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Trans-free Margarine Fat Produced Using Enzymatic Interesterification of Rice Bran Oil and Hard Palm Stearin

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Abstract *Trans*-free interesterified fats were prepared from blends of hard palm stearin (hPS) and rice bran oil (RBO) at 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, and 80:20 weight % using immobilized *Mucor miehei* lipase at 60°C for 6 h with a mixing speed of 300 rpm. Physical properties and crystallization and melting behaviors of interesterified blends were investigated and compared with commercial margarine fats. Lipase-catalyzed interesterification modified triacylglycerol compositions and physical and thermal properties of hPS:RBO blends. Slip melting point and solid fat contents (SFC) of all blends decreased after interesterification. Small, mostly β' form, needle-shaped crystals, desirable for margarines were observed in interesterified fats. Interesterified blend 40:60 exhibited an SFC profile and crystallization and melting characteristics most similar to commercial margarine fats and also had small needle-like β' crystals. Interesterified blend 40:60 was suitable for use as a *trans*-free margarine fat.

Keywords: margarine, *trans*-free, rice bran oil, hard palm stearin, enzymatic interesterification

Introduction

Margarine is a popular table spread as a substitute for butter. Margarine is a water-in-oil emulsion comprising at least an 80% lipid phase. Traditionally, margarine fats are obtained via a partial hydrogenation process resulting in *trans* fatty acids (TFAs) which, in turn, provide desirable physical and textural characteristics for margarine (1). However, TFAs have been associated with an increased risk of coronary heart disease (CHD) due to increased serum LDL cholesterol and decreased HDL cholesterol levels (2). Recent studies have revealed a connection between increased intake of total TFAs and an increases risk of CHD events (3), leading to a demand for healthy *trans*-free fat products.

One of the best methods for production of *trans*-free fats is the interesterification process that alters the position of fatty acids (FAs) in the structure of triacylglycerol (TAG) molecules and leads to changes in the physical properties of fats (4). Interesterification can either decrease or increase the melting temperature and solid fat content (SFC) of fat blends. Interesterification can be divided into chemical interesterification and enzymatic interesterification (EI). The former category involves high temperatures and catalysts whereas the latter uses enzymes as biocatalysts. EI has been widely used because it is a mild, region-and stereo-specific, and easily controlled reaction and is an effective method for changing physical and chemical properties of fats and oils without creating undesirable TFAs (5).

Production of a *trans*-free margarine fat from rice bran oil (RBO) and hard palm stearin (hPS) was investigated in this study. RBO is healthy edible oil that is an excellent source of tocopherols, tocotrienols and γ-oryzanol (6). However, the oil is liquid at room temperature due to a high content of unsaturated FAs that prevents use as a sole oil ingredient in many margarines, spreads, and shortenings because products would be too soft with a too low melting temperature. Palm stearin (PS) is the hard fraction of palm oil that is obtained via palm oil fractionation. Due to a high proportion of saturated FAs, PS is a natural source of solid fat that provides strength and structure to products (7). However, simply adding PS to RBO cannot create a good fat blend with a sharp melting characteristic. Therefore, an additional method, like EI, is necessary for improvement of the blend quality.

There have been a number of recent studies regarding EI of PS and RBO blends (8-13), where PS was used as a source of saturated FAs that were transferred to the TAG structure of RBO in order to create low *trans* or *trans*-free fat blends with improved crystallization and melting characteristics for shortenings and margarines. However, in this study, PS was solvent-fractionated and only the solid hard palm stearin (hPS) fraction was used. RBO and hPS were blended in different proportions and the structure of blends was modified via EI

to produce a *trans*-free margarine fat. Melting and crystallization profiles, SFC, polymorphism, and crystal morphologies of interesterified blends were investigated and compared with physical blends and margarine fats extracted from commercial margarines. Then, the best blend ratio was determined as a *trans*-free margarine fat for use in production of *trans*-free margarines.

Materials and Methods

Materials and reagents PS and RBO were obtained from Lam Soon Public Company Limited, Samutprakarn, Thailand in August of 2014. Lipozyme RM IM (1,3-selective lipase) from *Mucor miehei* was purchased from Sigma-Aldrich (St. Louis, MO, USA). Molecular sieves (3A, beads, 4-8 mesh) were purchased from Sigma-Aldrich. Five different commercial margarines were purchased from local supermarkets in Bangkok, Thailand in September of 2014. Margarine fats were extracted from commercial margarines following the methods provided by Kim *et al*. (1). Standard fatty acid methyl esters for FA analysis using GC were purchased from AccuStandard Inc. (New Haven, CT, USA). 1,2,3-Trioleoyl-glycerol (OOO) and 1,2-dipalmitoyl-3-stearoyl-glycerol (POP) as standards for TAG analysis using HPLC were purchased from Sigma-Aldrich. Organic solvents and chemicals were purchased from Labscan Asia Co. Ltd. (Bangkok, Thailand) and Macron Fine Chemicals (Center Valley, PA, USA). All chemicals and reagents for analysis were of analytical or chromatography grade.

Fractionation of palm stearin PS was solvent-fractionated using 5 mL/g of PS acetone at 35°C for 3 h (14) to obtain a 19.8±1.8% weight of the solid fraction (hPS) and an 81.2±2.2% weight of the liquid fraction.

Fat blending The hPS was melted and blended with RBO into the 7 proportions of hPS:RBO=20:80, 30:70, 40:60, 50:50, 60:40, 70:30, and 80:20 weight %. Immobilized lipozyme RM IM was conditioned in a desiccator using lithium chloride to reduce the water activity (a_w) content to 0.12.

Fatty acid compositional analysis of initial oils The hPS and RBO were converted into fatty acid methyl esters (FAMEs) using AOAC official method 969.33 (15). FAME analysis was performed using a Shimadzu (Kyoto, Japan) GC with a flame ionization detector (GC-FID). The system had a 50 m AT^{TM}-WAX capillary column (0.25 mm internal diameter and 0.20 μm film thickness, Heliflex; Grace, Columbia, MD, USA). Compound identification was carried out using FAME external standards. Helium was used as a carrier gas at a flow rate of 0.5 mL/min with a controlled initial pressure of 93.2 kPa at 120°C. N_2 and air were makeup gases. The injection temperature was 210°C, and the oven temperature program was holding at 120°C for 3 min before increasing at a rate of 10°C/min to 220°C, holding at this temperature for 30 min, increasing at a rate of 5°C/min to 240°C, followed by holding at 240°C for 30 min. The split ratio was 100:1, the injection volume was 1 mL, and the detector temperature was 280°C. All FA contents were reported as percentage areas.

Lipase-catalyzed interesterification Preliminary EI experiments were performed for all hPS:RBO blends in screw-cap glass vials to obtain an optimal reaction time that resulted in the highest conversion rate of TAGs for each ratio of fat blend. EI reactions were carried out at 60°C using conditioned immobilized lipozyme RM IM (10% weight of substrate) and molecular sieves (10% weight of substrate) for 2, 4, 6, 8, and 12 h. The mixing speed was set at 300 rpm using a magnetic stirrer. After reaction, the enzyme and molecular sieves were removed from the mixture via vacuum filtration. Free FAs formed during the reaction were removed using a previous procedure (16). TAG profiles of the reacted samples obtained at 2, 4, 6, 8, and 12 h were then analyzed using an LC-20AD HPLC (Shimadzu). Reactions were carried out in duplicate for each hPS:RBO blend ratio. Quantity changes of main TAG components in blends were plotted against reaction time and the optimum reaction time for each blend was determined from the time when an equilibrium or a plateau was reached. Then, a scale-up reaction was performed for each blend in a 100 mL double-jacket batch type reactor using the determined optimal reaction time. Interesterified blends together with physical blends and commercial margarine fats were then characterized.

Analysis of triacylglycerol compositions TAG compositions of physical and interesterified blends were determined using an LC-20AD HPLC (Shimadzu) with a CBM-20A system controller and an SPD-M20A diode array detector. Two Inertsil ODS-3 reverse C-18 columns (4.6x250 mm; 5 mm particle size) (GL Sciences Inc., Tokyo, Japan) were used in series. The mobile phase consisted of acetone and acetonitrile (63.4:36.4, v/v) with a flow rate of 1 mL/min. The column temperature was set at 25°C with a CTO-10AS column heater (Shimadzu). The injection volume was 20 mL. TAG peaks were identified based on retention time of TAG standards and the report of Tan and Che Man (17). All TAG contents were reported as percentage areas.

Characterization of solid fat contents Changes in solid fat contents (SFC) of physical and interesterified blends and commercial margarine fats as a function of temperature between 15 and 40°C, and melting behaviors, were determined using a pulse-nuclear magnetic resonance (p-NMR) spectrometer (Minispec-mq20; BRUKER, Karlsruhe, Germany) following American Oil Chemists' Society (AOCS) Method Cd 16-81 (18).

Characterization of crystallization and melting profiles Melting and crystallization characteristics of physical and interesterified blends and commercial margarine fats were determined using a Perkin-Elmer Model D8000 differential scanning calorimeter (DSC) (Perkin-Elmer Co., Shelton, CT, USA) following AOCS recommended

procedure Cj 1-94 (18). Each 3-5 mg analytical ample was placed in a 30 mL capacity aluminum pan and hermetically sealed using a pan crimper press. Analytical samples were heated from 20 to 80°C at a rate of 30°C/min and held at this temperature for 10 min to destroy the memory effect, then cooled to −60°C at a rate of 5°C/min. After a 30 min hold at −60°C, analytical samples were heated to 80°C at a rate of 5°C/min. Crystallization and melting profiles were generated during cooling and heating, respectively. Profiles were analyzed using software provided with the DSC (Pyris software; Perkin-Elmer).

Polymorphic characterization Crystal polymorphic forms of interesterified blends and commercial margarine fats were determined using a TTRAX III, X-ray diffractometer (Rigaku Corporation, Tokyo, Japan). Scans were performed in wide angle X-ray scattering (WAXS) mode from 14°2θ to 30°2θ with a scan speed and a step width of 4°2θ/min and 0.01°2θ, respectively. Analytical samples were melted at 80°C for 10 min and poured into 20 mmx25 mmx3 mm rectangular plastic moulds, then left to crystallize at 4°C for 24 h (1), followed by analysis. Generally, short spacings for the β' form of fats are identified with 2 strong spacings at 3.88 and 4.20 Å or 3 strong spacings at 3.71, 3.97, and 4.27 Å (19). In contrast, the β polymorph always displays a strong d-spacing at 4.6 Å (19). In this study, contents of β' and β structures in analytical samples was estimated based on relative intensities of the short spacing of the β form at 4.6 Å and of the β' form at 4.20 Å following a method described by Kim *et al*. (1).

Crystal microstructure The crystal network microstructure of physical and interesterified blends and extracted commercial margarine fats crystallized under static conditions at 4°C was observed under polarized light microscopy (PLM) using an Olympus BX51 (Olympus Optical Co., Ltd., Tokyo, Japan) equipped with a digital camera (Olympus C-7070; Olympus Optical Co., Ltd.). All fat samples were melted at 80°C for 10 min to totally eliminate the memory effect, then 20 mL of each molten fat sample was placed on a glass slide, which was heated to 80°C prior use, and covered using a cover slip. Fat samples were transferred to and stored in a temperature-controlled cabinet maintained at 4±0.2°C for 24 h. A 10x lens was used to acquire gray scale photomicrographs of fat crystals.

Statistical analysis All experiments were performed in duplicate and results were analyzed using an analysis of variance (ANOVA) with the least significant difference test (ANOVA/LSD) at $p<0.05$ using the SPSS software package (SPSS Inc., Chicago, IL, United States).

Results and Discussion

Fatty acid compositions of initial oils The major FA component in hPS was palmitic acid (C16) at 79.90±0.19% and the main FAs in RBO were oleic (C18:1) at 44.81±0.13%, linoleic (C18:2) at 32.14±0.30%, and palmitic (C16) at 18.6±0.31%. The FA composition of RBO reported herein was inconsistent with previous reports (12,20,21).

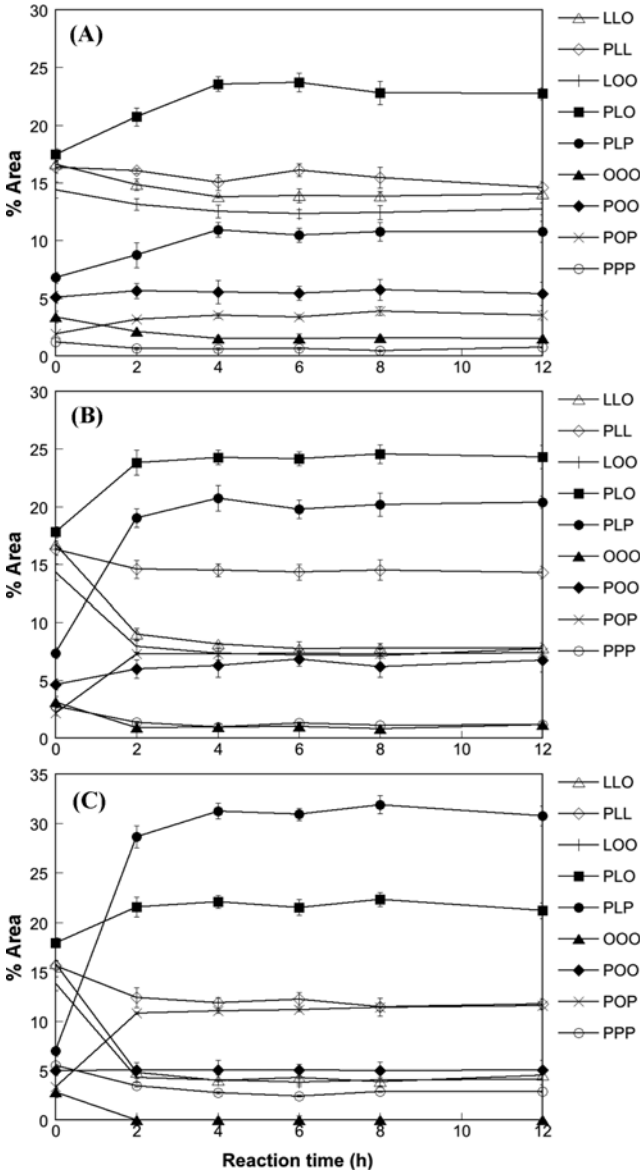

Fig. 1. Changes in relative quantities of triacylglycerol species with reaction time during lipase-catalyzed interesterification of selected hPS:RBO blends; (A) 20:80, (B) 40:60, and (C) 60:40. (P=palmitic acid, O=oleic acid, L=linoleic acids).

Changes in triacylglycerol compositions with time during enzymatic interesterification Changes in relative quantities of different TAG species as a function of reaction time during EI at 60°C were determined for blends 20:80 (Fig. 1A), 40:60 (Fig. 1B), and 60:40 (Fig. 1C). Increasing the reaction time resulted in an increase in PLO, PLP, and POP contents and a concurrent decrease in LLO, PLL, LOO, OOO, and PPP contents. Thus, exchange of palmitic (P), oleic (O), and linoleic (L) acids in TAG species of hPS and RBO occurred on the TAG backbone. In most blends, TAG species changed rapidly during

interesterification and reached equilibrium within 2 h of reaction for blends 30:70, 40:60 (Fig. 1B), and 50:50, 70:30, and 80:20. However, for blends 20:80 (Fig. 1A) and 60:40 (Fig. 1C), exchange of FAs in TAG species required 4-6 h before equilibrium was reached. Once equilibrium was attained, prolonging the reaction time did not result in any further exchange of FAs in TAG species.

In order to attain the highest conversion rate of FAs into new TAGs in scale-up experiments, 6 h was used as an optimal reaction time in this study. Scale-up EI of all fat blends was then performed in a double-jacket batch type reactor using the same conditions as for preliminary experiments (at 60°C with 10% enzyme load and a mixing speed of 300 rpm) and interesterified fats were then analyzed.

Triacylglycerol compositions TAG compositions of fat blends before and after scale-up interesterification reactions are shown in Table 1. Before interesterification, quantities of PLO, PLL, LLO, and LOO decreased. On the other hand, quantities of PPP, POP, and PLP increased as the amount of hPS in unreacted blends increased. After a lipase-catalyzed reaction, amounts of LLO, PLL, LOO, PPP, and OOO in all interesterified blends decreased significantly (p<0.05), compared with physical blends. On the contrary, amounts of PLP increased significantly (p<0.05). Amounts of POP and PLO did not increase significantly, compared with physical blends. Amounts of POO also increased slightly for blends 20:80 to 50:50, but decreased for blends that contained >50% hPS. A progressive increase in PLP amounts in interesterified blends as the content of hPS in physical blends increased was consistent with results from small-scale EI (Fig. 1). PLP contents increased 5.2%, from 6.3 to 11.5%, for the 20:80 blend and increased 24.6%, from 7 to 31.6%, for the 60:40 blend. Exchanging the position of FAs in TAG molecules resulted in formation of some TAG species at the expense of others, leading to changes in crystallization and melting behaviors of interesterified fats.

Slip melting point The slip melting point (SMP) of a fat is the temperature at which a column of fat in an open capillary tube becomes sufficiently fluid to slip or run up the tube when subjected to controlled heating. Prior to interesterification, the SMP value of hPS was 60.3°C. Addition of increasing amounts of hPS gradually increased the SMP value of physical blends from 47.1 (20:80) to 51.1 (30:70), 54.3 (40:60), 56.1 (50:50), 57.1 (60:40), 58.0 (70:30), and 58.6°C (80:20).

After interesterification, SMP values of interesterified blends were lower than for unreacted blends, ranging from 30.4 (20:80) to 36.6 (30:70), 39.1 (40:60), 43.8 (50:50), 48.8 (60:40), 51.3 (70:30), and 53.6°C (80:20). The SMP value was lowest for blend 20:80 and highest for blend 80:20. SMP value increases in blends 20:80 to 80:20 were likely due to increases in PLP, POP, and PPP contents in interesterified blends (Table 1).

Solid fat content (SFC) The SFC is the percentage of lipid that is solid at selected temperatures. Since the SFC influences physical properties of spreadability, hardness, mouthfeel, and stability, SFC values are required for characterization of properties of plastic fats and are considered as a qualitative parameter of margarine texture (22). Refrigeration, room, and body temperatures, respectively, are related to spreadability, product stability, and texture and mouthfeel (23).

SFC profiles as a function of temperature for hPS, RBO, and unreacted hPS:RBO blends are shown in Fig. 2A. The hPS exhibited high SFC values at all temperatures between 15 and 40°C whereas the SFC value of RBO was almost 0 within the same temperature

Table 1. Triacylglycerol compositions of hPS:RBO blends before (unreacted) and after (reacted) lipase-catalyzed interesterification

hPS:RBO (wt.)	Triacylglycerol compositions (area %)								
	LLO[1)]	PLL	LOO	PLO	PLP	OOO	POO	POP	PPP
Unreacted									
20:80	16.9±0.4$^{a2)}$	16.6±0.4^{ab}	14.6±0.3^{ab}	17.7±0.3^{e}	6.3±0.7^{a}	3.2±0.2^{a}	5.0±0.2^{abcd}	2.0±0.1^{a}	1.2±0.1^{abc}
30:70	16.6±0.2^{a}	17.0±0.6^{a}	14.7±0.0^{a}	17.9±0.1^{e}	6.4±0.4^{a}	3.0±0.3^{abc}	4.6±0.9^{abc}	1.9±0.4^{a}	1.8±0.1^{bcd}
40:60	16.6±0.1^{a}	16.4±0.2^{bcd}	14.4±0.0^{bc}	17.7±0.2^{e}	6.8±0.9^{a}	3.0±0.2^{abc}	4.8±0.3^{abc}	2.4±0.4^{ab}	2.6±0.3^{de}
50:50	16.2±0.2^{b}	16.2±0.4^{cde}	14.2±0.2^{bcd}	17.4±0.1^{e}	6.3±0.2^{a}	3.1±0.3^{ab}	5.2±0.2^{bcde}	2.9±0.1^{ab}	3.7±0.1^{ef}
60:40	15.8±0.0^{c}	15.9±0.3^{def}	13.6±0.3^{cd}	17.7±0.3^{e}	7.0±0.0^{ab}	2.8±0.0^{bcd}	5.0±0.0^{abcd}	3.2±0.2^{ab}	5.4±0.1^{g}
70:30	15.0±0.3^{d}	15.4±0.5^{fg}	13.4±1.3^{de}	18.1±0.1^{e}	7.8±0.1^{bc}	2.7±0.2^{cd}	5.4±0.9^{cde}	4.2±0.4^{bc}	6.3±2.4^{gh}
80:20	12.3±0.2^{f}	13.1±0.4^{h}	10.4±0.9^{f}	15.4±1.5^{g}	8.1±0.8^{c}	2.5±0.3^{d}	6.8±1.3^{f}	8.8±3.1^{f}	12.5±0.3^{i}
Reacted									
20:80	14.0±0.2^{e}	15.6±0.0^{efg}	12.6±0.2^{e}	24.3±0.4^{b}	11.5±0.1^{d}	1.3±0.2^{e}	5.5±0.6^{cde}	3.4±0.2^{bc}	0.0±0.0^{a}
30:70	10.8±0.1^{g}	15.6±0.1^{efg}	9.9±0.1^{f}	25.3±0.1^{a}	15.7±0.0^{e}	1.1±0.0^{ef}	6.1±0.1^{def}	5.2±0.1^{c}	0.0±0.0^{a}
40:60	7.8±0.0^{h}	15.0±0.0^{g}	7.4±0.1^{g}	25.5±0.0^{a}	20.4±0.2^{f}	0.8±0.1^{fg}	6.0±0.1^{def}	6.8±0.1^{d}	0.9±0.0^{ab}
50:50	5.6±0.2^{i}	13.4±0.2^{h}	5.3±0.0^{h}	23.6±0.4^{bc}	24.7±0.1^{g}	0.5±0.1^{g}	6.2±0.3^{ef}	9.6±0.1^{ef}	1.9±0.0^{bcd}
60:40	3.8±0.1^{j}	12.0±0.6^{i}	4.0±0.1^{i}	23.1±0.6^{c}	31.6±0.9^{h}	0.0±0.0^{h}	4.7±0.0^{abc}	10.8±0.2^{f}	2.4±0.4^{cde}
70:30	2.4±0.0^{k}	9.9±0.1^{j}	2.5±0.4^{j}	20.3±0.2^{d}	36.4±0.4^{i}	0.0±0.0^{h}	4.7±0.3^{abc}	13.5±0.2^{g}	4.1±0.1^{f}
80:20	1.1±0.0^{l}	7.2±0.1^{k}	1.2±0.0^{k}	16.4±0.0^{f}	40.5±0.4^{j}	0.0±0.0^{h}	4.1±0.2^{a}	16.2±0.1^{h}	7.0±0.2^{h}

[1)]P=palmitic acid, O=oleic acid, L=linoleic acid

[2)]Values followed by the same letters within the same column are not significantly different (p>0.05)

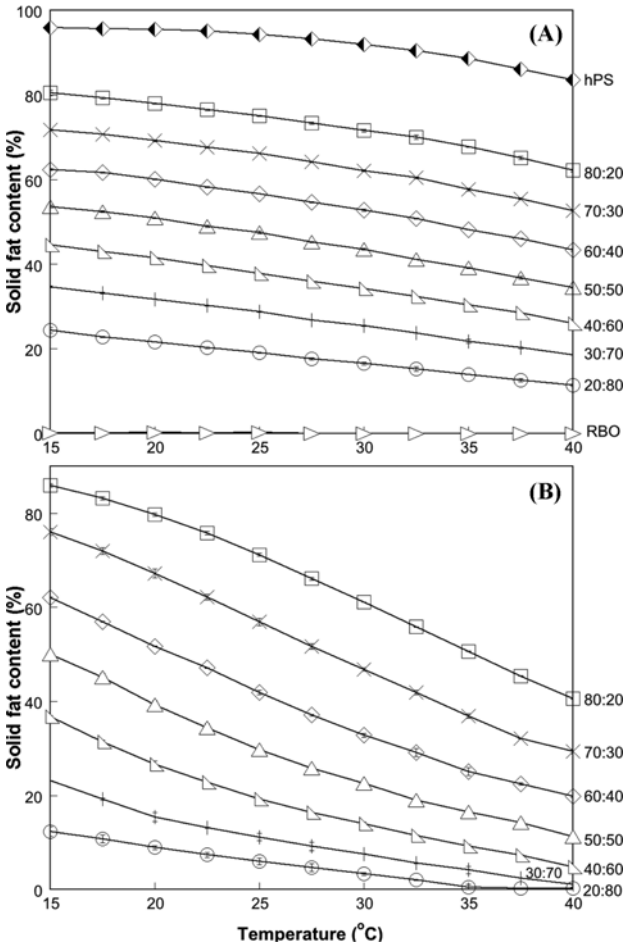

Fig. 2. Solid fat content profiles of hPS, RBO, and hPS:RBO blends (A) before and (B) after lipase-catalyzed interesterification.

range. High contents of monounsaturated and polyunsaturated FAs in RBO must have been a contributing factor. Only a modest SFC reduction was observed for all fat samples when the temperature increased from 15 to 40°C. SFC values of unreacted blends were 24.42-80.52% at 15°C, which then decreased to 11.39-62.15% at 40°C. At all temperatures, SFC values increased as the content of hPS in blends increased, likely due to a progressive increase in the content of high-melting TAGs, such as PPP and POP, in unreacted blends (Table 1).

SFC profiles of interesterified hPS:RBO blends are presented in Fig. 2B. EI significantly changed characteristics of SFC profiles of all fat blends. Below 20°C, SFC value of interesterified blends 80:20 and 70:30 were higher than for physical blends whereas SFC values of all other interesterified blends were either the same as blend 60:40, or slightly below values of physical blends. However, at 20°C and above, SFC values of all interesterified blends decreased significantly to below physical blend values. At 35°C, the average drop in SFC values from before to after interesterification for all blends was approximately 20%. Changes in SFC values after interesterification were due to exchange of FAs in TAG species in blends, leading to changes in the relative quantities of TAG components of interesterified lipids. SFC

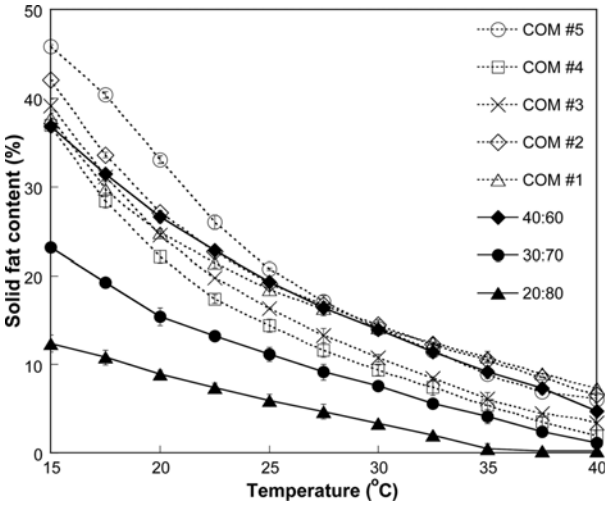

Fig. 3. Solid fat content profiles of selected interesterified hPS:RBO blends compared with commercial margarine fats (COM #1, #2, #3, #4, and #5).

increases at low temperatures in blends 80:20 and 70:30 after EI were likely due to increases in the contents of di-saturated TAGs PLP and POP and content decreases in the lower-melting polyunsaturated TAGs LLO, PLL, and LOO (24) (Table 1). Decreases in SFC values at high temperatures following interesterification for all blends could be related to a content decrease in the tri-saturated TAG PPP.

SFC values of margarines at 35°C should be below 10% in order to allow complete melting in the mouth without leaving any waxy mouthfeel (25). Only interesterified fats of 20:80, 30:70, and 40:60 blends with SFC values of 0.47, 4.14, and 9.15%, respectively, at 35°C met the requirement. Therefore, SFC profiles of only interesterified blends 20:80, 30:70, and 40:60 were compared with 5 commercial margarine fats (Fig. 3). Only interesterified blend 40:60 exhibited an SFC curve that fit within the range of SFC curves for all 5 commercial margarine fats, indicating that the blend can be used as a suitable *trans*-free margarine fat. In addition, the SFC profile of interesterified blend 40:60 was most similar to SFC curves of commercial margarine fats #1 and #2 (Fig. 3), which were from the 2 most well known margarines in Thailand. Hence, only commercial margarine fats #1 and #2 were used for subsequent comparisons.

Crystallization and melting characteristics Crystallization thermograms of hPS, RBO, and physical and interesterified hPS:RBO blends are shown in Fig. 4A. The hPS showed a sharp crystallization peak at 38.4±0.21°C whilst RBO exhibited a broad crystallization peak at −6.2±0.16°C. Crystallization of physical blends showed 2 peaks representing high-melting (I) and low-melting (II) point TAGs. As the amount of hPS in unreacted blends increased, both DSC peaks moved gradually toward higher temperatures.

Melting thermograms of all fat samples are shown in Fig. 4B. Fat and oil do not have a distinct melting point but rather a melting range due to different FAs present (26). Melting characteristics of margarine fats are important for flavor release and consumer

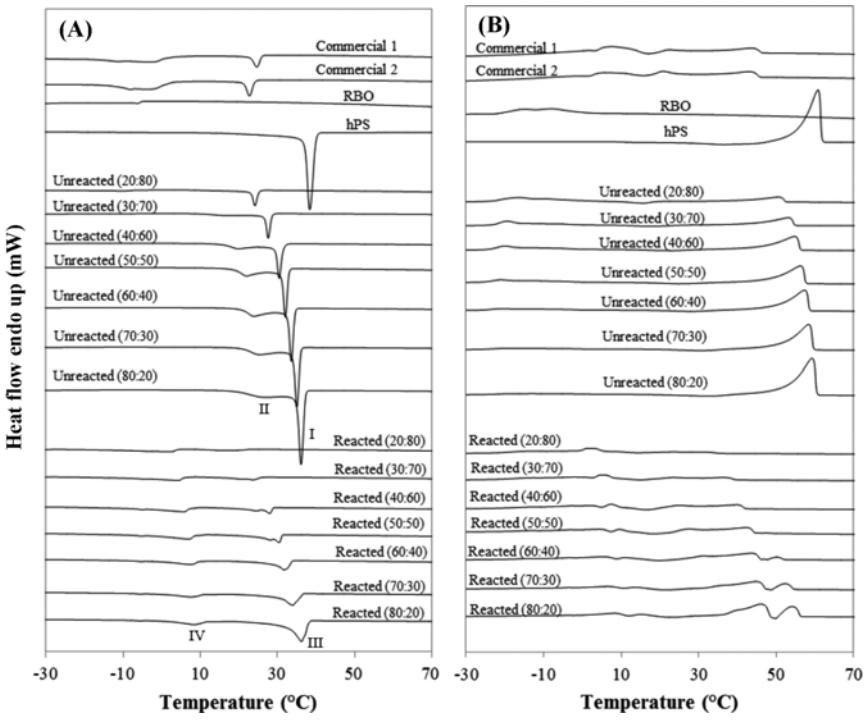

Fig. 4. DSC thermograms for (A) crystallization and (B) melting of hPS, RBO, and hPS:RBO blends before (unreacted) and after (reacted) lipase-catalyzed interesterification, compared with selected commercial margarine fats.

acceptance. The hPS had a single sharp melting peak at 60.8±0.24°C and RBO displayed 2 broad and overlapping melting peaks at −14.9±0.27 and −9.0±0.32°C. Exothermic thermograms of unreacted blends displayed a mixture of the melting characteristics of both hPS and RBO. As the content of hPS in blends increased, the peak representing melting of high-melting TAGs from hPS in blends moved slowly towards high temperatures and the 2 broad and overlapping peaks relating to melting of low-melting TAGs from RBO gradually diminished and finally disappeared in blend 80:20.

The melting end temperature (T_{end}) is the temperature where a fat melts completely. The T_{end} value of RBO was lowest at 8.40±0.18°C whilst the T_{end} value of hPS was highest at 64.5±0.35°C. T_{end} depends on the amount and type of TAG molecular species and also types of FAs contained in a fat. RBO was comprised mainly of the unsaturated FAs oleic and linoleic acids, while hPS consisted of mostly the saturated FA palmitic acid, resulting in a low T_{end} value for RBO and a high T_{end} value for hPS. The low value of T_{end} for RBO also explains why the SFC value was close to 0 in a temperature range of 15-40°C (Fig. 2A). For unreacted blends, T_{end} values increased with the content of hPS from 52.9±0.19°C for blend 20:80 to 60.8±0.28°C for blend 80:20.

Crystallization thermograms of interesterified fats together with the 2 commercial margarine fats #1 and #2 could be divided into 2 areas (Fig. 4A). The first area was the high-temperature side of the thermogram representing crystallization of high-melting TAGs (III) and the second area was the low-temperature side of the diagram representing crystallization of low-melting TAGs (IV). As the quantity of hPS in physical blends increased, both peaks III and IV of interesterified blends moved slowly toward the high temperature side. Although the shape of crystallization thermograms of all interesterified blends was similar to thermogram shapes of commercial margarines, interesterified blends 30:70, 40:60, and 50:50 exhibited both shape and DSC peak locations that were most comparable to the 2 commercial margarine fats.

Melting properties of fats can be changed via enzymatic interesterification. DSC melting thermograms of interesterified fats and 2 commercial margarine fats showed broad and overlapping peaks (Fig. 4B). Interesterified blends 30:70, 40:60, and 50:50 displayed melting thermograms that were closest to thermograms of commercial margarine fats. The T_{end} value of blend 20:80 was lowest at 36.84±0.24°C and increased as the hPS content in blends increased. Comparison of T_{end} values of unreacted and interesterified blend 40:60 with the same hPS:RBO ratio showed that the T_{end} value of the interesterified blend at 42.56±0.29°C was lower than for the unreacted blend at 56.67±0.11°C.

Before interesterification, each physical blend had unique melting and crystallization characteristics that depended on high and low TAG melting compositions. However, with interesterification using lipase-catalysis, rearrangements of FAs within or between TAGs occurred, leading to production of new TAG molecules and, in turn, crystallization and melting behaviors were changed (22). Based on crystallization and melting characteristics, interesterified blends 30:70, 40:60, and 50:50 had both DSC crystallization and DSC melting profiles most comparable with commercial margarine fats. However,

SFC studies showed that only interesterified blends 20:80, 30:70, and 40:60 would leave no waxy mouthfeel. Therefore, only interesterified blends 30:70 and 40:60 were suitable for use as suitable *trans*-free margarine fats. Therefore, subsequent studies focused on comparison of different characteristics of interesterified blends 30:70 and 40:60 with commercial margarine fats #1 and #2.

Polymorphism Polymorphic forms of fat crystals are a crucial criterion for determination of funtional properties of margarines and can greatly influence physical properties and processing conditions of final products (23,27). The 3 main polymorphs of fat crystals are α, β', and β forms. Each polymorph showed different characteristics. The α form was unstable with the lowest melting point while the β' form was metastable with an intermediate melting point. The β form was stable with the highest melting point. β' crystals are small at 5-7 mm in length with a shiny surface and a smooth texture, providing good spreadability for margarines (28,29). The β form is unfavorable for use as a margarine fat due to association with a sandy texture (19,30). β crystals are initially small, but grow into large needle-like agglomerates of 20-30 mm, leading to a grainy and hard texture and low spreadability (29,31-33).

Both unreacted and reacted blends 30:70 and 40:60 and commercial margarine fats #1 and #2 were crystallized at 4°C for 24 h. Polymorphs were determined based on short spacings obtained from X-ray diffraction spectra (data not shown). Physical blends exhibited a mixture of β' polymorphs with diffraction peaks at 3.8 and 4.2 Å and β polymorphs with a diffraction peak at 4.6 Å. After interesterification, the diffraction peak representing the β' structure became slightly dominant over the β polymorph. Commercial margarine fats #1 and #2 exhibited mainly β' polymorphs with diffraction peaks at 3.8 and 4.2 Å.

Contents of β' and β structures in all blends were estimated using diffracted intensities of short spacings of both β' and β forms (1). All fat blends both before and after EI and commercial margarine fats exhibited higher contents of β' than β ($\beta'>\beta$). Domination of β' over β polymorphs for all blends was likely due to a high degree of heterogeneity in TAG molecules (34) (Table 1). The dominant presence of β' is necessary for desirable margarine textural properties, appearance, and fluidity (1,21).

Crystal microstructure Microstructures of both unreacted and reacted blends 30:70 and 40:60 and commercial margarine fats #1 and #2 obtained using PLM after 24 h of crystallization at 4°C are shown in Fig. 5. Both physical blends showed densely-packed large rod-like crystals (Fig. 5A and 5C) of average size=40-50 mm. This morphology was similar to crystal microstructures previously reported for fully hydrogenated oil (22) and palm stearin (11). Interesterified blends displayed crystal microstructures different from physical blends with a significantly smaller crystal size of <10 mm. Blend 30:70 showed small needle-like crystals with some degree of aggregation (Fig. 5B), whereas in blend 40:60, only individual needle-like crystals were observed with no presence of any crystal aggregates (Fig. 5D). In comparison, both commercial margarine fats exhibited randomly-oriented and loosely-packed rod-like or plate-like crystals (Fig. 5E and 5F).

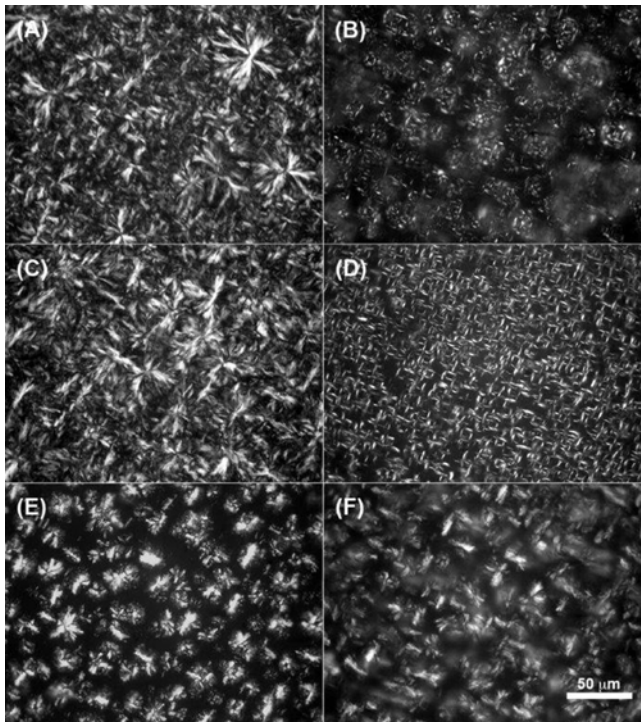

Fig. 5. Crystal microstructure of selected hPS:RBO blends before (unreacted) and after (reacted) lipase-catalyzed interesterification and selected commercial margarine fats obtained after crystallization at 4°C for 24 h: (A) blend 30:70 (unreacted), (B) blend 30:70 (reacted), (C) blend 40:60 (unreacted), (D) blend 40:60 (reacted), (E) commercial margarine fat #1, and (F) commercial margarine fat #2.

Apart from margarines, fats containing small compact crystals are also desired for use in baked goods since these crystals can surround and stabilize air bubbles produced during the creaming stage to produce a fine and smooth texture (22). The small needle-like microstructure of interesterified blend 40:60 suggested a shiny surface, smooth texture, and good spreadability for final products. Interesterified blend 30:70 with small, mostly β' crystals, did not show an SFC profile that was similar to most commercial margarine fats (Fig. 3). Criteria for selection of the best interesterified blend for production of a *trans*-free margarine fat were 1) physical properties and crystallization and melting behaviors most similar to commercial margarine fats, 2) a tendency to crystallize in the β' form, and 3) small needle-shaped crystals. Interesterified blend 40:60 had highest potential for use as a *trans*-free margarine fat.

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