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Fractionation of Linseed Oil Fatty Acids by Crystallization

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Linseed oil fatty acids or their "acid" sodium soaps were crystallized from absolute or aqueous methanol, using temperatures as low as −40°C. The solid and liquid fractions were separated by centrifuge filtration and their fatty acid compositions analyzed by gas-liquid chromatography.

"Acid" soap crystallization was found to give fractions with higher linolenic acid content than the crystallization of free acids under the same conditions. In aqueous methanol, fractionation was more efficient than in absolute methanol at the same temperature and soap concentration. Using absolute methanol, an increase in the soap concentration led to an increase in the linolenate content of the liquid fraction.

Fractional crystallization of "acid" sodium soaps in two stages (−25° and −40°C) gave best results when water was added to the first liquid fraction before the second crystallization. The linolenic acid content of the best fractions varied from 76 to 80 %, the total polyenoic acid content being about 95 %.

Vegetable oils serve as a natural source of the unsaturated C_{18}-fatty acids, but many difficulties are encountered in the separation of such acids from the oils. In laboratory conditions adsorption chromatography,[1,2] urea fractionation,[3–5] low-temperature crystallization,[6–8] and liquid-liquid extraction methods [9–12] can be used to produce small amounts of oleic, linoleic, or linolenic acids with a high degree of purity. On an industrial scale, however, no satisfactory methods for their separation are so far available.

The present paper deals with crystallization experiments of linseed oil fatty acids carried out as a part of a larger research project.[13,14] The purpose of this work was to investigate the possibilities for separating linolenic acid from linseed oil by crystallization without the use of very low temperatures and large volumes of solvents, two factors which hinder the economic separation of unsaturated C_{18}-fatty acids. The main emphasis in this work is directed to "acid" alkali soap crystallization, a method which has been used by certain workers [15,16] when fractionating different natural fatty acid mixtures.

EXPERIMENTAL

Gas-liquid chromatography of fatty acids. The fatty acids were determined as methyl esters with a PYE argon chromatograph.[13] Glass columns were packed with 10 % Craig polyester succinate (BDS) (Wilkens Instrument & Research, Inc.) on 100–120 mesh Celite 545 (Gas Chromatography, Ltd.) which had been treated with acid and alkali. The operating temperature was 195°C, the voltage of the detector 1000 V and the rate of carrier gas about 50 ml/min. Samples of 0.05 or 0.10 μl of methyl esters were injected into the column.

In the quantitation of the chromatograms, the measurements of the peak areas were made by multiplying the peak height by the peak width at half height.

Preparation of linseed oil fatty acids. 300 g of refined linseed oil (purity: Ph. F. VII) was refluxed under nitrogen for 3 h in a mixture containing 250 ml absolute ethanol and 70 g of potassium hydroxide dissolved in 35 ml of water. To remove the unsaponifiable matter, the soap solution was treated with peroxide-free ether and the fatty acids were then liberated by 10 % hydrochloric acid, taken into ether, washed with water and finally dried with anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the fatty acids thus obtained were stored under nitrogen at + 4°C.

The analytical values of the linseed oil acids were as follows: neutralization value 199; fatty acid composition: palmitic acid 8.3 %; stearic acid 5.1 %; oleic acid 23.1 %; linoleic acid 15.3 %; linolenic acid 48.3 %.

Preparation of "acid" sodium soaps. A known volume of methanolic potassium hydroxide was added to the weighed amount of linseed oil fatty acids to neutralize half of the acids. The "acid" sodium soap solution thus obtained was further diluted with methanol to the proper concentration.

Experimental method. 20 ml of the solution to be crystallized was pipetted into a centrifuge filtration tube [13] and allowed to crystallize at constant temperature. To separate the solid and liquid fractions, the tubes were centrifuged 8 min at 2400 rpm at the crystallization temperature (CHRIST, Universal Junior I KS refrigerated centrifuge usable to −50°C). The fatty acid composition as well as the dry matter content of both fractions were determined.

RESULTS

Solutions containing 10 % linseed oil fatty acids or "acid" sodium soaps were crystallized at different temperatures using absolute methanol or methanol-water (90:10 w/w) as solvents. As seen in Table 1, stearic acid-free filtrates were easy to obtain, but small amounts of palmitic acid were present even after crystallization at −40°C. Separation of oleic acid from linoleic and linolenic acids was difficult in absolute methanol but more efficient in aqueous. In the best case, the "acid" soap crystallization lowered the oleic acid content to about 5 %.

The most difficult problem was the separation of linoleic and linolenic acids from each other. Only in aqueous methanol at −40°C was there some tendency to separate. The highest linolenic acid percentage, about 77 %, was reached as "acid" sodium soaps, the yield of this concentrate being about 25 %. From the results obtained, it can be concluded that "acid" soap crystallization seems to be favorable when low working temperatures and large volumes of solvent are avoided.

To investigate the effect of soap concentration on the fractionation, solutions containing different levels of "acid" sodium soaps were crystallized at −25°C in absolute methanol; this solvent makes it possible to work with high soap concentrations. The results are presented in Table 2. With an increase of the soap concentration from 10 to 40 %, the linolenic acid content of the liquid

Table 1. Crystallization of linseed oil fatty acids (A) and their "acid" sodium soaps (B) at a concentration of 10 % (w/w).

	Solvent	Temp. (°C)	Fraction	Yield (%)	Fatty acid composition (w/w %) 16:0	18:0	18:1	18:1	18:3
A	Absol. MeOH	−25	Solid	14	29.5	31.0	13.3	6.7	19.5
		−40	»	26	24.2	19.2	31.4	6.2	19.2
		−25	Liquid	86	2.7	0.2	23.4	19.0	54.7
		−40	»	74	0.7	—	18.5	20.2	60.6
	MeOH-water 90:10 (w/w)	0	Solid	10	24.0	38.6	12.0	5.9	19.5
		−25	»	25	28.2	24.0	29.2	4.2	14.4
		−40	»	68	10.8	7.8	24.5	15.1	41.8
		0	Liquid	90	4.4	0.9	21.6	18.3	54.8
		−25	»	75	0.9	—	17.8	19.4	61.9
		−40	»	32	0.6	—	14.0	18.9	66.5
B	Absol. MeOH	0	Solid	10	30.6	40.0	9.5	4.6	15.3
		−25	»	23	24.5	24.6	31.5	5.1	14.3
		−40	»	30	20.0	17.4	43.7	5.2	13.7
		0	Liquid	90	3.6	0.9	22.7	17.8	55.0
		−25	»	77	1.0	—	18.0	20.1	60.9
		−40	»	70	0.8	—	10.7	21.7	66.8
	MeOH-water 90:10 (w/w)	+ 20	Solid	3	17.3	56.0	11.4	4.4	10.9
		0	»	15	32.4	33.1	11.5	5.5	17.5
		−25	»	30	17.0	14.0	35.6	9.0	24.4
		−40	»	79	10.2	7.5	27.7	13.7	41.0
		+ 20	Liquid	97	6.0	3.2	20.9	16.7	53.2
		0	»	85	1.8	—	20.2	18.8	59.2
		−25	»	70	0.5	—	11.0	20.1	68.4
		−40	»	21	0.3	—	5.1	17.8	76.7

Table 2. The effect of concentration on the composition of the fractions in crystallizing linseed oil fatty acids as "acid" sodium soaps from absolute methanol at −25°C. Crystallization time 24 h.

"Acid" soap Conc. (w/w %)	Fraction	Yield (%)	Fatty acid composition (w/w %) 16:0	18:0	18:1	18:2	18:3
10	Solid	19	32.5	25.2	23.1	4.7	14.5
15	»	26	26.9	16.6	36.0	6.5	14.0
20	»	32	20.9	15.1	37.4	7.4	19.2
30	»	59	12.4	8.4	27.3	12.6	39.3
40	»	93	9.5	5.8	22.7	12.0	50.0
10	Liquid	81	1.2	—	24.0	17.2	57.6
15	»	74	0.8	—	15.0	19.0	65.2
20	»	68	0.6	—	13.2	18.9	67.3
30	»	41	0.7	—	13.0	17.7	68.6
40	»	7	0.5	—	9.7	17.1	72.7

fractions increased from about 58 to 73 %. At the same time the oleic acid content decreased from 24 to about 10 %, but the percentage of linoleic acid remained practically unchanged. From this experiment it can be concluded that the improved selectivity obtained by adding water to the methanol may be partly compensated in absolute methanol by increasing the soap concentration and thus reducing the volume of solvent.

In order to get concentrates with higher linolenate content than by single crystallizations, a simple fractional crystallization procedure (Fig. 1) was used

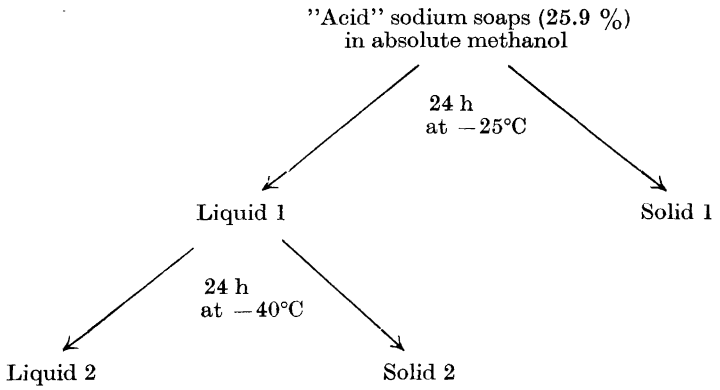

Fig. 1. Fractional crystallization of linseed oil fatty acids as "acid" sodium soaps.

to fractionate "acid" sodium soaps. Absolute methanol was used as the solvent. As seen from Table 3 the linolenate contents of the liquid fractions at −40°C were almost equal to those at −25°C (Table 2), amounting to about 70 %. Concentration of the first liquid fraction (Liquid 1) before crystallization at −40°C increased the linolenate content to about 75−77 % (Table 3 A) while the oleic acid content decreased to 6.6−4.9 %, respectively. No marked changes occurred in the percentages of linoleic acid.

The highest linolenic acid percentages were obtained by adding water to Liquid 1 and then crystallizing at −40°C (Table 3 B). Depending on the amount of water (5 to 15 %) in the methanol, concentrates with about 76−80 % linolenate were obtained, with the oleic acid content about 4−5 %. Some decrease in the linoleic acid percentage also occurred in this case. When the methanol contained 5 and 10 % water, the yields of the liquid fractions were about 20 % of starting material. With a water content of 15 %, which gave the best fractionation, the yield, however, was only 12 %.

DISCUSSION

Among the laboratory methods used for the separation of C_{18}-unsaturated acids from their mixtures, liquid-liquid extraction and crystallization are evidently best suited for large-scale fractionation. Promising results have been obtained by countercurrent extraction,[10,11] and it seems possible to develop methods also on a commercial scale for producing linoleic and linolenic acids

Table 3. Fractional crystallization of linseed oil fatty acids as "acid" sodium soaps (*cf.* Fig. 1) and the effects of concentration (A) and addition of water (B) on the further crystallization of Liquid 1 at −40°C.

		Fraction	Yield (%) of total	Fatty acid composition (w/w%)					
				16:0	16:1	18:0	18:1	18:2	18:3
		Solid 1	44.4	15.8	±	11.6	30.6	12.4	29.6
		Liquid 1	55.6	0.5	±	—	11.3	18.4	69.8
		Solid 2	9.3	2.0	±	±	30.8	18.3	48.9
		Liquid 2	46.3	0.5	±	—	10.0	20.0	69.5
	Concentration of liq. 1 (w/w %								
	25	Solid 2	30.6	0.9	±	—	18.7	19.5	60.9
A	25	Liquid 2	25.0	±	±	—	6.6	18.9	74.6
	30	Solid 2	39.7	0.8	±	—	17.7	19.8	61.7
	30	Liquid 2	15.9	±	±	—	4.9	17.8	77.3
	Water in liq. 1 (w/w % in) MeOH)								
	5	Solid 2	35.2	1.0	±	±	18.9	21.1	59.0
	5	Liquid 2	20.4	±	±	—	4.2	17.6	78.2
B	10	Solid 2	36.1	1.0	±	±	18.0	20.6	60.4
	10	Liquid 2	19.5	±	±	—	5.3	18.4	76.3
	15	Solid 2	43.5	0.8	±	—	16.9	20.5	61.8
	15	Liquid 2	12.1	±	±	—	3.9	16.1	80.0

from vegetable oils with a purity of 90 % or more. By using low temperature crystallization[6], concentrates containing up to 91—92 % linolenic acid have been obtained from linseed oil, but this has required a temperature as low as −75°C and large amounts of solvents. Crystallization without solvents or with only small amounts of them does not lead to marked fractionation of unsaturated C_{18}-acids (*cf.* Ref.[13]).

Very low temperatures can be avoided by using soaps instead of free fatty acids in the fractionation methods. Notevarp *et al.*[17] have fractionated fish oil fatty acids by extracting them as sodium soaps. Other workers [15,16,18] have crystallized different kind of oils, such as soybean, cottonseed, and tall oils, as their "acid" alkali soaps (RCOOM·RCOOH). In the present work, linseed oil fatty acids were crystallized as "acid" sodium soaps. To obtain concentrates with about 80 % of linolenic acid, relatively simple procedures can be employed. The crystallization temperature, however, is as low as −40°C.

"Acid" sodium soap crystallization of linseed oil fatty acids is evidently suited for the production of 70—80 % pure linolenate concentrates with a total polyenoic acid content of about 90 %. Higher concentrations of linolenic acid are obviously not easily obtained by this procedure alone. However, combined with other methods, *e.g.* countercurrent extraction, crystallization might be of practical value. It could be used as a preliminary step in a process

for the commercial separation of C_{18}-unsaturated acids with a purity of 90 % or more.

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