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Physicochemical characteristics of *Moringa peregrina* seeds and oil obtained by solvent and cold-press extraction

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SUMMARY: This study evaluated the quality of oil extracted from *Moringa peregrina* (MP) seeds. The seeds oil was extracted by Soxhlet and cold-press methods and named MPSO and MPCO, respectively. The physicochemical properties of the extracted oil were determined and the results showed that the Soxhlet technique had higher extraction efficacy than the cold-press technique (41.24 % vs. 22.5 %). In general, MPSO had higher acidity, oxidative stability, smoke point, and refractive index, with lower peroxide, iodine index, Cox index, and density than MPCO ($p < 0.05$). The antioxidant activity of MPSO was higher than MPCO (19.36 % vs. 12.88 %) ($p < 0.05$). In general, *oleic acid* and *palmitic acid* were found to be prominent in both samples with a 78 % omega-9 fatty acid content. The most abundant sterol compounds in the MP oil were stigmasterol, Δ^5-avenasterol, and campesterol. In addition, γ-, α- and, δ-tocopherol were the most abundant tocopherols, respectively.

KEYWORDS*: Antioxidant; Fatty acid; Moringa peregrina; Oil extraction; Seed oil.*

RESUMEN: ***Características fisicoquímicas del cultivo de Moringa peregrina y su aceite obtenido por extracción por solventes y presión en frío***. Este estudio evaluó la calidad del aceite extraído de las semillas de *Moringa peregrina* (MP). El aceite de las semillas se extrajo mediante los métodos Soxhlet y prensado en frío y se denominaron MPSO y MPCO respectivamente. Se determinaron las propiedades fisicoquímicas del aceite extraído. Los resultados mostraron que la técnica Soxhlet tuvo una mayor eficacia de extracción que la técnica de prensado en frío (41,24 % frente a 22,5 %). En general, el MPSO tuvo mayor acidez, estabilidad oxidativa, punto de humo e índice de refracción y menor peróxido, índice de yodo, índice de Cox y densidad que el MPCO ($p < 0,05$). La actividad antioxidante del MPSO fue mayor que la del MPCO (19,36 % frente a 12,88 %) ($p < 0,05$). Se encontró que los ácidos oleico y palmítico fueron los mayoritarios en ambas muestras, con un contenido de ácidos grasos omega-9 del 78 %. Los esteroles más abundantes en el aceite de MP fueron estigmasterol, Δ^5-avenasterol y campesterol. Además, el γ-, α- y δ-tocoferol fueron los tocoferoles más abundantes, respectivamente.

PALABRAS CLAVE*: Aceite de semillas; Ácido graso; Antioxidante; Extracción de aceite; Moringa peregrina.*

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1. INTRODUCTION

Moringa peregrina (MP) is a tropical tree belonging to the family Moringaceae, which includes 14 species. (Ghebremichael, 2004). A species of this tree known as MP (locally called *Khar-e-Arous*) is native to Iran and grows in the desert areas of the Sistan and Baluchestan provinces (Carabias-Martínez *et al*., 2005).

The study of the physicochemical characteristics of seeds is effective in the development of seed processing, and the moisture content in the seeds can affect the efficiency of devices such as dryers and silos. The difference in density can be applied to evaluate the quality of fruits, vegetables, cereals, and various crops. When mixing, transporting, storing, and packing materials such as beans, oilseeds, and flour, it is important to know the density characteristics of the fluid mass (Mohsenin, 2020). The density of agricultural products is usually measured in terms of both bulk and absolute density. They are important in the design of dryers, aeration, storage systems, impurities, and nutrient contents (Akbarpour *et al*., 2009).

The Romans, Greeks, and Egyptians extracted edible oil from *Moringa* seeds with an extraction efficiency of 34.80%. *Moringa* oil is rich in oleic, palmitic, stearic, behenic, and arachidic acids and belongs to the group of oleic acid-rich oils. According to these results, *Moringa* oil can be used as a food and commercial product (Mahmood *et al*., 2010). The seed of this plant contains 70.5 % oleic, 1.5 % gadoleic, and 8.9 % palmitic and 3.82 % stearic acid. In MP, the mature seed contains about 53.9 % oil. The oil is high quality and, like olive oil, contains 70 % oleic acid, is tasty and palatable, and is used for cooking and deteriorates very slowly (Homapour *et al.*, 2014). MP seed oil is a good source of behenic acid, which is used as a preservative in the food industry (Mahmood *et al*., 2010).

Antioxidants help the human body reduce oxidative damage. Food industry professionals are looking to replace synthetic antioxidants with natural antioxidants due to food safety and health concerns (Goli *et al*., 2005).

Phenolic compounds such as flavonoids, tannins, and anthocyanins are a large group of natural plant substances found in fruits and vegetables. These substances have significant advantages due to their wide range of desirable biological effects including antioxidant activities, antitumor, antimicrobial effects, and maintaining human health (Fathiazad *et al*., 2010).

Tsaknis (1998) studied the oil content in MP seeds from Saudi Arabia, and it was reported to be 53.9 %. It was also reported that MP seeds contain 35-40% oil. MP seed oil with a light-yellow color has a pleasant taste and is comparable to olive oil in quality.

As the *Moringa* plant, especially the MP species is native to Asia and has limited growth in Southwest Asia, not much research has been conducted on this species in Iran which is native to the southern regions of Iran. The use of MP seed oil in the country's food industry can be considered as an appropriate option. For this purpose, the present study was conducted to provide comprehensive information about the MP seeds (harvested in the south region of Iran) and their oil quality.

2. MATERIALS AND METHODS

2.1. Preparation of raw materials

Mature seeds of *Moringa peregrina* were collected from Fanuj County (26°43′44″N 59°30′30″E) in the Sistan and Baluchestan provincea, Iran. The chemicals used in this study were of analytical grade (Merck, Germany). alpha-glucosidase enzyme and substrate pNPG (*Saccharomyces cerevisiae* yeast origin) were purchased from Sigma Aldrich Co. with laboratory purity.

2.2. MP seed experiments

2.2.1. Seeds physical characteristics

Seeds dimension. Large, medium, and small diameters of seeds were measured with a caliper with an accuracy of 0.01 mm. For this purpose, 75 seeds were randomly selected, and their three main dimensions were measured. Then, the measured data were reported as the length, width, and thickness of the samples, for which the arithmetic mean of the diameter or dimensions of the object and the geometric mean of the dimensions were calculated (Razavi *et al*., 2007).

Bulk density. A measuring cylinder (100 mL) was used to measure the bulk density. The seeds were dropped into the cylinder until a certain volume

of the cylinder was filled by the MP seeds. The seeds were weighed and the bulk density of the samples was determined according to Eq. 1:

$$\rho_b = M/V_b \quad \text{Eq.1}$$

Where ρ_b is the bulk density; M is the mass of seeds (in grams), and V_b is the volume (mL) of the graduated cylinder.

Absolute density. The principle of liquid displacement was used to measure the absolute density of the seeds, which used Archimedes' law of buoyancy and the absolute density of the seeds was calculated according to the Eq. 2 and Eq. 3 (Mohsenin, 2020).

$$VK = \frac{(MPT-MP)-(MPTS-MPS)}{PT} \quad \text{Eq. 2}$$

$$\rho_k = \frac{(MPS-MP)}{VK} \quad \text{Eq. 3}$$

Where MT: toluene weight, MP: weight of the blank graduated cylinder, MPTS: weight of graduated cylinder with MP seeds and toluene, MPS: weight of the graduated cylinder with MP seeds, PT is toluene density at laboratory temperature, VK is MP seed volume and Kρ is seed absolute density.

2.2.2. Measurement of protein percentage

Crude protein content was calculated as a mass percentage in dry matter. To calculate the content of crude protein in the dry matter, the measured nitrogen content was multiplied by the conversion factor of 6.25 (Coates, 2011).

2.2.3. Measurement of fiber percentage

This experiment was performed according to the ISO 5498:1981 standard for the determination of crude fiber content.

2.2.4. Measurement of the lipid percentage

This experiment was performed according to the ISO 11085:2015 standard. The experimental method is the hydrolysis of the test sample by hydrochloric acid in the presence of ethyl alcohol and formic acid.

2.2.5. Measurement of the ash content

Five grams of the test material were burned in a furnace at 550 °C until complete combustion of the organic matter, and the rest of the material was weighed. This experiment was performed according to ISO 2171:2007 standard.

2.3. Oil extraction from seeds

MP seed oil was extracted by two methods of Soxhlet and cold press at 52 °C (Díaz *et al*., 2021). The extracted oil was centrifuged (S5810, Eppendorf, Germany) at 4000 rpm. The oil extraction percentage in both methods was calculated according to Eq. 4.

Extraction percentage =
(mass of raw material/mass of oil)×100 Eq. 4

2.3.1. Oil extraction by Soxhlet

Oil extraction was performed by Soxhlet using n-hexane as the solvent. The solvent was continuously circulated between the extraction chamber and the solvent reservoir until the oil was completely extracted (Özcan *et al*., 2019). The obtained oil was stored in airtight, dark containers at 4 °C until analysis.

2.3.2. Oil extraction by cold press

The seed oil was extracted by a mechanical press machine. Ambient temperature was controlled during oil extraction of 27 °C, helix speed was 15 rpm and oil extraction temperature was controlled at 50 °C.

2.4. MP seed oil experiments

2.4.1. Measurement of free acidity

The ISO 660: 2009 standard was used to measure free acidity. The sample was titrated with potassium hydroxide (0.01 N) and in the presence of phenolphthalein until the appearance of a purple color.

2.4.2. Measurement of peroxide index

The ISO 3960:2017 standard was used to measure the peroxide index. The basis of oil titration with sodium thiosulfate (0.01 N) was in the presence of potassium iodide and starch adhesive reagent. Finally, the result was expressed as milliequivalents of reactive oxygen per kilogram of oil sample.

2.4.3. Measurement of oxidative stability

The method established in ISO 6886:2016 was used to measure oxidative stability using 743 Ranci-

mat apparatus from Metrohm, Herisau, Switzerland (Maszewska *et al*., 2018). Rancimat or OSI is an oxidative stability-measuring device. A sample of 2.50 ± 0.01 g was used for each run. All the samples were assessed at the temperatures of 120 °C under a constant airflow (20 L/h).

2.4.4. Iodine index

The iodine index of oil samples was calculated from the percentage of fatty acids obtained by gas chromatography according to AOCS standard cd 1c-85 (Maszewska *et al*., 2018).

2.4.5. Cox Index

This index indicates the oxidation rate of oils based on the percentage of unsaturated fatty acid composition and is calculated according to Eq. 5 (Fatemi and Yang, 1998).

$$Cox = ((C18:1)\times 1=(C18:2)\times 10.3+(C18:3)\times 21.6)/100 \quad \text{Eq. 5}$$

2.4.6. Determination of smoke point

The AOCS standard number cc 9a-48 was used to determine the oil smoke point using YD-1 fully automatic Oil Smoke Point instrument. A sample of each oil was heated in a cup until a continuous bluish smoke appeared.

2.4.7. Determination of relative density

This experiment was performed according to the AOCS standard number CC-Loa-25 (Wang *et al*., 2018). The weight of a certain volume of sample was measured at 25 °C relative to the water of its entire volume at 25 °C. For this purpose, a 50 mL glass pycnometer with a sealed lid was employed.

2.4.8. Determination of the refractive index

The ISO 6320: 2000 standard was used in this experiment, and a digital refractometer was used, after first cleaning and then calibrating the lens using distilled water. Then, a sample of oil was poured onto the desired part of the device and the refractive index was recorded.

2.4.9. Determination of the fatty acids

To determine fatty acids, the sample was prepared as a methyl-ester derivative. The oil samples were esterified with 2N methanolic potassium hydroxide for 30 minutes at 50 °C and analyzed using gas chromatography based on ISO 24363: 2023. The gas chromatograph was equipped with a flame ionization detector (FID) and a hydrogen flow rate of 40 mL/min and an airflow rate of 450 mL/min. An HP-88 fused silica capillary column (length:100 m; internal diameter: 0.25 mm, film thickness: 0.2 μm) was employed. Nitrogen was used as the carrier gas (flow rate: 2 mL/min). The oven temperature was initially maintained at 120 °C for one minute. It was then increased to 175 °C at a rate of 10 °C per minute and held at this temperature for 10 minutes. Subsequently, the temperature was raised to 210 °C at a rate of 5 °C per minute and maintained for 5 minutes. Finally, the temperature was increased to 230 °C at a rate of 5 °C per minute and held constant for 5 minutes.

2.4.10. Determination of sterol compounds

Sterol compounds were determined by the IOC standard (COT/T.20/DOC NO.10/REV.1.2001). Unsaponifiable compounds were obtained through saponification with an ethanolic potassium hydroxide solution, followed by extraction with diethyl ether. These compounds were then spotted onto a thin-layer chromatography (TLC) plate, and the different components were separated and identified using a rhodamine detector at a concentration of 0.01 % in ethanol. The sterol-related fraction was isolated from the TLC plate, and sterols were extracted using chloroform. The analysis was performed using an Agilent model 7890A gas chromatograph equipped with FID. The chromatograph operated with a hydrogen flow rate of 40 mL/min, an airflow rate of 450 mL/min, and an HP-88 fused silica capillary column (length: 100 m, internal diameter: 0.25 mm, film thickness: 0.2 μm). The split ratio was set at 1:50. Nitrogen was used as the carrier gas (flow rate: 2 mL/min). The oven temperature was initially maintained at 120 °C for one minute. It was then increased to 175 °C at a rate of 10 °C per minute and held at this temperature for 10 minutes. Subsequently, the temperature was raised to 210 °C at a rate of 5 °C per minute and maintained for 5 minutes. Finally, the temperature was increased to 230 °C at a rate of 5 °C per minute and held constant for 5 minutes.

2.4.11. Determination of tocopherols

Tocopherol analysis was performed using HPLC according to the ISO 9936:2016 standard. The mobile phase used methanol–acetonitrile (30:70 v/v) solvent. Detection was achieved using a UV-Visible detector. Single-tocopherols were isolated, and their concentrations were determined using calibration curves.

2.4.12. Determination of antioxidant activity

The antioxidant activity was determined using the ABTS free radical scavenging method. ABTS radical was obtained by exposing potassium persulfate for 16 h. The absorbance at a wavelength of 734 nm in the presence of oil for 5 min was determined by ELISA-Reader (Re *et al*., 1999).

2.4.13. Determination of the total phenol content

The Chan *et al.* (2010) method was used to measure the total phenol content by the Folin-Ciocalteu reagent. The results were analyzed using linear regression from the standard gallic acid curve and the equivalent concentration of gallic acid was determined (Chan *et al*., 2010).

2.5. Statistical analysis

In this study, the data obtained from the experiments were analyzed using SPSS-16 software. The means were compared based on Duncan's test at 0.05 level.

3. RESULTS AND DISCUSSION

3.1. MP Seed characteristics

3.1.1. Seeds physical characteristics

The mean of the data obtained from the measurement of the physical characteristics of 75 MP seeds used in the present study was calculated by a caliper. Arithmetic mean diameter and geometric mean diameter were calculated using the results of measuring the large, medium, and small diameters of samples (in three dimensions). The results of these measurements are shown in Table 1.

The values of 100-seed weight, 33-seed weight, bulk density, absolute density, and porosity were calculated with 7 replications and are shown in Table 1.

The gravitational characteristics of MP seeds are important in the processes of transfer, washing, processing, oil extraction, and storage of this product. Designing equipment for harvesting, transferring, sorting, grading, processing, and other industrial processes requires information about the physical and geometric characteristics of the seeds.

The porosity of the crops used in this study was less than the porosity rate reported (41.85% vs. 45.4%). This difference can be due to differences in the growing area of the two plants, crop moisture content, and test error.

Table 1. Physico-chemical characteristics of MP* seeds

Characteristic	Unit	Mean**
Large diameter (length)	mm	17.91 ± 2.29
Medium diameter (width)	mm	10.08 ± 0.88
Small diameter (thickness)	mm	9.68 ± 0.81
Arithmetic mean diameter	mm	12.56 ± 0.99
Geometric mean diameter	mm	12.02 ± 0.90
100-crop weight	g	58.37 ± 1.23
33-crop weight	g	19.20 ± 0.86
Bulk density	g/cm^3	0.47 ± 0.00
Absolute density	g/cm^3	0.81 ± 0.00
Porosity	%	41.85 ± 0.26
Dry matter	%	96.76 ± 0.06
Protein	%	16.93 ± 0.03
Fiber	%	26.14 ± 0.06
Ash	%	2.66 ± 0.02
Lipid	%	41.12 ± 0.69
Carbohydrate	%	9.86 ± 0.86

* MP: *Moringa peregrina*
** Data mean ± standard error (SE) of three replicates

3.1.2. Seeds chemical characteristics

The dry matter percentage, protein, fiber, lipid, and ash content obtained from the analysis of MP seeds are presented in Table 1. The lipid content in MP seeds of 41.12 % was the highest composition in MP seeds based on dry matter. MP seeds harvested from the Kohat forests showed protein, fiber, moisture, and ash content of 31.65, 7.54, 8.90, and 6.53 %, respectively. The high percentage of protein in MP seeds makes them a valuable nutrient in the diet. Recommended Dietary Allowance (RDA) for a person weighing 70 kg should contain 56 g of protein. For

adults weighing 50 kg, the value is 46 g/day. Most of the fiber in food is in the form of cellulose and lignin. Water-insoluble carbohydrates, such as fiber, are separated by various methods (Whitney and Rolfes, 2018). Raw fiber weight has no significant nutritional value, it plays a major role in facilitating bowel movements. The percentage of fiber in the seeds was higher than in the leaves of MP ($p < 0.05$).

Another study reported MP seed compounds native to Sudan with a moisture content of 2.9 % and the content of protein, ash, lipid, and carbohydrate equal to 30.16, 2.60, 77.40, and 57.23 %, respectively. The lipid and ash contents measured in the present study were consistent with the values reported by (Sulaiman *et al*., 2017). Scientists reported that the dry matter content in the leaves and seeds of MP was 98.4 and 97.8 %, respectively, which was approximately equal to the contents measured in the present study. The protein content in the leaves and seeds of MP was 8 and 12 %, respectively, which was less than that of the present study. The ash content in the leaves and seeds of MP measured by these researchers was 10.3 and 6.1 %, respectively, higher than the content measured in the present study. Differences in the measured content of compounds depend on many factors, including differences in a plant growth area, accuracy of measurement methods, and test error.

Ash usually indicates the number of minerals in the sample. The ash content in natural foods is about 5%, which can be more than 10% for processed foods. Minerals are usually required in very small amounts for the human body. The high ash content in food indicates very low food quality, while the low ash content indicates high food quality (Pomeranz, 2013). This study revealed that the seeds and leaves of MP as natural food had acceptable levels of ash. The ash content in MP leaves was higher than in its seeds ($p < 0.05$). The percentage of lipids or oil in MP seeds was about 10 times higher than the oil in its leaves, which showed a significant difference ($p < 0.05$). Somali *et al*., (1984) expressed the values of compounds in MP seeds as 54.3 % lipid, 22.1 % protein, 3.6 % fiber, and 2.5 % ash (Somali *et al*., 1984). These values were measured with seed moisture content of 1.8 %. The ash content measured in the present study was lower than the value reported by these researchers. Lipid, protein, and fiber contents were much higher than the reported data. Factors such as moisture percentage, sampling time, etc. can be the reason for the differences in the values measured in the two experiments.

3.2. The percentage of oil extraction from MP seeds

The percentage of oil extraction from MP seeds by the Soxhlet method (MPSO) with hexane solvent was 41.25% and the percentage of oil extraction in the cold-press method (MPCO) at 27 °C was 22.5 %. In a study conducted in Saudi Arabia on the characteristics of MP oil, the oil content in the seeds of this plant was reported to be 53.9 % (Tsaknis, 1998). Elsewhere, MP seeds oil was reported to be 35-40 % consistent with our results (Babekir, 2014). The percentage of seeds oil extraction can vary based on factors such as seed ripeness, geographical location, harvest time, and other factors. Salaheldeen *et al.* (2015) stated that the percentage of MP seeds oil extraction with the Soxhlet method was 26%. In another study, the percentage of oil extraction from *M. oleifera* crops –native to India– based on the Soxhlet method with n-hexane solvent was reported to be 39.22 % (Bhatnagar and Krishna, 2013).

3.3. Experiments related to MP seed oil

3.3.1. Characteristics of seed oil

Some characteristics of MPSO and MPCO are presented in Table 2; such as acidity, iodine index, and density of MP oil were 0.7 (mg KOH/g), 67.73 (gI_2/100g), and 896 (kg/m^3), respectively (Salaheldeen *et al*., 2014). The results reported by Salaheldeen were consistent with our results.

Table 2. Characteristics of MPSO[1] and MPCO[2].

Characteristic	Soxhlet extraction	Cold-press extraction
Acidity as oleic acid (%)	0.28 ± 0.007^a	0.23 ± 0.008^b
Peroxide (meq O_2/1000 g)	0.01 ± 0.001^b	0.09 ± 0.005^a
Oxidative stability (h)	5.00± 1.300^a	4.00± 0.02^a
Iodine index	74.49± 0.001^a	74.53± 0.002^a
Cox index	1.15± 0.001^a	1.16± 0.002^a
Smoke point (°C)	250 ± 0.000^a	220 ± 0.000^b
Relative density (kg/m^3)	0.80 ± 0.000^b	0.91 ± 0.000^a
Refractive index	1.45 ± 0.00^a	1.47 ± 0.00^a

Mean ± standard error (SE) of three replicates
Different letters in each row indicate a significant difference at the level of $p < 0.05$
1. *Moringa peregrina* by the Soxhlet method (MPSO)
2. *Moringa peregrina* by cold-press method (MPCO)

The percentage of free fatty acids was expressed as the acid with the highest content in the sample, such as oleic acid, where the acidity of the sample was determined by dividing the acid number by 1.99. The permissible acidity limits for refined oils and virgin oils (oils extracted by cold press) were 0.3 and 2%, respectively. The peroxide number was obtained by measuring the iodine content released from potassium iodide and was expressed as meq O_2/kg oil. The permissible numbers for peroxide in refined and virgin oils (oils extracted by cold press) are 10 and 15 meq O_2/kg, respectively (Homapour *et al.,* 2014). MP seed oil had the standard acidity and peroxide in the permitted range. The acidity of the MPSO was higher than the MPCO. Also, as expected, the peroxide content in MPCO was higher than MPSO ($p < 0.05$).

Oxidative stability is the induction time, which is determined using the Rancimat device. Temperatures of 100-120 °C are commonly used to measure oxidative stability. The optimal induction time was between 6-24 h. The oxidative stability of MPSO and MPCO was 5 h and 4 h, respectively. The longer the induction period, the higher the oil quality. Low-quality oils usually have a shorter induction period. Oxidation leads to unpleasant odors and reduced food quality.

The iodine number indicates the degree of unsaturation of the lipid or oil, expressed as grams of iodine absorbed by 100 g of lipid. The iodine number is often accurate for unpaired double bonds. The lower the number of double bonds, the lower the iodine number and therefore lower oxidation. The Cox index indicates the oxidizability of oils based on the composition of unsaturated fatty acids. The Cox index was 1.15 for MPSO and 1.16 for MPCO. The lower the Cox index, the greater the resistance of the oil to oxidation. The Cox index for olive oil was reported to be 1.47 (Homapour *et al*., 2014). The smoke point is the temperature at which the oil begins to smoke and indicates the beginning of the breakdown of triglycerides in the presence of air. The smoke point for oils is normally in the range of 200-230 °C, which is slightly higher in real frying (outside of laboratory devices). The measured smoke point for MPSO was higher than the measured smoke point for MPCO. As the unsaturation of fatty acids and oils increases, the oil density increases, i.e., as the number of carbons in the fatty acid chain increases, the density decreases. The relative density of MPCO was higher than the relative density of MPSO, which meant a significant difference between the two extraction methods ($p < 0.05$).

3.3.2. Determination of the fatty acid composition

The fatty acid profiles of MPCO and MPSO are presented in Table 3.

The highest amount of fatty acid in the sample of MPSO was related to oleic acid at 78.1 % and palmitic acid at 11.47 %. Saturated fatty acids in this oil were 15.74 % including butyric, caproic, lauric, myristic, palmitic, stearic, and arachidic acids. In addition. 84.20 % of the fatty acids in this oil were unsaturated fatty acids including palmitoleic, elaidic, oleic, linoelaidic, linoleic, α- and γ-linolenic acids.

Oleic acid is one of the ω9 fatty acids with a content of 78.01% in this oil. The ω6 fatty acid content in this oil was 1.52 %, including linoleic acid and γ-linolenic acid. α-linolenic acid was one of the ω3 fatty acids with a content of 1.60 % in this oil. The ratio of ω6 fatty acid to ω3 fatty acid was calculated to be 0.95 %.

The highest value for fatty acid in the sample of MPCO was related to oleic acid at 77.73 % and palmitic acid at 11.49 %. The saturated fatty acids in this oil were equal to 15.9% including butyric, caproic, lauric, myristic, palmitic, stearic, and arachidic acids. Moreover, 84.08% of the fatty acids in this oil were unsaturated fatty acids including palmitoleic, elaidic, oleic, linoelaidic, linoleic, α- and γ-linoleic acids. Oleic acid content was equal to 77.73 %, while the ω6 content was 1.65%, including linoleic acid and γ-linolenic acid. α-linolenic acid as ω3 fatty acids was equal to1.60 %. The ratio of ω6 fatty acid to ω3 fatty acid was calculated to be 1.03 %.

MP *seed* oil belongs to the oleic-linoleic acid oils among vegetable oils. The saturated fatty acid contents in oils such as corn, olive, sesame, and canola oils was reported to be less than 20 % (Pantazaki et al., 2010; Kurt, 2018).

In one study, the fatty acid content in MPSO was reported to be oleic acid at 72.19 %, palmitic acid at 9.9 %, saturated fatty acids at 18.78 %, and unsaturated fatty acids at 81.22 %. The results reported in previous studies were consistent with the results obtained in this study (Salaheldeen *et al*., 2014). According to the results reported by Anwar and Rashid (2007), the content and type of fatty acids in MP native to Iran measured in this study were close

Table 3. Percentage of fatty acids of MPSO[1] and MPCO[2].

No.	Profile	Common name	MPSO Value (%)	MPCO Value (%)
1	C4:0	Butyric acid *	0.05±0.000[l]	0.02±0.000[k]
2	C6:0	Caproic acid *	0.01±0.000[m]	0.01±0.000[l]
3	C8:0	Caprylic acid *	N.D	N.D
4	C10:0	Capric acid *	N.D	N.D
5	C12:0	Lauric acid *	0.03±0.001[k]	0.02±0.000[k]
6	C14:0	Myristic acid *	0.11±0.000[j]	0.10±0.000[j]
7	C16:0	Palmitic acid *	11.47±0.002[b]	11.49±0.000[b]
8	C16:1	Palmitoleic acid **	2.93±0.001[c]	3.01±0.003[c]
9	C18:0	Stearic acid *	1.64±0.002[e]	1.88±0.001[e]
10	C18:1t	Elaidic acid **	0.14±0.000[i]	0.09±0.000[i]
11	C18:1c	Oleic acid **	78.01±0.001[a]	77.73±0.000[a]
12	C18:2t	Linoelaidic acid **	N.D	N.D
13	C18:2c	Linoleic acid **	0.25±0.000[h]	0.37±0.000[h]
14	C18:3n6	γ-linolenic acid **	1.27±0.001[g]	1.28±0.000[g]
15	C18:3n3	α-linolenic acid **	1.60±0.000[f]	1.60±0.002[f]
16	C20:0	Arachidic acid *	2.43±0.002[d]	2.43±0.001[d]
17	C21:0	Heneicosanoic acid *	N.D	N.D
18	C22:0	Behenic acid *	N.D	N.D
19	C23:0	Tricosanoic acid *	N.D	N.D
20	C24:0	Lignoceric acid *	N.D	N.D

N.D: Not detected, * Saturated, ** Unsaturated
1. *Moringa peregrina* oil extracted by the Soxhlet method (MPSO)
2. *Moringa peregrina* by extracted by cold-press method (MPCO)
Mean ± standard error (SE) of three replicates
Different letters in each column indicate a significant difference at the level of $p < 0.05$

to the content and type of fatty acids in *M. oleifera* oil native to Pakistan (76 % oleic acid, 6.5 % palmitic acid) (Anwar and Rashid, 2007).

3.3.3. Sterol compounds of seed oil

Sterols exist in all oils and are the most important components of substances which are non-saponifiable, neutral crystalline alcohols with a high melting point. The most abundant plant sterol (phytosterol) is β-sitosterol, which makes up the majority of sterols along with campesterol, stigmasterol, and Δ^5-onasterol. Generally, phytosterols reduce the absorption of total cholesterol, and low-density lipoprotein (LDL) and thus reduce the risk of heart disease. The sterol compounds in MPSO and MPCO are presented in Table 4.

The most abundant sterol compound in MPSO was stigmasterol with 31.75%. Δ^5-avenasterol and campesterol are the other two major constituents of this oil with 26.30 and 17.20 %, respectively. The most abundant sterol compound in MPCO was stigmasterol with 30.73%. The content of Δ^5-avenasterol and campesterol were equal to 26.22 and 17.08 %, respectively. The major sterols in MP oil were stigmasterol, Δ^5-avenasterol, campesterol, and β-sitosterol, while the major sterol composition of olive oil was β-sitosterol with a concentration of 68% (Tsaknis, 1998). The sterol compounds of Pakistan *M. oleifera* seed oil

mainly included β-sitosterol (46.16 %), stigmasterol (18.80 %), campesterol (17.95 %), and Δ^5-avenasterol (9.26 %) (Anwar and Rashid, 2007).

Table 4. Sterol compounds in MP seeds oil

Sterol compound	MPSO[1] (%)	MPCO[2] (%)
Cholesterol	0.13± 0.010[b]	0.11± 0.001[a]
Campesterol	17.20± 0.750[a]	17.08± 0.080[a]
Stigmasterol	31.75± 0.011[a]	30.73± 0.002[b]
β-Sitosterol	16.89± 0.002[a]	16.77± 0.007[b]
Δ^5-Avenasterol	26.30± 0.002[a]	26.22± 0.004[b]
Δ^7-Avenasterol	1.50± 0.030[a]	1.12± 0.002[b]
Other sterols*	2.07± 0.002[a]	1.97± 0.005[b]

1. *Moringa peregrina* by the Soxhlet method (MPSO)
2. *Moringa peregrina* by cold-press method (MPCO)
Mean ± standard error (SE) for three replicates
Different letters in each row indicate a significant difference at the level of $p < 0.05$

3.3.4. Tocopherols in seed oil

Figure 1 shows the type and content of tocopherol compounds in both MPCO and MPSO.

According to the results obtained in this experiment, the highest content of tocopherols in MP oil was related to γ-tocopherol, α-tocopherol, and δ-tocopherol, respectively. The content of β-tocopherol was not detectable in the experiments. Anwar and Rashid (2007) studied the physical and chemical characteristics of seeds and seed oil of *M. oleifera* native to Pakistan and measured α-, γ- and δ-tocopherols in oil at 140.5, 18.68 and 61.70 mg/kg, respectively, which decreased after gumming (Anwar and Rashid, 2007). Differences in *Moringa* species were considered to be due to the differences in the values of other tocopherols.

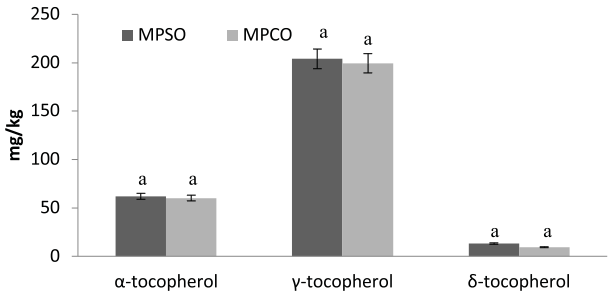

Figure 1. Content and type of tocopherols measured in MP seed oil (*Moringa peregrina* by the Soxhlet method (MPSO) and *Moringa peregrina* by cold-press method (MPCO)).

Mean ± standard error (SE) of three replicates.
Different letters indicate a significant difference for each tocopherol ($p < 0.05$)

3.3.5. Antioxidant activity

Figure 2 shows the antioxidant activity of MPCO and MPSO based on the percentage of ABTS radical scavenging with 3 replicates.

The results showed that MPSO had higher ABTS radical scavenging activity (antioxidant activity) than MPCO (19.36 % vs. 12.88 %). According to the statistical analysis, the antioxidant activity of MPSO was significantly different from that of MPCO ($p < 0.05$).

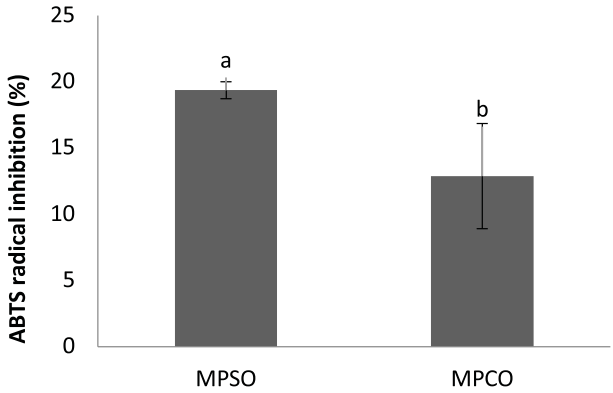

Figure 2. Antioxidant activity of *Moringa peregrina* by the Soxhlet method (MPSO)
and *Moringa peregrina* by cold-press method (MPCO)
Mean ± standard error (SE) of three replicates
Different letters indicate a significant difference at the level of $p < 0.05$

3.3.6. Phenolic content

According to the standard gallic acid curve, y=0.0012x+0.0254 (R^2=0.98), equivalent concentrations of gallic acid were obtained for each sample (Table 5).

Table 5. The equivalent concentration of gallic acid in MP oil in mg/mL gallic acid

Oil concentration (g/ mL)	MPSO[1]	MPCO[2]
0.1	0.02571 ± 0.00[c]	0.02591 ± 0.00[a]
0.05	0.02567 ± 0.00[d]	0.02588 ± 0.00[b]
0.025	0.02550 ± 0.00[f]	0.02571 ± 0.00[c]
0.0125	0.02549 ± 0.00[g]	0.02551 ± 0.00[e]
0.00625	0.02548 ± 0.00[h]	0.02544 ± 0.00[j]
0.003125	0.02546 ± 0.00[i]	0.02541 ± 0.00[k]

Mean ± standard error (SE) of three replicates
Different letters in each row and column indicate a significant difference at the level of $p < 0.05$.
1. *Moringa peregrina* by the Soxhlet method (MPSO)
2. *Moringa peregrina* by cold-press method (MPCO)

Phenolic compounds play an important role in the oxidative stability of oils. According to the results obtained from the study of phenolic content based on the equivalent concentration of gallic acid, it was found that MPSO at the concentration of 0.1 g/mL had the highest concentration of equivalent gallic acid (phenolic content) while MPCO at a concentration of 0.003125 g/mL depicted the lowest concentration equivalent to gallic acid (phenolic content). There was no significant difference between MPSO (0.1 g/mL) and MPCO (0.025 g/mL) ($p < 0.05$). The results for other concentrations were significantly different from each other ($p < 0.05$). There was a significant difference between the phenolic contents in MPSO and MPCO ($p < 0.05$). Phenolic compounds are sensitive to heat, so they are predicted to be destroyed during prolonged exposure to heat, such as extraction by Soxhlet; therefore, the content of total phenol in MPCO was higher than MPSO.

4. CONCLUSIONS

This study revealed that native oils from plants such as MP can be used as dietary supplements that are rich in essential nutrients such as protein, antioxidant compounds, and essential fatty acids such as ω3 and ω6. This study also depicted that the use of the MP plant as a low-cost source of food can be very desirable in the food industry. Due to the lack of awareness and negative approach to wild, indigenous plant resources, paying attention to public awareness for accepting these plants as beneficial food can pave the way for extensive and targeted cultivation of these plants in the region. Increasing the consumption and processing of these plant species is predicted to improve the diet and reduce nutrient deficiencies. The results of the present study suggested that the seeds of MP can be used as a source of edible oil like soybeans due to the content of 41 % oil and 16 % protein. Phenolic compounds play an important role in the oxidative stability of oils.

Phenolic compounds are heat sensitive, so they are better preserved in cold-press oil extraction. The percentage of oil extracted by cold press was much less than the content of oil extracted by solvent. In general, MP seed oil extracted by solvent had higher acidity, oxidative stability, smoke point, and refractive index and the contents of peroxide, iodine index, Cox index, and density were less than that of the cold-press process. Because this tropical plant is native to the southern regions of Iran, the use of MP oil is recommended as an appropriate source of edible oils. Refining such oil is supposed to produce better quality. Finally, further studies are required to identify, measure, and isolate the bioactive compounds present in this plant species for food and medicinal purposes as a treatment for diseases caused by free radicals. While this study focused on the beneficial properties of *Moringa peregrina* oil, future research should also investigate potential cytotoxic effects to provide a comprehensive safety profile.

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DATA AVAILABILITY

All data from the journal are available on demand.

DECLARATION OF COMPETING INTEREST

The authors have no financial, professional, or personal conflicts of interest that could have inappropriately influenced this work.

AUTHORSHIP CONTRIBUTION STATEMENT

1. A. Jafari: Literature search, Formal analysis, Data analysis; Writing the original draft. 2. M. Moslehishad: Idea & Conceptualization; Supervisor; Project administration; Reviewing & Editing; Data analysis; Methodology. 3. Z. Ghanavi: Design and implementation of the research, Reviewing & Editing.

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