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Original Article

Comparison of liberica and arabica coffee: chlorogenic acid, caffeine, total phenolic and DPPH radical scavenging activity

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Abstract

Information on the composition of chlorogenic acid, caffeine and antioxidant activity of *Coffea liberica* is scarce, albeit the importance of this species in some parts of the world. This study assessed the composition of chlorogenic acid, total phenolic, caffeine and free radical scavenging activity in green and roasted *C. liberica* in comparison to *C. arabica*. The compositions of these compounds were also investigated in *C. liberica* at different roasting degree. We found a comparable amount of chlorogenic acid in green *C. liberica* and *C. arabica*. However, roasted *C. arabica* had a significantly higher chlorogenic acid content than roasted *C. liberica* ($p<0.05$). Chlorogenic acid content significantly reduced in *C. liberica* after roasting when compared to green beans ($p<0.05$). There was an insignificant difference of caffeine content between the green and roasted beans of both coffee varieties. Total phenolic content were of comparable value between *C. liberica* and *C. arabica* for both green and roasted beans. There was a trend for higher total phenolic content in roasted *C. liberica* when compared to green beans, although significant difference was observed only in medium-dark roast ($p<0.05$). DPPH scavenging activity was comparable between *C. arabica* and *C. liberica* for both green and roasted beans, and was significantly reduced in *C. liberica* after roasting ($p<0.05$). Both green *C. arabica* and *C. liberica* had similar DPPH scavenging activity to the standards (BHT and α-tocopherol). These data can aid in promoting the production of *C. liberica* alongside *C. arabica* that has been regarded as a premium quality coffee.

Keywords: Antioxidant, Chlorogenic acid, *Coffea arabica*, *Coffea liberica*, Polyphenols

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Introduction

Coffee consists more than 90 different species, with *Coffea arabica* (Arabica coffee) and *Coffea canephora* (Robusta coffee) being the most important species economically in the world market (Briandet et al., 1996). *C. arabica* has higher commercial value

due to its favorable sensory and aromatic characteristics while *C. canephora* is commonly employed in coffee industry due to its high extractability of soluble solids (Clarke, 1987). Chlorogenic acids are the most prevalent phenolic compound found in coffee (Farah et al., 2005). Besides the contribution of this phenolic to the bitterness in coffee, chlorogenic acid has been associated with the possible health benefits from drinking coffee. Being a rich source of various bioactive compounds, coffee can be considered as functional food. The antioxidant properties from the bioactive compounds are reported to reduce the prevalence of cancer, liver and diabetes, fight against Parkinson's disease and even lower the risk of mortality (Bhupathiraju et al., 2013; Cano-Marquina et al., 2013).

Liberica coffee (*C. liberica)* which is considered to have less commercial value is grown at warm tropical area occurring in lowland such as Liberia, Surinam and Malaysia (Lim, 2013). Previous studies have mainly compared the composition of chlorogenic acid and caffeine between *C. arabica* and *C. canephora* (Robusta coffee), being the two most demanded coffee species in the world. However, the information on the composition of chlorogenic acid, caffeine and antioxidant activity in *C. liberica* coffee is scarce, albeit the importance of this species in some parts of the world. This study assessed the composition of chlorogenic acid, caffeine and free radical scavenging activity in green and roasted *C. liberica* as compared to *C. arabica*. The compositions of these compounds were also investigated in *C. liberica* at different roasting degree (medium-dark roast, dark and heavy roast).

Material and Methods

Plant material and chemicals

Unroasted and roasted *C. arabica* and *C. liberica* beans were obtained from a coffee plantation in Kg. Bukit Batu, Johor, Malaysia. Coffee beans obtained were roasted at three different roasting conditions (medium-dark roast: 222-226°C, dark roast: 230-234°C, heavy roast: 235-238°C for 14 to 16 minutes). Diphenyl picryl hydrazyl (DPPH) radicals, butylated hydroxyl toluene (BHT), α-tocopherol and Folin Ciocalteu's reagent were obtained from Sigma Aldrich (Malaysia).

Extraction of phenolic and caffeine

Coffee beans were ground with Krupps coffee grinder. Five grams of the grounded coffee was brewed with 100 ml of distilled water, then an aliquot (3 ml) was eluted with methanol through solid phase extraction cartridge (C18, 3 ml cartridge, Phenomenex®). This extraction was done in triplicate for each coffee sample. Extracts were diluted accordingly for each analysis based on the comparison to the authentic standard.

High Performance Liquid Chromatography (HPLC)

The chlorogenic acid and caffeine profile of the coffee samples was determined using High Performance Liquid Chromatography (HPLC). The system was equipped with an Agilent 1100 Series Solvent Delivery System and autosampler and a PDA detector (Agilent 1100). Results were analysed using Chemstation software. A Pursuit C18 (5µ; 250 x 4.6 mm) column eluted with a mobile-phase (1 mL/min) of both high performance liquid chromatography grade solvent A (2% (v/v) formic acid) and solvent B (acetonitrile) was used for separation. 20 µL of the extracts were injected and the elution program was as follows: 10% B to 15 min, 50% B to 35 min, 80% B to 38 min and 100% B to 42 min. Detection was set 350 nm. Chlorogenic acid and caffeine was quantified by comparing peak areas against a calibration curve of the standards.

Total phenolic content

Total phenolic concentration was measured in coffee extracts using Folin Ciocalteu's method (Folin and Ciocalteu, 1927) in triplicates. Gallic acid with concentration range between 0 and 200 µg.mL−1 was used as standard in this assay. Coffee extracts were diluted to fit in the standard curve linear range. 20 µL of standards, blank and extracts prepared were loaded into a cuvette. 100 µL of Folin Ciocalteu's reagent (diluted 1:10 with ultrapurified water) was added to the cuvette and mixed. The microplate was incubated at room temperature for 5 min. Then, 80 µL of 7.5% sodium carbonate was added to the microplate and mixed well. The microplate was covered and then incubated at 45°C for 30 min in dark. After the incubation, absorbance was read at 765 nm using the spectrophotometer. The area under the curve was calculated and results were expressed in mg.kg−1 fresh weight gallic acid equivalents (GAE).

Radical scavenging activity using DPPH assay

DPPH assay was performed according to Vignoli et al. (2011) with some modifications. The assay measures free radical scavenging activity using diphenyl picryl hydrazyl (DPPH) radicals in triplicates with butylated hydroxyl toluene (BHT) and α-tocopherol. A solution of 0.1 mM DPPH in methanol was prepared fresh for the assay. Then, 3 ml of sample was added to 1 mL of DPPH solution, shaken and incubated for 30 min in the dark at room temperature. Absorbance was monitored at 517 nm. Reaction mixture containing control and reference standard (BHT and α-tocopherol) were also measured. The radical scavenging activity is calculated with the following equation:

$$DPPH\ scavenging\ effect\ (\%\ of\ inhibition) = \frac{A_0 - A_i}{A_0} \times 100$$

where A_0 = absorbance value of control reaction

A_i = absorbance value of sample or standard

Results and Discussion

Chlorogenic acid content in unroasted *C. liberica* and *C. arabica*

Green (unroasted) coffee beans contains phenolic compound such as chlorogenic acids and its derivatives. In this study, chlorogenic acid content was expressed in milligram per gram of weight sample (mg/g). The result from the study (Figure 1) displayed comparable values of chlorogenic acid for both unroasted *C. liberica* and *C. arabica* with the mean of 1.6 and 2.2 mg/g, respectively.

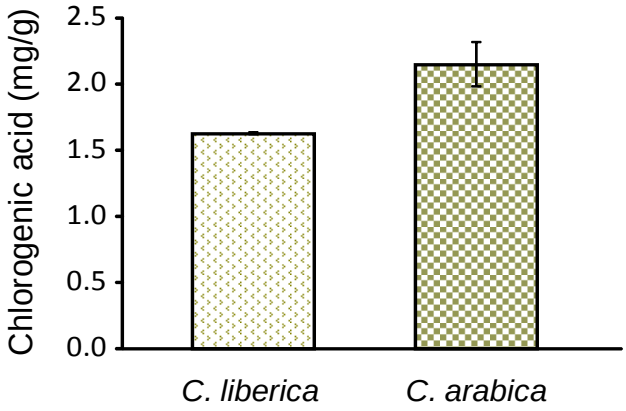

Fig. 1. Chlorogenic acid in unroasted *C. liberica* and *C. arabica*. Data are presented as mean ± SE.

According to Watanabe et al. (2006) and Shimoda et al. (2006), chlorogenic acid content in unroasted (green) bean shown positive effects for metabolic disorders condition when ingested. The content of chlorogenic acid in the coffee extract is dependent on the species, the variety, as well as the processing conditions of the coffee beans (Daglia et al., 2000; Moreira et al., 2005). Green coffee beans has been marketed as dietary supplements and this would suggest a favorable utilization of the unroasted *C. liberica* beans if it can be further proven to benefit human health.

Chlorogenic acid content in medium-roasted *C. liberica* and *C. arabica*

C. arabica is commonly roasted to the degree of medium roasting prior brewing for consumption. In order to observe the difference of chlorogenic acid content in roasted *C. arabica* and *C. liberica*, we therefore assess the two coffee beans roasted at the same degree of roasting (medium-roasted). The chlorogenic acid content was found significantly higher in medium-roasted *C. arabica* when compared to *C. liberica* ($p<0.05$) (Figure 2).

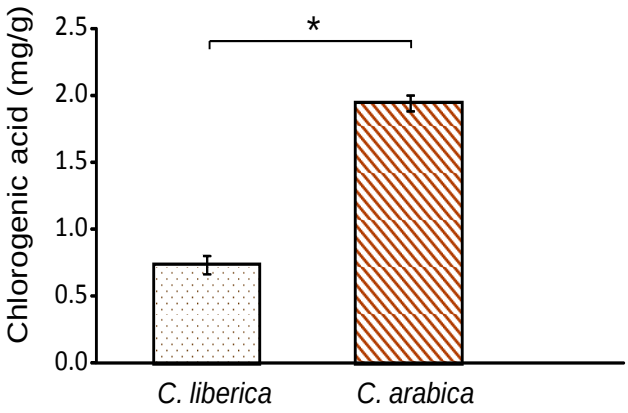

Fig. 2. Chlorogenic acid in med-dark roasted *C. liberica* and *C. arabica*. Data are presented as mean ± SE. (*) $p < 0.05$.

Medium-dark roast *C. arabica* had nearly 3 fold higher chlorogenic acid than *C. liberica*. Information on the different amount of chlorogenic acid between these two coffee species from previous studies is scarce. However, the composition of this compound has been reported for its variability across different wild coffee species (Campa et al., 2005). Chlorogenic acid was previously reported to be higher in *C. canephora* than *C. arabica* (Ky et al., 2001).

Effect of different roasting degree on the chlorogenic acid content in *C. liberica*

We thereafter assessed the effect of different roasting degree in *C liberica.* Figure 3 shows a significantly ($p<0.05$) reduced chlorogenic acid content of roasted *C. liberica* when compared to green beans.

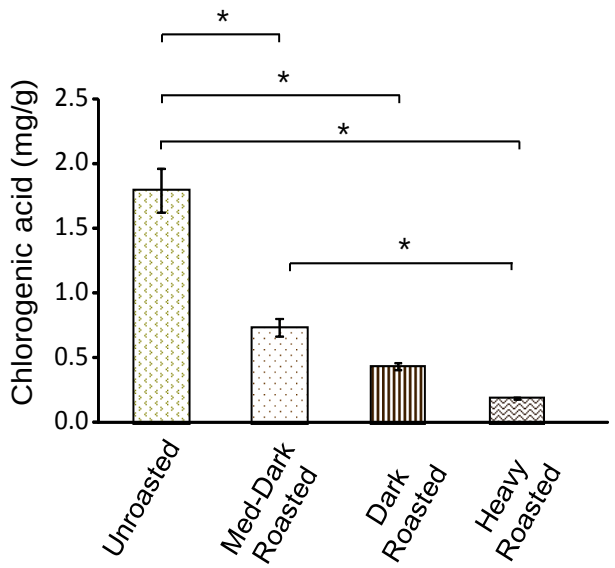

Fig. 3. Chlorogenic acid in unroasted and roasted *C. liberica*. Data are presented as mean ± SE. (*) $p < 0.05$.

The high temperature of the roasting process induces a breakage of the carbon-carbon bonds of chlorogenic acids which ends up resulting in isomerization in the early phase of roasting and later followed by epimerization, lactonization, and degradation (Trugo and Macrae, 1984; Moon et al., 2009; Perrone et al., 2008). Chlorogenic acids break down into smaller-molecule phenols and other chlorogenic acid lactones such as 3-caffeoylquinic-1,5-lactone (3-CQL), 4-caffeoylquinic-1,5-lactone. Trugo and Macrae (1984) reported that increasing degradation of chlorogenic acids during roasting provides the final formation of aroma of the roasted coffee, which may explain the stronger aroma of coffee in high-roasted coffee beans. As stated by Vignoli et al. (2014), roasting process *reduces* the amount of chlorogenic acid content in *C. arabica* and *C. canephora*, which supports our observation. The chemical composition and biological activity of the coffee beans changes during the roasting process.

Total phenolic content in unroasted *C. liberica* and *C. arabica*

Total phenolic content (TPC) is an assay conducted to determine the secondary metabolites phenolic compound in plants and food and beverages derived from plants. Using electron transfer-based assay as its principle, this method evaluates the antioxidant contents of particular constituents by measuring the decrement of oxidants. Figure 4 illustrates the total phenolic content of unroasted *C. liberica* and *C. arabica*. The result shows a comparable value between both samples with the mean of 18.8 and 14.4 mg GAE/g, respectively. Phenolic compounds have been acknowledged as potentially defensive determinant against cancer, cardiovascular disease as well as human chronic degenerative diseases such as cataracts, macular degeneration, neurodegenerative diseases, and diabetes mellitus (Scalbert et al., 2005). The comparable value of the phenolic content in *C. liberica* to the *C. arabica* can be a promoting point to further expand the market of this coffee species.

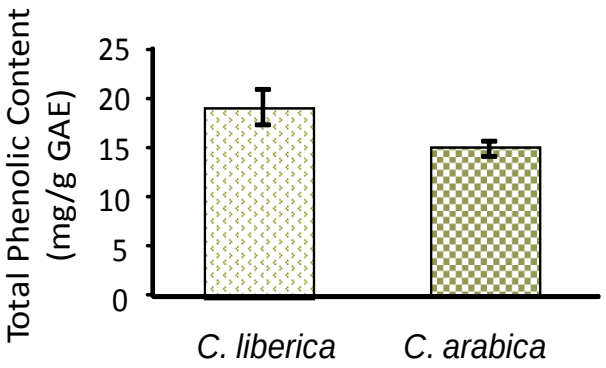

Fig. 4. Total phenolic content in unroasted *C. liberica* and *C. arabica*. Data are presented as mean ± SE.

Total phenolic content in in medium-roasted *C. liberica* and *C. arabica*

Comparison between total phenolic content in *C. liberica* and *C arabica* is illustrated in Figure 5. In this study, we found no significant difference between medium-dark roast *C. liberica* and medium-dark roast *C. arabica* ($p>0.05$). This further supports the importance of *C. liberica*, a less commercial value coffee due to its comparable level of the bioactive compound when compared to the *C. arabica* which is known for its superior quality.

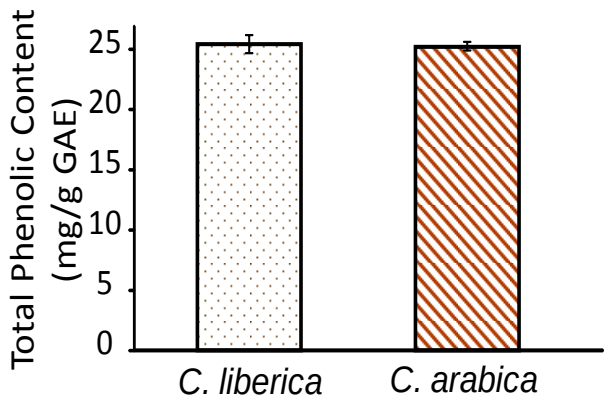

Fig. 5. Total phenolic content in roasted *C. liberica* and *C. arabica*. Data are presented as mean ± SE.

Effect of different roasting degree on the total phenolic content in *C. liberica*

The amount of total phenolic content in unroasted and roasted *C. liberica* is presented in Figure 6. Based on the result, medium-dark roasted *C. liberica* showed a significantly increased of total phenolic content when compared to the unroasted *C. liberica* ($p<0.05$). This might be the consequence of the depletion of other compounds that are more susceptible to heat, causing a relative increment in levels of the remaining ones.

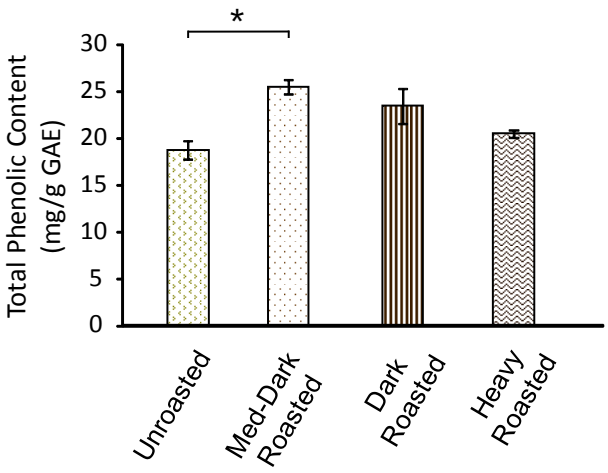

Fig. 6. Total phenolic content in unroasted and roasted *C.liberica*. Data are presented as mean ± SE. (*) $p < 0.05$.

The condition of high temperature and low water activity during roasting favoured the occurrence of Maillard reaction. This reaction involved some participation of phenolic compound which then becoming part of the melanoidins (Bekedam et al., 2008; Nunes and Coimbra, 2001; Wang et al., 2011). The level of melanoidins is directly proportional to the degree of roasting. Melanoidins which formed during roasting can react with the Folin-Ciocalteu's reagent (López-Galilea et al., 2007). This explains the significant increase in the total phenolic content of medium-dark roasted bean. A severe degree of roasting results in a reduction of total phenolic content. A loss of up to 60% were reported when mild roasting condition was used and nearly 90% of the initial phenolic compound could be degraded after a severe roasting process (Trugo and Macrae, 1984). Therefore, roasting process is crucial to retain the crucial bioactivities in coffee beans.

Caffeine content in *C. liberica* and *C. arabica* in both roasted and unroasted

As for the caffeine content in green (unroasted) bean, *C. liberica* showed no significant difference ($p<0.05$) when compared to *C. arabica* (Figure 7). However, when roasted beans were evaluated, it was found that caffeine content of roasted *C. liberica* was higher than *C. arabica*. Variation of caffeine content was also observed in previous study by Kreicbergs et al. (2011) that compared 18 different coffees available in the local market in Latvia.

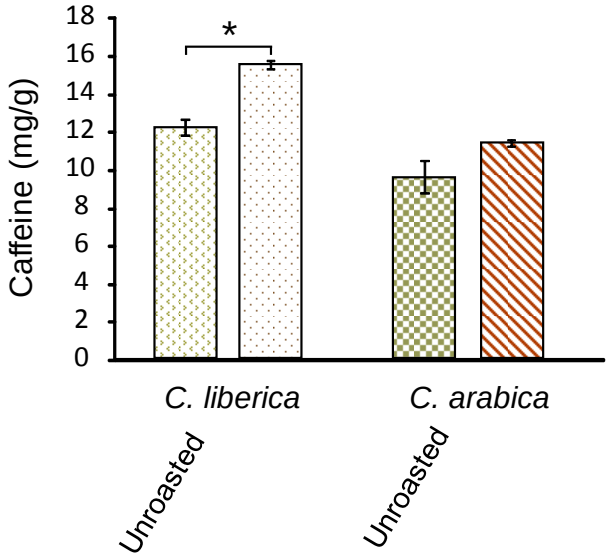

Fig. 7. Caffeine in unroasted and roasted *C. liberica* and *C. arabica*. Data are presented as mean ± SE. (*) $p < 0.05$.

This study reported a higher amount of caffeine in coffee brands containing *C. canephora* than *C. arabica*, which supports our observation of lower

caffeine content in *C. arabica*. Caffeine varies considerably relying on the coffee species, the method of bean-roasting and beverage preparation. Caffeine has numerous pharmacological effects, including on central nervous system and vascular tissue (Echeverri et al. 2010).

Conclusion

The result obtained in this study provide the data on the composition of chlorogenic acid, caffeine and DPPH free radical scavenging activity in green and roasted *C. liberica* as compared to *C. arabica*. Along with, an overview of the effect of different roasting conditions on the polyphenolic and caffeine composition, as well as the free radical scavenging activity of *C. liberica* are laid out. The results revealed that the bioactive compounds and antioxidant properties of different coffees vary depending on the coffee variety and are affected by the roasting conditions. In this study, roasting process decreased the amount of chlorogenic acid in *C. liberica* beans. Chlorogenic acid content of both beans showed a significant reduction after going through the roasting process while caffeine content showed no significant difference in chlorogenic acid content. Roasted *C. arabica* exhibited higher chlorogenic acid content than roasted *C. liberica* despite a comparable amount of this compound that was discovered initially in both green (unroasted) beans. Total phenolic content and DPPH scavenging activity showed a comparable value exerted by *C. liberica* and *C. arabica* for both green and roasted beans. This study fills the gap of information on the composition of the biological active compounds and the antioxidant capacity in the less demanded coffee species (*C. liberica*). In that respect, *C. liberica* can be potentially promoted to be utilized as a dietary source of the beneficial compound like polyphenols alongside *C. Arabica.*

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Contribution of Authors

Mubarak A: Design of work, data collection, data analysis and interpretation, drafting the article, critical revision of the article and final approval of the version to be published
Croft KD: Data collection, data analysis and interpretation and drafting the article.
Bondonno CB: Data collection, data analysis and interpretation and drafting the article.
Din NS: Data collection, data interpretation and drafting the article.

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