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Characterization of *Moringa oleifera* seed oil from drought and irrigated regions of Punjab, Pakistan

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RESUMEN

Caracterización del aceite de semilla de *Moringa oleifera* procedente de regiones de secano y de regadío del Punjab paquistaní.

La intención del presente estudio ha sido la de investigar la composición del aceite obtenido de la semilla de *M. oleifera* cultivada en regiones de secano y de regadío Paquistaní. El contenido de aceite extraído de estas semillas cosechadas en una región de secano (Layyah) y dos de regadío (Rahim Yar Khan, Jhang) del Punjab paquistaní resultó ser, respectivamente, de 30.36, 35.26 y 38.37 %. Los resultados de los parámetros físico-químicos fueron: Índice de yodo, 65.86, 70.50 y 67.86; índice de refracción (40 °C), 1.4570, 1.4582 y 1.4581; densidad (24 °C), 0.9059, 0.9069 y 0.9002 mg mL^{-1}; índice de saponificación, 181.1, 183.7 y 183.1; materia insaponificable, 0.84, 0.85 y 0.97 % y acidez (referida a oleico) 0.28, 0.35 y 0.33 %.

El periodo de inducción (Rancimat 20L/h, 120 °C) del aceite procedente de regiones de secano fue significativamente mayor (9.63 h) que el obtenido en las regiones de regadío (8.74 y 8.33 h). Los coeficientes de extinción a 232 y 270 nm fueron respectivamente 1.92, 1.98 y 1.68; 1.02, 0.97 y 0.75. El contenido total de tocoferoles (α, γ y δ), los cuales no variaron de manera significativa en los aceites según su procedencia de secano o regadío, se mantuvieron en los rangos de valores siguientes: 95.85-103.80, 80.26-85.56 y 55.75-64.55 mg kg^{-1}, respectivamente. Estos resultados ponen de manifiesto que la procedencia de secano es el factor que más visiblemente influyen en el aumento del periodo de inducción y del contenido de C22:0 en los aceites, así como de la reducción del peso de la semilla, de la producción de aceite, del índice de yodo y del contenido en C18:1.

PALABRAS-CLAVE: Ácidos grasos - Alto oleico - Caracterización - Moringa oleifera - Periodo de inducción - Secano - Tocoferoles.

SUMMARY

Characterization of *Moringa oleifera* Seed oil from Drought and Irrigated Regions of Punjab, Pakistan

The aim of the present study was to investigate the composition of *M.oleifera* seed oil from drought and irrigated regions of Pakistan. The hexane-extracted oil content of *M.oleifera* seeds harvested from one drought (Layyah) and two irrigated regions (Rahim Yar Khan, Jhang) of Punjab, Pakistan was found to be 30.36 and 35.26, 38.37% respectively. Results of physical and chemical parameters of the extracted oils were as follows: iodine value, 65.86 and 70.50, 67.86; refractive index (40°C), 1.4570 and 1.4582,1.4581; density (24°C), 0.9059 and 0.9069, 0.9002 mg mL^{-1}; saponification value, 181.1 and 183.7, 183.1; unsaponifaiable matter, 0.84 and 0.85, 0.97%; acidity (as oleic acid) 0.28 and 0.35, 0.33%.

The induction period (Rancimat 20L/h, 120 °C) of the *M.oleifera* oil from the drought region was significantly higher (9.63 h) as compared with those of irrigated regions (8.74, 8.33 h). Specific extinctions at 232 and 270 nm were 1.92 and 1.98, 1.68; 1.02 and 0.97, 0.75 respectively. The overall contents of tocopherols (α, γ and δ), which did not differ significantly in the Moringa oils from both regions ranged from 95.85-103.80, 80.26-86.56 and 55.75-64.55 mg kg^{-1} respectively. Fatty acid profiles of the *M.oleifera* oils from drought and irrigated regions of Punjab consisted in a high level of oleic acid (up to 72.68 and 75.55, 74.66 %) followed by palmitic and behenic acid (up to 9.26 and 8.76, 9.20 and 5.46 and 3.72, 4.53 %) respectively. Results of various physical and chemical parameters of the investigated *M.oleifera* seed oils revealed that drought is one of the most visible factors that have amplified the induction period and C22:0 content of the oils and reduced seed weight, oil yield, iodine value and C18:1 content.

KEY-WORDS: Characterization - Drought - FAs - High-oleic - Induction period - Moringa oleifera - Tocopherols.

1. INTRODUCTION

"Moringaceae" is a single genus family of Magnoliopsida with 14 known species of which *Moringa oleifera* (Syn. *Moringa pterygosperma* Gaertn.) is the most widely known and utilized specie (Sengupta and Gupta, 1970; Morton, 1991). The tree originated from Agra and Oudh in the northwestern region of India, south of the Himalayas and is now cultivated across the whole of the tropical belt (Mughal *et al.,* 1999; Anhwange *et al*., 2004). In Pakistan *M.oleifera* is locally known as "Sohanjna" (Qaiser, 1973). Only two species of *Moringa*: *M. concanensis* and *M. oleifera* are reported from Pakistan. The former species are not common and perhaps confined to only a remote locality (Tharparkar) in the Sindh province. The latter *M.oleifera* is grown and cultivated in the plains of the Punjab province, Sindh province, Balochistan and North Western Frontier Province (N.W.F.P) of Pakistan (Qaiser, 1973).

M.oleifera has received a great amount of attention as "Natural Nutrition of the Tropics". The leaves, fruits, flowers and immature pods of this tree are locally used as vegetables (Anwar and Bahnger, 2003). The tender pods are cooked and

pickled and used in culinary preparations. The fresh beans after roasting make a palatable dish (The Wealth of India, 1962). Seeds are also consumed after frying and reported to taste like peanuts. The leaves of *M.oleifera* are known to be a good source of protein, vitamin A, B and C and minerals such as calcium and iron and are used as a spinach equivalent (Dahot, 1988).

In addition to its myriad of uses and super charged nutritional benefits, *M. oleifera* also has surprising medicinal qualities. 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 (Dahot, 1988; Hartwell, 1995). The roots of the young tree and also root bark are rubefacient and vesicant. The root decoction is used in Nicaragua for dropsy and the flower decoction is used as a cold remedy (Hartwell, 1995).

M.oleifera seeds have antimicrobial activity and are utilized for waste water treatment. In Sudan, dry *M.oleifera* seeds are used in place of alum by rural women to treat highly turbid Nile water (Muyibi and Evison, 1994). The seeds of *M.oleifera* are considered to be antipyretic, acrid and bitter (Sutherland *et al.,* 1994). *Moringa* seed kernels contain oil that is commercially known as "Ben oil"or "Behen oil". Ben oil has also been used by watchmakers for illumination and lubrication of delicate mechanisms. The oil was erroneously reported to be resistant to rancidity and used extensively in the process of enfleurage (a process of extracting perfumes by exposing absorbents to the exhalations of flowers) (Ndabigengeser and Narasiah, 1998). An ihe interest in the composition of *Moringa* oil has increased over the years. Somali *et al.* (1994) reported the chemical composition and characteristics of *M.peregrina* seed oil. Tsaknis *et al.,* (1999) investigated *M. oleifera* seed oil (Mbololo variety) from Kenya. The oil concentration varied from 25-35.7 % depending on different extraction methods. The oil was found to contain high levels of oleic acid (up to 75 %). Lalas and Tsaknis (2002) characterized *M.oleifera* seed oil variety Periyakulam-I. Ibrahim *et al.* (1974) and reported that Ben seed oil content and its properties show a wide variation depending mainly on species and environmental conditions.

Oils and fats being a natural source have extensive applications in our modern industrial world. Global industrialization and the increasing demand for environmentally acceptable materials has led to the investigation and exploitation of more vegetable oils as a renewable feed stock in the preparation of oleo chemicals in order to meet the growing needs of human society.

Although Pakistan is an agrarian country it is unable to produce vegetable oils in sufficient quantities for its domestic needs. Edible oil is Pakistan's largest single food import with consumer demand steadily increasing at 7.7% per year. Per capita consumption of oil and fats was 12.2 kg per year in 1996/98 and increased to more than 14 kg per year in 2004. The total domestic requirement of edible oil is 2 million tons of which about 29% comes from local production and the remaining 71% has to be imported every year, which is imposing a severe drain on the supply of foreign exchange reserves (Bhambhore, 2003; Anjum, 2005). In order to reduce the out flow of huge amounts of foreign exchange, there is an severe need to accelerate efforts in the agricultural sector to steadily increase the local production of oil seeds.

Genetic and environmental factors are important for the economic outcome of a vegetable oil seed crop and can affect oil yield and the quality of its grain production. Environmental factors affect seed and kernel composition more than genetic factors while the opposite is true for hullability. Trials under favorable conditions have indicated that unfavorable conditions, especially drought, might alter the seed composition and related quality (Nel, 2001). The production of oil seeds is often limited by a large variation in the amount of rainfall. The lack of water during vegetative and /or reproductive growth stages is one of the most limiting factors for seed growth. Plant tolerance to drought results from both morphological adaptation and responses at the biochemical and genetic levels and thus can affect the composition of lipids (Carvalho *et al.,* 2005; Gigon *et al.,* 2004).

Pakistan has been divided into different agro-ecological zones based on physiography, climate, land use and water availability. According to a report issued by United Nations Food and Agriculture Organization (FAO) and the World Food Programme (WFP), prolonged drought in parts of Pakistan has decimated livestock and severely affected fruit and rain fed cereal production (http://www.europaworld.org/issue44/cropandlivestock27701.htm). The hardest hit are Balochistan, and parts of the Sindh and Punjab provinces. The specter of drought that has been looming over the horizon of this country, still remains with the possibility of dire consequences for the future. It has had a significant impact on the economy of the country, especially in the agriculture sector**.** According to the economic survey report of United Nations about Drought-Pakistan (http://www.un.org.pk/drought/rereport15.htm), the drought caused a decrease in the production of cotton by 1.1 %.

Until now, a full characterization and comparison of the oil produced from seeds of *M. oleifera,* native to drought and irrigated regions of Punjab, Pakistan has not been reported. This has prompted us to initiate this study. The primary objective of the present work was to evaluate and quantify the effects of drought on the chemical composition and quality of *M.oleifera* oil seeds native to Punjab, Pakistan. In this context, we have assayed *M.oleifera* seeds from drought and irrigated regions of Punjab, Pakistan and a comprehensive analysis has been conducted. The study would contribute to the understanding of the response of drought and irrigated regimes to the characterization and

especially the oil yield and fatty acid composition of *M.oleifera* oils.

2. MATERIALS AND METHODS

2.1. Seed Collection

The seeds of *M.oleifera* were assayed from two differently adopted regions which included three districts i.e. Layyah, Jhang and Rahym Yar Khan of Punjab, Pakistan. Samples of dry seeds from mature fruits were harvested from drought (Layyah) and irrigated [Jhang (Chenab Nagar), Rahym Yar Khan (Sadiqabad)] regions of the Punjab province of Pakistan. Three samples of seeds from each district were harvested. The globular, three-winged seeds were covered with off-white seed coat, average weight *ca* 0.096 g (drought region), *ca* 0.130 g (irrigated region), with off-white kernel constituting 65-75% of the seed weight. After the removal of seed coats, seed kernels were preserved until further analysis. All reagents and chemicals used were from E. Merck or Sigma-Aldrich. Pure standards of tocopherols [Dl-α-tocopherol, (+)-δ-tocopherol, -γ-tocopherol], and fatty methyl esters (FAMEs) were obtained from Sigma Chemical Co. (St. Louis, MO).

2.2. Oil Extraction

After removal of the seed coat, the seeds were crushed and flaked in an oven at 100°C for 1 hour. After flaking the seeds (~60g) in each batch of *M.oleifera* they were then fed into a Soxhelt extractor fitted with a 500 mL round-bottom flask and a condenser. The extraction was executed in a water bath for 8-9 h with 300 mL of *n*-hexane. After extraction, 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) and stored under refrigeration until used for further analysis. A small quantity (used for the Rancimat analysis), of the recovered oil from different batches was further degummed.

2.3. Degumming of Oils

The oil to be degummed was heated to 70 °C in a water bath, and hot water was added to reach a final volume of 18 %. The mixture was mixed for 10 minutes with the aid of a glass rod. After cooling, the oil was centrifuged (3000 rpm i.e. 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 (– 4°C) until used for the determination of induction periods (Rancimat method).

2.4. 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 a semi-automated FOSFA official method (FOSFA, 1982). Samples of meal were digested for 10 min. with a digestion mixture of sulphuric acid/ hydrogen peroxide/ potassium sulphate, using mercuric oxide as a catalyst. The final end point in the ammonia titration was measured photometrically.

Fiber content was determined according to the ISO method (ISO, 1981). 2.0-g of finely ground defatted sample were weighed and boiled with a sulfuric acid solution (0.255 mol/L) for half an hour followed by separation and washing of the insoluble residue. The residue was then boiled with a sodium hydroxide (0.313 mol L^{-1}) solution followed by separation, washing and drying. The dried residue was weighed and ashed in a muffle furnace at 600°C and the loss in mass was determined.

Ash content was determined according to the ISO method (ISO, 1977). Two grams of the test portion were 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.

2.5. Analysis of Extracted Oil

2.5.1. Physical and chemical parameters of oil

Determinations of iodine value, saponifcation value, unsaponifibale matter, acidity, peroxide value, density and refractive index of the extracted oil were carried out according to the standard AOCS methods (AOCS, 1989). The color of the oil was determined by a Lovibond Tintometer (Tintometer Ltd., Salisbury, U.K Wiltshire, United Kingdom), using a 1-inch cell. Specific extinctions ($^{1\%}\varepsilon_{1cm\ (\lambda)}$) at 232 and 270nm were determined following the IUPAC method (IUPAC, 1987). Samples of the oil were diluted with iso-octane and spectrum were recorded in the ultraviolet region and absorbance values were noted at 232 and 270 nm using a Hitachi, U-2001, model 121-0032 spectrometer.

2.5.2. 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 oil and ???(Metrohm Application Bulletin,1993). Testing was carried out at 120 ± 0.1 °C and oxidative stability was measured following the procedure described elsewhere (Anwar *et al.,* 2003). Briefly, oil (2.5 g) was carefully weighed into each of the six reaction vessels and analyzed simultaneously. The IP of the samples was automatically recorded and corresponded to the break point on the plotted curves.

2.5.3. Fatty acid composition

Fatty acid methyl esters (FAMEs) were prepared following the IUPAC standard method 2.301and analyzed on a SHIMADZU gas chromatograph model 17-A fitted with a SP-2330 (SUPELCO, INC., Supelco Park, Bellefonte, PA, 16823- 0048 USA) methyl lignoserate-coated (film thickness 0.20 µm), polar capillary column (30m x 0.32 mm) and an FID. Oxygen-free nitrogen gas at a flow rate of 5 mL min^{-1}. Other conditions were as follows: initial oven temperature, 120 °C; ramp rate, 5 °C min^{-1}; final temperature, 240 °C; injector temperature 250 °C; detector temperature, 275 °C. FAMEs were identified by comparing their relative and absolute retention times with those of authentic standards. All the quantification was done with a Chromatography Station for Windows (CSW32) data-handling program, Data APEX LTD. CZ-158 00 Pague 5, The Czech Republic.

2.5.4. Tocopherol Content

Tocopherol (α,γ, and δ) analysis of the non-degummed oils was carried with HPLC following the method of Thompson and Hatina (Thompson and Hatina, 1979) with slight modifications. One gram of oil was accurately weighed and brought 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 20-µL sample was injected into a LiChrosorb SI-60 (Supelco Park Bellefonte, PA 16823-0048, USA) column (250x4.6 mm) packed with LiChrosorb SI 605 (5µm), which was fitted with a 50 x 50 mm (i.d.) guard column with He-Pellosil packing. A mobile phase of dry heptane/ water-saturated heptane / 2-propanol (50.0/48.5/1.5) was used at the rate of 1.2 mL min^{-1}. Detection was conducted at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Tocopherols were identified by comparing the retention times and quantified on the basis of the peak area percent of the unknowns with those of pure standards of α-,γ-, and δ- tocopherols (Sigma Chemical Co. (St. Louis, MO). A Hitachi Chromatointegrator model D-2500 with a built-in computer program for data handling was used for the quantification.

3. RESULTS AND DISCUSSION

The data from the analyses of *M.oleifera* oil seeds and extracted oils from drought (Layyah) and irrigated [Jhang (Chenab Nagar), Rahym Yar Khan (Sadiqabad)] regions of Punjab, Pakistan have been summarized in Table 1-5. Values for the present analyses are given as mean ± SD, for three *M.oleifera* oil seed samples from each district, analyzed individually in triplicate.

Table 1 shows the proximate analyses of *M.oleifera* oil seeds from both regions of Punjab, Pakistan. The samples of seeds assayed from drought were significantly (P = 0.005) lower in weight compared to those of the irrigated region. The average weight of *M.oleifera* seeds from drought (Layyah) and irrigated (Jhang, Rahym Yar Khan) regions was found to be 0.096 and 0.140, 0.120 g respectively. Triboi-Blondel and Renard (1999) reported that seed weight decreased under conditions of water stress. The hexane-extracted oil content of *M.oleifera* seeds from drought and irrigated regions of Punjab, Pakistan varied significantly (30.36 - 38.37%) (Table 1). The oil concentration was high (38.37%) in the seed samples of Jhang (Chenab Nagar), which were assayed from the *M.oleifera* plants harvested in the vicinity of river "Chenab". The high oil yield from this region might be attributed to the sandy soil texture and favorable environment for Moringa growth in the vicinity of the river 'Chenab'. Literature revealed that the Moringa tree is plentiful in or near sandy beds and streams (The Wealth of India, 1962). The oil content of *M.oleifera* seeds from Rahym Yar Khan was found to be 35.2%. Whereas, *Moringa* seeds native to Layyah (a drought region) had a lower oil concentration i.e., 30.36%. The variation observed in the Moringa seeds with regards to oil content may have been due to either a different genetic make up

Table 1
Analysis of *Moringa oleifera* seeds

Constituents	Drought	Irrigated		Literature		
	***M.o*-LYH**	***M.o*-JHG**	***M.o*-RYN**	**Anwar and Bhangar, 2003**	**Lalas and Tsaknis, 2002**	**Tsaknis *et al*., 1999**
Average Seed Weight (g)	0.096	0.140	0.120	Present work	Present work	Present work
Oil Content (%)	30.36 ± 0.70	38.37 ± 0.90	35.26 ± 0.74	40.39	38.3	35.7
Fiber (%)	9.00 ± 0.63	8.50 ± 0.55	6.60 ± 0.49	7.20	Present work	Present work
Ash (%)	8.46 ± 0.55	5.46 ± 0.65	6.63 ± 0.40	6.60	Present work	Present work
Protein (%)	30.97 ± 0.95	31.36 ± 1.19	29.63 ± 0.99	29.63	Present work	Present work

Values are mean ± SD of three seeds from each region, analyzed individually in triplicate.
***M.o*-LYH,** *M.oleifera* samples harvested from Layyah
***M.o*-JHG,** *M.oleifera* samples harvested from Jhang
***M.o*-RYN,** *M.oleifera* samples harvested from Rahim Yar Khan

of the plants or more probably due to environmental effects**.** It is reasonable to expect that Moringa plants from Layyah must have been exposed to drought conditions since water deficit and very low rainfall is characteristic of this region. Thus, it is plausible to conclude that the reduction in oil content may have been due to water deficit. Our results were agreeable with the work of Champolivier and Merrien (1996) who demonstrated a strong oil content reduction with water deficit. Carvalho *et al.,* (2005) reported that drought is a major factor accountable for any significant decrease in oil content. The mean oil content (36.81%) of *M.oleifera* seeds from the irrigated region of Punjab in the present analyses was quite comparable to those of our previous investigation of *M.oleifera* oil seeds from different regions of Pakistan (Anwar *et al.,* 2005).

The average oil content (34.66%) of *M.oleifera* seeds from both the drought and irrigated regions of Punjab was significantly lower than that (40.39%) reported for *M.oleifera* seeds from Sindh (Anwar and Bhanger, 2003). *M.oleifera* grows abundantly in the Sindh province of Pakistan, whereas, it only grows sparsely in the Punjab province. The former region is close to the tropics where the variation in seasonal temperature is not as large as in the latter. Furthermore, the soil texture of the Sindh province is sandy, while in the Punjab province it is generally loamy. The large variation in seasonal temperature along with specific soil texture might have been the major factors responsible for reducing the oil seed content in the Punjab provenances.

The average oil content (34.66%) of *M.oleifera* seeds, from drought and irrigated regions of Punjab, Pakistan was quite a bit lower than that reported for *M.oleifera* seeds from India (38.30%) (Lalas and Tsaknis, 2002), although it was comparable with *M.oleifera* seeds from Kenya (35.7 %) (Tsaknis *et al.,* 1999). Ibrahim *et al*., (1974) reported that such variation in oil content across countries might be attributed to the environmental and geological conditions in the regions.

The range of oil content (30-36-38.37%) of *M.oleifera* seeds in the present analyses was found to exceed those of cotton seed (15.0-24.0 %) and soybean (17.0-21.0 %) and comparable with those of safflower (25.0-40.0 %), and mustard (24.0-40.0 %), grown in the United States, Brazil, China and some other Asian and European countries (Pritchard, 1991).

Analyses of the *M.oleifera* oil seed residue revealed a high protein content in the seeds, ranging from 29.6 to 31.3%. Our analyses showed no significant variation in the contents of protein from drought to irrigated regions. The literature revealed some contentious reports about the effect of drought on protein content in the seeds. Carvalho *et al.,* (2005) reported that water stress did not affect protein contents. However, Nel (2001) affirmed that the contents of protein is affected by genetic as well as environmental factors. Fiber and ash contents were in the range of 6.6-9.0% and 5.4 to 8.4% respectively. The values of these parameters of the investigated *M.oleifera* oils were quite comparable to those reported for *M.oleifera* seeds from Sindh, Pakistan (Anwar and Bhanger, 2003). There were no previously reported data on the *M.oleifera* oil seeds residue analysis from other countries to compare the results with our present work. Present analyses also show the meal to be a good source of protein, which could be added to poultry feed as a source of calories and may replace soybean meal in the local poultry industry. It could also be utilized as a fertilizer, a potential animal foodstuff (following saponins detoxification if proven necessary) and a source of water treatment chemicals, all of which provide value added by-products.

The results of various physical and chemical parameters of the extracted *M.oleifera* oils from drought (Layyah) and irrigated (Jhang and Rahim Yar Khan) regions of Punjab, Pakistan are given in (Table 2). The value of iodine (65.86 g of iodine/ 100 g of oil) of the *M.oleifera* oil from drought was somewhat lower than those of irrigated regions (67.86, 70.50 g of iodine/ 100 g of oil). The mean iodine value (69.18 g of iodine/ 100 g of oil) of *M.oleifera* oils from the present irrigated region of Punjab was well in line to those of (69.06 g of iodine/ 100 g of oil) our previous investigation of *M.oleifera* oils from different regions of Pakistan (Anwar *et al.,* 2005).

The values determined for refractive index at 40°C (1.4570 to 1.4582), density at 25°C (0.9002 to 0.9061 mg mL^{-1}) and saponification value (181.1 to 183.7 mg of KOH/g of oil), which did not significantly vary from drought to irrigated regions of Punjab, were in close agreement with those of *M.oleifera* oils investigated from Sindh, Pakistan (Anwar and Bhanger, 2003). The average values of these parameters were also in good agreement with those of *M.oleifera* seeds oils reported in the literature (Lalas and Tsaknis, 2002; Tsaknis *et al.,* 1999). There were no significant variations in color values of Moringa oils from drought and irrigated regions of Punjab province of Pakistan. However, the color (2.1-2.3R + 21-23 Y) values of *M.oleifera* oils from the investigated regions of Punjab were significantly lower in their yellow and higher in their red units than those reported in the literature (Anwar and Bhanger, 2003; Tsaknis *et al.,* 1999; Lalas and Tsaknis, 2002; Anwar *et al.,* 2005). 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 values of color index are more suitable for edible and domestic purposes.

No significant difference was noted in the values of acidity of the oils from drought and irrigated regions of Punjab. The average value of acidity i.e., 0.32% in the present analyses of Moringa oils was not as varied as those of *M.oleifera* oils from Sindh, Pakistan (Anwar and Bhanger, 2003). However, it was significantly lower than *M.oleifera* oils reported

Table 2
Physical and Chemical Characterization of *Moringa oleifera* oil

Constituents	Drought	Irrigated		Literature		
	***M.o*-LYH**	***M.o*-JHG**	***M.o*-RYN**	**Anwar and Bhangar, 2003**	**Lalas and Tsaknis, 2002**	**Tsaknis *et al*., 1999**
Iodine value (g of I/100 g of oil)	65.86 ± 0.41	67.86 ± 1.10	70.50 ± 0.64	69.45	65.58	66.83
Refractive index (40°C)	1.4570 ± 0.001	1.4581 ± 0.002	1.4582 ± 0.001	1.4608	1.457	1.4549
Density (g/cm^3) 24°C	0.9059 ± 0.003	0.9002 ± 0.002	0.9069 ± 0.003	0.9057	0.909	0.8809
Saponification value (mg of KOH/g of oil)	181.1 ± 1.50	183.1 ± 1.31	183.7 ± 0.30	186.67	188.36	178.11
Unsaponifiable mater (%)	0.84 ± 0.10	0.97 ± 0.18	0.85 ± 0.10	0.90	Present work	Present work
Color (1" cell) (red Unit)	2.3 ± 0.50	2.3 ± 0.50	2.1 ± 0.40	1.00	0.80	1.9
Yellow unit	23 ± 1.40	23 ± 1.40	21 ± 1.15	29.00	35.00	30
Acidity (% as oleic acid)	0.28 ± 0.02	0.33 ± 0.02	0.35 ± 0.02	0.40	1.12	0.85

Values are mean ± SD of three oils from each region, analyzed individually in triplicate.
***M.o*-LYH,** *M.oleifera* samples harvested from Layyah
***M.o*-JHG,** *M.oleifera* samples harvested from Jhang
***M.o*-RYN,** *M.oleifera* samples harvested from Rahim Yar Khan

from India (Lalas and Tsaknis, 2002) and Kenya (Tsaknis *et al.,* 1999) and other regions of Pakistan (Anawar et al 2005). A low value of acidity is indicative of a high resistance to hydrolysis of *M.oleifera* oils. The unsaponifiable matter (0.84-0.97%) and saponifiable value (181.1-183.7 mg of KOH/g of oil) of the investigated *M.oleifera* oils which did not vary from drought to irrigated regions of Punjab, were in close agreement with those of *M.oleifera* oils reported from Sindh, Pakistan (Anwar and Bhanger, 2003).

The refractive indexes (1.4570-1.4582) of the investigated *M.oleifera* oils were within the range of cotton seed, palm and mango kernel oils (Rossell 1991). The saponification values and unsaponifiable matter of the investigated *M.oleifera* oils were in close agreement with those of olive, corn, low erucic acid rapeseed, soybean, sunflower and safflower oils (Rossell, 1991; Norman, 1979). The acidity and iodine values were significantly lower than those of olive oil (Norman 1979) and could not be compared with other common vegetable oils available in the literature (Rossell 1991).

The oxidation parameters of *M.oleifera* oils native to the drought and irrigated regions of Punjab, Pakistan are shown in Table 3. The specific extinctions at 232 nm (1.68-1.98) and 270 nm (0.75-1.02), which reveal the oxidative deterioration and purity of the oils (Yoon *et al*, 1985), were comparable to those of *M.oleifera* oil reported from Sindh (Anwar and Bhanger, 2003). However, these values varied significantly from those of *M.oleifera* oil from India (Lalas and Tsaknis, 2002) and Kenya (Tsaknis *et al.,* 1999). The peroxide value (mean 1.30, range 0.81-

Table 3
Determination of Oxidative State of *Moringa oleifera* oil

Determinations	Drought	Irrigated		Literature		
	***M.o*-LYH**	***M.o*-JHG**	***M.o*-RYN**	**Anwar and Bhangar, 2003**	**Lalas and Tsaknis, 2002**	**Tsaknis *et al*., 1999**
Conjugated diene $\varepsilon^{1\%}_{1cm}$ (λ232)	1.92 ± 0.04	1.68 ± 0.05	1.98 ± 0.04	1.70	3.001	3.1536
Conjugated triene $\varepsilon^{1\%}_{1cm}$ (λ270)	1.02 ± 0.05	0.75 ± 0.07	0.97 ± 0.05	0.31	Present work	1.1333
Peroxide value m.eq/kg of oil)	0.81± 0.04	1.73 ± 0.06	1.40 ± 0.03	0.59	1.83	1.80
Oxidative stability, degummed oil Rancimat method (h)	9.63 ± 0.20	8.33 ± 0.17	8.74 ± 0.18	8.63	8.70	10.8

Values are mean ± SD of three oils from each region, analyzed individually in triplicate.
***M.o*-LYH,** *M.oleifera* samples harvested from Layyah
***M.o*-JHG,** *M.oleifera* samples harvested from Jhang
***M.o*-RYN,** *M.oleifera* samples harvested from Rahim Yar Khan

1.73 meq/kg), which measures hydroperoxide products in the oils (McGinely, 1991) was significantly higher than those of *M.oleifera* oils from the Sindh province of Pakistan (Anwar and Bhanger, 2003), but lower than those of *M.oleifera* oils from India (Lalas and Tsaknis, 2002) and Kenya (Tsaknis *et al.,* 1999). The induction periods (Rancimat: 20L/h, 120 °C), which are a characteristic of the oxidative stability of oils and fats (Anwar et al., 2003) of the degummed *M.oleifera* oil from the drought (Layyah) region was significantly higher (9.63 h) compared to those (8.33, 8.74h) of the irrigated (Jhang, Rahym Yar Khan) region of Punjab. The mean induction period of the oils from the irrigated region (8.53 h) of Punjab in the present analysis was in good agreement with those of our previous investigations of *M.oleifera* oils from different regions of Pakistan (Anwar *et al.,* 2005). The average value (8.90 h) of induction period of the investigated *M.oleifera* oils native to both regions of Punjab, Pakistan was in close agreement with those of *M.oleifera* oils from Sindh, Pakistan (Anwar and Bhanger, 2003) and India (Lalas and Tsaknis, 2002) but varied to some extent from the *M.oleifera* oils from Kenya (Tsaknis *et al.,* 1999). A good oxidative state of *M.oleifera* oils as exhibited in the present analysis might be attributed to a significantly higher level of monoenoic fatty acids, particularly C18:1 and a high amount of α-, γ-, and δ-tocopherols.

Table 4 shows the amount of different tocopherols in the non-degummed *M.oleifera* oils from the drought (Layyah) and irrigated (Jhang, Rahym Yar Khan) regions of Punjab, Pakistan. The overall content of α-, γ-, and δ-tocopherols in the *M.oleifera* oils from both the drought and irrigated regions of Punjab, Pakistan ranged from 95.85-103.80, 80.26-86.56 and 55.75-64.55 mg kg^{-1} respectively. It is understandable that the contents of α-, γ-, and δ-tocopherols of Moringa oils from the drought region did not significantly vary from those of the irrigated region. Regarding the effect of drought on the content of lipid soluble antioxidants, Price and Hendry (1989) have reported that drought enhances the synthesis of α-tocopherol in the leaves of number of grasses. However, this response has not been observed in mosses (Seel *et al*., 1992) or in the higher plants.

The average content of α-tocopherols (99.42 mg kg^{-1}) and γ-tocopherols (82.60 mg kg^{-1}) of the *M.oleifera* oils, indigenous to both the investigated regions of Punjab, Pakistan was lower than those reported for *M.oleifera* native to Sindh, Pakistan (Anwar and Bhanger, 2003). The content of α-tocopherol, which has the greatest vitamin E potency (Rossell, 1991) in the investigated *M.oleifera* oils, was significantly higher than that of *M.oleifera* seed oil from India (Lalas and Tsaknis, 2002) and Kenya (Tsaknis *et al.,* 1999). Furthermore, the level of α-tocopherols of the investigated *M.oleifera* oils was in close agreement with the values reported for soybean, groundnut and palm oils (Rossell, 1991; Norman, 1979). The average concentration (60.26 mg kg^{-1}) of δ-tocopherols, which has a greater antioxidant activity than either γ-, or α-tocopherols in the investigated Moringa oils was significantly higher than the values of *M.oleifera* seed oil reported from Sindh, (Anwar and Bhanger, 2003) and India (Lalas and Tsaknis, 2002).However, it was lower than the values reported from Kenya (Tsaknis *et al.,* 1999).

Table 5 shows the fatty acid composition (FAC) of *M.oleifera* oils from the drought (Layyah) and irrigated (Jhang, Rahym Yar Khan) regions of Punjab, Pakistan. The content of saturates, that is, palmitic-, stearic-, arachidic and behenic-acids, in the investigated *M.oleifera* oils from both the drought and irrigated regions of Punjab ranged from 8.76-9.20, 2.27-3.30, 1.98-2.39 and 3.72-5.46 % respectively. The oils were found to contain a high level of oleic acid (C18:1 ω-9) which accounted for 72.68-75.55 % of the total fatty acids. A small amount of linoleic acid (C18:2 ω-6), gadoleic acid (C20:1) and C22:1 were also detected and ranged from 0.27-0.60, 1.99-2.32 and 0-0.53% respectively. The results of the fatty acid profiles of Moringa oils in the present analysis signified a lower content (72.68%) of C18:1 from drought compared to those (74.66, 75.10%) from the irrigated region. In the contrary, the content of behenic acid was higher (5.46%) in the former region compared to those of the latter (4.53, 3.72%). There have been reports in the literature which reveal reduction of C18:1 under conditions of water stress (Triboi-Blondel and Renard, 1999). The average concentration of C18:1 from the irrigated region

Table 4
Tocopherol contents of *Moringa oleifera* oil

Determinations	Drought	Irrigated		Literature		
	***M.o*-LYH**	***M.o*-JHG**	***M.o*-RYN**	**Anwar and Bhangar, 2003**	**Lalas and Tsaknis, 2002**	**Tsaknis *et al*., 1999**
α-tocopherol (mg kg^{-1})	95.85 ± 1.83	98.60 ± 1.95	103.80 ± 1.99	134.42	15.38	98.82
γ-tocopherol (mg kg^{-1})	86.56 ± 1.65	81.00 ± 1.60	80.26 ± 1.40	93.70	4.47	27.90
δ-tocopherol (mg kg^{-1})	55.75 ± 2.75	60.50 ± 2.34	64.55 ± 2.59	48.00	15.51	71.16

Values are mean ± SD of three oils from each region, analyzed individually in triplicate.
***M.o*-LYH,** *M.oleifera* samples harvested from Layyah
***M.o*-JHG,** *M.oleifera* samples harvested from Jhang
***M.o*-RYN,** *M.oleifera* samples harvested from Rahim Yar Khan

Table 5
Fatty acid composition (grams per 100 g of fatty acids) of *Moringa oleifera* oil

Fatty Acids	Drought	Irrigated		Literature		
	***M.o*-LYH**	***M.o*-JHG**	***M.o*-RYN**	**Anwar and Bhangar, 2003**	**Lalas and Tsaknis, 2002**	**Tsaknis *et al*., 1999**
$C_{16:0}$	9.26 ± 0.10	9.20 ± 0.10	8.76 ± 0.15	6.50	6.46	6.04
$C_{16:1\ cis\ (n-7)}$	2.96 ± 0.10	3.70 ± 0.15	3.65 ± 0.12	1.00	1.36	1.46
$C_{18:0}$	3.30 ± 0.14	2.27 ± 0.10	2.47 ± 0.09	5.67	5.88	4.14
$C_{18:1}$	72.68 ± 0.50	74.66 ± 0.70	75.55 ± 0.42	76.00	71.21	73.60
$C_{18:2}$	0.60 ± 0.10	0.35 ± 0.10	0.27 ± 0.08	1.29	0.65	0.73
$C_{20:0}$	2.39 ± 0.20	2.04 ± 0.15	1.98 ± 0.10	3.00	3.62	2.76
$C_{20:1}$	2.32 ± 0.10	2.25 ± 0.13	1.99 ± 0.11	1.20	2.22	2.40
$C_{22:0}$	5.46 ± 0.23	4.53 ± 0.17	3.72 ± 0.19	5.00	6.41	6.73
$C_{22:1}$	0.05 ± 0.02	Not detected	0.53 ± 0.10	Not detected	0.12	0.14

Values are mean ± SD of three oils from each region, analyzed individually in triplicate.
***M.o*-LYH,** *M.oleifera* samples harvested from Layyah
***M.o*-JHG,** *M.oleifera* samples harvested from Jhang
***M.o*-RYN,** *M.oleifera* samples harvested from Rahim Yar Khan

(75.10%) of Punjab in the present analysis was quite comparable to those (74.26%) of our previous investigation of *M.oleifera* oils from different regions of Pakistan (Anwar *et al.,* 2005).

The average concentration of C18:1 (74.29%) and C22:0 (4.57%) of the *M.oleifera* oils from the investigated drought and irrigated regions of Punjab, Pakistan did not significantly differ from those of *M.oleifera* oil reported from Sindh (Anwar and Bhanger, 2003). However, the oils were significantly (P=0.005) different in their contents of C16:0 and C18:0 compared to those native to Sindh. The average content of principal fatty acid i.e.C18:1 in the present analysis was also well in line with that reported for *M.oleifera* oils from India (Lalas and Tsaknis, 2002) and Kenya (Tsaknis *et al.,* 1999). The *M.oleifera* oils from Punjab, Pakistan, however, varied significantly in their contents of other component fatty acids compared to those of Moringa seed oils from India (Lalas and Tsaknis, 2002). The FAC of the investigated *M.oleifera* oils was found to be quite agreeable in the contents of C18:1 and C18:0 with those of olive oil (Norman, 1979), but varied with respect to other component fatty acids and it could not be compared to other common vegetable oils (Rossell 1991).

From the results of the present comprehensive analysis it could be concluded that drought might be considered one of the most visible factors which affected the chemical composition of some parameters of *M.oleifera* seed oils. Seed weight, oil yield, degree of unsaturation, oxidative stability and fatty acid composition are the parameters which are most vulnerable to drought. More importantly, it is evident that oil yield is the most affected by drought when compared to w other parameters.

As the Punjab and Sindh provinces of Pakistan comprise vast fertile lands and irrigated plains, *M.oleifera* appears to be a potentially valuable oil seed crop to be cultivated in such irrigated regions to benefit from its potential oil. *M.oleifera* oil might be an acceptable substitute for high-oleic oils like olive and high-oleic sunflower oils as our dietary fats and it also could be used for various commodities of commercial attributes**.**

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