A total characterisation of *Moringa oleifera* Malawi seed oil

J. TSAKNIS, S. LALAS
V. GERGIS, V. SPILIOTIS

DEPARTMENT OF FOOD TECHNOLOGY
TECHNOLOGICAL EDUCATIONAL INSTITUTE (T.E.I.)
ATHENS - GREECE

CARATTERIZZAZIONE DELL'OLIO DI SEMI DI MORINGA OLEIFERA MALAWI

È stato prodotto olio dai semi di Moringa oleifera Malawi usando tre diversi sistemi: pressione a freddo (CP), estrazione con n-esano (H) ed estrazione con una miscela 50:50 di cloroformio:metanolo (CM). L'olio prodotto andava dal 25.1% con (CP) al 41,4% con (CM). Sono stati determinati densità, indice di rifrazione, colore, punto di fumo, viscosità, acidità, numero di saponificazione, numero di iodio, metilesteri degli acidi grassi, steroli, tocoferoli (per HPLC), numero di perossidi. $E^{1\%}_{1cm}$ a 232 nm e 270 nm e la sensibilità all'ossidazione con il metodo Rancimat.
Si è trovato che l'olio contiene alti livelli di acidi grassi insaturi, ed in modo speciale oleico (oltre il 67.80%). I principali acidi saturi sono il behenico (oltre il 6.81%) e lo stearico (oltre il 5.86%). È stato anche determinato un alto contenuto di β-sitosterolo (superiore al 47,10%), campesterolo (sup. a 23,83%) e stigmasterolo (sup. al 17,40%). α-, γ- e δ-tocoferoli sono presenti rispettivamente con le seguenti quantità: 226,9, 71,47 e 216,57 mg/kg. Il periodo di induzione (a 120°C) dell'olio di Moringa oleifera è stato ridotto dal 49% al 74% dopo demucillagginazione. L'olio presenta alta stabilità all'ossidazione. In confronto con l'olio di oliva, l'olio dei semi di Moringa oleifera presenta una maggiore stabilità, un livello inferiore di insaturazione e composizione acidica simile (eccettuati C18:2 e C18:3).

The oil from the seeds of *Moringa oleifera* Malawi was produced using three different ways. Cold pressure (CP), extraction with n-hexane (H) and extraction with a mixture of chloroform:methanol (50:50) (CM). The oil produced ranged from 25.1 (CP) to 41.4% (CM). The density, refractive index, colour, smoke point, viscosity, acidity, saponification value, iodine value, fatty acid methyl esters, sterols, tocopherols (by HPLC), peroxide value, $E^{1\%}_{1cm}$ at 232 nm and 270 nm and the susceptibility to oxidation measured by the Rancimat method were determined.
The oil was found to contain high levels of unsaturated fatty acids, especially oleic (up to 67.80%). The dominant saturated acid was behenic (up to 6.81%) and stearic (up to 5.86%). The oil was also found to contain high levels of β-sitosterol (up to 47.10%), campesterol (up to 23.83%) and stigmasterol (up to 17.40%). α- γ- and δ-tocopherols were detected up to levels of 226.9, 71.47 and 216.57 mg/kg respectively. The induction period (at 120°C) of *Moringa oleifera* seed oil was reduced from 49% to 74% after degumming. Moringa oil showed high stability to oxidative rancidity. Compared to olive oil, *Moringa oleifera* seed oil showed higher stability, lower degree of unsaturation and similar fatty acid composition (apart from C18:2 and C18:3).

INTRODUCTION

The Moringaceae family consists of 10 [25] or 12 [20] species which belong to only one genus called Moringa. All Moringa species are native to India from where they have been introduced into many warm countries [24]. Morton [20] reported that the most common species are *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* and *A. Berger, Moringa drouhardii, Moringa hildebrantii* [4].

The best known and most widely distributed species is *Moringa oleifera* (syn. *M. pterygosperma* Gaertn.) [20, 24] which is a native of the Western [15] and sub-Himalayan tract [24], India and other countries of Asia [15, 20, 24], Africa [1, 3, 4, 20], Middle East [15], Philippines, Cambodia, Central America, Northern South America and the Caribbean Islands [20].

The tree ranges in height from 5 to 10 m and sometimes even 15m [20]. Sengupta et al. [24], Morton [20] and Jamieson [14] 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 [15, 20, 23, 24], while the trunk is used in the paper industry [15, 27]. The fruits are usually 25 to 45 cm long. Although Ramachadran et al. [23] reported fruits up to 120 cm in length. They contain about 20 seeds [24] which are globular, about 1 cm in diameter, 3-winged with wings produced at the base of the apex, 2-2.5 cm long, 0.4-0.7 cm wide and scarious [23]. Sengupta et al. [24] reported that the seeds are three-angled and on average weigh about 0.3 g with the kernel bringing 70-75% of the weight. Ibrahim et al. [12] 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 [20], Anonymous [1], Ramachadran et al. [20]. These are Jaffna, Chauakacheri Murunga, Chem, Kadu, Palmurungai. Anonymous [1] also reported Periyakulam 1 (PKM 1).

Until now a full characterisation of the oil produced from the seeds of *Moringa oleifera* Malawi using three different ways of extraction has not been reported. The oils were also compared with virgin olive oil.

MATERIALS AND METHODS

Materials

The seeds of *Moringa oleifera* wild local variety of Malawi (Blantyre area) were obtained from Malawi. The virgin olive oil "Horio" was purchased from Minerva S.A. (Athens, 14452, Greece).

Reagents

All the reagents (analytical and HPLC grade) were obtained from Sigma Chemicals Company Co. (St. Louis, MO 63178, USA) and the standard solutions for the determination of tocopherols were purchased by Merck Ltd (Darmstand, D-64271, Germany) (dl-α-tocopherol), Sigma ((+)-δ-tocopherol), British Greyhound Chromatography and Allied Chemicals (Birkenhead, Merseyside, L434X, UK)(Fatty acid methylesters standards) and Larodan AB (Malmö, S-21616, Sweden) (sterol standards).

Oil extraction

The seeds of *Moringa oleifera* were divided into three portions and the oil was produced by cold pressure (CP) and extracted by the use of n-hexane (H) and a mixture of chloroform : methanol (50:50) (CM), as solvents.

The extraction procedure for the cold pressure 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) prior to extraction which was done with a O.M.F.B. pm 25-S/1 simple hydraulic hand press (Costruz. Mecc. Oleodinamiche Provaglio D'Iseo, Brescia, Italy) with a max. pressure of 300 kg/cm^2.

The solvent extractions were executed using a two litre soxhlet apparatus (i.e. "cold" solvent).

Before the beginning of the measurements the oil was refined (degummed) apart from a small quantity of about 40 ml which was kept for the induction time determination (Rancimat) and other methods in order to compare the unrefined with the refined oil. The solvent was evaporated under reduced pressure, and the oil from different batches were combined and kept in sealed bottles under refrigeration (0 –4°C) for further processing and analysis.

Degumming

The oil was heated at 75 °C and 20% boiling water was added. The mixture was mixed for 10 minutes with the aid of a glass rod. After cooling, the oil was centrifuged for 10 minutes in 3,500 rpm in tubes of 200 cm^3 using a Sorvall General-Purpose RC-3 Automatic Refrigerated Centrifuge (Ivan Sorvall Inc., Newtown, Connecticut 06470, USA).

Determination of the physical characteristics

The determination of the physical characteristics was as follows: the density (relative density 40 °C/20 °C), refractive index (at 40 °C), color (measured with a Lovibond tintometer) (The Tintometer Ltd., Salsbury, England) and smoke point (according to the method described by British Standards Methods of Analysis, BS 684: Section 1.8) were measured.

Determination of the chemical characteristics

The determination of the chemical characteristics was as follows: the acidity (measured according to the method described by IUPAC [13], the saponification value (determined according to the method described by AOCS method Cd 3-25, described in Bailey's Industrial Oil and Fat Products), the iodine value (measured according to the Wijs method as described by Pearsons [22].

Determination of the fatty acid composition

The determination of the fatty acids composition was done by gas-liquid chromatography according to the method described by Tsaknis [26].

The FAMES preparation was done using the following procedure: about 25 mg of oil were accurately weighed into a screw cap tube, and 1.5 cm^3 methanolic sodium hydroxide was added, mixed and heated at 100 °C for 7 minutes. After cooling, 2 cm^3 of boron trifluoride were added and heated at 100 °C for 5 minutes. The tube was cooled to 30 - 40 °C and 1 cm^3 of iso-octane was added, capped and shaken using whirli mix for 30 seconds. 5 cm^3 of saturated sodium chloride solution was immediately added and the tube was shaken again. The tube contents were allowed to separate and the top (iso-octane containing fatty acid methyl esters) layer was removed and the lower layer was extracted again with an addition of 1 cm^3 iso-octane. The two iso-octane extracts were combined (dried over anhydrous sodium sulfate) and concentrated to approximately 1 cm^3 with a stream of nitrogen.

Analysis of fatty acid methylesters was performed on a Varian 3600 Gas chromatograph (Varian, Palo Alto, California, USA) equipped with a Carbowax 20M (Supelco, Inc. Supelco Park, Bellefonte, PA 16823-0048) 10'x1/8" (5% on Chromosorb W 80/100 mesh) column. The temperature program was 60°C for 10 min and then 2°C min^{-1} up to 220°C. Injector and FID temperatures were set at 160°C and 280°C respectively, sample volume was 0.2 μl, the carrier gas was N_2 at a flow of 30 ml min^{-1}, chart speed was set at 0.5 cm min^{-1} and the attenuation at 10^{-10}x32. In total three samples were prepared and measured separately.

Determination of the sterol composition

The identification and determination of sterols by GLC was done according to the method described by the Official Journal of the European community, No. L 248, 5.9.1991.

Analysis of sterols was performed on a Hewlett Packard 5890 Gas Chromatograph (Hewlett-Packard, San Diego, CA, USA) equipped with a DB-5 FSOT capillary column (30 m x 0.25 mm x 0.25 μm) (J & W, 91 Blue Ravine rd., Folson, 95630-4714, California, USA). The pressure of the carrier gas (H_2) was 75 kpa. Injector and FID temperatures were 280°C and 300°C respectively. The temperature program was isothermal 260°C for 40 min at least.

In total three samples were prepared and measured separately.

Determination of the tocopherol composition

The method used for the determination of tocopherols was a modification of that reported by Carpenter's [6].

(a) 1 g of oil was accurately weighed into a 3 gram sample vial wrapped in foil paper to prevent oxidation. The oil was dissolved in a 5 cm^3 n-hexane before injection.

(b) A 20 μl sample was injected into the Waters 600E HPLC pump (Millipore Corporation, Waters Chromatography Division, Massachusetts, MA 01757, USA) fitted with a Waters μ-Polarsil, 125 Å, 10 μm, 3.9x300 mm column.

Detection was performed with a Waters 486 Tunable Absorbance Detector set at 295 nm. Iso-propanol:n-hexane:absolute ethanol (2:97.5:0.5) at 1 cm^3/min was used as the mobile phase. A total of 5 min was necessary to assay the tocopherols. In total three samples were prepared and measured separately.

Determination of the oxidative state

For the determination of the oxidative state the peroxide value as well as the specific extinction ($E^{1\%}_{1cm}$) at 232 and 270 nm were measured. The peroxide value was measured using the method adapted from Lea (1952). The determination of specific extinction ($E^{1\%}_{1cm}$ at 232 and 270 nm) was carried out using the method of IUPAC (1987) and by the use of a Hitachi U-3210 spectrophotometer (Hitachi Ltd. Tokyo, Japan).

Determination of the susceptibility to oxidation with the Rancimat method

Two and a half grams (2.5 g) of oil were accurately weighed into each of the six reaction vessels and the following procedure was carried out. The "Metrohm Rancimat 679" (Metrohm Ltd., CH-9101, Herisau, Switzerland) was switched on until the temperature of the oil batch reached the temperature of 120°C. Then 50 cm^3 of distilled water was placed into each of the six conductivity cells and the air flow rate was set at 20 L h^{-1}. The temperature was checked to ensure it had a constant value. The air supply was connected to the tubes containing 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 *Moringa oleifera* wild local variety of Malawi (Blantyre area) seeds were compared not only with those reported in the literature but also with virgin olive oil "Horio". The European Union founded this research in order to check the possible use of this oil as valuable edible oil like the olive oil, due to the similarity of the fatty acid composition of the Moringa oil to that of the olive oil.

The oil content of the *Moringa oleifera* Malawi seeds are shown in Table I. The extracted oils were liquefied at room temperature. The results fell in the range of previously reported data. Among the methods used for the extraction of oil, that with the mixture of chloroform:methanol (50:50) reported the highest yield of oil followed by extraction with n-hexane and extraction with cold pressure.

Due to the low free fatty acid content there was no need for neutralisation. 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. After the completion of the process the percentage of the oil recovered was measured. The mixture of chloroform:methanol (50:50) extracted more gums followed by cold pressure and n-Hexane. Results are shown in Table II.

Results of the physical characteristics of the oils are shown in Table III. The density of Moringa oils was higher than that reported in literature and lower compared to olive oil (apart from that of CM which was equal). The refractive index of Moringa oils was lower compared with the previously reported data and olive oil. There were no previously reported data to compare colour, smoke point and viscosity. The smoke point of the oils under examination was 12 °C(H) to 16 °C(CM) higher from that of the olive oil. The CM oil had the higher smoke point followed by CP and H. There are no previously reported data to compare colour and viscosity.

TABLE I - Oil content

Determination	CP	H	CM	Literature
Oli content, %	26.3 (2.5)	35.3 (2.3)	39.5 (2.3)	40.0[1], 37.7-39.72[3], 37.2-38.16[7], 21[8], 30-49[9], 24.0[10], 47.6[12], 37.7[14], 21[16,25], 34.7[23], 34.4[24], 27.0[27]

Values are means of triplicate determinations and percentage coefficient of variation is given in parenthesis. Numbers in brackets in the literature column, represent the number of reference in the references section.

TABLE II - Degumming

Way of extraction	CP	H	CM
Oli recovered (%)	98.7	98.9	91.2

TABLE III - Physical characteristics of the degummed oils

Determination	CP	H	CM	Olive oil	Literature
Density at 24°C (mg/ml)	0.9016 (0.006)	0.8882 (0.005)	0.9152 (0.006)	0.915 (25°C) (0.007)	0.919 (15°C) [16], 0.8984 [23] 0.8768 (25°C) [24]
Refractive index ($n_0$40°C)	1.4598 (0.002)	1.4559 (0.004)	1.4592 (0.003)	1.4689 (20°C) (0.005)	1.4650 (25°C) [8], 1.4650 (25°C)[9] 1.4681 (20°C) [10], 14610 [12], 1.4668 (25°C) [14], 1.4600 (40°C) [24] 1.46 (25°C) [27]
Colour (red units, yellow units)	2.0r (0.1) 28y.(6.5)	1.2r (0.1) 70y.(9.2)	1.5r (5.1) 79y.(10.7)	— —	present work present work
Smoke point (°C)	204 (2.0)	202 (2.0)	206 (2.1)	190 (1.9)	present work
Viscosity (mPa·s)	91 (0.21)	62 (0.17)	71 (0.18)	—	present work

Values are means of triplicate determinations and percentage coefficient of variation is given in parenthesis. Numbers in brackets in the literature column, represent the number of reference in the references section.

TABLE IV - Chemical characteristics

Determination	CP	H	CM	Olive oil	Literature
Acidity* (% as oleic acid)	1.20 (0.12	0.82 (0.01)	0.98 (0.02)	0.98 (0.02)	6.5[8], 0.7-13 [9], 0.53 [12], 0.9-2.3 [14], 9.50 [16], 3.5 [23], 15.5 [24], 0.26 [27]
Saponification value** (mg of KOH/g of oil)	188.70 (4.32)	184.16 (4.23)	186.70 (5.01)	188 (4.99)	188.1 [8], 186.4-188 [9], 189 [10], 171.7 [12] 186-187.7 [14], 189.73 [16], 182.2 [23]
Iodine value** (g of I/100 g of oil)	65.83 (0.46)	65.74 (0.50)	65.59 (0.50)	80.01 (0.71)	67.1 [8], 66-68 [9], 69 [10], 64.2 [12], 67.03 [16] 64.2 [23], 66.1 [27], 67.2 [24]

*) Not degummed oil, **) degummed oil. # Values are means of triplicate determinations and percentage coefficient of variation is given in parenthesis. Numbers in brackets in the literature column, represent the number of reference in the references section.

TABLE V - Fatty acid composition of the degummed oils

Determination	% (GC)				Literature
Fatty acid	CP	H	CM	Olive oil	%
C8:0	0.03 (0.01)	0.02 (0.01)	0.02 (0.02)	n.d.	present work
C12:0	n.d.	n.d.	n.d.	n.d.	12.5 [27]
C14:0	0.10 (0.05)	0.10 (0.06)	0.10 (0.06)	< 0.01	0.1-1.4 [3], <0.01 [10], 1.53 [14]
C16:0	5.40 (0.345)	5.51 (0.358)	5.46 (0.302)	11.2 (0.663)	3.5-9.3 [3], 5.5 [8,9], 6.18 [10], 3.59 [14], 9.3 [23], 3.5 [24], 9.6 [27]
C16:1*cis*ω9	0.11 (0.04)	0.11 (0.05)	0.11 (0.04)	0.60 (0.09)	1.1 [3], 0.9 [8,9], 1.42 [10], 1.6 [27]
C16:1*cis*ω7	0.98 (0.68)	1.10 (0.74)	1.11 (0.73)	n.d.	present work
C17:0	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)	0.1 (0.01)	<0.01 [10]
C18:0	5.80 (0.22)	5.86 (0.33)	5.82 (0.29)	2.80 (0.11)	4.3-5.1 [3], 7.7 [8], 7.8 [9], 5.68 [10], 10.84 [14], 10.50 [16], 7.4 [23], 8.2 [24], 3.0 [27]
C18:1	67.65 (0.66)	67.79 (0.67)	67.80 (0.65)	72.21 (0.78)	65.7-76.5 [3], 75 [8], 75.9 [9], 70.01 [10], 68.05 [14] 67.48 [16], 65.7 [23], 72.8 [24], 73.3 [27]
C18:2	0.69 (0.33)	0.71 (0.41)	0.69 (0.35)	4.2 (0.49)	0.6-3.5 [3], 0.8 [8], 0.8 [9], 0.78 [10], 3.77 [14] 3.40 [16], 0.7-1.1 [24]
C18:3	0.20 (0.06)	0.21 (0.07)	0.19 (0.05)	0.5 (0.10)	0.1 [3]
C18:4	n.d.	n.d.	n.d.	n.d.	0.26 [10]
C20:0	3.72 (0.28)	3.78 (0.29)	3.77 (0.51)	0.6 (0.29)	2.7-3.6 [3], 2.6 [8], 2.7 [9], 3.94 [10], 7.2 [24]
C20:1	2.64 (0.29)	2.60 (0.28)	2.61 (0.31)	0.2 (0.06)	2.3 [3], 2.13 [10]
C22:0	6.74 (0.41)	6.81 (0.56)	6.78 (0.33)	< 0.01	4.6-8.6 [3], 1.2 [8], 1.2 [9], 7.74 [10], 6.29 [14] 8.6 [23], 3.0 [24]
C22:1 *cis*	0.12 (0.04)	0.11 (0.01)	0.11 (0.06)	n.d.	present work
C24:0	n.d.	n.d.	n.d.	n.d.	1.0-3.2 [3], 5.3 [8], 5.3 [9], 0.13 [14], 4.6 [24]
C26:0	0.90 (0.09)	0.98 (0.10)	0.96 (0.08)	n.d.	present work

Values are means of triplicate determinations and percentage coefficient of variation is given in parenthesis. Numbers in brackets in the literature column, represent the number of reference in the references section.

Results of the chemical characteristics of the oil are shown in Table IV. The oil produced with cold pressure had the highest free fatty acid content. The results fell in the range of previously reported data. The acidity of all the Moringa oils is higher (apart from H) than the olive oil. This can be attributed to that the seeds where stored, at their place of origin, under high temperature and level of humidity, and also to the presence of lypolitic enzymes [24]. The oil extracted with cold pressure had the highest acidity possibly because during the extraction remained in contact with air and higher temperature longer than the other two oils. The iodine value is also lower compared to olive oil because the Moringa oil is less unsaturated than the olive oil (see also fatty acid composition, Table V). There was no statistical difference (at the level 0f 95%) in iodine value of the oils produced from the three different ways of extraction These results also fell in the range of previously reported data. The saponification values were in line with those reported in literature and with olive oil apart from the case of H which was lower.

Total unsaturated fatty acids were more than 72%, while the major fatty acid was oleic (C18:1) in a concentration of 67.65% (CP), 67.79% (H) and 67.80% (CM), followed by gadoleic (C20:1), 2.64% (CP), 2.60% (H) and 2.61% (CM). Behenic acid (up to 6.81%) was found to be the dominant saturated fatty acid, followed closely by stearic acid (up to 5.86%) and both fell in the range of previously reported data. Guerere et al. [10] reported a 0.26% of C18:4. This fatty acid was not detected in present work. The concentrations reported in literature for miris-

TABLE VI - Sterol composition of the degummed oils

Determination	Present work				Literature
Sterols by GC	%				
	CP	H	CM	Olive oil	
Cholesterol	0.19 (0.040)	0.09 (0.019)	0.16 (0.036)	n.d.	0.23 [10]
Brassicasterol	0.12 (0.033)	0.08 (0.012)	0.14 (0.039)	n.d.	<0.01 [10]
$\Delta^{5,25}$-Ergostadienol	0.08 (0.022)	0.09 (0.010)	0.07 (0.006)	n.d.	present work
24-methylene cholesterol	0.91 (0.160)	0.96 (0.116)	0.91 (0.111)	n.d.	present work
Campesterol	23.68 (2.131)	23.83 (2.996)	23.34 (2.096)	2.20 (0.098)	16.6 [10]
Campestanol	0.47 (0.071)	0.40 (0.069)	0.35 (0.036)	n.d.	present work
Stigmasterol	17.40 (1.325)	17.03 (1.026)	16.49 (1.123)	0.40 (0.036)	23.3 [10]
$\Delta^{5,24}$-Ergostadienol	0.53 (0.105)	0.30 (0.096)	0.30 (0.088)	n.d.	present work
Clerosterol	0.65 (0.130)	0.62 (0.121)	0.72 (0.101)	1.00 (0.009)	present work
β-Sitosterol	46.73 (3.546)	47.07 (2.998)	47.10 (3.026)	68.00 (4.351)	48.6 [10]
Stigmastanol	0.87 (0.111)	0.77 (0.199)	0.91 (0.123)	n.d.	present work
Δ^{5}-Avenasterol	2.87 (0.614)	2.94 (0.598)	1.15 (0.265)	18.02 (1.235)	10.2 [10]
$\Delta^{5,23}$-Stigmastadienol	1.23 (0.299)	1.23 (0.301)	1.31 (0.401)	n.d.	present work
$\Delta^{7,14}$-Stigmastedienol	0.52 (0.223)	0.39 (0.102)	0.40 (0.098)	n.d.	present work
28-Isoavenasterol	0.27 (0.121)	0.25 (0.112)	0.40 (0.096)	n.d.	present work
Δ^{7}-Avenasterol	0.45 (0.065)	0.19 (0.012)	0.31 (0.099)	n.d.	present work
Δ^{7}-Stigmastenol	0.81 (0.096)	0.51 (0.078)	0.43 (0.065)	n.d.	<0.01 [10]

Values are means of triplicate determinations and percentage coefficient of variation is given in parenthesis. Numbers in brackets in the literature column, represent the number of reference in the references section.

tic (C14:0) and palmitoleic (C16:1 cis ω9) were relatively higher than those in the present determination and relatively lower in the case of C17:0. Parinaric (C18:4) and lignoceric (C24:0) acids although reported in literature were not detected during present determination. Small to trace amounts of C8:0, C16:1 cis ω7, C22:1 cis and C26:0 were also found in *Moringa oleifera* seed oil. These were detected for the first time. There was no statistical difference (at the level of 95%) in the fatty acid composition of the oil produced from the three different ways of extraction. Based on results obtained, the fatty acid composition of *Moringa oleifera* seed oil showed that it falls in the oleic acid oil category. The *Moringa oleifera* Malawi seed oil had about the same content of C18:1, but much less C18:2 than olive oil. Moringa oil was less unsaturated than the olive oil.

The composition of the sterol fraction, analysed by GLC, is shown in Table VI. The sterol fraction of the *Moringa oleifera* seed oil consisted mainly of campesterol, stigmasterol, β-sitosterol, clerosterol and Δ^{5}-avenasterol, among of which β-sitosterol was the most predominant, followed by campesterol (up to 23.83%) and stigmasterol (up to 17.40%) and accompanied with trace to minute amounts of 24-methylenecholesterol, cholesterol, $\Delta^{7,14}$-stigmastadienol, brassicasterol, stigmastanol, 28-isoavenasterol,Δ^{7}-stigmastanol, Δ^{7}-avenasterol,

TABLE VII - Tocopherol composition of the not degummed oils

Determination	mg/kg				Literature
Tocopherols by HPLC	CP	H	CM	Olive oil	
α-Tocopherol	226.9 (10.23)	131.03 (13.51)	166.81 (12.69)	88.5 (6.33)	present work
γ-Tocopherol	71.47 (5.69)	70.43 (6.33)	68.11 (7.89)	9.9 (0.65)	preset work
δ-Tocopherol	216.57 (11.65)	53.98 (4.12)	54.44 (8.99)	1.6 (0.10)	present work

Values are means of triplicate determinations and percentage coefficient of variation is given in parenthesis.

TABLE VIII - Determination of the oxidative state of the oils

Determination	CP	H	CM	Olive oil	Literature
Peroxide value (meq O2/kg oil)	0.19 (0.12)	0.23 (0.13)	0.15 (0.09)	15.58 (0.987)	1.3 [12], 80.0 [16]
$E^{1\%}_{1cm}$ at 232 nm	1.3011 (0.18)	1.4401 (0.20)	1.1925 (0.16)	—	present work
$E^{1\%}_{1cm}$ at 270 nm	0.1222 (0.06)	0.1621 (0.07)	0.1239 (0.07)	—	present work

Values are means of triplicate determinations and percentage coefficient of variation is given in parenthesis. Numbers in brackets in the literature column, represent the number of reference in the references section.

$\Delta^{7,14}$-stigmastadienol, and campestanol. The previously reported data agree with those of the present determination only in the case of β-sitosterol. Also, Δ^7-stigmasterol reported in literature was not detected during present determination. The sterol composition of the olive oil was different from that of *Moringa oleifera*. The dominant sterol of the olive oil was also -sitosterol but in a concentration of 68%. 24-methylenecholesterol, campestanol, $\Delta^{5,24}$-ergostadienol, clerosterol, stigmastanol, $\Delta^{5,23}$-stigmastadienol, $\Delta^{7,14}$-stigmastadienol, 28-isoavenasterol, and Δ^7-avenasterol were detected for the first time in *Moringia oleifera* seed oil.

Table VII shows the tocopherol composition as determined by HPLC. A high tocopherol content was found in *Moringa oleifera* seed oil, consisting of α- γ- and δ-tocopherol. The oil produced with cold pressure had the highest content of α-, γ- and δ-tocopherol. Most vegetable oils contain α-, β- and γ-tocopherols. δ-Tocopherol exists in few oils like cottonseed, peanut, wheat germ, soybean and castor oil. The antioxidant activity of δ-tocopherol exceeds that of γ-, β-, and α-tocopherol. Thus, tocopherols present in high concentrations in *Moringa oleifera* seed oil are expected to offer some protection during storage and processing. There were no previously reported data to compare the tocopherol composition. Considering the tocopherol content, olive oil was expected to be less stable than Moringa oil.

The oxidative state of *Moringa oleifera* seed oil was determined using the peroxide value (PV), and specific extinction ($E^{1\%}_{1cm}$) at 232 nm and 270 nm.

The results are shown in Table VIII. The PV of *Moringa oleifera* seed oil were 0.19 (CP), 0.23 (H) and 0.15(CM)meq/kg and fell in the range adopted as satisfactory. The mixture of chloroform:methanol (50:50) produced oil which had the lower PV followed by cold pressure and the n-hexane. The results of the $E^{1\%}_{1cm}$ at 232 nm, $E^{1\%}_{1cm}$ at 270 nm determination seem to follow the same trend. The peroxide values were much lower than those reported in literature and that of the olive oil. For the $E^{1\%}_{1cm}$ at 232 nm and 270 nm there were no available published results for comparison.

TABLE IX - Determination of susceptibility to oxidation of the degummed and not degummed oils

Determination	CP	H	CM	Olive oil	Literature
	Rancimat method (h) at 120°C				
Before degumming	68.4 (0.6)	83.2 (1.0)	123 (7.2)	7.88 (0.5)	present work
After degumming	34.7 (0.8)	23.7 (0.5)	31.8 (0.6)	—	present work

Values are means of triplicate determinations and percentage coefficient of variation is given in parenthesis.

The susceptibility to oxidation of the *Moringa oleifera* Malawi seed oils, as it was measured by the Rancimat method, is shown in Table IX. A 49 to 74% 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 (123 h) before the degumming process followed by n-hexane (83.2 h) and cold pressure (68.4 h). The cold pressure produced oil had the longest induction period after the degumming process, followed by the mixture of chloroform:methanol (50:50) and n-hexane. There were no available published results for comparison to the data of the present work. The induction period of Moringa oil was 9 to 16 times longer than that of olive oil before degumming and up to 4.4 times longer after degumming.

The oxidative stability of olive oil is related to some extent to the presence of α-tocopherol [17]. Kiritsakis and Min [18] reported that olive oil contains between 15-150 mg/kg á-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 though water soluble are present in the olive oil and considerably increase the oxidation stability of the oil [18]. Hudson and Ghavani [11] reported that although most sterols are ineffective as antioxidants, Δ^5-avenasterol (which is present in Moringa oil but in much less quantity than 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 isomeration of to a relatively stable tertiary allylic free radical represents the mode of action of the sterol antioxidants. Δ^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 α-, γ- and δ-tocopherol content of the *Moringa oleifera* Malawi seed oil 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 Moringa oleifera Malawi seed oil and which are more easily undergone oxidation and degradation than C18:1. The long induction period of Moringa oil might be explained by the presence of Δ^5-avenasterol. However, the olive oil, which had sorter induction period, had higher content of that sterol. Furthermore, the higher oxidative stability of *Moringa oleifera* seed oil over olive oil should be attributed to other constituents of the non glyceride fraction of the oil, which posses antioxidant properties.

CONCLUSIONS

The fatty acid composition of *Moringa oleifera* seed oil was

similar to that of olive oil. Therefore the oil could be useful for edible purposes and for some industrial applications like hydrogenation, shortening production and others. This agrees with Morton [20], who reported that Moringa oil can be an acceptable substitute for olive oil.

The physical and chemical characteristics of the oil from the seeds of *Moringa oleifera* variety Malawi showed that this oil can be utilised successfully as source of edible oil for human consumption.

It contains high ratio of monounsaturated to saturated fatty acids, and might be acceptable substitute for highly monounsaturated oils such as olive oil in diets.

The induction period measurements demonstrated a great resistance to oxidative rancidity. The oxidative stability of the Moringa oil is related to some extent to α-, γ- and -δ tocopherols which are natural antioxidants and possibly to other constituents of the non glyceride fraction of the oil, which posses antioxidant properties.

Additional research on possible toxicity should be carried out.

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