Measurement of the Size Distribution of Fat Crystals Using a Laser Particle Counter

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A new method that uses a laser particle counter has been developed to measure the size of fat crystals. The fat crystals were suspended in isobutanol in the ratio of 1:25,000. The solid and liquid fat recovery after being in contact with isobutanol for 24 hr, fatty acid composition of the solid and liquid fractions, DSC melting behavior and polarized light microscopy proved that isobutanol did not affect the structural integrity of the fat crystals. The mean crystal size of commercial shortenings ranged from 5 to 9 μm. The isobutanol suspension method makes it possible to quantitatively separate the solid and liquid components of a fat. It also provides a convenient way of preparing fat crystals for scanning electron microscopy.

Plastic fats consist of a three-dimensional network structure of crystals in which liquid oil is trapped. The plasticity of a fat is determined by the shape, average size and size distribution of the crystals. Factors affecting the consistency of plastic fats include the content of solid material, crystal size, mechanical treatment, thermal history and polymorphism (1). The functional properties of fats in different foods are determined mainly by the dispersion of the fat, the balance between solid and liquid phase, the crystal structure and by polymorphic transitions occurring during the manufacture and storage of these products (2).

A major factor influencing the rheological properties of the fat crystal network is crystal size. The softness and grainy consistency of slowly chilled fats are well-known, and lard, which has a coarse crystal structure, is softer at a given solids content than fine-grained fats such as vegetable shortenings (3). However, until now, data on crystal size distribution of fats had been difficult to obtain because suitable methods were lacking.

Polarized light microscopy was the first method to give information on the crystal size, but size distribution analysis is time-consuming and inaccurate (4). Since the analysis of crystal particles requires observation between crossed polars in polarized light, this method is not suitable for size distribution measurements. Permeametry was a method developed by de Jager *et al.* (5) in which the specific surface area of the particles was measured. The method was not very successful and as a result did not find a wide application. Recently, light or X-ray scattering and sedimentation methods coupled with computer analysis have provided new and improved techniques for measuring particle size distribution.

The main problem to overcome in the use of light scattering is dilution of the sample to reduce the number of particles in a given volume to a level where measurement becomes possible. A recent paper (6) on crystallization kinetics of palm oil reported the use of the Malvern laser light scattering instrument. However, dilution with palm oil as reported is not a satisfactory method. Palm oil is highly viscous and might itself give rise to crystals.

In this paper we report on a new method of preparation of dilute fat crystal dispersions and the measurement of particle size distribution with a laser light scattering instrument.

MATERIALS AND METHODS

Shortenings were obtained from the local supermarket. They were all of vegetable origin. Reagent grade solvents were supplied by Fisher Scientific Co., Toronto, Ontario. A Bruker minispec PC/20 series NMR analyzer was used to determine solid fat content (7). Fatty acid composition was determined by GLC of the fatty acid methyl esters. Fats were transesterified with sodium methoxide, and the methyl esters were analyzed by GLC on a 2 m column packed with 10% SP 2330 (7).

The liquid oil of the shortenings was obtained by placing Whatman #1 chromatography paper strips into the shortenings and allowing the oil to rise. The liquid oil was then extracted from the filter paper with hexane and analyzed for fatty acid composition by GLC.

The melting point of the shortenings was determined with a model 99 duPont Thermal Analyzer. The samples were heated from 25°C to 80°C at a rate of 5°C/min.

Crystal structure of the shortenings was viewed by polarized light microscopy using an Olympus model BH polarizing microscope with PM-6 camera attachment. Photo-micrographs were taken at 400 × magnification.

The effect of isobutanol on the structural integrity of the fat crystals was established as follows. The shortenings were suspended in isobutanol in the ratio of 1:50 using a glass stirring rod to obtain a uniform suspension. The fat + isobutanol mixture was allowed to stand for 24 hr at 10°C. It was observed that this mixture separated in 2 layers—an upper layer of isobutanol and liquid oil, and a lower layer of solid fat crystals suspended in the isobutanol-liquid oil mixture.

Some of the clear upper layer was withdrawn with a pipet, and the liquid oil was obtained by evaporation of the isobutanol using a rotary evaporator. From the lower phase, the crystals were separated by vacuum filtration. The fat crystals were then dissolved in hexane; the hexane evaporated using the rotary evaporator to obtain the solid fat.

The amount of solid and liquid phase recovered after being in contact with the isobutanol for 24 hr was determined. The solid content of the original fat at 10°C was determined by pNMR. Recovery of solid or liquid phase was expressed as: amount recovered after contact with isobutanol for 24 hr/amount present in original sample as determined by pNMR × 100.

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The suspension for particle counting was prepared in the following way. Fat was suspended in isobutanol in the ratio of 1:50, adequately mixed using a glass stirring rod to obtain a uniform suspension. This mixture was sonicated for 10 sec using a low volume, high intensity ultrasonic processor (Vibracell VC 50, Sonics and Materials Inc., Danbury, CT) to complete the dispersion of the fat crystals. An aliquot of this suspension was added to isobutanol so that a final suspension of fat:isobutanol of 1:25,000 was obtained which was then used for particle counting. The final suspension was contained in a bottle which was placed in the pathway of the revolving laser beam generated by the model ILI 1000 Laser Particle Counter (Spectrex Corp., Redwood City, CA). The particle counter was attached to an IBM compatible computer, and by using the Supercount software (Spectrex Corp.), results were obtained as mean size and size distribution of fat crystals. The measured particle size ranges from 1 μm to 92 μm. Particles were measured in a 1 μm size interval from 1-16 μm and in a 5 μm size interval from 17-92 μm.

Shortening samples were prepared for scanning electron microscopy (SEM) as follows. The shortenings were suspended in isobutanol in the ratio of 1:50 and allowed to stand overnight at 23°C. Separation into 2 layers was observed. The isobutanol and the liquid oil of the shortening formed the upper layer while the solid crystals formed a sediment layer. The upper layer was discarded, and the sediment was resuspended in isobutanol. The process was repeated three times to ensure complete removal of the liquid oil phase. The fat crystals were then filtered and resuspended in isobutanol to obtain a suitable dilution of crystals. This suspension was applied to aluminum stubs, the isobutanol was allowed to evaporate, and the stubs were exposed to vapors of 0.25 g osmium tetroxide crystals (99.95%, Fisher Scientific) overnight. The samples were then sputter-coated with 200 A gold-palladium using the Hummer VII Sputter Coater, and scanned in the SEM (Hitachi S-570 SEM). Samples were scanned at an accelerating voltage of 10 kV, and the micrographs were recorded on Ilford FP4 120 mm film.

RESULTS AND DISCUSSION

In order to conduct particle size analysis of fat crystals, it is important that these fat crystals be dispersed and diluted in an appropriate solvent which is miscible with the liquid oil and in which the fat crystals are not soluble. To establish the solvent to be used, various solvents were tested for their miscibility with liquid canola oil. The solvents tested ranged from methanol, with the highest polarity, to octanol, with the lowest polarity. Liquid canola oil was miscible with propanol and higher alcohols and was immiscible with lower alcohols. Isobutanol was intermediate in polarity between methanol and octanol and hence was chosen as the solvent for further investigation.

The recovery of solid and liquid fraction of shortenings A-D after being in contact with isobutanol for 24 hr showed that, on an average, 95.2% of the solid fat and 94.8% of the liquid oil was recovered. This indicated that the fat crystals were not being adversely affected by the isobutanol. It also suggests that isobutanol can be used for separation of solid and liquid fractions of fat. Up to the present time separation of the solid phase of fats was a difficult and laborious task, accomplished by a detergent fractionation technique (8).

The fatty acid composition of the solid and liquid fractions showed that the solid fraction was higher in 16:0 and 18:0, and lower in 18:1, 18:2, 18:3 and 20:1 than both the original sample and the liquid fraction (Table 1). These results were verified with an unrelated method of absorption of the liquid portion of the fats into filter paper. The fatty acid composition of the liquid oil absorbed by the filter paper (Table 2) was comparable to that obtained by the isobutanol separation process.

Determination of the melting point of the original shortening versus the solid fraction by Differential Scanning Calorimetry (DSC) revealed that removal of liquid oil raised the melting point of the original shortening by 8-10°C.

The lack of interaction between fat crystals and isobutanol was observed under the polarized light microscope immediately after suspension and after 24 hr of contact of fat crystals with isobutanol. No change in the size of the fat crystals was observed, which

TABLE 1

Fatty Acid Composition of the Shortenings and the Solid and Liquid Fractions Separated with Isobutanol at 10°C[a]

	Fatty acid (wt %)				
Product	16:0	18:0	18:1	18:2	18:3
Shortening A					
Original	12.8	10.7	54.8	19.3	1.3
Solid	26.0	31.9	39.4	1.9	0.6
Liquid	9.4	5.3	59.5	23.6	1.3
Shortening B					
Original	14.5	11.2	44.9	26.6	1.8
Solid	30.4	38.1	26.7	3.2	0.7
Liquid	10.6	5.8	49.3	31.5	1.9
Shortening C					
Original	11.9	10.2	65.0	9.3	1.2
Solid	36.8	33.0	27.7	0.8	0.7
Liquid	6.6	5.5	73.4	11.1	1.3
Shortening D					
Original	12.3	11.8	61.2	10.9	0.9
Solid	27.7	35.4	33.4	1.2	0.8
Liquid	8.3	5.9	68.0	13.8	0.8

[a]Fatty acids occurring at less than 1% are not reported.

TABLE 2

Fatty Acid Composition of the Liquid Fraction of Shortenings Obtained by Absorption into Filter Paper at 10°C.[a]

	Fatty acid (wt %)				
Shortening	16:0	18:0	18:1	18:2	18:3
A	9.2	5.2	60.0	24.0	1.2
B	9.5	4.5	49.2	33.3	2.2
C	5.2	3.9	73.2	13.9	1.4
D	9.4	7.4	65.7	13.3	1.0

[a]Fatty acids occurring at less than 1% are not reported.

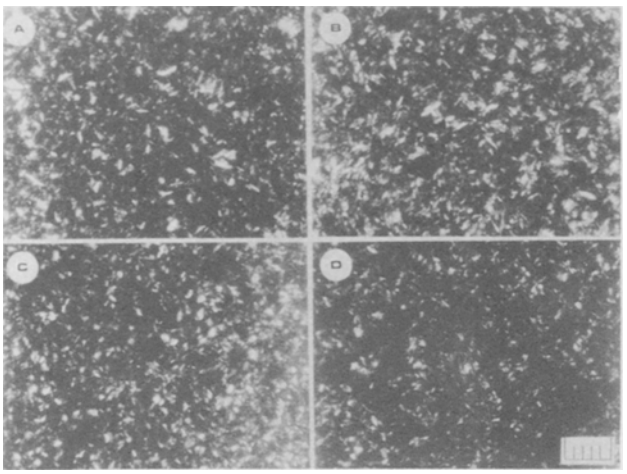

FIG. 1. Polarized light micrographs of: a) Shortening A immediately after suspension in isobutanol; b) Shortening A 24 hr later; c) Shortening B immediately after suspension in isobutanol; d) Shortening B 24 hr later (one scale division = 10 μm).

TABLE 3

Mean Crystal Size and the Height of Sediment and Supernatant of Different Shortenings Suspended in Isobutanol

Shortening	Mean crystal size (μm)	Sediment height (%)	Supernatant height (%)
A	8.51 ± 1.17	41.2	58.8
B	5.61 ± 0.23	65.9	34.1
C	5.60 ± 0.17	70.1	29.9
D	5.58 ± 0.19	69.2	30.8

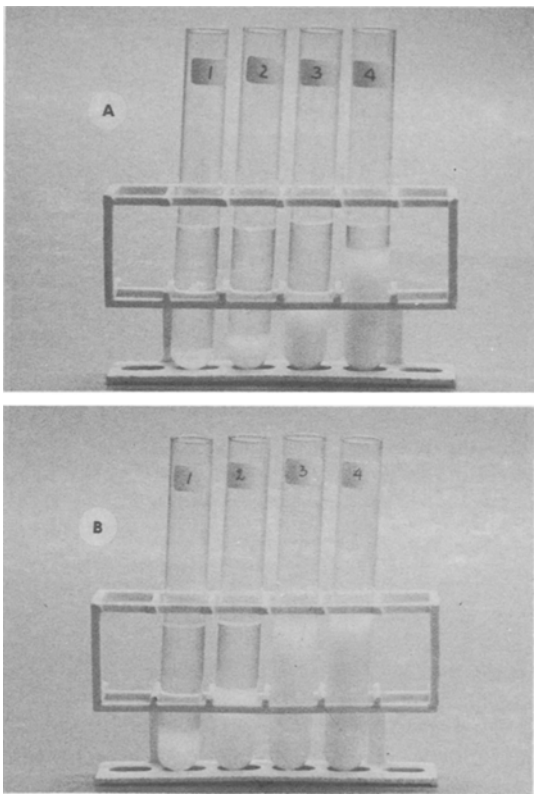

FIG. 2. Isobutanol suspensions of fats: A—Shortening with particle size over 10 μm: 1. 100 mg fat in 10 ml isobutanol; 2. 200 mg fat in 10 ml isobutanol; 3. 500 mg fat in 10 ml isobutanol; 4. 1000 mg fat in 10 ml isobutanol. B—Shortening with particle size under 5 μm: 1. 100 mg fat in 10 ml isobutanol; 2. 200 mg fat in 10 ml isobutanol; 3. 500 mg fat in 10 ml isobutanol; 4. 1000 mg fat in 10 ml isobutanol.

confirmed that the structural integrity of the fat crystals was not being affected by isobutanol (Fig. 1).

Isobutanol has been found to be an appropriate dilution medium with such plastic fats as margarines and shortenings. However, we have observed that isobutanol is not suitable for lauric fats which have a high content of lower chain fatty acids. It is suggested that for such fats, more polar solvents such as methanol or ethanol can be used as a dilution medium.

The rate at which the separation of the solid crystals and the liquid oil suspended in isobutanol takes place is a function of the size of the fat crystals. Fine crystals settle at a slower rate than coarse crystals. Fine crystals form a more voluminous network resulting in a larger sediment. This occurs because such crystals due to the presence of more contact points are able to immobilize more liquid. Coarse crystals settle at a faster rate and form a more closely packed sediment. Table 3 shows the height of the sediment and supernatant formed after 24 hr of contact of the shortening with isobutanol (1:50). This observation provides the basis for a rapid method of estimating the relative size of the fat crystals in a fat. The isobutanol dilution test can provide a rough estimation of crystal size without the use of sophisticated equipment. As seen in Figure 2, coarser crystals formed a more tightly packed sediment than the finer crystals. Also the height of the sediment increased as larger weights of fat were used in the suspension.

The mean crystal size of the shortenings ranged between 5-9 μm (Table 3). The size distribution indicated that the fat crystals ranged from 4-16 μm in size (Fig. 3 and 4). Albanese (9) reported that the fat crystals in shortenings are predominantly present in the form of needles ranging from 0.5 to 5.0 μm in size. The size as measured with the particle counter was compared with that seen under the polarized microscope using a stage micrometer. The results were comparable (Fig. 3 and 4).

Fat crystals obtained by the isobutanol separation technique from a shortening were examined by scanning electron microscopy. Figure 5 shows the appearance of these crystals as rod-like particles with a diameter of 1-2 μm and 5-8 μm in length. Further work is required to interpret the relationship between the traditional polarized light photomicrographs and the electron micrographs obtained by this procedure.

The isobutanol suspension method described in this paper enables the preparation of stable suspensions of fat crystals for particle counting by laser light scatter-

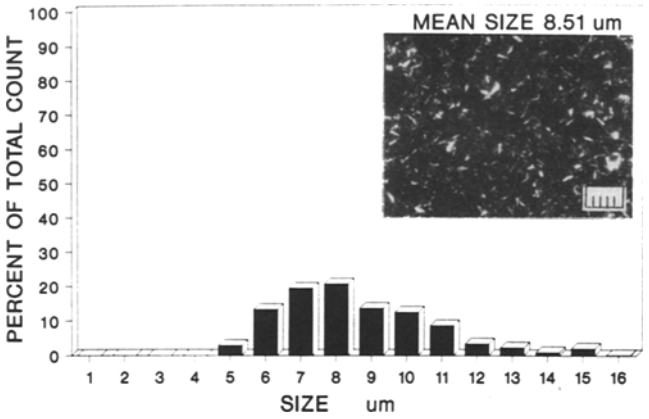

FIG. 3. Size distribution of fat crystals in Shortening A (1 division on scale = 10 μm).

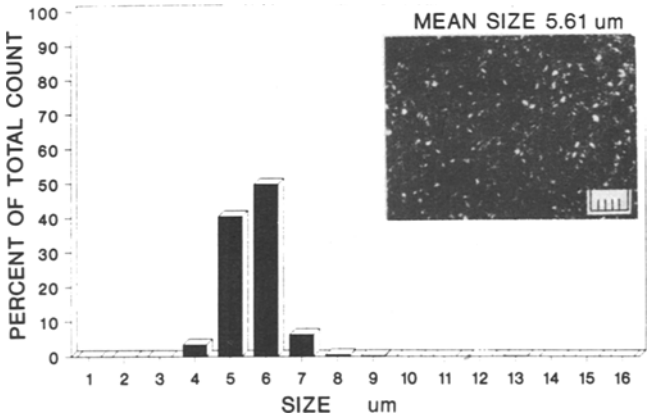

FIG. 4. Size distribution of fat crystals in Shortening B (1 division on scale = 10 μm).

ing. An additional advantage of this technique is that it makes it possible to quantitatively separate the solid and liquid phases of a fat. The separated solid phase can be conveniently examined by scanning electron microscopy, its fatty acid composition analyzed by GLC, and the polymorphic form determined by X-ray diffraction.

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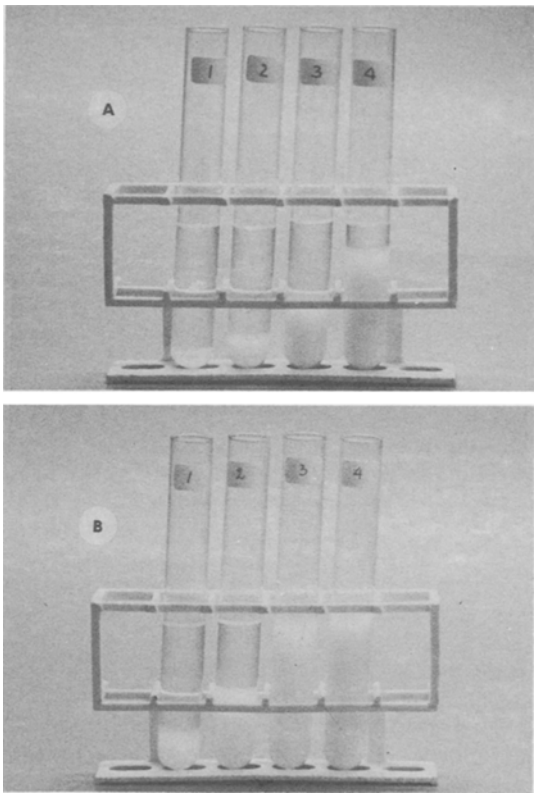

FIG. 5. Scanning electron micrograph of the solid fat from a shortening at 2,000 × magnification.

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