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Sol gel transitions during acid gelation of milk containing modified waxy maize starch. Differences between chemical and bacterial acidification measured using rheological and spectroscopic techniques

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ABSTRACT

Modified waxy maize starch (1%, w/v) was added to skim milk and the mixtures were heated and homogenized. Acidification was conducted at 40 °C, using either glucono-δ-lactone (GDL) or a commercial starter culture. The physico-chemical changes occurring during acidification were monitored using small oscillatory rheology, diffusing wave spectroscopy and ultrasound spectroscopy. A similar gelling behaviour was noted for GDL and bacterial-induced gels; however, a difference was noted in the values of storage modulus (G′). The presence of starch did not seem to affect the development of the gel structure, nor the mobility and positional correlations of the casein micelles during acidification. On the other hand, starch increased the final storage modulus, G′ of the acid milk gels. These results indicate the absence of direct interactions between micelles and the modified starch granules.

1. Introduction

Polysaccharides are often used in dairy-based gelling systems to modify the flow and texture properties of the final gel (Williams, Glagovskaia, & Augustin, 2003). Amongst the most popular non-dairy ingredients, starch is typically used in yogurts to impart viscosity, extend milk solids and prevent wheying-off (Lucey, 2002). When used alone or as part of a stabilizer blend, starch is the preferred thickening agent in yoghurt due to its creamy texture, processing ease and low cost when compared with other hydrocolloids.

Starch granules are supramolecular structures composed of long chain polysaccharides. Starch consists of two types of molecules: amylose, a long linear chain of D-glucose joined together by $\alpha(1 \rightarrow 4)$ glycosidic bonds and amylopectin, formed by non-random α-$(1 \rightarrow 6)$ branching of the amylose-type α-$(1 \rightarrow 4)$-D-glucose structure (Chamberlain, Rao, & Cohen, 1999). Waxy maize starch consists wholly or largely (99%) of amylopectin. When heated, native starch granules hydrate and swell and eventually break, thereby releasing amylose and amylopectin polymers into solution. Modified starches are chemically crosslinked to avoid rupture of the granule at high temperatures during processing and are commonly employed in dairy processes, as native granules could not withstand the shear applied during heating and homogenization.

During processing of yoghurt, the acid gelation of milk is achieved by addition of bacterial cultures, which convert lactose to lactic acid resulting in a decrease in pH. With acidification, the colloidal calcium phosphate, which is part of the micellar structure, is dissolved into the serum (Dalgleish & Law, 1988; Le Graet & Gaucheron, 1999). Once the pH reaches the isoelectric point of the caseins, the casein micelles begin to interact and eventually aggregate forming a self supporting network (Horne, 1999; Lucey, 2002). The chemical acidification of milk using GDL has often been used to study the acid gelation of milk as a simplified and more controlled model of the fermentation (Girard & Schaffer-Lequart, 2006; Lucey, 2002). Lucey, Tamehana, Singh, and Munro (1998) reported that the physico-chemical properties of GDL-induced gels are different from those of fermented milk. The difference between the rates of acidification of milk by GDL and bacteria influences the dissolution of colloidal calcium phosphate, the dissociation of casein micelles, the rate of aggregation as well as the time available for rearrangement of the aggregating protein particles (Laligant, Famelart, Brulé, Piot, & Paquet, 2003; Lucey et al., 1998).

The proprieties of the acid gels can be manipulated by using heat treatment of the milk before acidification (Lucey, 2004). The heat treatment of milk at temperatures above 70 °C results in the denaturation of whey proteins (Anema & McKenna, 1996). These

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denatured whey proteins can undergo a complex series of aggregation reactions with other denaturated whey proteins and the casein micelles (Corredig & Dalgleish, 1996). The occurrence of aggregates in heated milk has been linked to acid gels having an earlier onset of gelation, higher firmness, and lower syneresis than gels of unheated milk (Guyomarc'h, Law, & Dalgleish, 2003).

Heating is also necessary to gelatinize starch. The critical temperature of gelatinization is dependent on starch type (Appelqvist & Debet, 1997). Starch gelatinization encompasses hydration, swelling, disruption of the granular structure, and solubilisation of starch molecules (Appelqvist & Debet, 1997). The physico-chemical changes occurring in milk because of the presence of starch granules during heating and acidification have yet to be reported. Heating of a mixed system of milk and starch may lead to different characteristics of the final acid gel compared with acid gels made from milk proteins alone.

There have been very few studies comparing the properties of gels prepared either using GDL or bacterial fermentation. The studies that are available investigate the rheological and macroscopic differences between the two systems (Lucey et al., 1998; Van Marle & Zoon, 1995), or the differences in calcium release (Laligant et al., 2003). Moreover, the effect of starch addition on the aggregation behaviour of casein micelles during acidification by lactic bacterial cultures has never been investigated.

The aim of this work was to investigate the acidification process of a mixture of heated milk and modified waxy maize starch with two different acidifying agents, GDL or starter culture. These two modes of acidification will affect the rate of solubilisation of calcium phosphate and the dissociation of the caseins from the micelles, potentially affecting the early stages of aggregation of the casein particles. The gelation behaviour was observed using diffusing wave spectroscopy (DWS), ultrasound (US) and rheology, as these techniques probe at three different length scales and provide information about particle size and mobility, interparticle interactions and macromolecular developments that might occur during the primary stages of acid gelation.

2. Materials and methods

2.1. Preparation of acidified milks

Bulk skim milk was obtained from Gay Lea Foods Cooperative (Guelph, ON, Canada). A crosslinked and stabilized waxy maize starch was obtained from National Starch (Thermtex, Bridgewater, NJ, USA). A commercial culture composed of *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, *Lactobacillus acidophilus*, *Bifidobacterium* and *Lactobacillus paracasei* strains ("Yofast", Chr. Hansen, Hoersholm, Denmark) as well as GDL from Sigma–Aldrich Inc (St. Louis, MO, USA) were used for the acidification experiments. Starch was added at 1% (w/v) and milk mixtures were then heat-treated using a pilot plant HTST/UHT equipment (Microthermics, North Carolina, US). Milks with and without starch were preheated at 65 °C, then homogenized at a pressure of 11.7 MPa with back pressure ~0.69 MPa, followed by a final heating at 95 °C for 5 min, at a flow rate of 1 L min^{-1}. The heated milk exited the system at 20 °C and was collected and equilibrated for 2 h at room temperature.

The heat-treated samples were acidified with 1% (w/v) GDL at 40 °C or inoculated with 0.02% (w/w) of the mixed culture at 40 °C. Sufficient sample quantities were prepared to fill the sample chamber of the ultrasonic spectrometer, the rheometer cup, the DWS cuvette and to allow at least 25 mL to be reserved for bulk measurements of pH at 40 °C at intervals of 10 s. All experiments were started simultaneously at approximately 10 min after addition of GDL or culture.

Plots of the measured pH against time for each run were analyzed by curve-fitting, using the program Sigmaplot 8.0.2 (SPSS, Chicago, IL, USA). The fitted parameters were used to provide interpolated values of pH for all of the experimentally measured points from the rheometer and DWS experiments, so that it was possible to calculate the pH at any time during the reactions. In the ultrasonic spectrometer, the pH was directly measured in the spectrometer cell by a pH probe.

All experiments were carried out at least three times (different milk batches). All measurements were plotted as a function of pH using the corresponding acidification curve. Statistically significant differences were determined with the one way analysis of variance procedure (ANOVA) using Splus 8.0 (TIBCO, Palo Alto, California, USA). Significance was considered for $p < 0.05$.

2.2. Sediment volume test

The sediment volume test was performed in milk/starch mixtures after heat treatment to evaluate the quality of the gelatinization of the added starch. Processed milk (100 mL) with 1% starch (w/w) was placed in a graduated beaker. The solution was kept 24 h at 4 °C, after which the height of the sediment, mainly composed of swollen starch granules, was measured. Few drops of Congo red (Sigma–Aldrich Inc, St. Louis, MO, USA) (0.1%, w/v, in water) were added to the solution to improve the contrast between the sediment and the supernatant.

2.3. Rheology

Changes in the rheological proprieties were observed by dynamic oscillation measurements of the storage modulus (G′) as well as the phase angle (δ). A controlled stress rheometer (Physica MCR 301, Ostfildern, Germany) was used with a cup and bob geometry (CC27) consisting of two coaxial cylinders (diameters 26.66 and 28.92 mm). The cup and bob of the rheometer were cleaned with ethanol (95%) before the milk sample was added. An applied strain of 0.01 was used. Samples were oscillated at a frequency of 0.5 Hz and measurements were taken every 15 s at a temperature of 40 °C until a pH of 4.5 was reached. Gelation point was determined as the pH at which $G' = G''$.

2.4. Diffusing wave spectroscopy

Measurements were made using transmission DWS with a solid-state laser (532 nm and 100 mW power, Coherent, Santa Clara, CA, USA), two matched photomultipliers (HC120-03, Hamamatsu, Loveland, OH, USA) and a correlator (FLEX2K-12 × 2, Bridgewater, NJ, USA). The sample was loaded in a 5 mm glass cuvette (Hellma Canada Limited, Concord, Canada) and kept at a temperature of 40 °C by a circulating thermostatted water bath. The cuvette was disinfected with ethanol (95%) before inoculated milk was added. Each treatment was measured in triplicate (i.e., three separate milk batches). Each run was carried out for 2 h or 4 h for samples with GDL and bacterial fermentation, respectively. In all cases the measurement length was 2 min with 1 s intervals. Data were analyzed using Sigma Plot 10.0 (SPSS Inc., Chicago, USA). Standard latex spheres of 269 nm diameter (Portland Duke Scientific, Palo Alto, CA, USA) were used to calibrate the laser intensity daily.

DWS detects the intensity of the multiple scattered light, which fluctuates due to Brownian motion of particles. The scattered light is collected after it has traversed the whole length (L) of the scattering cell. Correlations in the scattered light are characterized by means of the correlation function (Weitz & Pine, 1993), and a value of characteristic decay time τ ($\tau = \tau_o(l^*/L)^2$) can be derived; where $\tau_o = (Dk_o^2)^{-1}$, and D is the particle diffusion coefficient and k_o, the