Determination of Chlorogenic Acids (CGA) in Coffee Beans using HPLC

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Abstract

Chlorogenic acids (CGA) are the main phenolic compounds in coffee and coffee has one of the highest concentrations of CGA of all plant constituents. In this study, the levels of CGA in certain coffee (Arabica Jimma (ArJM), Arabica Nekemit (ArNK), Arabica Sidamo (ArSD), Arabica Jimma (ArJM) raw, and Arabica Jimma (ArJM) Husk) brands found in Ethiopia were determined using High Performance Liquid Chromatography (HPLC). The levels of CGA in all the coffee brands were found to be within the documented range. The order of CGA concentration (mg/g) in coffee samples was found as follows: ArJM raw > ArJM > ArSD > ArNK > ArJM Husk. Generally, Arabica Jimma raw (46.144 mg/g) has the highest while Arabica Jimma husk (0.981 mg/g) has the least concentration of CGA.

Key words: Coffee, CGA, HPLC, Roasting, Extraction

Introduction

The word "coffee" comes from the name of a region of Ethiopia where coffee was first discovered ‘Kaffa’. The name ‘Kaffa’ is inherited from the hieroglyphic nouns ‘KA’ and ‘AfA’. ‘KA’ is the name of God; ‘AfA’ is the name of earth and all plants that grow on earth. So the meaning of Koffee (Coffee) from its birth-place bells on as the land or plant of God. Botanically, coffee is belonging to the family Rubiaceae in the genus Coffea. Although the genus Coffea includes four major subsections, 66% of the world production mostly comes from Coffea arabica L. and 34% from Coffea canephora Pierre ex Froehner (robusta type), respectively (Mekuria et al, 2004).

Ethiopia is the home and cradle of biodiversity of Arabica coffee seeds. More genetically diverse strains of C. arabica exist in Ethiopia than anywhere else in the world, which has lead botanists and scientists to agree that Ethiopia is the centre for origin, diversification and dissemination of the coffee plant (Mekuria et al, 2004).

The popularity and worldwide appeal of coffee, which stems from its unique flavour, make it currently one of the most desirable and frequently consumed beverages. Also, it has a strong historical, cultural, social and economic importance. Coffee is the single most important tropical commodity traded worldwide, accounting for nearly half of total exports of tropical products. Coffee beans found on the market are produced from two different species of Coffee genus: Coffee arabica and Coffee canephora syn. Coffee robusta. Both species present a rich source of biologically active compounds like caffeine, chlorogenic acid, nicotinic acid and some minerals like magnesium (Hecimovic et al, 2011).
The quality of coffee used for the preparation of a beverage is related to the chemical composition of the roasted beans, which, in turn, is affected by the chemical composition of green beans and by post-harvest processing conditions (drying, storage, roasting and grinding). During roasting, the green beans are heated at 200–240 °C for 10–15 min depending on the degree of roasting required, which is generally evaluated by colour. The characteristic flavour of coffee represents a combination of numerous chemical compounds produced by the chemical and physical changes that occur during roasting. The roasting process, especially at temperatures above 180–200 °C, leads to profound changes in the chemical composition and biological activities of coffee as a result of the generation of compounds deriving from the Maillard reactions (Hecimovic et al., 2011).

Chlorogenic acids (CGA) are the main phenolic compounds in coffee and coffee has one of the highest concentrations of CGA of all plant constituents (Farah et al., 2005). According to Farah et al. (2008) green (or raw) coffee contains (5–12 g/100 g) of CGA. CGA is being an ester of trans-cinnamic acids, such as caffeic acid, ferulic and p-coumaric acids with (-) quinic acid, Figure 1 shows the chemical structure of CGA. They are believed to have antioxidant properties which are suggested to play an important role in protecting food, cells and any organ from oxidative degenerative. Report indicate that diet rich in CGA compounds play a great role in preventing various diseases associated with oxidative stress such as cancer, cardiovascular, aging and neurodegenerative disease (Belay and Gholap, 2009).

Recent studies demonstrated that the consumption of green coffee extracts produced antihypertensive effect in rats and humans, improvement in human vasoreactivity, inhibitory effect on fat accumulation and body weight in mice and humans, and modulation of glucose
metabolism in humans. Such biological effects have been attributed to CGA present in green coffee (Farah et al, 2008).

On the other hand CGA contributes a great role in the formation of pigments, taste and flavor of coffee beans, which determines the quality and acceptance of the beverages. Previous research reports have indicated the relation between the composition of the CGA and quality of coffee beans (Belay and Gholap, 2009). CGA are known to be important determinants of coffee flavour. They contribute to the final acidity and confer astringency and bitterness to the beverage. As a result of maillard and strecker’s reactions bitterness increases during roasting due to release of caffeic acid and formations of lactones and other phenol derivatives responsible for flavour and aroma (Farah and Donangelo, 2006). According to Farah et al. (2006) the level of CGA has an inverse association with coffee quality with higher contents observed in lower quality coffee sample.

![Figure 1 The chemical structure of CGA](image)

Therefore, the objectives of this study was to determine the concentration of CGA content from different coffee samples by using HPLC after brewing the coffee samples with water and comparing the amount of CGA content among raw and roasted one and even with degree of roasting since degree of roasting affects the amount of CGA content.
Materials and Methods

Study setting

The experiment was conducted at McGill University (Canada), dietetics and human nutrition department research laboratory.

Experimental materials

Coffee samples: Five coffee samples (Arabica Jimma (ArJM), Arabica Nekemit (ArNK), Arabica Sidamo (ArSD), Arabica Jimma (ArJM) raw, and Arabica Jimma (ArJM) Husk) were brought from different regions of Ethiopia having different varieties and the experiments have a total of 15 treatments with three replications.

Chemicals: standard (CGA,), water, methanol, and acetic acid

Sample preparation, extraction of CGA from coffee beans

The coffee samples were prepared for HPLC analysis based on the following procedure according to the method, Wanika et al, 2010.

- 2 gm of each ground coffee sample were accurately weighed in 250 ml beakers,
- 100 ml distilled water were added to each samples,
- The samples were boiled for 5 minutes while stirring,
- The brewed coffee samples were cooled for some minutes and then the solution was filtered with 0.45 μm filter paper,
- The clear filtrate with some dilution was used for the HPLC analysis.
Standard solution preparation

0.01gm of CGA standard was dissolved in 100ml distilled water and 0.1, 1, 2.5, 5, 10, 50, and 100 ppm serial dilution of working standards were prepared. The following figure shows CGA calibration curve for HPLC.

![CGA Calibration curve for HPLC method](image)

Figure 2 CGA Calibration curve for HPLC method

Analyte determination, HPLC analysis

100µl of the filtrate were diluted with 900 µl of deionised water and pipetted into clean 1000 µl volumetric flasks. The standards and the samples were run in the HPLC system. The following were the HPLC conditions: Column, Reverse phase – ODS, 250 × 4.6 mm, flow rate, 1 ml/min, detector, photodiode array set at 278 nm, pressure, 150 khf/cm², mobile phase, water, acetic acid, methanol (799, 1 and 200ml) and sample volume, 20 µl. A calibration curve of peak areas versus concentration of the standards was plotted. The CGA level of the various samples was calculated using the regression equation of the best line of fit.

Statistical analysis

The data obtained from the HPLC analysis was analyzed with analysis of variance (ANOVA) using minitab 16 statistical software. When the p values (P< 0.05) were found significant the means were compared using Tekey’s method.
Results and Discussion

Chlorogenic acid (CGA):

Figure 3 shows the HPLC chromatogram of CGA standard measured at 278nm wave length. And Figure 4 shows the HPLC chromatogram of the different coffee samples. From this chromatogram ArJM raw sample has the highest peak area while ArJM has the second. But ArJM husk sample has the smallest peak area.

![Figure 3 Chromatogram of CGA standard](image-url)

(a)
The results obtained from the HPLC analysis for the concentration of CGA in mg/g of the different coffee samples are shown in table 1.
Table 1 The concentration of CGA for the different coffee samples

<table>
<thead>
<tr>
<th>Coffee sample</th>
<th>CGA concentration in mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArJM raw</td>
<td>46.144 A</td>
</tr>
<tr>
<td>ArJM</td>
<td>16.766 B</td>
</tr>
<tr>
<td>ArSD</td>
<td>2.749 C</td>
</tr>
<tr>
<td>ArNK</td>
<td>2.686 C</td>
</tr>
<tr>
<td>ArJM husk</td>
<td>0.981 D</td>
</tr>
</tbody>
</table>

Any two means (n=3) in the same column not followed by the same latter are significantly different (p< 0.05).

The data from Table 1 reveals that the amounts of CGA in ArJM raw coffee sample is maximum that is 46.144 mg/g while that of ArJM is minimum, 16.766 mg/g. This is because raw coffee sample contains high amounts of CGA but during roasting some amount is lost. That is why there is significant variation between ArJM raw and ArJM roasted coffee samples. This result is supported by Hecimovic et al. (2011), says that roasting results with the degradation of chlorogenic acids and its derivatives in coffee samples. Moon (2009) also reported that the total chlorogenic acids (CGA) present in coffee beans were reduced in accordance with the intensity of roasting conditions. Farah et al. (2005) also said that the loss of CGA during the roasting process of coffee has been previously described (45-49). The high temperature of the roasting process causes a breakage of the carbon-carbon bonds of CGA, resulting in isomerization and degradation. Longer periods of roasting resulted in a loss of total CGA. Besides isomerization and degradation, other chemical transformations may occur, the dominant being dehydration of the quinic acid moiety and formation of a lactone ring.

Table 1 also shows that there is significant difference between roasted samples; ArJM (light roasted), ArSD (dark roasted), and ArNK (dark roasted). Because roasting time and temperature
affects the amounts of CGA content from coffee samples. That is, dark roasting removes CGA content from coffee samples better than light roasting. That is why ArJM (light roasted) samples contains greater amount of CGA (16.766 mg/g) content than dark roasted samples; ArSD (2.749 mg/g) and ArNK (2.686mg/g). This result is supported by Moon (2009) said that, the total chlorogenic acids (CGA) present in coffee beans were reduced in accordance with the intensity of roasting conditions. When green coffee beans were roasted at 230 °C for 12 min and at 250 °C for 21 min, total chlorogenic acid content was reduced to nearly 50% and to almost trace levels. Farah et al. (2005) also reported that longer periods of roasting coffee samples resulted in a loss of total CGA.

Hecimovic et al. (2011) also reported that the contents of chlorogenic acid in various coffee samples are affected by degree of roasting. The content of the chlorogenic acid in the coffee beverage is dependent on the species, the variety, and the processing conditions of the coffee beans. Also Antonio et al. (2011) reported that during the roasting process, a series of transformations occurs in the chemical composition of the seeds as a consequence of pyrolysis, caramelization, Strecker degradation and Maillard reactions. Thus the contents of thermolabile compounds like the chlorogenic acids, trigoneline and diterpenes in roasted coffee are lower than those in green coffee, varying also according to the roasting degree. Chlorogenic acids are almost completely degraded when subjected to severe conditions of roasting due to its thermal instability. In the beginning of the process, bioactive lactones are formed reaching peaks in medium roasted seeds and degrading thereafter. Also during roasting a series of volatile compounds are formed and chlorogenic acids are partially incorporated into melanoidines’ backbones. Caffeine is not significantly altered during coffee roasting, but small losses may occur due to sublimation. In addition, roasting also degrades trigonelline, producing a variety of
compounds including nicotinic acid (3%). In summary, taking into account the roasting process, coffee chemical composition is truly modified, with some beneficial compounds degraded and some created.

Table 1 also reveal that coffee husk contains some amount of CGA even though its content is very small. That is ArJM husk has 0.981mg/g of CGA and there is significant difference with other coffee samples. This result is supported by Esquivel and Jimenez (2011) and it reported that coffee husks, skin and pulp can be a source of phytochemicals for the food and pharmaceutical industries. It contains chlorogenic acid (5-caffeoylquinic acid) (42.2% of the total of identified phenolic compounds), epicatechin (21.6%), 3, 4-dicaffeoylquinic acid, (5.7%), 3, 5-dicaffeoylquinic acid (19.3%), 4, 5-dicaffeoylquinic acid (4.4%), catechin (2.2%), rutin (2.1%), protocatechuic acid (1.6%) and ferulic acid (1.0%).

**Conclusion**

We can determine the concentration of CGA from different coffee beans using simple extraction methods using water by using HPLC.

The concentration of CGA from raw coffee sample is much greater than that of the roasted sample in addition the degree of roasting also affects the concentration of CGA; light roasting has relatively higher concentration than that of dark roasting.

There are also differences interns of the CGA content across different coffee verities.
Acknowledgements

The authors wish to acknowledge Jimma University, McGill University, and Nova Scotia Agricultural College for the arrangement of this short term training in Canada, at McGill University; CIDA Canada for funding the PHMIL project.

References


