Comparison of radical scavenging activity of commercial Arabica and Robusta coffee based on roasting method and brewing condition

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Abstract. Coffee is a pleasurable beverage which works widely consumed. Commercial coffee comes from two species of coffee plant which is Arabica and Robusta coffee. Currently, antioxidant activity in coffee is a popular topic which is obtained from drinking coffee that has passed through the complex stages of processes. In Banda Aceh, coffee drinking habits are obtained from coffee shops scattered throughout the nine districts, while drinking coffee is considered as a necessity and tradition that has been handed down over the centuries. Each traditional coffee shop has a coffee featured with unique roasting and brewing methods. This study aims to identify the antioxidant activity in commercial Arabica and Robusta grounded coffee based on roasting method (torrefacto and conventional) and brewing (manual and cooked) condition. This research was conducted using Factorial Random Block Design with 3 replications, then it continued with Least Significance Difference (LSD). The analysis of variance (ANOVA) showed coffee varieties and the brewing condition and interaction within three variables have very significant influence (P≤0.01) while roasting methods significantly influenced (P≤0.05) the total phenolic content of coffee drinks. The study also showed that brewed coffee drink has higher total phenolic content and antioxidant activity compared to cooked coffee drink. Commercial Arabica coffee, which is torrefacto roasted and manually brewed has 127.58 phenol GAE / ml total phenol, antioxidant activity 46.89% and IC50 value of 254.89 ppm. On the other hand, commercial Robusta coffee which accepted similar treatment is slightly higher, 141.10 GAE / ml total phenol, antioxidant activity 50.88%. To sum up in this research, commercial robusta coffee, which is torrefacto roasted and manually brewed has higher antioxidant activity than other variety.

1. Introduction

Coffee is a popular drink, which is commercially known as Arabica and Robusta coffee. In addition to its flavor, the benefits of coffee are also obtained from the content of the active compounds in it such as chlorogenic acid, nicotinic acid, trigonelline, quinolinate tanat, pyrogalat and caffeine [1]. This active compound together with other chemical components are responsible as a precursor of coffee flavour [2]. One of popular active compound in coffee is caffeine, which is also responsible for the
sense of bitterness in brewed coffee. The level of caffeine contained in robusta rice coffee is around 2.2%, while arabica coffee is around 1.2% [3].

Active compounds such as chlorogenic acid are reported as a source of antioxidants in coffee. Antioxidants are substances that can neutralize free radicals so that they can protect human biological systems from adverse effects arising from processes or reactions that cause excessive oxidation [4]. Sunarni [5] reported that Robusta coffee has more antioxidants than Arabica coffee. Furthermore, Antonio and Levacchia [6] stated that the antioxidant activity in Robusta coffee was 0.86-7.53%, where the extraction results of Robusta coffee beans which had been roasted on IC50 had an antioxidant of 140.42 ppm [7].

To have delicious coffee drink, the coffee fruits undergo through post-harvest processing, roasting and brewing. This complex process certainly affects the chemical composition and antioxidants of coffee, while the coffee flavor produced is a manifestation of these complex processes. Hernandez [8] reports that the roasting and grinding process will decrease coffee antioxidant activity, where Robusta coffee initially has higher antioxidant levels, but after roasting antioxidant levels of Arabica will be higher than Robusta [9].

In Banda Aceh, known as the city of a thousand coffee shops, drinking coffee is a necessity and a tradition that has been passed from generation to generation. Generally, each coffee shop has superior coffee with a unique processing in Aceh, such as the addition of other components during roasting called the torrefacto or the process of serving by cooking the coffee. According to Ludwig et al., [10], torrefacto roasting process is carried out by adding other components such as corn and sugar in the final stage of roasting. Torrefacto is a roasting method that adds sugar 10-15% of the weight of coffee at the end of the roasting process. Not only that, in traditional coffee shop, the presentation coffee is commonly done by cooked coffee instead of brewed, that will produce concentrate of black coffee. Therefore, based on the several mentioned facts above, the researchers find out that is necessary to examine the antioxidant activity in commercial Arabica and Robusta grounded coffee from some of the leading coffee shops in Banda Aceh city according to the roasting and brewing methods. The aim of this study is to obtained information about antioxidant activity of coffee that is presented by coffee shops in Banda Aceh.

2. Materials and methods
The laboratories works were done in Coffee Pilot Plant and Product Development Laboratory and Food Product Analysis Laboratory. All laboratories are part of Department of Agricultural Product Technology, Faculty of Agriculture – Universitas Syiah Kuala.

2.1. Materials
The main ingredients used in this research are commercial Arabica and Robusta grounded coffee. One day prior to analysis, commercial Arabica and Robusta coffee was roasted conventionally and torrefacto, then packed in aluminum foil and stored in a dark room temperature for analysis. The chemicals used include DPPH, methanol, a solution of folin-ciocalteau (Merck), sodium carbonate (Na2CO3) (Merck), gallic acid (Sigma), 90% ethanol. The equipment used in this study include drum air roaster, gas stove, teapot, spoon, UV-vis spectrophotometer, vortex, digital scales, separating funnel, measuring cup, beaker, test cube, volume pipette and filter paper.

2.2. Methods
This research is conducted using factorial randomized block design with three independent variables. The first variable is coffee variety being used (K1=Arabica and K2= Robusta), the second variable is roasting method (P1=conventional and P2=torrefacto), and the third variable is the presentation method (S1= brewed and S2= cooked). Then all the treatment combination has is done in three repetitions, which at the end the total experiments are 24 units. Moreover, the effect of the
independent factors are calculated by ANOVA then continued with Least Significance Difference (LSD) Test as the continuing test to determine the influenced treatment from independent variables. All statistical data was run in Microsoft Excell 2013 and SPSS 19.0.

2.3. Coffee roasting procedures
The sample used is grounded coffee both from Robusta and Arabica varieties. The green bean is roasted and ground a day before the analysis process. The conventional roasting process is carried out at temperatures between 160-180 °C with medium degree roasting. The hot air-roaster roasting machine is heated to a temperature of 160-170 °C, then the coffee beans are inserted and roasted for 7-8 minutes and cooled. As for torrefacto roasting, after being removed from the hot air-roaster roasting machine, roasted coffee beans are mixed with 11% sugar over a hot pot, stirred until blended and then cooled. Then grinded coffee beans with a size of 30 mesh, packed and ready to use.

2.4. Coffee brewing procedures
10 grams of each Arabica and Robusta coffee are weighed and prepared according to each treatment. The brewing process uses water temperature of ± 95 ° C with a ratio of coffee and water, 1: 9. Preparation of brewed by pouring the weighed grounded coffee with hot water ± 95 ° C then stirred and left for 4 minutes. Then the coffee is ready to be analyzed. On the other hand, cooked coffee preparation started by boiling the measured coffee samples with water by ratio 1 coffee: 9 water in gas stove, stirring while boiling it required until it reached water temperature of ± 100 ° C. The coffee sample ready to be analyzed after left for 4 minutes.

2.5. Coffee brewed analysis
The measured parameters in this study respectively are total phenol testing using the folin-ciocalteau method [11], antioxidant activity test by DPPH method [12] and IC50 value assignment for four samples with highest antioxidant activity [13].

Total phenol test is using UV-VIS Spectrophotometer, where 1 ml of coffee steeping samples was placed in a test tube containing 1 ml of 95% ethanol and 5 ml of ion-free water, and then 5 ml of the 50% folin-ciocalteau reagent was added. After 5 minutes, 1 ml of 5% Na2CO3 is added, and then it is vortexed until the mixture liquid is homogeneous and stored in a dark room for 1 hour. The absorbance is measured by a spectrophotometer at a wave length of 725 nm. The standard curve was prepared using tannic acid in 95% ethanol with a concentration of 0, 5, 20 and 25 ppm.

Measurement of antioxidant activity using DPPH method, which starts with preparation of DPPH solution by mixing 0.098 mg DPPH into a 100 ml measuring flask to the limit. Determination of the blank absorbance standard by mixing a solution of 3.8 ml DPPH (0.5 mm) with 0.2 ml of methanol 70%, then reading the absorbance on a spectrophotometer should be done first. The absorbance of the sample was carried out by taking 0.2 ml of the coffee steeping sample and adding 3.8 ml of DPPH solution then it was vented and let stand for 30 minutes, after which the absorbance was read on a spectrophotometer.

IC50 value assignment for four samples with highest antioxidant activity. IC50 is made with linear curves y = ax + b, made five sample concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm respectively. Then pipette from each concentration as much as 0.2 ml of sample and add each of the DPPH 3.8 ml reagents. After that, the samples are stood out for 30 minutes. All absorbance were measured using a wavelength 517 nm spectrophotometer, where the wavelength of 517 nm is the maximum wavelength of the DPPH wavelength which aims to get the maximum absorbance value in. the solution tested after the absorbance is read, then look for the percentage of inhibition of each concentration. Data on the percentage of resistance and concentration of the solution is used to find the IC50 value with the linear regression equation y = ax + b, where y is% inhibitory (worth 50) and x is the IC50 value.
3. Results and discussion

3.1. Total phenols of brewed coffee

Analysis of total phenols in coffee drinks is using folin-ciocalteau staining. This method is an easy method to measure the total phenol content of natural products [11]. This method uses a standard curve which prepared using gallic acid in the aquadest. Galic acid is made at concentrations of 5, 10, 15, 20, and 25 ppm.

The total phenol from resulted coffee drinks are ranged from 106.74 to 144.14 mg GAE / ml with an average of 129.06 mg GAE / ml. The results of variance on the total phenol of resulted coffee drinks showed that the roasting and presentation method variables had a very significant effect on the total phenolic content of coffee drinks (P≤0.01), the treatment of roasting conditions and the interactions among treatments have significantly affect (P≤0.05) the total content of phenol coffee drinks. The interaction between the three treatments can be seen in Figure 1.

Results showed the total phenol content obtained from conventional roasting robusta grounded coffee with brewed serving method which is 144.14 GAE / ml and was not significantly different from torrefacto roasting robusta grounded coffee with brewed serving method with 141.10 GAE / ml and conventional roasting robusta grounded coffee with cooked serving method which is 140.00 GAE / ml. However, this treatment is significantly different from all other unmentioned treatments.

Based on Figure 1 it can be seen that robusta coffee drinks have a higher total phenol compared to arabica coffee drinks. This result is in accordance with previous study [14], which states that the total phenol content of robusta coffee (38.8% ) steeping is higher than that of arabica coffee (36.9%). Furthermore, coffee processing processes such as roasting and serving steaming coffee certainly affect coffee chemical composition, especially reduce the coffee polyphenol content [15].

Furthermore, the method of serving with brewing produces a higher total phenol content compared to cooking in both Arabica and Robusta coffee. This is presumably due to the presentation with cooking using a higher temperature (> 100°C) than brewing (<100°C). This is in accordance with the previous statement [16], which claimed that the higher the temperature and pressure applied by brewing the coffee, the greater the number of water soluble components such as chloregenic acid, caffeine and nicotinic acid can be extracted, where these phenolic compounds are components the
main total phenol in coffee, which is up to 90% [16]. Other study [17] also reported that household or traditional brewing process managed to extract chlorogenic acid as much as 70-200 mg per 200 ml of Arabica coffee, while robusta, chlorogenic acid reached 70-300 mg per 200 ml, which according to Kristiningrum [14] the total phenol content of robusta coffee steeping was 38.3 mg equivalent to chlorogenic acid every 100 mg extract, whereas in arabica coffee steeping was 19.8 mg equivalent to chlorogenic acid every 100 mg extract.

Figure 1 also showed that conventional roasting also affects the total phenolic content of coffee drinks. For conventional arabica coffee and brewed it produces a higher total phenol content compared to torrefacto roasting arabica coffee. Likewise with robusta, torrefacto and cooked roasting robusta coffee produces higher total phenol content than conventional roasting coffee. This is consistent with the statement of Ludwig et al., [10] which states that the addition of sugar during roasting can increase antioxidant activity.

3.2. Antioxidant activity of coffee brewed drink
This analysis was carried out by measuring DPPH synthetic synthesis (2,2-diphenyl-1-pikrilhidrazil) in polar organic solvents such as ethanol at room temperature. This method is simple, easy, fast and sensitive and requires only a small sample using UV-Vis spectrometry. Huang et al. [18] stated that DPPH is a purple radical and when reduced by antiradical compounds will turn yellow. The process of inhibiting free radicals by antioxidants is expressed in the form of% inhibition. The higher percent inhibition of a sample shows the stronger the level of inhibition of free radicals by the antioxidants found in the sample.

The antioxidant activity produced ranged from 14.04% - 60.70% with a general average of 34.58%. The results of variance showed that variety of grounded coffee treatment and roasting conditions were not significant (P> 0.05) while the presentation method had a very significant effect on the value of the antioxidant activity of the coffee drinks produced (P≤0.01). The interaction between variety of grounded coffee and the presentation method had no significant effect on the value of antioxidant activity of coffee drinks produced (P> 0.05). For interaction between the three treatments had no significant effect on antioxidant activity (P> 0.05). The effect of interaction between coffee variety and presentation method can be seen in Figure 2 and the interaction effect between roasting conditions and presentation methods as seen in Figure 3.

![Figure 2. Interaction between coffee variety and presentation methods on the value of antioxidant activity of coffee drinks.](image-url)
Results (Figure 2) showed that the lowest antioxidant activity was obtained in cooked arabica grounded coffee (20.75%). This result was significantly different from all other treatments. While the highest antioxidant activity was obtained in brewed Robusta grounded coffee (55.79%). Based on Figure 2 it can be seen that the antioxidant activity of Arabica coffee is lower than Robusta coffee. This is in relevance with the analysis of the total phenol content (Figure 1), where the total phenol content of robusta coffee is higher than that of arabica coffee, so that the antioxidant activity produced is higher. According to the National Coffee Association [19], the best water temperature in making coffee ideally is 90-96°C (195 ° F -205 ° F), as implemented by brewing method. However, serving coffee by cooking or boiling the grounded coffee in water at 100°C as in this study is still commonly used in the community.

![Figure 3. Interaction between roasting conditions and presentation methods on the value of antioxidant activity of coffee drinks.](image)

Figure 3 shows that coffee drinks served by cooking with conventional and torrefacto roasting conditions produce antioxidant activity that is not significantly different from each other but this treatment is significantly different from coffee drinks served with brewing methods. Based on Figure 3 it is also known that brewed coffee drinks produce higher antioxidant activity than cooked coffee drinks. This is because the cooking process uses a higher temperature so that it can result in degraded antioxidant compounds [20]. In addition, Figure 3 also shows that the conventional roasted coffee produces coffee with higher antioxidant activity. In Figure 1 shows that both roasting conditions produce coffee drinks with the same total phenol, but with different antioxidant activities. Conventional roasting is generally used for good quality rice coffee while torrefacto roasting, generally used to cover quality defects in coffee [10]. This is allegedly influenced by the initial quality of rice coffee used. Lopez-Galilea et al., [21] reported that the antioxidant capacity in coffee is highly dependent on phenol compounds found in coffee beans and the formation of melanoidins during roasting.

### 3.3. Determination of IC50 Value.

IC50 is the concentration of antioxidants that inhibit 50% of free radicals. The smaller the IC50 value, the stronger the antioxidant activity produced, and vice versa, the greater the IC50 value, the weaker the antioxidant activity [22]. The smaller IC50 value shows greater antioxidant activity. In this study, the IC50 value of antioxidant activity was calculated on four samples of coffee powder according to the type of coffee treatment and roasting method. All samples are served by brewing
because of the analysis of total phenol and antioxidant activity it is seen that coffee brewing produces a better total phenol and antioxidant activity. The results of IC50 analysis in the four samples can be seen in Table 2.

| No  | Material                  | IC50 Value |
|-----|---------------------------|------------|
| 1   | Conventional arabica coffee steams | 274.03 ppm |
| 2   | Torrefacto arabica coffee brewing | 254.71 ppm |
| 3   | Conventional Robusta coffee | 150.00 ppm |
| 4   | Torrefacto robusta coffee steeping | 145.70 ppm |

Table 1 shows that conventional arabica coffee drinks have a value of IC50 274,03 ppm and arabica coffee torrefacto 254,71 ppm. When the value of IC50 obtained ranges from 200-1000 ppm, then the substance is less active but still potentially as an antioxidant substance [22]. This is thought to be influenced by the low total phenolic content of arabica coffee. In addition, the coffee used in this study is a commercial coffee originating from a coffee shop, so it is suspected many factors that affect the antioxidant activity of coffee, such as the condition and duration of coffee bean storage and so forth. Based on analysis of total phenol content and antioxidant activity, it is known that the total content of phenol and antioxidant activity in arabica coffee is lower than robusta coffee.

4. Conclusions
Based on the results of the study it can be concluded that the method of serving with brewing treatment on coffee drinks results in a higher total phenol content and antioxidant activity compared to cooking treatments. Torrefacto roasted arabica grounded coffee and brewed has a total phenol value of 127.58 GAE / ml, antioxidant activity 46.89% and IC50 254.89 ppm. The torrefacto robusta grounded coffee and brewed have a high total phenol content of around 141.10 GAE / ml, antioxidant activity 50.88%. Based on the test of total phenol and antioxidant activity, the best treatment was obtained in conventional robusta coffee drinks with brewed serving method. Further research is needed to determine the effect of the degree of neglect on antioxidant activity and also to determine the amount of chlorogenic acid and other components of the phenol chemical components that act as antioxidants in arabica and robusta coffee drinks in Aceh.

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