Size Distribution of Fat Globules in Goat Milk

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ABSTRACT

Milk from French-Alpine goats and Holstein cows was obtained from a bulk tank immediately prior to analyses. Fat globule size was determined by laser particle size analysis. Individual globules of fat in goat milk ranged from 0.73 to 8.58 μm in diameter. The average diameter of particles based on volume to surface area ratio (d_{vs}) was 2.76 μm and was less than the mean (d_{vs}) of 3.51 μm for bovine milk, in which fat globules ranged from 0.92 to 15.75 μm in diameter. The specific surface area of particles in caprine milk was 21,778 cm^2/ml, whereas the specific surface area of particles in bovine milk was 17,117 cm^2/ml. Ninety percent of the total particles found in goat milk were less than 5.21 μm in diameter, whereas 90% of the total particles in bovine milk were less than 6.42 μm based on the volume frequency distribution. Dissociation of casein micelles by urea in goat whole and skim milk caused larger d_{vs} values due to the effect of fat particles and reduced the specific surface area in both milks because the total number of detectable particles in both whole and skim milk was reduced.
(**Key words:** fat globule, size, particle, goat milk)

Abbreviation key: d_{vs} = volume/surface average diameter, **RI** = refractive index (indexes), **SSA** = specific surface area.

INTRODUCTION

Several reports of the size distribution of fat globules in bovine milk have been published (10, 17, 20), but the size of the fat globule in goat milk determined by sensitive techniques has not been reported recently. The creaming rate in milk and milk products is important in processing; the rate is less in goat milk than in bovine milk partly because of smaller fat globules (4, 10). Bovine milk creams more rapidly than goat milk because of agglutination, which causes clustering of fat globules. This agglutinin is apparently absent in goat milk and creaming is slower (4). Creaming rate is affected by several factors, among which the size of fat globules significantly influences this phenomenon.

Kulkarni and Dole (5) investigated the number and size of fat globules in buffalo, cow, and goat milk with a Naubeur Blood Counting Cell. The mean size of the fat globules was largest in buffalo milk and smallest in goat milk. Their results also indicated that total fat content, size of globules, and their relative abundance influenced the viscosity of milk. Total fat content and fat globule size distribution affects the viscosity of milk and has applications in the processing and manufacture of milk products (9). Smaller fat globules are usually better dispersed and provide a more homogeneous mixture of fat in milk. Puri et al. (11) applied Stokes' Law and, from fat globules rise, determined the fat globule size distribution in goat, cow, and buffalo milks. These investigators found that the average fat globule size is smallest in goat milk and largest in milk from buffalos, which is consistent with the results of Kulkarni and Dole (5).

The objectives of this research were to determine 1) the average fat globule size under different conditions in goat whole and skim milks, and 2) the contribution of casein micelles to the particle size distribution in goat whole and skim milks.

MATERIALS AND METHODS

Samples

Goat milk. Four replicates of morning milk were obtained from the bulk tank of a French-Alpine herd of 20 goats that were in midlactation at the International Dairy Goat Research Center at Prairie View A&M University. Milk was taken to the laboratory for analyses within 2 h of collection. Milk was heated to 63°C and homogenized at 0, 20, 40, 60, 80, and 100 MPa with a Rannie MINI-LAB type 8.30H homogenizer (APV Rannie Inc., St. Paul, MN). Milk was cooled to room temperature before analyses.

Goat skim milk. Skim milk was prepared by centrifugation of milk at 6000 $\times$ *g* for 10 min at 5°C (MR 18-12,

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Jouan Inc., Winchester, VA). Milk was centrifuged three times and the cream removed after each centrifugation.

Bovine milk. Four replicates of bovine milk from a herd of Holstein cows were obtained from the bulk tank at the Texas A&M dairy farm. The bovine milk was centrifuged at 6000 × *g* for 10 min at 5°C three times to obtain skim milk.

Percent fat and protein were determined by an automated infrared analyzer (Bentley 2000, Bentley Instruments, Inc., Chaska, MN). This instrument measures the energy absorption of milk components at specific wavelengths and determines concentration from comparison with reference samples in the mid-infrared region.

Particle Size Analysis

Light refraction is an additive property in a multicomponent system such as milk (3, 20). However, contributions from lactose, minerals, and soluble proteins are below the detection threshold of this system and only the refractive index (**RI**) of suspended particles that are in the detection range of this technique were applied in equations of optical models. Because the optical models are based on Mie theory of scattering of light by spherical particles with a homogeneous RI, fat globules and casein micelles most nearly fit these conditions (6). The RI of whole milk is primarily a measure of particles of fat globules and casein micelles. The RI of milk fat and casein in goat milk were determined by the following technique. Casein was collected by precipitation from goat milk as described by the Association of Official Analytical Chemists (1). The precipitated casein was lyophilized and the RI of serial dilutions of casein in double deionized and distilled water was determined by refractometer (Abbe-3L, No. 33.46.10; Spectronic Instruments, Rochester, NY). The RI of pure casein was obtained by extrapolating the concentration to 100% casein using linear regression (15). The RI of goat casein was determined to be 1.470. The RI of liquefied goat milk fat was 1.45, measured with a refractometer (Abbe-3L, No. 33.46.10; Spectronic Instruments). Fat for this determination was prepared from cream that was obtained by centrifugation of milk at 6000 × *g* for 10 min at 5°C and churned to butter. The butter was melted and centrifuged to separate butter oil from water. The RI of goat whole milk was determined to be 1.458, calculated from the average percent composition of its effective components as follows:

$$(3.31 \times 1.45) + (2.20 \times 1.47)/3.31 + 2.20$$

where

3.31 = mean % fat in goat milk,
1.45 = the refractive index of milk fat,
2.20 = mean % casein in goat milk
1.47 = the refractive index of casein.

Mean % casein was determined (1).

A few drops of milk, milk dissociated in 4 *M* urea, or milk dissociated in 8 *M* urea were suspended in the sample cell of the LS 130 Coulter Particle Size Analyzer (Coulter Corp., Miami, FL). The sample cell was filled with water, 4 *M* urea, or 8 *M* urea for the analysis of milk, milk dissociated in 4 *M* urea, and milk dissociated in 8 *M* urea, respectively. Milk was dissociated in 8 *M* urea buffer in 1:10 (vol/vol) ratio and then dispersed in water in the sample cell of the analyzer. The RI of goat whole milk (1.458) and pure water (1.333) were used in the equation of the optical model of the particle size analyzer that was used to determine the sizes of particles in goat whole milk suspended in water. When goat whole milk was dissociated and dispersed in 4 or 8 *M* urea buffers, the RI of these buffer solutions, rather than pure water, were used in the equations of optical models. The RI of 4 less and 8 *M* urea solutions, determined by refractometer, were 1.367 and 1.401, respectively. The dissociating buffer was prepared by mixing 8 *M* urea, 50 m*M* EDTA, and 10 m*M* beta mercaptoethanol (2, 16). The pH of this solution was adjusted to 7 with 1 *M* NaOH. It was filtered (Supor-450, 0.45 μm, Gelman Sciences, Ann Arbor, MI) to eliminate undissolved particles of urea or EDTA. For preparing the 4 *M* urea buffer, the concentration of 8 *M* urea solution was diluted by a factor of 2.

The RI values of goat milk casein and pure water were used in the equation of the optical model to calculate the size of particles when goat skim milk was dispersed in water. However, when the sample cell of the LS 130 Coulter contained 4 or 8 *M* urea, the RI of 4 and 8 *M* urea buffer solutions were used in the equations of optical models depending upon the buffer in which the skim milk was dispersed.

Bovine whole and skim milk samples were dispersed in water in the LS 130 Coulter particle size analyzer. Similar to goat whole milk, the RI value of bovine whole milk was calculated from the average percent composition of its components as 1.481. This value was used in the equation of optical model for bovine whole milk. Similar to goat milk casein, the RI of bovine casein was determined to be 1.503. This value was used in the equation of optical model for determining the particle size distribution in bovine skim milk.

Statistical Analyses

The ANOVA model used to analyze data from homogenized goat milk using the general linear model procedure of SAS (13) was:

Table 1. Average composition of milk samples.

	% Fat		% Protein	
	$\overline{X}$	SEM	$\overline{X}$	SEM
Goat skim milk	0.62	0.007	2.57	0.03
Cow skim milk	0.56	0.004	2.92	0.02
Goat whole milk	3.31	0.18	2.96	0.09
Cow whole milk	4.29	0.17	3.27	0.05

$$Y = \mu + P_i + C_j + (P*C)_{ij} + \varepsilon_{ij}$$

The following model was used to study the experimental variables in skim and whole milks separately.

$Y = \mu + C_j + \varepsilon$

μ = the population mean;

P_i = homogenization pressure (I = 20, 40, 60, 80, and 100 MPa);

C_j = treatments (j = 1 to 4; 1 = control, 2 = 4 *M* urea, 3 = 8 *M* urea, and 4 = 8 *M* urea dispersed in water);

P*C = interaction of homogenization and treatments;

ε = error term, the random variable assumed to be normally distributed with mean equal to zero and constant variance.

The least significant difference test on least squares means was used to determine significant differences between the treatment means (13).

RESULTS AND DISCUSSION

The composition of goat and bovine milks is shown in Table 1. The volume surface average diameters ($\mathbf{d_{vs}}$) and specific surface areas (**SSA**) in caprine and bovine whole milks were different (Table 2). However, the same parameters in the skim milks of these species were not different ($P < 0.05$). Walstra (17) and Mulder and Walstra (9) reported a mean d_{vs} of 3.4 μm and SSA of 2.0 m^2/g for fat globules in milk of Friesian cows which is in agreement with our results. However, the mean values of fat globules in caprine and bovine milks in this study were smaller than the values reported by estimation from direct microscopic count using a diluted sample of milk on Naubeur Blood Counting Cell (5, 7).

When goat whole milk was dissociated in 8 *M* urea and dispersed in water or dissociated and dispersed in 4 *M* urea, the d_{vs} and SSA did not differ from the control (Table 3). However, these parameters were different ($P < 0.05$) from the control when milk was dissociated and dispersed into 8 *M* urea. The 8 *M* urea solution probably caused complete dissociation of casein micelles and of some fat globule membrane proteins. The major particles in milk within the detection range of this instrument are fat globules and casein micelles; the fat globules are larger than the micelles. Casein micelles are spherical, ranging from 0.02 to 0.6 μm in diameter with an average diameter of approximately 0.14 μm. The number of casein micelles in milk decreases as the mean diameter of the micelles increase (14, 19, 21). Casein submicelles (0.008 to 0.02 μm) are below the detection limit of this system. Therefore, the number of casein micelles that were 0.1 μm or larger, which constituted the lower end of particle size distribution, probably decreased because dissociation and dispersion in 8 *M* urea reduced their size to below detection limit. This may have affected the particle size distribution and caused the larger d_{vs} and smaller SSA for the treatment. Additionally, some fat globules may have been released from their protective proteineaous membrane, which would allow coalescence and enlargement of fat globules. Thus, the number of total particles in the range of detection probably decreased and some larger fat particles may have been formed. These changes in number and size of particles were probably responsible for significantly altering d_{vs} and SSA. It appears that 4 *M* urea did not cause or caused only partial dissociation of casein micelles and fat globule membrane proteins. The effect of 4 *M* urea was not sufficient to change the d_{vs} and SSA values from the control. Likewise, the values obtained for the treatment with 4 *M* urea were not different ($P < 0.05$) from either treatment that was dissociated in 8 *M* urea.

When skim milk was dissociated in 8 *M* urea and dispersed in the sample cell of the analyzer in either 8 *M* urea or water, it exhibited a particle size distribution different from whole milk due to the predominance of casein micelles and the smaller fat globules that re-

Table 2. Mean diameter (d_{vs}), range, and specific surface area (SSA) of particles in milk.

	Whole milk					Skim milk			
	d_{vs}	SEM	Range	SSA	SEM	d_{vs}	SEM	SSA	SEM
	(μm)		(μm)	(cm^2/ml)		(μm)		(cm^2/ml)	
Bovine	3.51[a]	0.08	0.92–15.75	17,117[b]	550	0.213	0.03	282,100	7366
Caprine	2.76[b]	0.07	0.73–8.58	21,778[a]	476	0.225	0.02	266,411	4911

[a,b]Means in the same column without common superscripts differ ($P < 0.05$).

Table 3. Mean diameter (d_{vs}) and specific surface area (SSA) of particles in caprine milk treated with urea.

Treatments	Whole milk				Skim milk			
	d_{vs}	SEM	SSA	SEM	d_{vs}	SEM	SSA	SEM
	(μm)		(cm^2/ml)		(μm)		(cm^2/ml)	
Control	2.76[b]	0.07	21,778[a]	476	0.23[c]	0.02	266,411[a]	4911
4 *M* urea	2.92[ab]	0.08	20,587[ab]	550	0.29[c]	0.03	211,675[b]	7366
8 *M* urea	3.08[a]	0.07	19,581[b]	476	0.38[b]	0.03	158,866[c]	8506
H_2O[1]	2.73[b]	0.06	22,006[a]	426	0.49[a]	0.02	126,235[d]	6014

[a,b,c,d]Means in the same column without common superscripts differ ($P < 0.05$).

[1]Dissociated in 8 *M* urea, then dispersed in water medium.

mained after separation. The d_{vs} of the control and the treatment with 4 *M* urea were not different ($P < 0.05$), but the SSA between these treatments was different. Skim milk that was dissociated in 8 *M* urea and dispersed in urea or water had d_{vs} and SSA values different ($P < 0.05$) from the control. Unlike whole milk, the d_{vs} and SSA values of 4 *M* and 8 *M* urea treatments in skim milk were different ($P < 0.05$). This finding probably reflected a higher ratio of casein micelles to fat globules in skim milk. Dissociating casein micelles caused an increase in the d_{vs} which reflects the influence of the remaining fat globules in skim milk on the particle size distribution. The larger casein micelles (>0.1 μm), which are smaller than most fat particles probably do not exist after treatment of milk with 8 *M* urea and can not affect the d_{vs}. This is indicated because the SSA for the skim milk dissociated in 8 *M* urea and dispersed in either 8 *M* urea or water decreased significantly compared to samples treated with 4 *M* urea. When skim milk was dissociated in 8 *M* urea and dispersed in water the d_{vs} and SSA values were different from the other treatments. Because of the strong hydrophilic nature of water, after dissociation hydrophobic fat particles might have coalesced and contributed to the increased d_{vs} and reduced SSA. Robin and Paquin (12) used photon correlation spectroscopy to observe a 50% increase in d_{vs} caused by the use of dissociating buffer in a milk model emulsion system.

The sizes of particles in caprine and bovine milks that had d_{vs} values that represent the 10, 25, 50, 75, and 90 percent of volume frequency distribution are shown in Table 4. The mean d_{vs} of particles in each category in caprine and bovine whole milks were different ($P < 0.05$) with the caprine milk having smaller particles. This confirms the findings of earlier reports from microscopic techniques that goat milk has a larger proportion of smaller fat globules than bovine milk (10). However, the sizes of particles in skimmed milk from caprine and bovine milk were not different when compared within each percentage of the volume frequency distribution and probably reflects the contribution of mainly casein micelles rather than milk fat to the particle size distribution.

The SSA of particles in the control increased ($P < 0.05$) as the homogenization pressure increased (Table 5). This increased surface area of lipid is covered largely by casein micelles and some whey proteins (19). The SSA of the milk dissociated and dispersed in 4 *M* and 8 *M* urea increased ($P < 0.05$) as the homogenization pressure increased to 40 MPa as well as between 60 and 80 MPa. Further increases in homogenization pressure did not affect the SSA. The SSA was larger ($P < 0.05$) for the milk dispersed in 8 *M* urea compared to 4 *M* urea at each homogenization pressure. Most likely the 4 *M* and the 8 *M* urea treatments caused various degrees of dissociation of casein micelles and whey proteins which allowed some coalescence in the 4 *M* urea treatment and caused a smaller surface area. The SSA of the milk dissociated with 8 *M* urea and dispersed in water increased ($P < 0.05$) as the homogenization pressure increased to 80 MPa. As a result of urea dissociating protein membranes, the exposed fat particles with their hydrophobic characteristics in the strongly hydrophilic medium, water, probably formed larger fat particles which caused a

Table 4. The cumulative size distribution of particle diameter at 10, 25, 50, 75, and 90 percentile.

	Whole milk					Skim milk				
	(μm)									
	10	25	50	75	90	10	25	50	75	90
Bovine	2.23[a]	2.89[a]	3.84[a]	5.07[a]	6.42[a]	0.13	0.16	0.23	0.33	0.46
Caprine	1.69[b]	2.25[b]	3.09[b]	4.14[b]	5.21[b]	0.13	0.17	0.25	0.35	0.47

[a,b]Means in the same column without common superscripts differ ($P < 0.05$).

Table 5. Specific surface area of particles in homogenized caprine milk before and after treatment with urea.

Treatments	20MPa	40MPa	60MPa	80MPa	100MPA
	cm^2/ml				
Control	74,311^{eg}	109,839^{dg}	135,350^{cg}	153,375^{bh}	175,780^{ah}
4 *M* urea	62,899^{eg}	121,306^{bg}	145,300^{bg}	193,600^{ag}	203,500^{ag}
8 *M* urea	100,922^{ef}	170,650^{bf}	193,950^{bf}	244,100^{af}	253,300^{af}
H_2O[1]	60,036^{dg}	83,564^{ch}	110,375^{bh}	156,600^{ah}	174,866^{ah}

[a,b,c,d,e]Means in the same row without common superscripts differ ($P < 0.05$).

[f,g,h]Means in the same column without common superscripts differ ($P < 0.05$).

[1]Dissociated in 8 *M* urea then dispersed in water medium.

decrease in SSA at homogenization pressures greater than 20 MPa when compared with the other two dispersion treatments. However, this treatment when compared with the control indicates that self-association of caseins may have occurred at homogenization pressures of 80 and 100 MPa and probably self-association of caseins with increased internal hydration at lower homogenization pressures might have occurred, as was suggested in an earlier report (8). Walstra (18) showed that as pressure increased, d_{vs} of milk fat globules which is inversely proportional to the SSA decreased. Additionally, d_{vs} was decreased by repeated homogenization of milk under the same conditions.

Particle size measurements by laser light diffraction do not distinguish between different types of particles. Therefore, nonlipid particles in milk which are in the detection range of the instrument will be included in the measurements of fat globule sizes. Casein micelles greater than 0.1 μm in diameter are included in the measurements of undissociated milk and will introduce a small error in the calculations of fat globule sizes. On the other hand, it was not possible to obtain reliable counts for the fat globules smaller than 0.1 μm in diameter because this is the lower limit for the method used in this study. The number of fat globules smaller than the detection limit, which constitute only a small percentage of total fat, is unknown and hence the total number of fat globules is uncertain. This theoretically could cause a slight shift in the particle size distribution.

CONCLUSIONS

The average size of fat globules and the size distribution range of particles are smaller in goat milk than in bovine milk. When goat whole and skim milks were dissociated and dispersed in 8 *M* urea, the casein micelles were probably converted to submicelles that are below the detection limit and caused a decrease in total number of particles. This change caused an increase in the d_{vs} and a decrease in SSA of particles in milk. It appears that dissociation of skim milk in 8 *M* urea and dispersion of it in water might have caused coalescence of some fat globules.

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