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Polycyclic aromatic hydrocarbons in coffee brew: Influence of roasting and brewing procedures in two *Coffea* cultivars

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ABSTRACT

During coffee's roasting process undesirable compounds such as polycyclic aromatic hydrocarbons (PAHs) may be formed and later transferred to the brew. The influence of coffee cultivar, roasting degree and brewing procedure in the presence and transfer of four PAHs from ground roasted coffee to the brew was evaluated. Ground roasted coffees in three roasting degrees were obtained from *Coffea arabica* cv. Catuaí Amarelo IAC-62 and *Coffea canephora* cv. Apoatã IAC-2258 and their respective coffee brews were prepared by two brewing procedures (filtered and boiled). PAHs levels in the brews were determined by HPLC-FLD. At least one PAH was detected in all coffee brew samples. PAHs summed levels ranged from 0.015 to 0.105 μg/L (*C. arabica* brews) and 0.011 to 0.111 μg/L (*C. canephora* brews). The difference among the levels detected in different roasting degrees was not statistically significant, except between dark and roasted filtered brews. Coffee brews prepared with *C. arabica* ground roasted beans presented mean summed PAHs levels higher than the ones prepared with *C. canephora*, independently of the brewing procedure used. The caffeine levels in the beverages do not seem to influence the transfer.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms. They are formed during incomplete combustion or pyrolysis of organic matter and are present in the environment as pollutants. PAHs can be produced from natural and anthropogenic sources and generally occur in complex mixtures that may consist of hundreds of compounds with different composition, which may vary with the generating process (EFSA, 2008; WHO, 2006).

Food can be contaminated with PAHs through industrial food processing methods, by home food preparation and by environmental sources, where PAHs present in the air, soil, and water may contaminate food by transfer and/or deposition (EFSA, 2008; WHO, 2006). Thus, PAHs occur as contaminants in different food categories such as oils and fats, vegetables, fruits, seafood, tea, sugar, guaraná powder, sugarcane juice and smoked food products (Camargo, Antoniolli, Vicente, & Tfouni, 2011; Camargo, Tfouni, Vitorino, Menegário & Toledo, 2006; Camargo & Toledo, 2003; Garcia-Falcon and Simal-Gandara, 2005; Teixeira, Casal & Oliveira, 2007; Tfouni et al., 2009; Tfouni & Toledo, 2007; Vieira et al. 2010).

During the years, PAHs have attracted attention mostly due to their carcinogenic potential. Exposure to PAHs occurs through the airways, skin and digestive tract, and bioavailable fractions are absorbed through all three routes. The compounds have to be metabolically activated in order to the compounds toxic, mutagenic and carcinogenic effects take place (EFSA, 2008; IARC, 2010). The International Agency for Research on Cancer (IARC) has classified benzo(a)pyrene in the group 1, as carcinogenic to humans (IARC, 2012). During its 64th meeting, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that 13 of the 33 PAHs evaluated were clearly carcinogenic and genotoxic, including the four compounds selected for this study (WHO, 2005).

Coffee is a very popular beverage in many countries. With almost 1.57 million tons of green coffee exported, Brazil is the world's largest exporter, producing beans of the arabica (73.1% of the production) and canephora (26.8%) species (ABIC, 2010; CONAB, 2010). Ground roasted coffees commercially available in the Brazilian market are produced either exclusively with *Coffea arabica* species or with a blend of *C. arabica* and *Coffea canephora*, where dark roasted coffee is the most popular and main type commercialized and there are different procedures used for brewing.

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Coffee's roasting process is responsible for its characteristic flavour and final quality. In this process, several substances are formed or eliminated, providing flavour, acidity and body (Melo, 2004). On the other hand, undesirable compounds such as furan, acrylamide and PAHs may also be formed (Arisseto et al., 2008; Arisseto, Vicente, Ueno, Tfouni, & Toledo, 2011; Kruijf, Schouten, & Van der Stegen, 1987; Tfouni et al., 2012). The formation of these compounds may be related to coffee composition, which, as reported by different studies, varies according to species and cultivar. Differences in amino acids, caffeine and chlorogenic acids levels were described for different coffee species, cultivars and roasting degrees (Campa, Doulbeau, Dussert, Hamon, & Noirot, 2005; Farah, de Paulis, Trugo, & Martin, 2005; Ky et al., 2001; Martín, Pablos, & González, 1998; Murkovic & Derler, 2006; Perrone, Farah, Donangelo, de Paulis, & Martin, 2008).

Previous study has pointed coffee brew as a potential source of PAHs intake by the Brazilian population, contributing with approximately 0.88 μg to the dietary intake of these contaminants by the studied population (Camargo & Toledo, 2002). Nevertheless, there are few data regarding PAHs content in brewed coffee and the available studies usually involve coffee samples prepared with ground roasted coffee obtained in supermarkets and hardly ever inform the coffee species, cultivar or the roasting degree of the ground roasted coffee used for brewing.

Since there are many possible PAHs precursors and the composition of coffee beans vary among species and cultivars, the formation and composition of these compounds might vary according to the coffee beans species (or cultivar) and the roasting conditions. Also, roasting process could be a concern, especially taking into account the Brazilian popular dark roasted coffee. Furthermore, the PAHs transfer to the brew might be influenced by the brewing procedure.

Therefore, the objective of the present study was to evaluate the possible influence of coffee cultivar and roasting degree on the presence of four carcinogenic PAHs; the influence of brewing procedure on the PAHs transfer from ground roasted coffee to the brew; and verify if these factors would affect the intake of these compounds by the Brazilian population.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Two coffee samples (*C. arabica* cv. Catuaí Amarelo IAC-62 and *C. canephora* cv. Apoatã IAC-2258) developed by the Agronomic Institute of Campinas (IAC) and cultivated in the region of Campinas-SP, Brazil, were collected in September 2009.

Green coffee beans were obtained by the dry method, where coffee cherries were harvested, dried under the sun until achieving 12 g/100 g moisture content and then the dried outer parts were mechanically removed. Roasting process was performed in order to obtain samples with 3 roasting degrees: light, medium and dark. For this matter, batches of green coffee beans containing 1 kg each were roasted in a Probat roaster (Probatino model, Leogap, Curitiba, PR, Brazil) at 200 °C and roasting time of 7 min (for light roast), 10 min (medium roast) and 12 min (dark roast). The repeatability of the process was evaluated by performing the roasting process at least twice for each degree of roast. For *C. arabica* cv. Catuaí Amarelo the roasted samples obtained were: two light, four medium and three dark; while for *C. canephora* cv. Apoatã resulting samples were: four light, two medium and three dark roasted coffees.

Roasting degrees 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 the roasting degree as follows, no. 25–45: dark, no. 55–65: medium, no. 75–95: light.

Roasted beans were stored in aluminized valve bags at −18 °C and ground immediately before the preparation of the beverages. For grinding, a La Cimbali Special grinder (Cimbali, Milano, Italy) with ring nut number 4 was used, providing an average particle size of 400 μm or less.

All ground roasted coffee samples were then used to prepare coffee brews. Two brewing procedures were evaluated, using the same ground coffee/water ratio (50 g/500 mL): 1) *Filtered coffee* – water (92–96 °C) was left to drip onto ground coffee held in a paper filter; 2) *Boiled coffee* – water (25 °C) was added to the ground coffee, the mixture was boiled and then filtered in a paper filter.

Coffee brew samples were stored at −18 °C in glass flasks with 10 mL/100 mL N,N-dimethylformamide added until analyses in duplicate for the presence of 4 PAHs: benz(a)anthracene (BaA), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF) and benzo(a)pyrene (BaP).

2.1.2. Standards and reagents

BaA and BaP analytical standards were purchased from Supelco Inc. (Bellefonte, PA, USA), BbF and BkF were from Aldrich Chemical Co. (Steinheim, Germany). Hexane, cyclohexane and N,N-dimethylformamide (HPLC grade) were purchased from Tedia Company Inc. (Fairfield, OH, USA). Acetonitrile (HPLC grade) and reagent grade anhydrous sodium sulphate were from J.T. Baker (Phillipsburg, NJ, USA). Water was obtained from a Millipore Milli-Q water purification system (Milford, MA, USA). Millex HV 0.45 μm filters were purchased from Millipore and Bakerbond SPE silica columns (500 mg, 3 mL) were from J.T. Baker.

2.2. Method

2.2.1. Extraction and clean up

Extraction and clean up procedures were based on the method described by Tfouni et al. (2009). In a separating funnel, 50 mL of N,N-dimethylformamide–water (9:1, mL:mL) and 60 mL of a sodium sulphate aqueous solution (1 g/100 g) were added to a 10 mL sample. PAHs were successively extracted with three aliquots (25 mL) of cyclohexane. The combined extract was dried with anhydrous sodium sulphate, concentrated on a rotary evaporator to approximately 2 mL at 40 °C and dried under a flow of nitrogen. Clean up was performed by silica gel SPE. Cartridges were prepared by pre-washing with 12 mL of hexane followed by drying using a Vacuum Manifold from Supelco. The extracts were suspended with three aliquots (1 mL) of hexane, applied in the SPE cartridge and eluted with 7 mL hexane. Solvent was dried under a flow of nitrogen and the residue was dissolved in 1 mL acetonitrile, filtered through a 0.45 μm filter and analyzed by HPLC with fluorescence detection.

2.2.2. HPLC

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

2.2.3. Quantification and method validation

The external standard plot method was used for quantification. Duplicate HPLC injections of six concentration levels (0.1–2.0 ng/

mL) of PAHs standard solutions, in acetonitrile, were used to construct linear regressions lines (peak area ratios versus PAH concentration). 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.

Accuracy and precision data were obtained through recovery studies carried out by spiking a coffee brew sample with PAHs standard solutions at concentration levels of 0.3, 0.5 and 1.0 μg/kg in five replicates. Recoveries were calculated from the differences in total amounts of each PAH between the spiked and unspiked samples. Results reported were not corrected for recovery.

Precision of the method was evaluated through the relative standard deviation (RSD) associated to measurements of the PAHs performed during recovery analyses.

2.3. Statistical analysis

Data were processed using the software Statistica (Statistica 5.5, Stat Soft Inc.) by analysis of variance one-way ANOVA with means comparison (Tukey test) with 95% confidence.

3. Results and discussion

Mean recovery, RSD and LOD for BaA, BbF, BkF and BaP are presented in Table 1. Recoveries obtained ranged from 77% to 87% with RSDs varying from 9% to 30%. Limits of detection were from 0.006 to 0.01 μg/L. The calibration curves obtained for the PAHs studied were linear with correlation coefficients between 0.995 and 1.000. These results are satisfactory for determinations at μg/kg levels and comply with the performance criteria for methods of BaP analysis proposed by the European Union, where the LOD must be lower than 0.3 μg/kg and recovery must be in the range of 50–120% (CEC, 2007; Horwitz, Kamps, & Boyer, 1980). Therefore the analytical method used may be considered suitable for the analysis of BaA, BbF, BkF and BaP in coffee brew.

Tables 2 and 3 present the PAHs levels determined in the coffee brew samples prepared with ground coffees of two cultivars, in three roasting degrees and using two different brewing procedures.

At least one PAH was detected in all coffee brew samples analyzed. The most representative PAHs were BbF and BaA, detected in 94% and 83% of the samples, respectively, while BkF and BaP were detected in 17% and 14% of the analyzed samples. Levels of individual PAHs were from not detected to 0.062 μg/L (for BaA). These results are in accordance to the ones reported by Orecchio, Ciotti, and Culotta (2009) where a wide range of levels was shown for these four PAHs (0.001–0.161 μg/L) in coffee brew samples prepared from 13 commercial ground coffees available at the supermarket, in Italy. A study from Bishnoi, Mehta, Sain, and Pandit (2005) also reported a high variability of PAHs levels (not detected-0.46 μg/L) in coffee brews from Mumbai, India.

Table 1
Limits of detection, recovery and relative standard deviation.

PAH	LOD (μg/kg)	Spike level (μg/kg)	*R* (%)[a]	RSD (%)
BaA	0.01	0.3	80	30
		0.5	77	9
		1.0	86	29
BbF	0.01	0.3	80	29
		0.5	79	14
		1.0	84	29
BkF	0.006	0.3	79	23
		0.5	79	13
		1.0	85	29
BaP	0.006	0.3	77	22
		0.5	78	14
		1.0	87	28

BaA: benz(a)anthracene, BbF: benzo(b)fluoranthene, BkF: benzo(k)fluoranthene, BaP: benzo(a)pyrene.
PAH: polycyclic aromatic hydrocarbon.
LOD: limit of detection.
R: recovery.
RSD: relative standard deviation.
[a] $n = 5$.

According to Tables 2 and 3, in coffees brewed from *C. arabica* cv. Catuaí Amarelo beans, PAHs summed levels ranged from 0.015 to 0.105 μg/L and, in brews obtained from *C. canephora* cv. Apoatã beans, PAHs summed levels ranged from 0.011 to 0.111 μg/L.

In Brazil, there is no regulation regarding levels of PAHs in coffee or coffee brew. Maximum BaP levels are established for smoke flavourings (0.03 μg/kg, in the final product), drinkable water (0.7 μg/L) and olive-pomace oil (2 μg/kg) (Brasil, 2003, 2004, 2007). When using these levels for comparison, one can see that the values presented on Tables 2 and 3 are considerably low.

Results also show that the roasting degree has no apparent influence in PAHs levels in the brews, since the difference among the levels detected was not statistically significant ($P < 0.05$), except between dark and medium roasted filtered brews. Similar results were reported in a previous study by Tfouni et al. (2012) where no correlation was found between PAHs levels and the roasting degree of ground roasted coffee. This is due to the high variability of the process, as shown by the results obtained within the same cultivar and roasting degree, submitted to the same brewing procedure. The coefficients of variation of the process replicates ranged from 12% (*C. canephora* cv. Apoatã, dark roasted, boiled) to 62% (*C. canephora* cv. Apoatã, medium roasted, filtered). This high variability is probably due to the roasting process since, although the temperature of the roaster was set at 200 °C, when green coffee beans are placed inside, the equipment suffers a temperature variation that is inherent to the roasting process. The internal temperature drops and then starts to increase again throughout the process. Although there was an effort to maintain the same roasting profile for replicates of all processes, some differences were observed, with some samples reaching higher temperatures in a shorter/longer period of time than others (Tfouni et al., 2012).

Other authors presented results of PAHs levels in relation to coffee roasting process. Kayali-Sayadi, Rubio-Barroso, Cuesta-Jimenez, and Polo-Díez (1999) reported higher PAHs concentrations for brews made from commercial ground roasted coffees (2.87 ng/L) than the ones made from green or decaffeinated (1.99 and 1.65 ng/L, respectively), there was no mention on the samples roasting degree. Houessou et al. (2007) did not detect or detected only traces of BbF, BkF and BaP in coffee brews prepared from ground coffees roasted for 5 min under different temperatures. BaA was detected in the range of traces to 0.15 μg/L (260 °C/5 min).

The PAHs transfer to the coffee brew could be related to the known formation of a caffeine-PAHs complex (Kolarovic & Traitler, 1982; Moret & Conte, 2000; Navarro, Ishikawa, Morimoto, & Tatsumi, 2009). As *C. canephora* presents higher caffeine content than *C. arabica*, one should expect that the levels of PAHs in *C. canephora* brews would be higher due to the formation of the complex, which would facilitate the transfer of these lipophilic compounds to the brew. Nevertheless, in the present study, coffee brews prepared with *C. arabica* cv. Catuaí Amarelo ground roasted beans presented mean summed PAHs levels higher than the ones prepared with *C. canephora* cv. Apoatã, independently of the brewing procedure used (Fig. 1). *C. arabica* was contaminated with mean summed PAHs concentrations of 0.052 and 0.034 μg/L (filtered and boiled brews, respectively), while *C. canephora*

Table 2
Roasting degrees and PAHs levels in filtered and boiled coffee brew prepared from *Coffea arabica* cv. Catuaí Amarelo IAC-62.

Roasting degree		Mean PAHs levels (μg/L)[a] (range)				
		BaA	BbF	BkF	BaP	∑HPAs
Light ($n = 2$)	Filtered	0.019 (0.014–0.024)	0.025 (0.017–0.032)	nd	0.010 (nd–0.010)	0.054ab (0.031–0.066)
	Boiled	0.015 (nd–0.015)	0.017 (0.016–0.017)	nd	nd	0.024A (0.016–0.032)
Medium ($n = 4$)	Filtered	0.028 (0.014–0.042)	0.030 (0.014–0.048)	0.012 (0.011–0.014)	0.016 (nd–0.020)	0.075a (0.027–0.105)
	Boiled	0.017 (0.014–0.024)	0.016 (0.013–0.019)	nd	0.012 (nd–0.015)	0.037A (0.028–0.044)
Dark ($n = 3$)	Filtered	0.015 (nd–0.015)	0.015 (0.014–0.016)	nd	nd	0.025b (0.015–0.030)
	Boiled	0.016 (0.014–0.018)	0.019 (0.012-0.025)	0.013 (nd–0.013)	nd	0.039A (0.030–0.034)

n = Number of samples.
nd: Not detected (<limit of detection, from Table 1).
BaA: benz(a)anthracene, BbF: benzo(b)fluoranthene, BkF: benzo(k)fluoranthene, BaP: benzo(a)pyrene.
A, a, b: values in the same column with the same letter are not statistically different ($P < 0.05$).
[a] Mean of samples where the PAHs (polycyclic aromatic hydrocarbons) were detected.

Table 3
Roasting degrees and PAHs levels in filtered and boiled coffee brew prepared from *Coffea canephora* cv. Apoatã IAC-2258.

Roasting degree		Mean PAHs levels (μg/L)[a] (range)				
		BaA	BbF	BkF	BaP	ΣHPAs
Light ($n = 4$)	Filtered	0.014 (0.011–0.018)	0.016 (nd–0.020)	nd	nd	0.025ab (0.011–0.038)
	Boiled	0.015 (nd–0.018)	0.018 (0.012–0.020)	nd	nd	0.029A (0.012–0.040)
Medium ($n = 2$)	Filtered	0.040 (0.017–0.062)	0.049 (nd–0.049)	nd	0.010 (nd–0.010)	0.069a (0.026–0.111)
	Boiled	0.013 (nd–0.013)	0.029 (0.016–0.021)	0.010 (nd–0.010)	nd	0.029A (0.013–0.044)
Dark ($n = 3$)	Filtered	0.024 (nd–0.024)	0.022 (0.013–0.017)	nd	nd	0.023b (0.013–0.042)
	Boiled	0.013 (0.010–0.014)	0.015 (0.013–0.016)	0.010 (nd–0.010)	nd	0.031A (0.027–0.036)

n = Number of samples.
nd: Not detected (<limit of detection, from Table 1).
BaA: benz(a)anthracene, BbF: benzo(b)fluoranthene, BkF: benzo(k)fluoranthene, BaP: benzo(a)pyrene.
A, a, b: values in the same column with the same letter are not statistically different ($P < 0.05$).
[a] Mean of samples where the PAHs (polycyclic aromatic hydrocarbons) were detected.

presented 0.034 and 0.030 μg/L. This might be explained by the fact that the caffeine levels are much higher than the PAHs in both coffees (1195 mg/100 g, arabica; 1729 mg/100 g, canephora (Tfouni et al., 2012)), therefore the formation of caffeine-PAH complex could possibly be limited by PAHs levels, instead of caffeine content. Furthermore, the levels found follow the same pattern of the levels detected for the ground roasted coffee used for brewing, reported in a previous study (Tfouni et al., 2012), where *C. arabica* presented higher mean summed PAHs levels than *C. canephora*. Hischenhuber and Stijve (1987) also did not find correlation between caffeine levels and BaP extraction behaviour, with results showing no difference in extraction between canephora and arabica coffees.

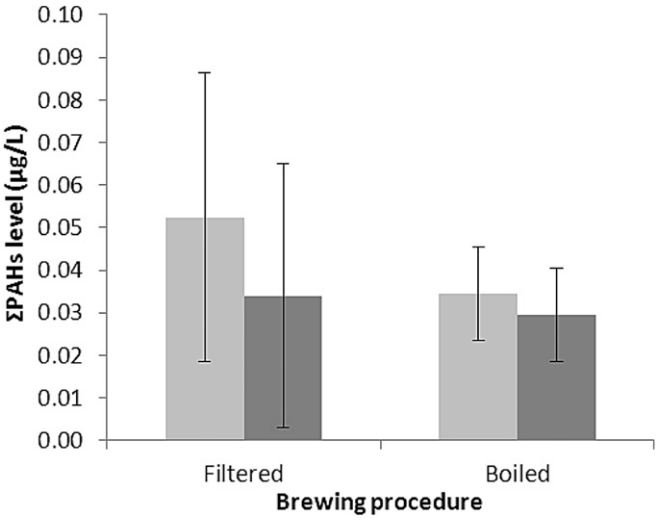

Fig. 1. Mean summed polycyclic aromatic hydrocarbons levels (∑PAHs) in *Coffea arabica* cv. Catuaí Amarelo IAC-62 (▒) and *Coffea canephora* cv. Apoatã IAC-2258 (▒) using two brewing procedures ($n = 9$).

Results in Fig. 1 also show that filtered coffee, for both cultivars, presented higher mean summed PAHs levels than the boiled brewed coffee. Although, taking in consideration the caffeine levels and the complex formation, it would be expected otherwise, since boiled coffee presents higher caffeine content than the filtered one (Camargo & Toledo, 1998).

Kruijf et al. (1987) analyzed BaP in coffee brew samples prepared by filtration (using a coffee maker) and boiling (addition of boiling water and heat at 90 °C for 15 min). As result there was no difference in the levels detected in both procedures: 0.0008 μg/kg for filtered coffee and 0.0010 μg/kg for boiled.

Camargo and Toledo (2002) evaluated PAHs levels in coffee brew samples prepared from commercial coffees available in Brazil. Authors detected higher PAHs levels in brews prepared by boiling than by filtration.

4. Conclusion

There was no correlation between the PAHs levels detected and the coffees roasting degree. A high variability of the results within the same cultivar and roasting degree, submitted to the same brewing procedure was verified.

Although the formation of a caffeine-PAH complex could facilitate PAHs transfer from the ground roasted coffee to the brew, the caffeine levels in the beverages do not seem to influence the transfer.

PAHs levels present in the coffee brew samples analyzed may be considered low when comparing with the maximum permitted levels in the Brazilian regulation or with those established in Europe for different foods (CEC, 2011). It is expected that these levels would not affect the intake of PAHs by the Brazilian population; however, it is important to have and provide information related to potentially carcinogenic compounds in highly consumed food.

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