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Original article

Caffeine and chlorogenic acids intake from coffee brew: influence of roasting degree and brewing procedure

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Summary The influence of coffee cultivar, roasting degree and brewing procedure in the presence and transfer of caffeine and caffeoylquinic acids (CQAs) from ground roasted coffee to the brew was evaluated. Two coffee cultivars were roasted in three roasting degrees and brewed using two different procedures. Compounds were determined simultaneously by HPLC-DAD. Caffeine levels ranged from 87.3 to 122.5 mg/100 mL for *Coffea arabica* cv. Catuaí Amarelo and from 123.3 to 192.0 mg/100 mL for *C. canephora* cv. Apoatã. The sum of CQA isomers ranged from 24.2 to 41.3 mg/100 mL for brews prepared with dark roasted coffee and from 187.7 to 295.6 mg/100 mL for light roasted ones. Brews prepared by boiling showed higher content of the compounds than the corresponding filtered ones. *C. arabica* cv. Catuaí Amarelo light roasted coffee brew presented the lowest caffeine/CQA ratio, regardless of the brewing procedure used, in comparison with the highest ratio of the dark boiled brews.

Keywords Caffeine, caffeoylquinic acids, coffee brew, intake, roasting degree.

Introduction

Coffee is one of the most popular beverages in the world. With an estimate production of 3.03 million tons of green coffee for the 2012 season, Brazil is the world's largest producer and exporter (ABIC, 2012a; CONAB, 2012). The ground roasted coffees available in the market are produced either exclusively with *Coffea arabica* beans or with a blend of *C. arabica* and *C. canephora*, with dark roasted coffee being the most popular. Brazilians consume daily a mean of 227 mL of coffee, which is brewed using different procedures (ABIC, 2012b).

Coffee beans contain a large variety of substances, which in many cases are biologically active, such as caffeine and chlorogenic acids (CGA) (Johnston *et al.*, 2003). These compounds are known to influence coffee flavour, contributing to the acidity and conferring astringency and bitterness (Clifford, 1985; Farah & Donangelo, 2006). Moderate caffeine consumption may have some benefits. Nevertheless, excessive intakes may increase the risks of dehydration, anxiety, headache and sleep disturbances (Ruxton, 2008). Over the years, different studies have been raising concerns regarding caffeine intake, especially among certain groups such as pregnant women, children and heavy coffee drinkers (Camargo *et al.*, 1999; Knight *et al.*, 2004; Crozier *et al.*, 2012; Fitt *et al.*, 2013). CGAs are phenolic compounds that due to their antioxidant activity present several beneficial health properties, including hypoglycaemic, antiviral, hepatoprotective and antispasmodic activities (Johnston *et al.*, 2003; Farah & Donangelo, 2006). One of the main groups of CGA found in green coffee beans is the caffeoylquinic acids (CQAs): 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA) and 5-caffeoylquinic acid (5-CQA) (Farah & Donangelo, 2006).

Roasting process is one of the factors responsible for coffee's characteristic flavour and final quality. In this process, several substances are formed or eliminated, providing flavour, acidity and body (Melo, 2004). Coffee cultivar and roasting are known to influence the levels of caffeine and CGA in ground roasted coffee. In the case of coffee brew, besides the previous factors, brewing procedure and grinding also affect the levels of these compounds in the beverage (Bell *et al.*, 1996; Campa *et al.*, 2005; Tfouni *et al.*, 2012). There are few data regarding the influence of roasting and brewing on the presence of caffeine and CGA in coffee brew, as most of the studies refer to coffee beans and ground roasted coffee.

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Therefore, the objective of the present study was to evaluate the influence of coffee cultivar and roasting degree on the presence of caffeine and CGA in coffee brew, the influence of brewing procedure on their transfer from ground roasted coffee to the beverage, and verify how these factors would affect the intake of these compounds.

Materials and methods

Samples

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

Green coffee beans were obtained by the dry method that involved coffee cherries harvesting, drying under the sun until reaching 12% moisture content and mechanical removal of the dried outer parts. Afterwards, batches of green coffee beans containing 1 kg each were roasted in 3 roasting degrees using 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). The repeatability of the process was evaluated by performing the roasting at least twice for each degree of roast. As a result, for *C. arabica* cv. Catuaí Amarelo, the roasted samples obtained were two light, four medium and three dark, while for *C. canephora* cv. Apoatã, samples were four light, two medium and three dark roasted coffees.

Roasting degrees were determined by the Agtron/SCAA Roast Color Classification System, using an E10-CP Agtron Coffee Roast Analyser (Agtron, Reno, NV, USA), analyses were performed in three replicates. 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 aluminised valve bags at −18 °C and ground immediately before brewing. 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.

Two brewing procedures were evaluated, using the same ground coffee/water ratio (50 g/500 mL): (i) *Filtered coffee*–water (92–96 °C) was left to drip onto ground coffee held in a paper filter and (ii) *Boiled coffee*–water (25 °C) was added to the ground coffee, the mixture was heated until coming to a boil and then filtered in a paper filter.

Coffee brew samples were stored at −18 °C in glass flasks until analyses in duplicate for the presence of caffeine and caffeoylquinic acids (3-CQA, 4-CQA and 5-CQA).

Standards and reagents

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). In this study, authors adopted the IUPAC numbering system for CGA.

Methanol (HPLC grade) was purchased from Tedia Company Inc. (Fairfield, OH, USA). HPLC-grade acetonitrile and reagent-grade glacial acetic acid, phosphoric acid and hydrochloric acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). 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.

Method

Sample preparation

The simultaneous determination of caffeine and caffeoylquinic acids (3-CQA, 4-CQA and 5-CQA) was performed by an extraction and clean-up method based on the one described by Trugo & Macrae (1984), previously used and validated by Tfouni *et al.* (2012).

Coffee samples were transferred (5 mL) to 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, 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 a 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. For separation, a C18 column (Lichrosphere 100, 250 × 4 mm, 5 μm particle size; Merck, Darmstadt, Germany) was used with a gradient elution at a flow rate of 1 mL min^{-1}. Mobile phase consisted 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 to 30 min increase to 80% of A, 30 to 35 min 80% of A, 35 to 40 min decrease to 8% of A, 40 to 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

The three isomer 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 three CQAs isomers, which was performed by adding up the areas of the three CQA peaks. Duplicate HPLC injections of caffeine and CQA standard solutions in water were used to construct linear regression 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.

Statistical analysis

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

Results and discussion

Figure 1 presents chromatograms (272 and 324 nm) of a sample to illustrate peaks separation. Table 1 presents caffeine levels detected in the coffee brew samples. Levels ranged from 87.3 mg/100 mL to 122.5 mg/100 mL for *C. Arabica* cv. Catuaí Amarelo and from 123.3 mg/100 mL to 192.0 mg/100 mL for *C. canephora* cv. Apoatã. As can be observed, *C. canephora* presented higher caffeine levels. Results obtained are in accordance with the ones that have been

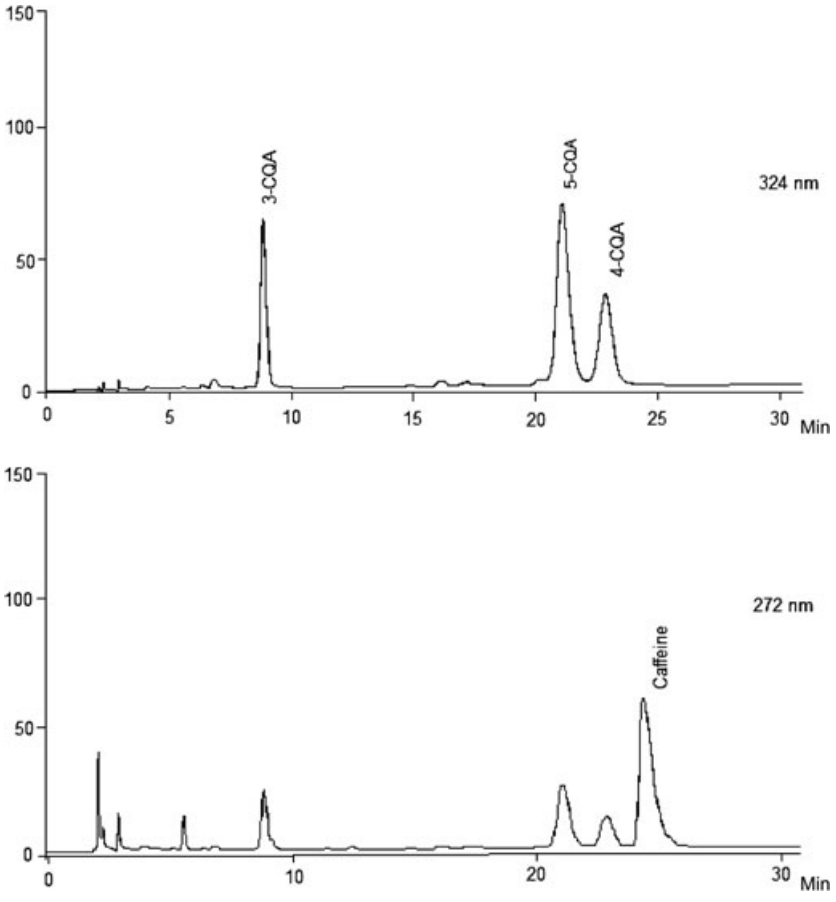

Figure 1 Chromatograms of a light roasted coffee brew (filtered). Detection at 324 nm for 3-CQA, 4-CQA and 5-CQA and at 272 nm for caffeine.

Table 1 Levels of caffeine in filtered and boiled coffee brews prepared from *Coffea arabica* cv. Catuaí Amarelo IAC-62 and *Coffea canephora* cv. Apoatã IAC-2258 ground roasted coffee with different roasting degrees

		Caffeine mean levels (mg/100 mL)* (SD)			
			Coffea arabica cv. Catuaí		*Coffea canephora* cv. Apoatã
Roasting degree		*n*	Amarelo IAC-62	*n*	IAC-2258
Light	Filtered	2	92.5 (16.3)a	4	129.0 (22.5)b
	Boiled	2	122.5 (17.7)a	4	171.3 (5.7)b
Medium	Filtered	4	87.3 (32.3)a	2	165.5 (4.9)b
	Boiled	4	110.8 (15.3)a	2	192.0 (14.1)b
Dark	Filtered	3	99.0 (43.1)a	3	123.3 (27.8)a
	Boiled	3	111.0 (2.6)a	3	176.3 (6.1)b

Values in the same row with the same letter are not statistically different ($P < 0.05$).
n, Number of samples in duplicate; SD, standard deviation.
*Mean of *n* samples.

frequently reported by other authors, where beans and ground roasted coffee of *C. canephora* contain higher caffeine levels than the *C. arabica* ones (Macrae, 1985; Casal *et al.*, 2000; Ky *et al.*, 2001; Tfouni *et al.*, 2012). Rodrigues *et al.* (2007) reported similar pattern for coffee brews; nevertheless, coffee cultivars studied were not informed. There was no statistical difference ($P < 0.05$) in caffeine levels between different roasting degrees, considering same cultivar and brewing procedure.

Caffeoylquinic acid values detected in the coffee brew samples are presented in Table 2. The sum of CQA isomers ranged from 24.2 mg/100 mL to 295.6 mg/100 mL for *C. arabica* and from 30.4 to 253.8 for *C. canephora*, with 5-CQA representing 33–43% and 34–41% of the sum, respectively. These results are in accordance with previous studies for ground roasted coffee, where 5-CQA represented 29–42% (Tfouni *et al.*, 2012).

Lower CQA levels were obtained for brews prepared with coffees of darker roasting degrees, with levels being reduced up to 91%. There are many data available regarding CQA levels in coffee, although studies are mainly performed with coffee beans or ground roasted coffee. Green coffee beans present high levels of CQAs in their composition; however, during roasting, these levels can be drastically reduced (Farah *et al.*, 2005; Perrone *et al.*, 2008; Tfouni *et al.*, 2012). Consequently, brews prepared from darker roasted coffees are expected to present lower CQAs content. No statistical difference ($P < 0.05$) was observed in CQA levels between the cultivars studied and within the same roasting degree and brewing procedure.

When considering brewing procedure, boiled coffees showed higher content of caffeine and CQAs than the corresponding filtered ones, although statistical analysis showed that for *C. arabica* brews, the difference was, in most cases, not significant ($P < 0.05$). Caffeine content in filtered and boiled brews ranged from 87.3 mg/100 mL to 165.5 mg/100 mL and from 110.8 mg/100 mL to 192.0 mg/100 mL, respectively. Comparing the procedures used, an increase of 12–43% in caffeine extraction was obtained in boiled coffee samples, while for the sum of CQA isomers, the levels detected were from 8% to 35% higher. Similar results were reported by Camargo & Toledo (1998). This can be explained by the fact that during boiled coffee procedure, ground roasted coffee stays in contact with water for a longer period, increasing the extraction yield. Different results were obtained by Bell *et al.* (1996), where boiled and filtered coffees presented different caffeine content only when boiling time was increased for 2 min; in this study, ground

Table 2 Levels of 5-CQA and sum of CQAs isomers in filtered and boiled coffee brews prepared from *Coffea arabica* cv. Catuaí Amarelo IAC-62 and *Coffea canephora* cv. Apoatã IAC-2258 ground roasted coffee with different roasting degrees

		Mean levels (mg/100 mL)* (SD)					
		Coffea arabica cv. Catuaí Amarelo IAC-62			*Coffea canephora* cv. Apoatã IAC-2258		
Roasting degree		*n*	5-CQA	ΣCQAs	*n*	5-CQA	ΣCQAs
Light	Filtered	2	92.5 (16.3)a	219.1 (31.1)a	4	77.3 (7.7)a	187.7 (16.7)a
	Boiled	2	126.5 (17.7)A	295.6 (46.2)A	4	104.3 (25.5)A	253.8 (57.6)A
Medium	Filtered	4	25.3 (11.5)b	67.3 (30.4)b	2	17.5 (0.7)b	50.3 (0.4)b
	Boiled	4	30.5 (9.0)B	81.9 (20.9)B	2	20.0 (0.0)B	58.1 (0.5)B
Dark	Filtered	3	8.0 (4.0)c	24.2 (11.7)c	3	10.7 (2.1)b	30.4 (6.7)b
	Boiled	3	8.7 (2.3)C	26.1 (6.7)C	3	14.7 (3.1)B	41.3 (9.3)B

Values in the same column with the same letter are not statistically different ($P < 0.05$).
5-CQA, 5-caffeoylquinic acid; ΣCQAs, sum of 3-caffeoylquinic acid; 4-caffeoylquinic acid and 5-caffeoylquinic acid; *n*, Number of samples in duplicate; SD, standard deviation.
*Mean of *n* samples.

coffee was only added to the water when this came to a boil.

As Brazilians are among the largest coffee consumers in the world, this product represents their most important source of caffeine in the diet (Camargo *et al*., 1999). Therefore, data obtained in Tables 1 and 2 were used to estimate the intake of caffeine and CQAs from *C. arabica* cv. Catuaí Amarelo IAC-62 and *C. canephora* cv. Apoatã IAC-2258 brews. For calculations, a daily mean consumption of 227 mL reported by the Brazilian Coffee Industry Association was used (ABIC, 2012b).

Filtered *C. arabica* brews are the ones that would least contribute to caffeine intake: 198.2–224.7 mg day^{-1}. The highest intakes would be provided by boiled *C. canephora* samples, with 388.9–435.8 mg day^{-1}, which are values almost twice as high. Based on a review, Narowt *et al*. (2003) reported that a daily caffeine intake of up to 400 mg day^{-1} (6 mg kg body $weight^{-1}$ per day) is not associated with adverse effects in healthy adults. As coffees available in the market are produced either exclusively with *C. arabica* beans or with a blend of both species, the consumption of pure *C. arabica* coffees will result in less caffeine ingestion than the one provided by a blend. Blends can be made by adding *C. canephora* in a proportion of up to 50% to *C. arabica* without being detected differences in sensory acceptance (Mendes, 2005). Hence, considering the highest arabica and canephora caffeine levels, and calculating the
daily intake for a 50% blend, result would be 357.0 mg day^{-1}. Therefore, results obtained with the cultivars studied are within the suggested limit for caffeine intake of 400 mg day^{-1}. Lower caffeine intake from coffee (175.3 mg day^{-1}) was reported by Camargo *et al*. (1999) for the population of Campinas, SP, Brazil. A study performed in the UK reported a mean intake from coffee of 49.5 mg day^{-1} (Fitt *et al*., 2013), however, the caffeine levels used for intake assessment were lower, probably due to different water/coffee ratio used for brewing.

As for CQAs, boiled brews prepared with light roasted coffee are the ones that most contribute to the sum of CQAs intake, 671.0 mg day^{-1} for *C. arabica* and 576.1 mg day^{-1} for *C. canephora*. CQA intakes are significantly reduced when the source of ingestion is medium and dark roasted coffee, with reduction rates of 69–77% and 73–84%, respectively. As in Brazil, the most popular and main type of ground roasted coffee commercialised is the dark roasted one, ingestions of CQAs provided by coffee brew may be among the lower ones for the majority of the population. Additionally, according to Duarte & Farah (2011), the simultaneous consumption of milk and coffee may produce a negative effect on CGA bioavailability in humans. This may be an important factor in CQA intakes by Brazilians, once it is very common in the country to consume coffee added with milk.

Considering health issues, the most beneficial coffee brew in terms of caffeine and CQAs content would be the one with the lowest caffeine:CQA ratio, which would mean high CQAs and low caffeine intake. The best brew in this aspect would be the *C. arabica* cv. Catuaí Amarelo IAC-62 light roasted coffee, which provides a ratio of 0.4, regardless of brewing procedure used, in comparison with the 4.3 ratio of the dark boiled coffees (independent of the cultivar).

Conclusion

Coffee species and brewing procedure were shown to have influence in the levels of caffeine in coffee brews, while the degree of roast played an important role in CQAs content. The highest caffeine levels were detected in boiled coffees prepared with *C. canephora* cv. Apoatã IAC 2258, while lower levels were found in filtered brews from *C. arabica* cv. Catuaí Amarelo IAC-62. Caffeoylquinic acids were detected in higher levels in boiled beverages made from light roasted coffee, while lower levels were detected in filtered brews of dark roasted coffee.

Results obtained with the cultivars studied are within the suggested limit for caffeine intake of 400 mg day^{-1}. However, it is important to emphasise that there are other sources of caffeine in the diet that may contribute to the total intake of this compound, such as soft drinks, tea, chocolate, energy drinks and guarana powder (Camargo & Toledo, 1999; Camargo *et al*., 1999; Tfouni *et al*., 2007).

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