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Changes in the total phenolic contents, chlorogenic acid, and caffeine of coffee cups regarding different brewing methods

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Abstract

Coffee is among the most popular beverages consumed worldwide due to its taste, flavor, and alertness effects on drinkers. Besides, caffeine and chlorogenic acid are known as featured compounds of coffee, and there are various phenolic compounds, that demonstrate many benefits for human health. However, the concentrations of those compounds ingested into the human body are greatly dependent on how coffee infusions are prepared. The extraction efficiency of phenolic compounds, caffeine, and chlorogenic acid in the Arabica and Robusta coffee obtained from the light, medium, and dark roasting levels, using the hot brew (at 50°C and 90°C) and cold brew methods was studied. The total phenolic contents (TPCs) were measured by the Folin-Ciocalteu assay, while the first -order derivative spectrophotometry was used to determine the caffeine and chlorogenic acid. Generally, light-roasted Robusta had the highest caffeine (2.10±0.017% w/w), chlorogenic acid (5.63±0.011% w/w), and TPCs (45.5±0.9 mg GAE/g). For brewing experiments, 10 g of coffee was brewed (100 mL of water) for different extraction times. The results show that hot brewing at 90°C was the most efficient method for extracting the TPCs and chlorogenic acid, while cold brewing was better for extracting caffeine. Extraction efficiency increased with longer extraction times, especially for the light roasting level.

1. Introduction

Coffee is a common beverage consumed globally due to its taste, aroma, and ability to provide a stimulating effect for drinkers (Górnaś *et al.*, 2016). Various volatile compounds, which can be formed or lost during the processing stages, have been reported to demonstrate a certain influence on the aroma and flavor of coffee beans (Murata, 2021). Coffee has a diverse chemical composition, with over 1000 identified compounds. Coffee contains various compounds, such as acids, alkaloids, alcohols, amines, esters, ketones, organosulfur compounds, phenols and triglycerides (Alves *et al.*, 2021). Among these components, phenolic compounds or polyphenols have been shown to demonstrate high biological activity and health benefits (Rana *et al.*, 2022). Coffee consumption can reduce the risks of different cancers (e.g., colorectal and oral cancers), inhibit LDL cholesterol oxidation, protect against Parkinson's disease, and even reduce mortality rates (Król *et al.*, 2019). Phenolic compounds have also been shown to be effective in preventing acute and chronic diseases (Cory *et al.*, 2018). The main phenolic compounds in coffee include caffeine, chlorogenic acid, diterpenes, and trigonelline (Yesil and Yilmaz, 2013). Specifically, chlorogenic acid, caffeine, and melanoidin contribute to the antioxidant activity of coffee (Santos and Lima, 2016). Chlorogenic acid is abundant in coffee beans, particularly in green, unroasted beans. Chlorogenic acid has been extensively studied for its potential health benefits and helps protect cells against oxidative stress caused by free radicals. It is also believed to have anti-inflammatory effects and may contribute to cardiovascular health by supporting healthy blood pressure levels as well as preventing cardiovascular diseases (Campa *et al.*, 2005). Additionally, coffee consumption can increase energy levels and promote alertness due to the presence of caffeine. This neurotransmitter inhibitor stimulates the central nervous system in the brain, enhancing the brain function (Fiani *et al.*, 2021). Caffeine is a natural

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stimulant, widely recognized as one of the primary bioactive compounds in coffee. It blocks adenosine receptors in the brain, leading to the increased alertness and reduced drowsiness (Hill *et al.*, 2007). It also stimulates the release of dopamine and norepinephrine, contributing to improved mood and cognitive function (Rao *et al.*, 2020). The common factor among phenolic compounds, caffeine, and especially chlorogenic acid, is their degradation under different temperature conditions during the roasting period (Liczbiński and Bukowska, 2022). Based on differences in temperature and time, the roasting can be classified as “light, medium, and dark” levels. Light roasting involves the lowest temperature and shortest time, typically around 200°C for 10 mins. On the other hand, dark roasting involves higher temperatures and longer time, reaching 240°C for 14 mins. Medium roasting falls between these extremes, typically at 220°C for 12 mins (Yeager *et al.*, 2022). Phenolic compounds are susceptible to temperature, starting to be degraded at around 180°C, i.e., these compounds could be more and more degraded regarding the longer roasting time and higher temperature (Ross *et al.*, 2011). A study by Awwad *et al.* (2021) reported a remarkable decline in phenolic compounds (from 7.3% to 32.1%) with longer roasting time. A prominent phenolic compound in coffee is chlorogenic acid. Its characteristic feature is its temperature sensitivity. During roasting, chlorogenic acid undergoes slow degradation, approximately 50%, to produce caffeic acid and quinic acid, contributing to the pronounced bitterness in coffee (Campa *et al.*, 2005). In 2017, Jeon *et al.* conducted a study investigating chlorogenic acid and caffeine levels in coffee brewed under different conditions. The results showed the highest chlorogenic acid in Ethiopian coffee samples ranging from 365.01 mg/L to 1020.4 mg/L. The caffeine content was found as the highest for Costa Rican coffee brewed under medium conditions (576.96 mg/L) and the lowest was observed in dark-roasted Brazilian coffee (460.74 mg/L) (Jeon *et al.*, 2017).

Unlike other phenolic compounds, caffeine content remains relatively stable during the roasting process, with variations mainly attributed to coffee bean varieties, with Robusta coffee containing higher caffeine content than Arabica coffee (Perrois *et al.*, 2015). Coffee beans naturally contain caffeine as a defense mechanism, acting as a natural pesticide to deter insects and pests (Fiani *et al.*, 2021). Robusta coffee plants are grown harsher than Arabica, leading to higher caffeine content. Similarly, the polyphenolic compounds and chlorogenic acid content of Robusta coffee are also superior to Arabica and Liberica coffee (Farah *et al.*, 2008). Chlorogenic acid is a phenolic acid with antioxidant, anti-inflammatory, neuroprotective, hypolipidemic, and hypoglycemic properties (Grosso *et al.*, 2016). Hečimović *et al.* (2011) found that Robusta coffee samples had higher caffeine content than Arabica or Liberica coffee, and the roasting process did not significantly impact caffeine levels. Previous studies on the variations of polyphenolic compounds, caffeine, and chlorogenic acid in coffee have reported similar findings (Thanh-Nho *et al.*, 2021; Anh-Dao *et al.*, 2022; Anh-Dao, Thanh-Nho, Huu-Trung *et al.*, 2023; Anh-Dao, Minh-Huy, Quoc-Duy *et al.*, 2023). While there are differences in the caffeine, chlorogenic acid, and phenolic compound levels among different coffee types, it is necessary to evaluate the actual concentrations of these compounds in each cup of coffee consumed. Hot water can extract caffeine, chlorogenic acid, and polyphenols from the ground coffee during the brewing period. The concentrations of these compounds in a cup of coffee can vary depending on the types of coffee beans, brewing methods, and ground coffee sizes. There are various brewing techniques, such as AeroPress, drip, espresso, French press, and simple infusion, but in general, the brewing can be categorized based on temperature and brewing time (Janda *et al.*, 2020). In addition to conventional brewing methods using a water temperature of above 50°C, the cold brew method has recently become increasingly popular and involves steeping coffee grounds in water at temperatures ranging from 20-25°C (or lower) for a longer period (Angeloni, Guerrini, Masella, Bellumori *et al.*, 2019; Cordoba *et al.*, 2019). With the diverse range of chemical compounds present in coffee, altering the brewing time and temperature can affect the extraction capabilities of these compounds, thereby influencing the quality of the produced beverage. Therefore, there has been increasing interest in exploring the relationship between the chemical composition of cold brew coffee and other brewing conditions (time, temperature, methods, coffee powder-to-water ratios, etc.) obtained from different coffee types at various roasting levels (Ginz *et al.*, 2000; Moon and Shibamoto, 2009; Vignoli *et al.*, 2014).

In 2019, Angeloni and co-workers surveyed the extraction effects on the caffeine and chlorogenic acid in espresso, Moka, Neapolitan, French press, filter, Turkish, cold brew, and capsule coffee. Espresso showed the highest caffeine, chlorogenic acids, melanoidins, and antioxidant activity. On the other hand, the cold brew had the lowest chlorogenic acids and melanoidins as well as antioxidant activity (Angeloni, Guerrini, Masella, Innocenti *et al.*, 2019). In another study, the impacts of roasting levels and brewing temperature (4°C, 22°C, and 92°C) on the caffeine and chlorogenic acid were investigated. The study found that the highest caffeine was obtained from medium-roasted coffee using the cold brew. Additionally, increasing roasting temperatures

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resulted in the decomposition of chlorogenic acid precursors (Cordoba *et al.*, 2019). Fuller and Rao (2017) investigated the effect of extraction time in different cold brew coffee types on the total phenolic compounds, which indicated that the highest was 1.50±0.37 g GAE/L in cold brew coffee, compared to 1.36±0.14 g GAE/L in hot brewed coffee.

Coffee contains various chemical compounds, e.g., phenolic compounds, chlorogenic acid, and caffeine. The present study aimed to explore the levels and variations of these compounds during the coffee brewing process. Brewing conditions, especially temperature and time, can significantly impact the concentrations of nutritional components, e.g., phenolic compounds, chlorogenic acid, and caffeine, which were demonstrated in the present study. Arabica and Robusta coffee products obtained from three different roasting levels, "light, medium, and dark", were used.

2. Materials and methods

2.1 Chemicals and reagents

Caffeine (98%, $C_8H_{10}N_4O_2$) and chlorogenic acid (≥ 95%, $C_{16}H_{18}O_9$) from Sigma-Aldrich, USA; gallic acid monohydrate (99%, $C_6H_2(OH)_3COOH.H_2O$), methanol (99.9%, CH_3OH), sodium carbonate anhydrous (99.0%, Na_2CO_3), and Folin-Ciocalteu reagent (2 N) were purchased from Merck, Germany.

2.2 Sampling and brewed coffee preparation

In the present study, two coffee varieties used were Arabica and Robusta, obtained from coffee suppliers in the Di Linh district, Lam Dong province. The coffee samples were roasted using three different roasting levels: light, medium, and dark. The light roast was conducted from 195°C to 210°C for 10 to 15 mins. The medium roast was performed between 210°C and 220°C for 20 mins. The dark roast lasted from 230°C to 240°C for 20 mins.

For the complete extraction of total phenolic compounds, caffeine, and chlorogenic acid, the reference extraction condition was applied, i.e., total phenolic compounds: 0.200 g of the coffee sample was extracted in 10 mL of a methanol: water mixture (70:30, v/v) for 20 mins at 70°C (Thanh-Nho *et al.*, 2021; Anh-Dao *et al.*, 2022); caffeine and chlorogenic acid: 0.200 g of coffee was extracted in 10 mL of deionized water at 90°C for 60 mins (Anh-Dao, Minh-Huy, Quoc-Duy *et al.*, 2023).

For coffee infusions, 10 g of ground coffee was weighed into a filter bag, and then placed into a beaker for brewing with 100 mL of water at temperatures of 50°C and 90°C for various time intervals, 5 to 60 mins. The cold brew method was conducted similarly, but the extraction process was carried out in a refrigerator at temperatures ranging from 4 to 9°C. The extraction efficiency was calculated as the ratio (%) between the concentrations found from each brewing condition (coffee infusions) and the reference extraction conditions (complete extraction).

2.3 Total phenolic contents

The measurement of total phenolic contents in coffee products was conducted following previous publications (Thanh-Nho *et al.*, 2021; Anh-Dao *et al.*, 2022). For the colorimetric reaction, the Folin-Ciocalteu reagent was utilized, with gallic acid as the standard compound for constructing the calibration curve. Initially, 0.5 mL of the sample was mixed with 2.5 mL of the Folin-Ciocalteu reagent (10%, v/v) for 3-8 mins. Subsequently, 2.0 mL of 7.5%, w/v sodium carbonate was added, followed by a 60-min incubation period. The same colorimetric assay was applied for the brewed coffee (different temperatures and times). The absorbance at 765 nm was then measured.

2.4 Caffeine and chlorogenic acid

The first-order derivative spectra were employed to determine the levels of caffeine and chlorogenic acid (Anh-Dao, Minh-Huy, Quoc-Duy *et al.*, 2023). The UV-Vis measurements were performed with the following parameters: scanning range of 200-400 nm, interval of 0.5 nm, and intermediate scanning speed. The "zero-crossing" method was employed to identify the quantification wavelengths for caffeine and chlorogenic acid based on the first derivative spectra. The zero-crossing point for chlorogenic acid was 261.5 nm, while for caffeine, it was 272 nm.

All samples were conducted in triplicates (n = 3) to ensure favorable precision.

3. Results and discussion

3.1 Total phenolic compounds, chlorogenic acid, and caffeine in different types of coffee

The results obtained from the complete extraction were used to compare the target compound concentrations among different coffee products. Table 1 shows that the Robusta had higher caffeine, chlorogenic acid, and total phenolic contents/compounds (TPCs) than the Arabica, with the highest levels observed in the Robusta-Light coffee sample. For caffeine, the concentration in Robusta coffee ranged from 1.81% to 2.10%, approximately twice as high as in Arabica coffee, where caffeine concentrations ranged from 0.78% to

Table 1. Total phenolic contents (TPCs), caffeine (CFI), and chlorogenic acid (CGA) in coffee products (complete extraction).

Coffee sample	CFI (% w/w)	CGA (% w/w)	TPCs (mg GAE/g)
Arabica-Light	1.30±0.034	4.11±0.060	35.7±0.3
Arabica-Medium	1.00±0.020	1.31±0.053	32.6±0.5
Arabica-Dark	0.78±0.015	0.30±0.027	27.8±0.7
Robusta-Light	2.10±0.017	5.63±0.011	45.5±0.9
Robusta-Medium	1.90±0.023	2.21±0.030	38.7±0.2
Robusta-Dark	1.81±0.020	0.85±0.010	35.3±0.1

1.30%. This could be attributed to the growth conditions of Robusta coffee plants in harsh environments, which results in higher caffeine content as a defense mechanism against pests and diseases (Liang and Kitts, 2014). The caffeine content was observed to be decreased according to the increasing roasting levels for the Arabica and Robusta coffee. However, the increase in roasting temperature did not severely affect the degradation of caffeine. The caffeine content decreased by 40% for Arabica and 13% for Robusta when comparing the dark roast condition to the light roast condition. Several published studies have demonstrated the observed trends mentioned above (Górecki and Hallmann, 2020; Muzykiewicz-Szymańska *et al.*, 2021).

Meanwhile, chlorogenic acid showed a significant decrease with increasing roasting conditions. The concentration of chlorogenic acid decreased by 93% and 85% in the dark roast condition compared to the light roast condition. This was due to the sensitivity of chlorogenic acid to heat, resulting in a gradual decrease according to the increasing roasting temperature. The heat sensitivity of chlorogenic acid has been demonstrated in previous studies (Moon *et al.*, 2009; Ludwig *et al.*, 2014; Awwad *et al.*, 2021; Misto *et al.*, 2022). Regarding total phenolic compounds, the trend was similar to caffeine and chlorogenic acid, with the highest levels observed in Robusta coffee and decreasing from light to medium to dark roast conditions.

However, during the roasting process, phenolic compounds undergo degradation and formation of other phenolic compounds, resulting in a slight decrease in the TPCs from light to medium roast. The TPCs decreased from 35.7±0.3 mg GAE/g in Arabica-Light to 32.6±0.5 mg GAE/g in Arabica-Medium. Similar trends in phenolic compounds have been observed in several publications for different types of coffee (Bobková *et al.*, 2020; Anh-Dao *et al.*, 2022; Anh-Dao, Thanh-Nho, Huu-Trung *et al.*, 2023).

3.2 Variation in total phenolic contents under different brewing conditions

Figure 1 presents the TPCs in brewed coffee from different time and temperature conditions (coffee infusions) for different types of coffee. The water temperature used for coffee brewing was set at 50°C (Figure 1a), 90°C (Figure 1b), and cold brew (Figure 1c). Overall, the TPCs increased with brewing time for all three temperature conditions. However, different

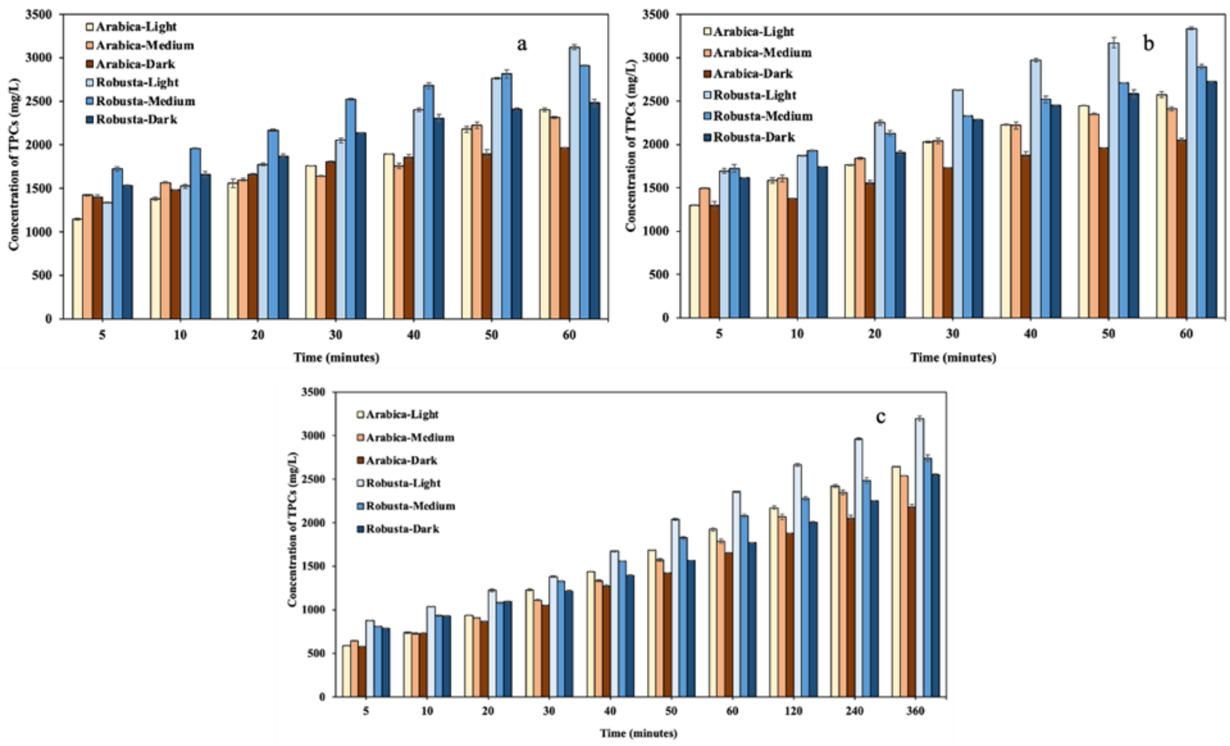

Figure 1. Total phenolic contents in coffee infusion at (a) 50°C, (b) 90°C and (c) cold brew.

temperature conditions showed variations in the rate of increase of phenolic compounds over time.

For the 50°C temperature condition (Figure 1a), the Arabica-Medium and Robusta-Medium coffee samples exhibited the highest TPCs within the 5-20-min range for Arabica and the 5-50-min range for Robusta. In the case of the light-roasted samples, the TPCs increased slowly in the initial stage. The extraction efficiency of the samples, as shown in Figure 1a, indicated that within the first 40 mins, the extraction efficiency increased by only 21% and 24% for Arabica-Light and Robusta-Light, respectively. However, in the next 20-min range (from 40^{th} min to 60^{th} min), the extraction efficiency increased by 14% (Arabica-Light) and 16% (Robusta-Light). In contrast, the TPCs in the coffee infusions of medium-roasted samples increased rapidly in the initial stage. Then, the TPCs increased at a slower rate in the later stage of the brewing process. Although the results in Table 1 shows that the Light-roasted samples had higher TPCs compared to the medium-roasted samples, the TPCs of the Arabica-Medium infusions within the 5-10-min range and the Robusta-Medium infusions within the 5-50-min range were higher than those of the corresponding Light-roasted samples. Similarly, the coffee infusions of Arabica-Dark within the 20-30-min range (1663.1-1803.1 mg GAE/L) had higher TPCs than Arabica-Light (1556.0-1760.9 mg GAE/L) and Arabica-Medium (1600-1641.1 mg GAE/L). This could be explained by the fact that the medium and dark roasted samples were processed at higher temperatures for a longer time, resulting in higher coffee ground porosity, which allowed for better extraction (Rao *et al.*, 2020). This trend was also observed in the coffee infusions at 90°C (Figure 1b). Within the 5-30-min range (for Arabica) and 5-10-min range (for Robusta), the Arabica-Medium and Robusta-Medium infusions had the highest TPC compared to the same variety. However, in the first 5 mins, the TPCs in the infusions were 1299.1-1495.6 mg GAE/L and 1615.9-1726.3 mg GAE/L for Arabica and Robusta, respectively. In contrast, these values were only 1146.4-1425 mg GAE/L (for Arabica) and 1335.4-1723 mg GAE/L (for Robusta) at 50°C.

Regarding the effects of the brewing temperature, the TPCs of the cold brew infusions increased very slowly in the initial 40-min period. However, the TPCs then increased rapidly in the remaining brewing time. However, at the end of the cold brew brewing process, the TPCs reached 2183.3-2642.9 mg GAE/L (Arabica) and 2553.1-3195.2 mg GAE/L (Robusta), which is similar to the results obtained at 50°C and 90°C. Some previous studies showed that the coffee extract of Robusta had higher phenolic compound levels than Arabica, and the hot brewing method was more efficient in extracting phenolic compounds compared to cold brew (Muzykiewicz-Szymańska *et al.*, 2021; Maksimowski *et al.,* 2022; Zhang *et al.*, 2022). Phenolic compounds performed high antioxidant capacity, so the hot brewing method helped increase the antioxidant capacity in the extract. Therefore, if the purpose is to enhance the antioxidant activity, the hot brewing is recommended.

3.3 Variation in chlorogenic acid and caffeine contents

Figure 2 indicates the highest caffeine for the medium roasted Robusta using the hot brewing method (90°C and 60 mins) and the lowest caffeine for the dark roasted Arabica coffee using the cold brew for 5 mins.

Generally, higher caffeine content was found at higher brewing temperatures (90°C) and with longer brewing times. The higher the brewing temperature and the longer the brewing time resulted in the higher caffeine in the brewed coffee. The hot brewing methods (50°C and 90°C) were suitable for shorter brewing times. The results in Figure 2a and Figure 2b show that after 30 mins of hot brewing, the caffeine content ranged from 305.1-931.4 mg/L (50°C) and 312.2-1132.1 mg/L (90°C). The cold brew required a minimum of 60 mins to extract over 50% of the caffeine in the coffee (Figure 2c). The caffeine in the cold brew coffee varied from 117.5 to 1178.4 mg/L. The medium and dark-roasted coffee still exhibited higher caffeine content in the infusions at all three brewing temperatures since caffeine can be still stable under the brewing temperature ranges (Ludwig *et al.*, 2014). Therefore, the caffeine in the brewed medium-roasted and dark-roasted coffee samples was higher than that of light-roasted coffee. Besides its ability to improve focus and mood, caffeine also contributes to the bitter taste of coffee. Therefore, shorter brewing times and lower temperatures will limit the caffeine in the coffee cups, and the cold brew did not remarkably reduce the caffeine extraction efficiency.

Figure 3 demonstrates the chlorogenic acid content and extraction efficiency under different extraction conditions. The highest chlorogenic acid concentration was found in Robusta-Light coffee when extracted at 50°C for 60 mins, 3311.6 mg/L. Except for the Arabica-Dark coffee infusion, which showed no detectable chlorogenic acid, the lowest chlorogenic acid was observed in the dark roasted Robusta coffee (cold brew, 5 mins), 89.2 mg/L, which was only 1/37 of the concentration achieved under the best extraction condition. Although the porous structure of medium-roasted and dark-roasted coffee can facilitate water permeation and dissolution of chlorogenic acid into the infusion, the pronounced difference in chlorogenic acid content among roasting levels allows light-roasted

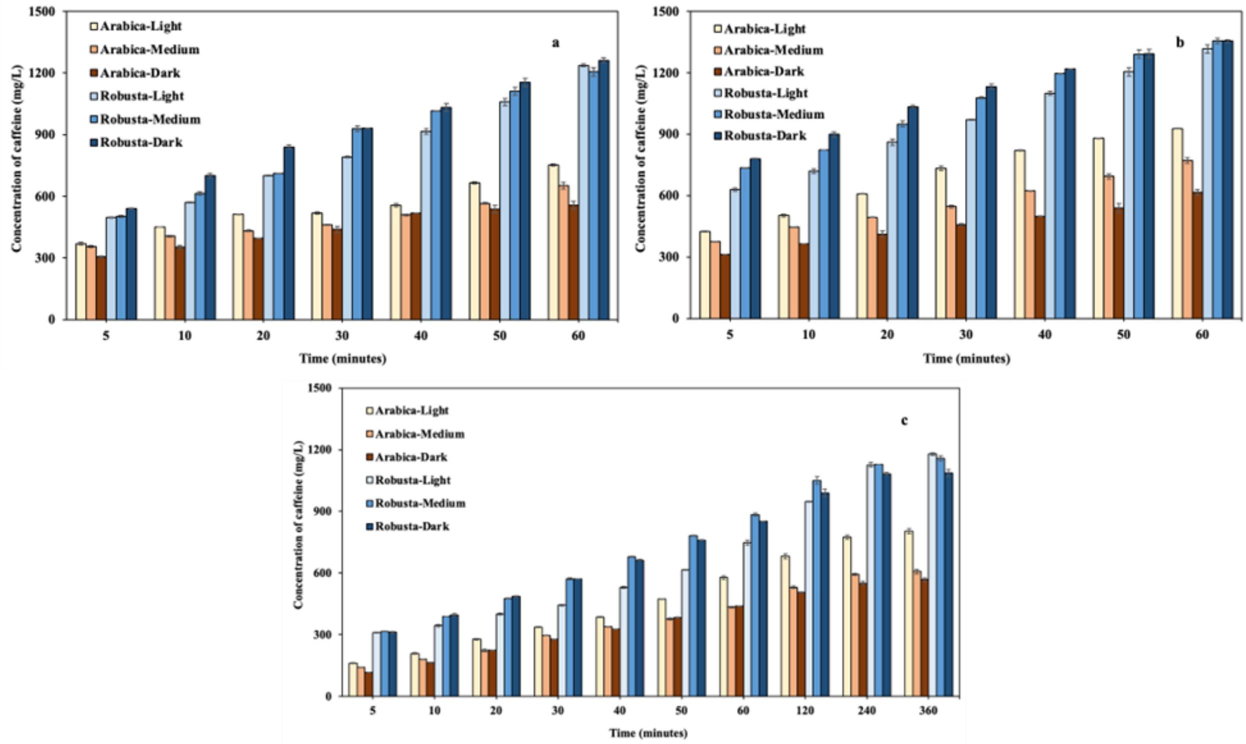

Figure 2. Caffeine in coffee infusion at (a) 50°C, (b) 90°C and (c) cold brew.

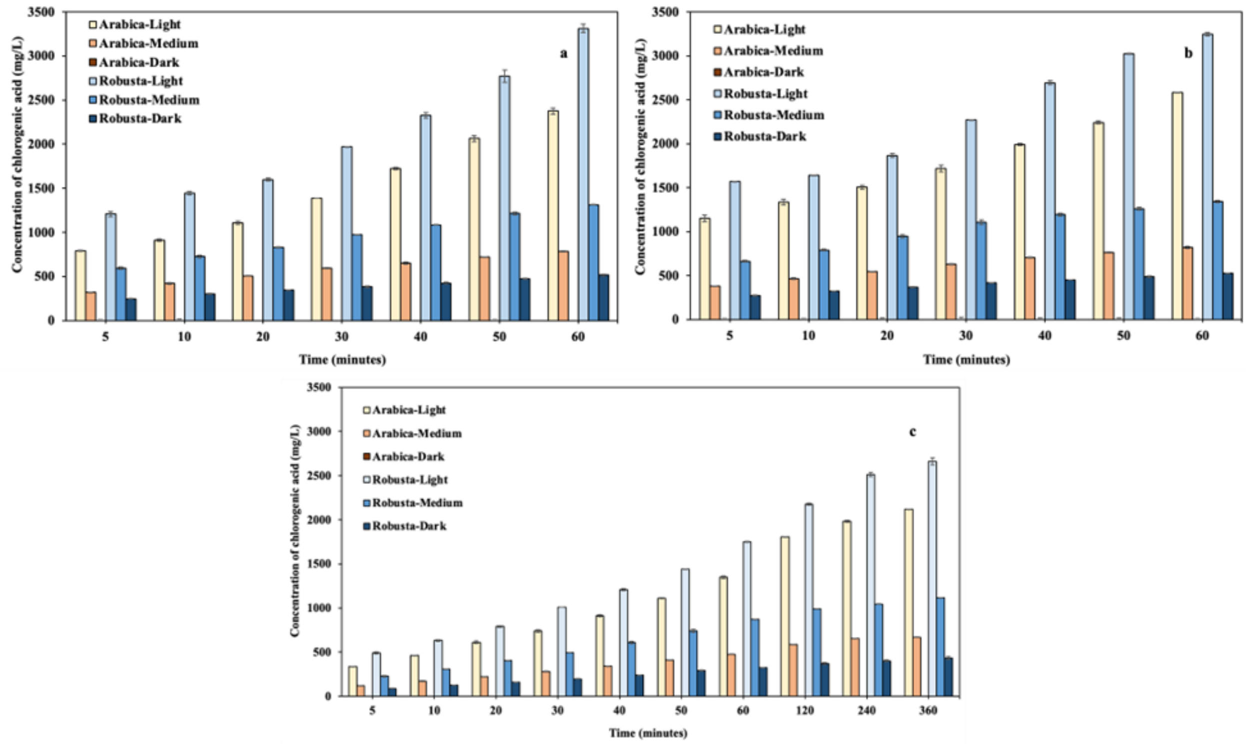

Figure 3. Chlorogenic acid in coffee infusion at (a) 50°C, (b) 90°C and (c) cold brew.

samples to maintain superior chlorogenic acid content in the infusions. Different extraction temperatures lead to different chlorogenic acid content in the coffee infusions, with chlorogenic acid concentrations ranging from 322.6 -3311.6 mg/L, 380.1-3246.7 mg/L, and 118.2-2660.0 mg/L for the 50°C, 90°C, and cold brew extraction conditions, respectively. Despite the six-fold longer extraction time of the cold brew method than the other two methods, the chlorogenic acid content extracted was lower by approximately 600 mg/L. Some previous studies have reported similar trends (Fuller and Rao, 2017; Jeon *et al.*, 2017). Chlorogenic acid is a crucial antioxidant compound in coffee, indicating that the cold brew method is inefficient when prioritizing antioxidant capacity in coffee extraction. However, chlorogenic acid contributes to the bitter taste, so the cold brew method can potentially reduce the bitterness in the coffee infusion.

4. Conclusion

The TPCs, chlorogenic acid, and caffeine were reported to be varied regarding different types (Arabica and Robusta) and roasting levels (light, medium and dark). Besides, the variation of those components in the coffee extracts prepared by hot brewing (at 50°C and 90°C) and cold brewing (at 4-9°C) was demonstrated. It was found that Robusta coffee had the highest TPCs, caffeine, and chlorogenic acid, which decreased from light to dark roasting levels. Hot brewing at 90°C extracted more phenolic compounds and chlorogenic acid than cold brewing, while cold brewing extracted slightly more caffeine than hot brewing. The TPCs, caffeine, and chlorogenic acid increased with longer brewing time, in which the medium and dark roasting levels required shorter brewing time than light roasting levels. Cold brewing can take a longer time to extract more target compounds than hot brewing. The present study can help users choose the optimal brewing temperature and time for their preferred concentrations of phenolic compounds, caffeine, and chlorogenic acid. Future research could explore other compounds, such as flavonoids, tannins, or antioxidants, and other factors, such as particle size and pressure-brewed coffee.

Conflict of interest

The authors declare no conflict of interest.

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