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THE INFLUENCE OF CHEMICAL INTERESTERIFICATION ON THE PHYSICOCHEMICAL AND MICROSTRUCTURAL PROPERTIES OF PALM STEARIN, PALM KERNEL OIL, RICE BRAN OIL AND THEIR BLENDS

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ABSTRACT

Blends of palm stearin (PS), palm kernel oil (PKO) and rice bran oil (RBO) were formulated using Design Expert 9.0 according to Simplex Lattice Mixture Design. Ten blends with 6 replicates were subjected to chemical interestererication (CIE) and the effects of CIE on the triacylglycerol (TAG) composition, slip melting point (SMP), solid fat content (SFC), polymorphism and microstructure were investigated. Chemical interesterification changed the TAG composition, microstructure and SFC profile of the blends. The polymorphism was not affected except for PKO and its binary blends. Interesterified blends had lower SMP than their respective non-interesterified blends except for PS, RBO and PKO:RBO blends. The optimised PS:PKO:RBO (22:16:62) blend formulated have similar SMP and SFC profile with the commercial soft table margarine.

Key words: Chemical interesterification, mixture design, Design Expert, palm stearin, palm kernel oil, rice bran oil

INTRODUCTION

Palm stearin (PS) is the solid fraction obtained from fractionation of palm oil at controlled temperature. It has limited applications due to its high melting point ranging from 44 to 56°C (Lin, 2011). The low plasticity and incomplete melting at body temperature make PS do not suitable for direct use in margarine production. Palm kernel oil (PKO) contains mainly of medium-chain triacylglycerols (MCTs) which are digested easily at a faster rate by the body compared to other fats (Ruan *et al*., 2014). Medium-chain triacylglycerols are also capable of increasing satiety and reduced energy intake, hence reducing obesity problem (Coleman *et al*., 2016).

Rice bran oil (RBO) is liquid oil as it is rich in polyunsaturated fatty acids. In addition, RBO contains oryzanol, tocopherols, tocotrienols and sterols which have good health benefits. Oryzanol, which is unique to RBO has antioxidant effects and is a potent hypocholesterolemic agent (Makynen *et al*., 2012). Thus, RBO could be a vital ingredient in producing nutritional margarine. In margarine production, the fat blends used must be able to impart plasticity, which can be achieved through modification techniques such as interesterification. Currently, interesterification is one of the important processes used to modify the physicochemical properties of oils and fats. Interesterification can be an alternative to partial hydrogenation process to avoid the formation of *trans* fatty acids. According to Micha & Mozaffarian (2009), *trans* fatty acid adversely affects the lipid and non-lipid factor of cardiovascular disease such as increase the LDL and decrease the HDL-cholesterol. Chemical interesterification (CIE) involves random re-arrangement of fatty acids on the glycerol backbone, normally catalysed by alkali metal or alkali metal-alcoholate.

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In this research, PS, PKO, RBO and their blends were subjected to chemical interesterification and the effects of CIE on the physicochemical properties of the blends was evaluated. The specific aim of this study was to find the most suitable interesterified ternary blends fats for the formulation of *trans*-free soft table margarine through optimisation process using Design Expert version 9.0 (2014).

MATERIALS AND METHODS

Materials

Refined, bleached and deodorised (RBD) palm stearin (PS) and palm kernel oil (PKO) were obtained from Golden Jomalina Sdn. Bhd., Klang, Selangor, Malaysia and rice bran oil (RBO) were obtained from the local supermarket. The oils were kept at 0°C prior to use. All chemicals and reagents used were of analytical or high-performance liquid chromatography (HPLC) grade.

Methods

Blending process

Palm stearin, palm kernel oil and rice bran oil were melted in an oven (Protech FSD-380, Malaysia) at 60°C prior to use. The liquefied PS, PKO and RBO were blended according to the ratio generated by Simplex-Lattice Mixture Design with 3 components (Design Expert 9.0, 2014). The mixture components were PS (X1), PKO (X2) and RBO (X3). The mixture components were expressed as follows: X1 + X2 + X3 =1. Ten different blend ratios were prepared in percentage (w/w) as in Table 1 which consists of 3 formulations of single blends, 3 formulations of binary blends and 4 formulations of ternary blends. There were 6 replicates, which make the total of 16 blends.

The Design Expert 9.0 software was used to determine the optimum ternary blend for soft table margarine formulation. Two responses, solid fat content (SFC) at 5 to 45°C and slip melting point (SMP) were selected based on their importance in determining the properties and acceptability of the formulated margarine. Graphical multiple responses optimisation tool in the software was used for optimisation of the soft table margarine formulation.

Chemical interesterification

The chemical interesterification was performed in accordance with MPOB Test Method (2004). The non-interesterified oil was abbreviated as NIE and interesterified oil as CIE. The oil blends were dried using nitrogen gas at 100°C for 60 min. Then, the temperature was lowered to 80°C, before the addition of 0.2% sodium methoxide as catalyst. The mixture was heated to 110°C while purging with nitrogen gas for 60 min with vigorous stirring. The temperature was then lowered to 70°C before citric acid solution (5%) was added to stop the reaction. The mixture was washed with warm water and stirred vigorously at 70°C for 15 min. The water was removed together with excess citric acid and catalyst. The interesterified oil was finally filtered using Whatman filter paper (#4).

Slip melting point (SMP)

The slip melting point was determined by using an open capillary tube according to the MPOB Test Method (2004). The capillary tubes were dipped into the completely liquid samples to obtain fat column of 10 mm high. The fat column was chilled using a piece of ice and then tempered at 10°C for 16 hours. After that, the capillary tube was attached to the bulb of thermometer and immersed into a beaker of water. The water bath was heated slowly at the rate of 1°C/min until the fat column started to rise. The temperature of water when each column rises was recorded. The analysis was carried out in triplicates.

Table 1. Percentage of the Blends (w/w)

Blend	Percentage (w/w)		
	Palm stearin (PS)	Palm kernel oil (PKO)	Rice bran oil (RBO)
PS	100.00	0.00	0.00
PKO	0.00	100.0	0.00
RBO	0.00	0.00	100.00
PS:PKO	50.00	50.00	0.00
PS:RBO	50.00	0.00	50.00
PKO:RBO	0.00	50.00	50.00
4PS:PKO:RBO	66.66	16.66	16.66
PS:4PKO:RBO	16.66	66.66	16.66
PS:PKO:4RBO	16.66	16.66	66.66
PS:PKO:RBO	33.33	33.33	33.33

Solid fat content (SFC)

The solid fat content of the blends was determined using pulsed Nuclear Magnetic Resonance (pNMR) spectrometer (Bruker Minispec, Karlsruhe, Germany). The analysis was carried out based on direct method as described by MPOB Test Method, (2004). The samples were tempered at 70°C for 30 minutes before chilling at 0°C for 90 minutes. The samples were then held at measuring temperature for 30 minutes before measurement was made. Measurements were carried out at a temperature range of 5 to 55°C with 5°C intervals. The analysis was run in triplicate for each sample and the average value was calculated.

Triacylglycerol composition

The triacylglycerol composition was determined according to AOCS Official Method Ce 5b-89 (2017) using reversed-phase high performance liquid chromatography (Agilent Technology, California, USA) equipped with refractive index detector. The column used was a Lichrosphere RP-18 (250mm x 4mm) of 5μm particle size (Merck, Darmstadt, Germany). A mixture of acetone and acetonitrile (75:25 v/v) was used as the mobile phase with a flow rate of 1.0 ml/min. The TAG was identified from comparison of the sample retention time with the commercial TAG standards.

Polymorphism

The crystal polymorphism of the oil blends were analysed by X-ray diffraction (XRD) spectrometry (Rigaku, Tokyo, Japan). The XRD was equipped with a rotating anode X-ray source. The sample was held by a flat stainless-steel plate with rectangular gaps. Samples were melted at 70°C and tempered at 20°C for 30 min. The short spacing of the β' form are at 4.2 and 3.8 Å, while for β form it is at 4.6 Å. Composition of β' and β crystals in the mixtures was determined by the relative intensity of the short spacing at 4.2 and 4.6 Å (D'Souza *et al.*, 1990).

Microstructure

Polarised Light Microscope (PLM) (Leica DMLP, Wetzlar, Germany) was used to analyse the microstructure of the samples. The sample was melted and about 10 μL was placed on a glass slide and covered with a glass slip. The sample was first heated to 70°C for 10 min and then cooled at a rate of 50°C/min to 5°C using liquid nitrogen. The sample was tempered at 5°C for 10 min before measurement. The photomicrograph was captured using a Leica colour video camera. The photomicrograph of the crystal was captured with a magnification factor of 200.

Statistical and data analysis

The quadratic and special quartic models in equation 1 and 2 were used to express the fitted response values. Analysis of variance at 5% was carried out to determine whether there is statistical significance between every equation.

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_1b_2X_1X_2 + b_1b_3X_1X_3 + b_2b_3X_2X_3 \quad (1)$$

$$Y = b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{1123}X_1^2X_2X_3 + b_{1223}X_1X_2^2X_3 + b_{1233}X_1X_2X_3^2 \quad (2)$$

where Y is the predictive dependent variable (SFC and SMP), b the equation coefficients (determined according to Cornell, 2002); and X the proportions of pseudo-components.

RESULTS AND DISCUSSION

Fitting for the best model

The independent and dependent variables were fitted to a series of models starting from linear up to quartic. The goodness of fit of the statistical model was checked using residual plot. According to Cornell (2002), low standard deviation, low predicted sum of squares, and high predicted R-squared are the characteristics of the best model. According to these guidelines, solid fat content (SFC) at 5°C was best fitted with quadratic model while the special quartic model was best fitted to the slip melting point (SMP) and SFC at 10 to 45°C.

Mixture component had been shown by analysis of variance (ANOVA) to have significantly ($p<0.05$) affect the response variations. ANOVA also been used to check the significance of all model fitting and there is significant different with $p<0.05$. From the analysis of response models (SFC and SMP ternary diagrams) the R^2 value calculated was always greater than 0.98, which indicates that relatively they are adequate for prediction purpose.

TAG composition

The TAG composition of palm stearin (PS), palm kernel oil (PKO), rice bran oil (RBO) and their blends before and after CIE are shown in Table 2a, 2b and 2c. For palm stearin, the major TAGs are POP, POO, PPP whereas the major TAG in RBO are PLO, POO and OOL where P is palmitic acid, O is oleic acid and L is linoleic acid. For palm kernel oil, the most abundant TAGs are LaLaLa, LaLaM and CLaLa where Ca is capric acid, La is lauric acid, and M is myristic acid.

Table 2(a). Triacylglycerol composition of palm stearin (PS), palm kernel oil (PKO), rice bran oil (RBO) and blends before (NIE) and after chemical interesterification (CIE)

TAG	PS		PKO		RBO	
	NIE	CIE	NIE	CIE	NIE	CIE
CCLa	–	–	6.7	5.1	–	–
CLaLa	–	–	8.7	6.5	–	–
LaLaLa	–	–	22.3	15.3	–	–
LaLaM	–	–	15.8	13.3	–	–
LaLaO	–	–	4.7	12.0	–	–
LaMM	–	–	8.7	9.7	–	–
LaLaP	–	–	0.7	0.7	–	–
LaMO	–	–	5.0	7.2	–	–
LaPM	–	–	4.2	5.3	–	–
LaOO	–	–	3.3	2.8	–	–
LaPO	–	–	4.3	4.0	–	–
LaPP	–	–	2.3	2.3	–	–
MOO	–	–	1.1	0.9	–	–
MPO	–	–	2.1	1.8	–	–
OLL	0.3	0.5	–	–	11.9	14.6
PLL	1.1	1.0	–	–	12.4	9.5
OOL	0.4	0.9	–	–	14.5	17.5
MMP	1.2	1.6	–	–		
PLO	6.1	6.7	–	–	18.0	18.4
PLP	7.7	6.7	–	–	6.3	4.4
OOO	3.7	4.2	2.4	1.7	12.3	7.9
POO	15.1	16.9	2.3	1.0	14.9	12.5
POP	37.3	29.9	1.4	1.1	4.5	6.3
PPP	14.9	17.1	0.3	1.3	0.6	1.5
SOO	1.3	1.3	0.4		1.1	1.6
POS	6.1	5.4	0.3	0.2	0.6	1.0
PPS	2.8	4.2	–		0.7	0.8
SSO	–	0.82	–	–		0.3

Note: NIE = Non-interesterified. CIE = Chemical Interesterification. C = Capric. La = Lauric. M = Myristic. P = Palmitic. O = Oleic. S = Stearic.

Table 2(b). Triacylglycerol composition of palm stearin (PS), palm kernel oil (PKO), rice bran oil (RBO) and blends before (NIE) and after chemical interesterification (CIE)

TAG	PS:PKO		PS:RBO		PKO:RBO	
	NIE	CIE	NIE	CIE	NIE	CIE
CCLa	3.3	1.1	–	–	3.2	0.9
CLaLa	4.7	1.8	–	–	4.9	1.7
LaLaLa	10.7	3.5	–	–	12.2	3.1
LaLaM	7.9	3.4	–	–	8.5	2.4
LaLaO	2.3	6.8	–	–	5.7	7.0
LaMM	4.2	7.9	–	–	5.5	4.0
LaLaP	0.3	1.6	–	–	0.4	7.2
LaMO	2.6	4.2	–	–	–	4.0
LaPM	2.6	5.9	–	–	–	2.7
LaOO	1.9	4.4	–	–	1.2	5.2
LaPO	2.3	11.4	–	–	1.8	7.4
LaPP	2.0	8.7	–	–	–	–
MOO	–	–	–	–	–	1.6
MPO	1.6	6.0			0.9	3.9
OLL	–	–	5.9	5.1	8.5	1.7
PLL	–	–	6.9	5.5	8.0	0.7
OOL	–	–	7.6	7.2	8.2	5.2
MMP	–	–	–	–	–	–
PLO	3.6	1.7	11.7	13.1	9.4	3.7
PLP	4.5	1.2	7.0	7.7	3.8	
OOO	3.0	5.3	7.7	7.2	6.6	3.3
POO	8.9	4.6	15.0	15.4	7.1	3.7
POP	19.4	6.5	22.4	19.9	2.0	2.9
PPP	7.7	3.7	8.0	7.7	0.4	1.6
SOO	0.7	0.5	1.4	2.7	0.9	1.3
POS	3.4	1.6	3.8	4.0	0.5	1.5
PPS	1.7	1.9	1.5	1.6	–	0.4

Note: NIE = Non-interesterified. CIE = Chemical Interesterification. C = Capric. La = Lauric. M = Myristic. P = Palmitic. O = Oleic. S = Stearic.

Table 2(c). Triacylglycerol composition of palm stearin (PS), palm kernel oil (PKO), rice bran oil (RBO) and blends before (NIE) and after chemical interesterification (CIE)

TAG	4PS:PKO:RBO		PS:4PKO:RBO		PS:PKO:4RBO		PS:PKO:RBO	
	NIE	CIE	NIE	CIE	NIE	CIE	NIE	CIE
CCLa	1.0	0.2	4.3	3.0	1.1	0.2	0.2	1.0
CLaLa	2.2	1.1	6.2	4.4	2.1	0.4	3.1	2.3
LaLaLa	3.8	0.7	15.5	11.8	4.3	0.6	6.9	4.1
LaLaM	2.8	0.5	10.8	8.5	2.8	1.8	4.8	3.5
LaLaO	1.7	1.6	4.1	4.9	4.4	2.9	3.4	3.9
LaMM	1.7	1.1	6.3	6.2	2.4	0.7	3.3	3.6
LaLaP	0.1	1.3	0.5	1.2		5.3	0.3	2.1
LaMO		1.0	1.9	3.4				2.0
LaPM	0.2		4.7	4.6				2.4
LaOO	0.2	2.5	2.5	3.0	0.6	3.2	1.2	2.6
LaPO	0.5	7.5	3.9	4.2		4.7	1.6	5.9
LaPP				4.1				
MOO								
MPO	1.2	3.0	1.5	1.9	0.5	1.8	1.0	2.4
OLL	2.4	0.6		1.3	7.9	6.1	5.3	2.4
PLL	2.9	2.5	2.0		8.4	4.9	5.2	2.3
OOL	3.2	7.0			9.5	9.6	5.6	6.7
MMP								
PLO	6.9	6.9	4.2	3.4	12.5	11.8	7.7	7.2
PLP	6.5	5.2	2.2	1.8	5.6	4.3	4.9	4.0
OOO	4.9	5.6	3.9	3.7	8.5	4.9	5.1	5.0
POO	13.0	11.0	6.2	5.5	11.9	9.4	9.4	8.6
POP	26.5	15.2	8.3	7.0	10.0	6.9	13.7	10.5
PPP	10.4	7.7	3.0	3.5	2.8	2.0	4.8	3.9
SOO	1.2	2.2	0.7	1.5	1.3	1.5	0.8	1.0
POS	4.5	3.1	1.3	2.8	1.7	1.6	2.2	1.6
PPS	2.2	2.9	0.7	1.8	0.7	0.6	9.1	1.1

Note: NIE = Non-interesterified. CIE = Chemical Interesterification. C = Capric. La = Lauric. M = Myristic. P = Palmitic. O = Oleic. S = Stearic.

After interesterification, prominent changes were observed in the TAG composition of PS, PKO and RBO. In CIE PS, TAG species such as POO, PPP and PLO had increased from 15.1%, 14.9% and 6.1% to 16.9%, 17.1% and 6.7%, respectively, while other TAG species such as POP, POS and PLP had decreased from 37.3%, 6.1% and 7.7% to 29.9%, 5.4% and 6.7%, respectively. Substantial changes in TAG species was observed in PKO after interesterification where LaLaO, LaMM, LaMO and PPP increased while other TAG species such as CLaLa, LaLaLa and CCLa decreased. For RBO, TAG species such as OLL, OOL, PLO and POP increased while TAG species such as PLL, OOO and POO decreased after CIE.

For the binary and ternary blends, as the amount of a component mixture increase, the TAG species associated with the respective fats and oils increased. As the amount of PS in the blend increase, the high melting TAG such as POP, PPP, POO and PLP increase as in NIE 4PS:PKO:RBO and NIE PS:PKO:RBO. The binary blend NIE PS:PKO contained high amounts of medium melting TAG (LaLaLa and LaLaM) and high melting TAG (POP, PPP, POO and POS). Interesterification cause prominent changes in TAG composition of CIE PS:PKO blend where TAG species such as POP, PPP, POS, LaLaLa and LaLaM decreased in proportion while TAG species such as LaPO, LaPP and OOO increased.

The main TAG for NIE PS:RBO blend are POP, POO, PLO, PPP, OOO and PLP. After interesterification, TAG such as POP and PPP decreased in proportion, while TAG such as PLO and POO increased. Table 2b shows that interesterification had reduced the trisaturated TAG (S_3) such as PPP and triunsaturated TAG (U_3) such as OOO, OLL and OOL in CIE PS:RBO blend. On the other hand, CIE increase the disaturated-monounsaturated (S_2U) TAG and monosaturated-diunsaturated (SU_2) TAG such as PLP and PLO, respectively.

For NIE PKO:RBO, the main TAG are LaLaLa, LaLaM, PLO, OLL and OOL. The medium chain fatty acids are from PKO while the unsaturated fatty acids are from RBO. Interesterification randomised the fatty acids on the glycerol backbone of CIE PKO:RBO which caused TAGs such as LaLaLa, LaLaM, OLL, OOO, PLO and PLL decreased while TAGs such as LaLaO, LaLaP, LaOO, LaPO, POP and PPP increased.

For ternary blends, the CIE 4PS:PKO:RBO showed a reduction in the trisaturated TAGs (S_3) such as LaLaLa, LaLaM and PPP while triunsaturated

TAG (U_3) such as OOL and OOO increased except for OLL. After CIE, randomisation also caused the S_2U TAGs such as LaLaO, POP and POS to decrease. In contrast, several SU_2 TAGs such as LaOO and SOO increased after CIE.

For CIE PS:4PKO:RBO, the interesterification process leads to reduction in S_3 and U_3 TAGs such as LaLaLa, LaLaM, LaMM and OOO. After CIE, there were increment in S_2U and SU_2 TAGs such as LaPO, LaMO, LaLaO, POS, LaOO and SOO. The CIE PS:PKO:4RBO blend shows reduction in S_3 and U_3 TAGs such as PPP, POS, LaLaLa, LaLaM, LaMM, OOO and OLL. The randomisation process also caused reduction in S_2U and SU_2 TAGs such as LaLaO, PLO, PLP, POP, PLL, PLO and POO.

For CIE PS:PKO:RBO the changes occurred after CIE is much similar with CIE PS:4PKO:RBO except for reduction in SU_2 TAGs such as PLL, POO, and PLO. After CIE, a well balance in TAGs composition was seen where more even peaks distribution obtained compared to the starting blends due increase in several TAGs and reduction in others. This finding is in agreement with Siti Hazirah *et al.* (2013).

Randomisation of fatty acids on the glycerol backbone also led to the synthesis of new TAGs where some of them were difficult to identify due to overlapped peaks and close retention times. According to Bessler & Orthoefer (1983), S_3 TAG species which melt at temperature between 54 and 65ºC, are important in contributing to the product structure while the U_3 TAG species which melted at low temperature ranges from -14 to 1ºC are responsible for softness of the product. On the other hand, the SU_2 TAGs are important for sensory and functional properties at room temperature, while the S_2U TAGs which usually melt near body temperature provide cooling effect in the mouth.

Slip Melting Point

Slip melting point for the NIE and CIE blends is shown in Figure 1, For original oil (Figure 1a), NIE PS had the highest SMP (49.9°C), followed by NIE PKO (29.0°C) and NIE RBO (5.0°C). After CIE, there were no significant ($p>0.05$) changes in the SMP of PS which could be due to only intraesterification occurred. Palm kernel oil shows a significant ($p<0.05$) reduction in SMP after CIE from 29.0 to 26.0°C. In contrast, SMP of RBO increased significantly ($p<0.05$) after CIE from 5 to 15°C. The increase in melting point may due to increase in high melting TAGs such as PPP, POP and POS in RBO as indicated in Table 2a.

For binary blends (Figure 1b), NIE PS:RBO had the highest SMP (44.8°C) followed by NIE PS:PKO (44.0°C) and NIE PKO:RBO (23.2°C). After randomisation, the binary blends CIE PS:RBO and CIE PS:PKO shows significant reduction in SMP to 34.0 and 39.7°C, respectively. In contrast, the CIE PKO:RBO had significant ($p<0.05$) increased in SMP from 23.2 to 24.0°C which may due to the increase in high melting TAGs such as PPP, POP, POS and PPS as shown in Table 2b.

After interesterification all ternary blends (Figure 1c) which were 4PS:PKO:RBO, PS:4PKO, RBO, PS:PKO:4RBO and PS:PKO:RBO show significant ($p<0.05$) reduction in SMP from 40.2 to 36.8°C, 30.0 to 27.3°C, 30.7 to 27.7°C and 33.3 to 28.0°C, respectively. The reduction of SMP after interesterification was due to the reduction in high melting TAGs such as POP and PLP and increase in intermediate melting point TAGs such as SOO, LaOO, MPO and LaPO. This findings were consistent with studies reported by Soares *et al.* (2012).

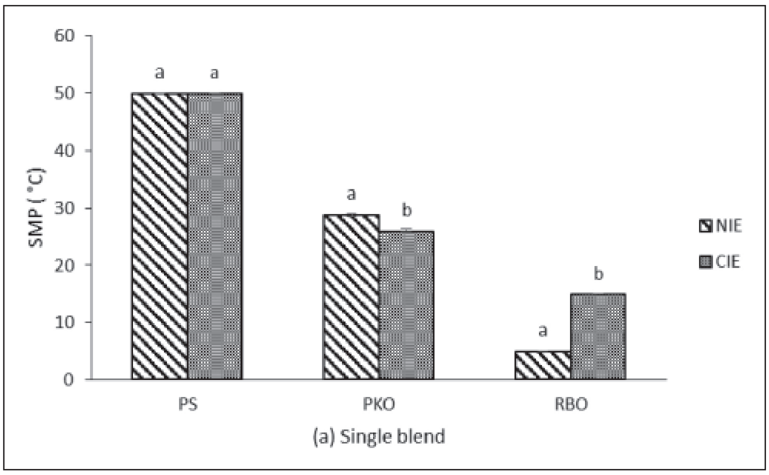


Fig. 1. (a) SMP of ternary blend.
Note: NIE = Non-interesterified, CIE = Chemical Interesterification.

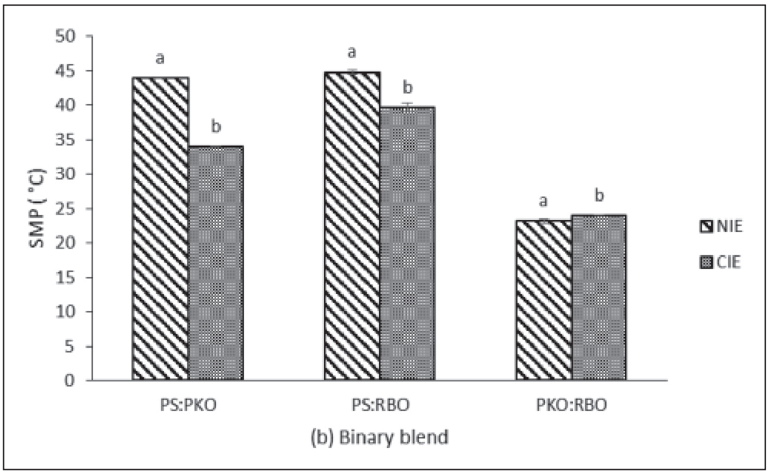


Fig. 1. (b) SMP of binary blend.
Note: NIE = Non-interesterified, CIE = Chemical Interesterification.

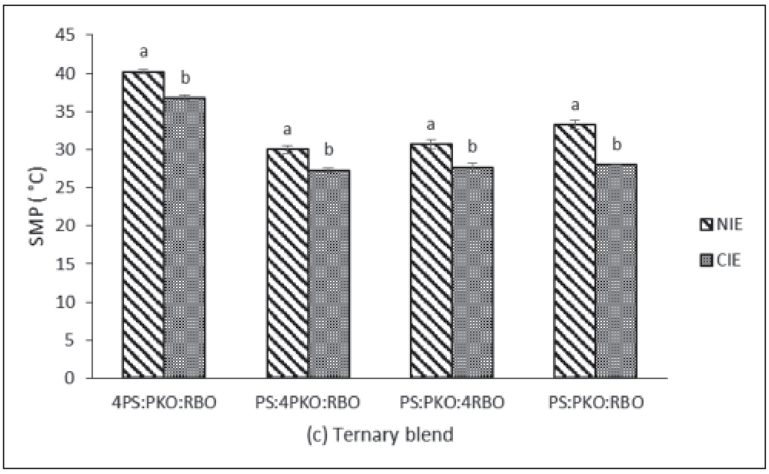


Fig. 1. (c) SMP of ternary blend.
Note: NIE = Non-interesterified, CIE = Chemical Interesterification.

Solid Fat Content (SFC)

Solid fat content of the NIE and CIE blends are shown in Figure 2a, 2b and 2c as a function of temperature. The SFC for single blend is shown in Figure 2a. Palm stearin possesses the highest SFC at all measured temperatures. This is due to high proportion of high melting TAG such as PPP. Palm kernel oil had a very sharp melting profile due to the high proportion of medium chain saturated fatty acid such as lauric acid. Rice bran oil had the lowest SFC at all measuring temperatures due to the high proportion of oleic and linoleic acids (Ornla-ied *et al.* 2016). After CIE, PKO showed reduction in SFC at all temperatures. This change may be due to increase in TAGs species with intermediate melting point such as LaLaO and LaMO. On the other hand, SFC for PS and RBO increased after CIE throughout the temperature range investigated. This is line with the SMP result (Figure 1a).

The SFC for binary blends is shown in Figure 2b. Interesterified blend of PS:PKO shows reduction in SFC starting from 35°C which may be due to reduction in S_3 TAGs such as PPP and LaLaLa. For CIE PS:RBO and CIE PKO:RBO, the SFC values are lower compared to their respective NIE blend starting from 10°C. A slight increase in SFC after CIE at temperature below 20°C and apparent decreased in SFC at higher temperature 35-40°C of interesterified PS/RBO and PS/CO blend was reported by Reshma *et al.* (2008) and Zhang *et al.* (2004), respectively. The SFC reduction in CIE PS:RBO was probably due to the increased in intermediate melting TAGs such as PLP, PLO and POO and for CIE PKO:RBO the SFC reduction may be attributed to the decreased in S_3 TAGs such as LaLaLa, LaLaM and CLaLa. The increase in proportion of intermediate melting TAGs may due to replacement of saturated fatty acids on the TAG

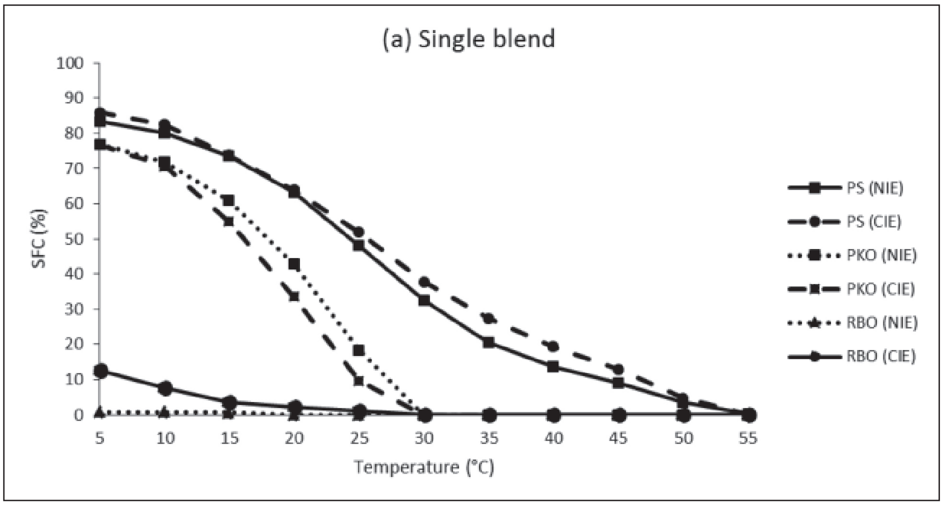


Fig. 2. (a) Solid fat content (SFC) of single blend – PS (palm stearin), PKO (palm kernel oil) and RBO (rice bran oil).

Note: NIE = Non-interesterified, CIE = Chemical Interesterification, PS = Palm Stearin, PKO = Palm Kernel Oil, RBO = Rice Bran Oil.

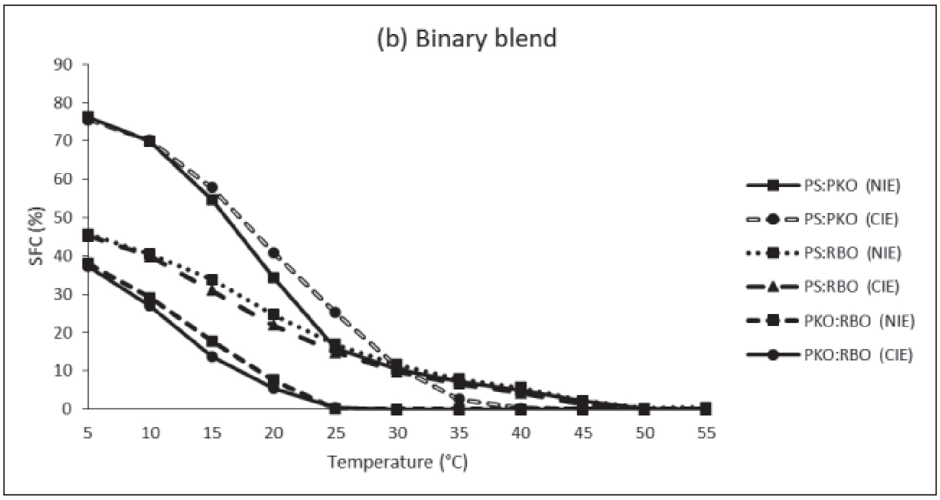


Fig. 2. (b) SFC of binary blends – PS:PKO, PS:RBO and PKO:RBO.

Note: NIE = Non-interesterified, CIE = Chemical Interesterification, PS = Palm Stearin, PKO = Palm Kernel Oil, RBO = Rice Bran Oil.

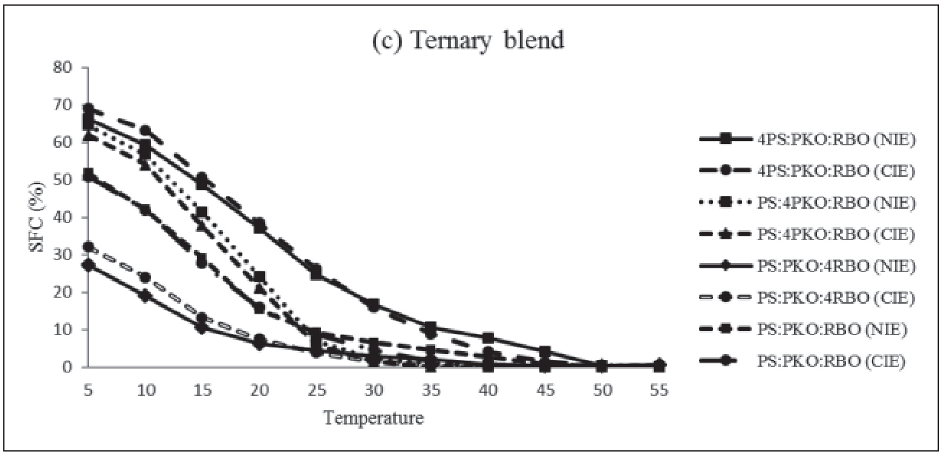


Fig. 2. (c) SFC of ternary blends – 4PS:PKO:RBO, PS:4PKO:RBO, PS:PKO:4RBO and PS:PKO:RBO.

Note: NIE = Non-interesterified, CIE = Chemical Interesterification, PS = Palm Stearin, PKO = Palm Kernel Oil, RBO = Rice Bran Oil.

of PS with unsaturated fatty acids from TAGs of RBO.

The SFC for ternary blends is shown in Figure 2c. After CIE, the PS:4PKO:RBO blend show reduction of SFC throughout the temperature range investigated while the other ternary blend show reduction in SFC starting from 30°C. According to Noor Lida *et al.* (2002), the SFC between 4 and 10°C is an important indicator for spreadability of the product at refrigeration temperature where SFC less than 32% at 10°C will give product with good spreadability. The SFC at 20 and 22°C is important to determine the product's stability and resistance to oil exudation at room temperature where SFC more than 10% is essential to prevent oiling off. The SFC between 35 and 37°C determines the thickness and flavour release properties of the fats in the mouth. The good margarines should have SFC less than 3.5% at 33.3°C and melt completely at body temperature to prevent waxiness in the mouth. From the properties stated, the CIE PS:PKO:4RBO will have good spreadability, stability, resistance to oil exudation and complete melting at body temperature.

Polymorphism

Table 3 shows the polymorphic form of the blends before and after interesterification measured by XRD. Before interesterification all blends show mixture of β and β' crystal form except for NIE PS:PKO:4RBO. For NIE RBO and NIE PKO:RBO, no crystal was detected as the sample were liquid at measured temperature. After interesterification, no changes in the polymorphic form of all CIE blend except for the formation of exclusively β' crystal form in PKO, binary blend containing PKO and formation of mixture of β and β' crystal in CIE PS:PKO:4RBO. The presence of exclusively β' crystal in CIE PKO and CIE PS:PKO may be due to the presence of fatty acids of varying chain length as reported by Timms (1984). Jin *et al.* (2008) reported that concentration of β' polymorph increase with the increase of PKO in the ternary blend containing tallow and palm olein. On the other hand, formation of exclusively β' crystal in CIE PS:RBO may be due to the dilution effect of RBO where β' polymorphic forms tend to dominate as the fat is diluted with a liquid oil, or the concentration of â polymorph is too low to cause any influence as observed by Siti Hazirah *et al.* (2013). Different polymorphic forms (α, β and β') could coexist at certain temperatures due to complex TAGs composition in fat blends (Chong *et al.,* 2007; Ribeiro *et al.,* 2009). β' crystal form is desirable for margarine production due to the smaller and thinner structure which provides smoother mouthfeel, increased firmness and can hold more air and liquid components compared to β crystals (Jennings & Akoh, 2010).

Table 3. Polymorphism of blends of PS, PKO, SBO and mixtures before (NIE) and after chemical interesterification (CIE)

	NIE	CIE
PS	β + β'	β + β'
PKO	β + β'	β'
RBO	–	–
PS:PKO	β + β'	β'
PS:RBO	β + β'	β + β'
PKO:RBO	–	β'
4PS:PKO:RBO	β + β'	β + β'
PS:4PKO:RBO	β + β'	β + β'
PS:PKO:4RBO	β	β + β'
PS:PKO:RBO	β + β'	β + β'

Note: NIE = Non-interesterified. CIE = Chemical Interesterification.

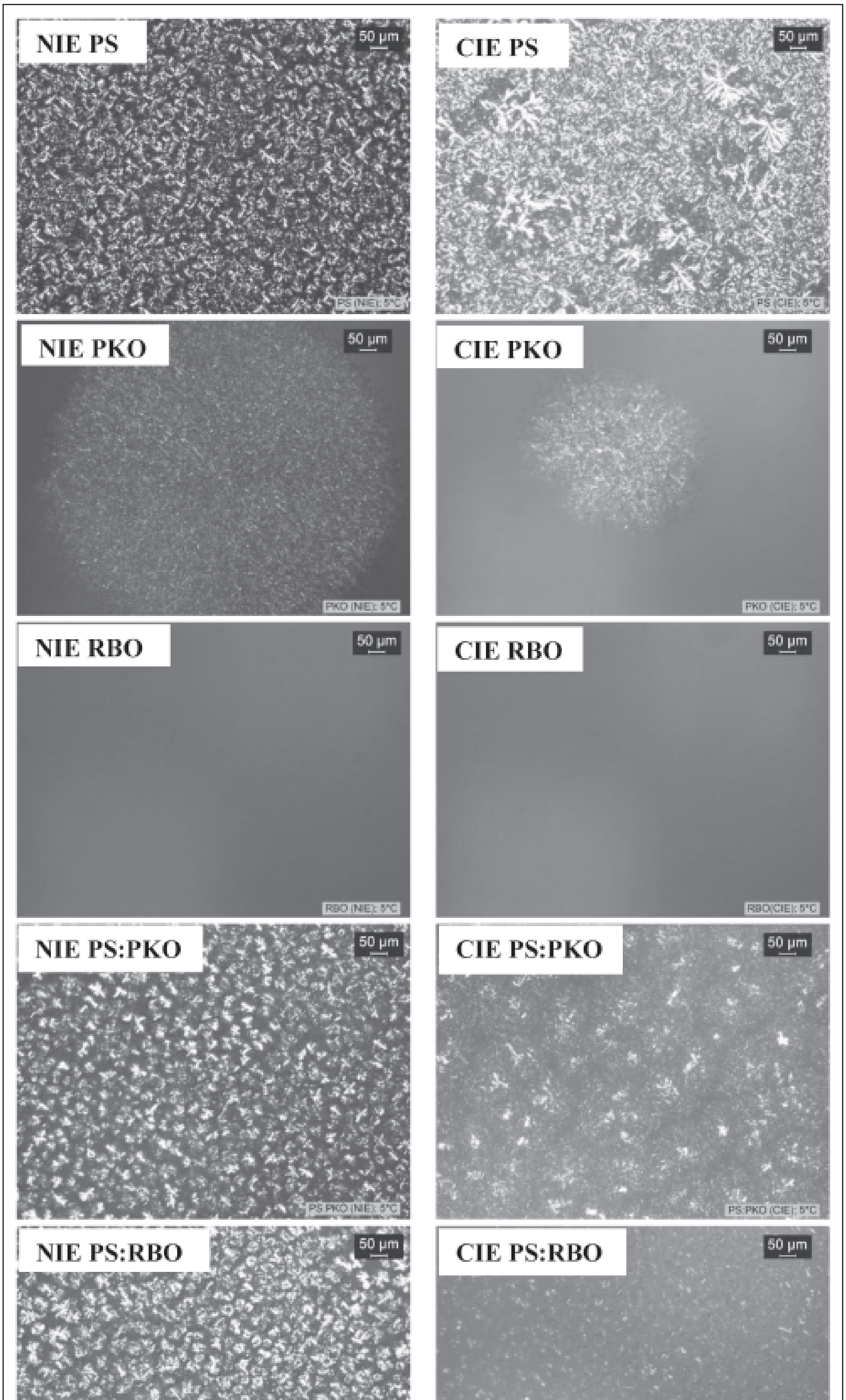
Microstructure

The microstructural images of the NIE and CIE blends at 20°C is shown in Fig. 3. The NIE PS shows dense crystal formation with fine plate-like structure while NIE PKO showed formation of small spherulite-shaped crystals. Chemical interesterification caused PS crystal to become large and loosely packed while PKO crystal decreased in size. No crystals were observed for both NIE and CIE RBO as they were liquid at measured temperature. According to Shi *et al.* (2005), the crystal morphology is largely dominated by the high melting triacylglycerols in the blends. Results show that NIE blends that contained 50 percent or more PS (PS:PKO, PS:RBO and 4PS:PKO:RBO) have dense and packed structure. After CIE, interesterified blend of PS:PKO, PS:RBO and 4PS:PKO:RBO showed smaller, finer crystals and loosely-packed structure.

For the NIE PKO:RBO the crystals is large spherulites-shape. After CIE, there were no crystals observed as the sample was liquid at measured temperature. The NIE PS:4PKO:RBO show dense, fine spherulite-shape crystals that were highly packed. After CIE, there was a reduction in number of crystals and the structure was loosely-packed. On the other hand, NIE PS:PKO:4RBO and NIE PS:PKO:RBO shows fine plate-like structure which turn into fine spherulite-shape crystals after interesterification. In general, CIE had promoted the formation of finer crystals than NIE blends.

Optimisation for soft table margarine formulation

Table 4 shows the predicted and observed values of SFC profile and SMP of formulated *trans*-free soft table margarine (STM) compared to soft table margarine (CSTM) available commercially. Results showed that optimisation of soft table


NIE PS
50 μm
CIE PS
50 μm
NIE PKO
50 μm
CIE PKO
50 μm
NIE RBO
50 μm
CIE RBO
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NIE PS:PKO
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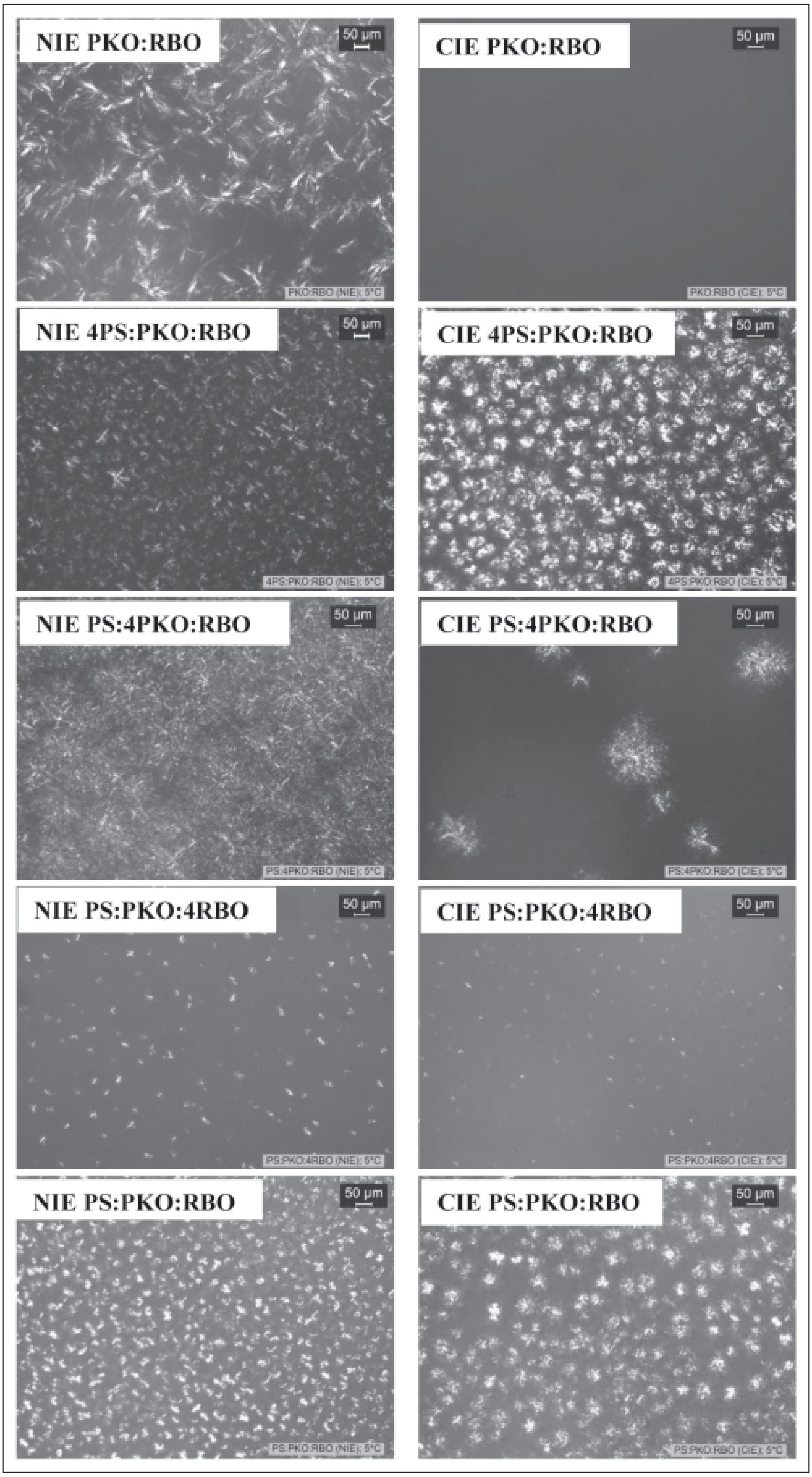

Fig. 3. Microstructural images under Polarized Light Microscope at 5°C and tempered for 10 min for single, binary and ternary mixtures of PS, PKO and RBO blends before (NIE) and after interesterification (CIE). Magnification at 200x and bar represent 50 µm.

Note: NIE = Non-interesterified, CIE = Chemical Interesterification, PS = Palm Stearin, PKO = Palm Kernel Oil, RBO = Rice Bran Oil.

Table 4. Solid fat content (SFC) and slip melting point (SMP) of trans-free table margarine formulation (TM) and commercial table margarine (CTM)

Sample	SFC (%)[a]									SMP (°C)
	5	10	15	20	25	30	35	40	45	
STM (Predicted)[b]	33.868	26.464	15.57	9.004	5.51	2.48	0.76	0.245	0	29.0
STM (Observed)	31.8 ± 0.25	22.7 ± 0.27	14.7 ± 0.29	9.6 ± 0.27	6.7 ± 0.25	4.3 ± 0.18	3.0 ± 0.22	1.4 ± 0.23	0.0 ± 0.00	32.5 ± 0.50
CSTM	29.2 ± 0.21	20.3 ± 0.08	12.0 ± 0.26	7.4 ± 0.20	4.4 ± 0.18	2.6 ± 0.09	0.5 ± 0.02	0.0 ± 0.00	0.0 ± 0.00	36.3 ± 0.58

Note: STM = Soft Table Margarine.
[a] Values show the mean ± standard deviation of triplicate.
[b] Predicted optimum ternary blends of PS/PKO/RBO with ratios of 22/16/62 (w/w) for trans-free soft table margarine (STM).

margarine formulation by using Design-Expert software was successful where the formulation have desirable SFC profile. The correlation coefficient obtained between predicted and measured SFC data was 0.998. Formulated *trans*-free soft table margarine (22:16:62) PS:PKO:RBO had almost similar SMP (32.5°C) with CSTM (36.3°C). Slip melting point (SMP) is considered an important factor in the formulation of *trans*-free soft table margarine where the formulated margarine must have SMP lower than body temperature. This is to ensure that it melts completely in the mouth and left no waxy sensation.

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REFERENCES

AOCS (2017). Triglycerides in Vegetable Oils By HPLC: Ce 5b – 89. In: *Official methods and recommended practices of the American Oil Chemist's Society*. 7th ed. AOCS Press. Champaign, Illinois.

Bessler, T.R. & Orthoefer, F.T. 1983. Providing lubricity in food fat systems. *Journal of the American Oil Chemists' Society*, **60(10)**: 1765-1768.

Chong, C.L., Kamarudin, Z., Lesieur, P., Marangoni, A., Bourgaux, C. & Ollivon, M. 2007. Thermal and structural behaviour of crude palm oil: Crystallisation at very slow cooling rate. *European Journal of Lipid Science and Technology*, **109(4)**: 410-421.

Coleman, H., Quinn, P. & Clegg, M.E. 2016. Medium chain triglycerides and conjugated linoleic acids in beverage form increase satiety and reduce food intake in humans. *Nutrition Research*, **36(6)**: 526-533.

Cornell, J.A. 2002. The original mixture problem: designs and models for exploring the entire simplex factor space. In: *Experiments with mixtures*. John Wiley & Sons, Inc(eds.) New York. pp. 22-95.

D'Souza, V., DeMan, J.M. & DeMan, L. 1990. Short spacings and polymorphic forms of natural and commercial solid fats: A review. *Journal of the American Oil Chemists' Society*, **67(11)**: 835-843.

Jennings, B.H. & Akoh, C.C. 2010. Trans-free plastic shortenings prepared with palm stearin and rice bran oil structured lipid. *Journal of the American Oil Chemists' Society*, **87(4)**: 411-417.

Jin, Q., Zhang, T., Shan, L., Liu, Y. & Wang, X. 2008. Melting and solidification properties of palm kernel oil, tallow and palm olein blends in the preparation of shortening. *Journal of the American Oil Chemists' Society*, **85(1)**: 23-28.

Lin, S.W. 2011. Palm Oil. In: *Vegetable Oils in Food Technology*. F.D. Gunstone (Eds.), Oxford: Wiley-Blackwell, New Jersey. pp. 25-58.

Makynen, K., Chitchumroonchokchai, C., Adisakwattana, S., Failla, M. & Ariyapitipun, T. 2012. Effect of gamma-oryzanol on the bioaccessibility and synthesis of cholesterol. *European Review for Medical and Pharmacological Sciences*, **16(1)**: 49-56.

Micha, R. & Mozaffarian, D. 2009. Trans fatty acids: effects on cardiometabolic health and implications for policy. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **79**: 147-52.

MPOB Test Method. 2004. Methods of Test for Palm Oil and Palm Oil Products. Malaysian Palm Oil Board, Malaysia.

Noor Lida, H.M.D., Sundram, K., Siew, W.L., Aminah, A. & Mamot, S. 2002. TAG composition and solid fat content of palm oil, sunflower oil, and palm kernel olein blends before and after chemical interesterification. *Journal of the American Oil Chemists' Society*, **79(11)**: 1137-1144.

Ornla-ied, P., Sonwai, S. & Lertthirasuntorn, S. 2016. Trans -free margarine fat produced using enzymatic interesterification of rice bran oil and hard palm stearin. *Food Science and Biotechnology*, **25(3)**: 673-680.

Reshma, M.V., Saritha, S.S., Balachandran, C. & Arumughan, C. 2008. Lipase catalyzed interesterification of palm stearin and rice bran oil blends for preparation of zero trans shortening with bioactive phytochemicals. *Bioresource Technology*, **99(11)**: 5011-9.

Ribeiro, A.P.B., Basso, R.C., Grimaldi, R., Gioielli, L.A., dos Santos, A.O., Cardoso, L.P. & Guaraldo Gonçalves, L.A. 2009. Influence of chemical interesterification on thermal behavior, microstructure, polymorphism and crystallization properties of canola oil and fully hydrogenated cottonseed oil blends. *Food Research International*, **42(8)**: 1153-1162.

Shi, Y., Liang, B. & Hartel, R.W. 2005. Crystal morphology, microstructure, and textural properties of model lipid systems. *Journal of the American Oil Chemists' Society*, **82(6)**: 399-408.

Siti Hazirah, M.F., Norizzah, A.R. & Zaliha, O. 2013. Effects of chemical interesterification on the physicochemical, microstructural and thermal properties of palm stearin, palm kernel oil and soybean oil blends. *Food Chemistry*, **137(1-4)**: 8-17.

Soares, F.A.S.D.M., Da Silva, R.C., Hazzan, M., Capacla, I.R., Viccola, E.R., Maruyama, J.M. & Gioielli, L.A. 2012. Chemical interesterification of blends of palm stearin, coconut oil, and canola oil: Physicochemical properties. *Journal of Agricultural and Food Chemistry*, **60(6)**: 1461-1469.

Timms, R.E. 1984. Phase behaviour of fats and their mixtures. *Progress in Lipid Research*, **23(1)**: 1-38.

Ruan, X., Zhu, X.M., Xiong, H., Wang, S.Q., Bai, C.Q. & Zhao, Q. 2014. Characterisation of zero-trans margarine fats produced from camellia seed oil, palm stearin and coconut oil using enzymatic interesterification strategy. *International Journal of Food Science and Technology*, **49(1)**: 91-97.

Zhang, H., Smith, P. & Adler-Nissen, J. 2004. Effects of degree of enzymatic interesterification on the physical properties of margarine fats: solid fat content, crystallization behaviour, crystal morphology, and crystal network. *Journal of Agricultural and Food Chemistry*, **52(14)**: 4423-4431.