Crystalline Fractionation of Hydrogenated Sunflowerseed Oil. II. Differential Scanning Calorimetry (DSC)

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This study explored the thermal behavior of hydrogenated sunflowerseed oil sample used in margarine manufacture, which had been previously fractionated by crystallization at different temperatures. Calorimetric diagrams showed that areas per gram were larger when solid samples, rather than liquids, were considered. Samples of high crystallization temperatures were found to have components with high fusion points that were not present in fractions of lower crystallization temperatures. This means that more saturated triglycerides are present in solids as well as in fractions crystallizing at high temperatures (above 30°C).

KEY WORDS: Crystallization, DSC, fractionation, hydrogenated sunflowerseed oil.

Dry fat crystallization behavior depends on various factors, such as the kind of starting material, compounds behaving as crystallization nuclei (mono- and diglycerides), agitation and cooling rate. Fractionation always results in a mixture of crystalline forms, the kind of crystal obtained depends on the cooling rate. When cooling is very fast, α and α' forms are present, whereas β and β' forms usually appear when it is slow. Thus, a slow cooling rate should be used if a crystallization leading to a regular and macro-crystalline granulometric distribution is desired (1-3). The nature of the fatty acid chains bound to glycerol and the wide range of fusion temperatures cause these triglyceride mixtures to have a complex thermal behavior (4).

A description is presented here of the thermal behavior of a hydrogenated sunflowerseed oil sample used in margarine manufacture that had been fractionated previously by crystallization at different temperatures.

MATERIALS AND METHODS

Starting oils. Samples utilized in this research were supplied by Molinos Rio de La Plata (Capital Federal, Argentina). They consisted of hydrogenated sunflowerseed oil (35°C), which is used for preparing the fatty phase of margarines. The oil used in the first experiment had an iodine value of 65 and a Mettler dropping point of 35.5°C; in the second experiment the iodine value was 68 and the Mettler dropping point was 35.4°C. All samples were fractionated as described previously (5).

Differential scanning calorimetry (DSC). A Dupont 910 programmed calorimeter, fitted to a cooling apparatus and a thermal analyzer (model 99) were used (Dupont, Wilmington, DE). Calorimetric diagrams were recorded with a Hewlett-Packard recorder (Hewlett-Packard, Palo Alto, CA). Samples ranging from 15 to 20 mg were placed in hermetically sealed aluminum pans, which were subjected to the following temperature program: −40°C isotherm for 5 min; heating process from −40°C to 80°C at a heating rate of 10°C/min; and a final isotherm at 80°C for 2 min. The apparatus was calibrated with indium as standard. Diagrams of dQ/dt were plotted as a function of time. The sensitivity varied from 1.038 mJoule/sec to 0.414 mJoule/sec, depending on the quantity and quality of the samples. Calorimetric run diagrams were measured according to the equation:

$$H = a^{*}\exp(-(t-b)^2/\sigma) + c^{*}\exp(-(t-d)^2/\sigma + \ldots \quad [1]$$

where a, c, etc., are the heights corresponding to the peak temperature (T_P); b, d, etc., are the peak temperatures, and σ is the standard deviation. All these parameters were obtained by means of an IBM personal computer, a basic manual program and the Systat method (6). In this way, fusion enthalpies corresponding to each endotherm and total enthalpies of samples were calculated for the areas, according to the equation:

$$H = \frac{A\ (cm^2)\ E\ (mW/mV)\ B\ (min/cm)\ S^y\ (mV/cm)\ 60\ (sec/min)}{m\ (mg)} \quad [2]$$

where S^y is the ordinate corresponding to sensitivity, E is the cell constant equal to 0.2080, B is the time base, A is the area corresponding to the endotherm, and m is the sample mass used. All these determinations were performed in triplicate for each experiment. Dispersion was less than 1% for all areas and 0.5°C for temperatures. Values are the average of three thermograms.

X-ray diffractometry. X-ray diffraction spectra were obtained by means of a Philips 1730 instrument fitted with a system for temperature control (Philips Argentina, S.A., Buenos Aires, Argentina). The sample holder placed within the refraction chamber was maintained at a constant temperature with a 3:1 solution of ethyleneglycol from a Lauda UK-30 cryostat (Messergate, Konigshapen, Germany). $K\alpha_1\alpha_2$ radiation from copper at 40 KV, 20 mA and a scanning velocity of 1°/min were used.

Infrared spectroscopy. A Beckman IR-10 double-beam spectrophotometer was used (Beckman Co., Munich, Germany) between 4,000 and 600 cm^{-1}. Samples were processed by using films between KBr windows; the standard was 99.95% triolein (Sigma Chemical Co., St. Louis, MO).

Nuclear magnetic resonance. A RMN-H_1 Varian EM-360 A apparatus (Varian, Palo Alto, CA) was used. Twenty mg of each sample were dissolved in CCl_4, and tetramethylsilane was used as internal standard. Run conditions were 0.05 filter, radiofrequency power 0.06 mGauss, scanning time 2 min and scanning width 10 Hz.

RESULTS AND DISCUSSION

Analysis of the original samples. Original samples were characterized by differential scanning calorimetry. This procedure was carried out by using the heating program

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described previously. Figure 1 shows the corresponding thermograms. The sample used for the first experiment exhibited two peaks, while the sample in the second experiment showed three peaks. The higher temperature peak in the first experiment contributes a higher percentage to the total endotherm than in the second experiment (53.0% and 45.0%, respectively). All these thermograms were mathematically analyzed—results are shown in Table 1. The first peak in sample 1 was asymmetric (Fig. 1) and could best be fitted by means of four components of different weights. The second peak consisted of three components, as can be seen in the original thermogram (Fig. 1). The first peak in sample 2 showed a fitting profile corresponding to four components, while the third peak [peak temperature (T_P) = 32.1°C] was assumed to have only one component. The fact that thermograms seemed to correspond to a number of components higher than the visible ones suggested the presence of triglyceride fractions with melting points too close to be differentiated under the conditions used. For example, the components of the first peak of sample 1 showed melting points higher than those corresponding to the first peak in sample 2. In sample 1, a component with a peak temperature of 41°C, which was not present in sample 2, was found within the peak of higher melting temperature. Although the total values of ΔH for both samples were similar, the liquid component in the first sample evidenced a slightly lower ΔH at room temperature. Therefore, solids were the major components contributing to the total enthalpy in sample 1, but not in sample 2. Hence, it was concluded that the percentage of saturated fatty acids was higher in the first sample than in the second one. This is consistent with previously obtained results (5).

In agreement with the triglyceride composition found for these hydrogenated oils (5): C16:0 C18:1 C18:1, C18:1 C18:1 C18:1, C18:2 C18:1 C18:1 and some others, such as

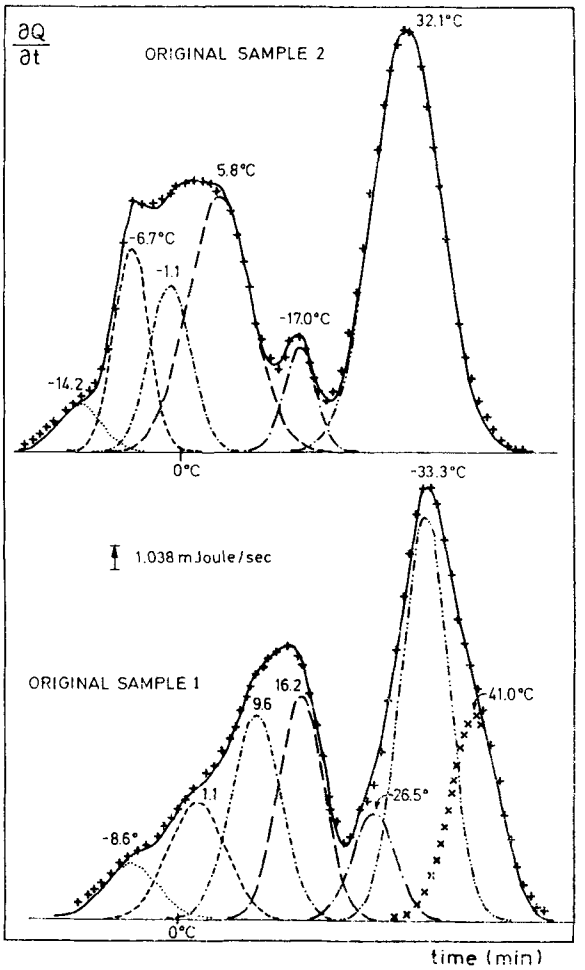

FIG. 1. Thermograms of original samples 1 and 2 fitted by computer.

TABLE 1

Thermograms of Original Samples Fitted by Computer

(1)	First peak				Second peak		
	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6	Comp. 7
T(°C)	−8.6	1.1	9.6	16.2	26.5	33.3	41.0
SA[a]	67.9	155.4	228.0	206.2	95.1	428.7	216.6
A%[b]	4.9	11.1	16.3	14.7	6.8	30.7	15.5
ΔH[c]	4.2	9.6	14.2	13.0	5.9	26.8	13.4
ΔHP[d]	41.2				46.0		
ΔHT[e]							

(2)	First peak				Second peak	Third peak
	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6
T(°C)	−14.2	−6.7	−1.1	5.8	17.0	32.1
SA	54.3	159.8	154.0	341.9	67.0	636.3
A%	3.8	11.3	10.9	24.2	4.7	45.0
ΔH	3.3	10.0	9.6	21.3	4.1	39.8
ΔHP	44.4				4.1	39.8
ΔHT	88.3					

[a]SA, specific area mm^2.
[b]A%, peak area (%).
[c]ΔH, component enthalpies (Joule/g).
[d]ΔHP, peak enthalpies (Joule/g).
[e]ΔHT, total enthalpy (Joule/g).

C18:0 C18:1 C18:1, C18:0 C18:0 C18:1 and C18:2 C18:2 C18:1 in lower amounts; were responsible for the behavior of the fat mixture. The melting points of the different components in the samples indicated the existence of *trans*-unsaturated fatty acids; this fact was confirmed by infrared spectroscopy. Values of 43.4% and 44.2% were found for samples 1 and 2, respectively. Figure 2 shows X-ray diffraction diagrams of the hydrogenated oils from both experiments. The original samples were found to be a mixture of different β' crystals. The results are consistent with the fact that the two samples presented a wide range of melting points.

Fraction analysis. The fractions obtained—solids and liquids—were analyzed by differential scanning calorimetry. Figure 3 shows three representative diagrams corresponding to the original sample, the solid fraction at 38°C and the liquid fraction at 38°C (second experiment). Both the solid and the liquid diagrams were different from that of the original sample. On the basis of the results obtained, three groups may be distinguished with components showing similar behavior—fractions crystallized at temperatures above 30°C and 22°C, and below 22°C. In order to further characterize the fractions, a representative component was chosen from each group. Liquid percentages corresponding to liquid and solid fractions at 14°C, 24°C and 38°C, and also those of the original sample at different temperatures are shown in Table 2. Table 3 shows the results obtained when the different thermograms were analyzed mathematically. Obviously, solid fractions have components with melting points lower than the fractionation temperatures. Thus, in spite of the fractionation procedure used, solids always hold a certain amount of liquid.

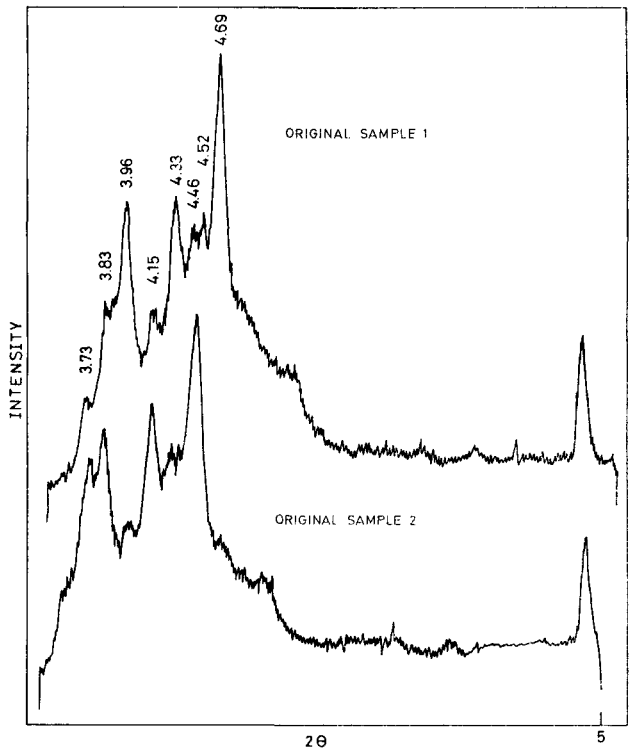

FIG. 2. X-ray diffraction diagrams of the hydrogenated oils employed in both experiments.

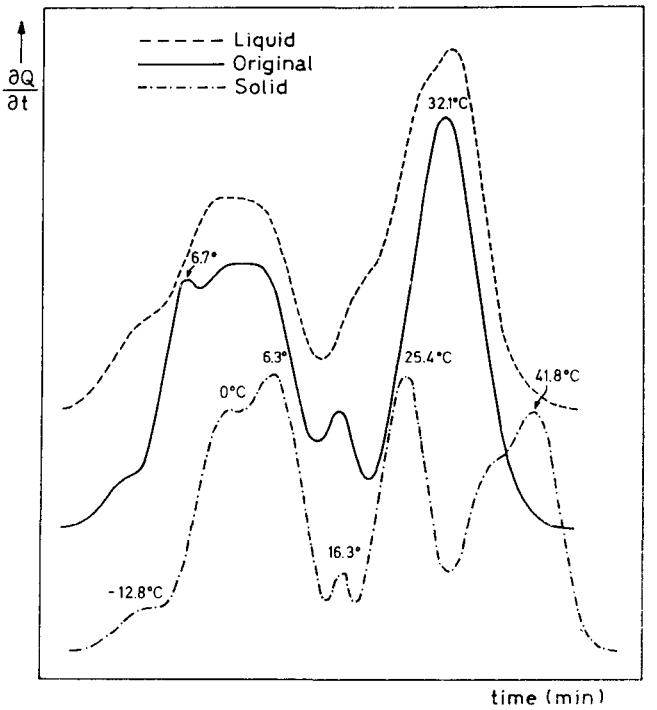

FIG. 3. Comparison of three thermograms corresponding to original sample 2, 38°C solid fraction and 38°C liquid fraction.

TABLE 2

Percentages of Liquid Oil in Solid, Liquid and Original Sample Fractions

	% of Liquid oil in each fraction[a]						
T(°C)	M02	S38	L38	S24	L24	S14	L14
0.0	20.8	13.3	20.4	24.9	29.0	28.2	34.0
5.0	37.9	31.3	36.3	35.2	31.6	43.2	45.4
10.0	41.8	35.0	39.7	39.7	44.3	48.5	62.6
15.0	52.5	45.0	50.6	43.5	59.5	59.0	78.4
20.0	53.3	55.4	54.7	53.7	74.1	59.9	82.4
25.0	55.0	57.6	59.9	55.0	79.7	66.5	86.3
30.0	73.0	59.8	75.8	62.1	82.5	72.3	94.6
35.0	82.0	60.2	86.0	72.3	94.1	89.0	98.9
40.0	84.2	61.0	88.6	84.4	98.8	—	—

[a]M02, original sample 2; S38, solid fraction separated after crystallization at 38°C; L38, liquid fraction remaining; etc.

Because of the similarity of both size and electrochemical nature between triglyceride molecules independent of melting point, those of lower melting can occupy places within the crystals of the higher-melting triglycerides, thus displacing them. In addition, liquid oil could be held between solid crystals. Solid fractions presented broader thermograms than liquid ones in the same range. Solid diagrams become narrower with decreasing temperatures until they are smaller than that of the original sample. Results from the first and second experiment are similar. Higher melting point peaks were found at higher fractionation temperatures. In this way, peak 9 (Table 3) only appeared in those fractions with crystallization

TABLE 3

Components with Different Fusion Points for Each Crystallization Temperature

Fraction		1	2	3	4	5	6	7	8	9
38°C										
Solid	(1)[a]	−13.4		−2.2	6.5	16.3	25.3		37.1	44.9
	(2)[b]	3.2		16.8	25.0	1.5	22.2		11.6	19.7
Liquid	(1)	−11.9		−1.7	6.7	20.9	28.3		34.8	
	(2)	9.0		18.5	17.1	10.6	18.0		26.8	
24°C										
Solid	(1)	−14.9	−6.4	0.8	7.7	17.9	24.4	29.1	35.7	42.0
	(2)	6.0	11.7	14.4	19.2	6.3	9.3	11.1	13.0	9.0
Liquid	(1)	−14.0	−5.2	0.2	5.1	13.8	28.4		35.0	
	(2)	7.5	11.5	14.0	25.3	29.8	10.1		1.8	
14°C										
Solid	(1)	−14.0	−6.0	2.1	9.8	19.5	27.4		36.8	
	(2)	7.4	14.3	16.2	21.2	19.7	19.2		2.0	
Liquid	(1)	−17.0	−8.2	0.2	8.7		23.8	29.5		
	(2)	7.9	14.6	22.9	34.1		9.8	10.7		

[a]Temperature (°C). [b]Peak area (%).

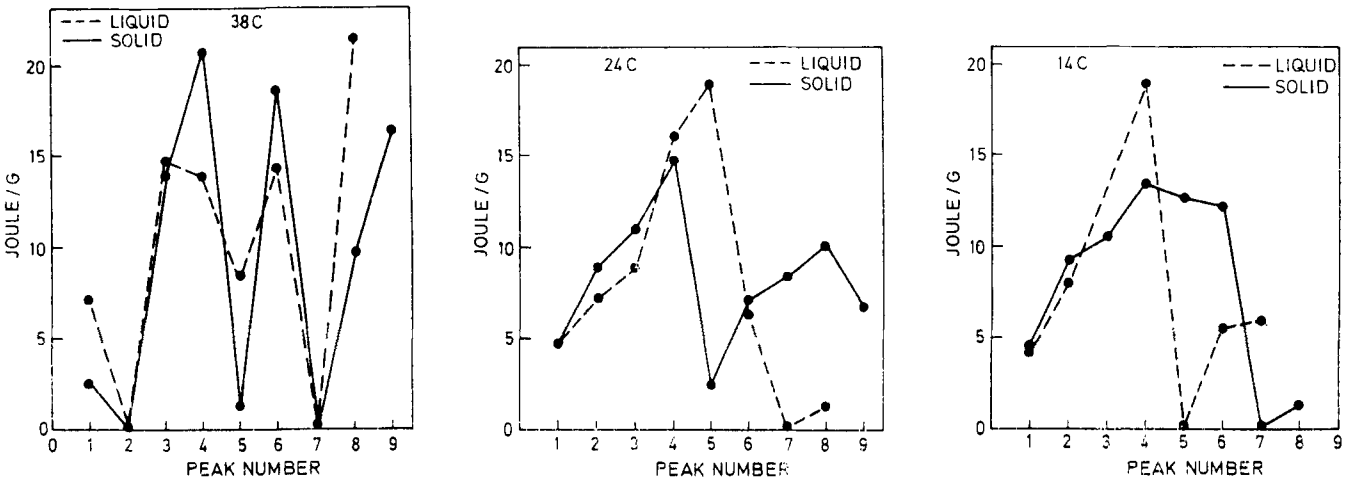

FIG. 4. Enthalpy of each peak as a function of temperature.

temperatures up to 24°C. Then, its percentage decreased when the temperature was lowered. The liquid fraction at 38°C should be similar to the original sample, because in this experiment the tank was loaded with 27 Kg, and only 333.0 g crystallized at this temperature. However, the selective extraction of the triglyceride mixture with high melting point only produced small changes in the melting curve. The total enthalpies of solid samples crystallized at 38°C, 24°C and 14°C were 82.9, 76.2 and 63.6 Joule/g, respectively; those corresponding to the liquids were 79.5, 62.8 and 54.8 Joule/g, respectively. Figure 4 represents the enthalpies of the peaks as a function of temperature. Solid samples showed total enthalpies and areas per gram higher than those of the liquid samples at the same temperature. Samples at high crystallization temperatures were found to have components of high melting point, which were not present in the fractions crystallizing at lower temperatures. This means that more saturated triglycerides can be found in solid fractions crystallizing at higher temperature. Solid fractions crystallizing at 38°C, 24°C and 14°C were then studied by X-rays and were compared to sample 2, while fractions at 21°C, 31°C and 41°C were compared to sample 1. In both cases, fractions showed diagrams that differ from the original sample. The three diagrams corresponding to samples at 38°C, 24°C and 14°C are shown in Figure 5. All of them are similar, but small differences can be observed regarding the relative sizes of the peaks. These three fractions crystallized with the β' shape. This was to be expected, because these crystals have the size and morphology adequate for filtration. As for the sample obtained at 14°C, it was statistically melted and crystallized at that temperature. Consequently, a mixture of β' and β crystals was obtained. The difference between static and dynamic

TABLE 4

NMR Analysis of Sample 2 and its Fractions

Sample	Number of olefinic protons	Number of total protons	Average molecular weight	Iodine value
38	4	84	746.4	69
24	4	82	710.0	71
14	6	96	696.0	80
MO	4	86	740.0	68

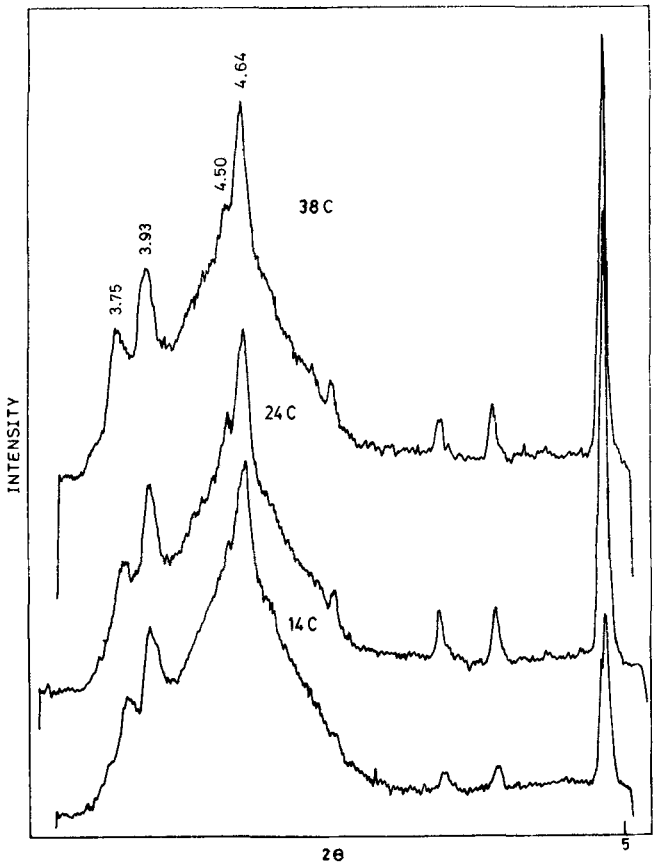

FIG. 5. X-ray diffraction diagrams of solid fractions crystallized at 38°C, 24°C and 14°C.

crystallization is evident within the same sample. At variance with this, fractions at 21°C, 31°C and 41°C showed β' crystals as reported in the second experiment. When the fractions were analyzed by means of infrared spectroscopy, *trans* isomer values of 43.8%, 42.4% and 42.7% were found for the solid fractions crystallized at 38°C, 24°C and 14°C, and values of 24.1%, 21.9% and 19.7% for the liquids. The content of *trans* unsaturated isomers was thus higher in the solids.

Nuclear magnetic resonance studies were performed to characterize the fractions and the original sample. Results obtained with a specimen of each group and of the original sample used in the second experiment can be seen in Table 4. According to the results obtained, the original sample has a composition similar to that of the higher temperature fractions; whereas the sample crystallized at 14°C is more unsaturated than fractions crystallized at 24°C and 38°C. The sample obtained at 14°C also shows a higher content of C18:2 C18:1 C18:1 and a lower content of C18:0 C18:0 C18:1 and C18:0 C18:1 C18:1.

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