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Evaluating Extraction Methods for Caffeine Content in Gayo Arabica Coffee Oil through Gas Chromatography-Mass Spectroscopy

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Abstract

This study aims to determine physicochemical properties, and caffeine analysis of green bean coffee essential oil (GBCEO) and roasted bean coffee essential oil (RBCEO) by maceration and soxhlet extraction methods. The results indicated that RBCEO by maceration method have higher percentage of yield compared to GBCEO. By the same to soxhlet extraction method, RBCEO also showed higher percentage of yield compared to GBCEO. The refractive index of the GBCEO*m* and GBCEOs have a lower acid value compared to RBCEO*m* and RBCEOs. The specific gravity obtained for GBCEO*m*, RBCEO*m*, GBCEOs, and RBCEOs ranged from 0.87 to 0.97. The results showed that GBCEO*m* has the highest saponification value followed by RBCEOs. GBCEO*m* has the highest iodine value followed by RBCEOs, while RBCEO*m* and GBCEOs have a similar iodine value. The peroxide value showed that GBCEOs, and RBCEOs by soxhlet extraction method have higher peroxide value. The GC-MS analysis revealed that GBCEO*m* has higher caffeine followed by GBCEOs with the percentages area of 9.31% and 7.36% respectively. Meanwhile RBCEO*m* has lower caffeine followed by RBCEOs with the percentages area of 7.36% and 4.28% respectively. This finding showed that GBCEO shows higher caffeine compound compared with RBCEO.

Introduction

Indonesia is one of the largest coffee producers in the world, after Brazil and Colombia [1]. Indonesian coffee has a unique and distinct taste coffee in each region of Indonesia [2]. Some of the Indonesian local coffees are already famous, such as Toraja, Lintong, Mandailing, Lintong, Kintamani, Flores, and Gayo (Aceh) coffee [3,4]. Gayo Arabica coffee is globally recognized for its distinctive flavor profile, characterized by a complex, full-bodied, and strong coffee aroma and taste [5].

Arabica coffee beans contain approximately 10-15% oil, which can be extracted from green or roasted beans to obtain green bean coffee essential oil (GBCEO) or roasted bean coffee essential oil (RBCEO) [6]. RBCEO is frequently used as a flavoring agent in food products. RBCEO has been shown to have various health benefits, such as antidepressants and aromatherapy [3]. GBCEO has been widely used in cosmetics because it maintains natural skin

humidity and absorbs UV radiation [7]. Solvent extraction is the most effective method for extracting oil from coffee beans. The maceration and soxhlet extraction methods are preferred due to their numerous advantages over other extraction techniques [7,8].

The extraction method employed influences the physicochemical properties of the resulting extract, which can be used to determine the quality of the coffee bean essential oil (CBEO) [9,10]. Some bioactive compounds, such as polyphenols, tocopherols, phytosterols, and caffeine, exist in CBEO [7,11,12]. Other bioactive compounds, such as palmitic acid, linoleic acid, stearic acid, sterols, phosphatides, diterpenes, and ceramides, have also been found in CBEO [9,13].

This study aims to extract CBEO using both maceration and soxhlet extraction techniques. Subsequently, it aims to compare the physicochemical properties and caffeine content of GBCEO and RBCEO obtained through these methods to determine their efficacy and potential applications.

Materials and Methods

Materials

The materials used in this research were Gayo Arabica coffee beans obtained from Bener Meriah Regeny, Aceh Province. The chemicals used in this study were *n*-hexane, distilled water, 95% ethanol, phenolphthalein indicator, methyl orange indicator, potassium iodide solution, KOH 0,1N, HCl 0,5N, $Na_2S_2O_3$ 0,1N, $Na_2S_2O_3$ 0,01N, chloroform, Hanus solution, starch solution, acetic acid, oxalic acid, $K_2Cr_2O_7$, and Na_2CO_3.

Plant Determination

Determination of Gayo Arabica coffee beans was conducted at the Biological Sciences Research Organization, National Research and Innovation Agency (BRIN), Bogor, Indonesia. The determination aimed to ascertain the identity of the plants used in this research.

Sample Preparation

A total of 1000g of green bean coffee is taken, cleaned, and ground using a grinder to produce green bean coffee powder. The green bean powder was then sieved using a 60-mesh sieve to obtain green bean coffee powder (GBCP). For the preparation of roasted bean coffee powder, 1000g of green bean coffee was taken and roasted using a roasting machine at a temperature of 195˚C for 20 minutes [9]. After roasting, the coffee beans were ground and sieved using a 60-mesh sieve to obtain roasted bean coffee powder (RBCP).

Maceration Extraction Method

One hundred of each GBCP and RBCP was placed into a beaker glass, and 600 mL of *n*-hexane solvent was added, heated to 67 67˚C, and stood for 24 hrs to obtain the macerates of GBCP and RBCP. The extracts of GBCP and RBCP were separated using a rotary vacuum evaporator to obtain green bean coffee essential oil by maceration (GBCEO*m*) and roasted bean coffee essential oil by maceration (RBCEO*m*).

Soxhlet Extraction Method

One hundred of each GBCP and RBCP was placed into a cylindrical filter paper and inserted into the Soxhlet apparatus equipped with a condenser and a boiling flask. A volume of 600 mL of n-hexane solvent is introduced into the boiling flask. The Soxhlet assembly is then positioned on a heater and heated for 3 hours at a temperature of 67˚C. The mixture of coffee bean oil and the solvent is separated using a rotary vacuum evaporator to obtain roasted bean coffee essential oil by soxhlet (GBCEOs) and roasted bean coffee essential oil by soxhlet (RBCEOs) [13].

Characterization of the Coffee Essential Oils

The physicochemical properties of GBCEOm, RBCEOm, GBCEOs, and RBCEOs were characterized, including organoleptic examination, moisture content, specific gravity, density determination, refractive index measurement, acid value determination, saponification value determination, iodine value determination, and peroxide value determination [14–16].

FT-IR Analysis

Functional group analysis of the oils was performed using Fourier Transform Infrared (FTIR) spectroscopy (Agilent resolution pro Cary 630 FTIR Spectrometer). The FT-IR spectrum analysis is conducted at wavenumbers ranging from 400 cm^{-1} to 4000 cm^{-1}.

GC-MS Analysis

One mL sample of the oils was taken and injected into a gas chromatography-mass spectrophotometer (Shimadzu GC-MS-QP2010). The operating conditions for the GC-MS spectroscopy are as follows: The GC-MS instrument (Shimadzu GCMS-QP-2010 plus) equipped with an RXI-5MS fused silica column (ID 30 m × 0.25 mm, film thickness 0.25 m) is operated under the following conditions: helium carrier gas (99.999%) at a flow rate of 1.0 mL/min and a split ratio of 1/10. The column temperature programming is raised from 50 °C (held for 3 minutes) to 100 °C at a rate of 50 °C/min, from 100 to 200 °C at 8 °C/min, and then to 290 °C (held for 10 minutes) at 100 °C/min. The injector and interface temperatures are 280 and 230 °C, respectively, with a pressure of 117.6 kPa, a total flow of 25.0 mL/min, a column flow of 2.0 mL/min, a linear velocity of 51.3 cm/s, and a purge flow of 3.0 mL/min.

Results and Discussion

Plant Determination

The determination of coffee beans was conducted to verify and identify the species the sample used. The determination results indicated that the sample used in this study was *Coffea arabica* L. from the Rubiaceae family.

Sample Preparation

The grinding and sieving processes aim to obtain smaller and more homogeneous particle sizes, enabling the solvent to penetrate more quickly into the sample's pores [8]. In this study, the grinding of the sample was aimed at determining the percentage of rendements of the sample. The percentage rendements of GBCP and RBCP are presented in Table 1**.** The results showed that GBCP has a higher percentage of rendements than RBCP. Ariga stated that the temperature and duration of roasting will influence the quality and quantity of the essential oils [9]. The previous study stated that the yield is very important due to the amount of the bioactive compound [4,17,18].

Table 1. The percentage yield of GBC and RBC simplicia.

No.	Simplicia	Sample weight (gram)	Simplicia weight (gram)	Rendements (%)
1	GBC	1000	370.72	0.37
2	RBC	1000	293.50	0.29

Extraction of Green Been Coffee Oil (GBCO) and Roasted Been Coffee Oil (RBCO)

Maceration and Soxhletation Extraction Methods

Maceration is an extraction method that involves soaking the material in a suitable solvent for extracting active compounds, with and without heating. The maceration method was chosen because of the simple equipment. In this extraction method, we used *n*-hexane as a solvent. *n*-

Hexane is a non-polar solvent that able to dissolve non-polar compounds. Additionally, *n*-hexane is stable, easily evaporated, and selective in dissolving substances. In this method, we have determined the percentage yield of the coffee oils (GBCEO*m* and RBCEO*m*). Table 2 shows the percentage yields of GBCEO*m* and RBCEO*m*. Table 2 shows that RBCEO, using the maceration method, has a higher percentage of yield than GBCEO. Using the same soxhlet extraction method, RBCEO showed a higher yield percentage than GBCEO.

Table 2. The percentage yields of GBCEO*m*, RBCEO*m*, GBCEO*s*, and RBCEO*s*.

No.	Extraction method		Extraction method	
	Maceration	**Percentages of yield (%)**	**Soxhletion**	**Percentages of yield (%)**
1	GBCEO*m*[a]	4.07	GBCEO*s*[a]	5.87
2	RBCEO*m*[b]	7.54	RBCEO*s*[b]	10.12

Soxhlet Extraction Method

Soxhlet extraction is the process of transferring the partially soluble components of a solid to the liquid phase using a Soxhlet extractor. In the method, the solvent (*n*-hexane) travels into the main chamber, and the partially soluble components are slowly transferred to the solvent [19]. According to Aziz, coffee bean oil extraction using 600 mL of n-hexane solvent yields the highest yield compared to ethanol solvent [14]. The temperature used is 67˚C for 3 hours. Based on the study by Lamona and Nurman, coffee bean oil extraction using the Soxhlet method produces an optimal yield at 180 minutes. Generally, the longer the extraction time, the better the results obtained [13]. The percentage of yields of coffee oils (GBCEOs and RBCEOs) is presented in Table 2. The results showed that RBCEO*s* by the soxhlet extraction method have a higher yield percentage than GBCEOm, with the percentages yield 10.12% and 5.87%, respectively. According to Aziz, coffee bean oil extraction using *n*-hexane solvent yields the highest yield compared to ethanol solvent [14]. Lamona and Nurman showed that coffee bean oil extraction using the soxhlet method produces an optimal yield of the oils [13].

The results also showed that the percentage yield of green bean oil is lower than that of roasted coffee bean oil for both the maceration and soxhlet methods. During roasting, water evaporation occurs, causing the cell pores in the plant to open, allowing secondary metabolites present in the cytoplasm to be easily extracted, resulting in higher yields of roasted coffee oil compared to green coffee oil. According to Mukhriani, the soxhlet extraction method has the advantage of being a continuous extraction process, and the sample is extracted by pure solvent from condensation, resulting in higher yields compared to the maceration method [21]. Heating during extraction causes damage to the cell walls, making them easily ruptured, and thus, the cell walls are easily penetrated by the solvent, leading to more oil being extracted. The yield indicates the effectiveness of the extraction process. The higher the yield obtained, the more effective the extraction method used, and the more compounds produced. Therefore, it can be said that the Soxhlet method is better for extracting coffee oil [22].

Characterization of the Coffee Essential Oils

The characterization of GBCEO*m*, RBCEO*m*, GBCEO*s*, and RBCEO*s* in this study includes organoleptic properties, density, refractive index, acid value, saponification value, iodine value, and peroxide value were determined.

Table 3 shows the oils' characterization. The main parameters for determining the quality of the oil are color, flavour, water content, refractive index, specific gravity, acid number, saponification number, iodine number, and peroxide number [23]. GBCEOm and GBCEOs were brownish

yellow, while RBCEOm and RBCEOs were blackish. The flavor of GBCEOm and GBCEOs was weak compared to RBCEO*m* and RBCEO*s*.

Table 3. The characterizations of GBCEO*m*, RBCEO*m*, GBCEO*s*, and RBCEO*s* by maceration and soxhletion methods.

No.	Parameters	Maceration Method		Soxhletation Method	
		GBCEO*m*	**RBCEO*m***	**GBCEO*s***	**RBCEO*s***
1	Color	brownish yellow	blackish brown	brownish yellow	Blackish brown
2	Flavor	weak	strong	weak	strong
3	Water content (%)	0.1	0.1	0.1	0.1
4	Refractive Index	1.463	1.472	1.472	1.474
5	Specific gravity	0.91	0.87	0.87	0.87
6	Acid number (mgKOH/g)	4.53	6.84	6.84	5.57
7	Saponification number (mgKOH/g)	185.747	138.466	138.466	159.729
8	Iodine number (g I_2/g)	11.51	7.51	7.51	9.70
9	Peroxide number (meq/kg)	5.32	8.25	8.25	6.74

The water content in oil is a parameter that determines the quality of the oil. The higher the water content in the oil, the lower the quality of the oil because water is one of the catalysts for the hydrolysis reaction of oil that produces free fatty acids [24]. The water content of all the oils by maceration and extraction does not affect the water content in coffee bean oil, as shown in Table 4.2. According to a study by Sanches, the water content of coffee bean oil extracted by the pressing method was 0.2% [15]. The water content is determined to assess the durability of food products and is related to the activity of microorganisms during storage. Products with high water content are more prone to spoilage because the product can become a conducive medium for the growth of microorganisms [25].

The refractive index is the ratio of the speed at which light travels through the air to the speed it travels through the test sample [26]. The refractive index reflects the oil's purity and indicates the chain length of fatty acid [26]. The refractive index of the GBCEO*m* and GBCEO*s* have a lower acid value than RBCEO*m* and RBCEO*s*. According to Kita and Figiel, heating at high temperatures and contact with oxygen can cause oxidation reactions in oil, causing the oil to break down into free fatty acids [25]. The higher the heating temperature applied, the more free fatty acids are formed, reducing the oil quality. The refractive index of four oil samples ranged from 1.463 to 1.474, similar to kernel oil [27].

Specific gravity or density is an important parameter to determine oil quality. Density is influenced by the number of components contained in the oil; the more components contained, the greater the molecular weight of the oil, resulting in higher density. The specific gravity obtained for GBCEO*m*, RBCEO*m*, GBCEO*s*, and RBCEO*s* ranged from 0.87 to 0.97. Based on the results, there is no significant difference in the specific gravity of all samples.

The acid value is correlated with the number of free carboxylic acid groups (fatty acid). The acid value is also an indicator of the degree of deterioration of oil. The results showed that the maceration extraction method found the lowest acid value. The GBCEO*m* has the highest saponification value, followed by RBCEO*s*. However, RBCEO*m* and GBCEO*s* have a similar saponification value. The saponification value is the milligrams of KOH required to saponify the fatty acids resulting from one gram of oil hydrolysis. The higher the saponification value, the smaller the fatty acids; conversely, the lower the saponification value, the larger the fatty acids and the lower the quality of the oil [28]. The iodine value indicates the degree of unsaturation of the compound. The iodine value of GBCEOm has the highest iodine value, followed by RBCEO*s*, while RBCEO*m* and GBCEO*s* have a similar iodine value. The peroxide value is an index of the amount of fat or oil that has undergone oxidation [29]. The peroxide value is very

important for identifying the oxidation level of oil. The results showed that GBCEOs and RBCEOs by the soxhlet extraction method have higher peroxide values. Higher temperatures used in the extraction method lead to the decomposition and oxidation of triacylglycerol, which further leads to an increase in free fatty acids [30].

FT-IR Analysis

Infrared spectroscopy is useful for identifying organic compounds because of its highly complex spectrum. The complex spectrum is due to many peaks indicating functional groups characterized by wavenumbers [31].

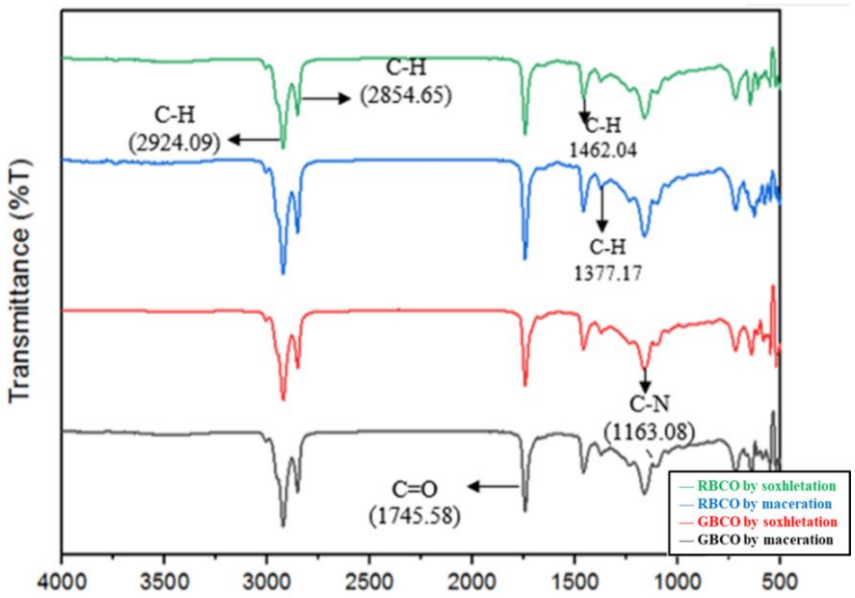

Figure 1. FT-IR spectrum of green Arabica coffee bean oil and roasted Arabica coffee bean oil extracted by maceration and Soxhlet methods.

Based on the FTIR spectrum analysis (Figure 1), it can be observed that there is no significant difference between the green coffee bean oil and roasted coffee bean oil obtained through maceration and soxhlet extraction methods. The absorption peaks at wavenumbers 2924 cm^{-1} and 2854.65 cm^{-1} indicate the presence of C-H groups. According to the literature, the wavenumber range of 3000-2850 cm^{-1} corresponds to C-H stretching vibrations. The wavenumber of 1745 cm-1 represents the absorption of the C=O group. As per Silverstein et al., the wavenumber range between 1870-1540 cm^{-1} exhibits strong C=O group absorption, which is observed at the wavenumber of 1462 cm^{-1}. The absorption at 1375 cm^{-1} indicates the presence of the C-N group [32]. The FTIR spectrum results obtained in this study are consistent with the findings of Lamona and Nurman for green coffee bean oil extracted via Soxhlet method, which showed absorption peaks at wavenumbers 2923 cm^{-1}, 2854 cm^{-1}, 1741 cm^{-1}, 1458 cm^{-1}, 1377 cm^{-1}, 1161 cm^{-1}, 1043 cm^{-1} and 719 cm^{-1} [12].

GC-MS Analysis

The composition of coffee bean oil was analyzed using Gas Chromatography and Mass Spectrometry (GC-MS). The GC-MS analysis revealed that GBCEO*m* has higher caffeine, followed by GBCEOs with a percentages area of 9.31% and 7.36%, respectively. Meanwhile, RBCEOm has lower caffeine, followed by RBCEOs, with 7.36% and 4.28%, respectively. This finding showed that GBCEO shows higher caffeine compounds compared with RBCEO. The GC-

MS analysis also showed that GBCEO has more compounds than RBCEO (Table 4). The caffeine content observed in this study is higher compared to the findings of Lamona & Nurman, which reported a caffeine content of 1.45% in coffee bean oil [13].

Table 4. Using GC-MS spectroscopy, the analysis of caffeine compounds in GBCEOm, RBCEOm, GBCEOs, and RBCEOs.

No.	Extraction method			Extraction method		
	Maceration	**Percentages of Caffeine**	**Number of Compound**	**Maceration**	**Percentages of Caffeine**	**Number of Compound**
1	GBCEO*m*[a]	9.31	41	GBCEO*s*[a]	7.36	27
2	RBCEO*m*[b]	7.30	34	RBCEO*s*[b]	4.28	36

Note: GBCEO[a], Green Bean Coffee Essential Oil; RBCEO[b], Roasted Bean Coffee Essential Oil

Caffeine is a characteristic compound found in coffee. Caffeine is one of the alkaloid types abundantly present in coffee beans. Caffeine belongs to the group of methylxanthine compounds (Figure 2). Methylxanthines are naturally occurring compounds classified as xanthine derivatives, a class of alkaloid compounds. This can be attributed to alkaloid compounds being not heat-stable during the roasting process [33]. Consequently, the roasting process leads to a reduction in the caffeine content of roasted coffee bean oil. Excessive caffeine intake can have negative health effects, such as increased psychomotor activity, gastric acid secretion, heart rate, urinary frequency, and muscle tension. Besides caffeine, coffee bean oil also contains other compounds like chlorogenic acids, trigonelline, carbohydrates, fats, amino acids, volatile compounds, and minerals [34].

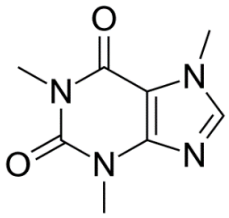

Figure 2. Structure of the chemical compound of caffeine.

The main acid in coffee that influences its acidity is chlorogenic acid. The chemical structure of chlorogenic acid consists of an ester of caffeic acid and quinic acid. Chlorogenic acid has the highest percentage in coffee beans, ranging from 6.7-9.2% in Arabica coffee and 7.1-12.1% in Robusta coffee [35]. The acidity of coffee is influenced by the maturity of the coffee beans and processing methods, particularly roasting.

Conclusions

This study comprehensively compared the efficacy of two extraction methods—maceration and Soxhlet extraction—in isolating CBEO from green and roasted Gayo Arabica coffee beans using n-hexane as a solvent. The findings indicate that the Soxhlet extraction method generally yielded higher percentages of essential oils than maceration, regardless of the coffee bean state (green or roasted). Notably, roasted bean coffee essential oil consistently demonstrated higher yields and more robust flavors across both extraction techniques, likely due to the enhanced solubility of oil-soluble compounds following the roasting process. Regarding caffeine content, GBCEO exhibited higher caffeine levels than RBCEO in both extraction methods. This result underscores the impact of roasting on caffeine levels, where the heat treatment during roasting likely degrades caffeine, reducing its concentration in the extracted oils.

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Ethical Clearance: Not applicable.

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Data Availability Statement: The data supporting this study's findings are available upon request from the corresponding author.

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Conflicts of Interest: All the authors declare that no conflicts of interest have been reported for this research.

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