Article

Effect of Roasting Degree on the Antioxidant Properties of Espresso and Drip Coffee Extracted from *Coffea arabica* cv. Java

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Abstract: Coffee roasting is the process of applying heat to green coffee beans to bring out flavors through chemical changes. This study aimed to investigate the effect of roasting degree on the antioxidant capacities of espresso and drip coffee extracted from *Coffea arabica* cv. Java in Laos. Green coffee beans were roasted under four conditions (Light-medium, Medium, Moderately dark, and Very dark), and espresso and drip coffee were extracted. The contents of total phenolics (TP), total flavonoids (TF), and chlorogenic acids (CGA) decreased as the roasting degree increased, whereas the caffeine content increased. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was lower in the Medium, Moderately dark, and Very dark compared to the Light-medium. The ferric reducing antioxidant power (FRAP) was lower in the Very dark than the Light-medium, Medium, and Moderately dark. Principal component analysis showed that TP, TF, CGA, caffeine, DPPH radical scavenging activity, and FRAP distinguish coffee extracts with various roasting degrees. Therefore, it is concluded that roasting degree is a modifiable factor for the use of coffee as an antioxidant material in the food industry, and TF, TP, CGA, and caffeine contents, DPPH radical scavenging activity and FRAP are good indicators for determining the antioxidant capacity of coffee.

Keywords: *Coffea arabica* cv. Java; espresso; drip; roasting degree; antioxidant capacity

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1. Introduction

Coffee is one of the most favored beverages in the world. The production of coffee beans in 2018/19 reached 170.22 million bags, 4.6% higher than in 2017/2018 [1]. A national survey in the United States shows that 75% of adults drink coffee, and 49% drink more than one cup daily [2]. Coffee consumption has beneficial effects on lowering the risk of Parkinson's disease, Alzheimer's disease, cardiovascular disease, type 2 diabetes mellitus, and liver cirrhosis [3]. These health-improving properties of coffee are related to its antioxidant content [3]. There are about 700 or more chemical compounds in coffee extracts [4]. Studies have reported that the antioxidant activity of coffee is attributed to phenolic compounds, mainly chlorogenic acids, and other compounds such as caffeine, tocopherols, or Maillard reaction products [5,6]. This bioactive concentration in coffee depends on many factors, such as origin, roasting, and brewing methods.

Coffee roasting is the process of producing coffee flavors by applying heat over 200 °C to green beans [7]. As the roasting progresses, temperature rise, moisture evaporation, volume increase, and dry matter decrease occur inside coffee beans, thereby roasting is one of the pivotal factors in determining coffee quality [7]. Coffee extraction refers to dissolving the soluble components of ground coffee beans in a liquid solvent [8]. The extracting procedure influences the composition of the final coffee extract. Significant differences are found in polyphenol extraction, caffeine content, total solids, antioxidant activity, and volatility profile [9]. Espresso and drip extraction is the most commonly used method of

coffee brewing [10]. The espresso method applies pressure above atmospheric pressure to the ground coffee beans [8]. In a drip method, coffee beans are ground into a powder, passing water through it and filtering the coffee extract simultaneously [10].

The antioxidant capacity of coffee extracts depends on coffee varietals and their extraction method [11,12]. However, previous studies on roasting degree and antioxidant capacity have mainly focused on coffee extracts of unknown species in local markets. Additionally, little has been reported on whether the effect of roasting degree on the antioxidant properties of coffee is the same for espresso and drip coffee. In a previous study, the amount of total phenolics and DPPH radical scavenging activity of early-roasted Java coffee bean powder have been reported [13]. However, the effect of roasting degrees on the antioxidant properties of *Coffea arabica* cv. Java coffee prepared by espresso and drip methods has not been studied yet. Several methods have been established for the determination of the antioxidant activity of plants. In addition, several analytic methods have been developed for its determination. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and Trolox equivalent antioxidant capacity (TEAC) are generally used techniques to evaluate antioxidant properties in vitro. However, it has been reported that the results of these analyzes are different and not correlated in some materials. Therefore, we aimed to investigate the effect of roasting degree on the antioxidant properties of espresso and drip coffee prepared using *Coffea arabica* cv. Java. This study applied various antioxidant assays to espresso and drip coffee with various degrees of roasting and statistically analyzed relationships among the variables.

2. Materials and Methods

2.1. Materials

Green coffee beans were *Coffea arabica* cv. Java species (Natural processed, Grade AA), collected in Laos from December 2017 to March 2018 and supplied by Moi Coffee Company (Yongin, Korea).

2.2. Roasting

Green coffee beans (250 g) were added to a roaster (Bullet R1, Aillio, Taipei, Taiwan) preheated to 170 °C. By varying the temperature at the end of roasting, four types of coffee beans with different roasting degrees were prepared. The Agtron value of coffee beans was determined using a coffee roast analyzer (CM-100, lighttells, Zhubei, Taiwan). The coffee beans used in this study corresponded to Light-medium, Medium, Moderately Dark, and Very Dark, according to the standard of Specialty Coffee Association (SCA) [14]. The roasted coffee beans were stored at −18 °C until use. The roasting temperatures, roasting time, and Agtron values after roasting are presented in Table 1.

Table 1. Roasting conditions for coffee beans.

Variables	Light-Medium	Medium	Moderately Dark	VERY DARK
Initial temperature (°C)	170	170	170	170
Final temperature (°C)	193	199	209	230
Weight loss (%)	12	12	16	20
Roasting time (min)	8.76 ± 0.12^{d}	9.25 ± 0.15^{c}	10.39 ± 0.11^{b}	15.26 ± 0.15^{a}
Agtron number	67.56 ± 0.84^{a}	58.61 ± 0.34^{b}	47.81 ± 0.11^{c}	27.64 ± 0.38^{d}

Data are expressed as mean ± standard deviation (n = 3). Means with different letters (a–d) in the same row denote significant differences according to the one-way analysis of variance (ANOVA) and Duncan's multiple comparison test ($p < 0.05$).

2.3. Espresso Extraction

The roasted coffee beans were ground to a size of 45 mesh using a semi-automatic grinder (900N, Yang-Chia Machine Works Co., Ltd., Taichung Hsien, Taiwan). From 7 g of coffee powder, espresso coffee was extracted with distilled water up to 30 mL using a semi-automatic espresso machine (E98 President A2, Faema, Milano, Italy). Samples were stored at −70 °C until analysis.

2.4. Drip Extraction

The roasted coffee beans were ground to a size of 25 mesh using a grinder (Fuji coffee mill R-440, Fujikouki Co., Ltd., Osaka, Japan). From 15 g of coffee powder, drip coffee was extracted with distilled water up to 210 mL using A Clever Dripper (Mr. Clever, EK, Int'l Co., Ltd., Taipei, Taiwan). Samples were stored at −70 °C until analysis.

2.5. Determination of Total Dissolved Solids

The value of °Brix was measured using a refractometer (HI 96801, HANNA Instruments, Woonsocket, RI, USA). The total dissolved solids (TDS) in coffee extracts was determined by multiplying the °Brix value by 0.85 [15]. All measurements were performed in triplicate, and the results were presented as average values.

2.6. Determination of Total Phenolic Content

Total phenolic content was analyzed by using the Folin-Ciocalteu method [16], with slight modification. The coffee extract was centrifuged at 842× *g* for 10 min. The supernatant was diluted 20 times for espresso or five times for drip so that the measured values for each sample were within the calibration curve range. A diluted sample of 100 µL was added to a mixture of 200 µL of 0.9 N Folin-Ciocalteu's reagent and 800 µL of 20% Na_2CO_3 and then incubated for 30 min at room temperature (RT). The absorbance was determined at 765 nm using a microplate reader (Spectramax M2E, Thermo Fisher Scientific, Waltham, MA, USA). A standard curve (50–500 µg/mL, R^2 = 0.9972) was constructed using gallic acid (Sigma Aldrich, St. Louis, MO, USA), and results were expressed as µg gallic acid equivalent (GAE)/mL.

2.7. Determination of Total Flavonoid Content

Total flavonoid content was analyzed by using the method of Chang et al. [17]. The coffee extracts were centrifuged at 842× *g* for 10 min. The supernatant was used as samples. 300 µL of the sample was mixed with 900 µL of ethanol, 60 µL of 10% $AlCl_3$, 60 µL of 1 M $C_2H_3NaO_2$, and 1700 µL of distilled water. The mixture was then incubated at RT for 30 min. The absorbance at 415 nm was measured using a microplate reader (Spectramax M2E, Thermo Fisher Scientific). A standard curve (100–1000 µg/mL, R^2 = 0.9916) was constructed using quercetin (Sigma Aldrich, St. Louis, MO, USA), and results were expressed as µg quercetin equivalent (QE)/mL.

2.8. Determination of Chlorogenic Acid and Caffeine

The contents of chlorogenic acid and caffeine were analyzed by the HPLC system (Ultimate 3000, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a UV–Vis detector (VWD-3100, Thermo Fisher Scientific) using the method of Jung et al. [18]. Coffee extracts were diluted 25 times for the espresso or 10 times for the drip. The diluted samples were filtered through a 0.45 µm syringe filter. The separation was conducted on a Capcell Pak C18 UG 120 column (5 µm, 4.6 × 150 mm, Shiseido, Tokyo, Japan) set at 40 °C. The injection volume was 20 µL. The mobile phase consisted of acetonitrile (13% A, Burdick & Jackson, Muskegon, MI, USA) and 0.4% H_3PO_4 (87% B, Samchun, Pyeongtaek, Korea) at a constant flow rate (0.5 mL/min). The calibration curves were prepared using chlorogenic acid (Sigma Aldrich, St. Louis, MO, USA) and caffeine (Sigma Aldrich, St. Louis, MO, USA). The results were expressed as mg/mL.

2.9. DPPH Radical Scavenging Activity Assay

The DPPH radical scavenging activity was analyzed using the method of Blois [19], with slight modifications. Coffee extracts were diluted 100 times for the espresso and 20 times for the drip before analysis. 40 µL of the sample was mixed with 160 µL of 0.45 mM DPPH solution and then incubated at room temperature (RT; 20–25 °C) for 30 min in the dark. The absorbance was measured spectrophotometrically at 517 nm. Ascorbic acid (Sigma Aldrich, St. Louis, MO, USA) was used to construct the calibration curve

(20–200 μg/mL, R^2 = 0.9734). The results were presented as mg ascorbic acid equivalent (AAE)/mL.

2.10. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP was analyzed as reported by Benzie and Strain [20]. The FRAP reagent consisted of 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), 20 mM $FeCl_3 \cdot 6H_2O$, and 0.3 M sodium acetate buffer (pH 3.6) in a ratio of 1:1:10, and warmed at 37 °C for 10 min at 37 °C. Coffee extracts were diluted 100 times for the espresso or 20 times for the drip before analysis. 5 μL of the sample was added to 145 μL of FRAP reagent and incubated in the dark at RT for 15 min. The absorbance at 593 nm was determined. The $FeSO_4 \cdot 7H_2O$ (Sigma Aldrich, St. Louis, MO, USA) was used to construct a standard curve (200–2000 μM, R^2 = 0.9993), and the results were expressed as μmol $FeSO_4$ equivalent/mL.

2.11. Trolox Equivalent Antioxidant Capacity (TEAC)

The TEAC was measured as the ability of test materials to reduce the cationic radical of ABTS (2,2-azinobis-(3-ethylbenzo-thiazoline-6-sulphonic acid)) as described Re et al. [21]. The ABTS radical was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate for 16 h in the dark and diluted with phosphate buffer saline (PBS) with an absorbance of 0.70 at 734 nm. Coffee extracts were diluted 50 times for the expresso and 10 times for the drip and used for analysis. After the addition of 150 μL of ABTS radical solution to 5 μL of the sample, the solution was incubated at RT for 3 min, and the absorbance at 734 nm was obtained. Trolox (Tokyo chemical industry, Tokyo, Japan) was used to prepare the standard curve (200–2000 mM, R^2 = 0.9929), and results were calculated as mg Trolox equivalent (TE)/mL.

2.12. Statistical Analysis

All measurements were carried out in triplicate. Results are expressed as mean ± standard deviation (SD). One-way ANOVA with Duncan's multiple comparison tests and Pearson's correlation tests were conducted using the SPSS software package (v.20; IBM Corporation, Armonk, NY, USA). In addition, principal components analysis (PCA) was applied to analyze the qualitative correlations between variables and demonstrate its applicability in determining the antioxidant capacity of coffee extracts by using GraphPad Prism (v.9; GraphPad Software Inc, San Diego, CA, USA). In PCA, principal components (PC) with eigenvalues above 1 were selected. p-values less than 0.05 are considered to be statistically significant.

3. Results and Discussion

3.1. Total Dissolved Solids

The TDS refers to the percentage of soluble components in coffee that determines the coffee strength. The TDS in coffee extracts of this study is shown in Table 2. As for the espresso coffee, the higher the roasting degree, the higher the TDS. The TDS was significantly higher in Very dark roasting compared to coffees in other roasting degrees. In the drip coffee, the TDS values in Medium and Moderately dark were lower than those in Light-medium and Very dark. Espresso uses finely ground coffee beans to increase the solubility of chemicals and flavors because of its short brewing time [22,23]. In contrast, when it takes several minutes to infuse coffee soluble in hot water, such as drip coffee, coarse particles are needed to slow diffusion and avoid the extraction of bitter compounds [23]. Previous studies have reported that the total soluble solids in espresso coffee increase with increasing roasting degrees [24]. In contrast, the TDS in drip coffee was decreased as the roasting time increases [25]. TDS in coffee depends on the structure of coffee beans, the size of coffee particle size, the temperature of extraction water, and the time of extraction [10]. Therefore, it is estimated that in espresso, an increase of TDS when roasting degree increases is related to the porous structure of coffee beans. However, the lack of difference in TDS between Light-medium and Very dark roasting in the drip extraction may be because the

TDS was not sufficiently eluted, presumably due to low water pressure and low surface area of the coffee powder.

Table 2. Total soluble solids in coffee extracts.

Variable	Extraction	Light-Medium	Medium	Moderately Dark	Very Dark
Total dissolved solids (%)	Espresso	4.51 ± 0.17 [b]	4.53 ± 0.10 [b]	4.62 ± 0.05 [b]	4.93 ± 0.00 [a]
	Drip	1.19 ± 0.00 [a]	1.02 ± 0.00 [b]	1.05 ± 0.05 [b]	1.13 ± 0.05 [a]

Data are expressed as mean ± standard deviation ($n = 3$). Means with different letters (a,b) in the same row denote significant differences according to the one-way analysis of variance (ANOVA) and Duncan's multiple comparison test ($p < 0.05$).

3.2. Total Phenolic Content

Phenolics are chemical compounds with aromatic benzene rings containing hydroxyl groups generated by plants to protect against stress [26]. They provide antioxidant activity due to their hydroxyl groups acting as electron donors [27]. Moreover, some of those phenolic compounds have been reported to trigger the synthesis of endogenous antioxidant molecules in the cell [28]. In this study, the total phenolic contents of coffee extracts with various roasting degrees are shown in Table 3. Results showed that the total phenolic contents of espresso coffee were significantly higher at the Medium roasting than the Light-medium roasting ($p < 0.05$). However, the total phenolic content in the espresso was significantly lower when prepared with the Moderately dark and Very dark roasted coffee beans compared to the Medium roasted coffee beans ($p < 0.05$). In the drip, the phenolic content was the highest at the Light-medium, whereas the phenolic content at the Very dark roasting was significantly lower compared to the Medium and Moderately dark roasting ($p < 0.05$).

Table 3. The contents of total phenolics and total flavonoids in coffee extracts.

Variables	Extraction	Light-Medium	Medium	Moderately Dark	Very Dark
Total phenolics (μg GAE/mL)	Espresso	5670.34 ± 30.74 [b]	5804.55 ± 40.04 [a]	4814.41 ± 11.44 [c]	4366.70 ± 79.25 [d]
	Drip	1224.43 ± 30.30 [a]	1022.84 ± 17.66 [b]	1048.64 ± 24.38 [b]	854.34 ± 14.15 [c]
Total flavonoids (μg QE/mL)	Espresso	554.78 ± 10.27 [b]	597.94 ± 3.77 [a]	580.14 ± 18.98 [a]	393.24 ± 1.75 [c]
	Drip	106.51 ± 0.79 [a]	87.61 ± 0.46 [b]	69.77 ± 0.56 [c]	30.44 ± 1.59 [d]

Data are expressed as mean ± standard deviation ($n = 3$). Means with different letters (a–d) in the same row denote significant differences according to the one-way analysis of variance (ANOVA) and Duncan's multiple comparison test ($p < 0.05$).

These results agreed with Kim and Han's results that the total phenolic compounds were 94.98–199.13 mg GAE/g and were the highest in water reflux extract of coffee beans (Columbia Valencia Supremo) roasted for 10 min and decreased with the longer roasting times [29]. It has been also reported by Cho et al. that phenolic compounds in freeze-dried coffee extracted by hot water immersion of coffee beans (*C. arabica* cv. Medellin) were 26.80–55.00 mg GAE/g and were the highest at the light roasting, decreasing along with an increase in roasting degree [30]. In a study by Górnaś et al., green coffee beans originating from Java were roasted for different roasting times, and coffee brews were prepared through immersion of the coffee beans in boiling water [31]. Total phenolic contents in coffee brews prepared from coffee beans with medium roasting (18 min) and dark roasting (22 min) were 90.22 and 37.60 mg/100 mL, respectively, and were lower compared to the green coffee beans (180.69 mg/100 mL) [31]. The decrease in phenolic compounds might be because phenolic acid is unstable with intense heat and is easily decomposed [31]. However, it has been reported that some phenolic acids, including caffeic, p-hydroxybenzoic, protocatechuic, vanillic, and ferulic, are increased in coffee beans with increasing roasting degree [32]. In this study, the content of TDS in espresso increased as the roasting degree increased, whereas the TDS content in the drip did not. Therefore, the slight increase of total phenolic contents in espresso coffee prepared using

the Medium roasted coffee beans may be partially due to the improved extractability of phenolic compounds.

3.3. Total Flavonoid Content

Flavonoids are a single group of phenolic compounds with a high antioxidant capacity, extensively distributed in plants [26]. Coffee is considered one of the primary sources of dietary flavonoids in adults [33,34]. In the espresso, the content of total flavonoids was significantly higher at the Medium and Moderately dark roasting compared to the Light-medium roasting ($p < 0.05$). However, the total flavonoid content at the Very dark roasting was significantly lowered compared to the Medium and Moderately dark roasting ($p < 0.05$). Total flavonoid content was the highest at the Light-medium roasting in the drip and significantly decreased as the roasting level increased ($p < 0.05$). In our results, the reduction rate of total flavonoid content according to the roasting degree was greater than that of total phenolic content, which indicates instability of flavonoids with heat. In the espresso, the total phenolic content of the Very dark was 23% less than that of the Light-medium, and the total flavonoid content of the Very dark was 29.1% less compared to the Light-medium. In the drip, the total phenolic content of the Very dark was 30.2% less than that of the Light-medium, while the total flavonoid content of the Very dark was 71.4% less than that of the Light-medium. In a previous study, the content of total flavonoid in coffee beans was the highest when roasted at 191 °C, followed by green beans and coffee beans roasted at 202 °C, 220 °C, and 233 °C [35]. In the study of Cho et al., the content of flavonoids was the highest in the light roasting, followed by green beans, Medium, dark, and very dark roasting [30]. Several factors affect the amount of bioactive compounds during the thermal procedure. The physical disruption of cellular constituents by thermal processing can enhance the release of antioxidants [36]. On the other hand, the amount of antioxidants can be reduced by hydrolytic enzymes activated thermal processing [36]. Therefore, it is estimated that the fluctuation in flavonoid content may be due to the difference in heating time required for the breakdown of cellular constituents to release the individual flavonoids and the activation of hydrolytic enzymes.

3.4. Chlorogenic Acid Content

Chlorogenic acid (CGA) is a polyphenolic compound found in many plants. CGA is most dominant among the phenolic acids in green beans and all roasted coffee beans [32] and is a well-known antioxidant [37]. Studies have reported that CGA has many therapeutic properties such as antibacterial, hepatoprotective, and anti-inflammatory activities [37]. In this study, the contents of chlorogenic acid of coffee extracts were significantly lowered as the roasting degree increased in both extracts ($p < 0.05$) (Table 4). Similarly, in the studies of Kim and Kim [25], Farah et al. [38], and Song et al. [39], the content of CGA decreased as the roasting degree increased. It has been reported that CGA is thermally unstable and easily decomposed into caffeic acid and quinic acid [40]. Therefore, it is assumed that the loss of CGA in roasting may be due to the breakdown of the ester bond of chlorogenic acid. In our results, the CGA content in coffee extracts was reduced sharply at the very dark roasting. The CGA content in espresso decreased by 42.5% in Light-medium (4.96 mg/mL) compared to Moderately dark (2.85 mg/mL), and decreased by 77.9% in Moderately dark compared to Very dark (0.63 mg/mL). In the drip, the CGA content decreased by 53.3% in Light-medium (1.37 mg/mL) compared to the content in Moderately dark (0.64 mg/mL) and decreased by 79.7% in Moderately dark compared to Very dark (0.13 mg/mL). Jeon et al. have reported that the CGA contents in Americano (diluted espresso) are 115 mg/serving (31–172.4), and the contents of CGA in the hand drip coffee prepared using commercially available roasted and ground coffee were 136.1 mg/serving (27–291.7) [23]. In our study, the serving size of espresso and drip was 30 and 210 mL, respectively. When the amount of CGA per mL is converted into CGA per serving, the CGA contents in the expresso were 148.8, 132.3, 85.5, and 18.9 mg/serving, and 287.7, 176.4, 134.4, and 27.3 mg/serving in the

drip. Therefore, to maintain the CGA level of commercially available coffee products, it is postulated that it would be better to set the roasting degree weaker than very dark.

Table 4. The contents of chlorogenic acid and caffeine in coffee extracts.

Variables	Extraction	Light-Medium	Medium	Moderately Dark	Very Dark
Chlorogenic acid (mg/mL)	Espresso	4.96 ± 0.13 [a]	4.41 ± 0.02 [b]	2.85 ± 0.06 [c]	0.63 ± 0.04 [d]
	Drip	1.37 ± 0.02 [a]	0.84 ± 0.01 [b]	0.64 ± 0.01 [c]	0.13 ± 0.00 [d]
Caffeine (mg/mL)	Espresso	0.52 ± 0.02 [d]	0.64 ± 0.01 [c]	2.23 ± 0.03 [b]	3.18 ± 0.10 [a]
	Drip	0.16 ± 0.00 [d]	0.53 ± 0.01 [b]	0.50 ± 0.00 [c]	0.66 ± 0.02 [a]

Data are expressed as mean ± standard deviation (n = 3). Means with different letters (a–d) in the same row denote significant differences according to the one-way analysis of variance (ANOVA) and Duncan's multiple comparison test ($p < 0.05$).

3.5. Caffeine Content

Caffeine (1,3,7-trimethylxanthine) is a heterocyclic compound having a purine base, known as xanthine [41]. Caffeine is also an alkaloid since it is a kind of secondary plant metabolite emanated from purine nucleotides, consisting of a heterocyclic nitrogen atom [41]. Caffeine exerts various physiological effects in the human body, most of them associated with enhancing functions of the Central Nervous System (CNS) and brain [42]. Several epidemiological studies have reported a positive relationship between moderate coffee consumption over the past few years and a reduced risk of chronic or degenerative diseases [42]. Although some studies have reported that caffeine has beneficial health effects for antioxidant properties, not all studies have observed such activity in physiological conditions [42]. In this study, we observed that caffeine content was the lowest at Light-medium roasting and was the highest at Very dark roasting, regardless of the extraction method (Table 4). The caffeine contents at Very dark roasting were 6.1-fold more in the espresso and 4.2-fold more in the drip than at the Light-medium roasting. According to a study by Song et al., caffeine content tended to increase as the roasting strength increased [39]. It has also been reported that the time of roasting and caffeine content was proportional [25]. The caffeine content is not significantly altered during coffee roasting due to its thermal stability, but slight losses may occur during intense roasting due to sublimation [42]. Therefore, in terms of percentage composition, an increase in caffeine could result from the loss of other thermolabile compounds, causing a relative increase in levels of the remaining compounds. In our results, as the roasting degree increased, the weight loss of coffee beans was increased, while the TDS in coffee extracts was increased. In a previous study, Lopes et al. have reported that the relative content of caffeine in coffee extracts depends more on the overall extraction of other compounds than caffeine itself [43].

3.6. DPPH Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay has been developed to measure antioxidant activity in natural products based on electron transfer. The DPPH radicals have a purple color in ethanol, but when antioxidants are present, DPPH radicals are reduced, resulting in a colorless solution [44]. As shown in Table 5, the DPPH radical scavenging activity was significantly lower in the espresso when prepared from the Medium, Moderately dark, and Very dark roasted coffee beans compared to those prepared from the Light-medium. Like the espresso, DPPH radical scavenging activity in the drip was significantly lower at the Moderately dark and Very dark roasting degrees than the Light-medium roasting degree. In a study by Górnaś et al., it has been reported that the DPPH radical scavenging activity is decreased as the roasting proceeds, which was similar to the results of this study [31]. Cho et al. have also reported that the DPPH radical scavenging activity of coffee extracts is increased due to Light roasting. In contrast, the DPPH radical scavenging activity decreased along with increasing roasting degrees [30]. Herawati et al. have reported that the DPPH IC_{50}, in coffee brew prepared from Robusta

coffee beans, increases as the roasting degree increases after the first crack is done [45]. Changes in the DPPH radical scavenging activity due to roasting were slightly different according to coffee beans' origin. Coffee beans cultivated in Brazil had the highest activity at the Medium roasting. In contrast, coffee beans from Ethiopia showed the highest value at Light roasting and decreased as the roasting level increases [46]. In most studies, the antioxidant capacity was reduced in intense roasting, but it has been reduced or increased in light and medium roasting. Sacchetti et al. have reported that the difference among the results is due to the lack of clear roasting degrees [47]. Since coffee roasting in this study followed the SCA standard, the results of this study may help determine antioxidant capacity depending on the roasting degree.

Table 5. TEAC, DPPH radical scavenging activity, FRAP of coffee extracts.

Variables	Extraction	Light-Medium	Medium	Moderately Dark	Very Dark
DPPH• scavenging activity (mg AAE/mL)	Espresso	10.20 ± 0.34^{a}	8.85 ± 0.60^{b}	8.13 ± 0.23^{b}	8.33 ± 0.40^{b}
	Drip	2.55 ± 0.01^{a}	2.44 ± 0.07^{ab}	2.17 ± 0.14^{b}	2.20 ± 0.26^{b}
FRAP (μmol $FeSO_4$/mL)	Espresso	107.56 ± 6.20^{a}	96.81 ± 8.65^{a}	98.12 ± 1.83^{a}	77.02 ± 6.21^{b}
	Drip	26.56 ± 1.12^{a}	24.18 ± 2.50^{a}	23.61 ± 1.74^{a}	17.41 ± 0.45^{b}
TEAC (mg TE/mL)	Espresso	66.86 ± 0.69^{ns}	62.36 ± 2.27^{ns}	63.47 ± 2.42^{ns}	63.68 ± 3.66^{ns}
	Drip	13.47 ± 2.20^{ns}	14.08 ± 0.17^{ns}	13.98 ± 0.65^{ns}	13.11 ± 1.20^{ns}

Data are expressed as mean ± standard deviation (n = 3). Means with different letters (a,b) in the same row denote significant differences according to the one-way analysis of variance (ANOVA) and Duncan's multiple comparison test ($p < 0.05$). ns, not significant.

3.7. Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay is based on reducing a ferric-tripyridyl-triazine complex to its ferrous, colored form under acidic conditions (pH 3.6) in the presence of antioxidants [48]. The FRAP analyzed in the espresso and drip is presented in Table 5. The FRAP was not significantly different at the Light-medium, Medium, and Moderately Dark, regardless of the extraction method. However, at the Very Dark roasting, the FRAP was considerably lower than the others in both the expresso and drip. Some studies have reported that the radical reduction power is deceased as the roasting degree increases, suggesting that lightly roasted coffee beans are better antioxidants than intensely roasted beans [30]. For example, in a study by Nam and Kang, it has been reported that the FRAP of coffee beans decreased with increasing roasting time and temperature [49]. Song et al. have also reported that green beans and Medium light roasted coffee beans have lower IC_{50} of reducing power than the Medium and Medium-dark roasted coffee beans [39]. On the other hand, thermal processing can enhance the antioxidant properties of naturally occurring compounds or generate new compounds containing antioxidant properties so that the antioxidant capacity increases or remains unaffected [50]. Borrelli et al. have reported that the free radical scavenging ability of coffee melanoidins is reduced with increasing roasting degrees [51], whereas Maillard reaction products produced through the amino-carbonyl reaction during roasting are increased; thereby, the antioxidant capacity of the non-phenolic fraction can be maintained or increased [47]. Since FRAP is a non-specific, redox-related, colorimetric analysis, the production and decomposition of various antioxidant components during roasting may be related to the fact that there are no significant differences in FRAP coffee extracts at the Light-medium, Medium, and Moderately dark roasting. However, according to the results, it is estimated that the decomposition of antioxidants proceeds overwhelmingly at Dark roasting than production.

3.8. Trolox Equivalent Antioxidant Capacity (TEAC)

The TEAC assay is another widely used method for assessing the antioxidant capacities using free radicals. This assay determines the ability of an antioxidant to scavenge the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical, a blue-green chromophore [52]. In this study, the roasting degree did not significantly affect the TEAC in

both the espresso and drip coffee extracts, unlike the FRAP and DPPH radical scavenging activity. In the espresso, the value of the TEAC at the Light-medium was slightly higher compared to the Medium, Moderately dark, and Very dark roasting, but there were no significant differences among the samples. In the case of drip coffee, TEAC slightly increased in Medium than in Light-Medium but showed a tendency to decrease as the degree of roasting increased. However, there were also no statistical differences among groups. In another study regarding hot water extract of *Colombian arabica* coffee beans, extracts with the light and medium roasted coffee beans had higher TEAC compared to the green bean extract, whereas the dark roasted coffee bean extract did not; however, the TEAC between light roasting and medium roasting did not significantly differ [53]. In the TEAC assay, the measurements are usually based on a fixed time (4–6 min) [21], which cannot consider antioxidants' different kinetic characteristics. It has been reported that several polyphenols, including ferulic acid, resveratrol, and others, did not complete their reaction at a fixed time [54]. A study by Pérez-Jiménez et al. also has reported that the antioxidants show a different reactivity towards ABTS and DPPH radicals. The caffeic acid, with a methoxy group, showed a higher antioxidant capacity with ABTS radicals than with DPPH radicals. In contrast, ferulic acid, which possesses a hydroxyl instead of the methoxy group, was a better antioxidant component with DPPH radicals than ABTS radicals. Therefore, TEAC did not decrease as the roasting proceeded, unlike the DPPH radical scavenging activity results, which might be because antioxidants with a methoxy group, showing higher antioxidant capacity with ABTS radicals, might not be significantly affected by roasting. Moreover, it is thought that there might be antioxidants that did not react sufficiently to ABTS radicals at the point of measurement (3 min after reaction); thus, the differences in the kinetic characteristics of radicals should be considered when comparing the antioxidant capacity of coffee brews, which could bring a more comprehensive understanding of the antioxidant capacity of coffee brews.

3.9. Correlations of TP, TF, CGA, and Caffeine and Antioxidant Activities

Correlations between the contents of TP, TF, CGA, and caffeine and antioxidant activities were analyzed with Pearson's correlation tests. In the espresso, the DPPH radical scavenging activity was positively moderately correlated with the contents of TP ($r = 0.664$) and CGA ($r = 0.679$), whereas the caffeine content showed a moderately negative correlation with the DPPH radical scavenging activity ($r = -0.691$) (Table 6). In particular, high positive correlations between the FRAP and the contents of TP ($r = 0.723$), TF ($r = 0.751$), and CGA ($r = 0.843$) were found in the espresso, while the caffeine content negatively correlated with the FRAP ($r = -0.761$) (Table 6). In the drip, we also found moderately positive correlations between the contents of TP ($r = 0.563$), TF ($r = 0.674$), and CGA ($r = 0.686$), but the caffeine content had negative correlation with the DPPH radical scavenging activity ($r = -0.638$) (Table 7). Moreover, the FRAP in the drip was found to be highly positively correlated with the contents of TP ($r = 0.878$), TF ($r = 0.915$), and CGA ($r = 0.885$), whereas the content of caffeine was negatively correlated ($r = -0.777$) (Table 7). A previous study has reported an interaction between caffeine and polyphenols in black tea [55]. They observed a strong positive relationship between DPPH radical scavenging activity and levels of EGCG, flavonoids, and total phenolics, but a very weak correlation between caffeine content and DPPH radical scavenging activity [55]. They have also reported that releasing gallic acid, EGCG, and caffeine into the tea brew is faster than polyphenols and flavonoids [55]. Astill et al. have shown a greater extraction efficiency of caffeine than that of the polyphenols, indicating that the release of polyphenols could be partially blocked by caffeine [56]. Therefore, TP, TF, and CGA contents might well account for the antioxidant capacity of both coffee extracts.

Table 6. Pearson's correlation coefficients (r) for the total phenolics, total flavonoids, chlorogenic acids, caffeine contents, and antioxidant activities in the espresso coffee.

Variables	TP	TF	CGA	Caffeine	DPPH• Scavenging Activity	FRAP	TEAC
TP	1						
TF	0.754 **	1					
CGA	0.955 ***	0.838 ***	1				
Caffeine	−0.987 ***	−0.758 **	−0.978 ***	1			
DPPH• scavenging activity	0.664 **	0.238 ns	0.679 **	−0.691 **	1		
FRAP	0.723 **	0.751 **	0.843 ***	−0.761 **	0.630 *	1	
TEAC	0.130 ns	−0.066 ns	0.224 ns	−0.215 ns	0.423 ns	0.406 ns	1

*** Significant correlation with $p < 0.001$; ** Significant correlation with $p < 0.01$; * Significant correlation with $p < 0.05$. TP, total phenolics; TF, total flavonoids; CGA, chlorogenic acids; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity; ns, not significant.

Table 7. Pearson's correlation coefficients (r) for the total phenolics, total flavonoids, chlorogenic acids, caffeine contents, and antioxidant activities in the drip coffee.

Variables	TP	TF	CGA	Caffeine	DPPH• Scavenging Activity	FRAP	TEAC
TP	1						
TF	0.927 ***	1					
CGA	0.966 ***	0.977 ***	1				
Caffeine	−0.951 ***	−0.854 ***	−0.941 ***	1			
DPPH• scavenging activity	0.563 *	0.674 **	0.686 **	−0.638 *	1		
FRAP	0.878 ***	0.915 ***	0.885 ***	−0.777 **	0.454 ns	1	
TEAC	0.198 ns	0.169 ns	0.116 ns	−0.001 ns	−0.156 ns	0.124 ns	1

*** Significant correlation with $p < 0.001$; ** Significant correlation with $p < 0.01$; * Significant correlation with $p < 0.05$. TP, total phenolics; TF, total flavonoids; CA, chlorogenic acids; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity; ns, not significant.

3.10. Principal Component Analysis

PCA was performed using the contents of TP, TF, CGA, and caffeine, DPPH radical scavenging activity, FRAP, and TEAC as variables (Figure 1). The first two principal components (PC) described 87.43% of the cumulative percentage of the total variation in espresso. The PC1 explained 69.68% of the total variance of the results. Variables including TP, TF, CGA, FRAP, and DPPH radical scavenging activity were highly negatively correlated with the PC1, but the caffeine had a strong positive correlation with this component. The PC2 accounted for 17.74% of the total variance and mainly accounted for the TEAC. In the drip, PCA showed an overall variation of 88.72%, explained by PC1 (72.72%) and PC2 (16.00%). Like the expresso, TP, TF, CGA, FRAP, and DPPH radical scavenging activity were highly negatively correlated with the PC1, and the caffeine had a strong positive correlation with this component. In both extracts, FRAP showed a close association with the PC1 compared to other antioxidant assays such as DPPH radical scavenging activity and TEAC. The DPPH radical scavenging activity and TEAC measure the relative antioxidant power of scavenging radicals [44], while the FRAP analyzes the antioxidant activity through electron-donating ability [48]. When PCA was performed in all extracts, TP, TF, CGA, FRAP, and DPPH radical scavenging activity were located close to each other in the same direction and had a strong negative loading on PC1. CGA and caffeine strongly correlated with PC1; however, CGA had a positive loading on PC2, whereas caffeine showed a negative loading on PC2. The calculated score plots show the position of the investigated samples in a

multivariate space consisted of the two PCs. It illustrates that calculated scores categorized coffee extracts in four types based on the roasting degree. In the Espresso, PC1 separated the Very dark, Moderately Dark, and Light-medium/Medium while, in the drip, PC1 separated the Very dark, Moderately Dark, Medium, and Light-medium, with almost all parameters (except TEAC) contributing for explained variance. It appears that PC2 separated Light-medium and Medium, with TEAC in espresso, but not in the drip coffee. From the PC scores of total extracts, it was estimated that higher values of CGA and lower caffeine values on PC2 correspond to lower roasting degrees. However, unlike the PCA results in the espresso and drip, other parameters did not sufficiently distinguish the coffee extracts with different roasting degrees.

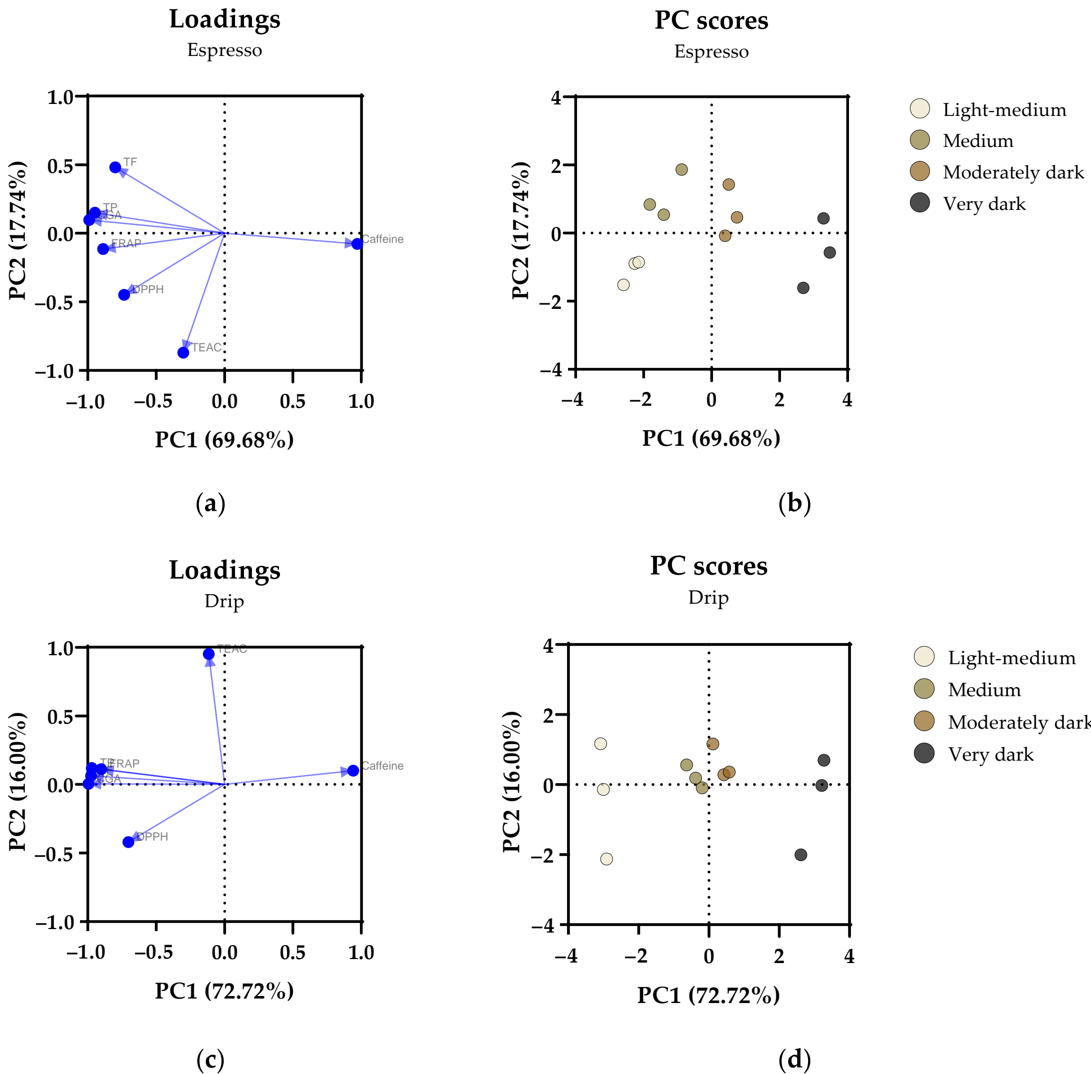

Figure 1. *Cont.*

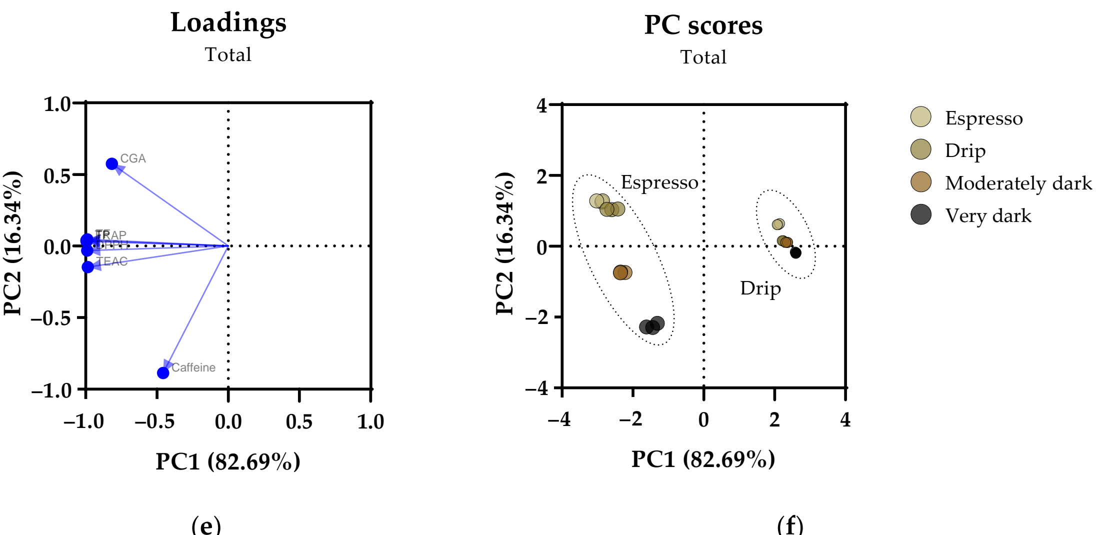

Figure 1. Results of principal component analysis (PCA). (**a**,**c**,**e**) Loading plot; (**b**,**d**,**f**) score plot. The contents of TP, TF, CGA, along with the estimations of antioxidant activities (TEAC, DPPH radical scavenging activity, and FRAP) of the espresso and drip coffee with various roasting degrees, were analyzed using PCA. TP, total phenolics; TF, total flavonoids; CGA, chlorogenic acids; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity.

4. Conclusions

This study investigated the influence of roasting degree on the antioxidant capacities of *Coffea arabica* cv. Java coffee extracted by espresso and drip methods. TP, TF, and CGA contents were decreased as the roasting degree increased, while the caffeine content was increased. As measured by the DPPH radical scavenging activity and FRAP, antioxidant capacities tend to be decreased with increasing roasting degree, whereas the TEAC was not. In each extract, TP, TF, CGA contents, and FRAP and DPPH radical scavenging activity were positively correlated with each other and essential variables that distinguish coffee extracts with different roasting degrees. However, when all extracts were analyzed simultaneously, CGA was the primary variable to distinguish coffee extracts with different degrees of roasting and was positively correlated with antioxidant activity. Therefore, it is concluded that roasting degree influences the antioxidant capacity of coffee extracts, presumably due to the alterations in the TP, TF, and CGA contents. In addition, we suggested for the first time that CGA would be an essential variable that distinguishes the antioxidant capacity of coffee extracts with different roasting degrees regardless of the extraction method.

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References

1. Annual Review 2019. Available online: http://www.ico.org/documents/cy2019-20/annual-review-2018-19-e.pdf (accessed on 13 April 2021).
2. Loftfield, E.; Freedman, N.D.; Dodd, K.W.; Vogtmann, E.; Xiao, Q.; Sinha, R.; Graubard, B.I. Coffee drinking is widespread in the united states, but usual intake varies by key demographic and lifestyle factors. *J. Nutr.* **2016**, *146*, 1762–1768. [CrossRef] [PubMed]
3. Janda, K.; Jakubczyk, K.; Baranowska-Bosiacka, I.; Kapczuk, P.; Kochman, J.; Rębacz-Maron, E.; Gutowska, I. Mineral composition and antioxidant potential of coffee beverages depending on the brewing method. *Foods* **2020**, *9*, 121. [CrossRef] [PubMed]
4. Jeszka-Skowron, M.; Sentkowska, A.; Pyrzyńska, K.; De Peña, M.P. Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: Influence of green coffee bean preparation. *Eur. Food Res. Technol.* **2016**, *242*, 1403–1409. [CrossRef]
5. Liang, N.; Kitts, D.D. Antioxidant property of coffee components: Assessment of methods that define mechanisms of action. *Molecules* **2014**, *19*, 19180–19208. [CrossRef]
6. Parras, P.; Martínez-Tomé, M.; Jiménez, A.M.; Murcia, M.A. Antioxidant capacity of coffees of several origins brewed following three different procedures. *Food Chem.* **2007**, *102*, 582–592. [CrossRef]
7. Baggenstoss, J.; Poisson, L.; Kaegi, R.; Perren, R.; Escher, F. Coffee roasting and aroma formation: Application of different time−temperature conditions. *J. Agric. Food Chem.* **2008**, *56*, 5836–5846. [CrossRef]
8. Andueza, S.; Vila, M.A.; Paz de Peña, M.; Cid, C. Influence of coffee/water ratio on the final quality of espresso coffee. *J. Sci. Food Agric.* **2007**, *87*, 586–592. [CrossRef]
9. López-Galilea, I.; De Pena, M.P.; Cid, C. Correlation of selected constituents with the total antioxidant capacity of coffee beverages: Influence of the brewing procedure. *J. Agric. Food Chem.* **2007**, *55*, 6110–6117. [CrossRef]
10. Cordoba, N.; Fernandez-Alduenda, M.; Moreno, F.L.; Ruiz, Y. Coffee extraction: A review of parameters and their influence on the physicochemical characteristics and flavour of coffee brews. *Trends Food Sci. Technol.* **2020**, *96*, 45–60. [CrossRef]
11. Wolska, J.; Janda, K.; Jakubczyk, K.; Szymkowiak, M.; Chlubek, D.; Gutowska, I. Levels of antioxidant activity and fluoride content in coffee infusions of Arabica, Robusta and green coffee beans in according to their brewing methods. *Biol. Trace. Elem. Res.* **2017**, *179*, 327–333. [CrossRef] [PubMed]
12. Jeewan, M.K.; Liyanage, T.; Roshana, M.R.; Mahendran, T. Determination and comparison of caffeine and other chemical constituents in Coffea arabica varieties grown in Sri Lanka. *Ceylon J. Sci.* **2020**, *49*, 151–158. [CrossRef]
13. Sunarharum, W.B.; Yuwono, S.S.; Aziza, O.F. Study on the effect of roasting temperature on antioxidant activity of early-roasted Java coffee powder (Arabica and Robusta). *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *230*, 012045. [CrossRef]
14. Pereira, L.L.; Debona, D.G.; Pinheiro, P.F.; de Oliveira, G.F.; ten Caten, C.S.; Moksunova, V.; Kopanina, A.V.; Vlasova, I.I.; Talskikh, A.I.; Yamamoto, H. Roasting Process. In *Quality Determinants in Coffee Production*; Food Engineering Series; Louzada Pereira, L., Rizzo Moreira, T., Eds.; Springer: Cham, Switzerland, 2021; pp. 303–372.
15. Converting Brix to TDS—An Independent Study. Available online: https://www.researchgate.net/publication/335608684_Converting_Brix_to_TDS_An_Independent_Study (accessed on 8 June 2021).
16. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.
17. Chang, C.C.; Yang, M.H.; Wen, H.M.; Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* **2002**, *10*, 3. [CrossRef]
18. Jung, S.; Kim, M.H.; Park, J.H.; Jeong, Y.; Ko, K.S. Cellular antioxidant and anti-inflammatory effects of coffee extracts with different roasting levels. *J. Med. Food* **2017**, *20*, 626–635. [CrossRef]
19. Blois, M.S. Antioxidant determinations by the use of a stable free radical. *Nature* **1958**, *181*, 1199–1200. [CrossRef]
20. Benzie, I.F.; Strain, J.J. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Meth. Enzymol.* **1999**, *299*, 15–27. [CrossRef]
21. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
22. Derossi, A.; Ricci, I.; Caporizzi, R.; Fiore, A.; Severini, C. How grinding level and brewing method (Espresso, American, Turkish) could affect the antioxidant activity and bioactive compounds in a coffee cup. *J. Sci. Food Agric.* **2018**, *98*, 3198–3207. [CrossRef] [PubMed]
23. Severini, C.; Derossi, A.; Ricci, I.; Fiore, A.G.; Caporizzi, R. How much caffeine in coffee cup? Effects of processing operations, extraction methods and variables. *Quest. Caffeine* **2017**, 45–85. [CrossRef]
24. Nunes, F.M.; Coimbra, M.A.; Duarte, A.C.; Delgadillo, I. Foamability, foam stability, and chemical composition of espresso coffee as affected by the degree of roast. *J. Agric. Food Chem.* **1997**, *45*, 3238–3243. [CrossRef]
25. Kim, S.H.; Kim, J.S. Chemical composition and sensory attributes of brewed coffee as affected by roasting conditions. *Culin. Sci. Hosp. Res.* **2017**, *23*, 1–11. [CrossRef]

26. Tungmunnithum, D.; Thongboonyou, A.; Pholboon, A.; Yangsabai, A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines* **2018**, *5*, 93. [CrossRef] [PubMed]
27. Bendary, E.; Francis, R.R.; Ali, H.M.G.; Sarwat, M.I.; El Hady, S. Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds. *Ann. Agric. Sci.* **2013**, *58*, 173–181. [CrossRef]
28. Côté, J.; Caillet, S.; Doyon, G.; Sylvain, J.F.; Lacroix, M. Bioactive compounds in cranberries and their biological properties. *Crit. Rev. Food Sci. Nutr.* **2010**, *50*, 666–679. [CrossRef] [PubMed]
29. Kim, J.Y.; Han, Y.S. Influence of roasting time on antibacterial and antioxidative effects of coffee extract. *Korean J. Food Sci. Technol.* **2009**, *25*, 496–505.
30. Cho, A.R.; Park, K.W.; Kim, K.M.; Kim, S.Y.; Han, J. Influence of roasting conditions on the antioxidant characteristics of colombian coffee (*Coffea arabica* L.) beans. *J. Food Biochem.* **2014**, *38*, 271–280. [CrossRef]
31. Górnaś, P.; Dwiecki, K.; Siger, A.; Tomaszewska-Gras, J.; Michalak, M.; Polewski, K. Contribution of phenolic acids isolated from green and roasted boiled-type coffee brews to total coffee antioxidant capacity. *Eur. Food Res. Technol.* **2016**, *242*, 641–653. [CrossRef]
32. Somporn, C.; Kamtuo, A.; Theerakulpisut, P.; Siriamornpun, S. Effects of roasting degree on radical scavenging activity, phenolics and volatile compounds of Arabica coffee beans (*Coffea arabica* L. cv. Catimor). *Int. J. Food Sci. Technol.* **2011**, *46*, 2287–2296. [CrossRef]
33. Fukushima, Y.; Tashiro, T.; Kumagai, A.; Ohyanagi, H.; Horiuchi, T.; Takizawa, K.; Sugihara, N.; Kishimoto, Y.; Taguchi, C.; Tani, M.; et al. Coffee and beverages are the major contributors to polyphenol consumption from food and beverages in Japanese middle-aged women. *J. Nutr. Sci.* **2014**, *3*, e48. [CrossRef]
34. Zujko, M.E.; Witkowska, A.M.; Waśkiewicz, A.; Mirończuk-Chodakowska, I. Dietary antioxidant and flavonoid intakes are reduced in the elderly. *Oxid. Med. Cell. Longev.* **2015**, *2015*, 843173. [CrossRef] [PubMed]
35. Lee, J.C. A study of compound changes in coffee beans by different roasting condition. *J. Culin. Sci. Technol.* **2016**, *22*, 114–119.
36. Sharma, K.; Ko, E.Y.; Assefa, A.D.; Ha, S.; Nile, S.H.; Lee, E.T.; Park, S.W. Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. *J. Food Drug Anal.* **2015**, *23*, 243–252. [CrossRef] [PubMed]
37. Naveed, M.; Hejazi, V.; Abbas, M.; Kamboh, A.A.; Khan, G.J.; Shumzaid, M.; Ahmad, F.; Babazadeh, D.; FangFang, X.; Modarresi-Ghazani, F.; et al. Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomed. Pharmacother.* **2018**, *97*, 67–74. [CrossRef]
38. Farah, A.; de Paulis, T.; Trugo, L.C.; Martin, P.R. Effect of roasting on the formation of chlorogenic acid lactones in coffee. *J. Agric. Food Chem.* **2005**, *53*, 1505–1513. [CrossRef]
39. Song, J.L.; Asare, T.S.; Kang, M.Y.; Lee, S.C. Changes in bioactive compounds and antioxidant capacity of coffee under different roasting conditions. *Korean J. Plant Res.* **2018**, *31*, 704–713. [CrossRef]
40. Murakami, M.; Yamaguchi, T.; Takamura, H.; Atoba, T.M. Effects of thermal treatment on radical-scavenging activity of single and mixed polyphenolic compounds. *J. Food Sci.* **2004**, *69*, FCT7–FCT10. [CrossRef]
41. Tarka, S.M.J.; Hurst, W.J. Introduction to the chemistry, isolation, and biosynthesis of methylxanthines. In *Caffeine*; Spiller, G.A., Ed.; CRC Press: Boca Raton, FL, USA, 1998; pp. 1–11.
42. dePaula, J.; Farah, A. Caffeine consumption through coffee: Content in the beverage, metabolism, health benefits and risks. *Beverages* **2019**, *5*, 37. [CrossRef]
43. Lopes, G.R.; Passos, C.P.; Rodrigues, C.; Teixeira, J.A.; Coimbra, M.A. Impact of microwave-assisted extraction on roasted coffee carbohydrates, caffeine, chlorogenic acids and coloured compounds. *Food Res. Int.* **2020**, *129*, 108864. [CrossRef]
44. Kedare, S.B.; Singh, R.P. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* **2011**, *48*, 412–422. [CrossRef]
45. Herawati, D.; Giriwono, P.E.; Dewi, F.N.A.; Kashiwagi, T.; Andarwulan, N. Critical roasting level determines bioactive content and antioxidant activity of Robusta coffee beans. *Food Sci. Biotechnol.* **2019**, *28*, 7–14. [CrossRef]
46. Suh, Y.S.; Lee, S.H.; Shang, Y.; Yoon, J.R.; Lee, W.J. Changes in antioxidant activities and flavor patterns of Coffea arabica beans during roasting. *Korean J. Food Preserv.* **2014**, *21*, 224–230. [CrossRef]
47. Sacchetti, G.; Di Mattia, C.; Pittia, P.; Mastrocola, D. Effect of roasting degree, equivalent thermal effect and coffee type on the radical scavenging activity of coffee brews and their phenolic fraction. *J. Food Eng.* **2009**, *90*, 74–80. [CrossRef]
48. Ou, B.; Huang, D.; Hampsch-Woodill, M.; Flanagan, J.A.; Deemer, E.K. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: A comparative study. *J. Agric. Food Chem.* **2002**, *50*, 3122–3128. [CrossRef] [PubMed]
49. Nam, S.; Kang, S. Changes of biochemical components and physiological activities of coffee beans according to different roasting conditions. *Korean J. Food Preserv.* **2015**, *22*, 182–189. [CrossRef]
50. Tomaino, A.; Cimino, F.; Zimbalatti, V.; Venuti, V.; Sulfaro, V.; De Pasquale, A.; Saija, A. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. *Food Chem.* **2005**, *89*, 549–554. [CrossRef]
51. Borrelli, R.C.; Visconti, A.; Mennella, C.; Anese, M.; Fogliano, V. Chemical characterization and antioxidant properties of coffee melanoidins. *J. Agric. Food Chem.* **2002**, *50*, 6527–6533. [CrossRef]
52. Dasgupta, A.; Klein, K. Methods for measuring oxidative stress in the laboratory. In *Antioxidants in Food, Vitamins and Supplements*; Dasgupta, A., Klein, K., Eds.; Elsevier: San Diego, CA, USA, 2014; pp. 19–40.

53. del Castillo, M.D.; Ames, J.M.; Gordon, M.H. Effect of roasting on the antioxidant activity of coffee brews. *J. Agric. Food Chem.* **2002**, *50*, 3698–3703. [CrossRef]
54. Pérez-Jiménez, J.; Saura-Calixto, F. Anti-oxidant capacity of dietary polyphenols determined by ABTS assay: A kinetic expression of the results. *Int. J. Food Sci. Technol.* **2008**, *43*, 185–191. [CrossRef]
55. Fernando, C.D.; Soysa, P. Extraction Kinetics of phytochemicals and antioxidant activity during black tea (*Camellia sinensis* L.) brewing. *Nutr. J.* **2015**, *4*, 74. [CrossRef]
56. Astill, C.; Birch, M.R.; Dacombe, C.; Humphrey, P.G.; Martin, P.T. Factors affecting the caffeine and polyphenol contents of black and green tea infusions. *J. Agric. Food Chem.* **2001**, *49*, 5340–5347. [CrossRef] [PubMed]