

Counting and Sizing Fat Globules Electronically

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Abstract

The particle volume and number of fat globules in milk has been successfully determined, using the electrical sensing-zone principle employed in the Coulter Counter. Changes occurring in the size distribution of these fat globules, as affected by the various steps in the production of cream, skim, homogenized milk, and milk concentrate have also been determined. Using raw milk, over 95% by weight of the fat was accounted for by globules with diameters ranging from 1 to 10 μ . Accuracy of the results is attested to by the fact that the fat content of the raw whole milk samples, as calculated from results obtained with the Coulter Counter, agreed with the standard Mojonnier analysis, with a relative error of less than 3%. The globules of raw milk, cream, HTST-pasteurized milk, and milk concentrate were counted with a 30- μ aperture. The skim and homogenized milks contained numerous small globules requiring the use of a 12- μ aperture. Milk samples homogenized at high pressures contained a significant number of globules too small to be counted.

The effect of pressure and shear on the constituents of milk moving through modern dairy equipment has been of continued interest to workers in our laboratory. When studying the changes in the fat phase of milk during product manufacture, we were struck by the difficulty of obtaining information pertaining to changes in the size distribution of fat globules. Classic methods employing either microscopy (4) or utilization of Stoke's law were tedious and time-consuming, microscopy being further limited by the resolution of the instrument and the visual acuity of the observer. The development of a commercial, electronic particle-sizing and counting device (Coulter Counter) seemed to offer a more acceptable solution to the problem of following changes in the size of the fat globules in milk. Whittlestone (7) has already reported on the use of a Coulter Counter in following changes in size distribution of fat globules in cow's milk throughout milking. How-

ever, as he used the equipment, no absolute value for fat globule sizes was obtained.

This paper presents our data pertaining to the applicability of electronic particle-sizing and counting, as done by the Coulter Counter, to studies of the fat globule size distribution in milk and related products.

Materials and Methods

The Coulter Counter. Berg (2) described the principles of the Coulter Counter in detail. The following is a brief summary: Figure 1 is an elementary sketch of the sensing unit of the Coulter Counter. A beaker contains the electrolyte and suspension to be counted. Immersed in the beaker is an aperture tube filled with electrolyte, with a precision micro aperture in its side. Two electrodes are suspended in the system; one in the beaker, the other within the aperture tube, with the micro aperture providing the only path for the conduction of electricity. A mercury manometer metering system (not shown) draws a measured volume of suspension to be counted through the aperture into the aperture tube. Each particle passage displaces electrolyte within the aperture, momentarily changing the resistance between the electrodes and producing a voltage pulse of a magnitude proportional to the particle volume. The resultant series of pulses is electronically amplified, scaled, and counted by the instrument, essentially a pulse-height analyzer. The proportionality between pulse height and par-

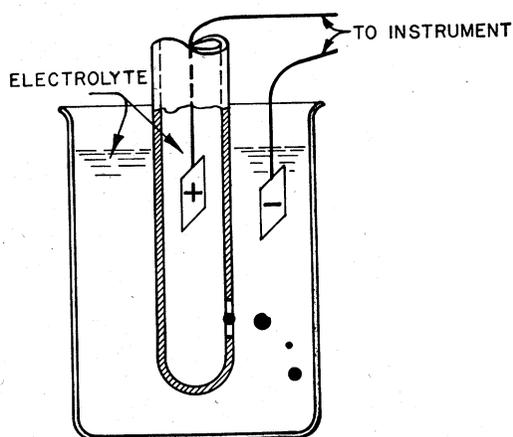


FIG. 1. Sensing mechanism of the Coulter Counter.

icle volume is established with the aid of a set of particles of known volume, thus giving the aperture calibration constant K .

The particles used in calibrating the instrument were mono-sized polystyrene latex spheres obtained from Particle Information Service, Los Altos, California. The size of these particles has been determined with the aid of an electron microscope. The calibration of each aperture was made with the manufacturer's recommended method, using clean electrolyte from the stock electrolyte to be used in the various size analysis. For the 30- μ aperture, either 3.59- or 2.04- μ -diameter spheres were used; for the 12- μ aperture either 1.30- or 0.871- μ -diameter spheres were used.

Milk processing. All the work reported here was done on mixed milk from a herd of 60-70 Holstein cows. Clarification and cream separation were done with a model 540B DeLaval cream separator, on milk warmed to 40 C. High-temperature—short-time pasteurization was done with a Mallory and a tubular heater at 77 C, followed by concentration to 50% T.S. in a Wiegand falling-film evaporator. One sample of milk was also pasteurized at 63 C for 30 min with gentle stirring. This last pasteurized milk was homogenized while hot, 60 C, at several pressures, using a Manton-Gaulin laboratory homogenizer model 15M8TA.

Fat globule size determination. Size distributions of the fat globules in the various milk samples were determined with a Coulter Counter Model A described above. For the raw milk, raw cream, and pasteurized milk samples a 30- μ aperture tube was used. The electrolyte was 2% potassium chloride in distilled water containing about 10^{-3} M phosphate buffer, 0.1% formaldehyde, and a few milligrams/liter of neomycin sulfate. The stock buffer solution was 0.1 M in each of mono- and disodium phosphate. The electrolyte was filtered successively through 8-, 1.2-, and 0.45- μ pore-size millipore filters. Electrolyte prepared in this way could be maintained at a low level of particulate contamination for several weeks. The pH of the electrolyte was kept above 6.5 with the phosphate buffer to prevent coagulation of the milk proteins.

Data were reduced in the manner recommended by the manufacturer, except for the method of choosing the average particle volume for each of the size intervals covered. The manufacturers use the average relative particle volume \bar{t} as the representative parameter for each size class, whereas we have chosen \bar{d}^3 , the cube of the average of the diameters defining each size class. The latter choice was made to reduce

the calculations involved in programming the counter.

The suspension to be counted was prepared by accurately weight about 1 g of milk into a 100-ml volumetric flask and diluting to the mark with the electrolyte solution. Dilution of 2 ml of this suspension to 200 ml in a volumetric flask gave the suspension on which counts of the globules above 2-3 μ were taken. To obtain an accurate count of the smaller globules, a further five- to tenfold dilution was used to reduce the noise level of the instrument at these more sensitive settings. In the data reduction step these counts were adjusted to the dilution used for the larger globules.

Counts were taken over the range of 0.6 to 14 μ on a metered volume of 50 μ l (0.05 ml) per count. To minimize the error in the approximation \bar{d}^3 , counts were taken at about 20 different size settings, thus giving a number of relatively narrow size classes.

Using the above data, the fat content of the original milk was calculated from the formula:

$$\text{Wt \% fat} = \frac{\sigma D}{M} \frac{\pi \Sigma \Delta \bar{n}^3}{6} \frac{100}{10^{12}}$$

where: σ = density of the fat phase
 D = dilution factor

$$\frac{\pi \Sigma \Delta \bar{n}^3}{6} = \text{total volume of fat in cubic microns per volume element } M$$

M = metered volume of dilute suspension in milliliters
 10^{12} = factor for converting cubic microns to milliliters
 100 = factor for converting from a fractional to a per cent basis.

For data reduced according to the manufacturer's procedure, the corresponding formula would be:

$$\text{Wt \% fat} = \frac{\sigma D \pi}{M 6} \frac{K^3 \Sigma \Delta n \bar{t}}{10^{12}} (100)$$

where:

K = aperture calibration constant
 \bar{t} = average relative particle volume for each size class.

Other terms are the same as those in the first equation.

For the work reported here, a value of 0.92 g/milliliter was used for the density of the fat. The dilution factor D is a volume-to-weight ratio (milliliters of diluent per gram of milk);

hence, the value for the density of the milk being analyzed is not needed.

The size distribution of the fat globules in homogenized and skimmilk was determined with a $12\text{-}\mu$ electrozone aperture, obtained from the Particle Data Instrument Company, Elmhurst, Illinois. The electrotype used was prepared as indicated above, except for the concentration, which was 7% in KCl, and the final filtration step for which a $0.22\text{-}\mu$ millipore filter was used.

For the homogenized milk samples fat recovery was determined by comparing the fat content of the undiluted milk as determined by the Coulter Counter with results of the standard Mojonnier fat test (6), according to the procedure outlined above. For this purpose a small quantity of the milk was weighed and diluted exactly, to give a suspension with a known concentration of about 1/10,000th that of the original milk.

Usually, no further dilutions were necessary, as electronically clean counts were obtainable even at the most sensitive settings for the $12\text{-}\mu$ aperture. As in the case with raw milk, the sensing range of the aperture, 0.3 to $6\ \mu$, was divided into about 20 intervals. A timer which started and stopped the count after a set time interval was used in place of the metering manometer. Counts were taken over a constant time interval of 5 or 10 sec, with a constant flow rate of suspension through the aperture. These settings correspond to volumes of about 2 and $4\ \mu\text{l}$, as determined according to the manufacturer's recommended method. The fat recovery figure thus obtained was used as the value for the weight per cent of fat with diameters larger than $0.30\ \mu$.

Materials used in the skimmilk analysis were the same as for the homogenized samples. Procedures used in the skimmilk analysis differed from the homogenized milk analysis only in the use of more concentrated suspensions, where the dilutions were approximately 2,000 to one.

Background counts were taken on the clean electrolytes used for all of the above systems, with the same instrument settings as those used in the corresponding size analysis. These counts were subtracted from the counts on the milk suspensions corrected for coincidence loss according to methods given by the manufacturer.

Results

Results of the various size analysis are presented in Figures 2-4. The comparison between the fat content of raw milk and cream, as calculated from the Coulter Counter results and the standard Mojonnier fat test, is presented in Table 1. The number of fat globules per mil-

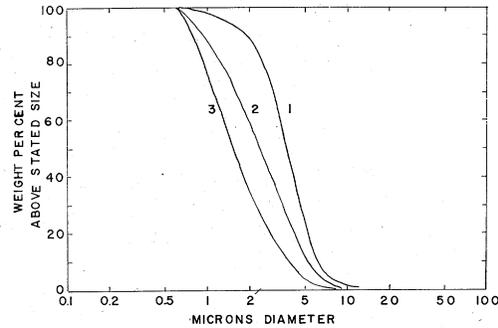


FIG. 2. Fat globule size distribution.
1. Raw whole milk
2. Pasteurized concentrate—tubular heater
3. Pasteurized concentrate—Mallory heater

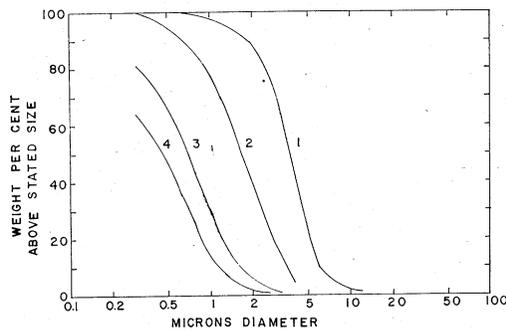


FIG. 3. Fat globule size distribution.
1. Raw whole milk. 2-4 homogenized milks.
2. $70\ \text{kg}/\text{cm}^2$
3. $176\ \text{kg}/\text{cm}^2$
4. $282\ \text{kg}/\text{cm}^2$

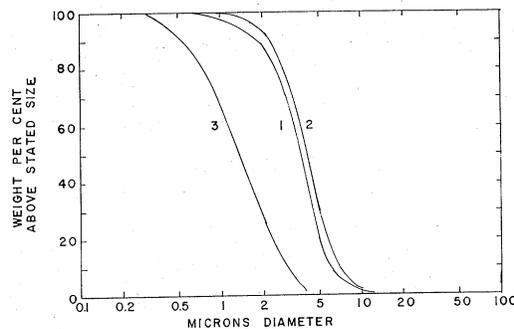


FIG. 4. Fat globule size distribution.
1. Raw whole milk
2. Raw cream
3. Skimmilk

liliter of raw and homogenized milk is presented in Table 2.

Figure 2 gives the cumulative weight distribution of the fat globules of raw and pasteurized milk. For raw milk, over 95% by weight of the fat consisted of globules with diameters between 1 and $10\ \mu$. The globules

TABLE 1
Comparison of Coulter Counter results with
Mojonnier fat analysis

Sample description	Wt. % fat		% Rel. error
	Mojonnier	C. Counter	
Raw whole milk	3.60	3.64	1.1
Raw whole milk	3.91	3.92	0.3
Raw whole milk	3.69	3.86	4.6
Raw whole milk	3.67	3.70	0.8
Raw whole milk	3.89	4.01	3.1
Raw cream	7.04	7.44	5.7
Raw cream	7.18	7.10	1.1
		Average	2.4%

TABLE 2
Fat globules/ml milk above stated diameter

Diameter (μ)	Raw ^a	Homogenized ^b	
		105 kg/cm ²	246 kg/cm ²
0.6	9.3×10 ⁸ (0.4)	6.9×10 ¹⁰	4.3×10 ¹⁰
1.0	4.2×10 ⁹ (0.2)	1.2×10 ¹⁰	3.0×10 ⁹
2.0	1.8×10 ⁹ (0.1)	1.2×10 ⁸	2×10 ⁷
3.0	7.5×10 ⁸ (0.4)	1.5×10 ⁷
8.0	4×10 ⁸ (2)

^a Average of six runs. Numbers in parentheses are mean deviations.

^b Data from single runs.

below 1 μ were quite numerous, but due to their small size they added little to the system. The fat globules above 10 μ were relatively few in number and the possibility of particulate contamination in the milk plasma itself made accurate counts increasingly difficult.

Pasteurization did not affect the size distribution of the fat globules, but subsequent concentration in a Wiegand falling-film evaporator caused a size reduction. The observed difference between the two concentrates is undoubtedly due to process variables, but further study is required to establish the exact cause.

In Table 1 the Mojonnier results are the averages of duplicate determinations (relative mean deviation less than 0.5%). The Coulter Counter results are from single determinations. The close agreement obtained attested to the validity of the Coulter technique for the determination of fat globule size distributions.

Results obtained on the homogenized milk sample (Figure 3) show the reduction in particle size as a function of homogenizing pressure. With the 12- μ aperture, essentially complete fat recovery was obtained on the milk samples homogenized at 70 kg/cm². These samples contained an appreciable fraction of globules larger than 2 μ . Homogenization at 176 and 282 kg/cm² reduced this oversize fraction to a few per cent by weight, and resulted in a sig-

nificant portion of the fat occurring as globules with diameters less than 0.3 μ . These globules could not be detected with the 12- μ aperture. Their presence was indicated by the incomplete fat recovery obtained with the Coulter Counter, as compared with the standard Mojonnier fat test.

Size distributions of the fat globules in cream and skimmilk are given in Figure 4. It can be seen that in the process of cream separation the smaller fat globules remained in the skimmilk, whereas the larger globules were separated out, as was expected.

Comparison between the result from the Coulter Counter and the Mojonnier fat test indicated that most of the particulate count obtained on skimmilk was due to the fat phase. The Mojonnier analysis of the above skimmilk (Figure 3) indicated 0.15% fat, whereas results from the Coulter Counter indicated 0.18% fat. The significance of the higher value obtained with the Counter has not been established and the difference should not, at this point, be construed as a measure of the nonfat particulate count. Size analysis on ammonium hydroxide-treated skim (1) gave essentially the same results as the untreated samples. Preliminary results showed that the addition of calcium to the electrolyte resulted in increased counts below about 0.5 μ (3). Presumably, the calcium stabilized particulate matter that would otherwise have been broken up by dilution in the potassium chloride electrolyte. The possible existence in milk of casein micelles and colloidal calcium phosphate particles with diameters above 0.3 μ has been discussed by Jenness and Patton (5). Further work is necessary to establish the cause and extent of this increased count.

The number of fat globules found in the raw whole milk and two of the homogenized samples is presented in Table 2. The data show the marked reduction in the number of globules above 2 μ as a result of homogenization. The fat globule count found for raw milk was higher than the range of 1.5 to 3 billion quoted by Jenness and Patton (4). The disagreement is not surprising, in view of the widely different conditions under which the data were taken. The microscope has poor resolving power below 1 μ and the detection of such small globules is a problem. The Coulter Counter data suffered from electronic noise, which contributed to the count at the 0.6 μ size setting. Attempts to determine the noise level with background counts on clean fat-free electrolyte were unsuccessful. Counts taken at 1 μ and above were found to be essentially free of such noise.

Discussion

Conversion of data obtained with the microscope (4) to weight distributions gave curves with the same general shape and range of fat globule diameters as found with the Coulter Counter. A faster and more reliable check of the instrument's performance was desired; hence, the fat content of raw milk and cream, as calculated from the experimental distributions, was compared with results from the Mojonnier fat test. The good agreement obtained attested to the validity of the Coulter technique for the determination of fat globule size distributions.

Homogenization, especially at high pressures, caused a reduction in the size of the fat globules to a point where a significant fraction of them could not be detected with the available equipment. Their presence was indicated by the incomplete fat recovery obtained with the Coulter.

For particulate systems which fall within the detection limits of the instrument, and where quantitative comparison with a standard is not required, exact preparation of the suspension to be counted is not necessary. The shape of a size-distribution curve is independent of the suspension concentration; hence, only approximate dilutions are required to keep the counts within reasonable limits.

The main experimental difficulties encountered in the size distribution determinations were the occurrence of electronic noise from external sources and the blockage of the apertures by large particles, especially dust, contaminating the electrolyte.

The electronic noise from external sources was virtually eliminated by placing a grounded shield around the counter. The shield consisted of aluminum foil wrapped around five sides of a lucite dust box, about the size of a laboratory hood. The front of the box was open, providing access to the instrument. The aluminum foil was grounded to a cold water pipe via a copper wire.

The problem of aperture blockage was com-

bated by rinsing the dust from all glassware coming in contact with electrolyte solution. A portion of the freshly filtered electrolyte was used for this purpose. The reservoir for the electrolyte came in contact with the solution only after the final filtration step. After several batches of electrolyte had been used, plugging ceased to be a problem with the 30- μ aperture. Plugging continued to occur with the 12- μ aperture even after a few batches of electrolyte had been used. This was probably a combination of the aperture's small size and particulate contamination in the milk itself. In all these cases, however, the simple flushing procedure recommended by the manufacturer removed the blockage and in very few cases was it necessary to interrupt an analysis.

Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

Acknowledgment

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