Polymorphic Behavior of High-Melting Glycerides from Hydrogenated Canola Oil[1]

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Canola oil was hydrogenated with a commercial nickel catalyst at 175°C and 15 psi hydrogen pressure. Samples were taken during the reaction starting at 15 min and thereafter at ten-minute intervals. The reaction was stopped after two hours. The high-melting glycerides (HMG) were obtained by fractional crystallization at 15°C with acetone as solvent. The HMG were analyzed for fatty acid and triglyceride composition by gas liquid chromatography and *trans* was determined by infrared spectroscopy. In the first 45 min of hydrogenation of canola oil, the 18:0 fatty acid increased at a low rate while the *trans* fatty acid content increased at a much faster rate. The 16:0 and 18:0 content of the HMG was highest and *trans* content the lowest during the period in which the triglyceride composition was the most diverse. The 54-carbon triglyceride content of the HMG increased from 64% to 78% during the two hours of hydrogenation. The short spacings for the HMG showed the presence of β crystals as well as several intermediate forms. The number of short-spacings increased with hydrogenation time. The differential scanning calorimetry (DSC) melting profile of the HMG showed one broad peak between 20 and 30°C and two peaks around 60°C and above. Crystallization temperatures of the HMG were in the range of 40–45°C.

KEY WORDS: Canola oil, high melting glycerides, hydrogenation, polymorphism.

Polymorphism is a well-known phenomenon associated with the crystallization of long-chain compounds. Fats can crystallize into sub α, α, β′ and β crystalline forms (1). The rheological and other physical properties of products such as margarine and shortening depend on the particular polymorphic form of the triglycerides present. In the manufacture of margarine, one strives to achieve crystallization in the β′ form and not the β form. The latter can easily grow in size resulting in an unacceptable product.

In Canada, canola oil is widely used in the manufacture of margarine, shortening and salad oil (2). Canola oil has a homogeneous fatty acid composition containing 95% of 18-carbon fatty acids. As a result, the β′ to β transition is rapid (3–6). Naguib-Mostafa and deMan (7) have shown that selectively hydrogenated canola oil with an iodine value of 70 is more stable in the β′ form than other samples obtained under nonselective conditions. The β′ stability was attributed to the *trans* isomers formed, which may sterically hinder the transformation to the β structure. deMan *et al.* (8) have shown that nonselective hydrogenation of canola results in high levels of trisaturates, which accelerate the β transformation.

When a fat is cooled, the high-melting glycerides (HMG) crystallize first. The composition of the HMG of a fat dictates the polymorphic form in which the solids will crystallize, and their future behavior on storage. In a related study (9) we investigated the chemical composition and the polymorphic behavior of the HMG obtained at different temperatures from commercial margarine with acetone as the solvent. The HMG contained higher levels of saturated fatty acids (16:0 and 18:0) and *trans* isomers than the original product. In the 15°C HMG of the β-tending margarine, the 16:0 content was less than 12%, whereas the 16:0 content of the β′-tending margarine was over 17%. The 54-carbon triglyceride content in the 15°C HMG of the β′-tending margarine was about 50%, whereas that of the β-tending margarine was 70%. The polymorphic behavior of the HMG was far more complex than that of the original product. This study was undertaken to investigate the chemical composition and the physical characteristics of the HMG of canola oil that was hydrogenated to different levels.

MATERIALS AND METHODS

Canola oil was industrially refined and bleached. Hydrogenation was carried out in a Parr Pressure reaction apparatus, series 4500, with a 2-L bomb and a charge of 1 L oil. The oil was hydrogenated at 175°C and 15 psi hydrogen pressure. A commercial nickel catalyst (Nysosel 222, Harshaw, Cleveland, OH) was used at a level of 0.1% by weight of the oil. The catalyst contained 22% nickel. Samples were taken during the reaction, starting at 15 min and thereafter at ten-minute intervals. The reaction was stopped after two hours. Samples were filtered to remove the catalyst before analysis. Iodine values were determined by the Wijs method (AOCS Cd 1-25).

Isolation of HMG. Fat was melted in the oven and then dissolved in acetone in a ratio of 1 part by weight of fat to 20 parts by volume of solvent. The solution was left overnight to crystallize in a water bath at 15°C. The fat crystals were filtered on a 0.45 μm Magna Nylon 66 filter paper, which was supported on a fritted glass vacuum filter. The yield of solids was recorded. They were labelled according to the time of removal from the hydrogenator, *e.g.*, HMG 25 refers to the high-melting glycerides crystallized from acetone from the sample removed after 25 min of hydrogenation.

Solid fat content (SFC) of the hydrogenated samples was measured by pulsed nuclear magnetic resonance (pNMR) in a Bruker PC/20 series NMR Analyzer (Minispec) (Ontario, Canada). Samples were heated at 60°C, cooled for 15 min in an ice-water bath, then tempered at 25°C for 30 min. They were then cooled again in ice and water for 15 min and left at 10, 20, 25 and 35°C for 30 min before measurements were made.

Chemical analyses. Fatty acid composition was determined by transesterification and analysis of the methyl esters by gas liquid chromatography (GLC) in a Shimadzu GC-8A gas chromatograph (Kyoto, Japan) with a two-meter glass column packed with 10% SP2330 on 100/200

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mesh chromosorb AW (Supelco, Bellefonte, PA) and operated at 170°C (10).

Analysis of triglycerides by carbon number was carried out according to the procedure described by Shehata *et al.* (11). A 30-cm glass column was used packed with 3% OV-1 on 80/100 Supelcoport (Supelco). Chromatography conditions were: detector (flame ionization detector—FID) and injector at 400°C, and oven temperature programmed from 270°C to 355°C at 5°C/min.

Isolated *trans* fatty acids were determined by infrared spectrophotometric method (AOCS Cd 14-61) (12) with a Beckman model 4230 infrared spectrophotometer (Fullerton, CA).

Crystallization analyses. Differential scanning calorimetry (DSC) was used to determine the melting and crystallization behavior of the HMG. Approximately 8 to 10 mg melted fat was weighed in aluminum pans. The pans were placed in aluminum dishes. Because the samples solidify rapidly in the pans, they were melted in the oven and cooled rapidly by placing them in a freezing cabinet at −16°C for 1 hr before scanning. Heating curves were recorded from 15°C to 75°C at a heating rate of 5°C/min. The cooling curves were recorded from 75°C to 15°C while using ice as a coolant. Temperature of crystallization was taken as the temperature at the start of the exothermic deflection of the curve.

The polymorphic forms of the HMG from hydrogenated canola oil obtained at 15°C with acetone as the solvent were established by X-ray diffraction in a Model FR 552 camera (Enraf Nonius, Delft, The Netherlands), which was operated at 23°C. The instrument was fitted with a fine-focus copper X-ray tube. The sample holders were flat stainless-steel plates of 1 mm thickness 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, Fullerton, CA). Short spacings on the X-ray film were measured with a Guinier viewer (Enraf Nonius).

All of the analyses were performed in duplicate, whereas solid fat content was run in triplicate. Correlation coefficients were obtained by linear regression analysis.

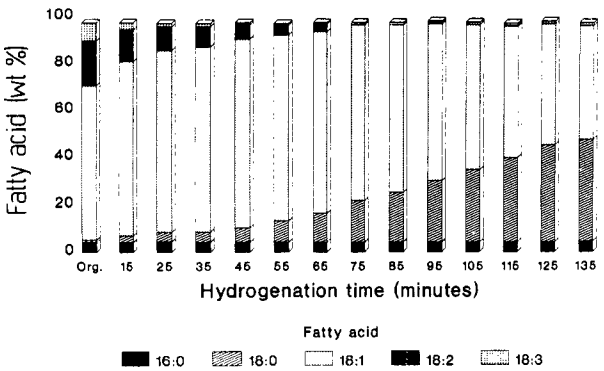

FIG. 1. Fatty acid composition of hydrogenated canola oil.

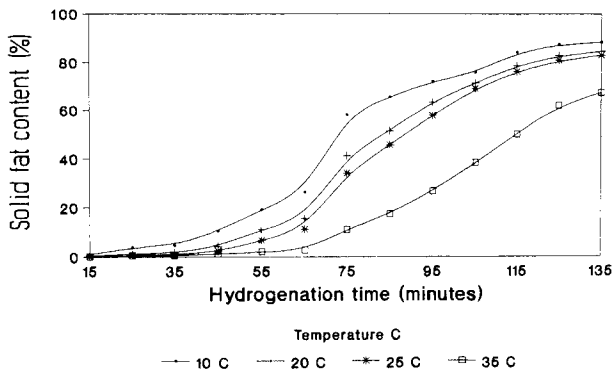

FIG. 2. Solid fat content of hydrogenated canola oil as a function of hydrogenation time.

RESULTS AND DISCUSSION

Samples taken up to 135 min hydrogenation time were analyzed, because samples taken thereafter were very hard. The average Δ iodine value (I.V.)/min was 0.57. The equation of the reaction was I.V. = 107 − 0.467 × (hydrogenation time) with a correlation coefficient = − 0.9945. The fatty acid composition of the original oil was 16:0 = 4.1%, 18:0 = 1.8%, 18:1 = 62.3%, 18:2 = 20.3%, 18:3 = 9.9%, 20:0 = 1.3% and 22:1 = .05%, respectively. The changes in the fatty acid composition at various stages of the hydrogenation process are displayed in Figure 1. There was a decrease in the levels of linolenic and linoleic acid and an increase in 18:0 and 18:1. The graph reveals that the hydrogenation was reasonably selective because in the early stages of hydrogenation linolenic acid was preferentially hydrogenated. After 35 min of hydrogenation (I.V. = 90.5), all of the 18:3 was reduced to trace amounts. In the second stage, *i.e.*, after 65 min (I.V. = 73.0), the 18:2 was converted to 18:1 and 18:0. The 16:0 content stayed the same during the hydrogenation process because only trace amounts of 16:1 were present in the original oil. The fatty acids with more than 18 carbons are not displayed in Figure 1 because they were present at low levels.

The solid-fat content (SFC) of hydrogenated canola oil is shown as a function of hydrogenation time in Figure 2. The solid fat content increased progressively as hydrogenation time increased. SFC increased at a slow rate during the first 55 min of hydrogenation. There was a rapid increase in SFC at 75 min and thereafter, due to the high levels of 18:0 and *trans*. The effect of solid fat content is reflected in the yield of HMG obtained at 15°C with acetone as solvent (Fig. 3). The yield was low during the early stages of hydrogenation. Also not shown in the figure is the yield for the sample taken after 15 min of hydrogenation because HMG was present in trace amounts only.

The fatty acid composition of the HMG is displayed in Figure 4. The HMG consisted mainly of the fatty acids 16:0, 18:0 and 18:1. The 16:0 content of the HMG decreased gradually with hydrogenation. It ranged from 8.8% after 25 min of hydrogenation to 4.6% after 135 min. The level of 18:0 increased with hydrogenation.

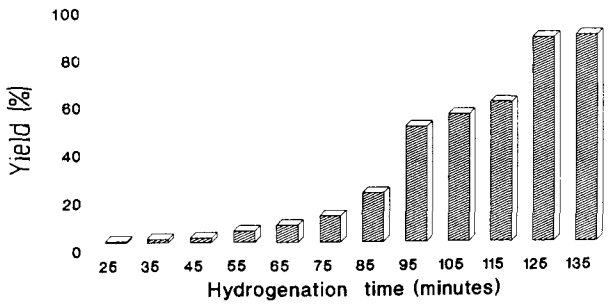

FIG. 3. Yield of high-melting glycerides from hydrogenated canola oil with acetone as the solvent at 15°C.

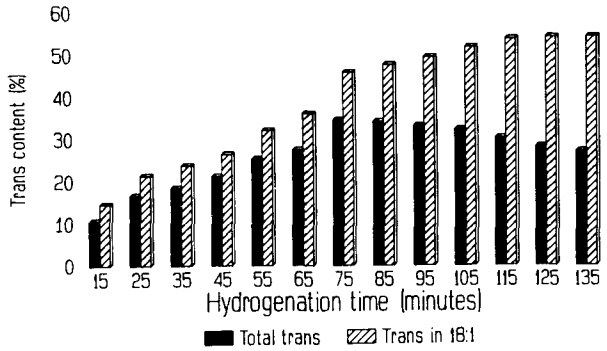

FIG. 5. *Trans* content in hydrogenated canola oil.

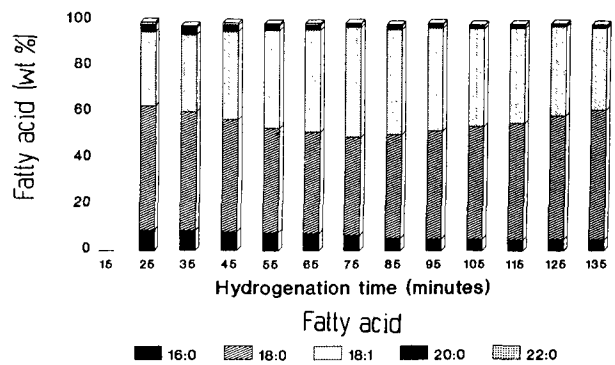

FIG. 4. Fatty acid composition of high-melting glycerides obtained at 15°C from hydrogenated canola oil.

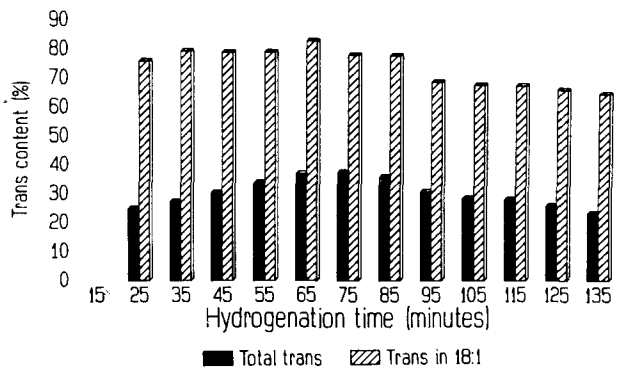

FIG. 6. *Trans* content in the high-melting glycerides obtained at 15°C from hydrogenated canola oil.

During hydrogenation of oil, two types of isomers are formed, *i.e.*, *trans* and positional isomers. The latter are formed by migration of double bonds along the carbon chain. The levels of *trans* isomers formed during hydrogenation of the oil and those present in the HMG are shown in Figures 5 and 6, respectively. The *trans* isomer content increased gradually and reached a maximum at 75 min. It declined thereafter as more *trans* isomers became saturated (Fig. 5). In the same figures (Figs. 5 and 6), the isolated *trans* content is compared with the level of *trans* calculated as a percentage of the 18:1 content. The reason for this comparison is that the majority of the *trans* fatty acids are located in 18:1. The *trans* calculated as percentage of the 18:1 content ranged from 15 to 55% in the hydrogenated samples. In the HMG samples the *trans* content (Fig. 6) was slightly higher than in the original hydrogenated samples (Fig. 5). In the HMG, however, the majority of the 18:1 fatty acids was mainly in the *trans* form and ranged from 65 to 83%.

Table 1 shows the triglyceride composition of the original oil and the HMG obtained at various stages of hydrogenation. Triglyceride composition was most diverse during the early stages of hydrogenation. The HMG consisted of triglycerides with carbon numbers ranging from 50 to 58. The predominating triglyceride was C54. Palmitic acid and erucic acid are located at the 1 and 3 positions of the glycerol molecule (13). Triglycerides containing palmitic acid are mainly 50-carbon (PSP or PEP, E = elaidic acid) and 52-carbon (PSS or PEE). They decreased in the HMG during the hydrogenation process, whereas 54-carbon triglycerides, containing mainly stearic

TABLE 1

Triglyceride Composition of Hydrogenated Canola Oil HMG

Hydrogenation time (min)	Carbon number					
	50	52	54	56	58	60
Original	0.9	13.0	78.9	4.7	1.3	0.8
HMG 25	2.9	20.6	64.4	8.2	2.6	1.2
HMG 35	2.7	20.3	65.8	7.7	1.9	1.1
HMG 45	2.6	20.2	65.6	7.8	2.4	1.1
HMG 55	2.2	19.4	68.4	7.0	2.0	1.0
HMG 65	2.0	18.6	69.5	6.8	2.0	1.0
HMG 75	1.3	16.0	73.6	6.5	1.7	1.1
HMG 85	1.2	15.2	74.6	5.8	1.6	1.1
HMG 95	1.2	14.9	75.4	5.7	1.6	1.1
HMG 105	1.1	14.4	76.4	5.7	1.5	1.1
HMG 115	0.9	13.3	78.1	5.1	1.4	1.1
HMG 125	0.9	12.9	78.9	4.9	1.3	1.1
HMG 135	0.9	12.9	78.2	5.2	1.3	1.1

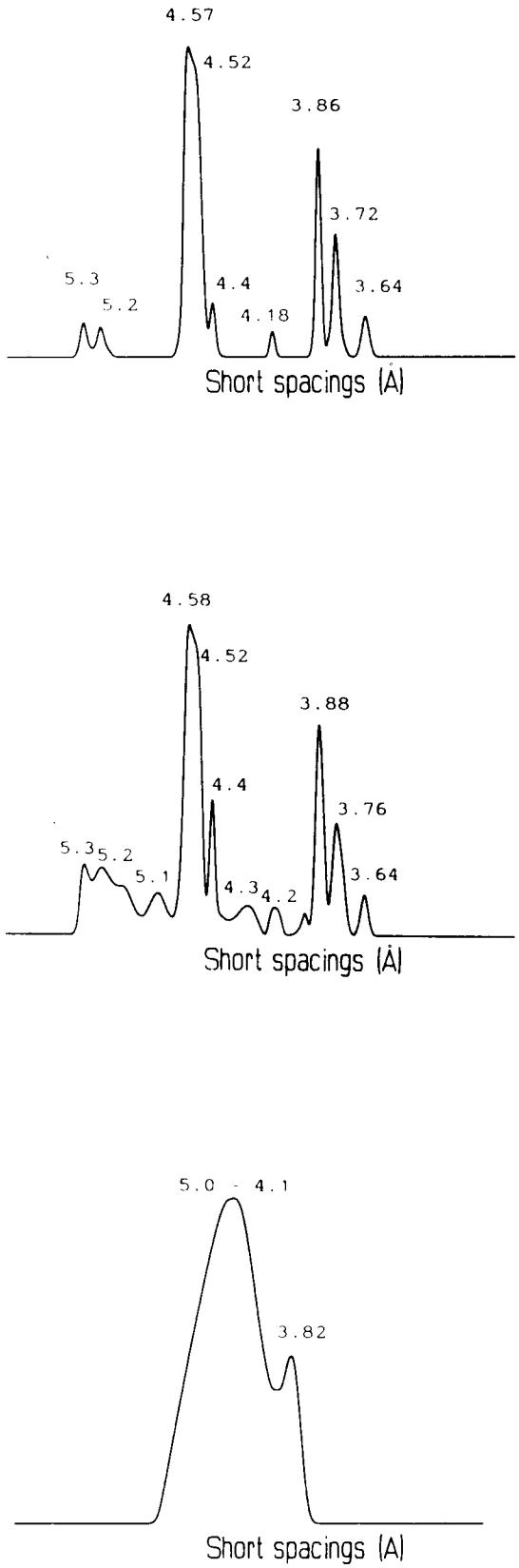

FIG. 7. Laser densitometer scans of X-ray film of the high-melting glycerides: A) solids crystallized from acetone (HMG 25); B) solids crystallized from acetone (HMG 95); C) solids heated at 70°C and cooled (HMG 95).

acid (SSS or SEE or SES) increased during hydrogenation. The 56-, 58- and 60-carbon triglycerides containing fatty acids of 20- and 22-carbon chain length also declined in the HMG with increasing hydrogenation time.

Results from X-ray diffraction analysis revealed that all the HMG crystallized from acetone showed short spacing corresponding to the major polymorphic forms as well as several intermediate forms (Fig. 7a and b). The major short spacing of the α form is 4.1 Å, that of β' is 4.2 and 3.8 Å of almost equal intensities. The β polymorphic form shows a strong line at 4.6 Å. β Crystals also exhibit a short spacing at 3.8 Å, but its intensity is less than that at 4.6 Å (14). The number of intermediate forms increased with hydrogenation time to 55 min, and thereafter there was no change in the number of short spacings (Fig. 7b). All of the HMG as crystallized from acetone exhibited a strong line at 4.6 Å, indicating the presence of β crystals. Lutton and Kolp (15) investigated the polymorphism of a series of *trans* octadecenoic acids. They observed that the presence of the double bond in the even position resulted in more short spacings than the presence of the double bond in the odd position. The additional short spacings were associated with the degree of tilt of the hydrocarbon chains. For instance, in the odd position structure the hydrocarbon chains are perpendicular, whereas in the even position the chains are tilted with respect to the carboxyl plane. It is possible that the additional short spacings were caused by positional isomers or because they were crystallized from acetone (16,17). X-ray analysis of the HMG of commercial margarine as crystallized from acetone also exhibited numerous short spacings (9).

In Figure 8 the melting profiles of the HMG that were heated and cooled before DSC analysis are shown. All of the HMG exhibited a broad endothermic peak between 20–30°C and two distinct endothermic peaks between 50 and 66°C. The melting temperature of the last two peaks increased with hydrogenation time. Some of the HMG also showed an endothermic peak (Table 2). The exothermic peak suggests a polymorphic transition. The other HMG did not show an exothermic peak.

X-ray diffraction analysis of the HMG that were heated and cooled showed a broad diffuse band extending from 4.1 to 5.0 Å and a distinct band at 3.82 Å (Fig. 7c). The broad diffuse band suggests that the HMG consist of a mixture of α, β' and β crystals. Similar findings were also observed for the HMG from commercial margarines (9), where the diffuse band extended from 4.1 to 4.4 Å. The short spacings of the HMG, either when crystallized from acetone or after heating and cooling, cannot be used to predict the polymorphic form of their hydrogenated fats. Their melting temperatures are high, like fully hydrogenated hard fats (18). The hard fats also crystallized mainly in the α and β' forms, but upon dilution with liquid oil they (except palm oil) crystallized in the β form.

Crystallization behavior was also investigated by DSC (Table 2). All the HMG crystallized between 40 and 45°C. The crystallization temperatures of the HMG of the commercial margarines were lower (33 to 37°C) as were the DSC melting temperatures (53–55°C) (9). These differences are probably caused by the higher palmitic acid content of the HMG in the commercial margarine, because these consisted of soybean, corn, canola-palm and canola.

In conclusion, the HMG consisted mainly of 16:0, 18:0

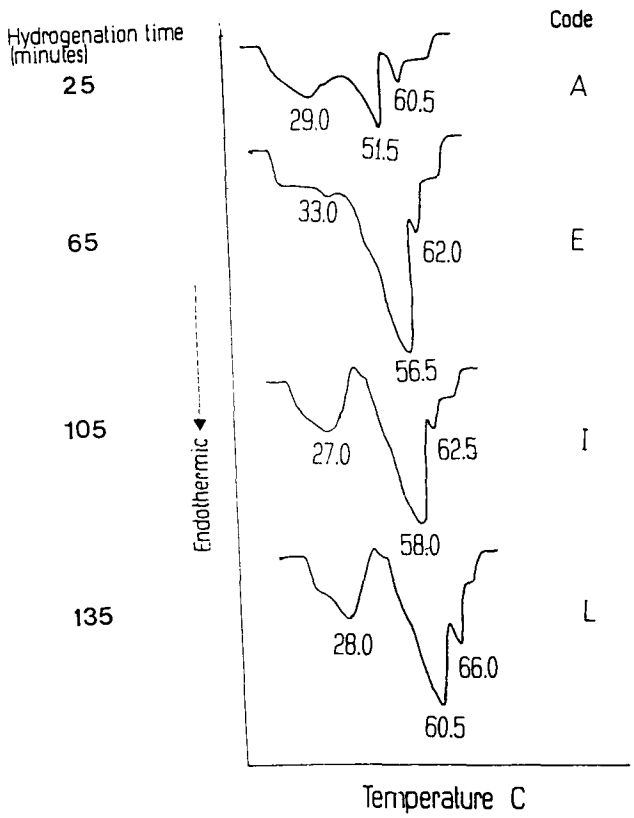

FIG. 8. DSC-melting curves of HMG which were heated and cooled before analyses: A = HMG 25; E = HMG 65; I = HMG 105; L = HMG 135.

and 18:1 fatty acids. The 18:1 consisted mainly of *trans* fatty acid. The triglyceride composition was most diverse at the start of hydrogenation. The HMG consisted mainly of 54-carbon triglycerides. From our previous paper (9) it was observed that stick margarines were in the β' form when their HMG (15°C crystallized from acetone) with 54-carbon content was 50% or less, whereas in β-tending margarine the level of 54-carbon of these HMG was 70%. In the present study, after 25 min of hydrogenation the 54-carbon triglyceride level in the HMG was 64%. This suggests that partially hydrogenated canola oil cannot be used exclusively for the manufacture of margarine. Incorporation of an oil with solids containing high levels of palmitic acid and triglycerides that are β'-tending is therefore necessary. This incorporation will increase the palmitic acid and decrease the C54 content in the HMG. Palm oil contains solids that are high in palmitic acid. When palm oil is lightly hydrogenated, the delaying effect of polymorphic transition has been shown to become more pronounced (19,20). This study has provided information on the reasons of polymorphic β' instability of hydrogenated canola oil.

TABLE 2

DSC-Melting Temperatures of Peaks and Crystallization Temperatures (°C) of the HMG of Hydrogenated Canola Oil

Hydrogenation time (min)	Melting: Endothermic peaks 1	2	3	Exotherm	Crystallization peaks 1	2
HMG 25	29.0	51.5	60.5	55.0	45.0	30.0
HMG 35	26.0	51.0	61.5	53.5	45.5	29.0
HMG 45	33.0	56.0	62.0		41.5	
HMG 55	33.0	56.5	62.0		40.0	
HMG 65	33.5	56.5	62.0		40.0	
HMG 75	25.0	57.5	62.0		39.0	
HMG 85	21.5	57.5	62.5		39.0	
HMG 95	27.0	57.5	62.5		39.0	
HMG 105	27.0	58.0	62.5	37.0	39.0	
HMG 115	27.0	60.5	66.0	37.0	41.0	
HMG 125	28.0	60.5	66.0	39.5	43.0	
HMG 135	28.0	60.5	66.0	43.5	44.5	

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