人工カゼインミセル濃縮液の低温保持による粘度上昇

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Increase in the viscosity of concentrated artificial casein micelle solution during storage at low temperature

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Abstract

An artificial casein micelle (ACM) solution was prepared, in which the concentrations of casein, calcium, phosphate, and citrate were close to those in skim milk. The viscosity of concentrated ACM solutions increased markedly at low temperature within several days. This phenomenon was similar to the one that takes place in highly concentrated skim milk indicating that casein micelles play a determinant role in the viscosity increment of concentrated milk. The addition of lactose to concentrated ACM solution efficiently decreased its viscosity, suggesting a stabilizing effect of soluble lactose on casein micelles against their aggregation. The contents of micellar casein, calcium, and phosphate increased with the calcium content of the ACM solution, while the voluminosity of casein micelles decreased and the viscosity increment of the concentrated ACM solution was accelerated. These results suggest that the increase in the interactions between casein micelles might accelerate the viscosity increment, and might overcome the suppressing effect of decreased voluminosity. The size distributions of ACM solutions were not influenced by their micellar calcium content, and almost no changes were observed after the significant increase in the viscosity, suggesting that interactions between casein micelles were weak and reversible.

Key words: casein micelle, skim milk concentrate, lactose crystallization, viscosity

Introduction

Concentrated skim milk is a dairy ingredient used in a wide variety of beverages and food products in Japan. It is produced mainly in Hokkaido and transported to areas of large-scale consumption by ship under the chilled condition. When highly concentrated skim milk is stored at low temperature for several days, its viscosity increases1) and lactose crystallization takes place2). These phenomena need to be controlled, because they restrict the utilization of concentrated skim milk in food processing.

A number of studies have been conducted to elucidate the rheological properties of concentrated milk; however, most of these experiments focused on the “age-thickening” phenomenon, wherein the rheological analysis of concentrated milk samples have been performed at room temperature or at higher temperatures3~6). Therefore, the rheological behavior of concentrated milk at low temperature is still a matter of particular interest.

In the previous work7), we demonstrated that the viscosity of concentrated skim milk stored at low temperature significantly increased with the formation of a number of fine lactose crystals, indicating that a decrease in supersaturated lactose following lactose crystallization may promote the viscosity increment of concentrated skim milk. In this
case, lactose crystallization and interactions of proteins proceed simultaneously, and their effects on viscosity changes might be co-operative, and their individual role remains to be experimentally elucidated.

An artificial casein micelle (ACM) is useful for the physicochemical study of casein micelle system because of its simple composition without whey proteins, lactose, and other minor constituents. Another experimental benefit of ACM is its flexibility in arranging its composition. Thus, in this paper, we prepared ACM solution, in which concentrations of casein, calcium, phosphate, and citrate were close to those in skim milk; we added CaCl$_2$, K$_2$HPO$_4$, and tri-potassium citrate to whole casein solution, and examined the changes in the viscosity of concentrated ACM solution during storage at low temperature. The effect of lactose addition and the level of calcium on the changes in the viscosity of the concentrated ACM solution were also examined. The size distribution of casein micelles in diluted concentrated ACM solutions, which may reflect its possible relevance to the change in viscosity, was estimated by using a laser diffraction particle analyzer.

The objective of this work was to determine the role of lactose and casein micelles in the viscosity changes in concentrated skim milk during storage at low temperature, by using an ACM solution.

Materials and Methods

1. Preparation of concentrated ACM solution

Fresh skim milk was obtained from a dairy factory of Meiji Co., Ltd. in Hokkaido and was used for preparing whole casein by performing isoelectric precipitation using HCl at pH 4.6. The precipitate was sufficiently washed with water until lactose and whey proteins were not detected in the flushed water and was dissolved by gradual addition of 1 N NaOH, while the pH was maintained below 7.0. The pH was finally adjusted to 6.7. The 3 kg of 5% casein solution was poured into 5 L plastic bags and kept at -30°C before use.

ACM solutions were prepared according to the method of Knoop et al.$^8$, with minor modifications.$^9$. Appropriate volumes of 1 M tri-potassium citrate, 0.2 M CaCl$_2$, and 0.2 M K$_2$HPO$_4$ were added to 5% casein solution to obtain the prescribed concentrations of citrate, calcium, and phosphate. Initially, the entire requisite volume of citrate solution was added to casein solution after which the calcium and phosphate solutions were added simultaneously in 10 equal portions at 10 min intervals. The volume was adjusted to provide a final casein concentration of 2.5%, and the pH was adjusted to 6.7. The mixture was kept at 35°C in a water bath and was stirred with T. K. Homo Mixer Mark-2 (Primix Corp., Osaka, Japan) during the preparation. The mixture was then homogenized at 15 MPa to disrupt the formation of small flocculates. The standard concentrations of Ca, inorganic phosphate (P$_i$), and citrate in the ACM solution were 30 mM, 22 mM (without the ester phosphate of casein), and 10 mM, respectively, which are close to those in bovine skim milk. Evaporation was carried out below 45°C by using a centrifugal thin film evaporator Evapol CEP-L (Okawara Manufacturing Co., Ltd., Shizuoka, Japan). Concentrated ACM solutions were stored at 4°C. The pH was measured every 24 h to evaluate the extent of microbial contamination, and no change was observed during storage.

2. Determination of nitrogen and mineral contents

The nitrogen content was determined by the Dumas combustion method using a Vario Max CN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany), and the amount of protein was calculated by multiplying the nitrogen content by 6.38.

Concentrations of calcium and phosphorus were determined by using the multi-type ICPE–9000 ICP emission spectrometer (Shimadzu Corp., Kyoto, Japan).

ACM solutions were ultracentrifuged at 100,000 g for 1 h at 25°C. The percentage of micellar casein to total casein was calculated on the basis of the total and serum nitrogen. Micellar calcium and micellar P$_i$ were calculated from their total and serum concentration.

3. Measurement of voluminosity

The voluminosity of casein micelles was determined using the method of Snoeren et al.$^{10}$. The
moisture content in the pellet was estimated from the difference between the weights before and after freeze-drying of the ultracentrifuged pellet, and the protein content of the dry pellet was determined by the method described above. The voluminosity of casein micelles was expressed as g water/g protein.

4. Measurement of viscosity
The viscosity was measured at 4°C using a Brookfield DV-E Viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA). Spindle No. 64 was adopted for all samples at 30 rpm for 30 s. The data shown are mean values obtained from 4 measurements, and the error bar represents the standard error.

5. Measurement of particle size distribution
The size distribution of ACM solutions was measured using a laser diffraction particle analyzer LS230 (Beckman Coulter, Inc., Miami, FL). The sample was added into the analytical circulating vessel filled with its corresponding ultrafiltrate as the suspension fluid which had been supplied with Pellicon 2 ultrafiltration cassette, with 10 kDa as the cutoff value (Millipore, New Bedford, MA, U.S.A).

Results and Discussion

The standard ACM solution was concentrated to 10.3-10.8 g casein/100 ml, and its viscosity was measured. Figure 1 shows changes in the viscosity of concentrated ACM solution during storage at 4°C. The viscosity apparently increased with storage time, and even a small increase in the concentration accelerated this rate of rise in viscosity. When the ACM solution was concentrated to more than 11 g protein/100 ml, a marked increase in viscosity occurred within 24 h followed by gelation after which the viscosity could not be measured. These observations were similar to those reported by us previously7, where the viscosity of highly concentrated skim milk increased markedly during storage at low temperature for several days. The increase in the viscosity of concentrated ACM solutions without whey proteins, lactose, and other minor constituents indicates that the casein micelles and their interactions play a determinant role in viscosity increment of concentrated milk. In order to confirm the influence of frozen storage and thawing of whole casein solution on the viscosity changes, the ACM solution was prepared from unfrozen whole casein solution and the viscosity changes of concentrated ACM solutions were compared. No differences were observed between the unfrozen and frozen samples (results not shown).

When the viscosity of concentrated skim milk increased to about 10,000 mPa·s after 72 h of storage at 4°C in our previous study7, the decrease in volume was estimated to be 1/5.3 of that of the original skim milk. On the other hand, concentrated ACM solution containing 10.8 g casein/100 ml, which had a similar viscosity increment, corresponds to that of 1/4.4. Thus, the viscosity of the concentrated ACM solution increased at lower concentration than that of the concentrated skim milk, suggesting that lactose may stabilize casein micelles against their aggregation in the latter case, because of the possibility of soluble disaccharide to have an effect on the stability of proteins11,12.

In the previous study7, we have proposed that the increase in the viscosity of concentrated skim milk during storage at low temperature might be suppressed by the presence of supersaturated lactose which remained in a soluble state before crystallization. To confirm the role of lactose in concentrated skim milk with respect to its possible relevance to the change in viscosity, a small amount of lactose was added to the concentrated ACM solu-
tion prepared from the standard ACM solution, so as to prevent the formation of lactose crystals. As shown in Fig. 2, the viscosity of the concentrated ACM solution decreased on adding increasing amounts of supplementary lactose. These results support our hypothesis that soluble lactose, which was included in skim milk originally and not crystallized under the supersaturated condition, stabilizes the casein micelles against their aggregation and suppresses the viscosity increment in the concentrated skim milk.

The influence of the formation and properties of casein micelles on the change in viscosity was examined by preparing concentrated ACM solutions with different levels of calcium, and measuring their viscosities after 72 h of storage at 4°C. The contents of micellar casein, calcium, and P_i along with the voluminosity of casein micelles in the ACM solutions are listed in Table 1. The contents of casein, calcium, and P_i in casein micelle increased with increasing the calcium concentration in ACM solutions, while the voluminosity decreased. In Fig. 3, the viscosity of concentrated ACM solutions is given as a function of protein content. The higher the calcium content, the lower is the protein concentration where a rapid rise in viscosity was observed for all the samples. This result suggests that the viscosity of concentrated ACM solution during storage at 4°C is promoted due to an increase in interactions between casein micelles, which might be caused by rise in micellar casein, calcium, and P_i in casein micelles. It is also possible that the diminished electrostatic repulsions between casein micelles with increasing calcium content accelerate the rise in viscosity. On the other hand, an increase in calcium content in the ACM solution decreased the voluminosity of casein micelles, which could decrease the viscosity of the ACM solution, even though the rate of viscosity increment of the concentrated ACM solutions was promoted. These results could offer a plausible explanation for the viscosity increment of the concentrated ACM solution due to its relevance to the formation and properties of casein micelles. According to Schmidt(14), casein micelles in ACM solution are considered to exist as colloidal particles with a submicellar structure. When the content of calcium in the ACM solution was increased, the distance between micelles could become smaller, accompanied by a decrease in the electrostatic

![Fig. 2 Effects of lactose addition on the changes in the viscosity of concentrated ACM solution (10.6 g/100 ml of casein content) prepared from an ACM solution with standard composition. Concentrated ACM solution without adding lactose. □ 1% of lactose addition, and △ 2% of lactose addition.](image)

![Fig. 3 Effects of calcium content on the changes in the viscosity of concentrated ACM solution. The samples were stored at 4°C and viscosities were measured after 72 h. Concentrated samples were prepared from ACM solutions of sample no. 1 (■), no. 2 (●), and no. 3 (▲), respectively. Sample numbers are the same as those shown in Table 1.](image)

### Table 1 Micellar casein, calcium and P_i contents, and the voluminosity of casein micelles in ACM solutions with different calcium concentration.

<table>
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<tr>
<th>Sample no.</th>
<th>Calcium (mM)</th>
<th>Micellar casein (g water/g protein)</th>
<th>Micellar Ca (mM)</th>
<th>Micellar P_i (mM)</th>
<th>Voluminosity (g water/g protein)</th>
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<tr>
<td>1</td>
<td>23</td>
<td>12</td>
<td>75</td>
<td>9</td>
<td>4.1</td>
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<tr>
<td>2^1</td>
<td>30</td>
<td>91</td>
<td>91</td>
<td>17</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>95</td>
<td>95</td>
<td>16</td>
<td>3.1</td>
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All samples contain 2.5% of casein, 22 mM P_i, and 10 mM citrate. a) Total calcium concentration in ACM solutions. b) Standard ACM solution.
repulsions caused by an additional binding of calcium to the negatively charged surface of casein micelles. We considered that this might decrease the voluminosity of casein micelles, which may weaken the interactions between casein micelles, since the distance grows due to the "shrinking" of each particle. However, soluble casein, calcium, and phosphate are transferred into a colloidal phase by adding more calcium. This leads to an increase in the amount of casein micelles. Furthermore, these interactions might be accelerated by evaporation, which increases the volume fraction of dispersed particles by the removal of water. Therefore, it is possible that the promoting effect of diminished electrostatic repulsions and increased interactions between the casein micelles on the viscosity increment overcomes the suppressing effect of decreased voluminosity. Consequently, the viscosity increment of concentrated ACM solution might be enhanced, as its calcium content increases in spite of the decrease in the voluminosity of casein micelles.

The particle size distributions in ACM solutions containing different concentrations of calcium are shown in Fig. 4. There was almost no effect of the calcium content on the size distribution of casein micelles, such that all micelles were in the size range of 40 to 400 nm, and their average diameters were around 130 nm in each sample, suggesting that changes in the viscosity of the concentrated ACM solutions might be influenced by the amount of casein micelles, but not by their size distribution. Furthermore, we found that the particle size distributions in the concentrated ACM solution stored for 72 h at 4°C, which was prepared from ACM solutions no. 1, 2, and 3, and measured after diluting with the ultrafiltrate obtained from their corresponding ACM solutions, coincided with those of the original ACM solutions. This suggests that the casein micelles in the concentrated ACM solution retained their original size distribution without irreversible aggregation even after a significant increase in the viscosity, and disaggregated reversibly after dilution. These findings support the conclusions drawn from previous studies on the concentrated skim milk7), and could explain more precisely the role of the casein micelles in concentrated system.

The experimental results obtained by using ACM solutions indicate possible roles of lactose and casein micelles in the viscosity changes in concentrated skim milk during storage at low temperature. This information is useful for developing a wide variety of dairy ingredients containing concentrated milk by assessing the stability and microstructure of the casein micelles.

![Size distributions of casein micelles in ACM solutions with different calcium concentrations, sample no. 1 (■), no. 2 (○), and no. 3 (▲). The measurements were performed by using a laser diffraction particle analyzer in the PIDS optical mode. Sample numbers are the same as those shown in Table 1.](image)

Fig. 4

References


4) Bienvenue, A., Jiménez-Flores, R., and Singh, H.: Rheological properties of concentrated skim milk: Importance of soluble minerals in the changes in viscosity-

人工カゼインミセル濃縮液の低温保持による粘度上昇

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人工カゼインミセル（ACM）溶液（カゼイン2.5％）を調製し、3.5倍以上に濃縮して冷蔵すると、時じた粘度の上昇が観られた。濃縮率を大きくすることで、冷蔵保存中の粘度上昇は著しかった。脱脂乳を4.5倍以上に濃縮して冷蔵すると同様の増粘現象が観られたことから、カゼインミセルが増粘の主要因であることが示唆された。ACM 濃縮液に1-2％の乳糖を添加すると増粘速度が小さくなり、乳糖が増粘を抑制する効果が確認された。ACM 濃縮液中のカルシウム濃度を上げてカゼインミセルの形成速度を上げると増粘が促進されたことから、カゼインミセルの相互作用の増加が増粘を促進したと考えられた。ACM 溶液中のカルシウム濃度は粒子径分布に影響せず、増粘した ACM 濃縮液中のカゼインミセルの粒子径分布は濃縮前の ACM 溶液の粒子径分布と一致した。脱脂濃縮乳と同様に、増粘に伴うカゼインミセル間の相互作用は、可逆的かつ弱い相互作用であると考えられた。