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Oleic Acid Enhancement of *Moringa oleifera* Seed Oil by Enzymatic Transesterification and Fractionation

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Abstract: Solvent-extracted *Moringa oleifera* seed oil was transesterified using immobilized lipase (Lipozyme IM 60) (Novozymes Bagsvaerd Denmark) at 1% (w/w) concentration, shaken at 60°C and 200 rpm for up to 24h. After transesterification, the oil was fractionated with acetone at -18°C and without acetone at 10°C to obtain two fractions, stearin and olein fractions. Incubation of the transesterified oil at 10°C for 24 h resulted in the formation of fat crystals, which settled at the bottom of the flask in sample transesterified for 24 h, while the control (0 h) sample became rather viscous with fat crystals in suspension. Transesterification resulted in a change in the triacylglycerol (TAG) profile of the oil, which in turn affected its solid fat content (SFC) and thermal behavior. The SFC value at 0°C after 24 h of reaction was 10.35% and significantly ($P<0.05$) higher than the control (0 h) (7.94%). The oil remained liquid at 20°C for all reaction times. The melting point shifted from 18.9°C for the unreacted oil to 20.5°C for oil transesterified for 24 h. Transesterification of the oil also resulted in a significant ($P<0.05$) increase in the crystallization temperature of the high melting glycerides from the original value of 1.6°C to 12.9°C after transesterification for 24 h. The olein and the stearin fractions obtained after the transesterified oil was fractionated with acetone at -18°C and without acetone at 10°C had different properties. The olein fraction of oil fractionated at -18°C had a significantly higher ($P<0.05$) oleic acid (75.2%) compared to that of fractionation without acetone at 10°C (70.5%) and the unreacted oil (67.9%). The relative percent unsaturated fatty acids in the olein fraction of the fractionated oil with acetone at -18°C (82.6%) was also significantly higher ($P<0.05$) than those of liquid fractions of reacted oil fractionated at 10°C (76.5%) and unreacted oil (71.6%), respectively.

Keywords: Enzymatic transesterification, *Moringa oleifera* seed oil, oleic acid enhancement and fractionation

INTRODUCTION

Production of high-oleic oils for use in food applications has been receiving increased attention due to the health benefits attributed to oils high in monounsaturated/oleic fatty acid and their high stability even in demanding applications such as deep-frying (Corbett, 2003). Monounsaturated fatty acids (MUFA) such as oleic (*cis*-9-octadecanoic acid), are known to reduce blood cholesterol levels in non-hypertriglyceridemic individuals (Mattson and Grundy, 1985; Mensink and Katan, 1990). Among the vegetable oils, those from olive, peanut, rapeseed, and canola have been identified as being rich sources of MUFA, and

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which comprise 50 to 80% of their fatty acid composition (Corbett, 2003). Due to the importance placed on dietary MUFA, it has been recommended that MUFA intake be as high as half of the total recommended dietary intake of calories from fat (30%) as a means to reducing the risk of coronary artery and heart disease (Nicolosi *et al.* 1991; Bockisch, 1998; Lee *et al.* 1998). The development of genetic modification techniques to increase the oleic acid contents of seed oils such as canola has been reported (Corbett, 2003). Oils that are high in monounsaturated/oleic acid can be used as a healthier alternative to the more saturated and hydrogenated oils used in frying because of their stability. Consumption of saturated and partially hydrogenated fats and oils have been shown to increase the risk of coronary heart disease (Mattson and Grundy, 1985; Mensink and Katan, 1990).

The major components of fats and oils are triacylglycerols (TAG), the composition of which is specific to the origin of each fat or oil. The physical properties of various fats and oils are different because of the structure and distribution of fatty acids in the TAG (Willis and Marangoni, 2002). *Moringa oleifera* seed oil is rich in triolein (OOO) about 36.72 - 38.81%, followed by POO+SOL (12.41 - 12.49%), SOO (10.65 - 11.49%), OOA (7.71 - 7.75%), POL (3.93 - 5.09%) and OOGa (4.12 - 4.15%) with small amounts of OOLn, OLA and OOL (Abdulkarim *et al.* 2005). The high amount of polyunsaturated TAG in the oil makes it liquid at ambient temperature.

During chemical and enzymatic interesterification, acyl groups are redistributed first intramolecularly, then intermolecularly until a random distribution is achieved (Willis and Marangoni, 2002). With enzymatic interesterification, better control of the final product composition is possible, and glyceride mixtures that cannot be obtained using chemical interesterification can be produced (Willis and Marangoni, 2002). Enzymatic interesterification is accomplished using lipases. The lipases catalyze the hydrolysis of carboxyl ester bonds in acylglycerols. Depending on the degree of hydrolysis, free fatty acids, monoacylglycerols, diacylglycerols and glycerols are produced. This happens in the presence of water, but under water limiting conditions, ester synthesis (the reverse reaction), can be achieved. Lipase-catalyzed interesterifications have been extensively studied in systems using organic solvents. However, when such processes are to be used in the food industry, solvent-free systems must be developed (Willis and Marangoni, 2002). In this study, *M. oleifera* seed oil was modified using lipase from *R. miehei* followed by fractionation in order to produce oil that is richer in oleic acid.

MATERIALS AND METHODS

Materials

Moringa oleifera seed oil (MoO) was extracted from dried and ground seeds using the Soxhlet method and stored at -20°C. Prior to use, the oil was melted at 60°C in an oven and then shaken gently to obtain a homogenous sample. Lipozyme IM 60 from *R. miehei* (an immobilized enzyme with 0.2-0.6 mm granules size and 2-3% moisture content) was obtained from Novozymes Industry (Bagsvaerd, Denmark). All chemicals used were of analytical or HPLC grade from BDH laboratories (Poole, England) and Merck (Darmstadt, Germany).

Transesterification Reaction

Ten grams of melted *M. oleifera* seed oil was weighed into a 50 mL conical flask. One percent (w/w) of the immobilized lipase powder was added, and 0.2 g molecular sieves (MS) 3Å previously dried at 100°C for 24 h was added to serve as a dehydrator in the reaction (Ghazali *et al.* 1995a). The flask was then flushed with oxygen-free nitrogen and sealed using two layers of parafilm. The reaction mixture was then agitated in an orbital shaker at 60°C at 200 rpm for 4 h. The same was carried out for 8, 12 and 24 h. After the reaction was completed, each mixture was filtered through a Whatman No. 1 filter paper to separate the lipase from the oil. The unreacted oil (0 h)

served as the control. Samples were then analyzed for TAG, solid fat content, and the melting and crystallizing behavior after the removal of free fatty acids (FFA; described below) using HPLC, wide-line NMR and DSC, respectively. All experiments were carried out in triplicates.

Fractionation of Enzyme-reacted Oil

After the removal of free fatty acid (FFA), fractionation of unreacted and enzyme-reacted oil was carried out using the method described by Lee and Foglia (2000). Fractionation was carried out with and without solvent (acetone), respectively, as follows: Five grams of oil was placed into a 50 mL polypropylene centrifuge tube and fractionally crystallized from acetone using 1:10 w/v oil to acetone ratio in a Sharp freezer Model SM-25 TAL (Sharp Appliances Corporation, Petaling Jaya, Malaysia) at –18 ± 1°C for 24 h. After the incubation period, crystals that formed were pelleted at 9820 *x g* for 10 min at 0°C using a Beckman centrifuge Model J2-12M/E (Beckman Instruments, Palo Alto, CA). The solid and liquid fractions were separated by decanting the liquid fraction from the crystal pellets. Acetone from the liquid fraction was evaporated using a rotary evaporator Model N-1 (Eyela, Tokyo Rakakikai Company Ltd, Tokyo, Japan) at 60°C under vacuum.

Another 10 grams of enzyme-reacted *M. oleifera* oil was placed into a 50 mL polypropylene centrifuge tube and incubated in a refrigerator at 10 ± 1°C for 24 h. The crystals that formed were pelleted using the same method described above but with the temperature adjusted to 10°C (i.e. the fractionation temperature). The liquid phase was decanted from the crystallized TAG. Both fractions were stored at -20°C until analyzed. The unreacted oil served as the control.

Removal of Free Fatty Acids (FFA)

FFA was removed from unreacted and enzyme-reacted oil samples using the method described by Long *et al.* (1997). The oil was placed in a 250 mL conical flask and 20 mL of acetone: ethanol (1:1 v/v) was added. The mixture was titrated with 0.1 N NaOH to a phenolphthalein end-point. After the addition of 5 mL distilled water, the mixture was transferred into a 250 mL separating funnel and shaken. After standing for several minutes, the bottom layer (aqueous phase containing the FFA) was separated from the top organic phase. The aqueous phase was discarded and the organic phase was dried at 60°C overnight. Confirmation of removal of FFA was carried out by thin layer chromatography (TLC) using a mixture of hexane: diethylether: acetic acid (70:30:1) and viewed in iodine vapor.

Determination of Solid Fat Content (SFC) and Thermal Behavior

The solid fat contents of the unreacted and reacted oil samples were 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 in a water bath. Frozen oil samples were melted at 80ºC and mixed by gentle shaking and then equilibrated at 60ºC 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 hr. 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.

The melting and crystallization behavior of the oil samples was determined using a Perkin-Elmer Diamond DSC (Shelton, CT, 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 samples were heated at 60°C in an oven until completely melted, and 5-7 mg were placed in individual aluminum volatile pans. Each sample was cooled to -70°C at the rate of 5°C/min and held for 2 min, followed by heating to 70°C at the rate of 5°C/min (Che Man and Swe, 1995) and held at 70°C isothermally for 2 min and cooled back to -70°C at the rate of 5°C/min. The heating and cooling thermograms were recorded and the onset, peak, and offset (end) 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.

Determination of Triacylglycerol (TAG) Profile

The TAG profile of unreacted and enzyme-reacted oils was determined 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 were separated using a commercially packed RP-18 column (250 x 4 mm) with a particle size 5μm (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 microliters 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 *et al.* (1995b), Swe *et al.* (1996) and Long *et al.* (1997). 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 appear after 12 min (time at which the first TAG peak appeared) in the chromatograph were included (Ghazali *et al.*, 1995b).

Analysis of the Solid and Liquid Fractions of the Enzyme-reacted Oil

The iodine value was determined using the AOCS standard method (Cd 1-25/93) (AOCS, 1989), while fatty acid (FA) composition was determined by conversion of oil to fatty acid methyl esters prepared by adding 950μL of n-hexane to 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 (1μl) 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 mm film thickness), (SGE Incorporated, Austin, TX) to obtain FA methyl ester peaks. The detector temperature was 240°C and column temperature was 110°C held for 1 min and increased at the rate of 8°C/min to 220°C and held for 1 min and a run time of 32 min. Individual peaks of FA methyl esters were identified by comparing their retention times with those of standards. Individual FA compositions were calculated using the peak areas of the FA species that appear in the chromatogram as a relative percentage of the total peak areas of all the FA in the oil sample.

Statistical Analysis

All values presented are means of triplicates and the standard deviations. Student's t-test statistical analyses were carried out using, SPSS Version 11 and ANOVA using SAS Version 8e. Significant differences between values are at $P<0.05$ levels using Duncan multiple range test.

Results and Discussion

The form and appearance of the unreacted and the lipase-reacted oil after incubation at 10°C for 24 h were found to be different. The unreacted oil sample became rather viscous with fat crystals in suspension, while for samples

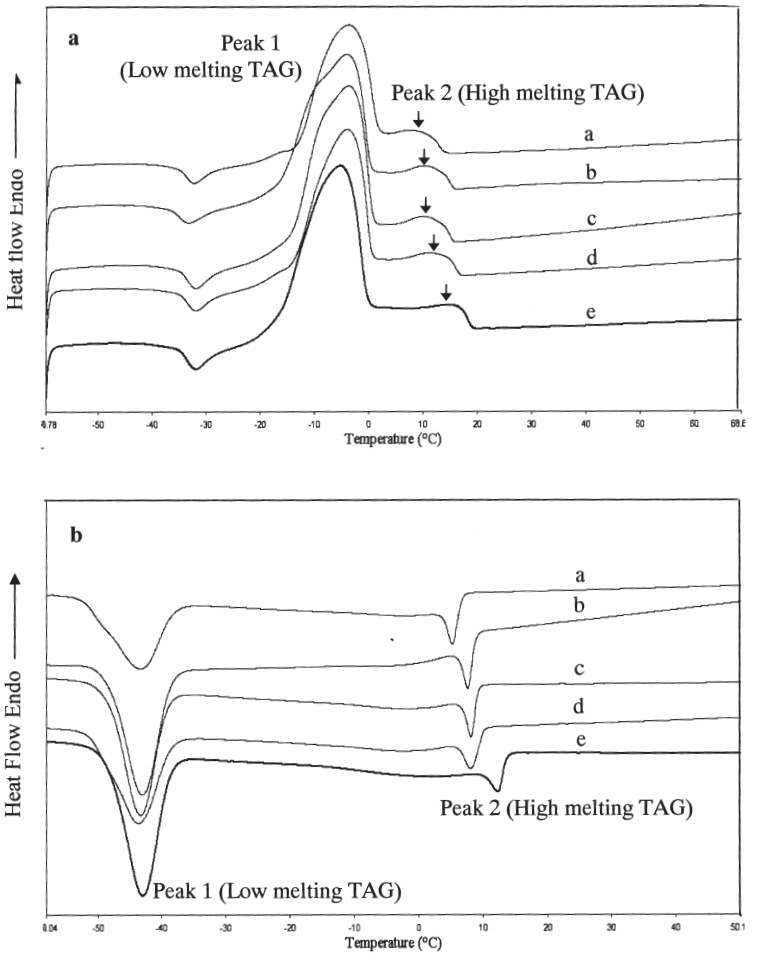

Figure 1: DSC (a) heating and (b) cooling thermograms of *M. oleifera* seed oil after transesterification using enzyme for (a) 0 h, (b) 4 h, (c) 8 h, (d) 12 h, and (e) 24 h

reacted with the enzyme, the fat crystals settled at the bottom of the flask, making the separation of the liquid and the solid fractions in the reacted oil sample much easier.

Effect of Reaction Time on Melting and Crystallization Behavior

Figures 1a and 1b show changes in the melting and crystallization behavior of the oil after reaction with enzyme at various times (0, 4, 8, 12, and 24 h). The peak temperatures of the last melting peak showed an increasing shift to a higher temperature, with increase reaction time, indicating an increase in the melting point of the oil from 18.9°C for the control to 20.5°C after 24 h of reaction with enzyme (Table 1). The shift in temperature values suggests that more high melting glycerides were synthesized during the transesterification reaction. The larger synthesis of higher melting glycerides hardened the oil and increased the SFC as the transesterification reaction proceeds. The advantage of this is that oil could be fractionated more easily by crystallizing out higher melting glycerides at selected temperatures. Other researchers have reported different changes. Zainal and Yusoff (1999), for example, observed a progressive decrease

Table 1: Crystallization behavior and melting points of original and enzyme-reacted *Moringa oleifera* seed oil (MoO)

Reaction time (hrs)	High melting TAG			Low melting TAG			Melting point (°C)
	Onset (°C)	Peak (°C)	End (°C)	Onset (°C)	Peak (°C)	End (°C)	
0 (Control)	2.65^{D}	1.61^{D}	-5.68^{D}	-37.98^{A}	-41.52^{B}	-46.03^{B}	18.90^{B}
4	7.37^{C}	6.07	4.82^{C}	-37.92^{A}	-42.08^{AB}	-48.11^{A}	19.39^{B}
8	9.45^{B}	7.46^{B}	6.02^{B}	-38.60^{A}	-43.13^{A}	-48.86^{A}	19.78^{AB}
12	10.04^{B}	7.75^{B}	6.03^{B}	-38.10^{A}	-43.54^{A}	-49.30^{A}	20.10^{A}
24	14.83^{A}	12.85^{A}	10.78^{A}	-37.91^{A}	-41.93^{B}	-47.10^{B}	20.52^{A}

Values within the same column followed by different superscript letters A, B, C, and D are significantly different ($P<0.05$)

in the SMP of interesterified blends of POs/PKOs (30:70) with increasing reaction time from 0-6 h. They found the SMP after 0, 2, 4, 5, and 6h of reaction to be 40, 38.5, 28.2, 28.6 and 29.9°C, respectively.

Table 1 also shows the onset, peak, and end temperature of crystallization for the original and enzyme-reacted oils. The peak and end temperature of crystallization for oil reacted with enzyme for 24 h (12.9 and 10.8°C), respectively, were significantly ($P<0.05$) higher than those of the original oil (1.6 and -5.7°C). The crystallization temperature of the high melting TAG (1.6°C) of the original oil was too low to be used to crystallize TAG, because even at 10°C the oil became semi-solid. This makes fractionation of the oil rather difficult. Transesterifying the oil resulted in a significant ($P<0.05$) increase in the crystallizing temperature of the high melting TAG from 1.6°C to 12.9°C. This shows that at 12.9°C almost all the high melting TAG will crystallize without the formation of semi-solid matrix. To ensure complete crystallization of the high melting TAG, and hence total separation from the low melting ones, a lower temperature (10°C) was used because the end temperature of the high melting TAG was 10.8°C, and was significantly ($P<0.05$) different from the crystallization temperature of the low melting TAG. However, after the transesterification reaction the crystallizing behavior of the low melting TAG was not significantly affected.

Effect of Transesterification on Solid Fat Content (SFC)

The SFC profiles, as a function of temperature for the *M. oleifera* seed oil (MoO) transesterified at various times (0, 4, 8, 12, and 24 h), are shown in Figure 2. A non-linear SFC profile was observed in all the cases. The oil became more solid as transesterification proceeded. The SFC value at 0°C after 24 h of reaction was 10.35% and significantly ($P<0.05$) higher than those at 0 h (control), 4, 8, and 12 h which were 7.29, 7.68, 7.93, and 7.94%, respectively. The SFC value as a function of temperature for MoO transesterified for 24 h was highest than at shorter times of reaction. This suggests that more glycerides of the higher melting class were being synthesized during the enzymatic reaction. This observation is the reverse to the case when mixtures of liquid and hard oil were used, wherein the resulting blends tended to have a lower SFC than the original harder mixture. Lai *et al.* (1998) while studying the effect of enzymatic transesterification on the fluidity of palm stearin-palm kernel olein mixture (POs-PKOo), found that the process reduced the SFC of the mixture. In their case, the concentrations of the higher melting TAG (POP and PPP) were reduced after the

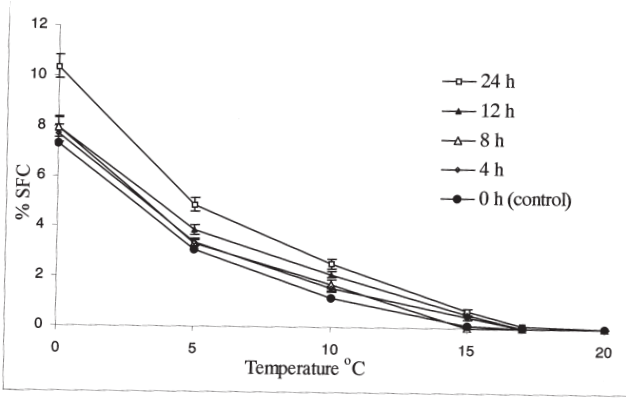

Figure 2: Changes in SFC (%) of *M. oleifera* seed oil after transesterification at various reaction times

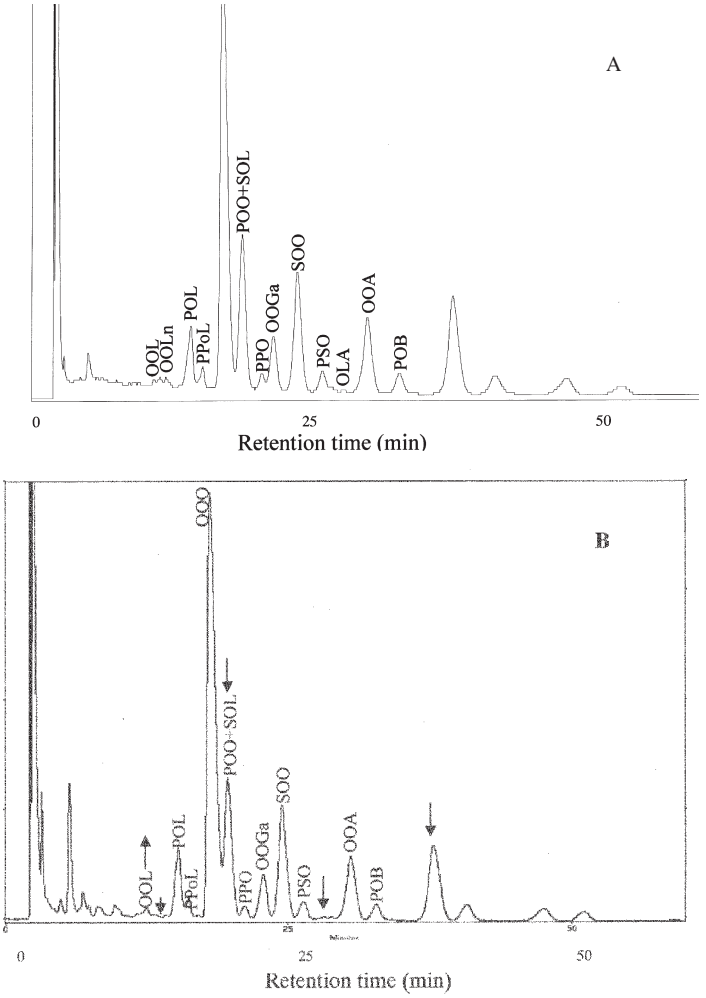

Figure 3: TAG profile of (A) unreacted (B) enzyme-reacted *M. oleifera* seed oil after 24 h. (↑) increase and (↓) decrease in amounts of TAG

Table 2: Fatty acid composition (%) of MoO fractionated with and without solvent

Fatty acids	Original oil	A		B	
		Liquid fraction	Solid fraction	Liquid fraction	Solid fraction
$C_{14:0}$	0.1^{A}	0.1^{A}	0.1^{A}	0.1^{A}	0.1^{A}
$C_{16:0}$	7.8^{B}	6.4^{C}	9.8^{A}	5.2^{D}	10.1^{A}
$C_{16:1}$	2.2^{B}	2.9^{AB}	1.8^{BC}	3.5^{A}	1.2^{C}
$C_{18:0}$	7.6^{BC}	6.9^{C}	8.4^{AB}	4.3^{D}	9.2^{A}
$C_{18:1}$	67.9^{C}	70.5^{B}	62.7^{D}	75.2^{A}	60.5^{E}
$C_{18:2}$	1.1^{BC}	1.8^{AB}	0.7^{C}	2.3^{A}	0.5^{C}
$C_{18:3}$	0.2^{A}	0.3^{A}	0.1^{A}	0.3^{A}	-
$C_{20:0}$	4.0^{A}	2.4^{C}	6.2^{A}	1.7^{C}	6.9^{A}
$C_{20:1}$	1.5^{B}	3.1^{A}	0.9^{B}	3.9^{A}	0.7^{B}
$C_{22:0}$	6.2^{C}	4.5^{D}	7.6^{B}	2.5^{E}	9.0^{A}
$C_{24:0}$	1.3^{A}	1.1^{A}	1.7^{A}	1.0^{A}	1.8^{A}
MUFA[a]	71.6^{C}	76.5^{B}	65.4^{D}	82.6^{A}	62.4^{E}
PUFA[a]	1.3^{B}	2.2^{A}	0.8^{BC}	2.6^{A}	0.5^{C}
SFA[a]	27.1^{C}	21.3^{D}	33.8^{B}	14.8^{E}	37.1^{A}

A = fractionation without solvent at 10°C, and B = fractionation with acetone at -18°C,
[a]MUFA = monounsaturated fatty acids, PUPA = polyunsaturated fatty acids and SFA = saturated fatty acids. Mean values in the same row followed by the same superscript letter A, B, C, D, and E are not significantly different (P>0.05).

transesterification, thus creating a substantially softer product. A similar observation was made by Zainal and Yusoff (1999) following the interesterification of POs/PKOs. Changes in TAG profiles of mixed oil substrates following enzymatic transesterification reaction have also been reported by Ghazali *et al.* (1995a and 1995b), Forssell *et al.* (1992), Foglia *et al.* (1993), Liew *et al.* (2001) and Lim *et al.* (2001) Such changes were often accompanied by changes in the SMP and SFC of the fats and oils blends (1995a). In the case of MoO, it is typically a case of cleavage and random rearrangement of fatty acids within the same or different TAG in the same oil. The TAG profiles of the original and enzyme-reacted oil (Figure 3A and 3B) may look similar but the disappearance of some of the low melting TAG such as dioleoyl-3-linoleoyl glycerol (OOL), dioleoyl –3-linolenyl glycerol (OOLn) and 1-oleoyl-2- linoleoyl-3 arachidyl glycerol (OLA) and an increase in peak size of dipalmitoyl-3-linoleoyl glycerol (PPL), which is a high melting TAG, created a harder oil.

Changes in the amounts of the TAG species after transesterification are normally accompanied by a change in the SFC depending on which type of TAG is synthesized. Generally, SFC of a fat, oil or mixture of fats/oils is responsible for many of the characteristics of a product, including its general appearance and plasticity.

Fatty Acid (FA) Composition of Fractionated Enzyme-reacted Oil

The FA compositions of the liquid and solid fractions of MoO following fractionation with acetone at -18°C and without acetone at 10°C are shown in Table 2. The results show that fractionation with or without the use of solvent resulted in a significant change in the FA composition of the fractions obtained in comparison with those of the unreacted oil (control). This is shown by the significant ($P<0.05$) differences in the major unsaturated and saturated FA in the isolated fractions. The oleic acid content of M. oleifera seed oil was significantly ($P<0.05$) enhanced from the

Table 3: MUFA, PUFA, SFA and total percent-unsaturated fatty acid contents of original and solvent fractionated MoO compared with some commercially available high-oleic oils

Fatty acids Composition (%)	*M.oleifera* seed oil (Liquid fraction)	High-oleic Sunflower oil*	High-oleic Canola oil*	High-oleic Safflower seed oil*	Olive oil
Oleic acid	75.2	75	~75	~77	73-75
MUFA	82.6	~76	~77	~78	76
PUFA	2.6	17	~17	~15	~8
SFA	14.8	~7	~6	~7	~16
Total unsaturation	85.2	~93	~92	~91	~84

*Genetically modified to increase oleic acid content (Corbett 2003)

original value of 67.9% to 75.2 and 70.5% following fractionation of the transesterified oil with and without solvent, respectively, resulting in a corresponding decrease in oleic acid content of the solid fractions to 60.4 and 62.7%, respectively. The percentage of oleic acid in the liquid fraction of the transesterified oil is comparable to those of high-oleic oils currently available in the market (Table 3).

Fractionation using acetone at -18°C was more effective compared to when no solvent was used. This is because the degree of separation of the liquid and solid components was higher, as reflected by the high amount of unsaturated FA contained in the liquid fraction (85.2%) of the solvent fractionated oil as compared to 78.7% in liquid fraction of oil fractionated without solvent. This observation agrees with that made by Lee and Foglia (2000) when fractionating chicken oil with and without solvent at varying temperatures. They observed no significant differences in the FA composition of the isolated fractions when temperature fractionation was used without solvent, and significant differences in the FA composition of the fractions isolated following fractionation with solvent (acetone). It is known that TAG at low temperatures generally form more stable crystals from a solvent than without solvent. Among solvents, acetone is regarded as the more suitable for promoting TAG crystal formation (Yikochi *et al.* 1990). However, compared to solvent-free fractional crystallization, solvent-fractionation processes are more complicated. They typically require lower temperatures for crystallization to occur, there is need to recover the solvent after the process and regulatory solvent residue levels must be met. Currently, the maximum permissible level for acetone is 30 ppm (Code of Federal Regulations 21CFR173.210). Fractionation without solvent was carried out at 10°C, the temperature at which crystal formation was practical without solidification of the whole oil.

Temperature fractionation of fat or oil can be regarded as a thermo-mechanical separation process where closely related TAG species for a given fat or oil are selectively crystallized from the melt or liquid phase (Lee and Foglia, 2000). During cooling of the liquid oil or melted fat, TAG species with the highest melting points preferentially crystallize, resulting in a solid phase within the liquid phase. To effectively achieve separation of the high and low melting TAG, an understanding of their crystallizing behavior is essential. DSC was used to determine the crystallization behavior of the original and enzyme-reacted oil. This study's main focus was on the high melting TAGs, which were to be crystallized during the fractionation method.

Table 3 also shows the relative composition of MUFA, PUFA and SFA of the liquid and solid fractions. Changes in the oleic acid content of the liquid fractions from both fractionations i.e. with and without the use of solvent (75.2 and 70.5%, respectively) were significant ($P<0.05$) from the original (67.9%). In all the cases, the total percentages of the MUFA and

Table 4: Iodine value and melting behavior of original, solvent fractionated Liquid and solid fractions of enzyme-reacted MoO

Sample	IV (gI_2/ 100g oil)	Low melting TAG			High melting TAG		
		Onset (°C)	Peak (°C)	End (°C)	Onset (°C)	Peak (°C)	End (°C)*
Original oil	65.9	-12.81	-4.43	3.39	7.55	16.1	19.01^{B}
Liquid fraction	72.8	-13.50	-4.52	0.32	3.00	9.83	14.64^{C}
Solid fraction	59.2	-14.89	-4.94	3.40	14.73	17.87	20.52^{A}

Values within the last column followed by different superscript letter were significantly different (P<0.05).
*Values represent the complete melting points of the samples.
IV= Degree of unsaturation of the oil.

PUFA increased significantly in the liquid fractions, with significant decreases in SFA content. In the solid fractions, however, the reverse is true with SFA content increasing significantly while the MUFA and PUFA contents were reduced. The increase in MUFA content was significantly (P<0.05) higher in liquid fractions from solvent fractionated oil (82.6%) than the liquid fraction of oil fractionated without solvent (76.5%). However, there was no significant (P>0.05) difference in the PUFA of the liquid fractions from the oils fractionated with and without solvent. Solvent fractionation of MoO was shown to be more effective than when temperature is used alone without solvent.

Melting Behavior and Iodine Value (IV) of Fractions from MoO

Table 4 shows the melting behavior of the liquid and solid fractions from the solvent fractionated MoO. It was observed that following the removal of high melting TAG from the enzyme-reacted oil, the complete melting point of the remaining liquid fraction (14.6°C) was significantly (P<0.05) lower than that of the original oil (19.0°C). On the other hand, the melting point of the solid fraction (20.5°C) was slightly higher than that of the original oil.

The iodine value (IV) indicates the degree of unsaturation of the oil and was determined to assess the degree of unsaturation of the two fractions. Its determination gives a reasonable quantification of lipid unsaturation if the double bonds are not conjugated with each other or with carbonyl oxygen (Shahidi and Wanasundara, 2002). As expected, the IV (g I_2/100g oil) of the liquid fraction increased to 72.8 and that of the solid fraction decreased to 59.2 from the IV of the original oil sample (65.9) before the fractionation (Table 4). Increase in IV in the liquid fraction was due to an increase in the percentage of unsaturated fatty acids caused by fractionation. Generally, non-drying oils (i.e. oils containing mono-unsaturated fatty acids/oleic acid) have typical IV of less than 100 (Van de Voort *et al.*, 1992). The IV of the liquid fraction (72.8) was lower than that of other oils such as olive (84.0) and high-oleic sunflower seed oils (87.0) because olive and high-oleic sunflower seed oils contain higher amounts of polyunsaturated fatty acids (9% and 17%), respectively, as compared to only 2.6% in liquid fraction of *M. oleifera* seed oil. The IV of the liquid fraction of the enzyme-reacted oil was therefore improved by fractionating the oil. Total percent-unsaturated fatty acids shown in Table 3 were 85.2 and 62.9% for the liquid and solid fractions, respectively.

CONCLUSION

The use of enzymatic transesterification followed by fractionation has been shown to

be successful in the enhancement of oleic acid content of *Moringa oleifera* seed oil making it high-oleic oil, which is desirable for health. The method can therefore be used with other oils to produce oils with high oleic acid content.

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