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Extraction and Characterization of Moringa Seed Oil Results from Soxhletation and Screw Pressing

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Abstract: Moringa seed oil is a vegetable oil that has many benefits for health and the pharmaceutical industry. Moringa seed oil can be extracted either chemically or mechanically. This research aims to characterize moringa seed oil's physicochemical properties and fatty acid composition using soxhletation chemical extraction and screw-pressing mechanical extraction with various pretreatment temperatures of 50, 60, and 70°C. The research showed that the highest yield of moringa seed oil, 41.74%, was obtained at an initial treatment temperature of 70°C using the soxhletation method for 6 hours. The acid value of moringa seed oil from soxhletation is 2.01 mg KOH/g, and screw pressing is 4.37 mg KOH/g. The iodine number from soxhletation was 66.62 mg iod/100g, and screw pressing was 60.27 mg iod/100g. The density of the oil from soxhletation and screw pressing is 0.922 mg/mL and 0.934 mg/mL, respectively, with an oil viscosity of 3.298 cP and 3.424 cP. The results of GC-MS analysis show that moringa seed oil from soxhletation has an oleic acid composition of 40.92%, while the screw pressing has an oleic acid composition of 22.50%. This finding demonstrates the influence of extraction methods on the fatty acid profile, particularly the concentration of oleic acid, a key component of moringa seed oil. These results contribute to understanding how specific extraction techniques can optimize the quality of moringa seed oil.

Keywords: Moringa Seed Oil; Screw Pressing; Soxhletation.

Introduction

The moringa plant (*Moringa oleifera*) can be found in almost every region in Indonesia. This plant can thrive even in a dry environment [1]. All parts of the moringa plant, such as leaves, seeds, flowers, roots, and even the bark, can be utilized in various fields for food, health, and industrial needs [2]. Moringa plants have been proven to have high nutritional value, so they have potential as medicinal plants [3]. According to [4] the moringa plant is one of the plants that has high antioxidant activity. Moringa leaves are widely used as a food source, as are vegetables and moringa leaf tea. Moringa leaf extract contains flavonoid compounds that have the potential to be antioxidants because they can ward off free radicals [5]. The results of [6] showed that the ethanol extract of moringa leaves has very strong antibacterial activity. Apart from moringa leaves, one part of the moringa plant that also has many benefits is the seeds, but until now, they have not been used optimally. The research of [7] stated that moringa seeds contain many minerals needed by the body as well as protein, lipids, ash, fiber and carbohydrates. According to [8] moringa seeds contain vegetable oil which is rich in oleic acid, more than 70%. Meanwhile, research by [9] stated that moringa seed oil has a composition of unsaturated fatty acids (47.4% oleic acid and 33.2% linoleic acid) and saturated fatty acids (12.7% palmitic acid and 5.5% stearic acid). Moringa seed oil has begun to be applied in the cosmetics industry, traditional medicines, and skin care formulations [10]. The research of [11] stated that moringa seed oil can be used as a cosmetic ingredient because it has an IC_{50} activity of 9.04%. In addition, moringa seed oil also contains polyphenols, β-carotene, sterols and tocopherols which have antioxidant activity [12].

Moringa seed oil can be obtained through the chemical soxhletation extraction method or the mechanical extraction method using a screw press. Soxhletation chemical extraction is a method of repeatedly separating oil from seeds using chemical solvent compounds. This research aims to study the effect of preheating and extraction time on the character and physicochemical properties of moringa seed oil using the soxhletation and screw pressing methods. Initial heating is carried out in an oven at certain temperature variations. Physico-chemical property tests include water content, acid value, iodine number, density, viscosity, yield and oil composition analysis using GCMS. The quality and characteristics of the extracted oil are greatly influenced by the extraction method used. Thus, it is very important to study effective extraction methods to obtain moringa seed oil's maximum yield and quality. This research provides important insights into selecting extraction techniques for producing high-quality moringa seed oil to meet industrial needs such as food, cosmetics, and medicine.

Research Methods

Soxhlet Extraction of Moringa Seed Oil

Moringa seed samples were peeled and cleaned. Then, the peeled samples were air-dried at room temperature

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to reduce the water content. The dried moringa seeds were then given various preheating treatments at 50, 60 and 70ºC temperatures. Then, the samples were crushed and wrapped in filter paper. Soxhletation is carried out at various predetermined times of 2, 4, and 6 hours. Soxhletation extraction results in oil and solvent were then evaporated using a rotary evaporator at a temperature of 40°C with a speed of 105 rpm. The obtained oil was weighed to determine the yield, and anhydrous sodium sulfate was added to remove the water content [17]. Oil yield can be calculated with the following equation [34]:

$$KM = \frac{m_1}{m_2} \times 100\%$$

Information:
KM = Oil Content
M_1 = weight of oil (g)
M_2 = sample weight (g)

Screw Press Extraction of Moringa Seed Oil

The second extraction method is carried out using the screw-pressing method. This method is based on the method of [18]. The screw press machine must be preheated and ensured that it is clean from dirt. This is to prevent the entry of foreign objects which can hinder the performance of the machine or can also affect the extraction results. Prepared moringa seeds are put into the press machine. The pressing results consist of moringa seed dregs and moringa seed pulp. The moringa seed pulp was then collected in a beaker and filtered using filter paper to obtain the oil yield. The weight of the oil obtained is then measured to get the yield.

Moringa Seed Oil Refining

Purification is done to reduce the free fatty acid content of the extracted moringa seed oil. 5 g of moringa seed oil was put into a vacuum chromatography column containing silica gel eluted using n-hexane eluent. The purification results are then separated from the solvent using a rotary evaporator at a temperature of 40°C with a speed of 125 rpm [17].

Characterization of the Physico-Chemical Properties of Moringa Seed Oil

Characterization of the physicochemical properties of moringa seed oil using a method previously carried out by [19]. Characterization of the physicochemical properties of oil consists of determining the acid value, determining the iodine number, density and viscosity.

Acid value

Moringa seed oil was weighed as much as 2.5 g and put into a 250 mL erlenmeyer, then added 12.5 mL of 96% methanol. The sample was added 10 drops of PP indicator, then titrated with 0.1 N KOH solution until the solution was pink and lasted for 30 seconds. The acid value is obtained using the equation of SNI 01-3555-1998 method:

$$BA = \frac{A \times N \times 56{,}1}{G}$$

Information:
BA = Acid value (mg KOH/g)
A = Amount of KOH for titration (mL)
N = Normality of KOH solution
G = Sample mass (g)

Iodine Number

Determination of the Iodine Number is carried out using the SNI 01-3555-1998 method. 0.1 g of moringa seed oil was put into a 250 mL Erlenmeyer flask, then 15 mL of CCl_4 and 25 mL of Wijs solution were added using a volume pipette. The sample solution was stirred until homogeneous, covered, and stored in a dark place for 1 hour. After 1 hour, 10 mL of 20% KI solution and 100 mL of boiled distilled water were added to the sample solution. The sample was then titrated with 0.1 N $Na_2S_2O_3$ solution until the brown color changed to pale yellow. 4 mL of starch solution was added to the sample, and then the sample was titrated again with 0.1 N $Na_2S_2O_3$ solution until the color disappeared. The same treatment was carried out for the blank solution. The iodine number is obtained using the equation of SNI 01-3555-1998 :

$$BI = \frac{(B - a) \times N Na_2S_2O_3 \times 12{,}691}{g}$$

Information:
B = Blank titration volume (mL)
A = Sample titration volume (mL)
g = Mass of oil (g)
12,691 = Equivalent weight of iodine number (g/mol)
BI = Iodine number (mg iod/100 g)

Density and Viscosity

An empty pycnometer is weighed, then moringa seed oil is put into the pycnometer until it is full, then closed measure the weight. Density can be obtained by the equation of AOAC 2005, 920.212 :

$$\rho = \frac{W_1 - W_2}{v\ (mL)}$$

Information:
W_2= weight of pycnometer + sample (g)
W_1= empty pycnometer weight (g)

Viscosity is measured using the Ostwald viscosity device. Moringa seed oil is put into pipe (A) of the viscometer until it is full, then sucked through pipe (B) until it rises to line (M) and is allowed to fall to line (N). Calculate the time used from line (M) to line (N). Viscosity is obtained by the equation of AOAC 2005, 920.212:

$$\eta = \frac{\rho_s \times t_s}{\rho_0 \times t_0} \times \eta_0$$

Information:
η_s = sample fluid viscosity (cP)
η_o= viscosity of reference fluid (cP)
ρ_o = density of the reference liquid (g/mL)
ρ_s = density of sample liquid (g/mL)
t_o = comparison fluid flow time (s)
t_s= sample liquid flow time (s)

Hydrolysis of Triglyceride

Measure 5 g moringa seed oil, then put into an erlenmeyer flask and saponified with 1.15 g KOH, 2 mL distilled water, and 14 mL 95% ethanol for 1 hour at 60 ˚C. The saponified oil was added with distilled water and then extracted with n-hexane using a separatory funnel until two layers formed. Two fractions were obtained, the saponified water fraction and the unsaponified hexane fraction. The water fraction (saponified) was acidified by adding a few drops of 6 N HCl until the pH was 1 and extracted again with n-hexane. The extraction results obtained two fractions, the water fraction and the hexane fraction, which contains free fatty acids. The hexane fraction was added with anhydrous sodium sulfate and evaporated using an evaporator to separate the solvent and free fatty acids. The same steps are carried out for moringa seed oil from screw pressing.

Esterification

Fatty acids obtained from triglyceride hydrolysis are converted into ethyl ester fatty acids. The oil sample was added with 95% ethanol (1:1). Then, 0.15 g of lipase enzyme and 10 mL of n-hexane were added. Then, the sample is placed in a water bath shaker for 24 hours at a temperature of 40°C and a stirring speed of 150 rpm, after which fatty acids can be identified using a GC-MS tool.

Results and Discussion

Research on the extraction and characterization of the physicochemical properties of *Moringa oleifera* seed oil resulting from soxhletation extraction and screw pressing has been completed.

The sample used was old moringa seeds. Old moringa seeds were chosen because they contain less water. High water content in the sample can cause hydrolysis reactions to occur. The old and dry moringa seeds that have been collected are then separated from the skin and flesh of the seeds. Calculating the percentage of seeds is carried out by weighing the whole, old, dried moringa seeds and then opening and weighing each moringa seed. The average weight of moringa seeds before and after peeling is 0.22 and 0.14 g.

Moringa Seed Oil Extraction Using Soxhletation and Screw Pressing

Soxhletation is commonly used to extract certain analytes from samples into a solvent. The sample is ground first to reduce the particle size and increase the surface area and contact area of the simplicia with the solvent so that extraction can occur optimally. The setup of the Soxhlet apparatus for extracting Moringa seed oil can be seen in Figure 1 and Figure 2.

In addition to the pressing process, the heating process is equally important. Heating in oil processing requires supervision because it determines the quality of the extracted oil. Preheating the moringa seed sample aims to collect the oil grains in the sample, reduce the oil's affinity on the surface, and reduce the oil's viscosity so that extraction takes place more effectively [20]. The oil produced from soxhletation extraction is bright yellow.

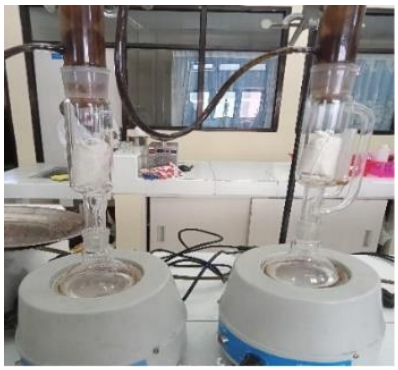

Figure 1. Soxhlet Extraction of Moringa Seed Oil

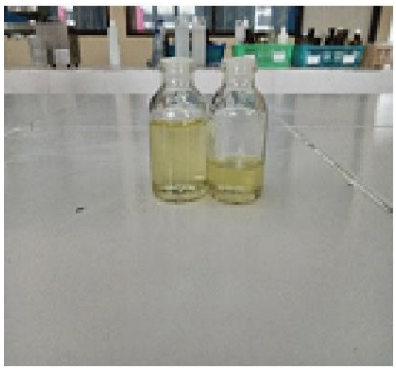

Figure 2. Moringa Seed Oil

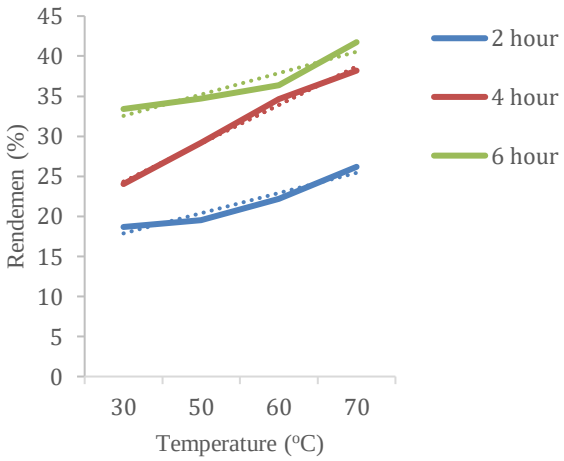

Figure 3. Effect of Preheating on Yield Using Soxhletation

Samples with an initial heating temperature of 70°C and an extraction time of 6 hours produced the highest oil yield, 41.73%. The yield obtained was even greater than previous research by [21] which made a yield of 36.33%. The research of [22] obtained a yield of moringa seed oil by soxhletation of 35.83% and [4] produced a yield of 38.64%. Figure 3 shows the effect of preheating on the oil yield percentage. The graph shows that increasing the moringa seed sample's preheating temperature improves the oil yield rate produced. The right extraction time can also affect the yield because the greater the opportunity for contact between the material and the solvent, the greater the yield will increase until the saturation point of the solution is reached.

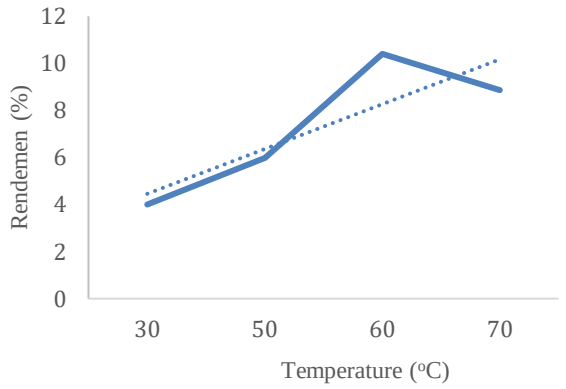

Figure 4. Effect of Preheating on Yield Using Screw Pressing

Mechanical extraction of moringa seeds using a screw press was also carried out by preheating the same sample using the soxhletation method. Screw pressing produces the highest oil yield at an initial heating of 60ºC by 10.40%, see in Figure 4. The low oil yield obtained from pressing compared to soxhletation can be caused by the pressing process which is only carried out once without re-pressing the first dregs. Based on the yield results obtained from both types of extraction, it is proven that the preheating temperature treatment and the length of sample extraction time affect the amount of yield produced.

Iod Number

The iodine number is a number that shows the degree of unsaturation in the oil. The results are usually expressed as the number of grams of iodine absorbed by 100 g of fat or oil. The results of the research, the value of the iodine number for moringa seed oil from soxhletation extraction with variations in room temperature pretreatment and 70ºC heating pretreatment were respectively 62.18 mg iod/100 g and 66.62 mg iod/100 g, while moringa seed oil from screw pressing with the same treatment variations had iodine values of 53.93 mg iod/100 g and 60.27 mg iod/100 g.

Density and Viscosity

Moringa seed oil from soxhletation has a density of 0.922 mg/mL and screw pressing of 0.934 mg/mL. The moringa seed oil has a density that tends to be greater than other oils. This shows that the specific gravity of moringa seed oil is higher due to the fatty acids having a larger molecular weight. Fatty acids with large molecular weights are composed of fatty acids with long carbon chains.

Moringa seed oil from screw pressing has a greater density than moringa seed oil from soxhleting. This is because the oil from screw pressing still contains a lot of impurities because the oil was not purified. [23] stated that oil density is influenced by molecular weight, the number of components in the oil, and the unsaturation of the oil's fatty acid components. The more components contained in the oil, the greater the molecular weight of the oil or fat.

Based on the viscosity test that was carried out using an Ostwald viscometer, the viscosity results for moringa seed oil were 3,298 cP for moringa seed oil from soxhletation and 3,424 cP for moringa seed oil from screw pressing.

Triglyceride Hydrolysis

Hydrolysis is breaking down triglycerides into glycerol and its constituent fatty acids. Hydrolysis reactions can use water, acids, bases, or enzymes as catalysts [24]. The hydrolysis process is carried out by saponification of oil together with KOH, which acts as a catalyst, distilled water, and ethanol. The purpose of adding ethanol to the hydrolysis reaction is to dissolve oil or fat, because ethanol can dissolve fat in hot conditions. The saponified fatty acids are then extracted using n-hexane. The water fraction obtained was then added with 6 N HCl to reach the pH. The function of adding HCl atoms to HCl will replace the bound potassium. The acidified water fraction is then extracted again with n-hexane to separate the fatty acids. The extraction results formed two fractions: the hexane fraction containing fatty acids at the top and the water fraction containing glycerol at the bottom [17].

Esterification

Fatty acids obtained from the triglyceride hydrolysis process are non-volatile, so they must be converted into their ethyl ester form. According to [25], this is very important because ethyl ester is a volatile component so it can be analyzed well by GC-MS.

The esterification process is a very slow reaction that requires a catalyst. The lipase enzyme is used as a catalyst to accelerate the reaction. This lipase enzyme is used because it is easily separated and can be reused.

GC-MS analysis

GC-MS analysis is an analytical instrument consisting of GC (gas chromatography) and MS (mass spectrometry). GC uses gas separation as a mobile phase that will pass through a stationary phase in the column. The results of the fatty acid analysis of moringa seed oil can be seen in table 1. The two types of extraction have very different percentages of fatty acids. In moringa seed oil resulting from soxhletation extraction, the largest percentage belongs to unsaturated fatty acids (oleic acid 40.92%, palmitic acid 10.32% and behenic acid 5.02%).

Table 1. Results of GC-MS Analysis of Moringa Seed Oil

Fatty acid	Molecule Formula	Area (%) Soxhlet	Area (%) Press
Myristic Acid	$C_{14}H_{28}O_2$	0.16	-
Palmitoleic Acid	$C_{17}H_{32}O_2$	0.28	1.34
Palmitic Acid	$C_6H_{32}O_2$	10.32	25.14
Stearic Acid	$C_{19}H_{38}O_2$	1.06	3.61
Oleic Acid	$C_{18}H_{34}O_2$	40.92	22.50
Arachidic Acid	$C_{20}H_{40}O_2$	4.77	10.44
Behenic Acid	$C_{22}H_{43}O_2$	5.02	10.30
Lignoceric Acid	$C_{25}H_{50}O_2$	0.13	-

Oleic acid is the highest fatty acid composition in moringa seed oil. This is in accordance with previous research by [4] [21]. moringa seed oil from screw pressing has a higher amount of saturated fatty acids compared to

moringa seed oil from soxhletation. This leads to a lower degree of unsaturation in the oil, an increase in molecular weight, and an increase in the density and viscosity of the oil.

Conclusion

The research results showed that the highest yield of moringa seed oil 41.74%, was obtained at an initial treatment temperature of 70°C using the soxhletation method for 6 hours. The acid value of moringa seed oil from soxhletation is 2.01 mg KOH/g and screw pressing is 4.37 mg KOH/g. The iodine number from soxhletation was 66.62 mg iod/100g and screw pressing was 60.27 mg iod/100g. The density of the oil from soxhletation and screw pressing is 0.922 mg/mL and 0.934 mg/mL respectively with an oil viscosity of 3.298 cP and 3.424 cP. The results of GC-MS analysis show that moringa seed oil from soxhletation has an oleic acid composition of 40.92%, while the screw pressing have an oleic acid composition of 22.50%.

Author's Contribution

Sri Seno Handayani: Designed the research concept, developed the methodology, and supervised the entire experimental process. Erin Ryantin Gunawan: Extracted moringa seed oil using the Soxhlet and Screw Press methods, and collected experimental data. Dedy Suhendra: Characterized the oil obtained, including analysis of physicochemical properties and fatty acid composition. Murniati: Analyzed and interpreted the experimental results and data processing. Rizka Dhia Khalilah Bali: Wrote and edited the results and discussion sections in the scientific article, and compiled the conclusions. Gita Sagita Sari: compiled tables and graphs of the research results.

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