Food Chemistry 252 (2018) 92–98

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

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

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ARTICLE INFO

Keywords:
Lactoferrin
Whey protein isolate
Gelation
Elastic modulus
Zeta potential
Size distribution

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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https://doi.org/10.1016/j.foodchem.2018.01.114
Received 5 September 2017; Received in revised form 12 January 2018; Accepted 16 January 2018

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

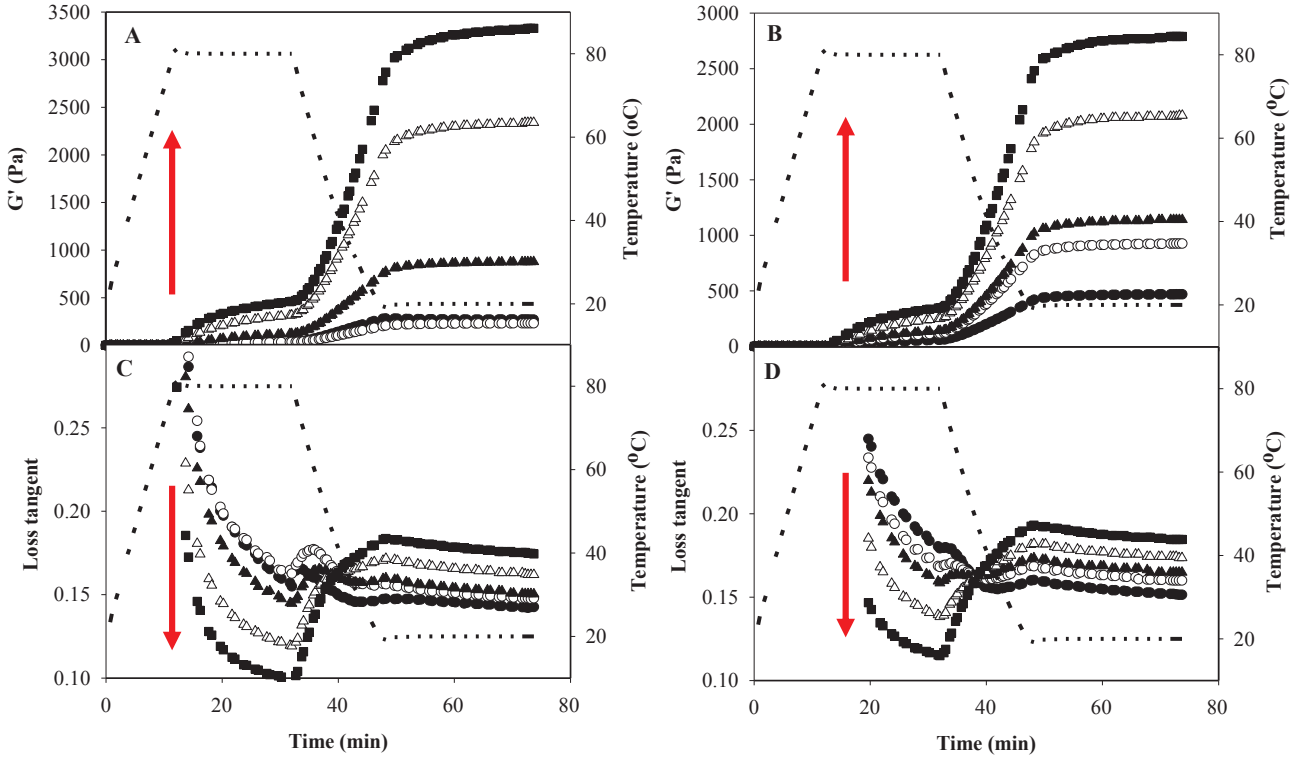

Fig. 1. Changes of G′ (A, B) and loss tangent values (C, D) of samples containing 0% (filled circles), 5% (empty circles), 10% (filled triangles), 20% (empty triangles), and 30% (filled rectangles) w/w LF, during temperature cycling in the range of 20–80 °C at pH 6.7 (A, C) and 5.8 (B, D). Dashed lines indicate temperature profile. Red arrows indicate the increase of LF concenration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

during storage. These typical gelation profiles for WPI samples have been published in previous researches (Tarhan et al., 2016; Yamniuk et al., 2009). Addition of low concentration of LF (< 10%) slightly decreased the development of gel during heating at pH 6.7 (A), while at pH 5.8 (B) the gel development increased significantly. At LF concentrations higher than 10%, the gel formation process was promoted significantly at both pH and this effect increased with the increasing of LF concentration. However, no significant difference in the gelation time (where tan δ = 1) was detected among different samples which all gelled at around 76 ± 3 °C, indicating that the structure of gel was dominated by whey proteins. It is important to note that LF molecules were also denatured at 80 °C, resulting in the exposure of buried residues and thus the increased interaction between LF and whey proteins (Li & Zhao, 2017). Therefore, LF molecules most probably played a role of "filler" and bridged the denatured whey proteins, which resulted in the faster increase of elastic modulus after gelation point.

In contrast to elastic modulus, the loss tangent decreased initially to a minimum for all samples. The minimum values decreased with the increasing of LF concentration, indicating the synergistic effect between LF and WPI, which was due to increased interaction between them (Havea et al., 2009). Our preliminary test indicated that LF could not form a gel structure at concentration lower than 6% (w/w), therefore, the results indicated stronger molecular interaction happened at higher concentration of LF. During the cooling stage (80 °C to 20 °C), the loss tangent increased to plateau values at around 60 min, which illustrated that the gels were more "liquid-like" probably due to the reorganization of gel. These findings have not been published before.

To better characterize the effect of LF on the structure of WPI, the final elastic modulus and loss tangent were summarized in Table 1. As shown, WPI gels formed at pH 5.8 had higher elastic modulus and lower loss tangents than those formed at pH 6.7, which is in agreement with previous researches (Bertrand & Turgeon, 2007; Stading, Langton, & Hermanson, 1993). The above pH effect can be ascribed to the changes of balance between protein-protein and protein-solvent interactions. At pH 5.8 which is close to the isoelectric point of WPI (pH 5.3), there is an increased protein-protein interaction, while the protein-solvent interaction increased at pH 6.7 (Stading et al., 1993). The optimal balance between protein-protein and protein-solvent interactions for WPI was shown at pH 5.5 (McGuffey & Foegeding, 2007). At the lowest LF concentration of 5%, no significant change of the gel strength was found at pH 6.7, while at pH 5.8 the elastic modulus increased from 413 ± 58 to 833 ± 106 Pa. When the LF concentration further increased from 10% to 30%, the elastic modulus increased rapidly from 824 ± 62 and 1217 ± 116 Pa to 3222 ± 105 and 2730 ± 131 Pa for pH 6.7 and pH 5.8, respectively. It needs to be noted that when LF concentration is higher than 20%, the gels formed at pH 6.7 became stronger (higher elastic modulus) than those formed at pH 5.8 (Table 1). Moreover, gels formed at pH 5.8 had higher loss tangent than those at pH 6.7, indicating that the gels formed at lower pH had higher flexibility. All those results indicated that the interaction between LF and WP were different at pH 5.8 and 6.7.

3.2. Frequency sweep

Since frequency dependency of the gel is determined by the interaction between denatured protein molecules, frequency sweep results could provide valuable information of the gels' structural properties (Stading & Hermansson, 1990). The frequency sweep results of samples with different LF concentrations of 0% (A, D), 5% (B, E) and 30% (C, F) are summarized in Fig. 2. It has been found that gels formed by non-covalent bonds are frequency dependent, while those formed by covalent bonds are elastic and frequency independent (Doucet, Gauthier, Otter, & Foegeding, 2001). In our study, all samples showed obvious dependence on the frequency, indicating the gels depended more on non-covalent hydrophobic and electrostatic interactions than disulphide bond (Creusot & Gruppen, 2007; Tarhan et al., 2016).

The elasticity of the gel at pH 6.7 was reduced in the presence of 5% LF, as shown in Fig. 2B. The elastic modulus only increased slightly

Table 1
Final elastic modulus and loss tangents of WPI gels in the presence of different concentrations of LF.

LF concentration (%)	Final elastic modulus (Pa)		Loss tangents	
	pH 6.7	pH 5.8	pH 6.7	pH 5.8
0	254 ± 31[a]	413 ± 58[a]	0.142 ± 0.001[a]	0.152 ± 0.001[a]
5	227 ± 31[a]	833 ± 106[b]	0.147 ± 0.00[b]	0.161 ± 0.001[b]
10	824 ± 62[b]	1217 ± 116[c]	0.152 ± 0.001[c]	0.165 ± 0.002[c]
20	2162 ± 191[c]	1993 ± 186[d]	0.163 ± 0.000[d]	0.174 ± 0.001[d]
30	3222 ± 105[d]	2730 ± 131[e]	0.175 ± 0.000[e]	0.185 ± 0.001[e]

Values are means of three measurements, ± standard deviation. Values in the same column with different superscript letter are significantly different ($p < .05$).

from 0 to 10 Hz. Further increase of frequency led to rapid decrease of the elastic modulus, and a crossover of loss modulus (G″) over elastic modulus (G′) was observed at 42 Hz, indicating a more liquid-like structure. In contrast, the gel at pH 5.8 increased with the increasing of frequency and no disruption was observed (Fig. 2E). These different behaviours were probably due to different complexation mechanisms between LF and whey proteins at different pH. At pH 6.7, which is closer to the pI of LF (8.9), LF molecules are less charged compared to pH 5.8 (Li & Zhao, 2017). Therefore, the interaction between whey proteins and LF is weak and the presence of low concentration of LF (5%) inhibited the formation of continuous gel structure. At pH 5.8, the electrostatic attraction between LF and whey proteins is stronger, which promoted the development of gel structure. At the highest LF concentration of 30%, gels at pH 5.8 and 6.7 both exhibited strong elasticity, which were due to the presence of high amount of molecular bonds between LF and whey proteins (Fig. 2C and F). Both elastic modulus and loss modulus increased with the increasing of frequency, indicating the increased hydrophobic interaction and more homogeneous structure in the presence of sufficient LF.

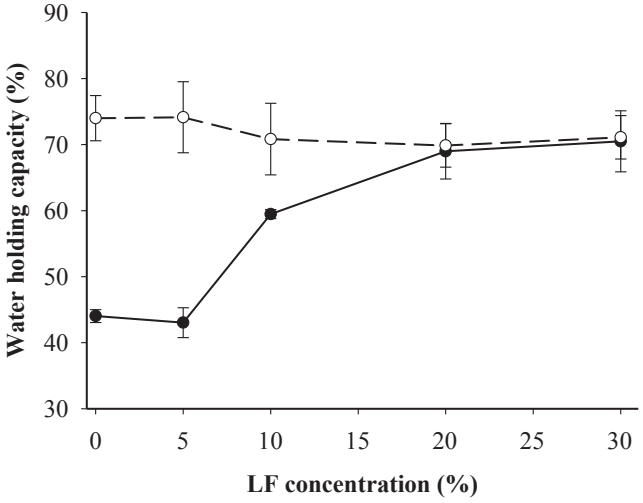

Fig. 3. Water holding capacity of gels with different concentrations of LF at pH 6.7 (filled circles) and 5.8 (empty circles).

3.3. Water holding capacity

The WHC is related to the structure of the gel, and that with a fine-stranded structure usually has better water holding capacity (Chantrapornchai & McClements, 2002). As shown in Fig. 3, The WPI gel control had a WHC value of 44 ± 1% at pH 6.7, while at pH 5.8 a much higher value of 74 ± 3% was determined. The higher WHC value at lower pH can be ascribed to the decreased electrostatic repulsion and increased hydrophobic interaction (Tet Teo, Munro, Singh, & Hudson, 1996). At pH 6.7, addition of 5% LF has no significant effect on the WHC (44 ± 1% to 43 ± 2%, $p > .05$), while further increase of the LF concentration from 10% to 30% significantly increased the WHC from 59 ± 1% to 70 ± 5% ($p < .05$). The increased WHC was obviously a result of the increased interaction between LF and whey protein molecules during heating. Moreover, the increased WHC could

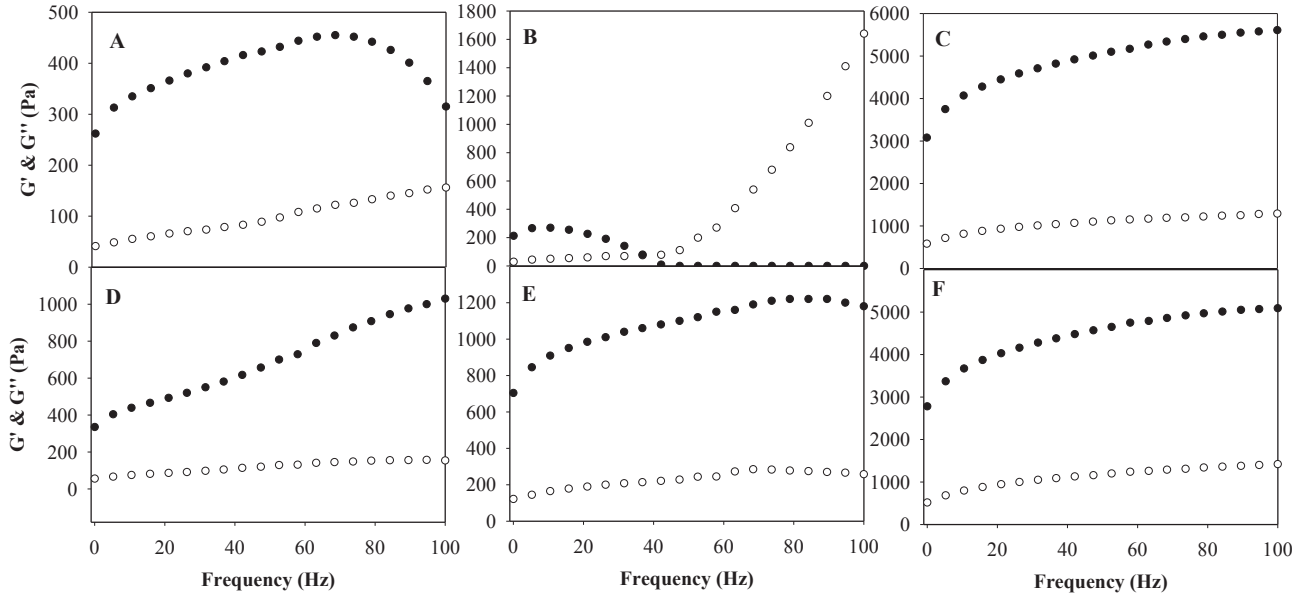

Fig. 2. Changes of G′ (filled circles) and G″ (empty circles) values during frequency sweep for gels containing 0% (A, D), 5% (B, E) and 30% (C, F) w/w LF at pH 6.7 (A-C) and pH 5.8 (D-F).

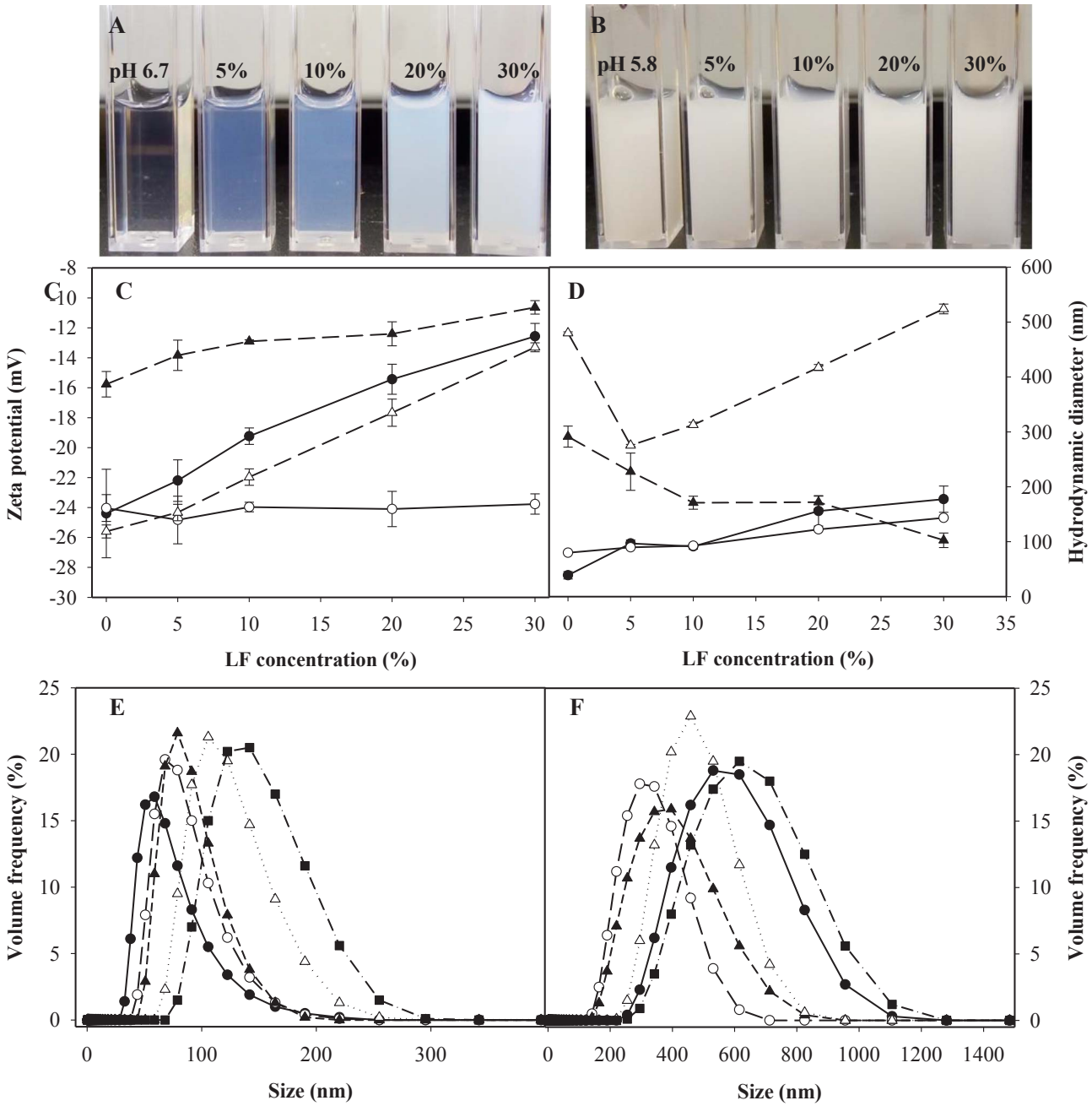

Fig. 4. Changes of optical appearance (A, B), zeta potential (C), hydrodynamic radius (D) for samples before (filled) and after (empty) heating at pH 6.7 (circles) and 5.8 (triangles), and the size distributions for samples containing 0% (filled circles), 5% (empty circles), 10% (filled triangles), 20% (empty triangles) and 30% (filled rectangles) w/w LF after heating at pH 6.7 (E) and 5.8 (F).

be used to explain the increased flexibility of the gel structure (Table 1). In contrast, at pH 5.8 the interaction between LF and whey proteins had no influence on the WHC which maintained at a value of 72 ± 3%. At higher concentrations of the LF (20% and 30%), no difference in the WHC was detected between pH 5.8 and 6.7. The results from this part further confirmed that the interactions between LF and whey proteins at pH 6.7 and 5.8 were different. Therefore, further characterization of their interactions is essential.

3.4. Optical appearance, zeta potential and hydrodynamic size

Fig. 4A and B show the optical appearance of different samples. The diluted samples are all transparent at both pH 5.8 and 6.7 before heating (photo not shown). After heating the WPI solution at pH 6.7 remained transparent (Fig. 4A), indicating the development of turbidity is mainly from the denaturation of LF. At LF concentrations of 5% and 10%, very low turbidity was observed. The solution became more turbid with the further increasing of LF concentration, indicating the increased denaturation of LF during heating. In contrast, at pH 5.8, all samples were turbid after heating (Fig. 4B).

Fig. 4C and D summarized the changes of zeta potential and hydrodynamic diameters as a function of LF concentration and heating. The WPI solutions before heating had an average zeta potential of −24 ± 3 and −16 ± 1 mV at pH 6.7 and 5.8, respectively, which is in agreement with previous research (Zhao & Xiao, 2017). Since LF is positively charged (pI = 8.9), the addition of LF to WPI solutions slightly decreased the zeta potential due to the effect of charge screening. When the LF concentration increased to 30%, the zeta

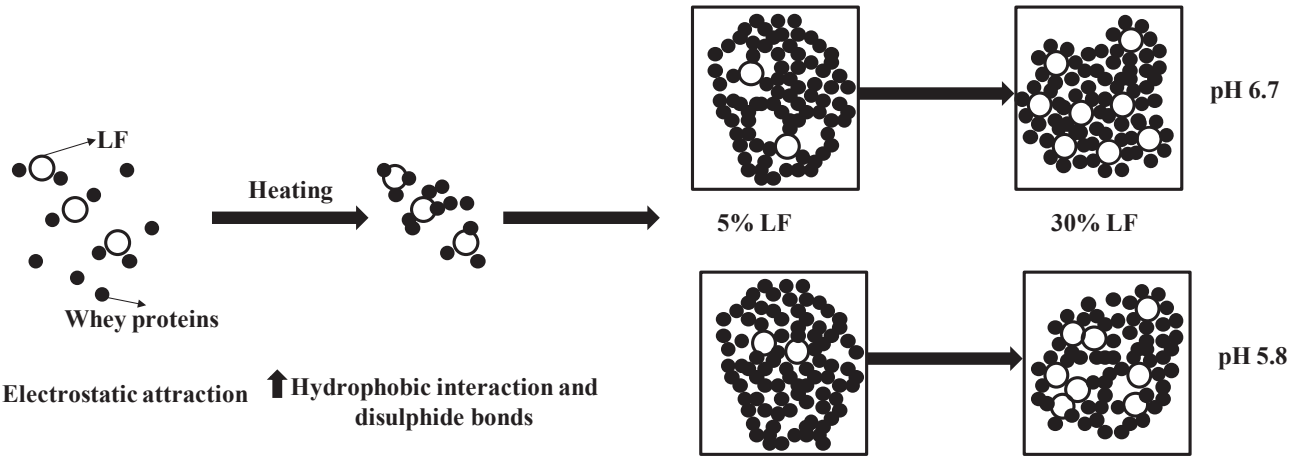

Fig. 5. A schematic representation of the heat gelation behaviour of WPI/LF mixtures at pH 6.7 and 5.8.

potential decreased to −13 ± 1 and −11 ± 1 mV at pH 6.7 and 5.8, respectively. After heating, a similar zeta potential value of −24 ± 1 mV was detected for all the samples at pH 6.7, which indicated that the LF molecules were surrounded by the denatured whey protein molecules. The denatured whey proteins could protect LF against the heat-induced denaturation and aggregation, thus resulting in the low turbidity after heating (Fig. 4A). The similar protection effect between NaCas and LF was also observed in our previous research (Li & Zhao, 2017). Therefore, less hydrophobic residues were available to form continuous gel structure, which led to the decreased elasticity of the gel (Table 1). In comparison, at pH 5.8 the zeta potentials increased after heating, probably due to the exposure of charged groups (Li & Zhao, 2017). Moreover, the zeta potential decreased in a faster speed with the increasing of LF concentration, indicating the enhanced interaction between LF and whey proteins.

Fig. 4D illustrates the hydrodynamic diameters before and after heating. Whey proteins had an average size of 39 ± 7 nm at pH 6.7. The addition of 5% LF immediately increased the size to 96 ± 7 nm, which was further increased to 156 ± 27 nm when 30% LF was added. Heating at 80 °C for 20 min resulted in the denaturation and aggregation of whey proteins, thus the average size increased to 80 ± 1 nm. In the presence of low concentrations of LF (5% and 10%), the average sizes were not influenced by heating, indicating the formation of stable complexes. Similar results have been observed when NaCas was mixed with LF at NaCas/LF ratios higher than 1:1 (Li & Zhao, 2017). At higher concentrations of LF (20% and 30%), the sizes decreased slightly after heating. At pH 5.8, WPI solution had a significantly higher size value of 291 ± 19 nm than pH 6.7. Nonetheless, the size decreased significantly with the increasing of LF concentration due to the increased electrostatic attractions. A much lower value of 102 ± 13 nm was obtained when 30% LF was added. After heating, the average size of the samples all increased. WPI control had an average size of 480 ± 2 nm, which dropped to a minimum value of 276 ± 2 nm in the presence of 5% LF. Further increase of the LF concentration gradually increased the average size. It is important to note that the native LF is also heat susceptible; the increased size after heating demonstrated the enhanced complexation between LF and whey proteins as a result of the increased disulphide bonds and hydrophobic interactions (Brisson, Britten, & Pouliot, 2007; Li & Zhao, 2017).

To better characterize the interaction between LF and whey proteins, the size distributions of different samples after heating are summarized in Fig. 4E and F. All samples had a unimodal size distribution. At pH 6.7, the WPI had a very narrow size distribution and the peak was shifted slightly to the larger size direction with the increasing of LF concentration. At pH 5.8, the WPI had a very wide size distribution and the peak was shifted to the smaller size direction when 5% LF was added. Further increase of LF concentration, the peak was gradually shifted to the larger size direction. All those changes are in accordance with the changes of average hydrodynamic size (Fig. 4B).

A possible gelation mechanism for the WPI/LF mixtures was proposed (Fig. 5). The interaction between LF and whey proteins was mainly through electrostatic attraction before heating. After heating, their interaction was greatly enhanced by the hydrophobic interaction and disulphide bond which significantly influenced the gelation process of whey proteins. At pH 6.7, in the presence of lowest LF (5%), the exposure of internal residues of LF was limited and the interaction between LF and denatured whey proteins was weak. Therefore, LF inhibited the continuity of whey protein gel. With the increasing of LF concentration, the denaturation of LF increased and complexation between LF and whey proteins enhanced. Whey protein aggregates were linked by denatured LF molecules homogeneously, resulting in the increased gel strength. In contrast, at pH 5.8 the electrostatic attraction and hydrophobic interaction between LF and whey proteins were stronger, thus the presence of LF even at the lowest concentration of 5%, significantly increased the gel strength. However, the formed LF/whey proteins complexes had a heterogeneous structure, which resulted in the lower gel strength compared to pH 6.7 at high concentrations (> 20%).

4. Conclusion

The aforementioned results clearly demonstrated that the incorporation of LF into WPI gel can change its structural properties, with these changes depending on pH and structural properties of LF. The denatured LF molecules could crosslink whey proteins through disulphide bonds and hydrophobic interactions. WPI gel obtained at pH 5.8 had higher elastic modulus and water holding capacity than that at pH 6.7. The addition of LF increased the gel strength at both pH values. However, stronger and more uniform gels were obtained at pH 6.7 compared to pH 5.8 in the presence of high concentrations of LF (20% and 30%). The findings from this research are of both practical and theoretical significance for the utilization of LF in gelled protein products.

Conflict of interests

All authors certify that there is no conflict of interest in this study.

Acknowledgements

This research was supported by the National Science Foundation of China (No. 31071516) and the talents program of Guangxi University (No.10001423).

References

Alting, A. C., Hamer, R. J., De Kruif, C. G., & Visschers, R. W. (2000). Formation of disulfide bonds in acid-induced gels of preheated whey protein isolate. *Journal of Agricultural and Food Chemistry, 48*, 5001–5007.

Bertrand, M., & Turgeon, S. L. (2007). Improved gelling properties of whey protein isolate by addition of xanthan gum. *Food Hydrocolloids, 21*, 159–166.

Brisson, G., Britten, M., & Pouliot, Y. (2007). Heat-induced aggregation of bovine lactoferrin at neutral pH: Effect of iron saturation. *International Dairy Journal, 17*, 617–624.

Chantrapornchai, W., & McClements, D. J. (2002). Influence of NaCl on optical properties, large-strain rheology and water holding capacity of heat-induced whey protein isolate gels. *Food Hydrocolloids, 16*, 467–476.

Creusot, N., & Gruppen, H. (2007). Enzyme-induced aggregation and gelation of protein. *Biotechnology Advances, 25*, 597–601.

Croguennec, T., Li, N., Phelebon, L., Garnier-Lambrouin, F., & Gésan-Guiziou, G. (2012). Interaction between lactoferrin and casein micelles in skimmed milk. *International Dairy Journal, 27*, 34–39.

Donato, L., & Guyomarc', h. F. (2009). Formation and properties of the whey protein/κ-casein complexes in heated skim milk – A review. *Journal of Dairy Science and Technology, 89*, 3–29.

Doucet, D., Gauthier, S., Otter, D., & Foegeding, E. (2001). Rheological characterization of a gel formed during extensive enzymatic hydrolysis. *Journal of Food Science, 66*, 711–715.

Foegeding, E., Davis, J., Doucet, D., & McGuffey, M. (2002). Advances in modifying and understanding whey protein functionality. *Trends in Food Science and Technology, 13*, 151–159.

Havea, P., Watkinson, P., & Kuhn-Sherlock, B. (2009). Heat-induced whey protein gels: Protein-protein interactions and functional properties. *Journal of Agricultural and Food Chemistry, 57*, 1506–1512.

Li, Q., & Zhao, Z. (2017). Formation of lactoferrin/sodium caseinate complexes and their adsorption behaviour at the air/water interface. *Food Chemistry, 232*, 697–703.

McGuffey, M., & Foegeding, A. (2007). Electrostatic effects on physical properties of particulate whey protein isolate gels. *Journal of Texture Studies, 32*, 285–305.

Nguyen, B. T., Chassenieux, C., Nicolai, T., & Schmitt, C. (2017). Effect of the pH and NaCl on the microstructure and rheology of mixtures of whey protein isolate and casein micelles upon heating. *Food Hydrocolloids, 70*, 114–122.

Nguyen, B. T., Nicolai, T., Chassenieux, C., Schmitt, C., & Bovetto, L. (2016). Heat-induced gelation of mixtures of whey protein isolate and sodium caseinate between pH 5.8 and pH 6.6. *Food Hydrocolloids, 61*, 433–441.

Perssin, K., & Gekas, V. (2000). Factors influencing aggregation of macromolecules in solution. *Process Biochemistry, 48*, 3555–3559.

Ren, F., Dong, D., Yu, B., Hou, Z., & Cui, B. (2017). Rheology, thermal properties, and microstructure of heat-induced gel of whey protein–acetylated potato starch. *Starch, 69*, 1600344.

Roesch, R., & Corredig, M. (2005). Heat-induced soy-whey proteins interactions: Formation of soluble and insoluble protein complexes. *Journal of Agricultural and Food Chemistry, 53*, 3476–3482.

Shimada, K., & Cheftel, J. (1989). Sulfhydryl group/disulphide bond interchange reactions during heat-induced gelation of whey protein isolate. *Journal of Agricultural and Food Chemistry, 37*, 161–168.

Stading, M., & Hermansson, A. (1990). Viscoelastic behaviour of β-lactoglobulin gel structures. *Food Hydrocolloids, 4*, 121–135.

Stading, M., Langton, M., & Hermanson, A. (1993). Microstructure and rheological behaviour of particulated β-lactoglobulin gels. *Food Hydrocolloids, 7*, 195–212.

Sun, W., Yu, S., Yang, X., Wang, J., Zhang, J., Zhang, Y., & Zheng, E. (2011). Study on the rheological properties of heat-induced whey protein isolate–dextran conjugate gel. *Food Research International, 44*, 3259–3263.

Surh, J., Decker, E. A., & McClements, D. J. (2006). Influence of pH and pectin type on properties and stability of sodium-caseinate stabilized oil-in-water emulsions. *Food Hydrocolloids, 20*, 607–618.

Tarhan, O., Spotti, J. M., Schaffter, S., Corvalan, C. M., & Campanella, O. H. (2016). Rheological and structural characterization of whey protein gelation induced by enzymatic hydrolysis. *Food Hydrocolloids, 61*, 211–220.

Tavares, C., & da Silva, J. A. L. (2003). Rheology of galactomannan-whey protein mixed system. *International Diary Journal, 13*, 699–706.

Tet Teo, C., Munro, P. A., Singh, H., & Hudson, R. C. (1996). Effects of pH and temperature on the water-holding capacity of casein curds and whey protein gels. *Journal of Dairy Research, 63*, 83–95.

Tobin, J. T., Fitzsimons, S. M., Chaurin, V., Kelly, A. L., & Fenelon, M. A. (2012). Thermodynamic incompatibility between denatured whey protein and konjac glucomannan. *Food Hydrocolloids, 27*, 201–207.

Tomita, M., Wakabayashi, H., Shin, K., Yamauchi, K., Yaeshima, T., & Iwatsuki, K. (2009). Twenty-five years of research on bovine lactoferrin applications. *Biochimie, 91*, 52–57.

Ward, P. P., Paz, E., & Conneely, O. M. (2005). Multifunctional roles of lactoferrin: A critical overview. *Cellular and Molecular Life Science, 62*, 2540–2548.

Yamniuk, A. P., Burling, H., & Vogel, H. J. (2009). Thermodynamic characterization of the interactions between the immunoregulatory proteins osteopontin and lactoferrin. *Molecular Immunology, 46*, 2395–2402.

Yang, T., Wu, K., Wang, F., Liang, X., Liu, Q., Li, G., & Li, Q. (2014). Effect of exopolysaccharides from lactic acid bacteria on the texture and microstructure of buffalo yoghurt. *International Dairy Journal, 34*, 252–256.

Yoshida, S., Wei, Z., Shinmura, Y., & Fukunaga, N. (2000). Separation of lactoferrin-a and -b from bovine colostrum. *Journal of Dairy Science, 83*, 2211–2215.

Zhao, Z., & Corredig, M. (2016). Influence of sodium chloride on the colloidal and rennet coagulation properties of concentrated casein micelle suspensions. *Journal of Dairy Science, 99*, 6036–6045.

Zhao, Z., & Xiao, Q. (2017). Effect of chitosan on the heat stability of whey protein solution as a function of pH. *Journal of the Science of Food and Agriculture, 97*, 1576–1581.

Zhu, H., & Damodaran, S. (1994). Heat-induced conformational changes in whey protein isolate and its relation to foaming properties. *Journal of Agricultural and Food Chemistry, 42*, 846–855.

Zhu, D., Damodaran, S., & Lucey, J. A. (2010). Physicochemical and emulsifying properties of whey protein isolate (wpi)-dextran conjugates produced in aqueous solution. *Journal of Agricultural and Food Chemistry, 58*, 2988–2994.