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Structural mechanisms leading to improved water retention in acid milk gels by use of transglutaminase

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ABSTRACT

Water retention in transglutaminase (TG)-treated acid milk gels was studied and linked with the gel formation dynamics. Heat-treated skim milk with and without pre-treatment by TG was acidified at 20 °C, 30 °C and 40 °C at constant glucono-δ-lactone (GDL) level to obtain different acidification rates. Formation dynamics and structural properties of acid-induced gels were followed by rheological and near-infrared light backscattering measurements as well as microscopy. TG-treated gels showed decreased tan δ values all through the acidification, which was pronounced around the gelation point. Backscattered light intensity was lowered in TG-treated gels compared to the controls indicating that TG-treated gels were comprised of smaller aggregates. Water holding capacity (WHC) was measured by using centrifugation at selected pH points (pH 5.2, 5.0, 4.8 and 4.6) during acidification. Both acidification temperature and TG treatment had significant effects on the water retention properties of the gels. Spontaneous syneresis observed at high acidification temperatures (≥30 °C) was prevented upon TG-treatment. WHC of TG-treated gels was significantly higher compared to the control gels at all pH points. TG-treated milk gels showed a homogeneous network formed of smaller aggregate and pore sizes at the gelation point and did not show any large-scale re-organisation thereafter. Transglutaminase is likely to act as a fixative of the protein network at an early stage of gelation and thereby limiting network rearrangements that take place in acid milk gels formed at high acidification temperatures leading to contraction and subsequent wheying off.

1. Introduction

Structure formation during acid-induced gelation of heated milk has been extensively characterized and involves the following phenomena: 1. Gradual loss of electrostatic repulsion between the proteins as the pH approaches the isoelectric point of whey proteins, 2. Solubilization of colloidal calcium phosphate (CCP) from casein micelles upon pH reduction and simultaneous dissociation of micellar caseins (more significant at temperatures < 20 °C) (Dalgleish & Law, 1988; Gastaldi, Lagaude, & De La Fuente, 1996), 3. Aggregation of casein micelles, serum casein and whey proteins, which eventually form a non-rigid particle gel network, and finally, 4) re-organization of the aggregated protein clusters/strands towards equilibrium (Horne, 1999; Lucey & Singh, 1998; Phadungath, 2005). A structure as energetic as a gel can only be retained as re-organization becomes kinetically impeded.

Water in milk gels is physically trapped within the gel network meaning that the tendency for whey separation is primarily linked to dynamics of the casein network rather than mobility of the water molecules (van Vliet & Walstra, 1994). Walstra, van Dijk, and Geurts (1985) stated that the proneness of the casein network to large-scale rearrangements during and after the gel formation is the most important factor determining the water retention in such gels. The higher rearrangement potential of a casein particle network can be inferred for example from a high loss tangent (tan δ) value which indicates that relatively more protein–protein bonds relax and/or yield-reform (van Vliet, van Dijk, Zoon, & Walstra, 1991). The formation of a local maximum in tan δ (tan δ_{max}) at pH values around the maximum CCP solubilisation (~pH 5.2) (Dalgleish & Law, 1988) can be regarded as typical for heat-treated acid milk gels when gelation pH is above 5.2–5.0 (depending on the acidification temperature). Removal of the residual CCP and casein

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dissociation cause loosening of the intra-micellar interactions while the micelle integrity is still preserved. The weakening of the intra-micellar structure leads to increasing tan δ values which then start to decrease due to the enhanced electrostatic interactions creating a local maximum in tan δ (Anema, 2009; Horne, 2003; Lakemond & van Vliet, 2008a; Lucey, 2002; Lucey & Singh, 1998; Lucey, Tamehana, Singh, & Munro, 1998). This behaviour is associated with high susceptibility of such gels (heated milk gels acidified at high temperatures) to large scale rearrangements at the initial stages of gel formation leading to spontaneous whey separation (Lucey & Singh, 1998; van Vliet & Walstra, 1994).

Structural properties of acid-milk gels can be modified by various factors such as pre-heat treatment temperature and duration, acidification temperature and rate or use of protein cross-linking enzymes. Modification of the protein matrix, which is mainly formed of weak physical interactions, directly by introducing strong covalent bonds within or between protein particles is one method to improve structural properties of skim milk gels. Use of transglutaminase (TG), a transferase which creates (γ-glutamyl)-lysine isopeptide linkages (Folk & Finlayson, 1977; Griffin, Casadio, & Bergamini, 2002), for that purpose has been extensively studied and reviewed (Buchert et al., 2010; Jaros, Partschefeld, Henle, & Rohm, 2006; Ozrenk, 2006). We have previously shown the effects of TG on texture and microstructure of acid-induced skim milk gels with the emphasis on the effect of pre-heat treatment (Ercili Cura et al., 2010). Clear indications on how cross-linking affects the rearrangement potential of the initial gel network was inferred through rheological measurements, i.e. loss tangent. However, the mechanism of how the covalent bonds introduced before or during the gel formation affect the aggregation dynamics of the micelles and the water retention properties of the subsequent gels was not studied. It has been stated that TG induces intermolecular cross-linking of the micellar caseins (Huppertz & De Kruif, 2008; Huppertz, Smiddy, & De Kruif, 2007; Schorsch, Carrie, & Norton, 2000). As a result, casein micelles are stabilized against dissociation (Huppertz & De Kruif, 2008; Moon, Hong, Huppertz, Fox, & Kelly, 2009; Mounsey, O'Kennedy, & Kelly, 2005) or disintegration due to removal of CCP from the micelles during acidification (Schorsch et al., 2000). As a consequence of modified casein micelle structure and micelle–micelle interactions, TG-treated milk gels are composed of altered gel particles organized in a lower mesh sized protein network and have increased mechanical strength (Ercili Cura et al., 2010; Schorsch et al., 2000).

In this study, the structure formation and water retention in heat-treated skim milk gels were elucidated by modulating the balance of contributing interactions. By combining the structural properties of the gels created by altered physical and chemical interactions to their ability to retain water, an attempt to identify particle and network properties that lead to better entrapment of water in acid-milk gels was made.

2. Materials and methods

2.1. Preparation of reconstituted skim milk

Low-heat skim milk powder (SMP) (34.5% protein, 53% lactose, 8% ash, 3.8% moisture, and 0.5% fat) was obtained from Valio Ltd. (Helsinki, Finland). Reconstituted skim milk was prepared by dispersing 9.57% (w/w) SMP in ultra-pure water by constant stirring with a magnetic rod at 50 °C for 1 h. It was tempered to room temperature (22–24 °C) with constant stirring in 30 min. Sodium azide (0.01% (w/w)) was added to prevent microbial growth. The reconstituted milk had a protein concentration of 3.3% (w/w). Prepared skim milk was heat-treated at 80 °C for 30 min. The heat treatment was performed in kimax tubes (inner diameter 13 mm), placed in a water bath at 82 °C. At the end of 30 min (+2 min for the centre of the tube to reach 80 °C), the tubes were directly immersed in ice and the sample was cooled down to refrigeration temperature in 7–10 min. Heat-treated reconstituted milk was stored in a refrigerator overnight before use.

2.2. Enzyme treatment and preparation of acidified gels

Transglutaminase (Activa® MP, Ajinomoto) was supplied by Ajinomoto Foods Europe SAS (France). The activity of the enzyme was determined according to Folk (1970) and was 2500 nkat g^{-1} of powder. Heat-treated reconstituted skim milk was tempered to 40 °C before addition of the TG. TG dosage was 100 nkat g^{-1} of substrate protein in all cases. All samples (with and without TG) were incubated for 1 h at 40 °C prior to the addition of GDL. Acidification was carried out at three different temperatures, 20 °C, 30 °C and 40 °C. GDL was added at a constant dosage of 1.3% (w/w) once the samples were tempered to the desired acidification temperatures. Acidification was followed until pH reached 4.6.

2.3. Small deformation oscillatory measurements

Small deformation oscillatory measurements were performed by using a stress-controlled rheometer (StressTech, Reologica Instruments AB, Lund, Sweden). Milk samples at 20 °C, 30 °C or 40 °C were placed into the concentric cylinder (CC 25) measuring system (pre-adjusted to desired temperature) 5 min after GDL addition. Silicon oil was applied to the sample surface to prevent evaporation. All measurements were carried out until the sample reached pH 4.6. Samples were oscillated at a constant frequency of 0.1 Hz. The strain was kept constant at 1% which was tested to be within the linear viscoelastic region by performing strain sweeps at the end of time sweep measurements for each gel sample. The final G′ and the final tan δ refer to the G′ and tan δ values attained at pH = 4.6. The gelation time and the gelation pH were considered as the time and pH at tan δ = 1. Each measurement was performed on three individual gels. The pH profiles were followed simultaneously with rheological measurements by using a pH meter with a data logger (pH 3310, WTW, Weilheim, Germany). The pH meter was calibrated for the studied temperature. Both the rheological measurement and the pH meter were started 10 min after GDL addition.

2.4. Near-infrared light backscattering

Gel formation was also followed by a multiple light scattering technique using a Turbiscan® Lab Expert (Formulaction, France) optical analyzer. The detection head of the instrument is composed of a pulsed near infrared light source ($\lambda = 880$ nm) and two detectors. The backscattering (BS) detector receives the light scattered by the sample at 135°. The gel samples were prepared as explained above and poured into special glass tube for gelation inside the Turbiscan®. The sample holder was pre-adjusted to the gelation temperature i.e. 20 °C, 30 °C or 40 °C. The measurements started 10 min after the addition of the GDL. A scan was performed every 5 min (10 min for the acidification temperature of 20 °C) until pH 4.6 was reached. The results represent the ΔBS% in the mid-point (20–20.1 mm) of the glass tube.

2.5. Large deformation tests (penetration)

The firmness of the milk gels was measured with a TA-HDi Texture Analyser (Stable Microsystems Ltd., Godalming, UK) equipped with a 5 kg load cell. For this test, after the addition of GDL and 5 min mixing, the milk samples were poured (20 g) into plastic