Research Article

Interaction between Casein and Soybean Soluble Polysaccharide during Acidification

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Abstract: Interactions between stabilizer and casein were believed to influence the texture and stabilizing behavior of fermented milk. In this study, interactions between Soybean Soluble Polysaccharide (SSPS) and casein were studied by investigated the properties of potentiometric titration, turbidity, ζ-potential and particle size. Attractive interaction of SSPS with caseins was initiated at pH 5.3. By adding SSPS, the values of turbidity and ζ-potential were much lower than casein alone at the same pH during acidification. It was confirmed that the process of desorption during neutralization was irreversible which resulted from the decrease of ζ-potential and the changes of particle diameter. The study of interactions between negatively charged SSPS and casein could have important theoretical and practical implications in the stabilizing behavior in acid milk drinks.

Keywords: Acidification, casein, interaction, soybean soluble polysaccharide, stability

INTRODUCTION

Caseins were the critical milk proteins which account for 76-86% of the milk proteins (McSweeney and Fox, 2009). In general, caseins were solubilized as the form of casein micelles under the neutral pH conditions, although which could aggregate and precipitate under acidic pH conditions. To avoid the undesirable phenomenon of phase separation, stabilizers were added into the system of acidified milk drinks, such as carrageenan, pectin and sodium carboxymethyl cellulose (Tuinier et al., 2002). Under acidic conditions, the negatively charged stabilizers could adsorb to positively charged caseins and thereby generate electrostatic repulsive forces and steric repulsive forces. Furthermore, that contributed to the stabilization of acidified milk drinks.

The previous studies had focused mainly on the interaction between casein micelles and some kinds of polysaccharides, such as carrageenan, pectin and sodium carboxymethyl cellulose (Cupeval et al., 2009; Du et al., 2009; Liu et al., 2006). Soybean Soluble Polysaccharide (SSPS) was an anionic polysaccharide. It contained 18% galacturonic acid originated from soybean cotyledons. Recently, SSPS had been commercialized to improve the stability and texture of acidified milk drinks (Pereyra et al., 1997). Compared to other stabilizers, such as guar gum and high methoxyl pectin pectin, SSPS had a globular structure which may be responsible for the lower viscosity and the optimal mouth feel (Wang et al., 2005). Meanwhile, SSPS had a high ability to stabilize proteins under the low acidic pH conditions (pH 4.4-4.6).

Nobuhara et al. (2014) reported that the protein stabilizing ability of SSPS-HMW (SSPS of High Molecular Weight) to disperse milk proteins because of the electrostatic repulsive force of polysaccharide chains and the steric repulsive force of the thicker layer (possibly a multi-layer) that formed on the surface of the particles. Some researchers (Mirhosseini et al., 2008; Pereyra et al., 1997) found that distribution of molecular weight affected the functionality of the SSPS. The ability of SSPS to stabilize milk proteins could lead to the production of new types of acidified milk drinks.

However, there has been no systematic research about the colloidal and interfacial properties of SSPS to stabilize protein dispersions. In this study, the potentiometric titration, turbidity, ζ-potential and particle size of casein alone and casein-SSPS complex were measured as a function of the pH during acidification. This investigation aimed to study the interaction of casein and SSPS during acidification and neutralization. It could have important theoretical and practical implications in the stabilizing behavior in acid milk drinks.

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MATERIALS AND METHODS

Material: Skim milk powder was obtained from Fonterra Co-operative Group (Wellington, New Zealand). SSPS was donated by Fuji Oil Co., Ltd. (Osaka, Japan). Citric acid was obtained from Zhanwang chemical Co., Ltd. (Wuxi, China).

Preparation of casein and casein-SSPS mixture: The reconstituted milk with a content of 2% (w/v) protein was prepared by mixing skim milk powder. Meanwhile, SSPS at 0.2, 0.4, 0.6, 0.8 and 1.0% (w/v), respectively were prepared in deionized water stirred at 70°C for 30 min and stored at room temperature to allow a complete hydration. The final concentrations in mixtures were calculated to obtain the samples with the contents of 1% (w/v) proteins and 0.0, 0.2, 0.3, 0.4 and 0.5% (w/v), respectively SSPS made from the reconstituted milk and SPSS hydrates.

Potentiometric titration: Titration curves up to pH 3.6 (from pH 6.8 to 3.6) were obtained by continuously adding 0.1 M citric acid with an automated titrator (MPT-2 AG, Herisau, Switzerland). The volumes of citric acid were recorded at 0.1±0.05 interval of pH.

Turbidity measurements: The sample was adjusted with citric acid (0.1 M) from pH 6.8 to 3.6. Each sample was diluted with deionized water. Turbidity measurements were carried out with a 722-type spectrophotometer (Shanghai Analytical Instruments, China). The turbidity was monitored by the absorbance at 600 nm. A Glass-stoppered quartz cuvette with 1-cm pathway was used.

Measurements of ζ-potential: The measurement of zeta potential (ζ-potential) was performed at 25°C using a Nano ZS Zetasizer (Malvern Instruments LTD., UK). The parameter of He-Ne laser was selected at 633 nm. PH pathway was used.

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Potentiometric titration of casein-SSPS mixtures: Potentiometric titration allowed the determination of ionic interactions between charged molecules. To reach the given pH value, acid was needed to add into the system (Mattison et al., 1998; Girard and Schaffer-Lequart, 2008). The curves of potentiometric titration obtained for the typical casein-SSPS mixture (0.5%) and the casein alone were illustrated in Fig. 1. It was showed that the interaction between casein and SSPS occurred at pH value of 5.3±0.1. The pKa of ionic groups of proteins was shifted by the interaction among the negatively charged groups of polysaccharide and the positively charged groups of proteins. The interaction of casein and the polysaccharide might result in proton migration from NH$_2^+$- to COO$^-$ groups. It had been demonstrated that electrostatic interactions between oppositely charged proteins or polysaccharides were the primary interactions in the associative system of biopolymer. Furthermore, hydrogen bonding or hydrophobic interaction could also cause the interaction among the macromolecules (Doublier et al., 2000).

Changes in turbidity during acidification: Turbidity was characterized by incident light attenuation caused by light scattering. According to Rayleigh scattering
theory (I \propto d^6, the intensity of the scattered light varies as the sixth power of the particle size), light scattering is influenced by the size of micelle particles. Turbidity could be evaluated to reflect the light scattering intensity (Pereyra et al., 1997). The effect of pH on turbidity (OD_{600}) of casein alone and casein-SSPS mixtures was shown in Fig. 2. For the sample of casein, turbidity significantly slowed down from 1.281 to 0.575 with the decrease of pH from 6.8 to 5.4. The changes in turbidity probability resulted from the collapse of outer “hairy” layer of casein micelles as well as the dissolution of $\alpha_s$-casein, $\beta$-casein and casein calcium phosphate (Rassis et al., 2002). At lower pH (pH<5.4), the turbidity of casein increased dramatically (0.570-1.257), which might due to the aggregation of casein micelles. When pH was below 4.8, the sample of casein without the addition of SSPS precipitated and no more data could be evaluated.

For casein-SSPS mixtures, values of turbidity ranged from 0.05-0.25 and pH had little effect on them with pH decreasing from 6.8 to 5.4. Meanwhile, values of turbidity were much lower than those determined from casein alone at the same pH. When pH was below 5.2, a significant increase of turbidity was observed ($p<0.05$). It could be concluded from the Rayleigh scattering theory that the increase of turbidity was due to the formation of larger particles by electrostatic interactions. Results of changes in particle size later in this study proved this point.

**Changes in $\zeta$-potential during acidification:** Values of $\zeta$-potentials of casein alone and casein-SSPS mixture as a function of pH (from 3.6 to 6.8) were illustrated in Fig. 3. It should be noted that the terms of ‘increase’ and ‘decrease’ used in the context of $\zeta$-potential, were not used algebraically but referred to the numerical value of $\zeta$-potential. For example, when $\zeta$-potential changed from -20 to -10 mV, it was referred as the terms of ‘decrease’.

At the range of pH from 6.8 to 5.0, $\zeta$-potentials of casein alone showed slightly increase from -29.5±0.5 to -21.4±0.3 mV, which probably resulted from the dissociation equilibrium among H+, the ionized protein, citrate buffer and dissolved casein calcium phosphate. With decreasing the pH, $\zeta$-potential increased obviously. The change from negative $\zeta$-potential to positive $\zeta$-potential was observed at pH 4.53. It could be interpreted that casein in milk could precipitate out of solution as pH was 4.6 because the overall charge at this pH was close to zero and it became positively charged as pH was below the point (Fox et al., 1998). It was observed that $\zeta$-potential of the sample of casein alone increased to 23.0±0.4 mV at pH 3.6.

The tendency of $\zeta$-potential of SPSS-casein mixture was similar to the sample of casein alone with decreasing of pH. Also the low values of $\zeta$-potential were found with addition of SSPS. That presumably caused by the adsorption of negatively charged SSPS molecules onto the positively charged casein micelles. The electrostatic interactions between anionic polysaccharides and anionic proteins in aqueous solutions or in emulsions had been reported previously.
Changes in ζ-potential and particle diameter during neutralization: During neutralization, ζ-potential decreased with pH increasing (Fig. 5). During neutralization the casein micelle structure was irreversible, the CCP couldn’t be re-formation. Also, α-casein and β-casein could not be re-integration, resulting in the increment of Ca\(^{2+}\) activity (Lucey et al., 1996). The loosen structure of casein micelles might cause more negative charge and decrease ζ-potential.

A very different change of particle diameter was shown during neutralization. The particle diameters suddenly changed twice. The first change was found at pH 5.25 and the second sudden change of particle diameter appeared at pH 6.0. The particle diameter of casein was higher than the initial acidification (210 nm). This probably reasoned from the structure of casein micelle once disintegrated by acidification and it could not reformed by neutralization (Lucey et al., 1996).

CONCLUSION

The potentiometric titration which allowed the initiation interactions between casein and SSPS occurred at pH 5.3. Below this pH value, turbidity of casein-SSPS increased dramatically, suggesting the formation of larger particles. ζ-potential of casein-SSPS mixture became more negative with increasing the content of SSPS, which was conductive to the stability of casein micelles. During acidification, SSPS was progressively adsorbed onto the casein micelles, which contributed to the large size of particles. Changes in particle diameters of casein-SSPS mixture at pH 5.25 and 6.0 were found during neutralization. These results of interactions between negatively charged SSPS and

Changes in particle sizes during acidification: Particle sizes of the samples with different contents of SSPS during acidification were shown in Fig. 4. Without the addition of SSPS, the average size of casein micelles was about 210 nm at the beginning of acidification. Particle sizes of casein micelles decreased (about 57 nm) at the pH range of 6.8-5.4, which mainly attributed to the progressive dissolution of Casein Calcium Phosphate (CCP), the collapse of the κ-casein and the solubility of α- casein and β-casein because those could cause casein micelles to shrink (Tuinier et al., 2002). Without the addition of SSPS, the particle size of the casein micelles showed a dramatic increase at pH 4.8, which indicated the aggregation of casein (Fox et al., 1998).

When the sample was acidified with the addition of SSPS, the initial size of casein micelles increased with increasing the contents of SSPS except the content of 0.5%. This indicated an association of SSPS to the casein micelles which was possibly due to the presence of calcium ions in the system. The decrease of particle size of the content of 0.5% might result from the repulsion among the particles. At the initial stage of acidification, the particle sizes of SSPS-casein mixtures showed no quite difference compared with the particle size of casein alone (p<0.05).

At pH was below 5.2, the precipitation of casein was inhibited by SSPS. This was due to the adsorption of SSPS onto the casein micelles, which also led to the larger sizes of casein micelles. Also, the increase of particle sizes occurred before the aggregation of casein micelle. These results were accorded with the report on pectin and casein micelles by Tuinier et al. (2002).

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casein could give an insight into the stabilizing behavior in acid milk products.

ACKNOWLEDGMENT

The authors acknowledge financial support from Food Science and Engineering-the most important discipline of Zhejiang province (JYTsp20141091), the natural science foundation of Zhejiang Province (LY15C200003) and the National Natural Science Foundation of the People’s Republic of China (Project Number 31401557).

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