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Chlorogenic acid and caffeine contents in various commercial brewed coffees

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Abstract

Twelve commercial brewed coffees (seven regular and five decaffeinated) were analyzed for chlorogenic acids (CGA) and caffeine by HPLC. Their pH and UV–Vis absorbances were also measured. The CGAs identified were three caffeolylquinic acids (3-CQA, 4-CQA, and 5-CQA), three feruloylquinic acids (3-FQA, 4-FQA, and 5-FQA), and three dicaffeoylquinic acids (3,4-diCQA, 3,5-diCQA, and 4,5-diCQA). The total CGAs ranged from 5.26 mg/g to 17.1 mg/g in regular coffees and from 2.10 mg/g to 16.1 mg/g in decaffeinated coffees. Among CGA, 5-CQA was present at the highest level, ranging from 2.13 mg/g to 7.06 mg/g coffee, and comprising 36–42% and 37–39% of the total CGA in the regular and decaffeinated coffees, respectively. CGA isomer contents were, in decreasing order, 5-CQA > 4-CQA > 3-CQA > 5-FQA > 4-FQA > 3-FQA > 3,4-diCQA > 4,5-diCQA, 3,5-diCQA. The caffeine content in regular and decaffeinated coffees ranged from 10.9 mg/g to 16.5 mg/g and from 0.34 mg/g to 0.47 mg/g, respectively. The pH of regular and decaffeinated coffees ranged from 4.95 to 5.99 and from 5.14 to 5.80, respectively. The relationship between the pH and the UV–Vis absorbance at 325 nm was moderately correlated ($R^2 = 0.7829$, $p < 0.001$, $n = 12$).

Keywords: Acid contents; Brewed coffee; Caffeine; Chlorogenic acids

1. Introduction

Coffee is one of the world's most popular beverages. It is also the most important traded commodity in the world after oil (Production & Consumption, 2006). World coffee production grew by over 100% from 1950 to 1990 and is projected to grow by 0.5–1.9% by 2010. Global output is expected to reach 7.0 million tons by 2010. World consumption of coffee is projected to increase by 0.4% annually from 6.7 million tons in 1998–2000 to 6.9 million tons in 2010 (Coffee, 2006).

There have been numerous reports on diseases associated with coffee consumption (Sandler, 1983; Schilter, Cavin, Tritscher, & Constable, 2001). Coffee drinking, however, does not always have exclusively non-beneficial results. One recent review article stated that epidemiological and experimental studies have shown positive effects of regular coffee drinking on various aspects of health, such as psychoactive responses (alertness, mood change), neurological conditions (infant hyperactivity, Parkinson's disease), metabolic disorders (diabetes, gallstones), and gonad and liver function (Dorea & da Costa, 2005).

The majority of consumers' concerns about coffee drinking are, however, the acid reflux symptoms caused by coffee's acidic components, such as chlorogenic acids (CGA), and doctors tend to recommend patients with acid reflux to limit their coffee intake. CGAs are well known secondary metabolites in green coffee beans and are known to contribute to coffee's bitterness (Campa, Doulbeau, Dussert, Hamon, & Noirot, 2005). There have been many reports on the presence of CGA in green coffee beans (Clifford, 1979; Van der Stegen & Van Duijn, 1980). For example, the content of CGA in various green coffee beans (21

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species) from Cameroon and Congo ranged from 0.8% to 11.9% on a dry matter basis (Campa et al., 2005). The CGA content in brewed coffee may be influenced by the kind of coffee beans used because Arabica beans contain less CGA than Robusta beans (Ky et al., 2001). Most commercial brands of coffee are, however, made up of both Arabica and Robusta beans. The roasting method might also play an important role in the CGA content of the final coffee product. For example, the light medium roasting condition was found to result in the highest amount of transformation from CGA to the corresponding lactones, suggesting that this process reduced the amount of CGA in coffee (Farah, de Paulis, Trugo, & Martin, 2005). The amounts of seven CGAs in green coffees were significantly reduced by the degradation occurring during the roasting process (Trugo & Macrae, 1984). The preparation processes, including roasting, may, thus, play an important role in the CGA content of the final product. In the present study, therefore, CGA levels in various commercial coffees were investigated.

2. Materials and methods

2.1. Coffee samples and chemicals

Various brands of commercial ground-roasted coffees were bought from a local market. Caffeine was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). Chlorogenic acids (CGA) were bought from Cayman Chemical Co. (Ann Arbor, MI) or a gift from TAKATA Koryo Co., Ltd. (Osaka, Japan). HPLC grade methanol and water were purchased from Fisher Co. (Pittsburgh, PA). All other chemicals and solvents were bought from reliable commercial sources.

Stock solutions of caffeine (20 mM) and CGA (20 mM) were prepared in methanol for preparation of standard solutions and spike analysis.

2.2. Preparation of brewed coffee samples

Ground-roasted coffee (12.5 g) was brewed with 450 ml of deionized water using a Mr. Coffee NCX-20 model coffee maker (Sunbeam Product, Inc., Boca Raton, FL) equipped with a paper filter. The brewed coffee was immediately cooled to room temperature in an ice bath, after which the samples were stored at 5 °C until required for pH determination and analysis for caffeine and CGA.

2.3. pH and color measurement

The pH of each brewed coffee sample was measured with a Corning pH meter 430 (Corning, NY). The brewed coffee samples (100 μl) were diluted 10-fold and 100-fold with purified water and the absorbances of the resulting solutions were measured at $\lambda = 276$, 325, and 420 nm with a Hewlett Packard 8452A Diode Array Spectrophotometer running UV–Visible Chemstation software (Agilent Technologies, 1995–2000). Water was used as a blank.

2.4. Analysis of caffeine and CGA in brewed coffee samples

Brewed coffee samples were treated with Carrez reagents I and II to eliminate polymeric components according to a previously reported method (Ito et al., 1983;Rincon, Martinez, & Delgado, 2003). Each brewed coffee sample (3 ml), along with 0.1 ml each of Carrez reagents I and II, and 0.8 ml of methanol, was vortex-mixed in a centrifuge tube and allowed to stand for 10 min. The precipitate was separated by centrifuging at 5000 rpm for 10 min. The solution was then decanted and filtered with a Acrodisc Syringe Filter with 0.2 μm HT Tuffryn membrane (Pall Corporation, Ann Arbor, MI).

Quantitative analyses of caffeine and CGA were performed using an Agilent 1100 model HPLC system equipped with a Zorbax Eclipse XDB C-18 5μ column (150 mm × 4.6 mm i.d.) and a multiple wavelength detector. Mobile phase A was 10 mM citric acid and mobile phase B was methanol. The gradient mode was initially set at A/B ratio of 85/15 from 0 to 5 min, then linearly increased to 60/40 at 40 to 85 min. The flow rate was 1.0 ml/min. The detector was set at 325 nm for CGA and at 276 nm for caffeine; injection volume was 5 μl.

Concentrations of caffeine, CGA, caffeic acid and ferulic acid were calculated using the regression equation of their

Table 1
pH and UV absorbance of brewed coffees

Brand	pH[a]		UV Absorbance at $\lambda =$ [a]					
			276 nm		325 nm		420 nm	
	Regular	Decaffeinated	Regular	Decaffeinated	Regular	Decaffeinated	Regular	Decaffeinated
A	5.99 ± 0.02	5.80 ± 0.01	0.496 ± 0.001	0.298 ± 0.001	0.306 ± 0.001	0.220 ± 0.001	0.273 ± 0.001	0.283 ± 0.004
B	5.22 ± 0.02	5.66 ± 0.01	0.720 ± 0.016	0.406 ± 0.003	0.513 ± 0.011	0.428 ± 0.002	0.397 ± 0.001	0.363 ± 0.003
C	5.26 ± 0.02	5.14 ± 0.01	0.882 ± 0.001	0.634 ± 0.001	0.712 ± 0.001	0.686 ± 0.001	0.458 ± 0.001	0.482 ± 0.005
D	5.17 ± 0.00	5.22 ± 0.01	0.738 ± 0.002	0.559 ± 0.001	0.577 ± 0.001	0.620 ± 0.001	0.384 ± 0.001	0.396 ± 0.003
E	5.12 ± 0.00	5.22 ± 0.01	0.872 ± 0.002	0.553 ± 0.001	0.774 ± 0.001	0.632 ± 0.001	0.370 ± 0.001	0.365 ± 0.001
F	4.95 ± 0.00	–	0.710 ± 0.003	–	0.621 ± 0.002	–	0.369 ± 0.001	–
G	5.21 ± 0.01	–	0.669 ± 0.001	–	0.575 ± 0.001	–	0.334 ± 0.001	–

–: Commercial samples were not available.
[a] Values are means ± SD ($n = 3$).

concentration and peak area. The limit of quantification was calculated as 10 times the standard deviation of the lowest concentration of standard solution (50 μM). The recovery efficient was determined with a coffee sample spiked with 250 μM each of caffeine, CGA, caffeic acid and ferulic acid. Measurements were done in triplicate.

Identification of caffeine and CGA in brewed coffee was confirmed by a Hewlett Packard 1100 liquid chromatograph interfaced to an Applied Biosystems API 2000 MS/MS *via* an atmospheric pressure chemical ionization (APCI) source operating in the positive ion mode at 475 °C.

Table 2
Caffeine concentrations in coffees

Brand	Concentration (μg/g ground coffee)	
	Regular	Decaffeinated
A	11.5 ± 0.22	0.40 ± 0.00
B	13.4 ± 0.19	0.39 ± 0.01
C	15.1 ± 0.12	0.34 ± 0.00
D	13.4 ± 0.97	0.47 ± 0.01
E	16.5 ± 0.24	0.43 ± 0.00
F	10.9 ± 0.37	–
G	11.3 ± 0.68	–

–: Commercial samples were not available.

3. Results and discussion

3.1. pH and color measurement of brewed coffees

The pH and UV–Vis absorbancies of various brewed coffees are shown in Table 1. The pH of the brewed coffees ranged from 4.95 (Coffee F) to 5.99 (Coffee A) for regular coffee and from 5.14 (Coffee C) to 5.80 (Coffee A) for decaffeinated coffee. Coffee A showed the highest pH and the least absorbance at the three different wavelengths. The difference in the pH values between lowest (4.95) and highest (5.80) was 0.85, which reflects a 7.09-fold difference in H^+ concentration. The relationship between the pH and the UV–Vis absorbance at 325 nm was moderately correlated ($R^2 = 0.7829$, $p < 0.001$, $n = 12$). The H^+ concentration in brewed coffee samples appears to be related to the presence of chlorogenic acids because their maximum UV absorbance is around 320–330 nm. The 420 nm wavelength has been widely used for assessing the color intensity of browning reaction mixtures as it relates to the palatability of heat-processed foods and beverages (Ajandouz & Puigserver, 1999). There is no significant difference in absorbance

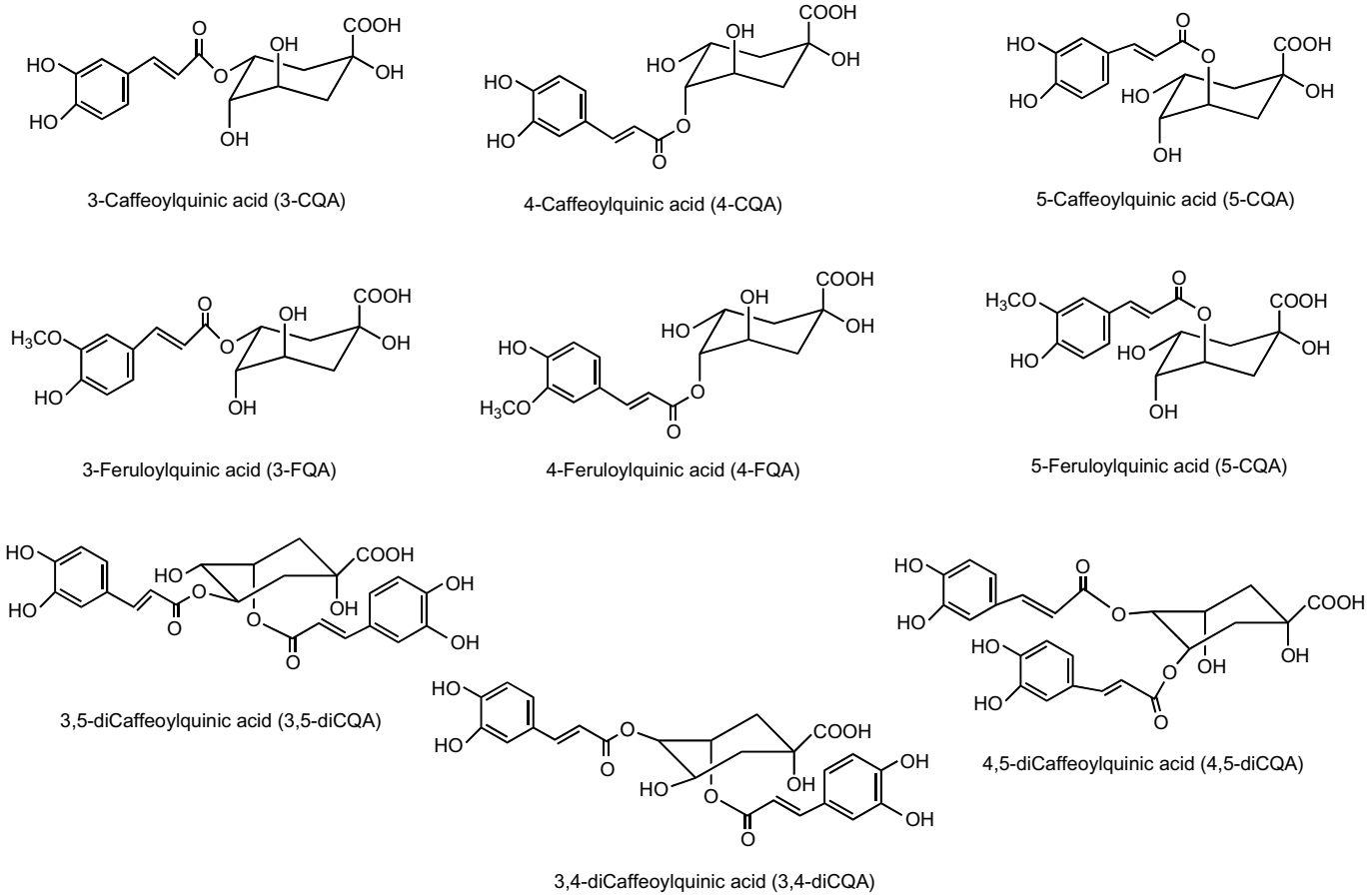

Fig. 1. Structures of chlorogenic acids (CGA) identified in the present study.

Table 3
Acid concentrations in different coffees

Brand	Concentration (mg/g of ground coffee)[a]									Total
	3-CQA	4-CQA	5-CQA	3-FQA	4-FQA	5-FQA	3,4-DCQA	3,5-DCQA	4,5-DCQA	
Regular										
A	1.32 ± 0.02	1.44 ± 0.02	2.13 ± 0.04	0.82 ± 0.00	0.84 ± 0.01	0.89 ± 0.01	0.47 ± 0.00	0.40 ± 0.00	0.28 ± 0.00	5.26 ± 0.09
B	2.01 ± 0.03	2.28 ± 0.03	3.23 ± 0.05	1.29 ± 0.00	0.21 ± 0.00	0.27 ± 0.00	0.11 ± 0.00	0.67 ± 0.00	0.73 ± 0.00	8.39 ± 0.12
C	2.93 ± 0.01	3.34 ± 0.01	4.62 ± 0.01	0.98 ± 0.00	0.53 ± 0.02	0.75 ± 0.03	0.22 ± 0.00	0.12 ± 0.00	0.16 ± 0.00	12.8 ± 0.08
D	2.77 ± 0.02	3.13 ± 0.02	4.48 ± 0.04	0.98 ± 0.00	0.34 ± 0.00	0.47 ± 0.01	0.18 ± 0.00	0.01 ± 0.00	0.11 ± 0.00	11.7 ± 0.10
E	3.95 ± 0.06	4.56 ± 0.07	6.27 ± 0.14	0.13 ± 0.00	0.62 ± 0.01	0.89 ± 0.03	0.30 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	17.1 ± 0.34
F	3.78 ± 0.01	4.27 ± 0.01	7.06 ± 0.01	0.12 ± 0.00	0.29 ± 0.00	0.54 ± 0.02	0.30 ± 0.00	0.22 ± 0.00	0.28 ± 0.00	16.9 ± 0.05
G	3.43 ± 0.01	3.89 ± 0.01	6.06 ± 0.01	0.13 ± 0.00	0.28 ± 0.00	0.45 ± 0.00	0.27 ± 0.00	0.19 ± 0.00	0.25 ± 0.00	15.0 ± 0.04
Decaffeinated										
A	0.45 ± 0.00	0.51 ± 0.00	0.82 ± 0.00	0.04 ± 0.00	0.09 ± 0.00	0.13 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	2.10 ± 0.01
B	0.83 ± 0.00	0.90 ± 0.00	1.43 ± 0.01	0.06 ± 0.00	0.16 ± 0.00	0.26 ± 0.00	0.07 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	3.80 ± 0.02
C	3.22 ± 0.01	3.54 ± 0.01	5.77 ± 0.01	0.11 ± 0.00	0.70 ± 0.00	1.22 ± 0.00	0.40 ± 0.00	0.26 ± 0.00	0.41 ± 0.00	15.6 ± 0.04
D	3.42 ± 0.02	3.78 ± 0.02	6.23 ± 0.03	0.10 ± 0.00	0.53 ± 0.00	0.99 ± 0.01	0.40 ± 0.00	0.27 ± 0.00	0.41 ± 0.00	16.1 ± 0.08
E	3.26 ± 0.05	3.59 ± 0.03	5.75 ± 0.03	0.12 ± 0.01	0.60 ± 0.02	1.09 ± 0.03	0.36 ± 0.01	0.23 ± 0.01	0.37 ± 0.00	15.4 ± 0.19

[a] Values are means ± SD ($n = 3$).

among coffee samples from different brands, although Coffee A showed the lowest absorbance at all three wavelengths. However, no significant differences in color among the coffee samples were observed.

3.2. Caffeine and chlorogenic acids (CGA) contents in various commercial brewed coffees

The recovery efficiencies (%) were 94.3 ± 0.23% for 3-CGA, 102 ± 0.64% for caffeic acid, 95.8 ± 0.05% for ferulic acid, and 97.7 ± 0.45% for caffeine.

Table 2 shows the caffeine contents of the different coffees. The caffeine contents in regular coffee ranged from 10.9 ± 0.04 mg/g (Coffee F) to 16.5 ± 0.24 mg/g (Coffee E). The caffeine contents in decaffeinated coffee ranged from 0.34 ± 0.00 mg/g (Coffees C) to 0.47 ± 0.01 mg/g (Coffee D). It is interesting that all decaffeinated brands of coffee contained caffeine levels of nearly 0.4 mg/g of coffee.

Fig. 1 shows the structures of CGA found in brewed coffees in the present study. Table 3 shows the results of CGA analysis in various coffees. Fig. 2 shows a typical chromatogram of a brewed coffee sample run for CGA analysis. The

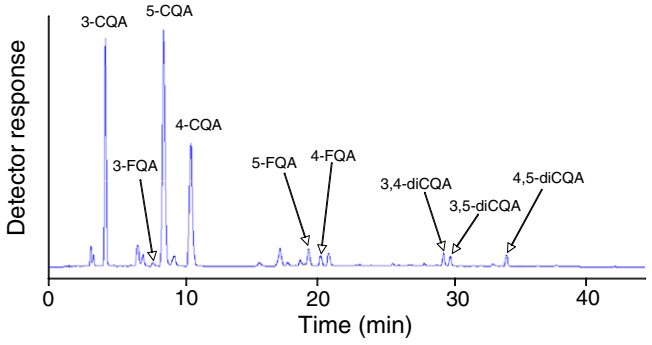

Fig. 2. Typical chromatogram of a brewed coffee sample (Coffee A) run for CGA analysis.

total acid content in the coffees ranged from 2.10 ± 0.01 mg/g (Coffee A, decaffeinated) to 17.1 ± 0.34 mg/g (Coffee E, regular) in the present study. Decaffeinated Coffees A and B contained much less total acid than their regular coffee counterparts, suggesting that the decaffeinating process reduces acid content. On the other hand, decaffeinated Coffees C, D, and E contained higher levels of total acid than their regular coffee counterparts. Coffee A contained the least total CGA and the fewest of the three CQA isomers both in regular and decaffeinated samples, followed by Coffee B. CGA isomer contents were, in decreasing order, 5-CQA > 4-CQA > 3-CQA > 5-FQA > 4-FQA > 3-FQA > 3,4-diCQA > 4,5-diCQA; 3,5-diCQA. The major CGA was 5-CQA, which comprised 36–42% and 37–39% of the total CGA in the regular and decaffeinated coffees, respectively, in the present study. 5-CQA was detected in the regular coffee sample prepared from Coffee F at the highest level of the 9 CGA isomers (7.06 ± 0.01 mg/g ground coffee).

5-CQA was also the major acid found in the Arabica and Robusta green beans, where it comprised 66% and 56% of total chlorogenic acids, respectively (Trugo & Macrae, 1984). It was also reported that the total CGA in green coffee beans was reduced significantly by roasting at 205 °C for four different time periods: light = 7 min for Arabica and 5 min for Robusta; medium = 10 min for Arabica and 7 min for Robusta; dark = 13 min for Arabica and 14 for Robusta or very dark = 19 min for Arabica and 16 min for Robusta (Trugo & Macrae, 1984). The rate of reduction was 60.9% for light, 67.7% for medium, 88.8% for dark, and 96.5% for very dark in Arabica and 59.7% for light, 76.4% for medium, 93.0% for dark, and 98.0% for very dark in Robusta. The total level of CGA was 68.8 mg/g of dry green bean in Arabica green bean and 88.0 mg/g in Robusta green bean. These levels reduced to 26.9 mg/g for light, 22.2 mg/g for medium, 7.71 mg/g for dark, and 2.42 mg/g for very dark in roasted Arabica coffee and 35.4 mg/g for light, 20.7 mg/g for medium, 6.15 mg/g for dark, and 1.76 mg/g

for very dark roasting conditions. In the present study, the total CGA ranged from 5.26 mg/g (Coffee A) to 17.1 mg/g (Coffee E) in regular brewed coffees, suggesting that these coffee beans were roasted under dark or very dark conditions.

4. Conclusions

Such reports indicated that the content of CGA is lowered most significantly by varying the roasting time/temperature curve. Changes to the roasting times and temperatures seem to affect CGA contents significantly in the final coffee products. This may explain the major differences in pH and CGA content found among the commercial coffees tested. However, vigorous treatment of food and beverages causes reductions in palatability factors, such as flavor and color (Ehling & Shibamoto, 2005), although the present study showed no significant color differences. Therefore, it is important to prepare a coffee with low acid content, yet one that does not lose biologically beneficial components. Investigation into the role of roasting conditions in content variation of beneficial chemicals, such as antioxidants, and in acid contents of coffees is in order.

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