LOW-TEMPERATURE CRYSTALLIZATION OF THE FATTY ACIDS AND GLYCERIDES[1]

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I. INTRODUCTION

Crystallization has been used in the study of the composition of the fats and oils since early in the nineteenth century. The objective of this method of investigation was the isolation of pure naturally occurring glycerides, thus affording definite information on glyceride structure, but the isolation of pure compounds was found to be extremely difficult because of the complexity of composition of the component glycerides of the fats. Only three reports have described the application of very low temperatures to the partial separation of the liquid glycerides of the fatty oils; of these, two have appeared within the past year. As applied to the fatty acids,

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crystallization has been used mainly as a method of final purification of the higher saturated acids and certain solid unsaturated acids. Owing to mixed-crystal formation, it was often necessary to carry out such crystallizations almost a prohibitive number of times. Only within the past five years have serious attempts been made to employ the method in the isolation of the liquid unsaturated acids. In this work temperatures as low as −75°C. were used in order to reduce the solubilities to values which are practical for crystallization methods and to work at temperatures at which the unsaturated acids are crystalline solids.

In the present discussion previous work on the crystallization of both fatty acids and the fats has been reviewed. More emphasis has been placed, however, on recent applications to fatty acid chemistry, because the low temperatures employed are somewhat unusual in this field of investigation and the possibilities of the method seem to be almost without limit in the study and separation of these compounds. Less attention has been devoted to the application of the method to the fats and oils, because most of the work reported in the literature has employed temperatures above 0°C. and because the separations which were achievable by this technic have been disappointing.

II. THE APPLICATION OF LOW-TEMPERATURE CRYSTALLIZATION TO THE SEPARATION OF THE FATTY ACIDS

Discussion of this subject will be confined primarily to work reported during the past five years on certain of the unsaturated acids which occur as major component acids in fats and oils. This work has included the separation and isolation of such acids as oleic and linoleic and the application of crystallization to the partial purification of certain other unsaturated acids. Further, it has applied the technic to the separation of the saturated and unsaturated acids and to the separation of isomeric linoleic and linolenic acids in mixtures resulting from the debromination reaction.

Generally, the naturally occurring unsaturated acids are liquid at room temperature; as a rule, they have much lower melting points than the saturated acid of the same series. Most of the unsaturated acids are infinitely soluble in organic solvents at temperatures above 0°C., while the saturated acids are sparingly soluble at this temperature in such solvents as acetone, petroleum ether, and alcohol. In other solvents, such as ether and chloroform, the solubility differences are not so marked.

Several factors have contributed to the slow progress in the application of crystallization methods to the separation of the fatty acids, and more particularly the unsaturated acids. In the first place, the impression has been general that fatty acids tend to exist in solution in an associated, dimeric state and that they crystallize as such from solutions. Thus with

mixtures, mixed crystals are formed. In the second place, the unsaturated acids have been regarded as oily liquids which are not adapted to this type of procedure. In the third place, the general lack of low-temperature apparatus in most laboratories has not encouraged research in this field.

The low melting points and almost infinite solubilities of the common unsaturated acids at temperatures above 0°C. led to the development of both physical and chemical methods of separation in which higher melting, less soluble derivatives were used. Among the physical methods, fractionation of the methyl esters affected the separation of acids of different carbon series. The use of soaps instead of the free acids served to raise the melting points and lower the solubilities, so that certain separations could be made at temperatures of 0°C. and higher. The most widely used soap method is the official lead soap–ether method (7) or the Twitchell lead soap–alcohol modification (9, 116). The chemical methods have employed high-melting derivatives of the fatty acids, such as the bromine addition compounds and the hydroxy acids. The disadvantages of these chemical methods are familiar to those who have used them. From the work which is reviewed below, it is evident that crystallization procedures for separating the fatty acids from one another and for actually preparing them in the pure state constitute the simplest and most direct methods so far available.

A. The isolation of oleic acid

At room temperature oleic acid is an oily liquid and is infinitely soluble in most organic solvents. Bertram (10) seems to have been the first investigator to crystallize oleic acid from an organic solvent. After the application of certain soap procedures designed to remove saturated acids, he cooled a mixture of equal volumes of oleic acid and acetone to −10° to −15°C. The crystals were further crystallized twice from the same solvent, thereby removing, as he claimed, traces of remaining saturated acids and linoleic acid. Raymond (103) used alcohol at −15° to −25°C. as a crystallizing medium. Both investigators employed concentrated solutions instead of much lower temperatures to reduce solubilities to practical levels.

In 1937 Brown and Shinowara (33) reported the preparation of oleic acid by direct crystallization of the fatty acids of olive oil and without the use of any soap separations. In this work the assumption was made that the fatty acids of olive oil belong to three solubility groups: the higher saturated acids, oleic acid, and linoleic acid. Such an assumption was necessary because of the complete lack of solubility data on oleic and linoleic acids. A solution of 225 g. of olive oil fatty acids in 3450 cc. of acetone was cooled to −20°C. overnight and filtered. The filtrate was cooled to

−60°C. in a dry ice–alcohol bath and again filtered. The resulting crystal fraction was subjected to three further crystallizations at −60°C., each time from 2000 cc. of acetone. By this procedure the more soluble linoleic acid was removed in the several filtrates. After these four crystallizations at −60°C., the product was made up to 1250 cc. with acetone and cooled slowly to the first appearance of crystals, about −35°C. Although this crystal crop was largely oleic acid, it contained some additional saturated acids which had not been removed by the first separation at −20°C. The product remaining in the filtrate was oleic acid, the analytical constants of which were identical for practical purposes with those of another specimen obtained by a similar procedure which included, however, six crystallizations at −60°C. At the time this work appeared, these specimens of oleic acid compared favorably with several of the best preparations previously reported, with the possible exception of the one reported by Bertram, who claimed a purity of 99.5 per cent. The actual purity of these crystallization products, in view of later work, was probably of the order of 96 per cent. Nevertheless the simplicity of this method in comparison with the combination of repeated soap separations, distillation, and crystallization previously used has recommended it to other investigators.

Hartsuch (54) in 1939 made a detailed study of the fractions which appeared during a similar fractionation and verified the fact that linoleic acid is easily removed by crystallization at −60°C. In a series of four crystallizations at this temperature from a 10 per cent solution in acetone, the iodine numbers of the consecutive filtrates were 144.5, 119.8, 92.5, and 89.0, respectively. That of the final crystal fraction was 87.6. Hartsuch removed the remaining saturated acids in part by the use of a modified Twitchell separation but mainly by a careful fractional distillation of the acid. His yield of oleic acid was relatively small; the claimed purity was 97.8 per cent.

J. C. Smith (113) prepared oleic acid by the following procedure: The methyl esters of a "high grade commercial" oleic acid were fractionally distilled to remove palmitic and some "stearic acid". The acids prepared from the resulting mixture of methyl oleate and linoleate were then crystallized from acetone solution to a constant melting point, 13.36°C. Stearic acid prepared from this product by catalytic reduction showed a depression of the melting point indicating the presence of less than 0.2 per cent of palmitic acid. Smith described several types of low-temperature filtration apparatus.

Five months later Wheeler and Riemenschneider (118) reported a detailed procedure for preparing very pure methyl oleate. Since the crystallization procedure failed to remove the palmitic acid entirely, they first made the pure C_{18} methyl esters from olive oil acids by a very careful

fractional distillation, and applied crystallization procedures afterwards. The following steps were described: (*1*) Fractional distillation of the methyl esters of the mixed acids of olive oil to remove palmitic and lower esters. (*2*) Removal of most of the linoleic ester (in the filtrate) by crystallization from acetone (15 cc. per gram) at −60°C. (*3*) Precipitation of the bulk of the saturated esters from acetone (10 cc. per gram) at −37°C. and removal by filtration. (*4*) Further elimination of methyl linoleate by two crystallizations from acetone (15 cc. per gram) at −60°C. (*5*) Fractional distillation *in vacuo* to remove small amounts of remaining methyl palmitate. (*6*) Two crystallizations (at −65°C.) from redistilled petroleum ether (6 cc. per gram; b.p. 30–45°C.) to remove the last traces of linoleate. The iodine number was not changed by the last two crystallizations. The purity was more than 99.6 per cent.

We have recently prepared very pure specimens of methyl oleate and oleic acid by a similar procedure (47), except that the C_{18} methyl esters of olive oil were repeatedly crystallized from methyl alcohol. These esters were composed of approximately 14 per cent linoleate, 83.5 per cent oleate, and 2.5 per cent stearate. A solution of 200 g. of these esters in 4 liters of methyl alcohol was cooled to −60°C. and filtered. The crystal fraction was dissolved in enough methyl alcohol to bring it up to the volume of solution employed in the first crystallization. Five successive crystallizations, each time from the same concentration of solution, were thus carried out. The filtrate fractions decreased from 15.0 g. in the first filtrate to 0.6 g. in the fifth filtrate. The first filtrate was made up of a mixture of methyl oleate and methyl linoleate containing about 88.7 per cent of methyl linoleate, calculated from the iodine number as a binary mixture of oleate and linoleate. The final crystal fraction contained 3.3 per cent of methyl stearate, which was almost completely removed in one step as follows: 100 g. of the oleate–stearate mixture in 4 liters of methyl alcohol was placed in the cold room at −22.5°C. for 2 days. A crop of large flat crystals came down and was removed. The yield was 3.2 g.; the iodine number of the product was 1.5. This was nearly pure methyl stearate. This experiment is reported here because it demonstrates an almost unbelievably sharp separation between two esters of the same carbon series. It is apparent that mixed-crystal formation under the conditions described is negligible.

B. The isolation of linoleic acid

The classic and only method available previously for the preparation of pure linoleic acid has been by the debromination of pure tetrabromostearic acid, obtained by bromination of the fatty acids of those semi-drying oils which do not contain linolenic acid (105). The debromination method

yields a product which is pure as evaluated by iodine number, but it is a wasteful method because more than half of the original acid is lost in the form of soluble, isomeric bromides. Furthermore, the method introduces the question of isomerism, both of the bromides and of the product (30, 96).

In 1935, in connection with some work on linoleic acid, it occurred to the writer that it should be possible to separate this acid from the oleic and saturated acids of cottonseed oil by precipitating the latter acids from solutions in organic solvents at very low temperatures. In our original report (34) of this work we described the separation of the saturated and unsaturated acids of this oil by cooling solutions in acetone and certain other solvents to −20°C. The unsaturated acids thus obtained were fractionally crystallized from acetone and methyl and ethyl alcohols. Typical results are shown in table 1. In this experiment 1 liter of methyl alcohol solution containing 100 g. of unsaturated acids was cooled, and

TABLE 1

Fractional crystallization of the unsaturated fatty acids of cottonseed oil from methyl alcohol

FRACTION	TEMPERATURE	WEIGHT	IODINE NO.	LINOLEIC ACID
	°C.	*grams*		*per cent*
Crystals	−50	25	134.8	50
Crystals	−55	16	159.2	76
Crystals	−60	28	157.7	75
Crystals	−65	15	168.2	86
Filtrate	−65	16	156.5	73

successive crystal fractions were taken off at progressively lower temperatures.

By various modifications of this procedure, crystallization fractions containing from 85 to 93 per cent linoleic acid were isolated from corn oil. Attempts to crystallize methyl esters gave less encouraging results. In a later study Brown and Frankel (30) crystallized the fatty acids of corn oil and obtained a good yield of 93.5 per cent linoleic acid in the fraction crystallizing between −50° and −70°C. Preparation of acid of this purity has made possible a comparison of the properties of linoleic acid, prepared by direct crystallization, with those of the purer acid obtained by debromination. The probable identity of the two products was thus established.[2] In a very recent report Brown and Frankel (31) have described the preparation of pure linoleic acid by a crystallization procedure.

[2] In a recent report from this laboratory (96), described later, debromination linoleic acid has been shown to contain about 12 per cent of an isomer, probably of the *cis-trans* type. The crystallization acid appears to be identical with the major

Platz and Steenbock (101) crystallized the acids of wheat germ oil from acetone. Fractions appearing at −45°, −50°, −55°, and −75°C. were converted into ethyl esters for feeding purposes in a study of rat acrodynia. The iodine numbers of the respective fractions were 117, 141, 149, and 153. The −75°C. fraction, containing about 86 per cent of ethyl linoleate, was found to be of about equal biological activity to ethyl linoleate obtained by debromination. G. O. Burr and coworkers (37) at Minnesota have found our linoleic acids obtained by crystallization to be of the same activity in curing the fat deficiency syndrome as debromination linoleic acid.

C. The use of low-temperature crystallization in the isolation of acids containing three and four double bonds

As in the case of linoleic acid, the usual method for the preparation of pure linolenic and arachidonic acids ($C_{18}H_{30}O_2$ and $C_{20}H_{32}O_2$) has been by the debromination of hexabromostearic and octabromoarachidic acids, respectively. In both cases losses through the formation of soluble, isomeric bromides are considerably greater than with linoleic acid.

Shinowara and Brown (111) attempted the isolation of linolenic acid from linseed and perilla oils. The problem with both oils is more complicated than the isolation of linoleic acid, because, in addition to the presence of the desired linolenic acid (40 to 50 per cent), three additional solubility classes of acids are present: the saturated acids, oleic acid, and linoleic acid. Thus, while it was comparatively easy to remove most of the saturated acids by crystallization from acetone at −20°C., it was more difficult to remove all of the oleic acid, and still more difficult to separate the linoleic and linolenic acids. Solubility differences between the latter two are relatively small. The fractionation was carried out, starting with 500-g. portions of the fatty acids made up to 6 liters with acetone. Data are given in table 2.

Approximate calculations of the solubilities of linoleic and linolenic acids may be made from the data in table 2. At −75°C. the filtrates were mixtures of about one part linoleic acid with three parts of linolenic acid. Assuming the volume of the filtrate at −75°C. to be about 4500 cc. (about one-quarter of the original 6 liters of solution has been removed with the four crystal fractions), the solubility of linoleic acid is 3.3 g. per liter of solution and of linolenic acid 10 g. per liter. At −60°C. the filtrate amounted to about 5000 cc., and there remained in this solution about 170 g. of acids containing about one-third linoleic acid and two-thirds linolenic

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constituent of the debromination acid. In the present discussion the expression "crystallization acid" refers to a product prepared exclusively by crystallization methods; "debromination acid" refers to an acid prepared by reduction of the addition compounds of bromine and the unsaturated acids.

acid. The solubilities of the two acids are 10 and 20 g. per liter of solution respectively. It should be emphasized again that these are rough estimates only, since in this early work accurate measurement of filtrate volumes was not made, and no account has been taken of the presence of oleic acid in these filtrates.

Several other attempts were made without success to increase the purity by varying the conditions of crystallization, the purity of the products ranging from 70 to 77 per cent. Later it was found that repeated crystallization of these products from petroleum ether removed additional amounts of linoleic acid. Thus, six successive crystallizations of 69 per cent linolenic acid from this solvent at −60°C. gave our best product,—namely, an acid of 88 per cent purity. From the preceding data (table 2) it was noted that the solubility ratio of linolenic acid to linoleic acid in acetone was 3 to 1 at −75°C. and 2 to 1 at −60°C. The improvement in purity as a result

TABLE 2

Fractional crystallization of the fatty acids of linseed and perilla oils

FRACTION	TEMPERATURE	LINSEED OIL			PERILLA OIL		
		Weight	Iodine No.	Linolenic acid*	Weight	Iodine No.	Linolenic acid*
	°C.	*grams*		*per cent*	*grams*		*per cent*
I	−23	65	7.8		40	63.9	
II	−45	124	165.2		20	142.3	
III	−60	137	217.6	38	235	220.3	41
IV	−75	118	242.1	65	130	242.8	66
Filtrate	−75	56	252.9	77	65	249.1	74

* Calculated from the iodine number as a binary mixture of linoleic and linolenic acids.

of recrystallization from petroleum ether at −60°C. may have been due to a more favorable solubility ratio, whereby crystal fractions at −60°C. were richer in linolenic acid.

Apparently crystallization procedures have not been applied to other trienoic acids, except in the case of eleostearic acid. Schumann (108) has reported a crystallization method for the estimation of this acid. A detailed procedure has been described by Ku (90), who employed the crystallization of tung oil acids from 76 per cent alcohol at 0°C. It is to be noted that both α- and β-eleostearic acids have relatively high melting points; hence they tend to behave in solubility like the higher saturated acids. Ku reported the solubility of several fatty acids. Some of his data are included in table 4. Ku (90) and Thomas and Thomson (114) prepared their eleostearic acid by recrystallization from alcohol.

The only attempt to isolate a tetraunsaturated acid by low-temperature

crystallization was reported by Shinowara and Brown (112) in 1940. The problem in this study was to separate arachidonic acid from a mixture of the acids of beef adrenal phosphatides. In addition to 20–30 per cent of arachidonic acid, these phosphatides contain oleic and saturated acids. The principal difficulty is due to the fact that both arachidonic acid and its methyl ester remain in the filtrate. Methyl arachidonate is a liquid at the temperature of dry ice. The acid melts at −49.5°C. Both are very soluble in organic solvents, even at −75°C. By the use of either the free acids or the methyl esters it was not possible to prepare a product containing above 75 per cent of arachidonic acid. In one experiment, for example, 175 g. of 56 per cent methyl arachidonate in 800 cc. of acetone, cooled to −75°C., gave 100 g. of filtrate esters, containing only 70 per cent of methyl arachidonate. The principal contaminant here is methyl oleate, which should have been rather completely removed by this treatment, but which was apparently much more soluble in the presence of large amounts of methyl arachidonate. Advantage was finally taken of the difference in boiling points to prepare methyl arachidonate of 95 per cent purity by careful distillation.

Any application of low-temperature crystallization to the preparation of the highly unsaturated acids of fish oils will apparently be limited by the considerations described above. It is likely that the solubilities of these acids with four, five, and six double bonds and of carbon series from C_{18} to C_{22} will be too great even at temperatures below their melting points to make it possible to isolate them in the form of crystal fractions. Actual crystallization is necessary to attain any high degree of purification.

D. The preparation of pure methyl ricinoleate

The isolation of several specimens of methyl ricinoleate of very high purity by low-temperature crystallization of the methyl esters of castor oil was reported in 1940 by Brown and Green (32). Both acetone and methyl alcohol were used as solvents, the latter being somewhat the better. The crystal fraction coming down between −40° and −65°C. from a 5 per cent solution in methyl alcohol was 98 per cent pure, as evaluated by the acetyl number. This degree of purity, as a result of a single crystallization, is believed to be due to the chemical dissimilarity of methyl ricinoleate from the other esters present,—oleate, linoleate, and saturated esters,—as a result of which the formation of mixed crystals is very slight.

E. Preliminary observations on chaulmoogric and erucic acids

Our work on these acids is still not complete. These brief descriptions are included to illustrate the application of the method in the partial purification of these solid unsaturated acids.

The high melting points of chaulmoogric and hydnocarpic acids, 71° and 59°C., respectively, and the low (not reported) melting point of gorlic acid, the three principal acids of chaulmoogra oil, suggest that crystallization from organic solvents might afford a convenient fractionation procedure. Preliminary work (11) shows that, starting with the 5 per cent solution of the fatty acids of chaulmoogra oil in acetone, chaulmoogric acid, along with the saturated acids, comes down principally in the crystal fraction at −20°C., hydnocarpic acid in the crystals at −50°C., and that gorlic acid remains in the filtrates at −50°C. Gorlic acid is still quite soluble in acetone at −60°C.

Erucic acid, which makes up 40 to 50 per cent of the acids of rape seed oil, has been prepared by Holde and Wilke (76) from the acids of the ether-insoluble lead soaps by recrystallization from alcohol in order to remove saturated acids. In preliminary experiments in our laboratory (6) we have found that erucic acid comes down from an acetone solution in the −20°C. crystal fraction along with the saturated acids. When this product in 5 per cent solution in acetone is cooled to about −8°C., a small crop of crystals, mainly saturated acids, forms and is removed. On cooling further to −20°C., the crystal fraction is crude erucic acid (iodine number 72 and molecular weight 336; theoretical values, 74.5 and 338, respectively).

It is likely that other solid unsaturated acids, such as isoöleic acid, may be conveniently separated from the saturated and liquid unsaturated acids by proper choice of solvent and temperature. Elaidic acid, the *trans*-isomer of oleic acid, may be easily separated from oleic acid by crystallization.

F. The determination of saturated acids

Lewkowitsch quotes Fachini and Dorta (45) as having first tried separating saturated and unsaturated acids by cooling petroleum ether solutions to −40 to −45°C. In our first paper (34) on low-temperature methods we described the separation of the saturated and unsaturated acids of cottonseed oil by cooling solutions of the acids in methyl or ethyl alcohol, acetone, and petroleum ether to −20°C.[3] Five out of seven trials with these solvents gave saturated acids of iodine number from 3.4 to 7.2. Ten to 12 per cent solutions were employed and the crystals were washed with cold solvent. We suggested then that the method might be used as a substitute for the official lead soap–ether or –alcohol procedures. Such a method could not be successful, however, either if association in solution between the two classes of acids were appreciable, or if they tended to form

[3] This method has been used by Dermer and Crews (41) in their study of the oil of *Sapindus Drummondii*.

mixed crystals. To minimize these effects, it seemed likely that solutions of lower concentration than 10 per cent would have to be used. On the other hand, with very dilute concentrations the solubility of the saturated acids would be an important error.

An excellent study of a crystallization method for the determination of the saturated acids in soybean oil has been described by Earle and Milner (43). In their method a 5-g. sample of the mixed fatty acids is dissolved in 50 cc. of acetone and cooled to −40°C. The crystals are filtered off and twice crystallized from the same volume of solvent at the same temperature. Results by their method agreed well with those they obtained by an oxidation procedure (Bertram's) as described by Pelikan and Von Mikusch (99) and were about 2 per cent higher than results by the Twitchell method. The liquid acids obtained by the Twitchell procedure were shown to contain long-chain saturated acids when examined by both of the other procedures. Earle and Milner stated that their method had been used with success on one specimen of cottonseed oil, but that it would probably have to be modified for use with highly unsaturated oils, such as perilla oil or for oils such as olive oil, which is high in oleic acid. Incidentally, they found the solubilities of stearic, palmitic, and myristic acids in acetone at −40°C. to be 1, 8, and 75 mg. per 100 cc., respectively.

We have analyzed eight specimens of fats and oils (102), including human fat, lard, beef tallow, cottonseed oil, and olive oil by a single crystallization procedure. A 20-g. sample of the acids is dissolved in 400 cc. of acetone and allowed to stand at −20° to −25°C. overnight. The crystals are filtered on a small Büchner funnel and washed several times with cold solvent. The crystal and filtrate fractions are quantitatively recovered and weighed. The results by this method were compared with those obtained by the lead soap–ether procedure. The iodine numbers of the saturated acids by the latter method ranged from 3.26 to 31.40, while those by the former gave values of 0.28 to 6.40. However, the contents of saturated acids by the crystallization method were generally 10 to 30 per cent lower than those by the soap method. The low iodine numbers by the former method indicate that mixed-crystal formation is relatively slight under these conditions, but, on the other hand, it seems likely that for quantitative results somewhat lower temperatures will have to be employed in order to reduce the solubilities of the saturated acids, especially myristic and palmitic. A comprehensive study of the application of this method to the analysis of a wide variety of fats and oils is desirable. It should be possible to work out a "single crystallization" method for most classes of fats and oils, thus avoiding the several crystallizations of the Earle and Milner method.

A quick low-temperature method for separating high- and low-titer fatty

acids has been reported recently by DeGray and DeMoise (40). The method involves cooling 1 to 2 per cent solutions of acids in petroleum ether to −45°C. or below. With one crystallization they claimed an efficiency of separation of 95 per cent. They described a jacketed funnel for low-temperature filtration.

G. The application of low-temperature crystallization to the separation of isomeric unsaturated acids

For many years it has been known that the *cis–trans* isomers, oleic and elaidic acids, may be separated by crystallization. The ease with which elaidic acid may be purified has been the basis of its use by Kass and coworkers as a standard for the determination of the thiocyanogen number

TABLE 3

Separation of debromination linoleic acid by crystallization at −60°C.

	WEIGHT	TETRA-BROMIDE NO.	$n^{20°}$	ISOMERIC ACID	IODINE NO.
	grams			*grams*	
Original acid*	170	90.6	1.4699	20.6	181.2*
Filtrate I	8.8	44.8	1.4700	5.0	179.0
II	7.4	45.8	1.4700	4.1	177.5
VIII	3.5	66.7	1.4700	1.2	179.5
X	5.2	93.9	1.4699	0.5	181.1
XII	5.1	97.9	1.4700	0.25	180.7
Final crystals†	92	102.9	1.4699	0.0	181.0

* Melting point −8.8 to −7.1°C.
† Melting point −5.2 to −5.0°C.

of the 9,10-octadecenoic acids (78) and, by hydrogenation, as a method for the preparation of pure stearic acid (77). From structural considerations, mixed-crystal formation between oleic and stearic acids and oleic and elaidic acids,—in fact, between *cis–trans* isomers generally,—should be slight. Crystallization, therefore, should constitute an excellent method for separating isomers of this type.

Very recently we have succeeeded in resolving debromination linoleic acid into two acids by repeated crystallization at −60°C. (96). The original debromination acid, containing about 12 per cent of an isomeric linoleic acid, in a 2 per cent solution in petroleum ether was crystallized twelve times from constant volumes of solution, the filtrates and final crystal fractions being recovered and analyzed. The results in part are shown in table 3.

In table 3 the amounts of isomeric acid were calculated on the assumption that its tetrabromide number is zero and that of true linoleic acid is 102.9. Filtrate I above is a 60–40 mixture of the isomeric acid with linoleic acid. Assuming the filtrate to be saturated with both acids, their relative solubilities are 3:2, provided also that at this high dilution mutual solubility effects are not important. Each of the twelve filtrates was saturated with linoleic acid, but each contained progressively less of the contaminating impurity. Thus, at the expense of about half of the wanted material, the impurity was practically quantitatively removed. Equally satisfactory results were obtained in the separation of debromination linolenic acid.

H. Discussion

From the preceding review of work already accomplished, it has been shown that low-temperature crystallization is a useful procedure for the separation and purification of fatty acids, especially the unsaturated acids. In the temperature range from 0° to −75°C., the solubilities of the common unsaturated acids vary from infinite solubility to one of the order of less than 0.1 per cent, a value comparable with those of the higher saturated acids at 20° to 0°C. Further, oleic, linoleic and linolenic acids, usually regarded as oils, are crystalline solids at low temperatures. Usually, but not always, crystal crops of these acids filter with great ease, and hence are well adapted to laboratory manipulation.

The possibilities of the crystallization procedure as a technic of research on the fatty acids are almost without limit. So far only a beginning has been made in this field. The greatest need at the present time is for accurate data on the solubilities of the acids in a large series of organic solvents and over a range of temperatures down to the lowest limit of dry ice. Such a study has been under way in our laboratory for the past year, but the difficulties are very great, more particularly the preparation of fatty acids of undoubted purity, and the development of low-temperature apparatus which can be held constant at the very low temperatures long enough to prevent supersaturation. We hope to have a preliminary report on this work soon.

In the meantime, it has been possible from the data above to make a number of *approximate* calculations of solubilities at the very low temperatures. These calculated values, along with the more important previously reported solubilities of myristic, palmitic, and stearic acids, are summarized in table 4.

Two important limiting factors in crystallization procedures are molecular association (compound formation in solution) and mixed-crystal formation. Waentig and Pescheck (117) in 1919, in a study of the mutual

solubility of the fatty acids, observed that palmitic acid is two and one-half times as soluble in carbon tetrachloride containing lauric acid as in

TABLE 4

Solubilities of the fatty acids

ACID	SOLVENT	TEMPERATURE, °C.	SOLUBILITY	REFERENCE
Myristic acid...	Petroleum ether	0	More than 1 per cent	(45)
	Petroleum ether	−18 to −20	0.092 g. per 100 cc. solvent	(45)
	Acetone	−40	0.075 g. per 100 cc. solvent	(43)
Palmitic acid...	94.4% alcohol	0	1.298 g. per 100 cc. solution	(55)
	100% alcohol	0	1.45 g. per 100 g. solvent	(106)
	95.0% alcohol	0	0.56 g. per 100 cc. solvent	(88)
	100% alcohol	10	2.8 g. per 100 cc. solution	(46)
	Petroleum ether	0	0.2578 g. per 100 g. solvent	(45)
	Petroleum ether	−18 to −20	0.0291 g. per 100 g. solvent	(45)
	90.0% alcohol	0	0.45 g. per 100 g. solution	(90)
	Acetone	−40	0.008 g. per 100 cc. solvent	(43)
Stearic acid.....	94.4% alcohol	0	0.155 g. per 100 cc. solution	(55)
	100% alcohol	0	0.37 g. per 100 g. solvent	(106)
	100% alcohol	5	0.51 g. per 100 g. solvent	(106)
	100% alcohol	10	1.10 g. per 100 g. solvent	(106)
	95.1% alcohol	0	0.1139 g. per 100 cc. solvent	(44)
	100% alcohol	10	0.90 g. per 100 g. solvent	(46)
	90.0% alcohol	0	0.88 g. per 100 g. solution	(90)
	Petroleum ether	0	0.047 g. per 100 g. solvent	(45)
	Petroleum ether	−18 to −20	0.010 g. per 100 g. solvent	(45)
	Acetone	−40	0.001 g. per 100 cc. solvent	(43)
Oleic acid.....	76.0% alcohol	0	28 g. per 100 g. solution	(90)
Methyl oleate...	Methyl alcohol	−22.5	Over 25 g. per liter	(47)
	Methyl alcohol	−60	0.15 g. per liter	(47)
Linoleic acid....	Acetone	−60	About 10 g. per liter	(96)
	Acetone	−75	About 3.3 g. per liter	(96)
	Petroleum ether	−60	About 1.6 g. per liter	(96)
Linolenic acid...	Acetone	−60	About 20 g. per liter	(96)
	Acetone	−75	About 10 g. per liter	(96)
	Petroleum ether	−70	About 1.0 g. per liter	(96)

the pure solvent. The formation of easily soluble compounds containing both acids was assumed to take place. Similar compound formation was observed in chloroform, benzene, toluene, and nitrobenzene, but not in

alcohol, ether, or ethyl acetate. Association tends to increase the solubility of the more insoluble constituent, and would be likely to increase the tendency to mixed-crystal formation. Other studies in this field have been made by Francois (50), Boutaric and Roy (26), and Broughton (29). Very recently, Brocklesby (28) in a study of stearic, oleic, and linoleic acids has shown that association decreases with increase in unsaturation.

The modern view of the crystal structure of the saturated acids is that the crystal layers are made up of units of two molecules of acids, bound together by residual valences at the carboxyl groups (48, 49, 58, 97, 98, 100, 115). The tendency to form mixed crystals is the greater, the more nearly alike the fatty acids, and is marked with the higher saturated acids. Bruni and Gorni (35, 36) and Mascarelli and coworkers (92–95) have shown, on the other hand, that such naturally occurring *cis*-forms as oleic and erucic acids do not form solid solutions with their respective saturated acids, stearic and behenic, while the *trans*-forms do form such solutions. Smith (113) found no evidence of compound formation between oleic and stearic acids. Many of the separations described above were so clear-cut that it seems likely that mixed-crystal formation under the conditions studied is not an important item in the separation of the saturated from the unsaturated acids and in the separation of the several classes of the unsaturated acids from each other.

Another important difficulty in crystallization procedures is contamination of the crystal fractions with mother liquor and, as a consequence, with filtrate acids. With the cottonseed oil acids the unwashed crystals, even with efficient suction filtration, contained four to five times their weight of solvent. This represents a tremendous contamination of the true crystal fraction with actual filtrate acids. This difficulty can be overcome in part either by the use of dilute solutions, by efficient washing with cold solvent, or by repeating the crystallization.

The crystallization procedure may be applied advantageously to the mixed fatty acids of most fats and oils without preliminary treatment. Thus, in most cases, the acids may be divided into three or four solubility groups: saturated, monoenoic, dienoic, and trienoic. In the case of the fish oils and the lipids of certain animal organs, such as the adrenal gland, the trienoic acids may be replaced by other highly unsaturated acids of the four, five, and six double bond types. In this very general classification each solubility group may include one or more minor component acids. With fats and oils of more complicated fatty acid composition, the method may be more effectively applied to individual carbon series of acids, thereby necessitating the preparation and efficient distillation of the methyl esters. If desirable, the esters themselves may be crystallized from solvents.

III. FRACTIONAL CRYSTALLIZATION OF THE FATS AND OILS

Before 1936 fractional crystallization was applied to the study of the fats and oils primarily as a method for the isolation of individual, naturally occurring glycerides. Reviews of this work have been given by Lewkowitsch (91), Grün (52), Schönfeld and Hefter (107), and Hilditch (61). Of these, the review by Hilditch (in reference 107) is the most complete. Furthermore, in this review Hilditch discusses two allied lines of investigation,—namely, the crystallization of the hydrogenated fats and the crystallization of the brominated fats and oils, both of which are not within the scope of the present paper. Since 1936 Hilditch and his coworkers have published a series of papers in which crystallization has been used as a preliminary step to the application of their chemical methods of study of fat structure. Because of the relatively low solubilities of the glycerides, and because the fats crystallize easily from solvents, most of the work so far reported has employed temperatures above 0°C.; hence it will be reviewed only briefly here. Only very recently have low temperatures been applied to the crystallization of the lower melting constituents of oils.

A. The isolation of naturally occurring glycerides by the fractional crystallization of fats

The isolation of naturally occurring glycerides by fractional crystallization, probably attempted before 1820 by Chevreul, was reported in detail by Duffy (42) in 1853. By a series of thirty-two crystallizations of mutton tallow from ether at 16°C., he succeeded in isolating 8 g. of tristearin from 2 kg. of the tallow. A similar experiment with beef tallow yielded only 2 g. of the same glyceride. Incidentally, he described double melting point phenomena which he called the "isomeric transformation of fats." Heintz (56) also used ether as a solvent in 1855. Blyth and Robertson (12) in 1889 crystallized butterfat from alcohol–ether and obtained the mixed triglyceride butyroöleopalmitin. When ether has been used as a solvent, the glycerides have been caused to crystallize by evaporation of the solvent or by the addition of alcohol, in which they are sparingly soluble. Other solvents which have been used are chloroform and ether by Fritzweiler (51), acetone by Klimont (81), and chloroform and acetone by Seitter (110).

Since about 1900 most of the researches on crystallization of fats have been reported by Klimont (80–87), Amberger (1–5), and Bömer (13, 16–21, 23–25), and their coworkers. Other important investigations, however, have been described by Heise (57), Henriques and Künne (59), Holde and Stange (75), Hansen (53), Kreis and Hafner (89), Fritzweiler (51), Seidenberg (109), Brash (27), Hilditch and Jones (64), and Kino (79). In Hilditch's review of this work (107), some twenty-five glycerides are

described as a result of the application of this technic. Among these are the simple triglycerides of lauric, myristic, palmitic, stearic, and oleic acids. The remainder are mixed triglycerides. About twenty fats and oils have been thus studied. In some cases literally hundreds of crystallizations were necessary in order to achieve the isolation of pure compounds.

The general procedure, used by Bömer and typical of the method (20, 24), is briefly described as follows: 1 to 2 kg. of the fat is dissolved in 2 to 3 volumes of ether, benzene, chloroform, or a similar fat solvent. The solution is allowed to stand at progressively lower temperatures for 1 to 24 hr., or the solubility is lowered by the addition of alcohol, and the resultant crystal fractions are removed by suction filtration. Each of the fractions is further divided by similar procedures into three or four subfractions, finally combining those that melt within a 5°C. range. Unsaturated glycerides are removed by saturating them with the Wijs reagent and crystallizing until they are free of halogen. As the refractionation proceeds, subfractions are united of 2°, 1°, and finally 0.5°C. melting range. Crystallizations are continued until the desired results are obtained.

Bömer and Limprich (22) have reported an ingenious crystallization method for the identification of certain glycerides, and Bömer (14, 15) has employed the technic for the detection of tallow in lard and of lard in vegetable fat and butterfat.

As a method of study of the composition of fats the method of crystallization has been disappointing, mainly on account of the complexity of composition of the naturally occurring glyceride mixtures.[4] Even with a fat containing only three fatty acids, and few so simple as this are known, there are theoretically possible eighteen triglycerides: three homogeneous simple triglycerides, twelve partly homogeneous glycerides, such as palmitodistearin, and three completely heterogeneous glycerides, such as palmitostearomyristin. It is clear that if all three of the fatty acids were saturated and of high molecular weight, the differences in solubility would be too slight to make practical separations possible. Even if one of the acids were unsaturated, such as oleic, if anything approaching the theoretical number of possible compounds were present, they would display such a gradually changing series of solubilities as to make separations very difficult.

The value of the crystallization technic as applied to the study of fat structure is excellently summarized by Hilditch (60) in the following tribute to Bömer and to the method, as follows: Bömer "subjected many fats to examination by the physical method of crystallization, supplemented in many cases by vacuum distillation. His methodical and sustained attack on this problem, involving several hundred crystallizations in the case of

[4] The complexity of the composition of fats and oils is further discussed in the paper by Dr. Longenecker (Chem. Rev. **29**, 201 (1941)) in this Symposium.

cocoanut and palm kernel oils, demonstrated beyond all doubt the mixed nature of naturally occurring triglycerides. The complexity of the mixtures of glycerides usually present and the close similarity in solubility and other physical properties made it impossible, as we now realize, to achieve adequate separation of most fats by physical methods alone and Bömer was rarely able to obtain more than fragmentary quantitative information; and when in due course the use of chemical methods of attack gave quantitative proof of this tendency for the different acyl groups to be distributed very evenly or indiscriminately amongst the glyceride molecules, the validity of Bömer's results was amply corroborated."

B. The separation of fats into crude fractions, preliminary to further chemical study

In 1936 Hilditch and coworkers (38, 39, 62, 63, 65–74) began to employ crystallization, usually from acetone at 0°C., as a method of dividing fats into a few simple fractions as a preliminary step before applying the chemical method to the study of the glycerides. Such fractions were intended to contain a relatively simple mixture of glycerides of similar solubilities. The number of fractions varied from two to seven, depending on the fat being studied. For example, Bushell and Hilditch (38) separated Borneo tallow into six fractions of iodine numbers 29.6, 33.9, 33.5, 33.3, 48.3, and 47.2. Likewise from cacao butter Hilditch and Stainsby (74) obtained five fraction of iodine numbers from 28.2 to 50.1.

The application of very low temperatures to the crystallization of an oil was reported in 1901 by Holde and Stange (75), who cooled an ether solution of olive oil to −40 to −45°C. The crystal fractions were recrystallized at 0°C., whereby an impure oleodipalmitin was obtained. They demonstrated for the first time the almost complete absence of triolein in this oil.

Within the past year two low-temperature crystallizations of cottonseed oil have been reported. Hilditch and Maddison (66) subjected a 33 per cent solution of the oil in acetone to a temperature of −10°C. The final crystallization of the liquid glycerides was from a 20 per cent solution at −35°C. The iodine numbers of the six fractions finally obtained were 38.3, 57.0, 97.0, 107.9, 124.7, and 134. Obviously this represents a wide range of unsaturation in the several fractions.

The most detailed application of the method to any oil has been reported by Riemenschneider, Swift, and Sando (104). One kilogram of cottonseed oil was crystallized according to the scheme in chart 1. Data on the several fractions are shown in table 5.

The separations achieved in this work are remarkable, especially fractions F and G which contained, respectively, 71.4 and 79.0 per cent of linoleic acid. Although these fractions represent only 19 per cent of the

original oil, they undoubtedly contain considerable amounts of trilinolein, thus demonstrating the occurrence of small amounts of this triglyceride

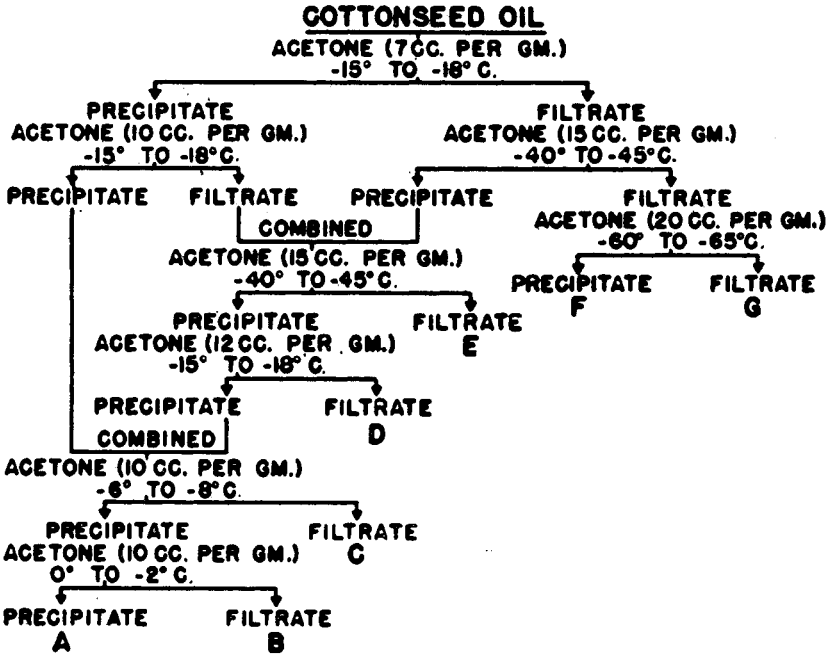

CHART 1

TABLE 5

Analysis of fractions obtained by crystallization of cottonseed oil

FRACTION	WEIGHT	IODINE NO. (WIJS)	THIOCYANOGEN NO. (3 HR.)	FATTY ACID COMPOSITION		
				Linoleic acid	Oleic acid	Saturated acids
	grams			*per cent*	*per cent*	*per cent*
A	149	52.5	30.8	25.0	10.6	64.4
B	11	58.2	33.5	28.5	10.2	61.3
C	18	66.3	38.3	32.3	12.0	55.7
D	451	107.1	62.1	51.9	19.9	28.2
E	154	133.4	74.8	67.6	18.8	13.6
F	145	140.6	78.8	71.4	19.7	8.9
G	48	148.3	79.9	79.0	13.3	7.7

in cottonseed oil. In comparison with the above results, it is interesting to note that the same oil, when subjected to molecular distillation, gave fifteen fractions, the linoleic acid content of which ranged from 48.0 to

59.6 per cent. The advantages of fractional crystallization in this instance are obvious.

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