ground coffees obtained from Catuaí Amarelo beans presented PAH summed levels that ranged from 0.052 to 0.814 μg kg^{-1}. On the other hand, in ground roasted coffees from Apoatã beans, PAH summed levels ranged from 0.142 to 0.392 μg kg^{-1}.

For *C. canephora* cv. Apoatã, the mean levels of the summed PAHs increased with darker roasting degrees, whereas ground roasted *C. arabica* cv. Catuaí Amarelo did not behave in the same manner and the samples with light roast presented the highest mean levels of the summed PAHs. Although it appears that roasting degree affected the presence of PAHs in a directly proportional way for *C. canephora* cv. Apoatã samples and inversely in *C. arabica* cv. Catuaí Amarelo, statistical analysis showed that the differences were not significant ($P < 0.05$). In this way, it was not possible to correlate PAH levels with the coffees' roasting degrees and likewise with the respective cultivars studied. This is a consequence of the high variability of PAH levels among samples of the same cultivars with the same roasting degree. The coefficients of variation of the repetitions for the roasting processes varied from 11%, for *C. canephora* cv. Apoatã light roasted, to 105%, for *C. arabica* cv. Catuaí Amarelo dark roasted samples. This might be attributed to the fact that although the temperature of the roaster was set at 200 °C, when green coffee beans are introduced, there is a variation inside the equipment that is inherent to the roasting process. The internal temperature decreases and then starts to rise again throughout the process. Although there was an effort to maintain the same roasting profile for replicates of all processes, some differences were observed, as some samples reached higher temperatures in a shorter (or longer) period of time than others. Furthermore, PAHs generally occur in complex

Table 4 Roasting degrees and mean PAH levels of *Coffea arabica* cv. Catuaí Amarelo IAC-62. Results expressed in dry matter basis

	Mean PAH levels (μg kg^{-1})[a] (range)				
Roasting degree	**BaA**	**BbF**	**BkF**	**BaP**	**ΣHPAs**
Green (*n = 1)*	0.053	0.033	nd	nd	0.086 a
Light (*n = 2)*	0.286 (0.072–0.500)	0.120 (0.109–0.130)	nd	0.092 (nd–0.185)	0.498 a (0.181–0.814)
Medium (*n = 4)*	0.088 (0.078–0.153)	0.145 (0.089–0.189)	0.015 (nd–0.060)	0.013 (nd–0.052)	0.261 a (0.089–0.453)
Dark (*n = 3)*	0.066 (0.052–0.145)	0.078 (nd–0.151)	0.028 (nd–0.083)	nd	0.172 a (0.052–0.379)

n = number of samples.

[a]Mean of *n samples.*

nd, not detected (<LOD from Table 1).

BaA, benz(a)anthracene; BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene; BaP, benzo(a)pyrene.

Values in the same column with the same letter are not statistically different ($P < 0.05$).

Table 5 Roasting degrees and mean PAH levels of *Coffea canephora* cv. Apoatã IAC-2258. Results expressed in dry matter basis

	Mean PAH levels (μg kg^{-1})[a] (range)				
Roasting degree	**BaA**	**BbF**	**BkF**	**BaP**	**ΣHPAs**
Green (*n = 1)*	0.049	0.059	nd	nd	0.108 a
Light (*n = 4)*	0.067 (0.052–0.087)	0.118 (0.103–0.150)	0.011 (nd–0.045)	nd	0.196 a (0.171–0.221)
Medium (*n = 2)*	0.103 (0.065–0.142)	0.097 (0.078–0.115)	0.031 (nd–0.063)	0.022 (nd–0.045)	0.253 a (0.142–0.364)
Dark (*n = 3)*	0.136 (0.061–0.182)	0.094 (0.079–0.120)	0.030 (nd–0.089)	nd	0.260 a (0.144–0.392)

n = number of samples

[a]Mean of *n samples.*

nd, not detected (<LOD from Table 1).

BaA, benz(a)anthracene; BbF, benzo(b)fluoranthene; BkF, benzo(k)fluoranthene; BaP, benzo(a)pyrene.

Values in the same column with the same letter are not statistically different ($P < 0.05$).

mixtures with hundreds of compounds in different composition, which may vary with the generating process (WHO, 2006; EFSA, 2008).

Similar results were described by Houessou *et al.* (2007) who evaluated *C. arabica* roasting conditions in the formation of PAHs. BbF presented levels from not detected (for samples roasted at 260 °C) to 0.31 μg kg^{-1} (roast at 180 °C), and only traces of BkF and BaP were detected. Under strong roasting conditions (250–260 °C), however, authors reported levels of BaA up to 12.67 μg kg^{-1} and the evidence of the formation of other PAHs (phenanthrene, fluoranthene and pyrene). A study conducted by Badolato *et al.* (2006) reported BaP contamination in *C. arabica* and *C. canephora* samples with levels ranging from not detected to 12.52 μg kg^{-1}; nevertheless, the highest BaP level was detected in a coffee sample submitted to the lighter roasting condition of the study. Additionally, BaP levels did not increase proportionally to the roasting process.

The amounts of BaA, BbF, BkF and BaP found in the present study are lower than the ones reported by Camargo & Toledo (2002) in samples of ground roasted coffee available in the Brazilian market. The PAH content determined was in the range of 0.25 μg kg^{-1}, for BkF, to 1.23 μg kg^{-1}, for BaP. The sample species, cultivars and degree of roast were not mentioned in the study, as this information is not provided in the products label.

Other authors studied the presence of PAHs in ground roasted coffee; however, only some studies describe the coffee species used, and in these cases, the cultivar is not mentioned. As different coffee compounds may lead to PAH formation and coffee composition varies among species and cultivar, it is important to identify the ones used during the studies.

Although the formation of BaA, BbF, BkF and BaP in ground roasted Catuaí Amarelo and Apoatã coffees did not show correlation with the roasting degree and cultivar, an increase in these PAH content was observed owing to roasting process, because green coffee presented the lowest summed levels of these compounds (although the difference was not statistically significant).

Different from PAHs, a correlation between the roasting degree and CQAs levels was showed. Unfortunately, it was not possible to establish a relation between these potential precursors and the amounts of PAHs detected in the samples.

Because *C. canephora* presents higher caffeine levels and the formation of caffeine–PAH complex may facilitate the transfer of these compounds to the brew, one can expect that beverages prepared from *C. canephora* could present higher potential for PAH transfer from the ground roasted coffee to the brew.

Principal component analysis and hierarchical cluster analysis

Hierarchical cluster analysis results are presented in Fig. 1. The dendogram shows three groups for similarity 1000 (Euclidean distance), where samples are mainly grouped according to their roasting degree.

When applying PCA to the autoscaled data matrix, up to 78% of the total variance in the data is explained by two principal components (Fig. 2). PC1 discriminates mainly green canephora (GC) and green arabica (GA) coffee samples, whereas PC2 was influenced by light arabica (LA), dark canephora (DC), medium canephora (MC) and light canephora (LC) coffee samples. Graphic distribution of the samples according to their factor scores in the PC's space has strong influence of the 5-CQA, sum of isomers, PAH and caffeine content in samples. Green coffee samples present higher 5-CQA and sum of CQA isomers, followed by light roasted samples. Roasted *C. arabica* presents decreasing PAHs for increasing intensity of roast. As for caffeine, *C. canephora* presents higher intensity than *C. arabica.*

Analysing both HCA and PCA results the larger cluster in the dendogram, formed by the dark and medium roasted coffees, is formed owing to caffeine and PAH levels, where caffeine is more important to distinguish *C. canephora* from *C arabica,* while the

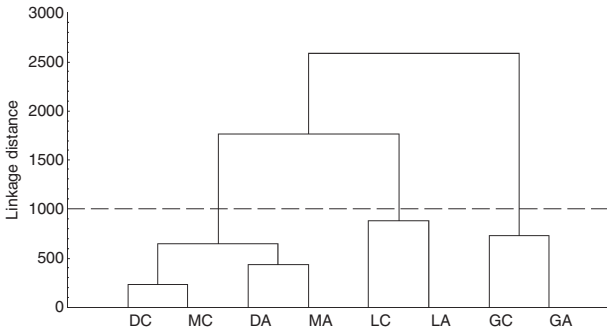

Figure 1 Dendogram (HCA) on 8 samples of coffee: dark canephora (DC), medium canephora (MC), dark arabica (DA), medium arabica (MA), light canephora (LC), light arabica (LA), green canephora (GC), green arabica (GA).

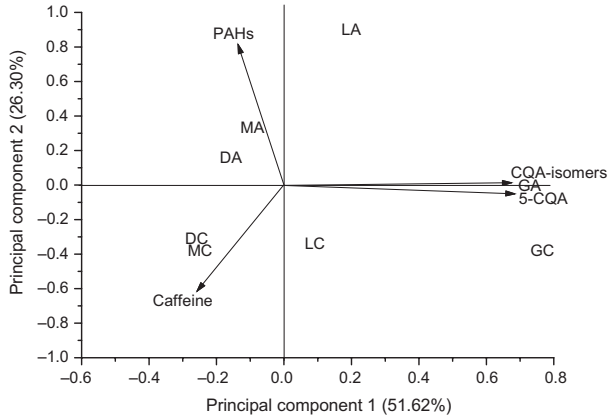

Figure 2 Principal component analysis for coffee samples: dark canephora (DC), medium canephora (MC), dark arabica (DA), medium arabica (MA), light canephora (LC), light arabica (LA), green canephora (GC), green arabica (GA).

sum of CQA isomers and 5-CQA contributed for the separation of the green coffees.

Conclusions

Roasting process, degree of roast and coffee species were shown to have influence in the levels of caffeine in ground roasted coffee. The cultivars studied played no role on CQAs content in the final product, although their levels were influenced by the degree of roast.

As for the PAHs, there was no correlation between the levels detected and both coffee roasting degree and cultivars used in the experiment. It was observed that there was a high variability in the PAHs levels for samples of the same cultivar and same degree of roast.

The multivariate methods, HCA and PCA, allowed the ground roasted coffee samples to be grouped according to their differences in caffeine, CQA and PAH content.

In Brazil, there is no regulation regarding limits of PAHs in ground roasted coffee. Maximum permitted levels are established for BaP only in smoke flavourings (0.03 μg kg^{-1} in the final product), drinkable water (0.7 μg L^{-1}) and olive pomace oil (2 μg kg^{-1}) (Brasil, 2003, 2004, 2007). European Commission, on the other hand, established, for different food products, BaP maximum levels ranging from 1 to 10 μg kg^{-1} (CEC, 2005). However, PAH levels present in the coffee samples evaluated may be considered low in comparison with the maximum levels for other kind of foods established in Europe and by the Brazilian regulation. However, owing to the carcinogenic potential of these compounds, all information related to PAHs in food should be addressed, especially in food products that are highly and regularly consumed, which is the case of coffee in Brazil and other countries.

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