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Effect of Roasting on the Formation of Chlorogenic Acid Lactones in Coffee

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Of all plant constituents, coffee has one of the highest concentrations of chlorogenic acids. When roasting coffee, some of these are transformed into chlorogenic acid lactones (CGL). We have studied the formation of CGL during the roasting of coffee beans in *Coffea arabica* cv. Bourbon; *C. arabica* cv. Longberry; and *C. canephora* cv. Robusta. Individual CGL levels were determined by comparison of HPLC peaks with those of synthetic CGL standards. Seven CGL were identified: 3-caffeoylquinic-1,5-lactone (3-CQL), 4- caffeoylquinic-1,5-lactone (4-CQL), 3-coumaroylquinic-1,5-lactone (3-*p*CoQL), 4-coumaroylquinic-1,5-lactone (4-*p*CoQL), 3-feruloylquinic-1,5-lactone (3-FQL), 4-feruloylquinic-1,5-lactone (4-FQL), and 3,4-dicaffeoylquinic-1,5-lactone (3,4-diCQL). 3-CQL was the most abundant lactone in *C. arabica* and *C. canephora*, reaching peak values of 230 ± 9 and 254 ± 4 mg/100 g (dry weight), respectively, at light medium roast (∼14% weight loss). 4-CQL was the second most abundant lactone (116 ± 3 and 139 ± 2 mg/100 g, respectively. The maximum amount of CGL represents approximately 30% of the available precursors. The relative levels of 3-CQL and 4-CQL in roasted coffee were reverse to those of their precursors in green coffee. This suggests that roasting causes isomerization of chlorogenic acids prior to the formation of lactones and that the levels of lactones in roasted coffee do not reflect the levels of precursors in green coffee.

KEYWORDS: Coffee; coffee roasting; chlorogenic acid lactones; quinides

INTRODUCTION

Phenolic acids occur widely in nature as mixtures of esters, ethers, or free acids. Caffeic, ferulic, and *p*-coumaric acids are phenolic compounds derived from cinnamic acid and occur naturally in the form of mono- or diesters with the aliphatic alcohols of (−)-quinic acid, under the common name of chlorogenic acids (CGA) (*1*). The main subgroups of chlorogenic acids are caffeoylquinic acids (CQA), dicaffeoylquinic acids (diCQA), feruloylquinic acids (FQA), and *p*-coumaroylquinic acids (*p*CoQA) (*2*, *3*).

Green coffee beans contain the largest amounts of CGA found in plants, ranging from 6 to 12% (*4*−*10*). Studies of these compounds have revealed they have several pharmacological properties, such as antioxidant activity (*11*−*13*), ability to increase hepatic glucose utilization (*14*−*18*), inhibition of the HIV-1 integrase (*19*−*21*), antispasmodic activity (*22*), and inhibition of the mutagenicity of carcinogenic compounds (*23*). In addition, their corresponding chlorogenic acid lactones (CGL), which are formed during the roasting process by the loss of a water molecule from the quinic acid moiety and formation of an intramolecular ester bond (*24*) (**Figure 1**), have received special attention due to their potential effects on brain function independent of the pharmacological effects of caffeine (*25*−*31*) (Chlorogenic acid and lactones nomenclature: the authors adopted the IUPAC numbering system (*36*) for chlorogenic acids. Although under IUPAC rules the numbering system for the lactones is different from that of the acids, to avoid confusion, in this paper, the authors used for lactones the same numbering of the carbon atoms as for the acid precursors. When citing other authors, their numbering has been changed for consistency.) De Paulis et al. reported that 3,4-dicaffeoylquinic-1,5-lactone (3,4-diCQL) inhibits the human adenosine transporter and could, therefore, potentially counteract the stimulant effect of caffeine in the brain (*29*). Using rat brain preparations, Boublick et al. reported that coffee exhibits opiate receptor binding activity with characteristics similar to those of opiate antagonists (*26*). Wynne et al. isolated an active fraction containing isomeric feruloylquinic acid lactones (FQL), which were later identified as 1-FQL, 3-FQL, and 4-FQL (*27*), based on their spectra. Clifford observed both agonist and antagonist behavior at opioid receptors from fractions known to contain CQL and FQL (*28*). In a study with cell homogenates expressing the human μ opioide receptor (MOR-1), de Paulis et al. (*31*) confirmed the affinities of synthetic 3-FQL and 4-FQL for MOR-1. In addition, they found that other synthetic CGL,

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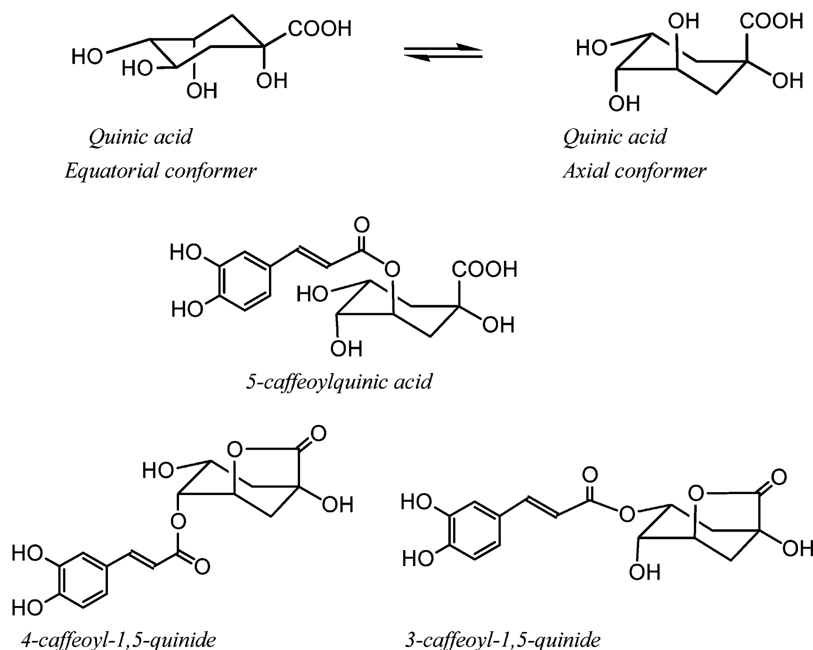


Figure 1. Lactone formation from CGA. Only those CGA that lack a substituent in the 5-position (i.e., 4-CQA and 3-CQA) are able to form a caffeoyl-1,5-quinide. Lactone formation from 3-CQA is favored relative to 4-CQA because of steric hindrance of the ester group in the axial position of the equatorial conformer.

especially those with a cinnamoyl substituent in the 4-position of the quinide (i.e., 4-CQL, 3,4-diCQL, 3,4-diFQL, and 3,4-di-*p*CoQL), have stronger affinities for MOR-1 than either FQL. In fact, the extract of instant decaffeinated coffee was able to reverse morphine-induced analgesia in mice at doses 1 order of magnitude less potently than that reported for naloxone (*31*).

While the main CGA, 5-caffeoylquinic acid, was shown to be at least partially bioavailable (*32*−*34*), there are no data to indicate whether other CGA constituents, such as CGL, are absorbed unchanged after coffee consumption. Furthermore, in light of the relatively weak in vitro affinities of these compounds at the μ opioid receptor and the adenosine transporter, acute pharmacological effects in the central nervous system seem unlikely to be produced by CGL acquired from normal coffee consumption. However, the future possibility of using such coffee constituents as nutraceuticals should not be discarded. Sensorially speaking, CGL were found to contribute considerably to the bitterness of the coffee beverage (*35*), an important attribute for coffee quality.

Data on the effects of roasting on the formation and prevalence of CGL in coffee are limited. The lack of commercial standards makes their identification and quantification in coffee difficult. In the present work, we studied the formation and prevalence of CGL during the roasting process in the two main species of marketed coffee, represented by *C. arabica* cv. Bourbon; *C. arabica* cv Longberry; and *C. canephora* cv. Robusta.

MATERIALS AND METHODS

Coffee Samples. *C. arabica* cv. Bourbon from Brazil; *C. arabica* cv. Longberry from Ethiopia; and *C. Canephora* cv. Robusta from Uganda, 2002 crop, were obtained from commercial sources.

Roasting. Samples were roasted in duplicate, in a Commercial Air Stream Roaster (Model 40001, Hearthware, Gumee, IL) operating at the maximum temperature of 230 °C. Samples were roasted from 5 to 15 min to obtain very light (5 min), light (6 min), light medium (7 min), dark medium (8 min), dark (9 min), and very dark (10−15 min) roasting degrees. Roasting degrees were determined by comparison with the Roast Color Classification System (Agtron-SCAA, 1995), following the standards used by the Brazilian Coffee Industries Association (ABIC), where disk #95 = very light roast; disk #85 = light roast; disk #65 = light medium roast; disk #45 = dark medium roast; disk #35 = dark roast; and disk #25 = very dark roast.

Extraction. Green coffee beans were frozen at −80 °C prior to being ground. Samples were ground to pass through a 0.046 mm sieve and extracted in triplicate according to a modification of the method of Trugo and Macrae (*37*), recently validated by Ky et al. (*38*). Half a gram of ground coffee was suspended in 60 mL of 40% aqueous methanol and shaken at room temperature for 20 min at 300 rpm. The mixture was filtered through filter paper (Whatman No. 1) and washed with 30 mL of water. For precipitation of proteins and other high molecular weight compounds, 1 mL each of Carrez's solutions, $K_2Fe(CN)_6$ (0.3 M) and $Zn(OAc)_2$ (1.0 M), were added, and the volume was made up with water to 100 mL. The mixture was shaken for 5 s and let stand for 10 min. The colloidal precipitation was then filtered (Whatman No. 1), and the filtrate was used directly for HPLC analysis.

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 AOAC (*39*).

Weight Loss. The percentual weight loss (%WL) was calculated using the following equation:

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

where WBR = weight before roasting and WAR = weight after roasting.

Standards. 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 of Trugo and Macrae (*37*). 3-CQL, 4-CQL, 3-FQL, 4-FQL, 3-*p*CoQL, 4-*p*CoQL, and 3,4-diCQL standards were synthesized using the low-temperature modification (*40*) of the method of Wynne et al. (*41*), starting with hydroxyl-protected quinide and the appropriate *p*-coumaric, caffeic, and ferulic acid chlorides. Melting points were taken on a Haake Buchler apparatus. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$−DMSO-d_6 (2:1) with tetramethylsilane as internal standard on a Bruker instrument operating at 300 and 75 MHz, respectively. Proton coupling constants of the cyclohexane ring were confirmed by simulation of proton spectra using MestRe-C, v 2.3 (courtesy of Dr. J. C. Cobas, University of Santiago de Compostela, Spain). Optical rotations were recorded on a Rudolph Autopol III using the sodium

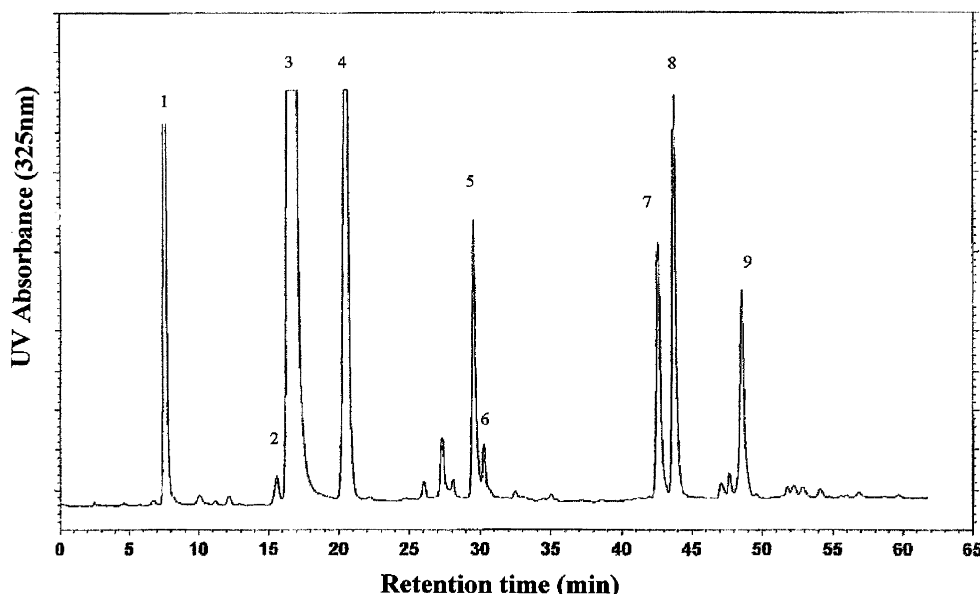


Figure 2. HPLC separation of chlorogenic acids in green *C. arabica* cv. Bourbon. 1 = 3-CQA; 2 = 3-FQA; 3 = 5-CQA; 4 = 4-CQA; 5 = 5-FQA; 6 = 4-FQA; 7 = 3,4-diCQA; 8 = 3,5-diCQA; and 9 = 4,5-diCQA. The three major peaks are shown off scale to show minor peaks.

emission line at 589 nm and a 10 cm cell (1.8 mL). 3-FQA, 4-FQA, and 5-FQA were synthesized from 3-FQL and 4-FQL, respectively, by hydrolysis in 50% aqueous tetrahydrofuran as reported (*40*). The identity and purity of the lactones were confirmed by proton and ^{13}C NMR spectroscopy and by HPLC. For diCQA, a mixture of 3,4-, 3,5-, and 4,5-diCQA from Roth (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 μm; 250 × 4.6 mm, Regis Technologies, Morton Grove, IL) coupled with a guard column (Rexchrom, 5 μm, 10 × 3 mm, Regis). Chromatographic data were recorded and integrated in the Easy Chrom Elite computer software (ESA). The chromatographic conditions for the gradient were as follows: eluent A: 80% 10 mM citric acid solution, acidity adjusted to pH 2.5 with 6 N hydrochloric acid and 20% methanol, which had previously been added to eluent A to avoid formation of air bubbles in the system. Eluent B: methanol. The flow rate was 1 mL/min and run time: 60 min. The gradient program was as follows:

time (min)	eluent A (% v/v)	eluent B (% v/v)
0.01	100	0
19	100	0
25	80	20
35	80	20
50	60	40
60	60	40
61	100	0

Identification of CQA and CGL was performed by comparison with retention time of the respective standards. Coffee samples were spiked with small amounts of the appropriate standards for confirmation of peak identity. The quantification of 5-CQA was accomplished by comparison of the area of the UV peak with that of the standard. Quantification of all other CGA was performed using the area of 5-CQA standard, combined with molar extinction coefficients as reported by Rubach (*36*), using the following equation:

$$C = \frac{RF\epsilon_1 MR_2 A}{\epsilon_2 MR_1}$$

where C is the concentration of the isomer in question in g L^{-1}; RF is the response factor of the 5-CQA standard (expressed in g L^{-1}); ϵ_1 is the molar extinction coefficient of 5-CQA; ϵ_2 is the molar extinction coefficient of the analogue or positional isomer in question; MR_1 is the molecular weight of 5-CQA; MR_2 is the relative molecular weight of the isomer in question; and A is the area of the peak of the isomer in question (*31*). Molar extinction coefficients (× 10^4) were as follows:

at λ_{max} 330 nm, 5-CQA = 1.95; 4-CQA = 1.80; 3-CQA = 1.84; 3.4-diCQA = 3.18; 3,5-diCQA = 3.16; and 4,5-diCQA = 3.32. At λ_{max} 325 nm, 5-FQA = 1.93; 4-FQA= 1.95; and 3-FQA = 1.90 m^{-1} cm^{-1} (*31*).

Because not all CQL standards were of analytical grade, the quantification of lactones was also performed using the area of 5-CQA combined with the molar extinction coefficient of the CGA that originated each particular lactone, except for the *p*CoQL for which the area of the 5-CQA standard was used due to lack of molar extinction coefficients. The use of molar extinction coefficients of the precursors for quantification of lactones was considered valid because the UV absorbances of the conjugated double bonds of the lactones are virtually identical with those of the corresponding chlorogenic acids. When compared with results from some of the lactone standards with high purity, this method was shown to be appropriate.

Statistical Analysis. The HPLC results were analyzed by Statistica software, version 6.0, using ANOVA. Differences were considered significant when $p < 0.05$.

Detection Limit. The detection limit for 5-CQA (4-fold baseline noise) under the conditions used in this work was 0.03 μg/mL.

RESULTS AND DISCUSSION

Green Coffee. A total of nine CGA were identified. A typical chromatogram obtained for green arabica coffee at 325 nm is depicted in **Figure 2**. The contents of individual CGA in green *C.arabica* cv. Bourbon, *C. arabica* cv. Longberry, and *C. canephora* cv. Robusta are presented in **Table 1**. The most abundant CGA group in green coffee samples was the CQA, which represented about 80 and 76% of the total CGA in arabica and Robusta coffee, respectively, with 5-CQA being the major CQA (62 and 56%, respectively) (**Table 1**). The diCQA isomers were the next three most-abundant CGA. Total diCQA represented 15 and 18% of the total CGA present in green arabica and Robusta samples, respectively. Total levels of FQA represented 5.2 and 6.2% of the CGA in arabica and Robusta coffee, respectively. The total contents of CQA and diCQA in

Table 1. Chlorogenic Acids Content in Green and Roasted *C. arabica* Cv. Bourbon, *C. arabica* Cv. Longberry, and *C. canephora* Cv. Robusta[a]

	roasting degree	roasting time (min)	3-CQ A	4-CQ A	5-CQ A	3-FQA	4-FQ A	5-FQA	3,4-DiCQ A	3,5-Di CQA	4,5-Di CQA
C. arabica cv. Bourbon (Brazil)	green	0	483.3 ± 6.1	543.5 ± 9.3	3126.1 ± 29.7	28.3 ± 0.9	40.1 ±1.8	210.7 ± 1.8	236.2 ± 4.0	254.9 ± 4.7	278.9 ± 12.5
	very light	5	996.0 ± 13.7	1277.7 ± 43.4	2808.5 ± 8.8	74.3 ± 2.7	73.2 ±2.7	167.0 ± 2.3	164.1 ± 5.1	152.3 ±20.9	190.5 ± 4.6
	light	6	816.1 ± 13.1	999.6 ± 45.1	1995.8 ± 19.9	47.8 ± 7.9	68.0 ±3.8	109.1 ± 8.2	119.7 ± 2.3	88.6 ± 3.8	127.8 ± 4.0
	light medium	7	458.5 ± 23.4	590.4 ± 51.2	1103.5 ± 40.9	34.2 ± 2.6	43.9 ±1.6	87.3 ± 5.9	51.7 ± 3.7	35.2 ± 1.4	50.1 ± 1.6
	dark medium	8	198.6 ± 10.1	281.6 ± 11.0	447.5 ± 22.9	17.6 ± 3.1	30.7 ±5.2	66.0 ± 4.8	14.0 ± 3.0	12.2 ± 2.0	16.6 ± 1.5
	dark	9	122.8 ± 3.0	155.8 ± 7.6	244.3 ± 17.9	9.7 ± 0.2	19.9 ±1.0	22.1 ± 0.8	7.4 ± 1.2	5.1 ± 2.7	9.2 ± 1.8
	very dark	10	80.1 ± 2.0	71.3 ± 3.3	159.6 ± 9.9	7.6 ± 0.5	13.7 ±0.0	12.6 ± 0.7	Nd[b]	2.2 ± 0.8	Nd
C. arabica cv. Longberry (Ethiopia)	green	0	478.2 ± 85.3	473.5 ± 12.7	3605.8 ±33.2	22.9 ± 2.1	34.5 ±0.3	234.9 ± 5.4	234.3 ± 3.0	263.5 ± 14.7	342.6 ± 4.9
	very light	5	950.7 ± 11.1	1234.3 ± 76.1	3102.2 ± 7.2	62.9 ± 3.5	65.2 ±2.0	165.8 ± 7.2	189.1 ± 5.1	176.0 ± 10.8	281.4 ± 11.6
	light	6	804.7 ± 11.6	933.0 ±20.4	1724.1 ± 19.0	42.2 ±10.9	56.8 ±3.0	107.0 ± 5.9	94.3 ± 7.0	72.5 ± 11.2	118.9 ± 29.5
	light medium	7	343.8 ± 23.8	494.8 ± 12.4	815.7 ± 37.6	41.1 ± 3.7	42.2 ±5.7	68.4 ± 6.2	40.9 ± 4.3	36.3 ± 9.0	54.1 ± 2.3
	dark medium	8	180.8 ± 12.6	265.0 ± 9.5	376.4 ± 8.5	14.6 ± 1.1	22.6 ±5.0	43.1 ± 2.3	11.3 ± 1.2	11.2 ± 0.4	15.8 ± 0.6
	dark	9	110.1 ± 9.6	156.2 ± 5.5	218.6 ± 5.2	9.2 ± 0.9	17.0 ±1.0	14.5 ± 0.9	3.4 ± 0.2	5.1 ± 0.2	6.1 ± 0.8
	very dark	10	53.0 ± 8.4	51.3 ± 1.7	104.7 ± 11.3	6.3 ± 1.1	12.2 ±0.4	7.4 ± 0.8	Nd	Nd	Nd
C. canephora cv. Robusta (Uganda)	green	0	924.8 ± 29.9	602.4 ± 12.1	4243.3 ± 15.3	34.1 ± 1.7	57.8 ± 3.0	379.2 ± 18.8	423.5 ± 6.3	400.7 ± 8.9	511.5 ± 2.7
	very light	5	1257.3 ± 21.4	1481.0 ± 28.5	3802.4 ± 10.5	89.7 ± 4.8	108.0 ±5.7	310.8 ± 17.9	337.2 ± 4.4	279.6 ±18.5	334.3 ± 13.8
	light	6	1086.7 ± 96.8	1342.9 ± 42.1	2520.8 ± 77.3	57.1 ± 0.4	110.0 ±0.9	241.7 ± 11.4	217.3 ±11.6	158.6 ± 7.6	205.0 ± 9.5
	light medium	7	623.4 ± 6.5	790.9 ± 35.9	1347.1 ± 7.9	79.2 ± 1.5	86.1 ± 4.1	169.8 ±8.0	81.8 ± 3.7	61.4 ± 2.9	91.9 ± 3.8
	dark medium	8	334.0 ± 14.0	308.3 ±14.2	448.0 ± 9.6	40.8 ± 1.8	70.1 ± 5.1	73.4 ± 4.3	23.1 ± 1.1	18.0 ± 1.3	19.0 ± 0.8
	dark	9	218.6 ±3.3	167.5 ± 3.4	225.8 ± 91.4	24.4 ± 4.4	39.4 ± 1.9	37.7 ± 2.7	6.9 ± 0.5	6.5 ± 0.4	6.0 ± 0.3
	very dark	10	108.0 ± 6.9	99.9 ± 10.8	111.0 ± 2.2	14.7 ± 0.7	18.2 ± 1.8	23.4 ± 1.6	2.5 ± 0.6	3.2 ± 0.2	1.9 ± 0.2

[a] Results are shown as the means of roasting in duplicates and extractions in triplicates ± standard deviation, expressed in mg/100 g of coffee, dry weight. [b] Nd = not detected.

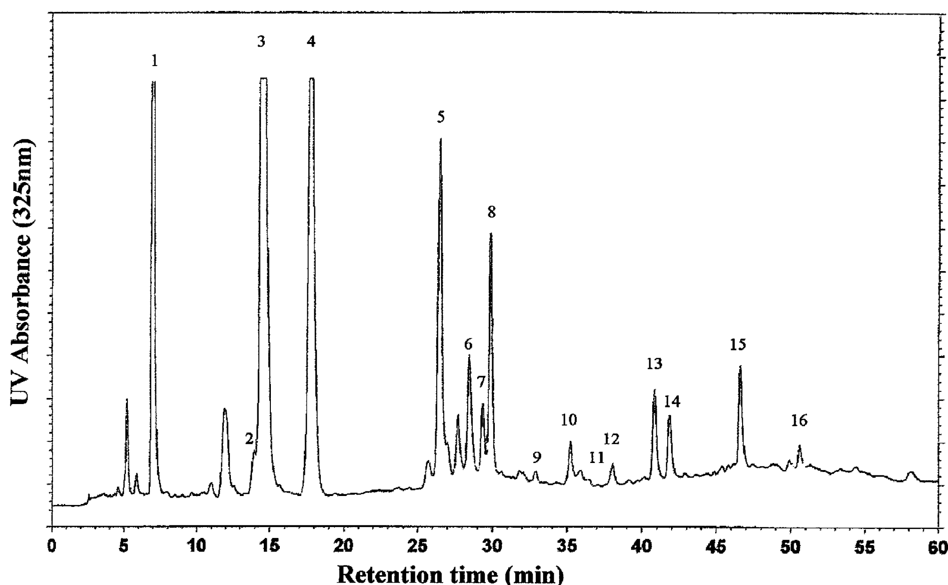


Figure 3. HPLC separation of chlorogenic acids and lactones in *C. arabica* cv. Bourbon roasted to light medium roast (i.e., ~14%weight loss) after 7 min of being roasted at a maximum temperature of 230 °C. The three major peaks are shown off scale to show the very minor peaks. 1 = 3-CQA; 2 = 3-FQA; 3 = 5-CQA; 4 = 4-CQA; 5 = 3-CQL; 6 = 5-FQA; 7 = 4-FQA; 8 = 4-CQL; 9 = 3-*p*CoQL; 10 = 3-FQL; 11 = 4-*p*CoQL; 12 = 4-FQL; 13 = 3,4-diCQA; 14 = 3,5-diCQA; 15 = 4,5-diCQA; and 16 = 3,4-diCQL.

arabica green coffee were 8.9 and 8.4%, respectively, higher in Longberry cultivar than in Bourbon. There was no significant difference between the total content of FQA in both arabica cultivars. The total CGA content in Robusta green coffee was 28% higher than the average CGA content in arabica cultivars. The difference between the contents of CGA in *C. arabica* and *C. canephora* has been extensively reported (*2*, *10*, *45*). No lactones were identified in green arabica coffee beans. However, small amounts of FQL and diCQL were identified in green Robusta beans. While Hucke and Maier (*46*) did not find any lactones in the analysis of green coffee samples by gas chromatography, Schrader et al. (*47*) have detected 4-CQL. The presence of lactones in green beans may be due to heating during primary processing.

Roasted Coffee. Samples were roasted from 5 to 15 min. The loss of CGA during the roasting process of coffee has been previously described (*45*−*49*). The high temperature of the roasting process causes a breakage of the carbon−carbon bonds of CGA, resulting in isomerization and degradation. After 5 min of roasting (~7% weight loss), the levels of 5-CQA had decreased substantially, while the levels of 3-CQA and 4-CQA had increased to twice their original values. This behavior was also observed for FQA. This is evidence that isomerization of CGA takes place at the beginning of roasting, as previously observed by Trugo and Macrae (*37*) and Leloup et al. (*43*). In addition, it is possible that partial hydrolysis of diCQA to monoester derivatives occurs in addition to isomerization (*43*). An initial rise in the total CGA amount was observed at 5 min of roast. This could be a result of the loss of other compounds that are more sensitive to heat, causing a relative increase in levels of the remaining ones. Longer periods of roasting resulted in a loss of total CGA. Besides isomerization

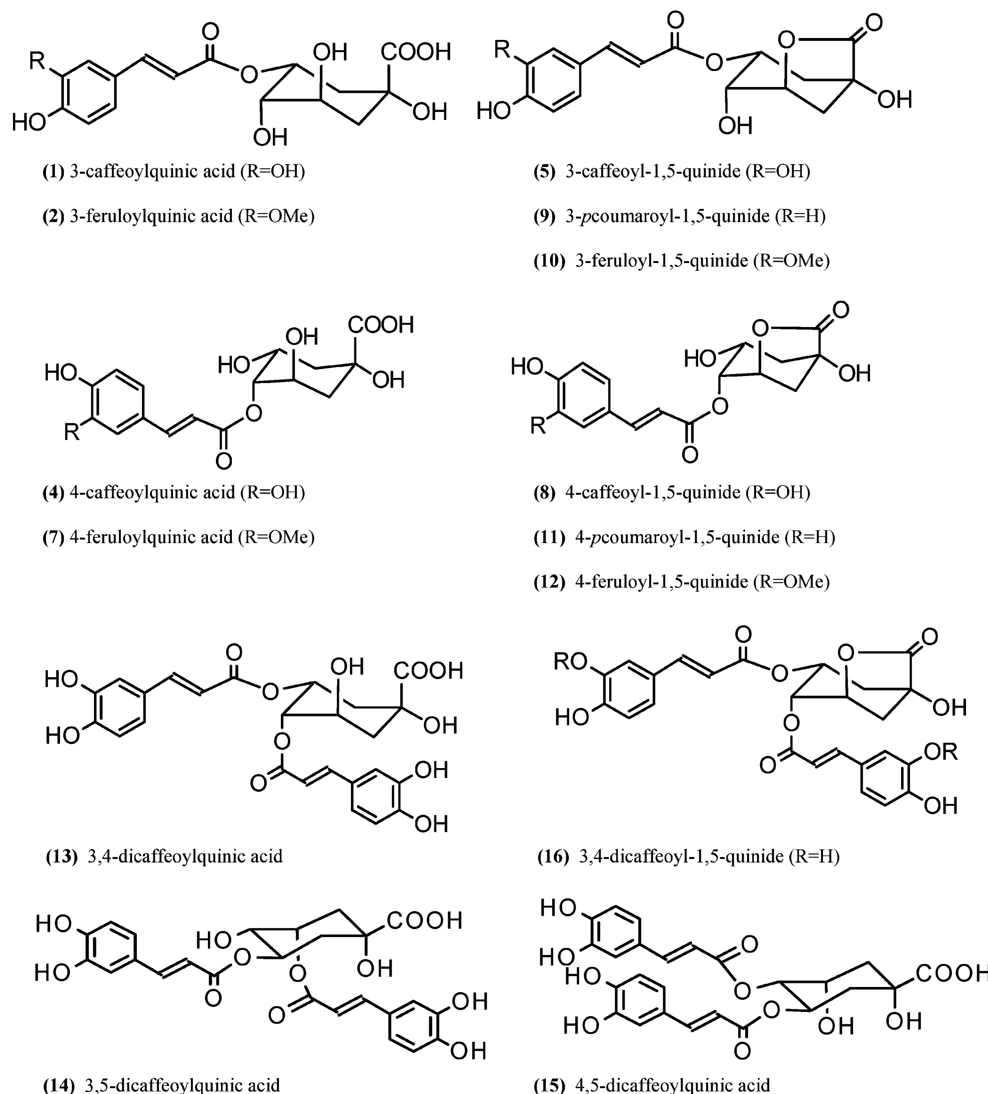


Figure 4. Structures of chlorogenic acid lactones and their precursors in roasted coffee. The numbers in parentheses refer to the peaks identified in the chromatogram (**Figure 3**).

Table 2. Chlorogenic Acid Lactone Content in Green and Roasted *C. arabica* Cv. Bourbon, *C. arabica* Cv. Longberry, and *C. canephora* Cv. Robusta[a]

	roasting degree	roasting time (min)	3-CQL	4-CQL	3-FQL	4-FQL	3,4-diC QL	3-*p*CoQ L	4-*p*CoQ L
C. arabica cv. Bourbon (Brazil)	green	0	Nd[b]	Nd	Nd	Nd	Nd	Nd	Nd
	very light	5	71.6 ± 8.7	25.6 ± 1.6	6.5 ± 1.3	1.9 ± 0.2	2.8 ± 0.2	2.1 ± 0.3	0.5 ± 0.0
	light	6	160.2 ± 2.3	92.1 ± 2.9	14.1 ± 1.2	7.0 ± 0.4	4.1 ± 0.4	4.6 ± 0.4	5.3 ± 0.2
	light medium	7	248.5 ± 9.4	115.3 ± 1.0	28.3 ± 2.0	13.4 ± 0.7	6.6 ± 1.2	7.5 ± 0.4	7.8 ± 0.2
	dark medium	8	146.6 ± 9.8	87.7 ± 2.1	29.8 ± 1.6	9.6 ± 0.4	2.1 ± 1.1	7.3 ± 0.7	6.6 ± 0.4
	dark	9	95.7 ± 4.7	53.1 ± 3.5	21.2 ± 1.9	7.8 ± 0.3	1.0 ± 0.2	4.4 ± 0.2	4.0 ± 1.6
	very dark	10	59.6 ± 10.0	24.8 ± 2.6	15.8 ± 6.0	6.2 ± 0.4	0.7 ± 0.06	2.3 ± 0.2	3.8 ± 0.6
C. arabica cv. Longberry (Ethiopia)	green	0	Nd	Nd	Nd	Nd	Nd	Nd	Nd
	very light	5	51.3 ± 1.9	22.5 ± 1.1	1.8 ± 0.0	Nd	3.1 ± 0.3	Nd	Nd
	light	6	161.2 ± 3.4	94.4 ± 3.1	20.2 ± 1.3	6.5 ± 0.3	6.8 ± 0.6	1.6 ± 0.1	1.9 ± 0.2
	light medium	7	210.5 ± 8.6	116.0 ± 4.7	29.7 ± 0.3	11.6 ± 0.6	7.8 ± 0.6	4.4 ± 0.3	2.6 ± 0.3
	dark medium	8	163.3 ± 8.1	72.4 ± 1.6	28.4 ± 1.3	9.7 ±0.4	6.1 ±0.5	3.0 ± 0.2	1.1 ± 0.1
	dark	9	94.5 ± 4.8	47.9 ± 1.7	15.5 ±2.8	5.6 ± 0.4	1.7 ± 0.4	3.5 ± 0.8	0.6 ± 0.1
	very dark	10	56.3 ± 7.7	37.1 ± 0.8	10.2 ± 1.2	3.5 ± 0.2	1.1 ± 0.1	2.0 ± 0.2	Nd
C. canephora cv. Robusta (Uganda)	green	0	Nd	Nd		4.0 ± 0.0	9.1 ±0.8		
	very light	5	57.7 ± 4.4	25.8 ± 1.4		6.4 ± 0.4	16.4 ± 1.7		
	light	6	198.8 ± 4.4	110.0 ± 2.1		18.4 ± 0.7	23.6 ± 1.0		
	light medium	7	253.6 ± 4.3	138.8 ± 2.0		31.1 ± 1.3	25.4 ± 1.0		
	dark medium	8	165.4 ± 10.6	125.9 ± 8.8		16.6 ± 0.4	10.0 ± 1.2		
	dark	9	87.6 ± 4.8	45.0 ± 2.0		8.4 ± 1.3	4.3 ± 1.4		
	very dark	10	54.4 ± 6.7	25.9 ± 2.9		5.1 ± 0.3	0.8 ± 0.2		

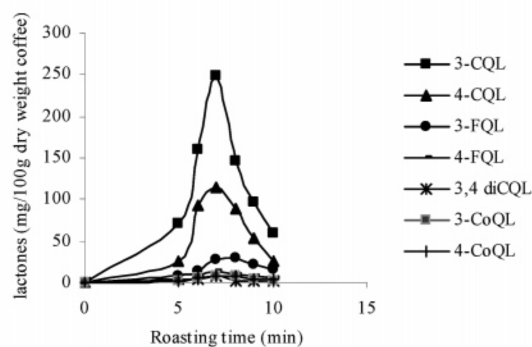
[a] Results are shown as the means of roasting in duplicates and extractions in triplicates ± standard deviation, expressed in mg/100 g of coffee, dry weight. [b] Nd = not detected.

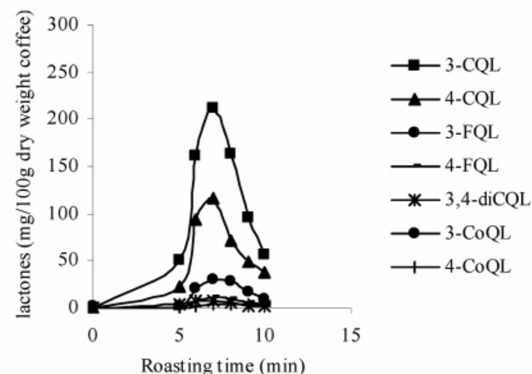
and degradation, other chemical transformations may occur, the dominant being dehydration of the quinic acid moiety and formation of a lactone ring (*24*) (**Figure 1**). Because the elimination of a water molecule from the six-membered ring of the quinic acid ((*R*,*R*,*S*,*R*)-1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid) requires a *syn*-1,3-diaxial configuration of the hydroxyl and carboxyl groups to form lactones, only those isomers that lack a cinnamoyl substituent in the 5-position of the quinic acid (i.e., 3-CQA and 4-CQA) are able to form a 1,5-quinide during roasting. The rate at which all these changes occur depends on both temperature and the amount of beans inside the roaster.

In addition to nine CGA identified in the green samples, seven CGL have been identified in roasted coffee. CGL reached their maximum levels approximately 7 min after the start of the roasting process (i.e., light medium roast equal to ∼14% weight loss). Longer roasting times resulted in lower amounts of both CGA and CGL. A typical HPLC chromatogram of CGA and CGL present in light medium roasted coffee is shown in **Figure 3**. Their chemical structures are shown in **Figure 4**. Arabica cultivars offered the clearest identification and quantification of lactones, except for one case in the Bourbon cultivar, in which *p*CoQL seemed to coelute with other compound(s). Because Robusta coffee is richer in phenolic compounds than arabica coffee (*10*, *44*, *45*), separation of lactones is more difficult due to coelution. The elution order of CGA and CQL was similar to that reported by Schrader et al. (*47*), who used mass spectrometry, NMR spectroscopy, and LC-MS for characterization. The exception was 3-CQL, which in our column eluted before 5-FQA and 4-FQA. Spiking the samples with synthetic standards and with green coffee extract made it possible to distinguish between CGA and CQL using a reverse phase column with a slightly different selectivity from that used by Schrader et al. Apparently, these seven CGL have not been previously separated in a single HPLC analysis.

The contents of individual CGA and CGL in green and roasted arabica and Robusta samples are shown in **Tables 1** and **2**, respectively. The maximum amount of total CGL was 398 and 424 mg/100 g of coffee for *C. arabica* (average) and *C. canephora*, respectively, which correspond to 7.3 and 5.6% of the contents of total CGA in green arabica and Robusta samples, respectively. This difference between arabica and Robusta coffees is not as high as expected, considering that Robusta showed 28% higher CGA levels than arabica. This fact has also been observed by Bennat et al. (*48*) for CQL. The total maximum amount of lactones in a Bourbon cultivar (427 mg/100 g) was higher than in Longberry cultivar (383 g/100 g), even though the total amount of CGA in green Longberry coffee (5690 mg/100 g) was higher than in Bourbon (5172 mg/100 g). However, for the total amounts of the precursors, Bourbon presented a higher content of corresponding precursors for CGL formation (1331 mg/100 g) than the Longberry cultivar (1244 mg/100 g). The lactone contents in Bourbon and Longberry cultivars correspond to 32 and 31%, respectively, of their precursors. In a similar evaluation of the Robusta sample, taking into account the exclusion of minor CGL from the table, the maximum amount of total CQL was equivalent to 26% of the corresponding precursors. This brings the percentages in both species closer, but the occurrence of a lower percentage of lactone formation in Robusta coffee still remains to be explained.

At a 10 min or longer time of roasting (i.e., dark roast equal to >20% weight loss), the amounts of total CGA and CGL decreased to less than 5.2 and 20%, respectively, of their maximal values. Most CGA could not be detected after 11 min, and only trace amounts of CGL could be detected after 15 min of roasting.





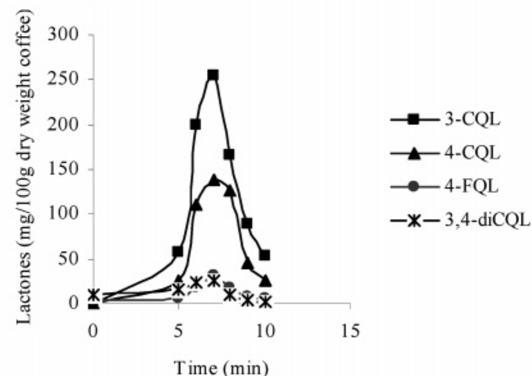


Figure 5. Lactones formation during roasting of **(a)** *C. arabica* cv. Bourbon; **(b)** *C. arabica* cv. Longberry; and **(c)** *C. canephora* cv. Robusta coffee beans. Maximum lactone levels were observed after 7 min of being roasted (i.e., ∼14% weight loss).

Caffeoyl-1,5-lactones (CQL). The identification and quantification of 3-CQL and 4-CQL in roasted coffee have been previously reported (*47*, *48*). Although the three major CGA in coffee are 5-CQA, 3-CQA, and 4-CQA, only the latter two compounds, having no substituent in the 5-position of the quinic acid, are able to form a 1,5-lactone (**Figure 1**). Therefore, 3-CQL and 4-CQL are expected to be the major lactones in roasted coffee extracts, which was confirmed in this study. 3-CQL was the most abundant lactone in all samples, reaching its maximum amount at 7 min of roasting (i.e., light medium roast (249 ± 9 mg; 211 ± 9 mg; and 254 mg ± 4 mg/100 g coffee for *C. arabica* cv Bourbon, *C. arabica* cv. Longberry, and *C. canephora* cv. Robusta, respectively) (**Table 2**)). 4-CQL

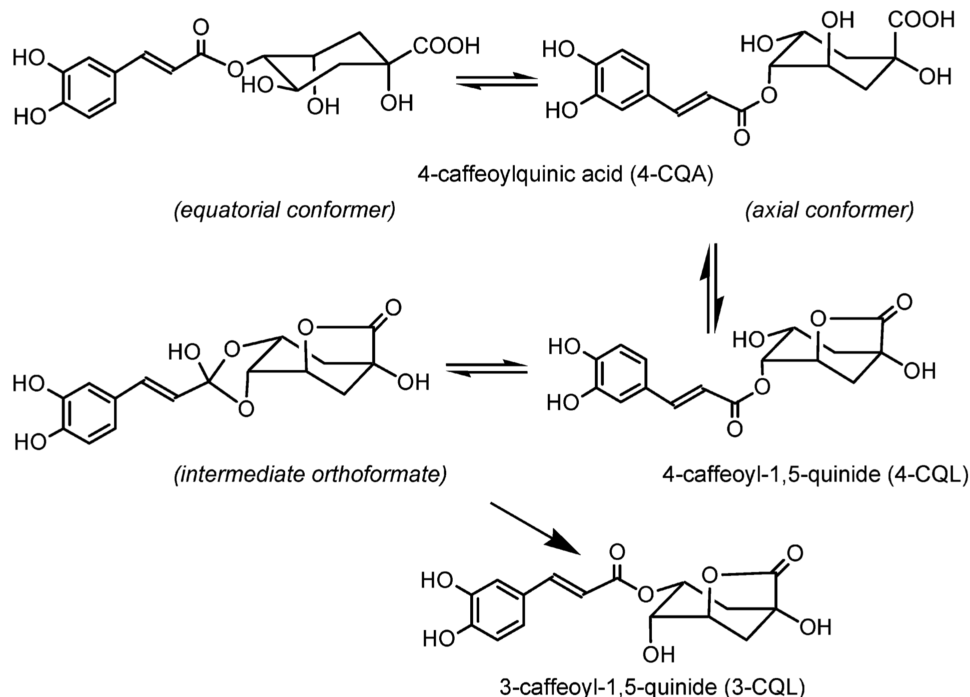


Figure 6. Formation of 3-CQL by thermal rearrangement of 4-CQA.

showed the second highest amount, reaching its maximum amount also after 7 min of roasting (115 ± 1 mg; 116 ± 5 mg; and 139 ± 2 mg/100 g coffee, respectively). These levels of lactones are in concordance with those from the analysis of commercial coffee brands reported by Schrader et al. (*47*), as well as with those reported by Bennat et al. (*48*).

The average amount of 3-CQL and 4-CQL at 7 min of roasting represented 37 and 23% of the average amounts of CGA in green coffee beans, respectively. Even though the amount of the precursor 4-CQA was higher than 3-CQA in arabica green samples, at light medium roast, when CGL reached their maximum levels, a 65:35 ratio between 3-CQL and 4-CQL was observed, similar to that of 60:40 observed by Bennat et al. (*48*). The ratio of 3-CQL/4-CQL in Robusta sample was 68:32. The higher levels of 3-CQL as compared to 4-CQL could be explained by assuming that 3-CQL is generated also from 4-CQL. During 4-CQL formation, elimination of a water molecule from the axial chair conformer of the cyclohexane ring of 4-CQA occurs. Because of the elimination of the axial conformer, the equilibrium between the equatorial and the axial chair conformers tends to be shifted until most of 4-CQA is transformed. After formation of both 3-CQL and 4-CQL from their corresponding precursors, 4-CQL undergoes an intramolecular migration of the caffeoyl substituent. The carbonyl group of the caffeoyl moiety can form an intermediate five-membered orthoformate ring with the hydroxyl group in the 3-position of the quinide (**Figure 6**). Breaking the bonds of this ring may occur at either of the two oxygen atoms. If the break occurs between the caffeoyl carboxylic carbon atom and the quinide oxygen atom in the 4-position, the intermediate returns to 4-CQL, and the process repeats. However, if the bond in the 3-position is broken, the intermediate is transformed into 3-CQL. This process is reversible, but the equatorial conformation of the caffeoyl group in 3-CQL is energetically favored over the axial conformation of 4-CQL. Further, because of the axial configuration of the 4-substituent in 4-CQL, the conformation of the cyclohexane ring necessary for lactone formation is less favored as compared to that of 3-CQL (**Figure 1**). As a result, 3-CQL is the dominant lactone in roasted coffee.

Feruloyl-1,5-lactones (FQL). The occurrence of FQL has been previously described in fractions of roasted coffee (*26*, *27*). We identified and quantified two major feruloyl-1,5-lactones, 3-FQL and 4-FQL. The highest amount of these compounds also occurred at 7 min of roasting, and the same pattern observed for CQL was observed for FQL. Although the amount of 4-FQA was higher than the amount of 3-FQA in all three samples, 3-FQL was the major FQL. The maximum levels of 3-FQL for Bourbon and Longberry samples were 29.8 and 29.7 mg/100 g, while the maximum levels of 4-FQL were 13.4 and 11.6 mg/100 g, respectively. 3-FQL could not be quantified in the Robusta sample due to its coelution with other peak(s). The formation of 3-FQL and 4-FQL mirrored that of CQL, as evidenced by the ratio of 70:30 between 3-FQL and 4-FQL in arabica samples.

***p*-Coumaroyl-1,5-lactones (*p*CoQL).** The occurrence of *p*CoQL in roasted coffee has not been reported previously, probably because they are present in very small amounts. The precursors, *p*CoQA, are known to be minor CGA in both green and roasted coffee (*2*, *28*), with typical amounts of 30−70 mg/100 g and 50−60 mg/100 g for green arabica and Robusta coffees, respectively (*28*). The total amount of 3-*p*CoQL in Bourbon and Longberry samples after 7 min of roasting was 7.5 mg and 4.4 mg/100 g of coffee, respectively. The total amount of 4-*p*CoQL was 7.8 and 2.6 mg/100 g of coffee, respectively. During the roasting process, many secondary compounds are formed. Robusta coffee is rich in minor unidentified phenolic compounds, and under these chromatographic conditions, it was not possible to quantify 3-*p*CoQL and 4-*p*CoQL, which coeluted with other compounds. A ratio of 63:37 between 3-*p*CoQA and 4-*p*CoQA was observed in roasted Longberry beans, which follows the pattern observed for CQL. However, this ratio was not seen in the Bourbon cultivar, which suggests that the quantification of 3-*p*CoQL or both *p*CoQL may have been confounded due to coelution with other minor compounds, as was the case for Robusta coffee.

3,4-Dicaffeoyl-1,5-lactone (3,4-diCQL). The maximum amount of 3,4-diCQL in Bourbon and Longberry cultivars was

6.6 and 7.8 mg/100 g of coffee, respectively. These represent 2.8 and 3.3% of the amount of the precursor 3,4-diCQA present in green beans, respectively. Green Robusta coffee reached a maximum value of 25.4 mg/100 g of coffee, also after 7 min of roasting. The green Robusta sample seemed to already contain a small amount of 3,4-diCQL.

The present work demonstrates that the formation of CGL is highly dependent on the degree of roasting. The optimum degree of roasting to achieve a maximum amount of lactones in coffee is light medium roast (∼14% weight loss), whereas darker roasts yield lower amounts. Despite favorable structural configurations of CGA, less than 10% of the total CGA and about 30% of the possible precursors in green coffee were converted to lactones. Despite the higher amounts of 4-CQA as compared to 3-CQA in green beans, 3-CQL was the major lactone followed by 4-CQL. The bioavailability and the physiological effects of these compounds in humans, in both normal and high coffee consumption, need to be determined. In addition, the fact that the hot water extraction method used for home coffee preparation would theoretically tend to extract lesser amounts of lactones than those obtained in this study raises the need for further investigation on the effect of preparation conditions.

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