

Effect of salinity on yield and quality of *Moringa oleifera* seed oil

By Farooq Anwar¹, Abdullah Ijaz Hussain¹, Muhammad Ashraf², Amer Jamail¹ and Shahid Iqbal³

¹ Department of Chemistry, University of Agriculture, Faisalabad-38040, Pakistan.

² Department of Botany, University of Agriculture, Faisalabad-38040, Pakistan.

³ Department of Chemistry, University of Sargodha, Sargodha-40100, Pakistan.

Tel: +92-41-9200161-67 Ext. 3309; Fax: +92-41-9200764; E-mail: fqnwar@yahoo.com

RESUMEN

Efecto de la salinidad en la producción y calidad del aceite de semilla de *Moringa oleifera*.

Ha sido examinada la variación en la producción y composición del aceite de semilla de *Moringa oleifera* extraído a partir de semillas cultivadas en Pakistán con diferente concentración salina en el cultivo. La producción obtenida por extracción con hexano fue de 33.50% y 32.79% respectivamente para las semillas cultivadas en áreas con alto y bajo contenido salino. El análisis de la varianza (ANOVA) reveló la inexistencia de diferencias significativas entre las características físicas (índice de refracción a 40 °C, color y gravedad específica) y las químicas (índice de yodo, acidez libre, índice de peróxidos, materia insaponificable, índice de saponificación, valores de dienos y trienos conjugados e índice de *p*-anisidina) de los aceites procedentes de ambas áreas. En el área de mayor salinidad las concentraciones de C_{18:1} y C_{16:0} de los aceites de semilla de *Moringa oleifera* fueron significativamente mayores ($P < 0.001$), mientras que el de C_{14:0} fue significativamente menor. El análisis de tocoferoles demostró que las concentraciones de α - y δ -tocoferol eran superiores significativamente ($P < 0.001$) en los aceites procedentes de las áreas con alta salinidad, mientras que el contenido en γ -tocoferol fue superior significativamente ($P < 0.001$) en los procedentes del área de baja salinidad. Los resultados de este estudio muestran que la salinidad no afecta sobre el contenido en aceite de estas semillas. Sin embargo, puede afectar al contenido en tocoferoles y el perfil de ácidos grasos.

PALABRAS-CLAVE: Calidad de aceite - Caracterización - Composición de ácidos grasos - *Moringa oleifera* - Salinidad - Tocoferoles.

SUMMARY

Effect of salinity on yield and quality of *Moringa oleifera* seed oil

Variation in the yield and composition of *Moringa oleifera* (*M. oleifera*) seed oil from two differently adopted (non-saline and saline) provinces of Pakistan was examined. Hexane-extracted *M. oleifera* seeds from saline and non-saline areas contained 33.50% and 32.79% oil yield, respectively. The analysis of variance (ANOVA) revealed no significant differences in the physical (refractive index (40 °C), color and specific gravity (24 °C) or chemical (iodine value, free fatty acid value, peroxide value, unsaponifiable matter, saponification value, conjugated diene and triene values and

p-anisidine value) characteristics of the oils obtained from both areas. The concentration of C_{18:1} and C_{16:0} was significantly ($P < 0.001$) higher whereas, that of C_{14:0} was lower in *M. oleifera* seed oils from the saline area. A tocopherol analysis demonstrated the concentration of α - and δ -tocopherol of *Moringa* seed oils to be significantly ($P < 0.001$) higher from the saline area. Whereas, the contents of γ -tocopherol was found to be significantly ($P < 0.001$) higher in the *Moringa* seed oils native to the non-saline area. Results from the present study revealed that salinity did not affect the oil content of *M. oleifera* seeds. Nevertheless, it might affect the tocopherol and fatty acid profiles of *M. oleifera* seed oil.

KEY-WORDS: Characterization - Fatty acid composition - *Moringa oleifera* - Oil quality - Salinity - Tocopherols.

1. INTRODUCTION

Soil salinity is a major environmental stress that drastically affects crop productivity. Due to the continuous build up of salinity in the soil, millions of hectares of useable land have now become unsuitable for cultivation. Nearly half of the irrigated surface is seriously affected by salinity and/or secondary alkalinity (Flagella *et al.*, 2002a). It is estimated that every year more than one million hectares of land are subjected to salinization (Munns, *et al.*, 1999). In Pakistan, the salt affected area is up to 6.67 million hectare (Khan, 1998) of which 60% is saline sodic. Soil salinity is thus eroding our civilization by persistently reducing the area for crop cultivation. But if this vast effected area is economically utilized it would be worth millions of rupees.

The adverse effect of salinity on crop growth results from disturbed metabolic processes (Taneja, 1988), which are most commonly manifested in stunted plant growth, poor productivity (Jin Woong and Choongsoo, 1998) and distinctly changed concentrations of key biomolecules. Plants grown under saline conditions are stressed and are characterized by increased levels of free proline in different tissues (Heuer, 1994) as a response to osmotic adjustment (Heuer and Nadler, 1998). The effects of salinity on plant growth include

modification of different, morphological, physiological and biochemical processes and anatomical changes (Tester and Davenport, 2003). Salinity is known to affect several aspects of plant metabolism, including lipid metabolism in many plants (Erdei *et al.*, 1980). In several cases, a change in lipid composition in response to salinity was correlated with adaptive properties of the species involved. However, its effect on the lipid metabolism of oilseeds has not been adequately studied.

Oilseed plants, if found suitable for irrigation with saline water and/or treated wastewaters, may be a highly economic alternative to traditional field crops. The quality of oils is associated with their fatty acid composition, mainly the percentage of oleic, linoleic, and linolenic acids. The literature revealed the effects of salinity on the yield and fatty acid compositions of oil (Bassil and Kaffka, 2002; Heuer *et al.*, 2002; Parti *et al.* 2003; Flagella *et al.* 2004).

The effects of salinity on quality of high oleic sunflower oil (Flagella *et al.*, 2004), chia, stock and evening primrose oils (Heuer *et al.*, 2002) and Arbequina olive oil (Royo *et al.*, 2005) have already been studied. Oleic acid showed an increase whereas; linoleic acid progressively decreased due to an increase in the salinity level (Flagella *et al.*, 2004). The amount of linoleic acid in cotton seeds has been reported to be reduced by salt stress and that of oleic acid remained unchanged (Smaoui and Cherift, 2000). With increased salt stress, both increases and decreases in chlorophyll content as well as changes in the mineral composition of different plant organs have also been reported (Jin Woong and Choongsoo, 1998).

Moringa oleifera (*M. oleifera*) Lam. is one of the most widely known and utilized species, belonging to the family *Moringaceae*. 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 (Anwar and Bhanger, 2003). A significant number of reports are available in the literature regarding the physico-chemical characteristics of *M. oleifera* seed oil (Tsaknis *et al.*, 1999; Anwar and Bhanger, 2003; Abdulkarim *et al.*, 2005; Anwar *et al.*, 2005). To our best knowledge no earlier reports are available regarding the effects of salt stress on the composition of *Moringa oleifera* seed oil indigenous to Pakistan or elsewhere. So the present investigation was carried out to examine and quantify the effects of salinity on lipid contents and the physico-chemical characteristics of *M. oleifera* seed oil. The preliminary objective of the present study was to characterize *M. oleifera* seed oil from the saline and non-saline areas of Pakistan.

2. EXPERIMENTAL

2.1. Materials

The seeds of *Moringa oleifera* were assayed from five different regions in the vicinity of the Pakka Anna

district Toba Tek Singh, Punjab, Pakistan (a saline area) and University of Agriculture, Faisalabad, Pakistan (a non-saline area) during May-June, 2005. Seeds, from both saline and non-saline areas, were triangular and covered with a thick, pale yellow to creamy seed coat; average weights were 0.133 and 0.120 g with the off-white kernels constituting 81.00% and 75.66 % of the weight, respectively. All reagents (analytical and HPLC) used were from E. Merck (Darmstadt, Germany) or Sigma Aldrich (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).

2.2. 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 Soxhelt extractor fitted with a 1-L round-bottom flask and a condenser. The extraction was executed in a water bath for 6 h with 0.5 L of *n*-hexane. The solvent was distilled off under vacuum in a rotary evaporator (EYELA, Rotary Vacuum Evaporator. N.N. Series equipped with an Aspirator and a Digital Water Bath SB-651, Japan).

2.3. Analysis of oilseed residues

The oilseed 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 a semi-automated FOSFA official method (1982). A sample of meal was digested for 10 min with a digestion mixture of sulfuric acid/hydrogen peroxide/potassium sulfate, with selenium dioxide as a catalyst. The final end point in the ammonia titration was measured photometrically.

Fiber content was determined according to the ISO method (1981). A 2.5-g sample of finely ground 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⁻¹), followed by separation and washing of the insoluble residue. The residue was then boiled with sodium hydroxide (0.313 mol L⁻¹), 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 in mass was determined.

Ash content was determined according to the ISO method (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.

2.4. Analysis of extracted oils

2.4.1. 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 following standard AOCS methods (1997). The color of the oil was determined by a Lovibond Tintometer (Tintometer Ltd., Salisbury, U.K.), using a 1-inch cell. Specific extinctions at 232 and 270 nm were determined using a Hitachi, U-2001, model 121-0032 spectrometer. Samples were diluted with iso-octane to bring the absorbance within limits (0.2-0.8) and ($\epsilon_{1\text{cm}}^{1\%(\lambda)}$) was calculated following the method of IUPAC standard method (1987).

2.4.2. Determination of *p*-anisidine value

The determination of *p*-anisidine value was carried out following an IUPAC standard method (1987). The oil samples dissolved in *iso*-octane were allowed to react with *p*-anisidine to produce colored complex and the absorbance values were noted at 350 nm by using a Hitachi U-2001, model 121-0032 spectrometer.

2.4.3. Tocopherol content

Tocopherol (α , γ and δ) analysis was performed by HPLC following the method described by Lee *et al.* (2003). An HPLC (Sykam GmbH, Kleinostheim, Germany) equipped with an S-1121 dual piston solvent delivery system and S-3210 UV/Vis diode array detector was used. One gram of oil was accurately weighed and brought up to volume with acetonitrile in a 10-mL volumetric flask wrapped in foil to inhibit oxidation. A 20- μ L of filtered sample was injected into an analytical Hypersil (Thermo Hypersil, GmbH, Germany) ODS reverse phase (C18) column (250 x 4.6mm; 5 μ m particle size) fitted with a C18 guard column. The mobile phase consisted of a mixture of HPLC grade methanol and acetonitrile (65:35 v/v). The chromatographic separation was performed by isocratic elution of the mobile phase at a flow rate of 1.3mLmin⁻¹ at 30 °C. Detection was performed at a wavelength of 292 nm. Tocopherols were identified by comparing the retention times and quantified on the basis of peak area percent of the unknowns compared to those of pure standards of α -, γ -, and δ - tocopherols (Sigma Chemical Co. (St. Louis, MO)). The peak areas were recorded and calculated by a computer with SRI peak simple chromatography data acquisition and integration software (SRI Instrument, Torrance, California, USA).

2.4.4. Fatty acid composition

Fatty acid methyl esters were prepared following the standard IUPAC standard method (1987) and analyzed on a SHIMADZU gas chromatograph model 17-A, fitted with a SP-2330 (SUPLECO, INC., Supelco Park, Bellefonte, PA, 16823-0048 USA) methyl lignoserate coated (film thickness 0.20 μ m), polar capillary column (30 m x 0.25 mm) and a flame ionization detector. Oxygen-free nitrogen

was used as a carrier gas at a flow rate of 5 mLmin⁻¹. Other conditions were as follows: initial oven temperature, 180 °C; ramp rate, 5 °C/min; final temperature, 220 °C; injector temperature, 230 °C; detector temperature, 240 °C. FAMES were identified by comparing their relative and absolute retention times with those of authentic standards of FAMES (Sigma Chemical Co. (St. Louis, MO)). The quantification was done by a Chromatography Station for Windows (CSW32) data handling software (Data APEX LTD. CZ-158 00 Pague 5, The Czech Republic). The fatty acid composition was reported as a relative percentage of the total peak area.

2.5. Soil analysis

2.5.1. Sampling and pretreatment

Five soil samples were collected from each trial area during the course of the experiment. 200g of soil was taken from each sample and dried in an oven at 90 °C for 5 days. An oven-dried soil sub-sample (100 g) was taken to make a saturation paste with distilled water. The soil extraction was obtained using a vacuum pump.

2.5.2. Determination of various parameters of soil

Nutrient Analysis

The determination of potassium, sodium and calcium in the soil saturation extract was carried out using a flame photometer model Jenway PFP-7. The concentration of magnesium in the soil extract was determined using a Perkin Elmer Atomic Absorption Spectrometer model Analyst-300. Chloride content was determined by Sherwood Chloride Analyzer 920. Electrical conductivity (ECe) and pH of the soil extract were measured by pH/Cond (Inolab), level 1 meter. Internationally, the electrical conductivity of the saturated extract is the most commonly used indicator of salinity in terms of plant growth (van de Graaff and Patterson, 2001).

2.6. Statistical analysis

Five *M. oleifera* seed samples were assayed from each of the saline and non-saline areas. Data is reported as mean \pm SD of five *M. oleifera* seed samples from each area, analyzed individually in triplicate. For all investigated parameters, the analysis of variance (ANOVA) was performed using the Minitab statistical software (version 13.20).

3. RESULTS AND DISCUSSION

Properties of soil samples from the investigated saline and non-saline areas are shown in table 1. The values of ECe, SAR and ESP and the contents of Na⁺ and Cl⁻ were found to be drastically higher in saline areas as compared with those of non-saline.

Table 1
Physico-chemical characteristics of soil from saline and non-saline areas

Characteristics	Saline area	Non-saline area
Saturation percentage	29.8 ± 1.65	29.4 ± 1.45
pH	9.7 ± 0.43	9.2 ± 0.50
ECe mS cm ⁻¹	16.8 ± 2.35	1.8 ± 0.10
Ca ²⁺ meq L ⁻¹	5.0 ± 0.70	2.8 ± 0.11
Mg ²⁺ meq L ⁻¹	0.20 ± 0.02	0.15 ± 0.01
K ⁺ meq L ⁻¹	4.1 ± 0.15	1.5 ± 0.01
Na ⁺ meq L ⁻¹	136.5 ± 10.5	3.0 ± 0.15
Cl ⁻ meq L ⁻¹	111.0 ± 8.53	11.9 ± 1.35
SAR (mmol) ^{1/2}	85.3 ± 4.55	2.5 ± 0.21
ESP	55.2 ± 5.05	2.4 ± 0.18

Values are means ± SD for five soil samples from each of the saline and non-saline areas, analyzed individually in triplicate.

ECe = Electrical conductivity of soil extract

SAR = Sodium adsorption ratio

ESP = Exchangeable sodium percentage

Furthermore, the concentrations of Ca²⁺ and K⁺ were also quite higher in the soil samples of saline areas as compared with those of its counterpart. A higher level of these values in soil from saline areas might be attributed to the high concentration of NaCl and thus indicated the area to be heavily salinized. Generally, at an EC above 2 or 3 dS m⁻¹, depending on soil type and crops grown, salt content in the soil will inhibit plant growth.

Table 2 shows the proximate analysis of *M. oleifera* seeds from saline and non-saline areas of Pakistan. As for as the oil yield of *M. oleifera* seeds, there was no significant ($P = 0.05$) variation in the oil content of samples collected from both areas. The mean values of oil yield were 33.50 and 32.79% in the samples collected from saline and

non-saline areas, respectively. In contrast to our present analysis, however, somewhat different results were reported by Flagella *et al.* (2004), who demonstrated a significant effect of salinity on the oil yield from the sunflower seed of a hybrid variety. Royo *et al.* (2005) reported a minor effect of soil salinity on the oil content of Arbequina olive oil. However, Heuer *et al.* (2005) reported no any change in the oil content due to effect of salinity.

The analysis of oilseed residue revealed no significant ($P > 0.05$) variation in the contents of protein (37.95 and 38.51 %), fiber (7.50 and 7.51 %) ash (9.00 and 8.96 %) and moisture (7.30 and 7.31 %) from saline and non-saline areas, respectively. The present analysis showed the meals to be a good source of calories and may be used 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 (Anwar and Bhanger, 2003).

Table 3 depicts the results of various physical and chemical characteristics of the extracted *M. oleifera* oils from the saline and non-saline areas of Pakistan. No significant ($P \geq 0.05$) differences were observed in the physical and chemical characteristics of the oils from saline and non-saline areas. The color (2.2R + 22Y, 2.10R + 21.0Y); refractive index at 40 °C (1.4586, 1.4579); density at 24 °C (0.88, 0.87 g cm⁻³); iodine value (68.63, 71.02 g of I/100 g of oil); free fatty acid content (0.50, 0.56 % as oleic acid); unsaponifiable matter (1.01, 1.10 %); saponification value (184.00, 186.30 mg of KOH/g of oil) of *M. oleifera* oil from saline and non-saline areas, respectively, were in good agreement with those reported for *M. oleifera* oil from temperate regions of Pakistan (Anwar and Bhanger, 2003) and Kenya (Tsaknis *et al.*, 1999). There are no earlier reports showing the effects of salinity on these physical and chemical characteristics of

Table 2
Proximate analysis of *M. oleifera* seeds from saline and non-saline areas

Constituents	<i>M. oleifera</i> (Saline)	<i>M. oleifera</i> (Non-saline)	ANOVA	
			F value	P
Oil content (%)	33.50 ± 1.50	32.79 ± 1.72	4.18 NS	0.050
Fiber (%)	7.50 ± 0.41	7.51 ± 0.30	4.14 NS	0.051
Ash (%)	9.00 ± 0.50	8.96 ± 0.64	3.18 NS	0.085
Moisture content (%)	7.30 ± 0.51	7.31 ± 0.37	3.60 NS	0.068
Protein (%)	37.95 ± 1.71	38.50 ± 2.12	4.07 NS	0.053

Values are mean ± SD of five *M. oleifera* seed samples from each of the saline and non-saline areas, analyzed individually in triplicate.

NS = non significant.

Table 3
Physico-chemical characteristics of *M. oleifera* oil from saline and non-saline areas

Constituents	<i>M. oleifera</i> (Saline)	<i>M. oleifera</i> (Non-saline)	ANOVA	
			F value	P
Iodine value (g of I/100 g of oil)	68.63 ± 2.00	71.02 ± 1.56	4.19 ^{NS}	0.050
Refractive index (40 °C)	1.4586 ± 0.001	1.4579 ± 0.001	4.04 ^{NS}	0.054
Density (gcm ⁻³) 24 °C	0.8800 ± 0.02	0.8700 ± 0.03	2.14 ^{NS}	0.154
Saponification value (mg of KOH/g of oil)	184.00 ± 3.10	186.30 ± 2.53	4.21 ^{NS}	0.510
Unsaponifiable matter (%)	1.01 ± 0.07	1.10 ± 0.09	4.19 ^{NS}	0.050
Color (red units)	2.20 ± 0.06	2.10 ± 0.08	4.08 ^{NS}	0.053
Color (Yellow units)	22.00 ± 0.66	21.00 ± 0.84	3.79 ^{NS}	0.061
Acidity (% as oleic acid)	0.50 ± 0.04	0.56 ± 0.03	4.07 ^{NS}	0.053

Values are mean ± SD of five *M. oleifera* oils from each of the saline and non-saline areas, analyzed individually in triplicate. NS = non significant.

vegetable seed oils to compare with our present findings.

Table 4 shows the results of some oxidation parameters of the extracted *M. oleifera* seed oils from the saline and non-saline areas of Pakistan. There were no significant ($P \geq 0.012$) variations in the values of specific extinctions at 232 and 270 nm, which reveal the oxidative deterioration and purity of the oils (Yoon, 1985), PV and *p*-anisidine values, which measure hydroperoxides and aldehydic secondary oxidation products of oils and fats, respectively (McGinley, 1991), of *M. oleifera* oils from saline and non-saline areas. The conjugated diene (1.85, 1.82); conjugated triene (0.52, 0.49); PV (1.97, 2.11 meq kg⁻¹ of oil) and *P*-anisidine value (1.84, 1.81) of *M. oleifera* seed oil from saline and non-saline areas respectively were

in good agreement with those reported in the literature (Anwar and Bhangar, 2003; Tsaknis, 1999). 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. Moreover, the high resistance of *M. oleifera* oil to oxidation might be attributed to the presence of a high content of α -, γ -, and δ -tocopherols. Somali *et al.* (1984) also reported that seed fat of the *Moringaceae* family is highly stable.

Table 5 shows the contents of different tocopherols in *M. oleifera* oils. The levels of α -, γ -, and δ -tocopherol of *M. oleifera* oils from saline and non-saline areas were 110.34, 10.95, 62.20 and 39.74, 14.11, 9.18 mg kg⁻¹ respectively. The levels of δ -tocopherol, which has the greatest vitamin E

Table 4
Determination of oxidative state of *M.oleifera* oil from saline and non-saline areas

Determinations	<i>M. oleifera</i> (Saline)	<i>M. oleifera</i> (Non-saline)	ANOVA	
			F value	P
Conjugated diene $\epsilon^{1\%}_{1\text{cm}} (\lambda_{232})$	1.85 ± 0.07	1.82 ± 0.10	3.09 ^{NS}	0.089
Conjugated triene $\epsilon^{1\%}_{1\text{cm}} (\lambda_{270})$	0.52 ± 0.05	0.49 ± 0.06	7.31 ^{NS}	0.012
Peroxide value (meq kg ⁻¹ of oil)	1.97 ± 0.12	2.11 ± 0.15	4.80 ^{NS}	0.037
<i>P</i> -anisidine value	1.84 ± 0.10	1.81 ± 0.15	4.85 ^{NS}	0.036

Values are mean ± SD of five *M. oleifera* oils from each of the saline and non-saline areas, analyzed individually in triplicate. NS = non significant.

Table 5
Tocopherol contents of *M. oleifera* oils from saline and non-saline areas

Determinations	<i>M. oleifera</i> (Saline)	<i>M. oleifera</i> (Non-saline)	ANOVA	
			F value	P
α -tocopherol (mg kg ⁻¹)	110.34 ± 3.31	39.74 ± 1.19	34488.61***	0.000
γ -tocopherol (mg kg ⁻¹)	10.95 ± 0.62	14.11 ± 0.50	641.24***	0.000
δ -tocopherol (mg kg ⁻¹)	62.20 ± 2.00	9.18 ± 0.60	14639.80***	0.000

Values are mean ± SD of five *M. oleifera* oils from each of the saline and non-saline areas, analyzed individually in triplicate.
*** = highly significant.

potency (Rossell, 1991), and δ -tocopherol, which has greater antioxidant activity than either γ or α -tocopherol (Tsaknis, 1998; Tsaknis *et al.*, 1999; Anwar *et al.*, 2005), of *M. oleifera* oils were significantly ($P < 0.001$) higher from saline areas. Whereas, the content of γ -tocopherol was lower in the *M. oleifera* oils from the saline area as compare to that of the non-saline area. No earlier studies were reported in the literature regarding the effect of salinity on the contents of tocopherol of vegetable seed oils.

Table 6 presents the fatty acid composition of *M. oleifera* oils from the saline and non-saline areas of Pakistan. Total saturates, i.e., myristic (14:0), palmitic (16:0), steric (18:0), arachidic (20:0), behenic (22:0) and (26:0) acids, in the saline and non-saline oils samples were 20.68 and 21.93%, respectively and thus revealed no considerable

variation. The investigated *M. oleifera* oils from saline and non-saline areas were found to contain high levels of monounsaturated fatty acids, up to 78.52 and 72.75%, respectively. Oleic acid (18:1 n-9) was the predominant fatty acid, accounting for 74.70 and 68.71% of the total fatty acids. The content of linoleic acid (18:2 n-6) was 0.63 and 0.69%, respectively. The only significant ($P < 0.001$) differences among saline and non-saline areas were observed for oleic, myristic and palmitic acids. The contents of oleic and palmitic acid were found to be higher (74.70, 9.49%) from saline areas as compare with those (68.71, 8.01%) from non-saline areas. The content of myristic acid was found to be significantly ($P < 0.001$) lower (0.46%) from saline areas as compare with those (3.13%) from non-saline areas. This variation in the concentration of oleic, myristic and palmitic acids of *M. oleifera* oils

Table 6
Fatty acid composition (grams per 100 g of fatty acids) of *M. oleifera* oils from saline and non-saline areas

Fatty Acids	<i>M. oleifera</i> (Saline)	<i>M. oleifera</i> (Non-saline)	ANOVA	
			F value	P
C _{14:0}	0.46 ± 0.04	3.13 ± 0.10	15246.29***	0.000
C _{16:0}	9.49 ± 0.24	8.01 ± 0.37	193.50***	0.000
C _{16:1}	1.58 ± 0.11	1.69 ± 0.13	6.85 ^{NS}	0.014
C _{18:0}	2.71 ± 0.20	2.58 ± 0.15	4.13 ^{NS}	0.052
C _{18:1}	74.70 ± 0.82	68.71 ± 1.25	101.27***	0.000
C _{18:2}	0.63 ± 0.10	0.69 ± 0.10	6.68 ^{NS}	0.015
C _{20:0}	2.10 ± 0.10	1.98 ± 0.15	5.92 ^{NS}	0.022
C _{20:1}	2.24 ± 0.12	2.35 ± 0.15	6.75 ^{NS}	0.015
C _{22:0}	4.33 ± 0.35	4.56 ± 0.20	4.87 ^{NS}	0.036
C _{26:0}	1.59 ± 0.11	1.67 ± 0.08	6.62 ^{NS}	0.016

Values are mean ± SD of five *M. oleifera* oils from each of the saline and non-saline areas, analyzed individually in triplicate.

*** = highly significant

NS = non significant.

from the investigated areas of Pakistan might be attributed to salt stress.

The literature also revealed the effects of salinity on the fatty acid composition of vegetable oils. In crambe (*Cartamus tinctorius* L.) an increase in oleic acid was observed in field trials carried out under saline irrigation ranging from 3.7 to 7.9 dS m⁻¹; concurrently, a decrease in linolenic acid but no changes in linoleic acid were observed (Francois and Kleiman, 1990). The increase in the oleic/linoleic acid ratio observed under saline conditions was also evident under water stress conditions occurring during grain filling for high oleic hybrids grown in fields (Baldini *et al.*, 2002; Flagella *et al.*, 2002b). Flagella *et al.* (2004) reported that salinity increases the oleic acid while it decreases the linoleic acid. Heuer *et al.* (2002) also reported changes in the oil composition due to salinity. Parti *et al.* (2003) reported the gradual increase in oleic acid with increasing salinity levels.

Thus, it could be concluded from the present studies that although salinity does not affect the oil yield it does affect the fatty acid and tocopherol profile of the *M. oleifera* oils. The results of the present analysis revealed that under the investigated conditions salinity did not affect the oil yield of the *M. oleifera*. Therefore, this species can be effectively grown in the saline areas of Pakistan and sub-continent to benefit from its oil potential for edible and industrial purposes.

REFERENCES

- Abdulkarim SM, Long K, Lai OM, Muhammad SKS, Ghazali HM. 2005. Some physico-chemical properties of *Moringa oleifera* seed oil extracted using solvent and aqueous enzymatic methods. *Food Chem.* **93**, 253-263.
- American Oil Chemist Society (AOCS). 1997. *Official and Recommended Practices of the American Oil Chemists Society*, 5th Ed., AOCS Press, Champaign.
- Anwar F, Ashraf M, Bhanger MI. 2005. Interprovenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan. *J. Am. Oil Chem. Soc.* **82**(1), 45-51.
- Anwar F, Bhanger MI. 2003. Analytical characterization of *Moringa oleifera* seed oil grown in temperate regions of Pakistan. *J. Agric. Food Chem.* **51**, 6558-6563.
- Baldini M, Giovanardi R, Tahmasebi-Enferadi S, Vannozzi GP. 2002. Effects of water regime on fatty acid accumulation and final fatty acid composition in the oil of standard and high oleic sunflower hybrids. *Ital. J. Agron.* **6**(2), 119-126.
- Bassil ES, Kaffka SR. 2002. Response of safflower (*Carthamus tinctorius* L.) to saline soils and irrigation: II. crop response to salinity. *Agric. Water Manage.* **54**, 81-92.
- Erdei L, Stuiver GEC, Kupier PJC. 1980. The effect of salinity on lipid composition and on activity of Ca²⁺ and Mg²⁺ simulated ATPase in salt-sensitive and salt-tolerant *Plantago* Species. *Physiol. Plant.* **49**, 315-319.
- Federation of Oils Seeds and Fats Association International (FOSFA International). 1982. *Manual of the Federation of Oils Seeds and Fats Association*, 277 (FOFSA) International. London.
- Flagella Z, Cantore V, Giuliani MM, Tarantio E, De Caro A. 2002a. Crop salt tolerance physiological, yield and quality aspects. *Rec. Res. Dev. Plant Biol.* **2**, 155-186.
- Flagella Z, Giuliani MM, Rotunno T, Di Caterina R, De Caro A. 2004. Effect of saline water on oil yield and quality of a high oleic sunflower (*Helianthus annuus* L.) hybrid. *Eur. J. Agron.* **21**, 267-272.
- Flagella Z, Rotunno T, Tarantino E, Di Caterina R, De Caro A. 2002b. Changes in seed yield and oil fatty composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to the sowing date and water regime. *Eur. J. Agron.* **17**, 221-230.
- Francois LE, Kleiman R. 1990. Salinity effects on vegetative growth, seed yield, and fatty acid composition of crambe. *Agron. J.* **82**, 1110-1114.
- Heuer B, Nadler A. 1998. Physiological response of potato plants to soil salinity and water deficit. *Plant Sci.* **137**(1), 43-51.
- Heuer B, Ravina I, Davidov S. 2005. Seed yield, oil content, and fatty acid composition of stock (*Matthiola incana*) under saline irrigation. *Aust. J. Agric. Res.* **56**, 45-47.
- Heuer B, Yaniv Z, Ravina I. 2002. Effect of late salinization of chia (*Salvia hispanica*), stock (*Matthiola tricuspidata*) and evening primrose (*Oenothera biennis*) on their oil content and quality. *Ind. Crop Prod.* **15**, 163-167.
- Heuer B. 1994. Osmoregulatory role of proline in water and salt stressed plants. In Pessaraki M. (Ed.) *Handbook of Plant and Crop Stress* 363-381. Marcel Dekker Inc, New York.
- International Organization for Standardization (ISO). 1977. *Oilseed Residues-Determination of Total Ash*, ISO, Geneva, Standard No. 749.
- International Organization for Standardization (ISO). 1981. *Animal Feeding Stuffs-Determination of Nitrogen and Calculation of Crude Protein Content*, ISO, Geneva, Standard No. 5983.
- International Union of Pure and Applied Chemistry (IUPAC). 1987. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th rev. enlarged Ed., edited by C. Paquot and A. Hautfenne, Blackwell Scientific, London.
- Jin Woong C, Choongsoo K. 1998. Effect of NaCl concentration on photosynthesis and mineral content of barley seedlings under solution culture. *Korean J. Crop Sci.* **43**(3), 152-156.
- Khan AH. 1998. Irrigation system performance assessment by decision support system and the role of a modern information transmission system in its operation. M. Phil Thesis, Report No.S44. International Water Management Institute. Lahore, Pakistan.
- Lee BL, New AL, Ong CN. 2003. Simultaneous determination of tocotrienols, tocopherol, retinol and major carotenoids in human plasma. *Clin. Chem.* **49**, 2056-2066.
- McGinley L. 1991. Analysis and quality control for processing and processed fats, in Rossell JB, Pritchard JLR, (Eds.), *Analysis of Oilseeds, Fats and Fatty Foods*, 460-470, Elsevier Applied Science, New York.
- Munns R, Cramer GR, Ball. 1999. Interactions between rising CO₂, soil salinity and plant growth. in Luo Y and Mooney HA.(Eds.), *Carbon dioxide and environmental stress*, 139-167, Academic Press, London.
- Parti RS, Deep V, Gupta SK. 2003. Effect of salinity on lipid components of mustard seeds. *Plant Food Human Nutr.* **58**, 1-10.
- Rossell JB. 1991. Vegetable oils and fats, in Rossell JB, Pritchard JLR, (Eds.), *Analysis of Oilseeds, Fats*

- and *Fatty Foods*, 261-319, Elsevier Applied Science, New York.
- Royo A, Gracia MS, Aragues R. 2005. Effect of soil salinity on the quality of Arbequina olive oil. *Grasas Y Aceites* **56**(1), 25-33.
- Smaoui A, Cherif A. 2000. Changes in molecular species of triacylglycerols in developing cottonseeds under salt stress. *Biochem. Soc. Trans.* **28**, 902-905.
- Somali MA, Bajnedi MA, Al-Fhaimani SS. 1984. Chemical composition and characteristics of *Moringa peregrina* seeds and seed oil. *J. Am. Oil Chem. Soc.* **61**, 85-86.
- Taneja R. 1988. Effect of cytokinin on growth, yield and metabolism of wheat (*Triticum aestivum* L.) grown under saline conditions. M.Sc. Thesis, Haryana Agricultural University, Hisar, India.
- Tester M, Davenport R. 2003. Na⁺ tolerance and Na⁺ transport in higher plants. *Ann. Bot.* **91**, 503-527.
- Tsaknis J, Lalas S, Gergis V, Dourtoglou V, Spilitois V. 1999. Characterization of *Moringa oleifera* variety Mbololo seed oil of Kenya. *J. Agric. Food Chem.* **47**, 4495-4499.
- Tsaknis J. 1998. Characterization of *Moringa peregrina* Arabian Seed Oil, *Grasas Aceites* (Sevilla) **49**, 170-176.
- Van de Graaff R, Patteson RA. 2001. Explaining the Mysteries of Salinity, Sodidity, SAR and ESP in Patterson R.A. and Jones M.J. (Eds). *On-site Practice in Proceedings of On-site 01 Conference: Advancing On-site Wastewater System*, 361-368. Published by Laboratories, Armidale.
- Yoon SH, Kim SK, Shin MG, Kim KH. 1985. Comparative study of physical methods for lipid oxidation measurement. *J. Am. Oil Chem. Soc.* **68**, 1487-1489.

Recibido: Marzo 2006

Aceptado: Julio 2006