International Dairy Journal 36 (2014) 65–73

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Temperature effect on calcium and phosphorus equilibria in relation to gel formation during acidification of skim milk

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ARTICLE INFO

Article history:
Received 4 October 2013
Received in revised form
8 January 2014
Accepted 8 January 2014

ABSTRACT

Levels of micellar, serum and free calcium and micellar and serum phosphorus were studied during gradual acidification of skim milk at 4, 20, 30 and 40 °C. From pH 6.0 to 5.4–5.2, calcium and phosphorus concentrations in milk serum increased for decreasing temperature. For pH < 5.0 the concentrations of serum phosphorus and free calcium were temperature independent, although the concentration of micellar calcium was decreased and the concentration of serum calcium increased with decreasing temperature. The molar ratio of serum calcium/free calcium was 1.71, that of serum calcium/serum phosphorus was 1.35. Gel formation (G') at pH 4.8, 4.7 and 4.6 showed that for constant pH, G' was higher for decreasing temperature. Moreover, G' was increased from pH 4.8 to 4.6 for constant temperature. These last observations could be important for controlling the properties of milk gels formed in low pH ranges and calcium could play a vital role.

1. Introduction

Salt equilibria in milk are important for understanding milk properties and for optimising milk processing in the dairy industr+y. Calcium and phosphorus are two of the most important mineral nutrients in milk with total concentrations between 25 and 35 mM (Fox & McSweeney, 1998). Both calcium and phosphorus in milk are in equilibrium between the micellar and the serum phase (Gaucheron, 2005). In the serum phase, calcium can be found as free calcium ions or associated with citrate, inorganic phosphate, some peptides and proteins and, to a lesser degree, with chloride. In the micellar phase, calcium is mainly present in the form of colloidal calcium phosphate (CCP), which is bound to certain regions of caseins micelles. In the serum phase, phosphorus can be found as free phosphate ions ($H_2PO_4^-$, HPO_4^{2-}) or associated with calcium or attached to small molecules like peptides, nucleotides and sugars. Calcium and phosphorus equilibria in milk are perturbed by changing conditions during milk processing, involving acidification, heating, cooling and addition of NaCl, $CaCl_2$ or possible chelators (Gaucheron, 2005).

During milk acidification calcium and phosphorus are solubilised, together with other minerals, as the net charge of casein micelles decreases and caseins dissociate from the micelles into serum (Dalgleish & Law, 1989; Le Graet & Gaucheron, 1999; Mekmene, Le Graet, & Gaucheron, 2010; Van Hooydonk, Hagedoorn, & Boerrigter, 1986b). Especially the change in calcium equilibrium due to acidification affects the final structure, texture and functionality of dairy products such as cheeses and yoghurts (Lucey & Fox, 1993; Lucey & Singh, 1998). Results presented by Guinee, Feeney, Auty, and Fox (2002) and by Joshi, Muthukumarappan, and Dave (2003) showed that the amount of calcium that remains bound to the caseins is critical for the structure of Mozzarella cheese, resulting in changes in fluidity of melted cheese. Lucey, Mishra, Hassan, and Johnson (2005) concluded that during Cheddar maturation, the decreasing concentration of micellar calcium was correlated with a decrease in storage modulus parameter (G') at high temperatures.

Milk acidification has been an important research area due to its importance for cheese yield. The knowledge of temperature effects on calcium and phosphorus equilibria during acidification is still of a great importance for both scientists and dairy industry. Dalgleish and Law (1989) and Law (1996) concluded that solubilisation of calcium and inorganic phosphate during milk acidification is independent of temperature in the region 4–30 °C. Law and Leaver (1998), however, showed that during milk acidification at 4, 20, and 30 °C, the amount of calcium and inorganic phosphate in the serum phase was greater at 4 °C than at 20 and at 30 °C. Anema (2009b) examined the effect of milk concentration, temperature and time on pH and the amount of serum calcium and inorganic

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http://dx.doi.org/10.1016/j.idairyj.2014.01.003

phosphate. The results of that study shown that the distribution of calcium and phosphorus between the micellar and serum phase in milk greatly depend on the temperature and milk concentration.

The majority of the studies dealing with calcium equilibria in milk only consider the total amount of serum calcium, without any further specification among different forms of calcium. According to the review by Lewis (2011), there is a need for further attention to the factors controlling the level of free calcium ions in milk. Quantification of free calcium ions in milk will depend on more sensitive and faster evaluation methods for different pH and temperature conditions. In the study of On-Nom, Grandison, and Lewis (2010), detailed information can be found on free calcium concentrations in milk at high temperatures. Moreover, Kocak, Zadow, and Purcell (1984) concluded that after heat treatment of milk the concentration of free calcium, initially decreased by the heating, needs time for complete recovery and the reestablishment of free calcium concentration during cooling is temperature dependent.

Visser, Minihan, Smits, Tjan, and Heertje (1986) showed that addition of 5 mM calcium in milk caused a doubling of the casein bound to whey proteins and that calcium ions had an important role for the binding of whey proteins to the micelle. Roefs and Van Vliet (1990) concluded that free calcium can bind to some of the negative charges of proteins and influence the protein conformation and structure of casein particles. Wolfschoon-Pombo and Andlinger (2013) studied the correlation between the micellar calcium and different levels of fat or protein, in cream cheese with pH < 5.2.

Accordingly, we found it timely to investigate the distribution of calcium and phosphorus between the micellar phase and the serum phase during milk acidification at different temperatures and present results not only for micellar and serum phase, but also for free calcium. Our study further investigates the effect on calcium distribution on gel formation.

2. Materials and methods

2.1. Materials

Sodium azide, glucono-delta-lactone (GDL), $CaCl_2$ and NaCl of analytical grade were all from Sigma–Aldrich Corporation (St Louis, MO, USA). All aqueous solutions were made from purified water (Mill-Q plus, Millipore Corporation, Bedford, MA, USA).

2.2. Milk and heat treatment

Full fat milk from Holstein Friesian cows was kindly donated by Osted Ost og Mejeri (Denmark) and was from a dairy farm located in Osted, Denmark. Upon arrival, milk was skimmed two times by centrifugation (Herolab, HiCen 21, Wiesloch, Germany) at 3000 × *g* for 45 min at 4 °C and then was filtered through glass wool fibre (Merck, Darmstadt, Germany). To prevent bacterial growth, 0.02% sodium azide was added to the milk before being stored at 4 °C for one day. The next day the milk was heated in a water bath to 72 °C for 15 s and then immediately cooled on ice. In addition, the pasteurised skim milk was divided into smaller samples each stored at 4, 20, 30 or 40 °C for one day.

2.3. Chemical analysis

Total nitrogen content in the pasteurised skim milk was determined by the Kjeldahl method according to the International Dairy Federation standard method (IDF, 1993). The protein content was estimated by multiplying the nitrogen content for casein by 6.36 (Van Boekel & Ribadeau Dumas, 1987). Fat content in the pasteurised skim milk was determined by the Gerber method (IDF, 1981), and total solid contents were estimated using the gravimetric method (IDF, 1987), while lactose content was determined using the lactose/D-galactose enzymatic method (Wehr & Frank, 2004). Ash content in the pasteurised skim milk was estimated according to standard method of dry ashing (Wehr & Frank, 2004).

2.4. Milk acidification and ultrafiltration

To obtain a certain level of pH, the pasteurised skim milk samples were divided into 36 × 50 mL portions and the pH of each sample was reduced by adding predetermined amounts of glucono-delta-lactone. The pasteurised skim milk samples were, after mixing with GDL for one minute, stored at 4 °C for seven days, 20 °C for two days and 30 or 40 °C for one day. During this period the GDL hydrolysed gradually to gluconic acid to give pasteurised skim milk samples with a pH ranging from 6.6 to 4.6 (Skibsted & Kilde, 1971). The final pH was measured directly by a pH meter (713 pH Meter, Metrohm, Copenhagen, Denmark) with a glass electrode (602 Combined Metrosensor glass electrode, Metrohm).

To separate the micellar phase from the serum phase, 15 mL from each 50 mL portion of pasteurised skim milk samples were centrifuged (Allegra 25R, Bekman Coulter, Copenhagen, Denmark) for 60 min at 2000 × *g* at 4, 20, 30, or 40 °C using centrifuge tubes fitted with ultrafiltration membranes (Vivaspin 20, GE Healthcare Bio-Sciences AB, Uppsala, Sweden) with a molecular mass cut-off of 10 kDa. Minerals that passed the membrane will be referred to as serum components.

2.5. Calcium and phosphorus determination

Total and serum contents of calcium were determined in pasteurised skim milk samples and in the corresponding serum phases using the atomic absorption spectrometric method (IDF, 2007). Micellar calcium was calculated as the total calcium minus the serum calcium. The contents of free calcium were determined in serum phase of pasteurised skim milk samples using an ion-selective electrode ISE25Ca with a reference REF 251 electrode (Radiometer Analytical SAS, Lyon, France). Before use, the calcium electrode was calibrated using standards solutions of $CaCl_2$ (0.1, 1.0, 10 and 24 mM) with 110 mM NaCl as background electrolyte. Free calcium contents were determined using the linear relationship (Nernst equation) between the electrode potential (mV) measured in the calibration solutions and the corresponding pCa value from the electrode potential measured in the serum phase of the pasteurised skim milk samples. All measurements of free calcium in serum samples and calibration solutions were done at 20, 30 or 40 °C. It was not possible to measure the amount of free calcium at 4 °C, due to lack of sensitivity of the ion-selective electrode at this low temperature. Total and serum contents of phosphorus were determined in pasteurised skim milk samples and in their serum phase using the standard absorption spectrometry method (IDF, 2006). Micellar phosphorus was calculated as the total phosphorus minus the serum phosphorus. Data are presented as the mean ± SD of two independent replicates. Furthermore equations of linear regression lines and correlation coefficients (R^2) at specific pH were for all temperatures calculated by Origin (Version 9.0, 2012, academic, Inno-Max, Aabybro, Denmark) from micellar, serum, free calcium, micellar and serum phosphorus concentrations.

2.6. Rheological properties

The rheological properties (storage modulus G') of pasteurised skim milk gels having a final pH of 4.8, 4.7 and 4.6 were evaluated using a rheometer (AR G2, TA Instrument, Elstree, UK) equipped with concentric cylinder geometry (Bob-cup) and a temperature