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 (Cucheval *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 titrometer (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 Instrument, UK). The parameter of He-Ne laser was selected at 633 nm. PH titration was performed directly in the measurement cell using an automatic titrator to control the addition of citric acid. PH was measured with the Ag-AgCl glass electrode. Titration was carried out with 1 M citric acid until aggregation occurred. PH was controlled from 6.8 to pH 3.6 at interval of 0.1±0.05 (Du *et al*., 2009; Kaufman *et al*., 2007).

ζ-potential was calculated by the Malvern software package using Henry's equation (Mirhosseini *et al*., 2008) that related electrophoretic mobility to ζ-potential as Eq. (1):

$$u_e = \frac{2\,\varepsilon\zeta f\,(ka\,)}{3\eta}$$

(1)

where,
ε = Dielectric constant
ζ = Zeta potential
η = Viscosity
u_e = Electrophoretic mobility
$f\,(ka)$ = Henry's function

Measurements of particle size: Different contents of soybean polysaccharide solutions (0-0.5%) were diluted to a measurable concentration (100-200 times) and the particle size was measured at 25°C with the Zetasizer Nano-ZS instrument (Malvern Instruments LTD., UK). Measurements were performed at a scattering angle of 90°. PH titration was performed when ζ-potential was determined. The titration was performed using 1 M HCl and 1 M KOH. The casein-SSPS mixtures were also titrated (with 1 M citric acid) from starting pH 6.8 to 3.6 at interval of 0.1±0.05 until aggregation occurred. For analyzing ζ-potential and particle size during acidification and neutralization, casein micelles with 0.5% SSPS were directly acidified with critic acid from pH 6.8 to 3.6 and then the sample was neutralized with NaOH to return to basic pH with continuous stirring.

Measurements of ζ-potential and particle size during neutralization: Casein micelles with 0.5% SSPS were directly acidified with critic acid from pH 6.8 to 3.6 and then neutralized with NaOH used to return the solutions to basic pH with continuous stirring. ζ-potential and particle diameters of casein-SSPS during neutralization were determined as the mentioned methods.

Statistical analysis: Experimental data were analyzed using Analysis of Variance (ANOVA). Data were expressed as mean value±standard deviation. Duncan's multiple range test was performed for post hoc multiple comparisons with the level of significance set at $p<0.05$. All statistical analyses were performed using SPSS 18.0 Software (SPSS Inc, USA).

RESULTS AND DISCUSSION

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_3^+- 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
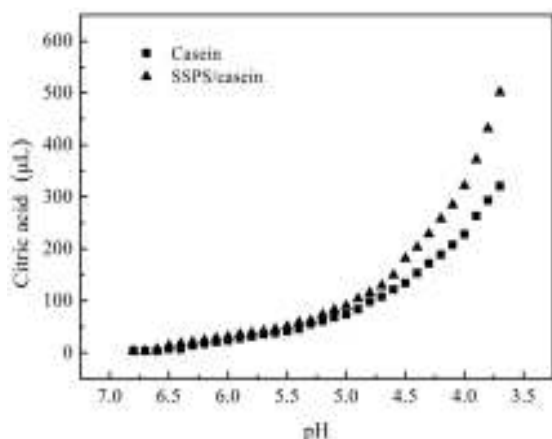
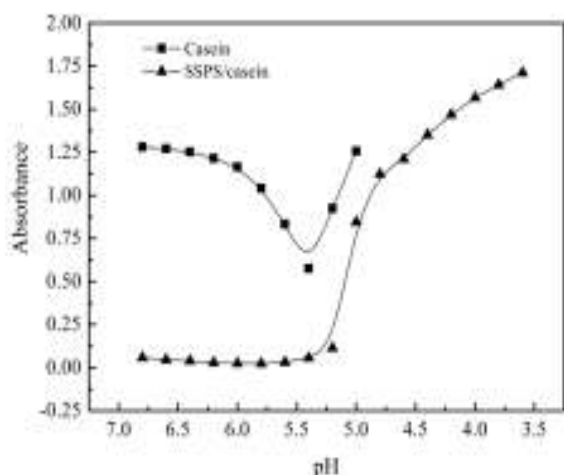Fig. 1: Typical titration curves of casein-SSPS mixtures

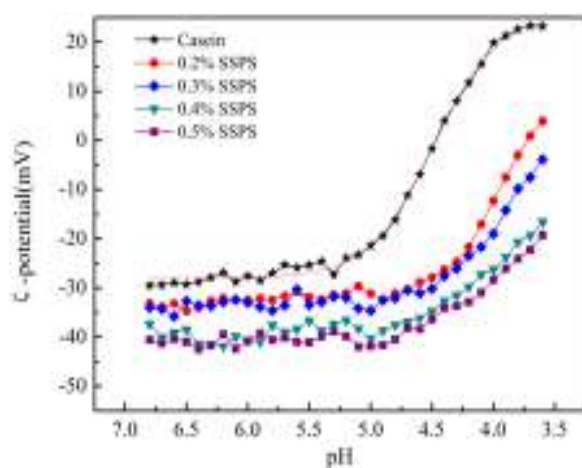Fig. 2: Turbidity of casein alone and casein-SSPS mixtures during acidification

Fig. 3: ζ-potentials of casein alone and casein-SSPS during acidification

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 α_s-casein, β-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 ζ-potential during acidification: Values of ζ-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 ζ-potential, were not used algebraically but referred to the numerical value of ζ-potential. For example, when ζ-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, ζ-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, ζ-potential increased obviously. The change from negative ζ-potential to positive ζ-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 ζ-potential of the sample of casein alone increased to 23.0±0.4 mV at pH 3.6.

The tendency of ζ-potential of SPSS-casein mixture was similar to the sample of casein alone with decreasing of pH. Also the low values of ζ-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
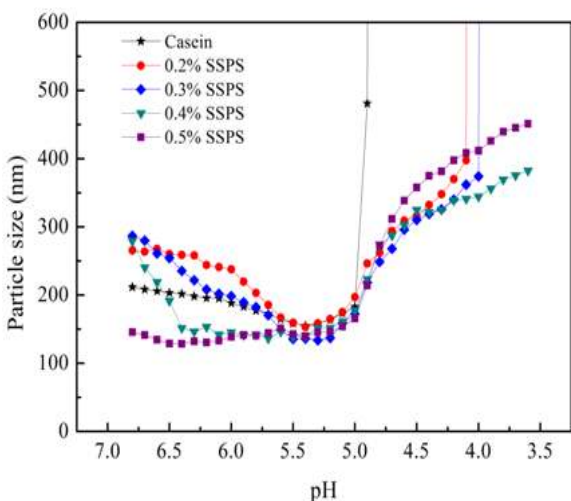
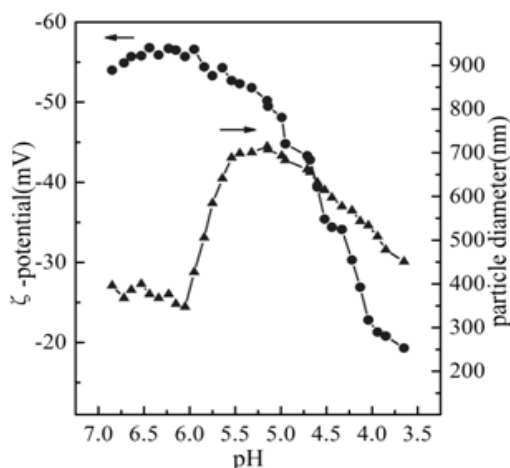Fig. 4: Particle size of casein alone and casein-SSPS during acidification

Fig. 5: Changes in ζ-potential and particle diameter during neutralization

(Surh *et al*., 2006; Tholstrup *et al*., 2007). ζ-potentials of SPSS-casein became more negative with increasing the content of SSPS. As pH 3.6, the final ζ-potentials of SPSS-casein mixture with the addition of SPSS were above 0.4% were significantly different with those of the addition of SPSS were below 0.3% (p<0.05).

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_s-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).

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_s-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

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

REFERENCES

Cucheval, A., M.A. Al-Ghobashy, Y. Hemar, D. Otter and M.A. Williams, 2009. Direct measurements of interfacial interactions between pectin and kappa-casein and implications for the stabilisation of calcium-free casein micelle mimics. J. Colloid Interf. Sci., 338(2): 450-462.

Doublier, J.L., C. Garnier, D. Renard and C. Sanchez, 2000. Protein-polysaccharide interactions. Curr. Opin. Colloid In., 5(3): 202-214.

Du, B., J. Li, H. Zhang, L. Huang, P. Chen and J. Zhou, 2009. Influence of molecular weight and degree of substitution of carboxymethylcellulose on the stability of acidified milk drinks. Food Hydrocolloid., 23(5): 1420-1426.

Fox, P.F., P.L.H. McSweeney and C.M. Lynch, 1998. Significance of non-starter lactic acid bacteria in Cheddar cheese. Aust. J. Dairy Technol., 53(2): 83-89.

Girard, M. and C. Schaffer-Lequart, 2008. Attractive interactions between selected anionic exopolysaccharides and milk proteins. Food Hydrocolloid., 22(8): 1425-1434.

Kaufman, E.D., J. Belyea, M.C. Johnson, Z.M. Nicholson, J.L. Ricks, P.K. Shah, M. Bayless, T. Pettersson, Z. Feldoto and E. Blomberg, 2007. Probing protein adsorption onto mercaptoundecanoic acid stabilized gold nanoparticles and surfaces by quartz crystal microbalance and ζ-potential measurements. Langmuir, 23(11): 6053-6062.

Liu, J., A. Nakamura and M. Corredig, 2006. Addition of pectin and soy soluble polysaccharide affects the particle size distribution of casein suspensions prepared from acidified skim milk. J. Agr. Food Chem., 54(17): 6241-6246.

Lucey, J., C. Gorry, B. O'Kennedy, M. Kalab, R. Tan-Kinita and P. Fox, 1996. Effect of acidification and neutralization of milk on some physico-chemical properties of casein micelles. Int. Dairy J., 6(3): 257-272.

Mattison, K.W., P.L. Dubin and I.J. Brittain, 1998, Complex formation between bovine serum albumin and strong polyelectrolytes: Effect of polymer charge density. J. Phys. Chem. B, 102(19): 3830-3836.

McSweeney, P.H.L. and P.F. Fox, 2009. Advanced Dairy Chemistry, Volume 3: Lactose, Water, Salts and Minor Constituents. 3rd Edn., Springer, New York.

Mirhosseini, H., C.P. Tan, N.S. Hamid and S. Yusof, 2008. Effect of Arabic gum, xanthan gum and orange oil contents on ζ-potential, conductivity, stability, size index and pH of orange beverage emulsion. Colloid. Surface. A, 315(1): 47-56.

Nobuhara, T., K. Matsumiya, Y. Nambu, A. Nakamura, N. Fujii and Y. Matsumura, 2014. Stabilization of milk protein dispersion by soybean soluble polysaccharide under acidic pH conditions. Food Hydrocolloid., 34: 39-45.

Pereyra, R., K.A. Schmidt and L. Wicker, 1997. Interaction and stabilization of acidified casein dispersions with low and high methoxyl pectins. J. Agr. Food Chem., 45(9): 3448-3451.

Rassis, D., I. Saguy and A. Nussinovitch, 2002. Collapse, shrinkage and structural changes in dried alginate gels containing fillers. Food Hydrocolloid., 16(2): 139-151.

Surh, J., E. Decker and D. McClements, 2006. Influence of pH and pectin type on properties and stability of sodium-caseinate stabilized oil-in-water emulsions. Food Hydrocolloid., 20(5): 607-618.

Tholstrup, S.M., T. Salomonsen, R. Ipsen, R. Clark, C. Rolin and E.S. Balling, 2007. Zeta potential of pectin-stabilised casein aggregates in acidified milk drinks. Int. Dairy J., 17(4): 302-307.

Tuinier, R., C. Rolin and D.C. Kruif, 2002. Electrosorption of pectin onto casein micelles. Biomacromolecules, 3(3): 632-638.

Wang, Q., X. Huang, A. Nakamura, W. Burchard and F.R. Hallett, 2005. Molecular characterisation of soybean polysaccharides: An approach by size exclusion chromatography, dynamic and static light scattering methods. Carbohyd. Res., 340(17): 2637-2644.