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Characterisation of *Moringa stenopetala* seed oil variety "Marigat" from island Kokwa

The oil from *Moringa stenopetala* seeds variety Marigat from the island Kokwa was extracted using 3 different procedures including cold press (CP), extraction with n-hexane and extraction with a mixture of chloroform:methanol (1:1) (CM). The yield of oil was 35.7% (CP) to 44.9% (CM). The density, refractive index, colour, smoke point, viscosity, acidity, saponification value, iodine value, fatty acid methyl esters, sterols, to copherols (by high-performance liquid chromatography), peroxide value, $E_{1cm}^{1\%}$ 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 76.40%). The dominant saturated acids were behenic (up to 6.01%) and palmitic (up to 6.21%). The oil was also found to contain high levels of β -sitosterol (up to 52.19% of total sterols), stigmasterol (up to 16.53% of total sterols) and campesterol (up to 14.26% of total sterols). α -, γ - and δ -tocopherols were detected up to levels of 98.00, 44.50 and 82.41 mg/kg of oil, respectively. The reduction of the induction period (at 120 °C) of *M. stenopetala* seed oil ranged from 29.4% to 54.7% after degumming. The *M. stenopetala* 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 and *Moringa oleifera* seed oil.

Keywords: Moringa stenopetala, Marigat, seed oil, composition, stability.

1 Introduction

The Moringaceae family consists of 12 species (Morton [1]), which belong to only one genus called Moringa (Lalas and Tsaknis [2]). The best-known and most widely distributed species is Moringa oleifera (syn. M. pterygosperma Gaertn.) (Sengupta and Gupta [3], Morton [1]). Moringa stenopetala is often referred to as the African Moringa Tree because it is native only to Ethiopia and northern Kenva. Though it does grow in many other parts of the old- and new-world tropics, it is not as widely known as its close relative, Moringa oleifera. Many parts of the plant have been used in medicinal preparations. Whole plants have been used as living hedges, fences, and windbreaks. The wood is very soft; useful for paper but makes low-grade firewood and poor charcoal. The crushed seeds are used as a coagulant similar to the chemical alum. M. stenopetala has large edible leaves and seeds and is more drought- but less freeze-resistant than M. oleifera. Freezes may cause it to die back to ground level, where new sprouts may be produced. The

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M. stenopetala has also lushest green foliage and continues to grow during exceptionally long dry seasons. It develops into a round shrub-like tree (*Meitzner* and *Price* [4]) and has been grown as an ornamental in private gardens in Kenya, reaching a height of 10-12 m and a trunk diameter of at least 2-3 times as that of *M. oleifera* in Sudan. In Ethiopia, *Moringa stenopetala* grows wild in elevations between 1000 and 1800 m, and it will grow in up to 2000 m (*Meitzner* and *Price* [4]).

To our knowledge, a full characterisation of the oil produced from the seeds of *Moringa stenopetala* seed variety Marigat from island Kokwa has not been reported. Additionally, the use of different ways of extraction and their effect on the composition and the characteristics of the oil have not been investigated. In addition, a comparison of Moringa oil with virgin olive oil was carried out.

2 Material and methods

2.1 Materials

The seeds were assayed 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 in 3 individual portions of 10 kg each. Virgin olive oil "Horio" (MINERVA S.A., Athens, Greece) was obtained commercially.

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All the reagents (analytical and high-performance liquid chromatography (HPLC) grade) were obtained from *Sigma* Chemicals Company Co. (St. Louis, MO, USA) and the standard solutions for the determination of to-copherols were purchased from *Merck* Ltd. (Darmstadt, Germany) (dl- α -tocopherol), *Sigma* (δ -tocopherol), *British Greyhound* Chromatography and Allied Chemicals (Birkenhead, Merseyside, UK) fatty acid methyl ester standards and *Larodan* AB (Malmö, Sweden) (sterol standards).

2.2 Oil extraction and degumming

Oil extraction and degumming were carried out using the method described by *Lalas* and *Tsaknis* [2]. 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. Degumming was also carried out using the method described by *Lalas* and *Tsaknis* [2].

2.3 Determination of the physical and chemical characteristics

The method used for the determination of the density and the refractive index (at 40 °C) was adapted from AOAC (method number 969.18) [5]. Colour was measured with a *Lovibond* tintometer (*The Tintometer* Ltd., Salsbury, England). Smoke point was determined according to British Standards Methods of Analysis (BS 684: Section 1.8) [6]. Acidity was measured according to IUPAC (method number 2.201) [7], the saponification value according to *Sonntag* [8], and the iodine value according to the *Wijs* method as described by *Pearsons* [9].

2.4 Determination of the fatty acid composition

Fatty acid composition was determined by gas-liquid chromatography (GLC) according to the method of *Lalas* and *Tsaknis* [2]. The analysis was performed on a *Varian* 3600 Gas chromatograph (*Varian*, Palo Alto, California, USA) equipped with a *Supelcowax* 10 (*Supelco*, Inc., *Supelco* Park, Bellefonte, PA, USA) fused silica capillary column 30 m × 0.32 mm ID, 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 flame ionisation detector (FID) temperatures were set at 160 °C and 280 °C respectively, sample volume was 0.2 µl, the carrier gas was N₂ at a flow of 1.3 ml/min, chart speed was set at 0.5 cm/min and the attenuation at $10^{-10} \times 32$.

2.5 Determination of the sterol composition

The identification and determination of sterols by GLC was according to the method described by *Lalas* and *Tsaknis* [2]. The analysis 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 × 0.25 mm × 0.25 μ m)(*J* & *W*, Folson, California, USA). The pressure of the carrier gas (H₂) was 75 kPa. Injector and FID temperatures were 280 °C and 300 °C, respectively. The column temperature was maintained at 260 °C and the run time was 40 min.

2.6 Determination of the tocopherol composition

The method used for the determination of tocopherols was that reported by *Lalas* and *Tsaknis* [2]. A *Waters* μ -Polarsil, 125 Å, 10 μ m, 3.9 \times 300 mm column fitted to a *Waters* 600E HPLC pump (*Millipore* Corporation, *Waters* Chromatography Division, Massachusetts, USA) and a *Waters* 486 Tunable Absorbance Detector were used.

2.7 Determination of the oxidative state and susceptibility to oxidation (*Rancimat* method)

The peroxide value was measured using the method adapted from *Lea* [10]. The determination of the specific extinction ($E_{1cm}^{1\%}$ at 232 nm) was carried out using the method of IUPAC (method number 2.505) [7] using 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 *Lalas* and *Tsaknis* [2].

2.8 Statistical analysis

Results, means and standard deviation (SD) (in parenthesis) were gathered by carrying out 3 simultaneous assays in cases of all methods applied. Statistical significance of the differences between mean values was assessed by ANOVA test.

3 Results and discussion

Characteristics of the oil produced from *M. stenopetala* marigat seeds were compared with those of virgin olive oil "Horio" and *M. oleifera* variety PKM 1 (described by *Lalas* and *Tsaknis* [2]). The extracted oils were liquid at room temperature. The oil content of *M. oleifera* PKM 1 seeds and level at which the differences are significant are shown in Tab. 1. H had the highest yield followed by CM. CP showed the lowest yield due to losses during the separation of the oil from the water. The yield of oil extracted using cold press and n-hexane from the seeds of *M. stenopetala* was higher compared to *M. oleifera*, while that extracted with chloroform: methanol was significantly lower.

al and chemical characteristics. Values are means of triplicate determinations and standard deviation is given in parenthesis. P ₁ : Level	veen methods of extraction. P_2 : Level of significant difference <i>Moringa stenopetala</i> vs. Virgin olive oil. P_3 : Level of significant difference	
_	of significant difference between methods of extraction	M. stenopetala vs. M. oleifera.

Determination	Cold	n-Hexane	Chloroform:	ጚ	Virgin	₽2	M. olei	<i>ifera</i> var. PKM ⁻	M. oleifera var. PKM1 (Lalas, Tskanis, [2])	s, [2])
	pressure		methanol		olive oil		Cold pressure	n-Hexane	Chloroform: methanol	٩
Yield of oil g oil/100 g seed]	35.7 (2.1)	44.9 (1.9)	40.5 (2.8)	0.05	1	1	25.1 (3.01)	38.3 (3.14)	41.4 (2.92)	0.05
Density at 24 °C [†] [mg/ml]	0.904 (0.008)	0.885 (0.006)	0.910 (0.009)	0.05	0.917 (0.009)	0.00	0.899 (0.006)	0.909 (0.004)	0.911 (0.006)	NS
Refractive index [†] [n _D 40 °C]	1.456 (0.003)	1.4538 (0.004)	1.4527 (0.007)	NS	1.4631 (0.004)	0.05	1.460 (0.004)	1.457 (0.002)	1.459 (0.005)	NS
Colour [†] [red/yellow]	2.0/37.0 (0.1)/(4.0)	0.0/44.0 (0.0)/(5.3)	3.1/75.0 (0.6)/(2.4)	0.05	0/48.00 (0.00)/(7.99)	0.05	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
Smoke point⁺ [°C]	200 (3.2)	199 (1.9)	201 (2.6)	0.00	189 (1.6)	0.05	203 (2.5)	200 (2.0)	206 (2.0)	0.05
Viscosity [‡] [mPa⋅s]	94.15 (0.19)	62.30 (0.43)	73.00 (0.21)	0.05	74.92 (0.19)	0.05	80.00 (0.92)	45.05 (0.13)	56.10 (0.12)	0.05
Free fatty acids [‡] [oleic acid %]	1.83 (0.12)	1.10 (0.08)	1.29 (0.10)	0.01	0.76 (0.10)	0.05	1.94 (0.21)	1.12 (0.20)	1.39 (0.19)	0.01
Saponification value [†] [mg KOH/g]	178.89 (2.88)	177.24 (3.25)	175.33 (2.40)	0.05	190 (5.1)	0.05	199.32 (3.99)	188.36 (4.02)	186.32 (3.66)	0.05
lodine value [g · l/100g]	65.93 (0.98)	65.80 (1.14)	65.69 (1.06)	NS	82.09 (0.65)	0.05	65.73 (0.49)	65.58 (0.48)	65.46 (0.47)	NS

degummed oil; [±] - not degummed oil; NS - not significant.

Cold n-Hexane Chloroform: P_1 Virgin P_2 pressure methanol 0.02 0.01 0.01 0.05 0.00 0.02 0.01 0.01 0.01 0.01 0.05 0.00 0.02 0.01 0.11 0.12 NS Not detected 0.05 0.01 0.02 0.01 0.11 0.12 NS 12.2 0.05 0.01 0.03 0.01 0.12 NS 12.2 0.05 0.01 0.77 0.042 0.033 0.12 NS 12.2 0.05 0.1 0.77 0.041 1.29 1.27 NS 1.12 0.1 0.05 0.71 0.07 0.06 0.03 0.03 0.01 0.0 0.0 0.87 0.74.4 0.55 0.03 0.05 0.1 0.0 0.98 0.71 0.75 0.75 0.74 0.05 0.1 0.98	M. stenopetala vs. M. oleitera.	M. oleitera.									
pressure methanol olive oil 0.02 0.01 0.01 Not detected 0.05 0.02 0.01 0.01 Not detected 0.05 0.02 0.01 0.12 NS <0.01 0.05 0.02 0.01 0.12 NS <0.01 0.05 0.02 0.03 0.02 NS <0.01 0.05 0.03 0.03 0.12 0.11 NS <0.05 0.05 0.07 0.04 0.03 0.01 0.05 0.05 0.05 0.03 0.04 0.05 0.06 NS 11.2 0.05 0.04 0.05 0.06 NS 12.2 0.05 0.05 0.03 0.07 0.06 NS 11.2 0.05 0.05 0.04 0.05 0.06 0.12 0.05 0.05 0.05 0.04 0.05 0.06 0.05 0.06 0.05 0.05 0.05 <th>Fatty acid</th> <th>Cold</th> <th>n-Hexane</th> <th>Chloroform:</th> <th>ቲ</th> <th>Virgin</th> <th>\mathbf{P}_2</th> <th>M. ole</th> <th><i>ifera</i> var. PKM</th> <th>M. oleifera var. PKM1 (Lalas, Tskanis, [2])</th> <th>s, [2])</th>	Fatty acid	Cold	n-Hexane	Chloroform:	ቲ	Virgin	\mathbf{P}_2	M. ole	<i>ifera</i> var. PKM	M. oleifera var. PKM1 (Lalas, Tskanis, [2])	s, [2])
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		pressure		methanol		olive oil		Cold pressure	n-Hexane	Chloroform: methanol	Ъ
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$.8:0	0.04 (0.02)	0.03 (0.01)	0.02 (0.01)	SN	Not detected	0.05	0.04 (0.01)	0.03 (0.01)	0.03 (0.01)	SN
	14:0	0.10 (0.05)	0.11 (0.08)	0.12 (0.03)	SN	<0.01	0.05	0.13 (0.08)	0.13 (0.08)	0.13 (0.06)	SN
js uad 0.09 0.12 0.11 NS 1.12 0.05 (0.07) (0.04) (0.08) (0.05) (0.06) Not detected 0.05 (0.71) (0.97) (0.66) (0.66) Not detected 0.05 (0.71) (0.97) (0.07)	16:0	6.01 (0.42)	6.21 (0.33)	5.98 (0.19)	SN	12.2 (0.76)	0.05	6.34 (0.41)	6.46 (0.32)	6.36 (0.25)	0.05
1.04 1.29 1.27 NS Not detected 0.05 (0.71) (0.97) (0.66) NS < 0.01 0.05 0.71 (0.97) (0.66) NS < 0.01 0.00 0.08 0.07 0.07 0.07 0.07 0.07 0.00 0.08 0.07 0.05 (0.06) 0.06 0.06 4.01 4.32 4.18 NS < 2.90 0.05 0.087 0.240 0.76 0.33 0.33 0.05 0.76 0.77 0.73 0.74 0.76 0.05 0.76 0.77 0.73 0.18 NS 0.05 0.76 0.77 0.73 0.148 NS 0.05 0.023 0.749 0.75 0.74 0.05 0.05 0.089 1.96 NS 0.091 0.074 0.05 0.089 0.34 <	16:1 <i>cis</i>	0.09 (0.07)	0.12 (0.04)	0.11 (0.08)	NS	1.12 (0.58)	0.05	0.10 (0.06)	0.09 (0.04)	0.09 (0.04)	SN
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	16:1 <i>cis</i>	1.04 (0.71)	1.29 (0.97)	1.27 (0.66)	SN	Not detected	0.05	1.28 (0.87)	1.36 (0.84)	1.40 (0.82)	SN
	17:0	0.08 (0.04)	0.07 (0.05)	0.07 (0.06)	SN	<0.01	0.00 1	0.08 (0.02)	0.08 (0.02)	0.08 (0.02)	NS
76.40 74.61 74.52 NS 74.44 0.05 (1.05) (0.94) (1.12) 0.75 $0.69)$ 0.05 0.76 0.77 0.75 NS 8.73 0.05 (0.22) (0.53) (0.18) 0.16 0.054 0.05 0.16 0.18 0.16 NS 1.02 0.05 0.16 0.18 0.16 NS 1.02 0.05 0.16 0.04 (0.04) (0.09) 0.16 0.054 0.08 (0.04) (0.09) 0.16 0.05 (0.46) (0.29) (0.34) NS <0.01 0.05 (0.46) (0.29) (0.34) NS <0.01 0.05 (0.27) (0.17) (0.74) NS <0.01 0.05 (0.27) (0.17) (0.74) NS <0.01 0.05 (0.20) (0.78) (0.78) NS <0.01 0.05 (0.20) (0.11) (0.78) NS NOt detected 0.05 (0.20) (0.11) (0.30) NS NOt detected 0.05 (1.47) 1.57 NS NOt detected 0.05	18:0	4.01 (0.87)	4.32 (0.58)	4.18 (0.33)	SN	2.90 (0.10)	0.05	5.70 (0.21)	5.88 (0.23)	5.74 (0.24)	0.05
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	18:1†	76.40 (1.05)	74.61 (0.94)	74.52 (1.12)	SN	74.44 (0.69)	0.05	71.60 (0.73)	71.21 (0.69)	71.22 (0.70)	0.05
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	18:2	0.76 (0.22)	0.77 (0.53)	0.75 (0.18)	SN	8.73 (0.54)	0.05	0.77 (0.38)	0.65 (0.32)	0.66 (0.33)	SN
2.34 2.58 2.52 NS <0.01	18:3	0.16 (0.08)	0.18 (0.04)	0.16 (0.09)	SN	1.02 (0.43)	0.05	0.20 (0.03)	0.18 (0.05)	0.17 (0.05)	SN
1.03 0.89 1.95 NS <0.01	20:0	2.34 (0.46)	2.58 (0.29)	2.52 (0.34)	SN	<0.01	0.05	3.52 (0.29)	3.62 (0.33)	3.60 (0.44)	0.05
5.62 6.01 5.87 NS <0.01 0.05 (0.90) (0.78) (0.61) 0.05 0.05 0.05	20:1	1.03 (0.27)	0.89 (0.17)	1.95 (0.74)	SN	<0.01	0.05	2.24 (0.26)	2.22 (0.26)	2.25 (0.20)	0.05
ois 0.65 0.64 0.65 NS Not detected 0.05 (0.20) (0.11) (0.30) (0.30) 1.57 NS Not detected 0.05	22:0	5.62 (0.90)	6.01 (0.78)	5.87 (0.61)	SN	<0.01	0.05	6.21 (0.49)	6.41 (0.46)	6.28 (0.47)	0.05
1.47 1.59 1.57 NS Not detected 0.05	22:1 <i>cis</i>	0.65 (0.20)	0.64 (0.11)	0.65 (0.30)	SN	Not detected	0.05	0.12 (0.07)	0.12 (0.07)	0.12 (0.08)	0.05
(0.40) (0.16) (0.24)	26:0	1.47 (0.40)	1.59 (0.16)	1.57 (0.24)	SN	Not detected	0.05	1.21 (0.16)	1.18 (0.20)	1.23 (0.21)	0.05

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The extracted oils were not neutralised due to low free fatty acid content, but were 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 characteristic unique odour and palatability. The chloroform:methanol mixture extracted the highest quantity of gums (6.7%) followed by cold pressure (3.0%) and n-hexane (1.1%). The different extraction rate should be related to the higher polarity of chloroform: methanol mixture. Lumney and Colwell [11] reported that Soxhlet extraction using chloroform and methanol extracted large amounts of non-fat 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 *M. stenopetala* seed oil depends on the method of extraction and was lower compared to olive oil (Tab. 1). There was no significant difference in 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 extracted using cold press 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 the virgin olive oil. The smoke point of the oils under examination was 10-11 °C (H and CM, 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 M. oleifera showed that there were significant differences in all physical characteristics apart from refractive index and density. Smoke point appears to be lower while viscosity higher.

The free fatty acid content of all M. stenopetala seed oils was significantly higher than that of virgin olive oil (Tab. 1). The oil extracted using cold press had the highest free fatty acid content. This can be attributed to the action of lipolytic enzymes, which was enhanced by the addition of water during milling of seeds prepared for cold press (Sengupta and Gupta [3]). The iodine value is also lower compared to olive oil because the M. stenopetala oil is less unsaturated than the olive oil (see also fatty acid composition, Tab. 2). There was no significant difference in the iodine value of the oils produced by the 3 different ways of extraction. The saponification values of the oils produced were lower than that of olive oil. The comparison of data with those of M. oleifera showed that there were significant differences in all chemical characteristics apart from iodine value. Saponification value and free fatty acid content appeared to be significantly lower.

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Total unsaturated fatty acids accounted for more than 78% (Tab. 2). M. stenopetala oil is characterised by a high content oleic acid (up to 76%) and belongs to the oleic acid oil category (Sonntag [8]). There are almost equal amounts of palmitic (C16:0) and behenic (C22:0) acids of about 6.0% and only trace to small amounts of other fatty acids in the oil. There was no significant difference in the fatty acid composition of the oils extracted by the 3 methods. Comparison with olive oil showed that M. stenopetala oil had similar levels of C18:1, much less C18:2 and C18:3, more C22:0 and saturation. The fatty acid composition of the *M. stenopetala* oil was similar to that of the *M.* oleifera variety PKM 1 seed oil (Lalas and Tsaknis [2]) and there were significant differences in the case of C16:0, C18:0, C18:1, C20:0, C20:1, C22:0, C22:1cis, and C26:0. Another interesting fact is the rather high content of behenic acid of the M. stenopetala oil. Due to its physical properties, addition of behenic acid can lighten chocolate texture and oily feel, prevent solid roux from been whitened and give excellent mouth feel and meltdown behaviour to semi-solid and solid fats (such as margarine, shortening, and foods containing semi-solid and solid fats) (Lalas and Tsaknis [2]). 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 [12]). Non-food uses of behenic acid include applications as surfactants and detergents, plastics and plastic additives, cosmetics, photographing and recording materials (Sonntag [13]). This could be of economic benefit for the industry.

There was no significant difference in sterol composition of the oils extracted by the 3 described methods (Tab. 3). On the contrary, the only resemblance between *M. stenopetala* and virgin olive oil was that β -sitosterol appeared to be the most predominant sterol of both. The comparison of data with those of *M. oleifera* 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 *M. stenopetala*.

The tocopherol profile of *M. stenopetala* seed oil consisted of α -, γ - and δ -tocopherol (Tab. 4). The oil extracted using CM had the highest content of α - and δ -tocopherol. The oil extracted using CP had the highest content of γ -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 (*Bourgeois* and *Czornomaz* [14], *Von Pongracz* et al. [15]). Therefore, tocopherols present in oil were expected to offer some protection during storage and processing (*Lalas* and *Tsaknis* [2]). There were sig-

ab. 3. Sterol composition (% of total sterols) of the degummed oils. Values are means of triplicate
P ₁ : Level of significant difference between methods of extraction. P ₂ : Level of significant difference <i>Moringa stenopetala</i> vs. Virgin olive oil. P ₃ : Level of significant
difference M. stenopetala vs. M. oleitera.

	2010	n-Hexane	Chloroform:	۵	Virain	പ്	M. ole	<i>ifera</i> var. PKM	M. oleifera var. PKM1 (Lalas. Tskanis. [2])	s. [2])
	pressure		methanol	-	olive oil	N	Cold pressure	n-Hexane	Chloroform: methanol	ے 2
Total sterols in oil [% w/w]	0.56 (0.02)	0.58 (0.01)	0.51 (0.02)	NS	0.66 (0.03)	0.05	0.52 (0.03)	0.56 (0.04)	0.48 (0.04)	NS
Cholesterol	0.11 (0.02)	0.10 (0.06)	0.13 (0.07)	NS	0.08 (0.02)	0.05	0.18 (0.04)	0.10 (0.02)	0.12 (0.03)	NS
Brassicasterol	0.03 (0.01)	0.05 (0.03)	0.07 (0.06)	NS	<0.1	0.05	0.06 (0.02)	0.05 (0.01)	0.05 (0.01)	NS
24, Methylene cholesterol	0.73 (0.15)	0.80 (0.21)	0.87 (0.22)	NS	Not detected	0.05	0.07 (0.01)	0.08 (0.01)	0.09 (0.01)	0.05
Campesterol	13.68 (0.93)	14.26 (0.55)	13.90 (0.73)	NS	3.11 (0.85)	0.05	15.81 (1.10)	15.29 (1.09)	14.60 (1.01)	NS
Campestanol	0.28 (0.11)	0.24 (0.09)	0.33 (0.43)	NS	0.40 (0.09)	0.05	0.36 (0.05)	0.33 (0.05)	0.33 (0.03)	NS
Stigmasterol	16.35 (1.18)	16.53 (0.97)	15.76 (1.25)	NS	0.54 (0.10)	0.05	23.10 (1.63)	23.06 (1.13)	22.50 (1.19)	0.05
Ergostadienol	0.22 (0.10)	0.34 (0.07)	0.26 (0.14)	NS	Not detected	0.05	0.30 (0.04)	0.35 (0.04)	0.36 (0.04)	NS
Clerosterol	1.15 (0.34)	1.43 (0.67)	1.60 (0.48)	NS	0.53 (0.22)	0.05	2.08 (0.12)	1.22 (0.09)	1.80 (0.09)	0.05
β-Sitosterol	52.19 (1.89)	51.60 (1.98)	51.48 (1.54)	NS	64.7 (4.15)	0.05	45.58 (3.66)	43.65 (2.79)	44.05 (3.02)	0.05
Stigmastanol	0.95 (0.38)	0.74 (0.22)	0.87 (0.19)	NS	0.38 (0.07)	0.05	0.76 (0.10)	0.64 (0.17)	0.74 (0.11)	0.05
Δ^5 -Avenasterol	11.45 (1.23)	10.67 (1.55)	12.02 (0.94)	NS	17.33 (1.24)	0.05	8.46 (0.92)	11.61 (1.14)	10.43 (1.01)	0.05
∆ ^{7,14} -Stigma- stadienol	Not detected	Not detected	Not detected	NS	Not detected	0.05	0.52 (0.22)	0.39 (0.10)	0.40 (0.09)	0.05
28, Isoavenasterol	0.98 (0.37)	1.37 (0.43)	1.11 (0.77)	NS	Not detected	0.05	0.27 (0.12)	0.25 (0.11)	0.40 (0.09)	0.05
$\Delta^{7,14}$ -Stigmastanol	0.72 (0.22)	0.33 (0.12)	0.40 (0.08)	NS	<0.1	0.05	0.35 (0.14)	0.85 (0.29)	0.51 (0.19)	0.05
Δ^7 -Avenasterol	1.01 (0.40)	1.18 (0.53)	1.11 (0.38)	NS	0.20 (0.07)	0.05	0.53 (0.07)	Not detected	1.15 (0.19)	0.05

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nificant differences in the tocopherol content of M. stenopetala compared to that of virgin olive oil. The α -tocopherol content of the oil extracted using the mixture of chloroform:methanol was more than 11% higher, while that of δ -tocopherol was up to 98% higher. So, the tocopherol content of the seed oil from the *M. stenopetala* variety Marigat from the island Kokwa was much higher than that of virgin olive and *M. oleifera* oils.

The oxidative state of *M. stenopetala* seed oil was determined using the peroxide value (PV) and specific extinction $(E_{1cm}^{1\%})$ at 232 nm (Tab. 5). The PV of the oil fell in the range selected as satisfactory. The H-produced oil had lower PV followed by CM and CP. The PV of the cold press-extracted oil was significantly higher than that of virgin olive oil. The determinations of $E_{1cm}^{1\%}$ at 232 nm showed that H-produced oil had a lower value, followed by CM and CP-extracted oil. The oxidative state of the oil from the seeds of M. oleifera was significantly different as determined by $E_{1cm}^{1\%}$ at 232 nm and the PV from that of *M*. stenopetala.

A 29.4%-54.7% reduction in the induction period was observed after degumming, which could be attributed to the high temperature used during the process (Tab. 5). The oil produced with CM had the longest induction period before the degumming process followed by CP and H. The CP-extracted oil had the longest induction period after the degumming process, followed by CM and H. The induction period of Moringa oil was up to 6-times longer than that of olive oil before degumming and up to 3-times longer after degumming. The unstable oxidation behaviour of the 3 oils could not be related to the ratio of tocopherol/C18:2 (tocopherol/C18:2 ratios of M. stenopetala oil were 290, 262 and 292 for the CP, H and CM, respectively). The oxidative stability of olive oil is related to some extent to the presence of α -tocopherol (Kiritsakis [16]). Kiritsakis and Min [17] reported that olive oil contains between 15-150 mg/kg α -tocopherol. However, the stability of olive oil could not be explained only on the basis of tocopherol action (Lalas [18]). 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 [17]). To the content of α -, γ - and especially δ -tocopherol (in significantly higher quantity than in virgin olive oil) of the M. stenopetala seed oils the resistance to oxidation could be partly attributed (Lalas and Tsaknis [2]). In addition, olive oil contained linoleic and linolenic acid which were contained in much lower quantity in *M. stenopetala* seed oil and undergo oxidation and degradation more easily than $C_{18:1}$. Furthermore, the higher oxidative stability of *M*. stenopetala seed oil than olive oil should be attributed to other constituents of the non-glyceride fraction of the oil, which possesses antioxidant properties (e.g. phenolic 29

Tocopherol	Cold	n-Hexane	Chloroform:	Ę	Virgin	\mathbf{P}_2	M. ole	<i>ifera</i> var. PKM	M. oleifera var. PKM1 (Lalas, Tskanis, [2])	s, [2])
[mg/kg]	pressure		methanol		olive oil		Cold pressure	n-Hexane	Chloroform: methanol	Ę
α-tocopherol	94.38 (1.84)	91.79 (2.07)	98.00 (2.24)	0.05	86.32 (5.29)	0.05	5.06 (0.67)	15.38 (0.68)	2.42 (0.37)	0.05
γ-tocopherol	44.50 (1.92)	32.75 (1.16)	38.39 (0.99)	0.05	10.33 (0.79)	0.05	25.40 (1.16)	4.47 (0.87)	5.52 (0.69)	0.05
ô-tocopherol	81.55 (3.33)	77.20 (2.96)	82.41 (3.84)	0.05	1.76 (0.69)	0.05	3.55 (0.45)	15.51 (0.99)	12.67 (0.55)	0.05

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Tocopherol	Cold	n-Hexane	Chloroform:	Ę	Virgin	P_2	M. ole	<i>ifera</i> var. PKM	M. oleifera var. PKM1 (Lalas, Tskanis, [2])	۶, [2])
[mg/kg]	pressure		methanol		olive oil		Cold pressure	n-Hexane	Chloroform: methanol	٩
Peroxide value [meq O ₂ / kg of oil]	2.40 (1.10)	1.65 (0.80)	1.95 (0.77)	0.05	1.4 (0.46)	0.05	0.11 (0.10)	1.83 (0.13)	1.48 (0.31)	0.05
$E_{1cm}^{1\%}$ at 232 nm	2.736 (0.114)	1.859 (0.123)	1.934 (0.212)	0.05	2.006 (0.187)	0.05	0.982 (0.13)	3.001 (0.86)	2.653 (0.81)	0.05
			Rancin	<i>nat</i> method i	Rancimat method induction period [h] at 120 °C.	[h] at 120 °C				
Before degumming	30.2 (1.2)	27.6 (0.9)	36.4 (0.7)	0.05	7.09 (0.29)	0.05	28.2 (0.91)	31.7 (1.24)	32.5 (0.97)	0.05
After degumming	21.3 (0.8)	12.5 (0.7)	19.3 (0.6)	0.05	I	0.05	16.20 (0.80)	8.70 (0.90)	14.30 (0.90)	0.05

compounds). The susceptibility to oxidation of the *M. stenopetala* seed oil was significantly higher than that of *M. oleifera*. This can be partly attributed to the lower to-copherol content of *M. oleifera* (see Tab. 4).

The characterisation of the oil from the seeds of *M. stenopetala* variety Marigat showed that this oil could be utilised successfully as a 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.

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References

- J. F. Morton: The horseradish tree, Moringa pterigosperma (Moringaceae) - A boon to arid lands? Econ. Bot. 45 (1991) 318-333.
- [2] S. Lalas, J. Tsaknis: Characterisation of Moringa oleifera, variety PKM1 seed oil. J. Food Comp. Anal. 15 (2002) 65-77.
- [3] A. Sengupta, M. P. Gupta: Studies on seed fat composition of *Moringaceae* family. Fette Seifen Anstrichm. **72** (1970) 6-10.
- [4] L. S. Meitzner, M. L. Price: Multipurpose trees. In: Amaranth to Zai Holes: Ideas for growing food under difficult conditions. ECHO (USA) 1999, pp. 115-150.
- [5] AOAC Official Methods of Analysis, Ed. D. Firestone, Association of the Official Analytical Chemists INC. Virginia (USA) 1990, pp. 951-952.
- [6] British Standard Methods of analysis of fats and fatty oils, Other methods BS 684, Part 2, Section 1.8, British Standards Institution, London (UK) 1987.
- [7] Standard methods for the analysis of oils, fats and derivatives. In: International Union of Pure and Applied Chemistry (IUPAC), 7th edn. Eds. *C. Paquot, A. Hautfenne*, Blackwell Scientific Publications, London (UK) 1987, pp. 212-213.
- [8] N. O. V. Sonntag: Analytical methods. In: Bailey's Industrial Oil and Fat Products-Vol. 2. Ed. D. E. Swern, 4th edition, John Wiley and Sons, New York (USA) 1982, pp. 407-527.
- [9] D. Pearsons: Chemical analysis of foods, 8th edn. Eds. H. Egan, R. S. Kirdm, R. Sawyer, Churchill Livingstone, Edinburgh (UK) 1981, pp. 520-547.
- [10] C. H. Lea: Methods for determination of peroxide in lipids. J. Sci. Food Agric. 3 (1952) 566-594.
- [11] I. D. Lumley, R. K. Colwell: Fats from fatty foods and determination of fat content. In: Analysis of fats and fatty foods. Eds. J. B. Rossell, J. L. R. Pritchard, Elsevier Applied Science, London (UK) 1991, pp. 238-247.
- [12] N. B. Carte, M. A. Denke: Behenic acid is a cholesterolrising saturated fatty acid in humans. Am. J. Clin. Nutr. 73 (2001) 41-44.

Eur. J. Lipid Sci. Technol. 105 (2003) 23-31

- [13] N. O. V. Sonntag: Erucic, behenic: Feedstocks of the 21st century. Inform 2 (1991) 449-463.
- [14] F. Bourgeois, A. M. Czornomaz. Utilisation du palmitate d' ascorbyle de l'α-tocopherol et des phospholipides pour stabiliser le saindoux. Rev. Fran. Corps Gras. 29 (1982) 111-116.
- [15] G. Von Pongracz, F. Hoffmann-La Roche A. G. Basel: γ-Tocopherol als natürliches Antioxidans. Fette Seif. Anstrichm. 12 (1984) 455-460.

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- [16] A. Kiritsakis: Tocopherols. In: The Olive Oil. Ed. A. Kiritsakis, Agricultural Co-operative Editions, Thessaloniki (Greece) 1998, pp. 61-64 and 234.
- [17] A. Kiritsakis, D. B. Min: Flavour chemistry of olive oil. In: Flavour Chemistry of Lipid Foods. Ed. D. B. Min, The American Oil Chemists' Society, Illinois (USA) 1989, pp.196-221.
- [18] S. Lalas: Ph. D. Thesis, University of Lincolnshire and Humberside, 1998.

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