Contents of chlorogenic acids and caffeine in various coffee-related products

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HIGHLIGHTS

• The total amount of CGAs and caffeine were quantified in different types of coffee-related drinks.
• The coffee with the highest content of CGAs was unblended roasted and ground coffee sold in supermarkets.
• The most caffeine-rich coffee was Americano coffee from coffee shops.
• The coffee with the highest value of caffeine/CGAs ratio was milk-added ready-to-drink coffee.
• According to caffeine/CGAs ratio, the good quality coffee was found to be the unblended ground coffee from market.

GRAPHICAL ABSTRACT

ABSTRACT

Coffee is the most popular beverage in the Republic of Korea, other than Korea’s traditional green tea. Coffee contains many physiologically active substances, such as chlorogenic acids (CGAs) and caffeine. Previous studies have focused on the content of CGAs and caffeine in brewed coffee. This study quantified the total amounts of CGAs and caffeine using high-performance liquid chromatography coupled with a diode array detector in 83 various coffee beverages, such as instant coffee, roasted and ground coffee sold in supermarkets, ready-to-drink coffee, and Americano coffee sold in franchise restaurants in the Republic of Korea. According to the results of this study, the coffee with the highest content of CGAs was unblended roasted and ground coffee sold in supermarkets, with a mean value of 194.1 ± 67.7 mg/serving, and the most caffeine-rich coffee was Americano coffee from coffee shops, with a mean value of 166.1 ± 37.5 mg/serving. The caffeine/CGA ratios were determined in various coffee beverages because they are useful parameters for estimating the human health. The lowest mean caffeine/CGAs ratio of 0.5 ± 0.1 was found in unblended ground coffee sold in supermarkets, and the highest mean ratio of 2.5 ± 1.4 was found in milk-added ready-to-drink coffee. Adult caffeine tolerance is defined as 400 mg a day in the Republic of Korea.
Introduction

Coffee has been the most commercialized food item for decades. Therefore, after water, coffee is the most widely consumed and traded beverage in the world [1,2]. Epidemiological and experimental studies have shown positive effects of regular coffee drinking on a variety of aspects of health, such as psychoactive response, neurological and metabolic disorders, and liver functions [3]. According to a recent prospective study on the association of coffee intake with total and cause-specific mortality in Japan, habitual coffee consumption is associated with a low risk of total mortality [4]. An interesting study showing that the frequent consumption of instant mix coffee by Korean women was associated with a high prevalence of obesity was published in 2017 [5]. The culture of drinking coffee is becoming popular in the Republic of Korea with the Westernization of diet and changes in lifestyle. Korea’s per capita coffee consumption is more than five times that of the Asia-Pacific region [6].

Instant mix coffee that contains non-dairy or dairy creamer and sugar was the most commonly consumed by Korean adults, with an increasing trend among middle-aged and older individuals and a declining trend among young men and women. Instant mix coffee causes weight gain and insulin resistance, and the potential benefits of coffee can be offset. Moreover, instant mix coffee still accounts for a substantial amount of coffee consumption, with approximately 10-fold higher consumption of instant mix coffee than instant coffee in the Republic of Korea [5,7]. Additionally, the consumption of ready-to-drink coffee (including traditional canned coffees and coffee sold in new metal bottles, polyethylene terephthalate (PET) bottles, and plastic cups) in Korean culture has increased rapidly in recent years [6]. Coffee contains over 2000 different ingredients, such as carbohydrates, fibers, nitrogen compounds, lipids, minerals, acids, and esters [8,9]. Among these compounds, chlorogenic acids (CGAs) and caffeine are recognized as healthful components [1,10–18], and their potential benefits of coffee can be offset. Moreover, instant mix coffee causes weight gain and insulin resistance, and the potential benefits of coffee can be offset. Instant mix coffee by Korean women was associated with a high prevalence of obesity was published in 2017 [5]. The culture of drinking coffee is becoming popular in the Republic of Korea with the Westernization of diet and changes in lifestyle. Korea’s per capita coffee consumption is more than five times that of the Asia-Pacific region [6].

The purpose of this study was to evaluate the contents of CGAs and caffeine in instant coffees (including instant mix coffee), ready-to-drink coffees, roasted and ground coffees from supermarkets, and Americano coffees sold in coffee shops, fast food restaurants, and bakery shops in the Republic of Korea. In addition, the caffeine/CGAs ratio, a good marker of the degree of roasting of coffee beans, was examined to compare the difference in the quality of coffee beans among various coffee beverages. The ratio is generally associated with brewed coffee; however, other studies have also reported the ratio in instant coffee [31]. The unit of mg/serving in this study indicates the amount in hot water to be measured up that can be manufactured in the laboratory (confined to the samples of the instant coffees, roasted and ground coffees from supermarkets in this study) and the very volume of the products as purchased, such as ready-to-drink coffees and Americano coffees sold in coffee shops, fast food restaurants, and bakery shops.

Material and methods

Material

The analytical standard 5-CQA (CAS Number: 327-97-9) was provided by Carl Roth GmbH (purity: >97%, Karlsruhe, Germany). 3-CQA (CAS Number: 906-33-2, purity >95%), 4-CQA (CAS Number: 905-99-7, >95%), caffeine (CAS Number: 58-05-2, purity >99%), glacial acetic acid, and potassium phosphate monobasic were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-FQA (CAS Number: 1899-29-2, purity 98%) was supplied by Chem Facies (Hubei, China). 3,4-DiCQA (CAS Number: 14534-61-3, purity >95.0%), 3,5-diCQA (CAS Number: 2450-53-5, purity >95%), and 4,5-diCQA (CAS Number: 32451-88-0, purity >95%) were obtained from Biopurity Phytochemicals (Sichuan, China). HPLC-grade acetonitrile and methanol were provided by J.T. Baker (Griesheim, Germany). A Barnstead Nanopure Diamond (Dubuque, IA, USA) was used to make refined deionized water.

Samples and coffee sample preparation

A total of 83 different coffee-related products from 38 brands and distinct production lots sold in Republic of Korea were collected and examined in this study. Nineteen instant coffee products (5 brands), 28 ready-to-drink coffee products (9 brands), and 18 roasted and ground coffee products (6 brands) were purchased from a supermarket. Additionally, 18 Americano coffees from different franchise shops (coffee shops: 11 brands, fast food restaurants: 4 brands, and bakery stores: 3 brands) were used herein. The instant coffee used in this experimental work consisted of 13 types of 100% coffee, which included two decaffeinated coffees and 6 instant mix coffees. In the Republic of Korea, instant coffee, including 100% powder coffee, is sold mainly in the form of sticks because of consumer convenience. Other studies have determined the CGAs and caffeine in instant coffees prepared from typical weights (e.g., 2.0 g of instant coffee, equivalent to one teaspoonful) of instant coffee dissolved in varied volumes of boiling water [26,31], which may lead to various concentrations of CGAs and caffeine. Therefore, in this study, we purchased products packaged in stick form, which better reflect the CGAs and caffeine intake by an individual per serving for use in our experiments. The
labels of instant coffees used in this study recommended dissolving approximately 1 g (net weight of one stick) of coffee or approximately 12 g (net weight of one stick) of instant mix coffee in 100–120 mL of boiling water. Each sample was prepared by dissolving the whole contents of a stick in 100 mL of boiling water. The 28 ready-to-drink coffees were divided into non-milk-added (7 products) and milk-added (21 products) groups. The roasted and ground coffees purchased from the supermarket were divided into 11 unblended roasted and ground coffees and 7 blended roasted and ground coffees. Brewed coffee was prepared as follows: approximately 10 g of a roasted and ground coffee sample was placed on a filter and extracted with a total of 200 mL of boiling water applied in 3 pours. Although the total brew time generally varies depending on the type of coffee, 2 min was used in the current study to standardize the sample preparation [35].

Sample preparation

Samples were prepared as reported in our previous study [35]. A 2.5 mL sample of prepared coffee was transferred to a 50 mL polypropylene centrifuge tube, and 0.1 mL each of Carrez solutions I (10.6 g of potassium ferrocyanide trihydrate dissolved in 100 mL of distilled water) and II (21.9 g of zinc acetate dihydrate and 2 mL of glacial acetic acid diluted with distilled water to 100 mL) was added to clarify the sample. The solutions were mixed by vortexing for 2 min. The total volume was brought to 50 mL by the addition of 10% methanol. The solution was then centrifuged at 3500 rpm for 10 min. A 0.2-μm PTFE syringe filter was used to filter the supernatant.

Analytical method

Instrumental analysis of the CGAs and caffeine was performed using a high-performance liquid chromatography coupled with diode array detector (HPLC-DAD) system (Dionex, UltraMate 3000, Sunnyvale, CA, USA) with an Accucore C18 (150 mm × 4.6 mm, 2.6 μm, Thermo Scientific, MA, USA). The mobile phase was composed of eluent A (20 mM KH₂PO₄ buffer containing 0.1% phosphoric acid) and eluent B (acetonitrile containing 0.1% phosphoric acid). The gradient mode was initially set at an A/B ratio of 97:3 from 0 to 5 min, the eluent was increased to 93:7 from 5 to 15 min, eluent B was slightly increased to 92:8 from 15 to 25 min, then eluent B was decreased to 75:25 from 25 to 35 min, and finally the eluent was returned to 97:3 from 35 to 45 min for column equilibration and system washing. The gradient program was carefully conducted to separate the 7 isomers of CGAs and caffeine (Fig. 1). The flow rate and injection volume were 1 mL/min and 10 μL, respectively. Detection wavelengths of 324 nm and 272 nm were used for the analysis of CGAs and caffeine, respectively [35].

Statistical analysis

Statistical analysis was performed using SPSS version 18 for Windows (SPSS, Chicago, USA). ANOVA followed by Tukey’s test were used to compare the values of CGAs and caffeine in three particular sample groups whenever a significant F-value was obtained (Tables 2, 5, and Fig. 2). A t-test was used to compare the mean values of CGAs and caffeine between the two groups (Tables 3 and 4).

Fig. 1. Identification of 3-CQA, 4-CQA, 5-CQA, 3'-FQA, 3,4-diCQA, 3,5-diCQA, 4,5-diCQA, and caffeine from chromatograms of the roasted and ground coffee samples.
In the same columns, from 3-CQA to total CGAs, different letters indicate significant differences among groups (100% coffee, decaffeinated coffee, and instant mix coffee) based on Tukey's test (P < 0.05).

Samples refer to 100% coffee (1–11), decaffeinated coffee (D 1 and 2), and instant mix coffee (M 1–6).

Data expressed as mean values. Standard deviation < 7% of the mean in all instances (n = 3).

NS denotes not significant based on Tukey's test.

### Table 2

**Chlorogenic acids (CGAs) and caffeine in instant coffees**

Instant coffees were purchased from grocery stores in Suwon-si in the Republic of Korea. The samples of coffee described in this section are instant coffees. As mentioned in the Introduction, however, more instant mix coffee than 100% coffee is sold in the Republic of Korea. The 100% coffee products were found to contain an average of 24.3 ± 12.6 mg/serving CGAs and 37.0 ± 14.5 mg/
serving caffeine (Table 2). The total CGAs in the decaffeinated coffee group averaged 40.1 ± 10.0 mg/serving, and caffeine was not detected. In the instant mix coffee group, the total CGAs averaged 53.8 ± 15.9 mg/serving, and caffeine averaged 45.3 ± 7.5 mg/serving. The mean value of the total CGAs in the instant mix coffee was more than twice that in the 100% coffee. Interestingly, the content of CGAs in the 100% coffee group varied from 8.7 mg/serving to 53.8 mg/serving, a range of approximately 45 mg/serving. From 3-CQA to total CGAs in Table 2, except for 3,5-diCQA and 3-FQA, there were significant differences among groups (100% coffee, decaffeinated coffee, and instant mix coffee) based on Tukey’s test (P < 0.05). Based on a t-test, there was no significant difference in the caffeine between the 100% coffee and instant mix coffee groups (P > 0.05). In contrast, the content of total CGAs in the instant mix coffee group varied from 38.5 mg/serving to 74.2 mg/serving, representing a range of approximately 33 mg/serving. In addition, the caffeine/CGAs ratio was more than twice as high in the 100% coffee group (1.9 ± 1.1) than in the instant mix coffee group (0.9 ± 0.3).

### Table 3

| No | Sample | 3-CQA (mg/L) | 5-CQA (mg/L) | 4-CQA (mg/L) | 3,4-diCQA (mg/L) | 3,5-diCQA (mg/L) | 4,5-diCQA (mg/L) | 3-FQA (mg/L) | Total CGAs (mg/serving) | Caffeine (mg/serving) | Caffeine/CGAs ratio |
|----|--------|-------------|-------------|-------------|-----------------|-----------------|-----------------|-------------|------------------------|---------------------|-------------------|
| 20 | NM 1   | 173.2       | 160.4       | 138.8       | 10.9            | 7.3             | 6.9             | 36.0        | 146.7                  | 95.1                | 0.6               |
| 21 | NM 2   | 124.6       | 124.6       | 105.7       | 4.0             | 2.9             | 2.6             | 22.9        | 106.5                  | 82.4                | 0.8               |
| 22 | NM 3   | 66.3        | 55.8        | 49.3        | 2.6             | 2.1             | 2.0             | 22.6        | 55.2                   | 123.9               | 2.2               |
| 23 | NM 4   | 54.2        | 65.1        | 52.9        | 4.4             | 2.7             | 2.9             | 19.7        | 48.4                   | 81.3                | 1.7               |
| 24 | NM 5   | 65.3        | 55.0        | 49.2        | 3.2             | 2.4             | 2.2             | 21.5        | 39.7                   | 64.9                | 1.6               |
| 25 | NM 6   | 62.3        | 52.9        | 51.7        | 3.0             | 2.5             | 2.2             | 24.0        | 39.7                   | 61.9                | 1.6               |
| 26 | NM 7   | 22.1        | 20.8        | 17.6        | 1.2             | 1.1             | 1.2             | 14.0        | 21.5                   | 127.5               | 5.9               |
| 27 | M 1    | 111.7       | 93.8        | 89.8        | 1.7             | 5.7             | 4.9             | 72.3        | 66.5                   | 64.6                | 1.0               |
| 28 | M 2    | 71.9        | 56.9        | 52.1        | 2.9             | 1.9             | 1.9             | 24.2        | 58.2                   | 125.4               | 2.2               |
| 29 | M 3    | 70.5        | 55.9        | 51.6        | 2.7             | 1.9             | 1.8             | 20.2        | 56.3                   | 87.5                | 1.6               |
| 30 | M 4    | 66.5        | 56.2        | 51.6        | 6.3             | 4.4             | 4.0             | 45.1        | 56.2                   | 109.3               | 1.9               |
| 31 | M 5    | 55.1        | 48.1        | 43.3        | 4.1             | 3.1             | 2.7             | 36.5        | 46.3                   | 112.7               | 2.4               |
| 32 | M 6    | 55.4        | 57.5        | 43.6        | 2.7             | 1.9             | 1.9             | 21.7        | 46.2                   | 106.4               | 2.3               |
| 33 | M 7    | 58.9        | 49.0        | 44.7        | 2.5             | 2.0             | 2.0             | 36.9        | 45.1                   | 82.9                | 1.8               |
| 34 | M 8    | 55.9        | 53.3        | 43.4        | 3.3             | 1.9             | 2.0             | 20.0        | 44.9                   | 96.5                | 2.1               |
| 35 | M 9    | 73.6        | 61.2        | 55.0        | 3.6             | 1.9             | 2.0             | 25.4        | 44.6                   | 98.1                | 2.2               |
| 36 | M 10   | 56.3        | 52.5        | 42.5        | 2.6             | 1.9             | 1.8             | 20.1        | 44.4                   | 102.4               | 2.3               |
| 37 | M 11   | 47.7        | 38.9        | 33.5        | 2.1             | 1.7             | 1.6             | 16.1        | 42.5                   | 124.2               | 2.9               |
| 38 | M 12   | 61.1        | 60.2        | 47.8        | 3.8             | 2.2             | 2.2             | 35.0        | 42.5                   | 98.2                | 2.3               |
| 39 | M 13   | 45.3        | 37.1        | 34.0        | 1.7             | 1.5             | 1.5             | 17.4        | 41.6                   | 131.7               | 3.2               |
| 40 | M 14   | 48.1        | 40.4        | 35.8        | 2.1             | 1.9             | 1.7             | 16.5        | 39.5                   | 59.2                | 1.5               |
| 41 | M 15   | 52.4        | 56.6        | 41.7        | 3.6             | 2.3             | 2.3             | 16.6        | 35.1                   | 50.8                | 1.4               |
| 42 | M 16   | 56.9        | 46.8        | 41.7        | 3.2             | 1.8             | 1.8             | 20.3        | 34.5                   | 60.7                | 1.8               |
| 43 | M 17   | 51.6        | 43.7        | 37.6        | 2.9             | 1.6             | 1.6             | 22.6        | 32.3                   | 119.2               | 3.7               |
| 44 | M 18   | 30.7        | 28.0        | 24.0        | 1.2             | 1.1             | 1.2             | 14.3        | 27.6                   | 101.1               | 3.7               |
| 45 | M 19   | 31.1        | 30.2        | 24.6        | 2.0             | 1.5             | 1.4             | 14.3        | 25.2                   | 60.1                | 2.4               |
| 46 | M 20   | 28.3        | 27.5        | 23.2        | 2.0             | 1.5             | 1.6             | 12.7        | 23.3                   | 61.5                | 2.6               |
| 47 | M 21   | 8.9         | 10.9        | 8.3         | 1.2             | 1.1             | 1.2             | 12.0        | 8.7                    | 68.4                | 7.9               |

Mean 54.2 ± 20.61 47.8 ± 16.81 41.4 ± 16.01 2.8 ± 1.11 2.1 ± 1.11 2.0 ± 0.95 24.8 ± 14.11 41.0 ± 13.21 91.5 ± 25.31 2.5 ± 1.41

Samples refer to as non-milk-added coffee (NM 1–7; serving size of 200–275 mL) and milk-added coffee (M 1–21; serving size of 175–300 mL). In the same columns, from 3-CQA to caffeine/CGAs ratio, there is no significant difference between 2 groups (non-milk-added coffee and milk-added coffee) based on t-test at 5% probability (P > 0.05). Data expressed as the mean value. Standard deviation < 7% of the mean in all instances (n = 3).
| No | Sample | 3-CQA (mg/L) | 5-CQA (mg/L) | 4-CQA (mg/L) | 3,4-diCQA (mg/L) | 3,5-diCQA (mg/L) | 4,5-diCQA (mg/L) | 3-FQA (mg/L) | Total CGAs (mg/serving of 200 mL) | caffeine (mg/serving of 200 mL) | Caffeine/CGAs ratio | Country of origin | Roasting degree | Types of coffee |
|----|--------|--------------|--------------|--------------|-----------------|-----------------|-----------------|--------------|----------------------------------|-------------------------------|------------------|----------------|----------------|----------------|
| 48 | RG 1   | 144.7        | 370.2        | 197.8        | 712.7           | 13.3            | 8.0             | 11.8         | 291.7                            | 111.7                      | 0.4              | Brazil         | Light Arabica | Arabica        |
| 49 | RG 2   | 130.1        | 300.6        | 172.5        | 603.2           | 11.1            | 6.4             | 9.4          | 246.7                            | 101.1                       | 0.4              | Colombia      | Light Arabica | Arabica        |
| 50 | RG 3   | 119.4        | 316.8        | 162.5        | 598.7           | 11.1            | 6.8             | 10.4         | 245.1                            | 97.2                        | 0.4              | Costa Rica    | Medium Arabica| Arabica        |
| 51 | RG 4   | 116.5        | 300.9        | 159.5        | 577.0           | 10.7            | 6.4             | 9.4          | 236.1                            | 102.1                       | 0.4              | Ethiopia      | Medium Arabica| Arabica        |
| 52 | RG 5   | 122.2        | 286.4        | 161.6        | 570.2           | 13.8            | 8.0             | 11.5         | 234.7                            | 100.9                       | 0.4              | Ethiopia      | Medium Arabica| Arabica        |
| 53 | RG 6   | 120.2        | 289.3        | 162.3        | 571.8           | 12.4            | 6.9             | 10.4         | 234.7                            | 107.0                       | 0.5              | Guatemala     | Medium Arabica| Arabica        |
| 54 | RG 7   | 102.2        | 218.8        | 135.7        | 456.7           | 9.1             | 4.7             | 6.9          | 186.8                            | 113.2                       | 0.6              | Honduras      | Medium Arabica| Arabica        |
| 55 | RG 8   | 73.5         | 161.5        | 97.8         | 332.8           | 6.5             | 3.4             | 5.1          | 136.1                            | 97.4                        | 0.7              | Indonesia     | Strong Arabica| Arabica        |
| 56 | RG 9   | 58.3         | 136.4        | 79.8         | 274.5           | 4.5             | 2.9             | 4.4          | 112.2                            | 68.4                        | 0.6              | Kenya         | Light Arabica | Arabica        |
| 57 | RG 10  | 56.6         | 132.2        | 76.9         | 265.7           | 5.2             | 3.1             | 4.8          | 108.9                            | 72.0                        | 0.7              | Uganda        | Medium Arabica| Arabica        |
| 58 | RG 11  | 54.4         | 121.6        | 72.4         | 248.3           | 4.5             | 2.8             | 4.0          | 101.6                            | 74.5                        | 0.7              | Peru          | Medium Arabica| Arabica        |

Mean 99.8 ± 33.0 239.5 ± 88.2 134.4 ± 44.6 473.8 ± 165.2 9.3 ± 3.5 5.4 ± 2.1 8.0 ± 3.0 194.1 ± 67.7 95.0 ± 15.9 0.5 ± 0.1

59 BRG 1 76.8 177.6 103.8 358.3 5.4 3.2 4.7 146.0 113.2 0.8 N.A. Medium Arabica 95%, Robusta 5%

60 BRG 2 75.3 151.7 99.4 326.3 6.5 3.4 4.8 133.5 100.3 0.8 N.A. Medium Arabica

61 BRG 3 66.3 135.4 87.7 289.3 6.6 3.5 5.1 118.8 92.8 0.8 N.A. Strong Arabica 90%, Robusta 10%

62 BRG 4 47.2 105.6 65.0 217.7 3.7 2.3 3.3 88.9 90.3 1.0 Colombia, Brazil Medium Arabica

63 BRG 5 42.7 87.7 56.9 187.3 2.9 1.8 2.5 76.4 136.9 1.8 Brazil, Honduras, Indorena Medium/ strong

64 BRG 6 14.9 30.7 20.1 65.7 1.2 1.1 1.2 27.0 124.0 4.6 N.A. Strong Arabica

Mean 53.8 ± 23.8 114.8 ± 52.2 72.1 ± 31.6 240.8 ± 107.3 4.4 ± 2.2 2.5 ± 1.0 3.6 ± 1.5 98.4 ± 43.8 109.6 ± 18.5 1.6 ± 1.5

65 RGB 7 106.8 235.7 140.5 483.0 9.3 5.0 7.4 197.5 below LOQ Colombia N.A. Arabica

Samples refer to as unblended roasted and ground coffee (RG 1–11), blended roasted and ground coffee (BRG 1–6) and blended roasted, ground, and decaffeinated coffee (RGB 7).

The serving size of 18 samples ranged from 270 to 410 mL.

In the same columns, from 3-CQA to caffeine/CGAs ratio except for caffeine and caffeine/CGAs ratio, there is a significant difference (P < 0.05) between 2 groups (unblended roasted and ground coffee (RG 1–11) and blended roasted and ground coffee (BRG 1–6)) based on a t-test at 5% probability (P < 0.05).

In the same columns, caffeine and caffeine/CGAs ratio, there is no significant difference between 2 groups (unblended roasted and ground coffee (RG 1–11) and blended roasted and ground coffee (BRG 1–6)) based on a t-test. P > 0.05.

* Data expressed as the mean value. Standard deviation < 7% of the mean in all instances (n = 3).
According to a t-test, there was a significant difference in the caffeine/CGA ratio between the 100% coffee and instant mix coffee groups (P < 0.05).

**Chlorogenic acids (CGAs) and caffeine in ready-to-drink coffees**

Due to the high convenience of being able to consume ready-to-drink coffee anywhere, the sales of ready-to-drink coffee are increasing [6]. While various types of ready-to-drink coffees described in the Introduction are available on the market, the ready-to-drink coffees were divided here only into a non-milk-added coffee group (NMG) and a milk-added coffee group (MG) to determine the contents of total CGAs and caffeine. The total CGAs were higher in the NMG (65.4 ± 44.6 mg/serving) than in the MG (41.0 ± 13.2 mg/serving) (Table 3). The standard deviation of the NMG was approximately 3.4 times greater than that of the MG. The caffeine content was identical in the two groups. The values for caffeine were 91.0 ± 26.2 mg/serving for the NMG and 91.5 ± 25.3 mg/serving for the MG. These results are attributed to the government’s regulation of the amount of caffeine in beverages and efforts to comply with it. In addition, there was little difference in the standard deviation between the two groups. The caffeine/CGAs ratio was 2.1 for the NMG and 2.5 for the MG. From the 3-CQA to caffeine/CGAs ratio in Table 3, there was no significant difference between the 2 groups (non-milk-added coffee and milk-added coffee) based on a t-test at a 5% probability (P > 0.05). It has to be noted that the effect of milk on measurements is outside the purview of the present study.

**Chlorogenic acids (CGAs) and caffeine in roasted and ground coffees from supermarkets**

Our previous study reported the contents of CGAs and caffeine in homemade brewed coffee under various conditions, such as the roasting degree of green coffee bean, coffee-ground size, and number of boiling-water pours [35]. While many people enjoy instant coffees and ready-to-drink coffees, a growing number of people also drink brewed coffee at home because of the idea that coffee promotes health condition reported by the mass media in the Republic of Korea. This section examines the contents of total CGAs and caffeine in roasted and ground coffees sold at supermarkets in the Republic of Korea. Information about the coffee of the Republic of Korea. This section examines the contents of total CGAs and caffeine in ready-to-drink coffees. The CGAs in coffees are composed of 3-CQA, 4-CQA, 5-CQA[12,13,32,33], more than three but fewer than nine isomers[22,26] or one isomer[31,37]. Studies have also reported experiments performed using HPLC, in which the full separation of these isomers was not carried out for various reasons[20,35]. In our previous study, we tried to quantify these substances using HPLC but found that it was not possible to separate 4-FQA and 5-FQA using this method[35]. Accordingly, in the samples' package labelling information and the results of the study. The reason is speculated to be that the content of CGAs is influenced not only by the roasting degree of coffee beans but also by the beans’ origin, species, and quality. The caffeine/CGAs ratios in the blended group were approximately three times higher than in the unblended group, averaging 1.6 and 0.5, respectively. The sample BRG6 had the highest ratio of 4.6 among the strongly roasted samples. The contents of total CGAs and caffeine of the sample BRG6 were 27.0 mg/serving, representing the lowest value, and 124.0 mg/serving, representing the highest value in this section. It should be noted that the information included on the package of sample BRG6 includes the roasting degree and coffee bean species without mentioning the country of origin.

**Chlorogenic acids (CGAs) and caffeine in franchise shops**

There were more than 12,000 franchise coffee shops in the Republic of Korea, including Starbucks and Cafe Benne. In addition to the franchise coffee shops, fast food restaurants such as McDonald’s and Lotteria also offered gourmet coffee from fresh beans at a lower price than other franchise stores [6]. With a growing population enjoying coffee, the beverage is also becoming increasingly available in franchise bakeries, such as Dunkin’ Donuts and PARIS BAGUETTE. The samples discussed in this chapter were divided into three groups according to the type of store where clients can easily obtain coffee: coffee shops, fast food restaurants, and bakeries, with 11.4, and 3 samples, respectively. All samples were of Americano coffee, which is sold in Republic of Korea more than other coffee-related beverages, such as cafe latte and cappuccino. The highest mean value of total CGAs among the three groups was found in coffee shops, with 99.4 ± 48.6 mg/serving, followed by fast food restaurants, with 87.7 ± 34.0 mg/serving, and bakeries, with 60.4 ± 14.0 mg/serving (Table 5). The amounts of caffeine showed a pattern similar to that of the abovementioned results: 166.1 ± 37.5, 107.7 ± 55.7, and 93.6 ± 14.3 mg/serving for coffee shops, fast food restaurants, and bakeries, respectively. However, the caffeine/CGAs ratios followed the order of coffee shops (2.1 ± 1.1), bakeries (1.6 ± 0.3), and finally, fast food restaurants (1.2 ± 0.3). According to Tukey’s test, the mean value of caffeine showed a significant difference (P < 0.05), and the rest were not significantly different (P > 0.05). The mean values of coffee shop coffee and bakery coffee showed significant differences.

The recommended consumption of caffeine for healthy adults in the Republic of Korea is up to 400 mg daily [36]. The highest and lowest caffeine concentrations found in coffees sold in franchise coffee shops in this experiment were 213.3 mg/serving and 91.7 mg/serving, respectively. Thus, consumers who are likely to be affected by caffeine should pay attention to their coffee consumption.

**A comprehensive evaluation of chlorogenic acids (CGAs) and caffeine in various types of coffee**

Many researchers have studied the contents of CGAs and caffeine in coffee beans, roasted and ground coffee and instant coffee. To our knowledge, this study is the first to quantify the contents of CGAs in instant coffee mix. The CGAs in coffees are composed of nine isomers, and some studies have measured all nine isomers [20,23,24,27,28], while others have measured three major isomers (3-CQA, 4-CQA, 5-CQA) [12,13,32,33], more than three but fewer than nine isomers [22,26] or one isomer [31,37]. Studies have also reported experiments performed using HPLC, in which the full separation of these isomers was not carried out for various reasons [20,35]. In our previous study, we tried to quantify these substances using HPLC but found that it was not possible to separate 4-FQA and 5-FQA using this method [35]. Accordingly, in the cur-
CGAs and caffeine contents of franchise coffees.*

In the Republic of Korea, with the development of coffee culture, the number of specialty coffee sales is also increasing. In this study, the contents of CGAs and caffeine were examined by collecting a variety of coffee-related products. The coffee-related culture, the number of specialty coffee sales is also increasing. In this study, the contents of CGAs and caffeine were examined by collecting a variety of coffee-related products. The coffee-related products were divided into four groups: instant coffees, ready-to-drink coffees, roasted and ground coffees sold in supermarkets and Americano coffees sold in franchise shops. The CGAs and caffeine were quantified based on their amount in coffee prepared in the laboratory (confined to the samples of the instant coffees, roasted and ground coffees from supermarkets in this study) and in the very volume of the products as purchased, such as ready-to-drink coffees, Americano coffees sold in coffee shops, fast food restaurants, and bakery shops. According to this study, the sub-group with the greatest content of CGAs was unblended roasted and ground coffee (mean value of 194.1 ± 67.7 mg/serving, range of 101.6–291.7 mg/serving) sold at the supermarket, and the sub-group with the lowest content was 100% instant coffee (37.0 ± 14.5 mg/serving, range of 23.7–71.2 mg/serving). Unusually, the standard deviation of these values was the highest in fast food restaurants (55.7 mg/serving) and the lowest in instant mix coffee (7.5 mg/serving). According to Tukey's test, the mean value of caffeine in the nine groups showed a significant difference (P < 0.05) (Fig. 2). No correlation between the total CGAs and caffeine was observed.

Table 5

| No | Sample | 3-CQA (mg/L) | 5-CQA (mg/L) | 4-CQA (mg/L) | 3,4-diCQA (mg/L) | 3,5-diCQA (mg/L) | 3,5-diCQA (mg/L) | 3-FQA (mg/L) | Total CGAs (mg/serving) | Caffeine (mg/serving) | Caffeine CGAs ratio |
|----|--------|-------------|-------------|-------------|----------------|----------------|----------------|-------------|------------------------|---------------------|-----------------|
| 66 | CS 1   | 86.9        | 191.3       | 11.2        | 6.6           | 4.2            | 5.5             | 24.2        | 127.2                  | 195.2               | 0.72            |
| 67 | CS 2   | 88.9        | 194.3       | 111.4       | 7.9           | 6.9            | 6.6             | 23.2        | 145.4                  | 174.2               | 0.72            |
| 68 | CS 3   | 79.4        | 201.4       | 111.5       | 6.1           | 5.9            | 6.6             | 21.0        | 141.6                  | 122.2               | 0.96            |
| 69 | CS 4   | 64.0        | 140.5       | 83.0        | 5.8           | 3.7            | 4.8             | 20.4        | 132.2                  | 205.7               | 0.73            |
| 70 | CS 5   | 69.7        | 145.9       | 89.2        | 6.0           | 3.8            | 4.9             | 19.5        | 120.1                  | 186.0               | 1.51            |
| 71 | CS 6   | 70.2        | 145.5       | 89.2        | 7.7           | 4.7            | 6.3             | 25.0        | 115.0                  | 192.1               | 1.74            |
| 72 | CS 7   | 54.2        | 108.9       | 68.6        | 4.6           | 2.9            | 3.6             | 18.2        | 86.1                   | 188.6               | 2.21            |
| 73 | CS 8   | 44.2        | 92.1        | 56.1        | 3.6           | 2.4            | 3.0             | 11.1        | 68.0                   | 153.2               | 2.31            |
| 74 | CS 9   | 27.6        | 57.1        | 34.6        | 2.5           | 1.9            | 2.1             | 10.4        | 42.2                   | 91.7                | 2.21            |
| 75 | CS 10  | 25.5        | 47.6        | 31.5        | 2.1           | 1.7            | 1.9             | 10.2        | 39.7                   | 106.2               | 2.22            |
| 76 | CS 11  | 19.5        | 37.6        | 23.8        | 1.8           | 1.5            | 1.5             | 8.1         | 31.0                   | 134.6               | 4.34            |
| Mean | 57.8 ± 25.5 | 123.8 ± 59.7 | 74.3 ± 33.6 | 5.2 ± 2.5 | 3.4 ± 1.5 | 4.4 ± 2.3 | 17.4 ± 6.3 | 99.4 ± 48.6 | 166.1 ± 37.5<sup>ab</sup> | 2.1 ± 1.1 |

Samples refer to as coffee shop coffee (CS 1–11), fast food restaurant coffee (FFR 1–4) and bakery coffee (B 1–3).

In the same columns, from 3-CQA to caffeine/CGAs ratio except for caffeine, there is no significant difference between the three groups (coffee shop coffee, fast food restaurant coffee, and bakery coffee) based on Tukey's test 5% probability (P > 0.05).

In the same column of caffeine, there is a significant difference between the three groups (coffee shop coffee, fast food restaurant coffee, and bakery coffee) based on Tukey's test (P < 0.05).<sup>ab</sup>

Data expressed as the mean value. Standard deviation < 7% of the mean in all instances (n = 3).
determined the amounts of caffeine and total CGAs in roasted coffee beans and calculated the caffeine/CGAs ratio, reported the smallest values, from 0.2 to 9.2.

In general, the higher the degree of roasting of coffee beans is, the lower the total CGA content becomes [13,24,38]. However, Table 5 shows that even when the roasting degree was the same, the caffeine/CGAs ratio varied. These results are attributed to the fact that the origin of the coffee bean was different even if the roasting degree was similar and the degree of coffee grinding was slightly different. While a recent study showed that instant mix coffee positively affected the obesity rate of middle-aged women in the Republic of Korea [5], our results show that the caffeine/CGAs ratio of instant mix coffee is the second lowest. Milk-added ready-to-drink coffee also showed the highest ratio. Such coffee is estimated to have a relatively high caffeine content and low CGAs. Although the process of manufacturing factory-made coffee drinks, which may affect the contents of CGAs and caffeine, was not considered in this study, it is believed that the results are meaningful with regard to the contents of CGAs and caffeine in coffee drinks sold in various forms.

### Conclusions

This study was carried out to evaluate the contents of total CGAs and caffeine in different types of coffee-related drinks.
Unblended ground coffee from the market contained the highest total CGA content, while the group with the most caffeine was the Americano coffee sold in coffee shops. According to the caffeine/CGA ratios, the best-quality coffee was unblended roasted and ground coffee from the market, which had the lowest ratio. In this study, the coffee with the highest caffeine/CGA ratio was milk-added ready-to-drink coffee, followed by non-milk-added ready-to-drink coffee. The high caffeine/CGA ratio means that the content of CGAs is relatively low compared with the content of caffeine. This result can be attributed to the high levels of caffeine in the ready-to-drink coffee sold in the Republic of Korea. The caffeine/CGA ratio is generally related to roasted coffee beans and brewed coffee. However, various coffee products are commercialized by processing coffee beans that have undergone a roasting process. Consumers do not have detailed information about what characteristics of coffee beans are used. Caffeine is more stable than CGAs during roasting, and as a result, the increase in the caffeine/CGA ratio is a good indicator of how much coffee beans were roasted [31]. As the caffeine content of some coffee drinks is stated on the product label, the consumer should be careful if that content exceeds the daily allowance. Coffee-related products suppliers should state the contents of CGAs, the one of the most available phenolic acid compound in foods, which demonstrates various therapeutics roles, such as antioxidant activity, antibacterial, hepatoprotective, cardioprotective, anti-inflammatory, antipycritic, neuroprotective, anti-obesity, antiviral, anti-microbial, anti-hypertension, free radicals scavenger, and a central nervous system (CNS) stimulator [40]. The consumers, in turn, should have the full right to select the products based upon caffeine/CGA ratio.

Conflict of Interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

Acknowledgements

The authors gratefully acknowledge the financial support received from the Gyeonggi Province Institute of Health and Environment (Suwon-si, Republic of Korea).

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