

## Chlorogenic Acids and Lactones in Regular and Water-Decaffeinated Arabica Coffees

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The market for decaffeinated coffees has been increasingly expanding over the years. Caffeine extraction may result in losses of other compounds such as chlorogenic acids (CGA) and, consequently, their 1,5- $\gamma$ -quinolactones (CGL) in roasted coffee. These phenolic compounds are important for flavor formation as well as the health effects of coffee; therefore, losses due to decaffeination need to be investigated. The present study evaluates the impact of decaffeination processing on CGA and CGL levels of green and roasted arabica coffees. Decaffeination produced a 16% average increase in the levels of total CGA in green coffee (dry matter), along with a 237% increase in CGL direct precursors. Different degrees of roasting showed average increments of 5.5–18% in CGL levels of decaffeinated coffee, compared to regular, a change more consistent with observed levels of total CGA than with those of CGL direct precursors in green samples. On the other hand, CGA levels in roasted coffee were 3–9% lower in decaffeinated coffee compared to regular coffee. Although differences in CGA and CGL contents of regular and decaffeinated roasted coffees appear to be relatively small, they may be enough to affect flavor characteristics as well as the biopharmacological properties of the final beverage, suggesting the need for further study.

**KEYWORDS:** Coffee; decaffeinated coffee; chlorogenic acids; chlorogenic acid lactones; quinides; coffee processing

### INTRODUCTION

Coffee is believed to be the most popular beverage in the world. Caffeine (1,3,7-trimethylxanthine) is an alkaloid generally responsible for ~0.9–2.5% of coffee dry matter composition (1, 2). Even though caffeine has been widely consumed and studied for centuries, research results are inconclusive about both adverse and beneficial relations of caffeine to several health outcomes (3). Low to moderate caffeine intake is generally associated with improvements in alertness, learning capacity, exercise performance, and perhaps mood (4, 5). Caffeine is also often used as an additive in pain medications (4, 6). However, its stimulatory effects may also adversely affect sensitive individuals by causing tachycardia, increase of blood pressure, anxiety, and insomnia (4, 7, 8). Research studies also suggest that caffeine intake may lead to calciuria (9, 10) and hypercholesterolemia (11, 12), although results are inconclusive. According to Shlonsky et al. (3), the search for a healthier lifestyle by some people and the effects of caffeine on various

illnesses may account for the increasing demand for decaffeinated coffee throughout the world. Today, decaffeinated coffee makes up ~10% of the coffee market (13).

Decaffeination is performed prior to the roasting process. The most common and least costly caffeine extraction methods in the coffee industry employ an organic solvent such as dichloromethane or ethyl acetate, associated with the use of water/vapor prior to and after extraction. Beans are then dried until they reach a moisture level similar to that prior to processing. Water alone has alternatively been used to replace organic solvents in the process. At the end of the process, caffeine content is usually reduced to 0.02–0.3% (14). During the decaffeination process, losses of key flavor components of coffee generally occur (13), especially when solvents that lack specificity, such as water, are used. Among the compounds lost may be the chlorogenic acids (CGA) and their related compounds.

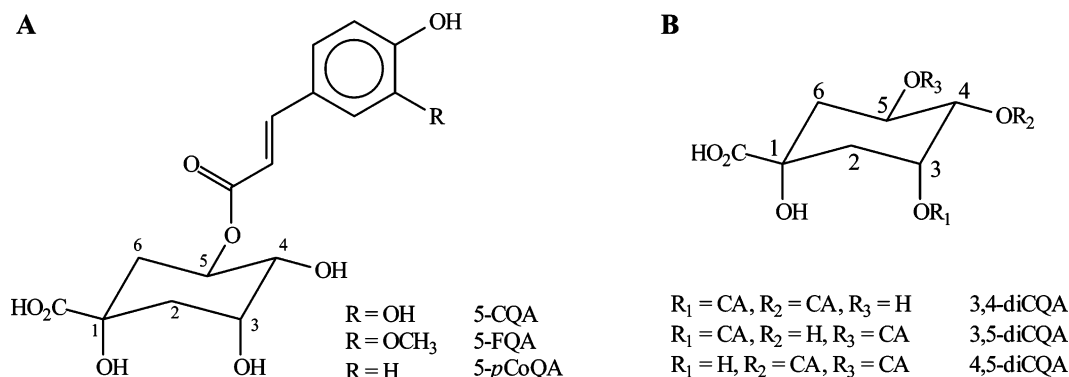
CGA are water-soluble phenolic components of coffee and other plants formed by the esterification of certain *trans*-cinnamic acids, such as caffeic (CA), ferulic (FA), and *p*-coumaric (CoA) acids, with (–)-quinic acid (15). The main subgroups of chlorogenic acid isomers in coffee are the caffeoylquinic acids (CQA), feruloylquinic acids (FQA), dicaffeoylquinic acids (diCQA) and, in smaller amounts, *p*-couma-

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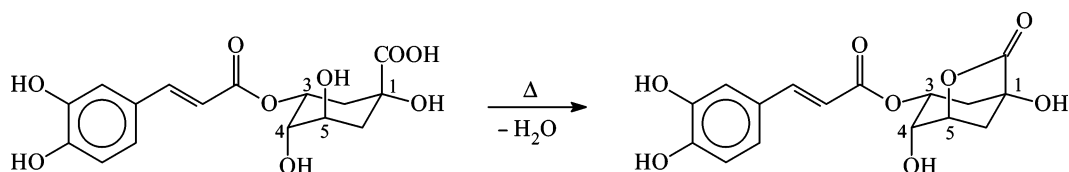
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**Figure 1.** Main chlorogenic acid monoesters (A) and diesters (B). Esterification also occurs in carbons 3 and 4 of the quinic acid. We have adopted the IUPAC numbering system (16) for chlorogenic acids. CA = caffeic acid.



**Figure 2.** Formation of a cinnamoyl-1,5- $\gamma$ -quinolactone from a chlorogenic acid isomer. Although under IUPAC rules (16) the numbering system for the lactones is different from that for the acids, to avoid confusion, in this work, we have used for lactones the same numbering of the carbons as for their precursors. When other authors are cited, the numbering has been changed for consistency.

roylquinic acids (*p*-CQA) (15, 16) (Figure 1). Responsible for 4–12% of the dry matter coffee composition (2, 18), CGAs are known for their contribution to the final acidity, astringency, and bitterness of the beverage (16, 19–21). During the roasting of coffee, as a result of the Maillard and Strecker reactions, bitterness increases due to the release of caffeic acid and the formation of lactones and other phenol derivative responsible for flavor and aroma (21–23).

CGAs and their lactones not only contribute to coffee flavor but also may be of potential biopharmacological importance in humans. The most studied pharmacological activities of phenolic compounds such as CGA have been related to their antioxidant properties, because they are thought to have positive effects on chronic degenerative diseases (24–31), cardiovascular diseases (32), and cancer (33–35). Lately, CGA have been receiving special attention due to their ability to increase hepatic glucose utilization (36–39).

Cinnamoyl-1,5- $\gamma$ -quinolactones (CGL) are the main CGA lactones in regular roasted coffee, being responsible for ~0.5% of its dry matter composition (18). They are generated from CGA through a loss of a water molecule and formation of an intramolecular ester bond during the roasting process (40) (Figure 2). In addition to their hypoglycemic effect demonstrated in rats (41), their potential biological activities have been related to their effects on brain functioning, specifically, antagonism of the  $\mu$  opioid receptor and adenosine transporter (6, 42–47). Recently, we have shown the formation and degradation of seven CGL in regular coffee during roasting process. We reported that light medium roasted coffee (~14% weight loss) produced the highest amount of lactones compared to other degrees of roasting (18).

In the present study we have evaluated the impact of the decaffeination process on the CGA and CGL contents of green and roasted arabica coffees due to the recognized sensorial and potential biological effects of these compounds, the lack of data on the levels of CGA and CGL in decaffeinated coffees, and the likelihood that these compounds are also removed by decaffeination. The importance of our findings needs to be viewed in light of the ever-increasing consumption of decaf-

feinated coffee and the importance of *Coffea arabica* species in the coffee market.

## MATERIALS AND METHODS

**Samples.** Four green *C. arabica* samples, cv. Bourbon (from Minas Gerais, Brazil), cv. Sumatra (from Mandhelim, Sumatra), cv. Sumatra (from Torajaland, Sulawesi), and cv. Herloom (from Harargue, Ethiopia), from the 2002 crop, were obtained from reliable commercial sources. The same lots were examined prior to decaffeination (reg) and after decaffeination (decaf). During the decaffeination process, water was used as the medium for caffeine removal.

**Roasting.** Two of the four reg and decaf arabica cultivars (Bourbon and Sumatra from Mandhelim) were roasted in duplicate, in a commercial bed fluid (hot air stream) roaster (model 40001, Hearthware, Gurnee, IL), operating at a maximum temperature of 230 °C, for 6, 7, and 8 min, respectively, to obtain light (6 min), light medium (7 min), and dark medium (8 min) roasts, including a 1 min cooling period. These are roasting degrees in which we have previously observed the largest amounts of CGA and CGL (18). Roasting degrees were determined by percent weight loss during roasting and by comparison with color disks from the “Roast Color Classification System” (Agtron-SCAA, Reno, NV; 1995), following the standards used by the Brazilian Coffee Industries Association (ABIC), where disk 75 = light roast, disk 65 = light medium roast, and disk 45 = dark medium roast.

**Water Content.** To express the amount of CGA and CGL per weight of dry matter, the water content of the freshly ground beans was determined according to the AOAC method (47).

**Weight Loss.** The percent weight loss (%WL) was calculated using the equation

$$\% \text{WL} = 100 - \frac{\text{WAR} \times 100}{\text{WBR}}$$

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

**Chlorogenic Acid and Lactone Extraction.** Green coffee beans were frozen in a -80 °C freezer prior to grinding. Samples were ground to pass a 0.046 mm sieve and extracted in triplicate with an aqueous solution of 40% methanol, according to a modification of the method of Trugo and Macrae (49), described in detail by Farah et al. (18).

**Standards.** 5-Caffeoylquinic acid (5-CQA) and caffeic acid (CA) were 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 of Trugo and Macrae (15), also described in Farah et al. (18). The lactones 3-CQL, 4-CQL, 3-FQL, 4-FQL, and 3,4-diCQL were synthesized in our laboratory from the corresponding cinnamic acid derivatives, according to Huynh Ba's (50) low-temperature modification of the method of Wynne et al. (51). 3-FQA was synthesized from 3-FQL by hydrolysis in 50% aqueous tetrahydrofuran as reported (50). The identity and purity of the lactones were confirmed by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy (Bruker, 300 and 75 MHz, respectively) and by HPLC (17). For diCQA, a mixture of 3,4-diCQA, 3,5-diCQA, and 4,5 diCQA from Roth (Deisenhofen, Germany) was used.

**HPLC Analysis.** Extracts of phenolic acids and lactones were analyzed by a HPLC gradient system using two high-precision pumps (model 582, ESA, Chelmsford, MA), a UV detector (model M 486, Waters Corp., Milford, MA), operating at 325 nm, and an ODS-C18 column (Rexchrom; 5  $\mu\text{m}$ , 250  $\times$  4.6 mm, Regis Technologies, Morton Grove, IL) coupled with a guard column (Rexchrom; 5  $\mu\text{m}$ , 10  $\times$  3 mm, Regis Technologies). Chromatographic data were recorded and integrated in the Easy Chrom Elite computer software (ESA). A gradient using a 10 mM citric acid solution and methanol was performed as described in detail by Farah et al. (18).

The identification of CGA and CGL was primarily performed by comparison with retention time of the respective standards and by spiking samples with small amounts of the appropriate standards. Their identity was later confirmed by LC-MS. The quantification of all CGA and CGL was performed using the area of 5-CQA standard combined with molar extinction coefficients of the respective CGA and direct CGL precursors, as previously described (18, 49). The detection limit for 5-CQA (4-fold baseline noise) under the conditions used in this study was 0.03  $\mu\text{g}/\text{mL}$ .

**HPLC-MS.** The confirmation of CGA and CGL was performed in a Shimadzu liquid chromatograph consisting of an LC-10AD vp quaternary pump and a SPD-M10A vp diode array detector. An LCMS-2010 mass spectrometer (Shimadzu, Kyoto, Japan) was used for spectrometric analysis. Five microliters of each sample (Rheodyne, Cotati, CA) was injected into a Magic C30 HPLC column (150  $\times$  20 mm, 5  $\mu\text{m}$ , 100  $\text{\AA}$ , Michrom Bioresources, Inc., Auburn, CA). The operating temperature was maintained at 40  $^\circ\text{C}$ . The LC mobile phase consisted of eluent A, an aqueous solution of 0.3% formic acid, and eluent B, methanol. The gradient was programmed as follows:

time (min)	eluent A (% v/v)	eluent B (% v/v)
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

The flow rate was 0.2 mL/min. Data were acquired by a LC-MS solution data system for both the mass spectrometer and diode array. UV spectra were acquired over the range of 190–370 nm.

The electrospray ionization source was operated in the negative mode to generate  $[\text{M} - \text{H}]^-$  ions and in the single ion monitoring (SIM) mode to detect CGA and CGL specific mass ions. The desolvation temperature was set to 250  $^\circ\text{C}$ , and the nebulizer gas ( $\text{N}_2$ ) flow was set to 2.0 L/min.

**Statistical Analysis.** HPLC results were analyzed by Statistica software, version 6.0, using ANOVA. Differences were considered to be statistically significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

**Chlorogenic Acids in Green Coffee Samples.** The levels of CQA, FQA, and diCQA in green samples of *C. arabica* cv. Bourbon, cv. Sumatra from two regions, and cv. Herloom before

and after decaffeination are represented in **Table 1** and **Figure 3**. Total CGA contents in reg arabica samples were 5.1; 5.4; 6.4, and 5.6 g%, on a dry matter basis (dm), for Bourbon, Sumatra from Mandelima, Sumatra from Sulawesi, and Herloom, respectively. It is interesting to note that the samples of *C. arabica* cv. Sumatra from different regions presented considerably different CGA contents. The higher content observed in the sample from Sulawesi was due mainly to higher amounts of 5-CQA and, to a lesser extent, 3,5-diCQA. An increase in the levels of 5-CQA and other phenolic acids in plants has been observed in severe weather conditions such as cold, high visible light, and water stress conditions (52–55) or in situations of boron deficiency and use of nitrogen-rich fertilizers (56, 57).

Total CGA contents in decaf samples were 6.1, 6.4, 7.3, and 6.4 g% (dm), for Bourbon, Sumatra from Mandelima, Sumatra from Sulawesi, and Herloom, respectively. Despite some loss in CGA, which may occur during decaffeination, a relative increase of 16.5% in average CGA levels of decaf compared to reg samples was observed, probably due to lixiviation of other water-soluble compounds such as carbohydrates, responsible for ~46% of *C. arabica* composition (dm), trigonelline (~1 g%), and other compounds (18, 58, 59). Washing has been successfully used as a means of lowering the content of oligosaccharides in legume seeds (60). As we have not controlled the decaffeination process, we were not able to measure the total decrease in mass of the beans resulting from their washing.

The decaffeination process produced average augmentations of 269, 187, 346, 252, 49, and 24% (dm) in the contents of 3-CQA, 4-CQA, 3-FQA, 4-FQA, 3,4-diCQA, and 4,5-diCQA, respectively. On the other hand, the average contents of 5-CQA, 5-FQA, and 3,5-diCQA decreased about 43, 42, and 35%, respectively. The increase observed in the levels of some of the CGAs may be partially due to the loss of other water-soluble compounds during the decaffeination process and partially due to the isomerization of the cinnamoyl substituent in the 5-position of the quinic acid to the 3- and 4-positions. This modification in the CGA molecules typically occurs in the presence of heat (15, 61) and suggests that decaf samples were exposed to high temperatures during processing, probably not only from the hot water used in the process but also from the heat used at the end of the process to dry the beans. A higher percentage increase of the cinnamoyl substituents in the 3-position of the quinic acid compared to those substituents in the 4-position is due to the hierarchical conformation, which has been previously explained (18). The higher percentage increase observed in 3,4-diCQA (49%), compared to 4,5-diCQA (24%), along with an average loss of 35% in 3,5-diCQA levels suggests that the caffeic acid moiety of diCQA esterified in the 5-position of the quinic acid becomes unstable during processing, forming 3,4-diCQA, and perhaps 3-CQA and 4-CQA, as previously suggested by Leloup et al. (61) in relation to roasting process. This may help to explain an average increase of 18% in total CQA, compared with the increase of 3% in diCQA as a consequence of decaffeination.

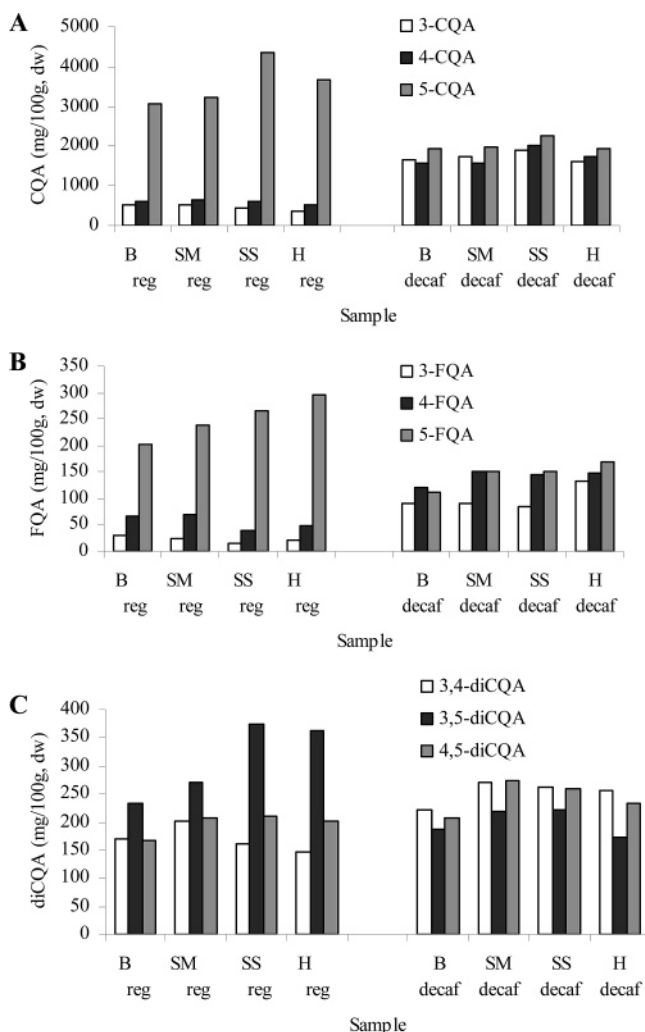
The fact that 3-FQA and 5-CQA elute very closely during chromatography may have caused an underestimation of 3-FQA levels in the presence of high levels of 5-CQA, as appears to be the case with reg green coffee beans. This probably explains the occurrence of the apparently higher percentage increase in 3-FQA than in 3-CQA levels in decaf samples, in which the levels of 5-CQA are lower and 3-FQA may be more precisely quantified, and, therefore, not underestimated.

Moreover, it is interesting to note in **Figure 3** the similarity of changes that occurred with CQA and FQA isomers and that

**Table 1.** Chlorogenic Acid (CGA) Contents in Green Regular and Decaffeinated Coffee<sup>a</sup>

cultivar	CGA								
	3-CQA	4-CQA	5-CQA	3-FQA	4-FQA	5-FQA	3,4-diCQA	3,5-diCQA	4,5-diCQA
	Regular Coffee								
Bourbon	542.0 ± 32.7	618.8 ± 66.3	3062.0 ± 113.0	28.8 ± 0.7	66.6 ± 5.4	200.9 ± 1.8	168.7 ± 2.1	232.8 ± 34.6	167.0 ± 17.7
Sumatra M	543.6 ± 19.7	647.9 ± 19.8	3224.6 ± 175.5	25.2 ± 0.2	68.0 ± 17.9	238.5 ± 16.5	201.1 ± 30.1	271.8 ± 29.9	208.2 ± 16.7
Sumatra S	436.7 ± 14.1	620.8 ± 23.4	4340.0 ± 277.6	14.5 ± 1.2	39.5 ± 2.8	264.1 ± 4.6	161.3 ± 22.9	374.2 ± 10.8	210.0 ± 13.0
Herloom	346.1 ± 21.9	520.5 ± 10.4	3662.7 ± 12.5	21.2 ± 1.5	49.3 ± 1.6	296.6 ± 4.2	146.7 ± 4.6	362.7 ± 10.1	200.4 ± 7.1
	Decaffeinated Coffee								
Bourbon	1672.3 ± 39.9	1585.6 ± 40.1	1931.5 ± 48.8	90.5 ± 5.8	120.8 ± 21.0	113.1 ± 13.1	222.1 ± 22.6	185.9 ± 22.2	206.6 ± 19.7
Sumatra M	1713.8 ± 54.7	1590.8 ± 96.8	1979.3 ± 153.4	90.6 ± 16.5	152.3 ± 23.9	150.2 ± 2.3	271.7 ± 34.2	219.6 ± 26.8	274.4 ± 33.3
Sumatra S	1884.0 ± 30.7	1996.7 ± 23.8	2264.0 ± 14.3	84.1 ± 0.7	143.4 ± 6.3	151.5 ± 1.9	262.9 ± 2.7	220.2 ± 7.1	260.0 ± 11.1
Herloom	1613.5 ± 85.0	1742 ± 100.0	1937.3 ± 58.0	134.2 ± 4.2	146.4 ± 7.7	169.8 ± 4.3	255.7 ± 2.1	173.2 ± 8.3	232.9 ± 7.1

<sup>a</sup> Results are shown as the means of roasting in duplicates and extractions in triplicates ± standard deviation, expressed as mg/100 g of coffee, dry weight. Sumatra M, Sumatra from Mandhelim; Sumatra S, Sumatra from Sulawesi.



**Figure 3.** Contents of caffeoylquinic acids (CQA) (A), feruloylquinic acids (FQA) (B), and dicaffeoylquinic acids (diCQA) (C) in green arabica coffee cultivars before (reg) and after (decaf) decaffeination in *C. arabica* samples: cv. Bourbon, from Brazil (B); cv. Sumatra, from Mandhelim (SM); Sumatra, from Sulawesi (SS); Herloom, from Ethiopia (H). Results are expressed in dry weight as averages of triplicates of extraction.

there seems to have been a certain balance in the levels of the isomers of all three subgroups of CGA after decaffeination.

Considering that part of the 5-CQA (and perhaps other CGA) adjacent to the cell walls of coffee beans seems to be associated with caffeine (62, 63), an absolute loss in 5-CQA and other CGA might have occurred during decaffeination, but the

lixiviation of other components along with the impossibility of knowing precisely how much of the CGA would be associated with caffeine makes it difficult to estimate losses of this kind.

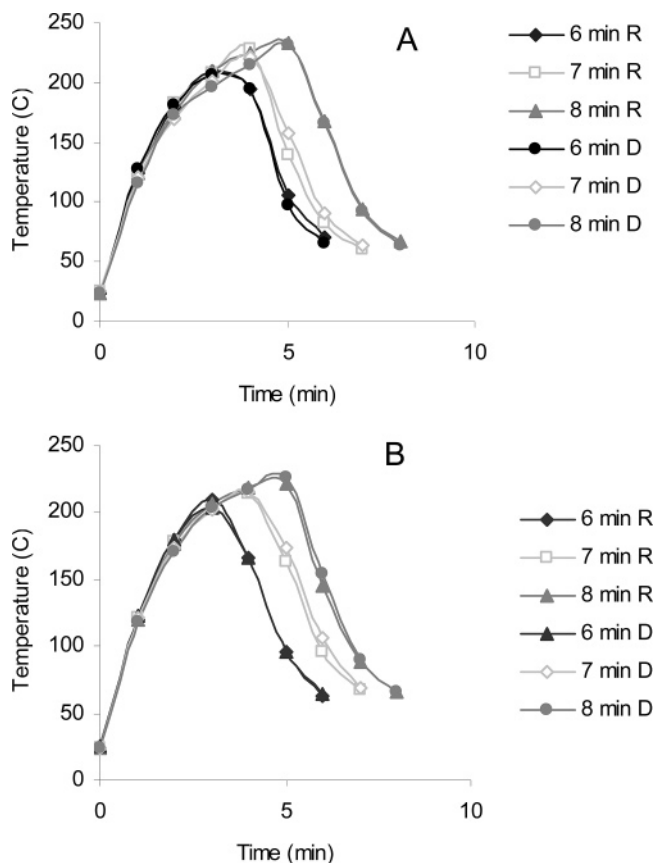
The levels of free caffeic acid varied from 12 to 21 mg% in reg coffee samples and increased differently between samples to ~32–51 mg% in decaf coffee, indicating some hydrolysis of CGA during decaffeination (21, 62).

No chlorogenic acid lactones were identified in any green decaffeinated coffee samples.

**Roasting.** Reg and decaf samples of *C. arabica* cv. Bourbon and *C. arabica* cv. Sumatra from Mandhelim were roasted in duplicates for 6, 7, and 8 min, to produce light, light medium, and dark medium roasting degrees. The average results of temperatures recorded inside the roaster for Bourbon and Sumatra reg and decaf coffees during the roasting process are shown in Figure 4. The temperature curves during roasting were similar for both reg and decaf coffees. Maximum average temperatures registered for 6, 7, and 8 min of roasting were, respectively, 208, 218, and 227 °C.

The weight loss during roasting, which is a consequence of the loss of water and a fraction of the organic material volatilized during pyrolysis, was used as an additional roasting degree determination tool. Average weight losses after 6, 7, and 8 min of roasting were, respectively, 12.5, 15, and 17.2% for reg coffee beans and 10.5, 13.1, and 15% for decaf beans. Although reg and decaf samples presented the same weight loss pattern, a small difference was observed between the weight losses of both reg samples, which could probably be explained by physicochemical differences between them. The difference between reg and decaf samples could probably be explained partially by the fact that decaf beans have undergone water loss during decaffeination, and therefore their initial water contents were lower than those of the reg beans (Table 2). A lesser drop in the mass during roasting also stems from the different composition of coffee beans after caffeine removal.

**Chlorogenic Acids and Lactones in Roasted Samples.** The average levels of total CGA after 6, 7, and 8 min of roasting were 3.4, 2.0, and 1.0 g% for reg coffee and 3.3, 1.8, and 0.9 g% for decaf coffee (dm), respectively. Losses of CGA during roasting have been extensively reported (16, 18, 62, 64). It is interesting to note that despite the large differences observed in the levels of CGA isomers between green reg and decaf coffees, roasting produced a very similar average distribution of CGA isomers in both reg and decaf coffees at 6, 7, and 8 min of roasting, with 3–9% lower CGA average contents in decaf, compared to reg coffees. Higher losses of CGA in decaf may be attributed to changes in the physicochemical characteristics of the beans during the decaffeination process.



**Figure 4.** Temperature changes inside the roaster during roasting of regular (R) and decaffeinated (D) Bourbon (A) and Sumatra (B) arabica coffees.

**Table 2.** Water Content in Green and Roasted Regular and Decaffeinated Coffee<sup>a</sup>

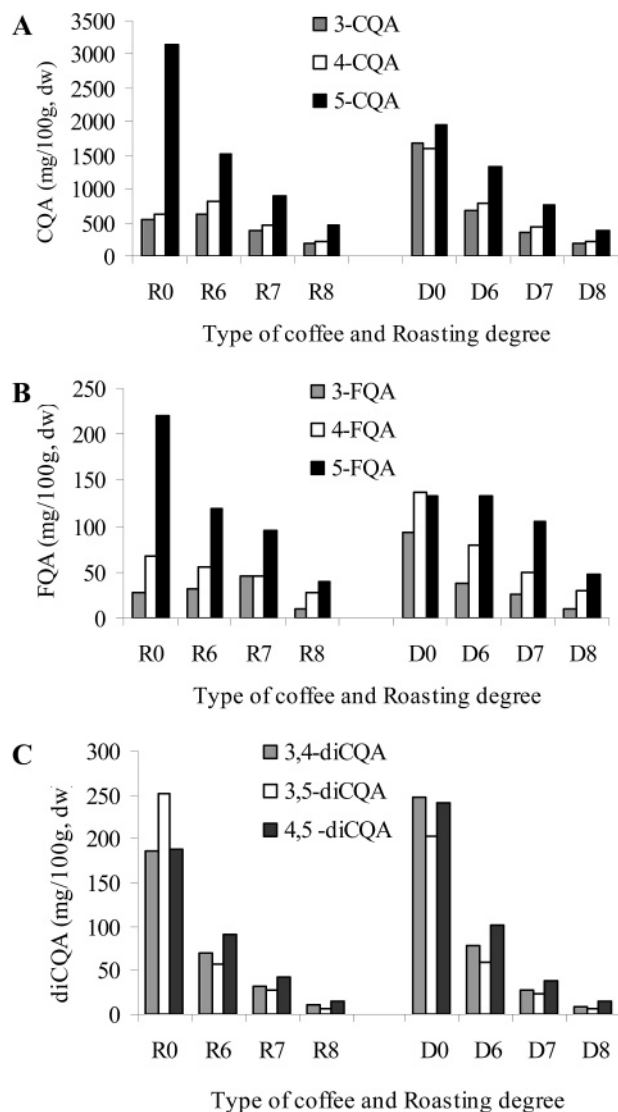
roasting time (min)	water (%)							
	B-R	B-D	SM-R	SM-D	SS-R	SS-D	H-R	H-D
0	9.61	8.53	11.00	8.51	10.30	8.90	10.70	9.87
6	4.74	3.85	6.40	5.35				
7	4.68	4.30	5.18	5.28				
8	3.95	3.20	4.04	4.62				

<sup>a</sup> Average of two determinations. B, Bourbon; SM, Sumatra from Mandhelim; SS, Sumatra from Sulawesi; H, Herloom; R, regular; D, decaffeinated.

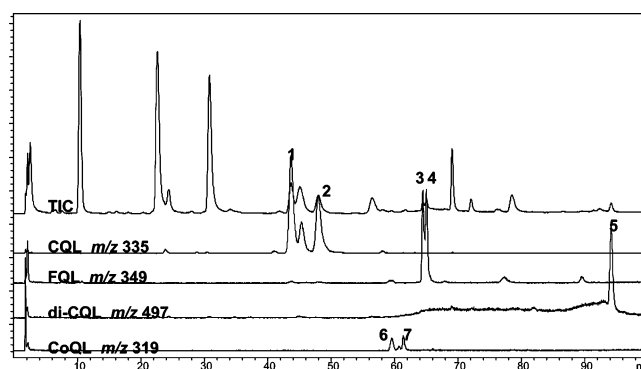
**Figure 5** compares the average contents of total CQA, FQA, and diCQA isomers in green and roasted reg and decaf samples. The production of a very similar distribution of CGA isomers in both reg and decaf coffees suggests that only a limited amount of CGA is isomerized when submitted to heat and that the isomerization may occur in steps, in this case, with decaffeination preceding roasting process.

In this study, the presence of the lactones 3-CQL, 4-CQL, 3-FQL, 4-CQL, and 3,4-diCQL identified with the aid of synthetic standards in our previous work (18) was confirmed by LC-MS (**Figure 6**). We also confirmed the presence of 1,5- $\gamma$ -quinolactones from *p*-coumaroylquinic acids, which we have not quantified here due to their very low levels in coffee and difficult integration of chromatographic peaks.

**Table 3** presents the levels of CGL in reg and decaf roasted coffee samples, as well as their individual percent weight loss during roasting. The maximum concentration of all lactones was observed after 7 min of roasting (light medium roasting degree). The average total CGL levels in reg and decaf coffee samples



**Figure 5.** Comparison of the average content of total CQA (A), FQA (B), and diCQA (C) in green and roasted regular (R) and decaffeinated (D) arabica Bourbon and Sumatra coffee cultivars. 0 = green coffee; 6, 7, and 8 = 6, 7, or 8 min of roasting.



**Figure 6.** Total ion chromatogram and mass chromatogram from LC-MS analysis of decaffeinated *C. arabica* cv. Bourbon, medium light roasted: 1, 3-CQL; 2, 4-CQL; 3, 3-FQL; 4, 4-FQL; 5, 3,4-diCQL; 6, 3-CoQL; 7, 4-CoQL.

were 432 and 455 mg% (dm), respectively, which correspond to about 8.2 and 7.1% of CGA average levels in green reg and decaf coffees, respectively. These results are in conformity with those previously obtained for reg coffee using HPLC and

**Table 3.** Chlorogenic Acid Lactones (CGL) in Regular and Decaffeinated Roasted Coffee (*C. arabica* Cv. Bourbon and *C. arabica* Cv. Sumatra)<sup>a</sup>

cultivar	roasting time (min)	weight loss <sup>b</sup> (%)	CGL				
			3-CQL	4-CQL	3-FQL	4-FQL	3,4-diCQL
Regular Coffee							
Bourbon	0	0	nd	nd	nd	nd	6.4 ± 1.2
	6	12.6	229.6 ± 2.4	118.1 ± 3.1	17.0 ± 1.6	10.9 ± 1.4	14.5 ± 1.0
	7	15.7	253.6 ± 11.5	109.8 ± 8.0	29.0 ± 1.5	12.3 ± 1.1	7.3 ± 0.8
	8	17.8	105.6 ± 3.7	66.0 ± 2.0	15.8 ± 1.3	8.0 ± 0.8	3.5 ± 0.6
Sumatra	0	0	nd	nd	nd	nd	5.6 ± 2.3
	6	11.6	183.0 ± 1.8	99.8 ± 1.8	12.0 ± 0.7	6.7 ± 2.4	16.3 ± 0.7
	7	14.3	257.1 ± 2.0	133.5 ± 2.4	30.4 ± 0.7	14.6 ± 0.9	16.3 ± 0.4
	8	16.5	150.5 ± 2.3	105.7 ± 9.3	26.5 ± 1.9	11.3 ± 0.7	8.3 ± 1.0
Decaffeinated Coffee							
Bourbon	0	0	10.1 ± 0.9	nd	nd	nd	11.1 ± 1.6
	6	10.0	237.2 ± 5.0	131.3 ± 3.0	17.6 ± 1.4	11.3 ± 1.6	14.5 ± 0.3
	7	13.1	249.5 ± 6.0	134.4 ± 9.5	40.8 ± 0.2	13.8 ± 0.9	11.6 ± 0.7
	8	14.9	176.4 ± 3.9	84.8 ± 2.1	23.7 ± 2.2	9.6 ± 1.1	7.4 ± 0.6
Sumatra	0	0	8.9 ± 1.2	nd	nd	nd	8.8 ± 1.5
	6	10.0	242.4 ± 5.0	133.4 ± 10.6	25.3 ± 0.3	13.0 ± 0.5	25.6 ± 1.4
	7	13.1	253.2 ± 5.5	129.8 ± 2.0	42.1 ± 1.6	22.1 ± 2.9	13.4 ± 2.5
	8	15.1	130.7 ± 2.1	87.3 ± 3.5	23.4 ± 1.2	9.7 ± 0.8	7.6 ± 1.2

<sup>a</sup> Results are shown as the means of roasting in duplicates and extractions in triplicates ± standard deviation, expressed as mg/100 g of coffee, dry weight. nd, not detected. <sup>b</sup> Weight loss during roasting process.

capillary gas chromatography (18, 64–66). Because the average levels of CGL direct precursors were 237% higher in green decaf compared to green reg coffee, one would expect the levels of CGL to be considerably higher in decaf compared to reg coffees. However, this was not observed (Table 3). The mean levels of CGL in decaf coffee were 18, 5.4, and 11.9% higher, respectively, for light, light medium, and dark medium roasts, compared to the levels in reg coffee. This is consistent with the hypothesis that isomerization of substituents in the 5-position of quinic acid to form isomers in the 3- and 4-positions may occur in steps. Also, the extent to which isomerization occurs appears to be limited and therefore will limit the formation of CGL direct precursors and, ultimately, the formation of CGL.

3-CQL was the most abundant 1,5- $\gamma$ -quinolactone in all samples, reaching average maximum levels of 255 and 251 mg% (dm) in reg and decaf coffees, respectively. 4-CQL showed the second highest content (respectively, 126 and 134 mg%, dm). Schrader et al. (64), after analysis of 10 commercial regular coffee brands, found 3-CQL and 4-CQL average levels of 213 and 96 mg% (dm), respectively. The small difference between these values and the ones found in the present study may probably be attributed to different degrees of roasting, because darker roasts are more commonly marketed than light medium roast, wherein maximum levels are observed. The maximum mean levels of 4-CQL represented about 48 and 52% of 3-CQL levels for reg and decaf coffees, respectively. A similar equilibrium between 3-CQL and 4-CQL has been previously observed in reg coffee (18). Bennat et al. (65) has observed an equilibrium of 60:40 for 3-CQA/4CQA in regular roasted coffees.

The maximum mean levels of 3-CQL and 4-CQL correspond, respectively, to 55 and 20% of the initial mean values of their direct precursors in reg coffee and to 15 and 8%, respectively, in decaf coffee. Only a 1.7% increase was observed in maximum CQL levels of decaf coffee (255.4 mg%), in comparison with reg coffees (251.0 mg%).

3-FQL mean levels were 29.7 and 41.5 mg% (dm) in reg and decaf coffees, respectively. Maximum levels of 4-FQL were 13.5 and 18.0 mg% in decaf coffee compared to reg coffee. It is interesting to note that decaf coffee samples presented a higher FQL increment (39.7% for 3-FQL and 33% for 4-FQL) than

CQL compared to reg coffee samples. Differences between FQA and CQA behaviors have been previously observed during roasting (16). A larger number of samples need to be investigated to confirm these results.

The maximum average contents of 3,4-diCQL in reg and decaf coffees were 15.4 and 20.1 mg% (dm), representing an increment of 30.5% in decaf samples, compared to reg coffee. Lower values were observed in our previous study (average of 7.2 mg% for arabica samples) (18). The possibility of coelution in the present work or spreading of the peak and underestimation of diCQL values in the previous study will be investigated.

In conclusion, decaffeination produced a relative 17% average increase in the levels of total CGA in green coffee, caused mainly by the lixiviation of other compounds during decaffeination. A 237% increase in 1,5- $\gamma$ -lactone direct precursors was observed due to isomerization of cinnamoyl substituents in the 5-position of the quinic acid. However, the average increment of 11% in lactone levels of roasted decaffeinated coffee, compared to regular coffee, was more consistent with total CGA levels than with the levels of lactone direct precursors in green samples. CGA levels in roasted decaffeinated coffees were 3–9% lower than in regular coffee, for different roasts. Results obtained in the present study indicate that the amounts of lactone direct precursors produced during roasting of coffee are a limiting factor for lactone formation and confirm that <10% of the amount of total chlorogenic acids in coffee is transformed into 1,5- $\gamma$ -lactones during the roasting process. Although differences in CGA and 1,5- $\gamma$ -lactone contents of regular and decaffeinated roasted coffees may appear to be relatively small, they may be enough to affect the flavor characteristics of the final beverage; moreover, the biopharmaceutical properties of decaffeinated coffee may differ from those of caffeinated coffee, not only because of the removal of caffeine but also because the pharmacological actions of CGA and CGL are “unmasked” by the absence of caffeine, suggesting the need of further specific investigations.

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