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Effect of addition of gelatin on the rheological and microstructural properties of acid milk protein gels

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ABSTRACT

To gain an understanding of the gelation mechanism of mixtures of milk proteins and gelatin, rheological and microstructural properties of the mixtures were characterized following four stages. During the acidification stage (at 45 °C), the presence of gelatin at sufficient concentration (higher than 1%) led to a lower storage modulus (G') than that of the pure milk protein gels and a more heterogenous microstructure with larger milk protein clusters was formed. During the cooling (from 45 to 10 °C) and annealing stage s (at 10 °C), the G' of the gels increased because of both milk gel enhancement and gelatin gelation. Higher concentrations of gelatin led to earlier formation of strand-like structures, seen in the micrographs. The gelation of gelatin changed the microstructure of whey protein isolate (WPI) gel dramatically, while gels of milk protein concentrate (MPC) and skim milk powder (SMP) maintained the typical milk gel network and gelatin formed strands and films without destroying the existing gels. During the heating stage (from 10 to 45 °C), gelatin strands were melted and the G' of the mixed gels tended to revert to the value at the end of the acidification stage, indicating that the changes caused by gelatin in the microstructure of milk protein gels after acidification are reversible. Additionally, gelatin enhanced the water holding capacity (WHC) of the gels (no serum expulsion was observed for gels containing $\geq$1% gelatin), without increasing gel firmness significantly.

1. Introduction

Gel formation by milk proteins is the crucial stage in the manufacture of acid gels such as yogurt and many other dairy-based products. To understand the gelation mechanism of milk proteins, considerable research has been carried out using a range of dairy ingredients such as skim milk powder (SMP), milk protein concentrate (MPC), whey protein isolate (WPI) and sodium caseinate (Cavallieri & Da Cunha, 2008; Cooney, Rosenberg, & Shoemaker, 1993; Graveland-Bikker & Anema, 2003; Hashizume & Sato, 1988). In addition to milk proteins, hydrocolloids are important ingredients in yogurt manufacture for producing a variety of mechanical and textural properties to cater for consumers' preferences and to improve product stability. Among the hydrocolloids used, gelatin, an animal protein produced from collagen (Boran, Mulvaney, & Regenstein, 2010), is still widely used to modify the texture of yogurt. It has high flexibility of the polypeptide chains and a non-random occurrence of imino acids (i.e., proline or hydroxyproline) in its sequence, which is unusual among gel-forming agents (Karim & Bhat, 2009). The intermolecular contacts in gelatin gels are hydrogen bonds, which make the gels thermally reversible. Specifically, a gelatin gel melts below human body temperature, which gives it the well-known "melt-in-mouth" property (Djabourov, 1988). The effects of added gelatin on the microstructure and rheology of acid milk gels have been previously reported (Fiszman & Salvador, 1999; Koh, Merino, & Dickinson, 2002; Walkenstrom & Hermansson, 1996). However, most of these studies were focused on the properties of the final mixed gel and some concerned heat-set milk gels. Therefore, the mechanism of interactions occurring in milk protein–gelatin systems during gelation (both of milk and gelatin) and melting of gelatin, which would be valuable to understand in relation to the function of gelatin during the manufacture process of yogurt and also consumption of yogurt, is still not clear.

Moreover, few studies have been published on the microstructure of milk protein–gelatin acid gels. In the 1970s, Kalab, Emmons, and Sargant (1975) reported that the gelatin in yogurt could not be detected by either scanning electron microscopy (SEM) or transmission electron microscopy (TEM), even at a very high

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concentration (10%). Fiszman, Lluch, and Salvador (1999) carried out a study using cryo-SEM, in which 1.5% gelatin was added to both a reconstituted milk gel and yogurt. They found that gelatin formed flat sheets or surfaces which interacted with the milk gel matrix and connected the granules and chains of milk proteins. Cryo-SEM is a powerful technique for observing samples which are difficult to observe by conventional SEM; however, attention must be paid to the possible formation of artifacts by this method. The formation of ice crystals can displace structural elements and destroy the original structure (Kalab, Allanwojtas, & Miller, 1995). SEM has been a very useful technique for determining the microstructure of milk protein gels with simple specimen preparation and to provide a three-dimensional image (Kalab & Harwalkar, 1973). The microstructure of milk gels with polysaccharides has been studied widely using SEM (Cavallieri & Cunha, 2009; Hood, Seifried, & Meyer, 1974; Sanchez, Zuniga-Lopez, Schmitt, Despond, & Hardy, 2000). The specimens in the study of Kalab et al. (1975) were prepared by freeze drying after fixation and observed at accelerating voltage of 20 kV. The relatively high accelerating voltage may induce structure damage, especially with the technique of freeze drying during specimen preparation, while at a low acceleration voltage, the freeze drying technique was reported to produce poor resolution (Trieu & Qutubuddin, 1994). Critical-point drying (CPD) has been used widely to produce dried specimens for SEM, which can provide distortion-free images (Bray, Bagu, & Koegler, 1993). Therefore, CPD was used in the present SEM study.

To understand how gelatin and milk components interact in yogurt, acid gelation of reconstituted WPI, MPC and SMP was studied individually, with and without gelatin. These products, with different compositions represent the ingredients in yogurt. Two important yogurt manufacturing stages were followed in this study: firstly, the solutions of gelatin and milk protein were heated at 95 °C for 10 min, by which gelatin was melted and whey proteins were denatured, and, secondly, fermentation of yogurt was simulated by using glucono-delta-lactone (GDL) for acidification. The convenience and reproducibility of the GDL method has already been proved (Kim & Kinsella, 1989; Vlahopoulou & Bell, 1995).

The objectives of this work were to determine the ability of gelatin to alter the physical and microstructural properties of acid-induced milk protein gels and to gain an understanding of the mechanism of gelation of milk protein–gelatin mixed systems.

2. Materials and methods

2.1. Materials

The gelatin used in this study was supplied by Gelita (Beaudesert, Australia). It was a light coloured edible beef skin (type B) gelatin powder with bloom 200, mesh 20 and isoelectric point of ~5.0, which is a commercial product commonly used in the food industry. The milk protein ingredients, whey protein isolate (WPI, protein 93.9%, moisture 4.7%, fat 0.3%, lactose 0.4% and ash 1.5%), milk protein concentrate (MPC, protein 85%, moisture 7%, fat 2.5%, lactose 5.5% and ash 8.5%) and skim milk powder (SMP, protein 33%, moisture 3.6%, fat 0.9%, lactose 54.7% and ash 7.8%) were obtained from Murray Goulburn Co-Operative Ltd (Melbourne, Australia). The chemical composition of these ingredients was provided by the supplier. The acidulant glucono-delta-lactone (GDL) was purchased from Sigma Chemical Co. (St. Louis, USA).

2.2. Methods

2.2.1. Preparation of protein solutions and acid protein gels

Milk protein solutions were prepared by dispersing the required amount of powders (WPI, SMP or MPC) in distilled water under continuous stirring for 30 min to obtain a milk protein concentration of 4.5% (w/w). To prepare the mixed solutions, milk ingredients were dispersed in water with gelatin. Three concentrations of gelatin (0.4, 1.0 and 2.5% [w/w]) were investigated. All solutions were stored at 4 °C overnight before use. The solutions were heated in a 95 °C water bath for 10 min at their natural unadjusted pH and then cooled to 45 °C immediately using cold water. For gel formation, an appropriate amount (0.6% for WPI, 1.2% for MPC and 1.5% for SMP [w/w]) of GDL was added to the solutions to decrease the pH to 4.6 in 4 h at 45 °C. During acidification the change in pH was monitored with a pH meter.

2.2.2. Small deformation rheological measurement

Dynamic oscillatory measurements were performed on a stress-controlled rheometer (Model AR-G2, TA Instruments, USA). Aliquots of protein solutions with or without gelatin were poured onto the bottom plate of the rheometer equipped with a 4 cm, 2° cone-plate measuring system immediately after GDL was added. The measurements were performed in a four-stage process as described by Pang, Deeth, Sopade, Sharma, and Bansal (2014), with some modifications:

Acidification stage: Measurement commenced at 45 °C and this temperature was maintained for 4 h, promoting formation of the milk protein gel;
Cooling stage: the temperature was lowered from 45 to 10 °C at a constant rate of 1 °C/min promoting gelatin gel formation;
Annealing stage: the oscillatory tests were performed at 10 °C for 2.5 h to observe the maturation of the gelling samples;
Heating stage: the melting characteristics of the gels were determined by increasing the temperature from 10 to 45 °C at 1 °C/min.

Preliminary experiments for strain sweep showed that a strain of 0.5% was within the linear viscoelastic region for all samples at a frequency of 1 Hz. The gelation point was defined as the point when a sharp increase in G' from the baseline occurred, according to a previous milk gel study (Matia-Merino, Lau, & Dickinson, 2004). Two independent repetitions were conducted for each sample.

2.2.3. Microstructure

2.2.3.1. Scanning electron microscopy (SEM). Milk protein solutions with or without gelatin were prepared as described above. After addition of glucono-delta-lactone (GDL), samples were transferred to a temperature-programmable water bath (Thermo Haake, C25P, Karlsruhe, Germany). The temperature profile was set as follows: Acidification: 45 °C for 4 h; Cooling: 45 to 10 °C, 1 °C/min; Annealing: 10 °C for 2.5 h and Heating: 10–45 °C, 1 °C/min. Six samples were taken for microscopy at the following points: Point 1 – end of acidification; Point 2 – 10 min from the start of annealing; Point 3 – 30 min from the start of annealing; Point 4 – end of annealing; Point 5 – at 35 °C during heating; Point 6 – at 45 °C during heating.

The microstructure of the gels was determined by SEM as described by Pang et al. (2014). Gel at each point as listed above was fixed immediately with glutaraldehyde at room temperature, dehydrated with ethanol at room temperature and then dried with a CO_2 critical point dryer (Tousimis Automatic). This procedure removed the soluble substances in the gels, such as lactose (Kalab & Harwalkar, 1973). Dried samples were platinum-coated and observed with a scanning electron microscope (JEOL 6610) at an acceleration voltage of 10 kV.

2.2.3.2. Confocal laser scanning microscopy (CLSM). Rhodamine B (0.1% [w/w]) was added to milk protein/gelatin solutions to dye the

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