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Enzymatic interesterification on the physicochemical properties of *Moringa oleifera* seed oil blended with palm olein and virgin coconut oil

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SUMMARY: The enzymatic interesterification (IE) of palm olein (PO) and virgin coconut oil (VCO) with the high oleic acid (86%) *Moringa oleifera* seed oil (MoO) could yield a good source of oleic acid fat stock that may contain desirable nutritional and physical properties. Lipozyme RMIM resulted in different functionalities for the MoO/PO and MoO/VCO blends due to inherent changes in triacylglycerol (TAG) compositions which, in turn, led to different trends in DSC thermograms and solid fat contents (SFC). The enzymatic IE of MoO/VCO increased U₂S and S₂U (up to 20% medium and long chain, MLCT) while it decreased U₃ (triunsaturated) and S₃ (trisaturated) TAGs. The IE of the MoO/PO blends increased U₂S and S₃ (MMP, myristic, myristic, palmitic) and decreased S₂U, resulting in a lowering of melting points and SFC for MoO/VCO, while showing an increase in them for MoO/PO. A 2.55% increase in S₃ after 24 h MoO/PO 30:70 IE revealed a 6.5% harder oil at 10 °C which may imply a wider application compared to the original liquid oils. Novel MLCTs with improved nutritional and physical properties were generated in the MoO/VCO blends after IE due to the incorporation of oleic acid and medium chain fatty acids. MoO/PO 50:50 and 70:30 w/w after 12 h IE and MoO/VCO 30:70 are suitable for incorporation into the fat phase in ice-cream formulations while, the spreadability and plasticity of MoO/VCO 70:30 improved at low temperatures. Both interesterified blends could be used as high oleic acid frying oils.

KEYWORDS: *Intesterification; Lipozyme RMIM; Moringa oleifera seed oil; Physicochemical properties; Structured lipids*

RESUMEN: *Intesterificación enzimática sobre las propiedades físico-químicas de las mezclas de aceites de semillas de Moringa oleifera con oleína de palma y aceite de coco virgen.* La interesterificación enzimática (IE) de la oleína de palma (PO), aceite de coco virgen (VCO) con alto contenido en ácido oleico (86%) y aceites de semilla de *Moringa oleifera* (MOO) podría ser una buena fuente de ácido oleico con propiedades nutricionales y físicas deseables. La lipozyme RMIM produce diferentes funcionalidades para las mezclas MoO/PO y MoO/VCO debido a los cambios inherentes en la composición de triacilglicerolos (TAG) que, a su vez, dieron lugar a diferentes perfiles en los termogramas de DSC y en los contenidos de grasa sólida (SFC). La IE de MoO/VCO aumentó los TAGs, U₂S y S₂U (hasta un 20% de cadena media y larga, MLCT), mientras que disminuyó los TAGs triinsaturados (U₃) y trisaturados (S₃). La IE de mezclas MoO/PO, aumentó los TAGs U₂S y S₃ (MMP, mirístico, mirístico, palmítico) y disminuyó S₂U, lo que da lugar a una reducción del punto de fusión y de SFC para el MoO/VCO mientras que estos parámetros aumentan para MoO/PO. El aumento de un 2.55% en S₃ después de 24 h de IE de MoO/PO 30:70 da lugar a aceites 6,5% más duros a 10 °C, que pueden tener unas aplicaciones más amplias en comparación con los aceites líquidos originales. Nuevos MLCTs con mejores propiedades

nutricionales y físicas se generaron con las mezclas MoO/VCO tras IE debido a la incorporación de ácido oleico y ácidos grasos de cadena media. MoO/PO 50:50 y 70:30 w/w después de 12 h de IE y MoO/VCO 30:70 son adecuados para su incorporación como grasa en las formulaciones de helados, mientras que la untabilidad y plasticidad de MoO/VCO 70:30 mejoró a bajas temperaturas. Ambas mezclas interesterificadas podrían ser utilizados como aceites alto oleico para fritura.

PALABRAS CLAVE: Aceites de semillas de *Moringa oleifera*; Interesterificación; Lípidos estructurados; Lipozyme RMIM; Propiedades fisicoquímicas

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1. INTRODUCTION

Several processes are used to modify and improve the properties of vegetable oils and therefore, the versatility to widen their commercial applications. Lipase-catalyzed interesterification has been extensively studied for this purpose (Liew *et al.*, 2001; Palla *et al.*, 2012). Among commercially available lipase preparations, immobilized Lipozyme RMIM (from *Rhizomucor miehei*) has been extensively used for the production of specifically structured lipids (Chen *et al.*, 2007). Structured lipids (SLs) are defined as triacylglycerols (TAGs) that have been modified by the incorporation of new fatty acids, restructured to change the positions of FA, or synthesized to yield novel TAGs (Xuebing *et al.*, 2002), aimed at obtaining some desirable nutritional or physicochemical properties (da Silva *et al.*, 2010).

Dietary consumption of high oleic acid oils has been reported to have physiological benefits such as lowering serum cholesterol levels, suppressing certain types of cancer, enhancing liver function, and reducing the effects of aging and coronary disease (Carluccio *et al.*, 2007). These nutritional advantages are attributed to the elevated level of monounsaturated fatty acids (MUFA) and reduced saturate contents (Gerhard *et al.*, 2004). According to Riveros *et al.* (2010), MUFA also contributes to a longer shelf life of products due to its high resistance against oxidation and rancidity at ambient storage and at high temperatures (Warner *et al.*, 1997) compared to polyunsaturated fatty acids (PUFA). Some high-oleic oils such as olive could be used for frying (Abdulkarim, *et al.*, 2007a); however, its high cost restricts its usage on a major scale. Therefore, the use of more stable frying oils of comparatively low price would be desirable. Among newer sources of edible oils, *Moringa oleifera* seed oil (MoO) is of interest and may play a major role in human nutrition and health, because of its resemblance to olive oil (Abdulkarim *et al.*, 2005; Ramachandran *et al.*, 1980).

With a high amount of MUFA (81.73%) and low PUFA (0.71%), MoO represents an alternative

source of superior oil with high stability and good characteristics concerning the prevention of cholesterol and heart disease (Abdulkarim *et al.*, 2005; Ramachandran *et al.*, 1980) and a fatty acid (FA) composition resembling olive oil (Abdulkarim *et al.*, 2005; Ramachandran *et al.*, 1980). *Moringa oleifera* is a tree growing rapidly even in poor soil and is little affected by drought (Tsaknis and Lalas, 2002) and can be easily grown in poor third world countries. The production of useful SLs from this oil interesterified with other vegetable oils could be of economic and nutritional benefits to the native population of the areas where the tree is cultivated. Two vegetable oils, palm olein (PO) and Virgin coconut oil (VCO) have been selected to be interesterified with MoO in order to evaluate the effect of lipase on their characteristics.

PO, which is the most commonly used oil for frying foods in the Southeast Asia region (Bansal *et al.*, 2010), is a liquid fraction of palm oil and contains a high proportion of saturated fatty acids that contributes to related health disorders. Because the eating habits of most Malaysian people are based on deep fried foods, oxidative-resistant low saturated oils are needed. It has been previously reported that more than 90% of the lipids in fried food came from the frying oil (Pérez-Camino *et al.*, 1991) due to the interactions between food and frying oil (Bansal *et al.*, 2010). Abdulkarim *et al.* (2007a) and Tsaknis and Lalas (2002) reported that MoO has greater frying stability over other conventional frying oils such as canola oil, soybean oil and palm olein. This requirement can be met through the interesterification process of PO with unsaturated oils like MoO.

VCO, as a main source of medium-chain triacylglycerol (MCT), has been used for years to treat patients with malabsorption of fat problems and provide instant energy to athletes (Marina *et al.*, 2009b). However, as MCT contain solely medium chain fatty acids (MCFA), it lacks essential fatty acids such as oleic acid. MCT is also unsuitable for cooking purposes such as frying as it has a lower

smoke point and high foaming tendency (Lee *et al.*, 2012; Matsuo *et al.*, 2001). Hence, long chain fatty acids (LCFA) should be incorporated into the MCT molecules to overcome these weaknesses. This will lead to the development of a new type of structured lipids, called medium-and long-chain triacylglycerol (MLCT). This can either be produced via a lipase-catalyzed reaction of acidolysis, esterification, or interesterification (Koh *et al.*, 2010; Lee *et al.*, 2012). MLCT-based oil blends can be used as healthy functional oils for daily consumption such as in salad dressing and for frying (Koh *et al.*, 2010). Thus, the objective of this study was to investigate the physical and chemical properties of enzymatically interesterified MoO/PO and MoO/VCO blends and to predict the food applications of the resulting products.

2. MATERIALS AND METHODS

Brown, mature *M. oleifera* pods were collected in the Serdang area in Selangor, Malaysia. The seeds were removed from the pods and crushed gently to obtain the seed kernels which were then sorted to remove any misshapen seeds. Well-formed seed kernels were dried in a convection oven at 60 °C for 12 h. PO was purchased from Lam Soon Edible Oil Sdn. Bhd., Malaysia, while VCO was produced from fresh coconut milk purchased from a local wet market. All solvents and chemicals used in this study were either of analytical or HPLC grade and purchased from BDH Laboratories (Pool, England), Merck (Darmstadt, Germany) and Sigma Aldrich Inc. (St. Louis, California, USA). Individual FA methyl ester standards of caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (18:2), arachidic (C20:0), eicosenoic (C20:1), behenic (C22:0) and lignoceric (C24:0) acids, approximately 99% pure, were purchased from Supelco (Sigma-Aldrich Tokyo, Japan). Immobilized Lipozyme RMIM (*Rhizomucor miehei*) purchased from Novozymes A/S, Bagsvaerd, Denmark, was used for the IE reaction in a solvent-free system.

2.1. Extraction of MoO

MoO was extracted from dried *M. oleifera* kernels that had been ground into a fine powder using a Waring blender Model 32BL 80 (Dinamic Corporation of America, New Hartford, Connecticut, USA). The ground kernel (500 g) was placed in a cellulose filter paper (Whatman No. 1) cone and the oil was extracted using light petroleum ether (b.p 40–60 °C) in a 5 L Soxhlet extractor for 8 hours (AOAC, 1984). The solvent was then evaporated off using a rotary evaporator Model N-1 (Eyela, Tokyo Rikakikal Co., Ltd., Japan) and the recovered oil

was heated in a convection oven at 60 °C for 1 hour to remove residual solvent. The oil was then flushed with 99.9% nitrogen and stored at –20 °C until further use (Abdulkarim *et al.*, 2005). The crude oil was used without further refining.

2.2. Production of VCO

VCO was produced using the natural fermentation method described by Bawalan and Chapman (2006). One kilogram of fresh coconut milk was placed in a 2 L beaker, covered with aluminum foil and left standing at room temperature (25–27 °C) for 24 hours for natural fermentation to take place. The upper layer (oil layer) was then filtered through a filter paper (Fisher QC100, Fisher Scientific, Leicestershire, UK) and heated at 60 °C in a convection oven overnight to remove extra moisture in the oil. The oil was kept in –20 °C for analysis.

2.3. Interesterification reaction

Sixty grams (60 g) of melted *M. oleifera* seed oil/palm olein (MoO/PO) and *M. oleifera* seed oil/virgin coconut oil (MoO/VCO) blends at ratios of 70:30, 50:50 and 30:70 (w/w), each dissolved separately in 100 mL of water-saturated n-hexane, were placed in 250 mL conical flasks. Lipozyme RMIM (10% w/w of oil mixture) was then added to initiate catalysis. The reaction mixtures were agitated at 60 °C at 200 rpm for 0, 3, 6, 12 and 24 h. The blend ratios were selected based on preliminary experiments that show desired changes after blending and IE. The reaction mixture at 0 h acted as the control. After the reaction, each oil mixture was filtered through a Whatman No. 1 filter paper to remove the enzyme and the oil mixtures were kept at –20 °C for further analysis. The reaction was carried out in duplicate. The stored mixtures were thawed at room temperature and then heated at 60 °C for 30 min to ensure complete melting before analysis.

2.4. Removal of free fatty acid

Free fatty acids (FFAs) were removed from the reacted oil using the method described by Long *et al.* (2003). The reacted oil (30 g) was placed in a 250 mL conical flask and 50 mL of acetone: ethanol (1:1 v/v) were added and mixed. The mixture was then titrated against 0.1 N NaOH to a phenolphthalein end-point. The titrated sample was diluted with 10 mL of boiling distilled water and transferred to a 250 mL separating funnel. The mixture was shaken and allowed to settle for several minutes. The aqueous phase which contained the FFA, was separated from the organic phase and discarded. The organic phase was then dried at 60 °C overnight.

2.5. Triacylglycerol profile

The triacylglyceride (TAG) profiles of the oil samples were obtained by injecting 10 μL of oil samples (10% in acetone, v/v) into a reversed-phase high performance liquid chromatograph (HPLC) comprising a Waters Alliance HPLC (Waters Corporation, Milford, Massachusetts, USA) equipped with a separation module (Waters Model 2695), an auto-injector and a refractive index detector (Waters Model 2414). The TAG were separated using a commercially packed RP-18 column (250 \times 4 mm) with particle size 5 μm (Merck, Darmstadt, Germany) held in a column oven set at 35 $^{\circ}\text{C}$. Elution of TAG from the column was achieved using a mixture of acetone/acetonitrile (63.5:36.5) as the mobile phase at a flow rate of 1 $\text{mL}\cdot\text{min}^{-1}$. The total run time was 100 min and TAG peaks were identified based on the retention time of available TAG standards and the results of Chen *et al.* (2007) and Marina *et al.* (2009a). Peak areas produced by the data integrator (Empower-Pro Version 2002, Waters Corporation, Milford, Massachusetts, USA) were used to quantify the components based on relative percentages.

2.6. Thermal properties

The thermal properties of the oil samples were investigated by differential scanning calorimetry (DSC) using a Perkin-Elmer Diamond DSC (Shelton, Connecticut, USA) based on the procedure described by Abdulkarim *et al.* (2005). The instrument was calibrated using indium and zinc. The purge gas used was 99.9% nitrogen with a flow rate of 100 $\text{mL}\cdot\text{min}^{-1}$ and a pressure of 20 psi. Samples (5–8 mg that had been completely melted at 60 $^{\circ}\text{C}$) were sealed in aluminum volatile DSC pans and placed in the instrument's sample chamber. An empty DSC pan was used as the reference. The sample chamber was heated to 70 $^{\circ}\text{C}$ and held for 2 min to erase crystal memory in the oil. It was then cooled to –60 $^{\circ}\text{C}$ at the rate of 10 $^{\circ}\text{C}\cdot\text{min}^{-1}$, and held at –60 $^{\circ}\text{C}$ isothermally for 2 min and heated from –60 $^{\circ}\text{C}$ to 70 $^{\circ}\text{C}$ at the rate of 10 $^{\circ}\text{C}/\text{minute}$. The heating and cooling thermograms were recorded and the values for onset, peak and end set temperatures tabulated. The onset temperature indicates the temperature at which the melting process starts and the end set temperature indicates the complete melting temperature of the oil.

2.7. Prediction of solid fat content

The solid fat contents (SFC) of all samples at different temperatures were calculated from their thermal profiles according to Adhikari *et al.* (2010), as obtained above. Each DSC melting thermogram was divided into different temperature sectors (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55 $^{\circ}\text{C}$) and the total crystallization energy (J/g) at each temperature was

then converted into percentage (%) fat based on the following equation:

$$(\%) \text{ Fat} = [\text{Partial (J/G)} / \text{Total (J/G)}] \times [100]$$

2.8. Statistical analysis

A statistical analysis was carried out using MINITAB 14 (Minitab Inc., State College, PA, USA) by subjecting all the data obtained to a univariate analysis of variance (ANOVA) to determine the significant differences among samples, monitored by Tukey's pairwise test, and significance was defined at $p < 0.05$. All measurements were carried out in triplicate and reported as the mean and standard deviation of independent trials.

3. RESULTS AND DISCUSSION

3.1. Degree of oil hydrolysis following interesterification

Intesterification (IE) requires the hydrolysis of oil by lipase in order to produce free fatty acids (FFAs) and a mixture of mono- and diacylglycerols. These products of hydrolysis will then be synthesized to generate more of either existing or new kinds of TAG. Since not all FFAs participated in the IE process, they may accumulate in the reaction mixture. The original amount of FFA in extracted MoO and VCO found to be 0.56% and 0.23% respectively where no FFA was recorded in refined bleached deodorized (RBD) PO. In this study, after the reaction, the FFA contents were found to be increased significantly ($p < 0.05$) with the time of reaction compared to the unreacted blends for both MoO/PO and MoO/VCO in which the highest FFA levels were produced after 24 h reaction time. Post-IE FFA levels for MoO/PO blends ranged between 1.85 and 2.14%, whereas the control values (pre-IE) ranged from 0.37 to 0.60%. For MoO/VCO blends, post-IE and pre-IE values of FFA content ranged from 1.75 to 3.24% and 0.23 to 0.42%, respectively. Prior to the analysis of other physicochemical properties, as many FFAs as possible were removed by solvent-solvent fractionation as they might act as an interference. The FFA levels of interesterified MoO/PO and MoO/VCO blends after their removal were between 0.15 and 0.18% and 0.12 and 0.18%, respectively.

3.2. Effect of enzymatic interesterification on triacylglyceride profile

Figure 1 shows the triacylglyceride (TAG) profiles of MoO/PO and MoO/VCO blends at the ratio of 50:50 w/w and their interesterified blends at zero (control) and 24 h reaction time. After Lypozyme RMIM IE, several TAG peaks increased in height indicating synthesis, while others decreased

TABLE 1. Triacylglycerol composition ($g \cdot 100 g^{-1}$) of *Moringa oleifera* seed oil interesterified with palm olein (PO) and virgin coconut oil (VCO)

TAG%	MoO/PO (30:70 w/w) Time of reaction (h)					MoO/PO (50:50 w/w) Time of reaction (h)					MoO/PO (70:30 w/w) Time of reaction (h)				
	0	3	6	12	24	0	3	6	12	24	0	3	6	12	24
	S ₃	2.24±0.20	2.96±0.23	3.12±0.06	3.76±0.85	4.79±0.13	2.22±0.06	2.74±0.20	3.05±0.15	3.38±0.11	4.05±0.07	2.19±0.13	2.59±0.13	2.95±0.07	3.12±0.17
S ₂ U	29.60±0.29	28.25±0.21	28.30±0.14	27.35±0.10	25.83±0.18	21.37±0.24	20.43±0.18	19.77±0.24	19.19±0.13	16.73±0.18	12.85±0.21	12.38±0.18	11.19±0.13	10.73±0.33	9.26±0.16
U ₂ S	41.48±0.26	42.73±0.18	43.2±0.28	44.96±0.37	46.90±0.28	37.76±0.09	39.02±0.17	39.80±0.28	40.41±0.16	42.04±0.34	32.16±0.23	33.33±0.33	34.79±0.27	35.12±0.17	35.77±0.24
U ₃	15.56±0.23	15.13±0.18	15.19±0.13	15.34±0.06	15.23±0.09	21.55±0.07	21.40±0.00	21.17±0.10	21.03±0.04	20.32±0.03	27.40±0.00	26.82±0.03	26.41±0.01	26.52±0.03	27.40±0.01
TAG%	MoO/PO (30:70 w/w) Time of reaction (h)					MoO/PO (50:50 w/w) Time of reaction (h)					MoO/PO (70:30 w/w) Time of reaction (h)				
	0	3	6	12	24	0	3	6	12	24	0	3	6	12	24
	S ₃	70.29±0.34	64.65±0.47	65.27±0.28	67.26±0.67	65.43±0.51	48.28±0.54	48.61±0.64	48.84±0.74	47.67±0.45	46.55±0.38	29.93±0.47	29.09±0.41	27.62±0.31	23.39±0.27
S ₂ U	2.63±0.33	3.69±0.16	4.06±0.23	5.48±0.24	7.53±0.23	1.39±0.27	2.77±0.11	3.65±0.16	5.46±0.16	7.55±0.18	1.24±0.20	2.98±0.11	4.29±0.13	7.81±0.07	8.71±0.16
U ₂ S	7.00±0.57	7.75±0.21	7.92±0.27	8.05±0.21	8.34±0.06	12.72±0.03	12.79±0.13	12.99±0.13	13.05±0.21	14.36±0.23	17.96±0.09	17.41±0.16	17.71±0.16	18.38±0.11	18.46±0.23
U ₃	10.38±0.11	11.03±0.04	8.55±0.07	8.10±0.14	7.23±0.04	19.19±0.13	18.00±0.00	16.50±0.14	13.55±0.07	13.52±0.30	25.48±0.11	24.44±0.57	23.27±0.10	20.54±0.06	18.75±0.07

Means for the determined values in the same row followed by the same superscript letter are not significantly different ($p > 0.05$).

Abbreviations: S₃: tri-saturated; S₂U: di-saturated-mono-unsaturated; U₂S: di-unsaturated-mono-saturated; U₃: tri-unsaturated.

MoO/VCO 70:30 w/w, while the lowest amount (no change in OOO value) was detected for the 24 h interesterified MoO/PO 70:30 w/w. In the case of MoO/VCO IE, other TAGs that showed corresponding decreases proportional to the reaction time belong to S3 TAGs consisting of CCLa, CLaLa, LaLaLa, LaLaM, and LaPM.

As shown in Table 2, the function of Lipozyme RMIM on MoO/VCO IE resulted in the increase in MLCT species (LaLaO, LaMO, LaPM and LaPO) concentration as well as the synthesis of a new TAG species (LaOO). In fact, the major MLCT group generated following IE was MLL (medium-long-long) comprising LaOO in all MoO/VCO ratios with a maximum value of 5.43% in 24 h interesterified MoO/VCO 70:30 w/w (Table 2). The most noteworthy increase in TAG concentration was observed for MML (medium-medium-long chain), the TAG group comprising LaLaO and LaMO in all MoO/VCO ratios (Table 2). The total amount of generated MLCT with incorporated oleic acid (LaLaO, LaMO, LaPO and LaOO) was maximized in 24 h interesterified MoO/VCO 50:50 with the value of 17.86%. It has been well established that substrate ratio is an important parameter to obtain the desirable yield in IE (Fomuso *et al.*, 1998).

In this study, IE resulted in the increase and synthesis of new TAGs, which was revealed by the emergence of several lower molecular weight TAGs, namely MLCT. The first commercially available MLCT sold in the market by Nisshin Ollio Ltd. Group, Ltd, uses the enzymatic IE process for its production involving coconut or palm kernel oil and an edible vegetable oil (rapeseed, cottonseed and soybean oil) (Lee *et al.*, 2012). Khatoun *et al.* (2005) reported on interesterified palm stearin with coconut oil, where LaLaLa decreased tremendously while that of LaLaO and LaOO experienced major increases. In other studies, the incorporation of medium chain saturated FAs occurred in safflower oil (Utsugi *et al.*, 2009) and anhydrous milk fat (Liew *et al.*, 2001). From the health benefit point of view, MLCT not only can provide us with nutritional properties from the essential fatty acids incorporated, but most importantly it can also help to reduce body weight and body fat accumulation in the body (Lee *et al.*, 2012).

During IE, Lipozyme RMIM catalyzes the cleavage of FAs from the glycerol backbone of some TAG species at the 1, 3 position (those that decreased in concentration after the reaction) and re-esterifies randomly at the free hydroxyl positions on the glycerol backbone of the existing acylglycerols. The difference in lipozyme RMIM functionality toward PO and VCO interesterified blends with MoO could be due to the processing methods undergone. As mentioned by O'Brien (2003), the PO

TABLE 2. Relative percentage of medium and long chain triacylglycerol (MLCT) in the control and interesterified MoO/VCO blends

TAG	Type of MLCT	Time of reaction (h)				
		0	3	6	12	24
30:70						
LaLaO	MML	2.63±0.25 ^a	3.69±0.13 ^b	4.29±0.13 ^b	5.48±0.11 ^c	6.99±0.13 ^d
LaMO	MML	1.05±0.07 ^a	1.92±0.03 ^b	2.87±0.10 ^c	3.49±0.13 ^d	4.17±0.10 ^e
LaPM	MLM	3.32±0.03 ^a	3.39±0.13 ^a	4.58±0.11 ^b	4.50±0.28 ^b	4.14±0.06 ^b
LaOO	MLL	0.15±0.07 ^a	0.86±0.08 ^b	2.49±0.13 ^c	2.67±0.10 ^c	3.31±0.01 ^d
LaPO	MLL	0.40±0.00 ^a	1.08±0.11 ^a	2.29±0.13 ^c	1.92±0.03 ^b	1.33±0.08 ^d
50:50						
LaLaO	MML	1.39±0.13 ^a	2.77±0.10 ^b	3.65±0.07 ^c	5.46±0.08 ^d	7.55±0.07 ^e
LaMO	MML	0.70±0.03 ^a	1.01±0.11 ^b	1.46±0.06 ^c	3.15±0.08 ^d	3.98±0.07 ^e
LaPM	MLM	2.33±0.01 ^a	2.25±0.04 ^b	2.26±0.01 ^b	3.53±0.20 ^c	3.25±0.06 ^c
LaOO	MLL	0.00±0.04 ^a	0.97±0.06 ^b	1.70±0.13 ^c	3.87±0.11 ^d	4.77±0.57 ^e
LaPO	MLL	0.23±0.28 ^a	0.88±0.13 ^a	1.21±0.10 ^b	2.63±0.47 ^c	1.33±0.01 ^c
70:30						
LaLaO	MML	0.90±0.14 ^a	2.24±0.06 ^b	3.12±0.03 ^c	5.13±0.04 ^d	5.78±0.11 ^e
LaMO	MML	0.34±0.06 ^a	0.74±0.06 ^b	1.17±0.10 ^c	2.68±0.11 ^d	2.93±0.04 ^d
LaPM	MLM	1.41±0.01 ^a	1.22±0.03 ^a	1.25±0.07 ^d	2.83±0.04 ^b	2.58±0.11 ^b
LaOO	MLL	0.00±0.00 ^a	1.15±0.07 ^b	1.96±0.08 ^c	4.51±0.01 ^d	5.43±0.04 ^e
LaPO	MLL	0.12±0.07 ^a	0.64±0.06 ^b	0.94±0.06 ^b	2.49±0.13 ^c	1.40±0.14 ^c

Means for the determined values in the same row followed by the same superscript letter are not significantly different ($p > 0.05$). Abbreviations: M: myristic acid; P: palmitic acid; S: stearic acid; O: oleic acid; L: linoleic acid; C: capric acid; La: lauric acid.

produced after the refining, bleaching and deodorizing (RBD) procedure with heating is applied especially during the deodorization process between 204 and 245 °C. However, VCO, known as a functional oil or mineral oil was produced in mild conditions (Chen *et al.*, 2007) in order to maintain the bioactive compounds. As indicated by khoramnia *et al.* (2011) and Ebrahimipour *et al.* (2011), the mineral contents of the cultures play an essential role in the function of lipases.

Generally, as indicated by Ribeiro *et al.* (2009), the increased U2S content in the MoO/PO and MoO/VCO blends produced by enzymatic interesterification lead to greater potential for applying these interesterified blends in foods. S2U TAGs are associated with enhanced technological functionality and improved sensorial properties while S3 TAGs are responsible for the structure of the fat (Rodrigues and Gioielli, 2003). In addition, the contribution of oleic acid from MoO into the interesterified blends made them a potential candidate to be used as frying oils. As demonstrated by Abdulkarim *et al.* (2007a), MoO revealed great resistance during the frying procedure due to its high oleic acid content. Therefore, it could be proposed that the frying quality of PO enhanced after IE with MoO due to a lower degree of saturation and higher resistance. It could be also indicated that interesterified

MoO/VCO fryng quality improved due to the MLCT generation and oleic acid contribution as the original VCO is not suitable for frying due to the high médium chain fatty acids and foam formation (Lee *et al.*, 2012).

3.3. Effect of enzymatic interesterification on DSC thermograms

Figures 2 and 3 show the melting and crystallization thermograms of the interesterified MoO/PO and MoO/VCO blends, respectively. Generally, by increasing the proportion of MoO, the melting profile of both IE blends is supposed to shift towards lower temperature regions indicating a generation of TAGs with lower melting points. However, as a result of the S3 increase in interesterified MoO/PO, the corresponding melting profile shifted towards higher temperature regions (Table 1).

In MoO/PO interesterified blends, by increasing MoO, a new peak (peak 3) was generated in 50:50 w/w and 70:30 w/w. As IE progressed, the enthalpy of the major peak 1 in MoO/PO 30:70 and 50:50 w/w blends increased, while peak 2 decreased followed by an overlapping with peak 1. This indicates a reduction in TAGs as represented by peak 2 and a possible formation of more TAGs, which was

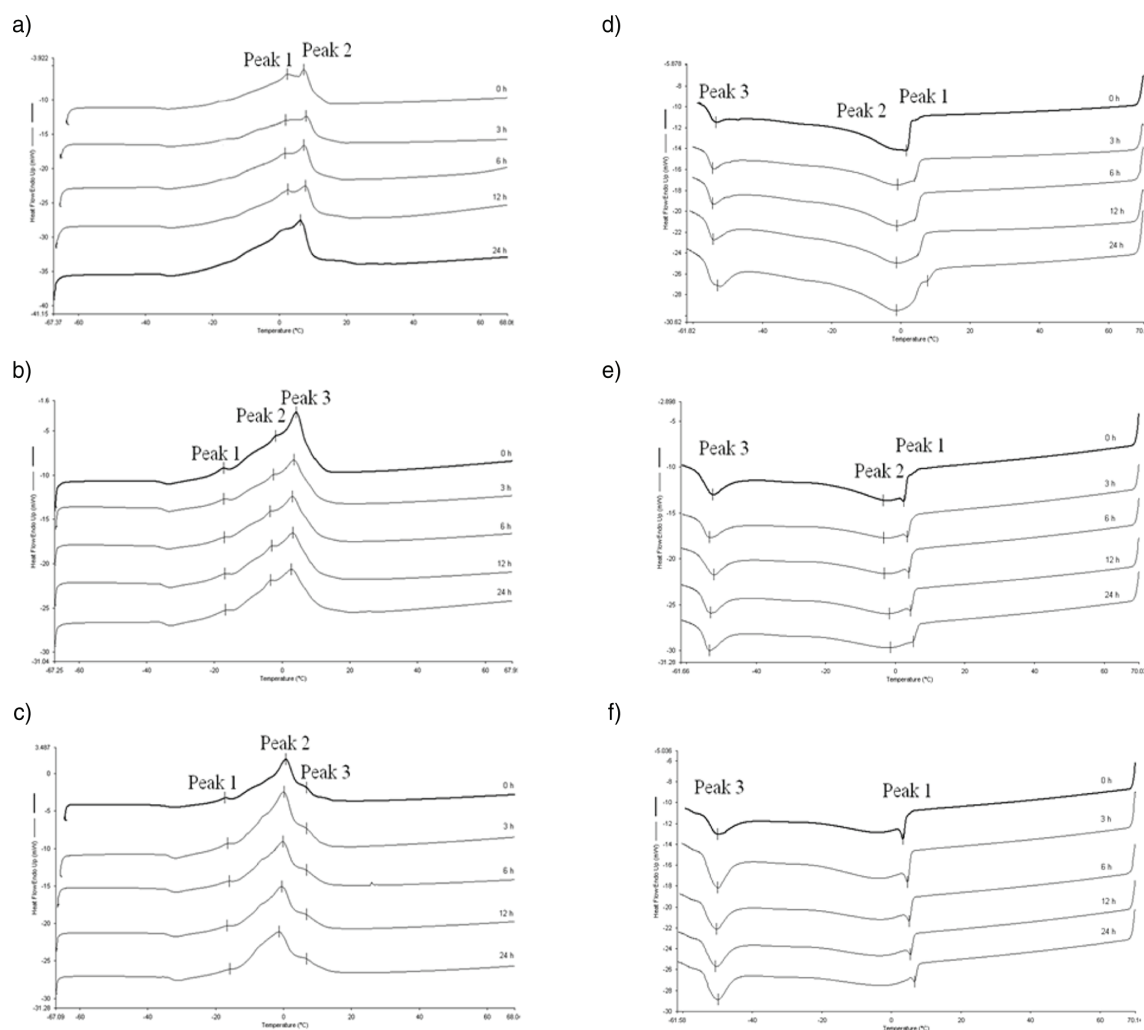


FIGURE 2. Melting and crystallization profile of Lipozyme RMIM interesterified MoO/PO blends. Melting profiles for (a) 30:70 w/w (b) 50:50 w/w (c) 70:30 w/w blending ratios and crystallization profiles for (d) 30:70 w/w (e) 50:50 w/w (f) 70:30 w/w blending ratios.

represented by peak 1. However, in the MoO/PO 70:30 blend, peak 1 gradually disappeared and the TAG represented by peak 2 became the dominant TAG. This is as expected as the blend contained a higher quantity of MoO which, when compared to PO, has a higher degree of unsaturated FA. These findings indicated that the blends were composed mainly of low melting TAGs, and the TAGs representing peak 3 were contributed by MoO. This result is expected as the main FA of the blends is oleic acid and OOO is the main TAG. da Silva *et al.* (2010) reported that triunsaturated TAGs have melting points from $-14\text{ }^{\circ}\text{C}$ to $1\text{ }^{\circ}\text{C}$, and triolein has a melting point of $-4\text{ }^{\circ}\text{C}$ (Hynes, 2010).

In the crystallization thermograms of unreacted MoO/PO blends (Figure 2), three crystallization peaks (peaks 1, 2 and 3) were observed. However, peak 2 gradually disappeared when the proportion of MoO was increased over the time of IE. Concurrently, it was observed that the size of peak 3

increased and as IE progressed it shifted to a lower temperature region, indicating the synthesis of lower melting TAGs. From the cooling thermograms of MoO/PO 70:30 blends, two obvious crystallization peaks were observed: TAGs representing Peak 1, which crystallized at $3.36\text{ }^{\circ}\text{C}$, and those representing Peak 2, which crystallized at $-49.97\text{ }^{\circ}\text{C}$. After 24 h IE, the TAGs in Peak 1 crystallized at a higher temperature ($6.69\text{ }^{\circ}\text{C}$), whereas there was little change in the peak temperature for the TAGs represented by Peak 2.

The above results show that the IE of different blends of MoO/PO would produce TAG species with wide ranging crystallization and melting temperatures. The resulting blends were found to have higher end set temperatures than the melting temperatures before IE. However, the melting temperatures were still lower than the original MoO, except for the blends containing 30% MoO which melted at $22.97\text{ }^{\circ}\text{C}$. Abdulkarim *et al.* (2007b) reported

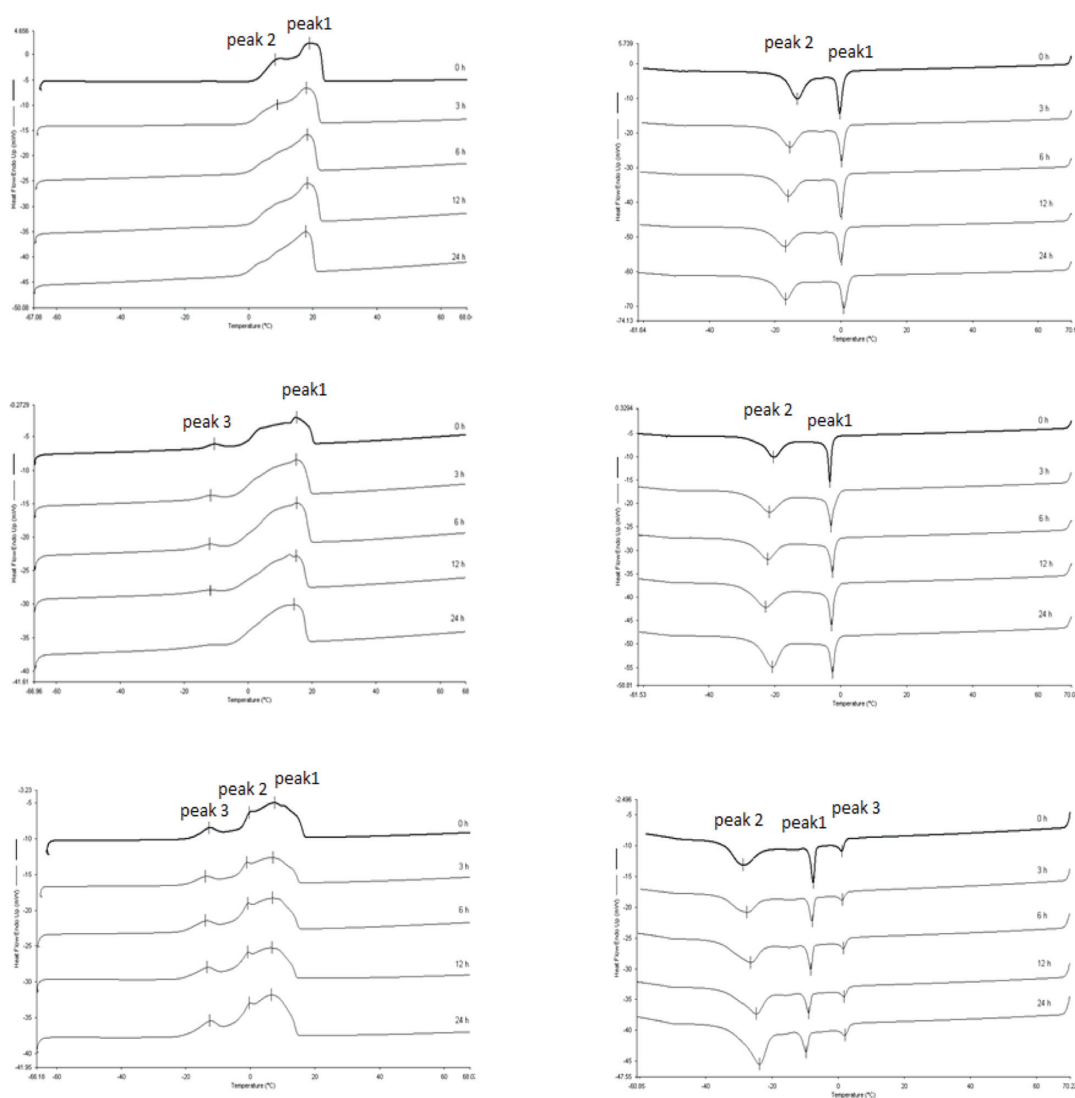


FIGURE 3. Melting and crystallization profiles of Lipozyme RMIM interesterified MoO/VCO blends. Melting profiles for (a) 30:70 w/w (b) 50:50 w/w (c) 70:30 w/w blending ratios and crystallization profiles for (d) 30:70 w/w (e) 50:50 w/w (f) 70:30 w/w blending ratios.

the increase in melting temperature from 18.9 °C (control) to 20.5 °C after 24 h self-IE of MoO using Lipozyme IM 60. This was reflected in the crystallization temperature increase from 1.6 °C to 12.9 °C. Long *et al.* (2003) reported that the slip melting point of palm olein/flaxseed oil blends (90:10 w/w) also increased from 14.1 °C to 19.9 °C after 6 h IE using Lipozyme IM, indicating that the synthesis of higher melting point TAGs took place during the rearrangement of the FA moiety.

The results for the IE of the MoO/VCO blends (Figure 3) provide several important observations concerning the melting and crystallization profiles. In the melting profile of the 30:70 blend, a low melting Peak 2 represented unstable TAG crystals of low melting points that melted earlier and this peak then became broader and finally overlapped with the major TAG peak (peak 1) after 6–24 h of IE, resulting

in peak 1 shifting to a lower melting region indicating the reduction of high melting TAGs due to oleic acid incorporation and a higher degree of unsaturation. The melting thermogram of the interesterified MoO/VCO 70:30 blend (Figure 3) displayed a well-defined peak which is related to the low, medium and high melting points of TAGs which melt in a wide temperature range. The major melting peak (peak 1) of 0 h blend occurred at 7.47 °C with shoulder peaks (peak 2) at -0.32 °C and a minor peak (peak 3) at -12.87 °C. After the IE of MoO/VCO 70:30 blend, the minor and major melting peaks shifted towards the low temperature region, resulting in a reduction in the melting temperature of the interesterified blend. This indicated that the rearrangement of FAs responsible for these peaks leading to the formation of lower melting TAGs after IE were most likely to be influenced by unsaturated FA (oleic acid

incorporation) from MoO. For blends with lower proportions of MoO, the thermal properties of the interesterified oil revealed an influence of saturated FA rich in VCO.

Generally, in the MoO/VCO crystallization thermogram, the major crystallization peak (peak 1) was observed with a minor peak (peak B) for the 30:70 blend ratio. During the IE of MoO/VCO 30:70, the major crystallization peak decreased and the minor peak increased. Both results from the melting and crystallization thermograms indicate that a softer mixture has been produced.

3.4. Effect of enzymatic interesterification on solid fat content

The solid fat content (SFC) profile as a function of temperature for the MoO/PO and MoO/VCO blends, interesterified at various times and ratios, is shown in Table 3. The SFC of the blends at 0 °C is related to the degree of saturation: the higher the amount of MoO in the blends, the lower the SFC is at 0 °C. Likewise, the greater the quantity of either PO or VCO in the blends, the higher the SFC is at 0 °C. The obtained result indicates that unlike MoO/VCO, IE caused the formation of more saturated TAGs which then contributed to an increase in melting temperature in the MoO/PO blends which is in accordance with the results obtained in previous sections. In this agreement, Long *et al.* (2003) and Abdulkarim *et al.* (2007b) demonstrated elevated SFC after IE indicating the ability of the enzyme to form higher-melting TAGs.

The findings show that for each MoO/PO blend ratio, the temperature at which SFC is zero increased after IE, compared to the control (0 h). However, it still remained below ambient temperature (25–27 °C) or body temperature (35–37 °C). After 24 h IE, the SFC% of all MoO/PO ratios increased where 30:70 w/w blend revealed more obvious changes compared to other ratios. The major difference was observed at 10 °C and 15 °C with SFC values of 4.6% and 0% in unreacted (0 h) MoO/PO 30:70 w/w blend which increased to 11.1% and 6% after 24 h IE, respectively. As stated in the TAG section, due to the TAG rearrangements after the IE of MoO/PO, the level of S3 (MML) increased by up to 2.55% in the 30:70 blend. It could be concluded that 2.55% of S3 enhancement brings about 6.5% and 6% more SFC% in 10 and 15 °C, respectively. According to Rodrigues and Gioielli (2003), S3 TAGs are responsible for the structure of fatty foods.

Consequently, interesterified MoO/PO revealed a harder structured fat blend produced from two liquid oils due to the function of Lipozyme RMIM. Changes in the amount of TAG species after IE are normally accompanied by a change in the SFC, depending on the type of TAG formed (Debnath *et al.*, 2011; Koh *et al.*, 2010).

In interesterified MoO/VCO blends the level of S3 decreased following the increase in the U2S level resulting in the production of softer fat. Soares *et al.* (2012) also reported SFC reduction in a coconut oil-canola oil interesterified blend. At 20 °C, the interesterified MoO/VCO 50:50 blend showed 0% SFC while it was higher for the 30:70 blend and lower for 70:30 due to the difference in the oleic acid incorporation associated with the MoO level. At low temperatures (4–10 °C), where SFC gives an indication of the fat's spreadability, VCO originally do not offer satisfactory characteristics with SFC values of 89.8%. Ribeiro *et al.* (2009), Wassell and Young (2007) and Lida and Ali (1998) demonstrated that for spreadability at refrigeration temperatures, the SFC should not exceed 32% at 10 °C in order to meet the minimum plasticity requirement. The binary blends of 70:30 w/w MoO/VCO can be regarded as offering good spreadability at low temperatures (10 °C) with SFC values of 23.1 to 11.2% after 0 to 24 h IE, respectively. The product's stability and its resistance to oiling-out could be characterized in fats with a minimum of 10% SFC between 20 and 22 °C (Lida *et al.*, 1998; Wassell and Young, 2007). The unreacted binary blend of MoO/VCO 30:70 w/w with 20.1% and 10.9% SFC at 20 and 22 °C, respectively and the interesterified blend after 6 h with 11.3% SFC fall within this group of fats.

Blends with zero SFC at 25 °C including MoO/PO 50:50 and 70:30 w/w after 12 h IE and MoO/VCO 30:70 are suitable for incorporation into the fat phase in ice-cream formulations as demonstrated by Liew *et al.* (2001). In addition, the MoO/VCO interesterified blends with elevated levels of MLCT may improve the quality of pastries as Ariffin *et al.* (2010) reported that cookies made from medium- and long-chain triacylglycerol-enriched margarines have good texture and sensory perception as well as excellent nutritional benefits.

4. CONCLUSIONS

This study has shown that enzymatic interesterification with MoO an effective way to modify the physical and chemical properties of PO and VCO. With regards to thermal properties, the broadening and shifting of Peaks were clearly seen, indicating the formation of TAG species with a wider melting range that have clearly affected the SFC of the interesterified blends. Interesterification of MoO/PO blends caused an increase in the concentration of high melting TAGs, thus increasing the product's melting temperature and SFC% at higher temperatures. All interesterified MoO/VCO blends melted at lower temperatures which allowed them to remain liquid at ambient temperature while improving their spreadability and plasticity at refrigerated temperatures. Healthier frying blends and ice-cream fat bases were produced from MoO interesterified

TABLE 3. Solid fat content of unblended and interesterified *Moringa oleifera* seed oil with palm oil and virgin coconut oil (MoO/PO and MoO/VCO blends)

MoO/PO blends (w/w)	Solid fat content (%)										MoO/VCO blends (w/w)	Solid fat content (%)									
	Temperature (°C)											Temperature (°C)									
	0	2	5	7	10	15	20	25	30	35		0	5	10	12	15	17	20	22	25	30
MoO	34.8	27.0	15.4	10.8	4.0	0.9	0.1	0.0	–	–	MoO	34.8	15.4	4.0	2.8	0.9	0.6	0.1	0.0	–	–
PO	60.5	50.3	34.9	24.1	8.0	0.5	0.0	–	–	–	VCO	94.9	91.9	89.8	84.6	76.9	66.5	50.8	32.0	3.7	0.0
30:70											30:70										
0	53.5	43.4	28.3	17.8	4.6	0.0	–	–	–	–	0	100.0	96.1	77.1	68.3	54.1	40.5	20.1	10.9	0.0	–
3	53.8	44.9	31.5	21.7	9.0	2.4	0.8	0.2	0.0	–	3	99.6	91.1	71.6	61.9	46.1	30.8	9.4	4.4	0.0	–
6	53.0	44.1	30.7	20.8	8.1	2.3	0.7	0.2	0.0	–	6	99.7	92.9	74.2	64.4	48.7	33.2	11.3	5.4	0.0	–
12	55.9	46.7	32.9	23.0	10.1	3.8	1.1	0.4	0.0	–	12	99.5	91.2	72.3	61.7	44.7	28.4	6.4	2.9	0.0	–
24	52.6	43.5	30.1	21.6	11.1	6.0	2.6	1.0	0.2	0.0	24	98.9	88.6	69.3	57.7	39.6	23.1	1.6	0.6	0.0	–
50:50											50:50										
0	47.0	35.5	19.7	11.8	3.6	0.5	0.0	–	–	–	0	90.3	73.9	51.0	40.3	24.5	14.5	0.0	–	–	–
3	45.9	34.8	19.4	11.9	4.2	0.8	0.0	–	–	–	3	90.3	74.0	49.5	37.5	20.6	12.1	0.0	–	–	–
6	45.7	34.3	18.7	11.5	4.3	0.7	0.0	–	–	–	6	91.1	76.4	51.0	38.6	21.1	11.7	0.0	–	–	–
12	45.3	34.4	19.3	12.3	5.1	1.3	0.3	0.0	–	–	12	90.6	75.0	48.7	35.1	16.6	8.8	0.0	–	–	–
24	45.2	34.7	20.4	13.7	6.9	2.5	0.7	0.2	0.0	–	24	91.6	75.0	48.3	24.7	16.5	8.7	0.0	–	–	–
70:30											70:30										
0	39.0	28.1	13.5	8.0	3.4	0.5	0.0	–	–	–	0	72.8	49.5	23.1	13.7	2.5	0.9	0.0	–	–	–
3	34.7	25.2	12.5	7.2	2.4	0.2	0.0	–	–	–	3	64.0	40.2	14.5	7.1	0.0	–	–	–	–	–
6	34.5	25.8	13.9	8.7	3.6	0.6	0.0	–	–	–	6	63.5	39.9	14.1	7.8	0.0	–	–	–	–	–
12	33.6	25.2	13.8	8.7	3.6	0.7	0.1	0.0	–	–	12	62.1	38.5	12.2	6.7	0.0	–	–	–	–	–
24	32.0	24.8	14.8	10.1	5.0	1.4	0.4	0.0	–	–	24	60.6	36.7	11.2	6.2	0.0	–	–	–	–	–

with PO and VCO using the Lipozyme RMIM. The findings indicate that the enzymatic IE of MoO with PO and VCO would enable the initial properties of the oils to be modified or altered and provide functional and nutritional attributes for use in various food applications, increasing the possibilities for commercial use of these oils.

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