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# LOW-TEMPERATURE DIRECTED INTERESTERIFICATION INCREASES TRIUNSATURATED AND TRISATURATED TRIACYLGLYCEROLS OF PALM OIL AND AFFECTS ITS THERMAL, POLYMORPHIC AND MICROSTRUCTURAL PROPERTIES

**NOOR LIDA HABI MAT DIAN*; LEE YEE YING**; NIK MOHD AZNIZAN NIK IBRAHIM*; NORAZURA AILA MOHD HASSIM*; SAW MEI HUEY*; TAN CHIN PING‡ and LAI OI MING‡‡**

## ABSTRACT

*Directed interesterification (DIE) is an interesterification technique performed at a sufficiently low temperature to allow higher melting triacylglycerols (TAG) to crystallise out as they are formed during the reaction. Thus, DIE is an excellent tool for changing the TAG composition of oils and fats and consequently extends their applications. DIE converts liquid oils into plastic fats, reduces the formation of graininess and improves the plasticity of lard, and increases the unsaturation level of the olein fraction of fats. This study explored the possibility of using lipase-catalysed DIE (EDIE) as a 'green' technique to produce palm oil (PO) which is high in triunsaturated ($U_3$) and trisaturated ($S_3$) TAG, which in turn, when fractionated, produce an olein fraction which is high in unsaturated (especially, oleic acid) and a stearin fraction that is high in saturated (especially, palmitic acid) fatty acids. EDIE resulted in a significant ($p<0.05$) increase in $U_3$ and $S_3$ TAG of PO, from 4.3% and 5.2% before EDIE to 27.6% and 31.9% after EDIE, respectively. The increased content of $U_3$ and $S_3$ TAG subsequently altered the physical properties of PO. Higher $U_3$ TAG content led to an increase in the proportion of the low melting fraction of PO. Meanwhile, the melting point, solid fat content at all temperatures and the proportion of high melting fraction of PO show to increase following the rise in $S_3$ TAG after EDIE. A differential scanning calorimetry melting curve showed that the low and high melting fractions of EDIE PO were distinctively separated, indicating ease of fractionation. EDIE also increased the proportion of β crystals in PO. Thus, its microscopic structure showed dense crystal aggregates with a coarse plate-like and orderly packed structure.*

**Keywords:** palm oil, directed interesterification, triacylglycerol, thermal profile, polymorphic behaviour, microscopic structure.

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## INTRODUCTION

Interesterification (IE) is an important technique currently employed to modify the physico-chemical characteristics of oils and fats. IE can take place within individual triacylglycerols (TAG) (intraesterification) or between TAG (interesterification) molecules. IE randomises the fatty acids (FA) at the *sn*-1, *sn*-2 and *sn*-3 positions (by a non-specific catalyst), or only at the *sn*-1 and *sn*-3 positions (by an *sn*-1,3 stereospecific

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[*] Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia.
E-mail: nlida@mpob.gov.my

[**] School of Science, Monash University Malaysia,
Jalan Lagoon Selatan, Bandar Sunway,
47500 Subang Jaya, Selangor, Malaysia.

[‡] Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia.

[‡‡] Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia.

catalyst, normally a lipase). Consequently, IE modifies the TAG composition which in turn alters physical characteristics such as crystallisation and melting behaviour, solid content and polymorphic behaviour of the oils and fats (Amir *et al*., 2012).

Typically, IE is conducted at a temperature of around 100°C-120°C for chemical IE (CIE), and 55°C-70°C for enzymatic IE (EIE). At these temperatures, all lipids are completely liquefied, and the reactions are, therefore, accomplished in a single liquid phase. Directed IE (DIE) is performed similarly, but at a much lower temperature, *i.e.,* below the temperature of the highest melting TAG (normally below 50°C) (De Lathauwer *et al*., 1983). This allows the high melting (more saturated) TAG, which are normally trisaturated ($S_3$), to crystallise as they form. The crystallised $S_3$ TAG will then be withdrawn from the reaction system (liquid phase) because in the crystallised form, they can no longer take part in the reaction. Such selective crystallisation disrupts the equilibrium system. To re-establish the reaction equilibrium, new $S_3$ TAG are continuously formed from the remaining liquid phase, which in turn also precipitate out. The formation and crystallisation of $S_3$ TAG continue until all TAG capable of crystallising have been eliminated from the reaction phase (Placek and Holman, 1957; Rousseau and Marangoni, 2008), or until the reaction is stopped. Segregation of saturated FA (SAFA) into $S_3$ TAG is accompanied by a corresponding tendency for unsaturated FA (USAFA) to form triunsaturated ($U_3$) TAG. DIE eventually results in a lipid mixture that contains a high proportion of $S_3$ and $U_3$ TAG, at the expense of disaturated ($S_2U$) and diunsaturated ($U_2S$) TAG (De Lathauwer *et al*., 1983; Placek and Holman, 1957).

DIE increases $U_3$ and $S_3$ TAG of lard (Rousseau and Marangoni, 2008; Chobanov and Topalova, 1979), tallow (MacKenzie and Stevenson, 1995) and palm oil (PO) (Lago and Hartman, 1986; Laning, 1985). Chemical DIE increases $U_3$ and $S_3$ TAG of PO to 12% and 30%, respectively (Laning, 1985). The olein and stearin fractions obtained from chemically DIEed PO are composed of about 75% $U_3$ and 62% $S_3$ TAG, respectively (Lago and Hartman, 1986). The ability of DIE to increase $U_3$ and $S_3$ TAG of oils and fats has been applied as a useful technique to change the physical characteristics of certain fats, making them more feasible for particular applications. DIE converts liquid oils into plastic fats (De Lathauwer *et al*., 1983), reduces the formation of graininess and improves the plasticity of lard (MacKenzie and Stevenson, 1995), increases the unsaturation level of the olein fraction of certain fats (MacKenzie and Stevenson, 2000), and produces oils and fats that are high in monoacylglycerols (MAG) and diacylglycerols (DAG) (Baur and Lange, 1951). Production of structured lipid containing medium chain SAFA and USAFA directly from coconut oil through DIE in a solvent has been reported by Nugrahini and Soerawidjaja (2015).

Evidence of effects of enzymatic DIE (EDIE) on the physico-chemical characteristics of PO is as yet unavailable. Hence, the present study was carried out to evaluate the physico-chemical changes in PO after EDIE. It is expected that utilising this 'green' EDIE process on PO (which consists of equal amounts of USAFA, mainly oleic, and SAFA, mainly, palmitic) will result in PO which is high in $U_3$ and $S_3$ TAG. Such PO is desirable, especially when the oil is subjected to fractionation, as it will produce an olein fraction which is high in USAFA and a stearin fraction that is high in SAFA, that is relatively much higher than the USAFA and SAFA content of olein and stearin fractions of refined, bleached and deodourised (RBD) PO. Both fractions have good potential to be used as oils and fats for healthy foods. The high oleic olein fraction could be used as a healthy high oleic frying or salad oil. The high SAFA stearin fractions could be used as an excellent alternative to *trans* fats. When blended or restructured with other vegetable oils via IE, and texturised, they could produce a *trans*-free hardstock with excellent oil binding capacity and could also perform effectively as a structural fat in formulating *trans*-free reduced saturated solid fat products.

## MATERIALS AND METHODS

### Materials

RBD PO was procured from Keck Seng (M) Berhad (Johor, Malaysia). Commercial immobilised *Thermomyces lanuginosus* lipase (Lipozyme TLIM) was purchased from Novozymes A/S (Bagsvaerd, Denmark). The TAG standards used for elucidation of the TAG species were purchased from Sigma Aldrich Inc. (Sigma Chemical Co., USA). All chemicals and solvents were of analytical and high performance liquid chromatography (HPLC) grades, respectively.

### Lipozyme TLIM Lipase Conditioning

Pre-conditioning of the immobilised lipase was carried out to remove any extra moisture before conducting EDIE of PO. Immobilised enzymes normally contain approximately 5% (w/w) of water, which need to be removed to prevent hydrolysis of the oil substrate which will lead to the formation of undesirable free FA (FFA) and partial glycerides such as MAG and DAG (Saw *et al*., 2009). Lipozyme TLIM lipase was conditioned by reacting the lipase (10% w/w) with PO at 70°C for 30 min before draining the PO from the reaction system. This step was repeated five times with fresh RBD PO. After the fifth reaction, the reacted PO contained only about 3% FFA as compared to 18% FFA in the reacted PO from the initial reaction. The FFA was assessed by a titration method defined in the AOCS Official Method Ca 5a-40 (Firestone, 2009). The FFA was expressed as the percentage of palmitic acid. The treated enzyme was then used for the subsequent EDIE reaction.

### EDIE of PO

Moisture-free PO was accurately weighed and melted at 80°C for 45 min in a double-jacketed and temperature-controlled stirred tank vessel under vacuum. The temperature was then lowered to 30.0°C before the addition of 10.0% (w/w) of pre-conditioned Lipozyme TLIM lipase. The mixture was left to react for 18.0 hr under stirring at 500 rpm. The reaction was then stopped by rapidly heating the reaction mixture to a high temperature in a microwave oven before being vacuum-filtered to separate the enzyme particles from the lipid mixture.

### Purification of Crude Enzymatically Directed Interesterified PO (EDIE PO)

The enzyme-free EDIE PO comprising TAG and by-products (a mixture of FFA, MAG and DAG) was subjected to purification before further analysis. Purification was performed using a short-path distillation (SPD) unit (KD6 System, UIC GmbH, Alzenau-Hörstein, Germany) in a two-step process, according to the method reported by Saberi *et al*. (2012). FFA and MAG were removed in the first step. The residue (a mixture of DAG and TAG) was purified again to remove DAG. The same conditions were used for both purification steps: evaporator vacuum of 0.001 mbar, feeding rate of 1.3 litres hr$^{-1}$, condenser temperature of 85°C, feeding tank temperature of 75°C, and roller speed of 280 rpm. Evaporation temperature employed in the first and second purification steps was 200°C and 250°C, respectively.

### Fatty Acid Composition (FAC)

FAC was determined as FA methyl esters (FAME) according to the MPOB Test Method p3.5:2004 (MPOB, 2004), which is technically equivalent to ISO 5508:1990(E) (IOS, 1990). FAME was prepared by dissolving 50 mg of a melted and homogenised fat blend in 950 µl hexane, and 50 µl of 1 M sodium methoxide. The mixture was then mixed vigorously with a vortex mixer for 10 s or until it became cloudy before centrifugation at 2000 rpm for 2 min to separate the mixture into two distinct layers. The upper clear supernatant consisting of FAME was decanted. One µl of the FAME was then injected and analysed using a Hewlett Packard HP 6890 Series (Palo Alto, USA) gas chromatography system equipped with a flame ionisation detector. Separation was performed using a Durabond DB-23 fused silica capillary column (60 m length x 0.25 mm i.d. x 0.25 µm film thickness) (Agilent, Santa Clara, USA). Injector and detector temperatures were set at 240°C, whereas the column temperature was isothermal at 185°C. The flow rate of the carrier gas helium was 0.8 ml min$^{-1}$. Peaks were identified by FAME standards and quantified using the peak area normalisation method.

### TAG Composition

TAG composition was determined using Waters HPLC Model Alliance e2695 (Waters, United Kingdom) equipped with Waters ELSD model ELSD 2424. A Merck KGaA column (Darmstadt, Germany) Purospher® Star RP-18e (250 mm × 4 mm) with 5 µm particle size was used to separate the TAG. The column temperature was set at 35°C. The sample was melted at 70°C, dissolved in acetone at a concentration of 10% (v/v) (100 µl of sample into 900 µl of acetone) and filtered through a 0.2 $\mu$m PTFE membrane filter to remove impurities. A 10 µl sample was then injected into the column. Separation was carried out with a mobile phase containing a mixture of acetone and acetonitrile (Merck, Darmstadt, Germany) at a gradient composition of 35%, 65%, 85%, 35% and 35% of acetone at 0, 10, 15, 20 and 25 min, respectively. The mobile phase flow rate was set at 1.5 ml min$^{-1}$ with a total run time of 25 min. Individual chromatographic TAG peaks were identified by comparing peak elution time with those of the TAG standard (OLL, OLO, OOO, PMP, PPP, PPS, PLL, PLO, POO, SOO, PLP, POP and POS) and quantified using the peak area normalisation method.

### Differential Scanning Calorimetry (DSC) Thermal Behaviour

DSC thermal behaviour was studied using a differential scanning calorimeter, DSC 7 (Perkin Elmer, Boston, MA). Approximately 7-8 mg (± 0.005 mg) of a precisely weighed molten sample was placed in a hermetically sealed DSC pan. The fat sample was held at 80°C for 10 min to erase its thermal memory. The sample was then cooled to -60°C at a cooling rate of -25°C min$^{-1}$, and held at this temperature for another 10 min. The melting thermogram of the sample was then recorded by heating the sample from -60°C to 80°C at a heating rate of 5°C min$^{-1}$. The sample was then held at 80°C for 10 min, and cooled to -40°C at a cooling rate of 5°C min$^{-1}$ to determine crystallisation behaviour. Total ($\Delta H_f$) and partial ($\Delta H_{i^{\circ}C}$) melting enthalpy, and total ($-\Delta H_f$) and partial ($-\Delta H_{i°C}$) crystallisation enthalpy, at various temperatures were calculated using the DSC Data Analysis software.

### Solid Fat Content (SFC)

SFC was determined with a Bruker Minispec MQ20 pulsed nuclear magnetic resonance (p-NMR) analyser (Karlsruhe, Germany) according to the MPOB Test Method p4.8:2004-Non-stabilised Serial procedures (MPOB, 2004), which is technically equivalent to MS 817: Part 9 Section 1:2003 (MS, 2003). Molten sample was filled up to 3 cm height of a p-NMR tube, re-melted at 70°C for 30 min, chilled at 0°C for 90 min, and held at the respective measuring temperatures for 30 min prior to measurement of total solid fat (%) at each measuring temperature from 5°C-50°C at intervals of 5°C.

### Slip Melting Point (SMP)

SMP of the sample was determined according to the MPOB Test Method p4.2:2004 (MPOB, 2004), which originated from the AOCS Official Method Cc 3-25 (Firestone, 2009). A molten sample was placed in three capillary tubes (50-60 mm length x 1.1-1.3 mm i.d. x 1.4-1.5 e.d.) with openings at both ends, filling each to a length of 10 mm. The sample in the tube was then solidified by rolling the tube on an ice cube. The capillary tube was then placed in a test tube, and held in a water bath equilibrated at 10°C for 16 hr. The tube was then attached to a thermometer (graduated in divisions of 0.1°C-0.2°C) with a rubber band, before immersing both into a beaker containing 400 ml of cold distilled water with a temperature of 5°C-10°C below the expected SMP of the sample. The water bath was agitated with a magnetic stirrer, and heat was applied continuously to increase the bath temperature at a rate of 0.5°C $\text{min}^{-1}$. The temperature at which the fat column rose due to hydrostatic pressure was recorded as SMP of the fat sample.

### Polymorphic Behaviour

Polymorphic forms of the fat crystals were determined using a Rigaku TTRAX III X-Ray diffractometer (Rigaku Inc, Tokyo, Japan). Fat sample was melted at 70°C for 30 min to erase the crystal memory. Subsequently, a small amount of the molten sample was placed in the X-ray sample pan and stored for 24 hr at 5°C, 10°C, 15°C, 20°C, 25°C and 30°C, prior to determination of the polymorphic form. The short spacings of the β′ form are at 4.2 and 3.8 Å and that of the β form is at 4.6 Å. The levels of β′ and β crystals in the sample were estimated by the relative intensities of the short spacings at 4.2 and 3.8 Å, and at 4.6 Å (D'Souza *et al.*, 1990).

### Microstructure

The microstructure of the fat crystals was determined using a polarised light microscope (PLM). A small droplet (about 10 µl) of molten fat was placed on a glass slide and covered with a glass slip, and then rapidly cooled to 5°C, 10°C, 15°C, 20°C and 25°C. The sample was stabilised at each temperature for 24 hr before measurement. A Leica DMLP polarised light microscope (Leica, Germany), equipped with a Linkam THMS 600 temperature controller stage and a JVC 3-CCD colour video camera, was used. Temperature was thermostatically controlled by a Linkam TP 94 multi-ramp temperature programmer and an LNP automatic cooling system (Linkam, United Kingdom). Liquid nitrogen was used as the coolant. A photomicrograph of the crystal was taken at 200X magnification.

### Statistical Analysis

Statistical analysis was conducted on triplicate measurements. Data were analysed with one-way analysis of variance (ANOVA), using the Minitab 16 (Minitab Inc, State College, PA, USA) statistical software. Data were expressed as means with standard deviations. Statistical significance was determined using Tukey's test at $p<0.05$.

## RESULTS AND DISCUSSION

### Purification of Crude EDIE PO

Purification of crude EDIE PO was done to remove the by-products (FFA, DAG and MAG) formed during the EDIE process. For a fair comparison, RBD PO was also purified. Purification of the crude EDIE PO is particularly vital as it contains about 20% by-products that will affect the quality and shelf-life of the EDIE oil. Purification by SPD resulted in EDIE PO and RBD PO with minute amounts of FFA, MAG and DAG.

### FA and TAG Composition

The EDIE process resulted in restructuring the FA distribution in TAG molecules. *Table 1a* shows the effect of EDIE on the FAC of PO. Following EDIE, a significant reduction in USAFA and a significant increase in the SAFA content of PO were observed. Small changes in FAC after EDIE were expected due to the removal of FFA, MAG and DAG from crude EDIE PO during the purification process. Reduction in USAFA and increase in the SAFA content after EDIE might be because, during EDIE, USAFA rather than SAFA was preferentially detached from the TAG molecules. Hence, there was more USAFA

**TABLE 1. FATTY ACID AND TRIACYLGLYCEROL COMPOSITIONS OF ENZYMATICALLY DIRECTED INTERESTERIFIED PALM OIL (EDIE PO) AND REFINED, BLEACHED AND DEODOURISED PALM OIL (RBD PO)**

| Characteristic | RBD PO | EDIE PO |
|---|---|---|
| (a) Fatty acid | Amount (%) | |
| C12:0 Lauric (La) | $0.2 \pm 0.01^{a}$ | $0.2 \pm 0.06^{a}$ |
| C14:0 Myristic (My) | $1.1 \pm 0.05^{a}$ | $1.1 \pm 0.06^{a}$ |
| C16:0 Palmitic (P) | $44.3 \pm 0.90^{a}$ | $47.2 \pm 1.60^{b}$ |
| C18:0 Stearic (S) | $4.2 \pm 0.05^{a}$ | $4.2 \pm 0.02^{a}$ |
| C18:1 Oleic (O) | $41.3 \pm 0.82^{a}$ | $39.5 \pm 1.52^{a}$ |
| C18:2 Linoleic (Ln) | $8.9 \pm 0.62^{a}$ | $7.8 \pm 0.51^{a}$ |
| Saturated fatty acids (SAFA) | $49.8 \pm 0.98^{a}$ | $52.7 \pm 0.81^{b}$ |
| Monounsaturated fatty acids (MUFA) | $41.3 \pm 0.83^{a}$ | $39.5 \pm 1.54^{a}$ |
| Polyunsaturated fatty acids (PUFA) | $8.9 \pm 0.62^{a}$ | $7.8 \pm 0.51^{a}$ |
| Unsaturated fatty acids (USAFA) | $50.2 \pm 0.56^{a}$ | $47.3 \pm 0.62^{b}$ |
| (b) Triacylglycerol | Amount (%) | |
| Triunsaturated ($U_3$) | | |
| OLL | $0.2 \pm 0.07^{a}$ | $1.3 \pm 0.14^{b}$ |
| OLO | $1.4 \pm 0.07^{a}$ | $9.7 \pm 0.49^{b}$ |
| OOO | $2.7 \pm 0.35^{a}$ | $16.6 \pm 0.85^{b}$ |
| Total | $4.3 \pm 0.28^{a}$ | $27.6 \pm 1.06^{b}$ |
| Trisaturated ($S_3$) | | |
| PMP | $0.5 \pm 0.14^{a}$ | $1.2 \pm 0.14^{b}$ |
| PPP | $4.5 \pm 0.21^{a}$ | $28.1 \pm 0.92^{b}$ |
| PPS | $0.2 \pm 0.07^{a}$ | $2.5 \pm 0.42^{b}$ |
| Total | $5.2 \pm 0.35^{a}$ | $31.9 \pm 0.97^{b}$ |
| Diunsaturated-monosaturated ($U_2S$) | | |
| PLL | $0.9 \pm 0.14^{a}$ | $1.5 \pm 0.28^{b}$ |
| PLO | $8.9 \pm 0.21^{a}$ | $7.7 \pm 0.49^{a}$ |
| POO | $27.8 \pm 0.42^{a}$ | $17.2 \pm 0.64^{b}$ |
| SOO | $0.6 \pm 0.21^{a}$ | $0.6 \pm 0.21^{a}$ |
| Total | $38.2 \pm 0.70^{a}$ | $27.0 \pm 0.82^{b}$ |
| Disaturated-monounsaturated ($S_2U$) | | |
| PLP | $7.2 \pm 0.64^{a}$ | $2.1 \pm 0.21^{b}$ |
| POP | $41.1 \pm 0.49^{a}$ | $10.2 \pm 0.71^{b}$ |
| POS | $4.0 \pm 0.14^{a}$ | $1.2 \pm 0.21^{b}$ |
| Total | $52.3 \pm 1.20^{a}$ | $13.5 \pm 0.67^{b}$ |

Note: Means within each row of a similar fatty acid or triacylglycerol (for the two forms of palm oil) bearing different superscript letters are significantly different ($p<0.05$).

that failed to be repositioned at the MAG and DAG molecules to form new (modified) TAG molecules. Free USAFA was then removed from EDIE PO during the purification process, resulting in the oil having lower USAFA and higher SAFA content than RBD PO.

TAG is a glycerol moiety consisting of three FA esterified to the glycerol backbone. Through IE, FA moieties are shuffled within (intraesterification) and among (IE) the TAG molecules until thermodynamic equilibrium is established (Marangoni and Rousseau, 1995). Therefore, IE of oils and fats, be it random, specific, non-directed or directed, results in the formation of new TAG species (Amir *et al*., 2012). The TAG structure, as well as the content of SAFA in the TAG itself, will indirectly determine the physical behaviour of the oils and fats, such as the crystal structure, hardness, and plasticity, which subsequently affect the properties of the final product made from the oils and fats (Szydłowska-Czerniak *et al*., 2005).

There was a substantial change in the TAG composition of PO following EDIE (*Figure 1* and *Table 1b*). The amount of $U_3$ and $S_3$ TAG increased remarkably at the expense of $S_2U$ and $U_2S$ TAG. The highest increase for $U_3$ TAG was in OOO (6.1-fold) and OLO (6.9-fold), while for $S_3$ TAG, PPP showed the highest increment (6.2-fold), where *O* is oleic, *L* is linoleic, and *P* is palmitic acids. The effect of EDIE on TAG composition of PO is in line with earlier findings on DIE of other oils and fats (Lago and Hartman, 1986; MacKenzie and Stevenson, 2000). The change in the TAG composition of PO as affected by EDIE was reflected in the physical characteristics of PO, *e.g.*, its visual appearance, thermal melting, crystallisation and polymorphic behaviour, and microscopic structure.

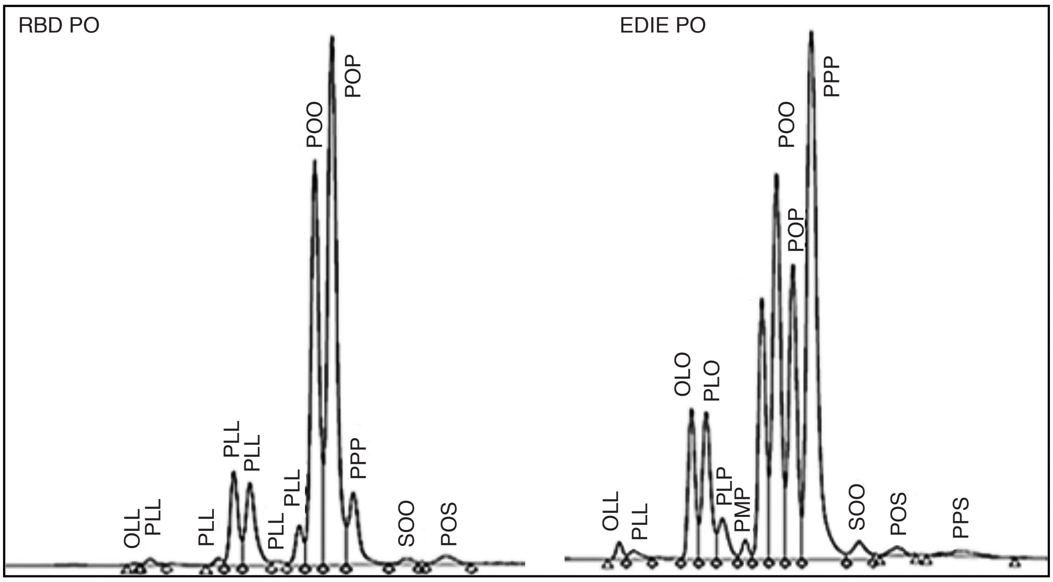

*Figure 1. Triacylglycerol chromatograms of enzymatic directed interesterified palm oil (EDIE PO) and refined, bleached and deodourised palm oil (RBD PO).*

## DSC Melting and Crystallisation Profiles

DSC is a widely used tool to verify any alteration to the thermal profile of oils and fats by monitoring the changes in melting and crystallisation enthalpies. When a sample is melted or crystallised, the amount of energy absorbed or released will be measured to provide quantitative and qualitative data on endothermic (heat absorption) and exothermic (heat evolution) processes. Changes in the TAG composition of PO as a result of EDIE is conjoined with alterations to its DSC thermal profiles. The DSC melting profile gives valuable information on the melting behaviour of fats. The $\Delta H_f$ and $\Delta H_{i^{\circ}C}$ can be used to monitor the physical interaction of fats in blends resulting from thermal changes. DSC $\Delta H_f$ of an oil or fat represents the amount of energy required to bring the substance from a solid state to a complete melt. Meanwhile, DSC $\Delta H_{i^{\circ}C}$ of an oil or fat is the energy required to bring the substance from a solid state to a partial melt at a specified temperature (Ali and Dimick, 1994). DSC melting thermograms of EDIE PO and RBD PO are shown in *Figure 2a*. Both thermograms showed two well-separated components, the low (olein) and high melting (stearin) components, which appear in the low and high temperature endothermic regions, respectively. There were also multiple endotherm peaks with shoulder peaks for both fractions of the fats. The multiple shouldered peaks in the DSC melting profiles of PO were due to its unique features of complex TAG distribution in PO (Tan and Che' Man, 2002). Melting of TAG complexes occurs simultaneously during heating and, therefore, results in broad or overlapping melting transitions. The highest melting TAG, *i.e.*, $S_3$, melted in the high temperature region, the lowest melting TAG ($U_3$) melted in the low temperature region while the intermediate melting TAG ($S_2U$ and $U_2S$) melted in between these two extreme regions. The second primary cause of the multiple endotherm peaks is the melting-recrystallisation of the original TAG crystallites and their subsequent melting, which is known as the polymorphism phenomenon or polymorphic transition (Frederick *et al.*, 2008).

The endothermic peak of the low melting component of RBD PO had initial and complete melting temperatures at -30°C and 12°C, respectively, while that of the high melting component were at about 15°C and 42°C, respectively. The two endothermic peaks of RBD PO are separated by an exothermic peak which appears in the temperature region of 12°C-15°C, parallel with the findings by Nassu and Gonçalves (1999). The exothermic valley between these two endotherms indicates that significant TAG complex recrystallisation and structural reorganisation (solid-to-solid transformation) occurred during the DSC scans. The endothermic peak in the low temperature region of RBD PO undoubtedly belonged to its low melting $U_3$ and $U_2S$ TAG, while the endothermic peak in the high temperature region belonged to the medium ($S_2U$) and high melting ($S_3$) TAG of PO. The broad and multiple endothermic peaks of RBD PO were due to its broad TAG distribution (Zhang *et al.*, 2013).

Owing to the altered TAG composition, EDIE PO had a melting curve which was different from that of RBD PO. EDIE PO had two distinct well-separated low and high temperature endothermic peaks, indicating ease of fractionation. The low and high melting components of EDIE PO showed a major shift in their initial and complete melting

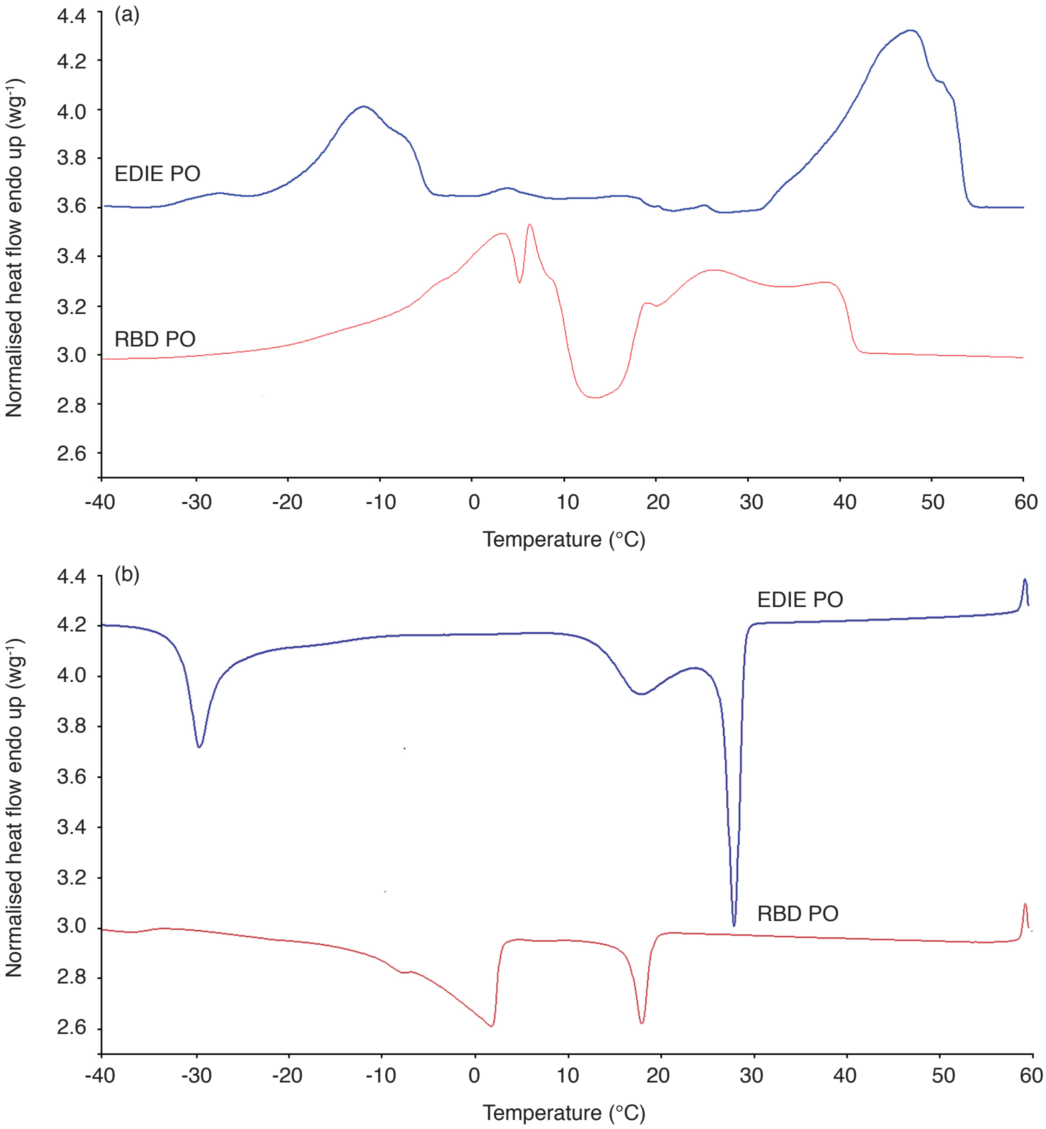

*Figure 2. Differential scanning calorimetry melting (a) and crystallisation (b) thermograms of enzymatic directed interesterified palm oil (EDIE PO) and refined, bleached and deodourised palm oil (RBD PO).*

temperatures. These melting temperatures of the low melting component shifted towards the lower temperature region, *i.e.*, to -35°C and -3°C, respectively, while those of the high melting component shifted to 30°C and 55.0°C, respectively. The transition in the initial and complete melting temperatures as affected by EDIE was due to the formation and accumulation of $U_3$ TAG in the low melting component and $S_3$ TAG in the high melting component, at the expense of $U_2S$ and $S_2U$ TAG. The high temperature endothermic peak of EDIE PO was broader, taller and sharper, with initial and complete melting temperatures greater than those of RBD PO, due to a greater amount of high melting $S_3$ TAG in EDIE PO than in RBD PO. EDIE PO also exhibited numerous small endothermic peaks in the temperature region of -3°C to 30°C, which most certainly belonged to the $U_2S$ and $S_2U$ TAG.

DSC $\Delta H_f$ and $\Delta H_{i°C}$, and partial liquid (%) of EDIE PO and RBD PO at various temperatures are presented in *Table 2*. Melting enthalpy of EDIE PO and RBD PO corresponded to the melting of the major TAG components present in the oils. Despite having a higher amount of low melting TAG than RBD PO, EDIE PO required significantly higher energy than RBD PO to melt its TAG components completely. That is because EDIE PO had a higher amount of $S_3$ TAG, which absorbed a large amount of energy to bring them to a complete melt. The higher $\Delta H_{i°C}$ values in the lower melting region below 0°C showed that the low melting TAG component of EDIE PO also absorbed more energy than that of RBD PO, as EDIE PO contained more $U_3$ TAG that melted in that temperature region. In the temperature region below 0°C, 35.3% of the TAG component of EDIE PO had already melted, compared to only 26.5% for RBD PO. There were not many changes in $\Delta H_{i°C}$ values for EDIE PO in the temperature region of 0°C to 30°C, unlike for RBD PO, due to the low amount of the TAG component

TABLE 2. DIFFERENTIAL SCANNING CALORIMETRY TOTAL ($\Delta H_f$) AND PARTIAL ($\Delta H_{i^{\circ}C}$) MELTING ENTHALPY, TOTAL (-$\Delta H_f$) AND PARTIAL (-$\Delta H_{i^{\circ}C}$) CRYSTALLISATION ENTHALPY, PARTIAL LIQUID (%) (calculated from crystallisation thermogram) AND PARTIAL SOLID (%) (calculated from melting thermogram) OF ENZYMATIC DIRECTED INTERESTERIFIED PALM OIL (EDIE PO) AND REFINED, BLEACHED AND DEODOURISED PALM OIL (RBD PO)

| **Temperature** | **Melting enthalpy (J g$^{-1}$)** | | **Crystallisation enthalpy (J g$^{-1}$)** | | **Partial liquid (%)** | | **Partial solid (%)** | |
|---|---|---|---|---|---|---|---|---|
| **(°C )** | **RBD PO** | **EDIE PO** | **RBD PO** | **EDIE PO** | | | | |
| | $\Delta H_f$ | | -$\Delta H_f$ | | | | | |
| | **65.8 ± 3.54$^{a}$** | **84.4 ± 4.30$^{b}$** | **-63.9 ± 3.33$^{a}$** | **-87.9 ± 3.02$^{b}$** | **RBD PO** | **EDIE PO** | **RBD PO** | **EDIE PO** |
| | $\Delta H_{i^{\circ}C}$ | | -$\Delta H_{i^{\circ}C}$ | | | | | |
| -40 | - | - | -63.4 ± 4.07$^{a}$ | -87.8 ± 2.54b | - | - | 99.2 ± 0.11$^{a}$ | 100.0 ± 0.00$^{a}$ |
| -35 | 0.0 ± 0.00$^{a}$ | 0.0 ± 0.00$^{a}$ | -62.6 ± 3.63$^{a}$ | -87.6 ± 3.09b | 0.0 ± 0.00$^{a}$ | 0.0 ± 0.00$^{a}$ | 97.9 ± 2.56$^{a}$ | 99.7 ± 2.12$^{a}$ |
| -30 | 0.0 ± 0.00$^{a}$ | 1.9 ± 0.86b | -62.3 ± 1.67$^{a}$ | -82.3 ± 1.88b | 0.0 ± 0.05$^{a}$ | 2.2 ± 0.40$^{a}$ | 97.4 ± 2.01$^{a}$ | 93.7 ± 1.91$^{b}$ |
| -25 | 0.2 ± 0.09$^{a}$ | 2.4 ± 0.18b | -61.3 ± 2.98$^{a}$ | -65.5 ± 2.00$^{a}$ | 0.3 ± 0.06$^{a}$ | 2.8 ± 1.20$^{a}$ | 95.9 ± 2.33$^{a}$ | 74.6 ± 2.02$^{b}$ |
| -20 | 0.6 ± 0.06$^{a}$ | 3.1 ± 1.04b | -59.1 ± 2.01$^{a}$ | -59.3 ± 3.45$^{a}$ | 0.9 ± 0.10$^{b}$ | 3.7 ± 0.73$^{a}$ | 92.5 ± 1.98$^{a}$ | 67.6 ± 3.52$^{b}$ |
| -15 | 2.0 ± 0.69$^{a}$ | 6.5 ± 1.70b | -55.9 ± 2.22$^{a}$ | -55.6 ± 1.86$^{a}$ | 3.1 ± 0.71$^{a}$ | 7.7 ± 2.32$^{b}$ | 87.5 ± 1.02$^{a}$ | 62.8 ± 2.90$^{b}$ |
| -10 | 4.7 ± 1.10$^{a}$ | 15.6 ± 2.33b | -50.8 ± 3.21$^{a}$ | -52.6 ± 2.22$^{a}$ | 7.1 ± 1.34$^{a}$ | 18.6 ± 1.69$^{b}$ | 79.4 ± 2.87$^{a}$ | 59.9 ± 1.49$^{b}$ |
| -5 | 9.2 ± 1.14$^{a}$ | 25.8 ± 1.62b | -41.4 ± 2.73$^{a}$ | -51.1 ± 2.76$^{b}$ | 14.0 ± 1.72$^{a}$ | 30.6 ± 2.13$^{b}$ | 64.8 ± 3.09$^{a}$ | 58.2 ± 2.54$^{b}$ |
| 0 | 17.4 ± 2.04$^{a}$ | 29.8 ± 2.12b | -26.8 ± 2.09$^{a}$ | -50.1 ± 1.99$^{b}$ | 26.5 ± 0.37$^{a}$ | 35.3 ± 1.76$^{b}$ | 41.9 ± 2.56$^{a}$ | 57.0 ± 3.03$^{b}$ |
| 5 | 29.9 ± 1.91$^{a}$ | 31.4 ± 1.88$^{a}$ | -13.5 ± 1.65$^{a}$ | -49.3 ± 3.22$^{b}$ | 42.7 ± 1.24$^{a}$ | 37.2 ± 1.72$^{a}$ | 21.2 ± 2.32$^{a}$ | 56.2 ±1.53$^{b}$ |
| 10 | 38.9 ± 1.62$^{a}$ | 34.1 ± 2.42b | -11.4 ± 1.12$^{a}$ | -48.9 ± 2.05$^{b}$ | 59.2 ± 1.63$^{a}$ | 40.4 ± 2.06$^{b}$ | 17.9 ± 1.06$^{a}$ | 55.8 ± 1.76$^{b}$ |
| 15 | 37.1 ± 1.49$^{a}$ | 35.6 ± 2.07$^{a}$ | -9.0 ± 1.03$^{a}$ | -46.7 ± 2.87$^{b}$ | 56.4 ± 2.57$^{a}$ | 42.1 ± 1.58$^{a}$ | 14.2 ± 1.13$^{a}$ | 53.2 ± 2.00$^{b}$ |
| 20 | 36.2 ± 2.31$^{a}$ | 35.9 ± 1.42$^{a}$ | -0.1 ± 0.07$^{a}$ | -34.7 ± 2.01$^{b}$ | 55.1 ± 1.50$^{a}$ | 42.6 ± 2.16$^{a}$ | 0.2 ± 0.03$^{a}$ | 35.9 ± 1.02$^{b}$ |
| 25 | 43.7 ± 3.92$^{a}$ | 35.8 ± 1.44$^{b}$ | 0.0 ± 0.0$^{a}$ | -25.2 ± 1.06$^{b}$ | 66.4 ± 4.00$^{a}$ | 42.4 ± 1.79$^{b}$ | 0.0 ± 0.00$^{a}$ | 28.7 ± 1.11$^{b}$ |
| 30 | 51.4 ± 2.12$^{a}$ | 36.6 ± 2.30$^{b}$ | 0.0 ± 0.0$^{a}$ | 0.0 ± 0.0$^{a}$ | 78.1 ± 1.75$^{a}$ | 43.4 ± 2.51$^{b}$ | 0.0 ± 0.00$^{a}$ | 1.8 ± 0.12$^{b}$ |
| 35 | 58.3 ± 1.81$^{a}$ | 41.8 ± 1.09$^{b}$ | - | - | 88.7 ± 3.46$^{a}$ | 49.5 ± 1.84$^{b}$ | 0.0 ± 0.00$^{a}$ | 0.0 ± 0.00$^{a}$ |
| 40 | 65.0 ± 2.39$^{a}$ | 52.6 ± 1.32$^{b}$ | - | - | 98.9 ± 0.11$^{a}$ | 62.3 ± 2.62$^{b}$ | - | - |
| 45 | 65.0 ± 2.39$^{a}$ | 66.2 ± 4.14$^{a}$ | - | - | 100.0 ± 0.00$^{a}$ | 78.5 ± 1.32$^{b}$ | - | - |
| 50 | 65.0 ± 2.39$^{a}$ | 81.4 ± 2.95$^{b}$ | - | - | 100.0 ± 0.00$^{a}$ | 96.3 ± 1.20$^{a}$ | - | - |
| 55 | 65.0 ± 2.39$^{a}$ | 84.4 ± 1.98$^{b}$ | - | - | - | - | - | - |

Note: Means within each row of the two forms of palm oil bearing different superscript letters are significantly different ($p<0.05$).

($U_2S$ and $S_2U$) that melted in that temperature region. RBD PO experienced a significant increase in $\Delta H_{i°C}$ values in the temperature region of 0°C to 10°C, most probably because of the melting of $U_2S$ type TAG. A decline in $\Delta H_{i°C}$ values was observed in the temperature region of 10°C to 20°C; most probably because of polymorphic changes that resulted in crystallisation of some of the TAG component that released heat during the heating process in that temperature region. Due to the heat release upon melting, the liquid TAG component in RBD PO also decreased from 59.3% at 10°C to 55.1% at 20°C. In the temperature region of 25°C to 40°C, the energy absorbed by EDIE PO was significantly lower than that absorbed by RBD PO. That is because RBD PO contains a higher amount of solid $S_2U$ TAG that melt in this temperature region than EDIE PO. In the temperature region above 40°C, the TAG component of EDIE PO absorbed significantly more energy than RBD PO, as EDIE PO contained a much higher amount of $S_3$ TAG. This observation is in line with the findings of Tan and Che' Man (2002), who demonstrated that more energy is needed to melt an oil that contains high amounts of long-chain saturated TAG.

Crystallisation properties of oils and fats, *e.g.*, crystal habit, crystal size and crystal number, indirectly influence the physical properties of the end-products. Crystallisation properties, in turn, largely depend on the chemical composition of a particular oil or fat, *e.g.,* the type of FA and the FA position on the TAG molecules (Zhang *et al.*, 2013). A DSC crystallisation curve of an oil or fat often has multiple exothermic peaks which are less complicated than its corresponding melting curve. The crystallisation curve can be subdivided into various exothermic regions, with each region associated with the various types of TAG. The higher melting TAG component will crystallise first; thus, the exothermic peak will appear in the high temperature region. Once the higher melting TAG component has completely crystallised, the lower melting TAG component will enter into the crystallisation process until all TAG components of the oil sample have crystallised, with the exothermic peak appearing in the low temperature region (Tan and Che' Man, 2002). *Figure 2b* illustrates the DSC crystallisation thermograms of EDIE PO and RBD PO. The thermogram of RBD PO exhibits two distinct exothermic peaks, *i.e.*, one sharp peak at the high (> 10°C) and one broad peak at the low (< 10°C) temperature regions, corresponding to the crystallisation of its major TAG components, the high and low melting TAG, respectively. This finding is in agreement with that of Busfield and Proschogo (1990). The high melting component of RBD PO which comprises mainly $S_2U$ and some $S_3$ TAG exhibited rapid crystallisation, as depicted by the sharp crystallisation peak with initial and complete crystallisation temperatures of 21°C and 12°C, respectively. The sharp and narrow exothermic peak indicates that crystallisation of the high melting TAG mixture proceeded interactively, and this is probably due to the presence of a highly homogeneous TAG component (Tan and Che' Man, 2002). The low melting TAG component, comprising mainly $U_2S$ and a small amount of $U_3$ TAG, crystallised over a wide temperature range, with initial and complete crystallisation temperatures of about -5.5°C and -34°C, respectively. The broad crystallisation peak is due to a wide TAG distribution (Liu *et al.*, 2010).

The thermogram of EDIE PO exhibits three distinct exothermic peaks, two at the high (> 10°C) and one at the low (< -10°C) temperature regions. The sharp exothermic peak of EDIE PO with an initial crystallisation temperature of 31°C and complete crystallisation temperature of 24°C is most probably due to the rapid crystallisation of the very high melting $S_3$ TAG which were present in high amounts in EDIE PO. A broader peak with initial and complete crystallisation temperatures of 24°C and 8.5°C may be due to the crystallisation of $S_2U$ and $U_2S$ TAG which also existed in quite large amounts. Another exothermic peak of EDIE PO, which is not present in the thermogram of RBD PO, shows crystallisation in the temperature region of -18°C to -38°C, corresponding to the crystallisation of the low melting $U_3$ TAG, which were present in substantial amounts in EDIE PO.

EDIE PO had significantly ($p<0.05$) higher crystallisation enthalpy (the energy to transform from a liquid state into a solid state) than RBD PO (*Table 2*), as EDIE PO had a higher content of $S_3$ TAG (particularly PPP) than RBD PO which had more $S_2U$ (particularly POP) and $U_2S$ (particularly POO) TAG. This finding is in agreement with that of Da Silva *et al.* (2017) who stated that the crystallisation enthalpy of PPP is high, *i.e.*, 66.99 J $g^{-1}$. The $-\Delta H_{i°C}$ values suggest that EDIE PO started to release energy to initiate crystallisation earlier than RBD PO. This can be explained by its high content of $S_3$ TAG. For instance, about -25.2 J $g^{-1}$ or 28.7% of crystallisation energy was released from EDIE PO when the crystallisation temperature dropped from 30°C to 25°C, corresponding to crystallisation of its $S_3$ TAG. RBD PO did not release any energy in the same temperature range as none of its TAG had yet to crystallise. Additionally, EDIE PO released about 40% more energy in the temperature range of -10°C to -40°C, which corresponded to crystallisation of its $U_3$ TAG. Most of the crystallisation energy of RBD PO (about 64.9% or -42.4 J $g^{-1}$) was released in the temperature range of 5°C to -15°C, which most certainly corresponded to crystallisation of its $S_2U$ and $U_2S$ TAG.

The DSC partial solid at various crystallisation temperatures for EDIE PO and RBD PO presented in *Table 2* show that EDIE PO crystallised much faster than RBD PO in the temperature region of 30°C to 20°C, due to crystallisation of its $S_3$ TAG. RBD PO showed rapid and almost complete crystallisation in the temperature region of 15°C to -20°C, indicating that most of its TAG ($S_2U$ and $U_2S$) crystallised in this region. There was not much of an increase in the percentage of solid for EDIE PO in this region as it contained a low amount of $S_2U$ and $U_2S$ TAG. Conversely, EDIE PO showed a rapid increase in the percentage of solid in the temperature region below -20°C, indicating that it contained a high amount of $U_3$ TAG. It has previously been established that $U_3$ TAG crystallise in a very low-temperature region (Bahadi *et al.*, 2016).

## SFC and SMP

SFC is an important index used to measure the amount of solid fats at a particular temperature. SFC is responsible for the end-product characteristics, such as physical appearance, ease of packing, spreadability, oil exudation and organoleptic properties of fat-based products (Krawczyk *et al.*, 1996). SFC also determines compatibility between fats by elucidating the alteration in percentage of solids at various fat proportions. The outcome is a graphical indication of the loss of solids as a result of dilution of existing solids with liquid oil, or of incompatibility due to eutectic interaction (Bigalli, 1988). Melting point, or in this study SMP of a fat, is the temperature at which a solid fat becomes liquid oil (Hidalgo and Zamora, 2006). SFC and melting point of a fat are affected by its FA chain length and composition, the ratio of saturation and unsaturation, the complexity of the TAG components and the FA position on the glycerol backbone (Rasor and Duncan, 2014).

An alteration in the TAG composition of PO following EDIE significantly increased its SFC, especially in the temperature region above 15°C (*Figure 3*), and SMP. SMP of EDIE PO and RBD PO was 52.2°C and 38.5°C, respectively. The higher SFC and SMP values of EDIE PO than those of RBD PO were due to the increase in high melting TAG content, particularly $S_3$ TAG (PPP and PPS), following EDIE, while RBD PO was high in medium ($S_2U$, mainly POP) and low melting ($U_2S$, mainly POO) TAG. The melting points of PPP and PPS TAG in their $\alpha$, $\beta'$ and $\beta$ polymorphic forms are at 45.0°C, 56.6°C and 66.1°C, and at 47.4°C, 50.9°C and 62.9°C, respectively. The melting points of POP and POO TAG in their $\alpha$, $\beta'$ and $\beta$ polymorphic forms are at 18.1°C, 30.5°C and 35.3°C, and at -4.0°C, 2.5°C and 19.2°C, respectively (Hidalgo and Zamora, 2006).

## Polymorphic Behaviour and Microstructure

Understanding the morphology of fat crystals is important for practical applications. The morphology of fat crystals determines the suitability of the fat for a given purpose. Many factors influence the polymorphic behaviour of a fat, most particularly the way in which the fat is cooled from the melt such as the cooling and agitation rate, as well as the FA and TAG composition of the fat (Martini *et al.*, 2002). The X-ray diffraction is the most widely used method for studying lipid polymorphism. Typical X-ray diffraction patterns of fats exhibit two

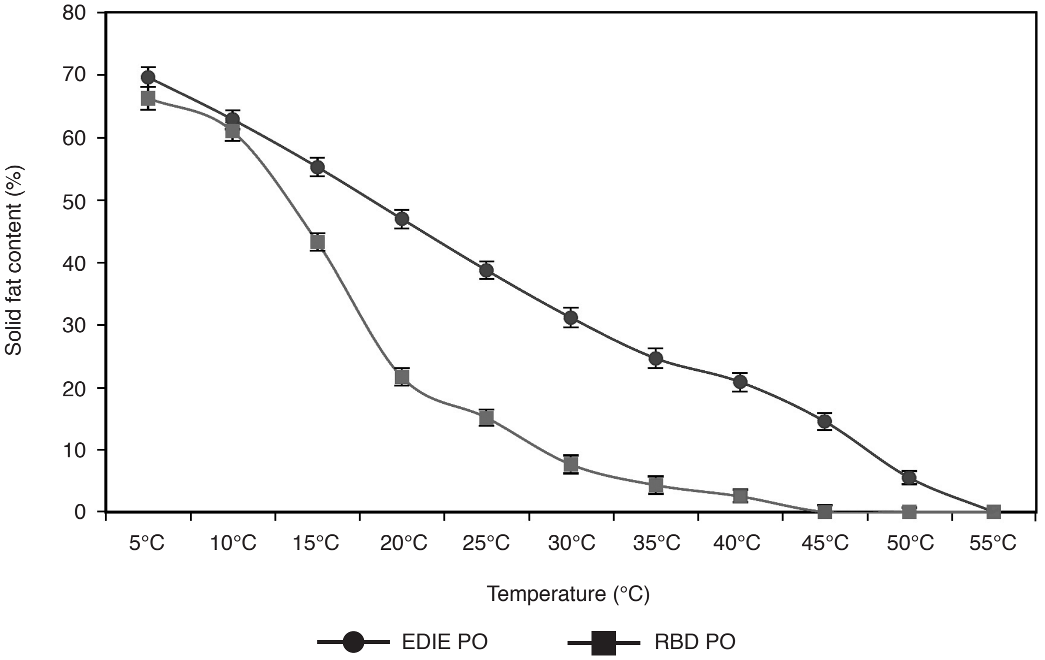

*Figure 3. Solid fat content profiles of enzymatic directed interesterified palm oil (EDIE PO) and refined, bleached and deodourised palm oil (RBD PO).*

groups of diffraction lines corresponding to long and short spacings. Short spacings are used mainly to characterise the various polymorphic forms of the fats, namely α, β and β′. Short spacings are attributed to the cross-sectional packing of the hydrocarbon chains (Zhang *et al*., 2013). The least stable α form, which has the lowest melting polymorph, will transform to the β′ form (which has intermediate stability and melting point) before being further changed into the β form, the most stable polymorph with the highest melting point (Chong *et al*., 2007).

The X-ray diffraction crystallography and the polymorphic crystal forms of EDIE PO and RBD PO at storage and determination temperatures of 5°C, 10°C, 15°C, 20°C and 25°C are shown in *Figure 4* and *Table 3*, respectively. The dominant polymorph of RBD PO stored at 5°C and 10°C was almost purely β′. According to Toro-Vazquez *et al*. (2000), RBD PO that is high in POP TAG tends to generate strong β′ polymorphs. However, as the storage temperature rose to 15°C, 20°C and 25°C, the relative content of β polymorphs in RBD PO increased, as shown by the emergence of a peak with spacing close to 4.6 Å. The higher the storage temperature, the stronger is the intensity of the peak at spacing 4.60 Å, because high temperatures instigate the transformation of

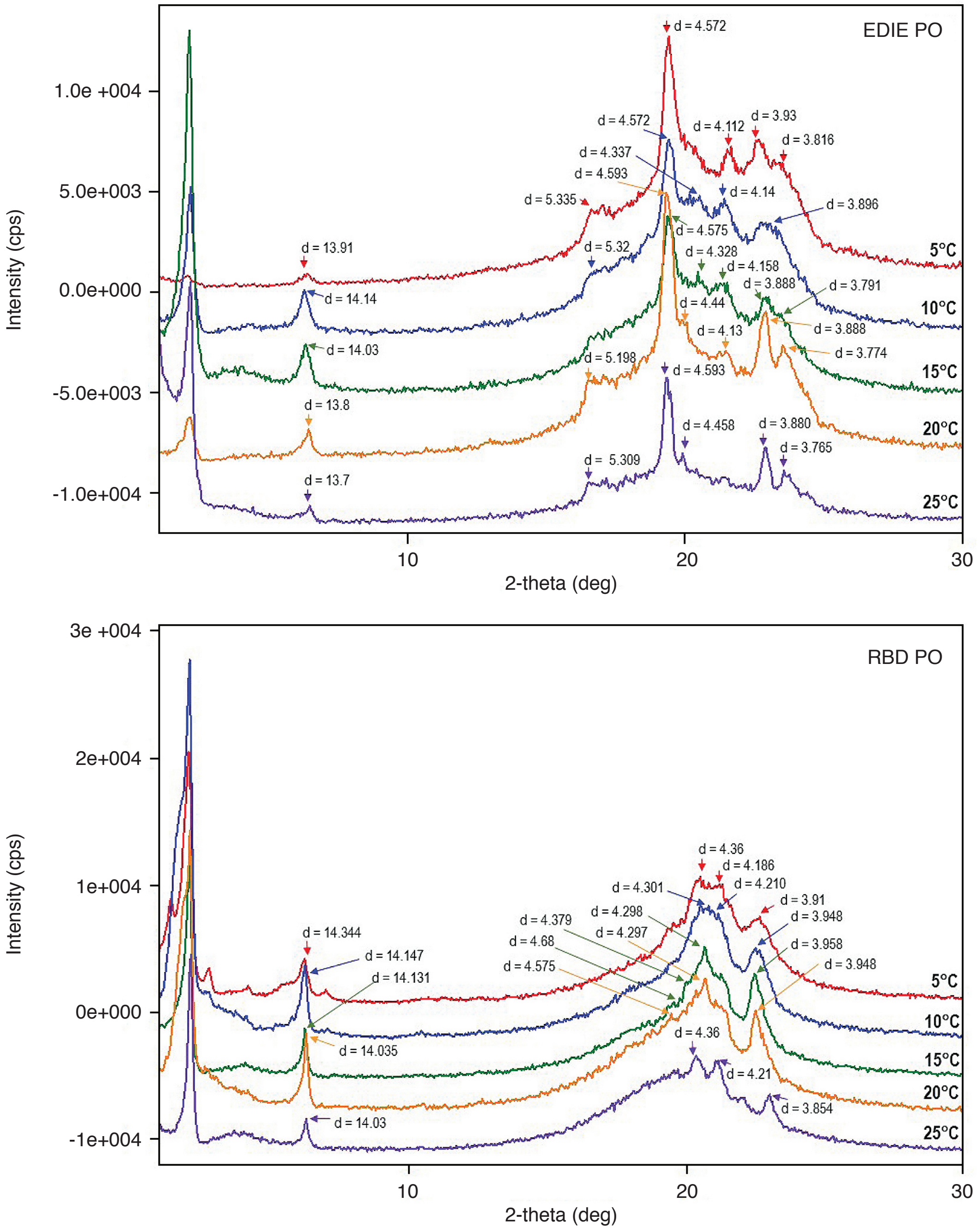

*Figure 4. The X-ray diffraction crystallography of enzymatic directed interesterified palm oil (EDIE PO) and refined, bleached and deodourised palm oil (RBD PO) at various storage and determination temperatures.*

TABLE 3. CRYSTAL POLYMORPHIC FORMS OF ENZYMATIC DIRECTED INTERESTERIFIED PALM OIL (EDIE PO) AND REFINED, BLEACHED AND DEODOURISED PALM OIL (RBD PO) AT VARIOUS STORAGE AND DETERMINATION TEMPERATURES

| Storage and determination temperature (°C) | Polymorphic form | |
|---|---|---|
| | EDIE PO | RBD PO |
| 5 | β >> β′ | β′ >>>> β |
| 10 | β >> β′ | β′>>>> β |
| 15 | β >> β′ | β′ >>> β |
| 20 | β >>>> β′ | β′ >> β |
| 25 | β >>>> β′ | β′ > β |

the POP polymorphs from the metastable β′ to the stable β form. As storage temperature increased, the degree of transformation of POP TAG from β′ to stable β also increased. This transformation from metastable β′ to stable β at high temperature may be because a higher storage temperature provides the energy required for the polymorphic transition as suggested by Shiota *et al*. (2011). The β′ form with small crystals of RBD PO is desirable for providing good texture and properties in fat-based products such as most margarines and shortenings (Zhu *et al*., 2017). EDIE PO stabilised in a mixture of β and β′ crystals with β polymorphs dominating at all storage temperatures, as shown by the strong intensity of peaks close to 4.6 Å for β crystals, and weaker intensity of peaks at 4.2 and 3.8 Å for β′. The intensity of the peak at 4.6 Å was remarkably high, especially at storage temperatures of 20°C and 25°C. This observation indicates that the relative content of β polymorphs increased following a rise in storage temperature and that the transformation rate from the unstable β′ into the stable β polymorphs was higher when the fat was kept at high storage temperature. Stabilisation of EDIE PO with β crystals dominating was to be expected as it is rich in $S_3$ TAG (PPP), which stabilised in the β form upon storage, in agreement with findings by Bhaggan *et al*. (2018) and Zhang *et al.* (2013). The EDIE PO which was β stable has a high melting point. According to ADM (2018), high melting fats are suitable to be used as coating agents for ingredients and additives, stabiliser or crystallisation agent for oil-rich products, *e.g*., peanut butter and spreads, texturisers for shortenings and liquid margarine, and plasticiser in confectionery.

The polymorphic behaviour of RBD PO and EDIE PO is harmonious with their crystal morphology (*Figure 5*). At low temperatures (5°C-15°C), RBD PO crystallised into very fine spherulite-shaped crystals, showing the dominance of β′ crystals. At higher stabilisation temperatures (20°C-25°C), small and loose plate-like crystals were observed with the disappearance of the spherulite-shaped β′ crystals, showing an increase in the amount of β crystals. On the other hand, at all stabilisation temperatures, from 5°C-25°C, EDIE PO crystallised into crystal aggregates with a dense, coarse plate-like and orderly packed structure, showing the occurrence of a high concentration of β crystals. These findings are coherent with that of Da Silva *et al.* (2017), Zhu *et al.* (2017), Zhang *et al.* (2014), Ribeiro *et al.* (2009) and Shin *et al.* (2009).

## CONCLUSION

EDIE can be considered as a mild and 'green' approach in oils and fats modification. Rearrangement of the FA distribution in the TAG molecules of PO, as a result of EDIE, caused a significant increase in $U_3$ and $S_3$ TAG at the expense of $S_2U$ and $U_2S$ TAG. This increase in $U_3$ and $S_3$ TAG, in turn, modified the physical characteristics of PO. The rise in $U_3$ TAG increased the proportion of the low melting fraction of PO as depicted by the larger endothermic and exothermic peaks in the very low temperature region, and the shift of endothermic and exothermic peaks towards the lower melting region, as reflected by the DSC melting and crystallisation curves. The elevation of $S_3$ TAG resulted in a higher proportion of the high melting fraction of PO, as reflected by the larger DSC endothermic peak in the high temperature region, the DSC endothermic peak shifting towards the high temperature region, the higher proportion of solid fat in the high temperature region, the higher SMP, and higher proportions of β polymorphs and coarser crystals. In all, EDIE can be considered as one of the methods of choice to increase $U_3$ and $S_3$ TAG in RBD PO. This process is essential especially when further fractionation is to be carried out to separate the olein fraction high in oleic acid from the stearin fraction high in palmitic acid for use as a high oleic oil and as a *trans*-free structural fat, respectively.

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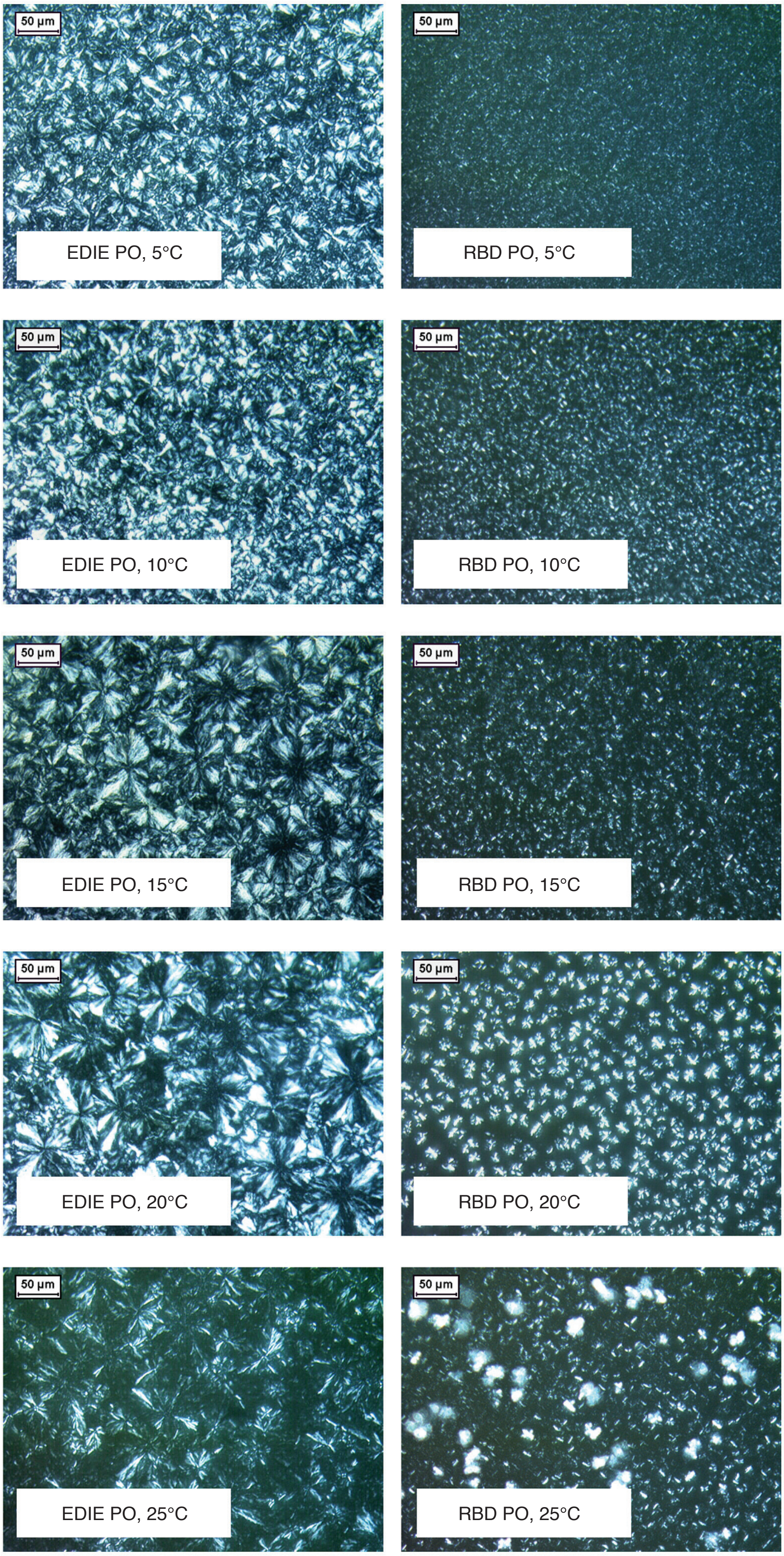

*Figure 5. Microstructural images (at 200X magnification) under a polarised light microscope of enzymatic directed interesterified palm oil (EDIE PO) and refined, bleached and deodourised palm oil (RBD PO) at stabilisation (24 hr) and various determination temperatures.*

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