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; pyrogallic 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, pyrogallic 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 falvanoids [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, pyrogallic 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 °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® (5 µm, 250 × 4.6 mm) reverse phase column (Grace Davison Discovery Sciences, Melbourne, Australia) fitted with a Phenomenex® SecurityGuard® 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 pyrogallic 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 pyrogallic 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 ± SD (min-max) quantity in mg/kg of each of the analytes of interest in caffeinated and decaffeinated coffee.

| Analyte               | Caffeinated                     | Decaffeinated                   | p Value       |
|-----------------------|---------------------------------|---------------------------------|---------------|
| **Caffeine (mg/kg)**  |                                 |                                 |               |
| Brand 1               | 56,209.73 ± 19,601.52 (37,534.6–76,622) | 1976.2 ± 96.99 (1914.6–2088) | p < 0.0001   |
| Brand 2               | 38,426.53 ± 10,157.26 (28,521.6–48,818.6) | 1710.93 ± 324.93 (1373.4–2021.6) |               |
| Brand 3               | 33,319 ± 14,046.25 (25,000–49,536.4) | 954.93 ± 487.07 (492.8–1463.6) |               |
| **Average**           | 42,651.95 ± 12,016.06 (25,001.9–76,621.93) | 16,791.13 ± 529.92 (9083.42–20,879.04) |               |
| **Caffeic acid (mg/kg)** |                                 |                                 |               |
| Brand 1               | 1550.87 ± 178.37 (1444.6–1756.8) | 1755.93 ± 130.01 (1625–1885) | p > 0.05     |
| Brand 2               | 2459.6 ± 410.62 (2095–2904.4)  | 2804.93 ± 343.55 (2454–3140.6) |               |
| Brand 3               | 1863.33 ± 335.71 (1625.4–2247.4) | 2140.67 ± 310.85 (1960.2–2499.6) |               |
| **Average**           | 1957.95 ± 461.69 (1444.63–2904.39) | 2233.83 ± 530.67 (1625.04–3140.53) |               |
| **Chlorogenic acid (mg/kg)** |                               |                                 |               |
| Brand 1               | 41,735.07 ± 4157.29 (37,405–45,694.8) | 36,560.2 ± 4240.49 (33,261–41,343.2) | p > 0.05     |
| Brand 2               | 33,605.53 ± 5368.33 (30,171–39,791.8) | 41,795.13 ± 3803.19 (38,722.2–46,048.6) |               |
| Brand 3               | 41,477.8 ± 6999.30 (36,541.2–49,488) | 14,993.8 ± 2412.20 (13,005.4–17,677.2) |               |
| **Average**           | 38,932.81 ± 4621.11 (30,171.02–49,488.03) | 31,115.23 ± 14,205.78 (13,005.32–46,048.63) |               |
| **Ferulic acid (mg/kg)** |                               |                                 |               |
| Brand 1               | 1852.2 ± 95.08 (1752.6–1942)    | 1235.07 ± 172.83 (1132.2–1434.6) | p > 0.05     |
| Brand 2               | 1388.2 ± 367.46 (1137.4–1810)  | 1925.27 ± 196.86 (1765–2145)  |               |
| Brand 3               | 1714.96 ± 215.25 (1519.4–1945.6) | 352.87 ± 247.88 (159.8–632.4) |               |
| **Average**           | 1651.13 ± 238.36 (1137.50–1941.99) | 1171.09 ± 788.15 (159.82–2145.0) |               |
| **Pyrogallic acid (mg/kg)** |                              |                                 |               |
| Brand 1               | 2007.6 ± 150.41 (1834–2099)   | 2666 ± 369.93 (2419.8–3091.4)  | p > 0.05     |
| Brand 2               | 2288.87 ± 294.58 (1964–2538.6) | 2972.07 ± 247.99 (2770.8–3248.4) |               |
| Brand 3               | 2511.4 ± 92.95 (2442–2617)    | 2344.2 ± 1059.22 (1490.6–3529.6) |               |
| **Average**           | 2269.33 ± 252.47 (1843.07–2616.99) | 2660.74 ± 313.97 (1490.50–3529.62) |               |
| **Trigonelline (mg/kg)** |                              |                                 |               |
| Brand 1               | 41,798.87 ± 12,084.62 (29,217.8–53,316.8) | 34,389.4 ± 4457.58 (31,106.2–39,464) | p > 0.05     |
| Brand 2               | 31,255.4 ± 4699.26 (28,559–63,647) | 28,965.27 ± 4181.40 (24,137–31,379.4) |               |
| Brand 3               | 31,235 ± 13,289.1 (23,401.8–46,578.8) | 17,499.47 ± 29,35.08 (14,551–20,406.2) |               |
| **Average**           | 34,070.33 ± 6093.17 (22,324.84–53,316.71) | 28,639.2 ± 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, pyrogallic 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, pyrogallic acid and trigonelline when decaffeinated coffee was compared to caffeinated coffee. There was however a non-significant increase in the quantity of caffeic and pyrogallic 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]. Pyrogallic 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 pyrogallic 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 pyrogallic 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.

**Author Contributions:** This study was conceptualized by S.H., J.W.Y. and G.D.G.; methodology was developed by S.H. and G.D.G.; experiments were performed by S.H.; data analysis was performed by S.H.; the original draft was prepared by S.H.; the manuscript was reviewed and edited by S.H., J.W.Y. and G.D.G.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Hall, S.; Desbrow, B.; Anoopkumar-Dukie, S.; Davey, A.K.; Arora, D.; McDermott, C.; Schubert, M.M.; Perkins, A.V.; Kiefel, M.J.; Grant, G.D. A review of the bioactivity of coffee, caffeine and key coffee constituents on inflammatory responses linked to depression. *Food Res. Int.* **2015**, *76*, 626–636. [CrossRef] [PubMed]
2. Lara, D.R. Caffeine, mental health, and psychiatric disorders. *J. Alzheimer's Dis.* 2010, 20, S239–S248. [CrossRef] [PubMed]

3. Mallik, S.B.; Mudgal, J.; Nampoothiri, M.; Hall, S.; Anoopkumar-Dukie, S.; Grant, G.; Rao, C.M.; Arora, D. Caffeic acid attenuates lipopolysaccharide-induced sickness behaviour and neuroinflammation in mice. *Neurosci. Lett.* 2016, 632, 218–223. [CrossRef] [PubMed]

4. Boudreau, L.H.; Maillet, J.; LeBlanc, L.M.; Jean-François, J.; Touaiiba, M.; Flamand, N.; Surette, M.E. Caffeic acid phenethyl ester and its amide analogue are potent inhibitors of leukotriene biosynthesis in human polymorphonuclear leukocytes. *PLoS ONE* 2012, 7, e31833. [CrossRef] [PubMed]

5. Horrigan, L.A.; Kelly, J.P.; Connor, T.J. Caffeine suppresses TNF-α production via activation of the cyclic AMP/protein kinase A pathway. *Int. Immunopharmacol.* 2004, 4, 1409–1417. [CrossRef] [PubMed]

6. Kang, N.J.; Lee, K.W.; Shin, B.J.; Jung, S.K.; Hwang, M.K.; Bode, A.M.; Heo, Y.-S.; Lee, H.J.; Dong, Z. Caffeic acid, a phenolic phytochemical in coffee, directly inhibits Fyn kinase activity and UVB-induced COX-2 expression. *Carcinogenesis* 2008, 30, 321–330. [CrossRef] [PubMed]

7. Shen, W.; Qi, R.; Zhang, J.; Wang, Z.; Wang, H.; Hu, C.; Zhao, Y.; Bie, M.; Wang, Y.; Fu, Y. Chlorogenic acid inhibits LPS-induced microglial activation and improves survival of dopaminergic neurons. *Brain Res. Bull.* 2012, 88, 487–494. [CrossRef] [PubMed]

8. Ruusunen, A.; Lehto, S.M.; Tolmunen, T.; Mursu, J.; Kaplan, G.A.; Voutilainen, S. Coffee, tea and caffeine intake and the risk of severe depression in middle-aged Finnish men: The Kuopio Ischaemic Heart Disease Risk Factor Study. *Public Health Nutr.* 2010, 13, 1215–1220. [CrossRef] [PubMed]

9. Guo, X.; Park, Y.; Freedman, N.D.; Sinha, R.; Hollenbeck, A.R.; Blair, A.; Chen, H. Sweetened beverages, coffee and tea intake and risk of depression in midlife and older US adults. *PLoS ONE* 2014, 9, e94715. [CrossRef] [PubMed]

10. Eaton, W.W.; McLeod, J. Consumption of coffee or tea and symptoms of anxiety. *Am. J. Public Health* 1984, 74, 66–68. [CrossRef] [PubMed]

11. Shanahan, M.; Hughes, R. Potentiation of performance-induced anxiety by caffeine in coffee. *Psychol. Rep.* 1986, 59, 83–86. [CrossRef] [PubMed]

12. Tanskanen, A.; Tuomilehto, J.; Viinamäki, H.; Vartiainen, E.; Lehtonen, J.; Puska, P. Heavy coffee drinking and the risk of suicide. *Eur. J. Epidemiol.* 2000, 16, 789–791. [CrossRef] [PubMed]

13. Veleber, D.M.; Templer, D.I. Effects of caffeine on anxiety and depression. *J. Abnorm. Psychol.* 1984, 93, 120–122. [CrossRef] [PubMed]

14. Godos, J.; Castellano, S.; Ray, S.; Grosso, G.; Galvano, F. Dietary Polyphenol Intake and Depression: Results from the Mediterranean Healthy Eating, Lifestyle and Aging (MEAL) Study. *Molecules* 2018, 23, 999. [CrossRef] [PubMed]
22. Kim, J.; Kim, J. Green Tea, Coffee, and Caffeine Consumption Are Inversely Associated with Self-Report Lifetime Depression in the Korean Population. *Nutrients* **2018**, *10*, 1201. [CrossRef] [PubMed]

23. Lucas, M.; Mirzaei, F.; Pan, A.; Okereke, O.I.; Willett, W.C.; O’Reilly, É.J.; Koenen, K.; Ascherio, A. Coffee, caffeine, and risk of depression among women. *Arch. Intern. Med.* **2011**, *171*, 1571–1578. [CrossRef] [PubMed]

24. Fujioka, K.; Shibamoto, T. Chlorogenic acid and caffeine contents in various commercial brewed coffees. *Food Chem.* **2008**, *106*, 217–221. [CrossRef]

25. *Manual of Methods of Analysis of Foods: Beverages (Coffee, Tea, Cocoa, Chicory), Sugar and Sugar Products & Confectionary Products*; Food Safety and Standards Authority of India, Government of India: New Delhi, India, 2012.

26. Bragg, C.; Desbrow, B.; Hall, S.; Irwin, C. Effect of meal glycemic load and caffeine consumption on prolonged monotonous driving performance. *Physiol. Behav.* **2017**, *181*, 110–116. [CrossRef] [PubMed]

27. Narita, Y.; Inouye, K. Degradation kinetics of chlorogenic acid at various pH values and effects of ascorbic acid and epigallocatechin gallate on its stability under alkaline conditions. *J. Agric. Food Chem.* **2013**, *61*, 966–972. [CrossRef] [PubMed]

28. ICH Expert Working Group. *ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1)*; ICH: Geneva, Switzerland, 1994.

29. Kaleda, W.W.; Saleeb, F.Z.; Zeller, B.L. Coffee Decaffeination with Caffeic Acid. U.S. Patent US4767634A, 30 August 1988.

30. Upadhyay, G.; Tiwari, M.N.; Prakash, O.; Jyoti, A.; Shanker, R.; Singh, M.P. Involvement of multiple molecular events in pyrogallol-induced hepatotoxicity and silymarin-mediated protection: Evidence from gene expression profiles. *Food Chem. Toxicol.* **2010**, *48*, 1660–1670. [CrossRef] [PubMed]

31. Alonso-Salces, R.M.; Serra, F.; Reniero, F.; Heberger, K. Botanical and geographical characterization of green coffee (Coffea arabica and Coffea canephora): Chemometric evaluation of phenolic and methylxanthine contents. *J. Agric. Food Chem.* **2009**, *57*, 4224–4235. [CrossRef] [PubMed]

32. Mills, C.E.; Oruna-Concha, M.J.; Mottram, D.S.; Gibson, G.R.; Spencer, J.P. The effect of processing on chlorogenic acid content of commercially available coffee. *Food Chem.* **2013**, *141*, 3335–3340. [CrossRef] [PubMed]

33. Ramalakshmi, K.; Raghavan, B. Caffeine in coffee: Its removal. Why and how? *Crit. Rev. Food Sci. Nutr.* **1999**, *39*, 441–456. [CrossRef] [PubMed]

34. Decazy, F.; Avelino, J.; Guyot, B.; Hamon, S.; Noirot, M. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild Coffea arabica L. and C. canephora accessions. *Food Chem.* **2001**, *75*, 223–230. [CrossRef]

35. Tfouni, S.A.V.; Serrate, C.S.; Carreiro, L.B.; Camargo, M.C.R.; Teles, C.R.A.; Cipolli, K.M.V.A.B.; Furlani, R.P.Z. Effect of roasting on chlorogenic acids, caffeine and polycyclic aromatic hydrocarbons levels in two Coffea cultivars: Coffea arabica cv. Catuaí Amarello IAC-62 and Coffea canephora cv. Apoatã IAC-2258. *Int. J. Food Sci. Technol.* **2012**, *47*, 406–415. [CrossRef]

36. Tsiouri, S.A.V.; Serrate, C.S.; Carreiro, L.B.; Camargo, M.C.R.; Teles, C.R.A.; Cipolli, K.M.V.A.B.; Furlani, R.P.Z. Effect of roasting on chlorogenic acids, caffeine and polycyclic aromatic hydrocarbons levels in two Coffea cultivars: Coffea arabica cv. Catuaí Amarello IAC-62 and Coffea canephora cv. Apoatã IAC-2258. *Int. J. Food Sci. Technol.* **2012**, *47*, 406–415. [CrossRef]

37. Moos, J.K.; Yoo, H.S.; Shibamoto, T. Role of roasting conditions in the level of chlorogenic acid content in coffee beans: Correlation with coffee acidity. *J. Agric. Food Chem.* **2009**, *57*, 5365–5369. [CrossRef] [PubMed]

38. Farah, A.; de Paulis, T.; Moreira, D.P.; Trugo, L.C.; Martin, P.R. Chlorogenic acids and lactones in regular and water-decaffeinated arabica coffees. *J. Agric. Food Chem.* **2006**, *54*, 374–381. [CrossRef] [PubMed]

39. Ky, C.-L.; Louarn, J.; Dussert, S.; Guyot, B.; Hamon, S.; Noirot, M. Caffeine, trigonelline, chlorogenic acids and sucrose diversity in green Arabica coffee L. and C. canephora P. accessions. *Food Chem.* **2013**, *75*, 223–230. [CrossRef]

40. Decazy, F.; Avelino, J.; Guyot, B.; Perriot, J.; Pineda, C.; Cilas, C. Quality of different Honduran coffees in relation to several environments. *J. Food Sci.* **2003**, *68*, 2356–2361. [CrossRef]