Crystalline Fractionation of Hydrogenated Sunflowerseed Oil. I. HPLC Analysis

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Crystalline fractionation of hydrogenated sunflowerseed oil was performed and the chemical composition of the separated fractions at different temperatures was determined. The results show that the triglycerides obtained after a short retention time (less than 16.4 min) were enriched in the low-temperature fractions (lower than 22°C), the triglycerides of long retention time (more than 21.5 min) were concentrated in the higher-temperature fractions (higher than 30°C), and the triglycerides of medium retention time (between 16.4 and 21.5 min) were concentrated in the medium-temperature fractions (22°C to 30°C). The partition ratio of triglycerides with retention times of 8.8, 12.5, 16.5, 21.5 and 29.1 minutes was increased as a function of the fractionation temperature.

KEY WORDS: Crystallization, fractionation, high-performance liquid chromatography, hydrogenated sunflowerseed oil.

Fractionation by means of crystallization has long been a widely used method in fats and oils. The aim is to obtain materials different from natural fats in order to manufacture better products for the consumer economically. It is a simple procedure, but it has the disadvantage of being the least selective to obtain fractions with physical properties different from those of the starting oils or fats (1). Crystalline fractionation has been applied to various vegetable and animal oils (2,3). Other fractionation methods also have been used, such as short-path destillation (4), chromatography on hydrated silicic acid (5), urea fractionation in methanol (6) and crystallization from solvents (7).

Sunflower production is of great importance to Argentina. Due to the commodity's availability, this oil is used largely to produce margarine and shortenings. Our aim is to study crystalline fractionation of hydrogenated sunflowerseed oil and to determine the chemical composition of those fractions obtained at different temperatures so as to improve the formulation of final products. Consequently, we have designed a pilot fractionator that allows separation of solids by crystallization at different temperatures and collection of liquid fractions in equilibrium with such solids. Both solids and liquids were thoroughly studied regarding their chemical composition by means of gas and liquid chromatography.

MATERIALS AND METHODS

Starting oils. Fractionation was carried out twice. In both cases, the sample was supplied by Molinos Rio de La Plata S.A. (Capital Federal, Argentina) and was composed of hydrogenated sunflowerseed oil (Sunflower 35°C), which is used to prepare the fatty phase of margarines. For the first experiment, sample 1 (hydrogenated North Sunflowerseed oil), the fractionation tank was loaded with 50 kg of hydrogenated oil with an iodine value of 65 and Mettler dropping point of 35.5°C; and for the second, sample 2 (hydrogenated Centre Sunflowerseed oil), the fractionation tank was loaded with 27 kg of oil with an iodine value of 68 and a Mettler dropping point of 35.4°C.

Fractionation equipment. The equipment was made in our laboratory. Its design is shown in Figure 1. The liquid in the tank is pumped through a filter, the solids are retained, and the liquid is returned to the tank. The hydrogenated sunflowerseed oil being fractionated runs through a closed circuit. The agitation obtained by the pump and tank agitators results in a dynamic equilibrium between solids and liquids.

Methods. The first fractionation experiment was initiated at 41°C, and the second experiment at 38°C.

Melted hydrogenated oil was allowed to circulate after crystallization for no less than 12 hr; the total amount of solid accumulated on the filter and a representative portion of liquid in the tank were extracted. Afterwards, temperature was diminished by 2°C until reaching a temperature of 19°C and 12°C, respectively. Fractions crystallized at 7°C and 3°C were obtained statically in a bath-submerged crystallizer, in which temperature was maintained. Twelve fractions were obtained from the first fractionation, and 14 from the second one. They were all purified in a bath at a controlled temperature for 2 hr and pressed between filter paper to absorb any remaining liquid. The fractions were analyzed by high-performance liquid chromatography (HPLC) and gas-liquid chromatography (GLC) regarding their chemical composition.

Gas-liquid chromatography (GLC). Fatty acids were analyzed in a 5890 A chromatograph (Hewlett-Packard, Palo Alto, CA) and a column (170-cm long and 0.3-cm internal diameter) packed with 10% SP 2330 on 100/120 WAW chromosorb. A N_2 flow of 20 mL/min, 250°C injector temperature and 188°C column temperature were maintained. The chromatograms were recorded on a Hewlett-Packard 3392 recorder. Methyl esters were prepared by transesterification with a mixture of methanol/benzene (3:1) and 3% w/v sulfuric acid. The standard used consisted of an equimolecular mixture of palmitic, stearic, oleic, linoleic and linolenic acids, 10 mg/mL concentration. The corresponding determinations were carried out in triplicate. Values are the average of three determinations, and the standard deviation is 0.5%.

High-performance liquid chromatography (HPLC). Chromatographic runs were performed in a liquid chromatograph (Waters Associates, Milford, MA; model 6000 A) equipped with a solvent mixing system, universal injector for liquid chromatography model U6K, and a Waters reverse phase C18 microbondapack column (30-cm long and 4-mm diameter). The run was performed at room temperature. A mixture of triglycerides, about 40 μg dissolved in 2 μL chloroform, was injected. The solvent system consisted of a binary mixture of

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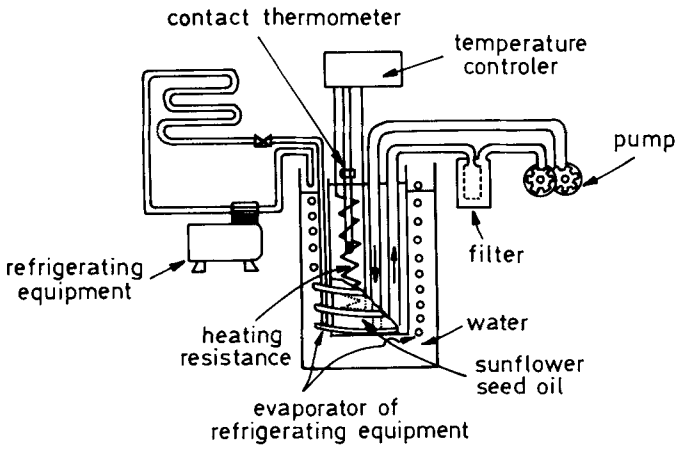

FIG. 1. Pilot fractionator diagram.

acetone/acetonitrile (1:2, v/v) at a flow of 1.1 mL/min. All solvents were of spectroscopic grade. Detection was carried out at 210 nm with a Waters UV-detector, model 450, with variable wavelength. The chromatograms were recorded and integrated with a Waters Data Module, model M 730. At least three determinations for each sample were done and the results were averaged; all data showed less than 1% dispersion. Chromatograms were analyzed by comparison with saturated triglyceride standards from Sigma Chemical Co., St. Louis, MO (tripalmitin, PPP, and tristearin, SSS), 99.99% purity. Pure oils were analyzed under the same conditions as those used by Dong and Di Cesare (8), which were suitable to tentatively identify chromatogram peaks. The results obtained were then compared to those found by Frede and Thiele (9), and tested by the matrix model of Takahashi *et al.* (10).

RESULTS AND DISCUSSION

Table 1 shows the values of total solids and liquids corresponding to each fractionation temperature in both experiments. It can be observed that the solid content was higher in the first experiment than in the second one. The fatty acid compositions of the original samples were as follows. The sample used in the first experiment (Hydrogenated North Sunflowerseed oil) contained 8.3% C16:0, 11.5% C18:0, 76.4% C18:1, 3.8% C18:2; only 0.1% was contributed by other acids, such as C12:0 and C14:0. The second sample (Hydrogenated Centre Sunflowerseed oil) showed a fatty acid content of 6.7%, 11.0%, 69.8% and 12.2% for C16:0, C18:0, C18:1 and C18:2, respectively. Results shown in Table 2 correspond to the same samples studied by HPLC. Only the most important triglycerides were included, those having areas less than 3% were not considered. In agreement with the fatty acid composition of both samples, the increase of C18:2 acid content (8% more in sample 2 than in sample 1) indicates the existence of triglycerides with lower ECN (even carbon number) and low retention times in sample 2 (Table 2). Figure 2 shows four chromatograms corresponding to the original sam-

TABLE 1

Total Solids and Liquids Obtained from Each Fractionation Temperature in Two Experiments

First experiment			Second experiment		
Temp. (°C)	Solid (g)	Liquid (g)	Temp. (°C)	Solid (g)	Liquid (g)
41	850.2	49,149.8	38	33.0	26,667.0
39	3,170.2	46,829.8	36	750.5	26,249.5
37	7,028.3	42,971.7	34	1,265.4	25,734.6
35	8,609.4	41,390.6	32	1,794.9	25,205.1
33	10,961.0	39,039.0	30	2,287.4	24,712.6
31	11,471.2	38,528.8	28	3,181.7	23,818.3
29	12,320.9	37,679.1	26	4,013.1	22,986.9
27	13,196.9	36,804.1	24	5,175.9	21,824.1
25	13,979.7	36,020.3	22	5,471.7	21,528.3
23	14,207.0	35,793.0	20	5,832.3	21,167.7
21	16,101.6	33,898.4	18	6,030.1	20,969.9
19	18,786.8	31,213.2	16	6,269.7	20,730.3
			14	6,529.7	20,470.3
			12	6,985.0	20,015.0

TABLE 2

Triglyceride Composition of the Original Samples

Retention times (min)	12.5	16.4	21.5	29.2	39.8
ECN	46	48	48	50	52
	Triglyceride content (%)				
Sample 1	4.2	7.3	25.5	48.4	12.0
Sample 2	18.1	39.5	29.4	7.0	0.8

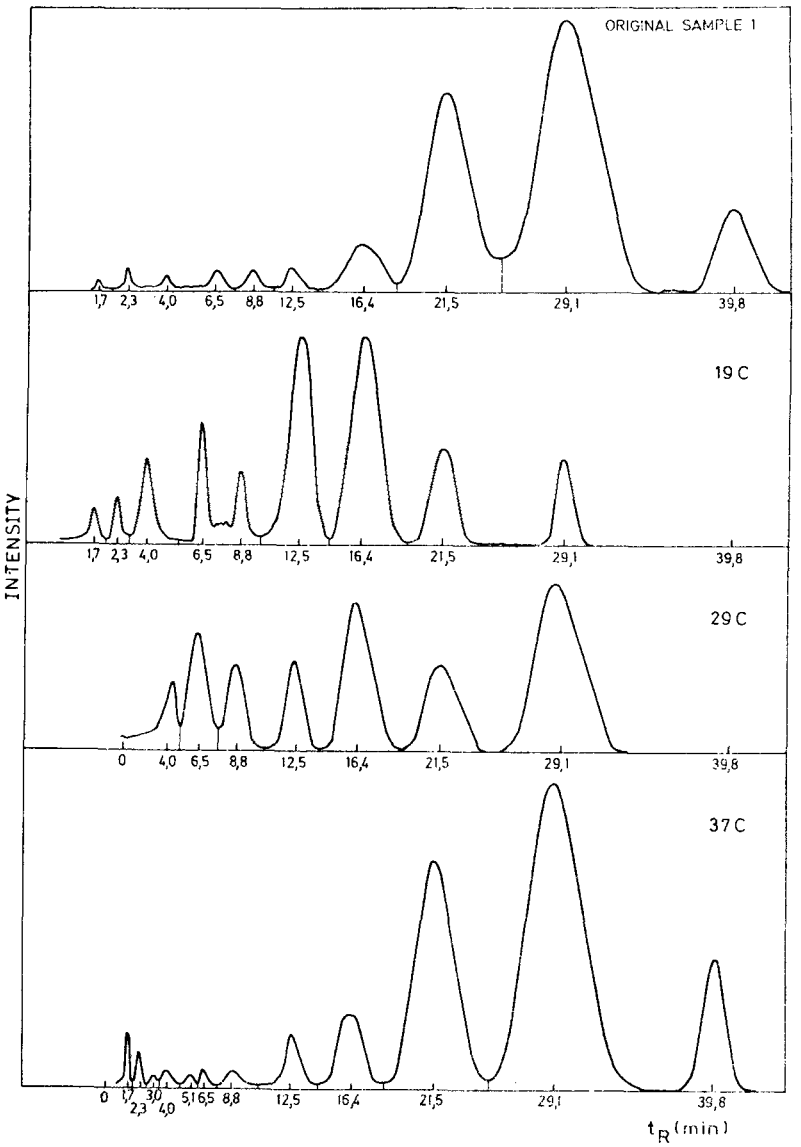

FIG. 2. Chromatograms of original sample and 37°C, 29°C and 19°C fractions (first experiment). Acetone/acetonitrile (1:2); flow, 1.3 mL/min.

ple and also to those fractions at 37°C, 29°C and 19°C of the first fractionation experiment (sample 1). Triglyceride components of the hydrogenated oil showed that glycerol had been replaced mainly by four fatty acids. If the approximate calculation of triglyceride content is determined by taking into account the fatty acids present as described by Nawar (11), it is obvious that triglycerides having a probability of formation at more than 3% are few. In Figure 2, it can be seen that the major peaks appeared at the following retention times (T_R): 12.5 min (C18:2 C18:1 C18:1); 16.4 min (C18:1 C18:1 C18:1); 21.5 min (C16:0 C18:1 C18:1); 29.2 min (C18:0 C18:1 C18:1) and 39.8 min (C18:0 C18:0 C18:1); the others were found in much lower proportion. Since two ECN triglycerides next to each other, *e.g.*, POO and PPO, have a different probability of formation, more than one triglyceride may exist in one peak, but in proportions parallel to their probability of formation. The triglyceride showing the highest probability was then assumed to be present in each peak. The corresponding analysis was performed on this basis. Some peaks in the chromatogram (Fig. 2) are not baseline-separated; consequently, it is not possible to determine the actual areas of such peaks. Figure 2 also shows integration of peaks, which was done by drawing lines perpendicular to the baseline. The obtained results are semi-quantitative.

Quantitative determination of fatty acid methyl esters by gas-liquid chromatography (GLC): Solid fractions. Percentages of fatty acids of samples shown in Figure 2 are represented in Figure 3a, and those corresponding to samples at 38°C, 26°C and 12°C are shown in Figure 3b. When the crystallization temperature was reduced, C18:2 acids increased, C16:0 and C18:0 acids slightly decreased, whereas C18:1 acid first increased and then de-

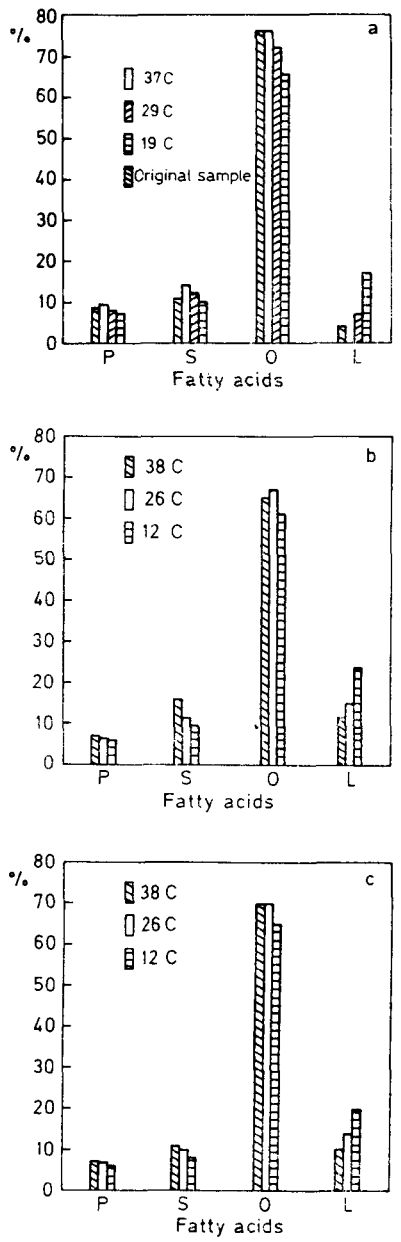

FIG. 3. a, Percentages for each fatty acid in 37°C, 29°C and 19°C fractions (first experiment). b, Percentages for each fatty acid in 38°C, 26°C and 12°C fractions (second experiment). c, Liquid fractions corresponding to the solid in 3b. Abbreviations of fatty acids: P, palmitic acid C16:0; S, stearic acid C18:0; O, oleic acid C18:1; L, linoleic acid C18:2.

creased. With regard to the first fractionation, C18:2 acid content was markedly lower in the original sample. In agreement with this fact, fractions at 41°C, 39°C and 37°C did not contain C18:2 acid. The fatty acid composition of the hydrogenated oil used in the second experiment was compared to that of the solid fractions. It was observed that fractions crystallizing at 28°C and 30°C were very similar to the original sample. The analysis of pure Centre Sunflowerseed oil showed 6.7% C16:0, 3.8% C18:0, 30.5% C18:1 and 57.8% C18:2, and the North Sunflowerseed oil showed 8.3% C16:0, 2.2% C18:0, 36.3% C18:1 and 52.1% C18:2; whereas the hydrogenated oil contained lower amounts of C18:2 acid and higher levels of C18:0 and C18:1 acids.

Liquid fractions. Figure 3c shows the fatty acid composition of liquid fractions corresponding to those solid at the same temperature. It may be observed that when the temperature was decreased, C16:0, C18:0 and C18:1 acids also decreased. This decrease is less marked in liquid than in solid fractions. Besides, C18:2 acid increased when the temperature was lowered. It should be noticed that the liquid fraction obtained at 38°C showed a fatty acid composition similar to that of the original hydrogenated sunflowerseed oil; then it underwent gradual changes as the temperature was lowered from 38°C to 12°C.

Quantitative determination of triglycerides by HPLC: Solid fractions. From the results obtained by HPLC analysis, fractions could be divided into three groups: the first one corresponds to samples crystallizing at temperatures higher than 30°C; the second to samples crystallizing from 22°C to 30°C; and the third corresponding to fractions crystallizing from 12°C to 22°C. In the case of higher temperatures, these fractions had a high ECN triglyceride content, similar to that of the original sample. In fractions crystallizing at lower temperatures, triglycerides of lower ECN were prevalent. Fractions obtained in the range of 22-30°C had a major proportion of intermediate ECN triglycerides (retention times of 21.5 min and 16.4 min).

Figure 4 represents the percentage of each species *vs.* retention time (T_R) for the fractions at 38°C, 26°C and 12°C in the second experiment. The most representative peaks in the total triglyceride composition are also shown in this Figure. Enrichment of triglycerides with low retention times is evident, *i.e.*, short-chain triglycerides or increasing unsaturations are found to be related to a decrease in the crystallization temperature.

Liquid fractions. Figure 4, corresponding to liquid fractions extracted at 38°C, 26°C and 12°C, also shows the percentage of each component related to its retention times. Liquid samples were analyzed; no triglyceride with retention times higher than 29.1 min could be detected at a percentage higher than 3%. It was observed that changes are more gradual in liquid samples. From the hydrogenated sunflowerseed oil compositions, fractions showed that the triglycerides C18:0 C18:1 C18:1 and C18:2 C18:2 C16:0 decreased, while C16:0 C18:1 C18:1 and C18:1 C18:1 C18:1 increased when the crystallization temperature decreased.

Calculation of the partition ratio. Since this is a solid-liquid system,

$$m_s + m_l = M \qquad [1]$$

where m_s is the solid mass, m_l is the liquid mass and M is the total mass.

Accordingly, for each temperature of crystallization considering the concentration of each component, the mass balance would be:

$$\sum_{T=Ti}^{T_c} m_{sT}\, C_{siT} + m_{lTc}\, C_{liTc} = M\, C_{oi} \qquad [2]$$

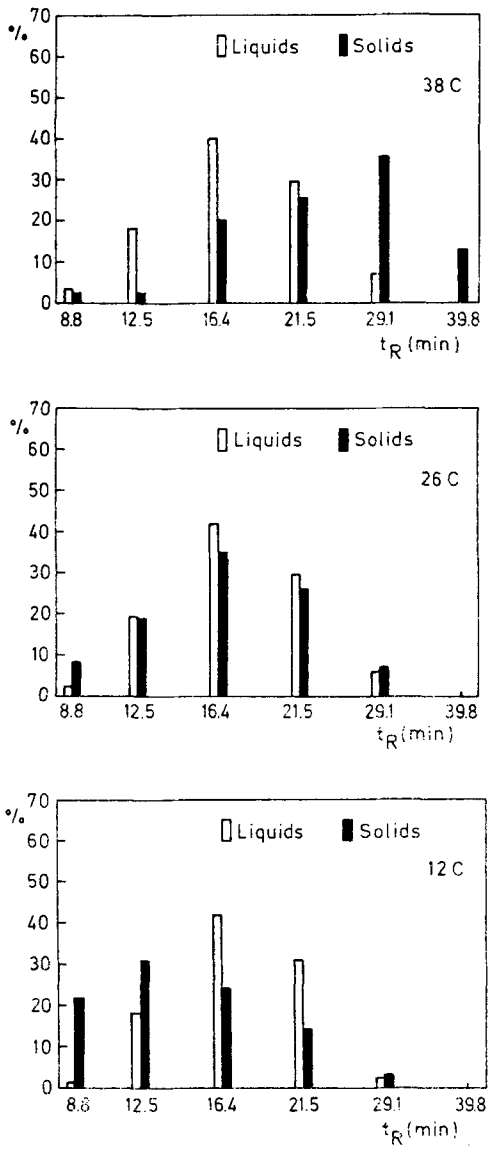

FIG. 4. Percentage for each species *vs.* retention time (T_R) for the fractions at 38°C, 26°C and 12°C in the second experiment.

C_{oi} is the concentration of each component in the original sample, C_{siT} is the concentration of each component in the solid for each range of temperature, m_{sT} is the solid mass crystallized at each range of temperature, m_{lTc} is the liquid mass, and C_{liTc} the liquid concentration of each component at crystallization temperature.

From each phase it is deduced that:

$$C_{sa} + C_{sb} + \ldots + C_{sn} = 1 \quad [3]$$

$$C_{la} + C_{lb} + \ldots + C_{ln} = 1 \quad [4]$$

and from the original sample:

$$C_{oa} + C_{ob} + \ldots + C_{on} = 1 \quad [5]$$

The partition ratio is defined as:

TABLE 3

Partition Ratios

Temperature (°C)	Retention times (min)				
	8.8	12.5	16.4	21.5	29.1
38	0.012	0.001	0.006	0.011	0.067
36	0.026	0.011	0.016	0.028	0.124
34	0.045	0.029	0.029	0.049	0.189
32	0.061	0.048	0.048	0.071	0.250
30	0.082	0.062	0.067	0.095	0.299
28	0.170	0.102	0.102	0.133	0.365
26	0.312	0.143	0.139	0.170	0.426
24	0.624	0.203	0.190	0.215	0.519
22	0.769	0.221	0.202	0.228	0.538
20	0.945	0.246	0.218	0.243	0.569
18	1.051	0.258	0.228	0.252	0.582
16	1.259	0.278	0.238	0.262	0.638
14	1.561	0.300	0.248	0.270	0.656
12	2.876	0.332	0.264	0.282	0.671

$$K_{Tc} = \frac{\sum_{T=Ti}^{T_c} m_{sT}\, C_{siT}}{m_{lTc}\, C_{liTc}} \quad [6]$$

Values obtained were semiquantitative. As has been explained earlier, areas corresponding to overlapping peaks were obtained by drawing a line perpendicular to the baseline. It has been assumed that the detector sensitivity was similar for the evaluation of the five triglycerides under study.

Table 3 shows the partition ratios calculated for triglycerides with retention times of 8.8, 12.5, 16.4, 21.5 and 29.1 min. It has been already mentioned that triglycerides of retention times higher than 29.1 min never amounted to more than 3% in liquids. Triglycerides of retention times lower than 8.8 min appeared mainly in the liquid fraction; in any case, their amounts were unimportant, both in the solid and liquid fractions.

In Figure 5, the partition ratio is represented as a function of the fractionation temperature. These ratios increase in different amounts as the crystallization temperature decreases. This means that the solid is enriched at 12°C in the C18:2 C18:2 C16:0 triglyceride with respect to the solid at 38°C. These data are consistent with those of Figure 4. C18:0 C18:1 C18:1 increases more than C18:2 C18:1 C18:1, C116:0 C18:1 C18:1 and C18:1 C18:1 C18:1 in solid fractions.

For a given temperature of crystallization the numerator in Equation [6] is equal to the total mass of solid multiplied by the concentration that the components in the solid should have at that temperature, calculated as the total content minus the content present in the liquid.

From Equation [2] follows:

$$C_{siTc} = \frac{M\, C_o - m_{lTc}\, C_{liTc}}{m_{sT}c} \quad [7]$$

and from Equation [6]:

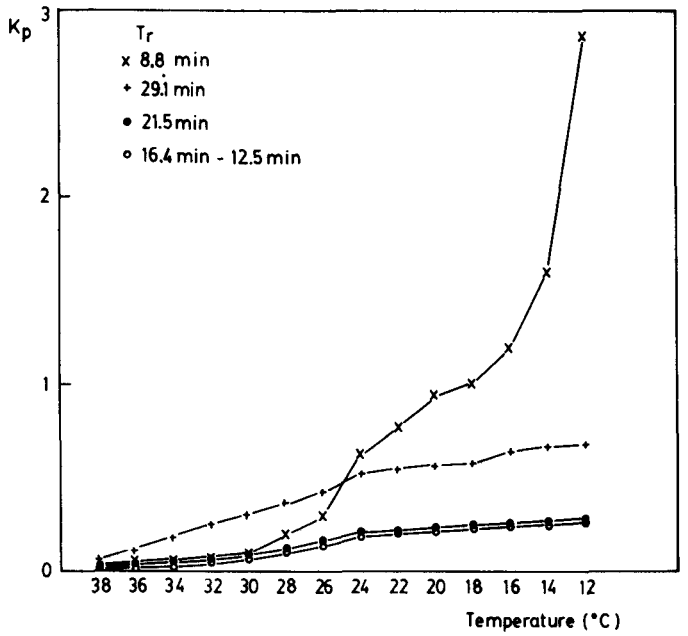

FIG. 5. The partition ratio as a function of the fractionation temperature.

$$\frac{C_{si}Tc}{K_{Tc}} = \frac{m_{lTc}\, C_{liTc}}{m_{sTc}} \qquad [8]$$

Replacing [8] in [7] and arranging gives:

$$C_{siTc} = \frac{M\, C_o}{m_{sTc}} - \frac{C_{siTc}}{K_{Tc}} \qquad [9]$$

Thus, for the liquids we can obtain:

$$C_{si}Tc = \frac{M\, C_o}{m_{sTc}} \quad \frac{K_{Tc}}{K_{Tc} + 1} \qquad [10]$$

$$C_{liTc} = \frac{M\, C_o}{m_{lTc}} \quad \frac{K_{Tc}}{K_{Tc} + 1} \qquad [11]$$

Knowing the total mass and the concentrations of the components in the original sample (C_{oa}, C_{ob},..., C_{on}) it is possible to find a value for m_{sTc} that would be in agreement with the values obtained for C_{siTc} (3). Equation [11] should be processed in the same way. For example, the following values of m_{sTc} were obtained from the mentioned equations for C18:0 C18:1 C18:1, C16:0 C18:1 C18:1, C18:1 C18:1 C18:1, C18:2 C18:1 C18:1 and C18:2 C18:2 C16:0 at 31°C (first experiment): 3765.5 g; 952.6 g; 581.1 g; 109.5 g and 66.3 g. From the solid mass and the percentage of triglycerides obtained by means of HPLC the values were: 3896.4 g; 952.6 g; 593.7 g; 257.1 g and 140.1 g. With this model we can obtain approximate values of triglycerides having a content in the sample higher than 10%.

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