Effect Solvent and Extraction Method on the Characteristics of Green Bean Robusta Coffee

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Abstract. One of the chemical elements found in coffee is caffeine, which is the second-largest secondary metabolite after chlorogenic acid. Robusta coffee has a higher caffeine content than Arabica. Low-caffeine coffee is an alternative choice for people who are aware to negative effects of caffeine. Process caffeine reduction was carried out in this reseerach by using the maceration method, soxhletation and microwave exposure with dichloromethane, ethyl acetate and water as solvents. Soxhletationee caffeine content was originally 2.2 % after caffeine was extracted using the soxhletation method using 15 % ethyl acetate solvent, coffee caffeine content was 0.87 %, antioxidant activity (IC 50) was 1 867 mg L^{-1}, total flavonoids were 420.3 mg L^{-1}, lightness 45.7, redness is 2.3 and yellowness 10. The reduction of caffeine content in coffee beans is influenced by the method and duration of extraction as well as the solvent used to dissolve the caffeine.

Keywords: Antioxidant activity**,** *Coffea canephora* Pierre ex A. Froehner, decaffeinated coffee, flavonoid, healthy drink.

1 Introduction

Coffee is one of the main plantation commodities, managed by farmers in Indonesia. Robusta coffee (*Coffea canephora* Pierre ex A. Froehner) is around 70.15 % [1]. It is adaptable and resistant to disease. Coffee has a distinctive taste and aroma caused by the chemical compounds contained. Caffeine is one of the chemical compounds contained in coffee and is the second largest secondary metabolite after chlorogenic acid. Caffeine is a group of methylxanthine compounds that are formed naturally and are included in the xanthine derivatives of the alkaloid compound group. The caffeine content in coffee beans varies depending on the type of coffee and the geographical conditions where the coffee is grown [2, 3]. Consumption of caffeine with the right dose can provide health benefits for the body [4, 5]. Naturally, coffee contains a bioactive component known as caffeine. Caffeine in coffee is useful as an antioxidant, anti-cancer, and is a special substituent that can stimulate brain performance [6, 7]. The dose of caffeine permitted by the FDA is

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100 mg d^{-1} to 200 mg d^{-1}, while according to SNI 017152-2006 it is 150 mg d^{-1} and 50 mg per serving. Excessive and regular consumption of caffeine can have a negative impact on the body, such as experiencing dependence, nausea, nervousness, anxiety, insomnia, increased heart rate, and even death [8].

Decaffeination is an attempt to reduce the caffeine content in coffee. Low-caffeine coffee is an alternative choice for people who are aware to negative effects of caffeine. According to decaffeination can be done using water (water decaffeination —Swiss method), solvent (solvent decaffeination) or super critical CO_2 (super critical carbon dioxide decaffeination [9, 10]. Wijaya and Yuwono [11] carried out the decaffeination process with a 2 h steaming system followed by reflux extraction for 1 h using 15 % ethyl acetate solvent was able to reduce the caffeine content and phenolic compound. Mursalin *et al.* [12] have reduced caffeine using the maceration method at a temperature of 50 ℃ to 65 ℃ for 120 min. Factors that affect the decrease in caffeine are the extraction method, solvent and extraction time. The purpose of this research was to examine other extractions methods to reduce green coffee caffeine.

2 Methodology

2.1 Materials

The main ingredients used in this research are canephora coffee cherries which are harvested and processed into coffee beans by coffee farmers in Karangploso village, Malang, East Java, Indonesia. The coffee used in this study is processed using the natural method. Coffee cherry was dried under sun rays until the moisture content reaches 12 %. The dried coffee dehulled to obtained the green bean. The supporting materials used were vitamin C, DPPH (2,2-diphenyl-1-picrylhydrazil) (sigma Aldrich), chloroform solvent, methanol, 80 % methanol, ethyl acetate and aquades. The chemicals used are pro-analytical E. Merck.

2.2 Reducing caffeine content of coffee bean

Materials are collected from coffee farmers in Karangploso, Malang, East Java, Indonesia (coordinate 7.8933°S 112.5956°E). Coffee is sorted, then steamed for 30 min. This process aims to rehydrate the coffee beans and make the pores of the bean tissue open, so as to facilitate the extraction process. The steamed coffee was extracted using maceration method for 24 h, 48 h, soxhletation (SOXH-6 SET made in Korea) 4 h, 8 h, and microwaves (Sharp Bioautotech - R-728(K) made in Indonesia) extraction for 4 min by aquades, 15 % ethyl acetate, and dichloromethane. The ratio of green been coffee and solvent is 1 : 3, after the extraction process is complete the coffee is separated from the solvent and steamed for 30 min to evaporate residu of solvent and dried using a cabinet dryer at 50 ℃ for 24 h. Coffee that has been dried to be ground is stored in a tight container stored in a cooler and analyzed.

2.3 Determination caffeine content

Amount of 0.050 g sample was dissolved with 100 mL distilled water, heated at a temperature 80 ℃ to 90 ℃, while stirring for 15 min. The hot sample solution filtered using Whatman filter No. 41 through a funnel into a dark glass bottle. Amount of 25 mL of sample solution was taken, put into a 100 mL Erlenmeyer, added with 25 mL dichloromethane, and homogenized for 10 min. The mixture was transferred to a separatory

funnel and allowed to stand for a few minutes to form two layers. The bottom layer was taken and accommodated in a 100 mL erlenmeyer, then the extract was transferred to a test tube. Measurement absorbance value using UV-Vis spectrophotometric (Shimadzu UV-VIS 1280 made in Japan) instrument at 275 nm. The caffeine concentration is determined basen on standard curve [13].

2.4 Determination flavonoid content

The coffee beans were ground then packaged in a closed container and stored in the refrigerator before being used for analysis. The ground bean (0.050 g) was dissolved with 96 % ethanol in a 10 mL volumetric flask. This solution was taken as much as 0.5 mL, then put into a test tube, added with 0.1 mL of 10 % $AlCl_3$, 0.1 mL of 1 M NaOH, and 2.8 mL of distilled water, incubated for 30 min at room temperature. Measure the absorption at 510 nm using UV-Vis spectrophotometer (Shimadzu UV-VIS 1280 made in Japan), then a curve of the relationship between concentration and absorbance was made [14].

Antioxidant activity was tested by the DPPH method which was expressed by IC50 value with units of g mL^{-1}. IC50 is a value that shows the ability to inhibit the oxidation process by 50 % for a sample concentration (mg L^{-1}). The smaller the IC50 value, the higher the activity antioxidants. A substance or compound is said to have a very strong antioxidant activity value if the IC50 value is less than 50 mg L^{-1}, a strong antioxidant if the IC50 value is between 50 mg L^{-1} and 100 mg L^{-1}, a moderate antioxidant if the IC50 value is 100 mg L^{-1}, a weak antioxidant if the IC50 value is 250 mg L^{-1} to 500 mg L^{-1}, and an antioxidant. Inactive if IC50 is more than 500 mg L^{-1} [15, 16].

2.5 Color intensity

Color determination is measured using the CIE lab model, which is designed to resemble human vision by using three components: L = indicates dark or light, a = red/green and b = blue/yellow [17].

3 Result and discussion

3.1 Caffeine content

Caffeine is an alkaloid compound that is naturally contained in coffee. The canephora coffee has a higher content when compared to other varieties of coffee for example arabica [18]. Canephora coffee that has been collected from farmers in the Karangploso, Malang area contain caffeine 2.2 %. Caffeine extraction using maceration, soxhletation and microwave exposure using dichloromethane, ethyl acetate and water can reduce caffeine. This study can decrease caffeine ranged from 14.0 % to 59.5 %.

Table 1 shows that the caffeine reduction using microwave exposure can reduce the caffeine content 45.45 % for 4 min. The results are equivalent to using the Soxhlet method for 8 h and maceration for 24 h, which its used ethyl acetate. Its means that caffeine reduction using microwave exposure can reduce time of extraction compared other conventional methods. When the sample is exposed to microwaves, the energy from the microwaves will be absorbed by the sample so that it will result in rapid heating of the solution due to friction between molecules that rotate through dipole reorientation under the influence of alternating electric field changes or through conductive migration of solvent ions [19, 20].

Table 1. Caffeine content, IC50 and flavonoid content of canephora green bean coffee.

Extraction methods	Duration of extraction	Solvent	Caffeine content (%)	IC 50 (mg L^{-1})	Flavonoid content (mg L^{-1})
Maceration	24 h	Diclorometane	1.46 g	1 105.3 a	426.8 j
		Etyl acetate	1.19 c	1 145.0 ab	420.3 i
		Water	1.63 i	1 555.2 c	389.7 h
	48 h	Diclorometane	1.29 e	1 838.3 c	383.8 h
		Etyl acetate	0.87 a	1 867.0 c	420.3 i
		Water	1.57 h	1 088.3 ab	309.7e
Soxhlet	4 h	Diclorometane	1.15 b	432.6 a	143.8 a
		Etyl acetate	1.89 j	1 145.1 ab	260.3 b
		Water	1.25 d	1 181.0 ab	292.6 d
	8 h	Diclorometane	1.25 d	1 242.8 b	336.8 f
		Etyl acetate	1.20 c	914.8 ab	366.8 g
		Water	1.69 i	1 977.8 c	276.2 c
Microwave	4 min	Diclorometane	1.35 f	706.8 ab	666.8 m
		Etyl acetate	1.20 c	1 077.1 ab	572.1 l
		Water	1.52 h	1 161.6 ab	475.0 k

The 15 % ethyl acetate was able to reduce caffeine better than dichloromethane and water. The solvent can dissolve the extracted component if it has similar polarity between the solvent and the extracted substance. Water has the highest polarity followed by ethylacetate and dichloromethane [21]. The 15 % ethyl acetate solvent can dissolved the most caffeine by maceration method for 48 h.

Rahmawati *et al.* [22] showed that the swiss water method with a duration of 24 h was able to reduce caffeine by 73 % while the results of this study showed the highest caffeine reduction in the 48 h maceration method in 15 % ethyl acetate, which reduced by 61 %. Extraction caffeine using 15 % ethyl acetate with the reflux method for 1 h resulting in the lowest caffeine of 0.02 %, before extracting coffee beans steamed for 3 h [11]. The different methods used to reduce caffeine have different impacts on caffeine reduction.

3.2 Antioxidant activity (IC50)

Antioxidant activity testing is an analysis to reflect the bioactive components in coffee. Antioxidant activity on green coffee bean using the DPPH radical scavenging method (2,2-diphenyl-1-picrylhydrazil) and then the IC50 value was measured which indicates antioxidant activity.

The bioactive components in coffee beans include caffeine, chlorogenic acid, and flavonoids, which are bioactive components that can act as antioxidants [23]. These components have been reduced by the decaffeination process, using different solvents.

Robusta coffee extracted caffeine using dichloromethane solvent had higher antioxidant activity than 15 % ethyl acetate and water in this study. This can be seen from the low IC 50 value owned by caffeine-reduced coffee using dichloromethane solvent, which is 432.6 when using the soxhlet method (Table 1). Dichloromethane is selective in dissolving caffeine while water is thought to dissolve components other than caffeine, such as flavonoid and phenolic. Compounds such as flavonoids and chlorogenic acid which are polar in robusta green coffee beans so that they have low antioxidant activity. The decaffeination method also affects the antioxidant activity. Decaffeinated coffee by microwave method for 4 min have higher antioxidant activity than maceration and soxhletation methods.

IC50 of arabica decaffeinated coffee green bean using dichloromethane is 66.000 µg L^{-1} [24], while in this study the highest value of IC50 is 1.977 mg L^{-1}. The difference in IC50 values can be expected to be influenced by the variety and decaffeination method used. The difference in IC50 value can be thought to be influenced by the variety and decaffeination method was used.

3.3 Flavonoid content

Based on the analysis of variance, it is known that the type of solvent has an effect on the flavonoid content, as well as the solvent used to reduce the caffeine content also affects the flavonoid content. In this study, it was found that the flavonoid content ranged from 143.8 mg L^{-1} to 666.8 mg L^{-1} GAE. Based on Table 1, it is known that the decaffeination process using water as a solvent leaves a low flavonoid content, but the decaffeination process using a diclomomethane solvent leaves the highest flavonoid content. Water and 15 % ethyl acetate dissolve phenolic compounds such as flavonoids and chlorogenic acid which are polar.

The maceration method can extract more flavonoid content but the yield is higher than the Sohklet method. The difference between these two methods are the temperature used in both. Maceration is an extraction process carried out at room temperature, while soxhletation uses high temperatures. So that high temperatures are thought to damage flavonoids. Different extraction method will produce different phytoconstituents [25].

The results of this study are greater than the results of other researchers, it is suspected that the standards used are different. Flavonoid results are equivalent to quercetin, which is (1.91 mg to 2.55 mg) QE mL^{-1} [26], while in this study using gallic acid.

3.4 Color intensity

Green coffee bean quality has been determined by color [27, 28]. Canephora coffee bean color intensity was analyzed using a color reader at the level of brightness, redness and yellowness. The results of the analysis of variance showed that the solvent had an effect on the brightness and yellowness of decaffeinated canephora coffee beans. Color intensity value from this study show at Table 2.

Table 2. Lightness, redness and yellowness of canephora green bean coffee.

Extraction methods	Duration of extraction	Solvent	Lightness		Redness	Yellowness	
Maceration	24 h	Diclorometane	47.9	bc	3.3	12.47	cb
		Etyl acetate	45.6	b	3.7	11.67	cb
		Water	41.0	a	1.2	5.83	a
	48 h	Diclorometane	48.0	bc	3.0	11.87	cb
		Etyl acetate	49.8	c	2.6	12.67	c
		Water	43.1	ab	3.3	9.40	b
Soxhlet	4 h	Diclorometane	47.2	bc	2.7	11.07	cb
		Etyl acetate	45.7	b	2.3	10.00	b
		Water	42.8	ab	1.5	7.67	b
	8 h	Diclorometane	47.7	bc	3.4	11.90	cb
		Etyl acetate	47.9	bc	3.3	11.73	cb
		Water	42.7	ab	2.1	8.30	b
Microwave	4 min	Diclorometane	48.8	bc	3.2	11.63	cb
		Etyl acetate	44.6	ab	2.6	10.10	c
		Water	41.6	a	1.6	7.23	b

The brightness level ranged from 41.0 to 48.8. The level of redness was not significantly different, ranging from 1.2 to 3.3, while the level of yellowness was significantly different due to the type of solvent treatment and the decaffeination method. Yellowness level ranges from 5.83 to 12.67. Decaffeination of coffee using dichloromethan and 15 % ethyl acetate solvents resulted in a brighter coffee color compared to decaffeinated coffee using water judging from the high lightness value. It is suspected that the pigments present in coffee beans are dissolved in both solvents used in the decaffeination process.

After decaffeination process using water as solvent, the brightness value of coffee beans decreased. The brightness value of coffee beans decreased between 25 % to 45 %, from 46.31 to 50.61 to 25.15 to 37.88. The results of caffeine dissolution research using 10 % ethyl acetate showed that the decaffeination process produced coffee beans with a decreased brightness value [29]. Discoloration of the coffee bean surface is caused by the Maillard reaction involving carboxyl group compounds from reducing sugars and amino acids [30]. The heat used in coffee preparation prior to decaffeination can break peptide bonds between amino acids in protein molecules or hydrogen bonds between amino acids and other compounds. Proteins in coffee beans are present in the cytoplasm in free form and in the cell wall. The increase of free amino acids and reducing sugars, the Maillard reaction will accelerate, causing the color of the coffee beans to darken, indicated by the decreasing brightness value (L, Lightness). The decaffeination process using water is thought to be able to bring proteins to the surface of the cell wall greater than other solvents so that the effect on the level of color produced will also be darker. This assumption is based on the basic theory that water is able to dissolve coffee bean protein [24].

4 Conclusion

Different extraction methods and solvents produce different characteristics of canephora coffee. Caffeine extraction using microwave exposure can reduce caffeine in a short time. Decaffeinated coffee using microwave with ethyl acetate 15 % produces coffee with characteristics of caffeine content of 0.87 %, antioxidant activity (IC50) of 1.867 mg L^{-1}, total flavonoids of 420.3 mg L^{-1}, whiteness of 45.7, redness of 2.3 and yellowness of 10.

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