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Effect of dextran and dextran sulfate on the structural and rheological properties of model acid milk gels

U. Pachekrepapol,* D. S. Horne,† and J. A. Lucey†[1]
*Department of Food Science, and
†Wisconsin Center for Dairy Research, University of Wisconsin–Madison, 1605 Linden Drive, Madison 53706

ABSTRACT

Various types of polysaccharides are widely used in cultured dairy products. However, the interaction mechanisms, between milk proteins and these polysaccharides, are not entirely clear. To explore the interactions between uncharged and charged polysaccharides and the caseins, we used a model acid-milk-gel system, which allowed acidification to occur separately from gelation. The effect of adding uncharged dextran (DX; molecular weight ~2.0×10^6 Da) and negatively charged dextran sulfate (DS; molecular weight ~1.4×10^6 Da) to model acid milk gels was studied. Two concentrations (0.075 and 0.5%, wt/wt) of DX or DS were added to cold milk (~0°C) that had been acidified to pH values 4.4, 4.6, 4.8, or 4.9. Acidified milks containing DX or DS were then quiescently heated at the rate of 0.5°C/min to 30°C, which induced gelation, and gels were then held at 30°C for 17 h to facilitate gel development. Dynamic small-amplitude-oscillation rheology and large-deformation (shear) tests were performed. Microstructure of gels was examined by fluorescence microscopy. Gels made with a high concentration of DX gelled at a lower temperature, but after 17 h at 30°C, these gels exhibited lower storage moduli and lower yield-stress values. At pH 4.8 or 4.9 (pH values greater than the isoelectric point of caseins), addition of 0.5% DS to acidified milk resulted in lower gelation temperature. At pH 4.4 (pH values less than the isoelectric point of caseins), addition of 0.5% DS to acidified milk resulted in gels with very high stiffness values. Gels made at pH 4.8 or 4.9 with both concentrations of DS had much lower stiffness and yield-stress values than control gels. Microstructural analysis indicated that gels made at pH 4.4 with the addition of 0.5% DX exhibited large protein strands and pores, whereas gels made with 0.075% DX or the control gels had a finer protein matrix. At higher pH values (>4.4), gels made with 0.5% DX had a finer structure. At all pH values, gels made with 0.5% DS exhibited larger pores than the control gels. This study demonstrated that low concentrations of uncharged DX did not significantly affect the rheological properties of model acid milk gels; high concentrations of DX resulted in earlier gelation, possibly caused by depletion-induced attractions between casein particles, which altered the microstructure and created weaker gels. At pH values <4.6, negatively charged DS produced stiff casein gels, which might be due to attractive crosslinking by electrostatic interactions between DS and caseins at pH values below the isoelectric pH of casein (i.e., positively charged casein regions interacted with negatively charged DS molecules).

Key words: dextran, dextran sulfate, exopolysaccharide, acid milk gel, stabilizer

INTRODUCTION

Important physical characteristics of yogurts for consumers include the lack of visual whey separation on the yogurt surface and its (perceived) viscosity, and these properties can influence the overall consumer acceptance of set-style yogurts (Lee and Lucey, 2010). In practice, these attributes can be controlled by the addition of dried dairy ingredients to increase the total milk solids content or by the addition of stabilizers, such as pectin, gelatin, or starch (Tamime and Robinson, 2007). Some lactic acid bacteria used in yogurt production are able to produce exopolysaccharides (**EPS**), which can affect the texture and viscosity of yogurts (Ruas-Madiedo et al., 2002). EPS can be uncharged, or negatively charged due to the presence of groups such as phosphate or acetate (Girard and Schaffer-Lequart, 2008).

When milk is fermented, lactose in milk is converted into lactic acid, leading to a reduction in pH. As pH decreases, the colloidal calcium phosphate of casein micelles is solubilized and the net negative charge of casein micelles is reduced, resulting in aggregation of caseins as they approach their isoelectric point (**pI**) around pH 4.6 (Lucey and Singh, 1997). At the beginning of fermentation, the pH of milk is higher than

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[1] Corresponding author: jlucey@cdr.wisc.edu

the pI of casein, so negatively charged or uncharged EPS do not associate with casein micelles, but at this pH the presence of both types of polysaccharides could cause phase separation (Tuinier and de Kruif, 1999). Depletion flocculation by uncharged polysaccharides can induce flocculation of casein micelles, which can cause phase separation (de Kruif and Tuinier, 2001). As the pH of milk decreases, casein particles and negatively charged polysaccharides may experience attractive electrostatic interactions due to increasing number of positive charges or patches on caseins (Girard and Schaffer-Lequart, 2008). Studies on the effect of uncharged and negatively charged EPS or polysaccharides on textural properties of yogurt are limited, however, and the results have been inconclusive (Purohit et al., 2009; Kristo et al., 2011; Mende et al., 2012). Acid-induced gelation of milk (by glucono-δ-lactone) was used by Aichinger et al. (2007) to kinetically trap a phase separating system of milk and xanthan gum into various types of mixed gel structures.

Acidification rate can greatly affect rheological and physical properties of acid milk gels (Lucey and Singh, 1997; Lee and Lucey, 2004). Each starter strain exhibits a specific acidification rate during growth, dependent on conditions, such as incubation temperature and type of growth medium.

In this study, dextran (**DX**) and dextran sulfate (**DS**) were selected as models for uncharged and negatively charged polysaccharides, respectively. Dextran was used in the study because it is a homoexopolysaccharide produced from *Leuconostoc mesenteroides* that can be obtained in purified form with various known molar masses. Dextran is composed of glucose subunits, which are linked by α(1→6) linkages on its main chain and α(1→3) linkages on its side chain. Dextran sulfate is a derivative of DX with added sulfate groups (SO_3^-) giving negative charges to the polymer molecules. Dextran sulfate has been reported to form complexes with caseins through electrostatic interactions even at very low pH levels; that is, pH 2 (Jourdain et al., 2008).

The objective of this research was to better understand the effect of uncharged and negatively charged polysaccharides on the rheological and microstructural properties of acid milk gels made at different pH levels. In this study, we used a system with the cold, acidified milk model developed by Roefs et al. (1990). This model gel system allows for the separation of the acidification and gelation processes, and gels can be prepared at various specific pH values (without the complication of differing acidification rates). Milks were cooled to ~0°C and acidified to low pH values in the cold to inhibit gelation. At low temperature (~0°C), acidified milks do not gel, probably because β-caseins protrude from the surface of casein particles providing steric repulsion (Roefs et al., 1990). We recently studied the interactions between caseins and DX and DS in cold, acidified milk dispersions (Pachekrepapol et al., 2014). High concentration of DX induced depletion flocculation. At pH > pI, DS caused loss of turbidity or greater dispersion of caseins. The pH range between 4.4 and 4.9 was selected in our study to have gels at pH values above and below the pI of caseins. At pH values higher than 4.9, (unheated) milk generally does not form an acid-induced gel, whereas at pH values lower than 4.4, gelation may occur spontaneously even at ~0°C (Roefs et al., 1990). In this study, we investigated gels made from cold, acidified milks and studied the effect of adding various concentrations of DX or DS to acidified milk on the gelation temperature, rheological properties, and microstructure of the acid milk gels.

MATERIALS AND METHODS

Sample Preparation

Reconstituted skim milk and DX and DS solutions were prepared as described by Pachekrepapol et al. (2014). Reconstituted skim milk was prepared by dispersing 12 g of low-heat, nonfat dry milk powder in 100 g of deionized water. The solution was stirred at ~25°C using a magnetic stirring unit overnight (16–20 h) before use. To inhibit bacterial growth, 100 mg/L of thiomersal ($C_2H_5HgSC_6H_4COONa$, Sigma-Aldrich, St. Louis, MO) was added as a preservative. Dextran (Sigma-Aldrich) with average molar mass of $\sim 2 \times 10^6$ Da from *Leuconostoc mesenteroides* and DS (MP Biomedicals, Solon, OH) with molar mass of $\sim 1.4 \times 10^6$ Da and charge density of 1.9 sulfate groups per glucosyl residue were prepared by dispersing in deionized water to make 10% (wt/wt) stock solutions. Thiomersal was added to inhibit bacterial growth. The solutions were stirred for 3 h at ~25°C and then heated in a water bath at 85°C for 5 min. These solutions were stored in an ice water bath until use.

Reconstituted skim milk was cooled in a water bath (with ice) to ~0°C for 30 min before acidification. Cooled milk was acidified by the addition of 3*N* HCl until the pH decreased to 4.9, 4.8, 4.6, or 4.4. The HCl was gradually 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). After acidification, DX or DS solution was added to give final concentrations of 0.075 or 0.5% (wt/wt), and the temperature was maintained at ~0°C. These concentrations were selected based on our previous study (Pachekrepapol et al., 2014).