Trans-Free Margarine from Highly Saturated Soybean Oil

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ABSTRACT: Highly saturated (HS) soybean oil (SBO), which contained 23.3% palmitic acid ($C_{16:0}$) and 20.0% stearic acid ($C_{18:0}$), was interesterified at 70°C in preparation for the processing of a *trans*-free margarine. High-performance liquid chromatography analysis of the triacylglycerides and analysis of the *sn*-2 fatty acid composition showed no further change after 10 min of interesterification. The interesterified HS SBO had a slip melting point of 34.5°C, compared with 9.5°C in the noninteresterified HS SBO, and increased melting and crystallization temperatures were found using differential scanning calorimetry. Analysis of solid-fat content by nuclear magnetic resonance revealed the presence of only a small amount of solids above 33°C. A 50:50 blend of interesterified HS SBO and SBO with a typical fatty acid composition was used to make the margarine. Compared to commercial soft-tub margarine, the maximal peak force on the texture analyzer of this blended margarine was about 2.3 times greater, the hardness about 2.6 times greater, and adhesiveness about 1.5 times greater. There were small but statistically significant differences ($\alpha = 0.05$) in the sensory properties of spreadability, graininess, and waxiness between the commercial and blended margarines at 4.5°C and, except for graininess, at 11.5°C. These very small differences suggest a potential use for HS SBO in margarine products.

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KEY WORDS: Differential scanning calorimetry, highly saturated soybean oil, high-performance liquid chromatography, interesterification, margarine, sensory evaluation, slip melting point, *sn*-2 position composition, solid fat content, texture analyzer.

Although the nutritional significance of *trans* fatty acids is controversial, it has been found that *trans* fatty acids may cause heart disease (1,2), and a comprehensive review concluded that *trans* fatty acids consumed at 4.0% or more of total calories may raise plasma lipid levels (3). An estimated 90–95% of *trans* fatty acids in foods is produced by hydrogenation, which imparts firmness to margarines and shortenings by saturating some double bonds and shifting others to a *trans* configuration (4). Margarines may have a *trans* fatty acid content as high as 21% (5). Enig *et al.* (6) estimated *trans* fatty acid consumption by typical U.S. consumers to be between 11.1 and 27.6 g/d.

Some plant oils also may be interesterified to provide firmness. Interesterification raises the melting range of these oils by introducing saturated fatty acids to the *sn*-2 position of glycerol and, thus, increasing the disaturated and trisaturated triacylglycerol (TAG) species (7). The fatty acid profile of the oil is not changed. Undirected interesterification is performed at temperatures higher than the melting points of possible TAG, and the fatty acids are randomly reassigned to the glycerol positions. In directed interesterification, which is performed at lower temperatures, high-melting TAG are selectively formed by crystallization and removed from the equilibrium. Interesterification can be viewed as a more "natural" process than hydrogenation because it does not change the acyl groups in the TAG (7). Sodium methoxide is the most commonly used catalyst and it can be easily washed out of the fat with water.

List *et al.* (8) noted that unmodified soybean oil (SBO) with 30 to 40% saturated fatty acids was not sufficiently solid enough to produce a margarine. But, SBO with elevated saturated fatty acids levels of 17 to 38% might be blended with high-melting oils, such as palm oil, interesterified palm oil, interesterified palm/SBO, cottonseed hardstock, or soybean hardstock to qualify as margarine oils (9). Undirected interesterification of a SBO containing 17% $C_{18:0}$ provided a solid-fat index and dropping point similar to that of liquid margarine oil (8), and SBO with a $C_{18:0}$ composition ranging from 20 to 33% was suitable for manufacture into soft-tub margarine after undirected interesterification. In addition, soft-tub margarine can be formed by interesterifying a blend of 20% fully hydrogenated SBO and 80% SBO (10).

The objectives of the current research were to interesterify a highly saturated (HS) SBO to produce a margarine, and to analyze the physical properties and sensory characteristics of the finished product.

EXPERIMENTAL PROCEDURES

Materials. The soybeans, a bulk of three sister lines with elevated $C_{16:0}$ and $C_{18:0}$ percentages, and termed Bulk ABC, were developed by the Departments of Agronomy and Food Science and Human Nutrition at Iowa State University. The three sister lines were selected from multiple generations of the progeny from a cross of FA 79 (28% $C_{16:0}$) with line A89-260026 (25% $C_{18:0}$). The harvested soybeans were

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*To whom correspondence should be addressed at 2312 Food Science Building, Iowa State University, Ames, IA 50011. E-mail: pjwhite@iastate.edu stored in 50-bushel boxes held at 3.3°C and 65% humidity in sealed plastic bags until used for processing. Crisco® (Procter & Gamble, Cincinnati, OH) soybean oil, and Blue Bonnet® and Chiffon® soft-tub margarines (Nabisco Inc., East Hanover, NJ) were purchased at a local grocery store.

Oil extraction. Seed preparation and oil extraction were carried out as previously described (11). The free fatty acid contents of the crude oils were determined according to AOCS official method Ca 5a-40 (12) and alkali refined following method Ca 9b-52 (12). The refined oils were bleached using method Cc 8b-52 (12). The SBO was steam deodorized on a laboratory scale (13,14) for all chemical and instrumental analyses. A pilot plant-scale continuous deodorizer (15) was used for the greater amounts of SBO needed in the actual processing of margarine. This deodorizer was run under 1 mm Hg or less of vacuum at 180°C with a flow rate of 600 mL/h. Citric acid was not added to the refined, bleached, and deodorized (RBD) oils at this point because acids destroy the sodium methoxide catalyst used during interesterification.

Fatty acid composition. The RBD oils were analyzed for fatty acid composition by converting the TAG to fatty acid methyl esters (FAME) with sodium methoxide in methanol, and analyzing the FAME by gas–liquid chromatography (16).

Interesterification. The RBD oils were interesterified according to List *et al.* (17) using 0.5% w/w sodium methoxide. Undirected interesterification was performed at 70°C for 10, 15, 20, and 25 min with stirring. Citric acid at 110% of the amount necessary to neutralize all catalyst was used to terminate the reaction. The neutralized oil was washed with water until the rinse water was clear, and the oil was dried in a rotary evaporator.

Monitoring interesterification. The composition of the *sn*-2 position of the TAG was observed after using pancreatic lipase hydrolysis according to Luddy *et al.* (18), except that chloroform was used in place of diethyl ether because the HS oil was more soluble in chloroform. The 2-monoglycerides were isolated and converted to methyl esters for gas–liquid chromatography analysis (16,19). Reversed-phase high-performance liquid chromatography (HPLC) of the TAG was done using AOCS official method Ce 5c-93 (12). Slip melting point was determined in accordance with method Cc 3-25 (12).

Solid-fat content. The nuclear magnetic resonance (NMR) procedure used for analysis of solid-fat content (SFC) was adapted from AOCS official method Cd 16-81 (12) and V. Rutar and E.G. Hammond (personal communication). The samples were tempered in a water bath set at 80°C (rather than the usual 60°C) to ensure complete melting of the interesterified HS oil. Peak areas for glycerol protons at the *sn*-1 and *sn*-3 (CH_2–O–) (4.0 to 4.4 ppm) positions, were compared with a chloroform standard (7.24 ppm) at 11 (the instrument could not be stabilized at 10°C), 21, 27, 33, and 38°C on a high-resolution NMR instrument (model AC 200; Bruker, Karlsruhe, Germany). Calibration graphs of the peak areas for tristearin (0, 5, 10, and 20%) in olive oil at each temperature were prepared. The chloroform reference was contained in melting-point tubes (Corning Inc., Corning, NY) centered in the NMR tubes. The melting-point tubes were selected for uniformity in outer diameter, height, and weight. Samples and chloroform filled the tubes to 5 cm. Proton signals were taken after 45° flip-angle pulses with a total spectra width of 18.5 kHz. The following NMR instrument parameters were used: number of scans, 16; sweep width, 3205 Hz; pulse duration, 8.7 μs; acquisition time, 2.56 s; data size, 16 K; and line broadening factor, 1 Hz. Data were transformed using DISNMR software (Bruker, Rheinstetten, Germany). The peaks were integrated using XWIN NMR software (SPECTROSPIN AG, Fällanden, Switzerland).

Differential scanning calorimetry (DSC). DSC was used to observe the melting and crystallization temperatures as described in AOCS method Cj 1-94 (12).

Margarine formulation and manufacture. The ratio of interesterified HS SBO and typical SBO to be used in the margarine was determined by preparing and evaluating small samples at ratios of 50:50, 67:33, and 75:25. The formula selected for the margarine (termed "experimental margarine") is listed in Table 1. The ingredients were dissolved completely in the appropriate fat or water phase. The oil phase was heated to 80°C and mixed in a commercial blender (Dynamic Corp. of America, New Hartford, CT) as the water phase was added over 30 s. To crystallize the margarine, the

TABLE 1
Formula for Preparation of the Experimental Margarine[a]

Ingredient	Product name	Source	Content (% weight)
Fat phase			**80.5**
Interesterified fat	Highly saturated SBO interesterified at 70°C	Pioneer Hi-Bred, Johnston, Iowa	40.0
Typical SBO	Crisco Pure Vegetable Oil	Procter & Gamble, Cincinnati, Ohio	40.0
Lecithin	Centrolex® F powdered soybean lecithin	Central Soya, Fort Wayne, Indiana	0.3
Mono- and diglycerides	Grinsted™ MONO-DI HV 52 A-K	Danisco Ingredients, New Century, Kansas	0.2
TBHQ	*Tenox* TBHQ	Eastman Chemical Co., Kingsport, Tennessee	0.008
Water phase			**19.4**
Water	Distilled water	Laboratory supply	17.4
Liquid imitation butter flavor	Imitation Butter	Tone's Bros., Inc., Des Moines, Iowa	0.03
Salt	Morton Salt	Morton International Inc., Chicago, Illinois	2.0
Total			**99.9**

[a] Formula from Chrysam (Ref. 22); TBHQ, tertiary butylhydroquinone; SBO, soybean oil.

emulsion was mixed manually in a beaker cooled in an ice bath. After the margarines were tempered at 4.5°C for 2 d, they were evaluated at 4.5 and 21°C by three judges.

An Armfield FT25B (Ringwood, England) margarine crystallizer was used for the pilot plant-scale preparation. The ingredient emulsion was fed into the crystallizer at 12 L/h. The A and B pin worker units were set at 350 rpm, and the margarine was processed at 0, −5, −10, −13, and −15°C to determine the best processing temperature. The product was filled into 237-mL polypropylene tubs and tempered at 4.5°C for at least two days before texture evaluation.

Texture analysis. Samples were tempered at 5°C overnight and texture was evaluated on a TA.XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY) with a 4-mm diameter cylindrical probe (20).

Sensory evaluation. A randomized complete block design was used for the sensory evaluation of the margarines by 13 trained panelists. The panelists judged the margarines on 15-cm line scales for spreadability (ease of product moving over cracker surface), graininess (amount of grain sensed between the tongue and palate), and waxiness (amount of sticky/thick fat on the mouth surface). Margarines were served at temperatures of 4.5 and 11.5°C during two replicate presentations. The panelists were trained in three sessions using several reference samples to anchor the ends of the line scale. Chiffon® soft margarine was used to anchor the 0-cm points of spreadability, nongraininess, and nonwaxiness. Refrigerated butter was used to anchor the end (15 cm) of the difficult-to-spread scale. Crystallized soybean hardstock blended in typical SBO at 33.3% w/w was used as the "very grainy" endpoint (15 cm), and lard portrayed the "very waxy" sample (15 cm). Replicates of the selected experimental margarine and two samples of the commercial Chiffon® Soft margarine with different lot numbers were evaluated in four separate panel evaluations.

Statistical analysis. Analysis of variance (ANOVA) (21) was used to analyze data from HPLC and sensory evaluation experiments. Differences in means among treatments were tested using least significant difference (LSD) at $\alpha = 0.05$ (21). All tests were done on duplicate oil samples from the same batch of RBD oil. The HS SBO used in the two replicated treatments of margarine were processed separately from different bags of soybeans.

RESULTS AND DISCUSSION

Monitoring interesterification by analysis of the sn-*2 position*. The FAME composition of a typical SBO (Crisco® oil) and of the HS SBO before interesterification are shown in Table 2. After interesterification of the HS SBO the amounts of each fatty acid on the *sn*-2 position were constant and very close to the overall composition of the HS SBO, although some of the FAME were different by LSD.

Monitoring interesterification by HPLC. To compare method efficiencies in detecting the interesterification endpoint, HS SBO interesterified at 70°C was analyzed by HPLC. At interesterification equilibrium, the amount of each TAG grouping should remain unchanged. The noninteresterified HS SBO and interesterified HS SBO were separated by HPLC into 21 and 23 peaks, respectively (Fig. 1). Perhaps peaks 22 and 23 of the noninteresterified HS SBO did not appear on the HPLC output because the concentration of trisaturates was too low to be detected. After interesterification, trisaturates were formed and detected in the HS SBO. The areas of peaks 1–4, 10–13, 16, 18, 19, and 21–23 were not significantly affected by interesterification. The areas of peaks 5–8 in the noninteresterified HS SBO were smaller than in all the interesterified samples. The areas of peaks 9, 15, 17, and 20 were greater in the noninteresterified HS SBO than in all the interesterified samples. Interesterification time did not significantly affect peak area, except for peak number 14, whose area decreased between 10 and 15 min and increased between 20 and 25 min.

In general, both the *sn*-2 position composition analysis and HPLC showed that interesterification at 70°C was completed within 10 min. Both HPLC and *sn*-2 fatty acid composition analyses might be used to monitor interesterification.

TABLE 2
Mean Fatty Acid Methyl Ester (FAME) Composition of Typical Soybean Oil[a] (SBO) and Highly Saturated (HS) SBO, Their Composition at the *sn*-2 Position, and the *sn*-2 Composition of HS SBO After Interesterification

	FAME (%)[b]				
Sample	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Typical SBO[c], mean FAME	12.5	3.3	21.4	53.7	9.1
Noninteresterified HS SBO, mean FAME[d]	23.3	20.0	10.5	39.7	6.5
Typical SBO[c], at the *sn*-2 position	4.5	0.0	26.3	63.7	5.5
Noninteresterified HS SBO, at the *sn*-2 position[d]	1.3	1.1	11.5	74.8	11.3
Interesterified HS SBO, at the *sn*-2 position	24.6	17.0	10.3	41.8	6.3
LSD ($\alpha = 0.05$)[e]	0.7	0.8	0.2	0.9	0.2

[a] Crisco® soybean oil (Procter & Gamble, Cincinnati, OH).
[b] $C_{16:0}$ = palmitic acid, $C_{18:0}$ = stearic acid, $C_{18:1}$ = oleic acid, $C_{18:2}$ = linoleic acid, and $C_{18:3}$ = linolenic acid.
[c] $n = 2$.
[d] $n = 3$.
[e] Least significant difference (LSD) comparison is between mean FAME of noninteresterified HS SBO and interesterified HS SBO at the *sn*-2 position.

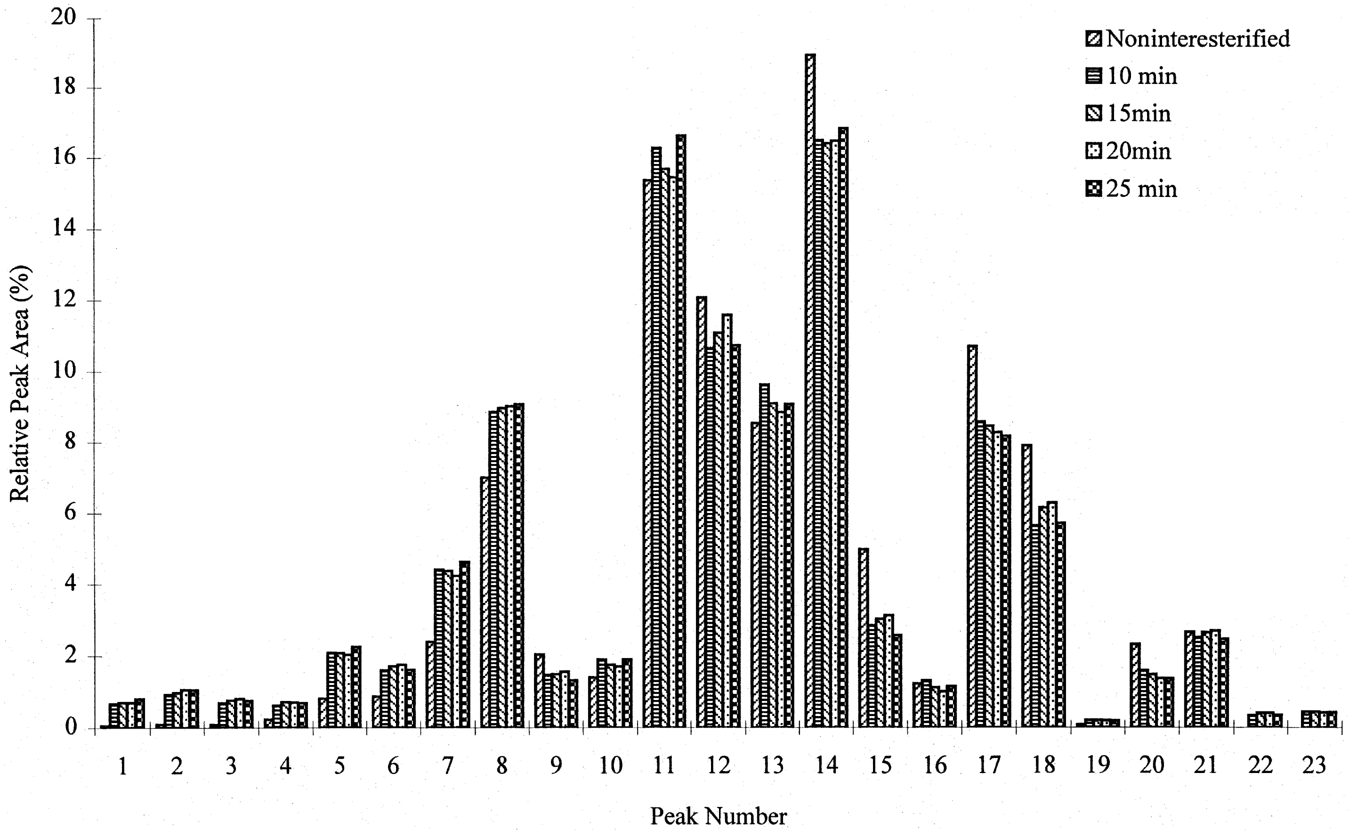

FIG. 1. Relative peak areas by high-performance liquid chromatography of noninteresterified highly saturated soybean oil (HS SBO) and interesterified HS SBO held for different times at 70°C ($n = 2$).

Analysis of slip melting point. Interesterification raised the slip melting point of HS SBO from 9.5 to 34.5°C, a nearly fourfold increase; solidification of the noninteresterified HS SBO was increased from 4.4 to 20.6°C. The higher melting point was probably a result of the increase in disaturates and trisaturates formed during interesterification.

SFC analysis. The SFC of a commercial soft-tub margarine (Chiffon®), noninteresterified and interesterified HS SBO, and HS SBO are indicated in Table 3. The NMR results indicated that the noninteresterified HS SBO melted completely between 11 and 21°C; however, the slip melting point measurement was 9.5°C. Melting ranges measured by NMR are higher than those measured by slip melting point because the latter does not measure complete oil melt. Samples at the slip melting point temperature contain about 4% solids (22).

G.R. List (personal communication) reported that Blue Bonnet® soft-tub margarine had low SFC by NMR at 27, 33, and 38°C, and relatively high SFC at 10°C. The SFC at 11°C

TABLE 3
Solid Fat Content Measured by NMR of Commercial Soft-Tub Margarines, and Noninteresterified and Interesterified HS SBO[a]

	Solid fat content (%)[b]				
Sample	11°C	21°C	27°C	33°C	38°C
Blue Bonnet® soft margarine[c]	24.8	10.8	5.5	1.8	0.4
Chiffon® soft margarine	9	7	5	3	0
Noninteresterified HS SBO	11	0	0	0	0
Interesterified HS SBO (70°C, 25 min)	14	9	9	7	5
LSD (α = 0.05)[d]	4	2	2	3	5

[a] NMR, nuclear magnetic resonance; see Table 2 for other abbreviations.
[b] $n = 2$.
[c] G.R. List (personal communication).
[d] LSD comparisons are between Chiffon® soft margarine, noninteresterified HS SBO, and interesterified HS SBO.

of Chiffon® soft margarine was considerably lower than that of Blue Bonnet®. The SFC profile of HS SBO interesterified at 70°C was similar to that of the SFC for Chiffon® soft margarine, although the SFC seemed too great above 27°C to be used for processing soft-tub margarines. Margarines without a waxy mouthfeel have less than 3.5% solid fat at 33.3°C and melt completely at body temperature (23). The exact melting temperatures of solids melting below body temperature are less important, provided enough solids are present to contribute to structure. Therefore, for margarine manufacture, interesterified HS SBO was blended at a 50:50 ratio with a typical SBO (commercial Crisco® SBO) to reduce the percentage of high-melting TAG.

DSC analysis. Representative cooling and heating curves of the noninteresterified and interesterified HS SBO are shown in Figure 2. Crystallization of high- and low-melting crystals appeared as multiple negative peaks in the cooling curve. The multiple melting phases in the melting curve were caused by the crystals of various types of fat molecules and by the less stable forms rearranging to more stable polymorphs. The heating curve of the DSC analysis showed that the interesterified HS SBO melted between −19.7 and 40.7°C (Fig. 2). At a body temperature of 37°C, the interesterified fat should melt almost completely in one's mouth. These data are consistent with the SFC values listed in Table 3 where the interesterified HS SBO contained 5% solids at 38°C. The melting range of the noninteresterified HS extended from −22.2 to 13.8°C. The DSC measurement of this oil generally agreed with SFC values in that there was 11% solid fat at 11°C and no solids measured at higher temperatures. Solidification of the noninteresterified HS SBO was higher than that noted in the literature for typical SBO, from −16 to −10°C (24), as a result of the higher content of $C_{16:0}$ and $C_{18:0}$ in HS SBO.

Preliminary selection of margarine manufacture conditions. To select a proper blend for further sensory analysis, laboratory-scale margarines made from blends of interesterified HS/typical SBO margarines at ratios of 50:50, 67:33, and 75:25 were evaluated by three observers for firmness at 4.5°C (refrigerated temperature) and 21°C (room temperature) by spreading on crackers. Samples with ratios of 67:33 and 75:25 were too firm to be spread at 4.5°C. The 50:50 blend had a suitable firmness at 4.5°C and was very soft at 21°C, which is similar to commercial soft-tub margarines. Therefore, the

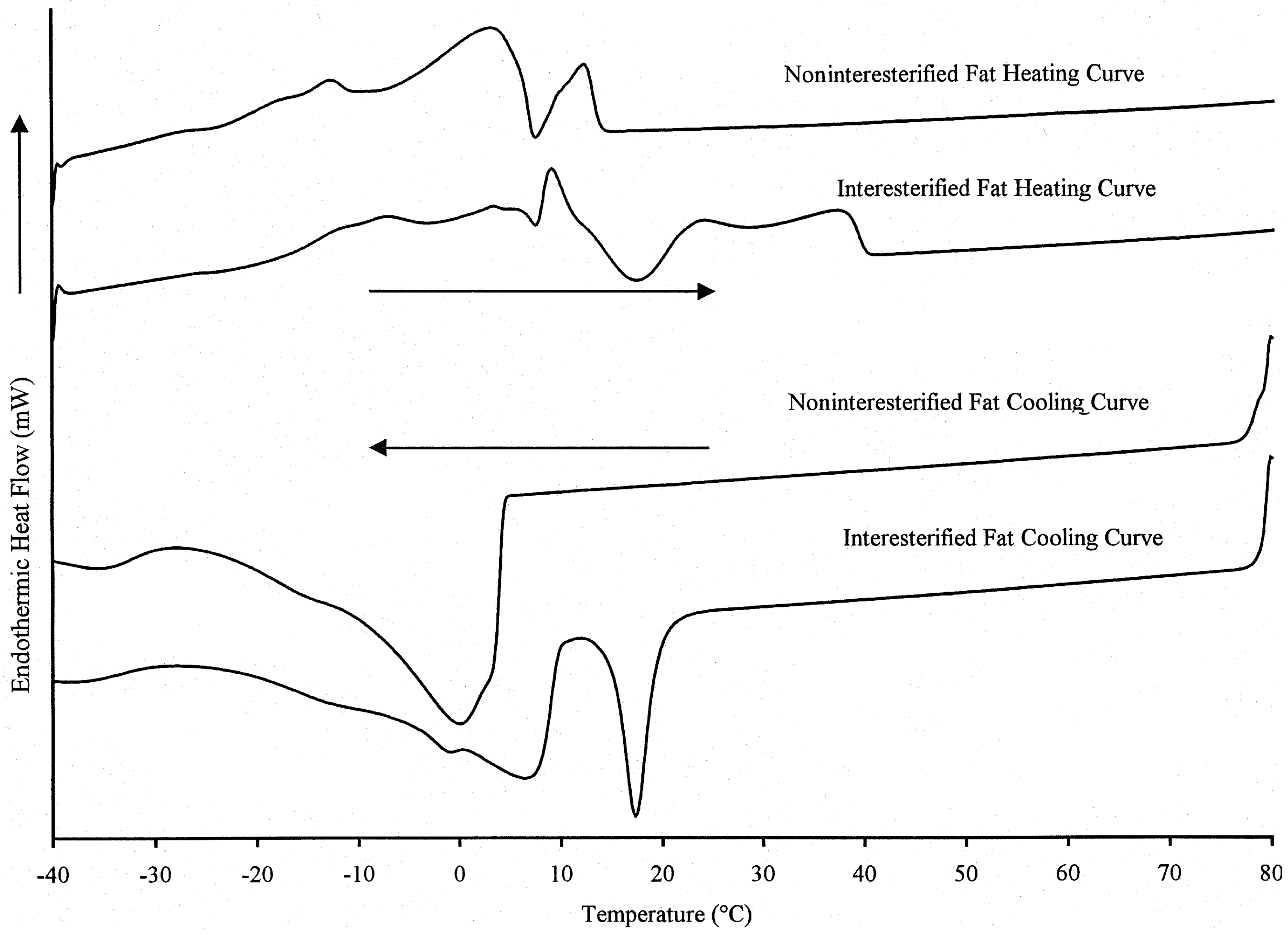

FIG. 2. Representative cooling and heating differential scanning calorimetry thermograms of noninteresterified HS SBO and HS SBO interesterified at 70°C (*n* = 2). See Figure 1 for abbreviation.

50:50 ratio (termed "experimental margarine") was selected for further evaluation.

In a randomized oil, the mole percentage of trisaturated TAG is shown in this equation (25): trisaturated TAG = (mole percentage saturates)^3/10,000. Since our oil had 43.3 mol% saturates, the randomized oil should contain 8.1 mol% trisaturates. Typical SBO trisaturate content is undetectably low (26), so the 50:50 blend of typical and randomized HS SBO should contain 4.0% trisaturates. The randomization of an oil containing 34.4 mol% saturates should also give 4.0 mol% trisaturated TAG. However, to get good spreadability at refrigeration temperatures we must also pay attention to the disaturatemonooleins, which crystallize below 20°C (27). In our randomized oil, this value was estimated at 59% using this equation (24): disaturatemonooleins = [3(mole percentage saturates)^2 (mole percentage oleate)]/10,000. The disaturatemonolinoleins probably do not crystallize at refrigeration temperatures; if they did, the randomized oil would be very hard. We could find no data in the literature on disaturatemonolinolein melting points.

Texture analysis of experimental and commercial margarines. The peak force, hardness, and adhesiveness of margarine processed at −5°C and below were similar among temperatures (data not shown). Therefore, a processing temperature of −5°C was selected, for which peak force was 24.5 g, hardness was 2412 gs and adhesiveness was −613 gs. The maximal force exerted by the experimental margarine on the probe was about 2.3 times greater, the hardness parameter about 2.6 times greater, and adhesiveness about 1.5 times greater than the commercial margarine. The greater content of high-melting TAG in the experimental margarine likely caused these higher values. Our initial goal of creating a product firm enough for consumer acceptance was obviously accomplished. Further dilutions of interesterified HS SBO with typical SBO would provide softer margarines, which might be closer to the texture of current commercial soft-tub margarines.

Sensory evaluation. Analysis of variance of the sensory scores for experimental and commercial margarines revealed small but significant differences between the margarines in spreadability, graininess, and waxiness when served at 4.5°C (Table 4). The difference in spreadability was supported by instrumental texture analysis at 5°C. There also were significant differences in spreadability and waxiness between the experimental and commercial margarines served at 11.5°C. Graininess did not differ significantly between the two margarines at 11.5°C.

For high-fat products, such as butter and margarine, rapid melting of fat between 10 to 26.7°C gives a cooling sensation in the mouth (23) and such products are likely to lack waxiness. The second peak in the DSC melting curve of the interesterified HS SBO started at about 9.5°C and ended at 17.8°C. The small amount of waxiness noted in the experimental margarine may have been caused by recrystallization of fat crystals to higher-melting fat crystals, as indicated by the presence of the third peak in the DSC melting curve (Fig. 2). Even though there were some significant differences between the scores of experimental and commercial margarines, all differences were small and average values for the various attributes were within 13% of the zero end of the line scale. Consumers likely would find the experimental margarine quite acceptable.

TABLE 4
Mean Sensory Evaluation Scores of Experimental[a] and Commercial Margarines

	Attributes (cm)[b]		
Margarine	Spreadability	Graininess	Waxiness
4.5°C Serving temperature			
Chiffon® Soft	0.85	0.01	0.65
HS/typical SBO	1.93	0.33	1.71
LSD (α = 0.05)	0.43	0.28	0.61
11.5°C Serving temperature			
Chiffon® Soft	0.10	0.00	0.17
HS/typical SBO	0.58	0.21	0.87
LSD (α = 0.05)	0.21	0.23	0.33

[a] Margarine made from a 50:50 blend of interesterified HS SBO and typical SBO at −5°C. See Table 2 for abbreviations.

[b] Distance of responses from 0 on 15-cm line scale, where 0 cm = easy to spread, no graininess, and no waxiness and 15 cm = hard to spread, very grainy, and very waxy. Duplicate samples of two margarine preparations were evaluated; *n* = 2.

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