ELSEVIER

International Dairy Journal 9 (1999) 201–205

INTERNATIONAL DAIRY JOURNAL

www.elsevier.com/locate/idairyj

Behaviour of casein micelles at conditions comparable to those in ice cream

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Abstract

The structure and stability of the casein micelle are fairly well known under conditions as in milk. However, much less is known and understood for conditions as are prevalent in ice cream. In this study it was observed that structure and behaviour of casein micelles in ice cream plasma at $-10^{\circ}C$ did not differ greatly from casein micelles in milk. A slightly increased amount of salts was associated with the micelles in ice cream plasma. Electron microscropy showed that casein in ice cream plasma exists in the form of micelles and submicelles. However, compared to milk, fewer submicelles and small micelles were present. Since the voluminosity was about the same as in milk, it was concluded that the increase in micelle size was primarily caused by the fusion of micelles and not by swelling. The properties of the casein micelles in ice cream plasma could be explained by a combination of the two conditions which differ significantly from those in milk: high concentration of milk components and low temperature.

Keywords: Casein micelles; Low temperature; Ice cream

1. Introduction

From a physical point of view, ice cream is a complicated disperse system. It is a solid foam of air cells, held together partly by emulsified fat, and partly by a network of small ice crystals dispersed in a sweetened, aqueous macromolecular solution (Dickinson & Stainsby, 1982). This solution (here called ice cream plasma) is mostly derived from milk and thus contains casein micelles, or fragments thereof.

The structure and stability of the casein micelle are fairly well described under conditions found in milk and several milk products (e.g. Holt & Horne, 1996; Walstra & Jenness, 1984; Walstra, 1990). However, there is little information on the casein micelle in ice cream. Casein micelles in ice cream are found in three locations: at the fat-plasma interface, at the air-plasma interface and in the plasma phase. The behaviour of casein micelles at these interfaces has been studied to some extent (e.g. Barford, Krog, Larsen & Buchheim, 1991; Buchheim & Dejmek, 1990; Gelin, Payen, Courthaudon, Le Meste & Lorient, 1994; Goff, Liboff, Jordan & Kinsella, 1987). So far, very little research has been carried out on the behaviour of casein micelles in the plasma phase of ice cream.

The understanding of the behaviour of casein micelles in milk is insufficient to predict what will happen with the micelles in the plasma phase of ice cream, especially because the conditions are so extreme: low temperature, high concentration of milk proteins and salts, high sugar concentration etc. All these variables affect the interaction forces between the components of the micelles, and often in different directions.

Thus, the behaviour of casein micelles in ice cream plasma is poorly described and is hard to predict. Nevertheless, this behaviour may well be as important in determining the properties (consistency, stability) of ice cream, as it is in many other milk products, and it should therefore be studied. Therefore the objective of this study was to determine and explain the behaviour of casein micelles in a model plasma phase of ice cream.

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0958-6946/99/$ - see front matter
PII: S0958-6946(99)00061-8

2. Materials and methods

A standard ice cream mix model system was made of demineralized water (71.0%), granulated sugar (16.7%) and low-heat skim milk powder (12.3%). After ageing at 2°C the mix was frozen in a scraped surface heat exchanger without aeration and stored at −10°C. After storage for at least one month at −10°C plasma was obtained from the frozen mass by removing ice and lactose crystals by pressing and centrifugation at −10°C. The water content of the ice cream plasma was 43%, the casein content was 8.4% and the whey protein content was 1.5%.

Reconstituted skim milk was prepared by dispersing 10.0 g of low-heat skim milk powder in 90.0 g demineralized water. To allow equilibration, the reconstituted skim milk was stirred for 24 h at 20°C.

For experiments at room temperature, 0.01% thiomersal (BDH Chemicals LTD) was added to ice cream plasma and milk to prevent bacterial growth.

2.1. Separation of the serum phase

To determine the fraction of salts present in the casein micelle and in the surrounding serum phase, the plasma had to be fractionated. The serum phase of ice cream plasma and milk was obtained by ultrafiltration (30 kDa cut-off) at −10°C and/or room temperature. Protein was completely retained by the membrane.

Milk was also fractionated by ultracentrifugation. Milk was centrifuged at 70 000 *g* at 20°C for 2 h in a Beckman XL-90 centrifuge with a 6 × 38 ml swing-out rotor. After centrifugation the supernatant was decanted and used for analysis.

The third way in which milk was fractionated was by renneting. 0.025% of a commercial calf rennet (0.025%; 10800 SU, CSK, Leeuwarden, The Netherlands) was added to the reconstituted skim milk. After 5 h the milk gel was centrifuged at 3330 g for 30 min and the whey was decanted and used for analysis.

2.2. Chemical analysis

Calcium and magnesium were determined, after wet digestion, by atomic absorption spectrometry at 422.7 and 285.2 nm, respectively. Lanthanum nitrate hexahydrate (0.1%; Merck 1.05326) was added to the samples to prevent interference of phosphate in the calcium determination.

Total phosphorus content was determined, after wet digestion, by a colorimetric method according to the International IDF Standard 42B : 1990. The phosphorus content was also estimated in the filtrate obtained after the precipitation of proteins by the addition of trichloroacetic acid (TCA; 12% final concentration). Total phosphorus minus phosphorus in the filtrate gave micellar inorganic **P**.

Citric acid concentrations were enzymatically determined using Boehringer Mannheim test kit (139076).

The amount of salts determined in permeate, supernatant, whey and filtrate were recalculated to values in ice cream plasma and milk by applying the corrections for non-solvent water and volume occupied by proteins.

2.3. Rheological measurements

Rheological measurements were performed at −10°C using a Bohlin CVO Rheometer, equipped with concentric cylinder geometry.

During viscometry the shear stress was varied from 0.02464 to 11.02 Pa in 23 steps with equal distance on a logarithmic scale and the corresponding shear rates were measured. Other instrumental settings were: constant delay time 120 s, integration time 60 s.

Dynamic measurements were also performed after a pre-shear of 300 Pa was applied for 1800 s. The instrumental settings were: target strain 0.001, frequency 0.1 Hz, delay time 10 s, wait time 900 s. A thin layer of silicone oil was applied to the samples to prevent drying out.

2.4. Microscopy

Transmission electron microscopy was used to study the appearance of casein micelles in ice cream plasma. Samples were prepared by freeze-fracturing followed by unidirectional shadowing using tantalum/tungsten at an angle of 45° and backed with rotary carbon at an angle of 90°. All micrographs were produced using a Jeol 1200EXII transmission electron microscope operated at 80 kV. Micrographs were obtained × 20 000 (microscope magnification) and printed × 4 (i.e. total magnification × 80 000).

Casein micelles on the micrographs were sized using General image analysis software.

3. Results and discussion

3.1. Salt distribution

The salt distribution present in the serum phase of milk obtained by ultrafiltration, renneting and ultracentrifugation is shown in Table 1. Compared to the proportions determined by other separation techniques, the percentage of salts present in the serum phase of milk obtained by ultrafiltration was rather low. However, the results obtained by ultrafiltration by Pouliot, Boulet and Paquin (1989) and Pouliot and Boulet (1995) were quite similar to the ultrafiltration results obtained in this study. The difference between the ultrafiltration and renneting results is probably due to precipitation of calcium phosphate on the surface of and inside the ultrafiltration

Table 1
Percentages of salt species present in the serum phase of milk and ice cream plasma

	Method	T	Ca	Mg	Cit	P	P_i
Milk	Uf	RT	26	58	84	40	55
Milk	Rennet	RT	29	67	89	46	63
Milk	Uc	RT	37	70	93	56	77
Ice cream plasma	Uf	−10°C	21	52	70	37	51
Ice cream plasma	Uf	RT	7.7	46	42	34	49

The percentage of calcium, magnesium, citrate, phosphorus and inorganic phosphorus was estimated in the serum phase of milk and ice cream plasma at −10°C and/or room temperature (RT). The serum phase was obtained by ultrafiltration (Uf) or renneting or ultracentrifugation (Uc).

membrane (Hanemaaijer, Robbertsen, van den Boomgaard & Gunnink, 1989), presumably as a result of the enhanced concentration of the salts especially near and in the membrane. Ultracentrifugation yielded higher fractions of salts in the serum phase (Table 1). Because the sedimentation velocity of submicelles was very low (calculated at about 2 mm/h), submicelles and small micelles, including their micellar calcium phosphate and counterions, would remain in the supernatant. Renneting of milk has little much influence on the calcium phosphate distribution (Schipper, 1961) and no casein and micellar calcium phosphate were supposed to be present in the whey. Therefore, renneting yields the most reliable results for the partition of salts between the micellar and serum phase in milk.

Because renneting and ultracentrifugation could not be applied at −10°C, the serum phase of ice cream plasma was obtained by ultrafiltration at −10°C and room temperature (Table 1). The percentages of all salts present in the serum phase were slightly lower for ice cream plasma than for milk. It is known that a reduction in temperature to about 5°C leads to dissociation of calcium phosphate from the micelles (e.g. Davies & White, 1960; Pouliot et al., 1989). The influence on the calcium phosphate distribution of a further decrease in temperature is not known. It is also known that concentration of milk leads to the association of calcium phosphate with the micelles (Nieuwenhuijse, Timmermans & Walstra, 1988; Pouliot & Boulet, 1995). If the observed lower percentage of salts observed in the serum phase is not due to differences in precipitation of salts on the membrane, it can be concluded that the total effect of concentration and lowering the temperature on the calcium phosphate distribution in ice cream plasma is a slightly enhanced association of calcium phosphate with the micelles.

Heating ice cream plasma to room temperature caused association of salts with the micelles (Table 1). In particular the percentage of calcium and citrate in the serum phase decreased. An explanation for this phenomenon may be that, in addition to precipitation of calcium phosphate in the micelles, precipitation of calcium citrate also occurred. In concentrated milk deposits of calcium citrate have been observed (Deysher & Webb, 1952) and computer calculations by Lyster (1979) showed that formation of tricalcium citrate was possible under these circumstances in a salt solution similar in ionic composition to an ultrafiltrate of milk.

3.2. Rheology

The viscosity as a function of shear rate was determined for ice cream plasma and its permeate at −10°C. It was observed that ice cream plasma was slightly shear thinning. The viscosity at a shear rate of 350 s^{-1} was for permeate (η_{serum}) 0.151 Pa.s and for ice cream plasma (η_{plasma}) 0.714 Pa.s.

Snoeren, Damman and Klok (1982) concluded that the Eilers relation predicts the viscosity of concentrated skim milks fairly well. Their results can be used to estimate the voluminosity of casein micelles in ice cream plasma. The viscosity of the ice cream plasma η_{plasma} can be expressed as a function of the volume fraction of the protein Φ; in ice cream plasma this is the volume fraction occupied by casein (Φ_c) and by native whey protein (Φ_{nw}). We thus have

$$\eta_{plasma} = \eta_{serum}[1 + 1.25\Phi/(1 - \Phi/\Phi_{max})]^2, \quad (1)$$

where

$$\Phi = \Phi_c + \Phi_{nw} \quad (2)$$

and generally

$$\Phi_x = v_x c_x. \quad (3)$$

In ice cream plasma the casein concentration c_c was 0.103 g ml^{-1} and the native whey protein concentration was c_{nw} 0.0183 g ml^{-1}. Snoeren et al. (1982) used a value of 0.79 for the maximum volume fraction Φ_{max} and, for the voluminosity of native whey protein v_{nw}, 1.07 ml g^{-1}. Substitution of these values in the equations yields a voluminosity of casein micelles in ice cream plasma v_c of 4.0 ml g^{-1}. Because a value of roughly 3.9 ml g^{-1} casein is usually found in milk (Walstra, 1979) we may conclude that the voluminosity of casein micelles in ice cream plasma at −10°C was similar to that in milk at room temperature.

Although reducing the temperature of milk to 5°C leads to a significant increase in voluminosity, the influence of milk concentration on the voluminosity of casein micelles is not known. Presumably it will lead to a decrease in voluminosity, since additional calcium phosphate is formed which would tend to make the micelles more compact. In our system, the effect of reducing the temperature appears to neutralise the effect of milk concentration on the voluminosity.

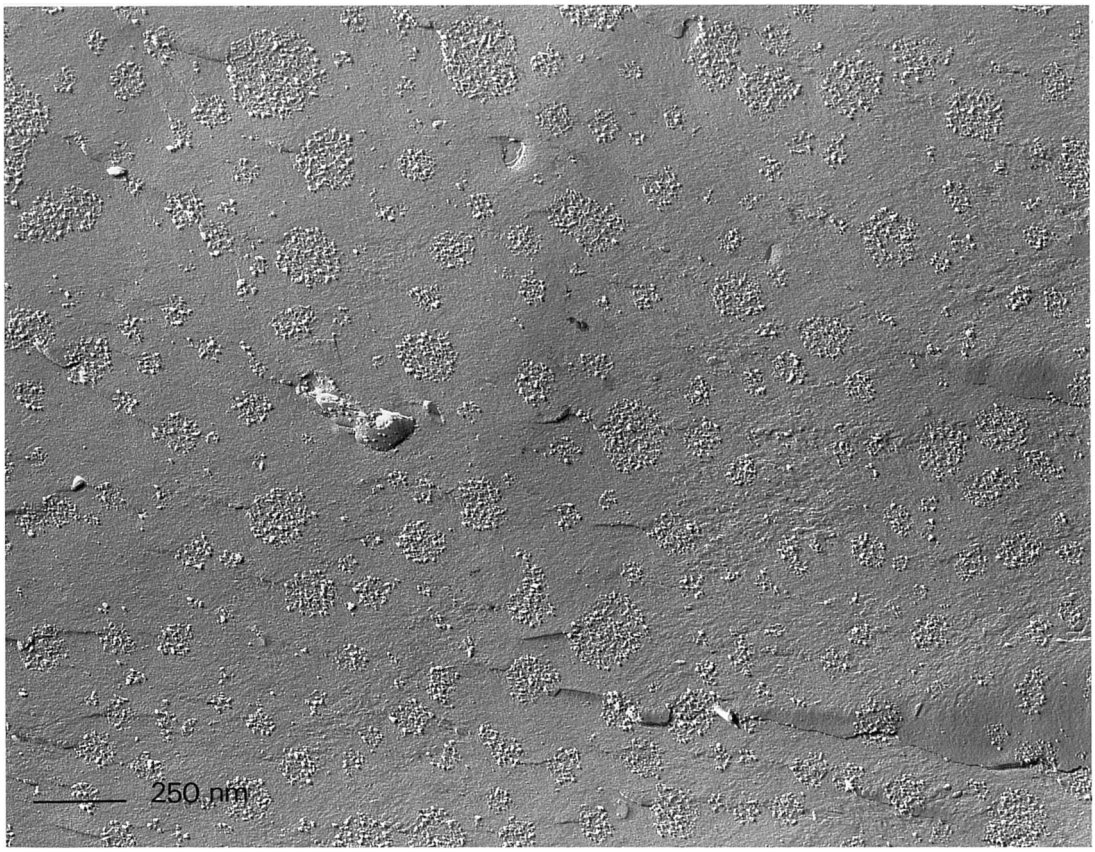

Fig. 1. Casein micelles in ice cream plasma (freeze-fracture electron microscopy).

Oscillation measurements showed no change in viscoelastic moduli in time during measurements for 100 000 s. Thus, network formation did not occur.

3.3. Microscopy

The appearance of micelles in ice cream plasma at $-10^{\circ}C$ is shown in Fig. 1. It can be seen that casein exists as micelles and as submicelles. When we compare this micrograph with micrographs of milk, fewer submicelles were present in ice cream plasma than in milk. No network could be observed on the micrograph.

Two hundred and seventy seven (277) micelles were sized on electron micrographs of ice cream plasma. Although the number of micelles counted was too low to derive a precise size distribution an estimate of size distribution parameters can be deduced from these data (Buchheim et al., 1995). The estimated surface average diameter, d_{vs}, was 101 nm and the number average diameter, d_{10}, was 39 nm. Schmidt, Walstra and Buchheim (1973) also used electron micrographs for sizing of micelles and found for micelles in milk at room temperature a d_{vs} of 86 nm and a d_{10} of 25 nm. The larger average micelle size in ice cream plasma may be due to fusion of micelles or to swelling. Because the viscosity measurements showed that the voluminosity of casein micelles in ice cream plasma at $-10^{\circ}C$ was about the same as in milk at room temperature, it can be concluded that the increase in size was primarily caused by fusion of micelles.

It is known that decreasing the temperature of milk to $5^{\circ}C$ causes a considerable increase in the number of small micelles and submicelles (Schmidt, van der Spek, Buchheim & Hinz, 1974). On the other hand, concentration of milk causes an increase in micellar size (Jonkman, Walstra, van Boekel & Cebula, unpublished results). Since the average micelle size increased, it appears that the effect of concentration is more important than that of temperature.

4. Conclusions

This study showed that structure and behaviour of casein micelles in ice cream plasma at $-10^{\circ}C$ did not differ greatly from casein micelles in milk. A slightly higher percentage of salts was associated with the micelles in ice cream plasma. The voluminosity of casein micelles was the same as in milk. The average size of micelles was increased. Fewer submicelles were observed and fusion of micelles must have occurred.

Most of the properties of the casein micelles in ice cream plasma could be explained by a combination of the two conditions which differ significantly as compared to milk: high concentration of milk components and low temperature. The effect of concentration on the micelle properties was slightly greater or neutralised the effect of a decrease in temperature.

Acknowledgements

The authors thank Mrs. J. Brigham (Unilever Research Colworth Laboratory) for making the electron micrographs and Mrs. M. A. H. Baltussen (Wageningen Agricultural University) for performing part of the salt analyses.

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