

Communication

# Bioactive Constituents in Caffeinated and Decaffeinated Coffee and Their Effect on the Risk of Depression—A Comparative Constituent Analysis Study

Susan Hall <sup>1,2,3,\*</sup> , John W. Yuen <sup>4</sup>  and Gary D. Grant <sup>1,2,3</sup> 

<sup>1</sup> Menzies Health Institute Queensland, Griffith University, Gold Coast, QLD 4222, Australia; g.grant@griffith.edu.au

<sup>2</sup> School of Pharmacy and Pharmacology, Griffith University, Gold Coast, QLD 4222, Australia

<sup>3</sup> Quality Use of Medicines Network, Griffith University, Gold Coast, QLD 4222, Australia

<sup>4</sup> School of Nursing, The Hong Kong Polytechnic University, Hung Hom, Kowloon 852, Hong Kong, China; john.yuen@polyu.edu.hk

\* Correspondence: s.hall@griffith.edu.au; Tel.: +61-075-552-9213

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**Abstract:** Coffee, a popular beverage throughout the world, has been shown to have numerous beneficial health effects, including reducing the risk of developing depression. This effect has only been shown with the consumption of caffeinated coffee and not decaffeinated coffee or caffeine alone and one of many hypotheses attributes this to the loss of key constituents during the decaffeination process. The aim of this study was to investigate whether any of the key bioactive coffee constituents with known anti-oxidant and anti-inflammatory effects are lost during the decaffeination process. The analysis of nine caffeinated and nine decaffeinated samples of various brands and batches of commonly consumed coffee in Australia using HPLC analysis found that, with the exception of caffeine, there were no significant differences in the quantity of other key bioactive coffee constituents in caffeinated and decaffeinated coffee. These results suggest that there may be an alternative explanation for the observed inverse correlation between caffeinated coffee consumption and the risk of developing depression.

**Keywords:** coffee; caffeine; caffeic acid; chlorogenic acid; ferulic acid; pyrogalllic acid; trigonelline; decaffeination; depression

## 1. Introduction

Coffee is one of the most widely consumed beverages worldwide and has been shown to have numerous health benefits ranging from reducing the risk of numerous cancers through to reducing the risk of developing depression [1,2]. These potential health benefits have been previously attributed to the bioactive constituents found in coffee with several studies showing that coffee contains compounds with anti-inflammatory and anti-oxidant activity [3–7]. After caffeine, the top five most abundant constituents are caffeic acid, chlorogenic acid, ferulic acid, pyrogalllic acid and trigonelline, which all have been shown to possess anti-inflammatory and anti-oxidant activity [1].

Numerous epidemiological studies have shown that caffeinated coffee consumption is inversely correlated with the risk of developing depression, an association that is not observed with decaffeinated coffee and caffeine alone [8–18]. In further support of these observations, a recent animal study assessing the effects of coffee consumption on both behavior and immunological biomarkers associated with depression have shown similar results with caffeinated coffee producing a significant positive

change in the behavior of treated animals in comparison to those treated with decaffeinated coffee and caffeine alone [19]. This supports the hypothesis that the observed association between caffeinated coffee consumption and the risk of developing depression is potentially due to differences in the individual bioactive constituents seen in coffee differing in the caffeinated and decaffeinated forms [19].

To date, a limited number of epidemiological studies have investigated the association between individual constituents and the risk of developing depression [9,10,20–23]. The constituents investigated were caffeine and classes of polyphenols found in coffee, including the hydroxycinnamic acids and the flavanoids [9,10,20–23]. Mixed results have been obtained, as seen in Table 1 below, suggesting that further investigation into the potential protective nature of these compounds is needed [9,10,20–23].

**Table 1.** Summary of the results of epidemiological studies investigating the association between depression risk and specific bioactive constituents found in coffee.

Constituent	Type of Study	Result	Reference
Caffeine	Cross-sectional study	Decreased risk of depression	[22]
Caffeine	Cohort study	No association with the risk of depression	[23]
Caffeine	Cross-sectional study	Decreased risk of depression	[9]
Caffeine	Cohort study	No association with the risk of depression	[10]
Polyphenols (hydroxycinnamic acids)	Cross-sectional	No association with depression risk	[21]
Polyphenols (flavonoids)	Cohort study	Decreased risk of depression	[20]

A limited number of previous studies have provided evidence that the decaffeination process may alter some of the key bioactive coffee constituents such as chlorogenic acid [24]. However, to date, comprehensive investigations assessing the effects of the decaffeination process on the key bioactive coffee constituents of interest has yet to be undertaken. Therefore, the aim of this study was to compare whether the quantity of the six most abundant constituents found in coffee differed significantly in commercially available decaffeinated coffee in comparison to caffeinated coffee.

## 2. Materials and Methods

Nine samples of caffeinated coffee and nine samples of decaffeinated coffee (three individual brands with three unique batches of each brand) were analyzed for their caffeine, caffeic acid, chlorogenic acid, ferulic acid, pyrogalllic acid and trigonelline content using HPLC analysis. All samples were prepared according to accepted food analysis guidelines with minor modifications [25]. Briefly, 50 mg samples of each of the samples of coffee were dissolved in 1 mL of hot distilled water. Samples were then diluted 1 in 20 before HPLC analysis was undertaken to ensure that the samples were within the upper limits of quantification. All analyses were undertaken on the same prepared sample, which were stored in aliquots at  $-20\text{ }^{\circ}\text{C}$  until the day of analysis to ensure consistency in the results obtained. HPLC analyses were run on a Shimadzu system consisting of a Shimadzu CBM-20A Prominence communications bus control module, two Shimadzu LC-20AD UFLC liquid chromatograph pumps fitted with a solvent mixer, a Shimadzu DGU-20A3 Prominence degasser, a Shimadzu SIL-20A HT UFLC Prominence chilled autosampler module, a Shimadzu CTO-20AC Prominence column oven, a Shimadzu SPD-M20A Prominence Diode array detector and LabSolutions software. The column used was a GraceSmart<sup>®</sup> (5  $\mu\text{m}$ , 250  $\times$  4.6 mm) reverse phase column (Grace Davison Discovery Sciences, Melbourne, Australia) fitted with a Phenomenex<sup>®</sup> SecurityGuard<sup>®</sup> guard

cartridge (Phenomenex, Lane Cove, Australia), using previously described methods for caffeine and trigonelline [26], and chlorogenic acid and caffeic acid [27]. The HPLC method for the quantification of ferulic acid and pyrogalllic acid was developed, and utilized a gradient elution method, run at 1 mL/min using 0.1% glacial acetic acid and acetonitrile (ACN). The gradient was run as follows: 0–10.0 min, 1–90% (*v/v*) ACN; 10.0–17.0 min, 90–1% (*v/v*) ACN; 17.0–20.0 min, 1% re-equilibration using ACN. The GraceSmart C18 reverse phase column was heated to 40 °C and the UV absorbance of the analytes of interest was monitored at 320 nm. All methods were validated according to the ICH Validation of Analytical Procedures guidelines [28]. All results were combined, that is all samples of caffeinated coffee were analyzed for the quantity of each of the six analytes of interest in comparison with the combined results of each of the analytes in decaffeinated coffee. Descriptive statistics were used to analyze all the analytical data. Unpaired, two-tailed t-tests were used in this study and were performed using GraphPad InStat version 3.06 (2003) (GraphPad Software, La Jolla, CA, USA).

### 3. Results

The results of the current study can be seen in Table 2. There is a significant decrease in the quantity of caffeine in decaffeinated coffee in comparison to caffeinated coffee, (16,791.13 (9083.42–20,879.04) vs. 42,651.95 (25,001.90–76,621.93) mg/kg,  $p < 0.0001$ ). Although a statistically significant difference was not achieved, caffeic acid was found to be at higher in mean and range concentrations in the decaffeinated coffee than the caffeinated coffee. Furthermore, the levels of pyrogalllic acid in caffeinated coffee were trending lower than in decaffeinated coffee but fall within the concentration range measured in the decaffeinated coffee. No significant differences were observed with any of the other analytes analyzed when decaffeinated coffee was compared to caffeinated coffee. However, for all constituents measured, it can be seen from the ranges of quantity of each of the analytes of interest, as seen in Table 2, that there is a degree of variability in the content of each of the analytes in the representative standards, although they did not differ significantly in decaffeinated coffee in comparison to caffeinated coffee.

**Table 2.** Summary of the average  $\pm$  SD (min-max) quantity in mg/kg of each of the analytes of interest in caffeinated and decaffeinated coffee.

Analyte		Caffeinated	Decaffeinated	<i>p</i> Value
Caffeine (mg/kg)	Brand 1	56,209.73 $\pm$ 19,601.52 (37,534.6–76,622)	1976.2 $\pm$ 96.99 (1914.6–2088)	<i>p</i> < 0.0001
	Brand 2	38,426.53 $\pm$ 10,157.26 (28,521.6–48,818.6)	1710.93 $\pm$ 324.93 (1373.4–2021.6)	
	Brand 3	33,319 $\pm$ 14,046.25 (25,000–49,536.4)	954.93 $\pm$ 487.07 (492.8–1463.6)	
	Average	42,651.95 $\pm$ 12,016.06 (25,001.90–76,621.93)	16,791.13 $\pm$ 529.92 (9083.42–20,879.04)	
Caffeic acid (mg/kg)	Brand 1	1550.87 $\pm$ 178.37 (1444.6–1756.8)	1755.93 $\pm$ 130.01 (1625–1885)	<i>p</i> > 0.05
	Brand 2	2459.6 $\pm$ 410.62 (2095–2904.4)	2804.93 $\pm$ 343.55 (2454–3140.6)	
	Brand 3	1863.33 $\pm$ 335.71 (1625.4–2247.4)	2140.67 $\pm$ 310.85 (1960.2–2499.6)	
	Average	1957.95 $\pm$ 461.69 (1444.63–2904.39)	2233.83 $\pm$ 530.67 (1625.04–3140.53)	
Chlorogenic acid (mg/kg)	Brand 1	41,735.07 $\pm$ 4157.29 (37,405–45,694.8)	36,560.2 $\pm$ 4240.49 (33,261–41,343.2)	<i>p</i> > 0.05
	Brand 2	33,605.53 $\pm$ 5368.33 (30,171–39,791.8)	41,795.13 $\pm$ 3803.19 (38,722.2–46,048.6)	
	Brand 3	41,477.8 $\pm$ 6999.30 (36,541.2–49,488)	14,993.8 $\pm$ 2412.20 (13,005.4–17,677.2)	
	Average	38,932.81 $\pm$ 4621.11 (30,171.02–49,488.03)	31,115.23 $\pm$ 14,205.78 (13,005.32–46,048.63)	
Ferulic acid (mg/kg)	Brand 1	1852.2 $\pm$ 95.08 (1752.6–1942)	1235.07 $\pm$ 172.83 (1132.2–1434.6)	<i>p</i> > 0.05
	Brand 2	1388.2 $\pm$ 367.46 (1137.4–1810)	1925.27 $\pm$ 196.86 (1765–2145)	
	Brand 3	1714.96 $\pm$ 215.25 (1519.4–1945.6)	352.87 $\pm$ 247.88 (159.8–632.4)	
	Average	1651.13 $\pm$ 238.36 (1137.50–1941.99)	1171.09 $\pm$ 788.15 (159.82–2145.0)	
Pyrogalllic acid (mg/kg)	Brand 1	2007.6 $\pm$ 150.41 (1834–2099)	2666 $\pm$ 369.93 (2419.8–3091.4)	<i>p</i> > 0.05
	Brand 2	2288.87 $\pm$ 294.58 (1964–2538.6)	2972.07 $\pm$ 247.49 (2770.8–3248.4)	
	Brand 3	2511.4 $\pm$ 92.95 (2442–2617)	2344.2 $\pm$ 1059.22 (1490.6–3529.6)	
	Average	2269.33 $\pm$ 252.47 (1843.07–2616.99)	2660.74 $\pm$ 313.97 (1490.50–3529.62)	
Trigonelline (mg/kg)	Brand 1	41,798.87 $\pm$ 12,084.62 (29,217.8–53,316.8)	34,389.4 $\pm$ 4457.58 (31,106.2–39,464)	<i>p</i> > 0.05
	Brand 2	31,255.4 $\pm$ 4669.26 (28,559.6–36,647)	28,965.27 $\pm$ 4181.40 (24,137–31,379.4)	
	Brand 3	31,235 $\pm$ 13,289.1 (23,401.8–46,578.8)	17,499.47 $\pm$ 29,35.08 (14,551–20,406.2)	
	Average	34,070.33 $\pm$ 6093.17 (22,324.84–53,316.71)	28,639.2 $\pm$ 8568.40 (14,551.02–39,463.89)	

#### 4. Discussion

The aim of this study was to investigate the effects the decaffeination process has on the quantity of the key bioactive coffee constituents, caffeine, caffeic acid, chlorogenic acid, ferulic acid, pyrogalllic acid, and trigonelline in coffee. This is of particular relevance in order to further investigate the observed results in epidemiological studies inversely correlating caffeinated coffee consumption with the risk of developing depression and identifying potential reasons why this may be the case [8–18,23].

Current hypotheses of depression focus on neuroinflammatory, neurogenesis and pro-oxidant pathways as important factors in the pathophysiology of depression [1,22]. Many of the constituents found in coffee, including the six constituents investigated in the current study, belong to the polyphenol and alkaloid classes of natural products [1]. These compounds have been evaluated in *in vitro* and *in vivo* studies and have been shown to possess anti-inflammatory, neurogenesis-promoting activity along with anti-oxidant properties [1]. Studies in humans (Table 1) have been limited, and to date, mixed results relating to the association between caffeine and polyphenols found in coffee and decreased depression risk have been found, suggesting that other constituents may be involved in the apparent relationship [9,10,19,21–23].

The current study investigated the differences observed when commonly consumed brands of caffeinated coffee were compared to commonly consumed brands of decaffeinated coffee. This method of analysis was chosen to best mimic the conditions of the epidemiological studies with data not being stratified according to particular brands of coffee, rather to caffeinated and decaffeinated coffee consumption. This study provides an overall picture comparing general caffeinated coffee composition with decaffeinated coffee composition.

The current study showed, as expected, that there was a significant decrease in the quantity of caffeine in decaffeinated coffee in comparison to caffeinated coffee. However, there was no significant difference in the quantity of the other bioactive coffee constituents, caffeic acid, chlorogenic acid, ferulic acid, pyrogalllic acid and trigonelline when decaffeinated coffee was compared to caffeinated coffee. There was however a non-significant increase in the quantity of caffeic and pyrogalllic acids present in decaffeinated coffee in comparison to caffeinated coffee. This may be due to complexes that form in caffeinated coffee dissolved in water between caffeine and caffeic acid which are not present in decaffeinated coffee resulting in increased caffeic acid quantities [29]. However, given chlorogenic acid has the same capacity to form complexes with caffeine, it would be expected that the quantity of chlorogenic acid also increased in decaffeinated coffee, which was not observed in the current study [29]. Caffeic acid is well-documented to have antioxidant, anti-inflammatory and neuroprotective properties and is expected to reduce the risk of depression [1]. This potential, however, has not been shown in a recent cross-sectional study investigating the association between dietary polyphenols and risk of depression [22]. In further support of the results, increased caffeic acid in decaffeinated coffee does not appear to afford any risk reduction in developing depression as evidenced by the epidemiological studies [8–18,23]. Pyrogalllic acid on the other hand, has been shown to have both protective and damaging properties in a concentration-dependent manner. At low concentrations it acts as an antioxidant and anti-inflammatory agent, while at higher concentrations it acts as a free-radical generator [30]. Although the increase in the quantity of pyrogalllic acid in decaffeinated coffee is not significant, it may play a role in the loss of protective effects against the risk of depression and warrants further investigations.

To date there have been limited studies undertaken in assessing the effects of the decaffeination process of constituents of coffee other than caffeine. However, two previous studies have investigated the effects of the decaffeination process on the quantity of chlorogenic acid in coffee and similarly found that there was no difference in the content after the decaffeination process [31,32]. There has, however, been one report of decreased chlorogenic acid after the decaffeination process [24]. This result is plausible given that there are many types of decaffeination processes available using a variety of chemicals [31,33], and this may influence the loss of specific constituents due to different chemical properties of these constituents. Given the commercial nature of the products tested, there is difficulty

in obtaining accurate and complete information on the decaffeination processes used. This further strengthens the justification to analyze the results of the current study as a combined result to provide a more complete picture from a population perspective.

Significant variability in the minimum and maximum quantities of each of the constituents analyzed in this study was observed. This is not unexpected given that three batches, from three separate brands of coffee, were analyzed. There are various possible reasons for this variability. Commercial coffee is usually prepared from one or both of *Coffea arabica* and *Coffea canephora*, also known as *Coffea robusta* [34]. Previous studies have shown that the quantities of caffeine and CGA are significantly lower in *Coffea arabica* than those seen in *Coffea canephora* [35,36]. Furthermore, studies have shown that the quantity of CGA and caffeine present in a final coffee preparation is dependent on the roast of the coffee and is either decreased or increased, respectively, as the roast increases [36–38]. Finally, climatic factors, including rainfall levels and air temperature during seed development have been shown to impact on the quantities of bioactive constituents found in the bean [39,40]. All of these factors have the potential to influence the range of bioactive constituents found in different brands of commercial coffee.

The results of this study indicate that, with exception to caffeine, there is no significant loss of key bioactive coffee constituents in the decaffeination process. There are, however, limitations in the current study that it only analyses a limited number of samples that are readily available in Australia and it is recommended that this study is repeated using a bigger sample size involving a range of brands available in various countries. Furthermore, only the most abundant of the many bioactive constituents found in coffee were assessed for loss in the decaffeination process in the current study. Future studies should characterize the changes that the decaffeination process has on a global constituent scale.

These results suggest that the loss of key bioactive constituents during the decaffeination process is not responsible for the observed differences in the risk reduction associated with depression as shown by numerous epidemiological studies [8–18]. Other possible explanations for the apparent antidepressant effects of coffee consumption now need to be investigated further. Another possible explanation for the observed risk reduction in developing depression with increased caffeinated coffee consumption could be due to the effects of caffeine in combination with other bioactive coffee constituents, such as those quantified in the current study, as previously hypothesized [19].

The findings suggest that the decaffeination process does not result in the loss of significant amounts of key bioactive coffee constituents compared to caffeinated coffee. It does show that there are non-significant increases in caffeic acid and pyrogalllic acid which require further investigation. These results suggest that the loss of bioactive coffee constituents does not lead to the benefits observed in caffeinated coffee in reducing the risk of depression. Given the results of a previous study suggesting that flavonoids are associated with a decrease in the risk of developing depression and the results of the current study, further studies are necessary to further elucidate the mechanisms by which caffeinated coffee consumption appears to afford protection against the development of the depression.

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