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trans-Free Margarines Prepared with Canola Oil/Palm Stearin/Palm Kernel Oil-Based Structured Lipids

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Structured lipids (SLs) for formulating *trans*-free margarines were synthesized by lipase-catalyzed interesterification of the blends of canola oil (CO), palm stearin (PS), and palm kernel oil (PKO) in weight ratios (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15. The atherogenicity was determined using fatty acid profiles. We also determined the physical properties (melting/crystallization profiles, solid fat content, polymorphism, and microstructure) of SLs and the textural properties of margarines made with the SLs. The SLs from the 50:30:20 and 60:25:15 blends had atherogenic indices similar to or lower than those of the commercial *trans* (CTMF) and similar to the *trans*-free margarine fats (CTFMF). SLs from the blends with PKO contained a wide range of fatty acids (C6−C20) and had more β' than β polymorphs. Margarines made with SLs from 50:30:20 and 60:25:15 blends possessed similar hardness, adhesiveness, or cohesiveness to margarines made with CTMF and CTFMF, respectively. Therefore, CO/PS/PKO-based SLs were suitable for formulating *trans*-free margarines with low atherogenicity and desirable textural properties.

KEYWORDS: Atherogenic index; canola oil; interesterification; Lipozyme TL IM; microstructure; palm stearin; palm kernel oil; polymorphism; structured lipids; *trans*-free margarine

INTRODUCTION

Margarine belongs to any of a wide range of table spreads that substitute for butter. The traditional margarine fats contain *trans* fatty acids (TFAs), which are defined as unsaturated FAs (USFAs) having one or more isolated double bonds in a *trans* configuration (*1*). TFAs present in traditional margarine fats are formed during a partial hydrogenation process used to impart desirable physical and textural properties to margarines. The amount of TFAs in margarines marketed in the United States is 10−36% (average = 14−24%) of total FAs (*2, 3*). The average intake of TFAs in the U.S. population is approximately 5.3 g/day (2.6% of total calories; 7.4% of fat calories) (*4*).

Dietary TFAs have recently raised many health concerns as a risk factor for coronary heart disease (CHD). High levels of dietary TFAs (>4.0% of total calories) have atherogenic effects by raising plasma low-density lipoprotein (LDL) cholesterol levels and lowering plasma high-density lipoprotein (HDL) cholesterol levels compared to diets high in *cis* USFAs or low in TFAs (*5*−*9*). Besides, a high intake of TFAs increases the levels of plasma lipoprotein(a) [Lp(a)] (*7, 10*) and plasma triacylglycerol (TAG) (*11*) and induces endothelial dysfunction (*12, 13*).

Accordingly, the U.S. Food and Drug Administration (FDA) made it mandatory to declare the TFA content in the nutrition label of food products containing TFAs ≥0.5 g/serving from January 2006 to reduce the consumption of TFAs (*1*). Margarine manufacturers have also been developing several alternatives to the partial hydrogenation process to reduce or eliminate TFAs in their products, resulting in low-*trans* or *trans*-free margarines. The use of structured lipids (SLs) is one of the most successful alternatives (*14*−*16*).

SLs are restructured fats or oils in which the composition and positional distribution of FAs are modified from the native state by chemical or enzymatic methods (*17*). In the present study, *trans*-free SLs were synthesized through lipase-catalyzed interesterification of the blends of canola oil (CO), palm stearin (PS), and palm kernel oil (PKO). CO is a good source of oleic acid (ca. 56% of total FAs; **Table 1**), having cholesterol-lowering effects (*18*). PS is a high-solid fraction obtained from palm oil. CO (liquid oil) turns into solid fat when esterified with PS. CO and PS are mostly composed of long-chain FAs (LCFAs) of C16−C18 (ca. 97% of total FAs; **Table 1**), whereas PKO contains a large amount of medium-chain FAs (MCFAs) that ranged from C8 to C12 (ca. 62% of total FAs; **Table 1**). Thus, the incorporation of PKO increases the diversity in the FA profile of SLs. Of the main polymorphic forms (α, β', and β) of fat crystals, the β' form is the most desirable in margarines because it gives smooth texture to the products. The high diversity in FA composition contributes to the predominant presence of the β' polymorph (*19*). However, PKO has a problem of high proportion of atherogenic saturated FAs (SFAs), such as lauric, myristic, and palmitic acids (ca. 75% of total FAs; **Table 1**). Therefore, SLs synthesized from the blends with proper ratios of CO, PS,

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Table 1. Total and Positional Fatty Acid Profiles of Lipid Substrates for Structured Lipid Synthesis (w/w, %)[a]

	canola oil			palm stearin			palm kernel oil		
fatty acid	total	*sn*-2	*sn*-1,3	total	*sn*-2	*sn*-1,3	total	*sn*-2	*sn*-1,3
C6:0							0.4 ± 0.0		0.6 ± 0.0
C8:0				tr[b]			5.1 ± 0.0	1.4 ± 0.5	6.9 ± 0.2
C10:0				tr			4.3 ± 0.0	1.7 ± 0.0	5.5 ± 0.0
C12:0				0.3 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	52.6 ± 0.3	49.8 ± 0.4	54.2 ± 0.6
C14:0				1.5 ± 0.0	1.0 ± 0.1	1.8 ± 0.0	15.3 ± 0.0	15.2 ± 0.1	15.4 ± 0.1
C16:0	4.7 ± 0.0	0.4 ± 0.1	6.8 ± 0.0	62.1 ± 0.1	46.5 ± 3.1	69.7 ± 1.5	6.8 ± 0.1	4.2 ± 0.2	8.1 ± 0.2
C16:1*n*-7	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	tr	0.2 ± 0.0			
C18:0	1.8 ± 0.0	0.3 ± 0.0	2.6 ± 0.0	4.6 ± 0.0	2.1 ± 0.1	5.9 ± 0.1	1.6 ± 0.0	0.5 ± 0.0	2.1 ± 0.1
C18:1*t*				tr		0.1 ± 0.0			
C18:1*n*-9	56.1 ± 0.1	64.1 ± 2.0	51.9 ± 1.1	24.3 ± 0.0	40.1 ± 2.6	16.4 ± 1.3	12.0 ± 0.2	23.4 ± 0.1	6.3 ± 0.2
C18:1*n*-7	2.8 ± 0.0	1.2 ± 0.0	3.7 ± 0.1	0.4 ± 0.0	0.1 ± 0.0	0.6 ± 0.0			
C18:2*t,t*									
C18:2*n*-6	21.1 ± 0.0	25.4 ± 1.3	19.0 ± 0.7	6.2 ± 0.0	9.9 ± 0.7	4.3 ± 0.4	1.9 ± 0.0	3.8 ± 0.1	0.9 ± 0.0
C18:3*n*-3	10.6 ± 0.4	7.2 ± 0.8	12.4 ± 0.6	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0			
C20:0	0.5 ± 0.0		0.8 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.4 ± 0.0			
C20:1	2.2 ± 0.3	1.3 ± 0.1	2.5 ± 1.0	tr					
SFA[c]	7.0 ± 0.0	0.7 ± 0.1	10.2 ± 0.0	68.8 ± 0.0	50.2 ± 3.3	78.2 ± 1.6	86.1 ± 0.2	72.8 ± 0.2	92.8 ± 0.2
USFA[d]	93.0 ± 0.0	99.3 ± 0.1	89.8 ± 0.0	31.2 ± 0.0	49.8 ± 3.3	21.8 ± 1.6	13.9 ± 0.2	27.2 ± 0.2	7.2 ± 0.2
TFA[e]				tr		0.1 ± 0.0			
atherogenic index (AI)	0.1[f]			2.2[f]			8.7[f]		

[a] Mean ± SD ($n = 2$). [b] Trace, <0.05%. [c] Saturated fatty acid. [d] Unsaturated fatty acid. [e] *trans* fatty acid. [f] AI = [C12:0 (w/w, %) + 4 × C14:0 (w/w, %) + C16:0 (w/w, %)]/USFA (w/w, %).

Table 2. Total Fatty Acid Profile of Commercial Margarine Fats and Structured Lipids (w/w, %)[a]

	commercial margarine fat[b]		structured lipids[c]					
fatty acid	CTMF	CTFMF	SL460	SL451	SL442	SL433	SL532	SL621
C6:0		tr[d]		tr	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	tr
C8:0		tr		0.5 ± 0.0	0.9 ± 0.1	1.5 ± 0.0	0.9 ± 0.0	0.7 ± 0.0
C10:0		tr		0.4 ± 0.0	0.9 ± 0.0	1.3 ± 0.0	0.8 ± 0.0	0.6 ± 0.0
C12:0		0.5 ± 0.0	0.1 ± 0.0	5.6 ± 0.0	11.1 ± 0.0	16.4 ± 0.0	10.5 ± 0.0	8.0 ± 0.1
C14:0	tr	0.8 ± 0.0	0.9 ± 0.0	2.5 ± 0.0	4.1 ± 0.0	5.6 ± 0.0	3.8 ± 0.0	2.9 ± 0.0
C16:0	12.1 ± 0.0	26.9 ± 0.0	39.9 ± 0.1	33.9 ± 0.0	28.1 ± 0.0	22.5 ± 0.0	22.3 ± 0.0	19.4 ± 0.0
C16:1*n*-7	tr	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
C18:0	8.5 ± 0.0	3.9 ± 0.0	3.4 ± 0.0	3.1 ± 0.0	2.9 ± 0.0	2.6 ± 0.0	2.7 ± 0.0	2.5 ± 0.0
C18:1*t*	14.9 ± 0.0							
C18:1*n*-9	23.2 ± 0.0	35.7 ± 0.1	36.7 ± 0.0	35.4 ± 0.0	34.1 ± 0.1	32.9 ± 0.0	39.0 ± 0.0	42.9 ± 0.0
C18:1*n*-7	2.0 ± 0.0	1.2 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.3 ± 0.1	1.2 ± 0.0	1.5 ± 0.0	1.7 ± 0.0
C18:2*t,t*	0.1 ± 0.0							
C18:2*n*-6	33.8 ± 0.1	25.9 ± 0.0	11.8 ± 0.0	11.5 ± 0.0	10.9 ± 0.0	10.5 ± 0.0	12.2 ± 0.0	13.8 ± 0.0
C18:3*n*-3	4.7 ± 0.1	3.7 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.1	3.9 ± 0.0	4.4 ± 0.0	5.3 ± 0.0
C20:0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
C20:1	0.4 ± 0.0	0.9 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.1	1.1 ± 0.0	1.3 ± 0.0	1.6 ± 0.0
SFA[e]	20.9 ± 0.0	32.4 ± 0.0	44.7 ± 0.1	46.4 ± 0.0	48.3 ± 0.1	50.2 ± 0.0	41.4 ± 0.0	34.5 ± 0.1
USFA[f]	79.1 ± 0.0	67.6 ± 0.0	55.3 ± 0.1	53.6 ± 0.0	51.7 ± 0.1	49.8 ± 0.0	58.6 ± 0.0	65.5 ± 0.1
TFA[g]	15.0 ± 0.0							
atherogenic index (AI)	0.2[h], 0.4[i], 1.1[j]	0.5[h]	0.8[h]	0.9[h]	1.1[h]	1.2[h]	0.8[h]	0.6[h]

[a] Mean ± SD ($n = 2$). [b] CTMF, partially hydrogenated fat separated from commercial *trans* margarine (Land O'Lakes); CTFMF, fat separated from commercial *trans*-free margarine (Smart Balance). [c] SL460, SL451, SL442, SL433, SL532, and SL621 indicate the structured lipids synthesized by Lipozyme TL IM-catalyzed interesterification reactions of the blends of canola oil (CO), palm stearin (PS), and palm kernel oil (PKO) in weight ratios (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15, respectively. [d] Trace, <0.05%. [e] Saturated fatty acid. [f] Unsaturated fatty acid. [g] *trans* fatty acid. [h] AI = [C12:0 (w/w, %) + 4 × C14:0 (w/w, %) + C16:0 (w/w, %)]/USFA (w/w, %). [i] AI = [C12:0 (w/w, %) + 4 × C14:0 (w/w, %) + C16:0 (w/w, %) + TFA (w/w, %)]/[USFA (w/w, %) − TFA (w/w, %)]. [j] AI = [C12:0 (w/w, %) + 4 × C14:0 (w/w, %) + C16:0 (w/w, %) + 4 × TFA (w/w, %)]/[USFA (w/w, %) − TFA (w/w, %)].

and PKO would be suitable for formulating margarines with reduced risk for CHD as well as desirable textural attributes.

The aim of our study was to examine the possibility of using CO/PS/PKO-based SLs for the formulation of *trans*-free margarines. To determine proper blending ratios of CO, PS, and PKO, we investigated the atherogenicity (evaluated by FA profile) and physical properties (melting/crystallization profiles, solid fat content, polymorphism, and microstructure) of SLs synthesized from the blends with different ratios (CO/PS/PKO) and the textural properties of margarines prepared with the SLs. We used fats separated from two kinds of commercial margarines (*trans* and *trans*-free) and margarines made with the fats as the reference standards.

MATERIALS AND METHODS

Materials. Commercial *trans* margarine (Land O'Lakes, Arden Hills, MN), commercial *trans*-free margarine (Smart Balance, GFA Brands, Cresskill, NJ), and CO (Wesson, ConAgra Foods, Omaha, NE) were purchased from a local grocery store. PS and PKO were donated by Fuji Vegetable Oil (Savannah, GA) and PGEO group (Johor, Malaysia), respectively. Lipozyme TL IM (immobilized *sn*-1,3 specific lipase from

Table 3. *sn*-2 Positional Fatty Acid Profile of Commercial Margarine Fats and Structured Lipids (w/w, %)[a]

	commercial margarine fat[b]		structured lipids[c]					
fatty acid	CTMF	CTFMF	SL460	SL451	SL442	SL433	SL532	SL621
C6:0								
C8:0				0.3 ± 0.0	0.5 ± 0.0	1.0 ± 0.4	0.5 ± 0.0	0.5 ± 0.0
C10:0				0.3 ± 0.0	0.6 ± 0.0	0.9 ± 0.0	0.6 ± 0.0	0.5 ± 0.0
C12:0		0.3 ± 0.0	0.1 ± 0.0	5.1 ± 0.1	9.1 ± 0.0	13.9 ± 0.1	9.3 ± 0.0	8.3 ± 0.4
C14:0		0.4 ± 0.0	0.8 ± 0.0	2.4 ± 0.1	3.6 ± 0.0	5.1 ± 0.0	3.6 ± 0.0	3.2 ± 0.1
C16:0	0.9 ± 0.0	10.5 ± 0.5	39.6 ± 0.3	36.7 ± 0.9	27.7 ± 0.1	22.4 ± 0.0	22.6 ± 0.0	22.9 ± 0.8
C16:1*n*-7	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
C18:0	6.9 ± 0.2	1.2 ± 0.1	3.6 ± 0.1	3.7 ± 0.1	2.9 ± 0.0	2.6 ± 0.0	2.8 ± 0.0	3.2 ± 0.1
C18:1*t*	27.4 ± 0.5							
C18:1*n*-9	33.7 ± 0.2	62.8 ± 1.9	37.8 ± 0.3	38.6 ± 0.3	37.0 ± 0.6	35.5 ± 0.0	40.9 ± 0.0	47.7 ± 0.7
C18:1*n*-7	1.7 ± 0.0	0.1 ± 0.0	1.4 ± 0.0	1.3 ± 0.0	0.9 ± 0.6	1.3 ± 0.1	1.4 ± 0.0	1.8 ± 0.0
C18:2*t*,*t*	0.3 ± 0.0							
C18:2*n*-6	27.4 ± 0.8	22.9 ± 2.3	11.7 ± 0.0	8.4 ± 1.0	12.1 ± 0.0	11.8 ± 0.1	12.6 ± 0.0	8.6 ± 1.5
C18:3*n*-3	1.4 ± 0.1	1.4 ± 0.3	3.5 ± 0.0	1.9 ± 0.5	4.0 ± 0.1	4.0 ± 0.0	4.0 ± 0.0	1.7 ± 0.6
C20:0		tr[d]	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0
C20:1	0.2 ± 0.0	0.3 ± 0.1	1.0 ± 0.0	0.8 ± 0.1	1.1 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	0.9 ± 0.1
SFA[e]	7.8 ± 0.2	12.4 ± 0.7	44.4 ± 0.4	46.4 ± 0.0	44.7 ± 0.1	46.2 ± 0.2	39.8 ± 0.0	39.1 ± 1.5
USFA[f]	92.2 ± 0.2	87.6 ± 0.7	55.6 ± 0.4	53.6 ± 0.0	55.3 ± 0.1	53.8 ± 0.2	60.2 ± 0.0	60.9 ± 1.5
TFA[g]	27.7 ± 0.5							

[a] Mean ± SD ($n = 2$). [b] CTMF, partially hydrogenated fat separated from commercial *trans* margarine (Land O'Lakes); CTFMF, fat separated from commercial *trans*-free margarine (Smart Balance). [c] SL460, SL451, SL442, SL433, SL532, and SL621 indicate the structured lipids synthesized by Lipozyme TL IM-catalyzed interesterification reactions of the blends of canola oil (CO), palm stearin (PS), and palm kernel oil (PKO) in weight ratios (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15, respectively. [d] Trace, <0.05%. [e] Saturated fatty acid. [f] Unsaturated fatty acid. [g] *trans* fatty acid.

Table 4. *sn*-1,3 Positional Fatty Acid Profile of Commercial Margarine Fats and Structured Lipids (w/w, %)[a]

	commercial margarine fat[b]		structured lipids[c]					
fatty acid	CTMF	CTFMF	SL460	SL451	SL442	SL433	SL532	SL621
C6:0					0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
C8:0				0.6 ± 0.0	1.1 ± 0.1	1.7 ± 0.2	1.1 ± 0.0	0.8 ± 0.0
C10:0				0.5 ± 0.0	1.0 ± 0.0	1.5 ± 0.0	0.9 ± 0.0	0.7 ± 0.0
C12:0		0.6 ± 0.0	0.1 ± 0.0	5.8 ± 0.0	12.1 ± 0.0	17.7 ± 0.1	11.1 ± 0.0	7.9 ± 0.1
C14:0		0.9 ± 0.0	1.0 ± 0.0	2.5 ± 0.0	4.3 ± 0.0	5.8 ± 0.0	3.9 ± 0.0	2.7 ± 0.0
C16:0	17.7 ± 0.0	35.1 ± 0.3	40.0 ± 0.1	32.5 ± 0.5	28.4 ± 0.1	22.5 ± 0.1	22.4 ± 0.0	17.7 ± 0.4
C16:1*n*-7		0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
C18:0	9.3 ± 0.0	5.3 ± 0.1	3.4 ± 0.0	2.8 ± 0.0	2.8 ± 0.0	2.5 ± 0.0	2.6 ± 0.0	2.1 ± 0.1
C18:1*t*	8.6 ± 0.2							
C18:1*n*-9	17.9 ± 0.1	22.2 ± 1.1	36.2 ± 0.1	33.9 ± 0.2	32.5 ± 0.1	31.6 ± 0.0	38.1 ± 0.0	40.4 ± 0.4
C18:1*n*-7	2.2 ± 0.0	1.8 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.7 ± 0.2	1.3 ± 0.1	1.5 ± 0.0	1.7 ± 0.0
C18:2*t*,*t*								
C18:2*n*-6	37.1 ± 0.5	27.3 ± 1.2	11.8 ± 0.0	13.0 ± 0.6	10.3 ± 0.0	9.9 ± 0.1	11.9 ± 0.1	16.5 ± 0.7
C18:3*n*-3	6.3 ± 0.1	4.8 ± 0.1	4.3 ± 0.0	5.1 ± 0.2	4.0 ± 0.1	3.8 ± 0.0	4.5 ± 0.0	7.1 ± 0.3
C20:0	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
C20:1	0.4 ± 0.0	1.3 ± 0.1	1.2 ± 0.0	1.4 ± 0.0	1.2 ± 0.1	1.1 ± 0.0	1.4 ± 0.0	1.9 ± 0.0
SFA[d]	27.5 ± 0.1	42.4 ± 0.3	44.8 ± 0.1	45.1 ± 0.6	50.0 ± 0.1	52.2 ± 0.1	42.3 ± 0.1	32.2 ± 0.7
USFA[e]	72.5 ± 0.1	57.6 ± 0.3	55.2 ± 0.1	54.9 ± 0.6	50.0 ± 0.1	47.8 ± 0.1	57.7 ± 0.1	67.8 ± 0.7
TFA[f]	8.6 ± 0.2							

[a] Mean ± SD ($n = 2$). [b] CTMF, partially hydrogenated fat separated from commercial *trans* margarine (Land O'Lakes); CTFMF, fat separated from commercial *trans*-free margarine (Smart Balance). [c] SL460, SL451, SL442, SL433, SL532, and SL621 indicate the structured lipids synthesized by Lipozyme TL IM-catalyzed interesterification reactions of the blends of canola oil (CO), palm stearin (PS), and palm kernel oil (PKO) in weight ratios (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15, respectively. [d] Saturated fatty acid. [e] Unsaturated fatty acid. [f] *trans* fatty acid.

Thermomyces lanuginosus) was provided by Novozymes North America (Franklinton, NC). Soy lecithin fluid was provided by Cargill Inc. (Minneapolis, MN). All other reagents were of analytical or HPLC grade.

Commercial Margarine Fat Preparation. Two kinds of margarine fats were separated from two commercial margarines (*trans* and *trans*-free). The commercial margarines were melted at 80 °C, and the top fat layers were decanted into a separatory funnel and washed five times with the same volume of warm water. The margarine fats were filtered through an anhydrous sodium sulfate layer with a Whatman filter paper (pore size = 0.45 μm) under vacuum to remove residual moisture. The obtained anhydrous margarine fats were designated commercial *trans* margarine fat (CTMF) and commercial *trans*-free margarine fat (CTFMF).

SL Synthesis. The SL synthesis was performed by interesterification reaction between CO, PS, and PKO blends in a 1 L capacity stirred tank batch reactor. Six kinds of substrate mixtures were prepared by blending CO, PS, and PKO in weight ratios (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15, respectively. The substrate mixture (500 g) was thoroughly melted in the reactor at 80 °C. Lipozyme TL IM (25 g) was then added to the substrate mixture, and the interesterification reaction was performed at 60 °C under an agitation speed of 200 rpm for 18 h. The reaction products were filtered through anhydrous sodium sulfate layer with a Whatman filter paper (pore size = 0.45 μm) under vacuum to remove the lipase and moisture. Short-path distillation was performed to purify the synthesized SLs from the reaction product using a KDL-4 unit (UIC, Joliet, IL). The reaction products were passed through the unit under high vacuum (<0.1 mmHg

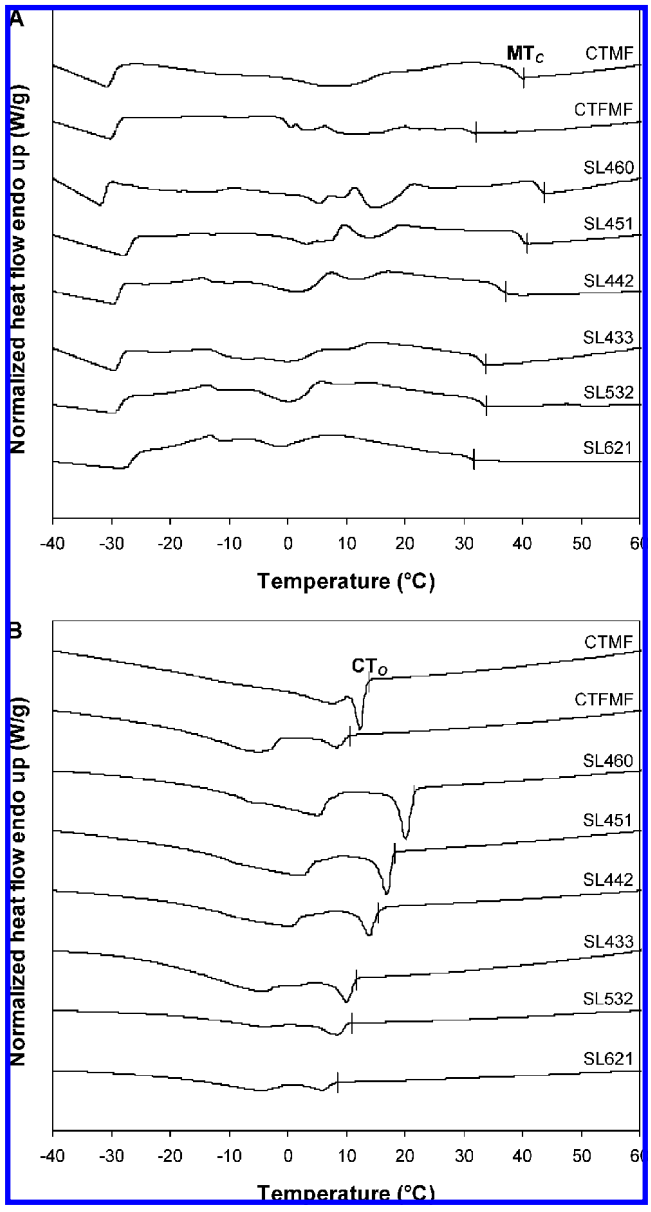

Figure 1. DSC melting (**A**) and crystallization (**B**) profiles of commercial margarine fats and structured lipids; MT_C and CT_O indicate melting completion temperature and crystallization onset temperature, respectively. CTMF indicates the partially hydrogenated fat separated from commercial *trans* margarine (Land O'Lakes). CTFMF indicates the fat separated from commercial *trans*-free margarine (Smart Balance). SL460, SL451, SL442, SL433, SL532, and SL621 indicate the structured lipids synthesized by Lipozyme TL IM-catalyzed interesterification of the blends of canola oil (CO), palm stearin (PS), and palm kernel oil (PKO) in weight ratios (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15, respectively.

absolute pressure) at the flow rate of ca. 500 mL/h. The evaporator and condenser temperatures were maintained at 185 and 45 °C, respectively. The SLs synthesized from the blends (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15 were designated SL460, SL451, SL442, SL433, SL532, and SL621, respectively.

Margarine Manufacture. Eight kinds of experimental margarine samples were produced using two kinds of commercial margarine fats (CTMF and CTFMF) and six kinds of SLs (SL460, SL451, SL442, SL433, SL532, and SL621), respectively. The margarine formulation (w/w, %) used in the present study is as follows: lipid phase, 80.5% (commercial margarine fats or SLs, 80%; soy lecithin fluid, 0.5%; and TBHQ, 0.01%); and aqueous phase, 19.5% (distilled water, 17.7%; and table salt, 1.8%). Both phases were poured into a tabletop blender and vigorously mixed for 2 min to emulsify them. The resulting liquid emulsion was then crystallized using an ice cream maker (type 358, Krups North America, 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. The resulting crystallized emulsion was tempered at room temperature for 4 h and then worked vigorously with a hand mixer until their textures were smoothed and all lumps were removed. The resulting paste-type margarine sample (120 g each) was placed into a crystal jar and stored for at least 2 weeks at 4 °C prior to use.

Total FA Profile Analysis. Each lipid sample (50 mg) was methylated in 3 mL of 6% methanolic HCl 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 (SP-2560, 100 m × 0.25 mm i.d. × 0.2 μm film thickness, Supelco, Deerfield, IL), was used for the analysis. The injection of sample (1 μL) was performed in the split mode (split ratio of 4:1). The carrier gas was helium, and its average velocity and flow rate were 30 cm/s and 2.5 mL/min, respectively. The injector and detector temperatures were maintained at 250 and 260 °C, respectively. The oven temperature was initially held at 80 °C for 5 min. It was then programmed to increase to 240 °C at the rate of 4 °C/min and held at 240 °C for 15 min. The FAMEs were identified by comparing their retention times with those of the reference standards (GLC-463, Nu-Check, Elysian, MN), and their relative contents were calculated as w/w, % with heptadecanoic acid (C17:0) as an internal standard.

Positional FA Profile Analysis. Each lipid sample (50 mg) was analyzed to determine the FA esterified at the *sn*-2 position according to the pancreatic lipase hydrolysis procedure described by Luddy et al. (*20*). The FA profile at *sn*-1,3 positions was obtained by calculation using the following equation: *sn*-1,3 (w/w, %) = [3 × total (w/w, %) − *sn*-2 (w/w, %)]/2.

Melting and Crystallization Profile Analysis. The melting and crystallization profiles of the lipid samples were determined using a Perkin-Elmer differential scanning calorimeter (DSC) (model DSC 7, Perkin-Elmer Co., Norwalk, CT) according to AOCS recommended procedure Cj 1-94 (*21*). Normal standardization was performed with indium (mp 156.6 °C, ΔH = 28.45 J/g) as a reference standard. Dry ice was used as the coolant. Each lipid sample (6−8 mg) was hermetically sealed in a 30 μL capacity aluminum pan (Perkin-Elmer), with an empty sealed pan used as a reference. Lipid sample was rapidly heated from room temperature to 80 °C and held at this temperature for 10 min to destroy any previous crystal structure, before being cooled to −40 °C at a rate of 5 °C/min to obtain the crystallization profile. After a 30 min hold at −40 °C, the sample was heated to 80 °C at a rate of 5 °C/min to generate the melting profile. The profiles were analyzed by the software provided with the DSC (Pyris software, Perkin-Elmer, Shelton, CT).

Solid Fat Content (SFC). The SFC was determined according to AOCS official method Cd 16-81 (*21*). Nuclear magnetic resonance (NMR) measurements were performed using a MARAN-20 pulsed NMR spectrometer (Resonance Instruments Ltd., Oxon, U.K.). Each lipid sample (3 g) was 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 used as the reference oil. The SFC was measured at 5 °C intervals from 5 to 45 °C.

Polymorphism. The crystal polymorphic forms of lipid sample at refrigeration temperature (4 °C) were determined by X-ray diffraction (XRD) spectroscopy using 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 35 mA. The 2θ range used was from 20 to 32°, and the scan rate was 4.0°/min. Each sample was melted at 80 °C and poured into a rectangular plastic mold. Sample was then crystallized at 4 °C for 24 h. Short spacings of the major polymorphs are as follows: α, one spacing

Table 5. DSC Melting Completion (MT_C) and Crystallization Onset (CT_O) Temperatures of Commercial Margarine Fats and Structured Lipids[a]

	commercial margarine fat[b]		structured lipids[c]					
	CTMF	CTFMF	SL460	SL451	SL442	SL433	SL532	SL621
MT_C (°C)	40.1 ± 0.6c	32.0 ± 0.4f	43.5 ± 0.0a	40.6 ± 0.3b	37.0 ± 0.1d	33.5 ± 0.1e	33.9 ± 0.2e	31.7 ± 0.1f
CT_O (°C)	13.7 ± 0.5d	10.4 ± 0.0f	21.4 ± 0.1a	18.0 ± 0.0b	15.2 ± 0.3c	11.5 ± 0.1e	10.8 ± 0.5f	8.3 ± 0.3g

[a] Mean ± SD (n = 3); means with the same letter in the same row are not significantly different (p < 0.05). [b] CTMF, partially hydrogenated fat separated from commercial *trans* margarine (Land O'Lakes); CTFMF, fat separated from commercial *trans*-free margarine (Smart Balance). [c] SL460, SL451, SL442, SL433, SL532, and SL621 indicate the structured lipids synthesized by Lipozyme TL IM-catalyzed interesterification reactions of the blends of canola oil (CO), palm stearin (PS), and palm kernel oil (PKO) in weight ratios (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15, respectively.

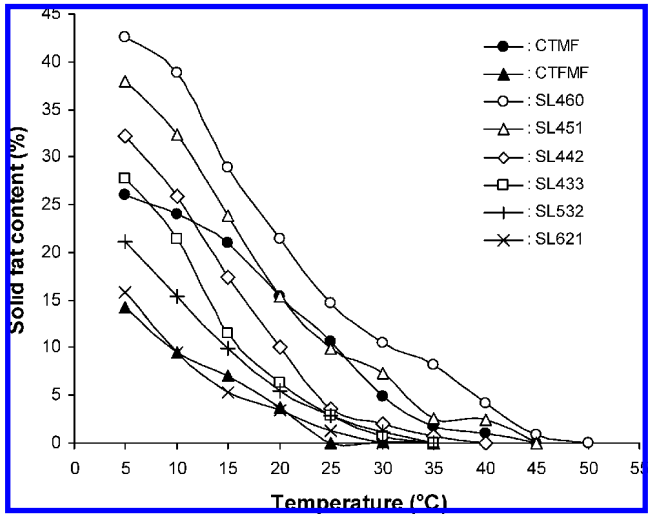

Figure 2. Solid fat contents of commercial margarine fats and structured lipids. CTMF indicates the partially hydrogenated fat separated from commercial *trans* margarine (Land O'Lakes). CTFMF indicates the fat separated from commercial *trans*-free margarine (Smart Balance). SL460, SL451, SL442, SL433, SL532, and SL621 indicate the structured lipids synthesized by Lipozyme TL IM-catalyzed interesterification of the blends of canola oil (CO), palm stearin (PS), and palm kernel oil (PKO) in weight ratios (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15, respectively.

Table 6. Crystal Polymorphic Forms of Commercial Margarine Fats and Structured Lipids

	polymorphic form	level of β' and β forms[a]
commercial margarine fat[b]		
CTMF	$\alpha + \beta' + \beta$	$\beta' \gg \beta$
CTFMF	$\alpha + \beta' + \beta$	$\beta' = \beta$
structured lipids[c]		
SL460	$\alpha + \beta' + \beta$	$\beta' = \beta$
SL451	$\alpha + \beta' + \beta$	$\beta' > \beta$
SL442	$\alpha + \beta' + \beta$	$\beta' > \beta$
SL433	$\alpha + \beta' + \beta$	$\beta' > \beta$
SL532	$\alpha + \beta' + \beta$	$\beta' > \beta$
SL621	$\alpha + \beta' + \beta$	$\beta' > \beta$

[a] Estimated using the equation β/β' = (intensity of short spacing at 4.6 Å)/(intensity of short spacing at 4.2 Å) as follows: $\beta' = \beta$ ($1.2 \geq \beta/\beta' > 0.8$); $\beta' > \beta$ ($0.8 \geq \beta/\beta' > 0.4$); and $\beta' \gg \beta$ ($0.4 \geq \beta/\beta' > 0$). [b] CTMF, partially hydrogenated fat separated from commercial *trans* margarine (Land O'Lakes); CTFMF, fat separated from commercial *trans*-free margarine (Smart Balance). [c] SL460, SL451, SL442, SL433, SL532, and SL621 indicate the structured lipids synthesized by Lipozyme TL IM-catalyzed interesterification reactions of the blends of canola oil (CO), palm stearin (PS), and palm kernel oil (PKO) in weight ratios (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15, respectively.

at 4.15 Å; β', two spacings at 3.8 and 4.2 Å; and β, a strong spacing at 4.6 and another one usually at 3.85 Å (*22*). The level of β' and β forms in the sample was estimated by the relative intensity of the short spacings at 4.2 and 4.6 Å.

Microstructural Analysis. Microstructural observation of lipid sample was conducted with a polarized light microscope (Leica Microsystem Inc., Allendale, NJ) attached to an Axiocam digital camera (Zess Inc., Göttingen, Germany). Lipid sample (10 μL) melted at 80 °C was placed on a preheated microslide, and then a preheated coverslip was placed over the sample. Microstructure at 4 °C was observed using the specimen, which was kept at 4 °C for 24 h. Microstructure at room temperature (23 °C) was observed using the specimen, which was kept at 4 °C for 24 h and then left at 23 °C for 24 h.

Texture Profile Analysis (TPA). Textural properties (hardness, adhesiveness, and cohesiveness) of margarine sample at 4 and 23 °C were determined, following the TPA procedure (*23*). A double-compression test was performed using a TA-X2 texture analyzer (Stable Micro Systems, London, U.K.). A 45° conical probe attached to a 5 kg compression load cell was penetrated into the sample at 1.0 mm/s to a depth of 10 mm from the sample surface (the height of sample was 66 mm) and then was withdrawn at the same speed. The maximum force (g) during the first compression was reported as hardness. The negative force area (g•s) for the first compression was reported as adhesiveness. The ratio of the positive force area during the second compression to that during the first compression was reported as cohesiveness. Textural properties at 4 °C were evaluated using the sample that had just been taken out of the refrigerator, and those at 23 °C were evaluated using the sample that had been left at 23 °C for 24 h after removal from the refrigerator. Three specimens were tested for each sample type.

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

RESULTS AND DISCUSSION

Total and Positional FA Profiles. Total and positional FA profiles of lipid substrates used for the synthesis of SLs are given in **Table 1**. Because the chain lengths of FAs present in CO, PS, and PKO ranged from C16 to C20, from C12 to C20, and from C6 to C18, respectively, the SLs synthesized from the ternary blends of substrates had a wide range of FAs from C6 to C20 as shown in **Table 2**. The total FA profile of commercial margarine fats and SLs is shown in **Table 2**. CTMF had TFA content of 15%, whereas no TFAs were detected in CTFMF and all SLs. The major FAs of SLs were oleic (32.9−42.9%), palmitic (19.4−39.9%), linoleic (10.5−13.8%), and lauric acids (5.6−16.4%; except SL460). *sn*-2 and *sn*-1,3 positional FA profiles of commercial margarine fats and SLs are shown in **Tables 3** and **4**, respectively. CTMF and CTFMF showed different contents of particular FAs according to their *sn* positions. In CTMF, for example, oleic acid predominantly existed at the *sn*-2 position (33.7%) compared to the *sn*-1,3 positions (17.9%), whereas palmitic acid were mostly located at the *sn*-1,3 positions (17.7%) compared to the *sn*-2 position (0.9%). TFA content was about 3 times greater at the *sn*-2 position (27.7%) than at the *sn*-1,3 positions (8.6%) in CTMF. However, SLs did not show a distinct difference in positional

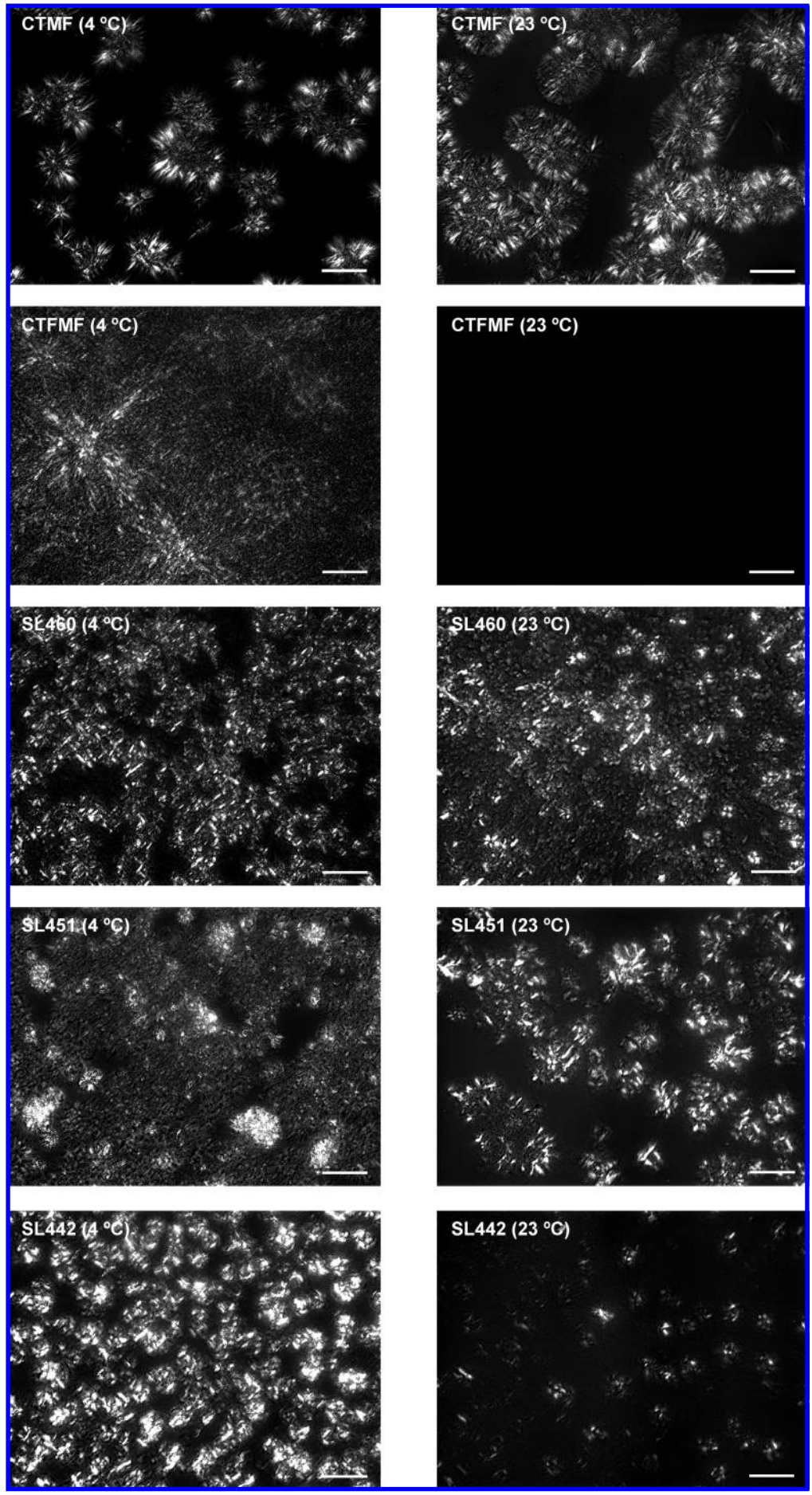
CTMF (4 °C)
CTMF (23 °C)
CTFMF (4 °C)
CTFMF (23 °C)
SL460 (4 °C)
SL460 (23 °C)
SL451 (4 °C)
SL451 (23 °C)
SL442 (4 °C)
SL442 (23 °C)

Figure 3

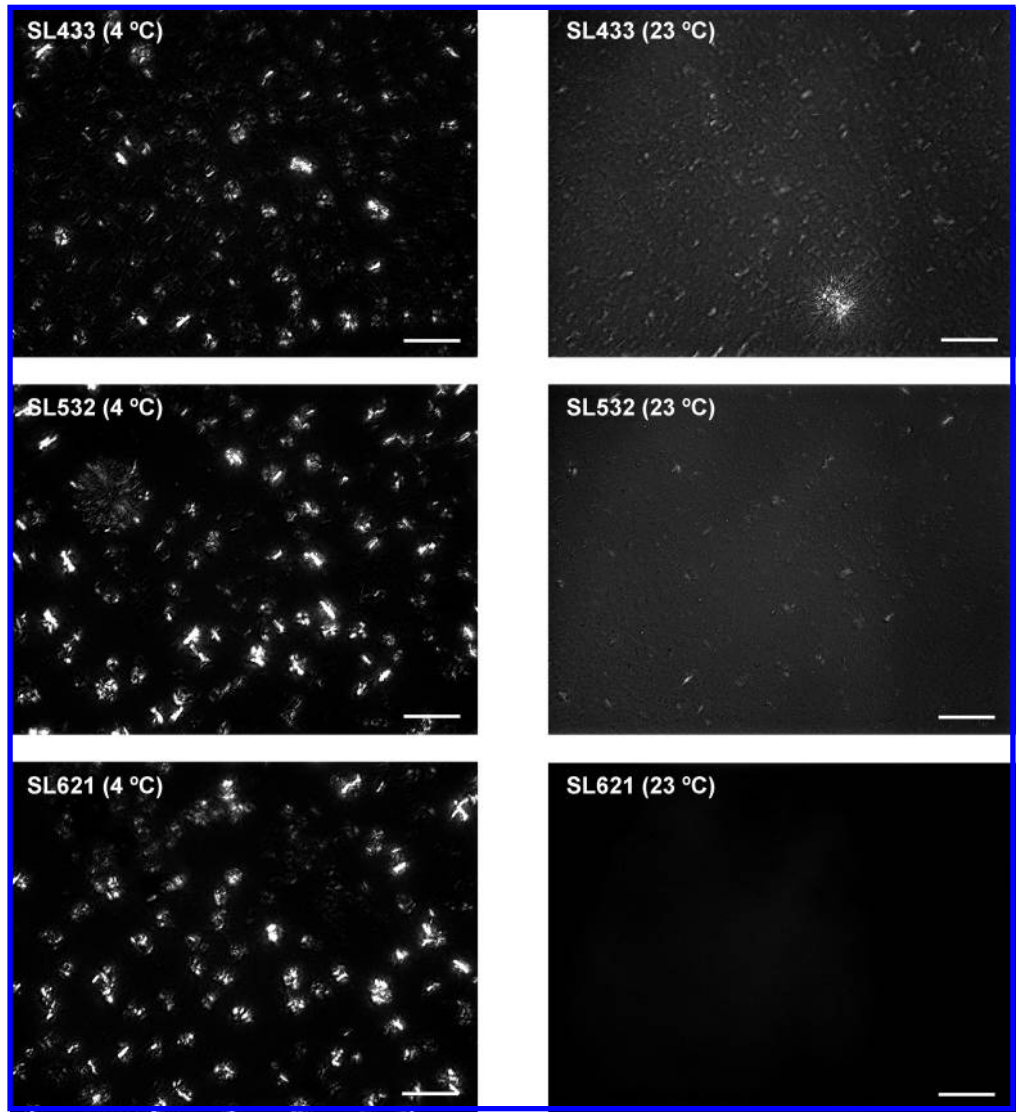

Figure 3. Morphology of fat crystal network of commercial margarine fats and structured lipids. CTMF indicates the partially hydrogenated fat separated from commercial *trans* margarine (Land O'Lakes). CTFMF indicates the fat separated from commercial *trans*-free margarine (Smart Balance). SL460, SL451, SL442, SL433, SL532, and SL621 indicate the structured lipids synthesized by Lipozyme TL IM-catalyzed interesterification of the blends of canola oil (CO), palm stearin (PS), and palm kernel oil (PKO) in weight ratios (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15, respectively. The bar represents 200 μm.

distribution of FAs (e.g., oleic acid, 35.5−47.7% at *sn*-2 vs 31.6−40.4% at *sn*-1,3; palmitic acid, 22.4−39.6% at *sn*-2 vs 17.7−40.4% at *sn*-1,3).

Atherogenic Index (AI). The risk of dietary lipid consumption for CHD can be evaluated by an AI, which is calculated by the contents of atherogenic SFAs (lauric, myristic, and palmitic acids) and USFAs present in the lipids as follows: AI = [C12:0 (w/w, %) + 4 × C14:0 (w/w, %) + C16:0 (w/w, %)]/USFA (w/w, %) (*25*). SLs synthesized from the blends with smaller amount of PKO or greater amount of CO had lower AI values, but all SLs had higher AI values than CTMF and CTFMF (**Table 2**). However, the AI is not a suitable measure of the atherogenicity of CTMF because the effects of TFAs are not considered in the AI. There have been conflicting findings regarding the comparison between atherogenic effects of dietary TFAs and SFAs. However, it is generally accepted that the effects of TFAs on plasma lipoprotein cholesterol profiles are at least as unfavorable as those of SFA (*11, 26*) despite some reports that TFAs have weaker hypercholesterolemic effects than SFAs (*27*). Thus, we modified the AI to include the effects of TFAs in the calculation as follows: if TFAs have similar atherogenic effects to lauric or palmitic acids, AI = [C12:0 (w/w, %) + 4 × C14:0 (w/w, %) + C16:0 (w/w, %) + TFA (w/w, %)]/[USFA (w/w, %) − TFA (w/w, %)]; if TFAs have similar atherogenic effects to myristic acid, AI = [C12:0 (w/w, %) + 4 × C14:0 (w/w, %) + C16:0 (w/w, %) + 4 × TFA (w/w, %)]/[USFA (w/w, %) − TFA (w/w, %)]. According to the modified calculations above, the AI value of CTMF was equal to or greater than 0.4 or 1.1 (**Table 2**). As a result, all SLs except SL442 and SL433 were evaluated to be of similar or less atherogenicity compared to CTMF.

Melting and Crystallization Properties. Melting and crystallization properties of lipid samples were evaluated by DSC thermal profiles (**Figure 1**). SLs synthesized from the blends that have smaller amounts of PS or greater amounts of CO have melting ranges that shifted from higher to lower temperature and lower melting peaks at higher temperature, indicating the presence of smaller portions of higher melting TAG species (**Figure 1A**). Similarly, the crystallization ranges of SLs shifted toward lower temperature and the crystallization peak sizes of SLs were diminished as the content of PS decreased or the content of CO increased in the blends (**Figure 1B**).

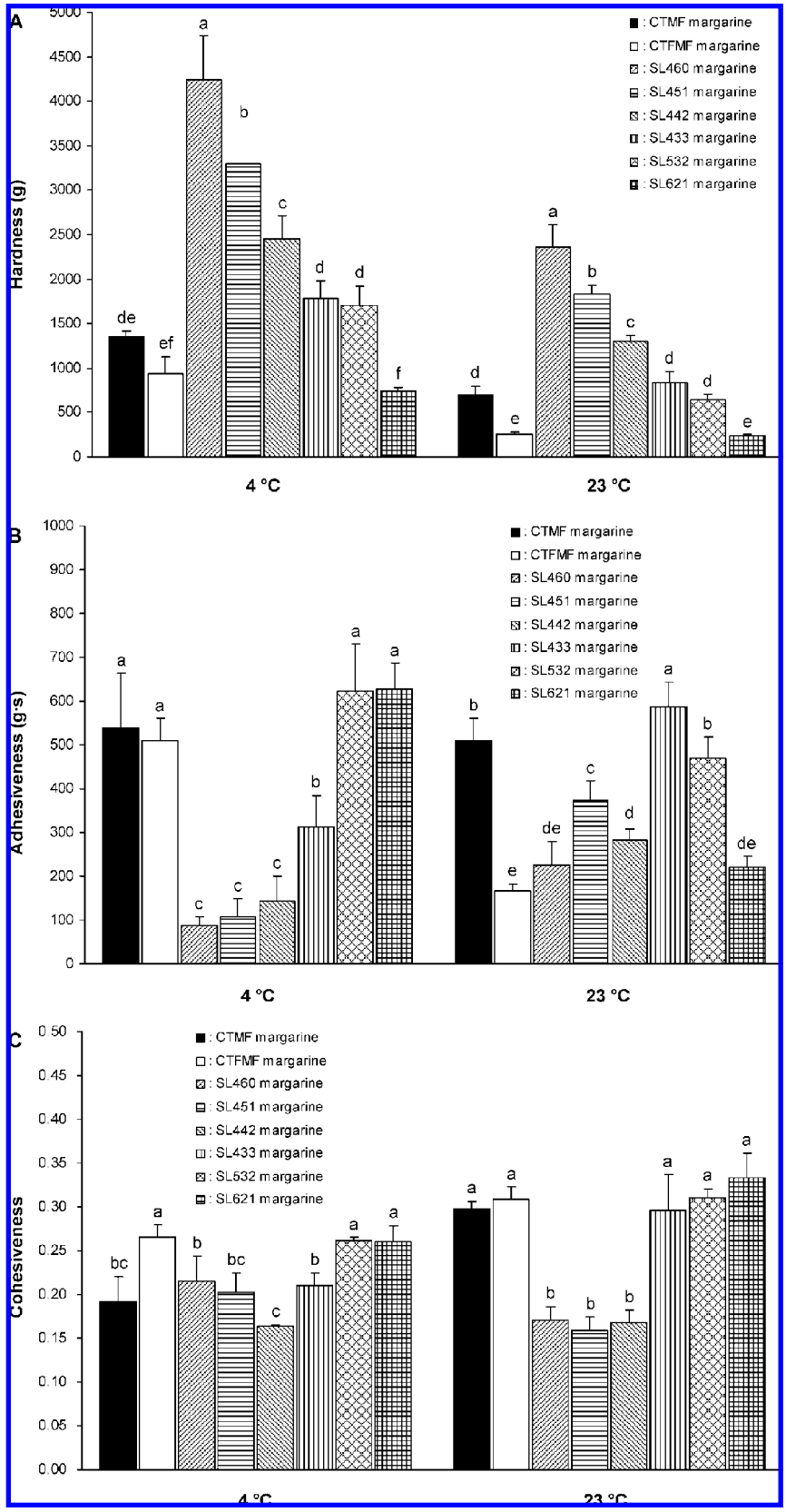

Figure 4. Hardness (**A**), adhesiveness (**B**), and cohesiveness (**C**) of margarines made with commercial margarine fats or structured lipids. Results are expressed as mean ± SD ($n = 3$). Means with the same letter on the bars are not significantly different ($p < 0.05$). CTMF margarine indicates the margarine prepared with the partially hydrogenated fat separated from commercial *trans* margarine (Land O'Lakes). CTFMF margarine indicates the margarine prepared with the fat separated from commercial *trans*-free margarine (Smart Balance). SL460, SL451, SL442, SL433, SL532, and SL621 indicate the structured lipids synthesized by Lipozyme TL IM-catalyzed interesterification of the blends of canola oil (CO), palm stearin (PS), and palm kernel oil (PKO) in weight ratios (CO/PS/PKO) of 40:60:0, 40:50:10, 40:40:20, 40:30:30, 50:30:20, and 60:25:15, respectively.

The melting completion (MT_C) and crystallization onset temperatures (CT_O) of the peaks found at the highest temperature of each lipid sample were considered to be the temperatures at which the melting ends and crystallization starts, respectively, and were compared to one another (**Table 5**). The melting of all SLs, except SL460 and SL451, was completed at temperature below body temperature as well as at significantly ($p < 0.05$) lower temperature than the CTMF. These results were correlated with the SFC at 30 or 35 °C described below. All SLs except SL621 completed the melting at significantly ($p < 0.05$) higher temperature than CTFMF. SL621 showed ($p > 0.05$) MT_C similar to that of CTFMF, leading to a similar change in the fat crystal network between CTFMF and SL621 at 23 °C and similar hardness between CTFMF and SL621 margarines at the same temperature, as described later. SL460, SL451, and SL442 started the crystallization at significantly ($p < 0.05$) higher temperature than CTMF, whereas SL433, SL532, and SL621 did so at significantly ($p < 0.05$) lower temperature than CTMF.

SFC. SFC is related to several physical and textural characteristics of table spreads including margarine. **Figure 2** shows the relationships between SFC and temperature of lipid samples. SFC should be <32% at 10 °C to have good spreadability at refrigeration temperature (*28*). CTMF, CTFMF, and all SLs, except SL460 and SL451, had SFC below 32% at 10 °C. The SFC of 7.6% is the minimal level necessary to maintain a crystal structure (*29*). CTMF, CTFMF, and all SLs had SFC >7.6% at 5 °C, whereas the SFC of CTFMF and all SLs, except SL460 and SL451, was lower than 7.6% at 20 or 25 °C. The SFC should not exceed 10% to prevent the oiling-off (the formation of visible oil at the surface of products) at room temperature (*28*). CTFMF and all SLs, except SL460 and SL451, had SFC <10% at 20 °C. Margarine fat should have SFC <3.5% at temperatures above 33 °C or should completely melt at temperatures below body temperature to eliminate waxy mouthfeel, which is one of the important textural drawbacks of margarine (*30*). CTFMF and all SLs, except SL460 and SL451, had SFCs below 3.5% at 30 °C or no SFC at 35 °C. The difference in SFC between 15 and 25 °C affects the coolness, which is the desired mouthfeel property for margarine, and the greater the difference, the greater the coolness (*31*). The differences between the SFC at 15 and that at 25 °C were SL460 (14.2%) > SL451 and SL442 (13.9%) > CTMF (10.5%) > SL433 (8.6%) > SL532 and CTFMF (7.0%) > SL621 (4.0%).

Therefore, these results suggest the possibility that all SLs, except SL460 and SL451, may be suitable for the formulation of margarines that (1) possess good spreadability at refrigeration temperature, (2) maintain a crystal structure at refrigeration temperature but almost melt at room temperature, (3) do not exhibit oiling-off phenomena, (4) do not have waxy mouthfeel, but (5) have poorer cooling mouthfeel than traditional *trans* margarine with the exception of SL442.

Polymorphism. The polymorphic forms of lipid samples crystallized at 4 °C were determined by XRD spectroscopy (**Table 6**). Three major types of polymorphs (α, β', and β) were present in all lipid samples. The α polymorph is the least thermally stable. The β polymorph, which is most stable, is unfavorable in margarine fats due to its association with grainy texture, whereas the β' polymorph, which is intermediate in stability, is responsible for the smooth texture of margarines (*32*). Thus, the predominant presence of β' over another polymorph (especially β) is necessary for imparting desirable textural properties to margarines. SLs prepared from the blends containing PKO had more β' than β polymorphs like CTMF. This is attributed to the high diversity in FA profile of the SLs as mentioned previously. The increased heterogeneity in FA composition is associated with the formation of heterogeneous and asymmetric TAG molecules, and such TAGs tend to preferentially crystallize in the β' polymorph (*19, 32, 33*).

Microstructure. Figure 3 shows the microstructural morphology of the fat crystal network of lipid samples obtained by polarized light microscopy. The fat crystals were observed as either individual crystals or crystal aggregates. In CTMF, only crystal aggregates were present, whereas both individual crystals and crystal aggregates were observed in SLs at 4 °C. The crystal aggregates in CTMF and SLs had the shape of spherulites. However, there was a distinct difference in the morphology of spherulites. Spherulites with radially oriented, orderly packed, needle-shaped crystals (spherulite type A) were found in CTMF, whereas spherulites with randomly oriented, disorderly packed, rod-shaped crystals (spherulite type B) were observed in SLs (*34, 35*). However, spherulite type A is not a typical shape of crystal aggregates from partially hydrogenated fats because the spherulite type B was also found in the fats (*36*). In CTFMF, no crystal aggregates existed and only individual crystals were present. The formation of granular crystals that impair smooth texture of margarines was also observed in CTFMF. The granular crystals are found in margarine fats containing palm oil (*37*).

The integrity of crystal aggregates in SLs became low as the temperature increased to 23 °C. In SL460, SL451, and SL442, the size of crystal aggregates (i.e., diameter of spherulites) was diminished, and a part of it disintegrated to individual crystals. In SL433 and SL532, all of the crystal aggregates lost their integrity and disintegrated to individual crystals. In SL621, the fat crystals completely disappeared, similar to CTFMF. These results were partly in accord with the findings that at 20 or 25 °C, CTFMF and all SLs except SL460 and SL451 had SFC below 7.6%, which is the minimal level for maintaining a crystal structure.

The distribution pattern of fat crystals and liquid oil differed according to the fat type. The interfacial boundary between crystal aggregates and liquid oil was much sharper in CTMF than in SLs. The diffuse interface of crystal aggregates in SLs was due to the presence of individual crystals around them. The size of crystal aggregates was diminished, and the portion of liquid oil was increased in SLs as the content of PS decreased or the content of CO increased in the blends. In SL460, SL451, and SL442, the liquid oil was entrapped within the network of crystal aggregates, which interacted with one another at 4 °C, whereas in CTMF, SL433, SL532, and SL621, the crystal aggregates were dispersed in the continuous liquid oil phase at the same temperature. The interaction phenomena between crystal aggregates were also observed in SL460 and SL451 at 23 °C. The interaction between crystal aggregates in SLs led to a stronger fat crystal network (microscopic structure) and substantially influenced their textural properties (macroscopic attributes) as described below (*38, 39*).

Textural Property. Textural properties (hardness, adhesiveness, and cohesiveness) of experimental margarine samples formulated with commercial margarine fats or SLs were evaluated by TPA (**Figure 4**). The hardness of margarine samples is shown in **Figure 4A**. SL532 margarine was slightly harder than CTMF margarine at 4 °C, but the difference was not significant ($p > 0.05$). SL532 margarine had hardness similar ($p > 0.05$) to that of CTMF margarine at 23 °C. SL621 margarine was slightly softer than CTFMF margarine at 4 °C, but the difference was not significant ($p > 0.05$). The hardness

of SL621 margarine was similar ($p > 0.05$) to that of CTFMF margarine at 23 °C.

The hardness of margarine fats is preferentially affected by their SFC. However, SFC is not a single factor to be correlated to the hardness. For example, although CTMF (15.3−10.6%) and SL451 (15.3−9.9%) have similar SFC at 20−25 °C, SL451 margarine is approximately 2.6 times harder than CTMF margarine at 23 °C. Likewise, CTMF and SL532 margarines have similar hardness at 4 °C despite lower SFC for SL532 (21.2%) than for CTMF (26.0%) at 5 °C (**Figures 2** and **4A**). Greater hardness of margarines formulated with SLs compared to CTMF margarine at the same level of SFC was attributed to the formation of stronger fat crystal network in SLs compared to CTMF. The composition of continuous medium, which connects the dispersed crystal aggregates, influences the strength of the fat crystal network and hence the macroscopic properties of the fats (*39*). In CTMF, the continuous phase consisted of only liquid oil, whereas it consisted of liquid oil and solid material of lower melting TAGs (i.e., individual crystals found around crystal aggregates) in SLs (**Figure 3**). The liquid oil type continuous phase is called a liquid bridge (link), and the continuous phase consisting of liquid oil and solid material is called a semisolid bridge. The semisolid bridge forms a stronger fat crystal network compared to the liquid bridge (*39*). The semisolid bridge was more clearly observed in SLs synthesized from the blends containing more PS or less CO.

The adhesiveness of margarine samples is shown in **Figure 4B**. CTMF, CTFMF, SL532, and SL621 margarines had similar ($p > 0.05$) adhesiveness at 4 °C. The adhesiveness of SL532 and SL621 margarines was not significantly ($p > 0.05$) different from that of CTMF and CTFMF margarines, respectively, at 23 °C.

The cohesiveness of margarine samples is shown in **Figure 4C**. The SL532 and SL621 margarines were similar ($p > 0.05$) in cohesiveness to one another and also similar ($p > 0.05$) to CTFMF margarine but had higher ($p < 0.05$) cohesiveness than CTMF margarine at 4 °C. However, CTMF, CTFMF, SL532, and SL621 margarines had similar ($p > 0.05$) cohesiveness to one another at 23 °C.

Therefore, these results indicate that among the six kinds of margarine samples formulated with SLs, the margarines prepared with SL532 and SL621 were most similar in textural properties to margarines prepared with CTMF and CTFMF, respectively.

In conclusion, CO/PS/PKO-based SLs synthesized in the present study were *trans*-free. SL532 has a similar or lower AI value compared to CTMF, and SL621 has a similar AI value to CTFMF. The β' crystal polymorphic form, which is desired in margarine fats, was predominantly present in the SLs. Although all SLs have different melting and crystallization properties, SFC, and microstructure compared to CTMF or CTFMF, the margarines prepared with SL532 and SL621 were similar in textural properties, such as hardness, adhesiveness, or cohesiveness, to margarines made with CTMF and CTFMF, respectively. Therefore, SL532 and SL621 were considered to be most suitable for the formulation of *trans*-free margarines that have low atherogenicity and desirable textural properties.

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