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Interaction between lactoferrin and whey proteins and its influence on the heat-induced gelation of whey proteins

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ABSTRACT

In this paper, the influence of lactoferrin (LF) on the structural development of whey protein isolate (WPI) gels during heating was investigated. The results demonstrated that the presence of sufficient LF could improve the strength and elasticity of WPI gels. When 30% LF was added, the elastic modulus of WPI gels increased from 254 ± 31 and 413 ± 58 Pa to 3222 ± 105 and 2730 ± 131 Pa at pH 6.7 and 5.8, respectively. The addition of LF improved the water holding capacity (WHC) of WPI gels at pH 6.7, while no improvement was observed at pH 5.8. LF interacted with whey proteins differently at pH 6.7 and 5.8 during heating. The LF/whey proteins complexes formed at pH 6.7 had smaller sizes and narrower size distributions than those formed at pH 5.8.

1. Introduction

Whey protein isolate (WPI) is one of the most widely used ingredients in food industry, attributing to its nutritional value and excellent functional properties, such as foaming, emulsifying and gelling properties (Alting, Hamer, De Kruif, & Visschers, 2000; Foegeding, Davis, Doucet, & McGuffey, 2002; Zhu & Damodaran, 1994; Zhu, Damodaran, & Lucey, 2010). As a by-product of cheese production, WPI is mainly consisted of β-lactoglobulin (β-lg) and α-lactalbumin (α-la) (Perssin & Gekas, 2000). Due to their well organized structure, both β-lg and α-la are very sensitive to heat treatment. Irreversible denaturation and aggregation of whey proteins occur at heating temperatures higher than 70 °C. In milk, denatured whey protein aggregates could attach to the surface of casein micelles, resulting in decreased stability and rennetability of the latter (Donato & Guyomarc', 2009). Heating at high concentrations (e.g., > 8% w/w protein, pH 6.9) and sufficiently high temperatures (e.g., 80 °C), denatured whey proteins interact with each other and form a gel network (Havea, Watkinson, & Kuhn-Sherlock, 2009). The special heat-gelation characteristic of whey proteins has been widely used in many food products, such as ice creams, confections and puddings, to achieve desired structural and sensorial properties (Ren, Dong, Yu, Hou, & Cui, 2017).

The formation of whey protein gels is a complicated process, which involves in sulphydryl-disulfide interchange interaction (Shimada & Cheftel, 1989), hydrophobic interaction, hydrogen bond and ionic interaction (Havea et al., 2009). One limitation of WPI gels is that they are usually brittle and susceptible to syneresis. Polysaccharide additives can be added to improve the gel strength by increasing the viscosity of protein solutions. Previous researchers reported that the addition of xanthan, even at a very low concentration of 0.01%, could significantly increase the strength of heat-induced WPI gel at pH 6.0 and 6.5 (Bertrand & Turgeon, 2007). In addition, Tavares and da Silva (2003) found that at pH 7.0 galactomannan could act as the filler of protein network and positively influence the structure of WPI gels, while at pH close to 5.3 (isoelectric point of whey protein), the galactomannan had a detrimental effect on protein network formed at low WPI concentration. Moreover, it had been demonstrated that the incorporation of konjac glucomannan into WPI gel resulted in the significant increase in gel strength, attributing to the segregative interactions between denatured whey proteins and konjac glucomannan (Tobin, Fitzsimons, Chaurin, Kelly, & Fenelon, 2012). Apart from the addition of polysaccharides, it was shown that structural modifications of whey proteins through glycosylation (Sun et al., 2011) and enzymatic treatment (Tarhan, Spotti, Schaffter, Corvalan, & Campanella, 2016) could successfully increase the gel strength and decrease the gelation time.

In comparison with polysaccharides, the effect of proteins on the formation of WPI gel is less studied. Roesch and Corredig (2005) investigated the heat-induced gelation behaviour of soy protein-WPI mixtures, and it was found that soy/WPI mixtures could form gels with much higher elastic modulus than WPI control. In addition, a more homogeneous gel structure was formed at soy/WPI ratios lower than 1:1. In a recent research, the influence of sodium caseinate (NaCas) on the heat-induced gelation of WPI was studied. The results indicated that WPI aggregation was inhibited at NaCas concentrations lower than 50%. However, at NaCas concentration higher than 50%, larger aggregates were formed and the required concentration of WPI to form a

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gel was decreased, but the hybrid gels had a lower elastic modulus than pure WPI gel (Nguyen, Nicolai, Chassenieux, Schmitt, & Bovetto, 2016). The same researchers further investigate the influence of micellar casein, and found that the addition of micellar casein could increase the elastic modulus and decrease the syneresis of WPI gels at pH 5.8–6.3 (Nguyen, Chassenieux, Nicolai, & Schmitt, 2017).

Lactoferrin (LF) is a glycoprotein, which is usually separated from milk colostrums (Yoshida, Wei, Shinmura, & Fukunaga, 2000). It has a high isoelectronic point (pI) of pH 8.9 and a molecular weight of 88 kDa (Yamniuk, Burling, & Vogel, 2009). In addition to its special iron-binding capacity, LF has a variety of biological functionalities, including antibacterial, antiviral, antifungal, antiparasitic, anti-inflammatory, anticarcinogenic and antitumor activities (Tomita et al., 2009; Ward, Paz, & Conneely, 2005). It has been reported that positively charged LF can combine with negatively charged whey proteins and caseins in milk through electrostatic attraction (Croguennec, Li, Phelebon, Garnier-Lambrouin, & Gésan-Guiziou, 2012). After heating, other forces, such as disulphide bond and hydrophobic interaction, contributed to the complexation between sodium caseinate (NaCas) and LF, resulting in the formation of soluble NaCas/LF complexes (Li & Zhao, 2017). It is therefore hypothesized that LF could complex with whey proteins and the formation of LF/whey protein complexes would influence heat-induced gelation behaviour of WPI.

The objective of this research was to study the heat-induced gelation behaviour of WPI in the presence of different amounts of LF. The gel formation process was monitored by measuring the changes of elastic modulus (G′) and loss modulus (tanδ). Water holding capacity and rheological properties were determined to characterize the structure of gels. Changes of zeta potential and hydrodynamic size were used to illustrate the complexation behaviour between LF and whey proteins.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) which has 90.5% protein, 1.4% ash, 0.8% fat and 4.8% moisture on a weight/weight basis, was purchased from Gallo Global Nutrition (Atwater, Canada). Native bovine LF with an iron saturation level of 10–20% (> 95% purity; isolated from cow milk) was purchased from Shanghai Yuanye Biotechnology Ltd. (Shanghai, China).

2.2. Sample preparation

Protein solutions with a fixed concentration of 10% (w/w) were prepared by dispersing protein powders in distilled water and stirring for 2 h at room temperature (22 °C). The final protein solutions contained different LF concentrations: 0%, 5%, 10%, 20%, and 30% (w/w). All prepared solutions were stored in refrigerator (4 °C) overnight to ensure complete hydration. After that, all samples were equilibrated at room temperature for at least 2 h prior to adjusting the pH to 5.8 and 6.7 with 1.00 mol L^{-1} HCl and NaOH.

2.3. Low amplitude dynamic oscillatory measurements

Dynamic oscillatory measurements (1 Hz, strain amplitude 1%) were used to monitor the gelation process by a controlled stress rheometer (Paar Physica MC 301, Anton Paar, Graz, Austria) equipped with a peltier temperature controller and concentric cylinder geometry. Aliquots of 17 mL samples were pipetted to the cylinder at 20 °C, allowed to equilibrate for 2 min, heated to 80 °C at 5 K/min, held at 80 °C for 20 min, cooled to 20 °C at 5 K/min, and then held at 20 °C for 20 min. Changes of elastic modulus (G′) and viscous modulus (G″) were monitored and the loss modulus (tanδ) was defined as the ratio of G″ to G′. Gelation time was determined as the time when tanδ = 1 (Zhao & Corredig, 2016).

2.4. Frequency sweep

After gel preparation, a frequency test was performed in the frequency range of 0.01–100 Hz at the constant strain of 1% and temperature of 25 °C. All measurements were performed in triplicate and the changes of G′ and G″ were determined.

2.5. Water holding capacity

Water holding capacity (WHC) was determined according to previous publication with slight modification (Yang et al., 2014). To prepare the gel, aliquots of 15 mL protein solutions were transferred to 20 mL Pyrex test tubes and capped. Subsequently, all samples were heated in water bath at 80 °C for 20 min and cooled to room temperature with running tap water. Then about 10 g of each gel was centrifuged at 6000*g* for 20 min. The water phase on the top was removed carefully and the weight of the remaining gel was determined. WHC was expressed as follows:

$$WHC(\%) = (m1/m2) \times 100$$

where m_1 is the weight of precipitate after centrifugation and m_2 is the weight of the gel used

2.6. Particle size measurements

To determine the apparent hydrodynamic size, protein solutions prepared as described in Section 2.1 were diluted 20 times using distilled water with pre-adjusted pH (5.8 or 6.7). The diluted solutions were then heated at 80 °C for 20 min in a water bath and cooled immediately to room temperature with running tap water. The hydrodynamic size of samples both before and after heating was determined at 25 °C using dynamic light scattering (Zetasizer Nano, Malvern Instruments, Worcestershire, UK). Radius values were reported as intensity-based average size using cumulants analysis. A backscattering mode (detection angle = 173°) was adopted and the particle size distribution was expressed on the basis of volume frequency.

2.7. Zeta potential measurements

The value of zeta potential represents the net charge on the surface of a particle, depending on not only the charge on the particles, but also the charge carried by any associated ions that move along with the particles in an electric field (Surh, Decker, & McClements, 2006). In this research, zeta potential was determined by a laser Doppler electrophoresis using the Nano-S Zetasizer with the DTS1060 capillary cell. Diluted samples (20 ×) both before and after heating (80 °C, 20 min), as described in Section 2.6, were used for the measurement. The Smoluchowski model was performed to calculate zeta potential from the mobility values. Samples were determined 200 times and the results were expressed in absolute values (mV).

2.8. Statistical analysis

At least three replicates were performed for each measurement. ANOVA and Turkey HSD were conducted (95% confidence level) to analyze the data using Minitab statistical package release 15 (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Heat-induced gelation profiles of WPI/LF mixtures

Fig. 1 illustrates the heat-induced gelation profiles of WPI/LF mixtures at pH 6.7 (A and C) and 5.8 (B and D). In all cases, elastic modulus (G′) increased slightly during the heating process, followed by a rapid increase during cooling from 80 °C to 20 °C, and then stabilized at 20 °C