Jurnal Teknologi

Full Paper

Effects of Chemical Interesterification on the Physicochemical Properties of Palm Stearin and Rice Bran Oil Blends

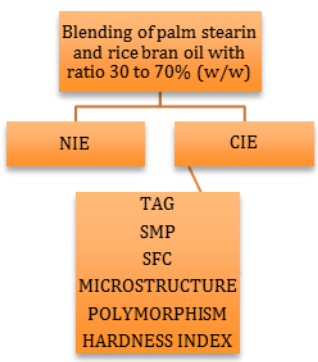
Norizzah Abd Rashid[a]*, Tunku Saidatul Sa'adiah Tunku Safuan[a], Amalyna Teja Kelana[a], Mohd Akram Zuher[a], Zaliha Omar[b]

[a]Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[b]Malaysian Palm Oil Board, No. 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

Article history
Received
21 August 2015
Received in revised form
13 November 2015
Accepted
20 January 2016

*Corresponding author
norizzah850@salam.uitm.edu.my

Graphical abstract



Abstract

Palm stearin (PS) and rice bran oil (RBO) were blended in the mass ratio of 100:0, 70:30, 50:50, 30:70 and 0:100. The oil blends were subjected to chemical interesterification (CIE) catalyzed by sodium methoxide (0.2% w/w). The following analysis were carried out before and after CIE: triacylglycerol (TAG) composition, slip melting point (SMP), solid fat content (SFC), microstructure, polymorphism and hardness index (HI). After chemical interesterification, there were decrease and increase in the amount of several TAG. Changes in TAG composition caused changes in the physical properties of the blends. Both SMP and SFC of all blends decreased significantly ($p<0.05$) after interesterification except for RBO. Chemical interesterification also reduced the eutectic interaction at 5°C. However, for hardness index, only blends with 50% and 100% PS decreased significantly ($p<0.05$) from 33.197 to 26.097 and 5.397 to 3.720, respectively. The crystals of the blends became smaller and reduced in number after interesterification. Interesterification promoted the formation of more β' crystals than β in all blends. The 30:70 PSRBO blend was the most suitable for margarine production as it melted close to body temperature.

Keywords: Blending, chemical interesterification, palm stearin, rice bran oil, physicochemical properties

1.0 INTRODUCTION

A demanding challenge that the food industry has been facing is replacement of trans fat in various food products. Trans fat increased low-density lipoprotein (LDL) levels and lowered high-density lipoprotein (HDL) plasma which increase the risk of coronary heart disease [1]. Consequently, effort had been made to replace trans fat through development of formulations and processes which are both beneficial towards functionality and economic viability.

Blending is the simplest method of fat modification whereby it involves mixing two or more different kinds of fat to produce fat with greater oxidative stability, higher nutritive value and desired melting behaviour with a low or zero-level of trans fatty acid. However, simple blending of hard and soft vegetable oils is not sufficient as the solid and liquid fractions separate during storage [2]. Hence, interesterification had become one of the alternatives to produce products without trans fatty acids.

Palm stearin (PS) is the solid fraction obtained by fractionation of palm oil after crystallisation at a controlled temperature. Palm stearin is not used directly for edible purposes due to its high melting point ranging from 45 to 55°C, giving the product low plasticity and incomplete melting at body temperature [3&4]. However it could be appropriately blended and/or interesterified with liquid oils in order to modify the physical characteristics to meet the functional properties and the quality required.

Rice bran oil is extracted from the germ and the inner husk of rice. Rice bran oil contains a range of fats with 47% of monounsaturated, 37% polyunsaturated, and 20% saturated fatty acids [5]. The oil may also offer some health benefits as it contains oryzanol, an antioxidant that help to prevent heart attacks; phytosterols, compounds believed to help lower cholesterol absorption; and relatively high amounts of vitamin E, sterols, γ-oryzanol and tocotrienol [5].

Interesterification is a process that involve the intramolecular and intermolecular rearrangement of fatty acid on a triacylglycerol backbone [6]. The rearrangement of fatty acids can occur randomly or specifically, depends on the type of catalyst used. Chemical interesterification utilised sodium methoxide as catalyst where rearrangement of fatty acids occurs randomly. According to Karabulut *et al*. [7], interesterification of liquid oil with hard fat is the most flexible way to produce trans-free fat, yielding fat base with desirable properties for production of margarines, shortening and spread.

The objective of this study is to evaluate the effect of chemical interesterification on selected physicochemical properties of palm stearin and rice bran oil blends and to identify the most suitable blend for certain food applications.

2.0 ISSUES OF THE STUDY

Refined, bleached and deodourised (RBD) palm stearin (PS) was obtained from Golden Jomalina Sdn. Bhd., Klang, Selangor, Malaysia and rice bran oil (RBO) was obtained from 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.

Palm stearin and rice bran oil were melted in an oven (Protech FSD-380, Malaysia) at 70°C prior to use. The liquefied PS was blended with RBO in the following ratio: PS:RBO = 100:0, 70:30, 50:50, 30:70 and 0:100. The chemical interesterification was carried out according to MPOB Test Method [8], where 0.2% sodium methoxide was added as the catalyst. The non-interesterified oil is abbreviated as NIE and interesterified oil as CIE.

The SMP determination for the NIE and CIE blends were conducted according to AOCS Method C.c.3.25 [9]. The analysis were conducted in triplicates. The solid fat content (SFC) was determined according to MPOB Test Method [8] using Bruker Minispec pulsed Nuclear Magnetic Resonance (pNMR) spectrometer (Karlsruhe, Germany). The SFC was measured in triplicates in the temperature range of 5 to 55˚C with 5 ˚C interval. The reported value is the average of the three readings.

The TAG profiles of non-interesterified and interesterified PS and RBO blends were analysed using High-Performance Liquid Chromatograph model Waters 2414 (Massachusetts, USA) using the refractive index (RI) Detector, Waters 600 pump and Waters 600 controller. A Lichrosphere RP-18 column (250 mm x 4 mm) of 5-μm particle size (Merck, Darmstadt, Germany) with acetone/acetonitrile (75:25% v/v) as the eluent at flow rate of 1.0 ml/min. Identification of TAG was done by comparison of retention time with those of commercial TAG standards.

The polymorphism of the fat crystals in the fat blends was determined by X-ray diffraction using the Enraf Nonius Model FR592 (Delft, The Netherlands). This instrument was fitted with fine copper X-ray tube. Flat stainless-steel plates with rectangular holes were used as sample holders. The samples were melted at 70˚C and tempered at 20˚C for 30 minutes. Short spacings of the X-ray film were measured with a Guinier viewer (Enraf Nonius Delft, The Netherlands). The levels of β'and β crystals in the mixtures were estimated by the relative intensity of the short spacings at 4.2 and 4.6 Å.

The microstructure of the blended fats was examined using Polarized Light Microscope Model Leica, DMLP (Wetzlar, Germany) which was equipped with a Linkam THMS 600 temperature controller stage and a JVC 3-CCD colour video camera. The sample was first heated to 70 ˚C for 10 minutes and then rapidly cooled (50˚C/min) to 20˚C. The sample was tempered at 20 ˚C for 30 minutes before measurement. About 10 μl of the melted fat was placed on a glass slide and covered with a glass slip to give homogeneous distribution. Temperature was thermostatically controlled by a Linkam TP 94 multiramp temperature programmer and LNP automatic cooling system (Linkam, Tadworth, Surrey, United Kingdom). Liquid nitrogen was used as the coolant. The photomicrograph of the crystal was taken at 200x magnification.

The hardness of the blends was determined according to MPOB Test Method [8], by cone penetrometer (Petrotest, PNR-12) using 45g cone with an angle of 20°.

3.0 RESULTS AND DISCUSSION

3.1 TAG Composition

Chemical interesterification (CIE) altered the TAG composition of the oil blends as shown in Table 1. The CIE generated a few changes in the TAG composition of PS and RBO. After interesterification, the relative concentrations of PLO, POP, POO and OOO in PS increased from 6.65%, 33.48%, 13.44% and 2.10% to 7.03%, 34.00%, 14.20% and 2.35%, respectively. Such considerable increase is due to random arrangement of the fatty acids during intraesterification.

The concentration of PPP and LaLaM decreased after interesterification in all of the blends except for RBO. The concentration of POP and OOO also decreased for all the blends except for PS. Chemical interesterification causes reduction in the amount of high-melting TAGs in the binary blends. However there were only few changes in TAG for PS and RBO after CIE. This is presumed to be due to intraesterification [4&10]. The POO concentration increased after CIE in all blends but decreased in the RBO. The amount of CaLaLa in all blends also decreased after CIE.

The most obvious changes were observed with significant reduction (p<0.05) of high-melting TAG (PPP and POP) in the binary blends after interesterification. The decrease in the amount of these high-melting TAG is accompanied by the increase in the amount of low-melting TAG such as OLL and PLL.

3.2 Slip Melting Point

The SMP of the non-interesterified and interesterified PS and RBO blends are shown in Figure 1. For both NIE and CIE, the SMP of the blends decreased significantly (p<0.05) with increase of RBO. This result is in line with the finding by [4,10,11&12], where the addition of soft oil decreased the SMP of PS blends due to the low amount of high-melting TAGs in the soft oil.

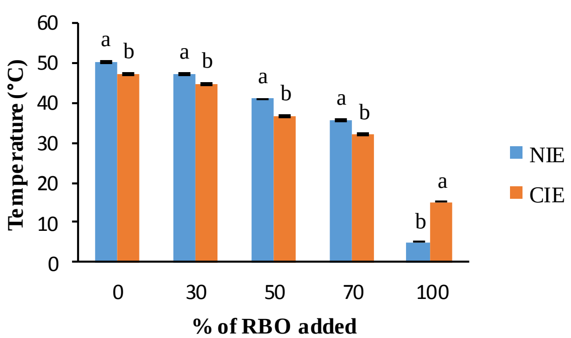


Figure 1 Slip melting point of NIE and CIE of palm stearin (PS), rice bran oil (RBO) and their binary blends

Figure 1 Slip melting point of NIE and CIE of palm stearin (PS), rice bran oil (RBO) and their binary blends
As shown in Table 1, the increase in the amount of RBO lowered the amount of high-melting TAG (PPP and POP) which in turn decreased the melting point of the blends. The CIE blends showed lower SMP than their corresponding NIE blends significantly (p<0.05), except for 100% RBO where CIE significantly increased the SMP. The SMP of PS decreased significantly (p<0.05) from 50.17°C to 47.23°C after interesterification.

In order to achieve the properties of fats suitable for manufacture of margarine and confectioneries with minimum waxiness to have a good oral-melt, it is important that the SMP is lower than the body temperature [10]. Thus, from the result, CIE is observed to be effective in reducing the SMP of the blends. With addition of 50% of RBO followed by interesterification, SMP below body temperature was achieved (36.77°C).

3.3 Solid Fat Content (SFC)

Solid fat content (SFC) is useful in determining the ratio of solid and liquid phases in a plastic fat. Changes in the triacylglycerols composition of the fats after interesterification were accompanied by changes in their solid fat content values [12]. Palm stearin (PS) had the highest SFC at all temperatures and melted completely at 55°C. The SFC of both non-interesterified (NIE) and chemically interesterified (CIE) blends increased with increasing amount of PS in the blends due to the presence of high-melting triacylglycerols. Rice bran oil is readily liquid at room temperature, thus had the lowest SFC at all temperatures. This is because RBO is rich in low melting triacylglycerols such as OOL, PLL, and POO as indicated in Table 1. Addition of RBO decrease the SFC of the binary blends.

Figure 2 indicated that all the interesterified blends were completely melted at lower temperatures compared to their corresponding non-interesterified blends except for RBO and this is in line with the SMP observation. This may be due to the decrease in high-melting TAG, such as PPP (S3) and POP (S2U) and the formation of low-melting TAG such as POO (SU2) and OLL (U3). Similar findings was reported by [13]. However for RBO, interesterification resulted in the increase of the SFC which may be due to the increase in high-melting TAG such as PPP and decrease in low melting TAG (OOO, POO and PLO) as depicted in Table 2.

According to Riberio *et al*. [14], temperature of 4-10°C indicates fats spreadability at refrigeration temperature, where blends with less than 32% SFC at 10°C will have good spreadability. Based on the results obtained both the NIE and CIE blends with 30% PS can be used in margarine production with good spreadability as the SFC values were less than 32% at 10°C which were 23.80% and 16.82%, respectively. The CIE blend with 50% PS also has SFC value of 30.79% at 10°C. Interesterification of PS, a hard fat with RBO, soft oil gives better properties in terms of melting behaviour and spreadability, as analysed by the pNMR for their SFC profiles. The CIE blends are more suitable to be utilised into products such as soft margarine and chocolates which require good spreadability and melts at body temperature.

Mixture of fats with different composition may show a eutectic effect. A mixture that exhibits eutectic effect has lower SFC than either one of the two pure fats, showing that the fats are not compatible with each other [15]. Figure 3a shows that eutectic interaction occurred in the non-interesterified PS and RBO blends at 5°C. The eutectic behaviour occurs in NIE blends because of the differences in the molecular size of the TAGs [4&12]. This shows that the PS and RBO are incompatible with each other at 5°C before interesterification. After interesterification, eutectic interaction at 5°C was reduced (Figure 3b). This indicated that CIE is effective in reducing the eutectic effects and improve miscibility between two fats. However, eutectic effects can be beneficial in products such as shortening and margarine eventhough these effects are usually undesirable [16].

3.4 Microstructure

Fat crystals examined under polarized light microscope (PLM) will appears bright while the liquid oil will remains dark due to its birefringent properties [17]. The shape and size of fat crystal clusters are very much influenced by temperatures, polymorphism, TAG profile and SFC. According to Marangoni and Tang [18], the shape of the crystal cluster can be spherulitic, blade or needle-shaped.

Figure 4 coded a, c, e and g show the microscopy image of fat crystals for the non-interesterified blends of 100% PS, 70% PS, 50% PS and 100% RBO taken at 20°C after tempered for 30 minutes while Figure 4 coded b, d, f and h show the microscopy image of fat crystals for chemically interesterified (CIE) blends. The NIE blends had smaller crystals and densely packed. On the other hand, the crystals in the CIE blends were longer, needle-like and are spherulite-shaped. This is probably due to the presence of β' crystals. This observation agreed with Norizzah *et al*. [4] and Adhikari *et al*. [19], who reported that chemical interesterification of oil blends leads to formation of finer spherulite crystals than that of the NIE blends.

In both NIE and CIE blends, the addition of RBO caused decrease in number of crystals formed due to dilution effects. The NIE blend with 50% PS still exhibits a large number of small crystals but after CIE, fewer large crystals were formed at the measured temperature. As the amount of RBO increased to 70%, no crystal formation were observed in both NIE and CIE blends due to dilution effects. A more compact and finer spherulite crystals indicate that the slip melting point of the blends were higher. This is in line with SMP where NIE blends have higher SMP than that of CIE except for 100% RBO.

3.4 Polymorphism

Table 1 shows all the NIE and CIE blends of PS:RBO contained mixtures of β and β'. All the NIE blends contained more β polymorphic form except for the NIE PS which had more β' polymorphic form in the mixture. This is due to the high amounts of POP triacylglycerol which is a β' former present in the palm stearin [20].

Addition of RBO caused the β polymorphic form to be more dominant. According to deMan [21], this is either due to the effect of dilution where the β polymorphic form tends to dominate as more fat is diluted with liquid oil or because the amount of β' polymorphic form is too low in concentration to exert any influence. The polymorphic forms of both NIE and CIE RBO could not be determined as both were liquid at the measured temperature.

Table 1 Polymorphic forms of the interesterified and non-interesterified palm stearin and rice bran oil blends

Blends (PS:RBO)	NIE	CIE
100:0	β' > β	β' > β
70:30	β > β'	β' > β
50:50	β > β'	β' > β
30:70	β > β'	β' > β
0:100	-	-

After CIE, the β' polymorphic form was dominant in all blends. This is due to the randomisation of fatty acids in PS glycerols that leads to the diversification of the fatty acids in the glycerols of the blends [4&22]. The main fatty acid in PS, the palmitic acid could have been interchanged with oleic acid which is present in large quantities in RBO. This result shows that CIE facilitates the formation of β' forms of oil blends and this agreed with the finding by Mayamol *et al*. [23].

Margarines with high fluidity and suspension stabilty requires β' formulation [24]. The β' crystals contribute in production of fat-based products with glossy surface and a smooth texture. The β crystals are not desired as it increase the hardness while decreasing the spreadability of margarines [19].

3.5 Hardness Index

The hardness index (HI) of PS, RBO and their blends were evaluated to measure the consistency of the fats with the addition of PS as well as the effect of CIE on the texture of the fats. Table 2 shows that the HI of NIE and CIE blends increased as the PS increased. Both the NIE and CIE PS had the highest ($p<0.05$) HI. This is due to the high amount of high-melting triacylglycerols (TAG) such as PPS, POP and POS as indicated in Table 1. Based on the result, addition of RBO reduced the HI of NIE and CIE blends significantly ($p<0.05$) except for CIE blends with 50% and 30% PS. The reduction in HI is due to dilution effect and the presence of low-melting TAGs contributed by the RBO in the blends.

In general, CIE blends had lower HI than those of NIE. This result is in accordance with findings by Adhikari *et al*. [19]. However, only blends with 100% and 50% PS differ significantly at $p<0.05$ from their NIE blends. This shows that CIE process is effective in softening the fat blends. The HI of both NIE and CIE RBO could not be detected because both were in liquid form at the measured temperature.

Therefore, from the observation, blends with 30% PS had the lowest hardness index which shows that the high amount of RBO oil has contributed to the softness of the blends. Softness of fat contributes to its plasticity which gives a good mouth feel suitable for manufacture of soft margarine and confectionery. According to Nusantoro [25], the softer fat could be utilised in the margarine formulation to give the best possible consistency requirements for composition, packing and handling. Hence, from the HI evaluation, CIE blend with 30% PS is the most suitable blend for these purposes due its soft texture.

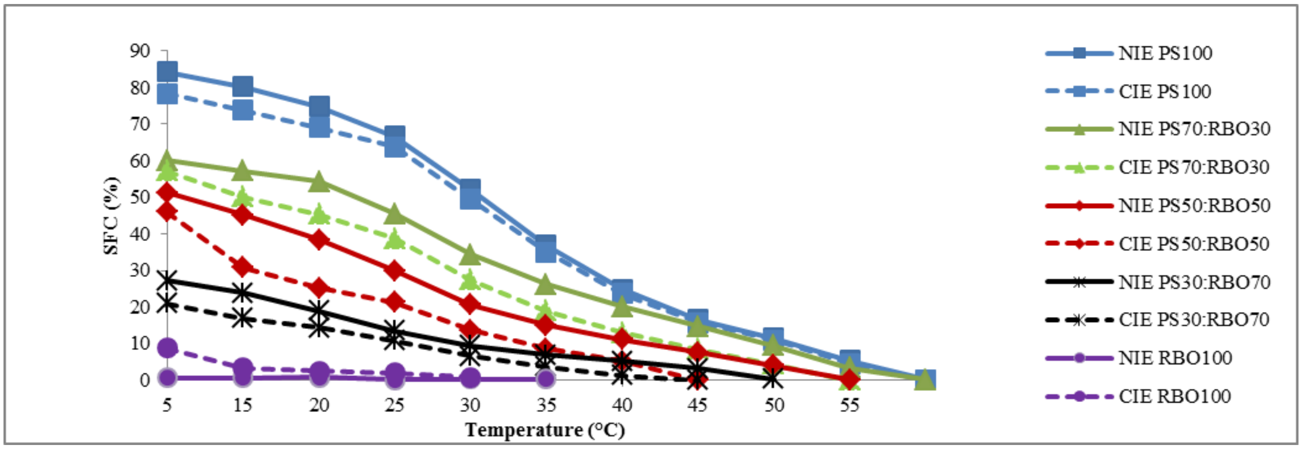


Figure 2 Solid fat content of NIE and CIE of palm stearin (PS), rice bran oil (RBO) and their binary blends

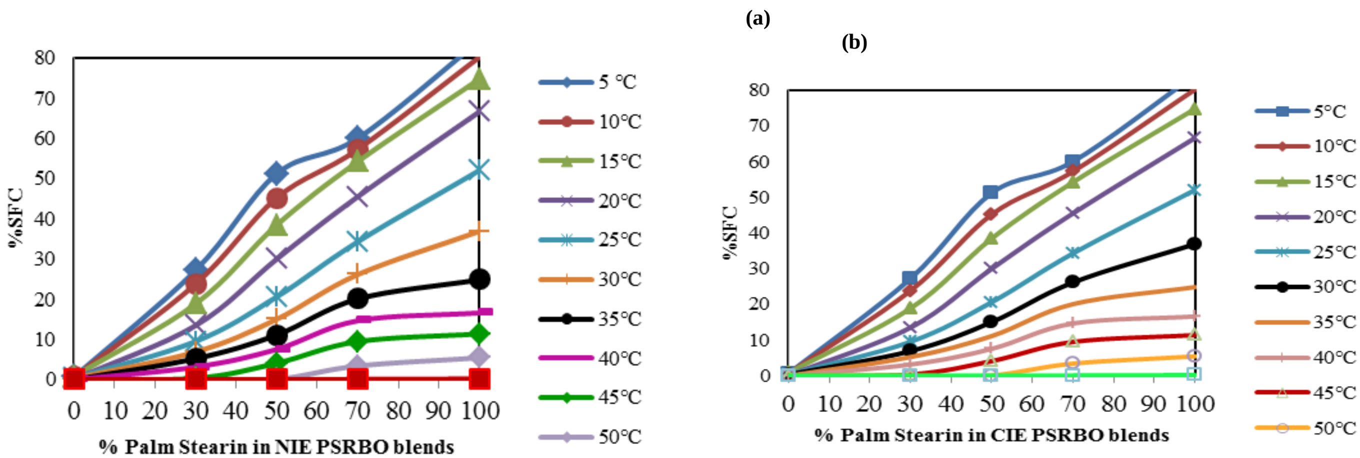


Figure 3 Eutectic effects of (a) NIE (b) CIE PS, RBO and their binary blends at different temperature

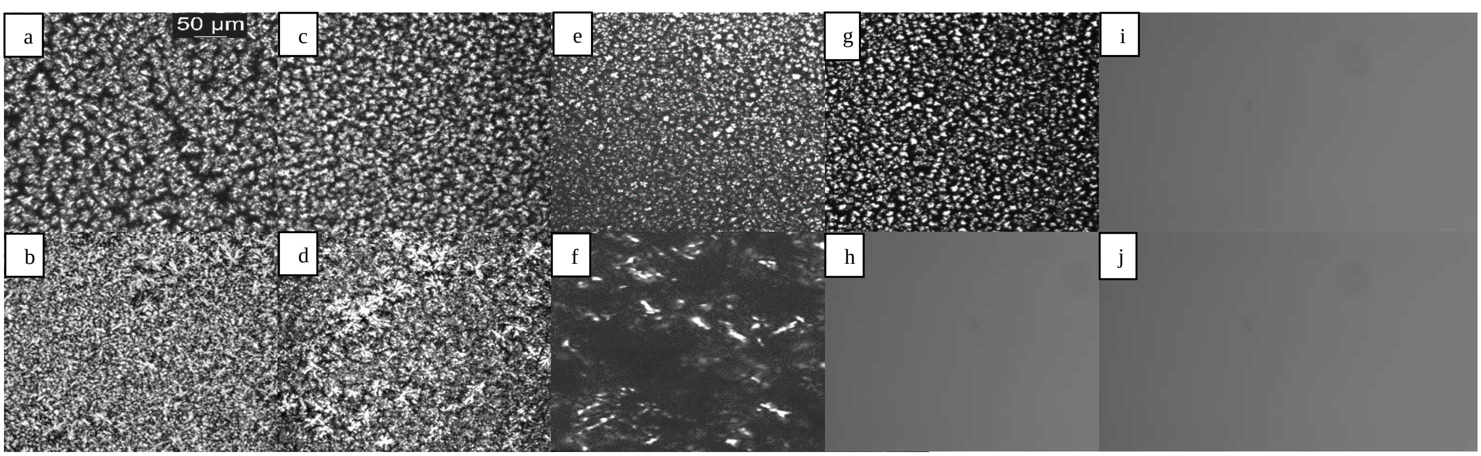


Figure 4 Microstructure images of PS:RBO blends; a) NIE PS100; b) CIE PS100; c) NIE PS70; d) CIE PS70; e) NIE PS50; f) CIE PS50; g) NIE PS30; h) CIE PS30; i) NIE RBO100; CIE RBO100 tempered for 20 minutes at 20°C at rate 5°C/min, Magnification 200x and bar represent 50µ

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

Blend PS:RBO	TAGs (%)																		
	CLaLa	**CaLaLa**	**LaLaLa**	**LaLaM**	**LaLaO**	**LaLaP**	**OLL**	**PLL**	**OOL**	**PLO**	**PLP**	**OOO**	**POO**	**POP**	**PPP**	**SOO**	**POS**	**PPS**	**SOS**
NIE 100:0	0.22	0.08	0.25	1.02	1.04	0.38	0.52	2.15	6.12	6.65	1.47	2.10	13.44	33.48	17.33	2.09	5.93	4.58	1.15
CIE 100:0	-	-	0.03	0.34	1.13	0.54	0.57	1.19	5.65	7.03	1.47	2.35	14.20	34.00	17.26	2.08	6.21	5.19	0.75
NIE 70:30	-	1.41	0.38	3.13	4.00	-	0.57	4.78	8.11	6.39	0.95	5.54	13.21	26.84	13.51	1.09	4.31	3.56	2.22
CIE 70:30	0.28	0.11	0.27	1.99	3.17	0.79	1.50	4.95	13.68	9.87	1.00	4.41	16.29	22.67	10.52	0.36	4.16	3.03	0.93
NIE 50:50	0.09	2.25	0.51	5.07	6.07	-	0.52	6.93	10.61	6.76	1.12	8.30	14.54	19.80	9.65	1.61	3.34	1.82	1.01
CIE 50:50	0.73	0.31	0.66	3.86	4.77	1.01	0.84	7.34	16.57	10.75	-	4.83	14.66	16.00	5.68	2.23	4.50	3.39	1.88
NIE 30:70	0.14	3.11	0.76	7.04	7.81	-	0.67	9.03	12.65	6.19	0.54	9.00	13.37	14.33	7.77	1.91	2.92	2.15	0.61
CIE 30:70	0.10	1.65	0.69	6.51	5.91	-	0.82	9.96	17.76	8.40	-	6.35	15.22	13.35	3.90	0.85	2.82	1.65	2.81
NIE 0:100	0.17	5.01	1.21	10.82	11.18	-	0.46	12.78	16.06	5.82	0.20	10.32	12.63	7.23	1.50	1.63	1.23	0.78	0.97
CIE 0:100	0.02	4.60	0.71	13.41	8.36	-	0.73	16.58	17.49	5.52	0.91	7.85	10.69	7.08	1.90	1.72	1.08	0.53	0.83

4.0 CONCLUSION

Blending of palm stearin with rice bran oil reduce the SMP, SFC and HI of all binary blends due to dilution effect. After chemical interesterification (CIE), SMP and SFC of all the blends were significantly ($p<0.05$) lower compared to their corresponding non-interesterified blends except for RBO. The hardness index (HI) also decreased significantly ($p<0.05$) for all the blends except for blends with 30% and 70% of PS. The further reduction in SMP, SFC and HI of the blends after CIE is due to decrease in high melting triacylglycerols. Chemical interesterification also led to formation of needle-like and spherulite-shaped crystals with more β' polymorphic form obtained. Blend containing 30% PS was the most suitable for margarine formulation as it melt closed to body temperature.

Acknowledgement

The authors gratefully acknowledge the financial support given by Ministry of Higher Education [600-RMI/FRGS 5/3 (11/2013)] and facilities provided by Universiti Teknologi MARA (UiTM) and Malaysian Palm Oil Board (MPOB).

References

[1] Mensink, R. P., Zock, P. L., Kester, A. D., and Katan, M. B. 2003. Effects of Dietary Fatty Acids and Carbohydrates on The Ratio of Serum Total To HDL Cholesterol and On Serum Lipids and Apolipoproteins: A Meta-Analysis of 60 Controlled Trials. *Am. J. Clin. Nutr.* 77(5): 1146-1155.

[2] Erickson, D.R. 1995. *Practical Hand Book of Soybean Processing and Utilization*. Champaign: American Oil Chemist's Society Press.

[3] Dian, N. L. H. M., Sundram, K., and Idris, N. A. 2007. Effect of Chemical Interesterification on Triacylglycerol and Solid Fat Contents of Palm Stearin, Sunflower Oil and Palm Kernel Olein Blends. *European Journal of Lipid Science and Technology*. 109(2): 147-156.

[4] Norizzah, A. R., Chong, C. L., Cheow, C. S., and Zaliha, O. 2004. Effects of Chemical Interesterification on Physicochemical Properties of Palm Stearin and Palm Kernel Olein Blends. *Food Chem*. 86: 229-235.

[5] Valantina, S. R., Sahayaraj, P. A., and Prema, A. A. 2010. Antioxidant Stability In Palm and Rice Bran Oil. *Rasayan Journal of Chemistry*. 44-50.

[6] Scrimgeour, C. 2005. *Bailey's Industrial Oil and Fat Products. 6th Edition*. New Jersey: John Wiley and Son Inc.

[7] Karabulut I., Turan, S., and Ergin, G. 2004. Effects of Chemical Interesterification On Solid Fat Content and Slip Melting Point Of Fat/Oil Blends. *Food Res. Technol*. 218: 224-229.

[8] Methods of Test for Palm Oil and Palm Oil Products (MPOB Test Method). 2004. Malaysian Palm Oil Board, Malaysia.

[9] Official and Tentative Methods of the American Oil Chemist's Society (AOCS). 1990. Champaign: American Oil Chemist's Society Press.

[10] Hazirah, S. F. M., Norizzah, A. R., and 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 Chem.* 137(1): 8-17.

[11] Farmani, J., Hamedi, M., Safari, M., and Madadlou, A. 2007. Trans-Free Iranian Vanaspati Through Enzymatic And Chemical Transesterification Of Triple Blends Of Fully Hydrogenated Soybean, Rapeseed And Sunflower Oils. *Food Chem*. 102(3): 827-833.

[12] Soares, F. A. S. D. M., da Silva, R. C., da Silva, K. C. G., Lourenço, M. B., Soares, D. F., and Gioielli, L. A. 2009. Effects of chemical interesterification on physicochemical properties of blends of palm stearin and palm olein. *Food. Res. Int*. 42(9): 1287-1294.

[13] Ribeiro, A. P. B., Basso, R. C., Grimaldi, R., Gioielli, L. A., dos Santos, A. O., Cardoso, L. P., and Gonçalves, L. A. G. 2009. Influence Of Chemical Interesterification On Thermal Behavior, Microstructure, Polymorphism And Crystallization Properties Of Canola Oil And Fully Hydrogenated Cottonseed Oil Blends. *Food. Res. Int.* 42(8): 1153-1162.

[14] Wassell, P., and Young, N. W. G. 2007, Food Applications Of Trans Fatty Acid Substitutes. *Int. J. Food Sci. Technol*. 42: 503-517.

[15] Bigalli, G. L. 1988. Practical Aspects Of The Eutectic Effect On Confectionery Fats And Their Mixtures. *Manuf. Confect*. 68(5): 65-80.

[16] Timms, R. E. 1984. Phase Behaviour Of Fats And Their Mixtures. *Progress in Lipid Research*. 23(1): 1-38.

[17] Liu, Y., Meng, Z., Zhang, F., Shan, L., and Wang, X. 2010. Influence Of Lipid Composition, Crystallization Behavior And Microstructure On Hardness Of Palm Oil-Based Margarines. *European Food Research and Technology*. 230(5): 759-767.

[18] Marangoni, A. G., and Tang, D. 2006. Microstructure And Fractal Analysis Of Fat Crystal Networks. *J. Am. Oil Chem. Soc*. 83: 377-388.

[19] Adhikari, P., Shin, J. A., Lee, J. H., Hu, J. N., Zhu, X. M., Akoh, C. C., and Lee, K. T. 2010. Production Of Trans-Free Margarine Stock By Enzymatic Interesterification Of Rice Bran Oil, Palm Stearin And Coconut Oil. *Journal of the Science of Food and Agriculture*. 90(4): 703-711.

[20] deMan, J. M. 1999. *Principles of Food Chemistry*. 3rd Edition. New York: Kluwer Academic/Plenum Publishers.

[21] deMan, L., and deMan, J. M. 1994. Functionality Of Palm Oil In Margarine And Shortening Formulations. *Malaysian Oil Science and Technology*. 4: 56-60.

[22] Gold, I. L., Ukhun, M. E., and Akoh, C. C. 2011. Characteristics Of Eutectic Compositions Of Restructured Palm Oil Olein, Palm Kernel Oil And Their Mixtures. *J. Am. Oil Chem. Soc*. 88(11): 1659-1667.

[23] Mayamol, P. N., Balachandran, C., Samuel, T., Sundaresan, A., and Arumughan, C. 2004. Zero Trans Shortening Using Palm Stearin And Rice Bran Oil. *J. Am. Oil Chem. Soc.* 81: 407-413.

[24] O'Brien, R. D. 2009. *Fats and oils: formulating and processing for applications*. 3rd Edition. Florida: CRC Press.

[25] Nusantoro, B. P. 2009. Physicochemical Properties of Palm stearin and Palm Mid Fraction Obtained by Dry Fractionation. *Agritech*. 29: 3.