JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical and Physical Properties of Butterfat−Vegetable Oil Blend Spread Prepared with Enzymatically Transesterified Canola Oil and Caprylic Acid

BYUNG HEE KIM AND CASIMIR C. AKOH*

Department of Food Science and Technology, The University of Georgia, Food Science Building, Athens, Georgia 30602-7610

Structured Lipid was synthesized from canola oil and caprylic acid with *sn*-1,3 specific lipase from *Rhizomucor miehei*. Cold spreadable butter was made by blending butterfat with the SL at a weight ratio of 80:20. Its chemical and physical properties were compared with pure butter and butterfat−canola oil 80:20 blend spread. The butterfat−SL blend had lower contents of hypercholesterolemic fatty acids (FAs) and the lowest atherogenic index (AI) as compared to the others. Melting and crystallization behaviors of butterfat−SL blend were similar to those of butterfat−canola oil blend above 0 °C. It showed solid fat contents (SFCs) similar to butterfat−canola oil blend but lower than pure butterfat. The butterfat−SL blend was shown to crystallize in the β' form. There were no differences between the hardness of butterfat−SL blend spread and butterfat−canola oil blend spread. Rheological analysis showed that butterfat−SL blend spread lost its elastic behavior at 5 °C, a lower temperature than pure butter.

KEYWORDS: Atherogenic index; canola oil; caprylic acid; cold spreadable butter; differential scanning calorimeter; dynamic stress rheometer; polymorphism; solid fat content; structured lipid; texture analyzer

INTRODUCTION

Butter is a kind of table spread, which is made from milk or cream and should contain at least 80% fat (weight per weight, w/w) according to U.S. FDA standards of identity. Butterfat has a wide range of fatty acids (FAs) from short chain FAs (SCFAs, C4−C6) and medium chain FAs (MCFAs, C8−C12) to long chain FAs (LCFAs, C16−C18), which mainly exist as saturated forms (*1*, *2*). Ney (*3*) reported that butterfat contains 10% myristic acid (C14:0) and 26% palmitic acid (C16:0) known as hypercholesterolemic FAs. The cholesterol content in butterfat is known to be 0.25−0.30% (*4*). Recently, butter has been criticized for the hypercholesterolemic attributes arising from its saturated fat and cholesterol content (*5*).

Butter also has innate physical limitations contrary to margarine. Butter exists as a solid state and has poor spreadability at refrigeration temperature (4 °C) while it melts and exhibits the exudation of oil (oiling off) at room temperature (23 °C) (*6*). Lately, growing consumers' demand for healthy fat spreads that are palatable and possess good textural properties has led researchers to attempt the development of several types of modified butters and butter-based table spreads.

The spreadability and nutritional properties of butter can be improved by blending with vegetable oils. Butterfat−vegetable oil blend spreads already appeared in the U.S. market in the early 1980s (*7*). They have improved spreadability at refrigeration temperature and have enhanced nutritional properties, such as desirable FA profile and lower cholesterol levels as compared to pure butter (*8*). They may also provide an acceptable alternative to some spreads made from partially hydrogenated vegetable oil, such as margarine, which has a high level of trans FA, raising plasma total and low density lipoprotein cholesterol levels and lowering plasma high density lipoprotein cholesterol levels (*9*, *10*). Canola oil may be a good source of vegetable oil to blend with butterfat in making cold spreadable butter due to its high content of oleic acid having a neutral effect on plasma total cholesterol level and flavorless properties, making its effect on butter flavor minimal (*6*, *11*).

Structured lipids (SLs) are fats and oils with improved nutritional or physical properties because of modifications to incorporate new FAs or to change the position of existing FAs on the glycerol backbone by chemically or enzymatically catalyzed reactions or genetic engineering (*12*). Canola oil transesterified with caprylic acid, which used in this study, is an SL containing MCFA (caprylic acid) at the *sn*-1 and *sn*-3 positions and LCFAs, such as oleic acid, at the *sn*-2 position of triacylglycerols (TAGs). This type of TAG is known to be more rapidly hydrolyzed and efficiently absorbed than typical long chain triacylglycerols (LCTs) (*13*) and to have a reduced caloric value and lower tendency to deposit as depot fat as compared with typical LCTs (*14*). It is also reported that such an SL made from vegetable oil and MCFA lowers both plasma cholesterol and tissue cholesterol in humans more significantly than conventional polyunsaturated oils (*15*). Therefore, SL made from

* To whom correspondence should be addressed. Tel: 706-542-1067. Fax: 706-542-1050. E-mail: cakoh@uga.edu.

10.1021/jf050059v CCC: $30.25
Published on Web 05/18/2005

Table 1. Formulation for Pure Butter, Butterfat–Canola Oil 80:20 Blend Spread, and Butterfat–SL[a] 80:20 Blend Spread (% w/w)

ingredient	pure butter	butterfat–canola oil 80:20 blend spread	butterfat–SL 80:20 blend spread
		lipid phase	
butterfat	80.0	64.0	64.0
canola oil		16.0	
SL			16.0
		aqueous phase	
skim milk	17.6	17.6	17.6
table salt	1.2	1.2	1.2
buttermilk powder	1.2	1.2	1.2

[a] SL prepared with enzymatically transesterified canola oil and caprylic acid.

caprylic acid and canola oil containing a high amount of LCFA can be used to produce healthy cold spreadable butterfat−vegetable oil blend spreads, which counterbalance the hypercholesterolemic attributes of butterfat as well as have improved spreadability at refrigeration temperature.

The objective of our study is to synthesize SL containing caprylic acid at the *sn*-1 and -3 positions and LCFAs at the *sn*-2 position from canola oil and caprylic acid using a *sn*-1,3 specific immobilized lipase as the biocatalyst and to use the resulting SL for the preparation of cold spreadable butter by blending butterfat with it. We evaluated several chemical and physical properties of the butterfat−SL blend spread and compared those to existing pure butter and butterfat−canola oil blend spreads to examine the possibility of its usage as cold spreadable and healthy butter spread products.

MATERIALS AND METHODS

Materials. Butter was obtained from a local grocery store and stored at −20 °C until experimental use. Canola oil was purchased from a local grocery store and used without further treatment. Caprylic acid (C8:0, purity > 98%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). A *sn*-1,3 specific immobilized lipase from *Rhizomucor miehei* (Lipozyme RM IM) was obtained from Novozymes North America Inc. (Franklinton, NC). All other reagents were purchased from Fisher Scientific (Fair Lawn, NJ).

Anhydrous Butterfat Preparation. Butter was melted at 60 °C, and the top butterfat layer was decanted into a separatory funnel and washed three times with its same volume of water. Anhydrous sodium sulfate (10%, w/w) was then added to the butterfat and stood for 30 min to remove residual moisture. The butterfat was filtered through a Whatman no. 1 under vacuum, and pure anhydrous butterfat was obtained and stored at −20 °C until needed.

SL Production. The SL was synthesized according to the apparatus setup and optimal conditions previously reported (*16*) for reacting canola oil and caprylic acid in a continuous packed bed bioreactor; substrate flow rate, 1 mL/min; temperature, 60 °C; and substrate mole ratio, 1:5 (canola oil:caprylic acid). The product was purified using a KDL-4 short path distillation unit (UIC Inc., Joliet, IL). The SL was passed through the distillation apparatus five times under the following conditions: holding temperature, 25 °C; heating oil temperature, 185 °C; cooling water temperature, 15 °C; and vacuum pressure, <50 mbar.

Lipid Blend Preparation. The 80:20 (w/w) blends of butterfat and canola oil or SL were prepared, respectively. The blends were prepared after completely melting butterfat at 60 °C and stored at −20 °C until needed.

Spread Manufacture. The spread was produced according to the method of Kaylegian and Lindsay (*17*) with a slight modification. The spread formulation used in the experiments is shown in **Table 1**. Each spread consisted of lipid phase (80%, w/w) and aqueous phase (20%, w/w). The kinds of lipid phases were as follows: 100% butterfat, 80:20% butterfat−canola oil blend, and 80:20% butterfat−SL blend. The aqueous phase consisted of skim milk (17.6%, w/w), table salt (1.2%, w/w), and buttermilk powder (1.2%, w/w), which was used as a natural emulsifier. The lipid phases were completely melted at 60 °C. The buttermilk powder and table salt were dissolved thoroughly in skim milk (4 °C). The lipid phase (45 °C) and the aqueous phase (8 °C) were poured into a tabletop blender and vigorously mixed for 2 min to emulsify them. The resulting liquid emulsion (40 °C) was then crystallized using an ice cream maker (type no. 358, KRUPS North America, Inc., Peoria, IL), which featured a double insulated bowl with a liquid refrigerant located between the walls. The bowl was frozen overnight and maintained at around 0 °C during the crystallization. Crystallization lasted for 10 min, and the final temperature of the spread was 7−8 °C. The spreads were tempered at 25 °C for 4 h and then worked vigorously with a hand mixer until their textures were smoothed and any lumps were removed. Spread samples were then tempered for at least 2 weeks at 4 °C prior to experimental usage.

FA Composition Analysis. Canola oil, SL, and each lipid phase (100 mg) of spread samples were methylated in 3 mL of 6% HCl in methanol at 75 °C for 2 h. The FA methyl esters (FAMEs) were extracted and analyzed by gas−liquid chromatography. An Agilent Technologies 6890N gas chromatograph (Agilent Technologies Inc., Palo Alto, CA), equipped with a flame ionization detector and a fused silica capillary column (DB-225, 30 m × 0.25 mm i.d., J & W Scientific, Folsom, CA) was used. The carrier gas was helium, and the total gas flow rate was 23 mL/min. The injector and detector temperatures were kept at 250 and 260 °C, respectively. The column was initially held at 40 °C for 3 min and programmed to increase to 130 °C at the rate of 10 °C/min. After holding at 130 °C for 3 min, the column was then programmed to increase to 215 °C at the rate of 20 °C/min. The FAMEs were identified, and their relative contents were calculated as weight% with heptadecanoic acid (C17:0) as an internal standard.

TAG Separation. The TAG was separated from pure butterfat, butterfat−canola oil 80:20 blend, and butterfat−SL 80:20 blend using AOCS Official Method Cd 11c−93 (*18*) with modification, respectively. Each lipid sample (2 g) was mixed with silica gel (8 g) in a mortar and rubbed with a pestle. This silica gel mixed with lipid sample was poured into the column (230 mm × 25 mm i.d., Bio-Rad Lab., Richmond, CA), and then, pure silica gel (10 g) was added onto the top of silica gel containing sample. After the silica gel was packing, an Econo-Column flow adaptor (Bio-Rad Lab.) was inserted into the column. To elute the TAG fraction from the lipid sample, 250 mL of diethyl ether in hexane solvent (10:90, v/v) was passed through the column from bottom to top at a flow rate of 1 mL/min. Eluted TAG fractions were thoroughly evaporated under vacuum and a stream of nitrogen to remove the solvent. The TAG fractions prepared from each lipid sample were used for thermal analysis and polymorphic forms study.

Thermal Analysis. The melting and crystallization profiles of TAG fractions separated from spread samples were determined by differential scanning calorimetry (DSC) on a Perkin-Elmer model DSC7 (Norwalk, CT). The analysis was performed according to the AOCS recommended procedure Cj 1−94 (*19*). Normal standardization was performed with Indium (mp 156.60 °C, ΔH 28.45 J/g) as a reference standard. Dry ice was used as the coolant. A sample of 8−10 mg was hermetically sealed in a 30 μL capacity aluminum pan (Perkin-Elmer), with an empty sealed pan used as a reference. Samples were rapidly heated from room temperature to 80 °C and held at this temperature for 10 min to destroy any previous crystalline structure, before being cooled to −40 °C at a rate of 10 °C/min to obtain the crystallization profiles. After they were held for 30 min at −40 °C, samples were heated to 80 °C at a rate of 5 °C/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). The SFC was determined by AOCS Official Method Cd 16−81 (*20*). NMR measurements were made using a MARAN-20 pulsed NMR spectrometer (Resonance Instruments Ltd., Oxon, United Kingdom). Samples were tempered at 100 °C for 15 min and then 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

Table 2. FA Composition in TAG of Pure Butterfat, Butterfat–Canola Oil 80:20 Blend, and Butterfat–SL 80:20 Blend (mol %)[a]

FA	canola oil	SL[b]	butterfat	butterfat–canola oil blend	butterfat–SL blend
C4:0			8.8 ± 0.1 A	7.5 ± 0.1 B	5.3 ± 0.1 C
C6:0			8.0 ± 0.0 A	7.2 ± 0.3 B	5.3 ± 0.2 C
C8:0		80.2 ± 0.1	4.9 ± 0.0 B	5.4 ± 1.7 B	24.5 ± 0.5 A
C10:0			8.9 ± 0.1 A	8.4 ± 0.3 A	6.3 ± 0.1 B
C12:0			8.1 ± 0.1 A	7.2 ± 0.1 B	5.7 ± 0.1 C
C14:0			17.6 ± 0.2 A	16.0 ± 0.1 B	12.8 ± 0.1 C
C14:1			1.7 ± 0.0 A	1.5 ± 0.0 B	1.2 ± 0.0 C
C16:0	9.8 ± 0.5	1.3 ± 0.0	25.2 ± 0.3 A	23.7 ± 0.9 A	19.7 ± 0.6 B
C16:1			0.3 ± 0.1 A	0.3 ± 0.1 A	0.2 ± 0.0 A
C18:0			3.3 ± 0.0 A	2.8 ± 0.1 B	2.5 ± 0.0 C
C18:1*n-9*	60.3 ± 0.0	11.8 ± 0.2	12.1 ± 0.2 B	16.1 ± 0.7 A	13.4 ± 0.3 B
C18:2*n-6*	23.1 ± 0.5	5.3 ± 0.0	1.1 ± 0.1 C	3.2 ± 0.1 A	2.6 ± 0.2 B
C18:3*n-3*	6.8 ± 0.0	1.4 ± 0.1		0.7 ± 0.0 A	0.5 ± 0.0 B
SFA	9.8 ± 0.5	81.6 ± 0.1	84.8 ± 0.3 A	78.2 ± 0.5 C	82.0 ± 0.2 B
USFA	90.2 ± 0.5	18.4 ± 0.1	15.2 ± 0.3 C	21.8 ± 0.5 A	18.0 ± 0.2 B
AI[c]	0.11	0.07	6.81	4.35	4.26

[a] Mean ± SD, $n = 2$; means with the same letter in the same row are not significantly different ($P < 0.05$). [b] SL prepared with enzymatically transesterified canola oil and caprylic acid. [c] AI = [(mol % of C12:0) + 4(mol % of C14:0) + (mol % of C16:0)]/(mol % of USFA).

used as the reference oil. The SFC was measured at 5 °C intervals from 5 to 45 °C.

X-ray Diffraction (XRD) Spectroscopy. The polymorphic forms of TAG fractions separated from spread samples were determined by ARL Scintag XDS 2000 (Ecublens, Switzerland) automated diffractometer. The diffractometer had a 2θ configuration, a solid state detector, and a cobalt tube as the X-ray source. The generation power for all sample runs was set at 40 kV and 40 mA. The 2θ range used was from 18 to 32°, and the scan rate was 2.0°/min. Samples were melted and poured into rectangular plastic molds on cold glass plates. They were solidified at room temperature and then kept at refrigeration temperature for 12 h. Short spacings of the major polymorphs are as follows: α, a single spacing at 4.15 Å; β', two strong spacings at 3.8 and 4.2 Å; and β, a very strong spacing at 4.6 and another one usually at 3.85 Å (*21*).

Texture Analysis. The hardness of each spread sample was measured at 4 and 23 °C using a TA-XT2 texture analyzer (Stable Micro Systems Ltd., London, United Kingdom). A 45° conical probe penetrated into the sample at 1 mm/s to a depth of 5 mm from the sample surface and then was withdrawn at the same speed. During penetration, the force increased up until the point of maximum penetration depth. The penetration force (g) was reported as hardness. Triplicate readings for each sample were obtained.

Rheological Analysis. Viscoelastic properties of each spread sample were analyzed using an SR5000 dynamic stress rheometer (Rheometrics Scientific, Piscataway, NJ). Parallel plate geometry was used with a diameter of 40 mm and a gap of 0.5 mm. Temperature control was carried out using a peltier element. Small amplitude oscillatory experiments were carried out within the linear viscoelastic region of each sample. A stress sweep ranging from 10 to 1000 Pa was used to determine the linear viscoelastic region for each sample. The storage modulus (G') was measured by an on-line computer for all samples. The temperature was set at 15 °C for samples. Oscillatory measurements as a function of temperature were also performed. The experiments were carried out by heating the samples from 5 to 40 °C in step of 5 °C. A stress of 100 Pa, a frequency of 0.5 Hz, and a gap of 0.5 mm were maintained. At each step, the sample was quickly heated to the desired temperature and allowed to equilibrate at that temperature before readings were obtained.

Other Analytical Methods. Free FA (FFA) contents of lipid samples were determined by AOCS Official Method Ca 5a−40 (*22*). *sn*-2 positional analysis of FA in TAG molecule was done using the pancreatic lipase hydrolysis method (*23*).

Statistical Analysis. Statistical analysis was conducted with the SAS software package (*24*). One-way analysis of variance (ANOVA) was performed to determine the differences among spread samples. When F values for the ANOVA were significant, differences in means were determined using Duncan's multiple range test as a procedure of mean separation ($P < 0.05$).

RESULTS AND DISCUSSION

FA Composition and Atherogenic Index (AI). After purification by short path distillation, SL contained 0.10% FFA. The FFA contents of commercial canola oil and anhydrous pure butterfat separated from commercial butter were 0.03%, respectively. Generally, fats and oils having below 0.10% FFA are accepted as ingredients for food products so they could be used in making our recombinant butter spread samples.

FA compositions of the total TAG and at the *sn*-2 position of the TAG of canola oil, SL, butterfat, butterfat−canola oil 80:20 (w/w) blend, and butterfat−SL 80:20 (w/w) blend are given in **Tables 2** and **3**, respectively. SL incorporated with 80.2% caprylic acid was successfully synthesized from canola oil (**Table 2**). About 16.4% caprylic acid was also found at the *sn*-2 position (**Table 3**), indicating that acyl migration occurred during the transesterification reaction, short path distillation, or *sn*-2 positional analysis (*25*, *26*).

Table 2 shows that the butterfat−SL blend had significantly ($P < 0.05$) lower contents of hypercholesterolemic FAs, such as lauric, myristic, and palmitic acids, than pure butterfat and butterfat−canola oil blend. Ulbricht and Southgate (*27*) proposed an AI, which is calculated by the contents of such hypercholesterolemic FAs and unsaturated FAs (USFAs) present in the lipids, as an indicator of dietary risk. The butterfat−SL blend showed the lowest AI as compared to pure butterfat and butterfat−canola oil blend. These hypercholesterolemic FAs also existed at the *sn*-2 position of the TAG of butterfat−SL blend at significantly ($P < 0.05$) lowest levels as compared to the pure butterfat and butterfat−canola oil blend (**Table 3**). The FAs present at the *sn*-2 position of the TAG are preferentially absorbed and stored in human bodies as compared to the FAs located at the *sn*-1 or *sn*-3 positions because TAGs are hydrolyzed into FAs and 2-monoacylglycerols (2-MAGs) by lipoprotein lipase and 2-MAGs readily form mixed micelles with bile salts (*13*, *28*). The difference in metabolic aspects of FAs by positional distribution in TAG also suggests that lower contents of hypercholesterolemic FAs at the *sn*-2 position of TAG of butterfat−SL blend may show a decreased hypercholesterolemic effect as compared to pure butterfat and butterfat−canola oil blend. These results suggest that SL can successfully

Table 3. FA Composition at *sn*-2 Position of TAG of Pure Butterfat, Butterfat–Canola Oil 80:20 Blend, and Butterfat–SL 80:20 Blend (mol %)[a]

FA	canola oil	SL[b]	butterfat	butterfat–canola oil blend	butterfat–SL blend
C4:0					
C6:0			0.4 ± 0.1 A	0.3 ± 0.0 A	0.3 ± 0.0 A
C8:0		16.4 ± 0.1	1.5 ± 0.2 B	1.4 ± 0.0 B	3.7 ± 0.1 A
C10:0			7.0 ± 0.5 A	6.2 ± 0.0 AB	6.1 ± 0.1 B
C12:0			8.8 ± 0.4 A	7.6 ± 0.0 B	7.3 ± 0.1 B
C14:0			26.4 ± 0.5 A	23.2 ± 0.1 B	22.2 ± 0.0 C
C14:1			1.6 ± 0.1 A	1.5 ± 0.0 B	1.4 ± 0.0 B
C16:0	0.7 ± 0.0	1.6 ± 0.0	33.0 ± 0.5 A	29.8 ± 0.1 B	28.3 ± 0.3 C
C16:1	0.3 ± 0.0		2.4 ± 0.1 A	2.2 ± 0.0 B	2.0 ± 0.1 B
C18:0			2.9 ± 0.2 A	2.5 ± 0.1 A	2.4 ± 0.1 A
C18:1*n-9*	52.9 ± 0.5	44.5 ± 0.2	13.3 ± 0.9 B	17.6 ± 0.2 A	17.8 ± 0.0 A
C18:2*n-6*	33.7 ± 0.4	27.1 ± 0.0	2.5 ± 0.1 C	6.0 ± 0.0 B	6.5 ± 0.1 A
C18:3*n-3*	12.4 ± 0.0	10.4 ± 0.0	0.2 ± 0.0 C	1.7 ± 0.0 B	2.0 ± 0.0 A
SFA	0.7 ± 0.0	18.0 ± 0.1	79.9 ± 0.8 A	71.1 ± 0.1 B	70.4 ± 0.1 B
USFA	99.3 ± 0.0	82.0 ± 0.1	20.1 ± 0.8 B	28.9 ± 0.1 A	29.6 ± 0.1 A

[a] Mean ± SD, $n = 2$; means with the same letter in the same row are not significantly different ($P < 0.05$). [b] SL prepared with enzymatically transesterified canola oil and caprylic acid.

Table 4. Comparison of DSC Melting Properties of TAGs Separated from Canola Oil and SL[a]

	T_o (°C)[b]	T_p (°C)[c]	T_c (°C)[d]	ΔH (J/g)[e]
canola oil	−34.95 ± 0.18 A	−33.11 ± 0.06 B	−28.25 ± 0.21 A	2.07 ± 0.25 B
SL[f]	−34.27 ± 0.25 A	−29.86 ± 0.18 A	−28.27 ± 0.14 A	70.17 ± 0.49 A

[a] Mean ± SD, $n = 2$; means with the same letter in the same column are not significantly different ($P < 0.05$). [b] Melting onset temperature. [c] Melting peak temperature. [d] Melting completion temperature. [e] Melting enthalpy. [f] SL prepared with enzymatically transesterified canola oil and caprylic acid.

counterbalance the hypercholesterolemic attributes of butterfat when used in making butter spread.

Melting and Crystallization Behaviors. Melting and crystallization behaviors of TAG fractions separated from each spread sample were evaluated by DSC. The approximate T_o (−34 °C) and T_c (−28 °C) of both canola oil and SL were very similar to each other (**Table 4**). It means that when SL is substituted for canola oil to blend with butterfat for making cold spreadable butter, it shows almost the same melting point as canola oil. However, SL exhibited about 35 times higher enthalpy values than canola oil, which suggested that much more energy was necessary to drive the melting of SL. However, because spread products are usually stored at refrigeration temperature and used above refrigeration temperature, their melting behaviors near the melting point are expected to have no effect on the melting behaviors of the spread products made with them.

Figure 1 shows melting curves of TAG fractions separated from each spread sample. The melting range of both butterfat−SL blend and butterfat−SL blend shifted from higher to lower temperature as compared to that of pure butterfat at the overall temperature range. The melting profile above 0 °C is especially important because, as mentioned above, spread products are usually stored and served at refrigeration or room temperatures. Therefore, the two melting peaks having similar patterns and shapes to each other above 0 °C were labeled as I (a narrow peak at lower temperature) and II (a broad peak at higher temperature), respectively, and their onset and peak temperatures were compared to each other in **Table 5**. Because both butterfat−canola oil blend and butterfat−SL blend have peak I with a small shoulder peak occurring at a slightly higher temperature, peak I is again differentiated into I-1 and I-2, respectively. In the case of peak I-1, both onset and peak temperatures of both butterfat−canola oil blend and butterfat−SL blend were significantly lower ($P < 0.05$) than those of peak I of pure butterfat. For peak I-2, only peak temperatures can be obtained and a significant ($P < 0.05$) difference was not found between those of butterfat−canola oil blend and butterfat−SL blend. For peak II, both butterfat−canola oil blend and butterfat−SL blend showed significantly ($P < 0.05$) lower onset temperatures as compared to that of pure butterfat, whereas there is no significant ($P < 0.05$) difference between peak temperatures of all of the samples.

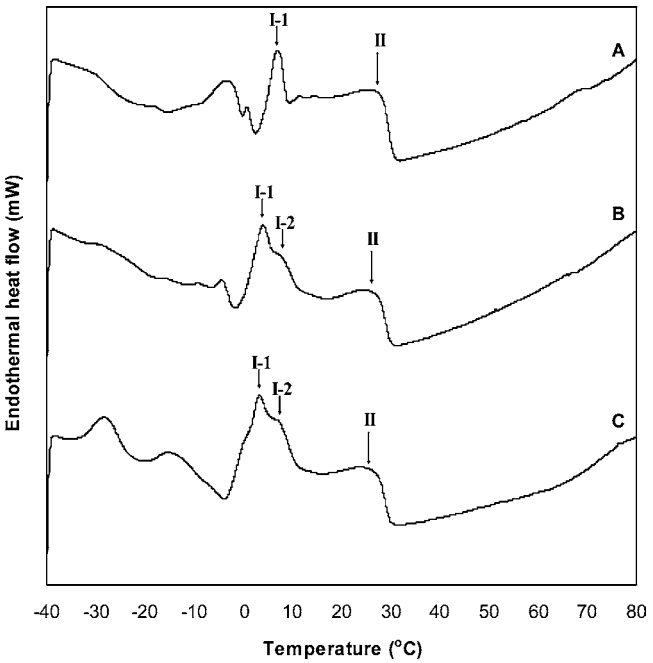

Figure 1. DSC melting thermograms of TAGs separated from each spread sample: (**A**) pure butterfat, (**B**) butterfat–canola oil 80:20 blend, and (**C**) butterfat–SL 80:20 blend; SL indicates the SL prepared with enzymatically transesterified canola oil and caprylic acid.

The crystallization curves of TAG fractions of each spread sample are shown in **Figure 2**. Similar to trends in their melting

Table 5. Comparison of DSC Melting Properties of TAGs Separated from Pure Butterfat, Butterfat–Canola Oil 80:20 Blend, and Butterfat–SL 80:20 Blend[a]

	peak I-1		peak I-2		peak II	
	T_o (°C)[b]	T_p (°C)[c]	T_o (°C)	T_p (°C)	T_o (°C)	T_p (°C)
butterfat	4.42 ± 0.68 A	7.02 ± 0.24 A			18.00 ± 0.26 A	27.68 ± 0.35 A
butterfat–canola oil blend	–0.16 ± 0.26 B	3.93 ± 0.12 B		7.89 ± 0.18 A	17.35 ± 0.20 AB	25.72 ± 1.00 A
butterfat–SL[d] blend	–1.01 ± 0.15 B	3.27 ± 0.00 C		7.43 ± 0.24 A	16.70 ± 0.23 B	26.56 ± 0.06 A

[a] Mean ± SD, $n = 2$; means with the same letter in the same column are not significantly different ($P < 0.05$). [b] Melting onset temperature. [c] Melting peak temperature. [d] SL prepared with enzymatically transesterified canola oil and caprylic acid.

Table 6. Comparison of DSC Crystallization Properties of TAGs Separated from Pure Butterfat, Butterfat–Canola Oil 80:20 Blend, and Butterfat–SL 80:20 Blend[a]

	peak I		peak II	
	T_o (°C)[b]	T_p (°C)[c]	T_o (°C)	T_p (°C)
butterfat	8.02 ± 0.04 A	6.38 ± 0.12 A	3.59 ± 0.19 A	0.38 ± 0.12 A
butterfat–canola oil blend	6.69 ± 0.55 B	4.88 ± 0.35 B	1.31 ± 0.10 C	–2.20 ± 0.24 B
butterfat–SL[d] blend	6.52 ± 0.09 B	4.97 ± 0.00 B	1.72 ± 0.04 B	–2.95 ± 0.12 C

[a] Mean ± SD, $n = 2$; means with the same letter in the same column are not significantly different ($P < 0.05$). [b] Crystallization onset temperature. [c] Crystallization peak temperature. [d] SL prepared with enzymatically transesterified canola oil and caprylic acid.

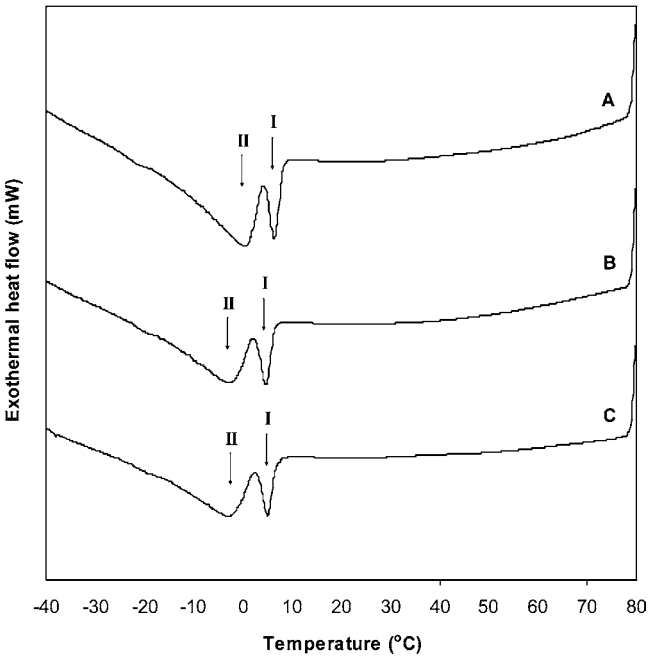

Figure 2. DSC crystallization thermograms of TAGs separated from each spread sample: (**A**) pure butterfat, (**B**) butterfat–canola oil 80:20 blend, and (**C**) butterfat–SL 80:20 blend; SL indicates the SL prepared with enzymatically transesterified canola oil and caprylic acid.

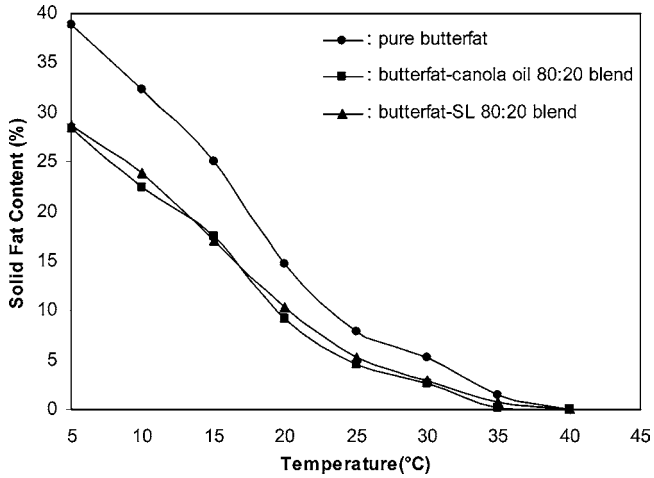

Figure 3. SFCs of pure butterfat, butterfat–canola oil 80:20 blend, and butterfat–SL 80:20 blend; SL indicates the SL prepared with enzymatically transesterified canola oil and caprylic acid.

curves, the crystallization ranges of both butterfat−canola oil blend and butterfat−SL blend shifted from higher to lower temperatures as compared to that of pure butterfat at overall temperature ranges. Each crystallization curve above 0 °C has two peaks showing similar patterns and shapes to each other: One is a broad peak at a lower temperature, and the other is a narrow peak at a higher temperature, respectively. Therefore, the two peaks were labeled as I and II, respectively, and then, their onset and peak temperatures were compared in **Table 6**. Both onset and peak temperatures of peak I in butterfat−canola oil blend and butterfat−SL blend were significantly ($P < 0.05$) lower than those in pure butterfat. However, there is no significant ($P < 0.05$) difference between those of both butterfat−canola oil blend and butterfat−SL blend. For the case of peak II, the onset temperature of butterfat−SL blend was slightly higher than that of butterfat−canola oil blend but its peak temperature was slightly lower than that of butterfat−canola oil blend. Both butterfat−canola oil blend and butterfat−SL blend also showed significantly ($P < 0.05$) lower onset and peak temperatures as compared to those of pure butterfat.

In summary, both butterfat−canola oil blend and butterfat−SL blend have lower melting and crystallization ranges as compared to those of pure butterfat, whereas similar patterns can be found between those of both butterfat−canola oil blend and butterfat−SL blend. These results suggest two facts as follows: First, SL can substitute for canola oil, which is already used in making commercial cold spreadable butter due to its similar melting and crystallization behaviors to those of canola oil above 0 °C; second, butter spread made with butterfat−SL blend can have improved spreadability at lower temperature because it would have lower melting and crystallizing temperatures as compared to commercial butter.

SFC. SFC is related with several physical characteristics of table spread, such as cold spreadability, which means how well spread products can be spread at refrigeration temperature. Cold

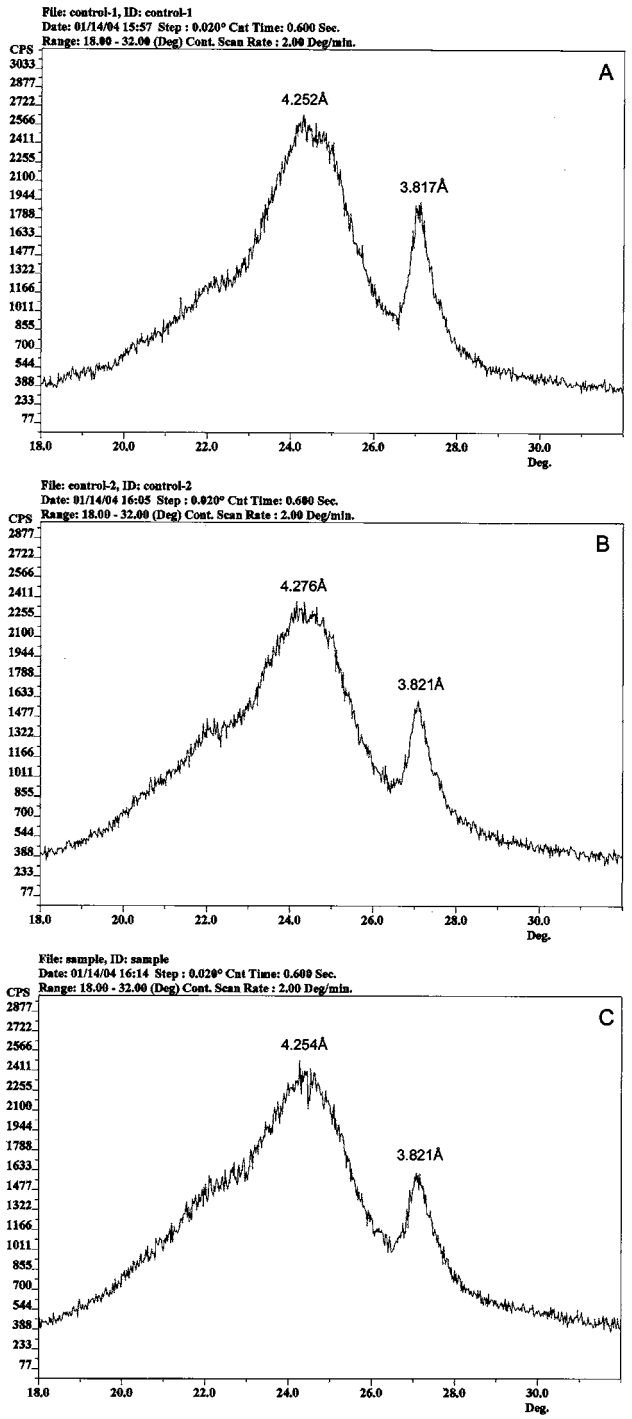

Figure 4. XRD spectroscopy of pure butterfat (**A**), butterfat–canola oil 80:20 blend (**B**), and butterfat–SL 80:20 blend (**C**); SL indicates the SL prepared with enzymatically transesterified canola oil and caprylic acid.

spreadability of butter spreads may be evaluated by SFCs in the temperature range of 4−10 °C. Lida and Ali (*29*) reported that SFC of table spread should be less than 32% at 10 °C to have good spreadability at refrigeration temperature. The relationships between SFC and temperature of the lipid fractions separated from each spread sample are shown in **Figure 3**. Pure butterfat shows 32.4% of SFC at 10 °C, whereas butterfat−canola oil blend and butterfat−SL blend represent 22.5 and 23.8% of SFC at 10 °C, respectively, which suggests that butterfat−SL blend can be successfully used for making cold spreadable butter. SFCs of butterfat−SL blend were also similar to those of butterfat−canola oil blend but lower than those of pure butterfat at 5−40 °C, which means that SL can substitute for canola oil for making cold spreadable butter.

Table 7. Comparison of Hardness of Pure Butter, Butterfat–Canola Oil 80:20 Blend Spread, and Butterfat–SL 80:20 Blend Spread at Different Temperatures (g)[a]

	4 °C	23 °C
butter	2107.1 ± 107.2 A	377.0 ± 22.6 A
butterfat–canola oil blend spread	1417.3 ± 71.7 B	256.4 ± 25.4 B
butterfat–SL[b] blend spread	1424.4 ± 49.1 B	279.7 ± 25.4 B

[a] Mean ± SD, $n = 3$; means with the same letter in the same column are not significantly different ($P < 0.05$). [b] SL prepared with enzymatically transesterified canola oil and caprylic acid.

Polymorphism. Most TAG molecules of lipid exhibit polymorphism, referring to their property to exist in several different crystalline forms (*29*). XRD spectroscopy data can provide the information about the polymorphism of TAG. TAG is known to primarily occur in any of three basic polymorphic forms: α, β', and β. The β' form is considered as the most desirable crystalline form of TAG existing in the table spreads, such as butter and margarine, because of its properties to give smooth mouth feel and to hold a large amount of liquid oil, which originated from a fine arrangement and a large surface area of solid fat crystals (*30*−*32*).

In the XRD analysis, all TAG fractions separated from butterfat, butterfat−canola oil blend, and butterfat−SL blend showed two strong peaks at 4.2 and 3.8 Å, indicating that their crystalline structures are β' forms (**Figure 4**). This means that butter spreads made with butterfat, butterfat−canola oil blend, and butterfat−SL blend would also have the desirable crystalline structure of β' forms. In addition, the butter spreads are expected to have the β' crystal forms because of using an ice cream maker, a type of scraped surface heat exchangers for making butter spreads. deMan et al. (*32*) reported that the α crystal was formed first and then the β' crystal was formed by scraped surface heat exchanger, which quickly cools the emulsion of the liquid state to below 10 °C, resulting in the formation of many nucleation sites during crystallization process.

Textural Property. The hardness of each spread sample was evaluated by texture profile analysis (TPA) done instrumentally. Wright et al. (*33*) reported that the hardness is inversely correlated with the spreadability in butter. Hardness has been also reported to predict the spreadability of some other types of spread products, such as margarine and peanut butter (*34*, *35*). There were no significant ($P < 0.05$) differences between the hardness of butterfat−SL blend spread and butterfat−canola oil blend spread at both refrigeration and room temperatures (**Table 7**). Both butterfat−canola oil blend spread and butterfat−SL blend spread were also significantly ($P < 0.05$) softer than pure butter at both refrigeration and room temperatures. At refrigeration temperature, butterfat−SL blend spread and butterfat−canola oil were 32.4 and 32.7% softer than butter, respectively. At room temperature, butterfat−SL blend spread and butterfat−canola oil blend spread were 25.8 and 32.0% softer than pure butter, respectively. These results indicate that butterfat−SL blend spread is potentially more spreadable than pure butter.

Rheological Property. Butter is a semisolid food; that is, it behaves as a viscoelastic material possessing both solidlike (or elastic) and liquidlike (or viscous) characteristics. Changes in viscoelastic properties of spread samples according to temperature changes were analyzed using a dynamic stress rheometer. The value of storage modulus (G') can be used as a measure of

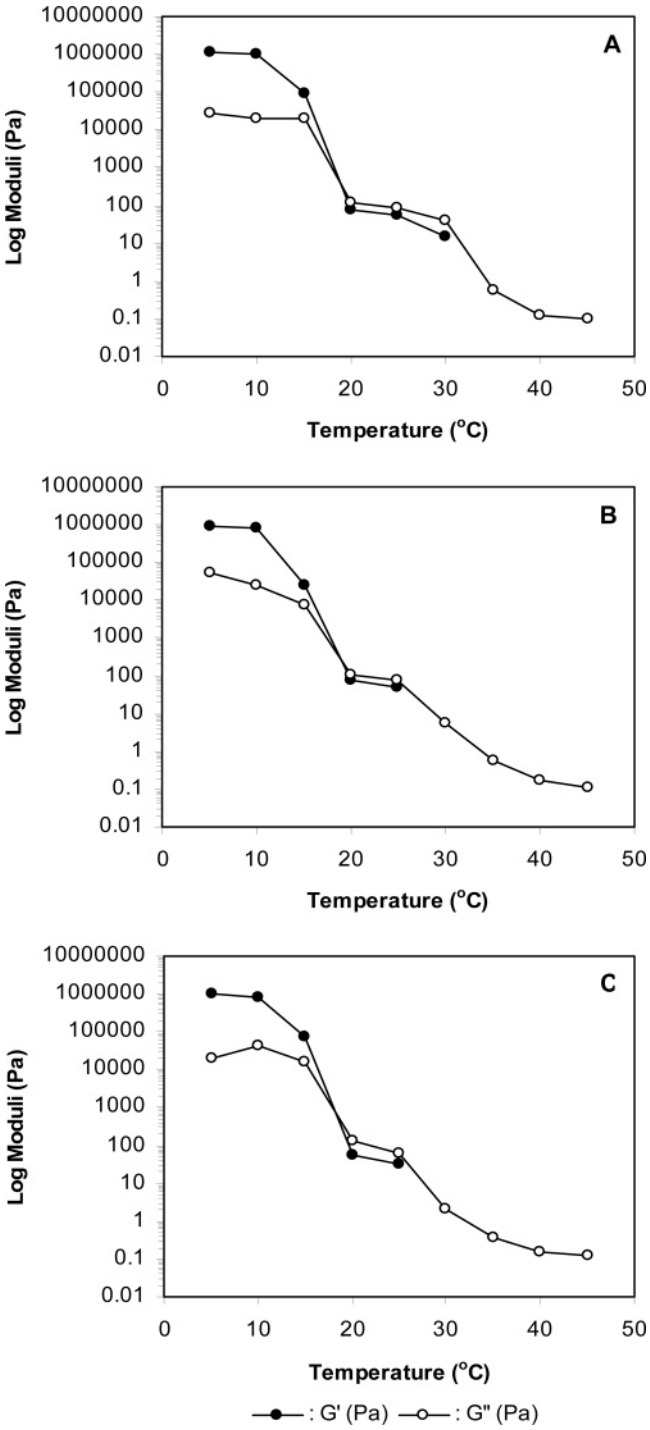

Figure 5. Rheological data, G', and G'' vs temperature of each sample: (**A**) pure butter, (**B**) butterfat–canola oil 80:20 blend spread, and (**C**) butterfat–SL 80:20 blend spread; SL indicates the SL prepared with enzymatically transesterified canola oil and caprylic acid.

elastic behavior, and loss modulus (G'') can be used for viscous behavior of spread samples. The values of both G' and G'' were found to decrease with increasing temperature for all of the spread samples, suggesting that, as expected, the butter spreads become less viscoelastic in character at higher temperature (**Figure 5**). Rheological analysis showed that G' values of butterfat−canola oil blend spread and butterfat−SL blend spread were below 0 Pa after 25 °C, whereas pure butter had G' value of below 0 Pa after 30 °C. This result indicates that both butterfat−canola oil blend spread and butterfat−SL blend spread lost their elastic behavior at lower temperature than pure butter. The G' value is expected to be affected by SFC (*34*). When this result is compared with SFC data (**Figure 3**), about 5% of SFC is shown to be the critical point where butter spreads can have elastic characteristics. After 20 °C, all of the spread samples showed higher G'' values than G', indicating that butter spreads show more viscous behavior than elastic behavior at room temperature.

In conclusion, butterfat−SL blend can be expected to successfully substitute for butterfat−canola oil blend in making cold spreadable butter because SL has similar physical characteristics, such as melting and crystallizing behaviors and SFCs above 0 °C, to those of canola oil. Butterfat−SL blend spread showed more desirable textural attributes, such as improved spreadability, which can be surmised from decreased hardness and more desirable nutritional properties, such as decreased hypercholesterolemic FA contents in both total TAG and the *sn*-2 position of the TAG as compared to pure butter. Therefore, SL prepared enzymatically from canola oil and caprylic acid can be used for making healthy and cold spreadable butter.

ABBREVIATIONS USED

AI, atherogenic index; DSC, differential scanning calorimetry; FA, fatty acid; FAME, FA methyl ester; FFA, free FA; LCFA, long chain FA; LCT, long chain triacylglycerol; MCFA, medium chain FA; SCFA, short chain FA; SFA, saturated FA; SFC, solid fat content; SL, structured lipid; TAG, triacylglycerol; USFA, unsaturated FA; w/w, weight per weight; XRD, X-ray diffraction.

LITERATURE CITED

(1) Podmore, J. Application of modification techniques. In *Recent Advances in Chemistry and Technology of Fats and Oils*; Hamilton, R. J., Bhati, A., Eds.; Elsevier Applied Science Publishers Ltd.: New York, 1987; pp 167−181.

(2) Grall, D. S.; Hartel, R. W. Kinetics of butterfat crystallization. *J. Am. Oil Chem. Soc.* **1992**, *69*, 741−747.

(3) Ney, D. M. Symposium: The role of the nutritional and health benefits in the marketing of dairy products−Potential for enhancing the nutritional properties of milk fat. *J. Dairy Sci.* **1991**, *74*, 4002−4012.

(4) LaCroix, D. E.; Mattingly, W. A.; Wong, N. P.; Alford, J. A. 7 Cholesterol, fat and protein in dairy products. *J. Am. Diet. Assoc.* **1973**, *62*, 275−279.

(5) Jiménez-Flores, R. Trends in research for alternate uses of milk fat. *J. Dairy Sci.* **1997**, *80*, 2644−2650.

(6) Marangoni, A. G.; Rousseau, D. Chemical and enzymatic modification of butterfat and butterfat-canola oil blends. *Food Res. Int.* **1998**, *31*, 595−599.

(7) Rodrigues, J. N.; Cioielli, L. A. Chemical interesterification of milkfat and milkfat-corn oil blends. *Food Res. Int.* **2003**, *36*, 149−159.

(8) Rousseau, D.; Forestiere, K.; Hill, A. R.; Marangoni, A. G. Restructuring butterfat through blending and chemical interesterification. 1. Melting behavior and triacylglycerol modifications. *J. Am. Oil Chem. Soc.* **1996**, *73*, 963−972.

(9) Michicich, M.; Vickers, Z.; Martini, M. C.; Labat, J. B. Consumer acceptance, consumption and sensory attributes of spreads made from designer fats. *Food Qual. Prefer.* **1999**, *10*, 147−154.

(10) Katan, M. B.; Zock, P. L.; Mensink, R. P. Trans fatty acids and their effects on lipoproteins in humans. *Annu. Rev. Nutr.* **1995**, *5*, 473−493.

(11) Mensink, R. P.; Katan, M. B. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. *Lancet* **1987**, *1*, 122−125.

(12) Akoh, C. C. Structured lipids. In *Food Lipids−Chemistry, Nutrition, and Biotechnology*, 2nd ed.; Akoh, C. C., Min, D. B., Eds.; Marcel Dekker: New York, 2002; pp 877−908.

(13) Jandacek, R. J.; Whiteside, J. A.; Holcombe, B. N.; Volpenhein, R. A.; Taulbee, J. D. The rapid hydrolysis and efficient absorption of triglycerides with octanoic acid in the 1 and 3-positions and long chain fatty acid in the 2-position. *Am. J. Clin. Nutr*. **1987**, *45*, 940−945.
(14) Babayan, V. K. Medium chain triglycerides. In *Dietary Fat Requirements in Health and Development*; Beare-Rogers, J., Eds.; American Oil Chemists' Society: Champaign, IL, 1988; pp 73−86.
(15) Stewart, J. W.; Wiggers, K. D.; Jacobson, N. L.; Berger, P. J. Effect of various triglycerides on blood and tissue cholesterol of calves. *J. Nutr*. **1978**, *108*, 561−566.
(16) Xu, X.; Fomuso, L. B.; Akoh, C. C. Synthesis of structured triacylglycerols by lipase-catalyzed acidolysis in a packed bed bioreactor. *J. Agric. Food Chem.* **2000**, *48*, 3−10.
(17) Kaylegian, K. E.; Lindsay, R. C. Performance of selected milkfat fractions in cold-spreadable butter. *J. Dairy Sci.* **1992**, *75*, 3307−3317.
(18) AOCS. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; American Oil Chemists' Society: Champaign, IL, 1998; Cd 11c−93.
(19) AOCS. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; American Oil Chemists' Society: Champaign, IL, 1998; Cj 1−94.
(20) AOCS. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; American Oil Chemists' Society: Champaign, IL, 1998; Cd 16−81.
(21) deMan, J. M. X-ray diffraction spectroscopy in the study of fat polymorphism. *Food Res. Int*. **1992**, *25*, 471−476.
(22) AOCS. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; American Oil Chemists' Society: Champaign, IL, 1998; Ca 5a−40.
(23) Luddy, F. E.; Barford, R. A.; Herb, S. F.; Magidman, P.; Reimenschneider, R. W. Pancreatic lipase hydrolysis of triglycerides by a semimicro technique. *J. Am. Oil Chem. Soc.* **1963**, *41*, 693−696.
(24) SAS Institute. *SAS Statistics Software*, release 8.2; SAS Institute: Cary, NC, 2000.
(25) Xu, X.; Skands, A.; Alder-Nissen, J. Purification of specific structured lipids by distillation: Effects on acyl migration. *J. Am. Oil Chem. Soc.* **2001**, *78*, 715−718.
(26) Xu, X.; Balchen, S.; Høy, C. E.; Alder-Nissen, J. Pilot batch production of specific-structured lipids by lipase-catalyzed interesterification: Preliminary study on incorporation and acyl migration. *J. Am. Oil Chem. Soc.* **1998**, *75*, 301−308.
(27) Ulbricht, T. L. V.; Southgate, D. A. T. Coronary heart disease: Seven dietary factors. *Lancet* **1991**, *338*, 985−992.
(28) Kubow, S. The influence of positional distribution of fatty acids in native, interesterified and structure-specific lipids on lipoprotein metabolism and atherogenesis. *J. Nutr. Biochem.* **1996**, *7*, 530−541.
(29) Lida, H. M. D. N.; Ali, A. R. M. Physicochemical characteristics of palm-based oil blends for the production of reduced fat spreads. *J. Am. Oil Chem Soc.* **1998**, *75*, 1625−1631.
(30) Krawczyk, G. R.; Buliga, G. S.; Bertrand, D. T.; Humphreys, W. M. Reviewing the technology of low fat spreads. *Inform* **1996**, *7*, 635−639.
(31) Borwankar, R. P.; Frye, L. A.; Blaurock, A. E.; Sasevich, F. J. Rheological characterization of melting of margarines and tablespreads. *J. Food Eng*. **1992**, *16*, 55−74.
(32) deMan, J. M.; Mostafa, A. N.; Smith, A. K. Thermal analysis microscopy for the study of phase changes in fats. *Food Microstruct.* **1985**, *4*, 233−239.
(33) Wright, A. J.; Scanlon, M. G.; Hartel, R. W.; Marangoni, A. G. Rheological properties of milkfat and butter. *J. Food Sci.* **2001**, *66*, 1056−1071.
(34) Fomuso, L. B.; Akoh, C. C. Enzymatic modification of high-laurate canola to produce margarine fat. *J. Agric. Food Chem.* **2001**, *49*, 4482−4487.
(35) Muego, K. F.; Resurreccion, V. A.; Hung, Y. C. Characterization of the textural properties of spreadable peanut based products. *J. Texture Stud.* **1990**, *21*, 61−73.

Received for review January 11, 2005. Revised manuscript received April 13, 2005. Accepted April 18, 2005. This material is based upon work supported by the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture, under Agreement 2001-35503-10037.

JF050059V