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Contribution of Chlorogenic Acids to the Iron-Reducing Activity of Coffee Beverages

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The iron-reducing activity of coffee beverages was determined by the ferric reducing antioxidant power (FRAP) assay. The influence on FRAP due to the degree of roasting (light, medium, and dark), species (*Coffea arabica* and *Coffea robusta*), and caffeine content (regular and decaffeinated) was investigated using ground and soluble coffee samples. The concentration of specific chlorogenic acids and caffeine in the beverages was determined by high-performance liquid chromatography and related to FRAP using Pearson correlation coefficients. All measurements were expressed per unit of soluble solids. Beverages prepared with ground coffee had, on average, 27% higher FRAP values than those prepared with soluble coffee ($p < 0.05$). In the former beverages, FRAP of *C. robusta* samples was significantly higher (on average, 50.3%) when compared to that of *C. arabica* samples, and FRAP values decreased with increasing degree of roasting ($p < 0.05$). A strong correlation ($r > 0.91$) was found between FRAP and the total content of chlorogenic acids, particularly that of the caffeoylquinic acid isomers. The iron-reducing activity of coffee beverages was not influenced by caffeine.

KEYWORDS: Coffee; iron-reducing activity; FRAP; chlorogenic acids; caffeine

INTRODUCTION

Several studies showed that chronic diseases such as cancer, cardiovascular, inflammatory, and neurodegenerative pathologies, and aging are associated with oxidative stress, a metabolic condition that causes cell degeneration (*1*, *2*). Antioxidant compounds present in fruits and vegetables appear to play a major role in the protection against oxidative stress (*3*). Besides fruits and vegetables, plant beverages such as coffee contribute to the dietary intake of antioxidants (*3*−*10*).

The antioxidant capacity of coffee beverages has been assessed by several studies, using different methods (*3*, *5*, *6*, *8*, *11*−*13*). It was shown that coffee has a high antioxidant activity compared to other beverages, 3 times higher than that of red wine and 5 times higher than that of green tea (*3*). The antioxidant properties of coffee in relation to species was investigated, and it was verified that *Coffea robusta* possesses significantly higher antioxidant capacity than *Coffea arabica* (*13*). A progressive decrease in antioxidant activity of coffee was observed with increasing degree of roasting associated mainly with the high content of chlorogenic acids (CGA) in the raw beans, partially degraded with heating (*11*). Coffee model systems prepared from combinations of chlorogenic acids, arginine, sucrose, and cellulose were also used to study the effect of roasting on the antioxidant activity (*14*). The antioxidant activity was positively and nonlinearly related to the amount of CGA remaining after roasting.

Several different methodologies have been applied to the measurement of the antioxidant capacity in foods. The ferric reducing antioxidant power (FRAP) assay, which measures iron-reducing activity (IRA), is a very simple and straightforward method, and it has been widely used for the screening of food samples (*4*, *15*), including coffee (*3*). However, the contribution of CGA and other compounds present in coffee such as caffeine to its antioxidant activity measured by the FRAP assay has not been yet identified.

The aim of this study was to elucidate the role of chlorogenic acids and caffeine to the IRA of coffee beverages measured by the FRAP assay. Because coffee composition is highly influenced by several factors such as species, degree of roasting, and industrial processing, these factors were taken into account for the proper identification of active compounds.

MATERIALS AND METHODS

Chemicals. 2,4,6-Tripyridyl-*S*-triazine (TPTZ) (Sigma-Aldrich), 5-caffeoylquinic acid (5-CQA) (Sigma-Aldrich), 3- and 4-CQA, prepared from isomerization of 5-CQA (*16*), a mixture of dicaffeoylquinic acids (3,4-, 3,5-, and 4,5-diCQA) (Roth), feruloylquinic acids (4- and 5-FQA) (*16*), and caffeine (Sigma-Aldrich) were obtained from the suppliers indicated.

Coffee Samples. The samples for preparation of coffee beverages were selected to represent the different products available in the Brazilian market (**Table 1**). Samples were divided into two major

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Table 1. Sample Characteristics and Ferric Reducing Antioxidant Power (FRAP) of the Coffee Beverages

sample	sample characteristics				brand	FRAP (mmol of Fe^{2+}/L of beverage)	FRAP (mmol of Fe^{2+}/g of SS[a])
1	ground	*C. arabica*	regular	light roasted	A	76.0	3.1
2	ground	*C. arabica*	regular	light roasted	B	94.8	4.4
3	ground	*C. arabica*	regular	medium roasted	A	60.3	2.6
4	ground	*C. arabica*	regular	dark roasted	A	39.6	1.8
5	ground	*C. arabica*	regular	dark roasted	B	51.2	2.2
6	ground	*C. arabica*	decaffeinated	light roasted	B	83.6	4.7
7	ground	*C. arabica*	decaffeinated	dark roasted	B	44.8	2.4
8	ground	*C. robusta*	regular	light roasted	A	120.3	5.2
9	ground	*C. robusta*	regular	medium roasted	A	75.1	3.4
10	ground	*C. robusta*	regular	dark roasted	A	62.5	2.9
11	soluble	blend[b]	regular	undetermined[c]	C	133.5	2.8
12	soluble	blend	regular	undetermined	D	114.2	2.4
13	soluble	blend	regular	undetermined	E	162.2	3.3
14	soluble	blend	regular	undetermined	F	122.4	2.6
15	soluble	blend	decaffeinated	undetermined	C	114.0	2.4
16	soluble	blend	decaffeinated	undetermined	D	105.8	2.2

[a] Soluble solids. [b] Mixture of unknown proportions of *C. arabica* and *C. robusta*. [c] Degree of roasting was not provided by the manufacturer.

groups: ground coffee ($n = 10$) and soluble coffee ($n = 6$). The ground coffee samples were divided into two subgroups according to species: *C. arabica* ($n = 7$) and *C. robusta* ($n = 3$). The *C. arabica* samples were either regular ($n = 5$) or decaffeinated ($n = 2$). The regular samples included light ($n = 2$), medium ($n = 1$), and dark ($n = 2$) roasted coffee, whereas decaffeinated samples included light ($n = 1$) and dark ($n = 1$) roasted coffee. The *C. robusta* samples included regular light ($n = 1$), medium ($n = 1$), and dark ($n = 1$) roasted coffee. The soluble coffee samples were divided into regular ($n = 4$) and decaffeinated ($n = 2$) coffee. These samples were blends of unknown proportions of *C. robusta* and *C. arabica* and of undetermined degree of roasting.

Preparation of Coffee Beverages. Beverages from ground coffee were prepared by stirring 2 g of sample in 20 mL of boiling distilled water followed by paper filtration. Beverages from soluble coffee were prepared by simply mixing 1 g of sample with 20 mL of boiling distilled water. All beverages were centrifuged at 1000*g* for 5 min (5 °C). The content of soluble solids present in the supernatant of the beverages was determined gravimetrically by oven-drying at 105 °C until constant weight.

FRAP Assay. The FRAP values were determined according to published procedures (*3*, *17*). 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 diluted beverage (1:320 in high-purity water); 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+} equivalents per liter of beverage and per gram of soluble solids in the sample. The final FRAP value of each sample was the mean value of five replications.

High-Performance Liquid Chromatography (HPLC). The concentration of chlorogenic acids in the coffee beverages was measured by HPLC according to a published procedure (*16*, *18*). In a final volume of 50 mL, 2.0 mL of coffee beverage was mixed with 1 mL of Carrez I solution, 1 mL of Carrez II solution, and 3 mL of glacial acetic acid and paper filtered after 5 min. The HPLC apparatus was a Shimadzu LC-10AD liquid chromatograph with a variable-wavelength UV detector. The stationary phase was Rexchrom ODS S5100 (Regis Technologies). For caffeine analysis, the mobile phase was water/methanol (60:40) at a flow rate of 1.0 mL/min, and the detection wavelength was 272 nm. For the CGA analysis, the mobile phase was a gradient of citric acid solution (10.0 mM) and methanol at a flow rate of 1.0 mL/min, and the detection wavelength was 325 nm. The content of each CGA was determined using the area of the 5-CQA standard combined with the respective molar extinction coefficient (*16*). The concentration of all components was expressed in milligrams per gram of soluble solids in the sample beverage.

Statistical Analysis. Data were analyzed with the statistical software Statistica (version 6.0, StatSoft Inc.). Student's *t* test assuming equal variance at a confidence level of 95% was used to test for significant difference between two groups of means. The Pearson correlation coefficient was calculated at a confidence level of 95%.

RESULTS AND DISCUSSION

All coffee beverages showed remarkable IRA using the FRAP assay, both when expressed per liter of beverage and when expressed per gram of soluble solids (**Table 1**). Beverages prepared from ground and soluble coffee had significantly different soluble solids, on average, 22.1 and 47.5 g/L, respectively. Therefore, to properly compare different groups of samples, FRAP values expressed per gram of soluble solids were used.

Beverages prepared with ground coffee generally had higher FRAP values than those prepared with soluble coffee, on average, 27% ($p < 0.05$). This may be due to the difference in the average degree of roasting between the two groups of coffee samples. Soluble coffee is usually prepared from beans with a high degree of roasting, whereas ground coffee may be prepared from light, medium, or dark roasted beans, as observed in our group of samples (**Table 1**). A slightly higher FRAP value of soluble coffee in comparison with ground coffee was found in a previous study (*3*), but in that study FRAP was expressed per liter of beverage. When the higher soluble solids content in soluble coffee in comparison with ground coffee is taken into account, the FRAP results of this and the previous study (*3*) are in agreement.

Beverages prepared with regular and decaffeinated coffee had, on average, similar FRAP values, 3.0 and 2.9 mmol of Fe^{2+} per gram of soluble solids, respectively (**Table 1**). In the soluble coffee samples, regular coffee had higher FRAP values than decaffeinated coffee, the decrease ranging from 5.9 to 19.3% according to the brand analyzed. In contrast, among ground samples, decaffeination led to a small increase in FRAP, ranging from 4.9 to 5.8% depending on the degree of roasting and sample brand. The opposite change in FRAP with decaffeination of soluble and ground samples suggests that caffeine itself is not related to the IRA of coffee beverages.

We found that the FRAP values of *C. robusta* samples were significantly higher than those of *C. arabica* samples, the difference ranging from 30.4 to 70.2% depending on the degree of roasting (**Table 1**). This result is in agreement with previous

Table 2. Chlorogenic Acids and Caffeine Composition of the Coffee Beverages[a,b]

sample[c]	caffeine	3-CQA	4-CQA	5-CQA	3,4-diCQA	3,5-diCQA	4,5-diCQA	4-FQA	5-FQA	total CGA
1	52.1	24.8	27.9	52.7	5.3	3.5	3.9	8.4	4.5	130.9
2	65.7	44.7	59.1	101.4	9.0	8.4	10.3	11.9	5.1	249.9
3	49.3	9.8	8.6	19.5	1.6	1.2	0.7	6.0	2.1	49.6
4	52.5	4.6	4.7	11.4	1.4	1.2	ND[d]	4.1	1.5	28.7
5	65.7	4.2	5.1	8.6	0.4	0.3	0.4	ND	ND	18.9
6	1.6	48.0	47.4	106.6	8.4	7.5	9.6	7.7	3.4	238.5
7	0.9	3.2	4.5	5.3	ND	0.2	0.4	ND	ND	13.6
8	85.9	49.8	77.9	83.7	6.7	5.5	5.1	13.1	11.0	252.9
9	98.3	14.6	16.5	34.3	2.7	2.0	1.4	13.6	7.5	92.7
10	101.8	3.2	2.9	5.8	1.7	ND	ND	4.4	1.9	19.9
11	29.1	13.6	13.6	15.8	1.8	1.7	1.6	5.6	2.3	56.1
12	28.2	6.5	5.7	6.9	0.9	1.0	0.9	3.8	1.5	27.1
13	28.8	9.4	8.9	10.0	1.2	1.0	0.8	6.2	2.5	40.1
14	32.9	4.1	3.5	4.0	0.6	0.3	ND	2.4	1.0	15.8
15	1.5	15.7	15.6	19.1	2.3	1.6	2.3	6.1	2.5	65.2
16	1.5	9.6	9.2	11.0	1.1	1.1	1.1	4.5	1.8	39.5

[a] See text for abbreviations. [b] All concentrations are given in milligrams per gram of soluble solids. [c] See **Table 1** for description. [d] Not detectable.

studies (*5*, *13*) and is probably due to the different CGA contents of the two coffee species. Moreover, for each species, a different percentage decrease in the CGA content after roasting has been observed (*18*, *19*).

The medium and dark roasted *C. arabica* samples had on average lower FRAP, respectively, 14.8 and 40.3%, when compared with the corresponding light roasted sample (**Table 1**). Similarly, samples of *C. robusta* had 34.7 and 44.0% lower FRAP values, for medium and dark degrees of roasting, respectively, compared to light roasting. The decrease in IRA with the degree of coffee roasting is probably due to the thermal degradation of CGA during this process (*5*, *11*, *13*, *14*) such as observed in our study (**Table 2**). Differently from our study, an increase in the antioxidant capacity during the early phases of coffee roasting has been reported (*11*), attributed to the formation of melanoidins and other Maillard reaction products. However, a decrease in the antioxidant activity was reported during the advance phases of coffee roasting (*11*, *20*). It should be noted that these studies used methods to assess antioxidant activity with different specificities and sensitivities for potentially active compounds in coffee beverages. An active antioxidant in an in vitro test may be inactive in an ex vivo test and vice versa. For instance, CGA were found to be very active in a chemical test based on coupled oxidation of β-carotene and linoleic acid and far less active in a biological lipid peroxidation assay in which compounds produced during roasting were found to be highly protective (*13*). Therefore, on the basis of our results it appears that compounds produced during early roasting, such as melanoidins and Maillard products, do not have iron-reducing activity and thus do not contribute to FRAP in coffee beverages.

To investigate which components present in the coffee beverages had influence in the IRA measured by the FRAP assay, we calculated the Pearson correlation coefficients between FRAP and the concentration of each of the different components in the beverages (**Table 3**). We also calculated the Pearson coefficient using the concentration of groups of compounds (CGA, CQA, FQA, and di-CQA). FRAP was significantly correlated with the concentration of all different specific and grouped CGA determined in our study, but not with caffeine. A strong correlation ($r > 0.91$) was found between FRAP and the total content of CGA, as well as between FRAP and total CQA. Significant correlations with FRAP were also found for total di-CQA and total FQA ($r > 0.78$). Among specific components, 3-CQA, 4-CQA, and 5-CQA were those that showed the highest correlations with FRAP ($r > 0.88$).

Table 3. Correlation between FRAP and Specific Components in the Coffee Beverages[a–c]

variable related to FRAP	Pearson correlation coefficient
caffeine	0.280
3-CQA	0.912
4-CQA	0.911
5-CQA	0.881
total CQA	0.912
3,4-diCQA	0.861
3,5-diCQA	0.845
4,5-diCQA	0.801
total diCQA	0.841
4-FQA	0.758
5-FQA	0.782
total FQA	0.784
total CGA	0.914

[a] Values expressed per gram of soluble solids. [b] See text for abbreviations. [c] Correlation coefficients are all significant at $p < 0.05$ except for caffeine.

The antioxidant activity of caffeine is still controversial. Caffeine has been reported to scavenge hydroxyl radicals in an electron spin resonance study (*21*). In addition, caffeine inhibited lipid peroxidation of rat liver microsomes when present at millimolar concentrations (*9*). In contrast, caffeine did not show antioxidant activity under physiological conditions (*22*) and using a radical trapping assay (*8*). Our study suggests that caffeine does not influence the IRA of coffee beverages, probably because there are no iron-reducing chemical groups in its molecular structure.

In a study with tea (*4*), a strong correlation was found between FRAP and the total phenolic content of the beverage. However, because the phenolic compounds were evaluated as a whole group in that study, it was not possible to determine the contribution of individual components to the IRA of tea measured by FRAP. In our study, we were able to identify that all CQA isomers are strong contributors to the IRA of coffee beverages measured by FRAP. Although 5-CQA was suggested to be the most active antiradical component present in coffee (*5*), it was not clearly distinguished from other CGA isomers that might have also contributed to the antiradical activity in that study as seen in our study.

In conclusion, we were able to identify the contribution of CGA to the iron-reducing activity of coffee beverages measured by FRAP. Further research is needed to determine the contribu-

tion of specific components of coffee to its antioxidant capacity measured by different in vitro, ex vivo, and in vivo methods.

ABBREVIATIONS USED

IRA, iron-reducing activity; FRAP, ferric reducing antioxidant power; CGA, chlorogenic acids; TPTZ, 2,4,6-tripyridyl-*S*-triazine; HPLC, high-performance liquid chromatography; CQA, caffeoylquinic acids; di-CQA, dicaffeoylquinic acids; FQA, feruloylquinic acids.

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LITERATURE CITED

(1) Beal, M. F. Aging, energy and oxidative stress in neurodegenerative diseases. *Ann. Neurol.* **1995**, *38*, 357−366.

(2) Mattson, M. P. Emerging neuroprotective strategies for Alzheimer's disease: dietary restriction, telomerase activation, and stem cell therapy. *Exp. Gerontol.* **2000**, *35*, 489−502.

(3) 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.

(4) Benzie, I. F. F.; Szeto, Y. T. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *J. Agric. Food Chem.* **1999**, *47*, 633−636.

(5) Daglia, M.; Racchi, M.; Papetti, A.; Lanni, C.; Govoni, S.; Gazzani, G. In vitro and ex vivo antihydroxyl radical activity of green and roasted coffee. *J. Agric. Food Chem.* **2004**, *52*, 1700−1704.

(6) Richelle, M.; Tavazzi, I.; Offord, E. Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. *J. Agric. Food Chem.* **2001**, *49*, 3438−3442.

(7) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.* **1996**, *44*, 3426−3431.

(8) Natella, F.; Nardini, M.; Giannetti, I.; Dattilo, C.; Scaccini, C. Coffee drinking influences plasma antioxidant capacity in humans. *J. Agric. Food Chem.* **2002**, *50*, 6211−6216.

(9) Devasagayam, T. P. A.; Kamat, J. P.; Mohan, H.; Kesavan, P. C. Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species. *Biochim. Biophys. Acta* **1996**, *1282*, 63−70.

(10) McAnlis, G. T.; McEneny, J.; Pearce, J.; Young, I. S. Black tea consumption does not protect low-density lipoprotein from oxidative modification. *Eur. J. Clin. Nutr.* **1998**, *52*, 202−206.

(11) 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.

(12) Anese, M.; Nicoli, M. C. Antioxidant properties of ready-to-drink coffee brews. *J. Agric Food Chem.* **2003**, *51*, 942−946.

(13) 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*, 1449−1545.

(14) Charurin, P.; Ames, J. M.; del Castillo, M. D. Antioxidant activity of coffee model systems. *J. Agric. Food Chem.* **2002**, *50*, 3751−3756.

(15) Halvorsen, B. L.; Holte, K.; Myhrstad, M. C. W.; Barikmo, I.; Hvattum, E.; Remberg, S. F.; Wold, A.; Haffner, K.; Baugerød, H.; Andersen, L. F.; Moskaug, J. Ø.; Jacobs, D. R., Jr.; Blomhoff, R. A systematic screening of total antioxidants in dietary plants. *J. Nutr.* **2002**, *132*, 461−471.

(16) Trugo, L. C.; Macrae, R. Chlorogenic acid composition of instant coffees. *Analyst* **1984**, *109*, 263−266.

(17) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.

(18) Trugo, L. C.; Macrae, R. Study of the effect of roasting on the chlorogenic acid composition of coffee using HPLC. *Food Chem.* **1984**, *15*, 219−227.

(19) Daglia, M.; Cuzzoni, M. T.; Dacarro, C. Antibacterial activity of coffee: relationship between biological activity and chemical markers. *J. Agric. Food Chem.* **1994**, *42*, 2273−2277.

(20) Nicoli, M. C.; Anese, M.; Manzocco, L.; Lerici, C. R. Antioxidant properties of coffee brews in relation to roasting degree. *Lebensm. Wiss. Technol.* **1997**, *30*, 292−297.

(21) Shi, X.; Dalal, N. S.; Jain, A. C. Antioxidant behaviour of caffeine: efficient scavenging of hydroxyl radicals. *Food Chem. Toxicol.* **1991**, *29*, 1−6.

(22) Lee, C. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. *Clin. Chim. Acta* **2000**, *295*, 141−154.

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