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Rheological and structural properties of differently acidified and renneted milk gels

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ABSTRACT

In this study we assessed the rheological and structural properties of differently acidified and renneted milk gels by controlling pH value and renneting extent. Skim milk were exactly renneted to 4 extents (20, 35, 55, and 74%) and then direct acidified to the desired pH (4.8, 5.0, 5.2, 5.5, 5.8, and 6.2), respectively. Rheological properties were assessed by dynamic rheological measurements, structural properties were studied by spontaneous whey separation and confocal laser scanning micrograph, and protein interactions were studied by dissociation test. Results showed that minimally renneted milk samples (20 and 35%) formed weak gels with low storage modulus, and the acidification range within which gels could form was narrow (pH $\leq$5.2). Highly renneted milk samples formed more gels with high storage modulus. The results of this study revealed that acidification determined the structural properties of highly renneted milk gels. As pH increased from 5.0 to 6.2, highly renneted milk gels had lower loss tangent, decreased spontaneous syneresis, and smaller pores. For both the low and high rennetings, divalent calcium bonds contributed less at low pH than at high pH. In conclusion, renneting increased the pH range suitable for gel formation; acidification determined the spontaneous syneresis and microstructure of highly renneted milk gels.
Key words: milk gel, acid, rennet, rheology

INTRODUCTION

Milk gelation is a crucial step in the manufacture of fermented dairy products and has been extensively studied (Lucey, 2002; van Vliet et al., 2004). Milk gels can be produced by acid or rennet, resulting in gels with different properties (Lucey, 2002). In acid-induced gels, colloidal calcium phosphate (**CCP**) is released from micelles and net-negative charges are lost with decreasing pH, thereby reducing the electrostatic and steric repulsions and favoring aggregation (Lucey and Singh, 1998). At pH 4.8, milk forms weak gels and shows gelation (Lucey et al., 1997). Hydrophobic, hydrogen, and electrostatic interactions are homogeneously distributed (Lefebvre-Cases et al., 1998). In rennet-induced gels, casein micelles aggregate extensively when more than 85% of κ-CN is hydrolyzed (Dalgleish, 1979). Hydrophobic interactions and calcium bonds are the main forces in rennet-induced gels (Lefebvre-Cases et al., 1998). Even though acid- and rennet-induced gels have been extensively studied, the gelation behavior of combined acid- and rennet-induced gels are not well understood.

Combined acid and rennet gelation is often used in the manufacture of certain types of cheese to improve gel draining and firmness (Jelen and Renzschauen, 1989). It has also been successfully used in soya milk and skim milk gels (Lin et al., 2012). Because it is an enzyme- and acid-induced process, the gelation behavior is quite complicated and largely depends on coagulation processes (Castillo et al., 2006a; Le Feunteun and Mariette, 2008; Cooper et al., 2010; Salvatore et al., 2011).

In previous studies, rheological and structural properties of combined acid- and rennet-induced milk gels were studied by simulating the coagulation process. Lucey et al. (2000) reported that the concentrations of acidulant and rennet significantly affect gelation behavior. The storage modulus (**G′**) of combined acid- and rennet-induced gels is higher than strictly acid- or rennet-induced milk gels. Researchers have reported that in partial renneting and continuous acidification, milk gel formation occurs in 2 stages based on the acidification degree (pH <5.0 and >5.0; Li and Dalgleish, 2006). Studies about the structural properties of combined acid- and rennet-induced milk gels reported that increasing inoculum levels induces a reduction in syneresis (Castillo et al., 2006b) and calcium release is responsible for the final structure (Salvatore et al.,

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2011). Therefore, the combined addition of acid and rennet can lead to different coagulation processes and gel products. However, based on these studies, acid and rennet act continuously and synergistically. The contribution of acid and rennet on gelation and gel properties is not clear. The objective of the current study was to assess how acid and rennet affect the behavior of combined acid- and rennet-induced gels by analyzing the rheological and structural properties.

MATERIALS AND METHODS

Materials

Fresh milk, obtained from San Yuan Dairy Co. (Beijing, China), was centrifuged at 4,000 × g for 20 min at 25°C and skimmed. Sodium azide (0.02% wt/vol) was added to prevent bacterial growth. Skim milk was stored at 4°C. Rennet (Chr-Max Powder Extra, 2,235 international milk clotting units/g) was supplied by Chr. Hansen Inc. (Hørsholm, Denmark). All chemicals and reagents were of analytical grade. Double distilled water (Millipore, Billerica, MA) was used throughout the experiments.

Preparation of Differently Acidified and Renneted Milk

As different pH needs different time to rennet κ-CN to the same hydrolysis, acidification before renneting would make it complex and difficult to control acidification and renneting. Therefore, we acidified after renneting. Controlled renneting was performed at 4°C by adding different amounts of rennet previously diluted in water (1:50 wt/vol) to skim milk. Four renneted milk samples (20, 35, 55, and 74%) were prepared by adding different amounts of rennet (20, 40, 60, or 80 μg/100 mL) to skim milk. After stirring for 1 min, the milk samples were stored at 4°C for 12 h. Milk was completely hydrolyzed with 2 mg/100 mL rennet. Renneting degree was determined by comparing the amount of casein macropeptide (**CMP**) released from different milk samples to that released from completely hydrolyzed milk (Gastaldi et al., 2003; Li and Dalgleish, 2006). The CMP content was determined by reverse-phase HPLC (Shimadzu, Kyoto, Japan) according to an established method (Thoma et al., 2006). Our preliminary results revealed that, as a result of the low rennet concentrations and the quickly direct-acidified gel formation, the renneting degree of the milk samples increased within 2% during gel formation. This increase, however, is unlikely to significantly affect the gel properties.

To control acidification, we used a direct acidification method, which keeps pH values constant (Roefs and van Vliet, 1990; Roefs et al., 1990; Hammelehle et al., 1997). The 4 renneted milk samples were acidified at 4°C to 6 different pH values: 4.8, 5.0, 5.2, 5.5, 5.8, or 6.2. In this experiment, pH was determined using a pH meter (YM Instrument Co., Jiangyan, China). The rate of acid addition was regulated with a constant flow pump (Qinpu Huxi Instrument Co., ShangHai, China); the pH values were controlled within ± 0.02. Particle size analysis confirmed that acidification with lactic acid (2% vol/vol) at 1 min/mL was adequate because it did not lead to a significant increase in casein micelle size.

Gel Formation and Dynamic Rheological Measurements

The acidified and renneted milk samples were transferred to a rheometer (AR1500 rheometer, TA Instruments Ltd., New Castle, DE) equipped with a 60-mm diameter plate. All samples were treated under the same conditions and the temperature was risen to 32°C in 5 min by the controlled heating system. The G′ and loss tangent (**tan δ**) were continuously measured. A cover was placed on the cylinder to prevent evaporation. Dynamic rheological measurements were performed to monitor the gelation process at 32°C. The measurements were performed at 1 Hz and 0.1 Pa of applied stress, which was within the linear viscoelastic region. Gelation point was defined as the point when gels had a G′ ≥1 Pa (Lucey et al., 2000; Castillo et al., 2006a). The effect of the time scale of deformation on the rheological properties was determined by frequency sweep after 1 h of acidification. Frequency varied from 0.01 to 1.0 Hz.

Spontaneous Whey Separation

Spontaneous whey separation was determined by volumetric flask experiment (Lucey et al., 1998). Following acidification, milk samples were filled below the volumetric flask neck and heated in a bath water to 32°C. After 2 h, the volumetric flasks were examined. Whey separation was expressed as the ratio of whey separated from the gels to the initial weight of the milk sample. Experiments were repeated 8 times.

Confocal Laser Scanning Microscopy

The microstructure of milk gels was examined under a Leica TCS SP2 confocal scanning laser microscope (Leica Microsystems CMS GmbH, Mannheim, Germany). Following acidification, 1 mg/mL of rhodamine B, a fluorescent dye, was added. After stirring for 1 min, 100 μL of the milk sample was placed in a concave slide. A