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Influence of colloidal calcium phosphate level on the microstructure and rheological properties of rennet-induced skim milk gels

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ABSTRACT

Colloidal calcium phosphate is an essential part of casein micelles and being responsible for their stability. Different mineralization of casein micelles was obtained by acidification of skim milk to pH 6.5, 6.0 or 5.5, followed by a dialysis method, using simulated milk ultrafiltrate without lactose, to obtain varying levels of micellar calcium and phosphorus but constant value of pH, serum and free calcium, and serum phosphorus. Bovine chymosin was added to the skim milk samples after dialysis and microstructural and rheological properties during gel formation were recorded at 30 °C. Samples after dialysis needed approximately 30 min after the addition of chymosin to form rennet gels. In addition, low micellar calcium and phosphorus values were both found to correlate with slightly less time for the gels to be formed. This information highlights the importance of CCP in the primary phase of rennet gel formation. The protein network of rennet gels after dialysis was more compact with many aggregates as demineralization decreased. The small protein particles are able to increase the potential connection points among proteins, support particle fusion and cause a compact structure.

1. Introduction

Gelation of milk is a crucial step for cheese production because the milk proteins, especially caseins, are destabilized as an initial step in gel network formation. Gelation can be induced by rennet (chymosin enzyme) action, acidification or combination of acid and rennet (Lucey, Johnson, & Horne, 2003). During renneting, chymosin cuts off the N-terminal part of κ-casein and para-casein micelles are formed (primary phase of rennet gel formation), while the hydrophilic parts of κ-casein is released to serum milk phase mainly as caseinomacropeptide (CMP). In addition, the protein system starts to be unstable and a gel is finally formed (secondary phase of rennet coagulation) as first steps in cheesemaking (Mellema, Heesakkers, van Opheusden, & van Vliet, 2000a). The structure development of rennet gels is one of the most critical steps during cheesemaking and may lead to and control specific structural and rheological properties of the final cheese products (Zoon, van Vliet, & Walstra, 1988a). The formation of rennet gels had been an issue for many studies with special focus on rheology and microstructural properties (Ong, Dagastine, Kentish & Gras, 2012; Mellema, Walstra, van Opheusden, & van Vliet, 2000b; Zoon et al., 1988a; Renault, Gastaldi, Cuq, & de la Fuente, 2000; Frederiksen et al., 2011).

Calcium is found in milk in equilibrium between the micellar and the serum milk phase. In the serum phase, calcium is present as free Ca^{2+} ions or associated in complexes mainly with inorganic phosphate and citrate and to lesser extent with chloride. In the micellar phase, calcium is present as a colloidal calcium phosphate (CCP) bound to the casein micelles (Gaucheron, 2005; Knudsen & Skibsted, 2010). CCP is suggested to be bound to phosphorylated serine residues of casein micelles, neutralizing their negative charge and giving them certain stability and structure (Tuinier & de Kruif, 2002; Horne, 2006; Dalgleish & Corredig, 2012).

The dissociation of CCP from caseins in milk has been a main topic for numbers of studies as a way to understand the structure of casein micelles (Pyne & McGann, 1960; Ozcan-Yilsay, Horne, & Lucey, 2011; Famelart, Gauvin, Paquet, & Brule, 2009; Ozcan-Yilsay, Lee, Horne, & Lucey, 2007; Udabage, McKinnon, & Augustin, 2001). An interesting approach from Silva et al. (2013) was published describing preparation of casein suspensions with different amount of CCP while keeping pH and ionic environment in the serum milk phase constant. Silva et al. (2013) studied different physicochemical

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and foaming properties of casein suspensions having different mineralization levels of primarily micellar calcium (18.5 mmol/L; 17.1 mmol/L; 15.1 mmol/L; 12.3 mmol/L and 9.5 mmol/L) and the results were addressed in relation to different amount of CCP. Demineralization of caseins was concluded to cause dissociation of casein micelles and reduced the foaming ability due to formation of small protein particles in the casein suspensions. Anema (2009) altered in similar way the amount of micellar calcium (CCP), keeping the amount of serum calcium constant, and the gelation properties of acid gels were studied. The results of that study demonstrated that the CCP was associated to the early stages of milk gelation during acidification at different temperature. Ong et al. (2011) studied the effect of increasing CCP in milk on the texture and structure of yogurt by adding NaOH. Their study showed that the yogurt texture was affected both by increasing the level of CCP and the addition of NaOH. Zoon, van Vliet, and Walstra (1988b) prepared rennet milk gels from dialyzing milk samples following the method of Pyne and McGann (1960), but the concentrations of micellar and serum calcium changed simultaneous hampering a direct relation with micellar calcium. Ozcan-Yilsay et al. (2007) add different amounts of trisodium citrate (5–40 mmol/L) to milk samples to modify both serum and micellar calcium. Then, yogurt culture was added and samples stored at 42 °C until pH decreased to 4.6. They concluded that high concentration of trisodium citrate caused a weak yogurt structure with large pores.

In our study, skim milk was acidified to pH 6.5, 6.0 or 5.5 followed by preparation of milk samples with different amount of micellar calcium and phosphorus while keeping the pH and the serum calcium and phosphorus constant, following dialysis method similar to the method described by Silva et al. (2013). The limited variation in pH (6.5, 6.0 and 5.5) was chosen in order to avoid micellar flocculation (Hinz, Huppertz, & Kelly, 2012) which is especially pronounced at pH around 5.2. In addition milk samples after the dialysis step were examined with respect to rheological and microstructural properties after addition of certain amount of chymosin (0.041 IMCU/ml). For rennet milk gels after dialysis, relations between the different CCP content and rheological and microstructure properties now become possible. Ultimately, understanding the changes that occur in the structure of rennet milk gels, due to micellar calcium and phosphorus, is important in order to optimize the cheese making process, and provide more insights to the properties and the structure of casein micelles.

2. Materials and methods

2.1. Chemicals

Reagent-grade chemicals and distilled-deionized water (Mill-Q plus, Millipore Corporation, Bedford, MA, USA) were used throughout.

2.2. Preparation of dialyzed milk and rennet gel

Pasteurized skim milk at 72 °C for 15 s (0.1 g/100 ml fat) was kindly donated by Arla Foods Amba and 0.02 g/100 ml sodium azide was added to prevent bacterial growth. In addition, the skim milk was divided into smaller samples each stored at 30 °C for one day. The next day the milk samples were acidified with 1.0 M HCl to pH 6.0, and 5.5 to be compare with a control sample (pH 6.5) without addition of HCl. The dilution caused by the HCl was kept constant for all samples by addition of distilled-deionized water. Normally the pH of milk is close to 6.7 at 20 °C but increasing temperature to 30 °C, the solubility of calcium and phosphorus decreases while decreasing CCP with a concomitant decrease in pH (Walstra & Jenness, 1984). Control and acidified milk samples were left at 30 °C for one day and then dialyzed, using a dialysis membrane with molecular weight cut-off 12–14 kDa (Spectrum Laboratories, Inc., Rancho Dominquez, Compton, CA, USA), at 4 °C for 60 h against a volume of 50 times of simulated milk ultrafiltrate without addition of lactose (Jenness & Koops, 1962). The simulated milk ultrafiltrate had the following composition: 18.3 mmol/L Sodium; 39.4 mmol/L Potassium; 9.0 Calcium mmol/L; 3.2 mmol/L Magnesium; 11.6 mmol/L Phosphorus; 32.4 mmol/L Chlorium; 9.6 mmol/L Citrate; 1.0 mmol/L Sulfate and 2.2 mmol/L Carbon dioxide. The dialysis at 4 °C for 60 h will not influence the rennet coagulation since hydrophobic interaction is known to be less important (Walstra, 1990). The simulated milk ultrafiltrate was changed 5 times in 60 h and then the dialyzed milk samples (milk after dialysis) were stored at 30 °C for one day. After dialysis step each sample had a volume of 20 ml. Bovine chymosin (Chymax plus, 0.041 IMCU/ml, Chr. Hansen, Hørsholm, Denmark) was added at the rate of 20 μL per 5 mL at milk after dialysis and all the samples were mixed for 1 min and incubated for 60 min at 30 °C to form a rennet gel.

2.3. Chemical and mineral analysis

Total nitrogen content in milk samples after dialysis was determined by the Kjeldahl method (IDF, 1993). The protein content was estimated by multiplying the nitrogen content for casein by 6.38 (van Boekel & Ribadeau Dumas, 1987). pH in milk samples after dialysis was measured directly by a pH meter (713 pH Meter, Metrohm, Copenhagen, Denmark) with a glass electrode (602 Combined Metrosensor glass electrode, Metrohm). For determination of calcium and phosphorus in serum milk phase, 10 mL of milk after dialysis were used for centrifugation (Allegra 25R, Bekman Coulter, Copenhagen, Denmark) for 60 min at 2000 g at 30 °C using centrifuge tubes with ultra-membranes Vivaspin 20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) with a molecular mass cut-off of 10 kDa. Total and serum contents of calcium were determined in milk after dialysis and in their serum phase using an atomic absorption spectrometric method (IDF, 2007). Micellar calcium was calculated by the following equation: Micellar calcium = Total calcium − Serum calcium. Free calcium contents were determined in serum phase of milk after dialysis using an ion-selective electrode ISE25Ca with a reference REF 251 electrode (Radiometer Analytical SAS, Lyon, France) at 30 °C. Before use, the calcium electrode was calibrated using standards solutions of $CaCl_2$ (0.10, 1.0, 10 and 24 mmol/L) with 80 mmol/L NaCl as background electrolyte. Determination of free calcium contents were done using the linear relationship (Nernst equation) between the electrode potential (mV) measured in the calibration solutions and the corresponding pCa value (electrode potential of calcium electrode, pCa = -log $[Ca^{2+}]$) measured in the serum phase of milk after dialysis.

Total and serum contents of phosphorus were determined in milk after dialysis and in their serum phase using the standard absorption spectrometry method (IDF, 2006). Micellar phosphorus was calculated by the following equation: Micellar phosphorus = Total phosphorus − Serum phosphorus.

2.4. Rheological properties

The rheological properties (storage modulus G′) of milk samples after dialysis and after addition of chymosin (Section 2.2) as a function of time (time values were varied from 0 to 60 min, with constant frequency of 0.16 Hz and strain of 0.50%) and as a function of frequency in the final rennet gels (frequency values were varied from 0.007 to 1.5 Hz with a constant strain of 0.50%) were estimated using a rheometer (AR G2, TA Instrument, Elstree, UK). In addition