LWT - Food Science and Technology 50 (2013) 526–530

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

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

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ARTICLE INFO

Article history:
Received 8 May 2012
Received in revised form
17 August 2012
Accepted 20 August 2012

Keywords:
Polycyclic aromatic hydrocarbons
Coffea arabica
Coffea canephora
Coffee brew
Roasting

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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0023-6438
http://dx.doi.org/10.1016/j.lwt.2012.08.015

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/