Laboratory Separation of Crystals from Plastic Fats Using Detergent Solution

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ABSTRACT

An improved laboratory technique for isolating the solid-fat fraction from plastic fats is described. The fat first is dispersed in an aqueous detergent solution; then the oil and solid fat fractions are separated by high speed centrifugation. To facilitate the separation, a 25% ammonia solution is added to adjust the density of the water phase to between those of the oil and the solid-fat phase. After rinsing with cold ethanol, a solid-fat fraction is obtained—contaminated with only ca. 5% oil—which can directly be used for differential thermal and X-ray analyses. The method also can be applied for a quick determination of the solid-fat content according to the dye dilution principle and for obtaining oil-free fat crystals for electron microscopy.

INTRODUCTION

The consistency of plastic fats and margarines is governed by important parameters, such as mp, crystal modification, and crystal size of the solid-fat fraction. The purer the solid fat the more accurately these parameters can be fixed. However, isolation of the solid-fat fraction from the fat phase by centrifugation techniques (1) has been rather defective, and the use of selective solvents often leads to partial dissolution of the fat.

Stein (2) developed a technical-scale hydrophilization method for liquid fats by which the fat is dispersed in an aqueous detergent solution, thus forming an oil-in-water emulsion. The water phase, which also contains the isolated, dispersed fat crystals, then is separated from the oil phase by centrifugation. Unfortunately, this technique is not readily applicable to plastic fats due to the huge amounts of submicron fat crystals present in such products (3), which made high demands upon the dispersion technique, the wetting ability of the detergent and on the separation by centrifugation.

To meet these requirements, we developed a laboratory method and will describe its suitability for preparing replicas for electron microscopic investigations (4) and for determining the solid-fat content according to Zobel's dye dilution method (5).

EXPERIMENTAL PROCEDURES

Apparatus and Materials

The apparatus in which the fat is dispersed consists of a water-jacketed vessel (Fig. 1) partly filled with glass beads (3-4 mm diameter). A thermostat is connected to the double wall of the vessel to maintain the operational temperature desired. A phase-contrast microscope allowing a 500-fold magnification is used to study the appearance of the emulsion for reasons that will be explained later. The emulsion phases are separated in a high speed centrifuge (containing 50 ml tubes), permitting temperature adjustment and a speed of 15,000 rev/min (corresponding with 25,000 g).

As dispersants, we used aqueous solutions of 5 and 0.5% sodium dioctylsulfosuccinate (AOT); as centrifugation aid, a 25% ammonia solution and 96% ethanol as rinsing aid.

Procedure

A sample of 20-25 g is mixed with a spatula into the glass beads of the vessel containing 10-30 ml 5% AOT solution; this amount depends upon the sample's solid-fat content. The glass beads are stirred with a spatula for 3-5 min; the dispersion formed is made up with water to 50 ml and subsequently drained. The vessel is rinsed with 50 ml ammonia solution and with a small amount of water, after which the rinsing liquids are added to the dispersion. The resulting diluted dispersion then is examined microscopically. When the dispersion shows the desired properties, it is centrifuged for 15 min at 15,000 rev/min, which results in the formation of separate layers of oil (upper), a water phase (intermediate), and a layer of fat crystals (lower). The oil layer is sucked off by a pipette and the water layer decanted. The residual fat crystals are transferred into the beads containing vessel, redispersed in a 0.5% AOT solution, and centrifuged again. This procedure is repeated until the dispersion is completely free from oil. After decantation of the water phase, the fat crystals finally are rinsed with cold ethanol (10-20 ml) a few times, repeatedly centrifuged (3000 rev/min for 1-2 min) and dried in air. During the separation procedure, all the equipment and

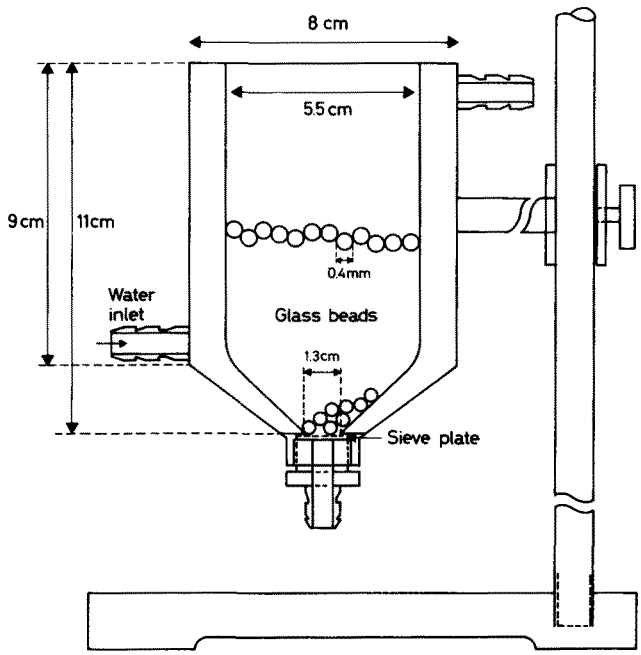

FIG. 1. Water-jacketed conical vessel partly filled with glass beads for dispersing margarine or fat in a detergent solution.

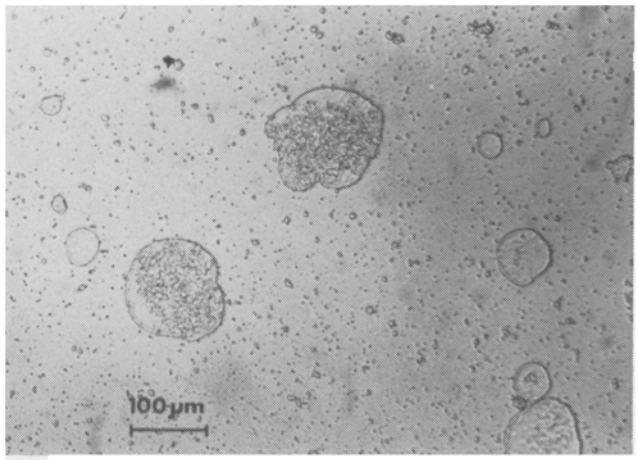

FIG. 2. Crystals still are present in the oil droplets and have, therefore, not been wetted sufficiently.

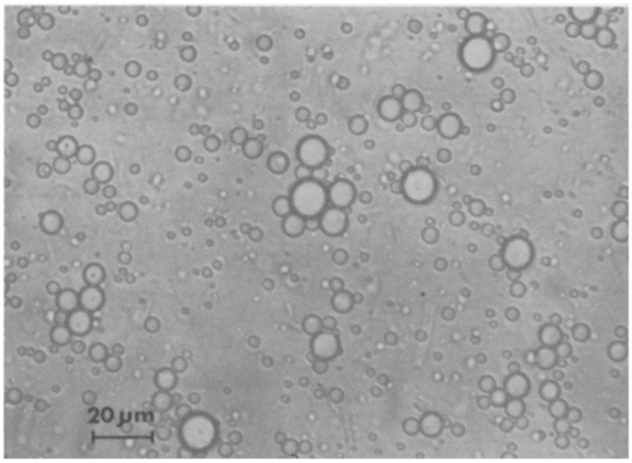

FIG. 3. Oil droplets are too small; emulsion is too stable and, therefore, difficult to separate by centrifugation.

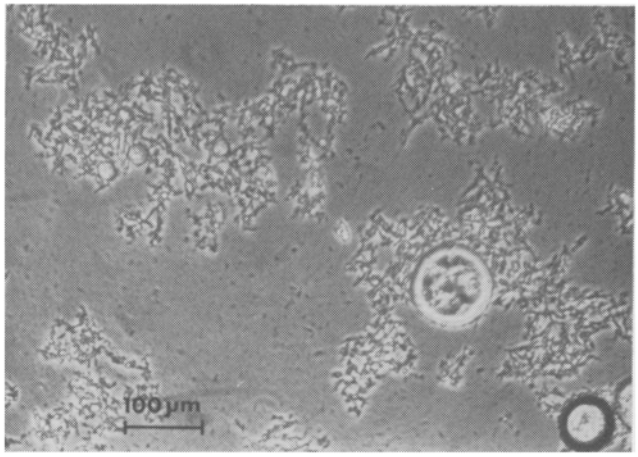

FIG. 4. Flocculation of the crystals. Initially, the correct concentration was applied, but, after dilution, the sodium dioctylsulfosuccinate (AOT) concentration appeared to be too low.

materials should be kept at the desired temperature.

Experiments

To find out to what extent liquid oil remains in the solid-fat fraction, we applied our technique to four margarines containing a large amount of triglycerides of unsaturated fatty acids (U3; U = unsaturated fatty acid). After separation (at 15 C), the U3 content of both the oil and the solid-fat fraction was determined according to a method developed by our laboratory which is based upon the thin layer chromatographic separation of the tri-azelaylglycerides obtained according to a method of von Rudloff (6). From these data, the amount of contaminating oil can be computed easily.

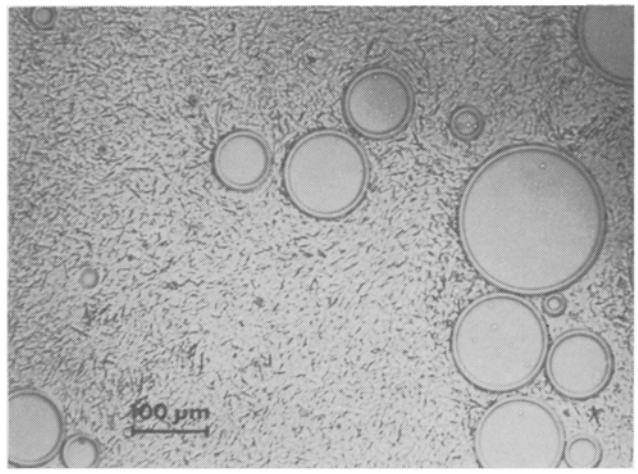

FIG. 5. Crystals are well wetted; no crystals present in oil droplets. The large droplets are easy to separate by centrifugation.

TABLE I

Estimates of Amount of Oil Present in Isolated Solid-Fat Fraction of Four Margarines

	Sample			
	1	2	3	4
U3[a] content oil (%)	32	39	40	28
U3 content solid fat (%)	1.6	1.6	2.5	2.1
Oil content solid fat (%)	5	4	6	7

[a]Glyceride with three unsaturated fatty acids.

RESULTS AND DISCUSSION

To obtain a suitable crystal separation, the dispersion should meet the following requirements: (A) the oil droplets may not contain any fat crystals, which can be achieved by using an appropriate amount of detergent; (B) the droplets may not be smaller than 12 µm—otherwise, the result of centrifuging will be affected greatly (too small droplets are formed when the emulsion is agitated too vigorously); and (C) the fat crystals may not flocculate, since flocculation would cause encapsulation of oil droplets (flocculation can occur on dilution of the dispersion with liquid ammonia, because too low a detergent concentration might result). For these reasons, the fat dispersion must be examined microscopically prior to centrifugation. Figures 2, 3, and 4 show typical examples of dispersions which do not allow a satisfactory separation. In these cases, the dispersing procedure should be repeated under varied conditions to obtain the dispersion pattern as shown in Figure 5, where all crystals are wetted satisfactorily and the oil droplets sufficiently large.

The ammonia to water ratio in the water phase of the dispersion has been chosen such that the density is ca. 960 kgm^{-3}. In most cases, this value is effective, since, at around room temperature, the densities of oil and solid fat may be put at 910 and 990 kgm^{-3}, respectively (7), so that, during centrifugation, the water phase acts as a buffer zone between the two fat phases to be separated (8).

Table I shows the results of the determination of the amount of oil in the isolated solid-fat fraction of four

TABLE II

Results of Solid-Phase Determinations by Modified Dye-Dilution Technique in Mixtures of Hardened Linseed Oil (HLO) and Winterized Sunflower Seed Oil

HLO added (%)	Solid fat content (%)	
	First determination	Second determination
5	4.5	5.0[a]
10	9.9	9.5
15	15.0	---
20	21.6	19.9
25	25.1	---
35	34.8	37.7

[a]5.3 in third determination.

TABLE III

Comparison between Results Obtained by Two Solid-Phase Determinations Techniques

Sample	Solid-fat content (%)	
	Modified dye dilution	Zobel's dye solution
I	14.0	14.4
Shortening II	15.6	14.7
III	17.9	20.0
IV	19.3	19.9
V	26.1	23.9
Margarine	34.1	35.7

[a]Correction required for water content.

margarines. These amounts appear to be only ca. 5% of the solid-fat fraction. The solid-fat fraction is, therefore, very suitable for DTA and X-ray investigations. It remains uncertain whether this oil is that adhering to the crystal surfaces (which is difficult to remove) or to U3 molecules encapsulated in the fat crystal matrix.

An important advantage of this hydrophilization method is that the solid-fat fraction is isolated from the liquid oil phase within a few min. This means that it is possible to interrupt crystallization processes, which do not proceed too fast, to determine and follow the increase in solid-fat content.

APPLICATIONS

Determination of Solid-Fat Content

According to Zobel's dye dilution method (5), an amount of dyed oil of known absorbance carefully is mixed with a fat sample. Using an ultracentrifuge, part of the liquid phase then is separated and its absorbance measured from which the percentage of solids is calculated. This method has two disadvantages: an expensive centrifuge is required and mixing the dyed oil with the plastic fat has to be done very accurately and slowly to prevent part of the crystals from melting as a result of an increase in sample temperature.

Our hydrophilization technique eliminates these drawbacks, because the dyed oil can be mixed roughly with the fat in the thermostated beads containing vessel prior to dispersion of the fat sample in the AOT solution. After centrifugation, part of the oil is removed, dried at 80 C under reduced pressure, and its absorbance measured after cooling to room temperature.

By this modified dye-dilution method, we determined the solid-fat content at 15 C of several mixtures of hardened linseed oil in winterized sunflower seed oil. Because hardened linseed oil almost completely consists of glyceryl tristearate, which may be regarded as insoluble in vegetable oil at 15 C, the experimentally found percentages

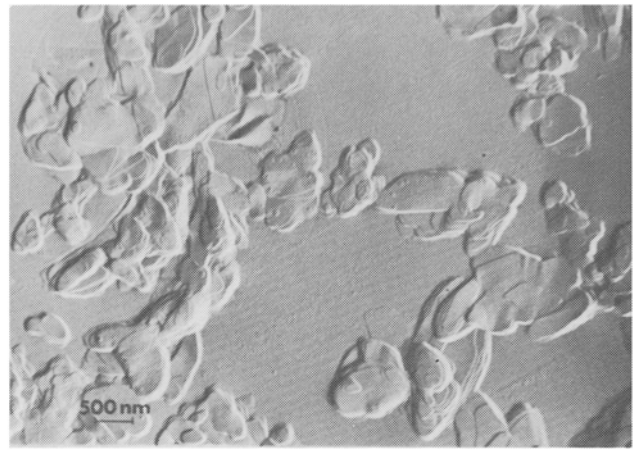

FIG. 6. Fat crystals (0.5-2 μm) from a shortening. Due to the dispersion spray technique, the crystals lie now free from the background. Magnification used: 20,000.

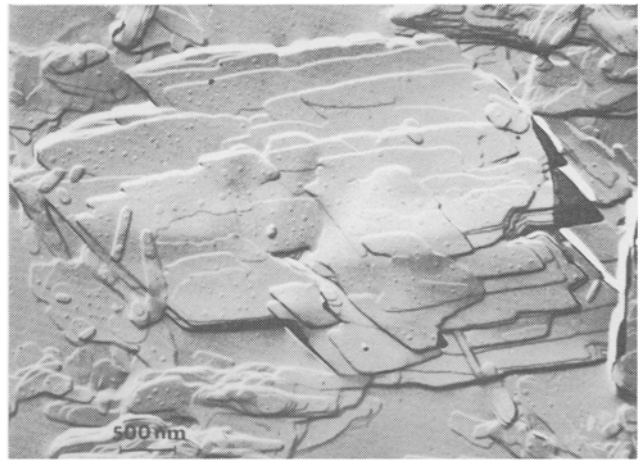

FIG. 7. Large fat crystal (≈5 μm) from a margarine. The layered structure and "pimples" are easily distinguishable. Magnification used: 30,000.

of solid should equal those of hardened linseed oil in the fat mixtures, which, indeed, proved to be the case (Table II). The dyed oil was a solution of 50 mg 1,4-bis-isopropyl aminoanthraquinone (5) in 100 g winterized sunflower seed oil, and its absorbance was measured at 599 nm using a Zeiss PMQII spectrometer.

Table III shows the results obtained for four shortenings and one margarine by Zobel's method and the modified technique. The absorbance obtained for the margarine concerns both the water and the solid-fat content, so that the water content must be corrected for.

We conclude that our modified technique gives reliable results compared with those obtained with Zobel's method. Therefore, we also used our method for investigations into the reliability of solid-fat determinations in plastic fats by wide-line NMR (9).

Determination of Crystal Size by Electron Microscopy

The method also can be used for electron microscopial investigations into the shape and size of fat crystals (4). The length of the cyrstals of plastic fats usually appears to be 0.1-3 μm, and the thickness then is ca. 0.05 μm. For this determination, the fat crystals first are separated as described in this paper until the dispersion is free from oil droplets. On redispersion, the crystal concentration should be maintained at ca. 1%, while, for this particular application, the AOT concentration must be kept high enough to prevent the crystals from flocculating. Because the fat

crystals would melt under the electron microscope, replicas should be made for which well known techniques are available (10).

One of the main features of our separation method is that the crystals have been freed from their surrounding oil. Crystallization of this oil occurring during the replica preparation (when the temperature temporarily is lowered to -90 C) might cover the original crystallization pattern. Jewell and Meara (11), indeed, showed that crystal patterns observed are greatly dependent upon the cooling conditions applied during replica preparation of crystals still surrounded by oil. The detergent itself has no influence upon the microscopic picture, which could be established by means of replicas made from Formvar substrates sprayed with an AOT solution (these showed no crystal structure whatsoever).

Figures 6 and 7 show that, after isolation of the crystal fraction by the hydrophilization technique, very small crystals can be observed (Siemens Elmiscop I with object cooling). The crystals were isolated from a shortening and a margarine. Sometimes, the crystals partly overlap, which can be diminished by diluting the dispersion to be sprayed onto the microscope slide. The large crystal of Figure 7 has a layered structure (layer thickness ca. 50 nm) with "pimples," the origin of which is unknown; both phenomena also have been observed by Jewell and Meara (11). Since the "pimples" do not occur in the background, it is improbable that they are due to an incorrect preparation of the replicas.

ACKNOWLEDGMENT

H.R. Kattenberg developed the triglyceride analysis technique, and M. Greep performed the analyses.

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