JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Influence of Coffee Roasting on the Incorporation of Phenolic Compounds into Melanoidins and Their Relationship with Antioxidant Activity of the Brew

Daniel Perrone,[†] Adriana Farah,*[,†,‡] and Carmen M. Donangelo[†,§]

[†]Laboratório de Bioquímica Nutricional e de Alimentos, Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, Brazil

[‡]Núcleo de Pesquisa em Café Prof. Luiz Carlos Trugo, Laboratório de Química e Bioatividade de Alimentos, Instituto de Nutrição, Universidade Federal do Rio de Janeiro, Brazil

[§]Escuela de Nutrición y Dietética, Universidad de la República, Uruguay

Supporting Information

ABSTRACT: In the present study, the influence of coffee roasting on free and melanoidin-bound phenolic compounds and their relationship with the brews' antioxidant activity (AA), evaluated by TRAP, TEAC, and TRAP, were investigated. Changes in the relative content of free chlorogenic acids (CGA), free lactones, and melanoidin-bound phenolic acids during roasting indicate that phenolic compounds were incorporated into melanoidins mainly at early stages of the process, being thereafter partly oxidized to dihydrocaffeic acid, and degraded. Although less than 1% of CGA in green coffee was incorporated into melanoidins during roasting, the relative content of melanoidin-bound phenolic acids increased significantly during this process, reaching up to 29% of total phenolic compounds in brews from dark roasted coffees. Regardless of the AA assay used and considering all roasting degrees, the overall contribution of CGA to the AA of the whole brews was higher than that of melanoidin-bound phenolic compounds. It was estimated that the latter compounds contributed to 25−47% of the AA, depending on the assay used.

KEYWORDS: *coffee, antioxidant activity, chlorogenic acids, melanoidins, phenolic acids*

INTRODUCTION

Coffee is one of the most traded food products and a highly popular beverage all over the world. In recent years, due to an increasing interest on health implications of foods, the relationship between coffee and health has been extensively studied. Epidemiological data indicate that moderate consumption of coffee is associated with a reduction in the relative risk of development of several diseases such as coronary heart disease, Alzheimer's disease, hepatic cirrhoses, and liver and colon cancers.[1−3] This effect has been mainly attributed to the brews' antioxidant activity.[4] Coffee possesses the highest in vitro antioxidant activity (AA) among commonly consumed beverages,[5,6] and its contribution to the overall intake of antioxidants from the diet may reach up to 70% in Western diets.[6,7]

The effect of roasting on the AA of coffee brews has been investigated in several earlier studies, but discordant results have been obtained. Compared to green coffee, while del Castillo et al.[8] reported an AA increase in brews from medium roasted coffee and a decrease in those from dark roasted coffee, Daglia et al.[9] reported AA decrease in brews from light roasted coffee and increase in brews from dark roasted coffee. Borrelli et al.[10] reported both increase and decrease of brews' AA caused by roasting depending on the assay employed. Such incongruence may possibly arise from lack of standard definition of roasting degrees (color, weight loss, etc.) and to the use of different methods to assay AA.[11]

The contribution of different coffee components to the AA of the brew is also a topic of great interest in the literature. Chlorogenic acids (CGA) are considered to be the major contributors to the AA of coffee brew, followed by melanoidins, which are end products of the Maillard reaction.[8,11−14] Additionally, low molecular weight volatile compounds formed during coffee roasting may also contribute to the AA of the brew.[15]

Although the possible participation of CGA and other hydroxycinnamates in the formation of coffee melanoidins has been under discussion for over three decades, consistent data on this incorporation have only recently been published.[16−21] However, data are limited and no studies evaluating the influence of coffee roasting on this phenomenon are available in the literature. It would be of particular interest to examine the extent of CGA incorporation into coffee melanoidins in a single sample roasted to increasing degrees. Such an approach would contribute to the investigation of the mechanisms and kinetics of CGA incorporation into melanoidins. Moreover, because CGA bound to the melanoidin backbone may partly explain the antioxidant activity of melanoidins in coffee brews,[19] it would be important to determine in the brew the amount of CGA bound to melanoidins relative to total CGA.

Received: September 8, 2011
Revised: March 27, 2012
Accepted: April 10, 2012
Published: April 10, 2012

dx.doi.org/10.1021/jf205388x | J. Agric. Food Chem. 2012, 60, 4265−4275

Considering the health implications of coffee drinking as well as the paucity of data on the detailed phenolic composition of melanoidins and on the relative contribution of CGA and melanoidins to the antioxidant activity of the brew, the aim of the present study was to investigate the influence of coffee roasting on phenolic compounds, both free and bound to melanoidins, and their relationship with the brew's antioxidant activity evaluated by different assays.

MATERIALS AND METHODS

Chemicals. Thirteen commercial standards of phenolic acids were acquired from Sigma-Aldrich (St. Louis, MO): 5-caffeoylquinic, caffeic, vanillic, ferulic, isoferulic, *p*-coumaric, gallic, *p*-hydroxybenzoic, dihydrocaffeic (DHCA), 3,4-dihydroxyphenylacetic, 3-[4-hydroxyphenyl]-propionic, *trans*-3-hydroxycinnamic, and benzoic acids. Basic lead acetate was purchased from Cromato Produtos Químicos (São Paulo, Brazil), and zinc acetate was obtained from Quimex (São Paulo, Brazil). 2,2′-Azobis-(2-methylpropionamidine) dihydrochloride (AAPH), 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), 5-caffeoylquinic acid (5-CQA), potassium persulfate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 2,4,6-tripyridyl-S-triazine (TPTZ) were purchased from Sigma-Aldrich (Steinheim, Germany). Heptahydrated ferrous sulfate and potassium hexacyanoferrate (II) were acquired from Merck (Darmstadt, Germany). Hexahydrated ferric chloride was purchased from Vetec Química Fina (Rio de Janeiro, Brazil). All solvents were HPLC grade from Tedia (Fairfield, OH, USA). LC grade water was used throughout the experiments (Milli-Q system, Millipore, Bedford, MA, USA).

Coffee Samples. Samples of good quality (no defects, "soft cup") green *Coffea arabica*, cv. Yellow Bourbon, cv. Red Catuai, and cv. Mundo Novo beans were obtained directly from producers in Guaxupé, Minas Gerais, Brazil. A good quality (no defects) green *Coffea canephora*, cv. Conillon sample was obtained from a cooperative of producers in Espírito Santo, Brazil. Coffee samples were roasted in a commercial fluidized bed roaster (i-Roast model no. 40009, Hearthware Home Products, USA) at 220 °C for 6, 7, 8, 9, 12, and 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). Instrumental color was determined using a coffee colorimeter Colorgap1A (Leogap, Curitiba, Brazil).

Coffee Brew Preparation. Green and roasted coffee beans were frozen in liquid nitrogen prior to grinding to pass through a 0.46 mm sieve. Coffee brews were prepared at 10% (w/v), which is the standard proportion of coffee to water generally used in Brazil as well as in other countries. For this, 20 mL of boiling water were added to 2 g of ground coffee, and the material was vortexed for 15 s and then filtered through a paper filter (Whatman no. 1). Soluble solids (w/v) of coffee brews were determined gravimetrically after oven drying at 105 °C according to AOAC.[22] Brews were kept at −20 °C until analysis.

Clarified Coffee Brew Preparation. Coffee brews were clarified in order to exclude high molecular weight compounds such as melanoidins. This was done by mixing 1 mL of coffee brew with 50 mL of water and 1 mL of each Carrez's solutions, $K_2Fe(CN)_6$ (0.3 M) and $Zn(OAc)_2$ (1.0 M). The volume was made up to 100 mL with water, and after 15 min the colloidal suspension was filtered through paper filter (Whatman no. 1), yielding the clarified coffee brew fraction, which was kept at −20 °C until analysis.

Isolation of High Molecular Weight Material from Coffee Brew. Ten milliliter aliquots of fresh coffee brew were dialyzed using a dialysis membrane (MW cutoff 12 kDa, Sigma-Aldrich, St. Louis, MO, USA) for 4 days against distilled water with five water renewals, according to the method described by Bekedam et al.[17] The retentates were lyophilized, yielding the high molecular weight fraction (HMWF) which was kept at −20 °C until analysis.

Spectroscopic Analysis. The absorption spectra of whole coffee brews (1:100 in water) and HMWF (0.1 mg/mL in water) were determined in the range 200−700 nm. The absorption of the HMWF at 280, 325, and 405 nm was measured and to guarantee linearity; if required, samples were diluted as to ensure that the absorbance at all three wavelengths were between 0.1 and 1.0. Spectroscopic analyses were performed using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

The specific extinction coefficient (K_{mix}) was calculated using the law of Lambert−Beer: Abs (−) = K_{mix} (L g^{-1} cm^{-1}) × concentration (g L^{-1}) × length of light path (cm). The use of the specific extinction coefficient (K) was preferred over the molar extinction coefficient (ε) because the molecular weight of melanoidins is unknown and probably variable. By applying K, the concentration parameter used in the law of Lambert−Beer was expressed as L g^{-1} cm^{-1}, which made it applicable to coffee.

Sample Preparation for the Determination of Unbound, Ionically Bound, and Covalently Bound Phenolic Acids in the HMWF. To investigate the content of free phenolic acids, specially CGA and derivatives, in the HMWF, the material was dissolved in water (10 mg/mL) and Carrez's solutions were added (1:100 v/v) for clarification. After centrifugation, the supernatant was analyzed by LC-DAD-MS.

A modification of the method by Delgado-Andrade and Morales[16] was used to assess the presence of ionically (noncovalently) bound phenolic acids, specially CGA, in the HMWF. Solutions containing 10 mg/mL of HMWF in 2 M NaCl were prepared and incubated overnight. Subsequently, Carrez's solutions were added to the mixture (1:100 *v*/*v*) and, after centrifugation, the supernatant was analyzed by HPLC-DAD.

For the determination of covalently bound phenolic acids in the HMWF, a modification of the saponification method by Bekedam et al.[19] was employed. The volume of 750 μL of 2 M NaOH solution containing 2% (w/w) ascorbic acid and 20 mM ethylenediaminetetraacetic acid was added to 750 μL of HMWF solution (10 mg/mL). After incubation for 1 h at 30 °C, the mixture was quenched to pH ≈ 1 with 330 μL of 5 M HCl and Carrez's solutions were added (1:100 *v*/*v*) to prevent precipitation of coffee material during further analysis. After centrifugation, the supernatant was analyzed by LC-DAD-MS.

Analysis of Chlorogenic Acids and Lactones in Coffee Brews and of Phenolic Acids in HMWF Preparations. The contents of CGA and CGA lactones (CGL) in the coffee brews at 10% were determined in triplicate using the extraction method described by Farah et al.[23] and the LC-MS conditions described by Perrone et al.[24] Phenolic acids contents in HMWF preparations to assess free and covalently bound components were determined by LC-MS according to Perrone et al.[24] and Farah et al.[25] The identity of analytes was confirmed by injection of commercial standards and spectroscopic data from the MS. To assess ionically bound phenolic acids in the HMWF, the HPLC-DAD method described by Farah et al.[23] was used.

Antioxidant Activity. The AA of whole coffee brews, clarified coffee brews, and HMWF (1 mg/mL in water) was evaluated by the ferric reducing antioxidant power (FRAP), Trolox equivalence antioxidant capacity (TEAC), and total radical-trapping antioxidant parameter (TRAP) assays. A blank solution, containing only Carrez's solutions, was also assayed in order to exclude the interference of the clarification procedure on AA.

FRAP assay was performed as described by Moreira et al.[26] The method is based on the reduction of the Fe^{3+}−TPTZ complex to the ferrous form at low pH. This reduction is monitored by measuring the absorption change at 593 nm. Briefly, 900 μL of working FRAP reagent prepared daily was mixed with 100 μL of appropriately diluted sample; the absorbance at 593 nm was recorded after a 30 min incubation at 37 °C. FRAP values were obtained by comparing the absorption change in the test mixture with those obtained from increasing standard concentrations of Fe^{2+} and expressed as millimoles of Fe^{2+} equiv per L (for whole and clarified coffee brews) or millimoles of Fe^{2+} equiv per 100 g (for HMWF).

TEAC assay was performed using adaptations of the methodology described by del Castillo et al.[8] The $ABTS^{\bullet+}$ radical was produced by reacting 7 mM ABTS and 2.45 mM potassium persulfate (final concentration in 10 mL of water) and keeping the mixture in the dark

Table 1. Chlorogenic Acids (CGA) and Lactones (CGL) Contents in Coffee Brews[a,b,c]

roasting time (min)	5-CQA	total CQA	total FQA	total diCQA	total CFQA	total CGA	total CGL
			C. arabica cv. Mundo Novo				
green	2226.8[1]	3029.9[1]	236.3[1]	198.6[1]	18.8[1]	3543.9[1]	ND[d]
6	567.9[2]	1359.6[2]	98.9[2]	33.9[2]	3.1[2]	1511.2[2]	59.2[1]
7	283.2[3]	725.8[3]	59.7[3]	12.6[3]	ND	809.0[3]	43.9[2]
8	86.9[4]	231.8[4]	25.2[4]	3.4[4]	ND	265.3[4]	16.8[3]
9	59.1[5]	158.9[5]	18.8[4]	2.2[4]	ND	183.8[5]	12.1[4]
12	19.2[6]	45.2[6]	7.2[5]	1.6[4]	ND	55.5[6]	4.0[5]
15	14.2[7]	24.5[7]	4.5[5]	2.4[4]	ND	32.3[7]	1.4[6]
			C. arabica cv. Red Catuai				
green	3198.2[1]	4471.7[1]	338.7[1]	198.1[1]	19.3[1]	5106.9[1]	ND
6	784.6[2]	1850.4[2]	110.3[2]	30.6[2]	14.1[1]	2024.9[2]	77.4[1]
7	407.2[3]	1066.2[3]	187.6[3]	28.3[2]	3.4[2]	1295.8[3]	103.0[2]
8	238.1[4]	629.3[4]	58.5[4]	9.8[3]	1.0[2]	708.5[4]	40.2[3]
9	104.3[5]	273.2[5]	30.5[5]	4.4[4]	ND	314.0[5]	19.2[4]
12	34.1[6]	85.0[6]	14.3[6]	3.0[4]	ND	104.7[6]	8.1[5]
15	15.4[7]	31.3[7]	6.9[7]	2.0[4]	ND	41.3[7]	1.8[6]
			C. arabica cv. Yellow Bourbon				
green	2132.5[1]	2770.0[1]	254.9[1]	93.0[1]	13.2[1]	3202.8[1]	ND
6	727.0[2]	1674.9[2]	159.6[2]	31.2[2]	3.6[2]	1925.2[2]	75.1[1]
7	224.2[3]	557.0[3]	52.8[3]	7.1[3]	ND	640.6[3]	44.7[2]
8	92.7[4]	235.7[4]	30.3[4]	3.4[4]	ND	283.1[4]	21.4[3]
9	42.7[5]	113.6[5]	19.3[5]	1.7[4]	ND	142.6[5]	15.5[4]
12	8.9[6]	22.0[6]	5.2[6]	ND	ND	28.7[6]	3.4[5]
15	5.9[7]	15.2[6]	3.3[6]	ND	ND	19.1[6]	2.4[6]
			C. canephora cv. Conillon				
green	3588.9[1]	5803.9[1]	423.3[1]	642.6[1]	116.1[1]	7026.8[1]	ND
6	946.3[2]	2048.5[2]	254.7[2]	84.5[2]	21.7[2]	2451.6[2]	290.6[1]
7	514.1[3]	1161.5[3]	185.5[3]	30.9[3]	11.6[3]	1417.5[3]	199.4[2]
8	218.1[4]	499.5[4]	101.3[4]	10.3[4]	5.4[4]	633.8[4]	106.8[3]
9	95.8[5]	220.4[5]	63.4[5]	2.3[5]	2.7[4]	302.0[5]	56.0[4]
12	18.2[6]	40.2[6]	18.7[6]	0.5[6]	ND	59.4[6]	15.4[5]
15	7.2[7]	16.0[7]	6.8[7]	0.4[6]	ND	23.2[7]	5.4[6]

[a]Results are presented as means of three true replicates, expressed in mg/L coffee brew; CV was lower than 5% for samples roasted for up to 9 min and lower than 10% for samples roasted for 12 and 15 min. [b]Different superscript arabic numerals in the same subsets indicate that samples are statistically different ($p < 0.05$). [c]5-CQA = 5-caffeoylquinic acid, CQA = caffeoylquinic acids, FQA = feruloylquinic acids, diCQA = dicaffeoylquinic acids, *p*-CoQA = *p*-coumaroylquinic acids, CFQA = caffeoylferuloylquinic acids. [d]Not detected.

at room temperature for 12−16 h before use. The aqueous $ABTS^{\bullet+}$ solution was diluted with ethanol (1:100) to an absorbance of 0.70 ± 0.02 at 734 nm in a 1 cm cuvette at 30 °C. Appropriately diluted samples (10 μL) were added to 990 μL of $ABTS^{\bullet+}$ solution. After mixing, the absorbance was measured at 734 nm after a 6 min incubation period at 37 °C and used to calculate the percent inhibition value. Absorbance values were corrected for the solvent as follows:

$$\Delta A_{\text{sample}} = \left(A_{t=0(\text{sample})} - A_{t=6\text{min}(\text{sample})}\right) \Big/ \left(A_{t=0(\text{sample})} - \left(A_{t=0(\text{solvent})} - A_{t=6\text{min}(\text{solvent})}\right) \Big/ A_{t=0(\text{solvent})}\right)$$

Percent inhibition values were obtained by multiplying ΔA_{sample} values by 100. Standard calibration curves were constructed by plotting percent inhibition values as a function of Trolox concentration. TEAC values were calculated using the calibration curves and expressed as millimoles of Trolox equiv per L (for whole and clarified coffee brews) or millimoles of Trolox equiv per 100 g (for HMWF).

TRAP assay was performed using an adaptation of the methodology described by Bartosz et al.[27] Briefly, 670 μL of 0.1 M sodium phosphate buffer, pH 7.0 were mixed with 30 μL of 5 μM ABTS solution and 100 μL of appropriately diluted sample. After a 15 min incubation period at 37 °C, the reaction was initiated by adding 200 μL of 400 mM AAPH. Then the absorbance of the solution at 428 nm was continuously monitored, and the induction time of the $ABTS^{\bullet+}$ formation reaction (i.e., the required time for a linear increase in the absorbance to be observed) was determined. Standard calibration curves were constructed by induction times as a function of the concentration of Trolox. TRAP values were calculated using the calibration curves and expressed as millimoles of Trolox equiv per L (for whole and clarified coffee brews) or millimoles of Trolox equiv per 100 g (for HMWF).

The final value of each AA assay was the mean of three replications.

Statistical Analyses. Data are presented as mean ± SD. The influence of roasting on the chemical composition and AA of coffee brews and their fractions was evaluated using analysis of variance (ANOVA) with Tukey multiple comparison post-test. The AA of whole coffee brews and clarified coffee brews were compared using Student's paired *t* test. Comparison between coffee species before roasting regarding chemical composition and AA was done using unpaired *t*-test. Considering that data were normally distributed, Pearson correlation analysis between CGA contents in the brews and phenolic acids in the HMWF and between different AA assays was performed. The relative contribution of CGA compounds in the brew and the phenolic acids bound to the HMWF to the AA of whole coffee brews was determined by multiple regression analysis. All statistical analyses were performed using Prism software for Windows, version 4 (GraphPad Software, Inc.). Differences were considered significant when $p < 0.05$.

Table 2. Contents (mg/100g) and Distribution (%) of Phenolic Acids Covalently Bound to the HMWF Isolated from the Roasted Coffee Brews[a,b]

		caffeic acid		ferulic acid		dihydrocaffeic acid	
roasting time (min)	total phenolics	content	%	content	%	content	%
		C. arabica cv. Mundo Novo					
6	179.5[1]	146.9[1]	81.8	10.7[1]	6	179.5[1]	146.9[1]
7	184.6[1]	139.8[1]	75.7	11.4[1]	7	184.6[1]	139.8[1]
8	153.6[2]	95.3[2]	62.1	11.8[1]	8	153.6[2]	95.3[2]
9	149.1[2]	83.1[2]	55.7	11.9[1]	9	149.1[2]	83.1[2]
12	117.6[3]	47.8[3]	40.7	10.3[1]	12	117.6[3]	47.8[3]
15	83.6[4]	24.5[4]	29.4	7.4[2]	15	83.6[4]	24.5[4]
		C. arabica cv. Red Catuai					
6	196.1[1]	167.2[1]	85.3	11.2[1,2]	6	196.1[1]	167.2[1]
7	195.9[1]	144.8[2]	73.9	14.5[1]	7	195.9[1]	144.8[2]
8	175.2[2]	124.8[3]	71.2	13.6[1,2]	8	175.2[2]	124.8[3]
9	153.5[3]	87.8[4]	57.2	12.6[1,2]	9	153.5[3]	87.8[4]
12	151.4[3]	65.3[5]	43.1	13.2[1,2]	12	151.4[3]	65.3[5]
15	94.0[4]	31.1[6]	33.1	10.0[2]	15	94.0[4]	31.1[6]
		C. arabica cv. Yellow Bourbon					
6	184.7[1]	153.7[1]	83.2	12.2[1]	6	184.7[1]	153.7[1]
7	174.0[1]	121.7[2]	69.9	12.9[1,2]	7	174.0[1]	121.7[2]
8	159.5[2]	95.4[3]	59.8	14.8[2]	8	159.5[2]	95.4[3]
9	149.0[2]	77.8[4]	52.2	14.7[2]	9	149.0[2]	77.8[4]
12	97.2[3]	33.4[5]	34.3	10.5[1,3]	12	97.2[3]	33.4[5]
15	67.6[4]	17.5[6]	25.9	7.2[3]	15	67.6[4]	17.5[6]
		C. canephora cv. Conillon					
6	370.3[1]	244.7[1]	66.1	62.5[1]	6	370.3[1]	244.7[1]
7	364.7[1]	215.1[2]	59.0	65.7[1]	7	364.7[1]	215.1[2]
8	341.9[2]	167.1[3]	48.9	61.8[1]	8	341.9[2]	167.1[3]
9	319.2[3]	121.8[4]	38.2	62.6[1]	9	319.2[3]	121.8[4]
12	213.8[4]	53.9[5]	25.2	42.3[2]	12	213.8[4]	53.9[5]
15	164.5[5]	24.2[6]	14.7	24.0[3]	15	164.5[5]	24.2[6]

[a]Results are presented as means of three true replicates; CV was lower than 5% for samples roasted for up to 9 min and lower than 10% for samples roasted for 12 and 15 min. [b]Different superscript arabic numerals in the same subsets indicate that samples are statistically different ($p < 0.05$).

RESULTS AND DISCUSSION

Characterization of Coffee Brews. Green coffee samples were roasted at 220 °C for 6, 7, 8, 9, 12, and 15 min, yielding from very light to very dark roasts (or color degrees), with percent weight loss ranging from 13.5% to 26.2% (Supporting Information data 1). The reproducibility of the roasting process and of CGA and CGL analysis was evaluated as a whole in a six replicate experiment. 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 and CGA and CGL analysis were performed in triplicate.

Eighteen CGA compounds were quantified in green coffee brews, where 5-CQA was the most abundant, representing from 51% to 63% of total CGA (Table 1). As expected, coffee roasting led to a reduction in the contents of CGA in comparison with green coffee brews, ranging from 56% to 99%, on average, for brews prepared with beans roasted for 6 and 15 min, respectively (Table 1). Even though the distribution of different CGA compounds was also affected by the roasting process, 5-CQA remained the most abundant CGA, representing, on average, 36% and 32% of total CGA in coffee brews prepared with beans roasted for 6 and 15 min, respectively (Table 1). In roasted coffee brews, in addition to the 18 CGA compounds present in green beans, seven CGL were quantified. The roasting-induced formation of CGL reached a maximum for brews prepared with coffee beans roasted for 6 or 7 min, and corresponded from 2.0% to 4.1% of the CGA contents observed in the respective green coffee brews (Table 1). CGL contents in coffee brews were greatly reduced after roasting for 15 min, with only an average of 2.3% of the maximum CGL contents remaining after this process (Table 1). These results, including differences between species, are in accordance with data published in the literature.[4,23,24,28−30]

Absorption spectra for the brews and the HMWF were obtained, and maximum absorption peaks occurred at 280 and 325 nm. The 280 nm peak is due to proteins, caffeine, CGA, and free cinnamic acids, while the 325 nm peak is due to CGA and free cinnamic acids.[17] The 405 nm wavelength is usually chosen to measure melanoidins[10,17] because other coffee components do not absorb light at this wavelength. The evaluation of the ratios between the absorption coefficients at these wavelengths (Supporting Information data 2) suggests the incorporation of CGA to the structure of coffee brew melanoidins.[18−20]

Phenolic Characterization of HMWF. To verify the efficiency of the dialysis process used to isolate the HMWF, we investigated the presence of unbound phenolic acids, specially CGA, in the HMWF. Because only trace amounts of free CGA could be found in HMWF (less than 0.3%), we considered the

isolation process to be efficient. This result was also important to correctly attribute the observed antioxidant activity of the HMWF to the bound components and not to any residual unbound CGA from the brew.

No ionically bound CGA derivatives were detected after overnight incubation of the HMWF with 2 M NaCl. In contrast, Delgado-Andrade and Morales[16] reported that considerable amounts of CGA were released from melanoidins after such high ionic strength treatment was employed. These contradictory results may be explained by the different methods used in both studies for the isolation of the melanoidins from the coffee brew. While Delgado-Andrade and Morales[16] employed ultrafiltration with three washing procedures, in the present work we used an extensive dialysis procedure with five water renewals, which could have been more efficient in eliminating the ionically bound CGA from the melanoidins' structure.

After saponification of HMWF to measure the covalently bound CGA, the release of caffeic (CA), ferulic (FA), and dihydrocaffeic (DHCA) acids from melanoidins' backbones was observed (Table 2). The possible presence of 11 other phenolic acids potentially derived from thermal degradation of CGA was also evaluated after saponification of HMWF but was not detected. Because the coffee samples did not contain significant amounts of unesterified CA and FA, the release of these compounds from the HMWF during saponification indicates the incorporation of CGA containing caffeoyl (CQA, diCQA, and CFQA) and feruloyl (FQA and CFQA) moieties to the melanoidins' backbones, respectively. The contents of CA released from the HMWF decreased with roasting from 155.9 to 24.7 mg/100 g (on average) and from 244.7 to 24.2 mg/100 g in *C. arabica* and *C. canephora* samples, respectively. Similarly, contents of FA released from the HMWF decreased from 11.4 to 8.2 mg/100 g (on average) and from 62.5 to 24.0 mg/100 g, respectively (Table 2). Bekedam et al.[19] reported the release of higher amounts of CA and FA after saponification of a HMWF (500 and 100 mg/100 g, respectively) based on the analysis of one roasted *C. arabica* sample. In the present study, the contents of DHCA released from the HMWF increased with roasting from 19.5 to 49.1 mg/100 g and from 63.0 to 116.2 mg/100 g in *C. arabica* and *C. canephora* samples, respectively (Table 2).

We observed a remarkable influence of the roasting process on the distribution of covalently bound phenolic acids in the HMWF (Table 2). In *C. arabica* samples, the content of CA in relation to total phenolics in the HMWF decreased, on average, from 83.4% (for beans roasted for 6 min) to 29.5% (for those roasted for 15 min). A similar decrease, from 66.1% to 14.7%, was also observed in the corresponding *C. canephora* samples. In contrast, the roasting process led to a slight increase in the relative content of FA in the HMWF. Such behavior of CA and FA bound to the HMWF during coffee roasting may be associated to that observed for free CQA and FQA during the same process, that is, the latter class is more thermally stable than the former.[23,24] A strong positive correlation ($r = 0.88$, $p < 0.0001$, $n = 24$, 4 coffee samples roasted at 6 conditions) was observed between the relative contents of CQA in the roasted coffee brews and of CA from the HMWF. Similarly, the relative contents of FQA in the roasted coffee brews and of FA in the HMWF were correlated ($r = 0.70$, $p < 0.0001$, $n = 24$).

The relative content of DHCA in the HMWF obtained from *C. arabica* and *C. canephora* samples increased substantially during roasting, from 10.5 to 60.4% and 17.0 to 70.7%, respectively. As DHCA was not present in the roasted coffee brews and increased in HMWF during roasting, its release from HMWF may be explained by the oxidation during roasting of previously incorporated CQA. Consistent with this hypothesis, Nunes and Coimbra[21] reported that the propenyl moiety of intact CQA is not altered during its incorporation into the structure of melanoidins. Moreover, the presence of oxidation products of hydroxycinnamates has been reported in roasted coffee and thought to be closely related to the role of phenolic compounds in coffee melanoidin formation.[21] To our knowledge, the present study is the first one to report the release of DHCA from melanoidins subjected to saponification.

Taken together, these results corroborate the hypothesis of Bekedam et al.[19] that the incorporation mechanism of CGA into melanoidins occurs via the CA (or FA) moiety, mainly through nonester linkages upon roasting of coffee beans. This raises the possibility of incorporation of CGL into melanoidins, considering that lactonization only modifies the quinic acid moiety of CGA. Therefore, when speaking of CGA incorporation, one should really consider both CGA and CGL incorporation. In the present study, hereafter, when referring to CGA incorporation into melanoidins, we will be also referring to the possible incorporation of CGL.

The kinetics of CGA incorporation into the HMWF, together with their degradation kinetics in the whole brew, indicates that CGA are incorporated in melanoidins mainly at the beginning of the roasting process and degrade thereafter. On the basis of the existing literature results,[19] it is probable that incorporation and degradation of CGA occur continuously, with higher degradation than incorporation rate.

Fate and Distribution of CGA during Coffee Roasting. CGA are major thermolabile constituents of green coffee but their fate during coffee roasting is still not completely understood. At early stages, Clifford[28] and Farah et al.[23] reported that CGA isomerization occurs accompanied by lactonization and partial hydrolysis, yielding quinic acid and various cinnamic acids, which in turn may be decarbolxylated, degraded to a range of simple phenols, and further transformed.

In the present study, the amount of free CGA and CGL in the brew, as well as the amount of phenolic acids covalently bound to the HMWF, in relation to the initial content of CGA in the green coffee brew were estimated (Table 3). To calculate the amount of phenolic acids covalently bound to the HMWF in a brew basis, we took into consideration that the amount of soluble solids in our coffee brew samples was on average 2.4% (w/v), from which it was estimated that 30% (w/w) corresponded to high molecular weight materials.[17,31,32] In brews from samples roasted for 6 min, on average, 47.4% of the CGA content in green coffee brews could still be found, mainly as nondegraded CGA in the brew, which accounted for 44.3% of the CGA content in green coffee brews. The remaining percentage of CGA was found as free CGL and CGA incorporated into the HMWF, which accounted for 2.4% and 0.7% of the CGA content of green coffee brews, respectively. The percentage of CGA incorporated into the HMWF was similar in both *C. arabica* and *C. canephora* (Table 3) despite the higher CGA content of the latter species (Table 1). Therefore, even though the incorporation of CGA into melanoidins was accounted for in the present study, the fate of most CGA lost during roasting remains largely unknown. This fact becomes even more evident when the fate of CGA in coffee samples roasted for 15 min (dark and very dark roasting degrees) is evaluated. For such samples, only the fate of 1.0% of

Table 3. Fate of CGA during Roasting in Relation to Total CGA Content in Green Coffee Brews

roasting time (min)	brew phenolics (% of total CGA in green coffee)		
	free CGA	free CGL	CGA covalently bound to the HMWF[a]
C. arabica cv. Mundo Novo			
6	42.6	1.7	0.7
7	22.8	1.2	0.7
8	7.5	0.5	0.6
9	5.2	0.3	0.6
12	1.6	0.1	0.4
15	0.9	0.1	0.4
C. arabica cv. Red Catuai			
6	39.4	1.5	0.5
7	25.6	2.0	0.6
8	13.9	0.8	0.5
9	6.1	0.4	0.4
12	2.1	0.2	0.4
15	0.8	0.1	0.3
C. arabica cv. Yellow Bourbon			
6	60.1	2.3	0.8
7	20.0	1.4	0.7
8	8.8	0.7	0.6
9	4.5	0.5	0.5
12	0.9	0.1	0.4
15	0.6	0.1	0.2
C. canephora cv. Conillon			
6	34.9	4.1	0.7
7	20.2	2.8	0.7
8	9.0	1.5	0.5
9	4.3	0.8	0.6
12	0.8	0.2	0.3
15	0.3	0.1	0.3

[a]Considering that coffee brew samples contained on average 2.4% (*w*/*v*) soluble solids, from which 30% (*w*/*w*) was estimated to correspond to the HMW fraction.

the CGA content of green coffee brews could be explained (nondegraded CGA in the brew and CGA incorporated into the HMWF contributed to 0.7% and 0.3% of the CGA content of green coffee brews, respectively).

Other compounds not considered in this study may explain, at least in part, the remaining fate of CGA during roasting. One might expect that a considerable part of the CGA present in the green bean would be transformed into volatile compounds and lost during coffee roasting. In a model roasting system, Moon and Shibamoto[33] reported that approximately 10% of CGA, CA, and quinic acid were recovered as volatile compounds, mainly phenols and derivatives. Moreover, other types of CGL besides the major cinnamoyl-1,5-γ-quinolactones quantified in this study (e.g., minor δ-lactones), di- and trihydroxybenzenes, simple phenols, and condensation products of high structural complexity, as well as lignan-like autoxidation products called caffeicins, have been characterized in roasted coffee[21,34,35] and might account for part of thermally degraded CGA. Finally, Maillard reaction products of molecular weight lower than 12 kDa, which were not retained by the dialysis procedure employed in this study, might also contain CGA incorporated into their structure, as reported by Gniechwitz et al.,[36] and should be investigated in future studies.

Even though incorporation into the HMWF evaluated in the present study was not a major fate of CGA during coffee roasting, the relevance of such incorporation in terms of relative content increased during the process. Considering the percent distribution of CGA and derivatives in the brew (Figure 1), while in coffee samples roasted for 6 min, on average, 93.2% of total CGA was found as nondegraded CGA and only 1.4% as CGA incorporated into the HMWF (66.6:1 ratio), in samples roasted for 15 min, total CGA distribution was shifted to 64.8% as nondegraded CGA in the brew and 28.6% as CGA incorporated into the HMWF (2.3:1 ratio). This result suggests that, in addition to following similar degradation profiles during roasting regardless of their incorporation, CGA continuously form covalent bonds with melanoidins. Regarding CGL, once formed their relative content was slightly affected by the roasting process, ranging from 5.4% of total initial CGA content in samples roasted for 6 min to 6.6% in those roasted for 15 min, on average.

If one considers that a large number of coffee consumers, specially in Brazil and Europe, tend to prefer medium-dark to dark roasted beans, the amount of CGA incorporated into the melanoidins might be relevant from a biological point of view, because they might possibly be released in the gastrointestinal tract and further absorbed.

Antioxidant Activity of Whole and Clarified Coffee Brews. The antioxidant activity of whole and clarified coffee brews, measured by different assays, are presented in Figure 2. Considering whole coffee brews, TEAC values were strongly correlated with those of FRAP ($r = 0.91$, $p < 0.001$, $n = 28$) and TRAP ($r = 0.88$, $p < 0.001$, $n = 28$), whereas the values of FRAP and TRAP showed a significant but lower correlation between each other ($r = 0.81$, $p < 0.001$, $n = 28$). This lower correlation could be explained by the different mechanisms of action of FRAP and TRAP, that is, single electron transfer (SET) and hydrogen atom transfer (HAT), respectively.[36] The TEAC assay, however, may act by both mechanisms, which was reflected in the strong correlation with both FRAP and TRAP assays.

The AA of whole coffee brews evaluated by different assays in this study was in agreement with previous studies on coffee's AA.[5,6,9,26] In whole coffee brews, the AA of the brew from green *C. canephora* was significantly higher than that of the average of brews from green *C. arabica* ($p < 0.001$) regardless of the assay considered. The higher AA of the green *C. canephora* brew in comparison to the green *C. arabica* brews may be explained by the higher content of CGA in the brew prepared with *C. canephora* beans in comparison to those prepared with *C. arabica* (Table 1), consistent with previous studies.[11,26]

The AA of coffee brews gradually decreased with roasting independently of the assay used (Figure 2). CGA content also decreased but in a more pronounced manner (Table 1). After the 15 min roasting period (dark and very dark roast), average reductions in FRAP, TEAC, and TRAP were 42%, 44% and 75%, respectively, when compared to the corresponding green brews (Supporting Information data 3), although the decrease in CGA content was on average >99% (Table 1). Even though a substantial reduction in AA of coffee brews was observed with increasing roasting (Figure 2), AA values were similar or higher, in all roasted samples, than those reported for green tea and red wine using similar assays.[5,6]

In the clarified coffee brews (Figure 2), there was also a gradual decrease in AA with increasing roasting time, independently of the assay used. AA values of clarified coffee brews were consistently lower than those of whole coffee brews at the same roasting time. Furthermore, the roasting-induced

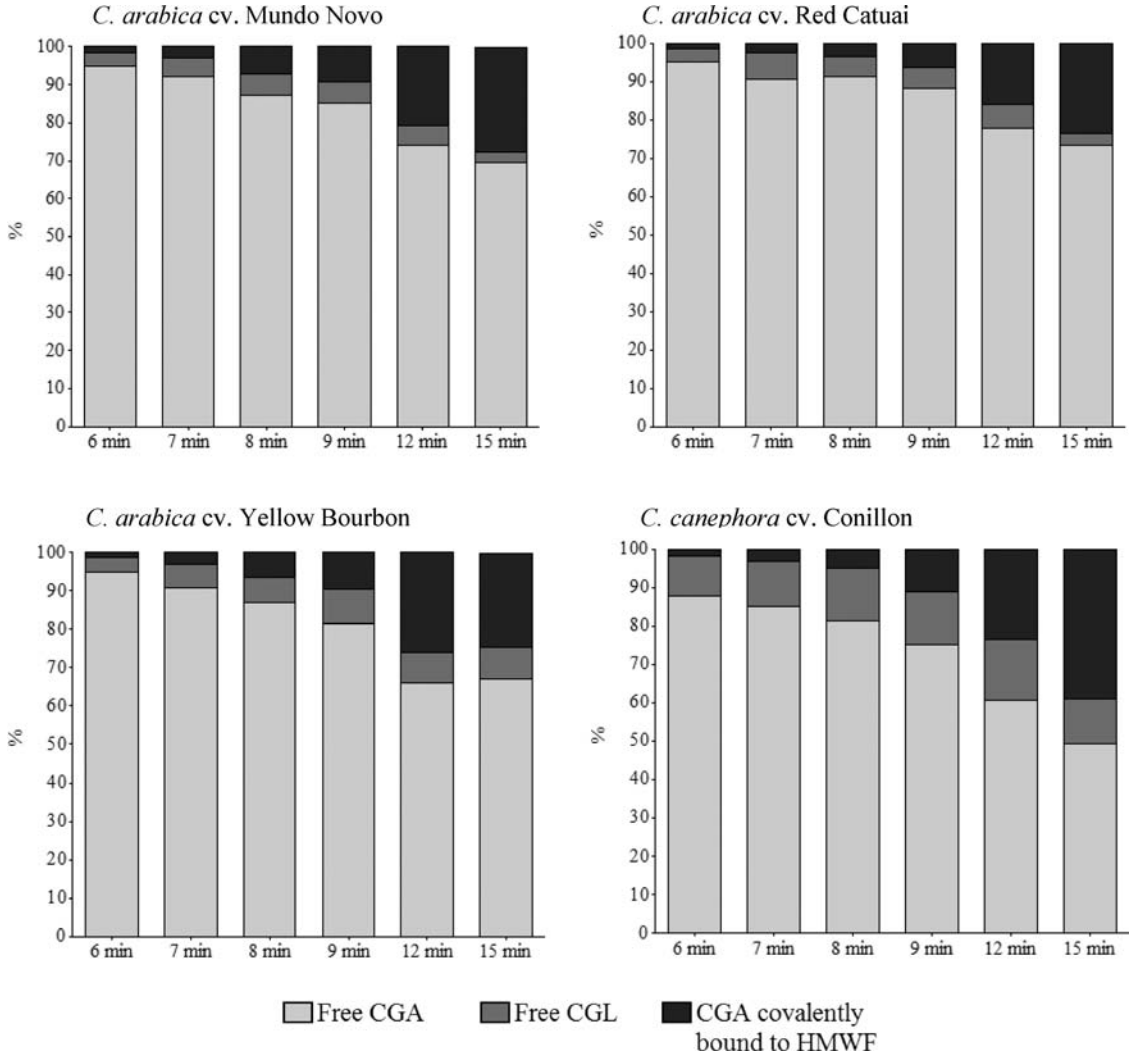

Figure 1. Percentage of phenolic acids in the brew distributed among free CGA and CGA covalently bound to HMWF, at increasing roasting times, considering that coffee brew samples contained, on average, 2.4% (w/v) soluble solids, from which 30% (w/w) was estimated to correspond to the HMW fraction.

decrease in AA was more pronounced in the clarified coffee brews than in the whole brews. These results may be attributed to the removal of potentially antioxidant high molecular weight compounds such as melanoidins during the clarification procedure. In 15 min roasted clarified coffee brews, in which melanoidins were removed and CGA contents were very low (<1% of initial content), the residual AA, on average 27% of initial value (Figure 2) indicates the presence of other low molecular weight components with AA which could be detected by the assays used. These compounds may include volatile heterocyclic compounds, such as pyrrols, furans and thiophenes,[15] indanes,[37] and hydroxybenzenes, such as catechol, ethylcathecol, and pyrogallol,[38] possibly present in the clarified coffee brews. It is noteworthy to mention that a blank solution containing only Carrez's reagents did not present any AA, which excludes the possible interference of the clarification procedure in the AA measured by the three assays.

Significant correlations were observed between AA values of whole coffee brews (Supporting Information data 3) and their CGA contents (Table 1), this correlation being higher for TRAP (r = 0.90, p < 0.001, n = 28), intermediate for TEAC (r = 0.80, p < 0.001, n = 28), and lower for FRAP (r = 0.60, p = 0.001, n = 28). It is relevant to point out that assays based in HAT and SET mechanisms provide conceptually different information: HAT is a kinetic measurement, while SET is a thermodynamic measurement based on the redox potential of the reagents.[10] Therefore, while HAT-based assays (e.g., TRAP) allow estimating the trapping capacity of most reactive compounds, SET-based assays (e.g., FRAP) provide an overall picture of the effective efficiency of oxidation/reduction of all antioxidants present in the sample, including the "slow" ones, which are not detected by kinetic methods. The higher sensitivity of TRAP assay to measure the AA of CGA indicates, therefore, that CGA are the most reactive free radicals trapping compounds in coffee brews. This higher reactivity is probably due to their low molecular weight and their structural simplicity when compared to the melanoidins.

Antioxidant Activity of the HMWF. The AA of HMWF isolated from roasted coffee brews was significantly higher than that isolated from green coffee brews (Figure 2). HMWF from green coffee probably contains polysaccharides, lignins, pectins, and proteins, and from these, based on chemical structure only lignin, which contains phenolic moieties, may contribute to the AA of this fraction. The maximum AA of HMWF from roasted coffee brews was on average 10, 6, and 7 times higher than that of HMWF from the green coffee brews when measured by the

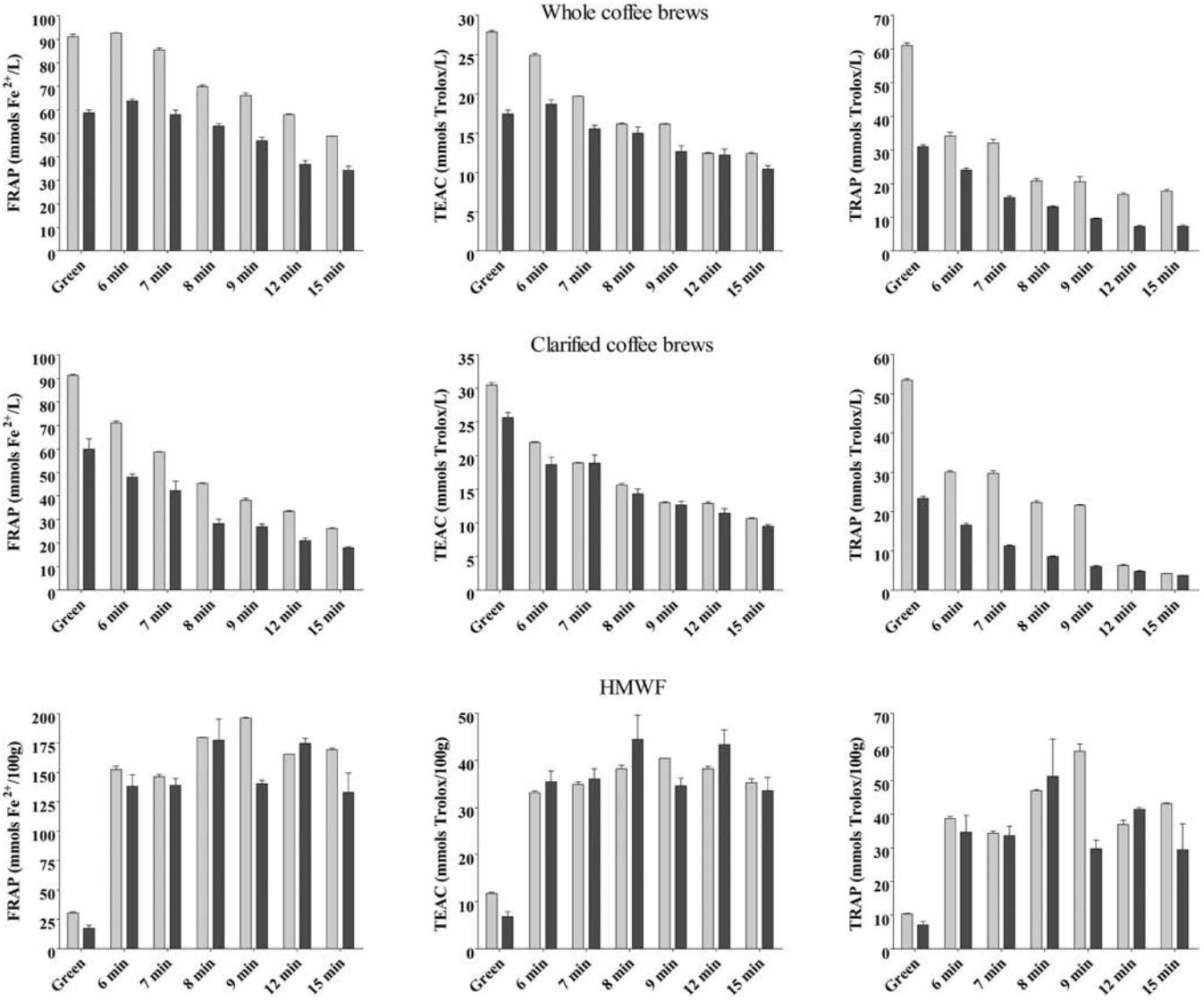

Figure 2. Antioxidant activity of whole and clarified coffee brews and HMWF measured by the FRAP, TEAC, and TRAP assays in *C. canephora* (light-gray bars) and *C. arabica* (average of three cultivars, dark-gray bars).

FRAP, TEAC, and TRAP assays, respectively. These results indicate that the melanoidins formed during coffee roasting, and present in high amounts in HMWF, play an important role in maintaining the expressive AA values observed in very dark roasted coffees. The influence of roasting on the AA of HMWF, as well as on the contents and distribution of phenolic acids in this fraction, show that melanoidins undergo drastic changes in their chemical composition and structure during this thermal processing.

A significant correlation was observed between the melanoidin content, expressed by the absorption at 405 nm (Supporting Information data 2), and the AA of HMWF, measured by FRAP ($r = 0.93$, $p < 0.0001$, $n = 28$), TEAC ($r = 0.91$, $p < 0.0001$, $n = 28$), and TRAP assays ($r = 0.85$, $p < 0.0001$, $n = 28$). The higher correlations with FRAP and TEAC indicate that these assays, specially FRAP, are more sensitive to measure the AA of the melanoidins, in contrast to TRAP, which seems to be more sensitive to measure the AA of CGA, as previously mentioned. The lower sensitivity of TRAP assay toward melanoidins may arise from the fact that this assay is based on the measurement of the *lag* phase, that is, the period of time necessary for all the antioxidant molecules present in the sample to be consumed. because it is known that not all antioxidant compounds possess a well-defined *lag* phase,[39] one may suppose that as a consequence of melanoidins' extremely complex structure, their *lag* phase might be difficult to measure. Moreover, as already mentioned, SET-based assays such as FRAP are capable of detecting the AA of all antioxidants present in a sample, including the "slow" ones.[10]

When taken together, our results on AA measured by different assays in whole coffee brews and in HMWF isolated from coffee brews, it appears that TEAC is the most appropriate assay to detect, simultaneously and more sensitively, the AA of both CGA and melanoidins present in coffee brews, because this assay accounts for both HAT and SET responses.

Contribution of CGA and HMWF to the AA of Coffee Brews. Multiple regression analyses were performed using FRAP, TEAC, and TRAP as dependent variables and the contents of CGA in the brew (Table 1) and of phenolic acids in the HMWF (Table 2) as independent variables in order to estimate the relative contribution of these components to the

Table 4. Multiple Regression Analysis Models to Evaluate the Contribution of Free CGA in Brew and Phenolic Acids Covalently Bound to HMWF to the AA of Coffee Brews Determined by FRAP, TEAC and TRAP assays (n = 24)

dependent variable	independent variables	p	β coefficients[a]	regression summary
$FRAP_{brew}$ (mmol Fe^{2+}/L)	CGA_{brew} (mg/L)	<0.0001	0.910	= 24.84989 + 0.00863 CGA_{brew} + 0.12875 $phenolics_{HMWF}$ adjusted R^2 = 0.9157 ANOVA p regression <0.0001
	$phenolics_{HMWF}$ (mg/100 g)	<0.0001	0.808	
$TEAC_{brew}$ (mmol Trolox/L)	CGA_{brew} (mg/L)	<0.0001	1.016	= 8.64408 + 0.00254 CGA_{brew} + 0.023657 $phenolics_{HMWF}$ adjusted R^2 = 0.9071 ANOVA p regression <0.0001
	$phenolics_{HMWF}$ (mg/100 g)	<0.0001	0.563	
$TRAP_{brew}$ (mmol Trolox/L)	CGA_{brew} (mg/L)	<0.0001	1.029	= 3.35238 + 0.00736 CGA_{brew} + 0.041228 $phenolics_{HMWF}$ adjusted R^2 = 0.9002 ANOVA p regression <0.0001
	$phenolics_{HMWF}$ (mg/100 g)	<0.0001	0.343	

[a] β coefficients allow the comparison of the relative contribution of each independent variable in the prediction of the dependent variable.

AA of the brews (Table 4). This estimation was made assuming that the total amount of melanoidins in roasted coffee remains unchanged during roasting. Although this may not be entirely true, we considered it to be a reasonable approximation based on a previous study.[19] Using this approach, more than 90% of the samples' variance could be explained by the developed models (adjusted R^2 > 0.90). For each AA assay, the percent contribution of the two evaluated components to the AA of the brews was calculated dividing the multiple regression's β coefficient of each component (Table 4) by their sum. The percent contributions of brew CGA and phenolic acids present in HMWF to the AA of the brew varied according to the assay used. Regardless of the AA assay, however, the contribution of brew CGA to the AA of the brews (53%, 64%, and 75%) was higher than that of the phenolic acids in HMWF (47%, 36%, and 25%), when FRAP, TEAC, and TRAP assays, respectively, were employed (Supporting Information data 4). Rufián-Henares and Morales[12] reported that melanoidins accounted for 26−38% of the overall AA of the coffee brew, depending on the assay applied, which is in line with our data. In addition, these authors also observed that the contribution of melanoidins to the AA of coffee brews was higher when FRAP assay was used in comparison to TEAC assay.

The contribution of high molecular weight melanoidins to TEAC values of coffee brews observed in the present study was estimated as 36%. Del Castillo et al.[8] observed that the proportion of the coffee brews' AA measured by the TEAC assay due to high molecular weight material increased with the degree of roasting, from 7% to 23% for light- to medium-roasted coffees, but no further increase was observed for the dark-roasted sample. Delgado-Andrade et al.[14] estimated that melanoidins contributed to less than 20% of the total AA of coffee brews measured by DPPH, TEAC, and FRAP assays. Sacchetti et al.[11] reported that the nonphenolic fraction (rich in melanoidins) of dark roasted coffee brews contributed with 19.3% of the AA measured by the TEAC assay. Very recently, Gniechwitz et al.[36] reported that, depending on the assay applied, the contribution of high molecular weight fractions to the total AA was ∼10% and ∼30−40%, for fractions >100 kDa and 3−10 kDa, respectively. It is worth noting that comparisons between studies should take into account differences in melanoidin isolation protocols and in approaches to calculate their contribution to AA.

The physiological relevance of melanoidins is still unknown because data on their absorption and metabolism in humans are scarce. Studies with isotope-labeled melanoidins indicated that only a small proportion of these compounds were absorbed through the intestinal wall, the vast majority being excreted in faeces.[40] Regardless of being or not bioavailable in humans, melanoidins could reach high concentrations in the gastrointestinal tract, where they may exert beneficial effects such as decrease in the absorption of cytotoxic lipid peroxidation products and protection against oxidative damages.[31] Moreover, Rufián-Henares and Morales[12] observed that low molecular weight compounds with intense AA were released from the melanoidins backbone after an enzymatic treatment, which simulated the gastrointestinal digestion, making them available for absorption. Such components could possibly be CGA and/or phenolic acids derived from CGA hydrolysis because they are incorporated into the melanoidins backbone and possess strong AA.[26] Considering that CGA are known to be bioavailable in humans[41,42] and that up to 39% of total CGA in the brew prepared with dark roasted beans is found covalently bound to the melanoidins (Figure 1), the current debate on whether the CGA incorporated into melanoidins and the AA they exert have an impact in vivo is of great relevance for the AA increase observed in human plasma after coffee intake.[43,44]

In conclusion, in the present study the hypothesis of incorporation of CGA into the melanoidins' skeleton was corroborated and this phenomenon was markedly influenced by the roasting process. The presence of DHCA in the structure of melanoidins was observed for the first time and probably originates from oxidation of previously incorporated CQA. The relative contents of individual hydroxycinnamates in the melanoidins, together with CGA contents in the whole brews, indicates that CGA are incorporated into melanoidins at the beginning of the roasting process and thereafter are continuously incorporated and degraded with a higher degradation rate. Considering the content of CGA initially present in the brews prepared from green beans, no more than 4% was lactonized and less than 1% was covalently bound to the HMWF regardless of the roasting degree. Nevertheless, in dark roasted coffee, CGA incorporation into melanoidins becomes relevant as CGA represented about 28% of total phenolic compounds present in the brews.

FRAP, TEAC, and TRAP values of whole coffee brews were correlated with each other, and the strength of these correlations reflected the specific mechanisms of action of these assays. Our results indicate that TRAP and FRAP were more sensitive assays to measure the AA of CGA and melanoidins, respectively, while TEAC was most appropriate to simultaneously and more sensitively detect the AA of both CGA and melanoidins present in coffee brews. Regardless of the AA assay employed and considering all coffee samples and roasting degrees, the contribution of CGA to the AA of the whole brews was higher than that of melanoidin-bound phenolic compounds. The latter contributed with 25%, 36%, and 47% of the AA of the brews measured by TRAP, TEAC, and FRAP, respectively.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Weight loss and colour of *C. arabica* and *C. canephora* samples roasted to different roasting degrees. Specific extinction coefficients at 405 nm (K_{mix} 405nm) and K_{mix} 280:405 nm and K_{mix} 325:405 nm ratios of the coffee brews and isolated HMWF. Percent contribution of CGA present in the coffee brews and of phenolic acids bound to the HMWF to the AA as determined by FRAP, TEAC, and TRAP assays. Antioxidant activity of whole coffee brews measured by the FRAP, TEAC, and TRAP assays. Antioxidant activity of clarified coffee brews measured by the FRAP, TEAC, and TRAP assays. Antioxidant activity of HMWF measured by the FRAP, TEAC and TRAP assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +55-21-2562-6449. Fax: +55-21-2280-8343. E-mail: afarah@iq.ufrj.br.

Funding

Consórcio Brasileiro de Pesquisa e Desenvolvimento do Café (CBP&D Café)−EMBRAPA, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (Brazil).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank José Mauro Costa Monteiro and Mônica Pinto (ABIC) for supplying the green coffee samples.

■ REFERENCES

(1) Bonita, J. S.; Mandarano, M.; Shuta, D.; Vinson, J. Coffee and cardiovascular disease: in vitro, cellular, animal and human studies. *Pharmacol. Res.* **2007**, *55*, 187−198.

(2) Lindsay, J.; Laurin, D.; Verreault, R.; Hebert, R.; Helliwell, B.; Hill, G. B.; McDowell, I. Risk factors for Alzheimer's disease: a prospective analysis from the Canadian Study of Health and Aging. *Am. J. Epidemiol.* **2002**, *156*, 445−453.

(3) Larsson, S.; Wolk, A. Coffee consumption and risk of liver cancer: a meta-analysis. *Gastroenterol.* **2007**, *132*, 1740−1745.

(4) Farah, A.; Donangelo, C. M. Phenolic compounds in coffee. *Braz. J. Plant Physiol.* **2006**, *18*, 23−36.

(5) Pellegrini, N.; Serafini, M.; Colombi, B.; del Rio, D.; Salvatore, S.; Bianchi, M.; Brighenti, F. Total Antioxidant Capacity of Plant Foods, Beverages and Oils Consumed in Italy Assessed by Three Different In Vitro Assays. *J. Nutr.* **2003**, *133*, 2812−2819.

(6) Torres, T.; Farah, A. Coffee is the most important contributor to the antioxidant capacity in Brazilian's diet. *FASEB J.* **2010**, *24*, 919.

(7) Svilaas, A.; Sakhi, A. K.; Andersen, L. F.; Svillas, T.; Ström, E. C.; Jacobs, D. R.; Ose, L.; Blomhoff, R. Intakes of antioxidants in coffee, wine, and vegetables are correlated with plasma carotenoids in humans. *J. Nutr.* **2004**, *134*, 562−567.

(8) del Castillo, M. D.; Ames, J. M.; Gordon, M. H. Effect of Roasting on the Antioxidant Activity of Coffee Brews. *J. Agric. Food Chem.* **2002**, *50*, 3698−3703.

(9) Daglia, M.; Papetti, A.; Gregotti, C.; Bertè, F.; Gazzani, G. In vitro antioxidant and ex vivo protective activities of green and roasted coffee. *J. Agric. Food Chem.* **2000**, *48*, 1449−1454.

(10) Borrelli, R. C.; Visconti, A.; Mennella, C.; Anese, M.; Fogliano, V. Chemical characterization and antioxidant properties of coffee melanoidins. *J. Agric. Food Chem.* **2002**, *50*, 6527−6533.

(11) Sacchetti, G.; Di Mattia, C.; Pittia, P.; Mastrocola, D. Effect of roasting degree, equivalent thermal effect and coffee type on the radical scavenging activity of coffee brews and their phenolic fraction. *J. Food Eng.* **2009**, *90*, 74−80.

(12) Rufián-Henares, J. A.; Morales, F. J. Effect of in Vitro Enzymatic Digestion on Antioxidant Activity of Coffee Melanoidins and Fractions. *J. Agric. Food Chem.* **2007**, *55*, 10016−10021.

(13) Gniechwitz, D.; Reichardt, N.; Ralph, J.; Blaut, M.; Steinhart, H.; Bunzel, M. Isolation and characterization of a coffee melanoidin fraction. *J. Sci. Food Agric.* **2008**, *88*, 2153−2160.

(14) Delgado-Andrade, C.; Rufián-Henares, J. A.; Morales, F. J. Assessing the Antioxidant Activity of Melanoidins from Coffee Brews by Different Antioxidant Methods. *J. Agric. Food Chem.* **2005**, *53*, 7832−7836.

(15) Fuster, M. D.; Mitchell, A. E.; Ochi, H.; Shibamoto, T. Antioxidative Activities of Heterocyclic Compounds Formed in Brewed Coffee. *J. Agric. Food Chem.* **2000**, *48*, 5600−5603.

(16) Delgado-Andrade, C.; Morales, F. J. Unraveling the Contribution of Melanoidins to the Antioxidant Activity of Coffee Brews. *J. Agric. Food Chem.* **2005**, *53*, 1403−1407.

(17) Bekedam, E. K.; Schols, H. A.; van Boekel, M. A. J. S.; Smit, G. High Molecular Weight Melanoidins from Coffee Brew. *J. Agric. Food Chem.* **2006**, *54*, 7658−7666.

(18) Nunes, F. M.; Coimbra, M. A. Melanoidins from Coffee Infusions. Fractionation, Chemical Characterization, and Effect of the Degree of Roast. *J. Agric. Food Chem.* **2007**, *55*, 3967−3977.

(19) Bekedam, E. K.; Schols, H. A.; Van Boekel, M. A. J. S.; Smit, G. Incorporation of Chlorogenic Acids in Coffee Brew Melanoidins. *J. Agric. Food Chem.* **2008**, *56*, 2055−2063.

(20) Bekedam, E. K.; Loots, M. J.; Schols, H. A.; Van Boekel, M. A. J. S.; Smit, G. Roasting Effects on Formation Mechanisms of Coffee Brew Melanoidins. *J. Agric. Food Chem.* **2008**, *56*, 7138−7145.

(21) Nunes, F. M.; Coimbra, M. A. Role of hydroxycinnamates in coffee melanoidin formation. *Phytochem. Rev.* **2010**, *9*, 171−185.

(22) AOAC. In *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990; p 757.

(23) Farah, A.; de Paulis, T.; Trugo, L. C.; Martin, P. R. Effect of roasting on the formation of chlorogenic acids lactones in coffee. *J. Agric. Food Chem.* **2005**, *53*, 1505−1513.

(24) Perrone, D.; Farah, A.; Donangelo, C. M.; de Paulis, T.; Martin, P. R. Comprehensive analysis of major and minor chlorogenic acids and lactones in economically relevant Brazilian coffee cultivars. *Food Chem.* **2008**, *106*, 859−867.

(25) Farah, A.; Monteiro, M.; Donangelo, C. M.; Lafay, S. Chlorogenic Acids from Green Coffee Extract are Highly Bioavailable in Humans. *J. Nutr.* **2008**, *138*, 2309−2315.

(26) Moreira, D. P.; Monteiro, M. C.; Ribeiro-Alves, M.; Donangelo, C. M.; Trugo, L. C. Contribution of chlorogenic acids to the iron-reducing activity of coffee beverages. *J. Agric. Food Chem.* **2005**, *53*, 1399−1402.

(27) Bartosz, G.; Janaszewska, A.; Ertel, D.; Bartosz, M. Simple determination of peroxyl radical-trapping capacity. *Biochem. Mol. Biol. Int.* **1998**, *46*, 519−528.
(28) Clifford, M. N. Chlorogenic acids and other cinnamates—nature, occurrence, dietary burden, absorption and metabolism. *J. Sci. Food Agric.* **2000**, *80*, 1033−1043.
(29) Perrone, D.; Donangelo, R.; Donangelo, C. M.; Farah, A. Modeling Weight Loss and Chlorogenic Acids Content in Coffee during Roasting. *J. Agric. Food Chem.* **2010**, *58*, 12238−12243.
(30) Tfouni, S. A. V.; Serrate, C. S.; Carreiro, L. B.; Camargo, M. C. R.; Teles, C. R. A.; Cipolli, K. M. V. A. B.; Furlani, R. P. Z. Effect of roasting on chlorogenic acids, caffeine and polycyclic aromatic hydrocarbons levels in two *Coffea* cultivars: *Coffea Arabica* cv. Catuaí Amarelo IAC-62 and *Coffea canephora* cv. Apoatã IAC-2258. *Int. J. Food Sci. Technol.* **2012**, *47*, 406−415.
(31) Tagliazucchi, D.; Verzelloni, E.; Conte, A. Effect of Dietary Melanoidins on Lipid Peroxidation during Simulated Gastric Digestion: Their Possible Role in the Prevention of Oxidative Damage. *J. Agric. Food Chem.* **2010**, *58*, 2513−2519.
(32) Vignoli, J. A.; Bassoli, D. G.; Benassi, M. T. Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: The influence of processing conditions and raw material. *Food Chem.* **2011**, *124*, 863−868.
(33) Moon, J.-K.; Shibamoto, T. Formation of Volatile Chemicals from Thermal Degradation of Less Volatile Coffee Components: Quinic Acid, Caffeic Acid, and Chlorogenic Acid. *J. Agric. Food Chem.* **2010**, *58*, 5465−5470.
(34) Scholz-Böttcher, B. M.; Ernst, L.; Maier, H. G. New stereoisomers of quinic acid and their lactones. *Liebig's Ann. Chem.* **1991**, *10*, 1029−1036.
(35) Müller, C.; Lang, R.; Hofmann, T. Quantitative Precursor Studies on Di- and Trihydroxybenzene Formation during Coffee Roasting Using "In Bean" Model Experiments and Stable Isotope Dilution Analysis. *J. Agric. Food Chem.* **2006**, *54*, 10086−10091.
(36) Gniechwitz, D.; Reichardt, N.; Ralph, J.; Blaut, M.; Steinhart, H.; Bunzel, M. Isolation and characterization of a coffee melanoidin fraction. *J. Sci. Food Agric.* **2008**, *88*, 2153−2160.
(37) Frank, O.; Blumberg, S.; Kunert, C.; Zehentbauer, G; Hofmann, T. Structure Determination and Sensory Analysis of Bitter-Tasting 4-Vinylcatechol Oligomers and Their Identification in Roasted Coffee by Means of LC-MS/MS. *J. Agric. Food Chem.* **2007**, *55*, 1945−1954.
(38) Müller, C.; Lang, R; Hofmann, T. Quantitative Precursor Studies on Di- and Trihydroxybenzene Formation during Coffee Roasting Using "In Bean" Model Experiments and Stable Isotope Dilution Analysis. *J. Agric. Food Chem.* **2006**, *54*, 10086−10091.
(39) Prior, R. L.; Wu, X.; Schaich, K. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290−4302.
(40) Finot, P. A.; Magnenat, E. Metabolic transit of early and advanced Maillard products. *Progr. Food Nutr. Sci.* **1981**, *5*, 193−207.
(41) Monteiro, M.; Farah, A.; Perrone, D.; Trugo, L. C.; Donangelo, C. Chlorogenic acid compounds from coffee are differentially absorbed and metabolized in humans. *J. Nutr.* **2007**, *137*, 2196−2201.
(42) Renouf, M.; Guy, P. A.; marmet, C.; Fraering, A.; Longet, K.; Moulin, J.; Enslen, M.; Barron, D.; Dionisi, F.; Cavin, C.; Williamson, G.; Steiling, H. Measurement of caffeic and ferulic acid equivalents in plasma after coffee consumption: Small intestine and colon are key sites for coffee metabolism. *Mol. Nutr. Food Res.* **2010**, *54*, 760−766.
(43) Natella, F.; Nardini, M.; Gianetti, I.; Dattilo, C.; Scaccini, C. Coffee drinking influences plasma antioxidant capacity in humans. *J. Agric. Food Chem.* **2002**, *50*, 6211−6216.
(44) Moura-Nunes, N.; Perrone, D.; Farah, A.; Donangelo, C. M. The increase in human plasma antioxidant capacity after acute coffee intake is not associated with endogenous non-enzymatic antioxidant components. *Int. J. Food Sci. Nutr.* **2009**, *11*, 1−9.