Stepwise Purification of Fatty Acids: Compared Fractional Crystallization with Urea or from Acetone Solutions of Palmitoleic, Heptadecenoic, and Oleic Acids

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ABSTRACT

Stepwise partition of a mixture of hexa- (C16:1ω7), hepta- (C17:1ω8), and octadecenoic (C18:1ω9) acids was thoroughly investigated. The efficiency of different rates of three fractionation procedures (i.e., fatty acid crystallization from acetone or fatty acid methyl esters crystallization from acetone or with urea) was compared. Fatty acid urea adducts formation is best suited for stepwise removal of the bulk of C18:1 (accumulated in the crystals) and fatty acid methyl ester crystallization from acetone to remove C16:1 as well as small quantities of C18:1 (both being removed in the filtrates). Whatever the technique, high crystallization rates were more efficient.

INTRODUCTION

Considerable interest was devoted to fractional crystallization techniques during the 1940s and 1950s. Purification of unsaturated fatty acids by very low temperature crystallization from acetone was first achieved by Brown and Stoner (1) and by Brown and Shinowara (2) in 1937. Similarly, urea adducts formation with aliphatic compounds was discovered by Bengen (3) in 1940, and the results of detailed investigations of the formation of complexes and steric limitations were reported by Bengen and Schlenk in 1949 (4-5). Both techniques are very appealing in that they allow the handling of great quantities of fatty acids and do not cause any damage to the molecular structure.

Combinations of both techniques have often been used successfully for the stepwise purification (up to 99 p.100) of various fatty acids (6-12). The potentially numerous applications of low temperature fractional crystallization in solvents, have been reviewed by Brown (10) and Brown and Kolb (13); while the interest in fractionation procedure in urea has been underlined by Schlenk and Holman (11) and by Iverson and Weik (14). Valuable information on fatty acid solubilities in acetone (10,13,15) or on urea complexes formation rate according to fatty acid structure (11,14) has been published. Nevertheless, the authors only stated that individual fatty acid behavior in stepwise crystallization was altered by the other fatty acids in the mixture (10,13) and did not document these findings. Moreover, numerous data applying to most fatty acids have never been published.

In a companion paper by Bauchart and Aurousseau (16), a method of purification of a large quantity of C17:1 from *Candida tropicallis* yeast was described. The characteristics of C17:1 crystallization with urea or from acetone at a low temperature, alone or in mixtures, had never been described before. Furthermore, this work brought to light some interesting crystallization features of monounsaturated fatty acids (C16:1, C17:1, C18:1) in mixtures of variable composition. These results may be considered as a model for behavioral studies of fractional crystallization for a given fatty acid compared to two others from the same sample whose physical properties differ very little from those of the first one, and moreover, in opposite and only slightly asymmetrical ways. These data thus seemed very valuable for other purification procedures.

MATERIALS AND METHODS

Detailed information on operating conditions for extraction of lipids, crystallization in acetone or in urea, and control of efficiency of each step are published in a companion paper (16).

Fractional crystallization steps, carried out in the course of preliminary work or actual C17:1 purification, provided a wide range of fatty acid mixtures. Their C16:1, C17:1, and C18:1 content ranged respectively from 0.5 to 28%, 15 to 99%, and 0.5 to 23%.

Concentrations for fractional inclusion complex formation with urea varied as follows: for 1 part of fatty acid methyl ester, 1 to 10 parts of urea and 10 to 25 volumes of methanol were used. Under these conditions, crystallization rates defined as the ratio of total amount of fatty acid incorporated in the crystals over the total amount in the corresponding treated fraction varied from 5% to 95% of total fatty acid methyl esters.

Concentrations used for crystallization from acetone ranged from 1% to 10% by weight fatty acid or fatty acid methyl ester, and the crystallization rates ranged from 10% to 95%.

The efficiency of each step of fractional crystallization of a given fatty acid was estimated by its partition coefficient which is the ratio of the amount of the fatty acid incorporated into the crystals over its total amount in the corresponding treated fraction.

RESULTS AND DISCUSSION

The results were all plotted together. On the one hand, when inclusion complexes were formed with urea, C18:1 was incorporated in the crystals more easily than C16:1 and C17:1 (Fig. 1). This was especially true for mild rates of crystallization (between 40 to 80%) which made this technique valuable for the elimination of the first compound from the mixture. Meanwhile, C16:1 was not incorporated into the crystals much less than C17:1.

On the other hand, fractional crystallization assays from acetone at -60 C revealed that C16:1 did not crystallize as easily as did C17:1 (Fig. 2). This was especially true for the highest rates of crystallization (90 to 95%) which made this second technique valuable for the partition of these two compounds.

The observed overall dispersion of the results was somewhat high. This was due to the wide conditions of substrate concentration or chemical structure (fatty acid or fatty acid methyl ester), samples composition, and volumes handled. The biggest dispersion of the results was related to the relative incorporation of C18:1 in the crystals during fractionation from acetone. With high concentrations of this acid in the mixture (10% and more), it was preferentially incorporated in the crystals, while with low concentrations, a crystallization pattern similar to that of C16:1 followed.

Careful analysis of the whole set of data allowed a

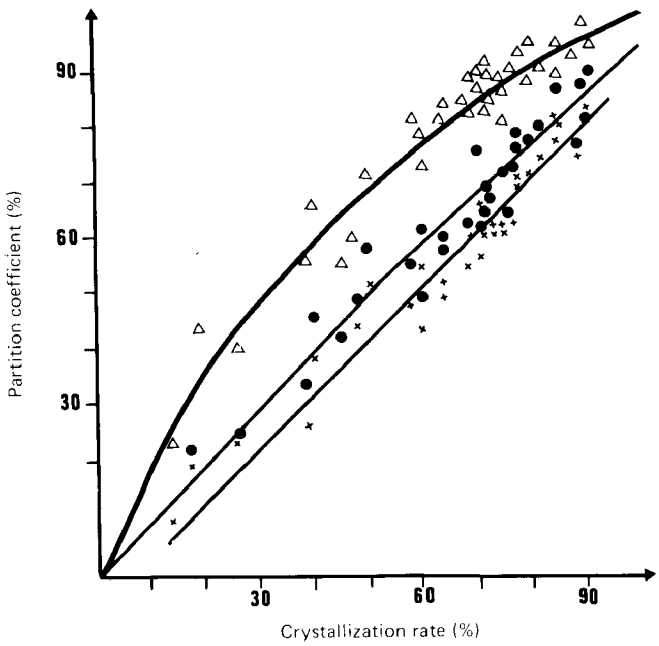

Figure 1

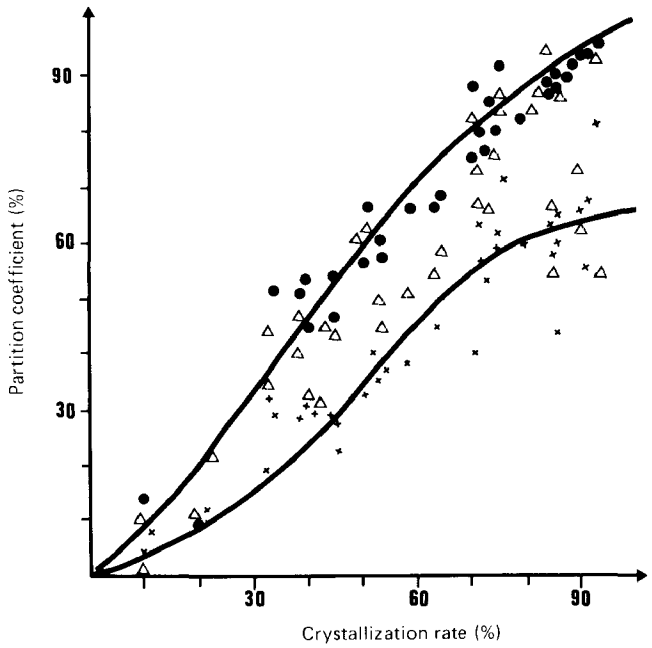

Figure 2

FIGS. 1 and 2. Comparative study of the behavior of monounsaturated fatty acids as related to the crystallization rate with urea at 4 C (FIG. 1, fatty acid methyl esters) and from acetone at -60 C (FIG. 2, fatty acids and fatty acid methyl esters). x = C16:1ω7; • = C17:1ω8; △ = C18:1ω9.

thorough comparison of the usefulness of crystallization techniques and of their limits. Inclusion in urea adducts of 70%, 80%, or 90% of total fatty acid methyl esters could thus be obtained, when operating at 4 C, by adding to 1 part of fatty acid methyl ester 20 volumes of methanol, and respectively about 3, 3.5, or 4 parts of urea. The partition of each fatty acid between filtrates and crystals was related to the crystallization rate (Table I) and was only slightly altered along with variations of their concentrations.

Similarly, under the operating conditions used (glass device shape and temperature, time of crystallization, filtration duration), crystallization rates of 70%, 80%, or 90% respectively, were obtained for substrate concentrations in acetone of 4%, 4.5%, or 6.5% by weight of fatty acid or 5%, 5.5%, or 10% by weight of fatty acid methyl ester. This makes it obvious that crystallization was not completed, and that the amounts of fatty acids or fatty acid methyl esters gathered in the filtrate were well above their solubility level. The partition coefficient of monounsaturated fatty acids (Fig. 3 and 4) was related to the crystallization rate and could be altered with the type of fatty acid mixture considered. On these figures, the curves (loaded with symbols) show the mean variations of partition coefficients in the case of a simple mixture of monounsaturated fatty acids (C16:1, C17:1, and C18:1). The hatched areas show the extent of the observed dispersion in the case of mixtures including saturated fatty acids. These areas would most likely extend under the curves in the case of mixtures including polyunsaturated fatty acids. In the case of fatty acid methyl ester crystallization from acetone, only simple mixtures of monounsaturated fatty acids were handled so that no dispersion of the results was observed. The partition coefficients for C17:1 were not plotted since they were relatively more dependent on the varying

TABLE I

Partition Coefficient (%) of the Three Monounsaturated Fatty Acids Considered during Fractional Crystallization with Urea

Crystallization rate (%)	Fatty acids		
	C16:1ω7	C17:1ω8	C18:1ω9
90	0.80	0.90	0.97
80	0.70	0.80	0.91
70	0.60	0.70	0.85

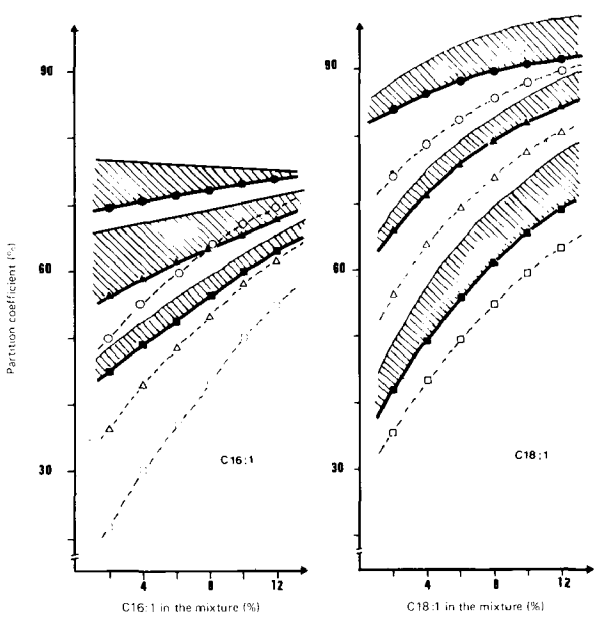

Figure 3

Figure 4

FIGS. 3 and 4. Crystallization of C16:1 (FIG. 3) and C18:1 (FIG. 4) from acetone at -60 C. Effect of the crystallization rate* (●,○ = 90%; ▲,△ = 80%; ■,□ = 70%). Effect of the chemical structure of the fatty acid* (black symbols = free fatty acids; white symbols = fatty acid methyl esters). *Hatched Areas: Variability of C16:1 or C18:1 partition coefficient in the case of mixtures including different amounts of saturated and polyunsaturated fatty acids (not studied in the case of fatty acid methyl ester crystallization).

amounts of C16:1 or C18:1 in the mixture. From those figures, it is readily obvious that for C18:1 concentrations higher than 8%, this acid has a partition coefficient very similar to the crystallization rate and thus no efficient partition between crystals and filtrates would occur, as observed in the course of C17:1 purification (16).

To better illustrate the meaning of these data, a set of figures was drawn comparing the results obtained with two rates of crystallization (80% and 90%) and with three techniques: crystallization of fatty acids in acetone, or of their fatty acid methyl esters in acetone or in urea. Repetitive rehandling of the fatty acids (or fatty acid methyl esters) eliminated in the crystals at constant crystallization rates was considered first, the successive filtrates obtained being gathered together after each step of the process. Figure 5 shows the theoretical variations of C18:1 concentration in the latter fraction, in the case of an initial mixture consisting of 10% C16:1, 80% C17:1, and 10% C18:1.

The best efficiency of C18:1 elimination would be obtained in the case of stepwise high rates of crystallization with urea (90%). It would lead, after 12 steps of the process, to a mixture of fatty acids amounting to more than 70% of the initial sample (including about 75% of the initial C17:1 amount) and comprising only 4.3% of C18:1. A lesser efficiency would be obtained with a lesser rate (80%) of fatty acid methyl ester crystallization with urea: after six steps, a similar quantity of fatty acids would be gathered, but their C18:1 content would be 5.9%.

Fatty acid crystallization from acetone would not lead to an efficient partition. For the recovery of a similar amount of fatty acids, C18:1 concentrations in the case of crystallization rates of 90% or 80% would be, respectively, 8.4% or 8.7%.

In the case of fatty acid methyl ester crystallization from acetone, there would be a slight increase in C18:1 content in the filtrates and a nonefficient decrease of C18:1 content in the crystals.

The theoretical concurrent behavior of C16:1 was worked out as well (Fig. 6). For a similar recovery of 70% of the initial sample, the content of C16:1 of the filtrate mixtures does not depend on the fractionation conditions very much and might vary from 10.9% to 14%, with a value of 11.4% in the case of high rates (90%) of urea crystallization.

The more efficient process, successive crystallization with urea at a rate of 90%, would thus lead to a mixture made of 11.4% C16:1, 84.3% C17:1, and 4.3% C18:1. Fractionation of this mixture was then considered. The efficiencies of the same three techniques, applied at similar rates (80 and 90%), were compared. Figure 7 shows the theoretical evolution of C16:1 content of the crystals after each step of repetitive crystallization.

The best efficiency of C16:1 elimination would be obtained with high rates (90%) of fatty acid methyl ester crystallization from acetone. It would lead, after seven steps of the treatment, to the recovery of about 50% of the initial sample with a content of C16:1 as low as 0.5%. Lower efficiencies would be obtained in that order in the case of high rates (90%) of fatty acid crystallization from acetone, low rates (80%) of fatty acid methyl ester crystallization from acetone, and low rates (80%) of crystallization with urea. For recoveries of 50% of the initial sample, the contents of C16:1 would be, in each of those cases, respectively, 2.6%, 3.4%, 4.9%, 5.3%, and 6.8%. Meanwhile, C18:1 should be eliminated from the crystals most efficiently in the case of fatty acid methyl ester crystallization in acetone (Fig. 8). A low content (0.5%) of this acid in the mixture would be obtained so that 30% of the initial sample might be recovered. In the case of fractionation with urea, even at low concentrations of this acid, C18:1 would keep being accumulated in the crystals (Fig. 8).

The results obtained in the case of a 70% crystallization rate were not shown on the figures since they fit in well with the above conclusions, the fractionation being less efficient for every technique considered than in the case of a 80% crystallization rate.

Stepwise fractionation of fatty acids is thus more efficient with high rates of crystallization whatever the technique may be, fatty acid crystallization from acetone or fatty acid crystallization from acetone or in urea, but more steps are required. The preceding analysis illustrates how the different techniques can compare in the case of the separation of three compounds whose properties differ very little from one anothers, and brings in light some clues for the choice of an efficient purification process combining

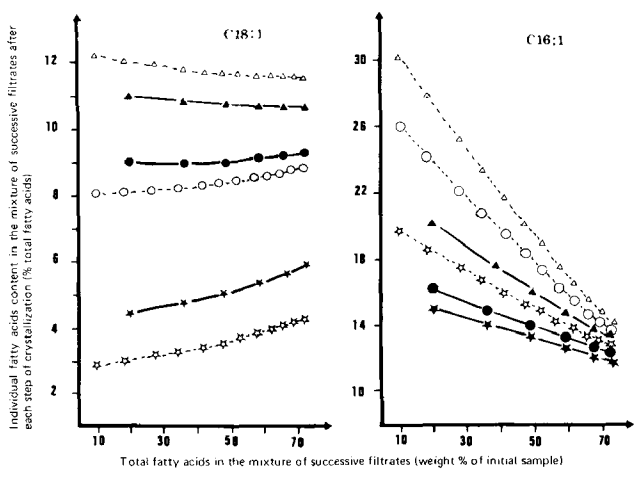

Figure 5 Figure 6

FIGS. 5 and 6. Efficiency of C18:1 elimination from a mixture consisting initially of 10% C16:1, 80% C17:1, and 10% C18:1. Evolution of C18:1 (FIG. 5) and C16:1 (FIG. 6) content of the mixture of successive filtrates in the course of various crystallization techniques; (★,☆ = crystallization of fatty acid methyl esters with urea at 4C; ○,● = crystallization of fatty acids from acetone at -60C; △,▲ = crystallization of fatty acid methyl esters in acetone at -60 C) repetitively applied at different rates (white symbols = 90%; black symbols = 80%) on the fatty acids recovered from the crystals.

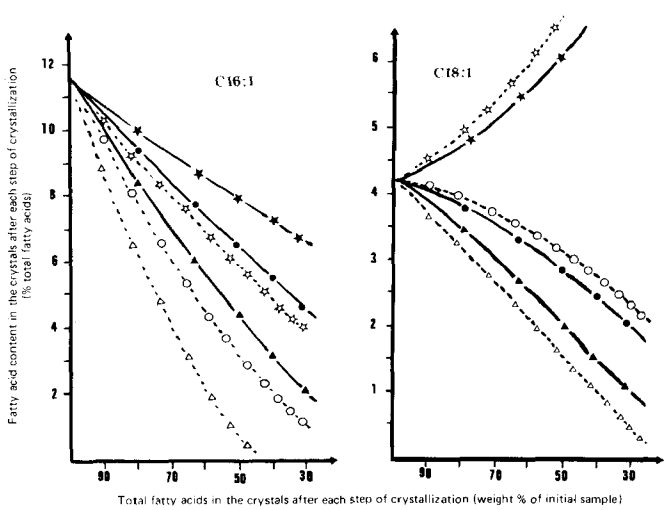

Figure 7 Figure 8

FIGS. 7 and 8. Efficiency of C16:1 and C18:1 elimination from a mixture consisting initially of 11.4% C16:1, 84.3% C17:1 and 4.3% C18:1. Evolution of C16:1 (FIG. 7) and C18:1 (FIG. 8) content in the crystals after repetitive steps of various crystallization techniques applied at different rates (same symbols as in Figures 5 and 6).

both of them.

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Letter to the Editor

Improved Method for the Quantitative Determination of Oil Content in Peanuts and Peanut Products

Sir: Although revised in 1979 to include roasted peanuts, the AOCS Official Method Ab3-49 for the quantitative determination of oil in peanuts (1) is not completely satisfactory for all peanut products. In the AOCS method, the sample is sliced with the Henry Nut Slicer, and then is mixed by a mechanical mixer. This may be a potential cause for loss of oil when slicing raw peanuts if the slicing blade is not properly adjusted. The Henry Nut Slicer may not always be readily available and is difficult to use for slicing roasted peanuts (fresh or rancid) particularly for inexperienced operators. In addition, in the AOCS method, solvent extraction is halted after 2 hr, the sample is removed from the Butt tube, petroleum ether is allowed to evaporate at room temperature, and the sample is carefully transferred to a mortar and reground with a pestle. The reground material is then returned to the same filter paper and extraction is continued for another 2 hr.

Our purpose was to develop an accurate, uniform procedure for determining the oil content of either raw or roasted, fresh or rancid peanuts, and peanut products such as peanut butter, and to offer an alternate procedure for grinding that reduces the potential for oil loss compared to the official AOCS method.

Raw and roasted Virginia and Spanish peanuts and peanut butter samples were obtained from commercial suppliers. Approximately 50 g of raw or roasted peanut

TABLE I

Oil Contents of Peanuts and Peanut Products

Sample no.	Variety	Treatment	% Oil[a]	% Deviation	% Coefficient of variation
1	Virginia	Raw	49.65	-0.05	
2	Virginia		49.66	-0.04	
3	Virginia		49.69	-0.01	0.12
4	Virginia		49.78	+0.08	
Avg.			49.70	±0.05	
1	Spanish	Fresh,	49.74	+0.03	
2	Spanish	lab	49.63	-0.08	
3	Spanish	roasted	49.73	+0.02	0.10
4	Spanish		49.73	+0.02	
Avg.			49.71	±0.05	
1	—	Commercially	47.94	-0.01	
2	—	roasted —	47.98	+0.03	
3	—	rancid	47.87	-0.08	0.11
4	—		47.99	+0.04	
Avg.			47.95	±0.05	
1	—	Commercial	48.72	+0.07	
2	—	peanut	48.67	+0.02	
3	—	butter	48.58	-0.07	0.13
4	—		48.62	-0.03	
Avg.			48.65	±0.06	

[a]Dry basis.