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Comprehensive analysis of major and minor chlorogenic acids and lactones in economically relevant Brazilian coffee cultivars

Daniel Perrone [a], Adriana Farah [a,*], Carmen M. Donangelo [a], Tomas de Paulis [b], Peter R. Martin [b]

[a] *Laboratório de Bioquímica Nutricional e de Alimentos, Universidade Federal do Rio de Janeiro, Instituto de Química, Departamento de Bioquímica, Ilha do Fundão, CT bloco A, 21949-900 Rio de Janeiro, RJ, Brazil*
[b] *Vanderbilt Institute for Coffee Studies, Department of Psychiatry, Vanderbilt University School of Medicine, Nashville, TN 37232, USA*

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Abstract

Coffee is the most consumed beverage in the world and a significant source of phenolic compounds, particularly chlorogenic acids (CGA). During coffee roasting, some CGA are partially transformed into chlorogenic acid lactones (CGL). Both CGA and CGL are important compounds for flavor and potentially beneficial to human health. In the present study, using LC–MS and synthetic standards, we investigated major and minor CGA and CGL isomers in green and roasted samples of economically relevant Brazilian *Coffea arabica* and *Coffea canephora* coffee cultivars. For the first time, in addition to nineteen previously identified CGA and CGL, 1-feruloylquinic acid, 1-feruloylquinic lactone and 3,4-diferuloylquinic acid were quantified in *C. arabica* and *C. canephora*, the contents of 3- and 4-*p*-coumaroylquinic lactones were reported in *C. canephora* and 3,4-di-*p*-coumaroylquinic acid was identified in *C. arabica*. Despite their low concentrations, the implications of these findings for flavor, cup quality and the biological properties of coffee merit further investigation.

Keywords: Coffee; Chlorogenic acids; Lactones; Quinides; LC–MS; Coffee roasting

1. Introduction

Coffee is one of the most widely consumed beverages in the world. In 2005, coffee production reached 6.4 million tons worldwide and, in this scenario, Brazil remains the largest green coffee producer and exporter, accounting for approximately 30% of the world market. Among the most economically relevant Brazilian coffee cultivars are *Coffea arabica* cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho, *C. arabica* cv. Bourbon and *Coffea canephora* cv. Conillon – similar to Robusta coffee. These cultivars are used in most commercial blends around the world.

Chlorogenic acids (CGA) are the main phenolic compounds in coffee, being esters of *trans*-cinnamic acids, such as caffeic, ferulic and *p*-coumaric acids, with (−)-quinic acid (QA) (Fig. 1) (Clifford, 2000). CGA may be subdivided according to the nature and number of cinnamic substituents and their esterification position in the cyclohexane ring of QA. The major classes of CGA in green coffee are caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), feruloylquinic acids (FQA), *p*-coumaroylquinic acids (*p*-CoQA) and caffeoylferuloylquinic acids (CFQA). Minor classes, such as diferuloylquinic acids (diFQA), di-*p*-coumaroylquinic acids (di-*p*-CoQA), dimethoxycinnamoylquinic acids and others, which together constitute less than 1% of total CGA content, have been recently identified (Clifford, Knight, Surucu, & Kuhnert, 2006a; Clifford, Marks, Knight, & Kuhnert, 2006b). Although CGA (primarily as 5-caffeoylquinic acid) are widely distributed in plant materials, their content in green coffee is among the highest found in plants, ranging from 4 to 14% (Farah & Donangelo, 2006; Trugo & Macrae, 1984), with 45 different CGA

* Corresponding author. Tel.: +55 2125627352; fax: +55 2125628213.
E-mail address: afarah@iq.ufrj.br (A. Farah).

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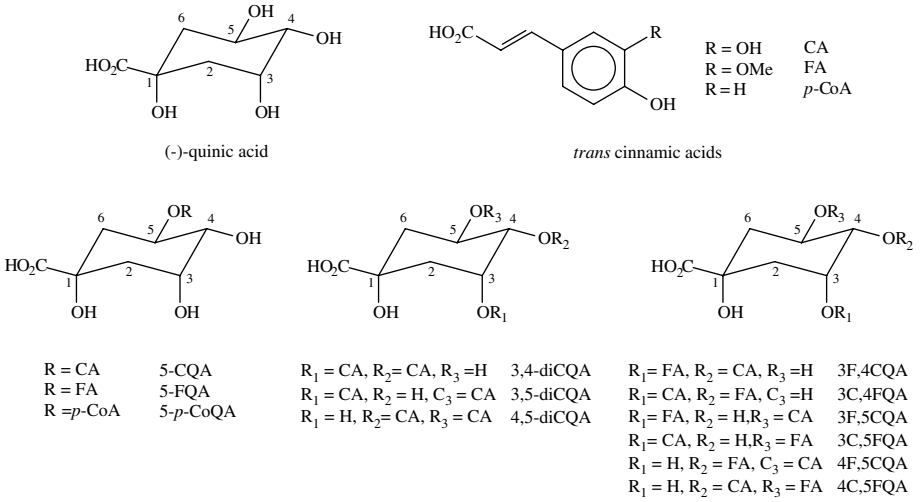

Fig. 1. Structure of chlorogenic acids precursors – quinic acid, caffeic acid (CA), ferulic acid (FA), *p*-coumaric acid (*p*-CoA) – followed by CGA main subclasses: caffeoylquinic acids (CQA), feruloylquinic acids (FQA), *p*-coumaroylquinic acids (*p*-CoQA) (example of 5-isomers for CGA monoesters), dicaffeoylquinic acids (diCQA) and caffeoylferuloylquinic acids (CFQA). We adopted the IUPAC numbering system (IUPAC, 1976) for chlorogenic acids.

compounds already identified (Clifford, Johnston, Knight, & Kuhnert, 2003; Clifford et al., 2006a; Clifford et al., 2006b).

CGA play an important role in the formation of roasted coffee flavor and have a marked influence in determining coffee cup quality (Farah, Monteiro, Calado, Franca, & Trugo, 2006). Moreover, several beneficial health effects have been attributed to CGA and may be largely explained by their potent antioxidant activities (Moreira, Monteiro, Ribeiro-Alves, Donangelo, & Trugo, 2005; Natella, Nardini, Gianetti, Dattilo, & Scaccini, 2002; Pereira, Pereira, Trugo, & Neto, 2003). Some *in vitro* and *in vivo* pharmacological properties of CGA are hypoglycemic, antiviral, hepatoprotective and immunoprotective activities (Basnet, Matsushige, Hase, Kadota, & Namba, 1996; Hemmerle et al., 1997; Robinson et al., 1996; Tatefuji et al., 1996).

During coffee roasting, CGA are partially degraded as a result of pyrolysis, generating phenolic lactones and other derivatives. Cinnamoyl-1,5-γ-quinolactones (CGL) are the main CGA lactones in roasted coffee, being produced through the loss of a water molecule and formation of an intramolecular ester bond between positions 1 and 5 of QA (Farah, de Paulis, Trugo, & Martin, 2005). Along with CGA, CGL also contribute to coffee flavor and, despite their low concentrations, their impact on the final cup quality may be significant (Ginz & Engelhardt, 2001). CGL have also been studied for their potential hypoglycemic effects (Shearer et al., 2003) and for their actions at opioid and adenosine brain receptors (de Paulis et al., 2004; de Paulis et al., 2002).

Using HPLC and synthetic standards, we have previously studied the formation of CGL isomers during roasting of *C. arabica* Bourbon and Longberry cultivars from Brazil and Ethiopia, respectively, and during roasting of *C. canephora* cv. Robusta from Uganda (Farah et al., 2005). In the present study, using LC–MS and synthetic standards, we investigated the occurrence of a greater number of major and minor CGA and CGL compounds in green and roasted economically relevant Brazilian samples of *Coffea arabica* cv. Mundo Novo, *Coffea arabica* cv. Catuaí Vermelho and *Coffea canephora* cv. Conillon.

2. Materials and methods

2.1. Standards

Although under IUPAC rules the numbering system for the lactones differs from that of the acids (IUPAC, 1976), to avoid confusion, in this study, the same numbering of the carbon atoms of QA was used for both the lactones and their acid precursors. 5-caffeoylquinic acid (5-CQA) was purchased from Sigma–Aldrich (St. Louis, MO). A mixture of 3-CQA, 4-CQA and 5-CQA was prepared from 5-CQA using the isomerization method described by Farah et al. (2005). The lactones 3-CQL, 4-CQL, 1-FQL, 3-FQL, 4-FQL, 3-*p*-CoQL, 4-*p*-CoQL, 3,4-diCQL, 3,4-di-*p*-CoQL and 3,4-diFQL were synthesized using the low temperature modification (Huynh-Ba, 1995) of the method of Wynne, Boublik, Drummer, Rae, and Funder (1985). The identity and purity of the lactones were confirmed by proton NMR spectroscopy and by HPLC. 1-FQA, 3-FQA, 4-FQA, 3-*p*-CoQA, 4-*p*-CoQA, 3,4-diCQA, 3,4-di-*p*-CoQA and 3,4-diFQA were obtained by hydrolysis of the corresponding CGL in 50% aqueous tetrahydrofuran, as previously described (Farah et al., 2005; Huynh-Ba, 1995).

2.2. Coffee samples

Good cup quality samples of green *C. arabica*, cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho (Red Catuaí) and *C. canephora*, cv. Conillon were obtained directly from producers in Guaxupé, Minas Gerais, Brazil.

2.3. Roasting

Coffee samples (100 g) were roasted in a commercial bed fluid roaster (i-Roast® Model No. 40009, Hearthware Home Products, USA). Mild roasting conditions were used to produce from very light to medium roasting degrees, so that the formation of CGA and CGL novel minor isomers could be observed: 170 °C for 6, 8, 12 and 15 min. We also selected a roasting condition to produce a dark roasting degree: 200 °C for 15 min. Roasting degrees were determined by percent weight loss during roasting and by comparison with color disks from the "Roasting Color Classification System" (Agtron-SCAA, Reno, NV, USA, 1995), following the standards used by the Brazilian Coffee Industries Association (ABIC). Roasting reproducibility based on percent weight loss was evaluated in an experiment using six replicates of the same sample (*C. arabica* cv. Mundo Novo) and roasting condition (170 °C for 6 min.).

2.4. Weight loss

The percent weight loss (%WL) of coffee beans after roasting was calculated using the following equation

$$\%\mathrm{WL} = \frac{(\mathrm{WBR} - \mathrm{WAR})}{\mathrm{WBR}} \times 100,$$

where WBR is the weight before roasting and WAR is the weight after roasting.

2.5. Water content

To express the amount of CGA and CGL on a dry weight basis, the water content of freshly ground green and roasted coffee beans was determined according to the AOAC method (AOAC, 2000).

2.6. Chlorogenic acids and lactones extraction

Green and roasted coffee beans were frozen in liquid nitrogen prior to grinding. Samples were ground to pass through a 0.46 mm sieve and extracted in duplicate according to the method described in detail by Farah et al. (2005).

2.7. LC–MS

Extracts containing CGA and CGL were analyzed according to the method recently described by Farah, de Paulis, Moreira, Trugo, and Martin (2006). The LC equipment (Shimadzu, Kyoto, Japan) comprised a LC-10AD*vp* quaternary pump, a CTO-10AS*vp* column oven, an 8125 manual injector (Rheodyne) with a 5 μL loop and a SPD-M10A*vp* diode array detector (DAD). This LC system was interfaced with a LC–MS 2010 mass spectrometer (Shimadzu, Kyoto, Japan) fitted with an electrospray ion source.

Chromatographic separations were achieved using a Magic C30 HPLC column (150 × 2 mm, 5 μm, 100 Å, Michrom Bioresources, Inc., Auburn, CA, USA) maintained at a constant temperature of 40 °C. The LC two-phase mobile system consisted of 0.3% aqueous formic acid (eluent A) and methanol (eluent B). The gradient was programmed with a flow rate of 0.2 mL/min, as follows.

Time (min)	Eluent A (%)	Eluent B (%)
0.03	90	10
30	83	17
45	83	17
55	65	35
75	65	35
93	50	50
93.01	90	10
100	Stop	Stop

Data were acquired by LCMS solution software (Shimadzu Corp., version 2.00, 2000) for both mass spectrometer and diode array detector (recording from 190 nm to 370 nm).

The electrospray ionization source was operated in the negative mode to generate $[M-H]^-$ ions, nebulizer gas (N_2) flow was set to 2.0 L/min and desolvation temperature was adjusted to 250 °C. The mass spectrometer was operated in the single ion monitoring (SIM) mode to detect CGA and CGL specific mass ions.

Identification of major CGA and CGL was primarily performed by comparison with retention time of the respective standard and by their molecular weight. In general, the quantification of CQA, FQA and diCQA and their respective lactones was performed using the diode array data for the peak area of 5-CQA standard corrected with molar extinction coefficients of the respective CGA and direct CGL precursors (in the case of CGL), as previously described (Farah et al., 2005). Because of the lack of molar extinction coefficient for 1-FQA, this compound was quantified using as the correction factor the mean molar extinction coefficients of the three major FQA isomers (Ruback, 1969). As molar extinction coefficients are unavailable for the whole *p*-CoQA class, no correction was employed for this class and their lactones. The CFQA class was quantified as a whole, using the mean molar extinction coefficient of the three diCQA isomers as the correction factor (Ruback, 1969). Because 3,4-diFQA and 1-FQL were only detected by MS, their quantification was based on MS data and their respective standards area.

3. Results and discussion

3.1. Green coffee

A total of fourteen CGA compounds were identified in green *C. arabica* and *C. canephora* samples, according to their molecular weight and retention time: 3-CQA, 4-CQA, 5-CQA, 3-FQA, 4-FQA, 5-FQA, 3-*p*-CoQA, 4-*p*-

CoQA, 5-*p*-CoQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, 3,4-diFQA and 3,4-di-*p*-CoQA. Moreover, 6 major CFQA isomers were observed and quantified as a whole class, since unequivocal individual identification was not possible due to lack of standards and unavailability of LC–MS^n. Despite this, the retention time and the relative abundance of CFQA isomers were similar to those identified by Clifford et al. (2003), suggesting that their order of elution is the same.

The contents of CGA compounds in green *C. arabica* cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho and *C. canephora* cv. Conillon are presented in Table 1. 3,4-di-*p*-CoQA was identified in both *C. arabica* cultivars only by MS and the quantification of this compound was not possible due to insufficient purity of the respective standard. Although 3,4-di-*p*-CoQA has been previously reported in green *C. canephora* cv. Robusta by Clifford et al. (2006b), in this study it was not detected in *C. canephora* cv. Conillon. Total CGA contents in *C. arabica* samples were 6.3 and 5.5 g/100 g on a dry weight basis (dwb), for Mundo Novo and Catuaí Vermelho cultivars, respectively. Total CGA content in *C. canephora* cv. Conillon was 8.6 g/100 g dwb. These results are in agreement with data previously reported for *C. arabica* and *C. canephora* (Correia, Leitão, & Clifford, 1995; Farah et al., 2006; Farah et al., 2005; Farah & Donangelo, 2006; Ky et al., 2001; Trugo & Macrae, 1984). The higher CGA content of *C. canephora* in comparison with *C. arabica* has been extensively reported (Clifford & Ramirez-Martinez, 1991; Farah et al., 2005; Farah & Donangelo, 2006; Trugo & Macrae, 1984). Considering that the beans of Conillon coffee differ physically from Robusta coffee (Conillon beans are smaller and generally darker than Robusta beans, regardless of the primary processing methods used) one would presume that their chemical composition might be different. In fact, comparing CGA content in both varieties of *C. canephora*, the total Conillon CGA content found in the present work is higher than the average CGA content reported elsewhere for Robusta (Correia et al., 1995; Farah et al., 2006; Farah et al., 2005; Farah & Donangelo, 2006; Trugo & Macrae, 1984), being in accordance with data observed for Conillon coffee in previous studies (Farah & Donangelo, 2006). These differences may be reflected in coffee flavor and other characteristics of these coffees and should be investigated.

Small differences in percentage distribution of major CGA were observed between both *C. arabica* cultivars. Such differences have been observed in previous studies investigating other *C. arabica* cultivars (Farah et al., 2006; Farah et al., 2005).

CQA was the most abundant CGA class, accounting for about 84% and 76% of the total CGA in *C. arabica* and *C. canephora* green coffee samples, respectively. Among CQA isomers, 5-CQA was predominant, followed by 4-CQA and 3-CQA. Their relative abundance in *C. arabica* (average) and *C. canephora* were 5.4:1.2:1.0 and 3.9:1.2:1.0, respectively. The second most abundant class was diCQA, which represented about 11% and 15% of total CGA in *C. arabica* and *C. canephora* samples, respectively. The relative abundance of 3,4-diCQA, 3,5-diCQA and 4,5-diCQA isomers in *C. arabica* (average) and *C. canephora* were 1.2:1.0:1.0 and 1.5:1.2:2.1, respectively. CQA and diCQA classes in *C. arabica* and *C. canephora* samples, represented together approximately 95% and 91% of total CGA, respectively. FQA, *p*-CoQA and CFQA accounted for the remaining amount of major CGA, where FQA was responsible for 4% and 7% of CGA in *C. arabica* and *C. canephora*, respectively, *p*-CoQA accounted for 1.0% and 0.6% of total CGA, while total CFQA accounted for only 0.4% and 1.6%, respectively. It is interesting to note that total CFQA content in *C. canephora* sample was four times higher than those in *C. arabica* samples. 3,4-diFQA, a minor CGA compound, recently reported by Clifford et al. (2006a) in green Robusta coffee, was identified and quantified for the first time in green *C. arabica* and *C. canephora* cv. Conillon. *C. canephora* samples presented a higher amount of this compound (2.9 μg/100 g dwb) in comparison to *C. arabica* (0.7 μg/100 g dwb). In both species 3,4-diFQA should be considered as a trace compound.

No lactones were observed in green coffee samples, with the exception of a small amount of 3-FQL present in Mundo Novo cultivar (Table 2). Farah et al. (2005) also identified low levels of 4-FQL and 3,4-diCQL in green coffee. The presence of lactones in green coffee may be explained by the heat applied during primary processing (Farah et al., 2005).

3.2. Roasted coffee

The reproducibility of the roasting process and of CGA and CGL analysis was evaluated as a whole, in a six replicate experiment, where the same coffee sample (*C. arabica* cv. Mundo Novo) was roasted at 170 °C for 6 min. Percent weight loss was calculated and CGA and CGL contents were analyzed. The obtained coefficients of variation for percent weight loss and for CGA and CGL LC–MS analysis results were low ($CV_{\%WL} = 0.6\%$; $CV_{CGA\ and\ CGL} = 3.3\%$), and suggested that chromatographic analysis rather than coffee roasting would be responsible for variations in the final result. Therefore, each coffee sample was roasted one time in each condition. Weight loss during roasting at different degrees ranged from 11.5% to 19.9%; 10.4% to 17.9% and 10.3% to 16.8% in *C. arabica* cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho and *C. canephora* cv. Conillon samples, respectively (Table 1). Differences among these cultivars were observed in both weight loss and color development, being color increasingly lighter and weight loss increasingly lower in *C. arabica* cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho and *C. canephora* cv. Conillon samples. Such differences during roasting of distinct varieties under the same conditions have also been observed in previous studies (Farah et al., 2006; Farah et al., 2005; Trugo & Macrae, 1984). They may be explained by differences in physical–chemical properties, such as initial water content in the green beans. The water

Table 1
Chlorogenic acids (CGA) contents in economically relevant Brazilian green and roasted coffee cultivars[a]

Roasting condition[b]	Weight loss (%)	3-CQA	4-CQA	5-CQA	3-FQA	4-FQA	5-FQA	3-*p*-CoQA	4-*p*-CoQA	5-*p*-CoQA	3,4-diCQA	3,5-diCQA	4,5-diCQA	3,4-diFQA	CFQA
C. arabica cv. Mundo Novo															
Green	0.0	667.2 ± 15.4	807.5 ± 13.2	3611.2 ± 63.1	9.6 ± 0.6	31.3 ± 0.9	192.0 ± 9.6	11.4 ± 0.2	8.0 ± 0.5	49.5 ± 2.3	262.1 ± 14.4	269.1 ± 15.9	316.6 ± 27.6	0.7 ± 0.1	31.2 ± 7.2
1	11.5	1096.6 ± 26.4	1344.7 ± 40.8	2736.7 ± 73.2	43.2 ± 0.8	58.3 ± 4.4	179.0 ± 3.8	12.6 ± 0.9	11.6 ± 1.0	44.2 ± 0.4	198.3 ± 4.5	154.6 ± 3.3	232.7 ± 11.8	0.8 ± 0.0	16.6 ± 0.1
2	13.6	720.8 ± 13.0	868.4 ± 18.2	1656.6 ± 29.3	31.5 ± 1.2	49.1 ± 0.6	143.8 ± 8.8	8.4 ± 1.5	6.8 ± 2.4	28.8 ± 1.4	103.4 ± 4.2	80.2 ± 3.2	118.6 ± 13.7	0.8 ± 0.0	10.3 ± 0.7
3	15.4	433.1 ± 2.5	493.3 ± 1.6	942.0 ± 11.4	18.9 ± 0.8	33.2 ± 0.3	104.1 ± 1.5	4.7 ± 0.8	5.9 ± 0.9	19.2 ± 1.0	48.1 ± 0.9	33.4 ± 1.3	49.6 ± 0.1	0.5 ± 0.0	5.4 ± 4.3
4	16.1	324.9 ± 2.4	388.4 ± 5.4	673.6 ± 3.1	13.6 ± 0.3	26.6 ± 0.7	80.7 ± 0.0	3.8 ± 0.3	ND[c]	15.8 ± 3.2	33.1 ± 1.2	21.9 ± 1.4	32.9 ± 0.5	0.4 ± 0.0	1.8 ± 0.0
5	19.9	51.8 ± 0.8	55.2 ± 1.5	93.3 ± 2.5	11.5 ± 0.4	7.2 ± 0.9	15.3 ± 1.0	ND	ND	ND	ND	1.4 ± 0.0	2.2 ± 0.5	ND	ND
C. arabica cv. Catuaí Vermelho															
Green	0.0	618.0 ± 1.7	770.9 ± 13.4	3357.4 ± 55.3	6.7 ± 0.2	25.7 ± 1.9	160.9 ± 2.2	10.3 ± 0.3	6.1 ± 0.3	38.8 ± 0.7	190.3 ± 2.0	156.9 ± 3.1	164.6 ± 4.2	0.9 ± 0.1	13.4 ± 1.0
1	10.4	1030.1 ± 10.5	1253.4 ± 20.2	2451.9 ± 16.6	20.2 ± 0.9	51.0 ± 0.3	148.6 ± 2.1	13.4 ± 0.6	11.2 ± 1.0	38.1 ± 1.4	146.9 ± 2.8	109.2 ± 2.2	161.2 ± 1.9	1.7 ± 0.2	7.8 ± 0.3
2	11.7	807.5 ± 1.1	971.0 ± 0.4	1905.1 ± 12.1	16.7 ± 1.2	38.2 ± 1.3	139.2 ± 4.3	9.4 ± 4.8	9.6 ± 4.1	39.0 ± 0.6	94.5 ± 5.3	69.9 ± 3.3	102.4 ± 7.2	1.4 ± 0.0	4.6 ± 0.3
3	14.1	495.3 ± 1.8	592.6 ± 1.3	1150.6 ± 8.2	9.4 ± 1.2	29.7 ± 1.2	109.9 ± 3.2	5.5 ± 0.4	ND	28.1 ± 1.2	46.8 ± 3.0	36.2 ± 3.8	54.8 ± 7.3	0.8 ± 0.1	6.0 ± 2.6
4	15.0	328.0 ± 18.9	393.4 ± 25.2	761.4 ± 54.5	7.1 ± 0.2	21.4 ± 1.0	77.4 ± 5.0	1.5 ± 0.6	ND	19.8 ± 1.7	26.2 ± 0.5	20.2 ± 2.5	29.7 ± 4.2	0.5 ± 0.2	1.3 ± 0.1
5	17.9	70.4 ± 3.8	82.4 ± 5.0	118.9 ± 29.4	ND	7.0 ± 2.9	18.0 ± 1.8	ND	ND	3.0 ± 0.4	ND	1.2 ± 0.2	4.4 ± 1.1	ND	0.3 ± 0.0
C. canephora cv. Conillon															
Green	0.0	1065.6 ± 9.5	1277.2 ± 13.6	4114.0 ± 39.7	32.6 ± 0.2	57.5 ± 1.0	519.7 ± 6.0	10.6 ± 0.3	11.8 ± 0.1	30.0 ± 6.2	429.1 ± 11.8	283.5 ± 7.9	586.4 ± 17.7	2.9 ± 0.1	136.5 ± 5.6
1	10.3	1308.4 ± 0.1	1666.5 ± 15.9	3175.5 ± 4.1	115.3 ± 2.3	139.0 ± 1.7	411.1 ± 2.7	9.4 ± 0.3	11.5 ± 0.5	20.6 ± 1.0	269.6 ± 5.0	195.8 ± 2.4	503.5 ± 0.8	5.9 ± 0.2	113.9 ± 1.0
2	11.7	1064.6 ± 38.8	1324.9 ± 47.4	2460.5 ± 85.7	108.1 ± 2.6	125.3 ± 4.1	362.6 ± 7.2	8.0 ± 0.3	9.2 ± 1.3	19.1 ± 1.5	181.2 ± 23.5	138.8 ± 12.2	370.9 ± 48.2	5.9 ± 0.3	76.7 ± 5.4
3	13.2	711.0 ± 27.2	894.4 ± 32.4	1534.3 ± 52.9	184.8 ± 2.8	105.3 ± 0.2	289.0 ± 2.6	5.9 ± 0.1	5.9 ± 0.4	16.3 ± 1.1	94.5 ± 3.8	73.8 ± 6.8	221.9 ± 8.6	7.2 ± 0.3	57.1 ± 2.0
4	13.9	563.4 ± 25.6	706.7 ± 21.4	1145.1 ± 37.5	155.3 ± 6.0	89.5 ± 4.2	223.5 ± 1.0	5.0 ± 0.1	ND	14.3 ± 0.7	61.1 ± 3.6	48.5 ± 8.0	163.4 ± 6.9	5.2 ± 0.7	39.0 ± 0.3
5	16.8	95.9 ± 2.5	106.9 ± 0.5	174.9 ± 1.6	49.6 ± 2.0	24.6 ± 0.3	51.4 ± 2.7	1.6 ± 0.3	ND	ND	3.9 ± 0.3	2.3 ± 0.2	30.0 ± 1.1	2.3 ± 0.1	ND

[a] Results are shown as the means of extractions in duplicates ± standard deviation, expressed as mg/100 g of coffee dry weight, except for 3,4-diFQA, which is expressed as mg/100 g of coffee dry weight.
[b] 1 = 170 °C, 6 min; 2 = 170 °C, 8 min; 3 = 170 °C, 12 min; 4 = 170 °C, 15 min; 5 = 200 °C, 15 min.
[c] Not detected.

Table 2
Chlorogenic acid lactones (CGL) contents in economically relevant Brazilian green and roasted coffee cultivars[a]

Roasting condition[b]	Weight loss (%)	3-CQL	4-CQL	3-FQL	4-FQL	3-*p*-CoQL	4-*p*-CoQL	3,4-diCQL
C. arabica cv. Mundo Novo								
Green	0.0	ND[c]	ND	5.6 ± 1.5	ND	ND	ND	ND
1	11.5	11.51 ± 3.5	77.6 ± 1.6	16.8 ± 1.1	15.0 ± 0.7	2.4 ± 0.3	1.6 ± 0.0	3.0 ± 0.9
2	13.6	170.1 ± 4.0	107.6 ± 6.5	25.2 ± 0.6	19.2 ± 0.1	3.5 ± 0.3	2.2 ± 0.9	9.4 ± 0.0
3	15.4	183.2 ± 4.6	118.8 ± 0.7	31.4 ± 2.8	23.8 ± 0.4	4.0 ± 0.2	4.5 ± 1.0	9.9 ± 0.6
4	16.1	161.4 ± 1.0	106.8 ± 1.4	27.3 ± 0.4	22.0 ± 1.2	3.9 ± 0.0	3.9 ± 0.6	8.4 ± 0.7
5	19.9	32.5 ± 0.4	18.0 ± 1.2	10.3 ± 1.4	7.6 ± 1.1	2.0 ± 0.1	0.9 ± 0.2	ND
C. arabica cv. Catuaí Vermelho								
Green	0.0	ND	ND	ND	ND	ND	ND	ND
1	10.4	106.4 ± 0.9	69.9 ± 1.8	14.5 ± 0.0	10.3 ± 0.0	1.6 ± 0.4	1.5 ± 0.4	8.3 ± 1.4
2	11.7	164.2 ± 0.2	109.7 ± 3.5	21.5 ± 5.6	18.8 ± 1.0	3.1 ± 1.9	2.0 ± 1.0	12.5 ± 1.8
3	14.1	215.8 ± 0.5	133.4 ± 0.3	36.8 ± 5.6	26.2 ± 4.0	3.6 ± 0.1	3.8 ± 0.7	11.3 ± 0.9
4	15.0	172.1 ± 7.9	107.6 ± 5.0	23.8 ± 0.3	30.7 ± 2.2	3.2 ± 0.2	3.1 ± 0.8	7.3 ± 1.1
5	17.9	47.7 ± 2.5	26.6 ± 3.5	15.0 ± 0.2	10.7 ± 0.1	2.0 ± 0.3	2.5 ± 0.4	2.6 ± 1.4
C. canephora cv. Conillon								
Green	0.0	ND	ND	ND	ND	ND	ND	ND
1	10.3	153.2 ± 2.1	111.4 ± 0.8	132.0 ± 0.4	31.5 ± 0.4	2.8 ± 0.1	4.8 ± 0.6	20.7 ± 0.1
2	11.7	210.4 ± 1.3	147.2 ± 0.9	143.7 ± 3.5	43.9 ± 0.9	3.7 ± 0.3	5.5 ± 0.5	17.5 ± 2.1
3	13.2	235.3 ± 5.7	163.9 ± 5.1	135.6 ± 4.3	56.6 ± 0.7	4.7 ± 1.1	5.4 ± 0.0	18.4 ± 0.0
4	13.9	218.3 ± 0.9	146.8 ± 1.8	133.4 ± 1.1	66.2 ± 10.8	4.4 ± 0.1	5.4 ± 0.1	10.2 ± 1.2
5	16.8	54.8 ± 0.2	31.6 ± 2.6	52.9 ± 0.6	28.5 ± 3.3	2.0 ± 0.8	3.2 ± 0.4	ND

[a] Results are shown as the means of extractions in duplicates ± standard deviation, expressed as mg/100 g of coffee dry weight.
[b] 1 = 170 °C, 6 min; 2 = 170 °C, 8 min; 3 = 170 °C, 12 min; 4 = 170 °C, 15 min; 5 = 200 °C, 15 min.
[c] Not detected.

contents in the green beans from *C. arabica* cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho and *C. canephora* cv. Conillon were 7.6%, 8.7% and 10.0%, respectively. Because coffee beans lose much of their water at the beginning of the roasting process (drying phase), a higher initial water content would result in a longer drying phase period and, consequently, a lower percent weight loss and color development. Moreover, other characteristics of green coffee beans, such as sucrose content, may also contribute to this phenomenon. It is known that *C. arabica* is usually richer in sucrose than *C. canephora* and that sucrose content is directly associated with color development during roasting of coffee (Trugo & Macrae, 1985).

In addition to the fourteen CGA compounds identified in green coffee, 1-FQA was identified in roasted *C. arabica* and *C. canephora* for the first time (Fig. 2). 3,4-di-*p*-CoQA, which has already been reported in green *C. canephora* cv. Robusta (Clifford et al., 2006b), was also identified in roasted *C. arabica* and *C. canephora* cv. Conillon for the first time.

The contents of CGA compounds in roasted coffee samples are presented in Table 1. In general, CGA contents in roasted samples are in agreement with those previously reported (Farah et al., 2005; Farah & Donangelo, 2006; Trugo & Macrae, 1984), and corresponded to 4.8%, 5.3% and 5.6% of average CGA losses for every 1% loss of dry weight for *C. arabica* cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho and *C. canephora* cv. Conillon, respectively, in comparison to green samples. The observed loss of CGA during roasting (up to 95% in dark roasted coffee), which has been extensively reported (Farah et al., 2006; Farah et al., 2005; Farah & Donangelo, 2006; Trugo & Macrae, 1984), is a consequence of the thermal breakage of carbon–carbon covalent bonds, resulting in isomerization in the initial roasting stages and epimerization, lactonization and degradation in the later stages. The contents of 1-FQA in *C. arabica* cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho and *C. canephora* cv. Conillon are presented in Table 3. 1-FQA was only detected in roasted coffee samples and was present at much lower levels than the other FQA isomers. In all roasting degrees, *C. canephora* presented higher levels of this compound than *C. arabica*, with maximum amounts of 0.7, 0.6 and 1.4 mg/100 g of coffee (dwb) in *C. arabica* cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho and *C. canephora* cv. Conillon samples, respectively. Such contents were found in samples with weight losses of 15.4%, 14.1% and 13.2%, respectively (light roasting degree). Comparing to other CGA, 1-FQA content may seem quite low, but it is comparable to the hydroxycinnamic acids content of other food sources such as different berries and apple juice (Murkovic, 2003). The quantification of 3,4-di-*p*-CoQA was not possible due to insufficient purity of the standard.

Eight individual CGL compounds were identified in the roasted samples: 3-CQL, 4-CQL, 1-FQL, 3-FQL, 4-FQL, 3-*p*-CoQL, 4-*p*-CoQL and 3,4-diCQL, being 1-FQL identified for the first time (Fig. 2). This is also the first report of 3-*p*-CoQL and 4-*p*-CoQL quantification in a *C. canephora* sample. Although 3,4-di-*p*-CoQA was identified in both green and roasted *C. arabica* samples, the corresponding lactone (3,4-di-*p*-CoQL) was not detected in the roasted samples.

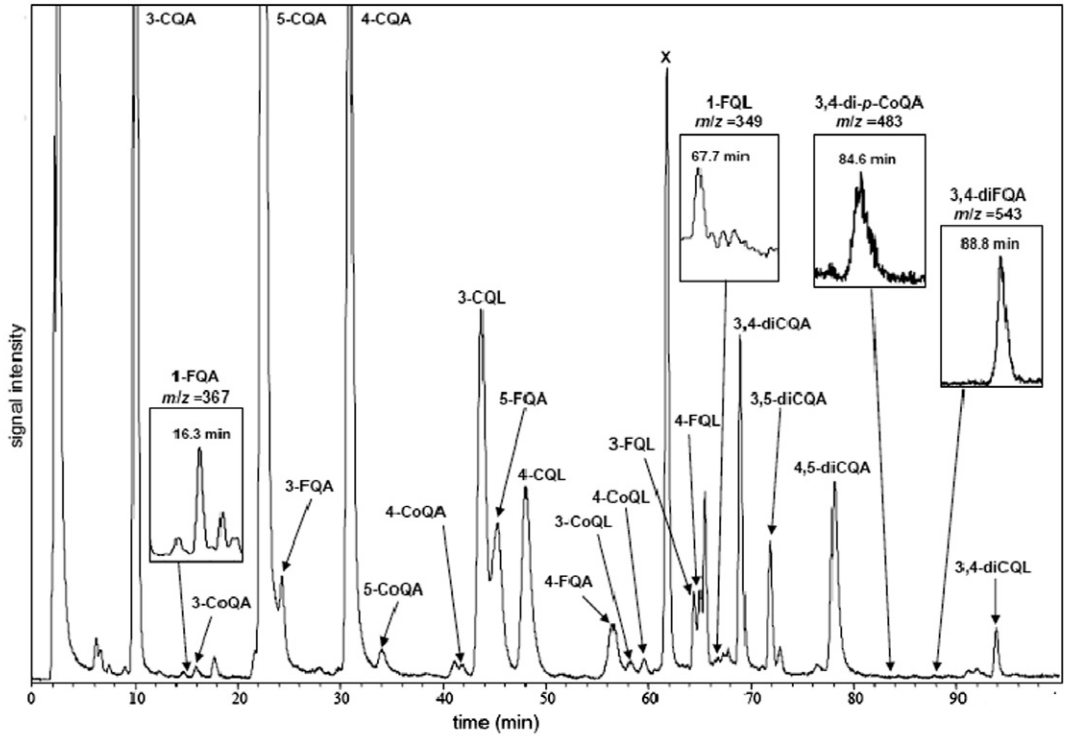

Fig. 2. Typical total ion chromatogram (TIC) of chlorogenic acids (CGA) and lactones (CGL) analysis represented by a light roasted *C. canephora* cv. Conillon sample. 3-CQA, 4-CQA and 5-CQA peaks are shown off scale to highlight small peaks. Note that signal intensity of different compounds cannot be directly compared, since ionization efficiency may vary among them. Selected areas of SIM chromatograms of mass-to-charge (*m*/*z*) 367, 349, 483 and 543 were inserted in the figure to illustrate the elution order of 1-FQA, 1-FQL, 3,4-di-*p*-CoQA and 3,4-diFQA, respectively. The peak marked "X" presented mass-to-charge ratio of 365, consistent with caffeoyl–tryptophan.

The contents of individual CGL compounds in the roasted coffee samples are presented in Table 2. The highest amounts of CGL in Mundo Novo and Catuaí Vermelho *C. arabica* cultivars (376 and 431 mg/100g dwb) were observed at 15.4% and 14.1% weight losses (light roasting degree), respectively. These results are in conformity with data recently published for *C. arabica* cultivars from Ethiopia and Brazil (Farah et al., 2005). The highest content of CGL in *C. canephora* (620 mg/100 g dwb) was observed at 13.2% weight loss, being this amount not only higher than all *C. arabica* reported CGL amounts but also higher than that observed by Farah et al. (2005) when analyzing a *C. canephora* cv. Robusta from Uganda. Even after subtraction of some CGL isomers, not quantified in Robusta by Farah et al. (2005), such as 3-FQL, 3-*p*-CoQL and 4-*p*-CoQL, the Brazilian *C. canephora* cultivar investigated in the present study still showed higher content of CGL in comparison with the African cultivar. 1-FQL contents in *C. arabica* cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho and *C. canephora* cv. Conillon are presented in Table 3. 1-FQL was only detected in roasted coffee samples and was present at trace levels, which were extremely lower than those of other FQL isomers. In all roasted samples, *C. canephora* presented higher levels of this compound than *C. arabica*, with maximum amounts of 1.2, 1.7 and 4.0 μg/100 g of coffee (dwb) in *C. arabica* cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho and *C. canephora* cv. Conillon samples, respectively. Such contents were found in samples with weight losses of 13.6%, 14.1% and 13.2%, respectively (light roasting degree).

In roasted samples with the maximum CGL formation, CQL was the most abundant CGL class, representing 81% and 64% of the total CGL in *C. arabica* and *C. canephora*, respectively. FQL was the second most abundant class, accounting for 15% and 31% of total CGL in *C. arabica* and *C. canephora* samples, respectively. The percentages of diCQL and *p*-CoQL classes, relative to total CGL, were similar in all samples, average of 3% and 2%, respectively. Dark roasted *C. arabica* cv. Mundo Novo, *C. arabica* cv. Catuaí Vermelho and *C. canephora* cv. Conillon (19.9%, 17,9% and 16.8% weight losses, respectively) still contained about 19%, 25% and 28% compared to the maximum amounts of CGL in each cultivar (71, 107 and 173 mg/100 g dwb, respectively) (Table 2).

In this study, we also found putative evidence for the existence of other sixty-four novel major and minor CGA and CGL compounds with mass-to-charge ratios corresponding to CQA, FQA, diCQA, CFQA, CQL, FQL and diCQL classes. Such compounds may originate from various mechanisms of isomerization, such as *cis*-isomerization of the cinnamic acid residue or possible racemization products of QA (Scholz-Böttcher, Ernst, & Maier,

Table 3
Contents of novel chlorogenic acids (CGA) and chlorogenic acid lactones (CGL) in economically relevant Brazilian green and roasted coffee cultivars[a]

Roasting condition[b]	Weight loss (%)	1-FQA (mg/100 g dwb)	1-FQL (μg/100 g dwb)
C. arabica cv. Mundo Novo			
Green	0.0	ND[c]	ND
1	11.5	ND	0.5 ± 0.0
2	13.6	0.6 ± 0.0	1.2 ± 0.0
3	15.4	0.7 ± 0.0	1.1 ± 0.1
4	16.1	0.5 ± 0.1	1.0 ± 0.1
5	19.9	0.4 ± 0.1	0.9 ± 0.2
C. arabica cv. Catuaí Vermelho			
Green	0.0	ND	ND
1	10.4	0.4 ± 0.0	0.6 ± 0.1
2	11.7	0.6 ± 0.1	1.7 ± 0.3
3	14.1	0.6 ± 0.2	1.7 ± 0.4
4	15.0	0.3 ± 0.0	1.7 ± 0.3
5	17.9	ND	1.1 ± 0.3
C. canephora cv. Conillon			
Green	0.0	ND	ND
1	10.3	0.7 ± 0.1	1.3 ± 0.4
2	11.7	1.2 ± 0.3	2.9 ± 0.3
3	13.2	1.4 ± 0.3	4.0 ± 0.2
4	13.9	1.2 ± 0.0	3.1 ± 0.7
5	16.8	0.5 ± 0.0	3.3 ± 0.5

[a] Results are shown as the means of extractions in duplicates± standard deviation.
[b] 1 = 170 °C, 6 min; 2 = 170 °C, 8 min; 3 = 170 °C, 12 min.; 4 = 170 °C, 15 min; 5 = 200 °C, 15 min.
[c] Not detected.

1991; Whitaker & Stommel, 2003). We also observed a peak with mass-to-charge ratio of 365, consistent with caffeoyl–tryptophan, a hydroxycinnamic acid derivative (Schrader, Kiehne, Engelhardt, & Maier, 1996). In the present work, they were not unequivocally identified due to lack of authentic standards, but their unequivocal characterization should be accomplished by means of LC–MS^n and/or NMR techniques.

In the present study, twenty-three major and minor CGA and CGL compounds were identified, being twenty-two quantified. *C. arabica* cv. Mundo Novo and cv. Catuaí Vermelho presented the same CGA and CGL compounds, with small differences in total content and percent distribution. Total CGA and CGL contents in *C. canephora* cv. Conillon were higher then those reported for other *C. arabica* and *C. canephora* cultivars. For the first time, 1-FQA, 1-FQL and 3,4-diFQA were quantified in *C. arabica* cultivars and *C. canephora* cv. Conillon, 3,4-di-*p*-CoQA was identified in *C. arabica* cultivars, and 3-*p*-CoQL and 4-*p*-CoQL contents were reported in *C. canephora*. Despite the low contents of the newly identified CGA and CGL, their influence on flavor and cup quality should be investigated, since threshold values may vary extremely. In addition, considering that the minimum concentrations of CGA and CGL necessary to produce beneficial biological activities are not yet established, their biological relevance should not be undermined, especially when taking into account the sum of all minor CGA and CGL.

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