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Enzymatic Interesterification of Palm Stearin and Palm Kernel Olein

Zaida Zainal* and Mohd Suria Affandi Yusoff

Palm Oil Research Institute of Malaysia, Persiaran Institusi, Bandar Baru Bangi, 43000, Kajang, Selangor, Malaysia

ABSTRACT: Palm stearin (POs) and palm kernel olein (PKOo) blends were modified by enzymatic interesterification (IE) to achieve the physical properties of margarine fats. POs and PKOo are both products of the palm oil industry that presently have limited use. *Rhizomucor miehei* lipase (Lipozyme IM 60) was used to catalyze the interesterification of oil blends at 60°C. The progress of interesterification was monitored by following changes in triacylglyceride composition. At 60°C interesterification can be completed in 5 h. Degrees of hydrolysis obtained through IE for all blends were decreased from 2.9 to 2.0 by use of dry molecular sieves. The solid fat contents of POs/PKOo 30:70 and 70:30 interesterified blends were 9.6 and 18.1 at 20°C, and 0 and 4.1 at 35°C, respectively. The slip melting point (SMP) of POs/PKOo 30:70 was 40.0°C before interesterification and 29.9°C after IE. For POs/PKOo 70:30, SMP was 47.7 before and 37.5°C after IE. These thermal characteristics of interesterified POs/PKOo blend ratios from 30:70 to 70:30 were comparable to those of commercial margarines. Results showed that IE was effective in producing solid fats with less than 0.5% *trans*.

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KEY WORDS: Enzymatic interesterification, palm kernel olein, palm stearin, *Rhizomucor miehei,* zero-*trans* solid fat.

Chemical modification of oils to alter textural and nutritional requirements is used in the manufacture of margarines, modified butters, shortenings, and other plastic fats. One of these processes, hydrogenation, results in the hardening of the oil and is accompanied by a rise in its melting point (1–3). Hydrogenation also leads to the formation of *trans* fatty acids (TFA), which have been shown to correlate positively with the development of coronary heart disease (4–6). This process is not applicable to hard fats such as stearins, and indeed palm stearin is not used directly for edible purposes because of its high melting point. The use in the process of palm kernel olein (PKOo) that contains a substantial amount of lauric acid or lauric fats confers a superior melting profile on hardstock fat or margarine (3). Palm kernel olein is the liquid phase from the fractionation of palm kernel oil whereas palm stearin (POs) is the hard fraction of palm oil. POs and PKOo are readily available products of the palm oil industry. Some blend ratios showed potential in food application as margarine base stock.

*To whom correspondence should be addressed at Chemistry and Technology Department, Palm Oil Research of Malaysia, No. 6, Persiaran Institusi, Bandar Baru Bangi, 43000, Kajang, Selangor, Malaysia.
E-mail: zaida@porim.gov.my

Fats used in margarine need to possess specific physical attributes. A convenient parameter related to these properties is the percentage solid fat content (SFC) at a range of temperatures. A firm margarine, for example, may have about 20% solid fat at 20°C, but may be completely liquid at 37°C. To achieve the functional properties required, fat blends may be chemically modified. With blends containing a substantial amount of POs the modification process should cause a decrease in the melting point and give a SFC profile typical of a plastic fat. The most appropriate modification is interesterification. In this process fatty acid rearrangement leads to changes in the triglyceride (TG) composition and hence in the physical characteristics.

Chemical interesterification successfully brings about this change, but this process is usually carried out above 100°C, and uses an inorganic catalyst. The use of high temperatures can lead to deterioration in finished product quality (7,8). This treatment is usually done with the oil under vacuum or a nitrogen blanket to prevent oxidative degradation. Another approach is physical blending with other oils and fats, but this tends to cause the formation of relatively coarse crystals (3).

Another approach is enzyme-catalyzed interesterification of solid fat and liquid oil or mixtures of edible oils to form new TG with modified physical and functional characteristics (9–12). In a typical case, a lipase from *Rhizomucor miehei* is employed as the catalyst, and the reaction is carried out at 60°C. Protection conferred by vacuum or nitrogen blanket is not necessary. Interesterification by this method may lead to the desired physical properties in an oil or blend containing a large proportion of stearin, as fatty acid rearrangement very often causes a lowering of the melting point. Enzymes are specific in their actions and also prevent TFA formation. In fact, reaction conditions are very mild, and no deleterious side reactions occur thus resulting in a purer product (13–15).

This preliminary laboratory-scale investigation was conducted on a few promising blends of POs and PKOo. The products were then analyzed for the effectiveness of the enzyme-catalyzed interesterification in producing an oil or fat of acceptable quality and the desired melting point and SFC.

MATERIALS AND METHODS

Interesterification. (i) Materials. Refined, bleached, and deodorized oils were obtained from local refineries. Hard palm stearin [slip melting point (SMF), 55°C, iodine value 36] was purchased from Felda (M) Sdn. Bhd., Semenyih, Selangor,

Malaysia, and palm kernel olein from Lam Soon Sdn. Bhd., Petaling Jaya, Selangor, Malaysia. Commercial immobilized lipase, Lipozyme IM 60 (*R. miehei*), was obtained from Novo Nordisk Industry A/S (Copenhagen, Denmark). Molecular sieves (MS) 3Å, used as a dehydrator in the reaction, were supplied by Sigma Chemical Co. (St. Louis, MO). All other chemicals and solvents used were of the highest purity available.

(ii) Method. Binary blends of POs/PKOo were made in the following mass (w/w) ratios: (i) 30:70, (ii) 40:60, (iii) 50:50, (iv) 60:40, and (v) 70:30. Interesterification was carried out by adding to a 50-mL conical flask 10.0 g of the substrate oil, 0.1 g Lipozyme, and 0.2 g molecular sieves which previously had been dried at 100°C for 24 h. The flask was covered with Parafilm and agitated in a shaking water bath at 250 rpm. The bath was maintained at 60°C to prevent solidification of the substrate. The reaction was allowed to proceed for 6 h for complete interesterification. Sampling was conducted at 0, 2, 4, 5, and 6 h to monitor the reaction. To stop the reaction, the sample mix was filtered through a double layer of cheesecloth. The enzyme and sieves were separated from the product, which was obtained as the filtrate.

Analysis of oils and interesterified products. (i) Determination of fatty acid composition and TFA. Fatty acid composition and TFA were determined by the rapid method of AOCS Official Method Cd 14c-94 (16). The fat was esterified into fatty acid methyl esters (FAME). The FAME were then analyzed on a Hewlett-Packard 5890 II gas chromatograph (Palo Alto, CA), fitted with a polar SP-2340 (Supelco, Bellefonte, PA) capillary column (0.25 mm i.d. × 60 m × 0.2 μm). The detector and injector port temperatures were 240°C. Carrier gas was helium at 0.8 mL/min. The column temperature was isothermal at 190°C. The injection volume was 1 μL. Chromatography parameters and column were chosen to optimize detection of TFA.

(ii) Determination of triacylglyceride composition. The TG profiles of the fat blends before and after transesterification were obtained by reversed-phase high-performance liquid chromatography using AOCS Method Ce 5C-93 (16). A commercially packed RP-18 column (250 × 4 mm i.d.) of 5-μm particle size (Merck, Darmstadt, Germany) was used to separate the TG. TG were eluted from the column using an acetone/acetonitrile (75:25 vol/vol) mobile phase at a flow rate of 1 mL/min. The TG were detected by a refractive index detector. The sample injection volume was 20 μL. Identification of the TG was made by comparison of retention times with those of TG standards, e.g., triolein (OOO), palmitoyldiolein (POO), oleodipalmitin (POP), and tripalmitin (PPP). Other peaks were identified by comparison with literature (17,18).

(iii) Determination of degree of hydrolysis. The degree of hydrolysis was measured by the amount of the free fatty acids (FFA) released. One unit of lipase activity is defined as the amount of enzyme which liberates 1 μmol FFA/min. At the end of the incubation, 5 mL of acetone/ethanol mixture (1:1) was added to 2-mL samples to stop the reaction. The FFA in the mixture were then estimated by direct titration with 0.05 M NaOH using phenolphthalein as the indicator. Palmitic acid was used as a reference standard.

(iv) Determination of SMP and SFC. To determine the SMP of the interesterified products, the FFA in the samples were removed using the method described by Foglia *et al.* (19). The SMP of the interesterified products was determined according to AOCS Official Method Cc 1-25 (16). This involved tempering a column of fat at 10 ± 1°C for 16 h in an open capillary tube. The tube was then heated slowly in a water bath until the fat column started to rise due to hydrostatic pressure. The temperature at which this occurs is the SMP.

The percentage SFC was determined by the AOCS recommended procedure Cd 16-18 (16) using a Minispec PC 120 pulsed nuclear magnetic resonance process analyzer (Bruker NMS, Karlsruhe, Germany). Prior to analysis, the fat was melted and tempered at 70°C for 30 min, and then chilled at 0°C for 90 min. SFC was then measured at 10, 15, 20, 25, 30, 35, and 37°C following 30 min of tempering at each temperature.

RESULTS AND DISCUSSION

The SMP and TG composition change progressively during enzymatic interesterification (IE). Sampling at 0, 2, 4, 5, and 6 h during the process indicated that at 5 or 6 h changes were maximal. Six hours was taken as the time to complete the reaction, and products were obtained by interesterifying the blends for this period.

The changes in triacylglyceride composition following interesterification are illustrated in Figure 1, which shows the TG profiles of PKOo, POs, a POs/PKOo 40:60 blend, and this blend after interesterification. Table 1 shows the composition of TG groups for blends 40:60 and 60:40 before and after interesterification. The TG can be grouped according to their equivalent carbon number (ECN), which is defined as ECN = cn − 2(db) where cn is the number of carbons in the constituent fatty acid and db the number of double bonds. The ECN of the TG palmitoyl-oleoyl-stearoyl-*rac*-glycerol, for example, will be [16] + [18 − 2(1)] + [18] = 50. Group identification is convenient, as the retention time of each peak in the chromatogram varies directly with the ECN.

The main TG in PKOo were in the following ECN groups, in descending order: 36, 38, 40, 44, and 34. The main TG in POs consisted of about 75% ECN 48 group, and the remaining peaks were ECN 46 and 50. In the untreated POs/PKOo 40:60 blend, the groups consisted of about 32% ECN 48, 14% ECN 36, and between 5 and 8% for each group of ECN 32, 34, 38, 40, 42, 44, and 46. After interesterification the profile showed a more balanced or even peak distribution than the untreated blend. TG of ECN 40 and 44 were roughly doubled, ECN 36 halved, and the ECN 48 group was decreased by 10%. TG of ECN 40 and 44 could be either elevated amounts of TG that in the uninteresterified blend were in minor quantities or new TG formed by the randomization of the fatty acids from the two stock oils.

Diacylglycerol (DG) and monoacylglycerol (MG) are expected to occur during enzymatic transesterification because of hydrolysis. In Figure 1, ECN 26, 28 and 30 are higher in the interesterified than the untreated blend. Most of these low

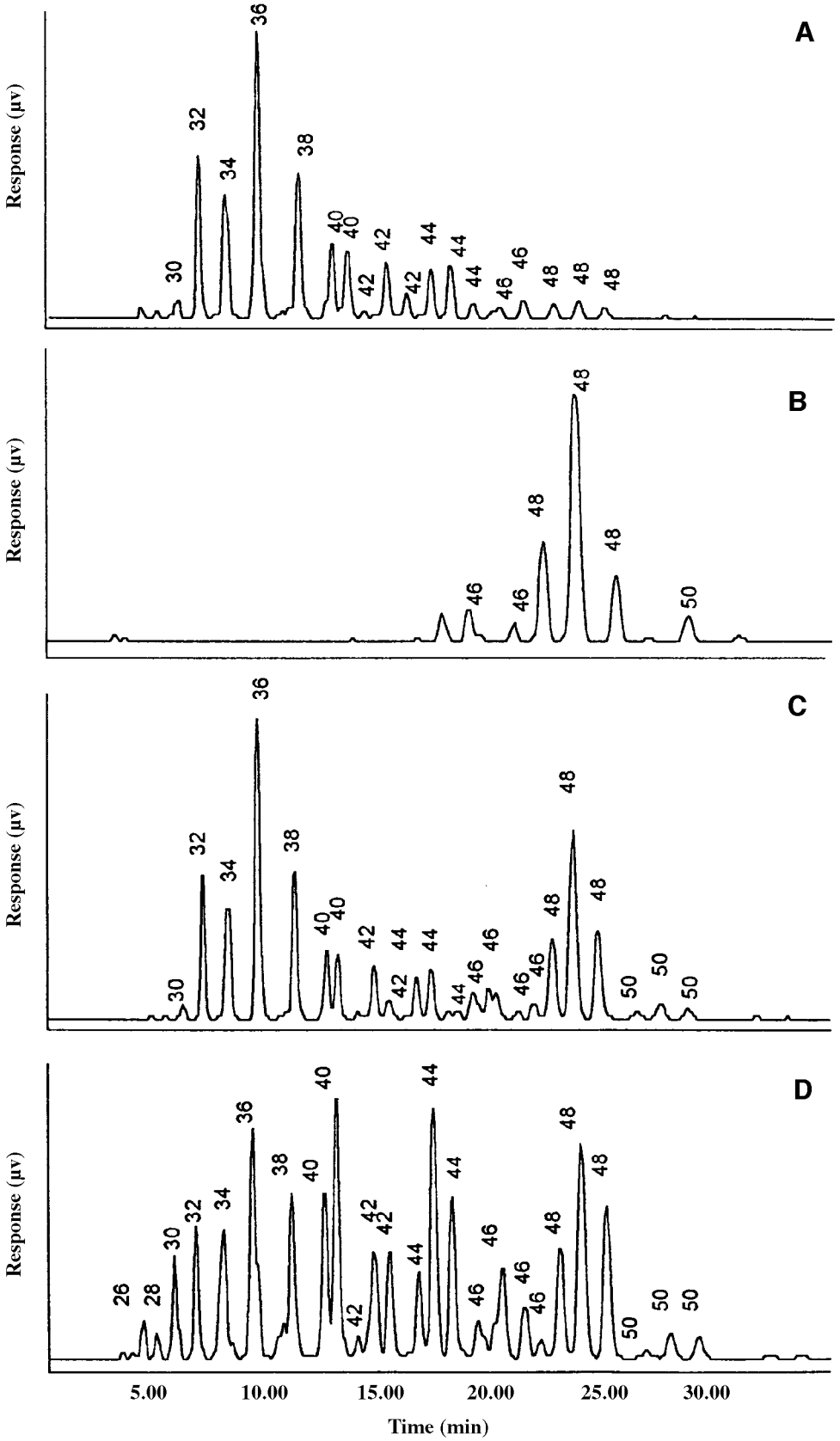

FIG. 1. Triacylglycerol profiles of palm stearin/palm kernel olein (POs/PKOo) (40:60) before and after enzymatic interesterification (IE) for 5 h at 60°C. A, PKOo; B, POs; C, POs/PKOo (40:60) before IE; D, POs/PKOo (40:60) after IE. Peaks are annotated by the equivalent carbon number values.

TABLE 1
Triglyceride Groups of Palm Stearin and Palm Kernel Blends Before and After Enzymatic Interesterification[a]

Equivalent carbon number (ECN) group	PKOo	POs	POs/PKOo (40:60) before IE	POs/PKOo (40:60) after IE	POs/PKOo (60:40) before IE	POs/PKOo (60:40) after IE
<30	2.4	—	1.4	2.0	1.0	2.6
30	1.5	—	0.9	4.0	0.6	1.4
32	8.5	—	5.1	4.1	3.4	1.5
34	10.5	—	6.9	5.6	5.1	4.5
36	22.6	—	13.6	7.7	9.0	7.2
38	13.3	—	8.0	4.8	5.3	3.1
40	12.7	—	7.6	13.4	5.1	5.4
42	8.3	—	5.0	9.5	3.3	9.8
44	10.7	1.1	6.8	16.1	4.8	18.6
46	4.1	13.8	7.9	8.7	9.8	16.2
48	4.5	74.7	32.1	20.9	45.9	26.9
50	0.9	9.2	4.2	3.1	5.8	4.1
52	—	1.3	0.1	0.1	0.8	0.1

[a]PKOo, palm kernel olein; POs, palm stearin; IE, enzymatic interesterification.

ECN peaks are likely to be DG. High moisture content could significantly contribute to an increase in DG (10,19). However, MG formation was not affected by this factor. With the use of dried enzyme it was possible to reduce the formation of by-products such as DG (20). A high content of DG can delay crystallization and lower the SFC of the interesterified products. On the other hand, DG can be regarded as beneficial since they can stabilize β-polymorphic crystals in margarine containing hydrogenated rapeseed and soybean oils according to Hernqvist *et al.* (21). Thus, the presence of a certain amount of DG in the interesterified oil can be an advantage in the commercial production of margarine stocks. The levels of MG in the interesterified products were very low probably due to rapid re-esterification. MG, however, are employed at low levels in the production of margarine, usually at 0.3% to stabilize the oil/water emulsions utilized in margarine (22).

Another product of lipase-catalyzed interesterification is FFA. Interesterification reaction usually occurs together with hydrolysis. For laboratory-scale interesterified products, the percentage of FFA, as shown in Table 2, was in the range of 2.0–2.9%. The percentage of FFA is an important quality criterion and according to PORIM Test Method (23), the acceptance level of FFA in palm oil and its products is in the range of 0.5–1.0%. Hence, unlike MG and DG, the presence of FFA in the product is a disadvantage. Hydrolysis of TG into FFA causes low yield of the desired product and contributes to the deterioration of product quality, such as rancidity.

Drying the enzyme and the substrate feedstocks before use can decrease FFA formation and improve the efficiency of interesterification. Dried molecular sieves were used to reduce the water content of the system (20,24,25). The degree of hydrolysis for the POs/PKOo 40:60 blend was 2.7% when interesterified in the presence of dried molecular sieves but 5.9% without. However, a small amount of water is needed in the reaction to maintain the enzyme activity. An optimal enzyme activity of 0.3 a_w was required to maximize product yield (10). A number of absorbents that can retain water, such as diatomaceous earth and silica gel, have been widely used to dehydrate fats during the interesterification process (26–27).

As the TG composition changed during interesterification, so did the SMP and the SFC as shown in Table 2 and Figure 2. For each of the five blends between POs/PKOo 30:70 to 70:30 there was a decrease of 10 to 12°C in the SMP after

TABLE 2
FFA, Slip Melting Point, and Solid Fat Content (%) of the Reference Products and the Fully Interesterified Blends of POs/PKOo

POs/PKOo blend ratio	FFA	Solid fat content (°C)							Slip melting point (°C)				
		10	15	20	25	30	35	37	0 h	2 h	4 h	5 h	6 h
30:70	2.9	31.5	17.3	9.6	3.3	1.1	—	—	40.0	38.5	28.2	28.6	29.9
40:60	2.7	31.8	18.7	11.1	5.8	1.5	—	—	42.7	40.4	37.3	32.8	31.5
50:50	2.5	33.6	19.4	10.7	6.8	3.5	0.3	—	45.6	37.4	35.0	34.1	33.0
60:40	2.0	34.5	21.9	14.2	11.5	6.4	3.9	2.1	46.9	41.4	40.2	35.8	34.1
70:30	2.2	41.3	28.0	24.1	12.2	6.8	4.1	2.4	47.7	45.1	43.3	39.4	37.5
Firm margarine[a] (POs:PKO)	N.A.	39.5	23.3	19.7	12.5	8.4	4.0	2.3	N.A.	N.A.		N.A.	36.8
Soft margarine[a] (POo:PKO:SFO)	N.A.	28.9	17.9	10.1	4.5	1.9	—	—	N.A.	N.A.		N.A.	32.6

[a]Reference 4. N.A., data not available; PKO, palm kernel oil; SFO, sunflower oil; FFA, free fatty acid; POo, palm olein; dashes, not detected. For other abbreviation see Table 1.

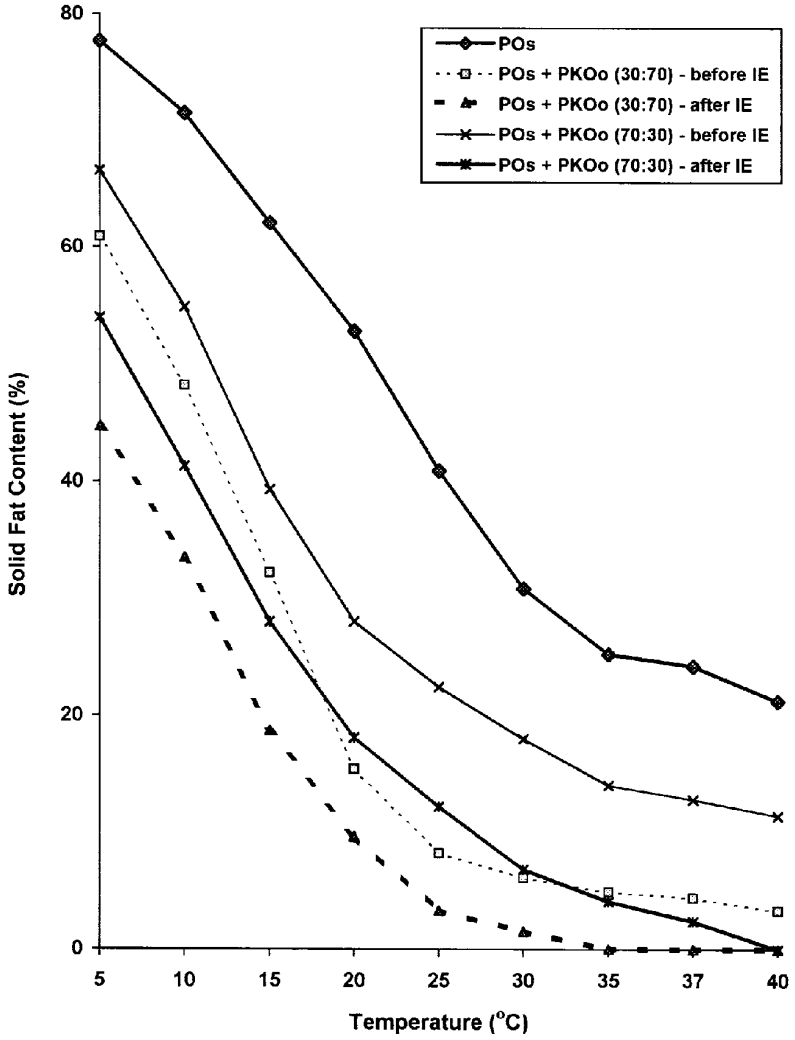

FIG. 2. Solid fat content profiles of POs/PKOo blends before and after enzymatic interesterification. For abbreviations see Figure 1.

TABLE 3
Fatty Acid Composition and *Trans* Content of Reference Fats, Stock Oils, and Enzymatically Interesterified POs/PKOo Blends[a]

	Fatty acid (wt%)											
Fat sample	8:0	10:0	12:0	14:0	16:0	18:0	18:1*c*	18:1*t*	18:2*c*	18:2*t*	18:3	*Trans* fatty acid
POs	—	—	0.4	1.2	52.6	4.7	32.7	—	7.6	0.1	0.4	—
PKOo	4.2	3.7	45.9	14.2	8.2	2.2	18.3	—	2.9	—	—	—
Commercial margarine[b] (HSBO, SBO)	—	—	—	Trace	9.9	7.3	54.7	16.2	24.9	2.1	2.4	18.3
Commercial margarine[c] (CO, HCO)	—	—	—	Trace	10.2	6.6	45.5	14.2	36.9	0.3	1.1	14.5
Interesterified, 70:30[d]	1.7	1.2	14.4	4.6	41.3	3.1	31.1	—	2.6	0.1	0.2	0.1
Interesterified, 60:40[d]	2.2	1.6	19.0	5.7	36.5	1.3	30.1	—	2.7	0.1	0.1	0.1
Interesterified, 50:50[d]	2.3	1.7	20.5	6.2	33.8	1.7	28.9	0.3	5.7	0.1	0.3	0.4
Interesterified, 40:60[d]	3.0	2.2	23.3	7.1	27.9	2.5	28.8	0.2	5.3	—	0.2	0.2
Interesterified, 30:70[d]	3.2	2.4	28.6	8.2	25.4	1.5	27.6	—	2.7	—	—	—

[a]HSBO, hydrogenated soybean oil; SBO, soybean oil; HCO, hydrogenated corn oil; CO, corn oil; dashes, not detected. For other abbreviations see Table 1.
[b]Reference 5.
[c]Reference 4.
[d]Enzymatically interesterified POs/PKOo blends.

complete interesterification. TG of ECN 40 and 44 should have lower melting points than ECN 48 and so, as ECN 40 and 44 increased, the SMP and the SFC decreased. Figure 2 shows that the SFC at the temperatures measured were lower after modification than before.

Table 2 shows that the SFC ranges of the interesterified products were 31.47–41.3%, 9.6–18.1%, and 1.1–6.8% at 10, 20, and 30°C, respectively, for these five blends. The SMP and the SFC values showed an unexpected increase for certain blend ratios. The SFC at 10°C for the POs/PKOo 30:70 blend was higher than for the 40:60, and the SMP of the 40:60 blend samples taken before complete interesterification were higher than the 50:50 blend. This break from the prevailing pattern was most likely due to eutectic formation of the oil mixture. This is not unexpected as the chemical nature of the oils, as evident from the fatty acid composition (Table 3) and the TG profiles of POs and PKOo, showed large differences between the two.

Based on the SFC results, these five interesterified blends may be suitable for the production of table margarine, spread margarine, and shortenings. Blends POs/PKOo 30:70 and 40:60, for example, showed close similarities with soft margarine and 70:30 with firm margarine.

As indicated in Table 3, a comparison between commercial and IE products shows that the latter were essentially free of TFA whereas the former had significant levels of them. The TFA in the IE products were less than 0.5% compared to hydrogenated fats or commercial margarine, which contain 18.3–14.5% fatty acid. IE of 30–70% POs with PKOo showed potential as a modification process in producing materials with the required physical properties for the production of plastic fats such as spread margarine and shortenings.

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