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## ORIGINAL ARTICLE

# Characterization of *Moringa oleifera* Seed Oil Variety "Periyakulam 1"

Stavros Lalas and John Tsaknis[1]

*Department of Food Technology, Technological Educational Institute (T.E.I.) of Athens, Ag. Spyridonos str., Egaleo, 12210, Athens, Greece*

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The oil from *Moringa oleifera* seeds variety Periyakulam 1 (PKM 1) from India was extracted using three different procedures including cold press (CP), extraction with n-hexane (H) and extraction with a mixture of chloroform:methanol (1:1) (CM). The oils were compared with those of a commercial virgin olive oil and *Moringa oleifera* var. Mbololo seed oil. The oil concentration 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 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 71.60%). The dominant saturated acids were palmitic and behenic (both up to 6.4%). The oil was also found to contain high levels of $\beta$-sitosterol (up to 45.58%), stigmasterol (up to 23.10%) and campesterol (up to 15.81%). $\alpha$-, $\gamma$- and $\delta$-tocopherols were detected up to levels of 15.38, 25.40 and 15.51 mg/kg of oil, respectively. *Moringa oleifera* seed oil showed a long induction period (at 120°C), which however was reduced from 42.56 to 72.56% after degumming. The *Moringa oleifera* seed oil showed high stability to oxidative rancidity. Among the methods used for extraction, the mixture of chloroform:methanol (1:1) (CM) showed the higher resistance to oxidation. 

*Key Words: Moringa oleifera*, PKM 1; seed oil; composition; stability; quality characteristics.

## INTRODUCTION

*Moringa oleifera* belongs to the Moringaceae family and *Moringa* genus, the best known and most widely distributed species (Morton, 1991; Sengupta and Gupta, 1970). There are a few known varieties namely Jaffna, Chauakacheri Murunga, Chem, Kadu, Palmurungai and Periyakulam 1 (PKM 1) (Tsaknis *et al.*, 1998). The edible oil was extracted, where the tree is cultivated, by boiling the seeds with water and collecting the oil from the surface of the water (Somali *et al.*, 1984). The seed oil contains all the fatty acids contained in olive oil, except linoleic and was used as its acceptable substitute (Morton, 1991).

Periyakulam 1 (PKM 1) is a new early variety released from the Horticultural Research Station of the Tamil Nadu Agricultural University (TNAU) in India. It

[1]To whom correspondence and reprint requests should be addressed. Fax: +30-10-5314874; E-mail: jtsaknis@teiath.gr

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is a selection of local types and is propagated only by seed. Seedlings were observed to grow to 4 m in height within 12 months of field planting and to have flowered and fruited after only 6 months from sowing (while other varieties need from 3 to 4 years) and would yield for 3–4 years. After 2 years the trees matured to 6 m tall productive specimens. The seedpods are left to mature and dry naturally on the tree before harvesting. The seeds are easily shelled, crushed and sieved using traditional methods.

Until now a full characterization of the oil produced from the seeds of *Moringa oleifera* PKM 1 has not been reported. Additionally, the use of different methods of extraction and their effect on the composition and the characteristics of the oil has not been investigated. The oil was compared to a commercially obtained virgin olive oil and *Moringa oleifera* var. Mbololo seed oil.

## MATERIALS AND METHODS

### *Materials*

The seeds were obtained from Kenya Forestry Research Institute (KE.F.R.I., Nairobi, Kenya). Thirty kilograms of seeds were harvested, air-dried for 1 week, mixed well and divided into three individual portions of 10 kg each.

Virgin olive oil "Horio" (MINERVA S.A., Athens, 14452, Greece) was obtained commercially.

### *Reagents*

All the reagents (analytical and HPLC grade) were obtained from Sigma Chemicals Company Co. (St Louis, MO, U.S.A), DL-$\alpha$-tocopherol from Merck Ltd (Darmstadt, D-64271, Germany), $\delta$-tocopherol from Sigma, standards of fatty acid methylester from British Greyhound Chromatography and Allied Chemicals (Birkenhead, U.K.) and sterols standards from Larodan AB (Malmö, Sweden).

### *Oil Extraction and Degumming*

Oil extraction and degumming were carried out using the method described by Tsaknis *et al.* (1998). The seeds were divided into three portions for cold press (CP) and solvent extractions using *n*-hexane (H) or a mixture of chloroform:-methanol (1:1) (CM). The scope of using the mixture of chloroform:methanol (1:1) was to estimate how a polar solvent affects the yield of extraction as well as the quality characteristics of the oil.

### *Determination of the Physical and Chemical Characteristics*

The method used for the determination of density and refractive index (at 40°C) was adapted from AOAC (method number 969.18) (1990). Colour was measured with a Lovibond tintometer (The Tintometer Ltd., Salsbury, England). Smoke point was determined according to British Standards Methods of Analysis (1976) (BS 684: Section 1.8). Acidity was measured according to IUPAC (method number 2.201) (1987), saponification value according to AOCS (method Cd 3-25) as described by Allen and Marvin (1982), and iodine value according to the Wijs method as described by Pearsons (1981).

*Determination of the Fatty Acid Composition*

Fatty acid composition was determined by gas–liquid chromatography (GLC) according to the method of Tsaknis *et al.* (1999). Analysis was performed on a Varian 3600 gas chromatograph (Varian, Palo Alto, CA, U.S.A.) equipped with a Supelcowax 10 (Supelco, INC., Supelco Park, Bellefonte, PA) fused silica capillary column 30 m × 0.32 mm i.d., 0.25 μm film thickness. The temperature program was 60°C for 10 min and then 2°C/min up to 220°C. Injector and FID temperatures were set at 160 and 280°C, respectively, sample volume was 0.2 μL, the carrier gas was $N_2$ at a flow rate of 30 mL/min, chart speed was set at 0.5 cm/min and the attenuation at $10^{-10} \times 32$. The internal standard used was nonadecanoic acid. Samples were prepared and measured separately in triplicate.

*Determination of the Sterol Composition*

The identification and determination of sterols by GLC was according to the method described by the Official Journal of the European Community (L248).

Analysis of sterols was performed on a Hewlett-Packard 5890 Gas Chromatograph (Hewlett-Packard, San Diego, CA, U.S.A.) equipped with a DB-5 FSOT capillary column (30 m × 0.25 mm × 0.25 μm, J & W, 91 Blue Ravine rd., Folson, 95630-4714, California, U.S.A.) using the method of the Official Journal of the European Community (L248). The pressure of the carrier gas ($H_2$) 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 α-cholestanol. Sterols were identified and quantified by comparing the retention times and peak area of the unknown components with those of known sterol standards (including all the sterols displayed in Table 3 plus corposterol and desmosterol). Samples were prepared and measured separately in triplicate.

*Determination of the Tocopherol Composition*

The determination of the tocopherols composition was carried out using a modified version of the method described by Carpenter (1979). An HPLC pump, Waters 600E (Millipore Corporation, Waters Chromatography Division, MA, U.S.A.), equipped with a Waters α-Polarsil, 125 Å, 10 μm, $3.9 \times 300\,mm^2$ column and a Waters 486 Tunable Absorbance Detector was used. The preparation of the samples was done as follows: 1 g of oil was accurately weighed into a 5 mL sample vial wrapped in foil paper to prevent oxidation. The oil was dissolved in 5 mL *n*-hexane before injection. A 20 μL sample was injected into the HPLC. Detector was set at 295 nm. Iso-propanol: *n*-hexane: absolute ethanol (2:97.5:0.5) at 1 mL/min was used as the mobile phase. A total of 10 min was enough to assay the tocopherols. Integration was done with Waters Baseline 815 software running on a computer. The samples were prepared and measured separately in triplicate.

*Determination of the Oxidative State and Susceptibility to Oxidation (Rancimat Method)*

Peroxide value was measured using the method of Lea (1952). Specific extinction ($E^{1\%}_{1cm}$ at 232 nm) was determined using the method of IUPAC (method number 2.505, 1987) and a Hitachi U-3210 Spectrophotometer (Hitachi Ltd. Tokyo,

Japan). The determination of the susceptibility to oxidation (Rancimat method) was carried out using the method described by Tsaknis *et al.* (1999).

*Statistical Analysis*

Results, means and standard deviation (S.D.) (in parenthesis), of three simultaneous assays were carried out in all the methods. Statistical significance of the differences between mean values was assessed by ANOVA test.

## RESULTS AND DISCUSSION

Characteristics of the oil were compared with those of virgin olive oil and *Moringa oleifera* variety Mbololo of Kenya (described by Tsaknis *et al.*, 1999). The extracted oils were liquid at room temperature. The oil content of *Moringa oleifera* PKM 1 seeds and the level at which the differences are significant are shown in Table 1. The oil extraction with CM had the highest yield, due to the increased ability of the polar solvent to overcome forces that bind lipids within the sample matrix (Lumley and Colwell, 1991). CP showed the lowest yield due to losses during the separation of the oil from the water. The yield of oil extracted with cold pressure from the seeds of the variety PKM 1 was lower compared to the variety Mbololo from Kenya, while the yield of oil extracted with *n*-hexane and chloroform:methanol was significantly higher.

The extracted oil was not neutralized due to low free fatty acid content, but degummed to reduce cloudiness and increase the smoke point, especially for CM extracted oil which extracted more gums than the other methods. The degummed oils were pale yellow liquids at ambient temperature with a characteristic unique odour and palatability. The chloroform:methanol mixture extracted the highest quantity of gums (7.2%) followed by cold pressure (2.5%) and *n*-hexane (0.7%). The different extraction rate should be related to the higher polarity of chloroform:methanol. Lumley and Colwell (1991) reported that Soxhlet extraction using chloroform and methanol extracted large amounts of nonfat material (10–20% by weight of fat extract), which was water soluble and had the appearance of gum. The results of the above authors agree with those of the present work.

The density of Moringa oil depends on the method of extraction and was higher compared to olive oil (Table 1). There was no significant difference in the refractive index of Moringa oils among the three methods of extraction and was lower compared to that of virgin olive oil. The viscosity of the oil obtained by CP was the highest, possibly because of the water that was absorbed by the gums (phospholipids) during extraction. The viscosity of the oils extracted by the other two methods was lower compared to that of virgin olive oil. The smoke point of the oils under examination was 10–16°C (oil extracted with *n*-hexane and chloroform:methanol, respectively) higher than that of olive oil. However, olive oil was not degummed, thus the direct comparison was not possible. The colour showed significant difference among the three methods of extraction and virgin olive oil. The comparison of data with those of the variety Mbololo from Kenya showed that there were significant differences in all physical characteristics apart from refractive index and density. Smoke point appears to be higher while viscosity is lower.

The free fatty acid content of all Moringa oils was significantly higher than that of virgin olive oil (Table 1). The oil obtained by cold pressure had the highest free fatty acid content. This can be attributed to the action of lypolytic

TABLE 1

Oil content, — physical and chemical characteristics[1]

| Determination | *M. oleifera* var. Periyakulam | | | | | | *M. oleifera* var. Mbololo from Kenya (Tsaknis *et al.,* 1999) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cold pressure | *n*-Hexane | Chloroform: methanol | $P_1$ | Virgin olive oil | $P_2$ | Cold pressure | *n*-Hexane | Chloroform: methanol | $P_3$ |
| Oil content (g oil/100 g seed) | 25.1 (3.01) | 38.3 (3.14) | 41.4 (2.92) | 0.05 | NS | NS | 25.8 | 35.7 | 31.2 | 0.05 |
| Density at 24°C (mg/mL) | 0.899 (0.006) | 0.909 (0.004) | 0.911 (0.006) | 0.05 | 0.915 (0.007) | 0.001 | 0.9037 | 0.8809 | 0.9182 | NS |
| Refractive index ($n_D$ 40°C) | 1.460 (0.004) | 1.457 (0.002) | 1.459 (0.005) | NS | 1.4620 (0.005) | 0.05 | 1.4591 | 1.4549 | 1.4581 | NS |
| Colour (red/yellow) | 1.90/30.00 (0.10)/(2.40) | 0.80/35.00 (0.20)/(3.14) | 2.00/35.00 (0.30)/(3.90) | 0.05 | 0/47,00 (0.00)/(7.91) | 0.05 | 1.9/30 | 0/40 | 3.3/72 | 0.05 |
| Smoke point (°C) | 203 (2.5) | 200 (2.0) | 206 (2.0) | 0.001 | 190 (1.9) | 0.05 | 201 | 198 | 202 | 0.001 |
| Viscosity (mPa s) | 80.00 (0.92) | 45.05 (0.13) | 56.10 (0.12) | 0.05 | 74.01 (0.17) | 0.05 | 103 | 57 | 66 | 0.05 |
| Free fatty acids[2] (oleic acid %) | 1.94 (0.21) | 1.12 (0.20) | 1.39 (0.19) | 0.01 | 0.98 (0.11) | 0.05 | 1.01 | 0.85 | 0.91 | 0.05 |
| Saponification value[3] (mg KOH/g) | 199.32 (3.99) | 188.36 (4.02) | 186.32 (3.66) | 0.05 | 188 (4.99) | 0.05 | 179.80 | 178.11 | 176.23 | 0.05 |
| Iodine value[3] (g I/100 g) | 65.73 (0.49) | 65.58 (0.48) | 65.46 (0.47) | NS | 80.01 (0.71) | 0.05 | 66.81 | 66.83 | 66.66 | 0.05 |

[1]*Note*: Values are means of triplicate determinations and standard deviation is given in parenthesis. $P_1$: Level of significant difference between methods of extraction. $P_2$: Level of significant difference PKM 1 versus virgin olive oil. $P_3$: Level of significant difference PKM 1 versus Mbololo.

[2]Non degummed oil.

[3]Degummed oil. NS: non significant.

TABLE 2

Fatty acid composition of the degummed oils[1]

| Fatty acid | *M. oleifera* var. Periyakulam | | | | | | *M. oleifera* var. Mbololo from Kenya (Tsaknis *et al.*, 1999) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cold pressure | *n*-Hexane | Chloroform: methanol | $P_1$ | Virgin olive oil | $P_2$ | Cold pressure | *n*-Hexane | Chloroform: methanol | $P_3$ |
| C8:0 | 0.04 (0.01) | 0.03 (0.01) | 0.03 (0.01) | NS | Not detected | 0.05 | 0.03 | 0.03 | 0.02 | NS |
| C14:0 | 0.13 (0.08) | 0.13 (0.08) | 0.13 (0.06) | NS | <0.01 | 0.05 | 0.11 | 0.11 | 0.11 | NS |
| C16:0 | 6.34 (0.41) | 6.46 (0.32) | 6.36 (0.25) | NS | 11.2 (0.66) | 0.05 | 5.73 | 6.04 | 5.81 | 0.05 |
| C16:1*cis* *n*-9 | 0.10 (0.06) | 0.09 (0.04) | 0.09 (0.04) | NS | 1.22 (0.71) | 0.05 | 0.10 | 0.11 | 0.10 | NS |
| C16:1*cis* *n*-7 | 1.28 (0.87) | 1.36 (0.84) | 1.40 (0.82) | NS | Not detected | 0.05 | 1.32 | 1.46 | 1.44 | NS |
| C17:0 | 0.08 (0.02) | 0.08 (0.02) | 0.08 (0.02) | NS | <0.01 | 0.001 | 0.09 | 0.09 | 0.09 | NS |
| C18:0 | 5.70 (0.21) | 5.88 (0.23) | 5.74 (0.24) | NS | 2.80 (0.12) | 0.05 | 3.83 | 4.14 | 4.00 | 0.05 |
| C18:1[2] | 71.60 (0.73) | 71.21 (0.69) | 71.22 (0.70) | NS | 74.53 (0.82) | 0.05 | 75.39 | 73.60 | 73.91 | 0.05 |
| C18:2 | 0.77 (0.38) | 0.65 (0.32) | 0.66 (0.33) | NS | 8.82 (0.79) | 0.05 | 0.72 | 0.73 | 0.71 | NS |
| C18:3 | 0.20 (0.03) | 0.18 (0.05) | 0.17 (0.05) | NS | 1.12 (0.40) | 0.05 | 0.20 | 0.22 | 0.20 | NS |
| C20:0 | 3.52 (0.29) | 3.62 (0.33) | 3.60 (0.44) | NS | <0.01 | 0.05 | 2.52 | 2.76 | 2.70 | 0.05 |
| C20:1 | 2.24 (0.26) | 2.22 (0.26) | 2.25 (0.20) | NS | <0.01 | 0.05 | 2.54 | 2.40 | 2.46 | NS |
| C22:0 | 6.21 (0.49) | 6.41 (0.46) | 6.28 (0.47) | NS | <0.01 | 0.05 | 5.83 | 6.73 | 6.38 | NS |
| C22:1 *cis* | 0.12 (0.07) | 0.12 (0.07) | 0.12 (0.08) | NS | Not detected | 0.05 | 0.15 | 0.14 | 0.14 | NS |
| C26:0 | 1.21 (0.16) | 1.18 (0.20) | 1.23 (0.21) | NS | Not detected | 0.05 | 0.96 | 1.08 | 1.06 | 0.05 |

*Note*: [1]Values are means of triplicate determinations and standard deviation is given in parenthesis. $P_1$: Level of significant difference between methods of extraction. $P_2$: Level of significant difference PKM 1 versus virgin olive oil. $P_3$: Level of significant difference PKM 1 versus Mbololo.

[2]Mixture of *cis* and *trans* $C_{18:1}$. NS: non significant.

enzymes, which was enhanced by the addition of water during milling of seeds prepared for cold press (Sengupta and Gupta, 1970). 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 2). There was no significant difference in iodine value of the oils produced from the three different ways of extraction. The saponification values of oils produced with cold pressure and *n*-hexane were higher than that of olive oil, while the oil extracted with chloroform:methanol showed lower values. The comparison of data with those of the variety Mbololo from Kenya showed that there were significant differences in all chemical characteristics. Iodine value appears to be significantly higher, while free fatty acid content and saponification value are significantly lower.

Total unsaturated fatty acids accounted for more than 76% (Table 2). Moringa oil is characterized by a high content of oleic acid (71%) and belongs to the oleic acid oil category (Sonntag, 1982). There are equal amounts of palmitic ($C_{16:0}$) and behenic ($C_{22:0}$) acids of about 6.40% and only trace to small amounts of other fatty acids in the oil. The fatty acid composition of the PKM 1 oil was similar to that of *Moringa oleifera* Mbololo variety seed oil (Tsaknis *et al*., 1999). There was no significant difference in fatty acid composition of the oils extracted by the three methods. Comparison with olive oil showed that *Moringa* oil had similar levels of $C_{18:1}$, less $C_{18:2}$, more $C_{22:0}$ and saturation. The comparison of data with those of the variety Mbololo from Kenya showed that there were significant differences in the case of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{20:0}$ and $C_{26:0}$. PKM 1 oil was less unsaturated than that of Mbololo. This agrees with the determined iodine value that was also lower. Another interesting fact is the rather high content of behenic acid of the PKM 1 oil. Due to its physical properties, addition of behenic acid can lighten chocolate texture and oily feel (Matsui *et al*., 2000), prevent solid roux from being whitened (Sakaguchi *et al*., 1997) and provide excellent mouth feel and melt-down behaviour to semi-solid and solid fats (such as margarine, shortening, and foods containing semi-solid and solid fats) (Cain and Moore, 1995; Cain *et al*., 1996). Also, behenic acid is poorly absorbed from the diet and can be used in low calorie foods. However, this led to the wrong assumption that it has no effect on serum lipid concentrations (Carte and Denke, 2001). Nonfood uses of behenic acid include applications as surfactants and detergents, plastics and plastic additives, cosmetics, photographing and recording materials (Sonntag, 1991). This could be of economic benefit for the industry.

There was no significant difference in sterol composition of the oils extracted from the three methods (Table 3). On the contrary, the only resemblance between *Moringa* PKM 1 and virgin olive oil was that $\beta$-sitosterol is the most predominant sterol in both. The comparison of data with those of the variety Mbololo from Kenya showed that there were significant differences in all sterols apart from cholesterol, brassicasterol, campesterol, campestanol and ergostadienol. In addition, $\Delta^{7,14}$ stigmastadienol was not detected in the oil of Mbololo variety.

The tocopherol profile of *Moringa oleifera* seed oil, consisted of $\alpha$-, $\gamma$- and $\delta$-tocopherol (Table 4). The oil extracted with *n*-hexane had the highest content of $\alpha$- and $\delta$-tocopherol and the lowest 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 few oils like cottonseed, peanut, wheat germ, soybean and castor oil. The antioxidant activity of $\delta$-tocopherol exceeds that of $\gamma$-, $\beta$-, and $\alpha$-tocopherol (Bourgeois and Czornomaz, 1982; Von Pongracz *et al*., 1984). Therefore, tocopherols present in *Moringa oleifera* seed oil were expected to offer some protection during storage and processing (Tsaknis *et al*., 1999). There were significant differences in tocopherol content of PKM 1

TABLE 3

Sterol composition of the degummed oils

| Fatty acid | *M. oleifera* var. Periyakulam | | | | | | | *M. oleifera* var. Mbololo from Kenya (Tsaknis *et al.*, 1999) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cold pressure | *n*-Hexane | Chloroform: methanol | $P_1$ | Virgin olive oil | $P_2$ | Cold pressure | *n*-Hexane | Chloroform: methanol | $P_3$ |
| Total sterols in oil (% w/w) | 0.52 (0.03) | 0.56 (0.04) | 0.48 (0.04) | NS | 0.57 (0.04) | NS | — | — | — | NS |
| Cholesterol | 0.18 (0.04) | 0.10 (0.02) | 0.12 (0.03) | NS | 0.15 (0.02) | NS | 0.13 | 0.13 | 0.12 | NS |
| Brassicasterol | 0.06 (0.02) | 0.05 (0.01) | 0.05 (0.01) | NS | <0.1 | 0.05 | Not detected | 0.06 | 0.06 | NS |
| 24, Methylene cholesterol | 0.07 (0.01) | 0.08 (0.01) | 0.09 (0.01) | NS | Not detected | 0.05 | 0.85 | 0.88 | 0.98 | 0.05 |
| Campesterol | 15.81 (1.10) | 15.29 (1.09) | 14.60 (1.01) | NS | 3.20 (0.95) | 0.05 | 14.03 | 15.13 | 14.12 | NS |
| Campestanol | 0.36 (0.05) | 0.33 (0.05) | 0.33 (0.03) | NS | 0.29 (0.03) | NS | Not detected | 0.35 | 0.35 | NS |
| Stigmasterol | 23.10 (1.63) | 23.06 (1.13) | 22.50 (1.19) | NS | 0.60 (0.09) | 0.05 | 17.27 | 16.87 | 16.78 | 0.05 |
| Ergostadienol | 0.30 (0.04) | 0.35 (0.04) | 0.36 (0.04) | NS | Not detected | 0.05 | Not detected | 0.39 | 0.28 | NS |
| Clerosterol | 2.08 (0.12) | 1.22 (0.09) | 1.80 (0.09) | NS | 0.54 (0.26) | 0.05 | 0.95 | 2.52 | 0.84 | 0.05 |
| $\beta$-Sitosterol | 45.58 (3.66) | 43.65 (2.79) | 44.05 (3.02) | NS | 64.3 (4.35) | 0.05 | 49.19 | 50.07 | 50.00 | 0.05 |
| Stigmastanol | 0.76 (0.10) | 0.64 (0.17) | 0.74 (0.11) | NS | 0.40 (0.08) | 0.05 | 1.05 | 0.86 | 0.80 | 0.05 |
| $\Delta^5$- Avenasterol | 8.46 (0.92) | 11.61 (1.14) | 10.43 (1.01) | NS | 16.77 (1.23) | 0.05 | 12.79 | 8.84 | 11.41 | 0.05 |
| $\Delta^{7,14}$ Stigmastadienol | 0.52 (0.22) | 0.39 (0.10) | 0.40 (0.09) | NS | Not detected | 0.05 | — | — | — | 0.05 |
| 28, Isoavenasterol | 0.27 (0.12) | 0.25 (0.11) | 0.40 (0.09) | NS | Not detected | 0.05 | 1.01 | 1.40 | 1.14 | 0.05 |
| $\Delta^{7,14}$ Stigmastanol | 0.35 (0.14) | 0.85 (0.29) | 0.51 (0.19) | NS | <0.1 | 0.05 | 0.83 | 0.44 | 0.52 | 0.05 |
| $\Delta^7$, Avenasterol | 0.53 (0.07) | Not detected | 1.15 (0.19) | NS | 0.29 (0.06) | 0.05 | 0.94 | 1.11 | 1.04 | 0.05 |

*Note*: [1]Values are means of triplicate determinations and standard deviation is given in parenthesis. $P_1$: Level of significant difference between methods of extraction. $P_2$: Level of significant difference PKM 1 versus virgin olive oil. $P_3$: Level of significant difference PKM 1 versus Mbololo. NS: non significant.

TABLE 4

Tocopherol composition of the nondegummed oils

| | *M. oleifera* var. Periyakulam | | | | | | *M. oleifera* var. Mbololo from Kenya (Tsaknis *et al.*, 1999) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| mg/kg | Cold pressure | *n*-Hexane | Chloroform: methanol | $P_1$ | Virgin olive oil | $P_2$ | Cold pressure | *n*-Hexane | Chloroform: methanol | $P_3$ |
| α-Tocopherol | 5.06 (0.67) | 15.38 (0.68) | 2.42 (0.37) | 0.05 | 88.50 (6.30) | 0.05 | 101.46 | 98.82 | 105.02 | 0.05 |
| γ-Tocopherol | 25.40 (1.16) | 4.47 (0.87) | 5.52 (0.69) | 0.05 | 9.90 (0.65) | 0.05 | 39.54 | 27.90 | 33.45 | 0.05 |
| δ-Tocopherol | 3.55 (0.45) | 15.51 (0.99) | 12.67 (0.55) | 0.05 | 1.60 (0.86) | 0.05 | 75.67 | 71.16 | 77.60 | 0.05 |

*Note*: [1]Values are means of triplicate determinations. Standard deviation is given in parenthesis. $P_1$: Level of significant difference between methods of extraction. $P_2$: Level of significant difference PKM 1 versus virgin olive oil. $P_3$: Level of significant difference PKM 1 versus Mbololo.

compared to virgin olive oil, α-tocopherol content of the cold pressure produced PKM 1 oil was up to 17 times lower, while δ-tocopherol content of the cold pressure produced PKM 1 oil was up to 2 times higher. *Moringa oleifera* variety Mbololo from Kenya seed oil tocopherol content was much higher than that of virgin olive and PKM 1 oils.

The oxidative state of *Moringa oleifera* seed oil was determined using the peroxide value (PV) and specific extinction ($E^{1\%}_{1cm}$) at 232 nm (Table 5). The PV of *Moringa oleifera* seed oil fell in the range selected as satisfactory. The cold pressure produced oil had lower PV followed by the mixture of chloroform: methanol (1:1) and *n*-hexane. This result was not expected, because cold pressure extracted oil remained in contact with air for a longer time and no satisfactory explanation can be given on that. The peroxide value of the cold pressure produced PKM 1 oil was significantly lower, while the PV of *n*-hexane and the mixture of chloroform:methanol (1:1) oils were significantly higher than those of virgin olive oil. The results of the $E^{1\%}_{1cm}$ at 232 nm determination show that CP had a lower value, followed by H and CM. The oxidative state of the oil from the seeds of *Moringa oleifera* var. Mbololo from Kenya was significantly different in the case of $E^{1\%}_{1cm}$ at 232 and PV from that of PKM 1.

A 42.6–72.6% reduction in induction period was observed, which could be attributed to oil degumming (Table 5). The oil produced with the mixture of chloroform:methanol (1:1) had the longest induction period before the degumming process followed by *n*-hexane and cold pressure. The cold pressure oil had the longest induction period after the degumming process (Table 5), followed by the mixture of chloroform:methanol (1:1) and *n*-hexane. The induction period of Moringa oil was more than 3 times longer than that of olive oil before degumming and up to 2 times longer after degumming. The unstable oxidation behaviour of the three oils could not be related to the ratio of tocopherol/$C_{18:2}$ (tocopherol/$C_{18:2}$ ratios of PKM 1 oil were 44.16, 54.40 and 31.23 for the cold pressure, *n*-hexane and chloroform:methanol, respectively). The oxidative stability of olive oil is related to some extent to the presence of α-tocopherol (Kiritsakis, 1988). Kiritsakis and Min (1989) reported that olive oil contains between 15 and 50 mg/kg α-tocopherol. However, the stability of olive oil could not be explained only on the basis of tocopherol action (Tsaknis *et al*., 1999). It is known that the olive mesocarp contains phenolic compounds, which are present in the olive oil and considerably increase the oxidation stability of the oil (Kiritsakis and Min, 1989).The content of α-, γ- and especially δ-tocopherol (in significantly higher quantity than in virgin olive oil) of the *Moringa oleifera* PKM 1 seed oils could be partly attributed to the resistance to oxidation (Tsaknis *et al*., 1999). In addition, olive oil contained linoleic and linolenic acid which were contained in much lower quantity in *Moringa oleifera* PKM 1 seed oil and more easily underwent oxidation and degradation than $C_{18:1}$. Furthermore, the higher oxidative stability of *Moringa oleifera* PKM 1 seed oil than olive oil should be attributed to other constituents of the nonglyceride fraction of the oil, which possess antioxidant properties (e.g., phenolic compounds) (Lalas, 1998). The susceptibility to oxidation of the *Moringa oleifera* Mbololo seed oil was significantly higher than that of PKM 1. This can be partly attributed to the lower tocopherol content of PKM 1 (Table 4).

## CONCLUSION

The characterization of the oil from the seeds of *Moringa oleifera* variety PKM 1 showed that this oil could be utilized successfully as a source of edible oil for

TABLE 5

Determination of the oxidative state of the degummed oils and the susceptibility to oxidation (Rancimat method) of the degummed and non degummed oils

| | *M. oleifera* var. Periyakulam | | | | | | | *M. oleifera* var. Mbololo from Kenya (Tsaknis *et al.*, 1999) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Determination | Cold pressure | *n*-Hexane | Chloroform: methanol | $P_1$ | Virgin olive oil | $P_2$ | Cold pressure | *n*-Hexane | Chloroform: methanol | $P_3$ |
| Peroxide value (meq $O_2$/ kg of oil) | 0.11 (0.10) | 1.83 (0.13) | 1.48 (0.31) | 0.05 | 0.76 (0.85) | 0.05 | 0.36 | 1.80 | 0.94 | NS |
| $E^{1\%}_{1cm}$ at 232 nm | 0.982 (0.13) | 3.001 (0.86) | 2.653 (0.81) | 0.05 | 2.011 (0.39) | 0.05 | 1.6648 | 3.1536 | 1.1658 | 0.05 |
| *Rancimat method (h) at 120 °C* | | | | | | | | | | |
| Before degumming | 28.2 (0.91) | 31.7 (1.24) | 32.5 (0.97) | 0.05 | 7.88 (0.53) | 0.05 | 34.1 | 36.8 | 46.2 | 0.05 |
| After degumming | 16.20 (0.80) | 8.70 (0.90) | 14.30 (0.90) | 0.05 | — | 0.05 | 18.9 | 10.8 | 16.5 | 0.05 |

*Note*: [1]Values are means of triplicate determinations and standard deviation is given in parenthesis. $P_1$: Level of significant difference between methods of extraction. $P_2$: Level of significant difference PKM 1 versus virgin olive oil. $P_3$: Level of significant difference PKM 1 versus Mbololo. NS: non significant.

human consumption. It contains high monounsaturated to saturated fatty acids ratio, and might be an acceptable substitute for highly monounsaturated oils such as olive oil in diets. *Moringa oleifera* is a tree growing rapidly even in poor soil and is little affected by drought (Sengupta and Gupta, 1970; Morton, 1991) and can be easily grown in poor third world countries. The production of useful oil from its seeds could be of economic benefit to the native population of the areas where the tree is cultivated.

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