

# The Fractionation of Milk Fat From a Solvent At Low Temperatures

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Any detailed study of milk fat is difficult because of the presence of numerous fatty acids, which occur as mixed glycerides. Reliable sources report as many as 16 or 17 individual fatty acids isolated from milk fat. Too generally the properties of the component fatty acids have been used to characterize a fat or oil, whereas the character actually results not only from the molecular composition of the fat but also from the molecular distribution of the fatty acids in the glycerides. The purpose of the present work has been to simplify milk fat into fractions more nearly homogeneous, for further study, while retaining the original glyceride configuration.

The glycerides comprising a fat may be fractionated by high-vacuum distillation, by molecular distillation, by partial solidification and filtering at suitable temperatures, or by fractional crystallization from solvents. This last method was chosen for the present study because of the difficulty of separating the adjacent fractions where the fat is solidified directly. Also, molecular and high-vacuum distillation subjects the fat to drastic heat treatment, with a greater possibility of decomposition. In the work of Riemenschneider, Swift, and Sando (11), low-temperature crystallization from solvent gave a greater degree of separation of the component glycerides of cottonseed oil than did molecular distillation.

Brown (4) has (1941) exhaustively reviewed the literature on crystallization of fats and fatty acids at low temperatures; he lists 118 references. This method has served to show that the natural fats are characteristically mixed glycerides. It has also been used for final purification of certain unsaturated fatty acids (5, 7). Recently the same technique has been employed for dividing fat into simpler fractions preliminary to chemical analysis (1, 8). It is also a means of removing some fractions from a fat in order to modify the fat for certain commercial uses (3).

Hilditch and Paul (10) have applied the technique of low-temperature fractionation from solvent to milk fat. Using acetone as the solvent, they separated the fat into three crude fractions, one separating on standing over night at room temperatures, another separating at 0°C., and a third being left in solution. These fractions were subjected to chemical analysis. From their analyses these workers calculated the percentage distribution of the individual acids among the glyceride molecules.

## Experimental

*Preparation of the Fat:* The milk fat for this work was obtained from commercial creamery butter taken from the churn before salting in three batches of 30 pounds each at 3-month intervals. This procedure was adopted to secure a representative fat in which seasonal, breed, and individual variations were minimized.

The fat was prepared for use by melting the butter and decanting the fat from the water and curd into

a filter at 50°C. to obtain a clear, dry product. After all the fat from the three lots had been prepared, these were combined by melting together in one container. The fat was then drawn off in 3-pound lots and stored at -10°C. until used. It was made ready in this large amount so that identical samples would be available for many types of investigations.

*Choice of Solvent:* Most of the previous investigators in the field of low-temperature crystallization of fats and fatty acids have used acetone as the solvent. Recent conditions, however, have made acetone somewhat less easy to obtain than formerly. DeGray and DeMoise (6), from their experience in the fractional crystallization of high- and low-titer fatty acids, have pointed out that petroleum ether is preferred to acetone and ethyl ether, because of the insolubility of condensed moisture in petroleum ether. They report that condensed moisture in the solvent is undesirable. Bailey and his co-workers, in discussing a choice of solvent for fractionation on a large scale, have set up the following specifications: that it be cheap, easily recoverable, and readily available in unlimited quantities. They found that a petroleum naphtha (Skelly Solve B) fulfilled these requirements. A lower-boiling naphtha (Skelly Solve A) was chosen for the work here reported, because it could be removed from the milk fat at lower temperature and more completely with less difficulty. According to preliminary experiments, separation of the desired degree could be obtained when this solvent was used in the proper concentration. Slight filtering difficulties, encountered with some of the fractions, will be discussed in detail later. They were not, however, deemed sufficient to overbalance the advantage of using this solvent.

*Freezing and Filtering Technique:* The fat was fractionated by cooling to different temperatures and filtering off the precipitated fat. Batches of 100 grams each were dissolved in 1500 ml. of solvent and frozen to -7°C., and the resulting precipitate and filtrate were treated as described later. Each lot consisted of 14 to 15 individual batches. The freezing and filtering equipment used was such that this amount of material could be handled in one day. The refrigerant for both the freezing and the filtering was ethyl alcohol and solid carbon dioxide.

Figure 1 shows the arrangement of the freezing apparatus.

The cooling bath was maintained at a temperature 3 degrees below the temperature to which the sample was to be frozen. In preliminary trials, fractions were taken at the following temperatures: -7° precipitate, -13° precipitate, -23° precipitate, -53° precipitate, and -53° filtrate. These proved to be fairly uniform in size, gave a desirable segregation of physical properties, and at the dilutions used were readily frozen and filtered.

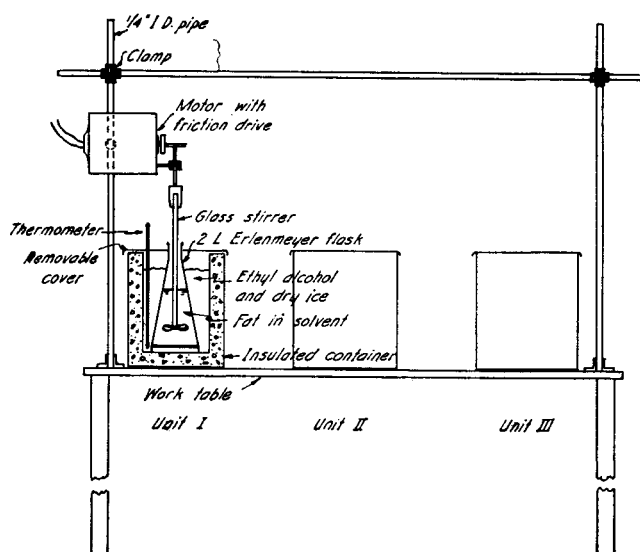


Fig. 1. Apparatus for freezing milk-fat fractions.

Figure 2 shows the apparatus used for filtering the fractions.

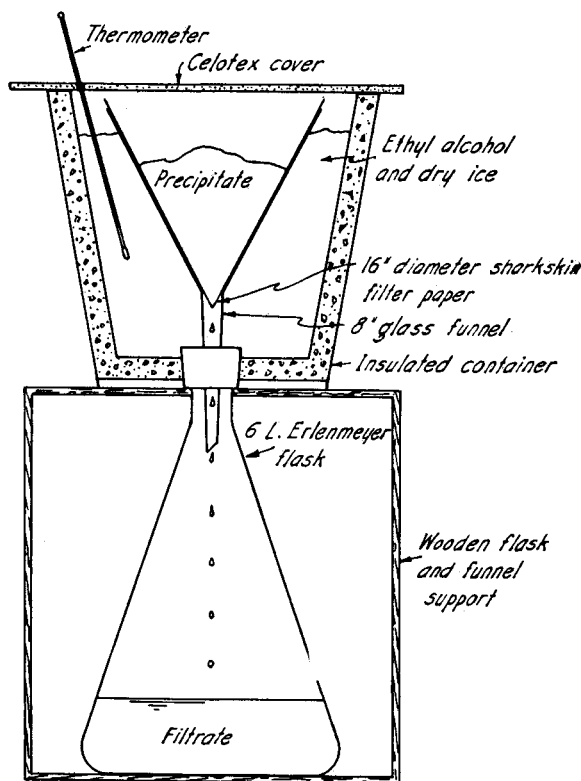


Fig. 2. Apparatus for filtering milk-fat fractions.

Contrary to the procedure for fatty-acid separation, gravity filtering proved much speedier than suction filtering; in fact, the latter was almost impossible. DeGray and DeMoise (6) also noted this fact. The filtering apparatus was used in a room kept at  $-13^{\circ}\text{C}$ . except for the  $-7^{\circ}$  precipitate. The cooling bath was maintained at the temperature of the precipitate. Eight units like the one shown here were used to filter the material from the three freezing units.

Figure 3 diagrams the scheme used in separating the different fractions.

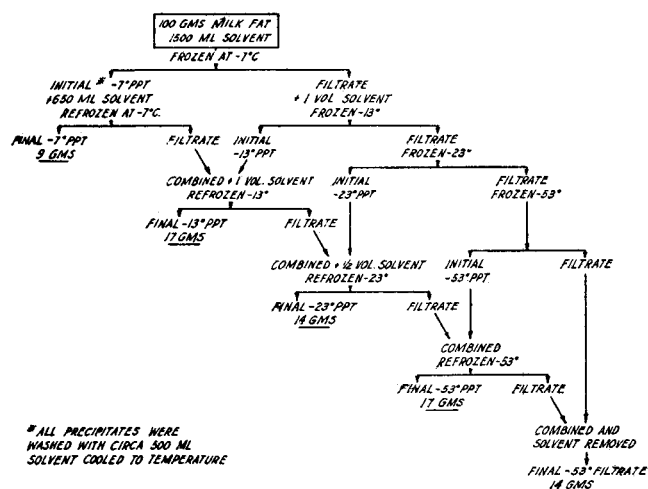


Fig. 3. Diagram of scheme for separating milk-fat fractions.

The  $-7^{\circ}\text{C}$ . precipitates filtered readily and after the second crystallization yielded a dry white powder. The occluded solvent evaporated readily under atmospheric conditions. The filtrates from this precipitation were cooled to  $-13^{\circ}\text{C}$ . according to the scheme shown in Figure 3. At this temperature the precipitate was voluminous, showing considerable solvation, and even after a long period of draining still held considerable solvent. This precipitate was filtered and allowed to drain overnight in a room maintained at  $-13^{\circ}\text{C}$ . The bulk of the solvent had to be removed by distillation, and the final traces by placing the material in a vacuum desiccator held at room temperatures. It was found desirable to remove the solvent from all the succeeding fractions in the same way.

The  $-23^{\circ}\text{C}$ . precipitate required handling very similar to the  $-13^{\circ}$  precipitate except that it was somewhat less solvated and required a shorter draining time, 5 to 6 hours usually being sufficient.

The  $-13^{\circ}$  and  $-23^{\circ}$  precipitates were similar in physical appearance, yielding a slightly yellow greasy solid, the  $-23^{\circ}$  being softer than the other.

The  $-53^{\circ}$  precipitate filtered quickly with only a slight amount of solvation and yielded a nearly white product, which was liquid at room temperature.

The  $-53^{\circ}$  filtrates, after solvent removal, were deep reddish-yellow oils, with a noticeable odor of melted fresh milk fat.

**Yield of Fractions:** The total yield for all the fractions was about 75 per cent of the original fat and was distributed approximately as shown in Figure 3. Most of the loss resulted from inability to remove all the precipitate readily from the filter paper; a little fat always adheres to the surface and pores of the paper and can be removed only by time-consuming extractions. As will be seen from Figure 3, numerous filtrations were necessary; and thus a yield of about 75 per cent is all that can be expected under these conditions.

The reproducibility of this method of fat fractionation when applied to different lots of the same fat is shown by the iodine numbers of the same fractions of separate lots. In nine individual lots the iodine numbers differed in value by not more than two within each fraction, and in most instances differed by less.

*Analysis of Fractions:* Nine different lots of the fractions were prepared, each lot representing 1400 to 1500 grams of milk fat. The different lots of the same fraction were combined and analyzed for iodine number, saponification number, Reichert-Meisel number, and melting point. The values were obtained by standard procedures (2).

Table 1 shows the results obtained, along with the same values for the original milk fat.

TABLE 1  
Some Chemical Constants of the Milk Fat and Milk-Fat Fractions

Sample	Iodine No. (Hanus)	Saponification		Reichert-Meisel No.	Melting point °C.
		No.	Equiv.		
Milk fat	32.93	236.3	237.2	32.48	32.8
—7° ppt.	8.29	213.4	262.8	7.30	53.0
—13° ppt.	22.15	232.4	241.0	35.28	31.1
—23° ppt.	23.46	238.4	235.2	36.70	23.5
—53° ppt.	31.18	229.1	244.5	33.00	11.4
—53° filtrate	58.37	230.3	243.3	42.50	—10.6

As may be noted from Table 1, milk-fat fractions from the —7°C. precipitate to the —53°C. filtrate have increasing iodine numbers. This difference in iodine numbers signifies that the unsaturated fatty acids are not uniformly distributed among all the glycerides, since they can be concentrated in greater quantities in one fraction than in another. Uniform distribution of unsaturated fatty acids might be expected because there are, in average milk fat, about 33 mol per cent (9, 10) of unsaturated acids that could supply one unsaturated fatty acid for each glyceride molecule.

The saponification equivalents, on the other hand, do not show the regularity of either the iodine numbers or the melting points. This results from a difference in the degree of segregation between the glycerides containing different proportions of short-chain and unsaturated fatty acids.

The melting points, in general, follow the iodine numbers. This is to be expected, since the unsaturated fatty acids are present in greater weight percentage than the short-chain fatty acids, and since both types lower the melting point. The —23 and —53 ppts. fractions do not show the same degree of reciprocal relationship that is exhibited by the other fractions.

The non-saponifiable matter is concentrated principally in the —53° filtrate. The analysis shows the milk fat to contain 0.28 per cent; and the —53° filtrate, which represents about 20 per cent of the fractions, contains 1.09 per cent of unsaponifiable matter.

### Summary

A procedure for fractionating milk fat from a solvent at low temperatures has been developed. This procedure consists of freezing out fractions of the fat from solvent (Skelly Solve A) at progressively lower temperatures —7°, —13°, —23°, —53°C., with the remaining filtrate taken as a final fraction. In physical appearance these fractions vary from a dry white powder to a reddish-yellow oil; in melting point from 53°C. to —10.6°C.; in iodine number from 8.29 to 58.37; and in saponification equivalent from 262.8 to 235.2. The saponification equivalents do not change in the same order as the other properties mentioned.

This fat fractionation effects a simplification of milk fat and makes available less complex portions of the natural glyceride mixtures for detailed study of the composition, configuration, and other properties. Some of these studies are now being carried out and will be reported later.

### REFERENCES

1. Anthony, D. S., Quackenbush, F. W., and Steenbock, Harry, *Oil & Soap*, **20**, 53, (1943).
2. Assoc. Official Agr. Chem., *Official and Tentative Methods of Analysis*, 5th ed., (1940).
3. Bailey, A. E., Feuge, R. O., Kraemer, E. A., and Bauer, S. T., *Oil & Soap*, **20**, 129, (1943).
4. Brown, J. B., *Chem. Rev.*, **29**, 333, (1941).
5. Brown, J. B., and Shinowara, G. Y., *J. Am. Chem. Soc.*, **59**, 6-8, (1937).
6. DeGray, R. J., and DeMoise, A. W., *Ind. Eng. Chem. Anal. Ed.*, **13**, 22, (1941).
7. Henderson, J. L., and Young, H. A., *J. Phys. Chem.*, **46**, 670-684, (1942).
8. Hilditch, T. P., "The Chemical Constitution of the Natural Fats," pp. 412-416, John Wiley & Sons, Inc., N. Y., (1941).
9. Hilditch, T. P., and Longenecker, H. E., *J. Biol. Chem.*, **122**, 497, (1938).
10. Hilditch, T. P., and Paul, S., *Soc. Chem. Indus. J.*, **59**, 138, (1940).
11. Riemenschneider, R. W., Swift, C. E., and Sando, C. E., *Oil & Soap*, **17**, 145, (1940).