*J. Agrobiotech.* **Vol 3**, 2012, p. 1–21.

ISSN 1985-5133 (Press)
ISSN 2180-1983 (Online)



# Enzymatic Synthesis of Structured Lipids through Interesterification of Palm Stearin (POs) IV 14, Cottonseed Oil (CSO) and Palm Olein (POo)

***Zarinah Zakaria[1,2], Nazaruddin Ramli[2] and Nor Aini Idris[3]***

[1]Faculty of Food Technology, Universiti Sultan Zainal Abidin, Gong Badak Campus, 21300 Kuala Terengganu, Terengganu Darul Iman, MALAYSIA.
[2] School of Chemical Sciences and Food Technology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, MALAYSIA.
[3]Malaysian Palm Oil Board, 43000 Kajang, Selangor Darul Ehsan, MALAYSIA.

***Corresponding author; E-mail: zarinah@unisza.edu.my**

## *ABSTRACT*

Structured lipids were produced from ternary blending of palm stearin (POs) with iodine value (IV=14), cottonseed oil (CSO) and palm olein (POo) through enzymatic interesterification (IE). Eighteen ternary blends of POs:CSO:POo that were produced from Mixture Procedure (D-Optimal) in Design-Expert® version 6 were enzymatically interesterified using TLIM lipase (10%, wt/wt substrate) for 24 hr at 55 ˚C. Blends without enzyme were used as comparison, while commercial vanaspati of Dalda brand was used as a control. Physico-chemical characteristics such as solid fat content (SFC), iodine value (IV), slip melting point (SMP) and melting/crystallization behavior of all blends and commercial samples were studied (Stage one). Blend samples with SFC similar to Dalda were chosen and their triacylglycerol (TAG), fatty acids compositions and peroxide value (PV) were determined. Results from the study showed that IV of blends without enzyme were in the range of 30.4 – 80.0, while IE blends were 33.5 – 74.4. The SMP of blends without enzyme were in the range of 50 – 59 ˚C and for IE blends, the value was decreased in the range of 44 – 53 ˚C. At stage two, the SFC profile for blends no. 3 (POs:CSO:POo; 20:23.33:56.67) and no. 15 (POs:CSO:POo; 20:10:70) with enzyme and without enzyme, had profiles quite similar to Dalda. SFC values for blend no. 3 with and without enzyme, at 20 ˚C were 23.53% and 17.99%, respectively. For blend no. 15, SFC with and without enzyme were 22.78% and 23.19%, respectively at 20 ˚C. Fatty acid composition for both blends showed no significant difference.

**Keywords:** Enzymatic synthesis, interesterification, cottonseed oil, Dalda (vanaspati)

## *ABSTRAK*

Lipid berstruktur dihasilkan dengan menggunakan adunan tigaan stearin sawit (POs) dengan nilai iodin (IV=14), minyak biji kapas (CSO) dan minyak olein sawit (POo) melalui proses interesterifikasi berenzim (IE). Sebanyak 18 adunan tigaan POs:CSO:POo yang terhasil dari Proses Pengadunan (D-Optimal) di dalam Design-Expert® versi 6 telah dilakukan interesterifikasi berenzim dengan menggunakan lipase TLIM (10%, b/b substrat) selama 24 jam pada suhu 55 °C. Adunan tanpa enzim telah digunakan sebagai perbandingan, manakala sampel vanaspati dagangan jenama Dalda telah digunakan sebagai kawalan. Kajian ciri-ciri fizik-kimia seperti kandungan lemak pepejal (SFC), nilai iodin (IV), takat lebur gelincir (SMP) dan kelakuan peleburan/penghabluran telah dilakukan ke atas kesemua adunan dan sampel dagangan (peringkat pertama). Sampel adunan yang mempunyai SFC menyamai Dalda dipilih dan ditentukan komposisi triasilgliserol (TAG), asid lemak dan nilai peroksida (PV). Hasil daripada kajian mendapati nilai IV untuk adunan tanpa enzim ialah antara 30.4 – 80.0 dan adunan IE ialah antara 33.5 – 74.4. Nilai SMP bagi adunan tanpa enzim ialah 50 – 59 °C dan nilai SMP bagi adunan IE menurun iaitu 44 – 53 °C. Pada peringkat kedua, profil KLP untuk adunan no. 3 (POs:CSO:POo; 20:23.33:56.67) dan no. 15 (POs:CSO:POo; 20:10:70) berenzim dan tanpa enzim, mempunyai profil yang hampir sama dengan Dalda. Nilai KLP bagi adunan no. 3 berenzim dan tanpa enzim, pada suhu 20 °C ialah 23.53% dan 17.99%, secara berturutan. Bagi adunan no. 15, nilai KLP berenzim dan tanpa enzim ialah 22.78% dan 23.19%, secara berturutan pada suhu 20 °C. Komposisi asid lemak bagi kedua-dua nisbah tidak menunjukkan perbezaan yang ketara.


**Kata kunci:** Sintesis berenzim, interesterifikasi, minyak biji kapas, Dalda (vanaspati)

## INTRODUCTION

Structured lipids (SLs) are defined as triacylglycerols (TGs) which are modified chemically or enzymatically to change the fatty acid (FA) composition and/or positional distribution in the glycerol backbone. The molecular structure of TGs influences their physical characteristics (*e.g.* melting points). Consequently, when designing SLs with particular chemical structure, it is possible to control the behavior of TGs, thereby improving the nutritional and pharmaceutical properties of TGs (Iwasaki & Yamane, 2000).

The mixing of oils and fats to produce blends with improved nutritional or physical properties has a long history. Most spreads contain blends of two or more oils in order to combine desirable nutritional properties with essential physical properties. Interesterification also is usually carried out on oil blends. Oils are also blended to obtain the desired mixture at minimum cost, and computer programs to give the best solution have been developed (Block *et al.*, 1997).

The interesterification process alters the distribution of the fatty acids in the triacylglycerols producing products with melting and crystallization characteristics different from the original oil or fat. Unlike hydrogenation, interesterification neither affects the degree of saturation nor causes isomerization of the fatty acid double bond. It does not change the fatty acid composition of the starting material but rearranges the fatty acids on the glycerol molecule (Gunstone, 2002).

Although chemical interesterification catalyzed by metal alkoxides is simple and inexpensive, it is not capable of modifying specific positions due to the random nature of the reactions. In contrast, the reactions catalyzed by *sn*-1,3-specific lipases are more promising for positionally specific modification of lipids (Iwasaki & Yamane, 2000).

The objectives of this study were to synthesize structured lipid from ternary blending of palm stearin (POs), cottonseed oil (CSO) and palm olein (POo), and to determine the changes in physicochemical characteristics of blends at 18 different ratio before and after enzymatic interesterification and Dalda such as solid fat content (SFC), iodine value, slip melting point (SMP), melting/crystallization behavior, triaclyglycerides (TAG) and fatty acids composition.

## MATERIALS AND METHODS

### Materials

Cottonseed oil (CSO) and commercial vanaspati of Dalda brand were obtained from the Malaysian Palm Oil Board (MPOB), Bangi, Selangor, Malaysia. Palm stearin (POs, IV=14) was obtained from Intercontinental Specialty Fats, Klang, Selangor, Malaysia, and Palm olein (POo) was bought from a retail shop.

### Direct Blending

The mixtures of 10 g of POs:CSO:POo were prepared at different ratios by using mixing procedure (D-Optimal) Design-Expert version 6. POs was first melted down at 80 °C in an oven for 1 h and then blended in predetermined ratios. Blends were homogenized using a magnetic stirrer (Nazaruddin *et al.*, 2005).

### Enzymatic Interesterification Reaction

The reaction mixtures composed of 10 g of POs, CSO and POo in a different ratio were dissolved in 10 mL hexane and placed in a 50 mL conical flask. 10% wt/wt substrate of immobilized *Thermomyces lanuginose* lipase (TL IM) was added to the oil samples. The reaction mixture was then agitated in a water bath shaker at 200 rpm and 55 °C. The sample flasks were removed after 24 hr. Lipase was removed from the mixture by simple filtration by using Whatman filter paper. Hexane was removed by using the rotary evaporator (Chen *et al.*, 2007).

## Solid Fat Content (SFC)

Solid fat content was determined by using a Bruker Minispec pulsed nuclear magnetic resonance (pNMR) analyzer Model 120 (Rheinstetten, Germany). The non-stabilized serial procedure of PORIM Test Methods (1995) was followed, whereby samples inside the pNMR tube were melted at 70 ˚C for 30 min, then cooled to 0 ˚C for 90 min, and subsequently held at the desired temperature for 30 min prior to the measurement. Predetermined temperatures for measurements were 0, 5, 10, 15, 20, 25, 30, 35 and 37.5 ˚C.

## Iodine Value (IV)

Iodine value was determined according to the PORIM test method (1993).

## Slip Melting Point (SMP)

Slip Melting Point (SMP) was determined according to the PORIM test method (1995).

## Melting/Crystallization Behavior

The instrument used was a Perkin-Elmer (Norwalk, CT) DSC model-4. Samples of about 6-12 mg were weighed in aluminium pans, and an empty pan was used as a reference. The samples were cooled at -60 ˚C, and then held for 5 min before melting to 90 ˚C at the rate of 5 ˚C/min and held at this temperature for 5 min for their cooling thermograms.

## Triaclyglicerol (TAG) Composition

The TAG composition was analyzed by non-aqueous reverse-phase HPLC. A commercially packed RP-18 column (240 mm × 4 mm) with 5 μ particle size (E. Merck, Darmstadt, Germany) was used to separate the TAG. TAG was eluted from the column using acetone/acetonitrile (63.5:36.5) mixture at a flow rate of 1 mL/min and detected with a Evaporate Fire Light Scattering Detector. The injected volume was 10 μL.

## Fatty Acid Composition

Fatty acid compositions were determined as fatty acid methyl esters (FAME). The oil (0.05 g) was weighed and dissolved in 1 mL of hexane, in a 2 mL screw-capped vial. Sodium methoxide solution (0.2 mL) was added and then mixed for 1 min by using a vortex mixer. After sedimentation of sodium glycerolate, 1 μL of the clear supernatant was injected into a SGE-BPX70 polar silica column (60 × 0.32 mm) and analyzed by using a Shidmazu-17A (Kyoto, Japan) Gas Chromatograph, equipped with a flame ionization detector (FID) and a C-R6A Chromatopac integrator. The oven temperature was programmed in two stages as follows: first from 50 ˚C to 180 ˚C (8 ˚C/min), and then from 180 ˚C to 200 ˚C (5 ˚C/min). The carrier gas (helium) flow rate was 6.8 mL/min. Correction responses factors were

determined by analysis of a RM-5 standard mixture of FAME (Supelco, Cat. No. 4-7024, Tokyo, Japan).

## Experimental Design

Two sets of blends were prepared in different ratios, with one set subjected to interesterification treatment. Both sets of samples were analyzed for their physico-chemical properties such as solid fat content (SFC), slip melting point (SMP), melting/crystallization characteristic, triaclyglycerides (TAG) and fatty acids composition. Ratios of blends were determined by using the Mixture (D-optimal) procedure, Design-Expert® version 6.0 software. A set of 14 different ratios have been determined with an addition of four ratios performed, to verify the accuracy of the experiment (Table 1).

Table 1. Sample codes at different ratios of blends.

| Sample no. | Palm stearin (POs) | Cottonseed oil (CSO) | Palm olein (POo) |
|---|---|---|---|
| 1 | 30.00 | 40.00 | 30.00 |
| 2 | 40.00 | 20.00 | 40.00 |
| 3 | 20.00 | 23.33 | 56.67 |
| 4 | 20.00 | 10.00 | 70.00 |
| 5 | 20.00 | 50.00 | 30.00 |
| 6 | 30.00 | 50.00 | 20.00 |
| 7 | 56.67 | 23.33 | 20.00 |
| 8 | 53.33 | 10.00 | 36.67 |
| 9 | 36.67 | 10.00 | 53.33 |
| 10 | 70.00 | 10.00 | 20.00 |
| 11 | 43.33 | 36.67 | 20.00 |
| 12 | 20.00 | 36.67 | 43.33 |
| 13 | 35.00 | 30.00 | 35.00 |
| 14 | 45.00 | 10.00 | 45.00 |
| 15 | 20.00 | 10.00 | 70.00 |
| 16 | 70.00 | 10.00 | 20.00 |
| 17 | 20.00 | 50.00 | 30.00 |
| 18 | 30.00 | 50.00 | 20.00 |

## Statistical Analysis

The results for total SMP & IV were subjected to ANOVA and Duncan analyses by using the Statistical Analysis System (SAS Institute Inc., Cary, NC). These analyses were carried out to determine whether or not the samples show any significant difference for different treatments at a level of $P < 0$.

## RESULTS AND DISCUSSION

### Stage 1

### Solid fat content (SFC)

Solid fat content, defined as the amount of fat crystals in the blends, is responsible for many product characteristics including general appearance, ease of packing, organoleptic properties, ease of spreading, and oil exudation. The SFC between 4 °C and 10 °C determines the ease of spreading of the product at refrigeration temperature. An SFC of not greater than 32% at 10 °C is essential for good spreadability at refrigeration temperature. The SFC at 20 °C and 22 °C determines the product's stability and resistance to oil exudation at room temperature; a value of not less than 10% is essential to prevent oiling off. The SFC between 35 °C and 37 °C determines the thickness and flavor release properties of reduced fat spread (RFS) in the mouth (Charteris & Keogh, 1991; Krawczyk *et al.,* 1996). The SFC profiles, a function of temperature for the POs:CSO:POo blends in different ratios before and after enzymatic interesterification (IE) are shown in Figures 1 and 2, respectively. The percentage of SFC decreased when temperature increased. Differences of %SFC at similar temperature were because of the different ratio of blends. Non-IE blends had a higher %SFC when compared to blends after IE. For example, %SFC blends no. 1 at temperature of 0 °C to 37.5 °C before IE was 44.13 – 19.69% and after IE, the value decreased slowly. After IE process, the value was in the range of 37.06 – 8.98%. This indicated the exit of more liquid fraction at these temperature ranges by an increase of unsaturated fatty acid at new TAG. This was also because of IE reaction that replaced the saturated fatty acids with unsaturated fatty acids.

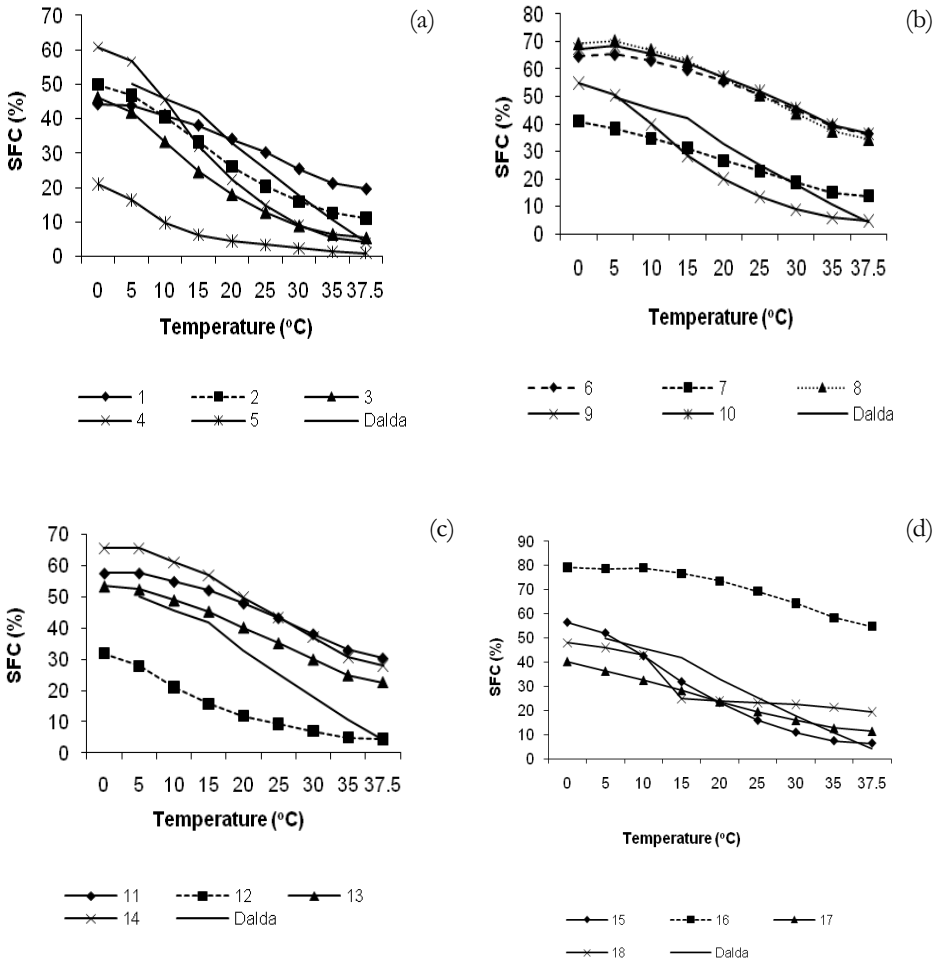


Fig. 1. Percentage of solid fat content for POs:CSO:POo in different ratios before enzymatic interesterification (IE).

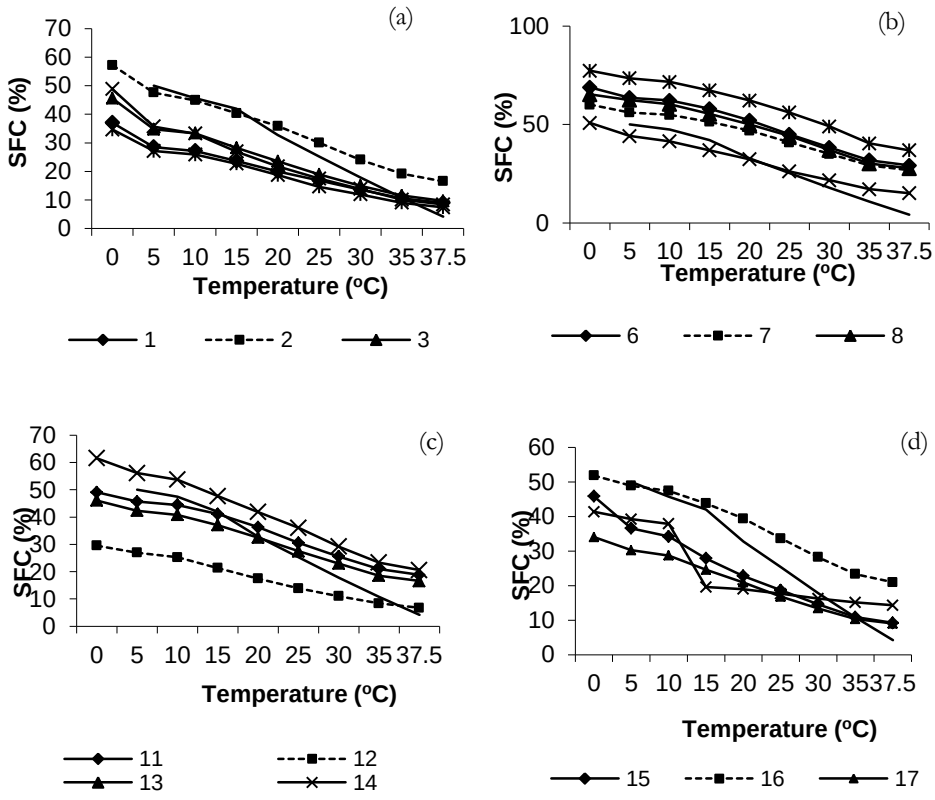


Fig. 2. Percentage of solid fat content for POs:CSO:POo in different ratios after enzymatic interesterification (IE).

## Iodine value (IV)

Figure 3 shows the iodine value blends of POs:CSO:POo before and after IE, and also the iodine value of Dalda. The iodine value is used to measure unsaturated fatty acid in oil or fat. High IV oil contains a greater number of double bonds than low IV oil. Range of iodine value for blends before IE was 30.39 – 80.05 while for IE blends; the iodine value was in the range of 33.51 – 74.43.

With a higher amount of palm olein in the blend, the iodine value could be higher. This is because palm olein consists of fatty acid unsaturated TAG such as POO, OOO, OLO, and OLL (Gunstone, 2004). From the analysis, this is true for blends before IE; blend no. 4 which consists of 70% of palm olein and blend no. 16 with 20% of palm olein both showed the iodine values at 58.63 and 30.39, respectively.

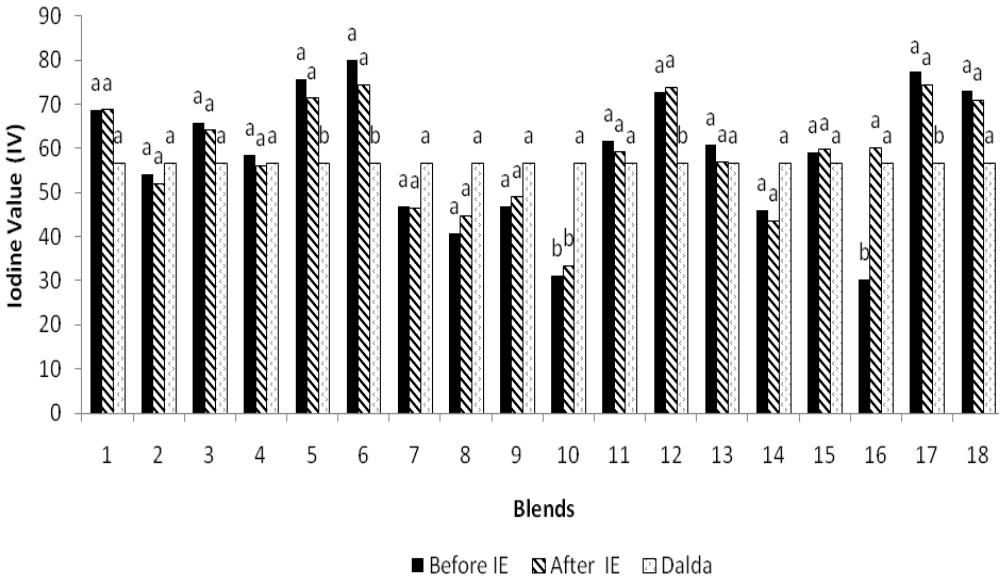


Fig. 3. Iodine value, IV, for POs:CSO:POo in different ratio before IE, after IE and Dalda sample.
Note: *Same alphabet showed no significant difference ($P > 0.05$) between non-IE and IE blends, and Dalda.

## Slip melting point (SMP)

Slip melting point is the temperature at which a fat in a capillary tube placed in water becomes soft enough to slip or rise up the tube. The fat will slip in the capillary tube where approximately 5% solid fat is present (Timms, 1985). Based on Figure 4, SMP of blends of POs:CSO:POo before IE, after IE and Dalda sample showed a significant difference ($P < 0.05$). During SMP measurements, the sample temperature is raised and the solid fat melts. The progressive reduction of crystalline matter means that, at a certain temperature, the fat crystal network lacks sufficient cohesion to hold onto its matrix and become sufficiently soft until it suddenly rises. Range of SMP values of blends before IE were 50 – 59 ˚C while after IE, the SMP values were in the range of 44 – 53 ˚C. The SMP value for Dalda was 40 ˚C. The decrease in SMP after interesterification by lipase TL IM indicated that the increase of TAG with a low melting point after fatty acid composition had been modified during this process.

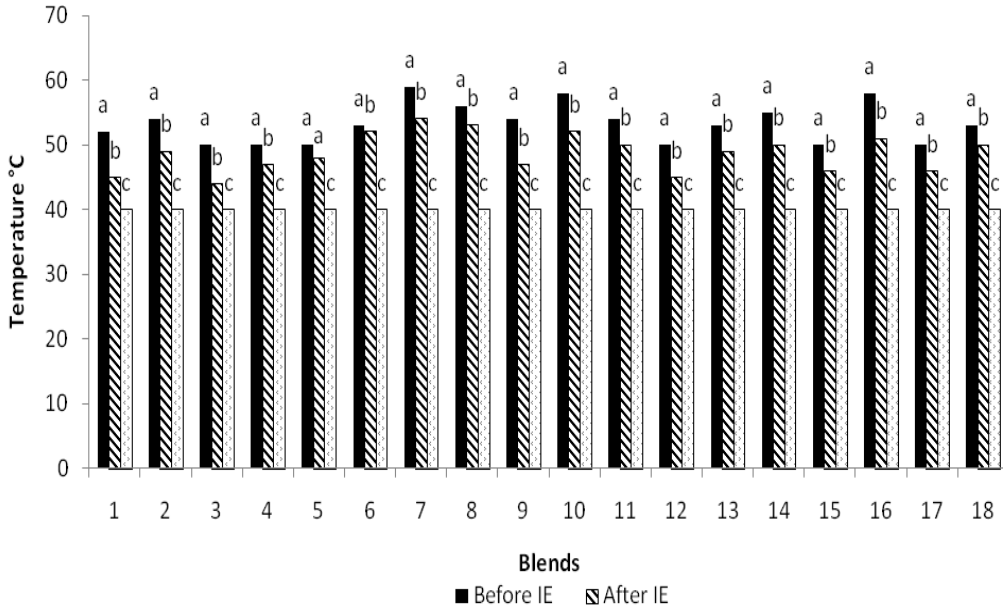


Fig. 4. Slip melting point, SMP for POs:CSO:POo in different ratios before IE, after IE and Dalda sample.
Note: *Same alphabet showed no significant difference ($P > 0.05$) between non-IE, IE blends and Dalda.

## Melting/crystallization behavior

Table 2 shows the melting and crystallization behavior for POS, CSO, POo and Dalda. POs started melting at 38.57 ˚C and completed the melting process at 62.37 ˚C. The melting process of POs produced two peaks at temperatures of 47.04 ˚C and 60.05 ˚C. Transition enthalpy and fusion enthalpy for POs were 134.14 (J/g) and 162.01 (J/g). Transition enthalpy and fusion enthalpy for CSO were 28.98 (J/g) and 114.95. CSO melted at -45.78 ˚C and completed at 0.32 ˚C. This melting value was different with the melting point of palm stearin (POs) because cottonseed oil was in a liquid form at room temperature while palm stearin was in a solid form at room temperature, and needs high temperature to melt it. Based on one analysis, CSO consists of TAG types PLL (26%), LLL (16%), POL (14%), LLO (13%) and PLP (9%) (Gunstone, 2004).

Palm olein (POo) starts melting at -0.28 ˚C with a peak at 5.36 ˚C and completes melting at 8.84 ˚C with fusion enthalpy of 72.48 (J/g). Palm olein was in a liquid form at room temperature and consisted of triunsaturated glycerides TAG such as OOO, OLO and OLL (Gunstone, 2004). Exotherm of palm olein produced two peaks, at 1.30 ˚C and -24.96 ˚C. Palm olein started to crystallize at 2.62 ˚C and completed at -40.18 ˚C with fusion enthalpy 121.67 (J/g). Dalda started

melting at -8.14 ˚C and completed the melting process at 42.29 ˚C. The melting process of Dalda produced two peaks at 11.61 ˚C and 38.02 ˚C. Transition enthalpy and fusion enthalpy for Dalda were 55.44 (J/g) and 81.21 (J/g). The crystallization process of Dalda produced one peak that is at 21.86 ˚C. Dalda was crystallized at 22.98 ˚C and completed the crystallization process at -19.41 ˚C.

Table 2. Transition point temperature and fusion enthalpy for POs, CSO, POo and Dalda.

| Sample | Melting behavior | | | | | Crystallization behavior | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Melting enthalpy (J/g) | Melting peak temperature (°C) | | | | Crystallization enthalpy (J/g) | Crystallization temperature peak (°C) | | | |
| | | Onset | 1 | 2 | Endset | | Onset | 1 | 2 | Endset |
| Palm stearin (Pos) | 134.14 | 38.57 | 47.04 | 60.05 | 62.37 | 163.01 | 37.93 | 36.77 | 3.06 | -10.51 |
| Cottonsee oil (CSO) | 28.98 | -45.78 | -32.37 | -6.40 | 0.32 | 114.95 | -4.30 | -11.44 | - | -49.95 |
| Palm Olein (POo) | 72.48 | -0.28 | 5.36 | - | 8.84 | 121.67 | 2.62 | 1.30 | -24.96 | -40.18 |
| Dalda | 55.44 | -8.14 | 11.61 | 38.02 | 42.29 | 81.21 | 22.98 | 21.86 | - | 19.41 |

Tables 3 and 4 present the more representative transition point and fusion enthalpy of 18 blends of POs:CSO:POo with different ratios for before and after IE process. Different ratios of oil and fat in blends gave different values of transition point and fusion enthalpy. Table 3 shows that range blends started melting at -26.15 ˚C to -9.30 ˚C for samples before IE. Endotherm for all blends produced three peaks, at temperatures of -18.38 ˚C to -3.78 ˚C, -2.4 ˚C to 58.89 ˚C and 52.40 ˚C to 58.96 ˚C. Blends that had more than one endothermic peak indicated that each oil and fat sample had a different transition point. All blends completed melting at temperatures of 52.40 ˚C to 58.96 ˚C. Exotherm of all blends produced two peaks that ranged at temperatures of 26.32 ˚C to 36.24 ˚C and completed at -17.96 ˚C to -40.60 ˚C. After IE process (Table 4), fat crystals of blends melt at low temperature compared with blends before IE. All blends after IE started melting at temperatures of -9.88 ˚C to -1.73 ˚C and completed melting at temperatures of 44.88 ˚C to 57.49 ˚C. After the IE process, blends melted at lower temperatures thus indicating an increase of low melting point glycerides. The melting process of IE blends produced four peaks from temperatures of -10.22 ˚C to 5.43 ˚C, 0.72 ˚C to 54.13 ˚C, 8.78 ˚C to 55.43 ˚C and 47.05 ˚C to 53 ˚C. All blends started to crystallize at the temperature range of 33.41 ˚C to 23.50 ˚C and completed at -5.34 ˚C to 44.62 ˚C, and produced two peaks from temperatures of 32.51 ˚C to 21.84 ˚C and 4.24 ˚C to -23.06 ˚C.

Table 3. Transition point temperature and fusion enthalpy for blends of POs, CSO, POo with different ratios before IE.

| Sample[a] | Melting behavior | | | | | | Crystallization behavior | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Melting enthalpy (J/g) | Melting peak temperature (°C) | | | | | Crystallization enthalpy (J/g) | Crystallization temperature peak (°C) | | | |
| | | Onset | 1 | 2 | 3 | Endset | | Onset | 1 | 2 | Endset |
| 1 | 44.81 | -26.15 | -5.74 | 50.55 | - | 53.59 | 43.59 | 27.46 | 26.01 | -1.42 | -21.98 |
| 2 | 54.76 | -24.60 | -6.40 | 52.17 | - | 54.98 | 52.72 | 28.85 | 27.76 | -0.74 | -20.34 |
| 3 | 53.66 | -14.38 | -7.04 | 7.62 | 52.40 | 55.45 | 46.41 | 29.15 | 27.50 | -0.43 | -17.95 |
| 4 | 39.55 | -14.31 | -1.74 | 47.23 | - | 52.29 | 39.50 | 26.32 | 24.84 | 0.59 | -18.21 |
| 5 | 44.26 | -22.03 | -8.37 | 51.05 | - | 53.77 | 42.56 | 27.67 | 25.66 | -2.93 | -30.38 |
| 6 | 111.77 | -22.81 | -6.88 | 57.48 | - | 59.83 | 103.02 | 35.15 | 33.60 | -1.60 | -35.13 |
| 7 | 56.65 | -26.10 | -17.89 | 53.49 | - | 55.77 | 56.44 | 29.85 | 28.58 | -4.93 | -40.60 |
| 8 | 99.60 | -16.26 | -11.55 | 5.26 | 56.52 | 58.65 | 93.03 | 33.28 | 32.09 | -0.42 | -23.17 |
| 9 | 79.31 | -14.39 | -10.72 | 5.76 | 54.48 | 56.94 | 66.04 | 31.08 | 30.17 | 0.24 | -18.09 |
| 10 | 131.10 | -9.30 | 2.62 | 58.89 | - | 61.15 | 123.75 | 36.24 | 35.46 | -0.44 | -25.09 |
| 11 | 79.84 | -23.56 | -20.05 | -6.05 | 55.45 | 57.68 | 73.43 | 32.21 | 31.35 | -4.77 | -32.63 |
| 12 | 44.70 | -21.97 | -10.39 | 51.23 | - | 53.95 | 40.02 | 26.70 | 25.34 | -2.10 | -22.05 |
| 13 | 70.46 | -20.85 | -15.73 | -2.40 | 53.98 | 56.38 | 63.83 | 30.65 | 29.45 | -2.26 | -34.16 |
| 14 | 97.45 | -6.99 | 3.78 | 56.76 | - | 59.27 | 91.33 | 34.37 | 32.99 | -0.44 | -35.64 |
| 15 | 42.82 | -17.48 | -3.07 | 49.72 | - | 53.08 | 41.79 | 27.02 | 25.51 | -3.10 | -18.91 |
| 16 | 120.80 | -25.48 | -3.71 | 13.96 | 58.96 | 61.32 | 112.92 | 36.04 | 34.45 | -1.45 | -31.97 |
| 17 | 38.77 | -24.74 | -17.23 | 50.39 | - | 53.04 | 35.84 | 26.40 | 24.83 | -4.59 | -34.96 |
| 18 | 55.26 | -25.88 | -18.38 | 53.52 | - | 55.81 | 54.26 | 29.84 | 28.57 | -4.94 | -40.38 |

[a] Sample blending of Palm stearin(POs):Cottonseed oil (CSO):Palm olein (POo).

Table 4. Transition point temperature and fusion enthalpy for blends of POs, CSO, POo with different ratios after IE.

| Sample[a] | Melting behavior | | | | | | | Crystallization behavior | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Melting enthalpy (J/g) | Melting peak temperature (°C) | | | | | | Crystallization enthalpy (J/g) | Crystallization peak temperature (°C) | | | |
| | | Onset | 1 | 2 | 3 | 4 | Endset | | Onset | 1 | 2 | Endset |
| 1 | 37.46 | -21.29 | -6.90 | 0.72 | 8.78 | 47.05 | 50.08 | 50.98 | 23.50 | 21.84 | -23.06 | -24.52 |
| 2 | 40.24 | -17.90 | 0.92 | 11.94 | 42.85 | 52.18 | 54.52 | 74.19 | 30.48 | 29.30 | 2.76 | -20.18 |
| 3 | 40.20 | -11.40 | -1.91 | 48.22 | - | - | 50.91 | 44.10 | 25.38 | 23.82 | -0.76 | -35.92 |
| 4 | 38.44 | -7.51 | 2.41 | 12.11 | 40.23 | - | 44.88 | 45.77 | 25.88 | 24.71 | 1.93 | -20.95 |
| 5 | 39.52 | -29.30 | -3.23 | 11.78 | 49.55 | - | 51.93 | 44.12 | 25.42 | 24.19 | -1.09 | -1.96 |
| 6 | 98.39 | -1.73 | 5.43 | 51.10 | - | - | 53.56 | 108.38 | 29.59 | 28.35 | -4.76 | -5.34 |
| 7 | 98.29 | -29.88 | -8.21 | 5.62 | 55.43 | - | 57.49 | 98.47 | 32.66 | 31.92 | 1.90 | -20.74 |
| 8 | 86.49 | -21.38 | -3.89 | 52.14 | - | - | 54.54 | 89.73 | 30.43 | 29.34 | 1.24 | -22.52 |
| 9 | 30.88 | -15.25 | -0.41 | 11.27 | 42.37 | 53.00 | 55.10 | 70.58 | 29.74 | 28.65 | 1.92 | -18.82 |
| 10 | 111.29 | -5.31 | -2.71 | 54.13 | - | - | 56.80 | 111.64 | 33.41 | 32.51 | 4.24 | -11.91 |
| 11 | 76.88 | -28.29 | -5.39 | 12.45 | 53.64 | - | 55.71 | 76.02 | 30.82 | 29.83 | 1.07 | -24.92 |
| 12 | 30.04 | -13.40 | -2.58 | 9.94 | 47.39 | - | 49.93 | 37.04 | 24.82 | 22.29 | -4.09 | -40.75 |
| 13 | 66.65 | -28.38 | -2.40 | 12.78 | 52.99 | - | 55.25 | 69.85 | 29.39 | 28.47 | 0.41 | -29.72 |
| 14 | 54.11 | -15.70 | 1.94 | 12.61 | 44.16 | 52.53 | 55.11 | 78.52 | 32.05 | 30.44 | 4.08 | -14.76 |
| 15 | 38.93 | -25.06 | -3.80 | 47.39 | - | - | 50.28 | 46.19 | 24.28 | 23.20 | -0.41 | -33.90 |
| 16 | 67.69 | -26.50 | -10.22 | 7.12 | 51.51 | - | 53.79 | 77.25 | 28.92 | 27.94 | -4.27 | -5.41 |
| 17 | 33.96 | -19.25 | -5.07 | 9.44 | 47.89 | - | 50.32 | 42.39 | 24.04 | 22.83 | -3.76 | -44.62 |
| 18 | 48.88 | -23.30 | -7.73 | 8.11 | 50.19 | - | 52.80 | 57.77 | 26.43 | 25.20 | -4.27 | -41.62 |

[a] Sample blending of Palm stearin(POs):Cottonseed oil (CSO):Palm olein (POo)

Figure 5 shows the melting enthalpy (J/g) for blends of POs:CSO:POo in different ratios; before and after IE. The results showed that melting enthalpy for blends before IE was higher than melting enthalpy of blends after IE, except for blend no. 7. Figure 6 shows crystallization enthalpy for blends of POs, CSO and POo with different ratios before and after IE. Crystallization enthalpy for blends before IE was in the range of 35.84 (J/g) to 123.75 (J/g) while after IE, the value was 37.04 (J/g) to 111.64 (J/g). After IE, blends no. 1, 2, 4, 5, 6, 7, 9, 11, 13, 15, 17 and 18 showed a high value of crystallization enthalpy if compared to before IE process. It might be due to the addition of unsaturated fatty acid and short chain fatty acids in the new TAG after IE.

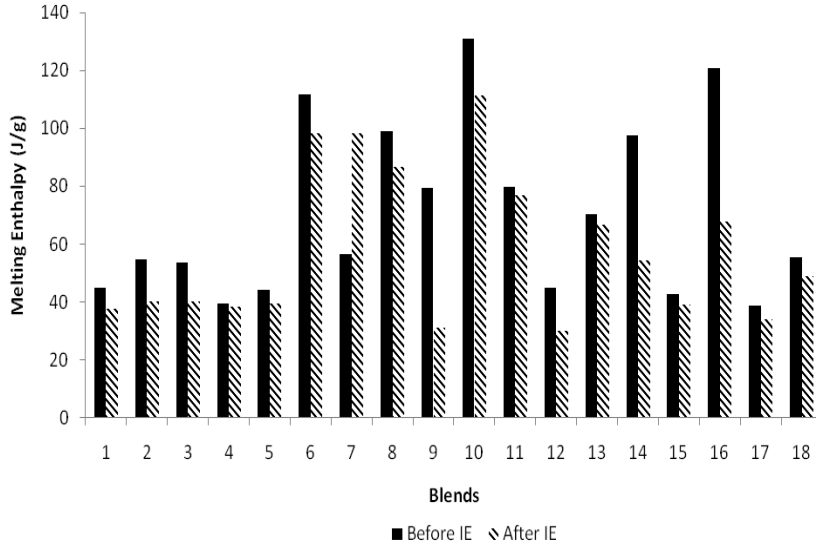


Fig. 5. Melting entalphy (J/g) for POs:CSO:POo in different ratios before IE and after IE.

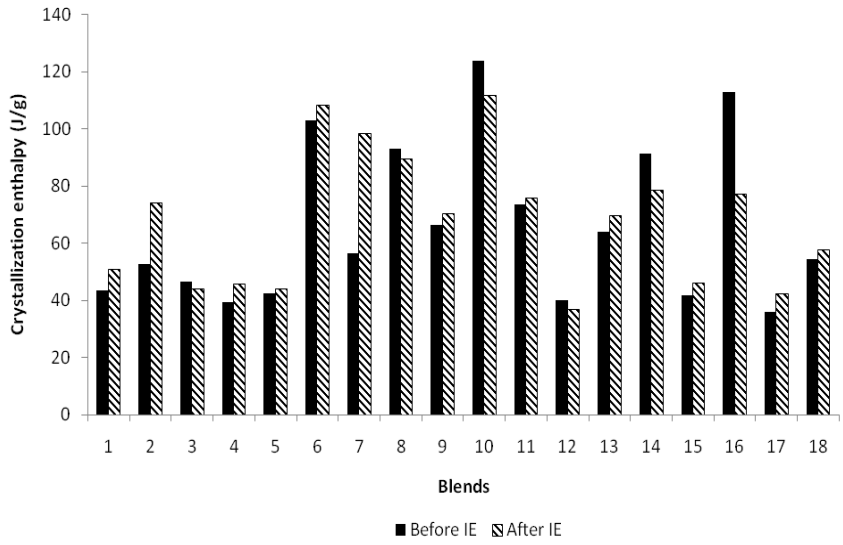


Fig. 6. Crystalization entalphy (J/g) for POs:CSO:POo in different ratios before IE and after IE.

**Stage 2**

Results for blend no. 3; (20% POs: 23.33% CSO: 56.67% POo) and blend no. 15; (20% POs: 10% CSO: 70% POo) which had profiles quite similar to control sample for vanaspati (Dalda).

**Solid fat content (SFC)**

Blends no. 3; (20% POs: 23.33% CSO: 56.67% POo) and no. 15; (20% POs: 10% CSO: 70% POo) were chosen based on SFC profiles that are similar with Dalda. Profile SFC showed the melting behavior for fats by calculating content of crystal fats at certain temperatures. SFC is one of the determining factors in the texture of plastic fats. The SFC profiles, a function of temperature for the Pos:CSO:POo blends for samples 3, 15 and Dalda were as shown in Figures 7 and 8.

Figure 7 shows % SFC for blends no. 3 and no. 15 before IE and Dalda. The % SFC for blends no. 3 and no. 15 at 0 ˚C were 46.19% and 56.37%, respectively. The % SFC for blend no. 3 was lower than SFC blend no. 15 because of the differences of the amount of fat and oil in these blends. Blend no. 3 consists of 23.33% cottonseed oil while blend no. 15 consists of 10% cottonseed oil only. The higher amount of cottonseed oil made the texture of blend no. 3 softer than blend no. 15. SFC value for blend no. 3 and no. 15 at 0 – 37.5 ˚C were 46.19 – 5.4% and 56.37 – 6.38%, respectively. For Dalda, SFC value at 5 – 37.5 ˚C was 50 – 4.2%. Figure 8 shows % SFC of blends no. 3 and no. 15 after IE and Dalda samples. The IE process produced softer fat blends. This was confirmed because after the IE process, there were significant decreases of SFC values from 0 – 35 ˚C for both blends no. 3 and no. 15 and these values were lower than in the Dalda sample. This result also indicated that both blends consisted of more liquid fraction at these temperature ranges, by increasing the unsaturated fatty acid in the new TAG. After IE, SFC profiles for both samples were quite similar. SFC value for blend no. 3 was 45.56 – 9.64% and blend no. 15 was 45.83 – 9.19%. SFC after IE values for both blends were lower when compared to before IE.

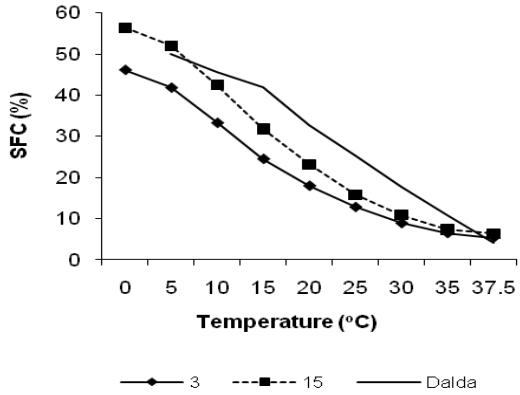


Fig. 7. Percentage of SFC for blends no. 3 and no. 15 at before IE and Dalda.

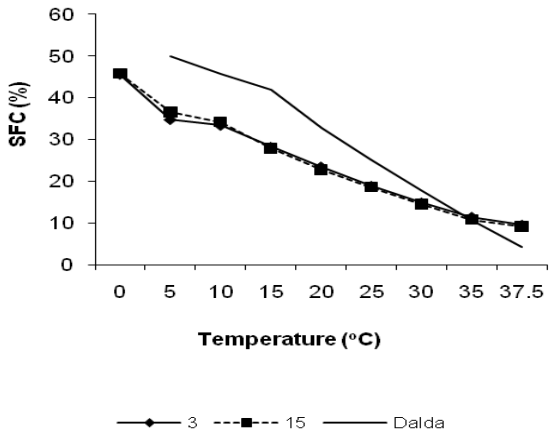


Fig. 8. Percentage of SFC for blends no. 3 and no. 15 at after IE and Dalda.

## Triacylglicerides composition (TAG)

Figure 9 shows TAG composition of POs, CSO, POo that were used in the blends and Dalda. CSO contained the highest value of TAG LLL (11.64%), followed by Dalda (8.11%), palm olein (7.76%) and palm stearin (0.56%). Palm stearin showed the lowest content of TAG LLL because it contained a high amount of saturated fatty acid such as palmitic acid (47 – 74%) (Pantzaris, 1987). Based on Figure 9, TAG types AAA, MMM, SSS, PPP, POS and CCC were shown to be absent in all samples. About 0.54% TAG type POP was found in palm olein and 9.47% TAG

type POS was found in the Dalda sample. Palm olein showed a high content of TAG type OOO (43.48%), followed by palm stearin (10.71%) and CSO (0.78%).

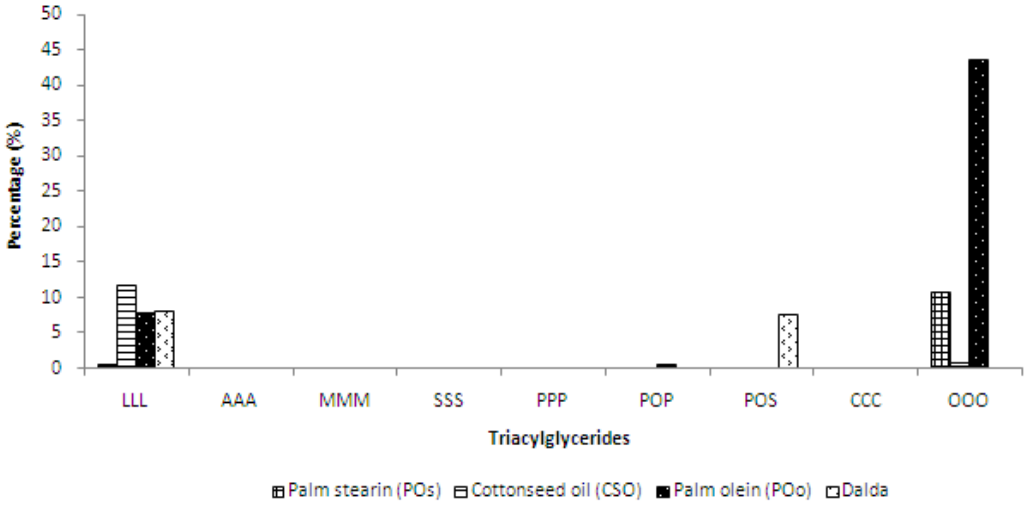


Fig. 9. Triacylglycerids composition (TAG) of Pos, CSO, POo and Dalda.

The effect of enzymatic interesterification changes for TAG in samples no. 3 and no. 15 are shown in Figures 10 and 11. Figure 10 shows triacylglicerides (TAG) composition for blend no. 3; (20% POs: 23.33% CSO: 56.67% POo) before and after IE. After IE, TAG type LLL was about 10.28% and absent at before IE. This possibility was due to the formation of a new TAG or the loss of a TAG as caused by a hydrolysis reaction after IE (Chen *et al.,* 2007). According to Chen *et al.,* (2007), an increase in the rate of hydrolysis (more than 5.20% FFA produced) will cause a high degree of TAG losses (more than 15%). TAG type AAA, MMM, SSS, PPP and POS were absent in blend no. 3; both before and after IE. Up to 0.39% TAG type CCC was present in blend no. 3 after IE. Before IE, approximately 1.35% TAG type OOO was present in blend no. 3 and it increases after IE, to approximately 28.35%.

TAG types AAA, MMM, SSS, PPP and POS were absent in blend no. 15; before and after IE. Based on Figure 11, about 6.32% TAG type LLL was present before IE and increased to 9.5% after IE. 17.20% TAG type POP was present in blend no. 15 before IE and decreased to 16.61% after IE. TAG type CCC was present in blend no. 15 only after the IE process that was about 0.16%. In blend no. 15, TAG type OOO was quite similar before and after IE, which were about 34.47% and 34.54%, respectively. The results showed that the values were quite similar for this blend at both before and after IE.

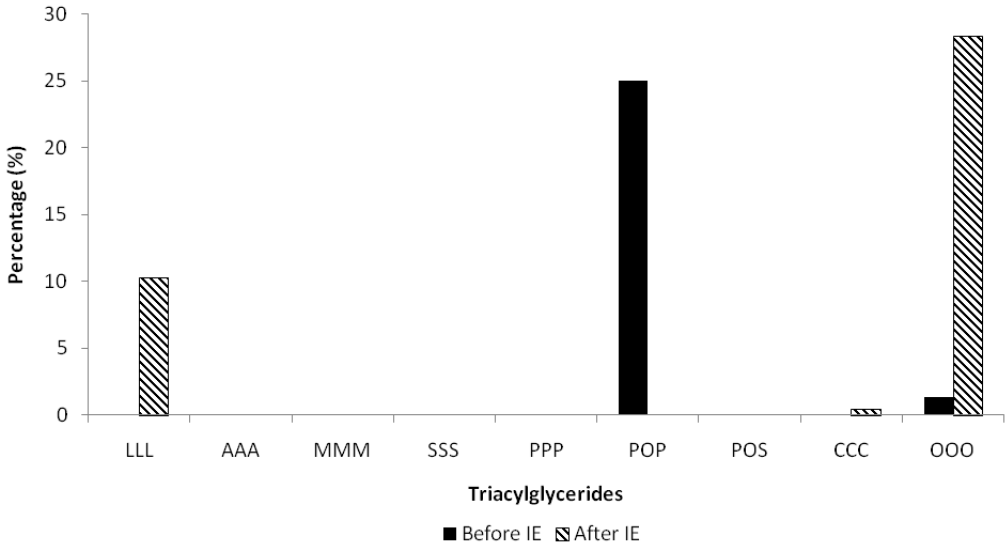


Fig. 10. Triacylglycerides composition of blend 3;(20%POs:10%CSO:70%Poo) before IE and after IE.

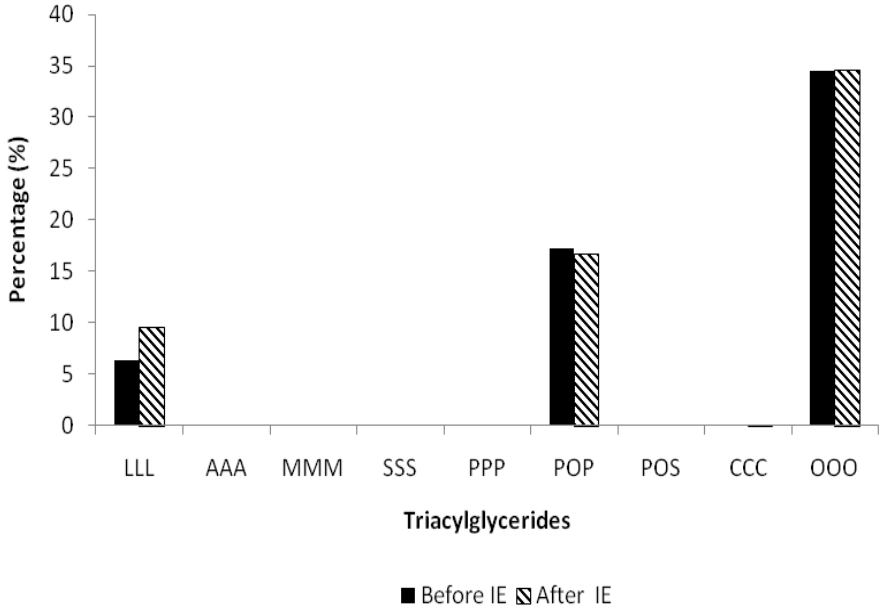


Fig. 11. Triacylglycerides composition of blend 15;(20%POs:10%CSO:70%Poo) before IE and after IE.

**Fatty acid composition**

Figure 12 shows the percentage of fatty acid composition in the commercial vanaspati, Dalda. Based on this figure, the highest fatty acid composition in Dalda was oleic acid (C18:1t) at 28.4% and palmitic acid (C16:0) at 27.9%, followed by 19.8% of C18:1c, 10.7% of stearic acid C18:0 and 6.8% of C18:1l. Only 0.2% lauric acid (C12:0) and 0.7% myristic acid (C14:0) was present in the Dalda sample.

Table 5 shows the precentage of fatty acid composition for blends no. 3 and no. 15 before and after IE. For blend no. 3, fatty acid composition before and after IE were quite similar. Enzymatic interesterification only modified the TAG through the changes of the fatty acid composition and/or their positional distribution in glycerol backbone. So, the fatty acid composition of the blend will not be changed after the IE process. Both blends no. 3 and no. 15 were rich in palmitic acids (43.7 – 47.2%) and oleic acids (30.7 – 34.8%). Both blends also contained high amounts of long chain fatty acids such as C18 and C20, and showed high content of saturated fatty acids which were more than 50%.

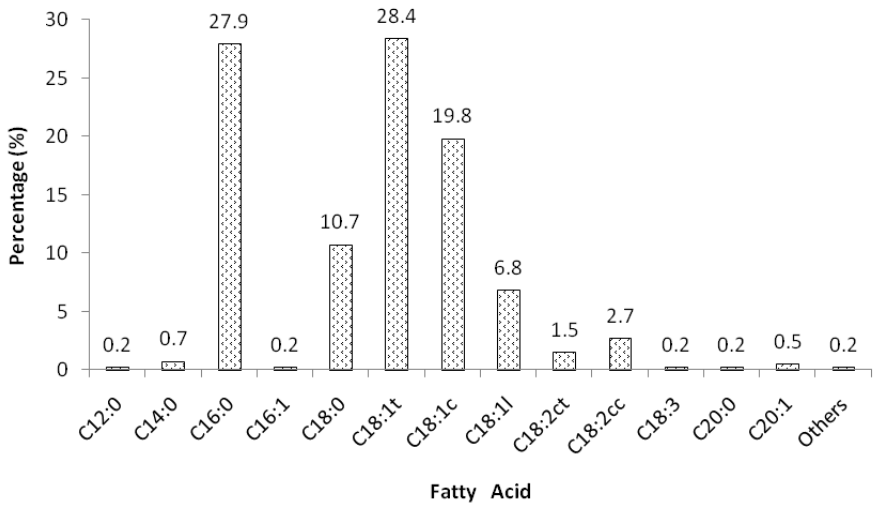


Fig. 12. Fatty acid composition of the Dalda sample.

Table 5. Fatty acid composition (%) for blends no. 3 and no. 15 with different composition of palm stearin (POs), cottonseed oil (CSO) and palm olein (POo) before IE[1] and after IE[2].

| Ratio[a] | Fatty acid composition (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C12:0 | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | C20:0 | Others |
| 3[1] | 0.3 | 1.0 | 43.7 | 0.2 | 3.8 | 30.7 | 19.5 | 0.2 | 0.3 | 0.3 |
| 3[2] | 0.3 | 1.0 | 46.3 | 0.2 | 3.9 | 31.1 | 16.4 | 0.2 | 0.3 | 0.3 |
| 15[1] | 0.4 | 1.1 | 46.2 | 0.2 | 4.0 | 33.8 | 13.7 | 0.2 | 0.3 | 0.1 |
| 15[2] | 0.4 | 1.1 | 47.2 | 0.2 | 4.1 | 34.8 | 11.6 | 0.2 | 0.3 | 0.1 |

Note: [a]no. 3; [20% palm stearin (POs): 23.33% cottonseed oil (CSO): 56.67% palm olein (POo)] no. 15; [20% palm stearin (POs): 10% cottonseed oil (CSO): 70% palm olein (POo)]

## CONCLUSIONS

Enzymatic interesterification with lipase TL IM significantly modified the physico-chemical and thermal behavior of the POs:CSO:POo blends. Results from SFC indicated that enzymatic interesterification of a ternary blends of POs:CSO:POo in the ratio no. 3 (20% POs: 23.33% CSO: 56.67% POo) and ratio no. 15 (20% POs: 10% CSO: 70% POo), respectively, gave a product which is suitable for vanaspati formulation.

## ACKNOWLEDGEMENTS

The first author wishes to thank colleagues at the Oils and Fats/Oleochemical Section, Universiti Kebangsaan Malaysia and those staff involved, for their assistance in this study.

## REFERENCES

Block, J. M., Barrera, D. Arellano, Figueiredo, M. F. & Gomide, F. A. C. 1997. Blending process optimization into special fat formulation by neural networks. *Journal of the American Oil Chemists' Society* **74**: 1537-1541.

Charteris, W. & Keogh, K. 1991. Fats and oils in table spreads. *Lipid Technology* **3**: 16-22.

Chen, C. W., Chong, C. L., Ghazali, H. M. & Lai, O. M. 2007. Interpretation of triacylglycerol profiles of palm oil, palm kernel oil and their binary blends. *Food Chemistry* **100**: 178-191.

Gunstone, F. D. 2002. Cottonseed oil . Gunstone F. D. (pynt.). In *Vegetable Oils in Food Technology, Composition, Properties, Uses*. p: 203-230.

Gunstone, F. D. 2004. Sources, composition, properties and uses. In *The Chemistry of Oils and Fats*. New York: Blackwell Publishing. 282 pp.

Iwasaki, Y & Yamane, T. 2000. Enzymatic synthesis of structured lipids. *Journal of Molecular Catalysis B. Enzymatic* **10**: 129-140.

Krawczyk, G. R., Buliga, G. S., Bertrand, D. T. & Humphreys, W. M. 1996. Reviewing the technology of low fat spreads. *INFORM* **7**: 635-639.

Nazaruddin, R., Mamot, S. & Loon, N. T. 2005. Physicochemical characteristics of binary mixtures of hydrogenated palm kernel oil and goat milk fat. *Journal of Food Lipids* **12**: 243-260.

Pantzaris, T. P. 1987. *Pocketbook of Palm Oil Uses*. Palm Oil Research Institute of Malaysia, Ministry of Primary Industries, Kuala Lumpur. p: 23-47.

PORIM. 1993. *PORIM Test Methods. Methods of Test For Palm Oil & Palm Oil Products*. Palm Oil Research Institute of Malaysia, Bangi.

PORIM. 1995. *PORIM Test Methods. Methods of Test For Palm Oil & Palm Oil Products*. Palm Oil Research Institute of Malaysia, Bangi.

Timms, R. E. 1985, Physical Properties of oils and mixtures of oils. *Journal of the American Oil Chemists' Society* **62**: 241-24.