J. Dairy Sci. 97:5371–5382
http://dx.doi.org/10.3168/jds.2014-8144

Interactions between acidified dispersions of milk proteins and dextran or dextran sulfate

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ABSTRACT

Polysaccharides are often used to stabilize cultured milk products, although the nature of these interactions is not entirely clear. The objective of this study was to investigate phase behavior of milk protein dispersions with added dextran (DX; molecular weight = 2×10^6 Da) or dextran sulfate (DS; molecular weight = 1.4×10^6 Da) as examples of uncharged and charged polysaccharides, respectively. Reconstituted skim milk (5–20% milk solids, wt/wt) was acidified to pH 4.4, 4.6, 4.8, or 4.9 at approximately 0°C (to inhibit gelation) by addition of 3 *N* HCl. Dextran or DS was added to acidified milk samples to give concentrations of 0 to 2% (wt/wt) and 0 to 1% (wt/wt) polysaccharide, respectively. Milk samples were observed for possible phase separation after storage at 0°C for 1 and 24 h. Possible gelation of these systems was determined by using dynamic oscillatory rheology. The type of interactions between caseins and DX or DS was probed by determining the total carbohydrate analysis of supernatants from phase-separated samples. At 5.0 to 7.5% milk solids, phase separation of milk samples occurred after 24 h even without DX or DS addition, due to destabilization of caseins in these acidic conditions, and a stabilizing effect was observed when 0.7 or 1.0% DS was added. At higher milk solids content, phase separation was not observed without DX or DS addition. Similar results were observed at all pH levels. Gelation occurred in samples containing high milk solids (≥10%) with the addition of 1.0 to 2.0% DX or 0.4 to 1.0% DS. Based on carbohydrate analysis of supernatants, we believe that DX interacted with milk proteins through a type of depletion flocculation mechanism, whereas DS appeared to interact via electrostatic-type interactions with milk proteins. This study helps to explain how uncharged and charged stabilizers influence the texture of cultured dairy products.

Key words: dextran, depletion flocculation, cultured dairy product

INTRODUCTION

Proteins and polysaccharides are 2 major components in food systems and contribute to formation of structure, texture, and stability of foods (Tolstoguzov, 1991; Doublier et al., 2000; Maroziene and de Kruif, 2000; Corredig et al., 2011). Polysaccharides used as stabilizers in production of fermented dairy products include starch and pectin. Some lactic acid bacteria that are used as yogurt starter cultures can produce exopolysaccharides (**EPS**), which can increase viscosity of stirred yogurt and decrease whey separation (Ruas-Madiedo et al., 2002). These EPS can be both uncharged and negatively charged (Girard and Schaffer-Lequart, 2008).

In mixtures of proteins and polysaccharides, the interactions between these 2 biopolymers can be segregative or associative (Tolstoguzov, 1991; Doublier et al., 2000; de Kruif and Tuinier, 2001). Segregative interactions result from thermodynamic incompatibility, which occurs when solvent-biopolymer interactions are favored over biopolymer-biopolymer interactions, whereas associative interactions are observed when interactions between 2 biopolymers are favored (Doublier et al., 2000).

Thermodynamic incompatibility between proteins and polysaccharides can induce phase separation due to depletion flocculation mechanisms (Doublier et al., 2000). A depleted layer of solvent (e.g., water) around protein particles is formed due to a loss of conformational entropy of polymers near an interface (Tuinier et al., 2003). As a consequence, osmotic potential difference causes the movement of solvent molecules from the depleted layer into the bulk. Protein particles then attract one another to reduce the volume of the depleted layer, which leads to a separation of the mixture into 2-phase system: a protein-rich phase and a polysaccharide-rich phase (McClements, 2000; Corredig et al., 2011).

In associative mixed-biopolymer systems, electrostatic interactions between oppositely charged proteins

Received March 17, 2014.
Accepted June 8, 2014.
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and polysaccharides are primary interactions; associative phase separation results from precipitation/sedimentation or bridging of proteins and polysaccharides (Doublier et al., 2000). During fermentation of milk, as the pH decreases from around pH 6.8 to 4.4, the carboxyl groups of caseins become protonated and induce attractive interactions between caseins and negatively charged polysaccharides (Girard and Schaffer-Lequart, 2008). Therefore, in cultured products, the types of interactions between caseins and polysaccharides depend on conditions such as the pH value of milk (Everett and McLeod, 2005).

Phase diagrams between mixtures of milk proteins and several polysaccharides [e.g., amylopectin and dextran (**DX**)] have been developed for neutral pH conditions (Grinberg and Tolstoguzov, 1997), but only a few studies have been done in acidic conditions [e.g., phase behavior of casein micelles and high-methoxyl, low-methoxyl, and low-methoxyl amidated pectins in natural (pH 6.7) and acidic conditions (pH 5.3); Maroziene and de Kruif, 2000]. They found that at pH 6.7, phase separation occurred between caseins and 0.2% high-methoxyl and low-methoxyl amidated pectins and 0.1% low-methoxyl pectins (Maroziene and de Kruif, 2000). At pH 5.3, all types of pectin adsorbed onto casein micelles and, upon increasing the concentration of pectin, casein micelles were fully coated by pectin molecules. However, phase behavior between milk proteins and polysaccharides in more acidic pH (pH level <5.0; i.e., in fermented dairy products) has not been extensively investigated. Gelation of caseins at these acidic pH values presents a significant challenge, obscuring the effects of incompatibility or other ongoing interactions with polysaccharides.

This study aims to understand the effect of addition of uncharged and negatively charged polysaccharides on acidified milk with different solids content at various pH levels. We focused on aggregation of caseins, as milk solids content varied from 5 to 20% (wt/wt). Dextran was used in the study because it is a homoexopolysaccharide produced from *Leuconostoc mesenteroides* that can be obtained with known molar masses. Dextran is composed of glucose subunits, which are linked by $\alpha(1\rightarrow6)$ linkages on its main chain and $\alpha(1\rightarrow3)$ linkages on its side chain. Dextran sulfate (**DS**) is a derivative of DX with added sulfate groups (SO_3^-), giving negative charges to the polysaccharide molecules.

To study these protein-polysaccharide interactions at acidic conditions, we used aspects of the cold milk acidification method developed by Roefs (1986). This allowed us to acidify milk and inhibit gelation due to the very low temperature. Polysaccharides could then be added to the mixtures to investigate the stability or instability of the acidified system.

MATERIALS AND METHODS

Preparation of Reconstituted Skim Milk Stock Solution

Low-heat nonfat dry milk was obtained from Dairy-America Inc. (Fresno, CA) with a whey protein nitrogen index of 7.94 mg of undenatured whey protein/g of powder (Bradley et al., 1992). Reconstituted skim milk stock solution was prepared at a total milk solids content of 25% (wt/wt). The solution was stirred at approximately 25°C using a magnetic stirring unit overnight (16–20 h) before use. To prevent bacterial growth, 100 mg/kg of thimerosal ($C_2H_5HgSC_6H_4COO$-Na; Sigma-Aldrich, St. Louis, MO) was added as a preservative.

Preparation of DX and DS Solutions

Dextran (Sigma-Aldrich) with a molar mass of approximately 2×10^6 Da from *Leuconostoc mesenteroides* and DS (MP Biomedicals LLC, Solon, OH) with a molar mass of approximately 1.4×10^6 Da and a charge density of 1.9 sulfate groups/glucosyl residue were prepared by dispersing them in deionized water to make 10% (wt/wt) stock solutions. Thimerosal (100 mg/kg) was added to prevent bacterial growth. The solutions were stirred for 3 h at approximately 25°C, and heated in a water bath at 85°C for 5 min. These solutions were stored in an ice water bath before adding to milk.

Preparation of Milk—DX or DS Dispersions

Reconstituted skim milk stock solution was cooled in an iced water bath to approximately 0°C for 30 min before use. The skim milk was diluted by adding deionized water and mixing with 10% DX or DS solution to have a milk solids content of 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, or 20.0% (wt/wt), and DX concentrations of 0.5, 1.0, 1.5, or 2.0% (wt/wt), or DS concentrations of 0.1, 0.4, 0.7, or 1.0% (wt/wt).

To prepare the dispersions using the method developed by Roefs (1986), diluted milks were cooled to approximately 0°C to inhibit gelation and then slowly acidified to pH 4.4, 4.6, 4.8 or 4.9 by adding 3 *N* HCl. The acid was added in 100-μL increments with 1-min intervals. The pH of milk was measured by a pH meter (Accumet Basic AB 15plus; Fisher Scientific, Pittsburgh, PA). When the diluted milk samples reached the required pH levels, DX or DS solution was immediately added to milk to reach the required concentrations. After adding DX or DS solution, the pH of samples could slightly increase, and additional HCl was added to the samples.