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Physicochemical characteristics of Hadhramaut *Moringa peregrina* seeds oil

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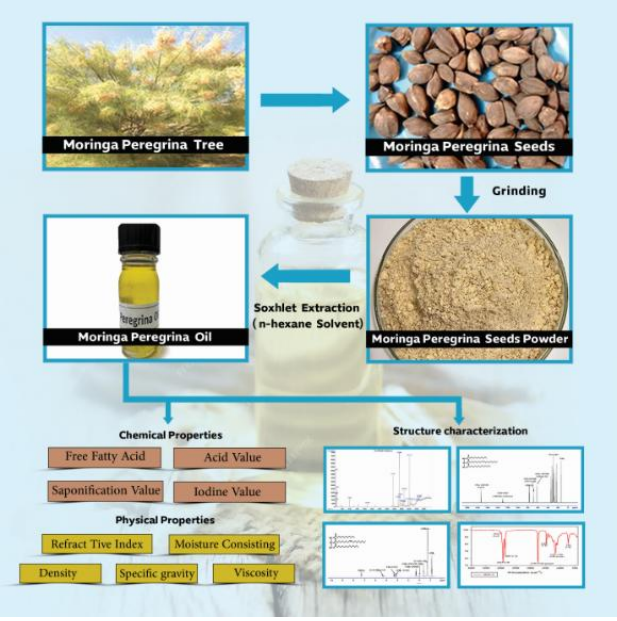
Abstract

The Hadhramaut *Moringa peregrina* seeds oil (HMPSO) composition was characterized by Fourier transform infrared, hydrogen and carbon nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectroscopy. The oil was extracted from the Hadhramaut *Moringa peregrina* seeds using the Soxhlet method with hexane as the solvent, which reached 34.0±0.2%. The physicochemical properties showed the free fatty acid consisted of 3.18±0.5%, an acid value of 7.0±0.5 mg NaOH/g, an iodine value of 69.4±0.2 g I_2/100g, saponification value of 185.1±0.1 mg KOH/g, refractive index of 1.47±0.01 at 25 °C, the moisture consisted of 0.48±0.03%, density of 0.92±0.03 g mL^{-1} at 25 °C, and a viscosity of 48±0.1 cP at 25 °C. The gas chromatography showed oleic acid (78.2±0.1%), palmitic acid (9.80±0.05%), stearic acid (3.6±0.1%), behenic acid (2.52±0.07%) and arachidic acid (1.83±0.05%). The major triacylglycerols of HMPSO, estimated by using high-performance liquid chromatography, were OOO (39.43%), POO (24.54%), SOO (8.18%), and AOO (6.74%). These findings provide important insights into the physicochemical properties of HMPSO; they could have significant implications for its utilization in various industries.



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Highlights

- To extract oil from Hadhramaut Moringa peregrina seeds using a Soxhlet method.
- To analyze the physicochemical properties of *Moringa peregrina* seed oil.
- To assess the oil's stability and nutritional value for potential applications.
- To compare Moringa oil with other edible oils in terms of quality and benefits.
- To explore the oil's potential as a biodiesel feedstock and its viability.

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1. Introduction

Moringa peregrina is a fast-growing and highly valuable tree with many beneficial applications in food, medicine, and industry. *Moringa peregrina* is a small genus comprising more than 13 species of trees (Elsorady *et al.*, 2023; Padayacheea and Baijnath, 2012). *Moringa peregrina* is spread in many areas in Yemen; however, in Hadhramaut, it is rarely scattered (Bataher, 2009). *Moringa peregrina* is a species of flowering plant in the family *Moringaceae* that is native to the Horn of Africa, Sudan, Egypt, the Arabian Peninsula, and as far north as Syria (Hegazy and Doust, 2016). It grows on rocky wadis and cliffs in drier areas (Shahin *et al.,* 2021). *Moringa peregrina* can attain heights between 3 to 10 meters within 10 months of planting. It has a grayish–green bark and long leaves; it is also distinguished by bisexual yellowish-white to pink showy, fragrant flowers. Its fruits are elongated and capsule-like, with a glabrous beak slightly narrowed amongst the seeds. The seeds have an orbicular to ovoid or trigonous shape (Al Khateeb *et al.,* 2013; Zaghloul *et al.,* 2010). In addition, *Moringa peregrina* seed oil is bright yellow with high nutritional value, comparable to olive oil, and it is a significant source of vitamins, minerals, proteins, and carbohydrates. This versatile oil can be used in many applications, including cosmetics, pharmacology, lubricants, biodiesel, and many industries (Al-Owaisi *et al.,* 2014).

The seeds of the *Moringa* plant contain a wide spectrum of fatty acids, esters, amides, and vitamins. The proportion of fatty acids was the highest, reaching 29.52%, followed by alcohol, which reached 3.57%. Then the hydrocarbon compounds 1.84% as well as the ketones and stearate, adenines, and amides, reached successive levels: 1.71, 0.13, 0.04, and 0.15%, respectively, plus vitamin E, which amounted to 0.27% (Senthilkumar *et al.,* 2020). So, the oil extracted from *Moringa peregrina* is recognized as Ben Oil; it is confirmed to comprise 70% oleic acid, a long-chain monounsaturated fatty acid with 18 carbons. Compared to polyunsaturated fatty acids, oleic acid exhibits better oxidative stability, which enables it to be used for extended storage and high-temperature frying in the food industry (Mariod and Salaheldeen, 2017). In a study in Saudi Arabia, the chemical composition, antioxidant activity, tannic acid content, mineral, fatty acid, and amino acid profiles of oil-extracted *M. peregrina* seed meal (OEMPSM) were determined where the neutral detergent fiber, acid detergent fiber, and hemicellulose were 40.2, 29.7, and 10.5%, respectively. About 15.41% of total FFAs were saturated and 84.57% unsaturated (Al-Harthi *et al.*, 2022). In a study conducted in Egypt, the extraction of Moringa peregrina oil was investigated using a Soxhlet apparatus with a 1:1 (v/v) mixture of dichloromethane and methanol. The result showed that the yield of *Moringa peregrina* oil was 42.23% (Hanaa and Gamal, 2013). In another study conducted in the United Arab Emirates, researchers employed response surface methodology to optimize the extraction of oil from *Moringa peregrina* seeds. The study aimed to identify the optimal conditions for maximizing oil yield by examining several variables. The optimal ranges determined for these variables were as follows: a liquid-to-solid ratio of 5–20 mL/g, an extraction time of 5–30 minutes, an ultrasound power of 348 W, and an extraction temperature of 30 °C. The findings indicated that response surface methodology is a robust approach for optimizing oil extraction processes from *M. peregrina* seeds, demonstrating that these optimal conditions can yield high-quality oil efficiently (Mohammadpour *et al.*, 2019). A study was conducted in Iran to analyze the physicochemical properties of *Moringa peregrina* seeds. The oil was extracted from the ground kernel using cold pressing after subjecting it to steam exposure and pressing with a screw pressing machine. The extracted oil was collected and stored at 4 °C. The physicochemical properties of the extracted oil showed that the acid value (0.06%), iodine value (91.7±1 gI_2/100 g oil), peroxide value (0.66 meq O_2/kg oil), saponification value (179.3±0.3 mg KOH/g oil) and unsaponifiable matter (0.32±0.01 g/kg), the oil refractive index, viscosity, and density were 1.4621, 52.05 mPa·s, and 0.9092 g/cm^3, respectively (Gharibzahedi *et al.,* 2013). In a study conducted by Mariod *et al.* (2022), the cold-pressing extraction of *Moringa peregrina* oil led to the following physicochemical characteristics: acid value 0.481, saponification value 206.4, free fatty acid 0.242, and peroxide value 0.394. Therefore, this study aims to extract oil from Hadhramaut *Moringa peregrina* seeds using the n-hexane solvent (Soxhlet) method and study the physicochemical characteristics of Hadhramaut *Moringa peregrina* oil.

2. Materials and methods

2.1. Sample collection

Hadhramaut *Moringa peregrina* seeds were selected from the Agricultural Research Station in Wadi Hadhramout-Al-Suwairi (Yemen). The sample was manually collected at room temperature. **Figure 1** illustrates the *M. peregrina* tree, its seeds, and the extracted oil.

(a) **(b)** **(c)**

Figure 1. Photos illustrate the *Moringa peregrina* tree **(a)**, seeds **(b)**, and seeds extracted oil **(c)**.
Source: Elaborated by the authors.

2.2. Chemicals and reagents

All chemicals, as well as the solvents, were used as received, and they included ethanol (99.8%), isopropanol (99.7%), hydrochloric acid (36.5%), potassium hydroxide (99%), potassium iodide (99.5%) and sodium sulfate (99%). The Wij's solutions, including cyclohexane (99.7%), sodium thiosulfate (99%), hexane (99%), and phenolphthalein, were purchased from Sigma-Aldrich.

2.3. Oil extraction

The HMPSO was extracted using the Soxhlet extraction method with hexane as a solvent, and a mild extraction temperature was chosen to avoid thermal degradation. The crushed seeds were placed in the drying oven at 100 °C for 30 min before extraction. 10 g of *Moringa peregrina* seeds were placed in an extraction thimble, which was placed in a Soxhlet extractor. The seeds were extracted with 200 mL of hexane for 4 h at 60 °C. The solvent was evaporated using a rotary evaporator, and the residual material was dried in an open-air, dark area. The oil yield was calculated, stored in hermetically closed dark bottles, and kept in a refrigerator for further physicochemical study (Azhari, 2020).

2.4. Physicochemical characteristics

2.4.1. Determination of free fatty acids and acid value (AV)

The free fatty acid content (FFA) was estimated by titrimetry as described in the AOCS Ca 5a-40 standard (Bahadi *et al.*, 2016a; Özcan *et al.*, 2019). First, 50 mL of isopropanol and 0.5 mL of the indicator phenolphthalein solution were put into a flask and neutralized with sodium hydroxide (NaOH, 0.1 mol/L) until a permanent pink color. The neutralized isopropanol was added to 5 g of *Moringa peregrina* oil in an Erlenmeyer flask. Until being dissolved at 40 °C, the mixture was first heated and then titrated with (NaOH, 0.1 mol/L) solution, forming a light pink color through 1 mL of phenolphthalein solution as the indicator. The FFAs percentage was calculated using **Eq. 1**.

$$\% \text{ FFA as oleic acid} = \frac{28.2 \times N \times V}{W} \quad (1)$$

where V is the volume of NaOH solution used in (mL); N is normality of NaOH solution in equivalent per liter (Eq/L); W is the weight of the sample in grams.

$$\text{Acid value} = \% \text{ FFA as oleic acid} \times 1.99 \quad (2)$$

where 1.99 is the conversion factor for oleic acid.

2.4.2. Determination of iodine value (IV)

The iodine value of HMPSO was determined using two methods, namely, the AOCS (1989) method Cd 1-25 and the method recommended by Al-Maqtari *et al.*, (2024) and Balaji (2022). In the first method, approximately 0.5 g of HMPSO was poured into a 500 mL flask. After that, 15 mL of cyclohexane was added to the flask. Next, 25 mL of Wijs solution was added; the flask was corked with a stopper. Later, the flask containing the mixture was gently shaken and left in the dark for 60 minutes. After incubating, 20 mL of 10% potassium iodide (KI) solution and 150 mL of distilled water were added to the mixture. Then the mixture was titrated with sodium thiosulfate (0.05 eq/L $Na_2S_2O_3$ solution or 0.025mol/L) until a yellow color was observed, indicating that the iodine almost disappeared. Subsequently, starch solution (1%) was added to the flask, and the titration continued until the blue color disappeared after shaking the flask. The blank was treated under the same conditions. The iodine value was determined using **Eq. 3**.

$$\text{I. V} = \frac{12.69 \times N(V_b - V_s)}{W} \quad (3)$$

where N is the normality of 0.05 N $Na_2S_2O_3$ solution (0.025 mol/L); V_b is the volume in mL of $Na_2S_2O_3$ solution used for the blank test; V_S is the volume in mL of $Na_2S_2O_3$ solution used to determine the sample; W is the weight in grams of the sample test portion.

2.4.3. Determination of saponification value

The HMPSO saponification value was calculated based on the methods suggested by Garba *et al.* (2023) and Derawi *et al.* (2014). Initially, 2 g of HMPSO were placed in a flask containing 25 mL of ethanolic potassium hydroxide solution (0.5 mol/L KOH solution) and some boiling stones. The boiling flask was connected to a condenser, and the mixture was gently boiled for one hour. Later, 1 mL of phenolphthalein solution (1%) was added to the mixture. Then, the mixture was titrated with 0.5 mol/L HCl solution until it became colorless, which indicated the endpoint. The saponification value was measured through **Eq. 4**.

$$\text{S. V} = \frac{56.1 \times N(V_b - V_s)}{W} \quad (4)$$

where V_b = mL of blank; Vs = mL of titrant; W = weight (g) of the sample; N = normality of the KOH (Eq/L).

2.4.4. Refractive index

The refractive index was determined as reported by Abdelwanis *et al.* (2023) and Salimon and Ahmed (2012). The HMPSO refractive index was determined in conjunction with the AOCS Official Methods (Cc7-25) using a refractometer (TAGO Co. Ltd. Series No.1211) linked to a Digital Thermometer (DTM-1T) at 25 °C.

2.4.5. Moisture content

The moisture content of HMPSO was identified using a Moisture Analyzer model and MX-50. About 5 g of the sample was weighed and put into a moisture dish. Then, it was dried in the moisture analyzer for 30 min at 101 °C, as Wiltshire et al. (2022) and Bahadi *et al.* (2016a) reported.

2.4.6. Viscosity

The viscosity of HMPSO was determined using a Brookfield viscometer model RV DV-I+ with a spindle size of 5. According to Tunit *et al.* (2022) and Bahadi *et al.* (2016a), after the sample was kept at 100 rpm/L/min at 25 °C, the viscosity was directly measured in centipoises (cP) using the viscometer.

2.4.7. Density

The density of HMPSO was measured using a balance. The weight of one milliliter of HMPSO placed on a balance was recorded at room temperature (Bahadi *et al.*, 2019; Lema, 2022).

2.4.8. Analysis of fatty acid composition in *Moringa peregrina* oil

The fatty acid composition of HMPSO was determined using a gas chromatography (Shimadzu GC-17A) equipped with a capillary column BPX 70 (30 m × 0.25 mm × 0.25 µm) and a flame ionization detector. The column temperature was programmed to 120 °C and was gradually raised as much as 3 °C every 1 min for 57 min. The injector and detector temperatures were set at 260 and 280 °C, respectively. The carrier gas was helium, with a flow rate of 0.3 mL min^{-1}. The parameters of GC were carried out according to Bahadi *et al.* (2016 b). Fatty acids methyl esters (FAME) were prepared with base-catalyzed transesterification using the method of Japir *et al.* (2018). One mL of hexane was added to 0.1 mL of HMPSO. One mL of sodium methoxide (1.55 g of NaOH in 50 mL methanol) solution was added to the oil mixture. The solution was stirred vigorously for 10 s using a vortex stirrer and then left for 10 min for phase separation of the clear FAME solution and the cloudy aqueous layer. The upper FAME layer was carefully decanted and dried with anhydrous sodium sulfate. The fatty acid content of HMPSO composition was determined using their respective FAME and then injected into gas chromatography for analysis. The peak identifications were through the retention time, i.e., by comparing them with genuine standards analyzed under the same conditions.

2.4.9. Triacylglycerols (TAGs) profile

Triacylglycerols of HMPSO were determined using high-performance liquid chromatography (HPLC Ultimate 3000 DIONEX) equipped with an evaporative light scattering (ELS) detector and an auto-injection. The separation of TAGs of HMPSO was carried out using a commercially packed C18 column 5 µm × 120 Å (4.6 × 250 mm) at room temperature. The parameters of HPLC were evaluated in line with Japir *et al*. (2017). The mobile phase consists of a mixture of acetone and acetonitrile (63.5%:36.5%, respectively), with a flow rate of 1 mL min^{-1}. The sample preparation entailed diluting 0.1 mL of the sample with 1.5 mL of an acetone-to-acetonitrile (63.5:36.5) mixture. The HPLC system was then auto-injected with this mixture, and the analysis was conducted over a total run time of 40 min. The TAG peaks were identified using the retention times of commercially obtainable TAG standards. The relative percentages of TAG peaks were evaluated from all peaks that emerged after 10 min.

2.4.10. Fourier transform infrared spectroscopy analysis of HMPSO

Fourier transform infrared spectroscopy (FTIR) was performed according to the methods described by Noor *et al*. (2022) and Mariod *et al*. (2022). The HMPSO FTIR spectrum was recorded using a Perkin Elmer Spectrum GX Spectrophotometer from 4000 to 500 cm^{-1}. The functional groups of HMPSO were measured, and a very thin film sample was covered on NaCl cells (25 mm ID × 4 mm thickness) and was used for analysis.

2.4.11. NMR analysis of HMPSO

Nuclear magnetic resonance (NMR) spectroscopy was employed to determine the molecular structure of triacylglycerols, using the methods adopted by Awang *et al*. (2007) and Aigbodion and Bakare (2005). ^{1}H and ^{13}C NMR analyses were conducted using a Joel FCP model at 400 MHz, with the solvent $CDCl_3$ from Sigma-Aldrich, which had a purity of 99.8%. After that, ^{13}C and ^{1}H NMR spectra of the products were recorded on a Bruker 400 NMR Spectrophotometer, where 10 mg of HMPSO was dissolved in 560 µL of $CDCl_3$. The sample was inserted into a glass tube before being introduced into the NMR tube.

3. Results and discussion

3.1. Oil extraction and physicochemical characteristics of *Moringa peregrina* seeds oil

Table 1 shows the physicochemical properties of the HMPSO studied. The yield of oil extracted was 34%, obtained from Hadhramaut Moringa peregrina seeds using the Soxhlet method with hexane as the solvent. The results of the extracted oil were like those of other studies (Lema *et al*., 2022). The color is an important factor that indicates product composition, purity, and degree of deterioration. Thus, it can be used to verify oil degradation, stability and suitability for a specific use (Rossi *et al*., 2001). The color test of HMPSO was light yellow and had a favorable fragrance. The percentage of FFAs in oil indicates their level of degeneration and quality. Also, the seeds' duration and storage conditions may affect the value of free fatty acids (Ibrahim, 2013). Accordingly, the result shows that the average value of FFA for HMPSO is 3.2±0.5%. Based on Ngozi *et al*. (2013) and Bale *et al*. (2015), FFA is one of the most significant quality parameters in the HMPSO as it specifies the level of oil deterioration. As shown in Table 1, the acid value of HMPSO was determined to be 6.3±0.5 mg NaOH/g. The acid value of the extracted oil (HMPSO) is higher, indicating lower quality, possibly due to an active lipase present in the seed oil, which is responsible for the hydrolysis of triacylglycerols to free fatty acids, diacylglycerol and monoacylglycerol (Albert *et al*., 2011). The iodine value of the HMPSO measured in this study was 69.4±0.2 g I_2/100g, as shown in Table 1. Chemical attributes like iodine value indicate the presence of unsaturated fatty acids, and a lower value marks the lower quantity of unsaturated fats and vice versa. Similarly, the saponification value of HMPSO was 185.1±0.1 mg KOH/g. The saponification value indicates the proportion of lower fatty acids in the oil. Therefore, the saponification value controlled the oil quality. The refractive index of HMPSO was determined to be 1.47±0.01, signifying a high concentration of carbon atoms in the fatty acid composition. The HMPSO moisture content was identified to be 0.48±0.03 at 101 °C, as illustrated in Table 1. The sample density highlighted a slight variation, with HMPSO falling within the range of 0.92±0.03 g/mL. This result is identical to the study performed in Saudi Arabia and Egypt. In Saudi Arabia and Egypt, the results for *Moringa peregrina* seed oil were, respectively, 0.30 and 0.01 equivalent to mg KOH/g for acid value, 69.6 and 67.9 g of I_2/100 g of oil for iodine value, 185 and 179 mg of KOH/g of oil for saponification value, 1.46 and 1.43 for refractive index, and 0.91 and 0.82 g/cm^3 for density (Hanaa and Gamal, 2013; Tsaknis, 1998). Thus, Ibrahim (2013) and Bahadi *et al*. (2019) emphasized that the data for viscosity indicated that HMPSO showed the highest resistance to flow with a viscosity of 48.0±0.1 cp.

Table 1. Physicochemical properties of HMPSO.

Components	Units	Values
Oil content	%	34.0±0.2
FFA (as oleic acid)	%	3.2±0.5
Acid value (AV)	mg NaOH/g	6.3±0.5
Iodine value (IV)	g I2/100 g	69.4±0.2
Saponification value (SV)	mg KOH/g	185.1±0.1
Refractive index	-	1.47±0.01
Moisture content	%	0.48±0.03
Density	g/mL	0.92±0.03
Viscosity	cp	48±0.1

Source: Elaborated by the authors.

3.2. Fatty acid composition of HMPSO

Moringa peregrina seeds have a novel fatty acid composition compared to other plant oils. In lipid science, fatty acid composition analysis is a widely used and common analytical technique. Nevertheless, the fatty acid composition is important for studying the characteristics of *M. peregrina* seed oil. Methylation is a method for preparing fatty acid methyl esters (FAMEs) from glycerolipids. Conventionally, FAMEs are prepared from base-catalyzed transesterification (Carvalho and Malcata, 2005). Base-catalyzed methanolysis proceeds much more rapidly under room temperature in 2 min. In this study, the fatty acid composition of *Moringa peregrina* seed oil is listed in Table 2 as a percentage of total FAME. Fatty acid methyl ester peaks were classified and quantified by comparing their peak area and retention times with standard methyl esters. There are three main types of fatty acid in *Moringa peregrina* seeds oil, which comprises saturated (Cn:0), monounsaturated with one double bond (Cn:1), and polyunsaturated with two double bonds (Cn:2). *Moringa peregrina* seeds oil consists of 82.25% unsaturated fatty acids and

17.75% saturated fatty acids as shown in Table 2. The results were like studies in Egypt, Saudi Arabia, and elsewhere (Robiansyah *et al.*, 2014; Tsaknis, 1998).

Table 2. Fatty acid composition of HMPSO using GC-FID analysis.

No	Fatty acids	Molecular formula	Percentage (%)
1	Palmitic Acid	$C_{16}H_{32}O_2$	9.80±0.05
2	Palmetoleic Acid	$C_{16}H_{30}O_2$	1.8±0.1
3	Stearic Acid	$C_{18}H_{36}O_2$	3.6±0.1
4	Oleic Acid	$C_{18}H_{34}O_2$	78.2±0.1
5	Linoleic Acid	$C_{18}H_{32}O_2$	0.43±0.02
6	Arachidic Acid	$C_{20}H_{32}O_2$	1.83±0.05
7	Eicosenoic Acid	$C_{20}H_{38}O_2$	1.4±0.1
8	Behenic Acid	$C_{22}H_{44}O_2$	2.52±0.07
9	Erucic Acid	$C_{22}H_{42}O_2$	0.42±0.09
	Total unsaturated fatty acids		82.25
	Total saturated fatty acids		17.75

Source: Elaborated by the authors.

3.3. Triacylglycerols composition of HMPSO

The triacylglycerol (TAG) profile of HMPSO was identified using high-performance liquid chromatography (HPLC). The result from the reversed-phase HPLC shows that HMPSO was composed of at least nineteen important TAGS. Table 3 shows the TAG composition of HMPSO. The main TAG of HMPSO contained a wide range of TAG species such as 1, 2,3-trioleoyl-glycerol, 1-stearoyl-2,3-dioleoyl-glycerol, 1-arachidoyl-2,3-dioleoyl-glycerol, 1,3-dioleoyl-2-linoleoyl-glycerol. The primary TAG composition of HMPSO was tri-unsaturated (45.31%), followed by di-unsaturated (42.73%), mono-unsaturated (6.87%) and tri-saturated (1.26%), as shown in Table 3. These results reflect the high unsaturation of HMPSO content and show good agreement with the fatty acids found in this study. It was expected that HMPSO would exhibit a notable content of FFAS, monoacylglycerols (MAG), and diacylglycerols (DAG), as shown in Table 3.

3.4. FTIR analysis of HMPSO

FTIR spectroscopy enables the deduction of molecular structures of substances by analyzing absorption bands associated with distinct functional groups, which are manifested as peak spectra (Lema *et al.*, 2022). In the case of HMPSO, the FTIR spectrum commonly exhibits prominent bands corresponding to triacylglycerol as shown in Fig. 2. Table 4 displays the distinctive bands of the principal functional groups found in HMPSO. Figure 3 represents an infrared spectrum of HMPSO spanning the range of 500 to 4000 cm^{-1}. The strong absorption band of HMPSO at 1750 cm^{-1} is probably due to the esterified carbonyl function, which is also accountable for the band at 1168 cm^{-1}. The band at 3012 cm^{-1} indicates =C-H stretch for Sp^2 (aliphatic), while the bands at 2925 cm^{-1} and 2863 cm^{-1} indicate C-H stretching vibration for Sp^3 (aliphatic) in HMPSO. The band at 1469 cm^{-1} is assigned to -CH deformation. The 1243-1168 cm^{-1} band denote -C-O-C stretching vibration (ester). The absorption band at 725 cm^{-1} indicates the presence of -(CH_2)-n, where n is greater than 3, representing an open-chain structure. Most of the remaining bands are absorption frequencies of the hydrocarbon chain.

Table 3. Triacylglycerol composition of HMPSO.

Triacylglycerol species	Composition (%)
Tri-unsaturated	
1,2,3-trioleoyl-glycerol (OOO)	39.43
1,3-dioleoyl-2-linoleoyl-glycerol (OLO)	4.94
1-oleoyl-2,3 dilinoleoyl- glycerol (OLL)	0.37
1,2,3-trilinoleoyl-glycerol (LLL)	0.34
1-eicosenoyl-2,3-dioleoyl-glycerol (EiOO)	0.23
Σ Tri-unsaturated	45.31
Di-unsaturated	
1-palmitoyl-2,3-dioleoyl-glycerol (POO)	24.54
1-stearoyl-2,3-dioleoyl-glycerol (SOO)	8.18
1-arachedoyl-2,3-dioleoyl-glycerol (AOO)	6.74
1-palmitoyl-2-oleoyl-3-linoleoyl-glycerol (POL)	1.28
1-palmitoyl-2,3 dilinoleoyl-glycerol (PLL)	0.58
1-palmitoyl-2-palmetoleoyl-3-linoleoyl-glycerol (PPL)	1.08
1-oleoyl-2-linoleoyl-3-arachedoyl-glycerol (OLA)	0.33
Σ Di-unsaturated	42.73
Mono-unsaturated	
1,3-dipalmitoyl-2-oleoyl-glycerol (POP)	4.12
1-palmitoyl-2-oleoyl-3-behenoyl-glycerol (POB)	1.89
1-palmitoyl-2-linoleoyl-3-stearoyl-glycerol (PLS)	0.42
1,2-dipalmitoyl-3-linoleoyl-glycerol (PPL)	0.23
1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS)	0.21
Σ Mono-unsaturated	6.87
Tri-saturated	
2,3-tripalmitoyl-glycerol (PPP)	1.26
Σ Tri-saturated	1.26
Total Triacylglycerol (TAG %)	96.19
Total Diacylglycerol (DAG %)	1.62
Mono Diacylglycerol (MAG %)	1.16
Free Fatty Acid (FFA%)	1.08

Source: Elaborated by the authors.

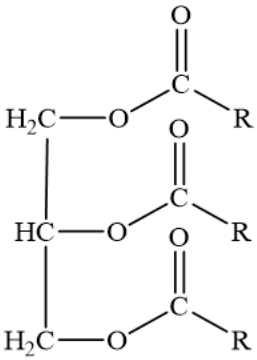

Figure 2. Structure of triacylglycerol.
Source: Elaborated by the authors.

Table 4. The functional groups of HMPSO in terms of the main wavenumber in the FTIR.

Functional Group	Wavenumber (cm^{-1})
C=C bending vibration (aliphatic)	3012
C-H stretching vibration (aliphatic)	2925, 2863
C=O stretching vibration (ester)	1750
C-H scissoring and bending for methylene	1469
=C–H (cis) unsaturated	1415
-CH3 sym deformation	1374
-C-O- stretching vibration (ester)	1243–1168
C-H group vibration (aliphatic)	725

Source: Elaborated by the authors.

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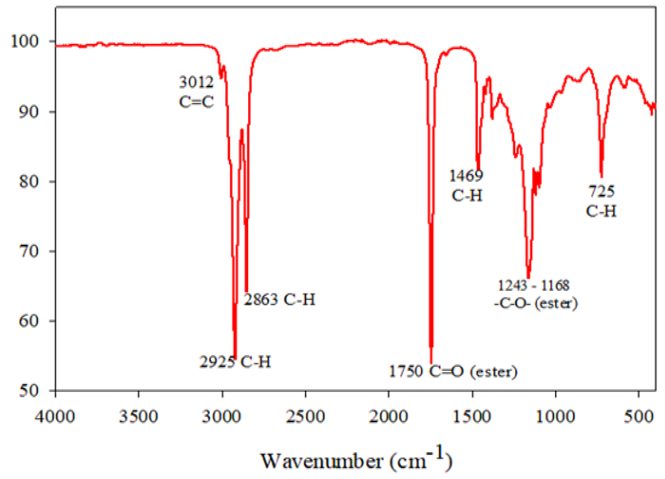

Figure 3. FTIR spectrum of HMPSO.
Source: Elaborated by the authors.

3.5. NMR analysis of HMPSO

Nuclear Magnetic Resonance (NMR) spectroscopy is useful in determining the chemical structure of HMPSO. In this study, the analysis of HMPSO was conducted through 1H NMR and 13C NMR. The findings were discussed in detail.

3.5.1. 1H NMR spectrum

The ^{1}H NMR results for HMPSO can be shown in **Table 5** and **Fig. 4**. The spectrum of ^{1}H NMR indicates the existence of proton peaks at a chemical shift of 5.32 ppm demonstrating protons attached to a double bond (vinyl protons) (-CH=CH), and the peaks at chemical shifts of 1.99-2.03 ppm signifying the presence of allylic protons attached to a carbon adjacent to a double bond (-CH_2-C=C-), where both represent the top of the double bond in the HMPSO. The chemical shift displacement of these two double bond peaks was close to the range of chemical shift displacement reported by Pavia *et al*. (2015); the chemical shift range of group -CH=CH is 4.5-6.5 ppm, and the chemical shift range of group -CH_2-C=C- is 1.6-2.6 ppm. For the molecular structure of an ester, two distinct types of protons exist. Those on the carbon atom attached to the oxygen atom in the alcohol part of the ester (-CH_2-O) have a chemical shift range of 3.5-4.8 ppm. The protons from the alpha carbon in the acid part of the ester (-CH_2-C=O) have a chemical shift of 2.1-2.5 ppm. **Figure 4** illustrates alcohol proton (glyceryl-CH_2-O-CO- (α–esterified glycerol)) identified at a chemical shift of 4.27-4.31 ppm and acid proton (-CH_2C=O) detected at chemical shift 2.28-2.32 ppm for HMPSO. The β-esterified glycerol (-CH–OCOR) is indicated by a chemical shift at 4.27-4.31 ppm for HMPSO. The chemical shift at 0.88-0.87 ppm corresponded to -CH_3 (terminal methyl in the alkyl chain) next to the terminal methyl -CH_2 at 1.25-1.29 ppm. The chemical shift at 7.28 ppm represented the solvent $CDCl_3$.

3.5.2. 13C NMR spectrum

The primary signal assignments in the ^{13}C NMR spectrum of the HMPSO are shown in **Table 6** and shown in **Fig. 5**. This spectrum was a feature of triacylglycerols and contains resonances arising from the methyl, methylene, glycerol backbone, and olefinic carbons of polyunsaturated fatty acids and monounsaturated fatty acids as well as carbonyl carbons. The ^{13}C NMR spectrum was divided into four groups of signals according to their chemical shifts. These four groups contain carbonyl carbons that resonate between 172 and 174 ppm, unsaturated carbons that resonate from 124 to 134 ppm, glycerol backbone carbons that resonate from 60 to 72 ppm, and aliphatic carbons that resonate between 10 and 35 ppm. The ^{13}C NMR of the triacylglycerols' acyl chains was assigned according to the extensive chemical shift data tabulated by Gunstone (2009).

The peak at 14.07 ppm corresponds to the carbon atoms of the methyl groups attached to the end of the acyl chains. The peaks ranged between 22.67 and 34.18 ppm, symbolizing the methylene carbon atoms in fatty acid moieties of HMPSO. Glycerol carbons in triacylglycerols ranged from 60 to 70 ppm. The glycerol carbons (β) were found at 68.87 ppm, and glycerol carbons (α) and (ά) were at 62.06 ppm. The olefinic carbons of unsaturated fatty acids of HMPSO triacylglycerols resonated between 127.86 and 130.14 ppm. The signals at 172.79 ppm and 173.21 ppm were observed in the carbon atom of the carbonyl group.

Table 5. The main signals present in the ^{1}H NMR spectrum of HMPSO.

Assignment	HMPSO δ(ppm)
–CH_3 (terminal methyl)	0.88–0.87
–(CH_2)n–(saturated alkyl chain)	1.25–1.29
–CH_2–CH=CH–CH_2-	1.99–2.03
–CH=CH–	5.32
-CH_2-COOR, acyl methylene	2.28–2.32
-CH_2-O-CO-R (α–esterified glycerol)	4.11–4.16
CH–OCOR (β-esterified glycerol)	4.27–4.31
-C=C-CH_2-C=C-	5.24–5.36
$CDCl_3$	7.28

Source: Elaborated by the authors.

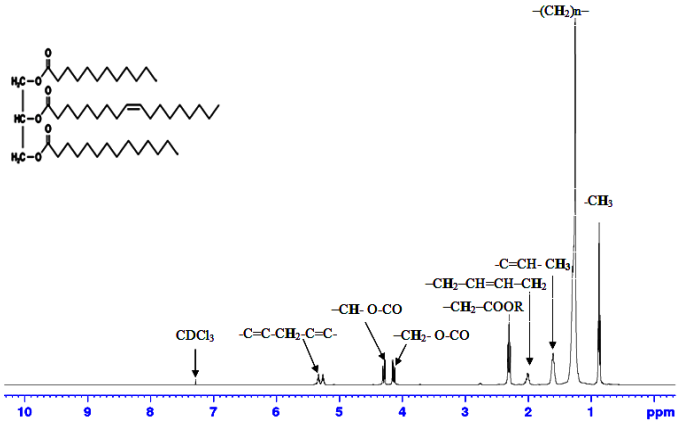

Figure 4. ^{1}H NMR spectrum of HMPSO.
Source: Elaborated by the authors.

Table 6. The main signals present in the ^{13}C NMR spectrum of HMPSO.

Assignment	HMPSO δ(ppm)
–CH_3	14.07
–(CH_2) n-	22.67–34.18
–CH_2-O-CO-R glycerol carbons (α) & (ά)	62.06
CH–OCOR glycerol carbons (β)	68.87
–HC=CH- (Olefinic Carbone)	127.86–130.14
CH_2–OCOR (carboxylic ester)	172.79–173.21
–CH_3	14.07
–(CH_2) n-	22.67–34.18
–CH_2-O-CO-R glycerol carbons (α) & (ά)	62.06

Source: Elaborated by the authors.

https://doi.org/10.26850/1678-4618.eq.v50.2025.e1527

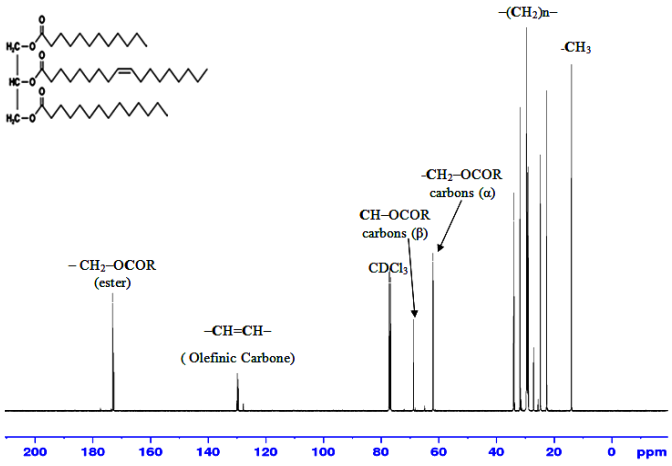

Figure 5. ^{13}C NMR spectrum of HMPSO.
Source: Elaborated by the authors.

4. Conclusions

This study demonstrated that the physicochemical characteristics of HMPSO were comparable to those of *Moringa peregrina* seeds oil from other countries. The GC-FID analysis revealed that the dominant fatty acids present in HMPSO were oleic acid, palmitic acid, stearic acid, behenic acid, and arachidic acid. The triacylglycerol composition of HMPSO contained a high concentration of unsaturated TAG and a low concentration of saturated TAG. The ^{1}H NMR, ^{13}C NMR and FTIR analyses performed on HMPSO confirmed the molecular structure of its components. Understanding the pattern of interactions in TAG can also have implications in numerous industries and medical treatments.

Authors' contribution

Conceptualization: Hani Mahfoodh Barfed; Murad Awadh Bahadi; **Data curation:** Maher Ail Al-Maqtari; Hussen Manaa Al-Maydama; **Formal Analysis:** Hani Mahfoodh Barfed; Murad Awadh Bahadi; **Funding acquisition:** Not applicable; **Investigation:** Maher Ail Al-Maqtari; Hussen Manaa Al-Maydama; **Methodology:** Murad Awadh Bahadi; **Project administration:** Hani Mahfoodh Barfed; Murad Awadh Bahadi; **Resources:** Not applicable; **Software:** Murad Awadh Bahadi; **Supervision:** Maher Ail Al-Maqtari; Hussen Manaa Al-Maydama; Murad Awadh Bahadi; **Validation:** Maher Ail Al-Maqtari; Hussen Manaa Al-Maydama; **Visualization:** Hani Mahfoodh Barfed; Murad Awadh Bahadi; Maher Ail Al-Maqtari; **Writing – original draft:** Hani Mahfoodh Barfed; Murad Awadh Bahadi; **Writing – review & editing:** Maher Ail Al-Maqtari; Hussen Manaa Al-Maydama.

Data availability statement

Data sharing is not applicable.

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Conflict of interest

The authors declare that there is no conflict of interest.

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