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PHYSICO-CHEMICAL CHARACTERISTICS OF *MORINGA OLEIFERA* SEEDS AND SEED OIL FROM A WILD PROVENANCE OF PAKISTAN

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Abstract

The purpose of the present study was to examine the physico-chemical characteristics of *Moringa oleifera* seeds and seed oil from a wild provenance of Pakistan. The Moringa seeds harvested from the forests of Kohat district of NWFP exhibited an oil yield of 34.80%. Protein, fiber, moisture and ash contents were 31.65, 7.54, 8.90 and 6.53%, respectively. The extracted *M. oleifera* seed oil revealed an iodine value of 68.63; refractive index (40°C), 1.4571; density (24°C), 0.9032 g cm^{-3}; saponification value, 181.4; unsaponifiable matter, 0.74%; acidity (as oleic acid) 0.81% and color (1-in. cell) 1.28 R + 31.00 Y. Determinations of oxidation parameters like induction period (Rancimat 20 L/h, 120°C), specific extinctions at 232 and 270 nm, peroxide- and *p*-anisidine values demonstrated a good oxidative stability of the investigated *M. oleifera* oil. Tocopherols (α, γ and δ) contents of the oil amounted to 140.5, 63.18 and 61.70 mg kg^{-1}, respectively and were reduced considerably after degumming. The major sterol components of the oil were β-sitosterol (46.16%), campesterol (17.59%), stigmasterol (18.80%) and Δ^5, avenasterol (9.26%). The wild *M. oleifera* seed oil was found to contain oleic acid up to 73.22%, followed by palmitic, stearic, behenic and arachidic acids 6.45, 5.50, 6.16 and 4.08%, respectively and fell in the category of high-oleic oils. The results of different quality attributes of *M. oleifera* oil from a wild provenance of Pakistan reveal that it could be employed for edible and commercial applications.

Introduction

Moringa oleifera is esteemed as a versatile plant due to its multiple uses. The leaves, fruits, flowers and immature pods of this tree are edible and they form a part of traditional diets in many countries of the tropics and sub-tropics (Siddhuraju & Becker, 2003; Anhwange *et al.*, 2004). The leaves of *M. oleifera* are a good source of protein, vitamin A, B and C and minerals such as calcium and iron (Dahot, 1988). In addition to its substantial uses and nutritional benefits, *M. oleifera* also has a great potential as a medicinal plant. The flowers, leaves and roots are used for the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulants in folk remedies. The roots of the young tree and also root bark are rubefacient and vesicant (Hartwell, 1995; Anwar & Bhanger, 2003; Anwar *et al*., 2007).

The seeds from this plant contain active coagulating agents characterized as dimeric cationic proteins, having molecular weight of 13 kDa and an isoelectric point between 10 and 11. The seeds also have antimicrobial activity and are utilized for waste water treatment. In some developing countries, the powdered seeds of *M. oleifera* are traditionally utilized as a natural coagulant for water purification because of their strong coagulating properties for sedimentation of suspended undesired particles (Kalogo *et al.*, 2000; Anwar *et al*., 2007).

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Moringa seed kernels contain a significant amount of oil that is commercially known as "Ben oil" or "Behen oil". The Ben oil was erroneously reported to be resistant to rancidity and used extensively in the "enfleurage" process (Ndabigengeser & Narasiah, 1998). Moringa seed oil content and its properties show a wide variation depending mainly on the species and environmental conditions (Ibrahim *et al.*, 1974).

In Pakistan, only two species of *Moringa*: *M. concanensis* and *M. oleifera* are reported. The former species is rare and confined to only a remote locality of Tharparkar, Sindh province of Pakistan The latter species is mostly cultivated in the Sindh province and irrigated plains of the country (Manzoor *et al*., 2007; Qaiser, 1973).

Wild *M. oleifera* grows naturally in forests of North West Frontier Province (N.W.F.P) of Pakistan. It is recognized as one of the main non-wood forest products of Pakistan. The wild fruit is collected by graziers from forests and sold in the local market. There has been reported a small supply about 10 tons, which is used domestically (Iqbal, 1991).

The increasing human population pressure together with the currently growing momentum of oleo-chemicals and oils/fats derived fuels (Biodiesel) has made it imperative to take advantages of more and more vegetable oils to meet the growing needs of the world. Although a number of under explored plants have been identified, lack of information on their chemical composition has limited their applications. The detailed scientific knowledge regarding the composition of *M. oleifera* (wild type) seed oil is of considerable importance for the development and commercialization of this potential oilseed crop.

To date, a detailed composition of the oil produced from seeds of *M. oleifera* plants native to a wild provenance of sub-Himalayan tract of NWFP, Pakistan has not yet been reported. The present research was therefore undertaken with the main objective to conduct a detailed analysis and to investigate the composition and quality attributes of *M. oleifera* seed oil extracted from seeds of wild *Moringa* plants native to NWFP of Pakistan.

Material and Methods

The seeds (each sample 1.5 kg) of wild *M. oleifera* were assayed from the forests in the periphery of Kohat city of NWFP, Pakistan. Samples of dry seeds from mature fruits were harvested from three different localities of the forests. The globular and three-winged seeds were covered with a thick pale yellow to whitish seed coat, average seed weight *ca.* 0.13 g, with off-white kernel constituting 72-76% of the seed weight.

All reagents (analytical and HPLC) used were from Merck (Darmstadt, Germany) or Sigma Aldrich (St. Louis, MO). Pure standards of sterols, tocopherols [Dl-α-tocopherol, (+)-δ-tocopherol, -γ-tocopherol], and fatty acid methyl esters (FAMEs) were obtained from Sigma Chemicals Co., (St. Louis, MO).

Oil extraction: After the removal of the seed coat, the seeds (350 g) in each batch of *M. oleifera* were crushed and then fed into a Soxhelt extractor fitted with a 1-L round-bottom flask and a condenser. The extraction was executed on a water bath for 5-6 h with 0.6 L of *n*-hexane. After extraction, the solvent was distilled off under vacuum in a rotary evaporator (EYELA, N.N. Series, Rikakikai Co. Ltd. Tokyo, Japan). Except for a small quantity (used for 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 water bath and hot water was added to a final volume of 18%. The mixture was mixed for 12 minutes with the aid of a glass rod. After cooling, the oil was centrifuged (1221 x g) for 10 minuets in tubes of 100 cm^3 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 the Rancimt and tocopherol analysis.

Analysis of oil seed residues: The oil seed residues (meal) remaining after the extraction of oil from the seeds were analyzed for protein, fiber and ash contents. Protein content was determined according to the Association of Official Analytical Chemists (AOAC) standard method 976.06 (Anon., 1990).

Fiber content was determined according to the ISO method 5983 (Anon., 1981). A finely ground 2.5g 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 (EYELA, TMF-2100, Tokyo, Japan) at 600°C and the loss of mass was determined.

Ash content was determined according to the ISO method 749 (Anon., 1977). 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 (EYELA, TMF-2100, Tokyo, Japan) at 550°C until constant mass was achieved.

Analysis of extracted oil

Physical and chemical parameters of oil: Determination of density, refractive index, iodine value, peroxide value, acidity, saponification value and unsaponifiable matter of the extracted oil was carried out following standard AOCS methods Cc 10a–25, Cc 7–25, Cd 1–25, Cd 8–53, F 9a–44, Cd 3–25 and Ca 61–40, respectively (Anon., 1997). The color of the oil was determined by a Lovibond Tintometer (Tintometer Ltd., Salisbury, Wiltshire, United Kingdom), using a 1-inch cell.

Specific extinctions at 232 and 270 nm were determined using a spectrophotometer (U-2001, Hitachi Instruments Inc. Tokyo, Japan). Samples of oil were diluted with *iso*-octane to bring the absorbance within limits (0.2-0.8) and ($^{1\%}\varepsilon_{1cm\ (\lambda)}$) was calculated following the IUPAC method II D.23 (Anon., 1987).

The *p*-anisidine value was determined according the IUPAC method II.D.26 (Anon., 1987). The oil samples were dissolved in *iso*-octane and reacted with *p*-anisidine solution in acetic acid (0.25% w/v) for 10 minutes to produce a colored complex, the absorbance of which was read at 350 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc. Tokyo, Japan).

Measurement of oxidative stability: An automated Metrohm Rancimat apparatus, Model 679, operating over a temperature range of 50-200°C, was used to measure the induction periods (IP) of the non-degummed and degummed oils (Anon., 1993). Determination was carried out at 120 ± 0.1°C following the procedure described by

Anwar *et al*., (2003). Briefly, oil (2.5 g) was weighed into each of the six reaction vessels and analyzed simultaneously. IP of the samples were recorded automatically and corresponded to the break point of the plotted curves.

Tocopherol content: Analysis of the tocopherol (α, γ and δ) was performed by HPLC following the Current Protocols in Food Analytical Chemistry method (Wrolstad, 2003). Oil (0.1g) and 0.05 g ascorbic acid were put in a 16×125-mm test tube. Five ml of 90% ethanol and 0.5 ml of 80% aqueous KOH solution were added to the test tube and vortexed for 30 s. The test tube was flushed with nitrogen, caped and incubated in a water bath (70°C) for 30 minutes with periodical vortexing. The tube was placed in an ice bath for 5 min, then 3 ml deionized water and 5 ml *n*-hexane were added and vortexed for 30 s followed by centrifugation at 1,000×*g* for 10 min, at room temperature. The upper hexane layer was transferred to another test tube. The aqueous layer and the residue were re-extracted by repeating the same practice. The upper hexane layers from both the extractions were combined and evaporated to dryness under nitrogen. One ml mobile phase was added to the tube and vortexed for 30 s to re-dissolve the extract and then transferred to an HPLC sample vial. A 20-µL sample was injected into a Supelcosil LC-Si column (250 × 4.6 mm, Supelco Inc.). A mobile phase of ethyl acetate/acetic acid/hexane (1:1:198, v/v/v) was used at the flow rate of 1.5 mL min^{-1}. Detection was performed at 295 nm. Tocopherols were identified by comparing the retention times with those of pure standards of α-, β-, γ- and δ- tocopherols and were quantified on the basis of peak areas of the pure standards (Sigma Chemical Co.). Quantification was based on an external standard method. A D-2500 Hitachi Chromatointegrator model with a built-in computer program for data handling was used for quantification.

Sterol composition: The determination of sterols was made following the Official method of the Association of Official Analytical Chemists (Anon., 1990). Analyses was carried out on a SHIMADZU gas chromatograph model 17-A, equipped with BPX5 (SGE Japan Inc. 23-4 Miyazaki-Cho, Nishi-Ku, Yokohama-Shi, Kanagawa 220, Japan) methylphenyl polysiloxane-coated capillary column (30m x 0.25 mm, 0.20 µm film thickness) and an FID. The column was isothermally maintained at 265°C. The injector and FID temperatures were set at 275 and 290°C, respectively. Oxygen-free pure N_2 at a flow rate of 3.5 mL min^{-1} was used as a carrier gas. The internal standard used was α-cholestanol. Sterols were identified and quantified by comparing the retention times and peak areas of the unknown components with those of known sterol standard mixture.

Fatty acid composition: Fatty acid methyl esters (FAMEs) were prepared by IUPAC standard method 2.301 (Anon., 1987) and analyzed on a SHIMADZU gas chromatograph model 17-A, fitted with SP-2330 (SUPELCO, INC., Supelco Park, Bellefonte, PA, 16823-0048 USA) methyl lignoserate coated (film thickness 0.20 µm), polar capillary column (30 m x 0.32 mm) and an FID. Oxygen-free nitrogen gas at a flow rate of 3.5 mL min^{-1}. The oven temperature was programmed from 180°C to 220°C at the ramp rate of 5°C/min. Initial and final temperatures were held for 2 and 10 minutes, respectively. Injector and detector temperatures were set at 230°C and 250°C, respectively. FAMEs were identified by comparing their relative and absolute retention times with those of authentic standards. All of the quantification was done by a Chromatography Station for Windows (CSW32) data-handling program (Data APEX Ltd., Pague 5, The Czech Republic). The fatty acid composition was reported as a relative percentage of the total peak area.

Table 1. Analysis of *Moringa oleifera* seeds[a].

Constituents	*M. oleifera* Wild (NWFP, Pakistan)	Literature			
		M. oleifera (Sindh, Pakistan)	*M. oleifera* var. Periyakulam (India)	*M. oleifera* var. Mbololo (Kenya)	*M. oleifera* var. Wild (Malawi)
Oil content (%)	34.80 ± 0.89	40.39	38.3	35.7	35.3
Moisture (%)	8.90 ± 0.20	5.70	Present work	Present work	Present work
Fiber (%)	7.54 ± 0.55	7.20	Present work	Present work	Present work
Ash (%)	6.53 ± 0.65	6.60	Present work	Present work	Present work
Protein (%)	31.65 ± 1.20	29.36	Present work	Present work	Present work

[a]Values are mean ± SD calculated as percentage of dry weight for three *M.oleifera* seed samples, analyzed individually in triplicate.

Table 2. Physico-chemical characteristics of *Moringa oleifera* oil[a].

Constituents	*M. oleifera* Wild (NWFP, Pakistan)	Literature			
		M. oleifera (Sindh, Pakistan)	*M. oleifera* var. Periyakulam (India)	*M. oleifera* var. Mbololo (Kenya)	*M. oleifera* var. Wild (Malawi)
Iodine value (g of I/100 g of oil)	68.63 ± 1.00	69.45	65.58	66.83	65.74
Refractive index (40ºC)	1.4571 ± 0.01	1.4608	1.457	1.4549	1.4559
Density (g/cm^3) 24ºC	0.9032 ± 0.03	0.9075	0.909	0.8809	0.8882
Saponification value (mg of KOH/g of oil)	181.4 ± 2.60	186.67	188.36	178.11	184.16
Unsaponifaiable mater (%)	0.74 ± 0.08	0.90	Present work	Present work	Present work
Color (1" cell) (red Unit)	1.28 ± 0.10	1.00	0.80	0	1.2
Yellow unit	31.00 ± 1.40	29.00	35.00	40	70
Acidity (% as oleic acid)	0.81 ± 0.05	0.40	1.12	0.85	0.82

[a]Values are mean ± SD for three wild *M.oleifera* oils, analyzed individually in triplicate.

Table 3. Determination of oxidative state of *Moringa oleifera* oil[a].

Constituents	*M. oleifera* Wild (NWFP, Pakistan)	Literature			
		M. oleifera (Sindh, Pakistan)	*M. oleifera* var. Periyakulam (India)	*M. oleifera* var. Mbololo (Kenya)	*M. oleifera* var. Wild (Malawi)
Conjugated diene $\varepsilon^{1\%}_{1cm}$ (λ232)	1.38 ± 0.06	1.70	3.00	3.15	1.44
Conjugated triene $\varepsilon^{1\%}_{1cm}$ (λ270)	0.79 ± 0.07	0.31	Present work	1.13	0.16
Peroxide value meq kg^{-1} of oil)	1.27 ± 0.05	0.59	1.83	1.80	0.23
P-anisdine value	2.50 ± 0.10	1.30	Present work	Present work	Present work
Oxidative stability, ndo Rancimat method (h)	9.60 ± 0.20	9.99	31.70	36.8	83.2
Oxidative stability, do Rancimat method (h)	8.40 ± 0.25	8.63	8.70	10.80	23.7

[a]Values are mean ± SD for three *M.oleifera* oils, analyzed individually in triplicate.
ndo, non-degummed oil
do, degummed oil

Statistical analysis: Analysis of three different *M. oleifera* seed samples was carried out and results were reported as mean ± SD. Samples were prepared and measured separately in triplicate.

Results and Discussion

Tables 1-6, depict the data for the analysis of *M. oleifera* seeds and extracted seed oils from a wild provenance of Pakistan, along with literature values of identical *Moringa*

species from different countries for comparative purposes. Values for the present analysis are given as mean ± SD of three different *M. oleifera* seeds, analyzed individually in triplicate.

The hexane-extracted oil content of *M. oleifera* seeds assayed from a wild provenance of Pakistan was found to be 34.80% (Table 1). The oil content varied among the seed samples collected from different sites, ranging from 32.90 to 36.40%. The average oil content 34.80% of wild *M. oleifera* seeds from wild provenance of Pakistan was considerably lower than 40.39% reported for *M. oleifera* seeds, cultivated in Sindh province of Pakistan (Anwar & Bhanger, 2003). This variation in oil yield of *M. oleifera* seeds between cultivated and wild provenance of Pakistan might be attributed to the diversity of natural habitats and agroclimatic constraints.

The oil content (34.80%) of *M. oleifera* seeds from wild provenance of Pakistan was not so different from those reported for *M. oleifera* (Mbololo variety) seeds from Kenya (35.7%) (Tsaknis *et al*., 1999) and *M. oleifera* (wild variety) (35.3%) from Malawi (Tsaknis *et al.,* 1998). However, the oil yield was lower by 3.53 and 6.75% as compared with those of *M. oleifera* (Periyakulam-1) seeds reported from India (38.30%) (Lalas & Tsaknis, 2002) and Nigeria (41.58%) (Anhwange *et al.,* 2004). The oil content (32.90-36.40%) in the present analysis of *M. oleifera* seeds was found to be quite higher than some commonly grown oil seed crops such as cotton (15.0-24.0%) and soybean (17.0-21.0%) and some what comparable with those of safflower (25.0-40.0%) and mustard (24.0-40.0%) (Pritchard, 1991).

Analysis of the oil seed residues revealed a high protein content of the seeds i.e., 31.65%, whereas fiber and ash contents were 7.54 and 6.53%, respectively. These values of *M. oleifera* seeds from a wild provenance of Pakistan were in agreement to those for *M. oleifera* seeds reported from Sindh, Pakistan (Anwar & Bhanger, 2003). There are no previously reported data on the *M. oleifera* oilseed residues analysis from other countries to compare the results with our present work. Our results showed that the *M. oleifera* oilseed meal being a good source of protein might be used in local poultry industry. The oilseed residue left after the extraction of oil could also be explored as a potential source of natural coagulants for water treatment. The current literature demonstrates that *M. oleifera* seeds contain active coagulant and antimicrobial agents, and thus could be utilized for water purification as a viable replacement of proprietary chemicals such as alum sulphate (Kalogo *et al.,* 2000; Anwar *et al.,* 2007).

Table 2 shows various physico-chemical characteristics of the extracted *M. oleifera* oil from the wild provenance of Pakistan. The values determined for iodine (68.63 g of iodine/ 100 g of oil), refractive index at 40°C (1.4571), density at 24°C (0.9032 g cm^{-3}) and saponification value (181.4 mg of KOH/g of oil) were in close agreement with those of *M. oleifera* oils reported earlier from cultivated provenance of Sindh, Pakistan (Anwar & Bhanger, 2003). The average values of these parameters were also not so different from those of *M. oleifera* (Mbololo var.) oil from Kenya (Tsaknis *et al.,* 1999), *M. oleifera* (wild var.) oil from Malawi (Tsaknis *et al.,* 1998) and *M. oleifera* (Periyakulam-1) from India (Lalas & Tsaknis, 2002). However, the color (1.28 R + 31.00 Y) values of the investigated *M. oleifera* oil were considerably lower in their yellow and higher in red units to those of *M. oleifera* oils reported in the literature (Tsaknis *et al.,* 1998; Tsaknis *et al.,* 1999; Lalas & Tsaknis, 2002; Anwar & Bhanger 2003). Color of the oils is mainly attributed to the presence of natural pigments which are extracted along with the oil during extraction and are effectively removed during the bleaching step of processing of oils.

Table 4. Tocopherol contents (mg kg^{-1}) of *Moringa oleifera* oil[a].

Constituents	*M. oleifera* Wild (NWFP, Pakistan)	Literature			
		M. oleifera (Sindh, Pakistan)	*M. oleifera* var. Periyakulam (India)	*M. oleifera* var. Mbololo (Kenya)	*M. oleifera* var. Wild (Malawi)
α-tocopherol, ndo	140.50 ± 3.80	134.42	15.38	98.82	131.03
α-tocopherol, do	113.00 ± 4.00	Present work	Present work	Present work	Present work
γ-tocopherol, ndo	63.18 ± 1.72	93.70	4.47	27.9	70.43
γ-tocopherol, do	49.40 ± 1.00	Present work	Present work	Present work	Present work
δ-tocopherol, ndo	61.70 ± 2.20	48.00	15.51	71.16	53.98
δ-tocopherol, do	51.50 ± 0.80	Present work	Present work	Present work	Present work

[a]Values are mean ± SD for three *M.oleifera* oils, analyzed individually in triplicate.
ndo, non-degummed oil
do, degummed oil

Table 5. Sterol composition of *Moringa oleifera* oil[a].

Sterols (%)	*M. oleifera* Wild (NWFP, Pakistan)	Literature			
		M. oleifera (Sindh, Pakistan)	*M. oleifera* var. Periyakulam (India)	*M. oleifera* var. Mbololo (Kenya)	*M. oleifera* var. Wild (Malawi)
24-Methylenecholesterol	0.99 ± 0.11	1.49	0.08	0.88	0.96
Campesterol	17.95 ± 0.50	16.00	15.29	15.13	23.83
Campestanol	0.53 ± 0.10	ND	0.33	0.35	0.40
Δ^7-campestanol	0.70 ± 0.07	0.50	Present wok	Present work	Present work
Stigmasterol	18.80 ± 0.50	19.00	23.06	16.87	17.03
Clerosterol	1.70 ± 0.10	1.95	1.22	2.52	0.62
Stigmastanol	0.53 ± 0.05	1.00	0.64	0.86	0.77
β-sitosterol	46.16 ± 0.40	46.65	43.65	50.07	47.07
Δ^7- avenasterol	0.84 ± 0.10	0.96	Present work	1.11	0.19
Δ^5-avenasterol	9.26 ± 0.15	10.70	11.61	8.84	2.94
28- isoavenasterol	1.04 ± 0.11	0.50	0.25	1.40	0.25
$\Delta^{7,14}$- stigmastanol	0.76 ± 0.12	ND	0.85	0.44	Present work

[a]Values are mean ± SD for three *M.oleifera* oils, analyzed individually in triplicate.
ND = Not detected

Table 6. Fatty acid composition (grams per 100 g of fatty acids) of *Moringa oleifera* oil[a].

Fatty acids	*M. oleifera* Wild (NWFP, Pakistan)	Literature			
		M. oleifera (Sindh, Pakistan)	*M. oleifera* var. Periyakulam (India)	*M. oleifera* var. Mbololo (Kenya)	*M. oleifera* var. Wild (Malawi)
$C_{16:0}$	6.45 ± 0.20	6.50	6.46	6.04	5.51
$C_{16:1}$	0.97 ± 0.07	1.00	1.36	1.46	1.10
$C_{18:0}$	5.50 ± 0.25	5.67	5.88	4.14	5.86
$C_{18:1}$	73.22 ± 0.50	76.00	71.21	73.6	67.79
$C_{18:2}$	1.27 ± 0.12	1.29	0.65	0.73	0.71
$C_{18:3}$	0.30 ± 0.07	ND	0.18	0.22	0.21
$C_{20:0}$	4.08 ± 0.10	3.00	3.62	2.76	3.78
$C_{20:1}$	1.68 ± 0.10	1.20	2.22	2.40	2.60
$C_{22:0}$	6.16 ± 0.15	5.00	6.41	6.73	6.81
$C_{22:1}$	ND	ND	0.12	0.14	0.11

[a]Values are mean ± SD for three *M.oleifera* oils, analyzed individually in triplicate
ND = Not detected

The value of acidity (0.81% as oleic acid) was considerably lower than *M. oleifera* oil from India (Lalas & Tsaknis, 2002), but comparable to those of *M. oleifera* oils reported from Malawi (Tsaknis *et al.*, 1998) and Kenya (Tsaknis *et al.*, 1999). Oils with lower values of acidity are more acceptable for edible applications. The content of

unsaponifiable matter of the investigated wild *M. oleifera* oils was 0.74%. There were no previously reported data on the unsaponifiable matter of the *M. oleifera* oils from other regions of the world to compare with our present data. The value of refractive index of the investigated *M. oleifera* oils was well in line to those of cotton seed, palm and mango kernel oils (Rossell, 1991). The saponification number and unsaponifiable matter of the investigated wild *M. oleifera* oils were in accord to those of olive, corn, low erucic acid rapeseed, soybean, sunflower and safflower oils (Norman, 1979; Rossell, 1991).

The results of different oxidation parameters of *M. oleifera* oil native to a wild provenance of Pakistan are shown in Table 3. The specific extinctions at 232 nm (1.38) and 270 nm (0.79), which are good indicative of magnitude of oxidative degradation of the oils (Yoon *et al.,* 1985) were comparable to that of *M. oleifera* oil reported from Sindh province of Pakistan (Anwar & Bhanger, 2003). However, these values considerably varied from those of *M. oleifera* oils from Kenya (Tsaknis *et al.,* 1999), Malawi (Tsaknis *et al.,* 1998) and India (Lalas & Tsaknis, 2002).

The peroxide value (1.27 meq. kg^{-1} of oil) and *p*-anisidine value (2.50), which measure hydroperoxide and α, β aldehydic secondary oxidation products of the oils (McGinely, 1991), were generally higher than those of *M. oleifera* oils reported from Sindh (Anwar & Bahnger, 2003) and Malawi (Tsaknis *et al.,* 1998). However, the peroxide value of the oil determined in the present analysis was quite lower than those of *M. oleifera* oils from Kenya (Tsaknis *et al.*, 1999) and India (Lalas & Tsaknis, 2002).

The investigated non-degummed *M. oleifera* oil exhibited an induction period (Rancimat: 20L/h, 120°C), of 9.60 h, that decreased to 8.40 h after degumming, a reduction of 12.5% in oxidative stability, which could be attributed to the process of degumming. Induction period (IP) is an important feature which describes the oxidative stability of oils and fats (Anwar *et al.,* 2003). The induction periods of the investigated non-degummed and degummed *M. oleifera* oils were somewhat comparable with those of *M. oleifera* oils reported from Sindh (Anwar & Bhanger, 2003), but significantly lower than those from Kenya (Tsaknis *et al.,* 1999), India (Lalas & Tsaknis, 2002) and Malawi (Tsaknis *et al.,* 1998).

The data for tocopherols analysis of the non-degummed and degummed *M. oleifera* oils from a wild provenance of Pakistan are presented in Table 4. The levels of α-, γ-, and δ-tocopherols in the non-degummed oil were 140.5, 63.18 and 61.70 mg kg^{-1}, respectively. The contents of these tocopherols in the degummed oil were reduced to 113.00, 49.40 and 51.50 mg kg^{-1}, reduction of 19.57, 21.81 and 16.53% in the α-, γ- and δ-tocopherols, respectively. This loss in tocopherols contents may be attributed to the degumming of the oils. Literature has shown that most of the steps involved in processing and storage of oils, generally reduce the level of tocopherols (Rossell, 1991).

The level of α-tocopherol of the investigated non-degummed *M. oleifera* oil from a wild provenance of Pakistan was well in line with those reported in the cultivated *M. oleifera* native to Sindh, Pakistan (Anwar & Bhanger, 2003) and to a wild variety of *M. oleifera* from Malawi (Tsaknis *et al.,* 1998). However, it was considerably higher to those of *M. oleifera* oils investigated from India (Lalas & Tsaknis, 2002) and Kenya (Tsaknis *et al.,* 1999). The content of α-tocopherol in the non-degummed *M. oleifera* oils was in close agreement with the values reported for soybean, groundnut and plam oils (Norman, 1979; Rossell, 1991).

The contents of γ-tocopherol in the investigated non-degummed *M. oleifera* oil was slightly lower than those of *M. oleifera* oil reported from Malawi (Tsaknis *et al.*, 1998)

but considerably higher than those from India (Lalas & Tsaknis, 2002) and Kenya (Tsaknis *et al.,* 1999). The concentration of δ-tocopherol was slightly lower than the values reported for *M. oleifera* oil from Kenya (Tsaknis *et al.*, 1999). However, it was higher than the values for *M. oleifera* oil reported from Sindh, Pakistan (Anwar & Bhanger, 2003) and India (Lalas & Tsaknis, 2002). Literature revealed that α-isomer of tocopherol has greatest vitamin E potency, whereas, δ-isomer of tocopherol has greater antioxidant efficacy than either γ- or α-tocopherols (Rossell, 1991; Tsaknis *et al.*, 1999).

The sterols profile of wild *M. oleifera* oil is shown in Table 5. The sterol fractions of *M. oleifera* seed oil from a wild provenance of Pakistan mainly consisted of β-sitosterol (46.16%), stigmasterol (18.80%), campesterol (17.95%) and Δ^5, avenasterol (9.26%) together with small amounts of clerosterol, 24, methylene cholesterol, Δ^7, campestanol, Δ^7, avenasterol, stigmastanol and 28, isoavenasterol. A small amount of cholesterol and brassicasterol components of sterols as reported in *M. oleifera* oil from Kenya (Tsaknis *et al.,* 1999) were not detected in the present analysis.

The contents of campesterol, stigmasterol, β-sitosterol and Δ^5 and avenasterol in the present analysis of Moringa oil from a wild provenance were rather comparable with the values for *M.oleifera* oil reported from Kenya (Tsaknis *et al*., 1999) and India (Lalas & Tsaknis, 2002), while these varied considerably, apart from Δ^5, avenasterol and campesterol to those of wild *M. oleifera* seed oil from Malawi (Tsaknis *et al.,* 1998). The sterol composition of the major constituents of the investigated *M. oleifera* oils generally varied to those of most of the conventional edible oils (Rossell, 1991) and thus could not be compared. Regional and inter-cultivar variations in the contents of phytosterols have been reported in the literature (Norman, 1979; Rossell, 1991).

Table 6 shows the fatty acid composition (FAC) of wild *M. oleifera* oils from Pakistan. The content of saturated fatty acids; that is palmitic-, stearic-, arachidic- and behenic-acids, in the investigated *M. oleifera* oil was 6.45, 5.50, 4.08 and 6.16%, respectively. The oil was found to contain a high amount of oleic acid (C18:1 n-9) up to 73.22%. A small quantity (1.27%) of linoleic acid (C18:2 n-6), gadoleic acid (1.68%) and palmitoleic acid (0.97%) was also detected. The concentration of C16:0, C16:1, C18:0 and C18:2 of the investigated wild *M. oleifera* oils were in close agreement with that of *M. oleifera* oil reported from a cultivated provenance of Pakistan (Anwar & Bhanger, 2003). However, the oil in the current analysis somewhat varied with regards to the contents of other component FAs to those of the latter.

The content (73.22%) of principal fatty acid, i.e. C18:1 was well in line with that reported for *M. oleifera* oil from Kenya (Tsaknis *et al*., 1999). It appreciably varied to those of *M. oleifera* oils from Malawi (Tsaknis *et al*., 1998), Sindh, Pakistan (Anwar & Bhanger, 2003) and India (Lalas & Tsaknis, 2002). The examined wild *M. oleifera* oil from Pakistan was considerably different in the contents of other component fatty acids to those of *Moringa* oils reported in the literature (Tsaknis *et al.,* 1999), Malawi (Tsaknis *et al.,* 1998) and India (Lalas & Tsaknis, 2002).

The results of the present study have demonstrated that most of the characteristics and quality attributes of *M.oleifera* oil from a wild provenance of Pakistan are quite identical with those of other Moringa oils reported in the literature. Its fatty acids composition revealed that it also fell in the category of high-oleic oils just like other Moringa oils. As Pakistan has vast wild provenances and forests in the NWFP provenance, so *M. oleifera* could be grown on wide scale production as a potentially valuable crop, yielding useful oil with high-oleic contents.

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