## Interprovenance Variation in the Composition of *Moringa oleifera* Oilseeds from Pakistan

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ABSTRACT: Interprovenance variation was examined in the composition of Moringa oleifera oilseeds from Pakistan. The hexaneextracted oil content of *M. oleifera* seeds harvested in the vicinity of the University of Agriculture, Faisalabad (Punjab, Pakistan), Bahauddin Zakariya University (Multan, Pakistan), and the University of Sindh, Jamshoro (Sindh, Pakistan), ranged from 33.23 to 40.90%. Protein, fiber, moisture, and ash contents were found to be 28.52-34.00, 6.52-7.50, 5.90-7.00, and 6.52-7.50%, respectively. The physical and chemical parameters of the extracted *M. oleifera* oils were as follows: iodine value, 67.20-71.00; refractive index (40°C), 1.4570–1.4637; density (24°C), 0.9012–0.9052 mg/mL; saponification value, 177.29-184.10; unsaponifiable matter, 0.60-0.83%; color (1-in. cell), 1.00-1.50R + 20.00-30.00Y; smoke point, 198-202°C; and acidity (% as oleic acid), 0.50-0.74. Tocopherols ( $\alpha$ ,  $\gamma$ , and  $\delta$ ) accounted for 114.50–140.42, 58.05–86.70, and 54.20-75.16 mg/kg, respectively, of the oils. The induction periods (Rancimat, 20 L/h, 120°C) of the crude oils were 9.64-10.66 h and were reduced to 8.29-9.10 h after degumming. Specific extinctions at 232 and 270 nm were 1.80-2.50 and 0.54-1.00, respectively. The major sterol fractions of the oils were campesterol (14.13–17.00%), stigmasterol (15.88–19.00%), β-sitosterol (45.30-53.20%), and  $\Delta^5$ -avenasterol (8.84, 11.05%). The Moringa oils were found to contain high levels of oleic acid (up to 76.00%), followed by palmitic, stearic, behenic, and arachidic acids up to levels of 6.54, 6.00, 7.00, and 4.00%, respectively. Most of the parameters of M. oleifera oils indigenous to different agroclimatic regions of Pakistan were comparable to those of typical Moringa seed oils reported in the literature. The results of the present analytical study, compared with those for different vegetable oils, showed M. oleifera to be a potentially valuable oilseed crop.

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**KEY WORDS:** Agroclimatic, composition, high-oleic, interprovenance, *Moringa oleifera*.

The Moringaceae is a single-genus family with 14 known species. Of these, *Moringa oleifera* Lam. (syn. *M. ptery-gosperma* Gaertn.) is the most widely known and utilized species (1,2). A native of the sub-Himalayan regions of northwest India, *M. oleifera* is also indigenous to many countries in Africa, Arabia, Southeast Asia, the Pacific and Caribbean islands, and South America (2–4). In some parts of the world, *M. oleifera* is referred to as the "drumstick tree" or the "horseradish tree," whereas in others it is known as the "kelor tree" (5,6).

In the Nile River valley, the tree is called "Shagara al Rauwaq," which means "tree for purifying" (7).

In Pakistan, *Moringa* is represented by only two species: *M. concanensis* and *M. oleifera*. The former species is not common and is confined to the remote locality of Tharparker, Sindh (8). The latter, locally known as "Sohanjna," is widely grown in the Punjab plains, Sindh, Baluchistan, and in the North-Western Frontier Province (8). *Moringa oleifera*, which is indigenous to the subcontinent, is a small or medium-sized tree found wild or cultivated throughout the plains (5,8). It thrives best in a tropical insular climate; it is plentiful near the sandy beds of rivers and streams and is often cultivated in hedges and private gardens (5). Fully mature, dried seeds are round or triangular shaped, and the kernel is surrounded by a lightly wooded shell with three papery wings (1,5,8).

As a traditionally important food commodity, *M. oleifera* has received attention as a "natural nutrition of the tropics." The leaves, flowers, fruits, and roots of this multipurpose tree are locally esteemed as a vegetable (8,9). Indonesians eat both the leaves and seedpods of this tree, which reportedly taste like asparagus (6). In addition to its myriad uses and superior nutritional benefits, *M. oleifera* also has surprising medicinal attributes and is used in the treatment of ascites, rheumatism, venomous bites, and as a cardiac and circulatory stimulant (5,10).

Although over the years researchers have shown interest in the composition of *M. oleifera* seeds and the extracted oil, known commercially as "ben oil" or "behen oil," little is known about use of the seeds in the production of an edible oil. Ben oil has been used extensively in the enfleurage process, whereby delicate fragrances are extracted from flower petals (5). Oliveira et al. (11) described the composition and nutritional attributes of Moringa seeds and suggested that these antipyretic, acrid, and bitter seeds could be utilized for the purification of contaminated water (5,6,12). Somali et al. (4) reported the chemical composition and characteristics of M. peregrina seed oil. Tsaknis et al. (13) investigated M. oleifera seed oil (Mbololo variety) from Kenya and found that the oil concentration varied from 25 to 35.7%, depending on the extraction method. The oil was found to contain a high level of oleic acid (up to 75%),  $\beta$ -sitosterol (up to 50%), and different tocopherol isomers. Lalas and Tsaknis (14) described the M. oleifera seed oil variety Periyakulam-1 and compared the physical and chemical characteristics of oils extracted by different methods with those of seed oil from the variety Mbololo. Ibrahim et al. (15) reported that the content of ben seed oil and

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its properties showed wide variation, depending mainly on the species and environmental conditions.

From the perspective of globalization, with the ever-increasing demand for seed oils and scientific awareness regarding the nutritional and functional properties of such oils (16,17), characterization of some nonconventional oilseed crops is of great value in their development and commercialization. Despite the fact that Pakistan has an agrarian economy, its production of oils is insufficient for domestic needs. As a result, every year an enormous amount of foreign exchange is devoted to importing vegetable oils and seeds.

Until now, a full characterization and comparison of the oils produced from seeds of *M. oleifera* indigenous to different agroclimatic regions of Pakistan have not been reported. In this context, as a part of our systematic studies characterizing non-conventional oilseed crops (18,19), we have assayed *M. oleifera* seeds from their natural habitats in Pakistan and conducted a comprehensive analysis. The primary objective of the present study was to conduct a detailed analysis and to investigate interprovenance variation in the composition of *M. oleifera* oilseeds native to different agroclimatic regions of Pakistan. Our analytical findings were correlated with those of literature reports, which will accelerate efforts to establish a global database for this valuable plant.

## MATERIALS AND METHODS

*Materials.* The seeds of *M. oleifera* were assayed from three different agroclimatic regions of Pakistan. Samples of dry seeds from mature fruits were harvested in the vicinities of the University of Sindh, Jamshoro (Sindh, Pakistan; sample JMS), the University of Agriculture, Faisalabad (Punjab, Pakistan; sample FSD), and Bahauddin Zakariya University (Multan, Pakistan; sample MUL). The globular, three-winged seeds were covered with a thick blackish seed coat, average weight *ca.* 0.30 g, with an off-white kernel constituting 65–75% of the weight.

All reagents used (analytical and HPLC) were from E. Merck (Darmstadt, Germany) or Sigma-Aldrich (Buchs, Switzerland). Sterol standards were from Fluka Chemie GmbH (Buchs, Switzerland) and Sigma-Aldrich (CH-9471). Pure standards of tocopherols [DL- $\alpha$ -tocopherol, (+)- $\delta$ -tocopherol, (+)- $\gamma$ -tocopherol], and FAME were obtained from Sigma Chemical Co. (St. Louis, MO).

*Oil extraction.* After removal of the seed coat, the seeds (500 g) in each batch of *M. oleifera* were crushed and then fed into a Soxhlet extractor fitted with a 1-L round-bottomed 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 (used for the tocopherol and Rancimat analyses), the recovered oil from different batches was further degummed.

Degumming of oils. The oil to be degummed was heated at 70°C on a water bath, and hot water was added to a final volume of 18%. The mixture was mixed for 10 min with the aid of a glass rod. After cooling, the oil was centrifuged (3000 rpm;

 $1221 \times g$ ) for 12 min in 100 cm<sup>3</sup> tubes in an automatic refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan). The degummed and centrifuged oil was left in contact (stirred) with the anhydrous sodium sulfate for *ca*. 5 min, filtered through a filter paper by gravity in a drying oven at 50°C, and kept in separate sealed bottles under refrigeration (0–4°C) until used for further analysis.

Analysis of oilseed residues. The oilseed residues (meal) remaining after the extraction of oil from the seeds were analyzed for protein, fiber, and ash content. Protein content was determined according to a semi-automated FOSFA official method (20). Samples of meal were digested for 10 min with a digestion mixture of sulfuric acid/hydrogen peroxide/potassium sulfate, with selenium dioxide as a catalyst. The end point in the ammonia titration was measured photometrically.

Fiber content was determined according to an ISO method (21). A finely ground 2.5-g sample of meal was weighed and freed from fat by extraction with 15 mL of *n*-hexane. The test portion was boiled with a 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 of mass was determined.

Ash content was determined according to an ISO method (22). 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 a constant mass was achieved.

Analysis of extracted oils. (i) Physical and chemical parameters of the oils. Determinations of the density, refractive index, iodine value, PV, acidity, saponification value, and unsaponifiable matter of the extracted oil were carried out by various standard AOCS methods (23). The color of the oil was determined by a Lovibond Tintometer (Tintometer Ltd., Salisbury, Wiltshire, United Kingdom), using a 1-in. cell. Specific extinctions at 232 and 270 nm were determined using a Hitachi U-2001 spectrometer, model 121-0032. Samples were diluted with isooctane to bring the absorbance within limits (0.2–0.8) and  $\epsilon_{1cm}^{-1\%}$  ( $\lambda$ ) was calculated following an IUPAC method (24). *para*-Anisidine values were also determined by following an IUPAC method (2).

(ii) Oxidative stability. An automated Metrohm Rancimat apparatus, model 679, capable of operating over a temperature range of 50–200°C, was used to determine induction periods (IP) of the degummed and nondegummed oils (25). Testing was carried out at  $120 \pm 0.1$ °C, and oxidative stability was measured following a procedure described elsewhere (26). Briefly, oil (2.5 g) was carefully weighed into each of the six reaction vessels and analyzed simultaneously. IP of the samples were recorded automatically and corresponded to the break point in the plotted curves.

(*iii*) Tocopherol content. Tocopherol ( $\alpha$ ,  $\gamma$ , and  $\delta$ ) analysis was carried out by HPLC following the method of Thompson and Hatina (27) 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 a LiChrosorb SI-60 column (250 × 4.6 mm) packed with LiChrosorb SI-605 (5 µm; Supelco, Bellefonte, PA), which was fitted with a 50 × 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  $\alpha$ -,  $\gamma$ -, and  $\delta$ -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.

(*iv*) Sterol composition. Sterols determinations were made following the official method of the Association of Official Analytical Chemists (28). Analyses were carried out on a Perkin-Elmer gas chromatograph, model 8700, equipped with an OV-17 methylphenyl polysiloxane-coated capillary column (30 m × 0.25 mm, 0.20 µm film thickness; Supelco) and an FID. The column was isothermally operated at a temperature of 255°C. The injector and FID temperatures were set at 275 and 290°C, respectively. Extra-pure N<sub>2</sub> at a flow rate of 3 mL min<sup>-1</sup> was used as a carrier gas. The internal standard used was 5- $\alpha$ cholestane, and unknown sterol components were identified and quantified using a pure sterol standard mixture.

(v) FA composition. FAME were prepared by standard IUPAC method 2.301 and analyzed on a Perkin-Elmer model 8700 gas chromatograph fitted with an SP-2340 methyl lignoserate-coated (film thickness  $0.22 \ \mu$ m) polar capillary column (60 m × 0.25 mm; SGE Japan Inc., Yokohama, Japan) and

TABLE 2

 TABLE 1

 Composition of Moringa oleifera Seeds<sup>a</sup>

Content (%)	FSD	MUL	JMS
Oil	$33.23 \pm 0.72$	$35.30 \pm 0.90$	$40.90 \pm 0.80$
Moisture	$7.00 \pm 0.18$	$6.60 \pm 0.14$	$5.90 \pm 0.20$
Protein	$34.00 \pm 1.00$	31.86 ± 1.15	$28.52 \pm 0.84$
Fiber	$7.50 \pm 0.45$	$6.80 \pm 0.50$	$6.52 \pm 0.65$
Ash	$7.00 \pm 0.57$	$5.90 \pm 0.64$	$6.00\pm0.42$

<sup>a</sup>Values are means ± SD, calculated as the percentage of dry seed weights for three *M. oleifera* seed samples, analyzed individually in triplicate. FSD, Faisalabad; MUL, Multan; JMS, Jamshoro.

an FID. 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. FAME were identified by comparing their relative and absolute retention times with those of authentic standards. All quantification was done by a built-in data-handling program provided by the manufacturer of the gas chromatograph (Perkin-Elmer, Norwalk, CT).

## **RESULTS AND DISCUSSION**

Data for the analysis of three *M. oleifera* oilseeds indigenous to three different agroclimatic regions of Pakistan are summarized in Tables 1–6. Values for the present analysis are given as mean  $\pm$  SD of three different *M. oleifera* seeds from each region, analyzed individually in triplicate. The hexane-extracted oil content of *M. oleifera* seeds from the vicinity of three universities, representative of three different agroclimatic regions, was found to

Physical and Chemical Characteristics of <i>M. oleifera</i> Oils <sup>a</sup>				
Determination	FSD	MUL	JMS	
Color				
Red units	$1.50 \pm 0.08$	$1.00 \pm 0.15$	$1.00 \pm 0.10$	
Yellow units	$25.00 \pm 1.5$	$20.00 \pm 1.15$	$30.00 \pm 2.00$	
Refractive index (n <sub>D</sub> 40°C)	$1.4570 \pm 0.002$	$1.4630 \pm 0.001$	1.4600 ± 0.001	
Density (24°C) (mg/mL)	$0.9012 \pm 0.003$	$0.9052 \pm 0.004$	0.9021 ± 0.002	
lodine value (g of I/100 g of oil)	67.20 ± 0.70	$71.00 \pm 0.48$	$69.00 \pm 0.62$	
Unsaponifiable matter (%)	$0.60 \pm 0.06$	$0.83 \pm 0.05$	$0.75 \pm 0.08$	
Saponification value (mg of KOH/g of oil)	$184.10 \pm 1.40$	177.29 ± 1.00	181.00 ± 1.15	
Smoke point (°C)	202 ± 1.20	198 ± 1.50	200 ± 1.18	
Acidity (% as oleic acid)	$0.62 \pm 0.03$	$0.74 \pm 0.04$	$0.50 \pm 0.04$	

<sup>a</sup>Values are means  $\pm$  SD of three oils, analyzed individually in triplicate. For abbreviations see Table 1.

Determination	FSD	MUL	JMS
$\epsilon_{1cm}^{1\%}_{(\lambda 232)}$	$1.80 \pm 0.07$	$2.07 \pm 0.05$	$2.50 \pm 0.10$
$\epsilon_{1cm}^{1\%}$ ( $\lambda 270$ )	$0.54 \pm 0.06$	$1.00 \pm 0.05$	$0.75 \pm 0.07$
PV (meq/kg of oil)	$0.80 \pm 0.04$	$1.35 \pm 0.03$	$1.57 \pm 0.05$
p-Anisidine value	$2.80 \pm 0.10$	$3.05 \pm 0.06$	$1.98\pm0.10$
Oxidative stability,			
Rancimat method (h)			
ndmo	$10.66 \pm 0.14$	$9.64 \pm 0.21$	$9.83 \pm 0.17$
dmo	$9.10 \pm 0.18$	$8.40 \pm 0.14$	$8.29 \pm 0.22$

 TABLE 3

 Determination of the Oxidative State of *M. oleifera* Oils<sup>a</sup>

<sup>a</sup>Values are means ± SD of three oils, analyzed individually in triplicate. ndmo, nondegummed oil; dmo, degummed oil; for other abbreviations see Table 1.

vary significantly (Table 1), ranging from 33.2 to 40.9%. The oil concentration was high in the seeds collected from the vicinity of the University of Sindh, Jamshoro, which is located in a dry region of Pakistan on the bank of the river Indus, whereas the seeds assayed from *Moringa* plants grown in the Botanical Garden of the University of Agriculture, Faisalabad (which lacks a moderate climate), were generally low in oil content. This variation in oil yield of seeds from different regions of Pakistan may be attributed to the diversity in natural soil texture and to climatic constraints.

*Moringa oleifera* grows abundantly in the Sindh province of Pakistan but sparsely in the Multan and Faisalabad regions. The former region is close to the tropics, where the seasonal variation in temperature is not so great as in the latter two regions. Furthermore, the soil texture in the Sindh province is sandy, whereas that in the Multan and Faisalabad regions is generally loamy. The large variation in seasonal temperature as well as the specific soil textures of the Multan and Faisalabad regions might have been the two major factors responsible for reducing the oil content of seeds in these regions.

The oil content (40.9%) of *M. oleifera* seeds collected from the vicinity of the University of Sindh, Jamshoro, was considerably higher than those reported for *M. oleifera* seeds (variety Mbololo) from Kenya (35.7%) (13) and *M. oleifera* seeds (variety Periyakulam-1) from India (38.3%) (14). However, the oil yield was lower by *ca.* 9% than that reported from *M. peregria* seeds from Saudi Arabia (29). Such variation in oil content across countries and species is attributable to the environmental and geological conditions of the regions (15). The oil content in *M. oleifera* seeds from different agroclimatic regions of Pakistan ranged from 33.2 to 40.9%, compared with four conventional oilseed crops grown in the United States, Brazil, China, and other Asian and European countries (30): cotton (15.0–24.0%), soybeans (17.0–21.0%), safflower (25.0– 40.0%), and mustard (24.0–40.0%).

Interprovenance analysis of the oilseed residues revealed a high protein content for the seeds, ranging from 28.5 to 34.0%, whereas the fiber and ash contents were low, at 6.5–7.5 and 5.9–7.0%, respectively. The oilseed samples high in oil content were generally found to be lower in protein, fiber, and ash contents. The analysis showed the meals to be a good source of

protein, which could be added to poultry diets as a source of calories and to replace soybean meal in the local poultry industry. It could also be used as a fertilizer, as a potential animal foodstuff (following saponin detoxification if necessary), and as a source of water treatment chemicals, all of which provide value-added by-products. There are reports in the literature that the *M. oleifera* husk and the oilseed residue remaining after oil extraction contain active fractions that could be used as water-purifying agents (12).

Various physical and chemical characteristics of the extracted oils are given in Table 2. The color, iodine value, refractive index, density, FFA content, unsaponifiable matter, saponification value, and smoke point determined for M. oleifera FSD, MUL, and JMS oils indigenous to Pakistan were in good agreement with those reported for M. oleifera (variety Mbololo) oil from Kenya and M. peregrina oil from Saudi Arabia (13,29). The color (1.0R + 20.0Y) of the *M. oleifera* oil from the Multan region of Pakistan was superior to that from other regions and to M. oleifera (variety Mbololo) oil from Kenya (13). The intensity of the color of vegetable oils depends mainly on the presence of various pigments such as chlorophyll, which are effectively removed during the degumming, refining, and bleaching steps of oil processing. Vegetable oils with minimum color index values are more suitable for edible and industrial purposes.

The saponification values (177–184 mg of KOH/g of oil) and unsaponifiable matter (0.60–0.83%) in the *Moringa* oils were in good agreement with those for olive oil (31) and were similar to those for corn, niger seed, low erucic acid rapeseed, soybean, sunflower, safflower, tomato seed, and winged-bean oils, as well as for mango kernel fat (31,32). The refractive indices of the oils

TABLE 4	
Tocophere	ol Content of Nondegummed <i>M. oleifera</i> Oils <sup>a</sup>
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Tocopherols (mg/kg)	FSD	MUL	JMS
α-Tocopherol	$114 \pm 2.60$	$129.82 \pm 3.00$	$140.42 \pm 4.00$
γ-Tocopherol	$58.05 \pm 2.10$	$70.90 \pm 2.00$	$86.70 \pm 3.50$
δ-Tocopherol	$75.16 \pm 2.25$	$54.20 \pm 2.30$	$62.00 \pm 4.15$

 $^{a}$ Values are means  $\pm$  SD of three oils, analyzed individually in triplicate. For abbreviations see Table 1.

Sterol Composition of <i>M. oleifera</i> Oils <sup>a</sup>				
Sterols (%)	FSD	MUL	JMS	
24-Methylenecholesterol	$0.90 \pm 0.05$	ND	$1.49 \pm 0.10$	
Campesterol	$14.13 \pm 0.40$	$16.56 \pm 0.30$	$17.00\pm0.28$	
Campestanol	ND	$0.29 \pm 0.10$	ND	
$\Delta^7$ -Campestanol	ND	$0.70 \pm 0.10$	$0.40\pm0.06$	
Stigmasterol	$15.88 \pm 0.40$	$17.32 \pm 0.30$	$19.00 \pm 0.21$	
Clerosterol	$2.05 \pm 0.10$	$3.00 \pm 0.15$	$1.95 \pm 0.07$	
Stigmastanol	$0.65 \pm 0.10$	$0.96 \pm 0.05$	$1.09\pm0.07$	
β-Sitosterol	$52.20 \pm 0.60$	$48.00\pm0.50$	$45.30 \pm 1.00$	
$\Delta^7$ -Avenasterol	$1.90 \pm 0.08$	$0.95 \pm 0.10$	$1.25 \pm 0.06$	
$\Delta^5$ -Avenasterol	$8.84 \pm 0.15$	$10.00 \pm 0.20$	$11.05 \pm 0.43$	
28-Isoavenasterol	$1.0 \pm 0.07$	$0.85 \pm 0.10$	$0.50\pm0.06$	

TABLE 5	
Sterol Composition of	of <i>M. oleifera</i> Oils <sup>a</sup>

TADLE

<sup>a</sup>Values are means ± SD of three oils, analyzed individually in triplicate. ND, not detected; for other abbreviations see Table 1.

were within the range for cottonseed, brazil nut, hazelnut, palm, and mango kernel oils as well as for dhupa fats (31,32), whereas the iodine value and FFA content of the oils were significantly lower than those for olive oil (31) and could not be compared with those of common vegetable oils available in the literature (32). A very low content of FFA for the different M. oleifera oils in the present analysis is indicative of the good resistance of these oils to hydrolysis.

Moringa oleifera oils from different regions of Pakistan were in a very good oxidative state, as indicated by the low PV and *p*-anisidine values shown in Table 3. The specific extinctions at 232 and 270 nm, which reveal the oxidative deterioration and purity of the oils (33), were more or less similar to those of *M. peregrina* oil (29) but varied to some extent from the M. oleifera oil reported from Kenya (13). The induction periods (Rancimat: 20 L/h, 120°C), which were used to characterize the oxidative stability (26) of the nondegummed M. oleifera FSD, MUL, and JMS oils, were 10.66, 9.64, and 9.83 h, respectively, indicating very good stability. After degumming, the IP of the respective oils decreased to 9.10, 8.40, 8.29 h, reductions of 14.63, 12.86, and 15.66% in oxidative stability. The PV (meq/kg of oil) and p-anisidine values, which measure hydroperoxides and aldehydic secondary oxidation products of the oils (34), were both quite low (i.e., FSD, 0.80, 2.80; MUL, 1.35, 3.05; JMS, 1.57, 1.98, respectively), showing high resistance to oxidation. The PV found in the present analysis were generally lower than those reported in the literature for *M. oleifera* (13). The high oxidative stability of *M. oleifera* oil, compared with conventional vegetable oils, could be attributed to the high level of monoenoic FA, particularly, 18:1, which is less prone to oxidation than polyenoics (27). Moreover, the high resistance of *M. oleifera* oil to oxidation might be attributed to the presence of a high content of  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherols. Somali et al. (4) and Sengupta and Gupta (1) also reported that seed fat of the Moringaceae family was highly stable.

Table 4 shows the content of different tocopherols in *M*. *oleifera* oils. The levels of  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherol in the FSD, MUL, and JMS oils ranged from 114.5 to 140.4, 58.0 to 86.7, and 54.2 to 75.1 mg/kg, respectively. The levels of  $\alpha$ - and  $\gamma$ tocopherols in the M. oleifera oils indigenous to Pakistan were significantly higher (P < 0.005) than those reported in the M. oleifera native to Kenya (13), whereas they were well in line with those reported in the *M. peregrina* from Saudi Arabia (29). The content of  $\alpha$ -tocopherol, which has the greatest vitamin E potency (32), was higher in *M. oleifera* oils than in soybean, palm, and coconut oils but was lower than those in cottonseed, maize, and sunflower oils (32). In the FSD oil, the concentration of  $\delta$ -tocopherol, which has greater antioxidant activity than either  $\gamma$ - or  $\alpha$ -tocopherol (29), was comparable to the values reported for Moringa seed oils from Kenya and Saudi Arabia (13,29), whereas it was lower in the MUL and JMS oils than in the Moringa oils reported in the literature (13,29). However, it was considerably higher than that in cottonseed, groundnut, coconut, palm, sunflower, and high and low erucic acid rapeseed and olive oils (31,32); thus, it would be expected to contribute excellent oxidative stability and protection to the *M. oleifera* oil during storage and processing.

TABLE 6			
FA Composition	of M.	oleifera	Oils <sup>a</sup>

FA	FCD	N 41-11	19.46
(g/100 g of FA)	FSD	MUL	JMS
14:0	$0.18\pm0.10$	ND	ND
16:0	$6.54 \pm 0.15$	$6.20\pm0.16$	$5.98 \pm 0.20$
16:1	$0.50\pm0.08$	$0.87 \pm 0.10$	$1.10 \pm 0.07$
18:0	$6.00 \pm 0.21$	$4.95 \pm 0.15$	$4.47 \pm 0.29$
18:1	$72.00 \pm 1.00$	$74.80\pm0.73$	$76.00\pm0.50$
18:2	$0.87 \pm 0.13$	$1.65 \pm 0.10$	$1.20\pm0.10$
18:3	ND	$0.20\pm0.06$	ND
20:0	$4.00\pm0.16$	$3.68 \pm 0.10$	$3.50\pm0.20$
20:1	$2.00\pm0.06$	$0.97\pm0.07$	$1.40 \pm 0.10$
22:0	$7.00\pm0.25$	$6.10\pm0.15$	$5.65 \pm 0.25$

<sup>a</sup>Values are means ± SD of three oils, analyzed individually in triplicate. For abbreviations see Tables 1 and 5.

The composition of different sterols in the *M. oleifera* oils is shown in Table 5. The sterol fractions of the FSD, MUL, and JMS oils consisted mainly of  $\beta$ -sitosterol (45.3–52.2%), stigmasterol (15.8–19.0%), campesterol (14.1–17.0%), and  $\Delta^5$ avenasterol (8.8–11.0%) together with small amounts (<3.0%) of clerosterol, 24-methylene cholesterol,  $\Delta^7$ -campestanol,  $\Delta^7$ avenasterol, stigmastanol, and 28-isoavenasterol. The minute amounts (<0.13%) of cholesterol and brassicasterol components reported in the *M. oleifera* oil from Kenya (13) were not detected in the present analysis.

The contents of the major sterols, i.e., campesterol, stigmasterol,  $\beta$ -sitosterol, and  $\Delta^5$ -avenasterol, in the oils investigated were comparable with the values reported for *M. oleifera* oil from Kenya (13) and to another *M. oleifera* oil (variety Periyakulam-1) (14), whereas apart from  $\Delta^5$ -avenasterol, they varied significantly from those of the *M. peregrina* seed oil from Saudi Arabia (29). The interprovenance sterol composition of major fractions of the *M. oleifera* oils investigated was significantly different from those of most conventional edible oils (32) and of virgin olive oil (31), and thus could not be compared. Regional and cultivar variations in the distribution of campesterol, stigmasterol,  $\beta$ -sitosterol,  $\Delta^5$ -avenasterol, and clerosterol have already been reported in the literature (31,32).

Table 6 shows the FA composition of the *M. oleifera* oils from different regions of Pakistan. Total saturates, i.e., palmitic (16:0), stearic (18:0), arachidic (20:0), and behenic (22:0) acids, in the FSD, MUL, and JMS oil samples were 23.5, 20.9, and 19.6%, respectively. The *Moringa* oils investigated were found to contain high levels of monounsaturated FA, up to 74.5, 76.6, and 78.0%, respectively. Oleic (18:1n-9) was the predominant FA, accounting for 72.0, 74.8, and 76.0% of the total FA in the respective oils. The content of the 18-carbon PUFA linoleic acid (18:2n-6) in the corresponding oils was 0.87, 1.6, and 1.2%, respectively, whereas linolenic acid (18:3n-3) was rarely detected.

The concentration of major FA, i.e., 18:1, 18:0, and 16:0, in the *Moringa* oils was in close agreement with that reported by Tsaknis *et al.* (13) for the *M. oleifera* oil indigenous to Kenya. The amounts of 20:0 in FSD, MUL, and JMS, respectively, (4.0, 3.6, and 3.5%), 22:0 (7.0, 6.1, and 5.6%), and 20:1 (2.0, 0.9, and 1.4%) in the corresponding oils did not vary to a significant extent from those reported in the literature (13). However, the *Moringa* oils investigated from Pakistan were devoid of the small amounts of 22:1 and 26:0 reported in the *M. oleifera* oil from Kenya (13).

The contents of 18:1 and 16:0 in the *M. oleifera* oil samples native to the Multan (Punjab) and Jamshoro (Sindh) regions of Pakistan varied somewhat from those reported in *M. oleifera* (variety Periyakulam-1) oil and the Saudi Arabian *M. peregrina* seed oil (14,29). The amount of gadoleic acid (20:1) was well in line with these reports, although 16:1, which was not reported for the *M. peregrina* oil, was detected in the present analysis.

In the *M. oleifera* oils investigated, the FA compositions were quite similar to olive oil in their contents of 18:1 and 18:0 (31) but varied with respect to other component FA and could not be compared with other conventional vegetable oils (34).

The FA composition of the *Moringa* oils from Pakistan was also in good agreement with that reported by Ferrao and Ferrao (35) but varied somewhat from that reported by Sengupta and Gupta (1).

The present FA compositions of *M. oleifera* oils from different agroclimatic regions of Pakistan show that this oilseed crop falls into the category of high-oleic oils and contains a high ratio of monounsaturated to saturated FA. High-oleic oils, although genetically hard to reproduce, have been gaining importance recently because of their superior stability and nutritional benefits (36,37).

Because Pakistan has vast fertile plains, agricultural lands, and a good irrigation system, *M. oleifera* appears to be a potentially valuable crop, yielding a useful oil that might be an acceptable fat substitute for high-oleic oils such as olive oil and high-oleic sunflower oil in our diets. Our investigation revealed that oil of *M. oleifera* seeds indigenous to Pakistan has very good potential for edible and industrial purposes. It could be incorporated into other high-linoleic oils to develop nutritionally balanced, high-stability blended formulations. The *M. oleifera* oils from Pakistan, which are naturally high in oleic acid and are coincident with oils produced from genetically modified oilseeds, might be well positioned for local and international oil trade, although further nutritional evaluation of the oil should be conducted.

## REFERENCES

- Sengupta, A., and M.P. Gupta, Studies on the Seed Fat Composition of Moringaceae Family, *Fette Seifen Anstrichm*. 72:6–10 (1972).
- Morton, J.F., The Horseradish Tree, *Moringa pterigosperma* (Moringaceae)—A Boon to Arid Lands? *Econ. Bot.* 45:318–333 (1991).
- Mughal, M.H., G. Ali, P.S. Srivastava, and M. Iqbal, Improvement of the Drumstick (*Moringa pterygosperms* Gaertn.)—A Unique Source of Food and Medicine Through Tissue Culture, *Hamdard Med.* 42:37–42 (1999).
- Somali, M.A., M.A. Bajnedi, and S.S. Al-Fhaimani, Chemical Composition and Characteristics of *Moringa peregrina* Seeds and Seed Oil, *J. Am. Oil Chem. Soc.* 61:85–86 (1984).
- Council of Scientific and Industrial Research (ed.), *The Wealth* of India (A Dictionary of Indian Raw Materials and Industrial Products): Raw Materials, Council of Scientific and Industrial Research, New Delhi, 1962, Vol. 6: L–M, pp. 425–429.
- Australian New Crops, 19.2: *Moringa oleifera* (the kelor tree), *Aust. New Crops Newslett. 9* (January 1998), http://www.new crops.uq.edu.au/newslett/ncn19192.htm (accessed June 12, 2003).
- Von Maydell, H.J., *Trees and Shrubs of the Sahal, Their Characteristics and Uses*, Deutsche Gesellschaft fur Technische Zusammenarbeit, Eschborn, Germany, 1986, pp. 334–337.
- Qaiser, M., Moringaceae, in *Flora of West Pakistan*, edited by E. Nasir and S.I. Ali, Department of Botany, University of Karachi, Karachi, Pakistan, 1973, No. 38, pp. 1–4.
- Siddhuraju, P., and K. Becker, Antioxidant Properties of Various Solvent Extracts of Total Phenolic Constituents from Three Different Agroclimatic Origins of Drumstick Tree (*Moringa oleifera* Lam.), J. Agric. Food Chem. 51:2144–2155 (2003).
- Dahot, M.U., Vitamin Contents of Flowers and Seeds of Moringa oleifera, Pak. J. Biochem. 21:21–24 (1988).

- Oliveira, J.T.A., S.B. Silveira, I.M. Vasconcelos, B.S. Cavada, and R.A. Moreira, Compositional and Nutritional Attributes of Seeds from the Multiple Purpose Tree *Moringa oleifera* Lamarck, J. Sci. Food Agric. 79:815–820 (1999).
- Ndabigengeser, A., and K.S. Narasiah, Use of *Moringa oleifera* Seeds as a Primary Coagulant in Wastewater Treatment, *Environ. Technol.* 19:789–800 (1998).
- Tsaknis, J., S. Lalas, V. Gergis, V. Dourtoglou, and V. Spilitois, Characterization of *Moringa oleifera* Variety Mbololo Seed Oil of Kenya, *J. Agric. Food Chem.* 47:4495–4499 (1999).
- Lalas, S., and J. Tsaknis, Characterization of *Moringa oleifera* Seed Oil Variety Periyakulam-1, *J. Food Compos. Anal.* 15:65–77 (2002).
- Ibrahim, S.S., M. Ismail, G. Samuel, E. Kamal, and T. El Azhari, Benseed, a Potential Oil Source, *Agric. Res. Rev.* 52:47–50 (1974).
- Neuza, J., and G. Lireny, Behaviour of High Oleic Sunflower Heated Under Thermal Oxidation and Frying Conditions, *Cienc. Tecnol.* 18:335–342 (1998).
- Delplanque, B., The Nutritional Interests in Sunflower Oil: Sunflower Seed with Linoleic and High Oleic Acid Contents, *Ol. Corps Gras, Lipides* 7:467–472 (2000).
- Anwar, F., and M.I. Bhanger, Analytical Characterization of Moringa oleifera Seed Oil Grown in Temperate Regions of Pakistan, J. Agric. Food Chem. 51:6558–6563 (2003).
- Anwar, F., M.I. Bhanger, M.K.A. Nasir, and S. Ismail, Analytical Characterization of *Salicornia bigelovii* Seed Oil Cultivated in Pakistan, J. Agric. Food Chem. 50:4210–4214 (2002).
- Federation of Oils Seeds and Fats Association International (FOSFA International), *Manual of the Federation of Oils Seeds* and Fats Association, FOSFA International, London, 1982, p. 277.
- International Organization for Standardization (ISO), Animal Feeding Stuffs—Determination of Nitrogen and Calculation of Crude Protein Content, ISO, Geneva, 1981, Standard No. 5983.
- International Organization for Standardization (ISO), Oilseed Residues—Determination of Total Ash, ISO, Geneva, 1977, Standard No. 749.
- American Oil Chemists' Society (AOCS), Official and Recommended Practices of the AOCS, 5th edn., AOCS Press, Champaign, 1997.
- 24. International Union of Pure and Applied Chemistry (IUPAC), Standard Methods for the Analysis of Oils, Fats and Derivatives, 7th rev. enlarged edn., edited by C. Paquot and A. Hautfenne, Blackwell Scientific, London, 1987.
- 25. Metrohm Application Bulletin No. 204/1e: Oxidative Stability of

*Oils and Fats—Rancimat Method*, Metrohm AG, Herisau, Switzerland, 1993, pp. 1–4.

- Anwar, F., M.I. Bhanger, and T.G. Kazi, Relationship Between Rancimat and Active Oxygen Method Values at Varying Temperatures for Several Oils and Fats, *J. Am. Oil Chem. Soc.* 80:151–155 (2003).
- Thompson, J.N., and G. Hatina, Determination of Tocopherols and Tocotrienols in Foods and Tissues by HPLC, *J. Liquid Chromatogr.* 2:327–344 (1979).
- Association of Official Analytical Chemists (AOAC), Official Methods of the Association of Official Analytical Chemists, 14th edn., AOAC, Arlington, VA, 1984, Method 28.110, p. 52.
- Tsaknis, J., Characterization of *Moringa peregrina* Arabian Seed Oil, *Grasas Aceites* (Sevilla) 49:170–176 (1998).
- Pritchard, J.L.R., Analysis and Properties of Oilseeds, in *Analysis of Oilseeds, Fats and Fatty Foods*, edited by J.B. Rossell and J.L.R. Pritchard, Elsevier Applied Science, New York, 1991, pp. 80–98,127.
- Norman, O.V.S., Composition and Characteristics of Individual Fats and Oils, in *Bailey's Industrial Oil and Fat Products*, 4th edn., edited by D. Swern, John Wiley & Sons, New York, 1979, Vol. 1, pp. 289–459.
- 32. Rossell, J.B., Vegetable Oils and Fats, in *Analysis of Oilseeds*, *Fats and Fatty Foods*, edited by J.B. Rossell and J.L.R. Pritchard, Elsevier Applied Science, New York, 1991, pp. 261–319.
- Yoon, S.H., S.K. Kim, M.G. Shin, and K.H. Kim, Comparative Study of Physical Methods for Lipid Oxidation Measurement, *J. Am. Oil Chem. Soc.* 68:1487–1489 (1985).
- 34. McGinely, L., Analysis and Quality Control for Processing and Processed Fats, in *Analysis of Oilseeds, Fats and Fatty Foods*, edited by J.B. Rossell and J.L.R. Pritchard, Elsevier Applied Science, New York, 1991, pp. 460–470.
- Ferrao, A.M.B., and J.E.M. Ferrao, Acidos gordos em oleo de moringuerio, Agron. Angolana 30:3–16 (1970).
- Neuza, J., and G. Lireny, Behaviour of High Oleic Sunflower Heated Under Thermal Oxidation and Frying Conditions, *Cienc. Tecnol.* 18:335–342 (1998).
- Delplanque, B., The Nutritional Interests in Sunflower Oil: Sunflower Seed with Linoleic and High Oleic Acid Contents, *Ol. Corps Gras, Lipides* 7:467–472 (2000).

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