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Caffeine Quantity and Phenolic Compounds in a Daily Cup of Coffee and Tea†

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Abstract: Coffee is a popular beverage enjoyed by millions of people across the globe. In the last few decades, the health benefits associated with drinking coffee beverages has listed coffee as a functional food. Apart from caffeine, other important bioactive compounds in coffee include chlorogenic acid and their derivatives, theophylline, and theobromine, cafestol, kahweol, tocopherols and trigonelline. The aim of this study was to quantify the caffeine content and identify the most abundant phenolic compounds which may be present in a daily cup of coffee. Since small amounts of caffeine usually remains in the coffee spent (waste product) which may potentially be harnessed for pharmaceutical and nutraceutical purposes, it was also included in the study. Additionally, for comparison purposes black tea leaves were also analysed for their caffeine content. Samples of coffee beans (CB), coffee spent (CS) and tea leaves (TL) were obtained from a Central Queensland University coffee shop in May 2020; no particular brand name or type were associated. A HPLC-DAD analytical method was utilized for caffeine quantification, while comparison to UV spectral data of phenolic standards was explored to tentatively identify the compounds in the sample extracts. No significant difference ($p < 0.05$) in the caffeine content in the CB and TL was noted (1.7 and 1.9%, respectively). However, a lower caffeine content was noted in CS (0.467 ± 0.062%), as expected. This indicated that about 27.5% caffeine from CB is lost in the CS. UV spectral data of the peaks obtained in the HPLC chromatogram were tentatively identified as glycosides of chlorogenic acid, catechin, chlorogenic acid and caffeine. These results as a proof of concept work suggests that a daily cup of coffee and tea contains reasonable amounts of caffeine and presence of bioactive compounds which offer antioxidant potential and health benefits. Large generation of CS as a by-product in the production of coffee beverage may potentially be utilised as a source of caffeine.

Keywords: Coffee; caffeine; antioxidant; phenolic compounds; tea; coffee spent

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1. Introduction

Coffee is a popular beverage enjoyed by millions of people across the globe. It is prepared from roasted seeds (beans) from the plant genus *Coffea* and is thought to have originated in Ethiopia, around 800 AD and then made its way to Yemen in the 15th Century, and by the 16th Century it was known in Persia, Egypt, Syria, and Turkey. Historically, it was immensely popular for its qualities to help improve mental alertness and wakefulness, allowing people to devote more time to spiritual matters and prayers [1], [2]. By an estimate, 400 billion cups are consumed annually, making coffee the second-most traded commodity in the world, after crude oil [3]. Brazil is known to produce about a third of the world's coffee supply, which is roughly twice as much as Vietnam, the second largest producers. There are 500 species of coffee, however coffee beans used in coffee production are mostly from species *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) [1].

Coffee beverages are popular for its caffeine (Figure 1) content, the main alkaloid in coffee beans responsible for the unique aroma and bitter taste. Caffeine content in coffee beans is usually around 1-4% (dry weight basis) amidst the 700 compounds found in coffee, with large variations within cultivars [1], [4]. Whereas roasted coffee beans usually have around 1.3-2.4% caffeine [4]. Other than its popularity for increasing alertness through simulation of the central nervous system, rising blood circulation and respiration, caffeine also offers other health benefits which include mood enhancement, better exercise performance and reaction time, and reduction of symptoms associated with Parkinson's disease and tremors [4], [5]. Apart from caffeine, other important bioactive compounds in coffee include chlorogenic acid and their derivatives, theophylline and theobromine, cafestol, kahweol, tocopherols and trigonelline [1], [6], [7].

Studies have shown that antioxidative activity of coffee beans are attributed to the high occurrence of characteristic phenolic compounds, particularly chlorogenic acid [1], [8], [9]. Hence in the last few decades, the health benefits associated with drinking coffee have listed coffee as a functional food as opposed to previous beliefs which claimed it causes liver diseases. Nevertheless, some negative effects of caffeine still exist which includes insomnia and mild addiction, anxiety, restlessness, tension, nervousness and psychomotor agitation (unintentional and purposeless motions) [4]. Therefore, moderate consumption of this beverage is recommended, usually 200 to 300 mg of caffeine per day, which is equivalent to four to seven cups of coffee [2].

Figure 1. Chemical structure of caffeine, an active ingredient in coffee beans.

Whilst the extraction of coffee is the final step in its production process before consumption, it is the most important step as it determines the final quality of the brew. The extraction process is carried out at different scales such as industrial extraction for instant coffee production or extraction in domestic devices for a single cup of coffee [4], [10]. Regardless of the technique, boiling ground roasted coffee beans in water then filtering is common in all processes. Hence the aim of this study was to simulate a simple water extraction of some coffee beans as performed in households and quantify the caffeine content and identify the phenolic compounds possibly present in a daily cup of coffee. High coffee consumption generates high amount of waste residue, referred to as the coffee spent, which usually contains small amount of caffeine and hence was also included in this study. Caffeine is also found in black tea, although the levels are usually claimed to be half that of coffee [11]. Consequently, we also investigated the caffeine content of tea leaves extracted using the same process for comparison.

2. Materials and methods

2.1. Samples

Samples were obtained from a Central Queensland University coffee shop in May 2020; no particular brand name or type were associated. Around 3-4g of ground coffee beans, coffee spent (CS) and tea leaves (TL) was procured.

2.2. Extraction protocol

Triplicate extracts were prepared from approximately 0.5 g of each sample, combined with 7 mL of water, and boiled at 94 °C for 10 mins. The extract was then filtered through 110 mm Whatman Filter paper and rinsed with additional 4 mL of water. The total volume of the extract obtained was 12 mL.

2.2. HPLC analysis of Caffeine

Caffeine content analysis was conducted on an Agilent 1100 system, comprising a G1313A autosampler, G1322A vacuum degasser, G1311A quaternary pump, G1316A thermostatted column compartment and G1365B multi-wavelength detector module. A reversed phase C_{18} column was used (Agilent Eclipse XDBC18; 150 × 4.6 mm; 5 µm pore size) with an injection volume of 10 µL. The mobile phase comprised Methanol (20%) and 2% Acetic acid (80%) at flow rate of 1 mL/min and a total run time of 10 mins. The detection wavelength was set at 272 nm for caffeine. Quantitative analysis was performed with external standardization by the measurement of peak areas. The standards were prepared in the range of 5-100 ppm (R^2= 0.9998; response factor=18.47).

2.3. Statistical analysis and data validation

All the parameters studied were subjected to statistical treatment using RStudio running version R-4.1.2 statistical software. The data were expressed as mean ± SD of triplicate analysis. One-way ANOVAs were used to compare data between different samples, followed up by post-hoc Tukey testing (at $\alpha = 0.05$) if a significant result was returned.

3. Results

The average percentage quantity of caffeine in the extracts are given in Table 1. No significant difference ($p < 0.05$) in the caffeine content in the CB and TL was noted (1.7-1.9%, respectively). The results obtained generally aligned with previous literature [4]. The thermolabile nature of most polyphenols contribute to their degradation after prolonged exposure to heat [12], hence why low caffeine content is reported in roasted beans compared to green coffee. There are also several other factors which influence caffeine content in a coffee beverage. These may include the species and variety, origin, the growing conditions, storage, process of roasting the beans, grinding and finally the brewing method [12]–[14]. Interestingly, the use of nitrogen fertilizer may also result in high caffeine in coffee beans [12].

A significantly lower caffeine content ($p > 0.05$) was noted in CS (0.467 ± 0.062%), as expected. It is coherent with previously reported value of caffeine in CS (0.36-0.81%) [15]. On an estimate 1 ton of green coffee generates 650 kg of CS which often end up landfills resulting in environmental issues mainly related to its organic material, high moisture content (80-85%) and high acidity [16], [17]. High quantities of this waste can therefore be a useful source of bioactive compounds, excellent candidate to produce bioenergy and can also be used as biofertilizers. Although, a study reported that fresh CS as fertilizer induced plant stress due to the presence of caffeine, whereas composted CS contained fewer toxic components which substantially improved plant growth [16].

Table 1. Percentage Caffeine concentrations in the samples on a dry weight basis.

Samples	Caffeine Content (%)
Coffee beans	1.721 ± 0.085^{a}
Coffee spent	0.467 ± 0.062^{b}
Tea leaves	1.924 ± 0.161^{a}

Data are given as mean ± SD (n = 3). Values followed by the same letters within a column are not significantly different ($p < 0.05$) according to the least significant difference test.

Moreover, the HPLC chromatograms obtained are given in Figure 2. The peaks of interest from Figure 2 (A) were tentatively identified by comparing spectral characteristics of authentic standards of some polyphenolic compounds. The UV spectral data in Figure 3 demonstrates peak 1 (Frame A) to most likely be glycosides of chlorogenic acid, peak 4 (Frame B) to be catechin, peak 3 (Frame C) to be chlorogenic acid and peak 4 (Frame D) to be caffeine. The occurrence of these antioxidant compounds coincides with the literature [1], [2], [8], [12]. Moreover, occurrence of these compounds in CS, demonstrates its potential as a viable source of bioactive compounds.

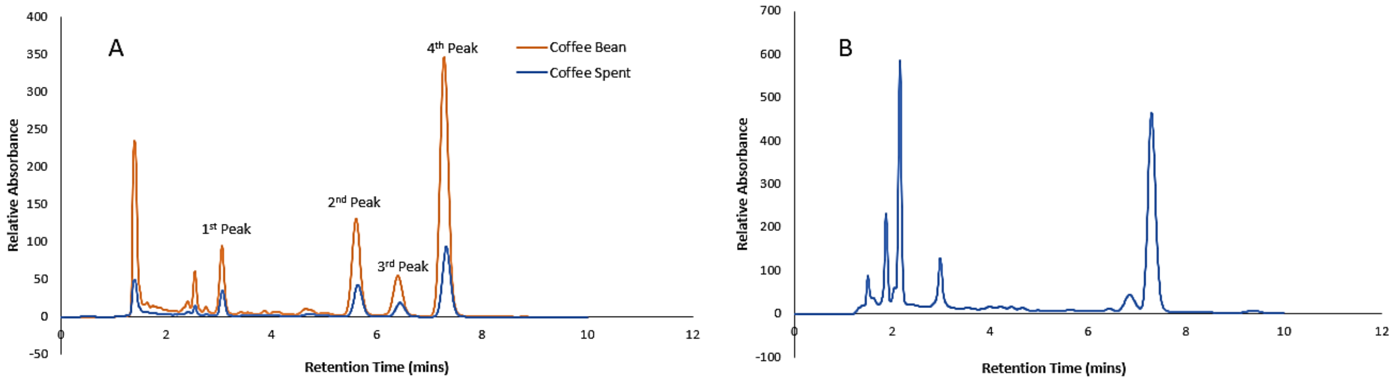

Figure 2. HPLC chromatograms of (A) Coffee Bean (CB) and Coffee Spent (CS); (B) Tea Leaves (TL). The UV spectra of peaks 1, 2, 3 and 4 are shown in Figure 3 as A, B, C and D, respectively.

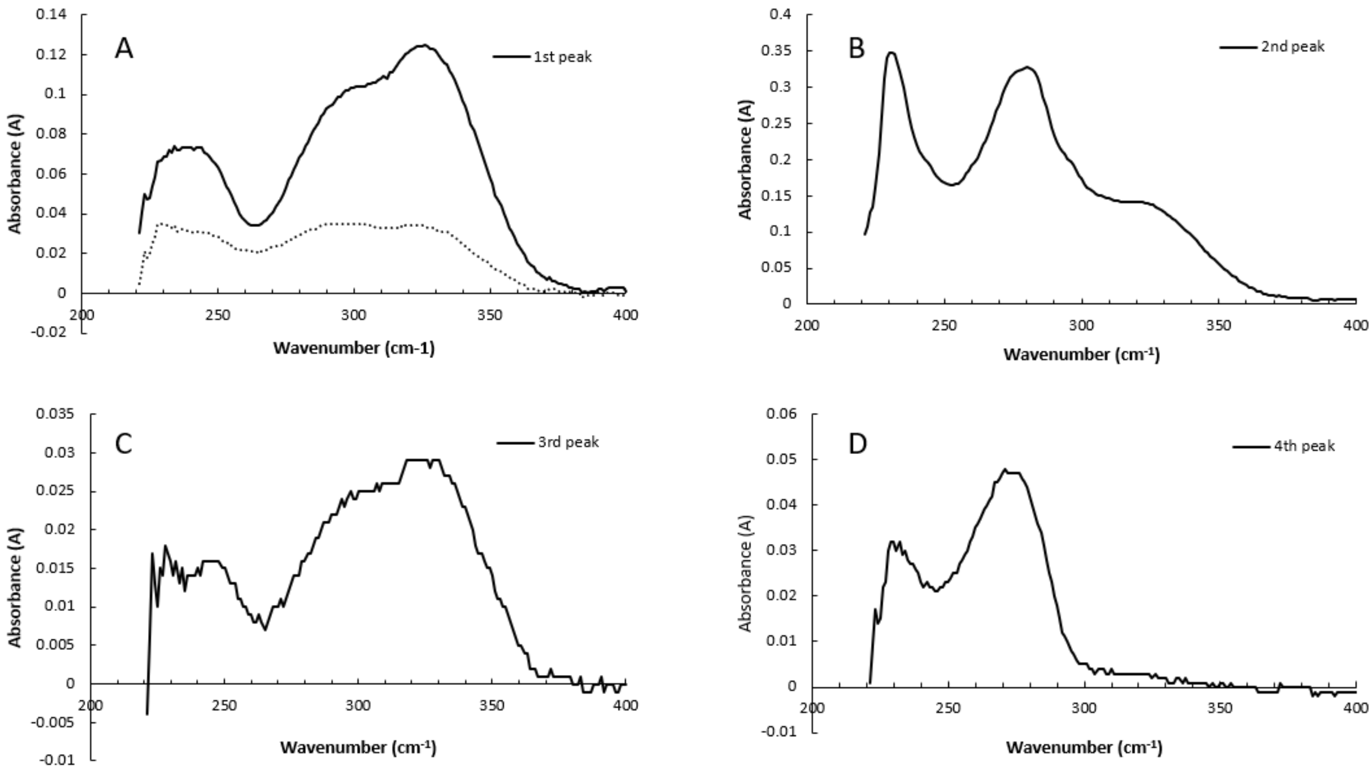

Figure 3. UV spectrums of the main peaks obtained in the chromatogram. (A) Glycosides of chlorogenic acid; (B) Catechin; (C) Chlorogenic acid; (D) Caffeine.

4. Conclusion

These results as a proof of concept work suggests that a daily cup of coffee and tea contains reasonable amounts of caffeine and presence of bioactive compounds which offer antioxidant potential and health benefits. Also, caffeine in some tea leaves may be higher than previously thought. Moreover, the large generation of CS as a by-product in the production of coffee beverage offers great potential as a source of bioactive compounds. Although, its use as an additive to fertilizers is mainly exploited, several other uses in pharmaceutics and nutraceuticals are only described in literature, however, are often not practiced on a commercial scale basis.

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