Chemical compounds and antioxidant activity in caffeinated and decaffeinated green robusta coffee beans enriched with ginger extract

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Abstract. Some detrimental effects of decaffeination process are attributed to the stability of the chemical composition of green coffee beans, include reducing the antioxidant activity. Ginger (Zingiber officinale) is widely used as an ingredient for herbal beverage due to its well-known antioxidant activity. The aim of this study was to evaluate the effect of decaffeination process on the chemical compounds and antioxidant activity of green robusta coffee beans without or with ginger extract. The result disclosed that the total phenolic contents of decaffeinated- and caffeinated- coffee were significantly higher up to 1.13 and 3.13 mg GAE/mL, respectively, as the increase of ginger extract concentration of 30%. Of the highest concentration of ginger extract, the total flavonoid contents of decaffeinated- and caffeinated- coffee increased up to 2.55 and 13.41 mg QE/mL, respectively, as well as the melanoidin contents, rose until 0.53 nm (decaffeinated coffee) and 1.19 nm (caffeinated coffee). The analysis also found that the highest antioxidant activity determined by 2,2-Diphenyl-1-picrylhydrazyl was observed in the decaffeinated- and caffeinated- coffee (0.20 and 0.46 mmol TEAC/mL, respectively) with the addition of 30% ginger extract. When 30% ginger extract was added into the decaffeinated- and caffeinated- coffee, there was a significant improvement in the antioxidant activity tested by ferric reducing antioxidant power (0.27 and 0.77 mmol TEAC/mL, respectively) and hydroxyl radical scavenging assay (2.10 and 2.66 mmol TEAC/mL, respectively). Regardless of the concentration of ginger extract, this study reveals that decaffeinated coffee has lower antioxidant activity since a lower content of polyphenol, flavonoid, and melanoidin.

1. Introduction
In many countries, coffee has been widely commercialized and has become of favorite drink since each coffee species has its own taste and aroma. Coffea arabica (arabica) and Coffea canephora (robusta) are the most commercially traded coffee species in the worldwide economy. Ref. [1] found that the caffeine content of the extract of robusta coffee was two-fold higher than that of arabica coffee and it was a common discriminator of both coffee species. Good quality of coffee can be attributed to the low percentage of caffeine level and it may be the reason why arabica coffee is more expensive compared to robusta.

Despite caffeine is one of the main contributors to the bitterness quality of coffee, it is also important to note that caffeine appears as a debatable compound related to human health. The European Food and Safety Authority recommends the caffeine consumption 3 mg/kg body weight/day is safe for healthy children and adolescents [2] but the safe dose is still vary depending on populations targeted. However, too much caffeine consumption may result in adverse effects such as depressive symptoms,
cardiovascular disease, as well as impairments in reproduction and sleep [3]. Hence, the market demand for decaffeinated coffee rose by about 10% in the last decade [4] due to the great interest of coffee drinkers on this product.

The decaffeination process is performed by using certain extraction methods completed with certain parameters determined to reduce the caffeine content in the coffee beans. Ref. [5] reported a significant depletion from 42,651.95 mg/kg to 16,791.13 mg/kg in the caffeine content of coffee by using a water extraction method. However, other bioactive compounds in coffee such as caffeic acid, chlorogenic acid, ferulic acid, pyrogallic acid, and trigonelline were comparable between caffeinated and decaffeinated coffee. Owing to the presence of other bioactive compounds in coffee beans with known antioxidant effects, this decaffeination process does not rule out the probability of the loss of those bioactive compounds. Polyphenol, flavonoid, and tannin are detected as antioxidant compounds in the green coffee beans [6], and they are susceptible to be decomposed during the water extraction process with high temperature.

With respect to the reduction of the bioactive compound and the stability of the antioxidant activity, this study added ginger extract to reconstitute the content of bioactive compound. Ginger has been studied to have remarkable antioxidant activity and many other health benefits from abundant bioactive compounds [7]. Therefore, this study aimed to assess the chemical compounds and antioxidant activity of green robusta coffee beans as affected by the decaffeination process, and with or without the addition of ginger extract.

2. Materials and Methods

2.1. Materials
Green robusta coffee beans were obtained from Argopuro mountain, Jember, East Java, Indonesia. Large white gingers were obtained from the traditional market in Jember, Indonesia.

2.2. Decaffeination of green coffee beans
Decaffeination process was performed as described by Ref. 1] with modification in pre-treatment of green coffee beans and water-to-beans ratio for extraction process. Steaming process for four hours was applied to green coffee beans to escalate the moisture content prior to extraction. The water extraction method was done with the water-to-beans ratio of 5:1 (v/w) and extraction temperature of 80 °C for three hours. Decaffeinated green coffee beans were dried at temperature of 100 °C for 21 hours.

2.3. Coffee roasting and brewing
Caffeinated- and decaffeinated- coffee beans were roasted according to the method of Haile and Kang [6] with modification in roasting time and temperature. The roasting was performed at 180 °C for 10 minutes. The roasted coffee beans were then ground to obtain a coffee powder. Approximately 100 g of coffee powder was brewed in 300 mL of water at temperature of 80 °C for 45 minutes. Hot brew extraction was equipped with a stirrer to corroborate the extraction process. A filter paper was used to obtain a coffee extract.

2.4. Ginger extraction
The extraction was carried out as described by Ref. [8] with modification in solvent type and solvent-to-ginger ratio. Briefly, about 100 g of peeled ginger was shredded. The shredded ginger was extracted in 50 mL of water at temperature of 100 °C for 15 minutes. A cheesecloth was used to obtain a ginger extract.

2.5. Crystallization
A ratio used between extract (a combination between coffee and ginger extract) and sucrose was 1:0.5 (v/w). The concentration of the coffee extract was made constant while the concentrations of the ginger extract were varied as to be 0, 10, 20, and 30% of the weight of the coffee extract. Sucrose was added
into the combined extract and the mixture was heated to reach a concentrated solution. The heating process was stopped but the stirring process was still performed until the crystal was formed. The crystal was ground to obtain powder form.

2.6. Determination of total phenolic content
Total phenolic content was examined according to the method of Slinkard and Singleton [9] with slight modification. An aliquot of approximately 0.05 mL of sample was pipetted into a test tube and mixed with 4.95 mL of distilled water. A volume of 0.5 mL of Folin-Ciocalteu’s phenol reagent was pipetted into the test tube and allowed to sit for five minutes. Approximately 1 mL of 7% sodium carbonate was pipetted into the mixture and incubated in the dark at room temperature for an hour. Absorbance was measured at 765 nm by using a UV spectrophotometer. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/mL.

2.7. Determination of total flavonoid content
Flavonoid content was spectrophotometrically assayed according to the method of Ref. [10] with slight modification. Approximately 0.05 mL of sample was pipetted into a test tube containing 2.35 mL of distilled water. A volume of 0.3 mL of 5% sodium nitrite and a volume of 0.3 mL of 10% aluminium chloride were pipetted into the mixture and homogenized. The mixture was allowed to sit for five minutes at room temperature and followed by the addition of 2 mL of 1M sodium hydroxide. Absorbance was measured at 510 nm by using a UV spectrophotometer. The total flavonoid content was expressed as mg quercetin equivalent (QE)/mL.

2.8. Determination of melanoidin content
The melanoidin content was measured according to the method described by Ref. [11] with slight modification. A sample was diluted 13 times using distilled water. The measurement was performed at absorbance of 420 nm. The melanoidin content was expressed as absorbance value.

2.9. Determination of DPPH radicals scavenging activity
Antioxidant activity was measured by the DPPH method as explained by Yamaguchi et al [12] with slight modification. Approximately 0.05 mL of sample was pipetted into a test tube and added with 2.95 mL of ethanol. About 3 mL of 300 µM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was also added into the mixture and homogenized for a moment. The mixture was allowed to stand for about 30 minutes. Absorbance was measured at 517 nm by using a UV spectrophotometer. The antioxidant activity was expressed as mmol trolox equivalent (TE)/mL.

2.10. Determination of ferric reducing antioxidant power
Determination of antioxidant activity by the FRAP method was also performed as explained by Benzie and Strain [13] with slight modification. The reagent was prepared by mixing 25 mL of 300 mM of an acetate buffer with a pH of 3.6; 2.5 mL of 2,4,6-tripyridyl-s-triazine (TPTZ) solution; and 2.5 mL of ferric chloride hexahydrate solution. A 0.05 mL volume of sample was pipetted into a test tube with the subsequent addition of a 2.95 mL reagent solution. The mixture was homogenized and stored in the dark for 30 minutes. Absorbance was spectrophotometrically measured at 593 nm. The antioxidant activity was expressed as mmol trolox equivalent (TE)/mL.

2.11. Determination of hydroxyl radical scavenging activity
Antioxidant activity was also measured by hydroxyl radical scavenging according to the method of Halliwell et al [14] with slight modification. A 0.05 mL volume of sample was pipetted into a test tube and followed by the addition of 0.05 mL of distilled water. Approximately 690 µl of 2.5 mM deoxyribose in 10 mM of phosphate buffer with a pH of 7.4 and 100 µl of the mixture of sodium calcium edetate (1.04 mM)-iron ammonium sulphate (1.0 mM) were also added into the test tube. About 200 µl of 1.0 mM ascorbic acid and 50 µl of 0.1 M hydrogen peroxide were subsequently added into the mixture. The
mixture was incubated at 37 °C for 10 minutes and it was then added by 1 mL of 2.8% trichloroacetic acid and 0.5 mL of 1% thiobarbituric acid. The mixture was heated in a water bath for eight minutes. Absorbance was measured at 532 nm by using a UV spectrophotometer. The antioxidant activity was expressed as mmol trolox equivalent (TE)/mL.

2.12. Statistical analysis
Data were statistically analyzed using a one-way analysis of variance (ANOVA) with SPSS 17 software to identify significant differences (α = 5%) among samples.

3. Results and Discussion
3.1. Chemical compounds
The total phenolic, total flavonoid, and melanoidin contents of the caffeinated- and decaffeinated- coffee with different additions of ginger extract concentration are shown in Figure 1, Figure 2, Figure 3, respectively. The total phenolic content (TPC) ranged from 2.89 mg GAE/mL in caffeinated coffee without the addition of ginger extract to 3.13 mg GAE/mL in caffeinated coffee with the addition of the highest ginger extract concentration as much as 30%. In contrast, the TPC of decaffeinated coffee only ranged from 1.01 mg GAE/mL (without ginger extract) to 1.13 mg GAE/mL (30% ginger extract). Despite a statistically significant difference among treatments was achieved by the decaffeinated coffee, the levels of TPC were still considerably lower than caffeinated coffee. This result is in line with the work reported by Ref. [15] that showed that the TPC of decaffeinated instant coffee (ranged from 7.182 to 7.727 g GAE/L) was lower than caffeinated instant coffee (ranged from 9.159 to 9.614 g GAE/L). Many of the compounds found in the coffee belong to the phenolic group [5] and they are heat-unstable compounds [16], thereby decomposing those compounds when the decaffeination process was performed at temperature of 80 to 100 °C.

In line with the result of the TPC levels of caffeinated- and decaffeinated- coffee, the total flavonoid content (TFC) also demonstrated a similar pattern. The TFC values of decaffeinated coffee (1.91-2.55 mg QE/mL) were extremely lower compared to caffeinated coffee (11.78-13.41 mg QE/mL). This may be due to thermal instability since flavonoids are also categorized into the phenolic group, so they undergo many changes during the decaffeination process. Ref. [17] presented a significant reduction on the TFC of coffee as a consequence of heating treatment, in which the loss of the TFC was found to be almost 30% of its initial content. In this study, the enormous loss of the TFC was recorded more than 85% and it can be speculated that the pre-treatment of green coffee beans (steaming for four hours) also enhances this loss in addition to decaffeination treatment. Hence, further analysis is needed to prove the TFC of pre-treated coffee and non-pretreated coffee.

Also, the melanoidin content of decaffeinated coffee showed a smaller level (in range of 0.44-0.43 nm) than the melanoidin content of caffeinated coffee (around 1.12-1.19 nm). This is similar to the study of Ref. [18]. The higher TPC the higher melanoidin content, as seen in the instant coffee samples that were processed under the strict conditions to maintain their components. In this study, the caffeinated coffee samples had higher TPC than the decaffeinated coffee, thus resulting in a higher level of melanoidin content in the caffeinated coffee. Many constituents such as phenols, carbohydrates, and proteins can be degraded when the coffee beans were subjected to decaffeination, while in the caffeinated coffee the constituents are unchanged and will promote to form melanoidin once they are intensively heated [18].

Regardless of the decaffeination process, the levels of chemical compounds observed in this study were significantly improved by the higher addition of the ginger extract. The highest value belongs to the 30% ginger extract, followed by 20% and 10% ginger extracts. Coffee without ginger extract appeared to perform the lowest value at all chemical compounds. Ref. [8] reported that the TPC and TFC of ginger extract were 314-504 µg/mL and 226-286 µg/mL, respectively. Their report supports the fact of this study that the higher concentration of ginger extract elevates the chemical compounds of the
coffee-ginger mixture. This is due to the ginger extract itself contains phenolic and flavonoid compounds.

3.2. Antioxidant activities

**Figure 1.** Total phenolic content of caffeinated- and decaffeinated- coffee without or with the addition of ginger extract. The bars with different letters denote statistically significant differences among treatments at $\alpha = 0.05$.

**Figure 2.** Total flavonoid content of caffeinated- and decaffeinated- coffee without or with the addition of ginger extract. The bars with different letters denote statistically significant differences among treatments at $\alpha = 0.05$.

**Figure 3.** Melanoidin content of caffeinated- and decaffeinated- coffee without or with the addition of ginger extract. The bars with different letters denote statistically significant differences among treatments at $\alpha = 0.05$. 
There were also significant differences in the antioxidant activities of caffeinated- and decaffeinated-coffee assayed by DPPH, FRAP, and hydroxyl radical methods, as shown in Figure 4A, Figure 4B, Figure 4C, respectively. All three antioxidant measurements showed a similar increasing trend along with the increase in ginger extract concentration. The antioxidant activities of caffeinated coffee were recorded in range of 0.40-0.46 mmol TE/mL (DPPH assay), 0.65-0.77 mmol TE/mL (FRAP assay), and 2.19-2.66 mmol TE/mL (hydroxyl radical assay). In contrast, the ranges of antioxidant activities of decaffeinated coffee were 0.17-0.20 mmol TE/mL (DPPH assay), 0.19-0.27 mmol TE/mL (FRAP assay), and 1.84-2.10 mmol TE/mL (hydroxyl radical assay).

**Figure 4.** Antioxidant activity of caffeinated- and decaffeinated- coffee without or with the addition of ginger extract tested by the methods of DPPH (A), FRAP (B), and hydroxyl radical (C). The bars with different letters denote statistically significant differences among treatments at $\alpha = 0.05$.

The caffeinated coffee exhibited a much higher activity, in particular, tested by DPPH and FRAP methods, than the decaffeinated coffee. This result is in agreement with Ref. [15]. The decaffeinated instant coffee showed a slightly weaker antioxidant activity than that of caffeinated instant coffee, as tested by DPPH, FRAP, and ABTS methods. In this study, all of the chemical compounds found in the caffeinated coffee accounted for higher contents compared to the decaffeinated coffee, thereby boosting the antioxidant activity performance of the caffeinated coffee. This antioxidative effect can be attributed to the presence of phenolic and melanoidin compounds [19].

Further, the maximum antioxidant activities were highlighted in the addition of 30% and the lowest activities were recorded in the coffee mixture without ginger. This result shows that the increase of antioxidant activity of the coffee-ginger mixture can be related to the synergistic effect of the chemical compounds in the coffee and ginger [20]. Phenolic compounds found in the coffee-ginger mixture donate their electron [6] to scavenge free radicals. On the other hand, melanoids act as an antioxidant
due to their functional groups such as ester, amine, and aromatic groups [19]. These compounds are possessed by either coffee extract or ginger extract, thus the combination of both extracts can raise the antioxidant activity.

4. Conclusion
In conclusion, the decaffeination process decreases TPC, TFC, melanoidin content, as well as the antioxidant activity of coffee and coffee-ginger mixture since this activity is influenced by the presence of those chemical compounds. The addition of ginger extract could be possibly used as a source of bioactive compounds for supporting the antioxidant activity of coffee.

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