Chemical and Physical Properties of the Solid Fats in Commercial Soft Margarines[1]

V. D'Souza, J.M. deMan* and L. deMan[2]
Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

The fats of ten Canadian soft margarines were crystallized from acetone at 15°C to obtain the high-melting glycerides (HMG). The solid fats in the margarines were extracted with isobutanol at 5°C. X-ray diffraction showed that the canola margarines were in the β crystal form, the soybean and sunflower-palm-palm kernel margarines in the β′ form, while those of canola-palm and another sunflower-palm-palm kernel margarines contained a mixture of β′ and β forms. X-ray diffraction of the isolated solids showed additional short spacings compared with those of the original margarines. Differential scanning calorimetry heating curves of the solids were compared with those of the HMG. The melting temperatures of the HMG were 10°C higher than the solids. It is suggested that the polymorphic behavior of soft margarines is related to the chemical composition of the HMG and the solids. Solids in margarines can also be provided by interesterification of palm oil products.

KEY WORDS: Fatty acids, fractionation, high-melting glycerides, palm oil products, physical properties, polymorphism, soft margarines, triglycerides.

Margarines in North America are generally produced from vegetable oils that have been modified by partial hydrogenation. During the hydrogenation process, positional and *trans* isomers are formed (1-3). Formation of *trans* isomers influences the chemical and physical characteristics of the final product because *trans* isomers have higher melting points than the corresponding *cis* fatty acid (4). Consumption of *trans* fatty acids may have an adverse effect on health (5). Hence, one of the alternatives for partial hydrogenation for the production of plastic fats is interesterification.

Interesterification involves rearrangement of fatty acids within and between triglycerides. The fatty acids on the glycerol molecule are rearranged in a random or directed manner depending on conditions used. This results in the formation of new triglycerides that do not exist in the original fat. In random interesterification, the fatty acids are distributed in a statistically random manner. Directed interesterification is carried out at low temperatures and form high-melting glycerides that crystallize from the reaction mixture. This does not occur in random interesterification (6-8).

The interesterification reaction of triglycerides has been extensively reviewed (6-8). It has been used for several years to modify the physical characteristics of lard. Chobanov and Chobanova (9) interesterified sunflower oil with completely hydrogenated lard and tallow. List and co-workers (10) interesterified sunflower oil with fully hydrogenated soybean oil to produce a plastic fat suitable for use in margarines. Lo and Handel (4) interesterified soybean oil with beef tallow to produce a plastic fat suitable for producing tub-type margarines. The possible use of hydrogenated, fractionated or interesterified milkfat in chocolate was investigated by Timms and Parekh (11). Hernqvist *et al.* (12) studied the polymorphic behavior of complex glycerides formed by interesterification of simple triglycerides, such as triolein, trielaidin and tristearin. These triglycerides were chosen as a model system for vegetable oils, so that they can be used in the manufacture of margarines. Lago and Hartman (13) investigated the fatty acid and triglyceride composition of interesterified Brazilian oil and its liquid and solid fractions. There is no published information regarding the composition and physical characteristics of the high-melting glycerides of these interesterified products. From the chemical composition of the high-melting glycerides, it is possible to predict the polymorphic behavior of margarines during storage (14). This study was undertaken to compare the chemical and physical characteristics of the high-melting glycerides (HMG) with those of the solids separated by isobutanol from soft margarines, with the objective to explain differences observed in polymorphic behaviors of various soft margarines.

MATERIALS AND METHODS

Samples were purchased from supermarkets in the provinces of Ontario and Quebec. All of the samples were transported in coolers containing ice and stored at refrigerator temperature (4°C) thereafter.

Solid fat contents (SFC) of the samples were measured by pulsed nuclear magnetic resonance (pNMR) in a Bruker PC/20 series NMR analyzer (Minispec, Milton, Canada, L9T 1Y6). NMR tubes were filled with margarine samples by means of a stainless-steel sampling tube with tight fitting plunger and kept overnight at 5°C (15). SFC measurements were then made at 5°C.

The solids were extracted with isobutanol at 5°C. About 2 g of sample was weighed into the glass tube of a pestle tissue grinder to which 12 mL of isobutanol was added. The crystals were dispersed by moving the tube up and down and rotating the tube for 3 min at low speed (200 rpm) and 2 min at high speed (400 rpm). The contents were transferred to a 50-mL stoppered tube. The tissue grinder was rinsed with 3 × 5 mL of isobutanol by rotating the tube and pestle. The crystal suspension was agitated on a vortex mixer and filtered through a 0.45-μm Magna nylon filter paper supported on a sintered Millipore glass filter (Millipore, Milford, MA). The stoppered tube and the filter were rinsed twice with 5 mL of isobutanol. Extraction and filtering were performed in a cold room. The solids were dried at room temperature in a vacuum oven. The yield of solids was recorded. Impurities in the solids, resulting from skim milk or whey powder in the margarines, were removed by dissolving the solids

[1]Presented at the Annual Meeting, Canadian section of AOCS, October 18-19, 1990, Toronto, Canada.

*To whom correspondence should be addressed.

[2]deMan Food Technology Services Inc., Guelph, Ontario N1H 6B5, Canada.

in chloroform and filtering through 0.45-μm Magna nylon filter paper.

The HMG were obtained as follows. The fat was isolated by melting part of the margarines in the oven. After removal of the water layer, the fat was filtered and dried. The fat was melted in the oven and dissolved in acetone at a ratio of one part by weight of fat to twenty parts by volume of solvent. The solution was left overnight in a waterbath to crystallize at 15°C. The fat crystals were filtered on a sintered glass filter with 0.45μm Magna nylon filter paper. The solvent was removed from the crystals under vacuum.

Fatty acid and triglyceride compositions were determined by gas-liquid chromatography (16). The polymorphic forms in the original product and the solids obtained from isobutanol were established by X-ray diffraction with a Model FR 552 camera (Enraf Nonius, Delft, The Netherlands), which was operated at 10°C for the margarines and at room temperature for the solids. The instrument was fitted with a fine-focus copper X-ray tube. The sample holders were flat stainless-steel plates (1 mm thick) with a rectangular hole. The samples were contained in this space with adhesive tape. The X-ray film was scanned with a Zeineh soft-laser scanning densitometer model SLR-504 XL (Biomed Instruments Inc., Fullerton, CA). Short spacings on the X-ray film were measured with a Guinier viewer (Enraf-Nonius, Delft, The Netherlands) (14).

Differential scanning calorimetry (DSC) was used to determine the melting profile of the high-melting glycerides obtained at 15°C (14). Approximately 8 to 10 mg of sample (HMG) was weighed in DSC pans. The pans were placed in closed aluminum dishes and left in a 70°C oven for 15 min. The fat was crystallized by placing the dishes in a freezing cabinet at −16°C for 1 h before transferring the pans to the DSC unit for scanning. Heating curves were recorded from 15 to 70°C at a heating rate of 5°C/min. The same heating program was used for the solids after isobutanol separation and drying in the vacuum oven.

RESULTS AND DISCUSSION

Samples A, B, C and D were manufactured from soybean oil, E and F from canola oil only, G and H from canola-palm, and I and J from sunflower-palm-palm kernel. These samples had been previously analyzed by deMan *et al.* (17) for their physical and textural attributes. The palmitic acid levels in the original product, the HMG and the solids extracted with isobutanol are shown in Table 1. Vegetable oils contain low levels of palmitoleic (16:1). Therefore, upon hydrogenation, the level of 16:0 remains almost the same. Canola margarines can be distinguished from soybean margarines by their palmitic acid contents (Table 1). Canola margarines contain 4.5% palmitic acid (16), whereas soybean margarine contains 10.5% (18). The fatty acid profiles of the original products are shown in Figure 1. The

TABLE 1

Palmitic Acid Content (%) in the Original Fats, High-Melting Glycerides (HMG) and the Solids

	16:0 (%)		
Product	Original	HMG 15°C (acetone)	Solids at 5°C (isobutanol)
A	10.0	14.5	14.6
B	9.5	14.0	13.9
C	9.7	14.4	14.6
D	10.3	15.8	16.0
E	4.4	7.6	7.1
F	4.1	6.2	6.1
G	5.9	14.7	14.2
H	5.7	15.5	13.7
I	9.2	32.8	26.2
J	12.2	74.1	52.9

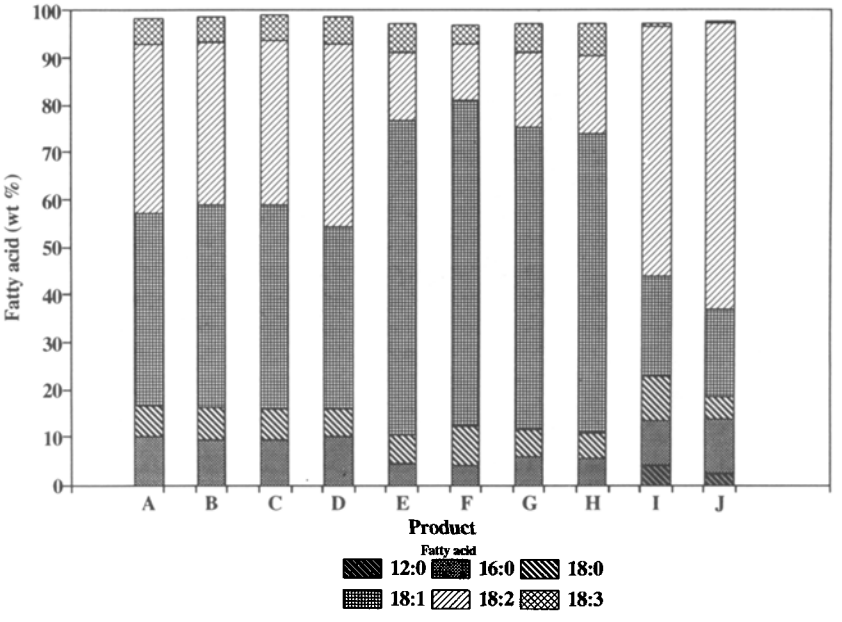

FIG. 1. Fatty acid profiles of the fats in the original soft margarines.

soybean margarines (A to D) had almost equal amounts of 18:1 and 18:2, whereas the canola margarines (E to H) had high levels of 18:1. Palm oil has a palmitic acid content of 44%. Additions of palm oil to canola oil products G and H did not change the palmitic acid contents to a large extent as compared with canola-only products E and F, indicating that only a small amount of palm oil was incorporated (approximately 5%). In addition to the long-chain fatty acids, products I and J also showed the presence of 12:0 and 14:0. These fatty acids are the result of incorporation of palm kernel oil.

The fatty acid profiles of the HMG and the solids extracted with isobutanol are shown in Figures 2 and 3, respectively. The HMG and the solids contained mainly 16:0, 18:0 and 18:1. The 16:0 levels in the HMG and the solids were almost identical except for the HMG of products I and J, which were higher in the HMG than in the solids (Table 1). The difference in 16:0 content between the HMG and the original margarines of samples I and J was large, especially for sample J (Table 1). The 12:0 content in samples I and J was higher in the solids than in the HMG. The 16:0 contents in the HMG and the solids were

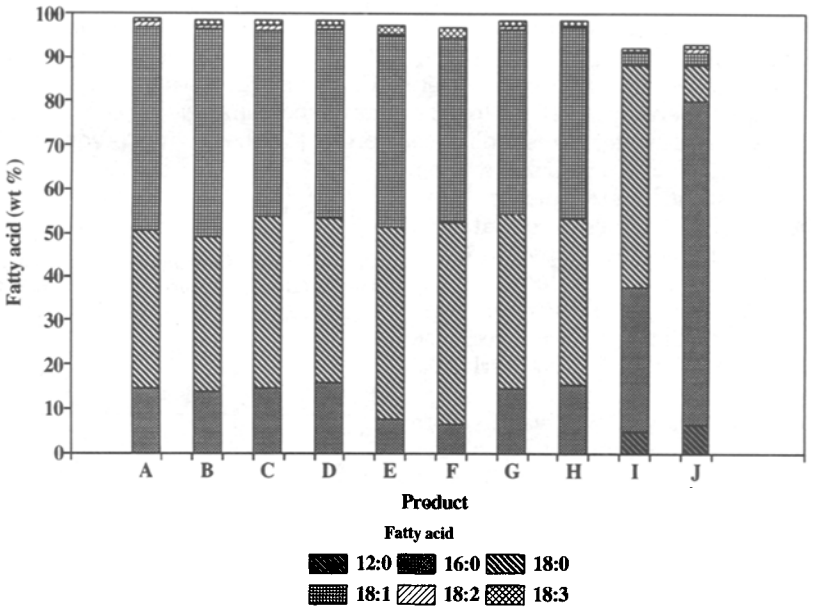

FIG. 2. Fatty acid profiles of the high-melting glycerides from soft margarines at 15°C. (Difference between total fatty acids listed and 100% is represented by 14:0.)

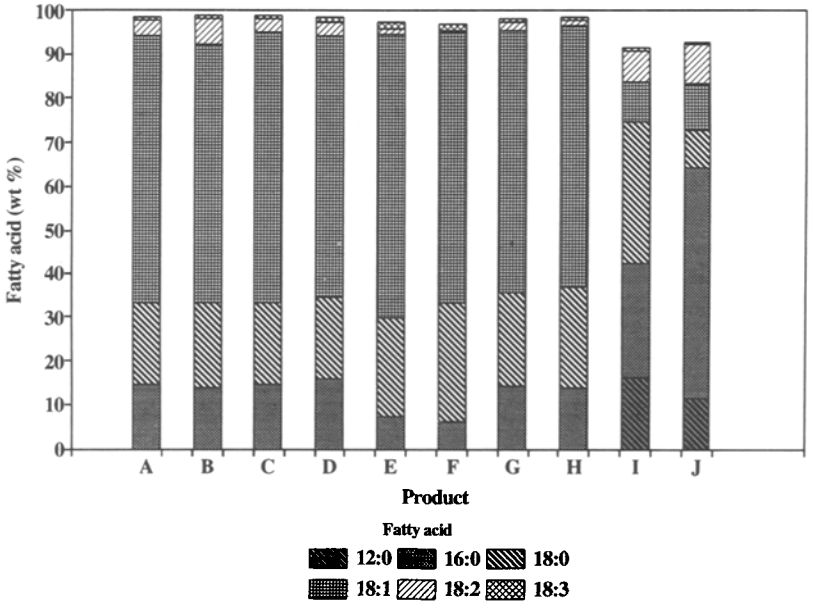

FIG. 3. Fatty acid profiles of solids from soft margarines at 5°C. (Difference between total fatty acids listed and 100% is represented by 14:0.)

lowest in the canola margarine. The 18:0 levels in the HMG (Fig. 2) ranged from 8.3 to 51.0%, whereas in the solids (Fig. 3) it ranged from 8.5 to 32.0%. The 18:1 levels were higher in the solids than in the HMG. The 18:2 levels in the solids ranged from 0.7 to 9.1%, whereas in the HMG they were present in trace amounts.

The yields of HMG and of solids extracted with isobutanol are shown in Figure 4. The yields of the solids were higher than those of the HMG, because the HMG represents only a part of the solids. Products I and J had the lowest yield due to a lower solids content. The solids obtained with isobutanol were compared with the solid fat content at the same temperature (5°C) by pNMR. The yield of the solids was slightly higher than the solids content determined by pNMR (Fig. 5).

The triglyceride composition by carbon number of the original products is tabulated in Table 2. The triglyceride composition was diverse in the sunflower-palm-palm kernel margarines, followed by the soybean and canola-palm margarines, and least diverse in the canola margarines. The triglyceride compositions of HMG and solids are shown in Tables 3 and 4, respectively. Comparison

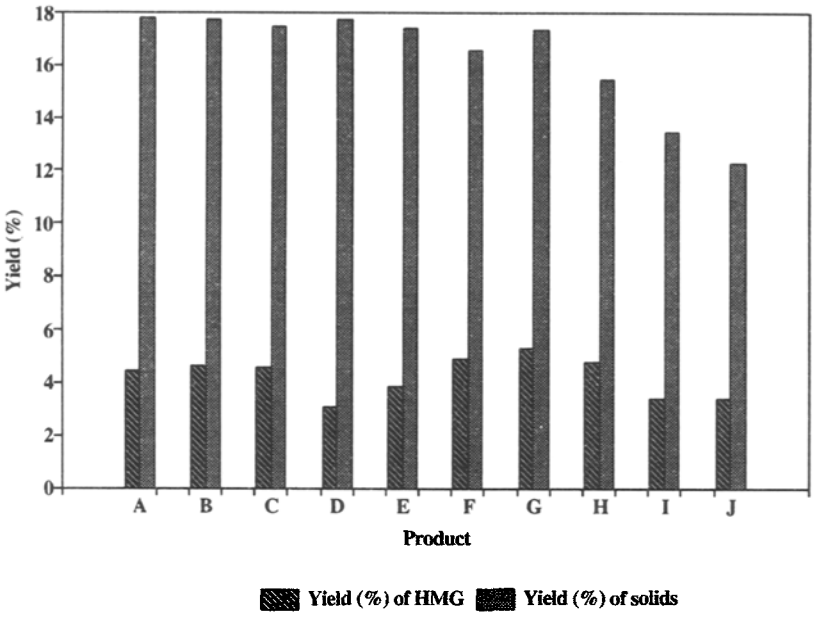

FIG. 4. Comparison of high-melting glycerides (HMG) yields (15°C) with solids (5°C).

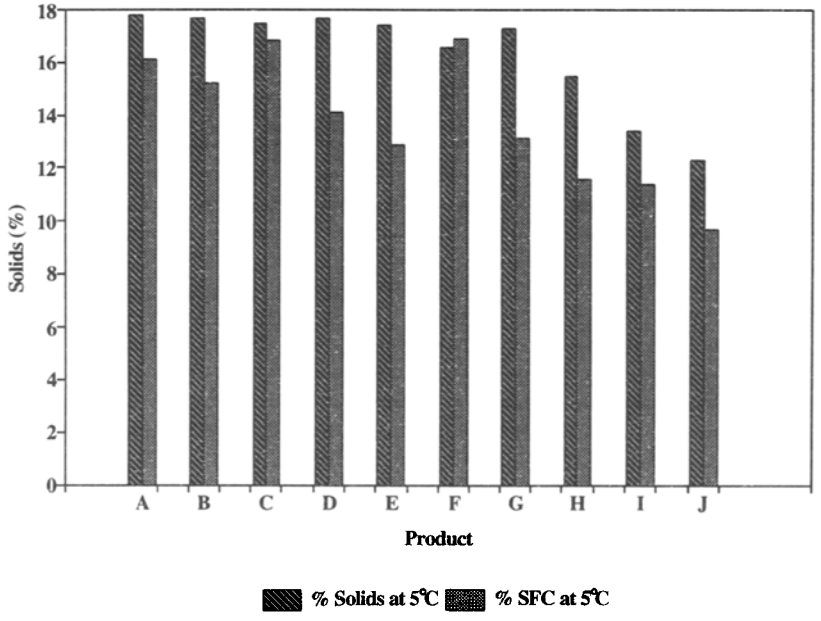

FIG. 5. Comparison of solids (5°C) with solid fat contents (SFC) (5°C) by nuclear magnetic resonance.

TABLE 2

Triglyceride Carbon Number Distributions of Soft Margarines

Product	Triglyceride carbon number												
	36	38	40	42	44	46	48	50	52	54	56	58	60
A	—	—	—	—	—	—	—	3.5	27.9	65.6	1.9	0.9	0.2
B	—	—	—	—	—	—	—	3.8	26.5	65.0	3.2	1.3	0.4
C	—	—	—	—	—	—	—	3.5	27.1	66.3	2.1	0.7	0.2
D	—	—	—	—	—	—	—	4.4	28.9	63.8	2.1	0.8	0.1
E	—	—	—	—	—	—	—	2.1	15.3	75.1	5.1	0.8	0.9
F	—	—	—	—	—	—	—	2.0	14.3	74.7	5.8	1.8	0.9
G	—	—	—	—	—	—	—	3.0	20.4	70.9	3.9	1.2	0.9
H	—	—	—	—	—	—	0.8	2.3	16.5	71.8	6.0	1.9	0.9
I	—	3.8	3.0	3.2	3.3	2.8	3.0	3.5	17.0	55.5	3.4	1.2	0.5
J	0.7	4.3	2.8	2.0	5.1	3.4	4.0	4.2	16.9	57.3	trace	trace	trace

TABLE 3

Triglyceride Carbon Number Distributions of High-Melting Glycerides from Soft Margarines

Product	Triglyceride carbon number												
	36	38	40	42	44	46	48	50	52	54	56	58	60
A	—	—	—	—	—	—	—	5.4	34.0	58.4	1.5	0.7	trace
B	—	—	—	—	—	—	—	5.1	33.9	56.8	3.0	1.1	trace
C	—	—	—	—	—	—	—	5.7	33.8	56.6	2.8	0.9	trace
D	—	—	—	—	—	—	—	7.4	36.3	53.0	2.6	0.5	trace
E	—	—	—	—	—	—	—	2.2	18.7	68.4	7.1	2.2	1.1
F	—	—	—	—	—	—	—	1.6	16.7	71.3	6.9	2.1	1.1
G	—	—	—	—	—	—	0.7	6.1	33.0	56.9	2.6	1.0	trace
H	—	—	—	—	—	—	0.9	6.0	32.7	59.0	0.6	0.5	trace
I	—	—	—	0.6	2.6	9.3	19.8	25.7	26.9	14.4	0.6	—	—
J	—	—	—	1.9	17.5	16.5	42.7	15.4	4.0	1.1	—	—	—

TABLE 4

Triglyceride Carbon Number Distributions of Solids from Soft Margarines

Product	Triglyceride carbon number												
	36	38	40	42	44	46	48	50	52	54	56	58	60
A	—	—	—	—	—	—	—	6.2	34.1	56.6	2.2	0.7	trace
B	—	—	—	—	—	—	—	5.4	33.1	58.9	1.9	0.6	trace
C	—	—	—	—	—	—	—	8.3	35.1	54.8	1.7	trace	trace
D	—	—	—	—	—	—	—	6.8	35.4	55.2	1.7	0.6	trace
E	—	—	—	—	—	—	—	2.2	18.0	71.7	5.4	1.5	1.1
F	—	—	—	—	—	—	—	1.7	16.4	73.1	5.8	1.7	1.1
G	—	—	—	—	—	—	0.5	5.6	32.4	58.3	1.9	0.6	trace
H	—	—	—	—	—	—	0.6	5.3	30.9	59.7	2.1	0.7	trace
I	—	—	6.2	10.7	11.6	15.2	14.0	11.0	14.9	0.8	0.4	—	—
J	—	—	5.2	6.0	19.8	13.1	19.2	15.9	10.5	9.3	0.6	0.4	—

between the triglyceride composition of the HMG and the solids showed no major differences (products A to H) with respect to carbon numbers 50, 52 and 54. Soybean margarines (A to D) contained higher levels of carbon 50 and 52 than the canola margarines (E and H), which were high in carbon 54. Addition of palm oil to products G and H increased the C50 and C52 levels. The carbon 54 content was lower in the HMG and the solids than in the original margarines, while the levels of carbon 50 and 52 were higher in the HMG and the solids. The HMG consisted mainly of 16:0, 18:0 and 18:1 fatty acids (Fig. 2). In a similar study on stick margarines, D'Souza *et al.* (14) analyzed the HMG for *trans* content and found that most of the 18:1 fatty acids were in the *trans* isomeric form. Therefore, the carbon 54 triglycerides consisted mainly of stearic and *trans* oleic acids. These triglycerides crystallize in the β form. The conclusion of the stick margarine study (14) was, that for a stick margarine to be in the β' form, the carbon 54 triglyceride content should not exceed 50%. In the present soft margarine study none of samples A to H could meet this target. At the same time, none of the solids of samples A to H as analyzed by X-ray diffraction (Table 5) was completely in the β' form. Palm oil decreased the C54 level in the HMG and solids

TABLE 5

Polymorphic Forms in Soft Margarines and the Solids Obtained at 5°C with Isobutanol as the Solvent

Product	Polymorphic form in the original product	Polymorphic form of the solids extracted with isobutanol
A	β'	$\beta' >>> \beta$
B	β'	$\beta' >> \beta$
C	β'	$\beta' >> \beta$
D	β'	$\beta' >>> \beta$
E	β	β
F	β	β
G	$\beta' >>> \beta$	$\beta' = \beta$
H	$\beta' = \beta$	$\beta' = \beta$
I	β'	β'
J	$\beta' = \beta$	$\beta' = \beta$

of samples G and F (Tables 3 and 4) as compared with canola-only samples E and F, thereby increasing the β' content in samples G and F (Table 5). Yap *et al.* (19) have demonstrated that hydrogenated palm oil resulted in a more stable product than unhydrogenated palm oil.

The triglyceride composition of the HMG from the canola soft margarines (E and F) was identical to the triglyceride composition of HMG from stick margarines analyzed by D'Souza *et al.* (14). Compared with the HMG from hydrogenated canola oil analyzed by D'Souza *et al.* (20), the HMG of canola soft margarine (E) corresponded to the triglyceride composition of HMG 55 (HMG 55 refers to the high-melting glycerides crystallized from acetone from the sample removed after 55 min of hydrogenation). The triglyceride composition of product F was between the triglyceride compositions of HMG 65 and 75.

The fatty acid compositions of the HMG and solids of sample I and J were unique (Figs. 2 and 3, Tables 3 and 4). In the first place, the 18:1 and 18:2 content was low, and the 16:0 content was high compared to those of original margarines I and J (Fig. 1). Secondly, the 16:0 contents of the solids and HMG were also high compared with those of all other margarines (Table 1). The solids were obviously derived from palm oil products, such as fractionated palm oil and palm kernel oil. Fatty acid and triglyceride compositions of these products are shown in Table 6. The 16:0 content of the HMG of sample J was much higher than that of I, the reverse was true for the 18:0 content. Obviously, different palm oil products were used in these samples. Palm stearin, for instance, can be fractionated to a higher degree to yield a stearin of a higher 16:0 content. Palm kernel oil can be hydrogenated to yield a higher 18:0 content. Judging from the triglyceride content of the palm products (Table 6) and from those of the HMG and the solids (Tables 3 and 4), it can be concluded that these products were interesterified because the carbon numbers of the triglycerides were more evenly distributed in the HMG and the solids than in either palm stearin or palm kernel oil (Table 6).

It is interesting that the solids contained more of the lower-carbon triglycerides (Table 4) than the HMG (Table 3). The lower-carbon triglycerides will solidify only at low temperatures. In this way, an SFC curve is obtained that is steeper than when fats of a narrow range of carbon number triglycerides are interesterified. The latter procedure would result in a flat SFC curve.

TABLE 6

Fatty Acid and Triglyceride Compositions (%) of Fractionated Palm Stearin and Palm Kernel Oil[a]

	Palm stearin	Palm kernel oil
Fatty acid		
8:0		4.4
10:0		3.7
12:0		48.3
14:0	1.3	15.6
16:0	54.0	7.8
18:0	4.7	2.0
18:1	32.3	15.1
18:2	7.0	2.7
Triglyceride		
C32		6.3
C34		8.4
C36		23.3
C38		17.0
C40		9.8
C42		9.1
C44		9.6
C46	2.2	5.1
C48	25.1	5.8
C50	39.1	2.3
C52	26.5	2.2
C54	7.1	2.4

[a]References 21, 22.

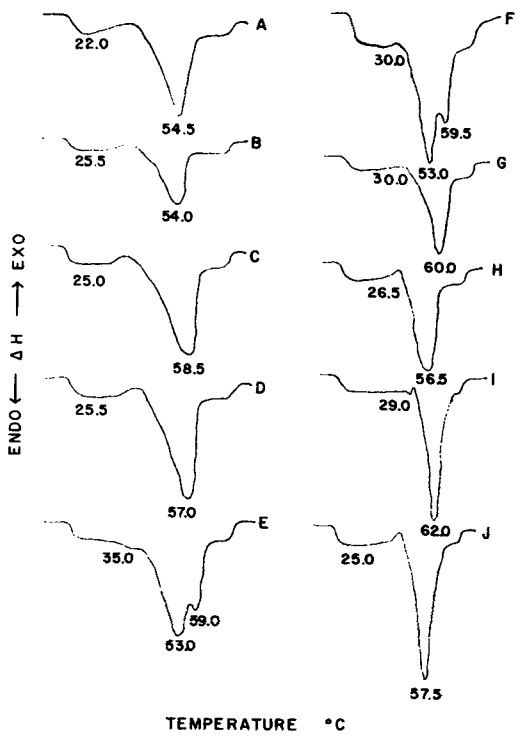

FIG. 6. Differential scanning calorimetry heating curves (5°C/min) of high-melting glycerides from soft margarines at 15°C. A) soybean, B) soybean, C) soybean, D) soybean, E) canola, F) canola, G) canola-palm, H) canola-palm, I) sunflower-palm-palm kernel, J) sunflower-palm-palm kernel.

Figure 6 shows the melting profile of the HMG from soft margarines. The melting temperatures of the major endothermic peak for soybean margarines (A to D) ranged from 54 to 58°C. Canola soft margarines (E and F) showed

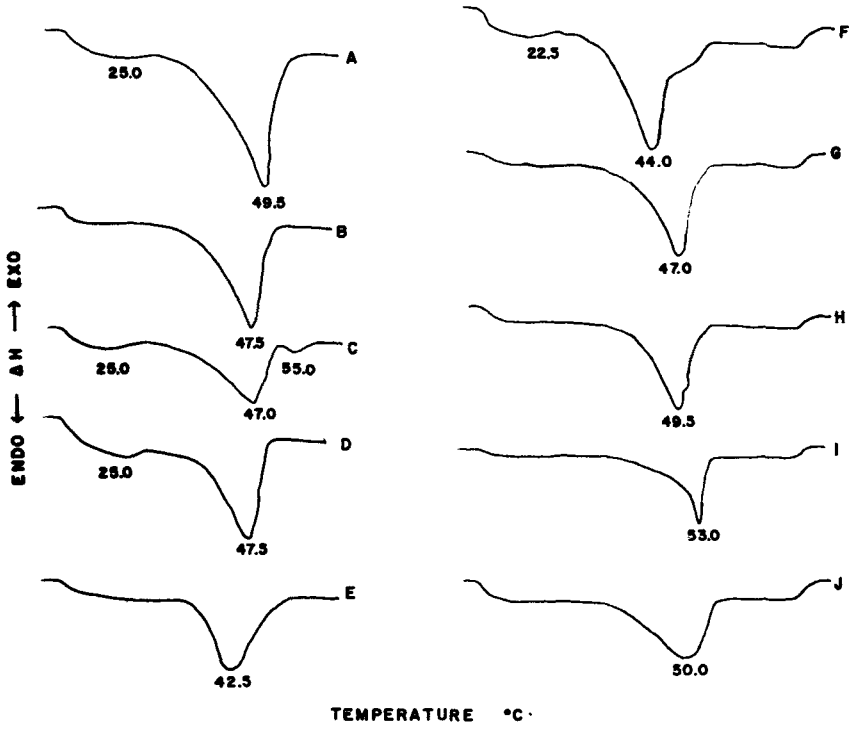

FIG. 7. Differential scanning colorimetry heating curves (5°C/min) of the solids from soft margarines at 5°C: A) soybean, B) soybean, C) soybean, D) soybean, E) canola, F) canola, G) canola-palm, H) canola-palm, I) sunflower-palm-palm kernel, J) sunflower-palm-palm kernel.

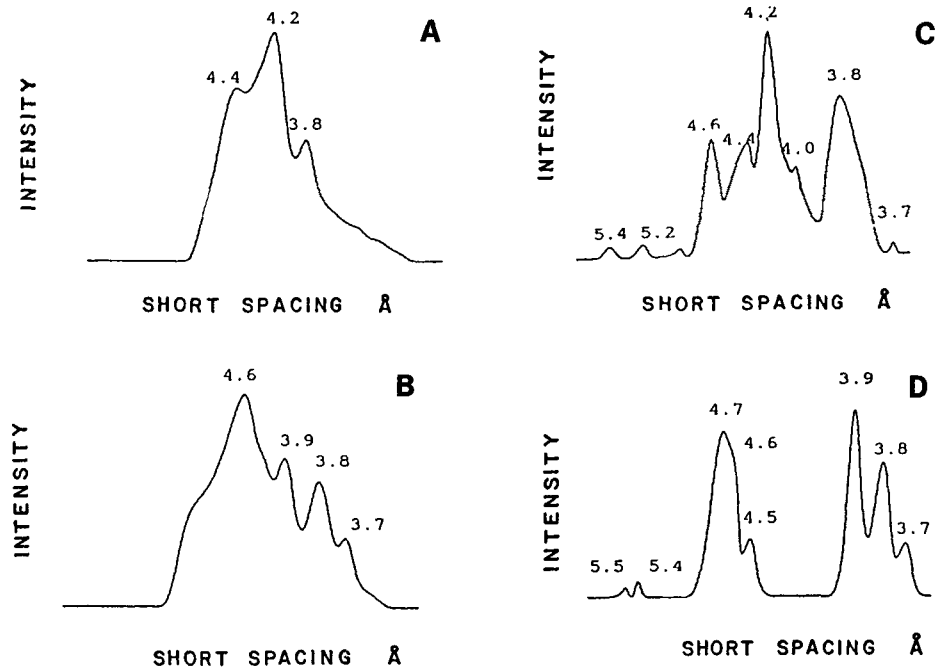

FIG. 8. Typical laser densitometer scans of X-ray film of the original fats and the solids: A) original fat of product B, B) original fat of product F, C) solids of product B, D) solids of product F.

a peak around 55°C and a shoulder peak at 60°C. A separate endothermic peak does not necessarily indicate a polymorphic transition (20). It may indicate a sub form of the major polymorphic form. Addition of palm oil to products G and H resulted in the disappearance of the shoulder peak. The melting temperature of the major endothermic peak of product I was 5°C higher than for product J. This difference may be attributed to the relatively higher levels of carbon 54 (14.4%) in the HMG of product I as compared with 1.1% in product J (Table 3). The melting profiles for the solids obtained with isobutanol are shown in Figure 7. Overall the melting temperatures of the solids were about 10°C lower than those of the HMG. The melting peaks of the solids were wider than those of the HMG. The lower melting temperatures for the solids can be attributed to the high levels of 18:1, part of which may be in the *cis* form, and the presence of 18:2 fatty acid.

The polymorphic behaviors of the original product and the solids extracted with isobutanol were investigated by X-ray diffraction analysis. X-ray analysis of the solids showed more diffraction lines than the original product (Fig. 8). Crystal concentration in the soft margarines was low while that of the isolated solids was high. Hence more detail of the diffraction pattern was shown by the solids. A typical laser scan of the original products (B and F) and solids (B and F) is also shown in Figure 8. Product B (Fig. 8A) displayed β' characteristics because of the short spacings at 3.8 and 4.2 Å, whereas product F (Fig. 8B) showed β crystals as indicated by the strong line at 4.6 Å. Solids extracted with isobutanol showed (Fig. 8, C and D) short spacings corresponding to the major polymorphic forms (β' and β) as well as several intermediate forms. Polymorphic forms of the original product and the solids extracted with isobutanol are tabulated in Table 5. All of the soybean margarines showed the presence of β crystals.

Results of this study indicate that the solids in margarines can be separated by isobutanol extraction of the liquid phase. Advantages are that the solids can be analyzed in more detail chemically as well as physically.

REFERENCES

1. Sahasrabudhe, M.R., and C.J. Kurian, *J. Inst. Can. Sci. Technol. 12*:140 (1979).
2. Carpenter, D.L., and H.T. Slover, *J. Am. Oil Chem. Soc. 50*:372 (1976).
3. Nazir, D.J., B.J. Moorecroft and M.A. Mishkel, *Am. J. Clin. Nutr. 29*:331 (1976).
4. Lo, Y.C., and A.P. Handel, *J. Am. Oil Chem. Soc. 60*:815 (1983).
5. Mensink, K.R., and M.B. Katan., *New Engl. J. Med. 323*:439 (1990).
6. Going, L.H., *J. Am. Oil Chem. Soc. 44*:414A (1967).
7. Sreenivasan, B., *Ibid. 55*:796 (1978).
8. Hustedt, H.H., *Ibid. 53*:390 (1976).
9. Chobanov, D., and R. Chobanova, *Ibid. 54*:47 (1977).
10. List, G.R., E.A. Emken, W.F. Kwolek, T.D. Simpson and H.J. Dutton, *Ibid. 54*:408 (1977).
11. Timms, R.E., and J.V. Parekh, *Lebensm. Wiss. U. Technol. 13*:177 (1980).
12. Hernqvist, L., B. Herslof and M. Herslof, *Fette, Seifen, Anstrichm. 86*:393 (1984).
13. Lago, C.A., and L. Hartman, *J. Sci. Food Agric. 37*:689 (1986).
14. D'Souza, V., L. deMan and J.M. deMan, *J. Am. Oil Chem. Soc. 68*:153 (1991).
15. Chawla, P., and J.M. deMan, *Ibid. 67*:329 (1990).
16. Shehata, A.A.Y., J.M. deMan and J.C. Alexander, *Can. Inst. Food Sci. Technol. J. 3*:85 (1970).
17. deMan, L., C.F. Shen and J.M. deMan, *J. Am. Oil Chem. Soc. 68*:70 (1991).
18. Vaisey-Genser, M., and N.A.M. Eskin, *Canola Oil: Properties and Performance*, Canola Council of Canada, Winnipeg, Manitoba, 1982, pp. 1-39.
19. Yap, P.H., J.M. deMan and L. deMan, *J. Am. Oil Chem. Soc. 66*:1784 (1989).
20. D'Souza, V., L. deMan and J.M. deMan, *Ibid. 68*:907 (1991).
21. Rossell, J.B., B. King and M.J. Downes, *Ibid. 62*:221 (1985).
22. Pantzaris, T.P., *Palm Oil Development 15*:15, Porim Publication, Kuala Lumpur, Malaysia.

[Received July 24, 1992; accepted September 23, 1992]