



## Analysis of Characteristics, Fatty Acid Composition, Antioxidant Activity, and Beta-sitosterol of Moringa (*Moringa oleifera* Lam) Oil from Three Different Regions in Indonesia

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### ABSTRACT

Moringa oil offers a range of health, nutritional, and cosmetic benefits. However, its characteristics can vary greatly depending on its geographical origin. This study aimed to compare the physicochemical properties, fatty acid composition, antioxidant activity, and beta-sitosterol content of moringa oil obtained from three regions in Indonesia: Central Java, Central Sulawesi, and East Nusa Tenggara. The physicochemical properties examined include organoleptic, specific gravity, viscosity, refractive index, iodine value, acid value, saponification value, and peroxide value. Furthermore, the fatty acid composition was analyzed using gas chromatography with flame ionization detection (GC-FID), antioxidant activity was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, and beta-sitosterol content was measured by thin-layer chromatography (TLC). Physical attributes, such as viscosity and specific gravity, do not exhibit significant differences between the regions. At the same time, chemical parameters, which include iodine value, acid value, and saponification value, show considerable variations. Fatty acid profiles revealed that oils from Central Sulawesi and East Nusa Tenggara have high oleic acid and low polyunsaturated fat content, providing better oxidative stability and longer shelf life. In contrast, Central Java oil showed higher levels of linoleic and behenic acids, making it more suitable for sensitive skin and hair care applications. Central Java oil exhibits the highest antioxidant activity, whereas Central Sulawesi oil contains the highest concentration of beta-sitosterol, suggesting a more significant potential as an anti-inflammatory agent. These findings highlight the influence of regional origin on the quality of moringa oil, presenting exciting opportunities for further research in this field.

**Keywords:** Physicochemical properties; Fatty acid profile; Antioxidant activity; Beta-sitosterol content; Geographic variation

### INTRODUCTION

In Indonesia, moringa oil production is spread across different regions, especially East Nusa Tenggara, East Java, Central Java, Sumatra, and Sulawesi.<sup>1,2</sup> The analysis of moringa oil from different regions is critical because

the nutritional content, safety, physical and chemical quality, and medicinal potential may vary. This analysis ensures that the oil meets quality standards and market preferences, promotes the development of new products, and provides a better understanding of the geographical factors

that influence the oil composition.<sup>3-5</sup>

Moringa oil, derived from the seeds of *Moringa oleifera*, belongs to the Moringaceae family. It offers numerous benefits for health, skincare, and nutrition. This versatile oil is rich in monounsaturated fatty acids, primarily oleic acid, which contributes to its stability and numerous health benefits.<sup>6,7</sup> Regarding skin benefits, the high oleic acid content helps retain skin moisture and provides deep hydration without clogging pores. Its antioxidants may delay aging by reducing fine lines and wrinkles.<sup>7</sup> Moringa oil also possesses anti-inflammatory properties.<sup>8</sup> Moreover, it may have anti-cancer properties.<sup>9</sup> The other content in Moringa oil is phytosterol, of which beta-sitosterol is the primary component responsible for its anti-inflammatory activity.<sup>10</sup>

Research on the characteristics of Indonesian moringa oil has been conducted. Ayu et al. demonstrated that the fatty acid content of moringa oil includes oleic acid (74.6-79.9%), palmitic acid (5.77-7.78%), stearic acid (4.71-5.48%), behenic acid (4.83-8.71%), and arachidic acid (2.76-6.50%). The results of the antioxidant activity test yielded an IC<sub>50</sub> value of 67.4-90.0 ppm.<sup>11</sup> Research on moringa oil from Central Sulawesi found that oleic acid is the dominant fatty acid, accounting for concentrations of 38.08% and 38.84%. The acid number was also found to be 10.68 mg KOH/g, while the free fatty acid (FFA) content was determined to be 5.36%. The saponification value was found to be 15.7108 and 7.2943 mg KOH/g.<sup>5</sup> The research on moringa oil from East Nusa Tenggara yielded data on the physical parameters, including a specific gravity of 0.912 g/mL and a viscosity of 1.054 cPs. Additionally, the chemical parameters yield a saponification value of 132.42 mg KOH/g, an acid value of 18.65 mg KOH/g, an iodine value of 66.46, and a peroxide value of 6.8 mEq/kg.<sup>12</sup>

Several studies on moringa oil from diverse geographical regions in Indonesia have been conducted. However, all studies

have incomplete data on its physicochemical characteristics, fatty acid profiles, antioxidant activity, and beta-sitosterol content. To address the gap, this study examined the characteristics, fatty acid profile, antioxidant activity, and beta-sitosterol content of moringa oil from Central Java, Central Sulawesi, and East Nusa Tenggara.

## METHODS

### Materials

**Sample:** Moringa oil from Central Java (Lansida), Moringa oil from Central Sulawesi (no brand) produced by UD Barokah, and Moringa oil from East Nusa Tenggara (Morifa).

**Chemical:** Ethanol (Merck), Ethanol (Smart Lab), KOH (Merck), Acetic acid (Merck), Chloroform (Merck), Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (Merck), Aquadestilata (Brataco), 2,2-diphenyl-1-picryl-hydrazyl (Sigma, Aldrich),<sup>z</sup> Beta-sitosterol (Sigma, Aldrich).

### Instruments

The equipment used in this research are Brookfield Viscometer, ABBE digital Refractometer SWYA2S, Gas Chromatography (CLARUS 690-IN254), Spectrophotometer UV-Vis (UV-800 Shimadzu), and TLC Scanner (Camag TLC Scanner 4).

## Procedures

### 1. Physicochemical Characteristics Organoleptic

Moringa oil was examined for shape, color, and odor using the five senses to determine its organoleptic properties.<sup>13</sup>

### Viscosity measurement

The viscosity was measured at 25°C with a Brookfield Viscometer.

### Determination of specific gravity

Specific gravity was determined using a pycnometer at 25°C and water as a standard.<sup>14</sup>

### Analysis of refractive index

The refractive index of moringa oil was analyzed with the ABBE digital Refractometer SWYA2S.<sup>15</sup>

### Determination of iodine value

Two grams of moringa oil were placed in a 250 mL Erlenmeyer flask, followed by the addition of 20 mL of Carbon tetrachloride (CCl<sub>4</sub>). Next, 25 mL of Wijs solution was added to the flask, and the mixture was stirred until it became homogeneous. Two blanks were prepared and treated in parallel with each group of samples under the same conditions. The flasks were stored in a dark environment for 30 minutes at a temperature of 25 °C ± 5 °C. After the reaction, 20 mL of 15% (w/v) potassium iodide (KI) solution was added to each flask, along with 100 mL of distilled water. The resulting mixture was titrated with 0.1 N sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) solution, which was added gradually with continuous stirring using a stirrer bar. The titration proceeded until the yellow color nearly vanished, at which time 1-2 mL of starch indicator solution was introduced. The titration continued until the blue color was no longer visible. The iodine value was subsequently calculated using the relevant formula.<sup>16</sup>

$$\text{Iodine value} = \frac{(B - S) \times N \times 12.69}{\text{weigh of sample}}$$

B = titration of the blank sample

S = titration of a sample

N = Normality of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>

### Determination of acid value

The moringa oil was weighed in a 250 mL Erlenmeyer flask, up to 5 grams. Then, 50 mL of 95% neutral ethanol was added, previously titrated using 0.1 N KOH and phenolphthalein as an indicator. The Erlenmeyer flask was equipped with a reflux condenser, heated to boiling, and shaken using a magnetic stirrer to ensure the complete separation of free fatty acids. After cooling, the solution underwent titration with 0.1N KOH, which was standardized using phenolphthalein. Titration was continued until a persistent pink color appeared and lasted for at least 0.5 minutes. The volume of KOH solution required for neutralization was recorded.

Determination of acid value using the appropriate formula.<sup>17</sup>

$$\text{Acid value} = \frac{\text{mL KOH} \times N \text{ KOH} \times 56.1}{\text{Sample weight (g)}}$$

### Analysis of Saponification Value<sup>17</sup>

Five grams of moringa oil sample were measured in a 250 mL Erlenmeyer flask, followed by the addition of 50 mL of KOH ethanol solution (4 g KOH/100 mL ethanol). A condenser was installed in the Erlenmeyer flask and then boiled for 30 minutes at 150°C while stirring using a magnetic stirrer. After cooling, several drops of phenolphthalein indicator were added to the Erlenmeyer flask, and it was titrated with a standardized 0.5 N HCl solution. Titration was also carried out on the blank. The saponification value was determined using the formula:

$$\text{Saponification value} = \frac{\text{mL HCl (Blank - Sample)} \times N \text{ HCl} \times 56.1}{\text{Sample weight}}$$

### Peroxide value

Moringa oil was carefully weighed in a 250 mL Erlenmeyer flask as much as 1 gram. Then, 30 mL of 3:2 acetic acid-chloroform solution was added and mixed until the oil was completely dissolved. Following this, 0.5 mL of saturated potassium iodide solution was introduced. The mixture was allowed to stand for 1 minute, with occasional shaking. Then, 30 mL of distilled water was added. The mixture was titrated with a standard Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> solution until the yellow color nearly disappeared. Afterward, 0.5 mL of starch solution was added, and titration continued until the blue color diminished. The peroxide value was subsequently calculated using the relevant formula.<sup>17</sup>

$$\text{Peroxide value} \left( \frac{\text{mEq}}{\text{kg}} \right) = \frac{\text{mL NO}_3 \times N \text{ Na}_2\text{S}_2\text{O}_3 \times 1000}{\text{Sample weight}}$$

### Fatty acid analysis

Fatty acid methyl esters (FAME) were prepared following AOCS Official Method Ce 1h-05.<sup>18</sup> The diluted FAME were separated on a gas chromatograph (Perkin Elmer Clarus 680 GC) equipped with a

Supelco SPTM 2560 10 capillary column (100 m × 0.25 mm × 0.20 μm) and a flame ionization detector (FID). Fatty acids were identified by comparing their retention times with those of authentic FAME standards. The calculation of fatty acid content was conducted using the normalization method. All components of the fatty acid in the sample are represented in the resulting chromatogram so that the total area under the peaks represents 100% of the fatty acid content.<sup>17</sup>

### Antioxidant activity

One mL of a 0.4 mM DPPH solution was combined with 4.0 mL of ethanol to serve as a control solution. This solution was then determined for its maximum wavelength utilizing a UV-Vis spectrophotometer (515-517 nm). The obtained wavelength was used to measure the absorbance of the control solution. A specific volume of moringa oil was mixed with 1.0 mL of 0.4 mM DPPH and ethanol to reach a total of 5.0 mL. The solution was then incubated for 30 minutes in a dark environment. The absorbance of the sample solution was then measured at the wavelength of maximum absorption.<sup>19</sup> Determination of IC<sub>50</sub> using the following formula:

$$\% \text{Inhibition} = \frac{(A \text{ blank} - A \text{ sample})}{A \text{ blank}} \times 100\%$$

The regression curve is created by calculating the sample concentration versus the percentage of inhibition. The IC<sub>50</sub> value is calculated using the regression equation that has been made.

### Beta-sitosterol content

One mL of moringa oil was placed into a 10 mL test tube, and then 1 mL of ethanol was added. The mixture was vortexed thoroughly and subjected to sonication for 10 minutes to facilitate extraction. After sonication, the sample was centrifuged, and the resulting ethanol phase was carefully collected. The ethanol extract was moved to a 5 mL volumetric flask and brought to volume with ethanol. A 2.0 μL

aliquot of resulting supernatant was placed onto a silica gel 60 F<sub>254</sub> TLC plate. For calibration, standard solutions of β-sitosterol 1000 μg/mL (purity of 83.10%) were applied to the same plate in volumes of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.5 μL. Chromatographic separation was carried out in a pre-saturated TLC chamber using a mobile phase consisting of toluene and ethyl acetate in a ratio of 80:20 (v/v). The plate was developed until the solvent front reached an appropriate height, then removed and air-dried. Post-development, the plate was treated with Liebermann-Burchard reagent and subsequently heated at 110° for 2 minutes to enable color development. The chromatogram was scanned at 340 nm using a TLC densitometer. The content of β-sitosterol in the sample was quantified by comparing the intensity of the sample spot to a calibration curve constructed from the β-sitosterol standards.<sup>20</sup>

### Statistical Analysis

The results of the study are presented as the mean ± SD, with a significance level set at p < 0.05. All statistical analyses were conducted using SPSS Version 26.0. Different groups were compared using One-way analysis of variance (ANOVA) followed by Tukey's HSD.

## RESULTS AND DISCUSSION

### 1. Physicochemical characteristics of Moringa Oil

The Moringa oil examined was obtained from the cold-pressed extraction of moringa seeds in Central Java, Central Sulawesi, and East Nusa Tenggara. These three regions have distinct soil structures and rainfall patterns, which impact the fertility and composition of their metabolites.

Table 1 presents the physicochemical properties of moringa oil from three regions in Indonesia: Central Java, Central Sulawesi, and East Nusa Tenggara. This is the result and discussion of this research.

## Physical Properties

*Shape, Color, and Odor:* Moringa oil from all three regions is a liquid at room temperature, golden yellow in color, and has a bitter almond-like odor. These attributes are consistent with established research, which notes that Moringa oil is typically liquid at room temperature and has a golden yellow color, with an odor similar to that of bitter almonds.<sup>7,21</sup>

*Viscosity:* The viscosity of the moringa oil from the three regions was not different ( $p > 0.05$ ). The viscosity values are consistent across regions, indicating uniform flow properties. This uniformity is particularly beneficial for applications in cosmetics, such as lotions and serums, where a consistent texture is essential. This result exceeds that of moringa oil from Turkey, which was obtained by pressing at 180°C. The extraction method affects the viscosity, with cold-pressed oils typically exhibiting higher viscosity due to the water content present during extraction.<sup>22</sup>

*Specific gravity:* The specific gravity of the moringa oil from the three regions did not differ significantly ( $p > 0.05$ ). Vegetable oils typically have specific gravity values that range from 0.91 to 0.93 g/mL.<sup>23</sup> The values of the three oils are slightly below the typical range but are acceptable. A lower specific gravity indicates a lighter oil, which is often preferred in cosmetic formulations for better skin absorption. These values align with previous findings where the specific gravity of moringa oil is reported to be around 0.901 g/mL.<sup>7</sup>

*Refractive Index:* The refractive index of moringa oil from Central Sulawesi and East Nusa Tenggara were not significantly different ( $p > 0.05$ ), but were higher than that of moringa oil from Central Java ( $p < 0.05$ ). For all in the range of 1.438-1.446. The Standard reference for the refractive index of vegetable oils typically ranges from 1.467 to 1.470.<sup>23</sup> The values are slightly below the standard range, indicating a lower degree of saturation compared to other vegetable oils. This could indicate better oxidative stability, which is advantageous for food

and cosmetic applications.

## Chemical Properties

*Iodine value:* The iodine value quantifies the level of unsaturation in fats and oils, reflecting the existence of double bonds in fatty acid chains.<sup>24</sup> The iodine value of the moringa oil from the three regions differed significantly ( $p < 0.05$ ). The order of Iodine value from the smallest to the largest was Central Java, East Nusa Tenggara, and Central Sulawesi. The iodine value of these three oils is less than 80, so they are classified as non-drying<sup>25</sup> and suitable for cosmetic and food applications. Central Java oil, with the lowest iodine value, is the most saturated, suggesting better oxidative stability. Higher iodine values, such as those found in Central Sulawesi and East Nusa Tenggara, indicate a higher degree of unsaturation compared to Central Java. Unsaturated fats, which are more prevalent in oils with higher iodine values, benefit cardiovascular health by reducing levels of harmful cholesterol.<sup>26</sup> However, oils with higher iodine values are also more prone to oxidation and rancidity, which affects their shelf life and stability.<sup>27</sup> Therefore, while the higher iodine values in Central Sulawesi and East Nusa Tenggara Moringa oils may indicate better nutritional benefits due to higher unsaturation, they also necessitate careful storage and handling to maintain oil quality.<sup>27</sup>

*Acid Value:* The acid value denotes the milligrams of potassium hydroxide (KOH) required to neutralize the fatty acids in 1 gram of the sample.<sup>28</sup> The acid values in this study were significantly different ( $p < 0.05$ ), with Central Java Moringa oil having the highest, followed by Central Sulawesi and East Nusa Tenggara. The resulting study was higher than that reported by Gharsallah et al.,<sup>29</sup> obtained from  $1.5 \pm 0.21$  mg KOH/g oil. Their acid value is higher than the reference standard for food and cosmetic grade oils; that acid value should be  $< 4.0$  mg KOH/g.<sup>30</sup> This study resulted in a higher free fatty acid content, which could affect the oil's stability and shelf life.

**Table 1.** Physicochemical properties of Moringa oil from three Indonesian Regions

Parameter	Central Java Oil	Central Sulawesi Oil	East Nusa Tenggara Oil
Shape	Liquid	Liquid	Liquid
Color	Golden-yellow	Golden yellow	Golden-yellow
Odor	Bitter almond	Bitter almond	Bitter almond
Viscosity (25°C) (mPa.s)	61.7±1.5	60.0±2.0	60.0±1.5
Specific gravity (25°C) (g/mL)	0.9080±0.0002	0.9085±0.0005	0.9061±0.0002
Refractive Index (29°C)	1.4639±0.0000	1.4644±0.0002	1.4638±0.0000
Iodine value	43.67±0.23	63.77±0.02	57.98±0.26
Acid value (mg KOH/g)	15.21±0.10	11.39±0.13	10.62±0.09
Saponification value (mg KOH/g)	256.27±1.04	207.62±1.03	235.82±1.69
Peroxide value mEqO <sub>2</sub> /0.1kg)	10.29±0.02	10.27±0.01	9.71±0.01

All three oils are cold-pressed, which means they are extracted using mechanical processes without adding heat and are not refined or fractionated. Due to several factors, cold-pressed moringa oil typically exhibits a higher acid value than solvent-extracted oil. Adding water during seed milling in the cold pressing process enhances lipolytic enzyme activity, increasing triglyceride hydrolysis and forming free fatty acids. Additionally, the prolonged exposure to air and temperature during milling and pressing promotes oxidation and hydrolysis reactions, further elevating acidity levels. Cold pressing yields approximately 61.4% oil from Moringa seeds, whereas hexane solvent extraction can achieve nearly 100%, concentrating free fatty acids in the pressing yield. Furthermore, cold pressing does not deactivate natural enzymes in the seeds, leading to the breakdown of triglycerides into free fatty acids over time. The minimal refining of cold-pressed oils also retains their natural acidity. Despite the higher acidity, cold-pressed moringa oil is valued for its high quality and retention of natural bioactive compounds.<sup>31</sup>

**Saponification Value:** The saponification value (SV) indicates the milligrams of NaOH or KOH necessary to saponify one gram of oil or fat under defined conditions.<sup>32</sup> The highest saponification value is found in Central Java, followed by East Nusa Tenggara, and the lowest is in Central Sulawesi ( $p < 0.05$ ). The saponification value in this study is higher

than that reported by Cevera-Chiner et al., who obtained a saponification value of moringa oil in the range of 192-222.<sup>33</sup>

A higher saponification value indicates that the oil contains a higher proportion of shorter-chain fatty acids. This can have several implications, including improved suitability for soap making and potential changes in taste and aroma; understanding the saponification value is crucial for determining the appropriate applications and quality of the oil.<sup>32</sup>

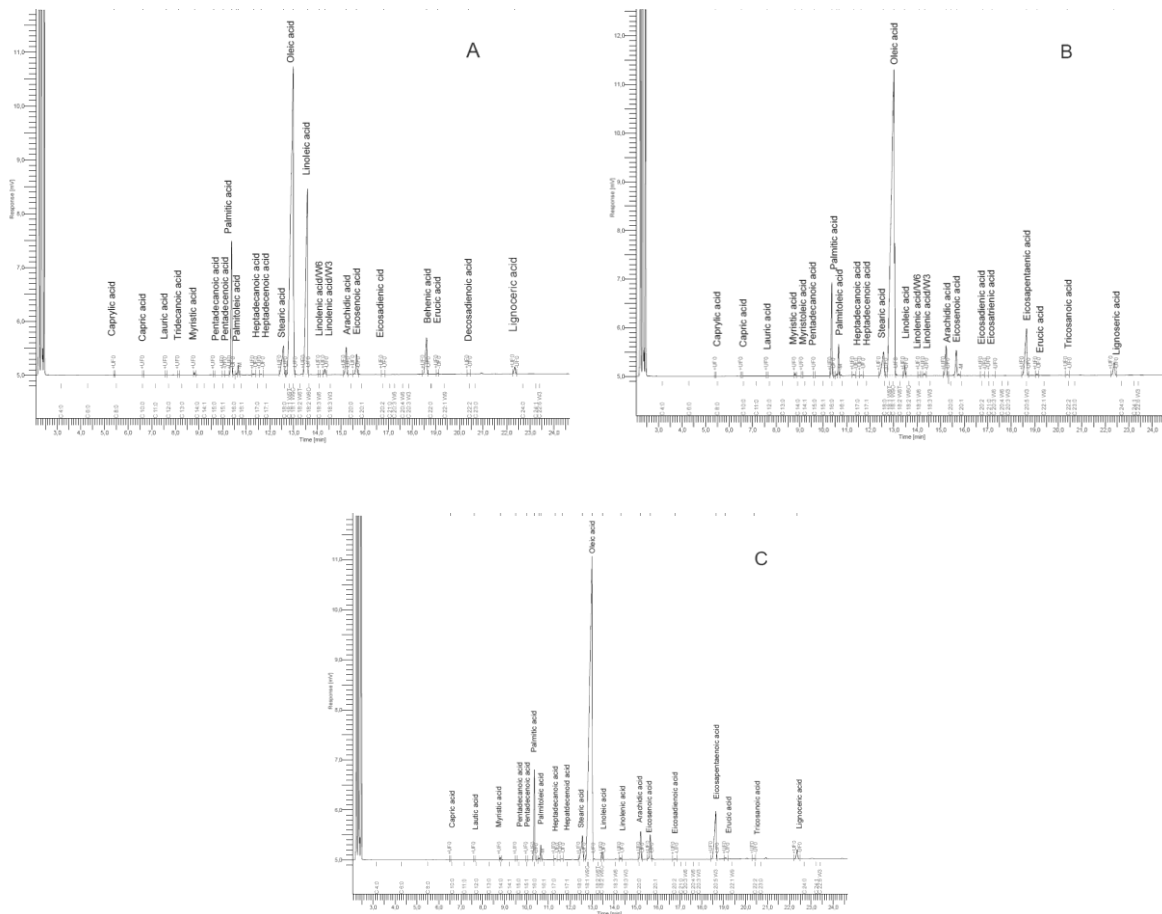
**Peroxide Value:** The peroxide value (PV) of an oil is a crucial indicator of its oxidative stability and shelf life. It indicates the degree to which the oil has undergone primary oxidation, the first stage of rancidity. This study's results show that the PV moringa seed oil from the three regions is not significantly different ( $p > 0.05$ ), with values ranging from 9.72 to 10.30.

Moringa seed oils are often categorized by their peroxide value (PV), which reflects their degree of oxidation and longevity. Oils with a peroxide value (PV) ranging from 3 to 5 meq/kg are classified as having low oxidation, those between 10 and 12 meq/kg are deemed to have moderate oxidation, and oils with a PV between 16 and 18 meq/kg are characterized by strong oxidation.<sup>34</sup> Based on this classification, the Moringa oils from Central Java, Central Sulawesi, and East Nusa Tenggara fall into the moderate oxidation category. This suggests that these

oils are moderately oxidized and thus possess a moderate shelf life.

**Table 2.** Fatty Acid Composition (%) of Moringa Oil from Three Indonesian Regions

No.	Fatty Acid	Central Java	Central Sulawesi	East Nusa Tenggara
1.	Caprylic acid (C8:0)	0.01295±0.00035	Not detected	Not detected
2.	Capric acid (C10:0)	0.00295±0.00007	0.00335±0.00007	0.0037±0.00000
3.	Lauric acid (C12:0)	0.0133±0.00014	0.01615±0.00007	0.0131±0.00028
4.	Tridecanoic acid (C13:0)	0.01725±0.00049	Not detected	Not detected
5.	Myristic acid (C 14:0)	0.10120±0.00028	0.13115±0.00078	0.11790±0.00000
6.	Pentadecanoic acid (C15:0)	0.0078±0.00000	0.00515±0.00007	0.00850±0.00000
7.	Pentadecenoic acid (C15:1)	0.0134±0.00014	Not detected	0.0061±0.00000
8.	Palmitic acid (C16:0)	8.44785±0.01167	6.28130±0.01952	6.30025±0.00361
9.	Palmitoleic acid (C16:1)	0.54855±0.00148	1.94495±0.00177	0.97400±0.00042
10.	Heptadecanoic acid (C17:0)	0.06975±0.00092	0.09905±0.00149	0.10025±0.00007
11.	Heptedecenoic acid(C17:1)	0.03560±0.00000	0.05820±0.00000	0.04535±0.00149
12.	Stearic acid(C18:0)	3.7868±0.00000	3.99835±0.00021	3.7262±0.01358
13.	Oleic acid (C18:1 ω9)	56.0953±0.01838	75.09645±0.03444	73.7291±0.01923
14.	Linoleic acid (C18:2 ω6)	21.86505±0.01110	0.70085±0.00446	0.60365±0.00149
15.	Linolenic acid (C18:3 ω6)	0.02035±0.00007	0.03480±0.00085	0.13210±0.00057
16.	Linolenic acid (C18:3 ω3)	0.3525±0.00000	0.18±0.00000	Not detected
17.	Arachidic acid (C20:0)	2.1950±0.0071	0.08±0.00	2.79 ±0.00
18.	Eicosenoic acid (C20:1)	1.61235±0.00643	2.90805±0.03698	2.50135±0.01138
19.	Eicosadienic acid (C20:2)	0.03335±0.00007	0.05430±0.00014	0.06095±0.00007
20.	Eicosatrienoic acid (C20:3 W6)	Not detected	0.00890±0.00000	Not detected
21.	Eicosapentaenic acid (C20:5 ω3)	Not detected	6.61980±0.02008	7.46015±0.01237
22.	Behenic acid (C22:0)	3.8932±0.00636	Not detected	Not detected
23.	Erucic acid (C22:1 W9)	0.0618±0.00057	0.11810±0.00000	0.13360±0.00057
24.	Docosadienoic acid (C22:2)	0.0409±0.00014	Not detected	Not detected
25.	Tricosanoic acid (C23:0)	Not detected	0.0776±0.00113	0.06690±0.00028
26.	Lignoceric acid (C24:0)	0.6855±0.00071	1.4891±0.00537	1.12850±0.00198
<b>Type</b>				
	Saturated fatty acid (SFA)	19.2328±0.0026	12.1834±0.0149	14.2524±0.0051
	Monounsaturated fatty acid (MUFA)	58.3668±0.0139	80.1279±0.0011	77.3897±0.0081
	Polyunsaturated fatty acid (PUFA)	22.3121±0.0112	7.5979±0.0138	8.2569±0.1320



**Figure 1.** GC-FID Chromatogram of the Moringa Oil from (A) Central Java, (B) Central Sulawesi, and (C) East Nusa Tenggara

## 2. The Composition of Fatty Acids in Moringa Oil

The fatty acid profile of moringa oil was examined using GC-FID, which identified twenty-three distinct fatty acids in Central Java, twenty-three in Central Sulawesi, and twenty in East Nusa Tenggara, as illustrated in Figure 1 and Table 2.

The molecular structure of moringa oil, particularly the carbon chain linkages, comprises saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). The content of SFAs, MUFAs, and PUFAs in moringa oil from the three regions differs significantly ( $p < 0.05$ ). These variations in fatty acid profiles among sources are evident.

Central Sulawesi has the lowest amounts of SFA and PUFA and the highest concentration of MUFA. In contrast, Central Java is characterized by the highest levels of both PUFA and SFA. Meanwhile, East Nusa Tenggara shows intermediate values for both SFA and PUFA.

Numerous studies have demonstrated that geographic origin significantly influences the fatty acid composition of various vegetable oil products. Environmental variables such as climate, soil characteristics, and agronomic practices specific to a region are crucial in shaping the resulting fatty acid profile. For instance, a study on hazelnut oil in Turkey reported significant regional differences in SFA, MUFA, and PUFA concentrations. The Central Black Sea region exhibited the highest levels of SFA (8.45%) and MUFA

(83.54%), whereas the Eastern Black Sea region was distinguished by a higher linoleic acid (C18:2) content (9.10%).<sup>35</sup> Similarly, research on olive oil in Turkey demonstrated that fatty acid composition can serve as a reliable indicator of geographic origin. Specific ratios, such as the linoleic-to-linolenic acid ratio, have been identified as practical geographical markers.<sup>36</sup> Moroccan argan oil was analyzed using stable isotope analysis and fatty acid profiling to trace its geographical origin.

Findings revealed that ecological parameters influenced isotopic values (e.g.,  $\delta^{13}\text{C}$ ) as well as concentrations of key fatty acids such as palmitic acid (C16:0) and linoleic acid (C18:2), enabling accurate geographic classification of the oils.<sup>36</sup> Research on camellia oil further supports this notion, indicating that oil quality is significantly affected by geographical variables, including local climate and soil composition, which are reflected in the variability of fatty acid profiles.<sup>36</sup>

The different compositions of fatty acids in moringa oil from three regions are implicated in its use. Moringa oil's emollient and non-comedogenic properties make it a desirable ingredient in skincare. Oils from Central Sulawesi and East Nusa Tenggara, with high oleic acid content (>73%), offer deep moisturization, enhance skin barrier repair, and promote anti-aging benefits.<sup>37</sup> Oleic acid is revealed to be anti-inflammatory for the skin.<sup>38</sup> It possesses pharmacological properties for managing metabolic syndrome, encompassing the mitigation of cardiovascular disease, dyslipidemia, central obesity, non-alcoholic fatty liver disease, and type 2 diabetes mellitus.<sup>39</sup> Conversely, Central Java oil, richer in linoleic acid (21.9%), is more suitable for acne-prone skin due to its lighter texture and anti-inflammatory nature.<sup>40</sup> Behenic acid, present only in Central Java oil, adds value to haircare formulations by acting as a detangling and smoothing agent.<sup>41</sup> Oleic acid has been demonstrated to possess anti-inflammatory properties for the skin.<sup>38</sup>

All three oils' high monounsaturated fat profile mirrors olive oil's, indicating suitability for heart-healthy diets. Oils from Central Sulawesi and East Nusa Tenggara contain notable levels of EPA (6.6–7.5%), are rare in plant oils, and suggest potential in vegan omega-3 supplements and anti-inflammatory diets.<sup>42,43</sup> Linoleic acid's presence in Central Java oil offers essential fatty acids but may reduce oxidative stability. In contrast, lower PUFA levels in Central Sulawesi and East Nusa Tenggara oils enhance their shelf life, making them ideal for high-temperature cooking.<sup>44</sup> Subsequently, lower linoleic acid and higher oleic acid percentages correlate with more excellent oxidative stability. Central Sulawesi and East Nusa Tenggara oils are less prone to rancidity, making them ideal for cosmetics and functional foods with extended shelf life.<sup>36</sup>

### 3. Antioxidant activity

Antioxidant measuring helps assess how well an oil can overcome oxidation. Measurement of the antioxidant activity can ensure that the oil remains rich in essential nutrients during storage and use. The antioxidant activity of moringa oil is represented by Inhibitor Concentration 50 (IC<sub>50</sub>). This means that the concentration of oil scavengers is 50% of the initial DPPH radicals. Therefore, the lower the IC<sub>50</sub> value, the more potent the oil's capacity as an antioxidant.<sup>45</sup>

**Table 3.** The antioxidant activity of moringa oil from three regions in Indonesia

Source	Average±SD IC <sub>50</sub> (mg/mL)
Central Java	28.405±2.573
Central Sulawesi	45.339±2.132
East Nusa Tenggara	58.562±1.827

Table 3 shows the result of examining the antioxidant activity of moringa oil. There are significantly different IC<sub>50</sub> of moringa oil from three regions ( $p < 0.05$ ). The moringa oil from Central Java exhibits the highest antioxidant activity, and its IC<sub>50</sub> value is better than that reported by other research, which reported an IC<sub>50</sub> value of

121.9 mg/mL.<sup>37</sup> It is revealed that this highlights regional variations that affect antioxidant properties.

Moringa oil's multifunctional properties, including its antioxidants, make it a promising ingredient for various applications. The oil has been used in formulations to enhance skin hydration and antioxidant activity <sup>37</sup> demonstrating its potential in cosmetic and pharmaceutical products.

#### 4. Beta-sitosterol content

It is vital to measure the beta-sitosterol content in moringa oil, as beta-sitosterol has several health benefits, including the ability to decrease cholesterol levels and exhibit anti-inflammatory effects.<sup>10,46</sup> Table 4 displays the findings of evaluating the beta-sitosterol content in moringa oil.

**Table 4.** The content of Beta-sitosterol in moringa oil

Source	Average±SD (µg/mL)
Central Java	1,120.68±47.44
Central Sulawesi	1,837.71±9.70
East Nusa Tenggara	1,648.00±5.66

The beta-sitosterol content in moringa oil from three regions differs ( $p < 0.05$ ). The moringa oil in Central Sulawesi has the highest beta-sitosterol content, which may have the most potential anti-inflammatory effect. It is revealed that the geography of the planting area influences the composition of its metabolites.

#### CONCLUSION

This study demonstrated that Moringa oil from Central Java, Central Sulawesi, and East Nusa Tenggara exhibits notable regional differences in physicochemical characteristics, fatty acid profiles, antioxidant activity, and beta-sitosterol content, all of which are influenced by geographical factors, including soil type and climate. While physical properties, such as viscosity and specific gravity, showed no significant differences, chemical properties, including iodine, acid, and saponification values, varied

significantly, impacting oil stability and usability. Central Sulawesi and East Nusa Tenggara oils were rich in oleic acid and exhibited better oxidative stability, making them suitable for cosmetic and food applications. In contrast, Central Java oil, which is higher in linoleic and behenic acid, is more suitable for acne-prone skin and haircare. The antioxidant activity was strongest in Central Java oil, whereas Central Sulawesi oil had the highest beta-sitosterol content, indicating superior anti-inflammatory potential. These findings suggest that regionally sourced Moringa oil possesses unique qualities that can be leveraged for targeted functional, nutritional, and cosmetic applications, emphasizing the importance of geographic origin in determining oil composition and potential use.

#### Conflict of Interest

The authors declare that they have no conflict of interest.

#### Authors' Declaration

The authors hereby declare that the work given in this article is original and that they will assume any liability for disputes over its content.

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