Enzymatic Interesterification of Lard and High-Oleic Sunflower Oil with *Candida antarctica* Lipase to Produce Plastic Fats

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ABSTRACT: Lard and high-oleic sunflower oil (Trisun® Extra) were interesterified at 55°C for 24 h with SP435 lipase from *Candida antarctica* to produce plastic fats. As the amount of trisun increased, percentage free fatty acid, unsaturated fatty acid/saturated fatty acid value, oxidizability, and the amount of 18:1 found at the *sn*-2 position of triglyceride products increased. Differential scanning calorimetry showed that the low-melting components in the product contained more 18:1 than the high-melting components. A 60:40 (w/w) ratio of lard to trisun had the widest plastic range (3–26°C). The scaled-up reaction to produce this blend resulted in a product that had 60.1% 18:1 at the *sn*-2 position compared to 44.9% for the physical blend. The solid fat content of the 60:40 interesterified mixture resembled soft-type margarine oil.
JAOCS 75, 1339–1345 (1998).

KEY WORDS: *Candida antarctica* lipase, differential scanning calorimetry of triglyceride, lard, oxidative stability of triglyceride, plastic fats, solid fat content, spreadability of triglyceride, triglyceride structure and properties,Trisun® Extra, lard.

Enzymatic interesterification has received considerable attention in recent years. Traditionally, chemical catalysts (usually sodium methoxide) have been used to perform interesterification reactions. By replacing the chemical catalysts with enzymes (biocatalysts, e.g. lipases), acyl exchange can proceed in a controlled manner. Enzymatic interesterification can be used to upgrade cheap and saturated fats or to add value to commercial fats and oils. For example, beef tallow was interesterified with rapeseed oil (1) and with sunflower oil (2) to produce fat mixtures with improved melting properties, or with soybean oil to produce plastic fat resembling tub-type margarine (3). Reactions between solid fat and liquid oil have been conducted to obtain fat mixtures with better melting behavior, such as enhanced spreadability of butter (4). Many researchers have investigated lipase-catalyzed reactions as an alternative to hydrogenation to improve the physical properties of vegetable oils (5). Hydrogenation is known to produce *trans* fatty acids often implicated in coronary heart disease (6). Interesterification was used to enrich melon seed oil with n-3 polyunsaturated fatty acids (n-3 PUFA) to improve its nutritional quality (7). Furthermore, owing to the positional and substrate specificities of lipases, several specialty fats or structured lipids have been produced (8,9) which may be potential products for dietetic applications.

Immobilized *Candida antarctica* lipase, SP435, was used as a biocatalyst to alter the fatty acid composition of evening primrose oil by incorporating n-3 PUFA such as eicosapentaenoic acid (EPA) (10). Huang and Akoh (8) optimized and scaled up the synthesis of structured lipids by transesterification of caprylic acid ethyl ester and soybean oil and high-oleic sunflower oil with SP435 lipase.

Lard is used extensively as a domestic frying medium as well as a fat for making short pastry dough. However, attempts to use it in a more general-purpose shortening showed that its very crystalline texture led to poor creaming (i.e., air incorporation) properties (11). Interesterification would randomize fatty acid residues in the triglycerides (TG) of lard, and the resulting fat could be used as a major ingredient in margarine formulations. This may be extended further by the interesterification of lard and liquid oil blends to produce good-quality plastic fats with improved unsaturated to saturated fatty acid (UFA:SFA) ratio.

Interesterification of lard and sunflower oil has only been briefly studied with a chemical catalyst (12). In the current report, lipase-catalyzed acyl exchange between lard and high-oleic sunflower oil was investigated. The products were analyzed in terms of fatty acid and TG composition, melting behavior, and oxidative stability. The purpose of this study was to produce interesterified blend of glycerides that would exhibit a wider range of melting properties compared to the individual fats and oils alone.

MATERIALS AND METHODS

Materials. Immobilized lipase, SP435 [7000 (Propyl Laurate Units) PLU/g) from *C. antarctica* was provided by Novo Nordisk Biochem North America, Inc. (Franklinton, NC). The activity of SP435 is expressed in PLU/g as defined by Novo Nordisk Biochem North America, Inc. Refined, bleached, winterized, and deodorized (RBWD) high-oleic sunflower oil (commercially known as Trisun® Extra) was obtained from SVO Enterprise (Eastlake, OH), and a com-

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mercially processed lard (Smithfield Packing Company, Inc., Smithfield, VA) was bought from a local supermarket. Porcine pancreatic lipase (Type II, crude), oleic acid (99% pure), monoolein, monostearin, and diolein standards were purchased from Sigma Chemical Company (St. Louis, MO). All organic solvents were obtained from Fisher Scientific (Norcross, GA).

Enzymatic interesterification. Interesterification was performed in a screw-capped test tube in which 1 g sample of the fat–oil mixture and 0.1 g of immobilized SP435 lipase (10% w/w of substrates) was dissolved in 3 mL *n*-hexane. The mixture was incubated in an orbital-shaking water bath at 200 rpm for 24 h at 55°C. The 60:40 (w/w) lard to Trisun® Extra mixture was also scaled up to 30 times its original weight and interesterified in a 250-mL Erlenmeyer flask with 3 g of SP435 lipase and 30 mL of hexane. A 60:40 mixture was physically blended and incubated for 24 h at 55°C for comparison. All reactions were performed in duplicate, and average values are reported.

TG separation procedure. After incubation, the reaction mixture was passed through a column (2–3 cm) of anhydrous sodium sulfate to remove enzyme particles. Then a portion of the sample was analyzed by thin-layer chromatography (TLC) on silica gel G plates (Fisher Scientific). Authentic glycerides and free fatty acid (FFA) standards were spotted on the TLC plates to determine relative migration of the reaction products. The developing solvent system was petroleum ether/ethyl ether/acetone (90:10:1, vol/vol/vol). The bands on the developed TLC plates were sprayed with 0.2% 2,7-dichlorofluorescein in methanol solution prior to scanning with a UNISCAN Video Densitometer (Analtech, Inc., Newark, DE) under ultraviolet light (245 nm). The relative percentage of monoglyceride (MG), diglyceride (DG), TG, and FFA was based on the total area of the spots as calculated by an on-line computer.

Fatty acid composition analysis. The fatty acid profile of the TG band was determined after TLC analysis. Bands corresponding to TG were scraped and methylated with 3 mL of 6% HCl in methanol at 75°C for 2 h to form fatty acid methyl esters (FAME), which were extracted with hexane (2 mL) and 0.1 M KCl solution (1 mL), centrifuged (1000 rpm or $200 \times g$ force, 3 min), and concentrated under nitrogen. FAME were analyzed with an HP 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a DB-225 fused-silica capillary column (30×0.25 mm i.d.) (J&W Scientific, Folsom, CA) and flame-ionization detector, FID (Hewlett-Packard) and operated in a splitless mode. The injector and detector temperatures were 250 and 260°C, respectively. The column temperature was held at 210°C for 10 min and total helium carrier gas flow was 23 mL/min. The relative fatty acid content of FAME as mol% was quantitated by an on-line computer with heptadecanoic acid (17:0) as an internal standard.

Hydrolysis by pancreatic lipase. Determination of the fatty acids at the *sn*-2 position of the TG isolated by TLC analysis was conducted using a modified method of Luddy *et al.* (13). Briefly, 1 mg of TG was first emulsified with 1 mL of 2% (wt) polyvinyl alcohol, and then mixed with 1 mL of 1 M Tris-HCl buffer (pH 7.6), 0.25 mL of 0.05% bile salts, 0.1 mL of 2.2% $CaCl_2$, and 5 mg pancreatic lipase. The mixture was incubated in a water bath at 40°C for 10 min, vortexed vigorously (1 min), centrifuged (1900 rpm or $727 \times g$, 3 min), extracted with 3 mL diethyl ether (twice), and dried by passing through a column of anhydrous sodium sulfate. TLC analysis was on silica gel G, and the developing solvent system was hexane/diethyl ether/acetic acid (50:50:1, vol/vol/vol). The band corresponding to 2-MG was scraped, methylated, and the fatty acid analyzed by gas chromatography.

Melting characteristics. Differential scanning calorimetry (DSC) on Perkin-Elmer Model DSC7 (Norwalk, CT) was performed to determine the melting profile and percentage solid fat content of the TG. A 4–5 mg TG sample was hermetically sealed in an aluminum pan with an empty pan serving as a reference. Analysis was performed according to the AOCS recommended DSC procedure Cj 1-94 (14). Briefly, the samples were initially and rapidly heated (200°C/min) from room temperature to 80°C and held at this temperature for 10 min to destroy crystal memory; cooled to –40°C at 10°C/min and held for 30 min; and heated to 80°C at 5°C/min to define the melting profile. Normal standardization of instrument was performed with *n*-decane (mp –30°C) and indium (mp 176°C) as reference standards. Liquid nitrogen (–196°C) was used as coolant.

Melting point determination. Melting point was determined by the capillary tube method according to AOCS official method Cc 1-25 (14). Briefly, a capillary tube was dipped in the completely liquid TG sample obtained from TLC analysis until the sample rose to about 10 mm high in the tube. The end of the tube was fused (where the sample was located) with a flame. After 16 h of refrigeration at 4°C, the capillary tube was attached to the lower end of the thermometer which was suspended in a 600-mL beaker half-filled with distilled water. Starting at 15°C, heat was gently applied until the fat inside the tube was completely clear. Two replicate analyses were performed on each sample, and average values are reported.

FFA value. FFA was determined on scaled-up reaction mixtures using the method described by Foglia *et al.* (2). FFA was quantitated with 0.1 and 0.01 N NaOH in ether/ethanol/water (3:3:2 vol/vol/vol) solvent mixture (30 mL/0.5 g sample with 4–5 drops of phenolphthalein indicator solution, 1% in 95% ethanol, added) by titrating the samples until the first pink color appeared (an end point of pH 12). The percentage of FFA was expressed in terms of oleic acid and calculated according to AOCS official method Ca 5a-40 (14).

Oxidative stability of TG. Oxidative stability index (OSI) of the fat/oil mixture was determined with an Oxidative Stability Instrument from Omnion Inc. (Rockland, MA) manufactured under license from Archer Daniels Midland Co. (Decatur, IL). TG sample (5 g) was obtained from the nonaqueous layer after FFA determination by diluting the titrated sample with 30 mL water. Air flow was continuous at 2.5 mL/s while the sample was heated at 110°C to induce oxida-

TABLE 1
Relative Percentages of Glycerides and Free Fatty Acids of Lard, Trisun® Extra, and Their Interesterified Blends[a]

	TG[b]	DG	MG	FFA
R_f value	0.8	0.4	0.1	0.5
Small scale (lard/trisun, w/w)				
Lard[c] (unmodified)	100	0.0	0.0	0.0
100:0	88.6	5.0	2.2	4.1
80:20	85.9	5.8	3.0	5.3
60:40	83.1	7.4	3.6	5.9
40:60	71.4	9.6	3.6	15.4
0:100	91.7	3.7	0.9	3.7
Trisun[d] (unmodified)	100	0.0	0.0	0.0
Scaled-up 60:40 (lard/trisun, w/w)				
Interesterified blend	64.7	24.3	8.8	2.2
Physical blend	80.7	12.8	4.7	6.9

[a]An indicated mixture of lard to trisun was incubated with 10% (w/w of substrate) SP435 lipase (Novo Nordisk Biochem North America, Franklinton, NC) in 3 mL hexane at 55°C for 24 h. Each product species in the interesterified blend was separated by thin-layer chromatography and analyzed with UNISCAN Video Densitometer (Analtech, Inc., Newark, DE).
[b]TG, triglycerides; DG, diglycerides; MG, monoglycerides; FFA, free fatty acids.
[c]Lard from Smithfield Packing Company, Inc. (Smithfield, VA).
[d]Trisun® Extra is from SVO Products, Eastlake, OH (17) and is designated as trisun throughout these tables.

tion according to AOCS official method Cd 12b-92 (15). Each test was continued until the cells were automatically inactivated after reaching maximum conductivity.

RESULTS AND DISCUSSION

Enzymatic interesterification of lard and Trisun® Extra by SP435 lipase was conducted using different proportions of fat and oil blends. Trisun® Extra is designated as trisun throughout this paper. Before interesterification can begin, partial hydrolysis of lard and trisun to several glycerides and FFA must occur (2). The relative amounts of unesterified partial glycerides and FFA were quantitated with the UNISCAN Video Densitometer. The separation and values obtained were comparable to those obtained from high-performance liquid chromatography (HPLC) (16). Table 1 shows the relative percentages of TG, DG, MG, and FFA present in the mixture after the reaction was stopped. With an increasing amount of trisun, a decreasing amount of TG (88.6 to 71.4%) was found. Also, more DG, MG, and FFA were found in the mixture. Foglia *et al.* (2) measured % FFA after the reaction as % hydrolysis occurring during the reaction. Since a high amount of FFA (15.4%) was observed in 40:60 lard to trisun interesterified blend, we conclude that maximal hydrolysis occurred with this blend. In the unesterified lard and trisun, no DG, MG, or FFA were detected. R_f values of each glyceride and FFA band are also reported in Table 1.

Table 2 shows the fatty acid profile and selected physical properties of the interesterified blends after DG, MG, and FFA were removed by TLC. Major fatty acid residues observed in the TG were: myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic acid (18:3). Interesterification of lard itself (100:0) with SP435 increased the relative proportion of SFA residues. Similar results were reported in the enzymatic interesterification of beef tallow (1) and chemically interesterified lard (12). Interesterification of trisun alone (0:100) also decreased the UFA to SFA residue ratio slightly from 15.4 to 13.6. The analyzed fatty acid profile of unmodified trisun was comparable to the data given by SVO Specialty Product, Inc. (17): 2.7% 16:0, 4.0% 18:0, 85.0% 18:1, 4.4% 18:2, and 0.1% 18:3. Interestingly, the UFA/SFA value of interesterified 80:20 lard to trisun decreased considerably owing to the

TABLE 2
Analysis of Interesterified Lard–Trisun® Extra Blends

	Fatty acid profile (mol%)									
Sample (lard/trisun, w/w)	14:0	16:0	16:1	18:0	18:1	18:2	18:3	UFA/SFA[a]	mp[b]	Ox[c]
Lard[d]	2.2	23.7	2.1	10.2	40.9	19.2	1.7	1.8	47.0	0.23
100:0	3.1	34.6	2.3	13.5	29.7	16.0	0.8	1.0	42.8	0.18
80:20	2.9	40.4	0.9	18.7	28.7	8.0	0.4	0.6	39.6	0.09
60:40	1.8	24.6	1.2	11.5	50.2	10.6	0	1.6	36.6	0.12
40:60	1.3	21.1	0.7	10.5	59.0	7.3	0.1	2.0	33.5	0.09
0:100	0	3.3	0	3.5	87.3	5.9	0	13.6	N.D.[e]	0.08
Trisun[d]	0	3.6	0.4	2.3	8.43	6.5	0	15.4	N.D.	0.08
At *sn*-2 position										
Lard	5.2	63.5	3.3	3.4	17.6	7.2	0	0.4	—	—
100:0	3.5	52.7	2.4	9.4	26.5	5.4	0	0.5	—	—
80:20	3.5	48.2	1.6	9.0	37.6	0	0	0.6	—	—
60:40	0	38.6	0.9	7.0	52.4	1.1	0	1.2	—	—
40:60	0.7	26.2	0.7	4.4	64.3	0.6	0	2.1	—	—
0:100	0	3.2	0	2.8	89.5	4.5	0	15.7	—	—
Trisun	0	1.2	0	0.4	92.2	6.2	0	62.1	—	—

[a]UFA/SFA = ratio of unsaturated fatty acid (16:1, 18:1, 18:2, and 18:3) to saturated fatty acid (14:0, 16:0, and 18:0).
[b]mp = melting point (°C) determined by recommended AOCS method Cc 1-25 (14).
[c]Ox = Calculated oxidizability = [0.02(18:1%) + (18:2%)]/100 (20).
[d]Unmodified sample. See Table 1 for company sources.
[e]ND = Not determined. Trisun and 0:100 were in liquid state at 0°C.

higher 16:0 and 18:0 and lower 18:2 obtained, compared to lard at 100:0. At higher trisun contents, the 16:0 content in the TG products was lowered and 18:1 was increased. This resulted in an increase in the value of UFA/SFA.

Major TG species in lard are SPS, OPO, and POS (where S = 18:0, P = 16:0, and O = 18:1) with P significantly concentrated at the *sn*-2 position; the S is primarily located at the *sn*-1 position and linoleic (L) at the *sn*-3 position; and a large amount of O occurs at positions 1 and 3 (18). When pancreatic lipase hydrolysis was conducted, the fatty acid residues at the *sn*-2 position were determined. Our result for unmodified lard (Table 2) is consistent with that reported previously (18). Interesterified lard (100:0), however, had lower P and higher O content. This led us to question the specificity of SP435 lipase. Novo Nordisk (19) stated that the positional specificity of SP435 (Novozym® 435) depends on the reactants. In some esterification reactions, SP435 shows 1,3-positional specificity whereas in other reactions the lipase functions as a nonspecific lipase. Thus, it is possible that SP435 was acting as a nonspecific lipase when catalyzing lard and trisun interesterification. It is also possible that more random distribution of fatty acid residues could be obtained if the reaction were to continue over 24 h.

The calculated oxidizability (20,21) of each sample based on its fatty acid composition is also reported in Table 2. Lard had the highest calculated oxidizability and therefore would be expected to have the lowest oxidative stability, whereas trisun should have the highest oxidative stability because it has the lowest calculated oxidizability. Neff *et al.* (21) reported that high oleic acid (O) content, especially at the *sn*-2 position, increased oxidative stability, and increased contents of L, at any position, led to reduced oxidative stability. Therefore, in theory, the 40:60 blend would have the lowest oxidative stability among the interesterified samples.

Since the fatty acid compositions of these interesterified fats have been altered, their melting properties would be expected to be different compared to the starting substrates. Newar (18) stated that the polymorphic structure of mixed TG is complicated by a tendency for carbon chains to segregate according to length or degree of unsaturation to form structures in which the long spacing is made up of triple chain lengths. Also, decreases in melting point (Table 2) and solid fat content are due to a decrease in the proportion of higher-melting TG as a result of interesterification (3). In lard, the preponderance of 16:0 at the *sn*-2 position leads to a β rather than β´ (the less stable polymorphic form) crystal type (11). Its high-melting component was found at 29.2°C (Fig. 1). This peak almost disappeared after lard was interesterified, although SFA in 80:20 blend increased. Several small and broad peaks were also observed (Fig. 1).

Similarly, when trisun was added to the reaction mixture, many endotherms at lower temperature range were observed compared to that of unesterified and interesterified lard alone. Major melting components are labeled in Figure 1. As the amount of trisun increased, the lowest-melting components seemed to become larger and sharper. This is expected since

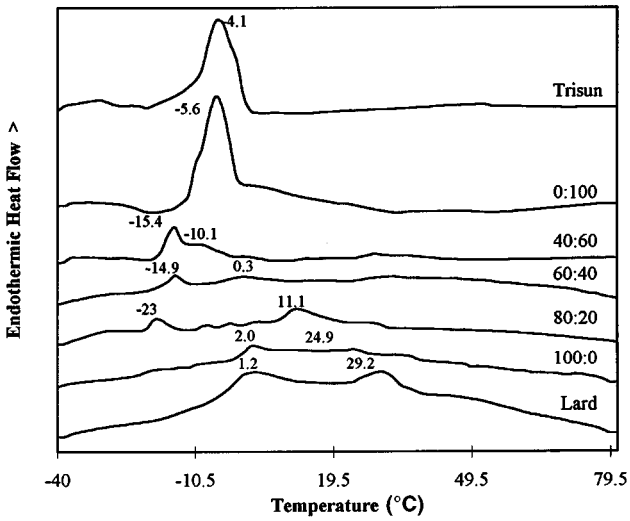

FIG. 1. Melting profile of lard, trisun, and their interesterified triglyceride products at an indicated proportion of lard and trisun. Heat treatment: heat to 80°C at 200°C/min; hold for 10 min; cool to –40°C at 10°C/min; hold for 20 min; and heat to 80°C at 5°C/min to define melting profile. Major endotherms are labeled.

the 18:1 content of the blends had increased. Unmodified trisun contains an extremely high amount of 18:1 and has a distinct peak at –4.1°C. HPLC analysis of trisun indicated that its TG contained primarily triolein (OOO) with a slight trace of LOO, SOO, and SLL (data not shown). Triolein (99% pure) has two sharp endotherms at –12 and –5°C (22). Interesterified trisun had its major endotherm at –5.6°C. Although almost comparable to that of unmodified trisun, the interesterified trisun peak was broader.

The factors that determine the range within which a material will be plastic include the percentage of solids and the characteristic continuous variation that occurs with change in temperature. DeMan (23) stated that the plastic range of fat for a desirable spreadability occurs in a narrow range of roughly 15 to 35% solid fat content. The *Handbook of Soy Oil Processing and Utilization* (24) explained that when a fat product remains "workable" or plastic as the temperature range increases, the plastic range is said to widen. Also a plastic range is desirable for shortenings and margarines that are to be used for their creaming ability. Sunnyland Refining Company prefers that the solid fat content of their margarine be between 8 and 25% in order to be called plastic (25).

Percentage solid fat content calculated from the melting profile (Fig. 1) is shown in Figure 2. Solid fat contents of the interesterified fats were between that of the substrates. Although SFA residues increased slightly when lard was interesterified from 10 to 30°C, its solid fat content was lower than unesterified lard as confirmed by Podmore (11). This also coincides with the diminishing high-melting components observed in Figure 1. Similarly, the 80:20 blend also had lower percentage solid fat content than the interesterified lard (100:0) and therefore was a softer fat blend. Overall, the blend with the widest plastic range resembling that of margarine was the 60:40 interesterified blend (3–26°C) as shown in Figure 2.

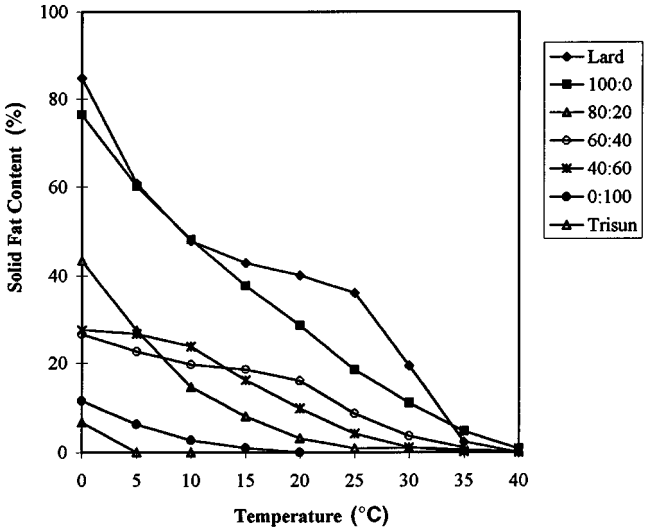

FIG. 2. Percentage solid fat content of lard, trisun, and their interesterified triglyceride product obtained from Figure 1. The calculation was performed with the software (UNIX7) in the DSC7, and the area under the curve was determined under sigmoid baseline.

The lard and trisun 60:40 interesterification reaction was scaled up to 30 times its original weight. Analysis showed that less TG and FFA were produced in the scaled-up enzymatic reaction compared to the small-scale and physical blends (Table 1). The level of DG and MG formed increased. The reason for these variations is not clear. It seems like the FFA were converted more effectively to the MG and DG in the scaled-up reaction of the 60:40 blend than in the small-scale reaction. Heat (55°C for 24 h) may have enhanced partial hydrolysis of the physical blend since no detectable DG, MG, or FFA were observed in the starting materials. The interesterified blend exhibited higher 18:2 and UFA/SFA values than the small-scale blend (Table 3). Previous researchers reported that a chemically interesterified 60:40 blend of lard and sunflower seed oil exhibited increased saturation, especially in S_2D, SM_2, S_2M, and SMD (where S = saturated acyl, D = diene acyl, and M = monoene acyl) (26). The enzymatically interesterified blends reported here had fatty acid profiles similar to the physical blend. However, the physical blend contained considerably less 18:1 at the *sn*-2 position than the interesterified blends.

In Figure 3, three major peaks were observed at –20.2, –8.0, and 5.6°C in the melting profile of the 60:40 interesterified blend. A slight variation was observed between the melting profile of scaled-up and small-scale interesterified blends (Figs. 1, 3). The physical blend had a low- and a high-melting component at –6.8 and 33.1°C, respectively. These peaks are similar to the major endotherms of trisun and lard, respectively. These two separated peaks indicate that hydrolysis was occurring to a lesser extent and virtually no interesterification was evident compared to when SP435 lipase was used.

The spreadability of margarine at refrigerator temperatures is related to its content of solid fats at 2 to 10°C (24). Also, the solid content at 25°C influences plasticity at room temperature, and the solid content between 33 and 38°C largely determines the mouthfeel (24). The percentage solid fat contents (at 10, 21.1, and 33.3°C) of two types of commercial margarine oils (stick and soft) are shown in Table 4. The 60:40 physical blend had a higher percentage solid fat content than that of the 60:40 interesterified scaled-up blend and therefore was a harder fat blend. The solid fat content of the 60:40 physical blend resembled that of stick margarine oil at 10–21.1°C, whereas the interesterified blend resembled soft margarine oil at 10–33.3°C.

Oxidative stability index (OSI) analysis was conducted on the two scaled-up blends (Table 4). Trisun containing 84.7% 18:1 showed a considerably higher OSI value than lard. The physical blend was more stable than the interesterified blend (11.6 vs. 3.9 h), which means that interesterification lowered the oxidative stability of the lard-trisun blend. This could be due to residual soap in the FFA determination and/or loss of tocopherols during the downstream processing of the product mixture. Adding back antioxidants such as citric acid or butylated hydroxy anisole (BHA) may increase the stability of this blend (3).

Percentage free fatty acid values (before DG, MG, and FFA were removed) are reported in Table 4. Since the reaction mixture contained a higher 18:1 than 16:0 content, %FFA was expressed as %18:1, and the calculation was as follows:

$$\%\text{FFA as } 18{:}1 = \frac{(\text{volume of NaOH}) \times \text{N} \times (28.2)}{\text{weight of sample}} \quad [1]$$

where volume of NaOH is milliliters of sodium hydroxide used to reach the end point, N is concentration (N) of NaOH used, and weight of sample is g of fat blends. Interesterification increased the free fatty acid level to 0.63%. Refining and deodorization can be used to reduce the acid level. We did not perform deodorization or refining operations.

TABLE 3
Analysis of Scaled-Up 60:40 Blends[a] of Lard and Trisun® Extra Blends

	Fatty acid profile (mol%)									
Sample	14:0	16:0	16:1	18:0	18:1	18:2	18:3	UFA/SFA[b]	mp[b]	Ox[b]
Interesterified blend	1.1	17.2	1.5	7.5	59.4	12.6	0.7	2.9	32.2	0.15
Physical blend	1.1	16.4	1.4	7.8	59.3	13.4	0.7	3.0	33.5	0.15
At *sn*-2 position										
Interesterified blend	0.5	18.3	0.8	9.7	60.1	5.7	0.0	2.3	—	—
Physical blend	9.3	33.8	1.0	3.4	44.9	6.0	0.0	1.1	—	—

[a]Scaled-up 60:40 (w/w) of lard to trisun mixture incubated at 55°C for 24 h with both lipase and solvent, and with neither lipase nor solvent (physical blend).
[b]See Table 1 for company sources and Table 2 for abbreviations.

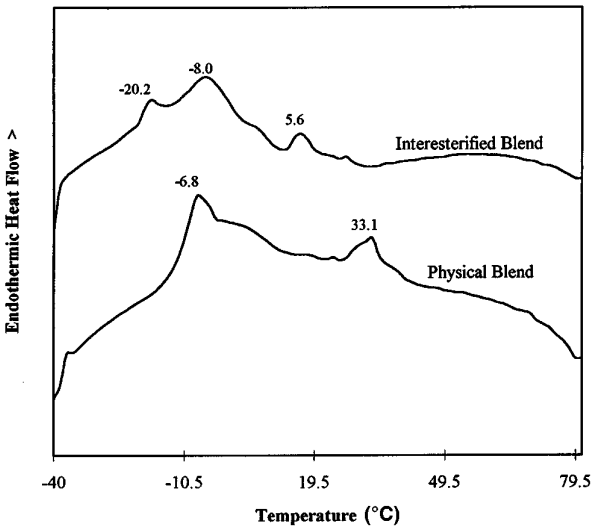

FIG. 3. Melting profile of physical and enzymatically interesterified scaled-up blends of 60:40 (w/w) lard and trisun. See Figure 1 for heat treatment.

This study has demonstrated that enzymatic interesterification (with SP435 lipase) of saturated fat (lard) with an unsaturated vegetable oil (trisun) can be used as an alternative to partial hydrogenation to produce plastic fats suitable for commercial application. Our results indicated that various plastic fats with different solid fat contents can be produced, depending on the proportion of lard and trisun in the starting mixture, by enzymatic interesterification using SP435 lipase. The monounsaturation content of the blend was improved by interesterification. Physical blending produced a harder fat with less monounsaturated fatty acid at the *sn*-2 position. Thus, more trisun would be required if a product with a lower solid fat content were needed. Moreover, studies have shown that fatty acids at the *sn*-2 position of the TG may be better absorbed (27,28) and consumption of monounsaturated fatty acids may be desirable. Thus enzymatic interesterification has advantages over physical blending for producing plastic fats intended for commercial use.

TABLE 4
Comparison of Interesterified Lard and Trisun® Extra (60:40) Blends with Commercial Margarine Oils[a]

Sample	Solid fat content (%) at °C			OSI[b]	
	10.0	21.1	33.3	(h)	%FFA[c]
Stick margarine oil[d]	23.0–27.0	15.0–18.0	2.5–4.0	—	0.05
Soft margarine oil[e]	8.0–11.0	4.0–7.0	0.5–2.0	—	0.05
Physical blend[f]	25.4	17.6	10.6	11.6	0.03
Interesterified blend[f]	11.3	2.0	0.2	3.9	0.63
Lard (unmodified)[g]	99.8	86.9	31.3	9.1	0.02
Trisun (unmodified)[g]	4.5	2.4	0.9	24.1	0.08

[a]Specification of the commercial margarine oils were obtained from Sunnyland Refining Co. (25).
[b]Oxidative stability index (OSI) was determined by the oxidative stability instrument method (15).
[c]Free fatty acid values as % oleic acid were determined according to AOCS Ca 5a-40 (14).
[d]Ingredients of stick margarine oil are minimum of 51% soybean oil and maximum of 49% partially hydrogenated soybean oil.
[e]Ingredients of soft margarine oil are approximately 70% soybean oil and approximately 30% partially hydrogenated oil.
[f]60:40 physical and enzymatically interesterified blends of lard and trisun, respectively.
[g]See Table 1 for company source.

ACKNOWLEDGMENTS

Contributed by the College of Agricultural and Environmental Sciences, The University of Georgia. Research supported by FoodPAC grant and Food Science Research.

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[Received October 10, 1997; accepted May 3, 1998]