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Influence of sodium chloride on the colloidal and rennet coagulation properties of concentrated casein micelles suspensions

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ABSTRACT

The research investigated the influence of NaCl on the colloidal and rennet coagulation properties of concentrated milk. Milk was concentrated to 1×, 3×, and 5× using ultrafiltration. Rennet gelation was followed by rheology and diffusing wave spectroscopy. Soluble protein, total and diffusible calcium and phosphate, size, and zeta potential were also measured as a function of concentration history. In the presence of 300 m*M* NaCl, colloidal calcium phosphate solubilized and pH and the negative charge on the surface of casein micelles decreased. Increasing the volume fraction caused the formation of stiffer gels for both samples with or without NaCl. The addition of NaCl caused a significant increase in the bulk viscosity of the milk concentrated 5× and a decrease in turbidity. The concentration had no effect on the gelation time of control samples, nor on the kinetics of caseinomacropeptide release. On the other hand, rennet gelation was retarded by the addition of NaCl, and the gels showed lower elastic moduli compared with those obtained with control milk.
Key words: casein micelles, sodium chloride, concentrated milk, rennet gelation

INTRODUCTION

Skim milk is a dispersion of proteins, minerals, and lactose. The major proteins in milk are caseins, which are present in the form of casein micelles. Casein micelles play an important role in the processing functionality of milk (Dalgleish, 2011; Dalgleish and Corredig, 2012). The structure of casein micelle has attracted the attention of scientists, and many models have been proposed to elucidate the structure of casein micelles over the past 50 yr (Holt, 1992, 1998; Horne, 2006; Dalgleish and Corredig, 2012). Colloidal calcium phosphate nanoclusters, with an average radius of 2.3 nm, are present in the inner core of the casein micelles and are surrounded by phosphorylated caseins (α_S- and β-CN; Holt, 1998, 2004). κ-Casein molecules are located on the surface of the casein micelle, providing a polyelectrolyte brush of great importance to the colloidal stability of these protein particles (De Kruif and Zhulina, 1996).

The structure of casein micelles is affected by changes of environment, such as pH, temperature, and the presence of other minerals (Grufferty and Fox, 1985; Le Graët and Gaucheron, 1999; Carr et al., 2002; Huppertz and Fox, 2006). It has been reported that the addition of sodium chloride (NaCl) causes the solubilization of colloidal calcium phosphate, increases dissociation of caseins, and improves the solubility of milk concentrates (Mao et al., 2012); however, it may influence the processing functionality of casein micelles (Famelart et al., 1999; Gaucheron et al., 2000). Furthermore, the addition of NaCl decreases milk pH (Huppertz and Fox, 2006) and increases the hydration of casein micelle (Van Hooydonk et al., 1986).

Recent research demonstrated that when NaCl is added to 1× and 2× concentrated milk, there is dissolution of the colloidal calcium phosphate and release of caseins, with consequent changes of viscosity and turbidity (Zhao and Corredig, 2014). However, few studies conducted on the influence of NaCl on concentrated milk can be found, and further research is needed to better understand the effect of charge shielding and, in particular, the addition of NaCl on the processing properties of casein micelles.

Rennet coagulation is a critical processing step in cheesemaking. The rennet gelation involves 2 overlapping stages. The primary stage is the cleavage of κ-CN; once enough κ-CN (about 85–90%) is cleaved, the second stage, aggregation of the casein micelles, occurs with the formation of a gel network (Lucey, 2002; Liu et al., 2014). Concentration of skim milk using ultrafiltration increases the proteins and colloidal minerals in the retentate and decreases the average distance between casein micelles (Sandra et al., 2011). Previous researchers found that concentrated milk has a longer coagulation time and higher gel firming rate than nonconcentrated milk (Dalgleish, 1980; Waungana et al.,

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1999). However, recent research (Sandra et al., 2011) showed that concentration has no significant effect on the release of caseinomacropeptide (**CMP**) and coagulation time when the same amount of rennet is added to milk. Addition of NaCl was reported to increase the coagulation time of milk (Sbodio et al., 2006). It is still unknown how NaCl will influence the rennet coagulation of concentrated milk.

This research aims to better understand the influence of addition of NaCl before concentration on the structure of casein micelles and on the rennet gelation behavior of the concentrated milk. The concentration (1×, 3×, and 5×) was achieved by ultrafiltration based on the volume fraction. In our study, the rennet gelation process was followed by rheology and diffusing wave spectroscopy (**DWS**). Before rennet gelation, the changes in the physicochemical properties of concentrated milk were characterized by measuring total and soluble protein, total and diffusible calcium and phosphate, hydrodynamic size, zeta potential, and light scattering properties using DWS.

MATERIALS AND METHODS

Preparation of Concentrated Milk

Skim milk was obtained from a local dairy company (Crown Dairy, Guelph, Ontario, Canada). A total of 0.01% sodium azide was added immediately to prevent the bacterial growth. Sodium chloride (300 mmol/L) was added to milk and stirred for 15 min at room temperature. This concentration was chosen based on prior results, which showed a significant change in the size of the casein micelles and turbidity parameter of milk (Zhao and Corredig, 2014). All the samples were then equilibrated overnight in the refrigerator. After equilibration, aliquots of samples (1 L) were concentrated to different concentrations (1×, 3×, and 5×) using an ultrafiltration cartridge (10-kDa Millipore CDUF001LG, Fisher Scientific, Mississauga, ON, Canada) based on the volume reduction. All permeates were collected.

Determination of Total and Soluble Protein Content

The total protein was determined directly using a Dumas nitrogen analyzer (FP-528, Leco Inc., Lakeview Avenue, St. Joseph, MI). The protein concentration was calculated using 6.38 as conversion factor.

The soluble protein was defined as the fraction that did not sediment after ultracentrifugation at 100,000 × g for 1 h at 20°C (OptimaTM LE-80K ultracentrifuge with rotor type 70.1Ti, Beckman Coulter Canada Inc., Mississauga, Canada). The supernatants were then filtered through the 0.45-μm membrane (low protein binding, Fisher Scientific) before analysis.

Determination of Total and Diffusible Calcium and Phosphate

In this research, the 861 Advanced Compact IC (Ω Metrohm ion analysis, Metrohm Ltd., Herisau, Switzerland) was used to measure the total and diffusible calcium and phosphate in milk. The diffusible calcium and phosphate were defined as those present in the supernatant but not combined with the serum proteins. The sample preparation for both total and diffusible calcium and phosphate has been described in our previous research (Zhao and Corredig, 2014).

The calcium was determined using nonsuppressed ion chromatography (Rahimi-Yazdi et al., 2010). On the other hand, a CO_2 suppressed ion chromatographic method was used to determine the total and diffusible phosphate. An anion exchange column (Metrosep A Supp5–150/4.0, Metrohm) packed with 5-μm polyvinyl alcohol with quaternary ammonium groups was employed. Sodium hydrogen carbonate and sodium carbonate solutions were used to prepare the mobile phase (1.0 mM sodium carbonate and 4 mM sodium hydroxide). Samples were eluted at a flow rate of 0.5 mL/min.

Addition of Rennet and κ-CN Hydrolysis

Before the addition of rennet, milk samples were equilibrated at 30°C for at least 20 min. The rennet used was Chymax Ultra (Chr. Hansen, Milwaukee, WI) with average strength of 790 (±5%) international milk clotting units (IMCU)/mL. The rennet was diluted 100-fold in MilliQ water (Thermo Fisher Scientific, Burlington, Canada) before addition to milk. The diluted rennet was added to milk with a proportion of 4 μL/mL of milk. The final concentration of rennet in milk was 0.031 IMCU/mL. Milk samples were stirred for 30 s after rennet addition before further analysis. The gelation process was carried out at 30°C.

The release of CMP by rennet was monitored using an established method (Lopez-Fandino et al., 1993). Rennet at the concentration defined above was added to the milk sample, which was then immediately divided into 2-mL aliquots in different test tubes. Samples were incubated at 30°C, and the reaction was stopped at designated times (every 10 min) by addition of 4 mL of 3% trichloroacetic acid. After overnight storage in refrigerator, the supernatant was collected and then centrifuged at 4,500 × g for 15 min at 22°C (Eppendorf centrifuge, 5415D, Mississauga, Canada). The obtained superna-