

# Characterization of *Moringa oleifera* Variety Mbololo Seed Oil of Kenya

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The oil from *Moringa oleifera* variety Mbololo seeds from Kenya was extracted using three different procedures including cold press (CP), extraction with *n*-hexane (H), and extraction with a mixture of chloroform/methanol (50:50) (CM). The oil concentration ranged from 25.8% (CP) to 31.2% (CM). The density, refractive index, color, smoke point, viscosity, acidity, saponification value, iodine value, fatty acid methyl esters, sterols, tocopherols (by HPLC), peroxide value, and  $E_{1\text{cm}}^{1\%}$  at 232 and 270 nm and the susceptibility to oxidation measured with the Rancimat method were determined. The oil was found to contain high levels of unsaturated fatty acids, especially oleic (up to 75.39%). The dominant saturated acids were behenic (up to 6.73%) and palmitic (up to 6.04%). The oil was also found to contain high levels of  $\beta$ -sitosterol (up to 50.07%), stigmasterol (up to 17.27%), and campesterol (up to 15.13%).  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherols were detected up to levels of 105.0, 39.54, and 77.60 mg/kg of oil, respectively. The induction period (at 120 °C) of *M. oleifera* seed oil was reduced from 44.6 to 64.3% after degumming. The *M. oleifera* seed oil showed high stability to oxidative rancidity. The results of all the above determinations were compared with those of a commercial virgin olive oil.

**Keywords:** *Moringa oleifera* var. *Mbololo*; seed oil; composition; stability

## INTRODUCTION

The Moringaceae family consists of 10 (Somali et al., 1984) or 12 (Morton, 1991) species that belong to only one genus called *Moringa*. All *Moringa* species are native to India, from where they have been introduced into many warm countries (Sengupta and Gupta, 1970). Morton (1991) reported that the most common species are *Moringa oleifera* (syn. *M. pterygosperma* Gaertn.), *Moringa peregrina* (forsk) *fiori* (syn. *M. aptera* Gaertn.; *M. arabica* (Lam.) Pers., *Moringa zeylanica* Sieb.; *Balanus myrepsica* Blackm), *Moringa stenopetala* Cufod, *Moringa borziana* Mattei, *Moringa longituba* Engl., *Moringa concanensis* Nimmo, *Moringa ovalifolia* Dinter & A. Berger, and *Moringa drouhardii*. Bianchini et al. (1981) also reported *Moringa hildebrandtii*.

The best known and most widely distributed species is *Moringa oleifera* (syn. *M. pterygosperma* Gaertn.) (Morton, 1991; Sengupta and Gupta, 1970), which is a native of the western (Kantharajah and Dodd, 1991) and sub-Himalayan tracts (Sengupta and Gupta, 1970) of India and other countries of Asia (Morton, 1991; Kantharajah and Dodd, 1991; Sengupta and Gupta, 1970), Africa (Bianchini et al., 1981; Morton, 1991), the Middle East (Kantharajah and Dodd, 1991), The Philippines, Cambodia, Central America, northern South America, and the Caribbean islands (Morton, 1991).

The tree ranges in height from 5 to 10 m and sometimes even 15 m (Morton, 1991). Sengupta et al. (1970), Morton (1991), and Jamieson (1939) reported that the tree grows rapidly even in poor soil and is little affected by drought. The leaves, flowers, fruits (which

are called “pods”), and roots of the tree are used as vegetables (Kantharajah and Dodd, 1991; Morton, 1991; Ramachadran et al., 1980; Sengupta and Gupta, 1970), and the trunk is used in the paper industry (Kantharajah and Dodd, 1991; Verma et al., 1976). The fruits are usually 25–45 cm long, although Ramachadran et al. (1980) reported fruits up to 120 cm in length. Fruits contain ~20 seeds (Sengupta and Gupta, 1970), which are globular, ~1 cm in diameter, and three-winged, with wings produced at the base of the apex, 2–2.5 cm long, 0.4–0.7 cm wide, and scarios (Ramachadran et al., 1980). Sengupta and Gupta, (1970) reported that the seeds are three-angled and on average weigh ~0.3 g, with the kernel responsible for 70–75% of the weight. Ibrahim et al. (1974) reported that the oil content and its properties show a wide variation depending mainly on the species and the environmental conditions.

There are a few known varieties. They can be distinguished largely by the color and the size of the fruit. Some of them are reported by Morton (1991) and Ramachadran et al. (1980). These are Jaffna, Chauakacheri Murunga, Chem, Kadu, and Palmurungai.

Until now a full characterization of the oil produced from the seeds of *Moringa oleifera* var. Mbololo using the three methods of extraction described here has not been reported. The oils were compared with virgin olive oil.

## EXPERIMENTAL PROCEDURES

**Materials.** Three individual seed samples of 10 kg each were assayed from February to March 1997 (all samples were harvested simultaneously) from Kenya Forestry Research Institute (KEFRI, Nairobi, Kenya). The oil from each sample was extracted separately.

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The seeds, before extraction, were air-dried for 1 week.

The virgin olive oil "Horio" (Minerva S.A., Athens, Greece) was obtained commercially.

**Reagents.** All of the reagents (analytical and HPLC grade) were obtained from Sigma Chemical Co. (St. Louis, MO), and the standard solutions for the determination of tocopherols were purchased from Merck Ltd. (Darmstadt, Germany) (*dl*- $\alpha$ -tocopherol), Sigma ( $\delta$ -tocopherol), British Greyhound Chromatography and Allied Chemicals (Birkenhead, Merseyside, U.K.) [fatty acid methyl ester standards (FAMES)], and Larodan AB (Malmö, Sweden) (sterol standards).

**Methods.** *Oil Extraction.* The seeds from each individual sample were divided into three portions, and the oil was extracted by cold press (CP) or by the use of either *n*-hexane (H) or a mixture of chloroform/methanol (50:50) (CM) solvents.

The extraction for the CP procedure was performed as follows: The seeds were milled to a fine paste with a Vorwerk Thermomix 3300 (Vorwerk France S.A., Paris) at a speed of 12 with the addition of water (in a ratio of 1 seed/2 water by weight) prior to extraction. Extraction was carried out using an OMFB pm 25-S/1 simple hydraulic hand press (Costruz. Mecc. Oleodinamiche Provaglio D' Ised, Brescia, Italy) with a maximum pressure of 300 kg/cm<sup>2</sup>.

The solvent extractions were executed using a 2-L Soxhlet apparatus for 10 h. The seeds were milled to a fine paste with a Vorwerk Thermomix 3300 at a speed of 12, placed in a paper ampule, and divided into smaller samples (which were combined afterward) prior to extraction. The temperature was adjusted to have a rate of 200 drops of the refluxing solvent per minute. The solvent was evaporated using a rotary evaporator at 35 °C and under reduced pressure (60 mbar), and the oils from different batches were combined and kept in sealed bottles under refrigeration (0–4 °C) for further processing and analysis.

**Degumming.** The extracted oil was refined (degummed) apart from a small quantity of ~40 mL, which was kept for induction time determination (Rancimat) and other methods to compare the unrefined with the refined oil.

The oil was heated to 75 °C, and 20% boiling water was added. The mixture was stirred for 10 min with the aid of a glass rod. After cooling, the oil was centrifuged for 10 min at 3500 rpm (3595.06g) in tubes of 200 cm<sup>3</sup> using a Sorvall general purpose RC-3 automatic refrigerated centrifuge (Ivan Sorvall Inc., Newtown, CT).

**Determination of the Physical Characteristics.** The method used for the determination of density and refractive index (at 40 °C) was adapted from AOAC Method 969.18 (AOAC, 1990). Color was measured with a Lovibond tintometer (The Tintometer Ltd., Salisbury, U.K.). Smoke point was determined according to the method described by *British Standards Methods of Analysis* (1976) (BS 684: Section 1.8).

**Determination of Chemical Characteristics.** The determination of the chemical characteristics was as follows: acidity (measured according to IUPAC Method 2.201) (IUPAC, 1990), saponification value (determined according to AOCS Method Cd 3-25 described in Allen and Marvin (1982), and iodine value [measured according to the Wijs method as given by Pearsons (1981)].

**Determination of the Fatty Acid Composition.** Fatty acid composition was determined by gas-liquid chromatography according to the method of Tsaknis (1991).

Oil (~25 mg) was accurately weighed into a screw-cap tube (15 mL), and 1.5 mL of methanolic sodium hydroxide was added, mixed, and heated at 100 °C for 7 min. After cooling, 2 cm<sup>3</sup> of boron trifluoride was added and heated at 100 °C for 5 min. The tube was cooled to 30–40 °C, and 1 cm<sup>3</sup> of iso-octane was added; the tube was capped and shaken using a Whirlmix for 30 s. Saturated sodium chloride solution (5 cm<sup>3</sup>) was immediately added, and the tube was shaken again. The tube contents were allowed to separate, and the top (iso-octane) layer was removed and the lower aqueous layer was extracted again with an addition of 1 cm<sup>3</sup> iso-octane. The two iso-octane extracts were combined (dried over anhydrous sodium sulfate) and concentrated to ~1 cm<sup>3</sup> with a stream of nitrogen.

Analysis of FAMES was performed on a Varian 3600 gas chromatograph (Varian, Palo Alto, CA) equipped with a Supelcowax 10 (Supelco, Inc., Bellefonte, PA) fused silica capillary column, 30 × 0.32 mm i.d., 0.25- $\mu$ m film thickness. The temperature program was 60 °C for 10 min and then 2 °C min<sup>-1</sup> up to 220 °C. Injector and FID temperatures were set at 160 and 280 °C respectively, sample volume was 0.2  $\mu$ L, the carrier gas was N<sub>2</sub> at a flow of 30 mL min<sup>-1</sup>, chart speed was set at 0.5 cm min<sup>-1</sup>, and the attenuation was set at 10<sup>-10</sup> × 32. The internal standard used was nonadecanoic acid. Methyl esters were identified and quantified by comparing the retention times and peak areas of the unknowns with known FAME standard mixtures. Samples were prepared and measured separately in triplicate, for each method of extraction (three ways in total), and initial samples (three initial samples in total). Total samples measured were 27 (3 × 3 × 3 = 27).

**Determination of the Sterol Composition.** The identification and determination of sterols by GLC were according to the method described by the *Official Journal of the European Communities* (L248).

Analysis of sterols was performed on a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, San Diego, CA) equipped with a DB-5 FSOT capillary column (30 m × 0.25 mm × 0.25  $\mu$ m) (J&W, Folsom, CA). The pressure of the carrier gas (H<sub>2</sub>) was 75 kPa. Injector and FID temperatures were 280 and 300 °C, respectively. The column temperature was maintained at 260 °C, and the run time was 40 min.

The internal standard used was  $\alpha$ -cholestanol. Sterols were identified and quantified by comparing the retention times and peak areas of the unknown components with those of a known sterol standard mixture. Samples were prepared and measured separately in triplicate, for each method of extraction (three in total), and initial samples (three in total). Total samples measured were 27 (3 × 3 × 3 = 27).

**Determination of the Tocopherol Composition.** The method used for the determination of tocopherols was a modification of that reported by Carpenter (1979).

One gram of oil was accurately weighed into a 3-dram sample vial wrapped in aluminum foil to prevent oxidation. The oil was dissolved in 5 cm<sup>3</sup> of *n*-hexane before injection. A 20- $\mu$ L sample was injected into the Waters 600E HPLC pump (Millipore Corp., Waters Chromatography Division, Milford, MA) fitted with a Waters  $\mu$ -Polarsil, 125 Å, 10  $\mu$ m, 3.9 × 300 mm column.

A Waters 486 tunable absorbance detector was used set at 295 nm. 2-Propanol/*n*-hexane/absolute ethanol (2:97.5:0.5) at 1 cm<sup>3</sup>/min was used as the mobile phase. A total of 5 min was necessary to assay the tocopherols. The external standards used were *dl*- $\alpha$ -tocopherol (Merck) and (+)- $\delta$ -tocopherol (Sigma). Tocopherols were identified by comparing the retention times and quantified on the basis of peak area percent of the unknowns with those of known standards. Samples were prepared and measured separately in triplicate, for each method of extraction (three ways in total), and initial samples (three initial samples in total). Total samples measured were 27 (3 × 3 × 3 = 27).

**Determination of the Oxidative State.** For the determination of the oxidative state the peroxide value as well as the specific extinction ( $E_{1\text{cm}}^{1\%}$ ) at 232 nm was measured. The peroxide value was measured using the method adapted from Lea (1952). The determination of specific extinction ( $E_{1\text{cm}}^{1\%}$  at 232 nm) was carried out using IUPAC Method 2.505) (IUPAC, 1987) using a Hitachi U-3210 spectrophotometer (Hitachi Ltd., Tokyo, Japan).

**Determination of the Susceptibility to Oxidation with the Rancimat Method.** Two and a half grams of oil was accurately weighed into each of the six reaction vessels, and the following procedure was carried out. The Metrohm Rancimat 679 (Metrohm Ltd., Herisau, Switzerland) was switched on until the temperature of the oil batch reached 120 °C. Then 50 cm<sup>3</sup> of distilled water was placed into each of the six conductivity cells, and the air flow rate was set at 20 L h<sup>-1</sup>. The temperature was checked to ensure that it had a constant value. The air supply was connected to the tubes containing

**Table 1. Oil Content<sup>a</sup>**

determination	CP	H	CM
oil content (% w/w)	25.8 ± 2.6	35.7 ± 2.4	31.2 ± 2.0

<sup>a</sup> Values are means calculated from the data for three oils individually analyzed in triplicate ± standard deviation. CP, oil produced with cold press; H, oil produced with solvent extraction with *n*-hexane; CM, oil produced with solvent extraction with chloroform/methanol (50:50).

**Table 2. Physical Characteristics of the Degummed Oils<sup>a</sup>**

determination	CP	H	CM	olive oil
density at 24 °C (mg/mL)	0.9037 (0.004)	0.8809 (0.005)	0.9182 (0.005)	0.915 (0.007)
refractive index ( <i>m</i> 40 °C)	1.4591 (0.002)	1.4549 (0.001)	1.4581 (0.004)	1.4620 (0.005)
color				
red units	1.9 (0.1)	0 (0.0)	3.3 (0.4)	0 (0.0)
yellow units	30 (6.0)	40 (7.12)	72 (9.9)	47 (7.91)
smoke point (°C)	201 (2.1)	198 (1.6)	202 (1.9)	190 (1.9)
viscosity (mPa·s)	103 (0.43)	57 (0.16)	66 (0.16)	74 (0.17)

<sup>a</sup> Values are means of triplicate determinations; percentage coefficients of variation are given in parentheses.

the oil samples, and the chart recorder was started. The determination continued automatically until the conductivity reached the maximum value and the induction period was read.

## RESULTS AND DISCUSSION

The results of the determinations of the oil produced from *M. oleifera* Mbololo seeds were compared with the virgin olive oil "Horio". The European Union funded this research to check the possible use of this oil as a valuable edible oil like olive oil, due to the similarity of the fatty acid composition of the oil to that of the olive oil.

The oil content of the *M. oleifera* Mbololo seeds is shown in Table 1. The extracted oils were liquefied at room temperature. Among the methods used for the extraction of oil, that with *n*-hexane reported the highest yield of oil (35.7%) followed by extraction with the mixture of chloroform/methanol (31.2%) (50:50) and cold press (25.8%).

Due to the low free fatty acid content there was no need for neutralization. However, there was a need for degumming because the oils were cloudy and the gums reduce the temperature at which an oil produces smoke. The degumming process produced transparent pale yellow liquids at ambient temperature with characteristic odor and palatability. The mixture of chloroform/methanol (50:50) (because of its increased polarity) extracted more gums (7.4%) followed by cold pressure (1.9%) and *n*-hexane (1.0%).

Results of the physical characteristics of the oils are shown in Table 2. The density of *Moringa* oil depends on the method of extraction and can be higher or lower compared to olive oil. The refractive index of *Moringa* oils was lower than that of olive oil. The viscosity of the oil produced with CP was the highest, possibly because of the water that was bound in the oil during extraction. The smoke point of the oils under examination was 8 °C (H) to 12 °C (CM) higher than that of olive oil. The CM oil had the higher smoke point followed by CP and H.

Results of the chemical characteristics of the oil are shown in Table 3. The oil produced with cold pressure had the highest free fatty acid content. The acidity of

**Table 3. Chemical Characteristics<sup>a</sup>**

determination	CP	H	CM	olive oil
acidity <sup>b</sup> (% as oleic acid)	1.01 (0.14)	0.85 (0.09)	0.91 (0.11)	0.98 (0.02)
saponification value <sup>c</sup> (mg of KOH/g of oil)	179.80 (4.10)	178.11 (3.99)	176.23 (3.86)	188.00 (4.99)
iodine value <sup>c</sup> (g of I/100 g of oil)	66.81 (0.51)	66.83 (0.63)	66.66 (0.54)	80.01 (0.71)

<sup>a</sup> Values are means of triplicate determinations; percentage coefficients of variation are given in parentheses. <sup>b</sup> Not degummed oil. <sup>c</sup> Degummed oil.

**Table 4. Fatty Acid Composition of the Degummed Oils<sup>a</sup>**

determination	% (GLC)				
	fatty acid	CP	H	CM	olive oil
total saturated	19.1	20.98	20.17	14.72	
C8:0	0.03 (0.01)	0.03 (0.01)	0.02 (0.010)	nd <sup>b</sup>	
C14:0	0.11 (0.07)	0.11 (0.07)	0.11 (0.05)	<0.01	
C16:0	5.73 (0.311)	6.04 (0.406)	5.81 (0.356)	11.2 (0.663)	
C16:1 cis $\omega$ 9	0.10 (0.05)	0.11 (0.06)	0.10 (0.06)	0.60 (0.09)	
C16:1 cis $\omega$ 7	1.32 (0.89)	1.46 (0.88)	1.44 (0.91)	nd	
C17:0	0.09 (0.03)	0.09 (0.03)	0.09 (0.03)	0.1 (0.01)	
C18:0	3.83 (0.16)	4.14 (0.19)	4.00 (0.20)	2.80 (0.11)	
C18:1 cis $\omega$ 9	75.39 (0.75)	73.60 (0.77)	73.91 (0.79)	72.21 (0.78)	
C18:2 cis (9,12)	0.72 (0.36)	0.73 (0.41)	0.71 (0.39)	4.2 (0.49)	
C18:3 cis (9,12,15)	0.20 (0.07)	0.22 (0.08)	0.20 (0.04)	0.5 (0.10)	
C20:0	2.52 (0.65)	2.76 (0.55)	2.70 (0.49)	0.6 (0.29)	
C20:1	2.54 (0.44)	2.40 (0.38)	2.46 (0.39)	0.2 (0.06)	
C22:0	5.83 (0.56)	6.73 (0.29)	6.38 (0.36)	<0.01	
C22:1 cis	0.15 (0.06)	0.14 (0.07)	0.14 (0.08)	nd	
C26:0	0.96 (0.11)	1.08 (0.12)	1.06 (0.13)	nd	

<sup>a</sup> Values are means of triplicate determinations; percentage coefficients of variation are given in parentheses. CP, oil produced with cold press; H, oil produced with solvent extraction with *n*-hexane; CM, oil produced with solvent extraction with chloroform/methanol (50:50). <sup>b</sup> Not detected.

all the *Moringa* oils is lower than that of olive oil apart from the one produced with cold press. This can be attributed to the water added during milling of seeds prepared for cold press, which enhanced the action of lipolytic enzymes (Sengupta and Gupta, 1970). The iodine value is also lower compared to that of olive oil because the *Moringa* oil is less unsaturated than the olive oil (see also fatty acid composition, Table 4). There was no statistical difference (at the level of 95%) in the iodine values of the oils produced from the three different ways of extraction. The saponification values were lower compared to that of olive oil. The oil extracted with cold pressure had the highest acidity, possibly because during the extraction it remained in contact with air and higher temperature longer than the other two oils.

Total unsaturated fatty acids were >80%; the major fatty acid was oleic (C<sub>18:1</sub>) at concentrations of 75.39% (CP), 73.60% (H), and 73.91% (CM), followed by gadoleic (C<sub>20:1</sub>) [2.54% (CP), 2.40% (H), and 2.46% (CM)]. Behenic

**Table 5. Sterol Composition of the Degummed Oils<sup>a</sup>**

determination sterols by GC	GC%			
	CP	H	CM	olive oil
cholesterol	0.13 (0.020)	0.13 (0.019)	0.12 (0.016)	0.15
brassicasterol	nd <sup>b</sup>	0.06 (0.010)	0.06 (0.029)	tr <sup>c</sup>
24-methylenecholesterol	0.85 (0.160)	0.88 (0.116)	0.98 (0.111)	
campesterol	14.03 (0.931)	15.13 (0.996)	14.12 (0.916)	3.20 (0.955)
campestanol	nd	0.35 (0.069)	0.35 (0.036)	0.29 (0.032)
stigmasterol	17.27 (1.225)	16.87 (1.01)	16.78 (1.133)	0.60 (0.099)
ergostadienol	nd	0.39 (0.071)	0.28 (0.066)	
clerosterol	0.95 (0.230)	2.52 (0.916)	0.84 (0.301)	0.54 (0.269)
$\beta$ -sitosterol	49.19 (3.896)	50.07 (3.998)	50.00 (3.106)	64.3 (4.351)
stigmastanol	1.05 (0.411)	0.86 (0.196)	0.80 (0.124)	0.40 (0.086)
$\Delta^5$ -avenasterol	12.79 (1.614)	8.84 (1.198)	11.41 (1.065)	16.77 (1.235)
28-isoavenasterol	1.01 (0.421)	1.40 (0.312)	1.14 (0.196)	
$\Delta^{7,14}$ -stigmastanol	0.83 (0.223)	0.44 (0.102)	0.52 (0.093)	tr
$\Delta^7$ -avenasterol	0.94 (0.095)	1.11 (0.089)	1.04 (0.099)	0.29 (0.062)

<sup>a</sup> Values are means of triplicate determinations; percentage coefficients of variation are given in parentheses. CP, oil produced with cold press; H, oil produced with solvent extraction with *n*-hexane; CM, oil produced with solvent extraction with chloroform/methanol (50:50). <sup>b</sup> Not detected. <sup>c</sup> Traces.

acid (C<sub>22:0</sub>) was found to be the dominant saturated fatty acid at concentrations of 5.83% (CP), 6.73% (H), and 6.38% (CM), followed by palmitic acid (C<sub>16:0</sub>). Small to trace amounts of C<sub>8:0</sub>, C<sub>16:1cis</sub>, C<sub>22:1cis</sub>, and C<sub>26:0</sub> were also found in *M. oleifera* seed oil. There was no statistical difference (at the level of 95%) in the fatty acid compositions of the oils produced from the three different ways of extraction. On the basis of the results obtained, the fatty acid composition of *M. oleifera* seed oil showed that it falls in the oleic acid oil category (Sonntag, 1982). The *M. oleifera* Mbololo seed oil had about the same content of C<sub>18:1</sub> but much less C<sub>18:2</sub> than olive oil. *Moringa* oil was less unsaturated than the olive oil. Results are shown in Table 4.

The composition of the sterol fraction, analyzed by GLC, is shown in Table 5. The sterol fraction of the *M. oleifera* seed oil consisted mainly of campesterol, stigmasterol,  $\beta$ -sitosterol, and  $\Delta^5$ -avenasterol, among which  $\beta$ -sitosterol was the most predominant, accompanied with trace or minute amounts of cholesterol, brassicasterol,  $\Delta^7$ -avenasterol, 24-methylenecholesterol, campestanol, ergostadienol, clerosterol, stigmastanol, 28-isoavenasterol, and  $\Delta^{7,14}$ -stigmastanol. The sterol composition of the olive oil was different from that of *M. oleifera*. The dominant sterol of the olive oil was also  $\beta$ -sitosterol, at a concentration of 68%. There was no similarity of sterol composition of *Moringa* oil with olive oil. The CP did not extract campestanol, brassicasterol, and ergostadienol. *n*-Hexane extracted 2.4 times more clerosterol and  $\sim 2$  times less  $\Delta^{7,14}$ -stigmastanol.

Table 6 shows the tocopherol composition as determined by HPLC. A high tocopherol content was found in *M. oleifera* seed oil, consisting of  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherol. The oil extracted with the mixture of chloroform/

**Table 6. Tocopherol Composition of the Non-degummed Oils<sup>a</sup>**

determination tocopherols by HPLC	mg/kg			
	CP	H	CM	olive oil
$\alpha$ -tocopherol	101.46 (10.23)	98.82 (6.32)	105.02 (5.09)	88.5 (6.33)
$\gamma$ -tocopherol	39.54 (4.51)	27.90 (1.23)	33.45 (3.33)	9.9 (0.65)
$\delta$ -tocopherol	75.67 (6.33)	71.16 (6.96)	77.60 (4.52)	1.6 (0.10)

<sup>a</sup> Values are means of triplicate determinations; percentage coefficients of variation are given in parentheses.

**Table 7. Determination of the Oxidative State of the Degummed Oils<sup>a</sup>**

determination	CP	H	CM	olive oil
peroxide value (mequiv of O <sub>2</sub> /kg of oil)	0.36 (0.19)	1.80 (0.21)	0.94 (0.25)	1.5 (0.18)
$E_{1\text{cm}}^{1\%}$ at 232 nm	1.6648 (0.25)	3.1536 (0.97)	1.1658 (0.21)	2.0 (0.39)
$E_{1\text{cm}}^{1\%}$ at 270 nm	0.1948 (0.08)	1.1333 (0.31)	0.8934 (0.11)	0.15 (0.7)

<sup>a</sup> Values are means of triplicate determinations; percentage coefficients of variation are given in parentheses.

methanol (50:50) had the highest content of  $\alpha$ - and  $\delta$ -tocopherol and the second higher content of  $\gamma$ -tocopherol. The oil produced with cold pressure had the highest content of  $\gamma$ -tocopherol. Most vegetable oils contain  $\alpha$ -,  $\beta$ -, and  $\gamma$ -tocopherols.  $\delta$ -Tocopherol exists in a few oils such as cottonseed, peanut, wheat germ, soybean, and castor oils. The antioxidant activity of  $\delta$ -tocopherol exceeds that of  $\gamma$ -,  $\beta$ -, and  $\alpha$ -tocopherol. Thus, tocopherols present in high concentrations in *M. oleifera* seed oil are expected to offer some protection during storage and processing. Considering the tocopherol content, olive oil was expected to be less stable than *Moringa* oil.

The oxidative state of *M. oleifera* seed oil was determined using the peroxide value (PV) and specific extinction ( $E_{1\text{cm}}^{1\%}$ ) at 232 and 270 nm. The results are shown in Table 7. The PVs of *M. oleifera* seed oil were 0.36 (CP), 1.80 (H), and 0.94 (CM) mequiv of O<sub>2</sub>/kg of oil and fell in the range adopted as satisfactory. The cold pressure produced oil that had the lower PV followed by the mixture of chloroform/methanol (50:50) and *n*-hexane. The results of the  $E_{1\text{cm}}^{1\%}$  at 270 nm determination seem to follow the same trend, but at 232 nm CM had the lower value, followed by CP and H. The PV of olive oil appeared to be higher than that of *Moringa* oil produced with cold pressure and the mixture of chloroform/methanol (50:50) but not *n*-hexane. The  $E_{1\text{cm}}^{1\%}$  at 232 nm of olive oil appears to follow the same trend. At 270 nm, the mixture of chloroform/methanol (50:50) had the lowest absorbance.

The susceptibility to oxidation of the *M. oleifera* Mbololo seed oils, as measured by the Rancimat method, is shown in Table 8. A 42–73% reduction in induction period was observed, which could be attributed to oil degumming. The oil produced with the mixture of chloroform/methanol (50:50) had the longest induction period before the degumming process followed by *n*-hexane and cold pressure. The CP-produced oil had the longest induction period after the degumming process, followed by the mixture of chloroform/methanol (50:50) and *n*-hexane. The induction period of *Moringa* oil was >9 times longer than that of olive oil before degumming and up to 2.5 times longer after degumming. The

**Table 8. Determination of the Susceptibility to Oxidation of the Degummed and Non-degummed Oils<sup>a</sup>**

determination	Rancimat method (h) at 120 °C			
	CP	H	CM	olive oil
before degumming	34.1 (0.9)	36.8 (0.4)	46.2 (0.5)	7.88 (0.5)
after degumming	18.9 (0.9)	10.8 (0.2)	16.5 (0.4)	

<sup>a</sup> Values are means of triplicate determinations; percentage coefficients of variation are given in parentheses.

oxidative stability of olive oil is related to some extent to the presence of  $\alpha$ -tocopherol (Kiritsakis, 1988). Kiritsakis and Min (1989) reported that olive oil contains between 15 and 150 mg/kg  $\alpha$ -tocopherol. However, the stability of olive oil could not be explained only on the basis of tocopherol action. It is known that the olive mesocarp contains phenolic compounds, which although water soluble are present in the olive oil and considerably increase the oxidation stability of the oil (Kiritsakis and Min, 1989). Hudson and Ghavani (1990) reported that although most sterols are ineffective as antioxidants,  $\Delta^5$ -avenasterol (which is present in *Moringa* oil but in a lesser quantity than in olive oil), fucosterol, and citrostadienol have been shown to exhibit antioxidant properties in oils heated at 180 °C. It has been suggested that the donation of a hydrogen atom from the allylic methyl group in the side chain, followed by the isomerization to a relatively stable tertiary allylic free radical, represents the mode of action of the sterol antioxidants.  $\Delta^5$ -Avenasterol appears to be increased in concentration in a layer at the surface, and it is ineffective at room temperature. These findings suggest that avenasterol acts as a chemical antioxidant, its effectiveness arising from its concentration in the surface where oxidation occurs.

To the high content of  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherol of the *M. oleifera* Mbololo seed oils could be attributed the higher resistance to oxidation. In addition, olive oil contained linoleic and linolenic acids, which were contained in much lower quantity in *M. oleifera* Mbololo seed oil and which more easily undergone oxidation and degradation than C<sub>18:1</sub>. The long induction period of *Moringa* oil might be explained by the presence of  $\Delta^5$ -avenasterol. However, the olive oil, which had a shorter induction period, had a higher content of that sterol. Furthermore, the higher oxidative stability of *M. oleifera* Mbololo seed oil over olive oil should be attributed to other constituents of the non-glyceride fraction of the oil, which possess antioxidant properties (Lalas, 1998).

The characterization of the oil from the seeds of *M. oleifera* variety Mbololo showed that this oil could be utilized successfully as source of edible oil for human consumption. It contains a high ratio of monounsaturated to saturated fatty acids and might be an acceptable substitute for highly monounsaturated oils such as olive oil in diets.

#### LITERATURE CITED

Allen, R. R.; Marvin, F. W. Analytical methods. In *Bailey's Industrial Oil and Fat Products*; Swern, D., Ed.; Wiley-Interscience: New York, 1982; Vol. 2.

AOAC. *Official Methods of Analysis of the Association of the Official Analytical Chemists*; Firestone, D., chapter Ed.; Association of the Official Analytical Chemists: Arlington, VA, 1990.

Bianchini, J. P.; Gaydou, E. M.; Rabarisoa, I. Fatty acid and sterol composition of the seed oils of *Moringa hildebrandtii*, *Brochoneura freneeii* and *Strychnos spinosa*. *Fette-Seifen-Anstrichm.* **1981**, *83*, 302–304.

*British Standards Methods of Analysis*. British Standards Institution: London, U.K., 1976; Fats and Oils, part 1, Physical Methods, Section 1.8.

Carpenter, A. P., Jr. Determination of tocopherols in vegetable oil. *J. Am. Oil Chem. Soc.* **1979**, *56*, 668–671.

Hudson, B. J. F.; Ghavani, M. Phospholipids as antioxidant synergists for tocopherols in the autoxidation of edible oils. *Lebensm. itten Wiss. Technol.* **1984**, *17*, 19–24.

Ibrahim, S. S.; Ismail, M.; Samuel, G.; Kamel, E.; El Azhari, T. Benseeds: A Potential Oil Source. *Agric. Res. Rev.* **1974**, *52*, 47–50.

IUPAC (International Union of Pure and Applied Chemistry). *Standard Method for the Analysis of Oils, Fats and Derivatives*; Paquot, C., Hautfenne, A., Eds.; Blackwell Scientific Publications: London, U.K., 1987.

Kantharajah, A. S.; Dodd, W. A. Rapid clonal propagation of *Moringa oleifera* lam. using tissue culture. *South Indian Hortic.* **1991**, *39*, 224–228.

Kiritsakis, A. Tocopherols. In *The Olive Oil*; Agricultural Cooperative Editions: Thessaloniki, Greece, 1988.

Kiritsakis, A. K.; Min, D. B. Flavour chemistry of olive oil. In *Flavour Chemistry of Lipid Foods*; Min, D. B., Ed.; The American Oil Chemists' Society: Champaign, IL, 1989.

Lalas, S. Quality and stability characterisation of *Moringa oleifera* seed oil. Ph.D. Thesis, Lincolnshire and Humberside University, England, 1998; p 123.

Lea, C. H. On the antioxidant activities of the tocopherols. II. Influence of substrate, temperature and level of oxidation. *J. Sci. Food Agric.* **1952**, *11*, 212–215.

Morton, J. F. The Horseradish tree, *Moringa pterigosperma* (Moringaceae)—A boon to arid lands? *Econ. Bot.* **1991**, *45*, 318–333.

*Official Journal of the European Communities* **1991**, No. L248, 5.9.

Pearsons, D. *Chemical Analysis of Foods*; Egan, H., Kird, R. S., Sawyer, R., Eds.; Churchill Livingstone: Edinburg, U.K., 1981.

Ramachandran, C.; Peter, K. V.; Gopalakrishnan, P. K. Drumstick (*Moringa oleifera*): A multipurpose Indian vegetable. *Econ. Bot.* **1980**, *34*, 276–283.

Sengupta, A.; Gupta, M. P. Studies on seed fat composition of *Moringaceae* family. *Fette Seifen Anstrichm.* **1970**, *72*, 6–10.

Somali, M. A.; Bajneid, M. A.; Al-Fhaimani, S. S. Chemical composition and characteristics of *Moringa peregrina* seeds and seeds oil. *J. Am. Oil Chem. Soc.* **1984**, *61*, 85–86.

Sonntag, N. O. V. Composition and characteristics of individual fats and oils. In *Bailey's Industrial Oil and Fat Products*; Swern, D., Ed.; London, 1982; Vol. 2, pp 352–413.

Tsaknis, J. Quality changes of olive oil and other selected vegetable oils during frying. M.Phil. Thesis, Humberside University, England, 1991; pp 88–89.

Verma, S. C.; Banerji, R.; Misra, G.; Nigam, S. K. Nutritional value of *Moringa*. *Curr. Sci.* **1976**, *45*, 769–770.

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