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The Effect of Extraction Time Variation on Caffeine Content in Robusta Coffee Extract using Ultrasound-Assisted Extraction Method

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Abstract: Robusta coffee (*Coffea canephora*) contains a higher caffeine content than other types of coffee. The ultrasound-assisted extraction (UAE) introduced extraction time variation, which can influence caffeine yield compared to conventional extraction methods. This study aims to evaluate the impact of different extraction times(15, 30, 45 minutes) on caffeine coffee extract. The qualitative assessment included an organoleptic test and alkaloid detection. The qualitative test included an organoleptic and alkaloid test. The extracts were measured for moisture content (% MC) and yield value. Quantitative analysis was performed using UV-Vis Spectrophotometry to determine the caffeine content with different extraction times, and then each group was assessed using SPSS software. The coffee bean powder was dark brown, with a characteristic coffee aroma, a bitter taste, and a smooth texture. Alkaloid presence was confirmed using the Mayer, Wagner, and Dragendorff reagents and wavelength scanning. The alkaloid of the extract was investigated at the selected caffeine wavelength of 273 nm. The yield, %MC of robusta coffee extract (RCE) and caffein extract (CE) at 30 (36.938%; 6.39%; 19.45%) were higher than at 15 (24.111%; 6.07%;11.22%) and 45 minutes (22.170%; 6.37%;17.29%).The CE 45 obtained 0.979 ± 0.026 mgCE/mg in significant ($P<0.05$) showed the highest compared to the CE 15 (0.977 ± 0.018 mgCE/mg) and CE 30 (0.792 ± 0.054 mgCE/mg). In conclusion, extraction time significantly influenced the caffeine content of RCE, demonstrating the importance of optimization for maximizing caffeine yield and minimizing %MC.

Keywords: *Coffea canephora*, Caffeine, UAE, Extraction Time, Robusta.

1. INTRODUCTION

Indonesia is one of the world's largest coffee exporters in the global market [1]. As the fourth-largest coffee bean exporter in the world, Indonesia exports approximately 400,000 tons of coffee annually, supported by a robust coffee farming community of 1.7 million farmers [2]. Domestically, coffee consumption reaches 250,000 tons per year, reflecting the strong cultural affinity for coffee among Indonesians. Twenty-one coffee-plantation regions in the existing country have earned certification under the Indonesian National Standard (SNI) for coffee production. These regions include Aceh, South Sumatra, West Kalimantan, Central Sulawesi, and others [3], [4], showcasing Indonesia's commitment to maintaining high-quality coffee production standards . The Indonesia's dual role as both a major exporter and a significant consumer of coffee is based on SNI for coffee [2].

Lampung Province is the second-largest coffee-producing region in Indonesia and has been designated as a National Coffee Plantation Area to contribute significantly to regional economic growth [5], [6].

Coffee is a plant that contains a source of caffeine. Caffeine is the most abundant compound in coffee beans and belongs to the xanthine alkaloid group. Caffeine levels depend on the type of coffee. Based on previous research [7]. Robusta coffee has a higher caffeine content than other types of coffee, namely 2.4-2.5%. Moreover, over 95% of coffee plant varieties cultivated in Indonesia are Robusta coffee types [8]. Apart from the coffee type, caffeine levels can also be influenced by factors such as the extraction method. The extraction time can increase moderately caffeine levels of 1.02 mg/mL at the 5 minutes, 1.13 mg/mL at the 15 minutes, and 1.10 mg/mL at the 25 minutes using the Ultrasound-Assisted Extraction (UAE) [9]. [10]

Ultrasound assisted extraction is effective extraction method for natural antioxidants [9]. The UAE method offers advantages, including enhanced extraction efficiency, yield value, reduced extraction time, and smaller solvent volumes [10]. Previous study observed that caffeine levels varied with UAE conditions, that coffee extract was extracted at 80°C for 5 minutes yielded the highest caffeine content (27.65 mg/g extract) compared than 10 minutes and 20 minutes [6]. In this study, a modification of the extraction time was carried out which was longer compared to the research by Putro et al., (2022) 15, 30, and 45 minutes which correlated with the research by Carciochi et al., (2021) that the optimum extraction time using UAE was in the 45 minute with a caffeine content of 26.4 g/kg compared to UAE in the 10th minute with a caffeine content of 21.2 g/kg [9], [11]. Based on this study, limited research on caffeine levels in robusta coffee samples from the West Lampung area, further analysis is necessary. Therefore, this study aims to analyze caffeine levels in Robusta coffee beans from West Lampung with time variations using the UAE method.

2. MATERIALS AND METHODS

2.1. Place and time of research

This study was carried out at the Pharmacy Laboratory, Universitas Jenderal Achmad Yani Yogyakarta, between May and August 2024.

2.2. Chemical and reagents

Caffeine p.a. (Sigma Aldrich), aluminium foil, distilled water (technical), bluetip, $CaCO_3$ (Merck), ethanol 70% (technical, Onemed), chloroform p.a. (Merck), and filter paper.

2.3. Tools and materials

Ultrasonicator (GT-Sonic), UV-VIS Spectrophotometer (Thermo Fisher Genesys 10S UV-VIS), analytical balance (Ohaus) with sensitivity of 0.1 mg, porcelain cup, separating funnel (Iwaki), Erlenmeyer flask (Iwaki), measuring flask (Iwaki), micropipette (Ohaus), volume pipette (Iwaki) 5 mL, and other glassware.

2.4. Sampling and plant determination

The Robusta coffee samples were sourced from the Pekon Kubu Perahu Plantation, Balik Bukit District, Liwa, West Lampung Regency (geographic coordinates: 40° 47' 16" – 50° 56' 42" S and 103° 35' 08" – 104° 33' 51" E). Plant identification was conducted at the Biology Learning Laboratory of the

Faculty of Science and Applied Sciences, Ahmad Dahlan University, Yogyakarta to confirm the authenticity of the coffee bean plants used in the research.

2.5. Preparation robusta coffee bean

Robusta coffee fruit was harvested at 8 months of ripeness, red skin, and fresh in the morning at 07.00-10.00 WIB for five kg. Coffee fruit was separated between coffee beans and coffee skin using a wet sorting method by washing the coffee beans with tap water to minimize dirt attached to the robusta coffee beans and air-dried, then ground with a pulping machine to separate the pulp of coffee beans [12]. Robusta coffee beans were weighed at 1.5 kg and dried until the dark color was used in an oven for 60 minutes at a temperature of 200°C [13]. After that, the robusta coffee beans were sorted by selected coffee beans. The selected coffee beans met the criteria that were not defective. The defects in coffee beans were broken, perforated, and black coffee beans [2]. The coffee beans were then ground with a grinder into powder to reduce the particle size and then filtered with a 60-mesh sieve [13].

2.6. Organoleptic test

Organoleptic test evaluated Robusta coffee beans and powder, including assessment of color, aroma, taste of coffee beans and uniformity of coffee powder [2].

2.7. Alkaloid test

A qualitative alkaloid test was conducted to ensure the presence of alkaloid compounds in robusta coffee using Mayer, Wagner, and Dragendorff reagents. 2 grams of coffee powder was added to 10 mL of ammonia and chloroform in a beaker glass, followed by the addition of 10 drops of H_2SO_4. The mixture was stirred until homogeneous and allowed to separate into two layers. The upper layer of the filtrate was taken, and separated 1 ml each in three test tubes. Each tube was added with 1 mL of Wagner, Mayer, and Dragendorff's reagents. If the filtrate contained alkaloids, the Mayer reagent would form a white precipitate. The filtrate was added with Wagner and Dragendorff's reagent to form a white precipitate [14].

2.8. Coffee extraction

2.8.1 Ultrasound Assisted Extraction (UAE) Method

The coffee bean extract was prepared using the UAE. Twenty grams of coffee powder were dissolved in 200 mL of 70% ethanol (1:10 w/v) in a 250 mL Erlenmeyer flask. The extraction was carried out at 30°C for 15, 30, and 45 minutes. Before sonication, the solution was preheated to ensure the temperature reached 30°C. The sample was then subjected to the UAE process. After sonication, the mixture was filtered using filter paper. The filtrate obtained was evaporated with a water bath at a temperature of 60°C until it thickened for 1-2 days. The thickened extract was transferred to a glass bottle, and the extraction yield was calculated. The extract was then stored in a refrigerator for further analysis [13].

$$\text{The extraction yield} = \frac{extract\ weight}{powder\ weight} \times 100\% \quad \text{.....(1)}$$

2.8.2 Measurement of Moisture Content (%MC)

Five grams of robusta coffee powder and one gram of coffee bean extract were weighed and prepared into the moisture analyzer at a temperature of 100°C, then generated until the green color

was on the device screen. The water content of the powder and coffee bean extract was evaluated for each extraction time variation following the procedure [13].

2.8.3 Separation of Caffeine Content in Samples Using Liquid-Liquid Extraction

One gram of thick extract was weighed for each variation of extraction time. 150 mL of hot distilled water and 25 mL of chloroform was added until homogeneous. Chloroform was replicated 2 times for each extraction. After the two phases were separated, 1500 mg of calcium carbonate ($CaCO_3$) was added to separate the caffeine compound from other base compounds and filtered using filter paper. The solution was evaporated into a water bath at 60 °C until all the chloroform evaporated for 1-2 days. The thick extract obtained would be stored in the refrigerator to be explained further (modified by [13]).

2.9. Determination of Caffeine Content by UV-VIS Spectrophotometry

A total of 20 mg of caffeine standard was weighed and dissolved in 100 mL of distilled water until fully homogenized, resulting in 200 ppm caffeine solution. A positive control containing 18 ppm caffeine was then prepared [13].

For sample preparation, 5 mg of coffee extract was added to 10 mL of distilled water, followed by dilution, where 1 mL of the mixture was further diluted to 10 mL with distilled water. The control and sample solutions were analyzed using a UV-Vis spectrophotometer within the wavelength range of 200–400 nm, using distilled water as a blank [13].

A standard caffeine series of 6, 9, 12, 15, and 18 ppm was prepared using distilled water. Each solution was diluted with distilled water up to the marked volume. Caffeine extract samples from various extraction time variations were weighed at 5.0 mg and diluted with 10 mL of distilled water. The absorbance of each standard and sample was measured using a UV-Vis spectrophotometer at the maximum wavelength [13].

2.10. Data Processing and Data Analysis Methods

The actual caffeine content (mg CE/mg) measures the caffeine content in the sample. The caffeine content in the sample could be calculated using the formula:

$$C = (M.V)/m \quad \text{.....(2)}$$

Information:
C = actual caffeine content (mg CE/mg)
M = concentration (mg/mL)
V = sample volume (mL)
m = sample weight (mg)

The dependent data variable was the caffeine content influenced by the independent variable of the difference in extraction time with statistical methods using SPSS software version 29. The data obtained were tested for normality using Shapiro-Wilk and homogeneity using Levene's confidence level of 95%. Normally and homogeneous data were continued with One-Way ANOVA with a significant level of 95% [15]. Therefore, the Pearson test evaluated each group's extraction time with several parameters, including % yield, moisture content and caffeine content [16].

3. RESULTS AND DISCUSSION

3.1. Determination Robusta Coffee

Determination of robusta coffee samples was carried out at the Biology Learning Laboratory of the Faculty of Science and Applied Sciences, Universitas Ahmad Dahlan, Yogyakarta, with the number SK 224 / Lab. Bio/ B/V/2024. The results of plant identification showed robusta coffee beans with another name Coffea canephora var. robusta (L. Linden) A. Chev.

3.2. Preparation and organoleptic test

Five kg of coffee fruit was categorized as cherry fruit, intact structure, and bright red color. The red color characteristics indicated that the fruit have reached optimal ripenes. The coffee fruit was ripe with wet sorting using running water to facilitate peeling with a pulper machine and washing the coffee fruit. The wet processing method would remove the remaining mucus on the skin of the coffee bean horns [12]. The green coffee bean was roasted in an oven at 200 °C for 60 minutes, typically resulting in a medium roast in Figure 1. This temperature generated complicated flavors while minimizing the bitterness at higher temperatures [17]. Medium roast coffee bean contained the highest caffeine content compared light or dark roasted with different ultrasound-assisted extraction (UAE) conditions [6]. Table 1 provides information about the organoleptic results of the coffee bean powder. Coffee bean powder showed medium dark, a distinctive coffee aroma, a bitter and slightly sour taste in the coffee beans, and uniformity in robusta coffee powder [18].

(a) (b) (c) (d)

Figure 1. Organoleptic results display on different coffee forms, including fruit (a), green beans (b), roasted beans (c) and coffee grounds (d)

3.3. General Alkaloid Test

Qualitative tests in this study determined the presence of general alkaloids in Robusta coffee powder. The alkaloid test data obtained compared to negative controls (distilled water) in Table 1. The results showed positive alkaloid detection, as evidenced by the formation of potassium alkaloid precipitates when tested with Mayer, Wagner, and Dragendorff reagents. [5][6]This indicates that Robusta coffee powder contains alkaloid compounds, primarily xanthine alkaloids such as caffeine, theobromine, and theophylline, which contribute to its stimulant effects. Additionally, other alkaloid groups may be present, influencing the potential bioactive properties of the Robusta coffee. These findings highlight the chemical complexity of Robusta coffee and its potential functional benefits [5], [6].

Table 1. General Alkaloid Test Identified the Robusta Coffee Powder

Sample	Alkaloid test			Theory
	Mayer	**Wagner**	**Dragendorff**	[14]
Coffe bean powder				**+ (**White precipitate**)**
Negative control (water)				- (Clear)

3.4. Measurement Yield Value and Moisture Content

In this study, coffee bean powder was extracted using the UAE method. This research extraction method aims to dissolve caffeine compounds allocated by ultrasonic vibrations [19]. The UAE method is a more efficient extraction method than other methods and requires less solvent [20]. Ethanol 70% was chosen as the extraction solvent because the caffeine content obtained in ethanol 70% has a higher value compared to ethanol 96%. Ethanol 70% is a semipolar solvent that can extract compounds that are either polar or semipolar [21].

In Table 2. % yield value of roasted coffee bean can decrease caused by high roasting temperature at 200°C for 60 minutes, as similar results from % MC of powder coffee obtained with 3.40% that met requirement of SNI Coffee (less than 5%). Wet sorting and roasting temperature impacted to % yield value, moisture content, acid level and flavour [22].

The yield values of green beans, roasted beans, and coffee powder are presented in Table 2, containing 30%, 4.01%, and 84.35%. The yield of roasted coffee beans tends to decrease due to high roasting temperatures at 200°C, consistent with findings from previous studies [22]. The moisture content (% MC) of the coffee powder met the SNI coffee standard, remaining below 5%. Additionally, factors such as wet sorting and roasting temperature influenced yield percentage, moisture content, acidity levels, and flavour profile [22].

Percentage yield value was obtained at the 15, the 30, and the 45 minutes of extraction time in Table 2. Based on these data, the highest % coffee powder yield result at RCE 30 was 36.94%, followed by RCE 15 and RCE 45, reaching 24.11% and 22.17%. Table 2 also illustrates the results of the percentage of caffeine extract (CE) yield. The higher caffeine yield % result at the CE 30 was 19.468% compared to the CE 15(11.224%) and CE 45 (17.291%). Moisture content of coffee powder, and several RCEs were displayed in Table 2. RCE met the requirements with % MC in coffee beans of <5% [2] and

in the extract of <10% [23]. The yield values at the 30 minute (extract and caffeine) indicated a direct association resulting in the water content difference at the 30 minute being higher than at the 15 and 45 minutes.

Table 2. Yield Value and Moisture Content of Robusta Coffee Extract and Caffeine Extract

Sample	Weight (g)	% Yield Value	% MC	Theory
Fruit	5000	-	-	Modified by [22] < 5% [24]
Green bean	1600	30.00	-	
Roasted bean	60.14	4.01	-	
Powder	50.73	84.35	3.40	
RCE 15	4.822	24.11	6.07	Modified by [13], [25] <10% [2]
RCE 30	7.388	36.94	6.39	
RCE 45	4.434	22.17	6.37	
CE 15	0.112	11.22	-	
CE 30	0.195	19.45	-	
CE 45	0.173	17.29	-	

Notes: RCE = Robusta coffee extract in 15, 30, and 40 minutes, CE= Caffein extract, MC= moisture content.

3.5. Determination of caffeine content using UV-VIS Spectrophotometry

Wavelength scanning was used to find the maximum caffein wavelength in a caffeine extract (CE). In this work, the positive control (caffeine standard) and CE samples at different extraction times had a maximum wavelength peak of 273 nm [13]. The determination of caffeine content in coffee bean extract at various extraction times with linear regression equation y=0.046x-0.0207 can be seen in Table 3. The average caffeine content of CE 15 was 0.977 ± 0.018 mgCE/mg, followed by CE 30 was 0.792 ± 0.054 mgCE/mg, and CE 45 was 0.979 ± 0.026 mgCE/mg. The caffeine content of CE 45 was higher than CE 30 and CE 15, while the inverse correlation with the highest water content was CE 30. Therefore, it could be necessary to re-thicken at CE 30 and then examine the water content of the CE 30 until the water content obtained was lower than CE 45. The caffeine content value obtained in this study was following the research of Carciochi et al., (2021), which stated that the highest caffeine content value was CE 45. Therefore, the extraction time can affect the caffeine content in robusta coffee beans extracted using the UAE method[11].

Table 3. Determination of Caffeine Content in Caffeine Extract (CE) using One-Way ANOVA

Time extraction (min)	Rep	Abs	Caffeine content (mgCE/mg)	$\bar{x}$ ± LE (mgCE/mg)	CV (%)	One Way ANOVA	*Post-Hoc*
CE 15	1	0.432	0.984	0.977±0.018	0.756	0.000*	0.000*
	2	0.430	0.980				0.903
	3	0.424	0.967				
CE 30	1	0.506	0.763	0.792±0.054	2.724		0.000*
	2	0.529	0.797				0.000*
	3	0.542	0.815				
CE 45	1	0.649	0.970	0.979±0.026	1.084		0.903
	2	0.650	0.972				0.000*
	3	0.665	0.994				

*Notes:*Significantly different data (p-value < 0.05)*

Caffeine content data was obtained with the Shapiro-Wilk and Levene's test. According to Table 3, data shows a p-value > 0.05 of homogeneous and normal distribution, then continued with the

One-Way ANOVA test. The results of the One-Way ANOVA analysis obtained significant data differences in time extraction($p < 0.05$). Statistical analysis is needed to determine whether the difference is significant. The observed difference in values may be attributed to a large standard deviation, suggesting that the small variation (0.002) is likely due to random variability rather than a true effect of extraction time [26]. The CE 45 is the extraction method with the highest caffeine content, but caffeine content at CE 15 and CE 45 are very close. In general, a shorter extraction time (15 minutes) would be preferred to the efficiency extraction method in applications [27].

The continued Pearson test determined the correlation coefficient between data with a confidence level of 95% in a range of relationship values from -1 to 1. The correlation of caffeine content with % yield value of RCE was found a strong negative correlation (r = -0.994). A strong negative correlation showed one variable rose, while the other fell dramatically. The factor of correlation due to the extraction procedure, materials, and experimental condition was critical for determining the extract [28]. Then, the Pearson correlation evaluated between the yield value of RCE and the % MC of RCE data (in Fig.2). The results of the Pearson test obtained a correlation value of 0.541 to indicate a moderate positive correlation, so the higher the % yield value as same as the water content. Overall, the Pearson correlation data established the relationship between caffeine content, % yield value, and % moisture content (MC).

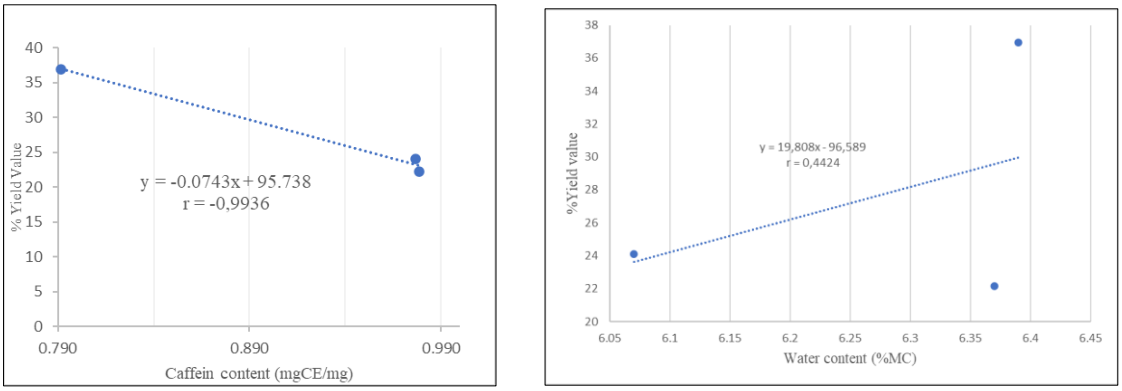

Figure 2. The Pearson test correlated between caffein content, % yield value and water content

4. CONCLUSION

The difference in extraction time with the UAE method affects the caffeine content, yield, and % moisture content in West Lampung robusta coffee beans. The caffeine content value obtained at 15, 30, and 45 minutes provided 0.977 ± 0.018 mgCE/mg, 0.792 ± 0.054 mgCE/mg, and 0.979 ± 0.026 mgCE/mg. However, it was inversely proportional to percentage yield value, which was the same as water content. However, the percentage yield value showed an inverse relationship, similar to the water content. Although the 45-minute extraction resulted in the highest caffeine content, the values at 15 minutes (0.977 mgCE/mg) and 45 minutes (0.979 mgCE/mg) were nearly identical. Therefore, a shorter extraction time of 15 minutes using UAE would be preferred for greater efficiency in real-world applications.

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