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EFFECT OF MORINGA (*MORINGA OLEIFERA* LAM.) SEED OIL EXTRACTION METHODS ON ITS PHYSICOCHEMICAL PROPERTIES

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SUMMARY

Moringa (*Moringa oleifera* Lam.) trees' successful growing in Iraq revealed vital seeds distinguished by their oil quantity and quality. A seed proximate analysis indicated its moisture (4.08%), ash (3.25%), protein (32.91%), fats (38.11%), fibers (7.55%), and carbohydrates (14.1%), respectively. Moringa seeds oil extraction employed different methods (mechanical pressure, Soxhlet extraction, cold solvent extraction, and soaking and mixing with hexane solvent for 24 hours). The results showed significant ($P \leq 0.05$) variations in the extracted oil percentage and all tests for physicochemical properties. The cold solvent extraction yielded the highest oil percentage (41.899%), followed by the Soxhlet extraction with petroleum ether (39.9%) and with hexane (38.04%), and the mechanical pressure extraction (12.97%). The results indicated substantial ($P \leq 0.05$) differences in the percentage of fatty acids (Palmitic, Arachidic, and Behenic) in the extracted oil for each extraction method. Significant ($P \leq 0.05$) differences were evident in their qualitative properties, such as peroxide value, free fatty acid content, and melting point. Overall, the soaking and mixing extraction method with a solvent displayed the highest quantity yield of oil with superior quality. Meanwhile, the mechanical pressing method produced high-quality oil and the lowest yield.

Keywords: Moringa (*M. oleifera*), seed oil, mechanical pressing extraction, cold extraction, Soxhlet extraction, oil content, physicochemical properties of oil

Key findings: Moringa oleifera seeds are one of the most important sources of oils, which are considered healthy oils due to the high percentage of essential fatty acids. It is important to find the best methods to extract them with high quality.

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INTRODUCTION

The moringa (*M. oleifera* L.) tree, also known as the 'Tree of Life,' is a fast-growing, drought-resistant tree of the family Moringaceae native to the Indian subcontinent and useful extensively in South and Southeast Asia. With its adaptability, it is a widely distributed tree in tropical and subtropical regions, including the Middle East (Lalas and Tsaknis, 2002; Saini *et al*., 2016; Ibrahim and Ameen, 2017).

Moringa seeds contain a high oil content (up to 40%), with enhanced concentrations of oleic acid, sterols, and tocopherols, contributing to their stability. Behenic and palmitic fatty acids also form the majority of saturated fatty acids (Tsaknis *et al*., 1999; Leone *et al*., 2016; Al-Taweel and Al-Anbari, 2019). *Moringa oleifera* seed oil can benefit food and non-food purposes, including human consumption, cosmetic products, and lubricants. Additionally, after oil extraction, the seed residues can serve as sewage treatment and fertilizer (Leone *et al*., 2016; Al-Karboli and Al-Janabi, 2024).

In moringa seeds, the oil content varies depending on the variety, climate, and extraction methods (Lalas and Tsaknis, 2002; Anwar and Bhanger, 2003). Organic solvent extraction is the most widely used chemical technique for extracting vegetable oil. Given its efficiency and high extraction rate, hexane was the most common solvent for extracting *Moringa oleifera* seed oil (Saini *et al*., 2016). However, the primary disadvantage of the solvent extraction method is the potential presence of residual solvents in the extracted oil.

The growing demand for oils as a food source, with the requirements of various industries, such as food, pharmaceuticals, and cosmetics, requires an imperative to explore new oil sources and develop more efficient extraction methods that will increase oil yields with superior quality standard (Crescente *et al*., 2023). One estimate for moringa oil yield from the seeds was around 250 L/ha (Radovich, 2009). The oil can become a base for cosmetics for hair and the skin. Moringa seeds can also favor biofuel production (Ayerza, 2012).

With the success of *Moringa oleifera* cultivation in Iraq and the abundant production of seeds with high oil content (Al-Taweel *et al.*, 2022; Al-Shebli and Al-Anbari, 2023), the presented study began to extract and characterize the seed oil of moringa (*Moringa oleifera* Lam.) cultivated in the fields at the College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq. The relevant study also aimed to investigate the seed components and the influences of different extraction methods on the percentage of extracted oil and its qualitative characteristics for fatty acid composition, saponification number, iodine number, refractive index, specific gravity, and melting point. The study also examined the effects of extraction methods on oil stability by studying free fatty acids, peroxide value, and thiobarbituric acid values.

MATERIALS AND METHODS

Moringa seeds

The collection of *Moringa oleifera* pods from its cultivated trees transpired in the fields at the College of Agricultural Engineering Sciences at the University of Baghdad, Baghdad, Iraq, during the summer of 2021–2022 at the physiological maturity. The seed separation from the pods and manual dehulling ensued, followed by grinding using an electric household mill (Moulinex, France).

Estimation of chemical composition

The percentage estimates of the total components of *Moringa oleifera* seeds were as follows: The moisture content determined using the Gallenkamp Vacuum 105 C oven drying method (AOAC- 2007 900.02); employing the Kjeldahl method estimated protein content in seed samples (Van-Dijk and Houba, 2000); and the fats estimation used a Soxhlet fat extraction apparatus (AOAC- 2007 927.09).

The estimated percentage of ash in the seed samples depended on the AOAC-2007 927.39 method. The percentage of total fibers in the seed samples approximation used the Muffle furnace 600 C (AOAC-2007 993.21). The total carbohydrates mathematical estimation resulted from calculating the sum of all components subtracted from 100 (Pearson, 1976).

Moringa seed oil extraction

The moringa seed oil extraction engaged four different methods as follows:

Cold mechanical pressing method

The oil extraction from previously dried *Moringa oleifera* seeds (using the Gallenkamp Vacuum 105 C oven drying method) employed the cold mechanical pressing method Spiral press (system with an oil press) (Pereira *et al*., 2016**)**. An oil pressing machine (Tina –China) also helped the extraction process **(**Al-Anbari and Ali, 2022).

Soxhlet extraction method (petroleum or hexane)

The oil extracted from ground *Moringa oleifera* seeds (using an electrical mill manufactured by Panasonic Malaysia) applied a Soxhlet device manufactured by Electrothermal-England, with Petroleum ether (boiling point: 60 °C–70 °C) and hexane (boiling point: 60 °C–70 °C) as the extraction solvents. Then, solvent evaporation utilized the Rotary evaporator (Gallenkamp-England) at 40 °C, with the extracted oil collected and stored in dark glass and containers at freezing temperatures until further use, following previous studies (AOAC, 1995- 921.08; Obikili, 2010).

Cold solvent extraction

Extracting the oil from the ground *Moringa oleifera* seeds used the cold solvent extraction method. Then, mixing and soaking the seed powder in hexane solvent took 24 h, with continuous stirring. The mixture reached filtering, with the solvent evaporated using a rotary evaporator (Gallenkamp - England) at low pressure and a temperature of 40 °C (Elsorady**,** 2023).

Total fatty acids determination

Total fatty acids of *Moringa oleifera* seed oil acquisition used a gas chromatography-mass spectrometry (GC-MS) (AOAC, 2007) instrument provided by Agilent Technologies (7820-A). The column specifications for the device were as follows. Length: 30 meters, Inner diameter: 250 microns, Thickness: 0.25 micron, Pressure: 11,933 psi, Temperature: 250 °C, and helium gas as an inert carrier gas. Comparing the spectra of unidentified components occurred with those of the identified components stored in the standards library of the National Institute (Uduman *et al*., 2017; Al-Hayali *et al*., 2023).

Physicochemical properties of the seed oil

The physicochemical properties determination of the moringa seed oils included the refractive index (RI) (defined as the ratio of the speed of light in air to the speed of light in oil, where samples typical measurement used a refractometer at 20 °C or 25 °C for oil and 40 °C for fat, as fat melts at this temperature) (AOAC, 2007-921.08) and saponification number (Saponification is the treatment that results in the breakdown or decomposition of fats into glycerol and fatty acids by treating the fat with an alkali) (AOAC, 2007-920.160).

The Iodine number is the iodine grams needed to saturate the pair bonds in 100 grams of sample. This estimation helped measure the degree of unsaturation and the percentage of unsaturated fatty acids in oil or fat. It also tracks the various stages of the hydrogenation process and detects adulteration of oils and fats (AOAC, 2007-993.20). Measuring free fatty acids (FFA) comprised taking a sample of liquid fat, 95% of the neutralizer, and the betovitalin indicator as a reagent mixed, then titrating with the NooH, and calculating the percentage of free fatty acids (AOAC, 2007-940.28) by the following equation:

$$\%\ FFA = \frac{(V.M.28.2)}{m}$$

Where:
F.F.A (%): represents the percentage of free fatty acid, V: is volume of solvent used, M: corresponds to the Molarity of the NaOH solution, and m: is the mass of crude oil sample (Vicentini-Polette *et al.,* 2021).

Peroxide value (PV) is the millimeters of peroxide in 1 kg of oil or fat. It also estimates the degree of oxidative rancidity of oils and fats, as the formation of hydro peroxides in the early stages gives an idea of the degree of suitability of the oils for human consumption (AOAC, 2007-965.33).

Thiobarbituric acid value (TBA) is the test that serves as an indication of the occurrence of the final oxidation of oils and fats and depends on measuring aldehyde compounds resulting from the breakdown of hydro peroxides, producing an odor that is unacceptable to the consumer. It measures the byproduct of malnoaldehyde oxidation of lipids. It involves reacting malnoaldehyde with TBA to give a colored compound measured using a spectrophotometer (AOAC, 2007-969.33).

The melting point is the degree to which the examined sample becomes completely clear and transparent. Water prepared with a mixer and a heating rate of half a minute determines the melting point by the closed capillary tube method (AOAC, 2007-920.156).

The total acidity is the degree of acidity expressed in the number of milligrams of potassium hydroxide needed to neutralize acidity in one gram of oil. An assumption is that it does not exceed 17 mg KOH per gram of oil in olive oil and 0.6 mg KOH per gram of oil for other purified and refined edible oils to make them usable by humans (AOAC, 2007-935.57). Specific gravity estimation of oils is at 20 °C, defined as the weight of a specific volume of oil divided by the weight of the same volume of distilled water at 20 °C (AOAC, 2007-965.33).

Statistical analysis

All the recorded data analyses used the SAS statistical analysis system (SAS, 2018), studying the impact of the factors on the studied properties according to a completely randomized design (CRD). Significant differences among the means' further comparison and separation employed the least significant difference ($LSD_{0.05}$) test.

RESULTS AND DISCUSSION

The laboratory analysis revealed that *Moringa oleifera* seeds have the highest total fat and protein percent, at 38.11% and 32.91%, respectively (Table 1). In addition, the moringa seeds also contain a considerable percentage of fiber (7.55%). The moringa seed oil is an important source that can treat chronic diseases like diabetes and high cholesterol, as the seed oil also contains a high ash content (3.25%). It indicates that moringa oil is rich, with the necessary mineral elements and the percentage of moisture and carbohydrates, at 4.08% and 14.1%, respectively.

The observed protein contents (31.4% and 31.7%), fat (36.7% and 38.4%), fiber (7.3% and 6.75%), and carbohydrate content (18.4%) of moringa seed samples were consistent with previous studies (Leone *et al*., 2016; Mahmud *et al*., 2017), but also different from those reported in other studies (Barakat and Ghazal, 2016). The results also showed moisture content (7.5%), ash (4.73%), protein (35.54%), fat (29.61%), fiber (10.92%), and carbohydrate content (20%). However, these values were inconsistent with previous findings by Ijarotimi *et al*. (2013), who also reported the moisture, protein, fat, and carbohydrate contents (10.6%, 18.86%, 13.35%, and 53.36%, respectively). The discrepancy may be due to the high moisture content in the moringa seed samples, environmental factors, harvesting time, and degree of ripeness (Adejumo and Abayomi, 2012).

The moringa seed oil percentage extracted with various extraction methods showed significant differences (Table 2). The oil extraction through the cold pressing method (mechanical) was the lowest (12.97%) compared with other seed oil extraction methods. It may refer to using cold pressing, which increases the oil viscosity, preventing the extraction of the oil found in the seeds. The seed oil percentage using hexane solvent

Table 1. Chemical composition of *Moringa oleifera* seeds.

Chemical Content	Percentage
Moisture	4.08±3
Total Ash	3.25±0.5
Protein	32.91±1
Total Fat	38.11±3
Fiber	7.55±2
Carbohydrates	14.1±2

Table 2. Effect of extraction methods on the percentage of extracted *Moringa oleifera* seed oil.

Treatments	Percentage
Soxhlet extraction (hexane)	38.04a
Soxhlet extraction (petroleum ether)	39.09a
Solvent extraction	41.90a
Mechanical pressing	12.97 b
$LSD_{0.05}$	7.284*

Means with the different letters in same column differed significantly, * ($P \leq 0.05$)

extraction reached 38.04%, the Soxhlet extraction (with petroleum ether) yielded 39.09%, while solvent extraction (hexane soaking for 24 h) caused a seed oil percentage of 41.899%. Notably, using solvents to extract oil is the best concerning the amount of oil extracted because fats are hydrophobic or nonpolar. Therefore, the fats dissolve in nonpolar solvents, such as petroleum ether and hexane, which are among the lowest polar solvents (Wang *et al*., 2023).

The results further indicated a substantial increase in the oil percentage extracted by the cold solvent method compared with other extraction methods. However, a significant ($P \leq 0.05$) decrease was evident in the moringa seed oil percentage extracted by the mechanical pressing method. The presented results were highly analogous to past findings (Aly *et al.*, 2016; Leone *et al.,* 2016; Oluwafunke, 2019; Idris *et al.*, 2020; Elsorady, 2023; Garba *et al.,* 2023). The increased oil production using cold solvent extraction may be due to the seed powder's extended soaking time (24 h) with continuous stirring, facilitating cell disruption, and the massive oil quantity extraction. The increased production of oil extracted with solvents (Soxhlet) can refer to the polarity of the solvents (non-polar) (Juhaimi *et al.,* 2019). Likewise, it can be the extraction temperature that leads to cell wall disruption, along with the organic solvents used and the continuous recycling of the hot solvent throughout the process, enhancing its efficiency by penetrating the seed cell walls and, eventually, dissolving all the available oil in the seeds (Li *et al.*, 2016; Daroch *et al.*, 2013). However, the reduced percentage of seed oil extracted by the cold mechanical pressing method might be due to a significant proportion of the oil attached to proteins and the high oil viscosity hindering its release from the cells, resulting in a lower oil yield (Cai *et al*., 2021).

In *Moringa oleifera* seed oil, the analysis of the total fatty acids revealed that oleic, palmitic, and stearic acids were the major components (Table 3). In the *Moringa oleifera* seed oil, oleic acid percentages were 74.72%, 73.41%, 72.46%, and 72.38%. It was also apparent that the Soxhlet extraction method (hexane) increased the oleic acid percentage, which may be due to the affinity of these fatty acids for the solvent. The results further revealed that the rest of the fatty acid components of the seed oil differ in proportions. The reason could be the same with the palmitic acid percentage ranges at 7.20%, 6.88%, 6.19%, and 6.17%. It was also evident that the Soxhlet extraction method (hexane) contained the highest palmitic and stearic acid percentages (6.07%, 6.29%, 6.3%, and 6.27%). These results were consistent with past findings of Pereira *et al.* (2016), Mohamed *et al.* (2021), and Elsorady (2023).

Table 3. Effect of extraction methods on fatty acid percentages of *Moringa oleifera* seed oil.

Treatments	Palmitic acid	Palmitolic	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Arachidic	Gadoleic	Behenic acid	lignoceric
Soxhlet extraction	7.20 a	1.40	6.07	74.72	0.52	0.18	3.10 b	2.02	4.79 b	0.97
Soxhlet extraction	6.88 ab	1.26	6.29	73.41	0.50	0.17	3.46 ab	2.13	4.64 b	0.87
Solvent extraction	6.19 b	1.10	6.3	72.46	0.45	0.16	3.83 a	2.25	5.90 a	1.00
Mechanical pressing	6.17 b	1.13	6.27	72.38	0.47	0.17	3.85 a	2.23	5.91 a	1.01
$LSD_{0.05}$ ($P \le 0.05$).	0.936*	0.459^{NS}	0.46^{NS}	4.702^{NS}	0.182^{NS}	0.077^{NS}	0.618*	0.371^{NS}	1.02*	0.16^{NS}

Means with the different letters in same column differed significantly, *($P \le 0.05$)

Significant ($P \le 0.05$) differences were remarkable among the extraction methods, which may be due to the distribution of fatty acid concentrations in *Moringa oleifera* oil, and the results align with previous findings (Aly *et al.,* 2016; Leone *et al.,* 2016). Various biological properties of distinct fatty acids found in Moringa seed oil were also prevalent. For instance, the palmitic and oleic acids displayed antioxidant activities (Asghar and Choudhary, 2011; Hema *et al.,* 2011). Additionally, reports stated palmitoleic acid had hepatoprotective effects, while oleic acid exhibited relevant anticancer potential (Astudillo *et al.,* 2018). The biological results obtained by researchers confirmed that *Moringa oleifera* seed oil helps to protect the liver from fibrosis (Hamza, 2010).

The chemical properties of *Moringa oleifera* seed oil also vary and depend on the different oil extraction methods (Table 4). Significant ($P \le 0.05$) variations manifested among the extraction methods for peroxide value and free fatty acids. The Soxhlet extraction showed higher values than the mechanical extraction, and this can be because of the occurrence of breakdown and oxidation of fatty acids (Pereira *et al*., 2016). It may be due to the heat used in the extraction by the Soxhlet method and the long extraction period compared with the mechanical extraction, leading to the extraction of effective oil-soluble compounds, which may become enzyme inhibitors, and reducing the peroxide and pH numbers. However, no significant differences were visible among the extraction methods for saponification values, acidity, iodine values, and thiobarbituric acid, and all these values were within the acceptable range for edible vegetable oil. The variations that occurred due to the extraction methods did not influence the extent of changing the oil properties for the degree of unsaturation or developing sizable oxidation, increasing the value of the thiobarbituric acid (Kim *et al*., 2013).

The physical properties of *Moringa oleifera* seed oil varied with different oil extraction methods (Table 5). Among these extraction methods, remarkable ($P \le 0.05$) differences were prominent for the melting point. The highest values appeared with the mechanical extraction, and the lowest values emerged in the Soxhlet extraction (hexane), which could be due to the presence of natural resins in the extracted oil caused by the mechanical pressing process, and these observations were consistent with past findings (Lalas and Tsaknis, 2002; Latif *et al*., 2011; Olaleye and Kukwa, 2018). However, nonsignificant differences were evident in the oil density and refractive index among the seed oil extraction methods. It may be due to some compounds extracted with the oil, as the cold press extraction method leads to extracting neutral gums due to increased viscosity and a change in melting point, color, and flavor, in addition to various active compounds. As for extraction methods with nonpolar solvents, limitations were on extracting compounds not dissolved in water, such as oil and nonpolar compounds (Satriana *et al*., 2019).

Table 4. Effect of extraction methods on the chemical properties of *Moringa oleifera* seed oil.

Treatments	Sapo (g/mg KOH)	P.V Meq.100gm	Acidity No.	FFA%	TBA (mg MDA/gm)	Iodine No.
Soxhlet extraction	189.2	0.40 a	0.9521	0.20 ab	0.06	119.2
Soxhlet extraction	188.5	0.38 ab	0.9590	0.22 a	0.07	118.8
Solvent extraction	191.4	0.27 bc	0.9550	0.18 ab	0.056	120.4
Mech. pressing	192.6	0.24 c	0.9541	0.16 b	0.051	120.8
$LSD_{0.05}$ ($P \leq 0.05$).	8.73 N.S.	0.127*	0.0336 N.S.	0.059*	0.025 N.S.	4.593 N.S.

Means with the different letters in same column differed significantly. * ($P \leq 0.05$)

Table 5. Effect of extraction methods on the physical properties of *Moringa oleifera* seed oils.

Treatments	RI (25 °C)	Density (25 °C)	Melting point
Soxhlet extraction (hexane)	1.479	0.9220	11.2 c
Soxhlet extraction (petroleum ether)	1.473	0.9234	11.6 bc
Solvent extraction	1.488	0.9201	12.6 ab
Mechanical pressing	1.499	0.9238	12.9 a
$LSD_{0.05}$ ($P \leq 0.05$).	0.166 N.S.	0.0281 N.S.	1.270 *

Means with the different letters in same column differed significantly, * ($P \leq 0.05$)

CONCLUSIONS

Moringa oleifera seeds are a source of nutritious, rich oils and proteins. The residual meal produced during oil extraction can benefit as a protein source for animal feed and other industrial applications. The moringa seed oil has a high oleic fatty acid content, benefitting heart health. It even has more oleic acid than olive oil. The study also demonstrates that the most efficient and high-quality method for extracting *Moringa oleifera* seed oil is cold solvent extraction (mixing and soaking with hexane).

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