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Hydrostatic Pressure Effects on Physical Properties of Ultrafiltrated Skim Milk in the Presence of EGTA

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EGTA를 첨가한 한외여과 탈지유의 물성에 미치는 초고압의 영향

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Abstract

The study investigated the effects of protein concentration, EGTA and strength of hydrostatic pressure on pH, viscosity and turbidity for ultra filtrated skim milk retentates. The results showed that hydrostatic pressure treatment up to 600 MPa did not affect the viscosity of skim milk, while the turbidity of skim milk increased at higher than 200 MPa. Addition of EGTA caused reduction in turbidity of skim milk, two times (2SR) and three times (3SR) concentrated skim milk retentates. Viscosity for 2SR and 3SR increased proportionally to the amount of EGTA, but viscosity of skim milk was not influenced by EGTA. High pressure treatment also did not cause any difference in viscosity and turbidity of skim milk. However, this treatment decreased viscosity and turbidity for 2SR and 3SR. In particular, 200 MPa treatment showed to induce a higher decrease in turbidity compared with 400 MPa.

Key words: hydrostatic pressure, skim milk, UF-retentate, viscosity, turbidity, EGTA.

Introduction

Casein micelles in milk are almost spherical colloidal particles, composed of a number of small subunits, referred to as casein submicelles, and colloidal calcium phosphate (CCP) plays a key role in maintaining the integrity of the casein micelles[18,22]. High pressure induces both reversible and irreversible conformational changes in proteins by disruption of hydrophobic, ionic and hydrogen bonds[11]. The degradation of the CCP, which may be accomplished by either treatment with chelating agent[4,16] or the application of high pressure results in a dissociation of micelles into submicelles[2,16,17,19], with a decrease in milk turbidity[2,19] and lightness,[1,4] and an increase in the viscosity[1] of the milk. The pH and calcium ion activity of the skim milk were not effectd up to 6 kbar[4], on the other side pH of skim milk was increased by high pressure[19]. In addition, turbidity of β-casein solution was decreased until 1,500 kg/cm^2 and then increased in pressure up to 3,000 kg/cm^2[12]. And the whey protein were preferentially participated in pressureinduced aggregatuion and gelation through S-S bonding[15]. As a consequence, the solubility and/or the functional properties of the protein can be altered, which would have further implications for the performance and marktability

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of a product containing the protein[4], such as preferential proteolysis of β-lactoglobulin[3,13,14], improving shelf-life of yogurt[21], and improving yield or coagulation time in cheese[6,7,8]. I considered that the behavior of forming gel in milk both high protein concentrations and higher pressure was linked to calcium content. By applying ultrafiltration and adding EGTA, it may be possible to induce different concentrations of calcium ion and protein in skim milk. This experiment was conducted to investigate the effects of the concentrations of casein and calcium ion, and the strength of pressure on pH, viscosity and turbidity of ultrafiltrated skim milk retentate.

Materials and Methods

Sample Preparation and Treatment

1) Ultrafiltrated Skim Milk

Skim milk was prepared by using a cream separator (Electric Cream Separator 80 L/h, Elecream Co., France). The skim milk was than concentrated in the ratios of 1.0 (skim milk), 2.0 (skim milk retentate, 2SR) and 3.0 (skim milk retentate, 3SR) of the initial volume using a ultrafiltrater (Amicon CH2PRS, Hollow Fiber Cartridge HIP10-20, cut-off MW 10,000). The ratios of retentate and permeate were 1:0, 1:1 and 1:2, respectively. The protein concentration for skim milk, 2SR and 3SR, determined according to the methods described by Markwell et al.[10] and Peterson[15] using bovine Albumin as a standard protein at 750 nm. The final protein concentration were 1.59±0.8, 3.18±0.17 and 4.59±0.03 %, respectively.

2) EGTA Treatment

1N-EGTA (Ethylene Glycol-bis(b-Aminoethyl Ether)-N.N.N.N.-Tetraacetic Acid, WAKO Chemical Co., Japan) stock solution (pH 8.1) was added to the prepared skim milk and skim milk retentates to the final concentration of 0, 10, 20 and 30 mM.

3) Hydrostatic Pressure

0.1, 200, 400 MPa was applied to each mixture in a capped-Teflon tube (10 ml) using a Ultra-Pressure Reactor (HR15-B2, Hikari Koatsu Co., Hiroshima, Japan) for 15 minutes at 30℃.

Measurement and Statistical Analysis

The pH was determined using a potable combination electrode (Horiba F-21, Japan). Turbidity of the treated samples was measured using a spectrophotometer (Hitachi U-2000, Japan) at 570 nm, according to the methods followed by Kanno et al.[5]. For the measurement, samples were diluted 50 times in volume with distilled water. Effect of the treatments on changes in viscosity was measured using a digital viscometer (DV-Me 20, Tokyo Keiki, Japan) at 30℃, and expressed as centi poise. Analysis of variance at the level of 5% was performed using a SPSS[29].

Results and Discussion

Effects of Hydrostatic Pressure on Viscosity and Turbidity

A preliminary exoeriment was performed to determined the effects on hydrostatic pressure on viscosity and turbidity of skim milk. High pressure treatment appeared to have a little effect on viscosity of skim milk, ranging from 1.9 to 2.2 cp(Fig. 1). However there was a tendency that, when the magnitude of pressure gradually increased from 0.1 to 600 MPa, viscosity increased at a hydrostatic pressure higher than 300 MPa after a marginal decrease at 100 and 200 MPa by 0.1~0.2 cp. The trend for the highest viscosity at 200 MPa was not clearly understood, but a limited effect of high pressure treatment on turbidity of skim milk was generally in agrement with the result of Kanno et al.[5].

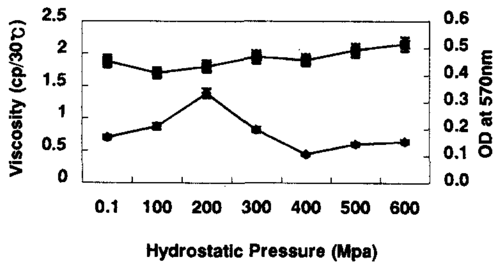

Fig. 1. Effects of hydrostatic pressure on the viscosity and turbidity of skim milk.
-■- viscosity, -▲- turbidity

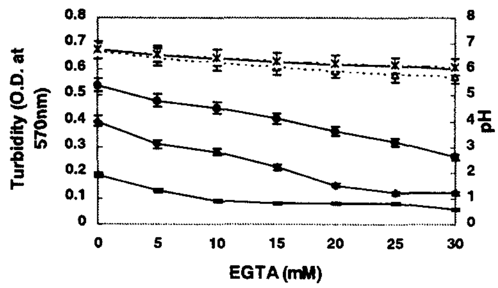

Fig. 2. Effects of EGTA on the turbidity and pH of UF-retentated skim milk. Turbidity : -▲- skim milk, -◆- retentate(conc. ratio ×2.), -●- retentate(conc. ratio ×3.), pH : -△- skim milk, -◇- retentate(conc. ratio ×2.), -○- retentate(conc. ratio ×3.).

The study showed that the influence of high pressure treatment up to 400 MPa on viscosity was negligible, when the concentration of whey protein isolate and whey protein concentrate was lower than 8%.

When turbidity of diluted skim (eg, 50 times) was determined by observance at 570 nm, that showed a quadratic relationship between magnitude of high pressure existed; turbidity increased up to 200 MPa, decreased up to 400 MPa, and again increased up to 600 MPa. The results were generally in similar tendency with the previous study of Schrader and Buchheim[19]. They showed that either 200 MPa or 350 MPa treatment increased turbidity, and then the treatment higher than 350 MPa caused decrease in turbidity. The authors concluded that the results were associated with denaturation of whey protein and subsequent reaggregation of other proteins. In the current study decrease in turbidity for higher than 300 MPa treatment was not clearly explained. In addition, the results under this experimental condition did not coincide with a number of previous studies[2,4,12]. In the study of Desobry-Banon et al.[4] turbidity of milk was stable up to 230 MPa high pressure and decreased up to 430 MPa. However, given to the slightly different sample and treatment conditions, it was not easy to reach conclusion.

Effects of EGTA Treatment on Turbidity and pH

Fig. 2 shows the effects of EGTA treatment on the turbidity and the pH of skim milk and UF-retentated skim milk(2SR and 3SR). As expected, initial turbidity was high for higher concentrations of skim milk retentate with 0.403 and 0.538 for 2SR and 3SR, respectively, compared to 0.192 for non-concentrated skim milk. Turbidity increased with increasing the concentration of skim milk, and decreased with increasing EGTA concentration ($p<0.05$). However, turbidity for skim milk and 2SR was not influenced even at the EGTA concentration higher than 25 mM ($p>0.05$). The results were contributed to a reductive effect of EGTA, which prevents the aggregation of casein micell to some extent. In the case of the 3SR sample, rich calcium ions due to a higher concentration of skim milk were considered to be linked to continuous decrease in turbidity. A previous study[4] showed that high pressure treatment up to 6 Kbar did not influence the calcium activity of pasteurized skim milk. In addition, disintegration of casein micelles induced by adding chelating agent did not showed a proportional increase in L* value of the aliquots.

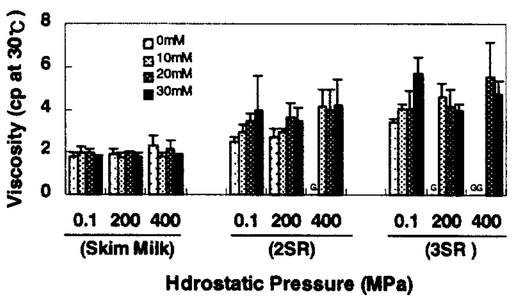

Fig. 3. Effects of hydrostatic pressure on the viscosity of UF-retentated skim milk added with EGTA. G : forming a gel and not measured. 2SR, 3SR : UF-retentate (The ratios of retentate and permeate were 1:1 and 1:2, respectively)

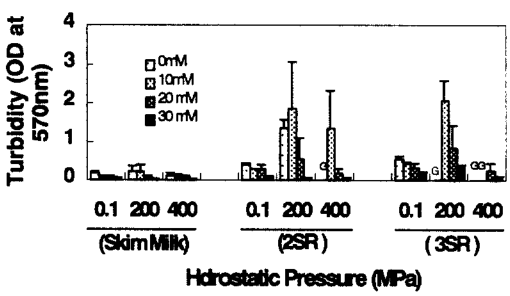

Fig. 4. Effects of hydrostatic pressure on the turbidity of UF-retentated skim milk added with EGTA. G : forming a gel and not measured. 2SR, 3SR : UF-retentate (The ratios of retentate and permeate were 1:1 and 1:2, respectively).

The process of concentrating skim milk did not affect pH as shown by similar pH of 6.76 to 6.79. Similarly, addition of EGTA(pH 8.13) up to 5 mM to the three different concentrations of skim milk showed a little effect on pH. However when the concentration of EGTA was higher than 10 mM, addition of each 1 mM EGTA induced 0.03 units of pH decline for skim milk, and 0.02 units for 2SR and 3SR ($p<0.05$). It was particularly noticeable that decrease in pH for 2SR was more prominent than other treatments after EGTA concentration higher than 10 mM.

Interaction between hydrostatic pressure and EGTA treatment on viscosity and turbidity

Fig. 3 shows the interaction between concentration of EGTA and strength of pressure on viscosity for skim milk, 2SR and 3SR. Initial viscosity (ie, 0.1 MPa) for a higher protein concentration showed higher viscosity with 1.80, 2.47 and 3.38 cp for skim milk, 2SR and 3SR, respectively, which was the increase of 37.2% and 36.8% in comparison to the initial levels for skim milk ($p< 0.05$).

It was noticeable that skim milk was not affected by pressure treatment, ranging 1.80 ~2.00 cp. even with addition of EGTA. On the other hand, when the magnitude of pressure increased, viscosity for the retentates raised. When 2SR and 3SR without the addition of EGTA were pressured under higher than 400 MPa and 200 MPa, the retentates formed complete gel respectively. For these samples, the measurement of viscosity was not possible.

As expectedly, EGTA treatment on 2SR and 3SR significantly increased viscosity ($p<0.05$). In the case of 2SR, 400 MPa resulted in a higher viscosity of EGTA treated sample than a 200 MPa ($p<0.05$). Non-EGTA treated samples formed gel under a 400 MPa treatment. However 400 MPa treatment for 10~30 mM of EGTA treated sample resulted in a similar viscosity without gel formation. For the 3SR sample, adding 10 mM and 20 mM of EGTA prevented the gelation even under 200 MPa and 400 MPa treatments, respectively. In contrast, when EGTA treatment was higher than those concentrations, viscosity decreased. On the basis of calcium activity[4] and dissociation and ressociation of casein micelles[12,19], the appropriately concentrations of EGTA to prevent the formation of gel were 0, 10 and 20 mM for skim milk, 2SR and 3SR, respectively. This also indicated that gel formation did not occur when calcium activity was controled even for high protein concentration.

Fig. 4 shows the effects of EGTA and hydrostatic pressure treatments on the turbidity of skim milk in three different concentrations. There was general tendencies that turbidity for a higher protein concentration resulted in a higher turbidity, and the increase in addition of EGTA reduced the turbidity. EGTA treatment showed little effect on viscosity of skim milk even for high pressure treatment. However high pressure and EGTA treatments for 2SR and 3SR were considerable. In these samples, the highest turbidity resulted from 200 MPa treatment for 10 mM-EGTA samples, and decreased dramatically from 20 mM, resulting in a lower turbidity than that for 0.1 MPa. 400 Mpa resulted in a lower turbidity than 200 MPa even at a same level of EGTA. In particular 3SR showed a lower turbidity than 0.1 MPa. These results demonstrated that reducing calcium activity by adding EGTA causes a dissociation of casein micelles, resulting in decrease turbidity. On the other hand, high pressure treatment induces re-association of casein micelles[4,12,19]. In addition, the current study indicated that 200 MPa induced more strong reassociation of casein micelles in compared with 400 MPa. However high pressure and EGTA treatments for 2SR and 3SR were considerable. In these samples, the highest turbidity resulted from 200 MPa treatment for 10 mM-EGTA samples, and decreased dramatically from 20 mM, resulting in a lower turbidity than that for 0.1 MPa. 400 Mpa resulted in a lower turbidity than 200 MPa even at a same level of EGTA. In particular 3SR showed a lower turbidity than 0.1 MPa. These results demonstrated that reducing calcium activity by adding EGTA causes a dissociation of casein micelles, resulting in decrease turbidity. On the other hand, high pressure treatment induces reassociation of casein micelles[4,12,19]. In addition, the current study indicated that 200 MPa induced more strong reassociation of casein micelles in compared with 400 MPa.

요 약

탈지유와 UF-retentate에 착염제인 EGTA를 첨가하여 Ca ion을 조절하였을 때 점도와 탁도의 변화에 미치는 고압처리의 영향을 조사하였다. 탈지유는 600MPa까지 가압하여도 점도의 변화는 없었으나 탁도는 200MPa에서 증가하였다.

탈지유, 2배, 3배 농축유에 첨가한 EGTA의 농도가 증가하면 탁도는 점차 감소하였으며 점도는 탈지유에서 변화가 없는 반면 2배와 3배 농축유는 EGTA의 농도가 증가할수록 증가하였다. 여기에 압력을 가하면 탈지유의 점도나 탁도에 압력과 EGTA의 영향은 없었으나 2배 또는 3배 농축유의 경우 점도와 탁도는 감소하였으며 특히 탁도는 200MPa 보다도 400MPa에서 더 감소하였다.

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