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Some physico-chemical properties of *Moringa oleifera* seed oil extracted using solvent and aqueous enzymatic methods

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Abstract

The physico-chemical properties of oil from *Moringa oleifera* seed were determined following extraction either with petroleum ether or 2% Neutrase 0.8L (a neutral bacterial protease from *Bacillus amyloliquefaciens*, Novozyme Bagsvaerd Denmark). The enzyme was chosen following a preliminary study conducted on the enzymatic extraction of *M. oleifera* seed oil using four commercial enzymes that showed Neutrase to be the best enzyme with the highest oil recovery value. The enzymes used were Termamyl 120L, Type L (α-Amylase), Neutrase® 0.8L (Neutral protease), Celluclast® 1.5 L FG (Cellulase) and Pectinex® Ultra SP-L (Pectinase), all from Novozyme, Denmark. The fatty acid compositions of solvent and enzyme-extracted oil from *M. oleifera* seed were determined. Results showed that the solvent-extracted oil has 67.9% oleic acid compared to 70.0% in enzyme-extracted oil. Results obtained following analysis of extracted oil showed that the oil is highly unsaturated because of the high percentage of oleic acid. Apart from oleic acid, other prominent fatty acids were palmitic (7.8% and 6.8%), stearic (7.6% and 6.5%), and behenic (6.2% and 5.8%) acids for solvent and enzyme-extracted oils, respectively. It was liquid at room temperature and pale-yellow in colour (0.7R + 5.9Y and 0.7R + 3.0Y for solvent and enzyme-extracted oils, respectively). Electronic nose analysis showed that it had flavor similar to that of peanut oil. The melting points estimated by differential scanning calorimetry were found to be 19.0 and 18.9 °C for the solvent- and enzyme-extracted oils, respectively. The oil contains 36.7% triolein as the main triacylglycerol. The extraction methods were found to slightly influence the relative amounts of the fatty acids in the oil. The oils extracted using these two methods were found to differ in the percentage composition of their fatty acids. Quality attributes such as relative percent of oleic acid, total percentage of unsaturated fatty acids, iodine value, free fatty acid and unsaponifiable matter contents and the colour of the enzyme-extracted oil were better than those of the solvent-extracted oil.
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Keywords: *Moringa oleifera*; Aqueous enzymatic extraction; Properties; Thermal behaviour

1. Introduction

Moringa oleifera belongs to the genus *Moringaceae*. A single genus with 14 known species, *M. oleifera* is the most widely known and utilized of these (Morton, 1991). It is commonly known as the horse-radish or drumstick tree and is a native of the sub-Himalayan region of northwest India. The tree ranges in height from 5–12 m and the fruits (pods) are around 50 cm long. Fully mature, dry seeds are round or triangular in shape, the kernel surrounded by a light wooded shell with three papery wings.

Almost every part of the tree is of value for food. In Malaysia, the young tender pods are cut into small pieces and added to curries. Almost all parts of the tree have been utilized within traditional medicine practices

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and the oil is applied externally for skin diseases (Foidl, Makkar, & Becker, 2001). In Haiti and elsewhere, the oil has been used as general culinary and salad oil (Price, 1986). *Moringa oleifera* seed oil is pleasant tasting, highly edible (Lowell, 1999) and resembles olive oil in its fatty acid composition (Ramachandran, Peter, & Gopalakrishnan, 1980). The seed oil contains all the main fatty acids found in olive oil, and therefore, can be used as a possible substitute to the expensive olive oil after some modifications. In addition it possesses behenic acid ($C_{22:0}$), lignoceric acid ($C_{24:0}$) and traces of lauric *n*-pentadecanoic and pentadecenoid acids (Dahot & Memon, 1985; Ferrao & Ferrao, 1970).

The characteristics of *M. oleifera* seed oil can be highly desirable especially with the current trend of replacing polyunsaturated vegetable oils with those containing high amounts of monounsaturated acids (Corbett, 2003). High oleic acid vegetable oils have been reported to be very stable even in highly demanding applications like frying (Warner & Knowlton, 1997). The press cake obtained after oil extraction has positively charged protein molecules that have coagulant properties (Sutherland, Folkard, Mtanali, & Grant, 1994). These properties have been exploited in water clarification and wastewater treatments. Previous studies on *M. oleifera* have been focused on its medicinal uses and nutritional aspects of the tree parts (Lowell, 1999) and on the use of the seed in the clarification of waste-water during treatment (Folkard, Travis, Sutherland, & Holmes, 1993); however, little or no studies have been done on the oil properties, such as the triacylglycerol profiles and other physico-chemical properties apart from the fatty acid composition. In this paper some physical and chemical properties such as thermal behavior, triacyglycerol profile and sensory attributes were determined following extraction using solvent and aqueous enzymatic methods.

2. Materials and methods

2.1. Materials

Mature *M. oleifera* pods were collected from neighborhood gardens and fences around Universiti Putra Malaysia and surrounding areas where the plant was found growing in abundance to obtain approximately 10 kg of seeds. The seeds were removed from the pods, sorted and sun dried. Only seeds that were not damaged were chosen and stored under cool dry storage conditions until needed. Solvents and chemicals used were of analytical grade obtained from BDH Laboratories England. Individual standard fatty acid methyl esters of lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), arachidic ($C_{20:0}$), eicosenoic ($C_{20:1}$), behenic ($C_{22:0}$) and lignoceric ($C_{24:0}$), acids, approximately 99% pure, were purchased from Supelco (Sigma–Aldrich Tokyo, Japan). Triacylglycerol (TAG) standards (purity approx. 99%) were obtained from Sigma-Aldrich, Inc. (St. Louis, USA) while Neutrase 0.8L, which according to the manufacturer contains only the neutral part of *Bacillus amyloliquefaciens* proteases and with a declared activity of 0.8 Anson unit per gram (AU/g), was donated by Novozymes Bagsvaerd Denmark.

2.2. Methods

2.2.1. Proximate analysis of M. oleifera seed

Moisture, crude protein (micro-Kjeldahl), crude fibre and oil (Soxhlet) contents were determined using the methods described by Pearson (1976), whereas the ash content was determined using the method of Pomeranz and Meloan (1994), and total carbohydrate was determined by difference. All determinations were done in triplicate.

2.2.2. Oil extraction

Dried *M. oleifera* seeds were ground into fine powder using a Waring blender Model 32BL 80 (Dynamic Corporation of America, USA). For solvent extraction, 150 g of ground seeds were placed into a cellulose paper cone and extracted using light petroleum ether (b.p 40–60 °C) in a 5-l Soxhlet extractor for 8 h (AOAC, 1984). The oil was then recovered by evaporating off the solvent using rotary evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and residual solvent was removed by drying in an oven at 60 °C for 1 h and flushing with 99.9% nitrogen. For aqueous enzyme extraction, the same amount of the ground seeds were mixed with distilled water at a ratio of 1:6 w/v. The mixture was then gently boiled for 5 min and allowed to cool down to room temperature. The pH was then adjusted to 6.8 (optimal pH for Neutrase 0.8L) with 0.5 N NaOH. Two percent v/w of Neutrase was added and the mixture incubated at 45 °C for 24 h in a water bath with constant shaking at 120 rpm (these are optimal conditions for enzymatic extraction of *M. oleifera* seed oil, unpublished results). Oil from enzymatic extraction was recovered by centrifugation of the aqueous mixture using a Beckman centrifuge model J2-21M/E at 8000 rpm and 20 °C for 20 min to separate the emulsion and the residue. The emulsion was decanted into a separating funnel and allowed to separate into the oil and water layer. The water layer was then drained off to obtain the oil. Residual moisture in the oil was removed by gentle heating (Aparna, Khare, & Gupta, 2002). The oil obtained from both extractions was stored at −20 °C until it was analyzed. All experiments were done in triplicates and the mean and standard deviations were calculated.

2.2.3. Physical and chemical analysis of crude oil

2.2.3.1. Thermal behaviour. The thermal property of the oil samples was investigated by differential scanning calorimetry using a Perkin–Elmer Diamond DSC (Norwalk, USA). The instrument was calibrated using indium and zinc. The purge gas used was 99.99% nitrogen with a flow rate of 100 ml/min and a pressure of 20 psi. Sample weights ranged from 5–7 mg and were subjected to the following temperature program: Frozen oil sample was heated at 60 °C in an oven until completely melted. Oil sample was placed in an aluminium volatile pan and was cooled to −70 °C and held for 2 min, it was then heated from −70 to 70 °C at the rate of 5 °C/min (normal rate) (Che Man & Swe, 1995) and 100 °C/min (past rate), and held at 70 °C isothermally for 2 min and cooled from 70 to −70 °C at the rate of 5 °C per minute. The heating and cooling thermograms for the normal and the fast (hyper DSC) scan rates were recorded and the onset, peak, and offset temperatures were tabulated. These values provide information on the temperature at which the melting process starts, the temperature at which most of the TAG have melted, and the complete melting temperature of the oil, respectively.

2.2.3.2. Determination of solid fat content. The solid fat content was determined using a Minispec Bruker wide line pulse nuclear magnetic resonance spectrometer Model mq20 NMR Analyser (Karlsruhe, Germany) using a direct measurement method advocated by the Palm Oil Research Institute of Malaysia (PORIM, 1995). Sample temperature treatment was done using a water bath (JEIO TECH Model VTRC-620 DESK TOP REF.CIR). Frozen oil samples were melted at 80 °C and mixed by gentle shaking and then equilibrated at 60 °C for 30 min. The oil was then placed into 6 solid fat content tubes up to at least one third of the tubes. All tubes were transferred into a water bath set at 0 °C and allowed to remain there for 1 h. Then five of the tubes were transferred, one each into a separate water bath set at 5, 10, 15, 17, and 20 ± 0.5 °C. The tubes were left to stand in the bath for 30 min and were inserted one after the other into the NMR analyzer. The signals were recorded and integrated by a computer to obtain the percent SFC.

2.2.3.3. Colour determination using lovibond tintometer. The oil colour was determined using a Lovibond tintometer Model E (Salisbury, England) Frozen oil samples were melted by placing them at 60 °C in an oven and were then gently shaken. The liquefied samples were placed into an inch cell up to three quarter full and the colour was determined at 30 °C by achieving the best possible match with the standard colour slides provided.

2.2.3.4. Flavour evaluation. The flavour from the solvent-extracted oil and peanut oil sample were analyzed and compared. Peanut oil was chosen for the comparison based on the similarity of the oil with *M. oleifera* seed oil in terms of their odour. Analysis was done using a Vapor Analyzer (Electronic nose) model 4100 (Electronic Sensor Technology Company, Newbury Park, CA, USA) equipped with an automatic injection unit and an array of non-specific sensors that mimic the human nose. The sensors gave a series of signals that were integrated by the electronic nose into a series of peaks used to compare the two samples. The working principle of this electronic nose and its commercial expression (zNose) is based on the principle of fast gas chromatography; chemical analysis of any odour is accomplished in 10 s by a very fast separation of chemical sampled vapours (Bartlett, Elliott, & Gardner, 1997). Liquid oil samples were placed in universal bottles filled up to half capacity. The bottles were then covered with parafilm and the samples heated in an oven at 40 °C for I5 min to allow emission of flavor compounds. The sample vapour was introduced into the electronic nose. The flow rate of the carrier gas (purified helium) was fixed at 30 cm^3/min and the sampling time was 10 s. The temperature was programmed from 40 to 160 °C, at the rate of 5 °C/s. Each cycle of analysis consisted of sampling, injection, and analysis phase. The system analysed compounds by drawing air via a pump during the injection phase after which the compounds were vapourised and transported by the carrier gas to the capillary column where the compounds are separated. Separated compounds sequentially exit the column where they are detected by the surface acoustic wave detector. The same procedure was performed on 100% pure, high quality refined peanut oil obtained from Lam Soon edible oil Sdn. Bhd. Malaysia.

2.2.3.5. Chemical analysis. Determinations for peroxide, iodine, and saponification values, unsaponifiable matter and free fatty acid contents were carried out using AOAC (1984) standard analytical methods.

The fatty acid composition was determined by conversion of oil to fatty acid methyl esters prepared by adding 950 μl of n-hexane 50 mg of oil followed by 50 μl of sodium methoxide using the method of Cocks and Van Rede (1966). The mixtures were vortex for 5 s and allowed to settle for 5 min. The top layer (1 μl) was injected into a gas chromatograph (Model GC-14A, Shimadzu Corporation, Kyoto, Japan) equipped with a flame-ionisation detector and a polar capillary column (BPX70 0.25), 0.32 mm internal diameter, 60 m length and 0.25 μm film thickness (SGE Incorporated, USA) to obtain individual peaks of fatty acid methyl esters. The detector temperature was 240 °C and column temperature was 110 °C held for one minute and increased at the rate of 8 °C/min to 220 °C and held for one minute. The run time was 32 min. The fatty acid methyl esters peaks were identified by comparing their

retention time with those of standards. Percent relative fatty acid was calculated based on the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample.

2.2.3.6. Triacylglycerol profile. Triacylglycerol (TAG) profile was obtained by reverse phase high performance liquid chromatography (HPLC) using a Shimadzu liquid chromatograph LC-10AD equipped with SCL-10A*vp* system controller, an auto-injector, and refractive index detector (Shimadzu model RID-6A). The chromatogram was processed using a Shimadzu CR4AX-integrator (Shimadzu Co., Kyoto, Japan). The TAG of both the enzyme- and solvent-extracted samples were separated using a commercially packed RP-18 column (250 × 4 mm) with a particle size of 5 μm (Merck, Darmstadt, Germany) and was eluted from the column with a mixture of acetone/acetonitrile (63.5:36.5) at the flow rate of 1 ml/min, the TAG was detected with a refractive index detector (Shimadzu Co., Kyoto, Japan). Ten μl of sample [6% concentration in chloroform (w/w)] was injected into the HPLC. The total run time was 1 h. TAG peaks were identified based on the retention time of available TAG standards and results of Ghazali, Maisarah, Yusof, and Yusoff (1995) and Swe, Che Man, and Ghazali (1996). Peak areas produced by the data integrator were used to quantify the components based on relative percentages. In calculating the relative percentage of the TAG all the peaks that appears after 12 min (time at which the first TAG peak appeared) in the chromatograph were included (Ghazali et al., 1995).

2.2.3.7. Statistical analysis. Values represented are the means and standard deviations for three replicates. Statistical analysis was carried out by Student's *t*-test using SPSS Version 11.0 software and ANOVA (Duncan multiple range test) using SAS system Version 8e. Significance was defined at $P < 0.05$.

3. Results and discussion

3.1. Proximate analysis of Moringa oleifera seed oil

Results obtained showed that the seeds contained 7.9% moisture, 30.8% crude oil, 38.3% crude proteins, 16.5% carbohydrate (by difference), 4.5% crude fiber and 6.5% ash (Table 1). The high percentage of oil makes this seed a distinct potential for the oil industry. According to Benthall (1946), Burkill (1966), and Irvine (1961), the mature seed yields 22–38% oil. Jamieson (1939) reported a 40% yield by weight of the seed. Variation in oil yield may be due to the differences in variety of plant, cultivation climate, ripening stage, the harvesting time of the seeds and the extraction method used.

Table 1
Proximate analysis of *Moringa oleifera* oil seed

Characteristic	Obtained values[a] (M ± S.D.)	Reported values[b]	
		1	2
Moisture content (%)	7.9 ± 1.00	nd	4.1
Crude protein[c](%)	38.3 ± 1.03	36.7	38.4
Fats/oils (%)	30.8 ± 2.19	41.7	34.7
Crude fibre (%)	4.5 ± 0.38	4.8	3.5
Ash content (%)	6.5 ± 0.15	3.8	3.2
Total carbohydrate[d] (%)	16.5	17.8	17.1

nd, not determined.
[a] M ± S.D. mean ± standard deviation.
[b] (1) Makkar and Becker (1997). (2) Duke and Atchley (1984).
[c] Crude protein = N(%) × 6.25.
[d] Carbohydrate obtained by difference.

3.2. Oil extraction

The percentage of oil extracted from *M. oleifera* seed by enzymatic method was found to be lower than that obtained by solvent extraction (enzymatic extraction yielded about 72% of the total oil extracted by the solvent). This is because the organic solvent used has a greater ability to extract most of the oil that is available in the oil seed. The advantage of enzymatic extraction over solvent extraction has been highlighted in several studies, for example, the extraction temperature is lower, explosive solvents not required and harmful waste are not produced (Hanmoungjai, Pyle, & Niranjan, 2001; Rosenthal, Pyle, & Niranjan, 1996). It also facilitates separation of extracted vegetable components, the mild condition of the process ensures extracted components remain unchanged and only few by-products that may affect taste and smell of the final product are produced (Ksenija, Zarko, & Miyjana, 1997). Enzymatic extraction processes are carried out in an aqueous medium, thus phospholipids are separated from the oil so that there is no need for degumming thereby reducing the overall cost of processing of the oil to the final product (Christensen, 1991). The disadvantage of using enzymes, although safer, is that they are expensive. Only recently the use of enzymes in commercial extractions of oil from olives have been reported (FAO, 1997).

3.3. Physical and chemical properties of oil

3.3.1. Physical properties

The complete melting points as derived from DSC measurement of oil extracted using solvent (19 °C) and enzyme (18.9 °C) (Table 2) were found to be not significantly different ($P > 0.05$). On the other hand, the solid fat content (SFC) at 0 °C for the solvent-extracted oil (11.1%) was significantly higher ($P < 0.05$) than that of the enzyme-extracted oil (10.2%). The higher SFC values of the solvent-extracted oil might be due to the pres-

Table 2
Physical and chemical properties of *Moringa oleifera* seed oil extracted using solvent and aqueous enzymatic methods

Properties	Obtained values		Reported values[a]
	Solvent extract	Aqueous enzymatic	Solvent extract
Oil[b] (%)	30.8 ± 2.19^{A}	22.6 ± 1.17^{B}	35.7
MP (°C)	19.0 ± 0.00^{A}	18.9 ± 0.01^{A}	nd
SFC[c] (%)	11.1 ± 0.23^{A}	10.2 ± 0.20^{B}	nd
Colour			
Red index	0.7 ± 0.00^{A}	0.7 ± 0.00^{A}	0.0
Yellow index	5.9 ± 0.20^{A}	3.0 ± 0.10^{B}	7.12
FFA (as % oleic acid)	2.48 ± 0.11^{A}	1.13 ± 0.08^{B}	0.85
IV (wijs)	65.4 ± 0.50^{A}	66.1 ± 1.32^{A}	66.8
Saponification value	164 ± 1.49^{A}	163 ± 0.98^{A}	nd
Unsaponifiable matter			
Content (%)	0.74 ± 0.03^{A}	0.59 ± 0.07^{B}	nd

Means for the determined values in the same row followed by the same superscript letter are not significantly different ($P < 0.05$).
[a] Tsaknis et al. (1999).
[b] Oil = weight of extracted oil × 100/weight of seed.
[c] Solid fat content values obtained at 0 °C.

ence of gums in the crude oil that were extracted by the solvent. On the other hand, the enzyme-extracted oil will contain no gums as the process of extraction was done in an aqueous medium and the water soluble gums were likely separated from the oil during the extraction process. The red colour index was found to be the same for the enzyme and solvent-extracted oil (0.7 units) while the yellow index for the solvent-extracted oil (5.9 units) was significantly higher ($P < 0.05$) than that of the oil enzyme-extracted oil (3.0 units). This gives the solvent-extracted oil a more yellowish colour than the enzyme-extracted oil (Table 2) and this may be due to the ability of the organic solvent to also extract pigments from the seed.

3.3.1.1. Flavour analysis. Fats and oils quality comprises those attributes that affect the acceptability of food prepared with it, such as texture, color and flavour. The flavour of fats and oils is one of the most critical factors influencing quality and the most important sensory characteristic associated with foods. Using a surface acoustic wave (SAW) electronic nose the flavour of *M. oleifera* seed oil was compared with that of peanut oil, which sensory wise appeared to be similar in their smell. The chromatograms of *M. oleifera* and peanut oils obtained from the electronic nose analysis (Fig. 1) showed overlapping of the major flavour compounds peaks, suggesting that the two oils samples have similar flavour. Flavour intensity was stronger in *M. oleifera* oil samples than the peanut oil and was indicated by the taller peaks of the former seed oil sample as compared to the smaller peaks of the peanut oil sample. The lower flavour intensity of the peanut oil was most likely due to the refining process that it underwent, during which strong flavours were removed at the deodorization stage.

3.3.1.2. Melting and crystallizing behaviour. Melting and crystallization, two commonly used physical events to

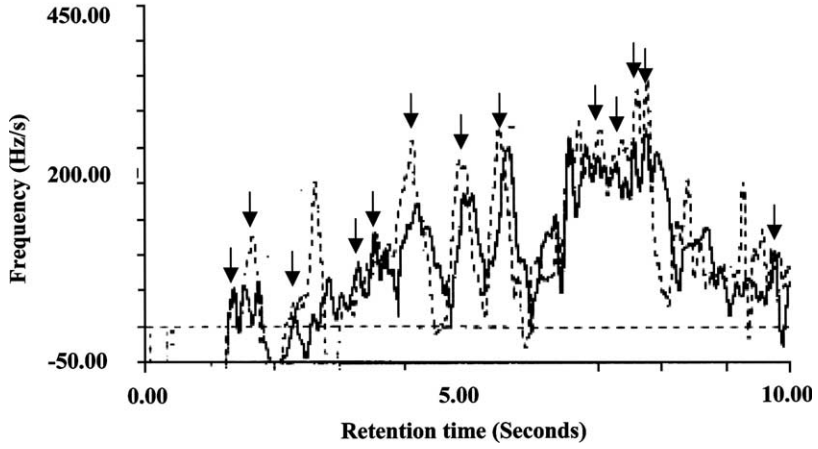

Fig. 1. Electronic nose chromatograms of *Moringa oleifera* and peanut oils.

characterize thermal behaviour of oil samples, require intake or release of thermal enthalpy. DSC is suitable to determine these physical properties. Results obtained from the heating and cooling regime with the DSC showed slight differences in both melting and crystallizing behaviour for the solvent- and enzyme-extracted oil samples when conventional temperature scanning (5 °C/min) was used. The differences were however eliminated using a fast scan rate of 100 °C/min (HyperDSC™). The heating profile using the conventional scan rate showed that there is one major peak **b** and **b′** and two small shoulder peaks **a**, **c** and **a′**, **c′** for both the enzyme- and solvent-extracted oil samples, respectively (Fig. 2(a)). The shoulder peaks **a** and **a′** represented the melting temperature of unstable crystals of the low melting TAG that pre-maturely melted. The more stable low melting unsaturated TAG crystals melted at a higher temperature shown as peaks **b** and **b′**.The higher melting, more saturated TAG peaks (**c** and **c′**) appeared at higher temperatures. The incomplete resolution of peaks at 5 °C/min scan rate was due to re crystallizing of the unstable low melting TAG crystals as slow scan rates allow the melting of unstable crystals that re crystallize into more stable crystal forms when the temperature was raised. This caused the formation of shoulder peaks that would have otherwise formed separate peaks. HyperDSC™ (Ford & Mann, 2002), in which fast heating scan rates (100–500 °C/min) were used, showed that the problem of re crystallization of unstable TAG during melting process could be prevented, and therefore, better separation of low and high melting TAG peaks. Fig. 2(b) shows the thermograms of the solvent- and en-

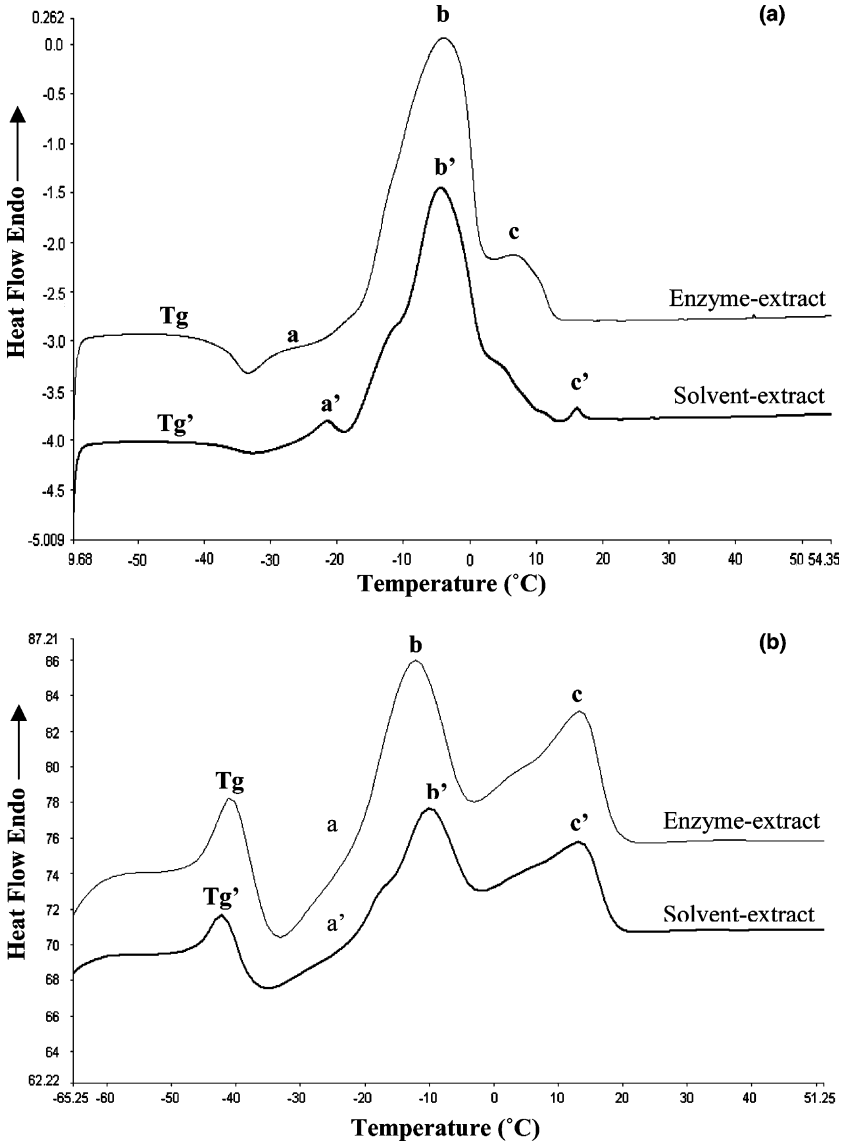

Fig. 2. (a) Heating profiles of enzyme- and solvent-extracted *Moringa oleifera* seed oils at 5 °C/min scan rate. (b) Heating profiles of enzyme- and solvent-extracted *Moringa oleifera* seed oils at 100 °C/min scan rate.

zyme-extracted oil samples obtained using HyperDSC™ (100 °C/min) scan rate. The shoulder peaks (**a** and **a′**) were completely eliminated and the two peaks (**b**, **b′** and **c**, **c′**) representing the low and high melting TAG of the enzyme- and solvent-extracted oil were completely separated, which makes characterization of the TAG species according to their melting behavior more accurate. Weak transitions like the glass transitions (T_g) of crystalline material with very low amorphous content are difficult to characterize using conventional scan rates (Fig. 2(a)), but the greatly increased sensitivity in HyperDSC™ allows for the clear characterization of this type of transition (Fig. 2(b)). The use of hyperDSC™ is not only advantageous in separating TAG species in terms of their melting points, but it is very sensitive and fast, which reduces analysis time by over 20 times. More samples can be handled and analysed with higher accuracy. The onset and offset temperatures, which indicate the start and the end of the melting process, for the peaks in the two oil samples analysed using the 5 °C/min scan varied but the peak temperatures for the major peaks in the two samples were similar. Variation in the onset and offset temperature was probably due to changes that occur in the sample because of the slow scan rate. Differences in the onset and offset temperature in the two oil samples suggest that the oil samples exhibit a different melting behaviour at slow scan rates. In the case of samples analysed using 100 °C/min scan rate these differences were eliminated because the faster scan rates do not allow for the changes that occur when slow scan rates were used (Table 3). The cooling profile of the two oil samples showed that the two sets of TAG crystallize at different temperatures (Fig. 3). The higher melting TAG shown as **d** and **d′** for enzyme- and solvent-extracted oil samplescrystallizes at 4.5 and −5.1 °C, respectively. The lower melting TAG shown as **e** and **e′** for enzyme- and solvent extracted oil samples crystallized at −38.1 and −37.5 °C, respectively. The peak temperature in the cooling profiles are considered the temperatures at which most of the oil has crystallized. The complete melting and crystallization points were determined as the offset

Table 3
Melting behaviour of *Moringa oleifera* seed oil using different scan rates

Peak Nos.[a]	5 °C/min			100 °C/min		
	Onset	Peak	Offset	Onset	Peak	Offset
(**a**)	–	–	–	–	–	–
(**a′**)	−24.95	−21.60	−19.52	–	–	–
(**b**)	−17.3	−4.4	1.4	−21.42	−12.09	−6.05
(**b′**)	−12.6	−4.4	3.4	−20.13	−11.79	−6.00
(**c**)	3.5	14.1	18.9	1.57	13.14	18.62
(**c′**)	14.7	16.1	19.0	1.46	13.10	18.93

[a] Peaks are shown in Fig. 2(a) and (b) (**a**, **b**, **c** and **a′**, **b′**, **c′** are peaks represent the melting TAG of enzyme-and solvent-extracted oil samples, respectively).

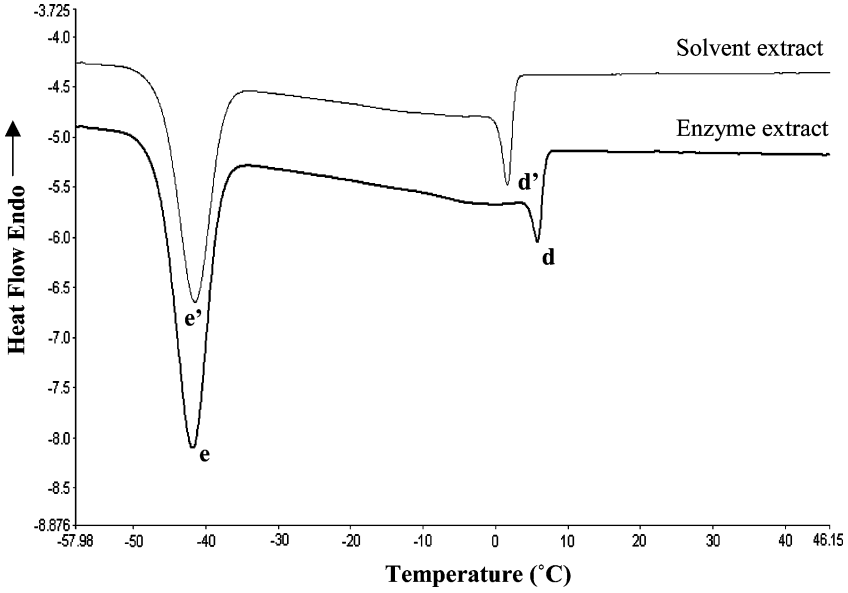

Fig. 3. Cooling profile of enzyme- and solvent-extracted *Moringa oleifera* seed oils at 5 °C/min scan rate.

Table 4
Relative percent composition of fatty acid in *Moringa oleifera* seed oil

Fatty acids	Determined values		Reported values[a]		
	Enzyme extract	Solvent extract	1	2	3
($C_{14:0}$)	0.2 ± 0.00^{A}	0.1 ± 0.05^{B}	–	1.4	–
($C_{16:0}$)	6.8 ± 0.60^{A}	7.8 ± 1.25^{A}	6.9	3.5	6.7
($C_{16:1}$)	2.9 ± 0.10^{A}	$2.2\ 0.06^{B}$	1.1	–	–
($C_{18:0}$)	6.5 ± 0.50^{B}	7.6 ± 0.05^{A}	8.3	8.3	4.3
($C_{18:1}$)	70.0 ± 1.90^{A}	67.9 ± 0.75^{A}	67.7	67.3	76.5
($C_{18:2}$)	0.9 ± 0.00^{A}	1.1 ± 0.10^{A}	0.4	3.5	0.7
($C_{18:3}$)	0.0 ± 0.00^{B}	0.2 ± 0.00^{A}	–	–	–
($C_{20:0}$)	4.2 ± 0.35^{A}	4.0 ± 0.06^{A}	4.7	2.7	2.7
($C_{20:1}$)	1.4 ± 0.06^{A}	1.5 ± 0.06^{A}	2.3	–	
($C_{22:0}$)	5.8 ± 0.00^{A}	6.2 ± 0.50^{A}	7.4	5.6	4.6
($C_{24:0}$)	1.3 ± 0.30^{A}	$1.3 \pm$	0.4	3.2	1.1

Means for the determined values in the same row followed by the same superscript letter are not significantly different ($P < 0.05$).
[a] (1) Sunga and Whitby (1995). (2) Dahot and Memon (1985). (3) Ferrao and Ferrao (1970).

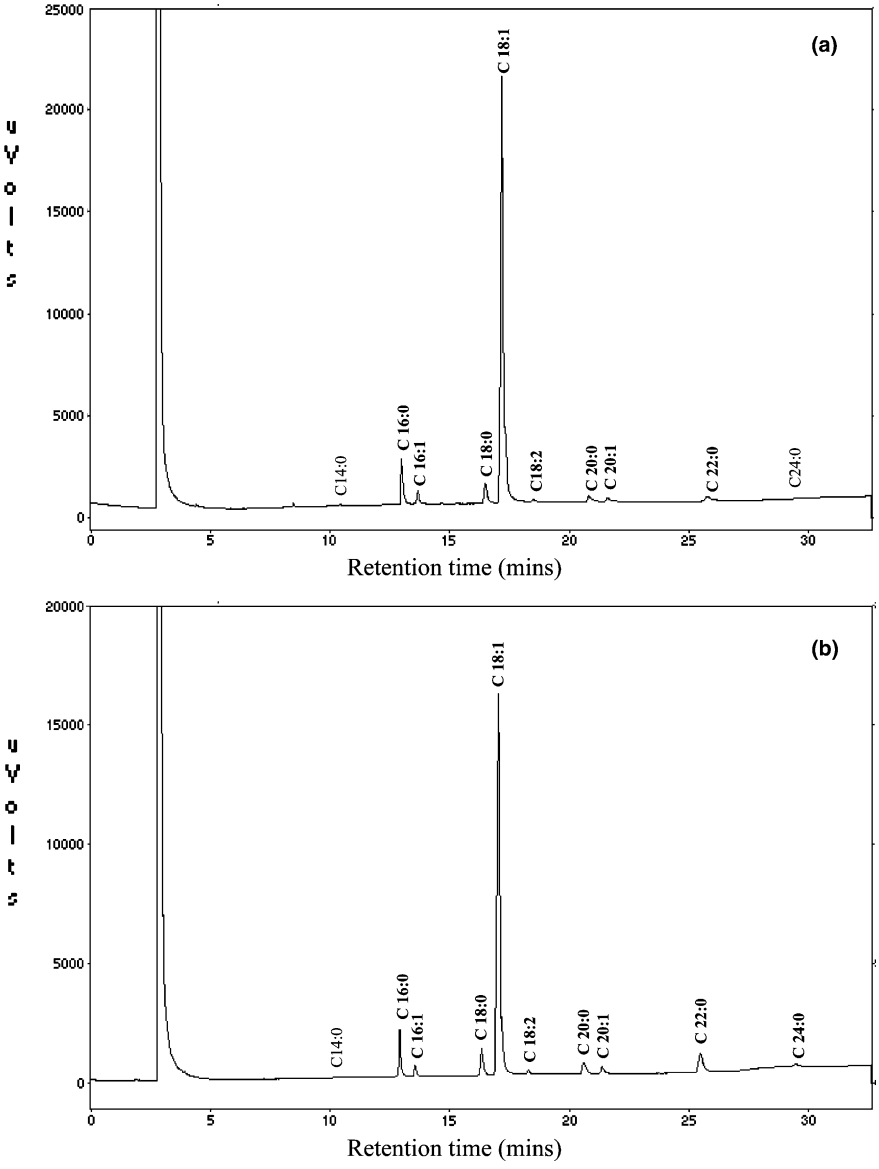

Fig. 4. (a) Fatty acid profile of enzyme-extracted *Moringa oleifera* seed oil. (b) Fatty acid profile of solvent-extracted *Moringa oleifera* seed oil.

Table 5
Triglycerides composition of solvent extracted *Moringa oleifera* seed oil

Triglyceride	Relative composition (%)
Polyunsaturated	
OOL	0.5
OOLn	0.8
POL	5.0
OOO	36.7
POO + SOL	12.4
OOGa	4.1
SOO	11.4
OLA	0.6
OOA	7.7
Monounsaturated	
PPO	1.3
PPL	1.5
PSO	2.0
POB	2.1
Others[a]	13.9

[a] These are triacylglycerol peaks that are yet to be identified.

temperature values of the last melting and crystallization peaks, respectively.

3.3.2. Chemical properties

The chemical properties of oil are amongst the most important properties that determines the present condition of the oil. Free fatty acid and peroxide values are valuable measures of oil quality. The iodine value is the measure of the degree of unsaturation of the oil. The free fatty acid and the unsaponifiable matter content of the solvent extracted oil were significantly higher ($P < 0.05$) than those of the enzyme-extracted oil (Table 2). There was no significant difference in the iodine and saponification values, of the solvent- and enzyme-extracted oil ($P > 0.05$). The slightly higher value of unsaponifiable matter in the solvent extracted oil may be due to the ability of the solvent to extract other lipid- associated substances like, sterols, fat soluble vitamins, hydrocarbons and pigments (Bastic, Bastic, Jabanovic,

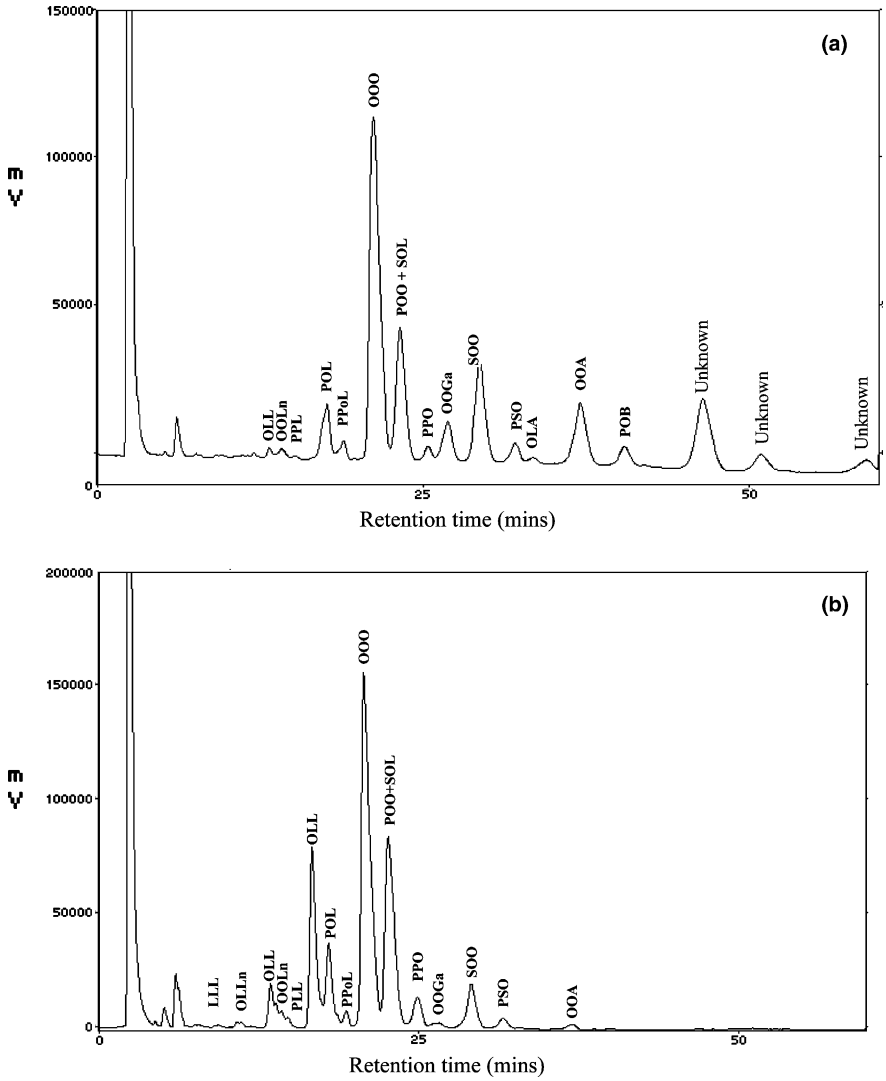

Fig. 5. (a) Triacylglycerol profile of solvent-extracted *Moringa oleifera* seed oil. (b) Triacylglycerol profile of olive oil.

& Spiteller, 1978; Salunke, Chavan, Adsule, & Kadam, 1992).

3.3.2.1. Fatty acid composition. The major saturated fatty acids in *M. oleifera* seed oil were palmitic, stearic, arachidic and behenic acids and the main unsaturated fatty acid is oleic acid (67.9–70.0%), with small amounts of palmitoleic, linoleic, linolenic, and eicosenoic acids (Table 4). Figs. 4(a) and (b) show the fatty acid profiles of enzyme- and solvent-extracted oils, respectively. There was no significant difference ($P > 0.05$) in the amounts of the major fatty acids in the enzyme- and solvent-extracted oil samples. The enzyme- and solvent- extracted oils contained a substantial amount of behenic acid (5.8% and 6.2%), respectively. The oil can, therefore, be used as a natural source of behenic acid, which has been used as an oil structuring and solidifying agent in margarine, shortening, and foods containing semi-solid and solid fats, eliminating the need to hydrogenate the oil (FDA, 2001). A small amount of linolenic acid (0.2%) was found in the solvent extracted oil, which was not found in the enzyme-extracted oil.

The high percentage of oleic acid in the oil makes it desirable in terms of nutrition and high stability cooking and frying oil. Many circumstances have focused attention on high-oleic vegetable oils. It has been demonstrated that a higher dietary intake of "bad" fats (saturated and *trans* fatty acids) is associated with an increased risk of coronary heart disease caused by high cholesterol levels in the blood (Mensink & Katan, 1990; Siguel & Lerman, 1993) whereas a higher intake of 'good' fats (monounsaturated/oleic) is associated with decreased risk (Corbett, 2003). High oleic-acid vegetable oils such as high-oleic corn, sunflower and canola have been found to have enough oxidative stability to be used in demanding applications such as frying (Petukhov, Malcolmson, Przybylski, & Armstrong, 1999; Warner & Knowlton, 1997). In addition high-oleic oils have low saturated fatty acid levels. Therefore high-oleic oils can be viewed as a healthy alternative to partially hydrogenated vegetable oils.

3.3.2.2. Triacylglycerol profile. HPLC analysis of the oil (Table 5 and Fig. 5) showed that the most prominent polyunsaturated TAG was triolein (36.7%), followed by POO + SOL (12.4%), SOO (11.4%), OOA (7.7%), POL (5.0%) and OOGa (4.1%) with small amounts of OOLn (0.8%), OLA (0.6%) and OOL (0.5%). The types and percentages of the triacylglycerides were not affected by the extraction method used to obtain the oil samples.

4. Conclusion

*Moringa oleifera seed*oil has the potential to become a new source of high-oleic acid oil, and its full potential should be exploited. Since solvent extraction used for oilseed processing poses environmental problems, the use of enzymes in oil extraction should be encouraged. Furthermore, there is the possibility of using enzymatic modification to obtain tailor- made oil with specific physical attributes and chemical composition for usage in various food applications.

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