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Trans-Free Plastic Shortenings Prepared with Palm Stearin and Rice Bran Oil Structured Lipid

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Abstract Rice bran oil structured lipid (RBOSL) was produced from rice bran oil (RBO) and the medium chain fatty acid (MCFA), caprylic acid, with Lipozyme RM IM as biocatalyst. RBOSL and RBO were mixed with palm stearin (PS) in ratios of 30:70, 40:60, 50:50, 60:40 and 70:30 v/v (RBOSL to PS) to formulate *trans*-free shortenings. Fatty acid profiles, solid fat content (SFC), melting and crystallization curves and crystal morphology were determined. The content of caprylic acid in shortening blends with RBOSL ranged from 9.92 to 22.14 mol%. Shortening blends containing 30:70 and 60:40 RBOSL or RBO and PS had fatty acid profiles similar to a commercial shortening (CS). SFCs for blends were within the desired range for CS of 10–50% at 10–40 °C. Shortening blends containing higher amounts of RBOSL or RBO had melting and crystallization curves similar to CS. All shortening blends contained primarily β' crystals. RBOSL blended with PS was comparable to RBO in producing shortenings with fatty acid profiles, SFC, melting and crystallization profiles and crystal morphologies that were similar. RBOSL blended with PS can possibly provide healthier alternative to some oils currently blended with PS and commercial shortening to produce *trans*-free shortening because of the health benefits of the MCFA in RBOSL.

Keywords Palm stearin · Rice bran oil · Shortening · Solid fat content · Structured lipid

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Introduction

Shortening provides desirable textural properties by lubricating, weakening, or shortening food components. Shortening used in frying allows uniform heat transfer and forms a moisture barrier. Other desirable functions of shortening in food include, imparting tenderness and mouth feel, providing structural integrity, incorporation of air, and extending shelf life [1].

Shortening is produced from hydrogenated vegetable oils. The hydrogenation process produces *trans* fatty acids, which when consumed can increase the risk for coronary heart disease (CHD) [2]. Fractionation and blending of palm oil with rice bran oil (RBO) and mahua oil [3], with palm stearin (PS) and RBO [4] has resulted in *trans*-free shortenings. Interesterification of RBO and PS has also produced *trans*-free shortenings [5, 6]. Another alternative to blending palm oil fractions with vegetable oil is to blend palm oil fractions with a structured lipid (SL).

Palm oil is high in palmitic and oleic acids and has a higher polyunsaturated fatty acid (PUFA) content than coconut and palm kernel oils. Palm oil is extracted from the mesocarp of the oil palm (*Elaeis guineensis*) fruit, is economical to produce and can be fractionated into liquid fraction (palm olein) and solid fraction (PS). The high oxidative stability of palm oil is due to its high tocopherol content. The carotenoids and tocopherols present in palm oil protect against certain cancers and lower serum cholesterol, respectively [7]. There is conflicting evidence as to the effects of palm oil on CHD [7–10]. However palm oil is becoming more widely used as an alternative to *trans* fats. The solid fat content (SFC) of palm oil gives consistency without hydrogenation and can be used in food products with variable plastic ranges [7].

A SL containing RBO and the medium chain fatty acid (MCFA) caprylic acid would have several health benefits and would contribute to the desirability of a shortening made by blending palm stearin with rice bran oil structured lipid (RBOSL). RBO contains γ-oryzanol which can lower plasma cholesterol [11], cholesterol absorption [12], and inhibit platelet aggregation [13]. The γ-oryzanol concentration in RBO is not significantly affected by enzymatic modification to incorporate caprylic acid with Lipozyme RM IM as biocatalyst [14]. MCFAs provide a quick energy source that can be rapidly oxidized and utilized. MCFAs are metabolized through the portal system instead of the lymphatic system as are long chain fatty acids. MCFAs have been used to treat patients with fat absorption abnormalities and by athletes with increased energy requirements [15, 16]. Previous animal and human studies have shown that the composition of triacylglycerols (TAGs) containing MCFA resulted in increased energy expenditure and decreased weight gain [17, 18]. Blending of RBOSL with PS is an option for producing *trans* free shortening without hydrogenation and with the added health benefit of MCFA contained in RBOSL.

The objectives of this study were: (1) to blend RBOSL or RBO with PS in various ratios to produce shortening, and (2) to determine the fatty acid profile, melting and crystallization profile, SFC and crystal morphology of the blended shortening and commercial shortenings (CS).

Experimental Procedures

Materials

RBO was purchased from California Rice Oil Company (Novato, CA). Caprylic acid was purchased from Sigma Chemical Co. (St. Louis, MO). Lipozyme RM IM (immobilized lipase on a macoporous anion exchange resin) from *Rhizomucor miehei* was purchased from Novo Nordisk Biochem North America, Inc. (Franklinton, NC). RBD PS was donated by Fuji Vegetable Oil, Inc. (Savannah, GA). CS#1 (palm oil shortening) was purchased from Loders Croklaan North America, LLC (Channahon, IL). CS#2 (hydrogenated vegetable oil shortening) was purchased from a local grocery store.

Synthesis of Rice Bran Oil Structured Lipid

The SL was produced in 1 kg quantities in a packed-bed reactor with a flow rate of 1 mL/min, 1:6 substrate mole ratio (RBO: caprylic acid) and a temperature of 45 °C [14, 19]. A bioreactor with a jacketed stainless steel column (47 × 500 mm) and a FMI Lab pump model QV from Fluid Metering, Inc. (Oyster Bay, NY) was used for SL synthesis. The bioreactor set up was as reported by Fomuso and Akoh [20]. A circulating water bath was used to maintain a constant column temperature. The column was packed with immobilized Lipozyme RM IM and plugged at both ends with approximately 3 cm of glass wool.

Short-Path Distillation

The SL reaction products were passed through a short-path distillation apparatus four times to remove free fatty acids to a level below 0.1%. A KDL-4 (UIC Inc., Joliet, IL) distillation unit was used. The heating oil temperature was 185 °C, the coolant temperature was 15 °C, and the vacuum pressure was below 1 Torr [20].

Determination of Shortening Fatty Acid Profiles

One hundred milligrams of the shortening sample was weighed into a culture tube, and 1 mL of internal standard (17:0 in hexane 20 mg/mL) was added to the culture tube and the mixture was then flushed with nitrogen. Two milliliters of 0.5 N NaOH in methanol solution was then added and the samples were placed in an oven at 100 °C for 5 min. Two milliliters of 14% BF_3 in methanol was added and the reaction mixture was vortexed for 1 min and again placed in an oven at 100 °C for 5 min. Two milliliters of hexane and 2 mL of saturated NaCl were then added to extract the fatty acid methyl esters (FAMEs). The samples were then vortexed for 2 min and centrifuged at 1,000 rpm for 5 min at room temperature [21]. The upper layer was then removed and analyzed by gas chromatography. The gas chromatograph was an Agilent 6890N (Wilmington, PA) equipped with a Supelco (Bellefonte, PA) SP-2560 100 m × 0.25 mm × 0.20 μm film thickness and a flame ionization detector operated in the split mode with a split ratio of 50:1. The injector and detector temperatures were both at 250 °C. The column temperature was at 140 °C. The carrier gas was helium and the flow rate was 1.1 mL/min. The relative concentrations of FAMEs were calculated by computer with 17:0 as internal standard. Retention times of GLC reference standard (Supelco 37, Bellefonte, PA) were used to identify detected FAMEs.

Shortening Blend Preparation

Palm stearin was melted at 65 °C and was blended with RBOSL in ratios of (RBOSL or RBO to PS) 30:70, 40:60, 50:50, 60:40 and 70:30, v/v, to produce shortening.

Differential Scanning Calorimetry

A Perkin-Elmer model DSC7 (Norwalk, CT) was used for the analysis according to AOCS recommended procedure

Cj 1–94 [22]. Indium was used as a reference standard and for standardization (mp 156.6 °C, ΔH 28.45 J/g); dry ice was used as a coolant. The 5–20 mg sample was hermetically sealed in a 30-μL capacity aluminum pan (Perkin Elmer); an empty pan was used for reference. Samples were heated rapidly from room temperature to 80 °C and held for 10 min (to destroy crystal memory). The sample was then cooled to −40 at 10 °C per min, held for 30 min, and then heated to 80 °C at a rate of 5 °C per min to generate melting profiles. The thermograms were analyzed by the software provided with the DSC (Pyris software; Perkin-Elmer, Shelton, CT).

Solid Fat Content

SFC was determined for shortening containing unmodified RBO blended with PS, and shortening containing RBOSL blended with PS and CS by AOCS Official method Cd 16-81 [22]. NMR measurements were made using a MARAN-20 pulsed NMR spectrometer (Resonance Instruments Ltd., Oxon, UK). Samples were tempered at 100 °C for 15 min and placed at 60 °C for 10 min followed by 0 °C for 60 min and finally 30 min at each chosen measuring temperature. Olive oil was used as the reference oil. The SFC was measured at 5 °C intervals from 5 to 60 °C.

X-Ray Diffraction Spectroscopy

The polymorphic forms of shortening containing RBOSL blended with PS, shortening containing unmodified RBO blended with PS, and CS were determined to characterize and differentiate the crystal structures among the shortenings. Samples were kept at 24 °C and spread over a glass X-Ray slide. A Rigaku Multiflex (Rigaku Corporation, Tokyo, Japan) automated theta-theta powder X-Ray diffractometer with a copper X-Ray tube was used for the analysis. The generation power was 40 kV and 44 mA. The X-Ray analysis was performed at room temperature from 0.9 to 25° C at a scan speed of 1° C/min and a step of 0.02. MIDI Jade 6.5 software was used for data analysis.

Results and Discussion

Fatty Acid Composition

Table 1 shows the fatty acid profiles of shortening blends, CS, PS, RBO and RBOSL. Only CS#2 contained *trans* fatty acids (11.42 mol%). The major fatty acids for the shortening blends, commercial shortening and palm stearin were palmitic acid (14.07–58.75 mol%), oleic (25.52–38.20 mol%) and linoleic acids (7.10–30.03 mol%). Caprylic acid contents in blends containing RBOSL and PS ranged from 9.92 to 22.14 mol%. CS#2 and PS contained 0.42 and 1.55 mol% caprylic acid, respectively.

The saturated fatty acid content for blends containing RBO and PS ranged from 35.31 to 53.44 mol% and blends containing RBOSL and PS had saturated fatty acid contents of 48.38–59.70 mol%. Commercial shortenings had saturated fatty acid contents of 51.44 and 27.61 mol% for

Table 1 Fatty acid profiles of shortening blends, commercial shortenings, palm stearin, RBO and RBOSL

Fatty acid (mol%)									
Shortening blend									
	8:0	14:0	16:0	18:0	18:1c	18:1t	18:2c	18:3	20:0
30:70 RBOSL:PS	9.92 ± 0.02	0.96 ± 0.01	44.27 ± 0.01	4.02 ± 0.05	26.49 ± 0.01	ND	13.58 ± 0.04	0.36 ± 0.02	0.53 ± 0.04
30:70 RBO:PS	ND	1.06 ± 0.03	47.49 ± 0.01	4.37 ± 0.01	30.39 ± 0.01	ND	15.72 ± 0.06	0.52 ± 0.13	0.52 ± 0.13
40:60 RBOSL:PS	12.44 ± 0.06	0.89 ± 0.06	39.58 ± 0.01	3.65 ± 0.01	28.30 ± 1.90	ND	15.71 ± 0.21	0.54 ± 0.01	0.42 ± 0.03
40:60 RBO:PS	ND	0.99 ± 0.03	44.10 ± 0.03	4.11 ± 0.01	31.86 ± 0.06	ND	17.87 ± 0.01	0.60 ± 0.14	0.69 ± 0.04
50:50 RBOSL:PS	15.92 ± 0.03	0.75 ± 0.05	33.04 ± 0.11	3.14 ± 0.01	27.80 ± 0.21	ND	18.39 ± 0.01	0.52 ± 0.06	0.61 ± 0.06
50:50 RBO:PS	ND	0.88 ± 0.04	38.78 ± 0.01	3.90 ± 0.03	33.74 ± 0.01	ND	21.61 ± 0.03	0.64 ± 0.04	0.76 ± 0.04
60:40 RBOSL:PS	19.12 ± 0.01	0.64 ± 0.06	28.06 ± 0.03	2.75 ± 0.06	27.85 ± 0.01	ND	20.55 ± 0.01	0.59 ± 0.05	0.60 ± 0.01
60:40 RBO:PS	ND	0.80 ± 0.01	38.89 ± 0.15	3.48 ± 0.01	34.68 ± 0.26	ND	23.72 ± 0.09	0.69 ± 0.01	0.78 ± 0.02
70:30 RBOSL:PS	22.14 ± 0.33	0.50 ± 0.01	22.84 ± 0.10	2.28 ± 0.01	28.19 ± 0.11	ND	22.84 ± 0.11	0.65 ± 0.04	0.62 ± 0.01
70:30 RBO:PS	ND	0.73 ± 0.04	30.72 ± 0.13	3.05 ± 0.06	35.94 ± 0.01	ND	27.97 ± 0.04	0.95 ± 0.21	0.81 ± 0.01
CS#1	ND	1.12 ± 0.01	45.26 ± 0.02	4.66 ± 0.01	38.20 ± 0.01	ND	10.23 ± 0.01	0.21 ± 0.04	0.45 ± 0.06
CS#2	0.42 ± 0.03	0.19 ± 0.01	14.07 ± 0.06	12.30 ± 0.11	28.53 ± 0.09	11.42 ± 0.24	30.03 ± 0.11	2.64 ± 0.01	0.63 ± 0.33
PS	1.55 ± 0.02	1.28 ± 0.01	58.75 ± 0.28	5.15 ± 0.02	25.52 ± 0.16	ND	7.10 ± 0.03	0.46 ± 0.05	0.28 ± 0.18
RBO	ND	0.43 ± 0.01	19.12 ± 0.11	2.26 ± 0.02	40.61 ± 0.16	ND	35.56 ± 0.11	1.06 ± 0.02	0.99 ± 0.01
RBOSL	28.89 ± 0.04	0.23 ± 0.04	9.88 ± 0.01	1.25 ± 0.01	29.61 ± 0.03	ND	28.70 ± 0.01	0.75 ± 0.06	0.75 ± 0.06

RBOSL rice bran oil structured lipid, *RBO* rice bran oil, *PS* palm stearin, *CS* commercial shortening, *ND* not detected

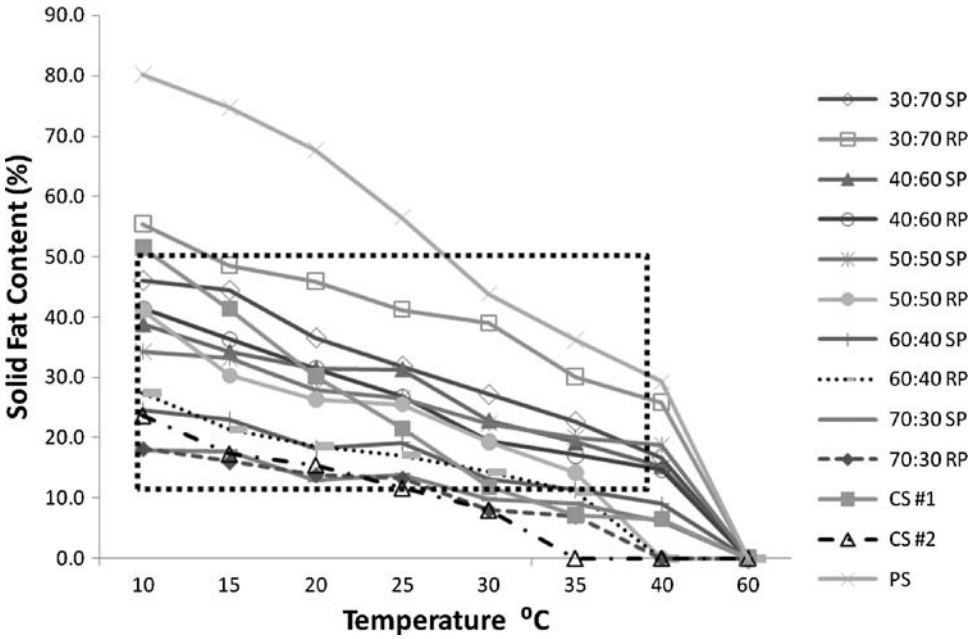

Fig. 1 Solid fat contents of shortening blends of RBO or RBOSL with palm stearin and commercial shortenings. RBOSL: palm stearin (*SP*), RBO: palm stearin (*RP*), commercial shortening (*CS*), palm stearin (*PS*)

CS#1 and CS#2, respectively, and PS saturated fatty acid content was 67.01 mol%. RBO and RBOSL saturated fatty acid contents were 22.80 and 41.00 mol%, respectively. Overall, palmitic acid content decreased in PS after blending with RBO and RBOSL. Myristic, palmitic and stearic acid contents for blends containing 30:70 RBO or RBOSL were similar to those of CS#1. A previous study [4] reported that a 50:50 blend of RBO:PS had a saturated and unsaturated fatty acid contents of 41.00 and 53.20%, respectively. Our findings showed 44.06 mol% saturated fat and 55.94 mol% unsaturated fat for the 50:50 RBO:PS blend. The 50:50 RBOSL:PS blend contained 53.46 mol% saturated fat and 46.71 mol% unsaturated fat. The 70:30 RBOSL:PS blend had very similar and almost balanced saturated and unsaturated fatty acid contents of 48.38 and 51.68 mol%, respectively.

PS is highly saturated and provides natural hardness to fats, but this texture does not provide a suitable plastic range to edible fats such as margarine and shortening. Vegetable oils such as RBO contain polyunsaturated fatty acids that are liquid at room temperature and when blended with PS helps to improve the fat plastic range.

Solid Fat Content

Determination of SFC allows a description of the amounts of solid fat crystals in relation to the amount of liquid oil from about 10–40 °C, which gives a measure of plasticity [23] and influences fat physical and sensory characteristics [24]. The plastic range of fat is the temperature range over which the fat can be molded and is neither too hard nor too soft. The 70:30 shortening blends containing 70:30 RBO or RBOSL and PS exhibited solid fat contents most similar to CS#1, which was a palm oil shortening (Fig. 1). The sharp decline in solid fat content for CS#1 shows a more limited plastic range than all other shortening blends; CS#2 exhibited a less steep decline in solid fat content. The solid fat contents shown at 40 °C for the shortening blends were very similar to those reported in a previous study [24] with blends of sunflower oil and PS in nearly the same ratios (Fig. 1). A wider plastic range occurs when there is a more diverse fatty acid content with variable melting points. High stability shortening with a SFC of 10–50% at 10–40 °C is most desired [25]. The SFC for shortening blends used in this study fell within this range, which indicates suitability for use in shortening applications requiring various consistencies. As the amount of PS increased in the shortening blend, the SFC also increased because of an increase in the saturated fatty acid content mainly from the palmitic acid content of palm stearin. The rate at which a fat solidifies can affect some bakery products such as pastries that must contain fats that become solid as the product cools to prevent fat loss which results in an oily paste [26].

Melting and Crystallization Properties

The melting profiles show that the peak 3 for blends containing RBO and PS blends is very similar and only slightly higher than the peak 3 for PS blends containing RBOSL (Fig. 2). As the content of RBO or RBOSL liquid oil

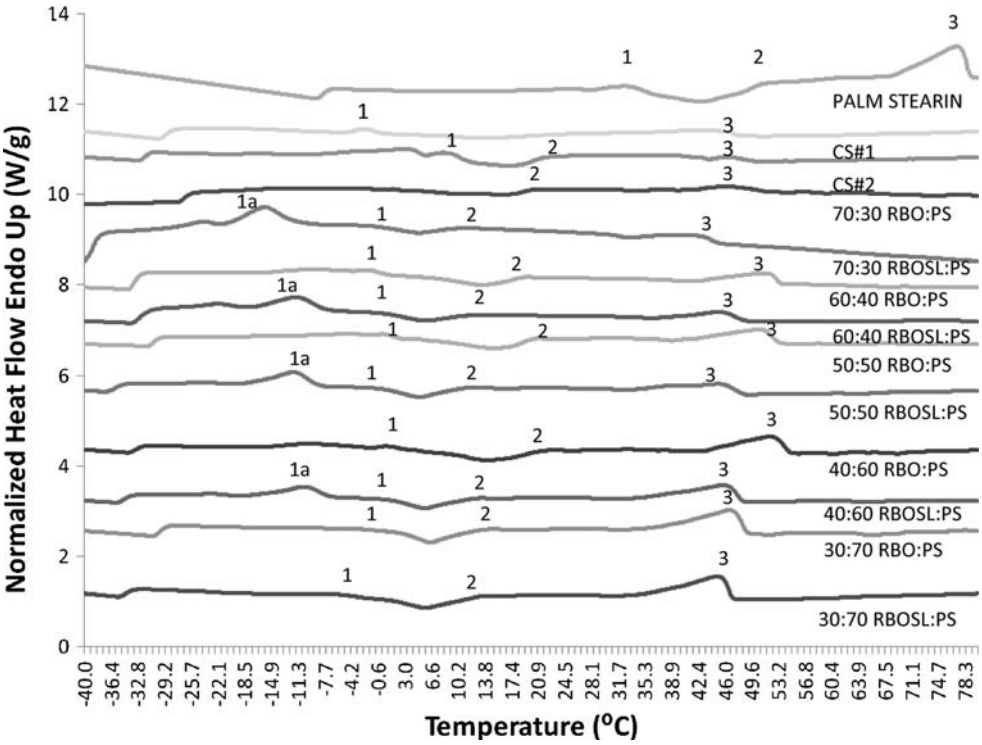

Fig. 2 Melting profiles of shortenings blends of rice bran oil (*RBO*) or rice bran oil structured lipid (*RBOSL*) with palm stearin (*PS*) and commercial shortenings (*CS*)

increased, the height of peak 3 decreased and melting profiles for the blends became more similar to the CSs (Fig. 2). This change was due to the decrease in high melting TAG concentration and an increase in low melting TAG concentration. Similar findings were reported in a previous study of shortening blends containing PS and RBO [3, 4]. Peak 1a in the melting curve of the blends containing RBOSL is caprylic acid, which is distinctly absent in the melting peaks containing RBO and different from CS and PS (Fig. 2). The depressed area between peaks 1 and 2 (Fig. 2) is a recrystallization peak, which probably occurred when the α polymorph transitioned to the β' form [27]. This recrystallization peak decreased as the amount of PS decreased.

Blends with RBOSL crystallized at slightly lower temperatures than blends containing RBO (Fig. 3). The lower crystallization was because of the lower viscosity of RBOSL, which did not inhibit nucleation as much as the higher viscosity RBO [28]. As the concentration of palm stearin decreased, the size of the crystallization peak also decreased and the crystallization profiles became more similar to CS (Fig. 3).

Polymorphism

The three major polymorphic forms of fats consist of α, β and β' also referred to as hexagonal, orthorhombic, and triclinic, respectively. Shortenings are composed of a solid phase consisting of solid fat crystals and a liquid phase consisting of oil held together by cohesive forces [23]. Fat crystal size, shape, number and bonding force determine fat characteristics such as hardness and plastic behavior [29]. The β' crystals, which have a smaller thinner structure, can hold more air and liquid component (an important functional quality of shortening) than larger, more stable, higher melting point β crystals. By comparison, the α crystal form has the lowest melting point and is the least stable of the polymorphs. The β' crystals provide a smoother mouthfeel and are more desirable than the β crystal forms providing increased firmness and air incorporation [30].

X-Ray diffraction spectroscopy analysis (XRD) produced d spacings at 4.20, 3.80 and at 4.15 Å. The peaks at 4.20 and 3.80 Å were much larger than those at 4.15 Å. The data showed that all shortenings and shortening blends tested in this study contained primarily β' crystals with $\beta' \gg \alpha$. These results demonstrate that RBOSL:PS blends form β' crystals as does the RBO:PS blend. The β' crystal form is more stable in shortenings with higher palmitic acid contents [31]. The PS used for this study contained 58.75 mol% palmitic acid and 30:70 and 40:60 blends containing RBO or RBOSL contained 39.58–47.49 mol% palmitic acid.

RBOSL blended with PS showed properties comparable to RBO:PS blends when the fatty acid profiles, solid fat content, melting and crystallization profiles, and crystal forms were compared. Blending of RBOSL with PS is an option for producing *trans*-free shortening without

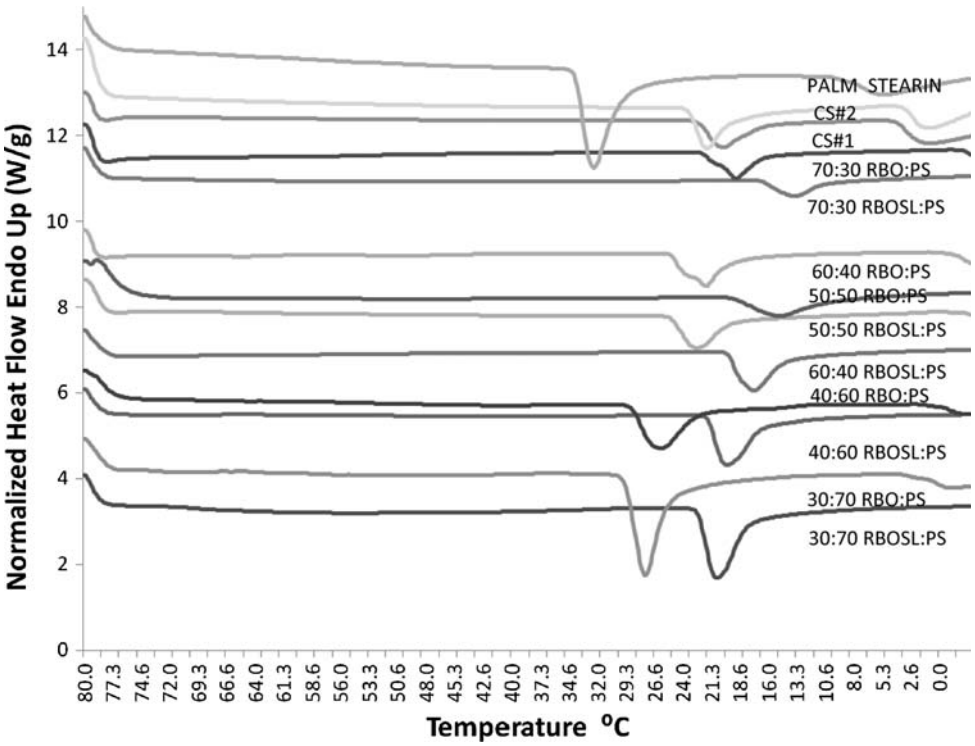

Fig. 3 Crystallization profiles of shortening blends of rice bran oil (*RBO*) or rice bran oil structured lipid (*RBOSL*) with palm stearin (*PS*) and commercial shortenings (*CS*)

hydrogenation and with the added health benefit of MCFA contained in RBOSL.

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