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Analytical Characterization of *Moringa oleifera* Seed Oil Grown in Temperate Regions of Pakistan

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The hexane-extracted oil content of *Moringa oleifera* seeds ranged from 38.00 to 42.00%. Protein, fiber, and ash contents were found to be 26.50−32.00, 5.80−9.29, and, 5.60−7.50%, respectively. Results of physical and chemical parameters of the extracted oil were as follows: iodine value, 68.00−71.80; refractive index (40 °C), 1.4590−1.4625; density (24 °C), 0.9036−0.9080 mg/mL; saponification value, 180.60−190.50; unsaponifiable matter, 0.70−1.10%; and color (1 in. cell), 0.95−1.10 R + 20.00−35.30 Y. Tocopherols (α, γ, and δ) in the oil were up to 123.50−161.30, 84.07−104.00, and 41.00−56.00 mg/kg, respectively. The oil was found to contain high levels of oleic acid (up to 78.59%) followed by palmitic, stearic, behenic, and arachidic acid up to levels of 7.00, 7.50, 5.99, and 4.21%, respectively. The induction period (Rancimat, 20 L/h, 120 °C) of the crude oil was 9.99 h and reduced to 8.63 h after degumming. Specific extinctions at 232 and 270 nm were 1.70 and 0.31, respectively. Many parameters of *M. oleifera* oil indigenous to Pakistan were comparable to those of typical *Moringa* seed oils reported in the literature. The results of the present analytical study were also compared with those of different vegetable oils.

KEYWORDS: ***Moringa oleifera*; analytical characterization; high oleic; tocopherols**

INTRODUCTION

Ten to twelve species of the Moringaceae family have been reported to belong to one genus called *Moringa* (*1*). Almost all *Moringa* species are native to India, from where they have been introduced in many warm countries (*2*). The best-known and most widely naturalized species is *Moringa oleifera* Lam. (syn. *Moringa pterygosperma* Gaertn.) (*2*, *3*), which is a native of the western and sub-Himalayan tracts, India, Pakistan, Asia Minor, Africa, and Arabia (*1*, *4*). It is also distributed in The Philippines, Cambodia, Central America, North and South America, and the Caribbean Islands (*3*). The tree ranges in height from 5 to 10 m and sometimes even 15 m (*1*). It can grow well in the humid tropics or hot dry lands, can survive destitute soils, and is little affected by drought (*3*, *4*). In some parts of the world *M. oleifera* is referred to as the drumstick tree or the horseradish tree, whereas in others it is also known as the kelor tree (*5*, *6*). In the Nile Valley, the name of the tree is "Shagara al Rauwaq", which means "tree for purifying" (*7*).

M. oleifera Lam., indigenous to the subcontinent, is a small- or medium-sized tree, ~10 m high, found wild and cultivated throughout the plains (*5*, *8*). It is often cultivated in hedges and in houseyards, thrives best under the tropical insular climate, and is plentiful near the sandy beds of rivers and streams (*5*). In Pakistan, *Moringa* is represented by only two species, *M. concanensis* and *M. oleifera.* The former species is not common and perhaps confined to only a remote locality (Tharparker) Sindh (*8*). The latter, *M. oleifera*, locally known as "Sohanjna", is grown and widely cultivated in the Punjab plains, Sindh, Baluchistan, and North Western Frontier Province (N.W.F.P) particularly, in temperate and tropical regions of the country (*8*). The flowers and fruits appear twice a year, and seeds or cuttings can propagate the tree; the latter is more preferred. Flowers are white and fragrant, and the fruits are usually 30−45 cm long (*8*).

M. oleifera represents a traditionally important food commodity as the leaves, flowers, fruits, and roots of this tree are locally used as vegetables (*8*, *9*). The tender pods are cooked or pickled and used in culinary preparations. The fresh beans after roasting also make a palatable dish (*5*, *10*). Seeds are also consumed after frying and reported to taste like peanuts (*8*). Indonesians eat both leaves and seedpods of this tree as vegetables, which are reported to taste like asparagus (*6*). Philippine women consume *Moringa* leaves mixed in chicken or shellfish soups to enhance breast milk production (*9*). In southern India, village people use the fresh leaves to prepare cow and buffalo ghee from butter fat, and recently *Moringa* fruits have also been added to curries, canned and sold in stores (*6*, *9*).

A number of medicinal and therapeutic properties have been ascribed to various parts of this multipurpose tree, which included the treatment of ascites, rheumatism, and venomous bites and use as cardiac and circulatory stimulants (*5*, *10*). The

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plant has been well positioned in Ayurvedic, Unani, and even allopathic systems of medicine (*4*). Several of its parts have been reported to show antitumor, antipyretic, antiepileptic, anti-inflammatory, and antiulcer effects and are used in native medicines and folk remedies (*9, 11*−*13*). The roots of the young tree and also root bark are rubefacient and vesicant. Leaves are rich in vitamins A and C and are considered to be useful in catarrhal afflictions, said to have purgative properties and to promote digestion, and are used as an external application for wounds (*5*). The flowers of this plant are considered to possess medicinal value as a stimulant, aphrodisiac, diuretic, and cholagogue, and they have also been reported to contain flavonoid pigments (*9*).

Interest in the composition of *M. oleifera* seeds, and the extracted oil, known commercially as "Ben" or "Behen", oil has existed over the years. Oliveira et al. (*14*) described the compositional and nutritional attributes of seeds. The seeds are considered to be antipyretic, acrid, and bitter (*5*). They are reported to show antimicrobial activity and are also utilized for wastewater treatment and purification of muddy river water (*6, 15*).

Ben oil has been used for illumination and is considered to be particularly suitable as a lubricant for fine machinery (*5, 8*). The oil was erroneously reported to be resistant to rancidity and used extensively in the "enfleurage" process whereby delicate fragrances are extracted from flower petals (*5*). Some studies have been reported in the literature that present the composition and characteristics of seed fat of the Moringaceae family (*1, 2, 16*); however, very little is known about its production as an edible oil. Ibrahim et al. (*17*) reported that the oil content and its properties are varied over a wide range, mainly depending on the species and environmental conditions.

The uncontrolled growth in world population coupled with industrialization has widened the gap between demand and production of vegetable oils. This has resulted in ever-increasing imports, requiring the expenditure of valuable foreign exchange, and a deficiency in people's fat intake in many developing countries (*18*). Now, when, sustainable socio-/agroforestry is gaining recognition as an appropriate means to improve national economies, the search for alternative sources of additional fats and vegetable oils has to play a crucial role.

In view of growing demand and scientific awareness about the nutritional and functional properties of oils (*19*), the quality assessment and composition of oils from some nonconventional oilseeds is of much concern. Until now, a full characterization and comparison of the oil produced from seeds of *M. oleifera*, indigenous to the subcontinent and, in particular, from Pakistan, have not been reported. This has prompted us to design an analytical protocol and further investigate this oil. We have already published a preliminary report of proximate characteristics of *M. oleifera* oilseed, indigenous to the temperate regions of Pakistan (*20*). The main objective of the present study was to look into the detailed analytical characterization and exploitation of this oil for edible as well as other commercial purposes.

EXPERIMENTAL PROCEDURES

Materials. The seeds of *M. oleifera* (12 individual samples of 1.5 kg each) were assayed in dry state from the mature fruits, over a period of two years during July−August and February−March (two crops/year, i.e., a total of four harvests). Samples of seeds in dry state of mature fruits were harvested from 12 different sites (18 km toward the city of Hyderabad and the river Indus; 15 km toward the southwest, Super Highway; 10 km toward the Jamshoro Power Station, and 12 km toward the north), in the vicinity of the University of Sindh, Jamshoro, Pakistan. Seeds were globular, three-winged, and covered with a thick blackish seed coat; average weight was ∼0.32 g with the off-white kernel constituting 69−76% of the weight. All reagents (analytical and HPLC) used were from E. Merck or Sigma Aldrich. Sterol standards were of Fluka Chemie GmbH, Sigma-Aldrich (CH-9471, Buchs, Switzerland). Pure standards of tocopherols [DL-α-tocopherol, (+)-δ-tocopherol, -γ-tocopherol] and fatty methyl esters were obtained from Sigma Chemical Co. (St. Louis, MO).

Oil Extraction. After removal of the seed coat, the seeds (500 g) of each batch of *M. oleifera* were crushed and then fed to a Soxhlet extractor fitted with a 1-L round-bottom flask and a condenser. The extraction was executed on a water bath for 4−5 h with 0.5 L of *n*-hexane. The solvent was distilled off under vacuum in a rotary evaporator. Except for a small quantity (for tocopherol and Rancimat analysis), the recovered oil from different batches was further degummed.

Degumming of Oil. The oil to be degummed was heated (70 °C) on a water bath. Water was added to a final volume of 18% and mixed well with a glass rod. After cooling, the mixture was centrifuged (3000 rpm) for 12 min in an automatic refrigerated centrifuge (Sorval RC-3). The degummed oil was dried over anhydrous sodium sulfate, filtered, and kept in separate sealed bottles under refrigeration (0−4 °C) before use.

Analysis of Oilseed Residues. The oilseed residues (meal) after the extraction of oil from the seeds were analyzed for protein, fiber, and ash contents. Protein content was determined according to a semi-automated FOSFA official method (*21*). Samples of meal were digested for 10 min with a digestion mixture of sulfuric acid/hydrogen peroxide/potassium sulfate, using mercuric oxide as a catalyst. The final end-point in the ammonia titration was measured photometrically.

Fiber content was determined according to the ISO method (*22*). Two and a half grams of finely ground sample of meal was weighed and freed from fat by extraction with 15 mL of *n*-hexane. The test portion was boiled with sulfuric acid solution (0.255 mol/L) followed by separation and washing of the insoluble residue. The residue was then boiled with sodium hydroxide (0.313 mol/L), followed by separation, washing, and drying. The dried residue was weighed and ashed in a muffle furnace at 600 °C, and the loss in mass was determined.

Ash content was determined according to the ISO method (*23*). Two grams of the test portion was taken and carbonized by heating on a gas flame. The carbonized material was then ashed in an electric muffle furnace at 550 °C until constant mass was achieved.

Analysis of Extracted Oil. *Physical and Chemical Parameters of Oils.* Determination of density, refractive index, iodine value, peroxide value, acidity, saponification value, and unsaponifiable matter of the extracted oil was carried out according to various standard AOCS methods (*24*). The color of the oil was determined by a Lovibond Tintometer (Tintometer Ltd., Salisbury, U.K.), using a 1 in. cell. Specific extinctions at 232 and 270 nm were determined using a Perkin-Elmer model Lambda 2 spectrometer. Samples were diluted with iso-octane to bring the absorbance within limits (0.2−0.8) and $\epsilon^{1\%}_{1cm(\lambda)}$ was calculated following the IUPAC method (*25*). The *p*-anisidine value was also determined following an IUPAC method (*25*).

Oxidative Stability. An automated Metrohm Rancimat model 679, capable of operating over a temperature range of 50−200 °C, was used for the determination of induction periods (IP) of the degummed and non-degummed oils (*26*). Testing was carried out at 120 ± 0.1 °C, and oxidative stability was measured following the procedure described elsewhere (*27*). Briefly, oil (2.5 g) was carefully weighed into each of the six reaction vessels and analyzed simultaneously. IP of the samples were automatically recorded and corresponded to the breakpoint of the plotted curves.

Tocopherol Content. Tocopherols (α, γ, and δ) analysis was carried by high-performance liquid chromatography (HPLC) following the method of Thompson and Hatina (*28*) with slight modifications. One gram of oil was accurately weighed and made up to volume with heptane in a 10-mL volumetric flask wrapped in foil to inhibit oxidation. A Hitachi L-6200 HPLC unit coupled with a Hitachi F-1050 fluorescence detector was used. A 25-μL sample was injected into an analytical column (250 × 4.9 mm) packed with LiChrosorb SI 605 (5 μm), which

Table 1. Analysis of *M. oleifera* Seeds

constituent	*M. oleifera*[a]	literature
oil content (%)	40.39 ± 1.15 (38.00–42.00)	35.7 (*30*)
moisture content (%)	5.70 ± 0.42 (4.86–6.09)	present work
protein content (%)	29.36 ± 1.50 (26.50–32.00)	present work
fiber content (%)	7.20 ± 0.90 (5.80–9.29)	present work
ash content (%)	6.6 ± 0.50 (5.60–7.50)	present work

[a] Values are means ± SD calculated as percentage of dry seed weights for 12 *M. oleifera* seeds analyzed individually in triplicate. Parentheses show range of data.

was fitted with a 50 mm × 50 mm (i.d.) guard column with He-Pellosil packing. A mobile phase of dry heptane/water-saturated heptane/2-propanol (50.0:49.0:1.0) was used at the rate of 1.2 mL/min. Detection was performed at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Pure standards of α-, γ-, and δ- tocopherols were used for identification and calibration. A Hitachi Chromatointegrator model D-2500 with a built-in computer program for data handling was used for the quantification.

Sterol Composition. The determination of sterols was made following the method of the Association of Official Analytical Chemists (*29*). Analysis was carried out on a Perkin-Elmer gas chromatograph model 8700, equipped with a methylphenyl polysiloxanes-coated capillary column OV-17 (30 m × 0.25 mm, 0.20-μm film thickness) and a flame ionization detector (FID). The column was isothermally operated at a temperature of 255 °C. Injector and FID temperatures were set at 275 and 290 °C, respectively. Extra pure N_2 at a flow rate of 3 mL min^{-1} was used as a carrier gas. The internal standard used was 5-α-cholestane, and identification and quantification of unknown sterol components were made using a pure sterol standard mixture.

Fatty Acid Composition. Fatty acid methyl esters were prepared according to standard IUPAC method 2.301 and analyzed on a Perkin-Elmer gas chromatograph model 8700 fitted with a methyl lignoserate coated (film thickness = 0.22 μm), polar capillary column SP-2340 (60 m × 0.25 mm), and a flame ionization detector. Oxygen-free nitrogen was used as a carrier gas at a flow rate of 3.5 mL/min. Other conditions were as follows: initial oven temperature, 130 °C; ramp rate, 5 °C/min; final temperature, 220 °C; injector temperature, 260 °C; detector temperature, 270 °C. The internal standard used was nonadecanoic acid. Fatty acid methyl esters were identified by comparing their relative and absolute retention times to those of authentic standards of fatty acid methyl esters. All of the quantification was done by a built-in data-handling program, provided by the manufacturer (Perkin-Elmer) of the gas chromatograph.

RESULTS AND DISCUSSION

The data for the analysis of 12 individually extracted *M. oleifera* oils, together with literature values of a hexane-extracted oil of an identical *Moringa* species reported from Kenya (*30*), have been summarized in **Tables 1**−**6**. Values for the present analysis are given as mean ± SD of 12 *M. oleifera* oils, analyzed individually in triplicate. Ranges are given in parentheses.

The hexane-extracted oil content of different *M. oleifera* seeds from the vicinity of the University of Sindh, Jamshoro campus, was found to be 40.39% **(Table 1)**. The oil content varied among the seed samples collected from different sites, ranging from 38 to 42.0%. The oil concentration was high in those seeds harvested from the belt of the river Indus and from cultivated healthy plants, to which a supply of water was adequate and regular. The seeds from plants native to the dry mountainous area, which lacks proper irrigation, were generally found to be low in their oil content. The minor variation in oil yield of seeds from different sites may be the result of differences in natural soil texture and other man-made cumulative effects.

Table 2. Physical and Chemical Characteristics of Oil

determination	*M. oleifera*[a]	literature
iodine value (g of I/100 g of oil)	69.45 ± 1.20 (68.00–71.80)	66.83 (*30*)
refractive index (n_D 40 °C)	1.4608 ± 0.001 (1.459–1.4625)	1.4549 (*30*)
density (24 °C) (mg/mL)	0.9057 ± 0.001 (0.9036–0.9080)	0.8809 (*30*)
saponification value (mg of KOH/g of oil)	186.67 ± 2.01 (180.6–190.5)	178.11 (*30*)
unsaponifiable matter (%)	0.90 ± 0.09 (0.70–1.10)	present work
acidity (% as oleic acid)	0.40 ± 0.04 (0.27–0.48)	0.85 (*30*)
smoke point (°C)	200 ± 1.80 196–203	198 (*30*)
color (red units)	1.00 ± 0.04 (0.95–1.10)	0 (*30*)
color (yellow units)	29.00 ± 3.00 (20.00–35.30)	40 (*30*)

[a] Values are means ± SD for 12 *M. oleifera* oils analyzed individually in triplicate. Parentheses show range of data.

Table 3. Determination of Oxidative State of Oils

determination	*M. oleifera*[a]	literature
$\epsilon^{1\%}_{1cm(\lambda_{232})}$	1.70 ± 0.12 (1.40–1.85)	3.15 (*30*)
$\epsilon^{1\%}_{1cm(\lambda_{270})}$	0.31 ± 0.07 (0.18–0.40)	1.13 (*30*)
peroxide value (mequiv/kg of oil)	0.59 ± 0.05 (0.53–0.80)	1.80 (*30*)
p-anisdine value	1.30 ± 0.12 (0.95–1.62)	present work
oxidative stability, non-degummed oil Rancimat method (h)	9.99 ± 0.17 (9.76–10.30)	36.8 (*30*)
oxidative stability, degummed oil Rancimat method (h)	8.63 ± 0.19 (8.40–9.00)	10.8 (*30*)

[a] Values are means ± SD for 12 *M. oleifera* oils analyzed individually in triplicate. Parentheses show range of data.

Table 4. Tocopherol Content of the Oils

determination	*M. oleiferea*, non-degummed oil[a]	*M. oleifera*, degummed oil[a]	literature
α-tocopherol (mg/kg)	134.42 ± 10.00 (123.50–161.30)	110.0 ± 2.60 (105.00–114.0)	98.82 (*30*)
γ-tocopherol (mg/kg)	93.70 ± 7.00 (84.07–104.00)	81.63 ± 2.83 (77.00–86.10)	27.90 (*30*)
δ-tocopherol (mg/kg)	48.0 ± 4.65 (41.00–56.00)	41.00 ± 1.96 (38.00–44.25)	71.16 (*30*)

[a] Values are means ± SD for 12 *M.* oleifera oils analyzed individually in triplicate. Parentheses show range of data.

The oil content of *M. oleifera* seeds determined in the present work was significantly higher than that reported for *M. oleifera* seeds (var. Mbololo) from Kenya (*30*). However, the oil yield was lower by 9% as compared with that of *M. peregrina* seeds, reported from Saudi Arabia (*16*)**.** Such variation in oil content within the countries and species is attributed to the possible change in environmental and geological conditions of the regions (*17*). The average oil content of *M. oleifera* seeds in the present analysis was found to exceed those of four conventional oilseed crops: cotton (15.0−24%), soybean (17.0−21.0%), safflower

Table 5. Sterol Composition (Percent) of the Oils

sterol	*M. oleifera*[a]	literature
cholesterol	not detected	0.13 (*30*)
brassicasterol	not detected	0.06 (*30*)
24-methylenecholesterol	1.49 ± 0.12 (0.73–2.0)	0.88 (*30*)
campesterol	16.00 ± 1.03 (14.23–18.30)	15.13 (*30*)
campestanol	not detected	0.35 (*30*)
Δ^7-campestanol	0.50 ± 0.07 (0.19–0.76)	present work
stigmasterol	19.00 ± 1.00 (17.10–21.50)	16.87 (*30*)
ergostadienol	not detected	0.39 (*30*)
clerosterol	1.95 ± 0.13 (1.50–2.46)	2.55 (*30*)
stigmastanol	1.00 ± 0.07 (0.71–1.23)	0.86 (*30*)
β-sitosterol	46.65 ± 1.50 (42.60–49.00)	50.07 (*30*)
Δ^7-avenasterol	0.96 ± 0.06 (0.80–1.300	1.11 (*30*)
Δ^5-avenasterol	10.70 ± 0.93 (8.85–12.00)	8.84 (*30*)
28-isoavenasterol	0.50 ± 0.06 (0.30–0.90)	1.40 (*30*)

[a] Values are mean ± SD for 12 *M. oleifera* oils analyzed individually in triplicate. Parentheses show range of data.

Table 6. Fatty Acid Composition (Grams per 100 g of Fatty Acids) of Oils[a]

fatty acid	*M. oleifera*[a]	literature
$C_{8:0}$	not detected	0.03 (*30*)
$C_{14:0}$	not detected	0.11 (*30*)
$C_{16:0}$	6.50 ± 0.40 (5.90–7.00)	6.04 (*30*)
$C_{16:1}$	1.00 ± 0.08 (0.90–1.20)	0.11 (*30*)
$C_{18:0}$	5.67 ± 0.39 (4.88–7.50)	4.14 (*30*)
$C_{18:1}$	76.00 ± 1.23 (74.05–78.59)	73.60 (*30*)
$C_{18:2}$	1.29 ± 0.10 (0.65–1.96)	0.73 (*30*)
$C_{18:3}$	not detected	0.22 (*30*)
$C_{20:0}$	3.00 ± 0.20 (2.95–4.21)	2.76 (*30*)
$C_{20:1}$	1.20 ± 0.06	2.40 (*30*)
$C_{22:0}$	5.00 ± 0.33 (4.17–5.99)	6.73 (*30*)
$C_{22:1}$	not detected	0.14 (*30*)
$C_{26:0}$	not detected	1.08 (*30*)

[a] Values are mean ± SD for 12 *M. oleifera* oils analyzed individually in triplicate. Parentheses show range of data.

(25−40%), and mustard (24−40%), grown in the United States, Brazil, China, and some other Asian and European countries (*31*).

Analysis of the oilseed residue revealed a high protein content of the seeds, ranging from 26.5 to 32.00%, whereas fiber and ash contents were low at 7.20 and 6.60%, respectively. The analysis showed the meal to be a good source of protein, which could be added to chicken diets as a source of calories and may replace soybean meal for the local poultry industry. It could also be utilized as a fertilizer, a potential animal foodstuff (following saponins detoxification if proven to be necessary), and a source of water treatment chemicals, all of which provide added value to what may be regarded as a byproduct. There are reports in the literature that *M. oleifera* oilseed residue, left after the oil extraction, and husk contain active fractions that could be utilized as water-purifying agents (*15*, *18*, *32*).

The results of various physical and chemical characteristics of the extracted oil are given in **Table 2**. The values determined for iodine (69.45), refractive index (1.4608), density (0.9057 mg/mL), smoke point (200), free fatty acid (FFA) content (0.40% as oleic acid), and saponification value (186.67) were in close agreement with those of *M. oleifera* and *M. peregrina* oils reported from Kenya and Saudi Arabia, respectively (*16*, *30*). The oil was superior in color measurement (1 R + 29 Y) to *M. peregrina* oil and also comparable with *M. oleifera* oil reported from Kenya. The intensity of the color of vegetable oils depends mainly upon the presence of various pigments such as chlorophyll, which are effectively removed during the degumming, refining, and bleaching step of oil processing. The vegetable oils with minimum values of color index are more attractive for edible and domestic purposes. The values for saponification number (180.6−190.5 mg of KOH/g of oil) and unsaponifiable matter (0.70−1.10%) of the investigated oil, which are in good agreement with those of olive oil (*33*), were not so significantly different from those of corn, niger seed, low erucic acid, soybean, sunflower, safflower, tomato seed, and winged bean oils and mango kernel fat (*33*, *34*). The refractive index of the oil was within the range of cottonseed, Brazil nut, hazelnut, palm oils, and mango kernel and dhupa fats (*33*, *34*), whereas the iodine value and FFA content of the oil were significantly lower than in olive oil (*33*) and could not be compared with common vegetable oils (*34*). A very low value of FFA as determined for *M. oleifera* oil in the present analysis is an indicative of good quality of the crude oil (*35*).

M. oleifera oil also exhibited a very good oxidative state as indicated by the determinations shown in **Table 3**. The specific extinctions at 232 and 270 nm, which revealed the oxidative deterioration and purity of the oils (*36*), were similar to those of *M. peregrina* oil (*16*) but vary to some extent with regard to *M. oleifera* reported from Kenya (*30*). The induction period (Rancimat; 20 L/h, 120 °C), which is a characteristic of the oxidative stability of the oil and fats (*27*), of the non-degummed oil was 9.90 h, indicating a very good stability. After degumming, the IP of the oil was decreased to 8.63 h, a reduction of 14% in oxidative stability, which could be attributed to the degumming process. The peroxide value (0.59 mequiv/kg of oil) and *p*-anisidine value (1.30), which measure hydroperoxides and aldehydic secondary oxidation products of the oils, respectively (*35*), were quite low even for palm oil (*35*), thus showing high resistance to oxidation. There are no previously reported data of *Moringa* oils to compare the results of peroxide value and *p*-anisidine values with our present work. A high IP and oxidative stability of *M. oleifera* oil, exhibited in the present analysis, compared with those of conventional vegetable oils (*27*), were attributed to a significantly higher level of monoenoic fatty acids, particularly C18:1, which is less prone to oxidation than polyenoics (*27*). Moreover, a high resistance to oxidation of *M. oleifera* oil might be explained due to the presence of high contents of α-, γ-, and δ-tocopherols. Somali et al. (*1*) and Sengupta et al. (*2*) also reported a high stability of seed fat of the Moringaceae family.

Table 4 shows the content of different tocopherols in the non-degummed (crude) and degummed *M. oleifera* oils as determined by HPLC. The levels of α-tocopherol, γ-tocopherol, and δ-tocopherol in the non-degummed oil were 134.42, 93.70, and 48.00 mg/kg, respectively. The levels of these tocopherols in the degummed oil were reduced to 110.00, 81.63, and 41.00 mg/kg, reductions of 18.17, 12.89, and 14.59% in the α-, γ-,

and δ-isomers of tocopherol, respectively. This loss in the content of tocopherols is attributed to the degumming of the oils, because most of the steps involved during processing and storage reduce the level of tocopherols (*34*). As with significant of the other traits, there were no previously reported data on the tocopherol contents of degummed *M. oleifera* oil.

The investigated levels of α- and γ-tocopherol of the non-degummed *M. oleifera* oil, indigenous to Pakistan, were significantly higher than those reported in *M. oleifera* native to Kenya (*30*), whereas they were well in line with those reported in *M. peregrina* from Saudi Arabia (*16*). The content of α-tocopherol, which has greatest vitamin E potency (*34*), in the investigated oil was in close agreement with the values reported for soybean, groundnut, and palm oils (*34*). The concentration of δ-tocopherol, which has a greater antioxidant activity than either γ- or α-tocopherol (*16*), in the oil was slightly lower than the values reported for *Moringa* seed oils from Kenya and Saudi Arabia (*16*, *32*). However, it was significantly higher than in the cottonseed, groundnut, palm, sunflower, and high- and low-erucic acid rapeseed and olive oils (*33*, *34*) and, thus, would be expected to contribute to excellent oxidative stability and protection to the *M. oleifera* oil during storage and processing.

The composition of the sterols of *M. oleifera* oil as determined by GLC is shown in **Table 5**. The sterol fractions of the oil mainly consisted of campesterol (16.0%), stigmasterol (19.0%), β-sitosterol (46.65%), Δ^5-avenasterol (10.70%), and clerosterol (1.95%) accompanied with minute amounts of 24-methylenecholesterol, Δ^7-campestanol, stigmastanol, and 28-isoavenasterol. Cholesterol, brassicasterol, and campestanol components of sterols were not detected. The content of major sterols, that is, campesterol, stigmasterol, β-sitosterol, Δ^5-avenasterol, and clerosterol, of the investigated oil was quite comparable with the values for *M. oleifera* oil reported from Kenya (*30*), whereas it varied significantly from those of *M. peregrina* in Saudi Arabian seed oil (*16*). The content of minute fractions of sterols varied to some extent with the values of *Moringa* oils reported in the literature (*16*, *30*). The sterol composition of the major fractions of *M. oleifera* oil was greatly different from those of most of the conventional edible oils (*34*) and virgin olive oil (*33*) and thus could not be compared. Regional and cultivar variations for the distribution of campesterol, stigmasterol, β-sitosterol, Δ^5-avenasterol, and clerosterol have been reported in the literature (*33*, *34*).

Table 6 shows the fatty acid (FA) composition of *M. oleifera* oil. The content of total saturates, that is, palmitic ($C_{16:0}$), stearic ($C_{18:0}$), arachidic ($C_{20:0}$), and behenic ($C_{22:0}$) acids, in the oil was 20.17%, of which $C_{16:0}$ was the dominant acid. The oil was found to contain a high level of monounsaturated fatty acids up to 78.20%. Oleic ($C_{18:1}\omega-9$) was the predominant fatty acid, which accounted for 76.00% of the total fatty acids. The content of 18-C polyunsaturated linoleic fatty acid ($C_{18:2}\omega-6$) was 1.29%, whereas linolenic acid ($C_{18:3}\omega-3$) was not detected.

The concentration of major fatty acids, $C_{18:1}$, $C_{18:0}$, and $C_{16:0}$ of the oil, investigated in the present study was in close agreement with that reported by Tsaknis et al. (*30*) for the *M. oleifera* oil indigenous to Kenya. The amounts of $C_{20:0}$ (3.0%), $C_{22:0}$ (5.0%), and $C_{20:1}$ (1.40%) in the oil varied to a small extent from those reported in the literature (*30*).

The contents of $C_{18:1}$ and $C_{16:0}$ in *M. oleifera* oil indigenous to Pakistan, however, varied significantly from those reported in *M. peregrina* Saudi Arabian seed oil (*16*). The oil was higher by 6% in $C_{18:1}$ and lower by 2.4% in $C_{16:0}$. The amount of gadoleic acid ($C_{20:1}$) was well in line, and $C_{16:1}$ was also detected in the present analysis, which was not reported in *M. peregrina* oil.

The fatty acid composition of the investigated *M. oleifera* oil was quite similar in the contents of $C_{18:1}$ and $C_{18:0}$ with those of olive oil (*33*), but varied with respect to other component fatty acids. The fatty acid composition of this potential oil seed crop was also in good agreement with that reported by Ferrao et al. (*37*). Sengupta and Gupta (*2*) also found a similar fatty acid profile in *M. oleifera* L. The present fatty acid composition of the oil shows that it falls in the category of high-oleic oils and contains a high ratio of monounsaturated to saturated fatty acids. High-oleic oils, although genetically hard to reproduce, recently are gaining importance because of their superior stability and nutritional benefits (*19*, *38*).

Despite the fact that Pakistan is an agrarian economy, it is unable to produce oils sufficient for domestic needs. Resultantly, an enormous amount of foreign exchange is being spent every year for the import of vegetable oils and seeds. As Pakistan has vast fertile plains, agricultural lands, and a good irrigation system, *M. oleifera* appears to be a potentially valuable crop, yielding useful oil that might be an acceptable substitute for high-oleic oils such as olive and high-oleic sunflower oils in the dietary fats. Our present investigations revealed that *M. oleifea* seed oil indigenous to Pakistan has a very good potential for edible and industrial purposes as well as for developing nutritionally balanced, high-stability blended formulations with other high-linoleic oils. The investigated *M. oleifera* oil, naturally high in oleic acid and coincident with genetically modified oilseeds, might be well positioned in the local as well as international oil trade, provided it can be cultivated in widescale production. Nutritional evaluation of the oil should be further done.

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