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Milk protein–gum tragacanth mixed gels: Effect of heat-treatment sequence

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ABSTRACT

The aim of this study was to investigate the role of the heat-treatment sequence of biopolymer mixtures as a formulation parameter on the acid-induced gelation of tri-polymeric systems composed of sodium caseinate (Na-caseinate), whey protein concentrate (WPC), and gum tragacanth (GT). This was studied by applying four sequences of heat treatment: (A) co-heating all three biopolymers; (B) heating the milk-protein dispersion and the GT dispersion separately; (C) heating the dispersion containing Na-caseinate and GT together and heating whey protein alone; and (D) co-heating whey protein with GT and heating Na-caseinate alone. According to small-deformation rheological measurements, the strength of the mixed-gel network decreased in the order: C > B > D > A samples. SEM micrographs show that the network of sample C is much more homogenous, coarse and dense than sample A, while the networks of samples B and D are of intermediate density. The heat-treatment sequence of the biopolymer mixtures as a formulation parameter thus offers an opportunity to control the microstructure and rheological properties of mixed gels.

1. Introduction

Both protein and polysaccharide polymers either are naturally present or added as ingredients in many manufactured products (Doublier, Garnier, Renard, & Sanchez, 2000). Thus, a mixed system containing these hydrocolloids is an active area of research. As well as the academic interest in the mechanisms of interaction, there are practical and commercial reasons for the study of such systems. In the food area there are at least two main factors driving such studies. Firstly, there is consumer pressure to reduce the level of 'additives' present in food. Secondly, there is a desire to develop 'low fat' structured products. Both problems can be addressed by exploiting mixtures of polysaccharide-protein to generate novel-textured foods (Morris & Wilde, 1997). Also knowledge of the protein–polysaccharide interaction is a valuable tool in the design of, cosmetics, and medicine. A mixed system in such products is used based on biopolymers' ability to form gel (Tolstoguzov, 1991).

The majority of milk protein-based gels depend on two characteristics of the casein and whey protein system: (1) the insolubility of casein in the region of its isoelectric point (pI = 4.6), and (2) denaturation of whey protein by heating to or above its gelling temperature generally around 80 °C for between 10 and 60 min (Harris, 1990). The overall interaction between these proteins is the sum of the different intermolecular forces arising between the various segments and chains of the two biopolymers (Goh, Sarkar, & Singh, 2008). When whey proteins and casein micelles are heated together, disulphide bonds can be formed between the whey-protein molecules that contribute to their aggregation process; and also between whey-protein and casein molecules that result in K-casein/β-lactoglobulin complex formation on the casein particles' surface. On lowering the pH, the surface charge, and consequently the repulsion forces of the casein molecules are reduced, allowing coagulation due to van der Waals' attractions. The interactions responsible for gel structure can be classified as either strong, permanent covalent bonds formed during the aggregation of protein particles (thiol/disulphide interchange) or weak, reversible non-covalent interactions that occur between the particles before the permanent bonds (Tamime & Robinson, 1999). The nature and amount of the bonds are presumably affected by the presence of polysaccharides, which alter the structure and stabilization of the protein gels.

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The polyelectrolyte character of milk proteins and ionic polysaccharides means that electrostatic interactions play an important role in determining mixed-biopolymer behavior (Dickinson, 1998). At pH values above the isoelectric point (pI), protein molecules have a net negative charge and behave as polyanions. At ranges between the neutral pH and the isoelectric point of milk proteins, an anionic polysaccharide and a protein carry same type of net charge. As a result, proteins and polysaccharides repel each other, leading to phase separation (McClements, 2006). But electrostatic interactions between anionic polysaccharides and positive charged subunits of oligomeric proteins can still occur (Ye, 2008). Thus, the preferential interaction between two proteins of milk and polysaccharide is affected by controlling internal (pH, ionic strength, biopolymer ratio and charge of the biopolymer) and external (pre-heat treatment, heat-treatment sequence of the biopolymer mixture, gelation temperature, and shear rate) factors (Hatami, Nejatian, & Mohammadifar, 2012). The effects of anionic polysaccharides on milk-protein gelation and mixed-gel properties have been previously investigated for both the internal and external factors (de Jong, Klok, & van de Velde, 2009; Matia-Merino, Lau, & Dickinson, 2004; Picone & da Cunha, 2010; Sanchez, Zuniga-Lopez, Schmitt, Despond, & Hardy, 2000).

While the effect of the heat-treatment sequence of casein–whey protein mixtures on rheological and microstructural properties of acid milk protein gels alone have been studied (Schorsch, Wilkins, Jones, & Norton, 2001) the effect of the heat-treatment sequence on the development of the mixed-gel network structure has not been systematically considered. The aim of the present work was to obtain further understanding of the effect of a formulation parameter (heat-treatment sequence) as an external variable on the properties of mixed-biopolymer gels. The study examined the possible preferential interactions between protein and polysaccharide molecules under different heat-treatment sequences in aqueous dispersion. Three biopolymers including caseinate (Na-caseinate), whey protein concentrate (WPC) and gum tragacanth (GT) were selected. GT, a dried exudate obtained from the stems and branches of Asiatic species of *Astragalus*, is a very complex heterogeneous anionic polysaccharide of high molecular weight and consists of two main fractions: a water-insoluble component called bassorin, which has the capacity to swell and form a gel, and a water-soluble component called tragacanthin (Balaghi, Mohammadifar, & Zargaraan, 2010). It consists of a linear 1,4 linked α-D-galacturonic acid backbone with three types of side chains: single β-D-xylopyranose and disaccharide units of 2-O-α-L-fucopyranosyl-D-xylopyranose and 2-O-β-D-galactopyranosyl-D xylopyranose (Yokoyama, Srinivasan, & Fogler, 1988). In particular, the study compared tri-polymeric systems containing (Na-caseinate), (WPC) and GT formulated using different heat-treatment sequences of three biopolymer mixtures, acidified to pH 4.6 by adding glucono-δ-lactone (GDL). For this purpose a combination of techniques, including small-amplitude oscillatory rheology and microscopy, was used.

2. Materials and methods

2.1. Materials

Iranian GT exuded by the species *Astragalus gossypinus* was collected from plants growing in Iran's Isfahan Province. Characteristics of this polysaccharide and nature of physicochemical properties were pointed out in our previous work (Balaghi, Mohammadifar, Zargaraan, Ahmadi Gavlighi, & Mohammadi, 2011). The raw gum was grounded and sieved. Powdered gum with mesh size between 200 and 500 μm was used in this study. WPC was supplied by DMV International (Veghel, Netherlands) and had the following ion composition (%, w/w): K^+ 0.46, Na^+ 0.38 and Ca^{2+} 0.41. Na-caseinate (6.25% moisture content, 86.29% total protein content) and glucono-δ-lactone (GDL) were purchased from Sigma Chemical Co (St. Louis, MO, USA). The ion content of the casein was (%, w/w): K^+ 0.08, Na^+ 0.16 and Ca^{2+} 0.14.

2.2. Preparation of dispersions and milk protein–polysaccharide mixtures

In order to prepare the samples, a stock solution of each biopolymer was prepared. The Na-caseinate solution (5.6%, w/w) was prepared by dispersing 5.6 g of protein powder in 94.4 g of distilled water under continuous stirring at 45 °C for 2 h. The 1.4% (w/w) WPC stock solution was prepared by dissolution of the powder in 98.6 g of distilled water. The 0.2% (w/w) GT stock solution was also prepared by dispersing the gum powder in distilled water at room temperature and stirring with a magnetic stirrer for 2 h. Binary Na-caseinate/WPC dispersion was obtained by dispersing the proteins powder in distilled water under continuous stirring at 45 °C for 2 h at a fixed concentration (5.6% Na-caseinate and 1.4% WPC w/w). Binary Na-caseinate/GT and WPC/GT dispersions were prepared in the same way containing (w/w) 5.6% Na-caseinate/0.2% GT and 1.4% WPC/0.2 GT, respectively. About 0.025% sodium azide was added to all dispersions to prevent bacterial growth. The dispersions were stored for 24 h at 5 °C to enable biopolymer hydration.

2.3. Heat treatment sequence of dispersions

Four types of experiments were carried out. The first consisted of the co-heating of all three biopolymers. Sample A was prepared by mixing one volume of the stock solutions of milk protein dispersions with one volume of GT in order to obtain the final concentration 2.8% Na-caseinate, 0.7% WPC (because the total protein concentration of milk is about 3.5%) and 0.1% GT. Sample A was co-heated for 30 min at 85 °C, then cooled in ice to 37 °C. In sample B, the milk-protein and GT dispersions were heated separately for 30 min at 85 °C; after cooling in ice, the polysaccharide dispersion was added to protein dispersion. In sample C, the whey protein was heated alone for 30 min at 85 °C; after cooling in ice, one volume of the whey protein solution was added to one volume of the stock dispersion containing Na-caseinate/GT (the final concentration 2.8% Na-caseinate, 0.7% WPC and 0.1% GT). In sample D, whey protein was co-heated with the GT (WPC/GT stock dispersion), and Na-caseinate was heated alone for 30 min at 85 °C; after cooling in ice to 37 °C, the two dispersions were mixed together, in order to obtain the final concentration 2.8% Na-caseinate, 0.7% WPC and 0.1% GT.

2.4. GDL-induced acidification

Glucono-δ-lactone (GDL) was used as a slow acidulant to give a final pH of 4.6. A constant GDL concentration of 0.55% (w/w) was added to each sample at 37 °C and the mixture was stirred for 5 min. Acidification of dispersions was carried out at 37 °C for 200 min. The reduction patterns of pH during the acidification mixtures were continuously monitored using a pH meter (Metrohm, 827 pH Lab, Switzerland) equipped with an electrode. The measurements were carried out after a two-point calibration at the incubation temperature.

2.5. Rheological properties

The rheological properties of the mixed gels during acidification were determined using low-amplitude dynamic oscillation. A Physica MCR 301 Rheometer (Anton-Paar, GmbH, Graz, Austria) and a

four-blade vane St14 geometry were used for all experiments. Following the addition of GDL, 37 mL of solution was transferred to the rheometer cup. A solvent trap was used to prevent evaporation of samples. Temperature was regulated by a Viscotherm VT2 circulating bath and controlled with a Peltier system (Anton Paar, GmbH) with an accuracy of ±0.01 °C. Rheological data were collected using Rheoplus software version 3.21 (Anton-Paar). The development of the storage modulus (G') and the loss modulus (G'') during gelation was recorded during 200 min at 37 °C at a frequency of 0.5 Hz and with an applied strain of 0.5%. After gelation a strain sweep test was performed at increasing strain from 0.01% to 1000% to verify the linear viscoelastic region, and to determine the limiting value of the linear viscoelastic range (LVE or γ_L); the resistance to mechanical force or yield stress (τ_y; which is also a measure of structure strength), calculated from limiting the value of LVE range in terms of shear stress; and the flow point (τ_f), the stress at which the internal structure breaks to the extent that it causes the material to flow. Frequency sweep tests were performed from 100 to 0.01 Hz at a deformation of 0.5%. The experimental data were fitted to a power-law model to determine structure strength (a) and type of structure (b) (Yoneya, Ishibashi, Hironaka, & Yamamoto, 2003).

2.6. Microscopy

Samples were prepared for scanning electron microscopy using the following protocol: 4 mm^2 blocks of gel were excised and rapidly frozen in liquid nitrogen at −196 °C. Freeze-fixed specimens were dried by sublimation of the frozen water in a vacuum at −90 to −85 °C (Christ, Alpha 2-4 LD Plus, Osterode, Germany) and then mounted on aluminum stubs and coated with gold in a sputter-coater (type SCD005, Baltec Inc., Balzers, Switzerland). Photomicrographs were recorded at magnification of 250, 500, and 1000× using a scanning electron microscope (Philips XL30, Eindhoven, Netherlands) operated at 17 kV.

2.7. Statistical analysis

The preparation of all dispersions and subsequent analysis on them were carried out in three replicates. Analysis of variance (ANOVA) was used for the data analysis (SPSS, 16). The treatment means comparison was performed with the Duncan test at $P < 0.05$.

3. Result

Time (t_{gel}) and pH (pH_{gel}) of gelation are arbitrarily defined as the time and pH of an abrupt increase in the elastic modulus from the baseline. This is judged apply when a modulus value becomes greater than the instrument noise level (ca. 1 Pa). The different heat-treatment sequences resulted in no significant change in t_{gel} (Table 1).

The pattern of pH reduction was not affected by the heat-treatment sequences, so pH_{gel} for all samples was the same (Table 1).

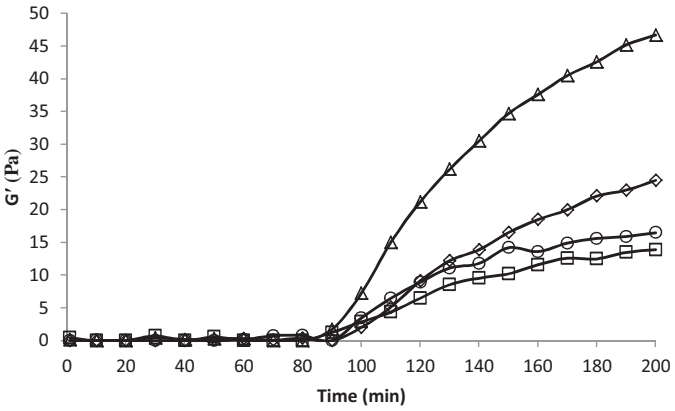

Fig. 1. Storage modulus, G', as a function of time for acid milk protein–GT mixtures under different heat-treatment sequences, sample A (□), sample B (◊), sample C (Δ) and sample D (○) as determined at 37 °C. (Frequency, 0.5 Hz; strain, 0.5%).

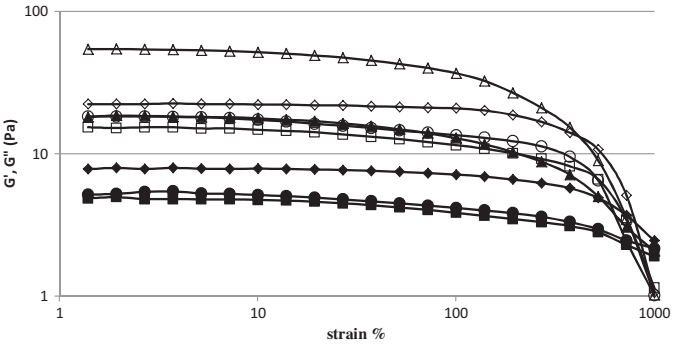

Fig. 2. Strain sweep at 1 Hz frequency modulus G' (open symbols) and G'' (filled symbols) for acid milk protein–GT mixtures under different heat-treatment sequences, sample A (□ and ■), sample B (◊ and ♦), sample C (Δ and ▲) and sample D (○ and •) as determined at 37 °C.

Fig. 1 shows the acid-gelation profiles for the milk protein–GT mixtures under different heat-treatment sequences.

The results show that co-heating the Na-caseinate–GT mixture at 85 °C for 30 min (sample C) imparts an increased final G' value to acid gels in comparison to gels made from other heat-treatment sequences. The G' value is in relation to the material's capacity to naturally retain shape, and is a measure of structural strength or firmness. Therefore the strength of the mixed-gel network decreased in the order: C > B > D > A samples. Table 1 also shows that the viscoelasticity of the C and A samples had the highest and least final loss tangent value respectively, while the B and D samples had the intermediate values. Thus the C sample has the highest viscous character contribution to the gel network's firmness of all the samples.

For all samples, G' was greater than G'' over the tested range of strain (Fig. 2).

Table 1
Gelation parameters of acid milk protein–gum tragacanth mixtures under different heat-treatment sequences as determined by time sweep measurements (frequency, 0.5 Hz; strain, 0.5%; 37 °C); values are means of triplicate samples (±SD).

Sequences	Gelation parameter			
	$Time_{gel}$ (min)	pH_{gel}	G' (Pa)	Tan δ_f
Sample A	91 ± 1a	5.15 ± 0.01a	14.00 ± 3.24a	0.30 ± 0.01b
Sample B	91 ± 1a	5.15 ± 0.01a	24.01 ± 2.27b	0.30 ± 0.01b
Sample C	89 ± 2a	5.16 ± 0.02a	46.41 ± 4.07c	0.35 ± 0.01c
Sample D	91 ± 1a	5.14 ± 0.01a	18.76 ± 1.95^{ab}	0.27 ± 0.01a

a–c means with different letters with in the same column differed significantly ($p < 0.05$).

Table 2
Limiting value of strain γ_L, yield stress at the limit of the LVE range τ_y, flow-point stress (τ_f), and flow-point stress with corresponding modulus G_f: $G' = G''$ of acid milk protein–gum tragacanth mixtures under different heat-treatment sequences as determined by strain sweep tests at 37 °C and a frequency of 1 Hz.

Sequences	Strain sweep parameters			
	γ_L (%)	τ_y (Pa)	τ_f (Pa)	G_f (Pa)
Sample A	32.33 ± 7.53a	3.83 ± 1.53a	17.50 ± 7.44a	1.57 ± 0.47a
Sample B	156.33 ± 6.03b	32.87 ± 2.15c	39.74 ± 1.69c	2.54 ± 0.49a
Sample C	30.10 ± 4.60a	14.57 ± 1.86b	32.00 ± 2.55b	2.74 ± 0.64a
Sample D	29.87 ± 3.45a	4.80 ± 0.86a	25.87 ± 2.55b	2.80 ± 1.16a

a–c means with different letters with in the same column differed significantly ($p < 0.05$).

This result is expected for solid viscoelastic systems. A significant increase in G' at LVE, which indicates improved structure strength, was observed in the co-heated Na-caseinate–GT mixture (sample C). The G'' modulus values of mixed-gel C were approximately equal to the G' of other mixed gels (A, B and D) in the LVE range (Fig. 2); this indicates that this sample has the highest viscous character. The limiting values of strain (γ_L), and τ obtained within the LVE range are presented in Table 2.

γ_L was the highest for sample B, which indicates longer LVE ranges, implying a higher stability of the viscoelastic material under the γ-amplitude. In addition, sample B had the highest τ_y value, which indicates that it requires higher mechanical forces to flow. In summary, the co-heated whey protein–caseinate mixture and the co-heated mixture containing all three biopolymers give the highest and lowest values respectively for strain sweep parameters.

Increasing the frequency increased both the G' and G'' values of all samples (Fig. 3), with the elastic component dominating over the viscous component throughout the entire frequency range examined.

The graph clearly shows G' and G'' values for the C sample are greater than those for other gels. A typical strong gel spectrum, also known as covalent gel, consists of two nearly horizontal straight lines, where G' is typically one to two orders of magnitude higher than G''. A biopolymer gel, however, usually shows a slight frequency dependence, with G' exceeding G'' at all frequencies; gels with this characteristic are called physical gels. Therefore, the mixed gels formed by different heat-treatment sequences are considered weak gels because their G' and G'' values showed a slight frequency dependence.

To determine the degree of the frequency dependence of the storage modulus, the power-law model ($G' = a\,\omega^b$) was fitted on the experimental data. Power-law parameters are given in Table 3.

Here, the coefficient a represents the magnitude of G' at a frequency of 1 Hz, and the exponent b represents the slope of G' versus the ω plot. The a-value and b-value are related to the strength and nature of the gel respectively. A b-value near zero means that G' does not change with frequency; for $b = 1$, the system behaves as a viscous gel. Low b values are characteristic of elastic gels. It is also known that $b = 0$ for a covalent gel, whereas for a physical gel $b > 0$ (Yoneya et al., 2003). As can be seen in Table 3, the C sample has the highest a-value ($a = 37.19$ Pa), which means that this gel has a stronger elastic structure than the others. The b values showed no difference between gels made from different heat-treatment sequences in the sensitivity to frequency variation. The similar b-value of all gels, on the one hand, and the higher a-value of the C sample on the other hand, indicate that this mixed gel, as a physical gel, has the highest structure strength.

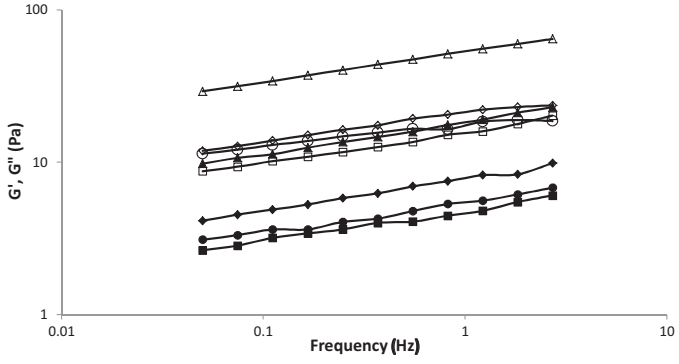

Fig. 3. Frequency sweep at 1% strain modulus G' (open symbols) and G'' (filled symbols) of acid milk protein–GT mixtures under different heat-treatment sequences, sample A (□ and■), sample B (◇ and ♦), sample C (Δ and ▲) and sample D (○ and •) as determined at 37 °C.

Examination of the gel structures by scanning electron microscopy showed that different types of structure were formed depending on the heat-treatment sequence (Fig. 4).

For the co-heated mixture of Na-caseinate–GT (sample C), scanning electron microscopy showed that the network is much more homogenous, coarse and dense (Fig. 4c) than that of the co-heated mixture of all three biopolymers (sample A), while co-heating the whey protein–caseinate mixture and whey protein–GT mixtures leads to a network of intermediate density (Fig. 4b and d). Sample D has the lowest homogeneous structure compared to the other samples (Fig. 4). Co-heating all three biopolymers (sample A) resulted in a less particulate microstructure.

4. Discussion

These results illustrate the effect of the heat-treatment sequence of mixtures containing three biopolymers on the subsequent formation of acid mixed gels. The different heat-treatment sequences caused no change in gelation time or pH. Schorsch et al. (2001) previously reported clear differences in gelation time and pH the effects of pre-heat treatment of whey protein before adding it to casein micelles, compared to co-heating whey with casein micelles. Thus, the polysaccharide plays an important role in the aggregation behavior of the protein during mixed-gel formation, and in interactions between them, showing the complexity of multi-compound systems with an increasing number of ingredients.

According to Lucey, Tamehana, Singh, and Munro (1998) when a caseinate–whey protein mixture is co-heated, heat treatment causes denatured whey proteins to complex with the casein micelle surface via k-casein/whey protein sulphydryl interchange, and the whey protein is then able to aggregate and form bridges between

Table 3
Power-law parameters for the elastic modulus of acid milk protein–gum tragacanth mixtures under different heat-treatment sequences as determined by frequency sweep tests at 37 °C and a strain of 1%. Values are means of triplicate samples (±SD).

Sequences	Power-low parameters	
	a	b
Sample A	10.17 ± 1.25a	0.192 ± 0.007b
Sample B	15.08 ± 1.23b	0.193 ± 0.006b
Sample C	37.19 ± 3.06c	0.204 ± 0.009c
Sample D	13.36 ± 2.19ab	0.163 ± 0.008a

a–c means with different letters with in the same column differed significantly ($p < 0.05$).

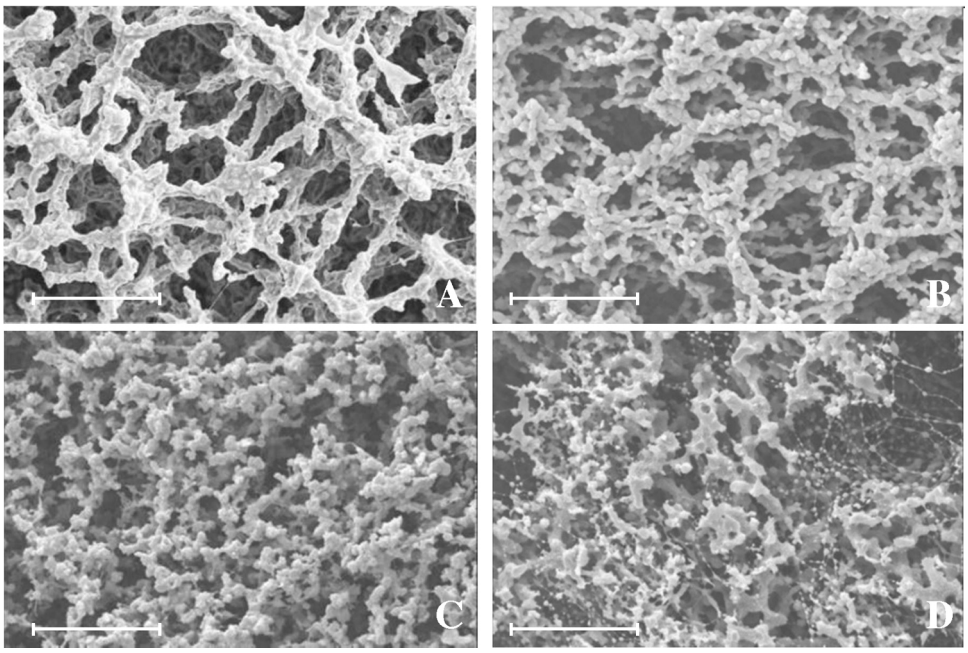

Fig. 4. SEM micrographs of acid milk protein–GT mixtures under different heat-treatment sequences, sample A (A), sample B (B), sample C (C) and sample D (D). Scale is 50 μ.

the micelles. During the acidification stage, the casein particles aggregate. This is due to the loss of the steric repulsion of the kappa-casein as well as the loss of electrostatic repulsion due to the decrease in pH. As the pH approaches to its isoelectric point (pH 4.6), the caseins aggregate. The casein micelles also have a strong tendency to aggregate because of hydrophobic interactions. The aggregation of the caseinate particles with associated whey protein leads to the formation of chains of proteins that are linked together to give a three-dimensional network. In addition, decreasing pH toward to the pI of caseinate, some whey protein aggregation also occurs since the protein was heat-denatured (cold-set gelation). When co-heating three biopolymers (sample A), GT satirically interferes in the interaction between caseinate and whey protein during the heating and cooling stages. GT is an anionic polysaccharide, as it has weakly dissociating surface acidic groups (carboxylic groups), and thus belongs to the adsorbing polysaccharide category. The mixtures were heated at pH 6.7. At pHs above the isoelectric point of the milk proteins (pH > 4.6–5.1), however, both caseinate and GT carry negative charges, but as shown by Ye (2008), electrostatic interactions between anionic carboxyl-containing polysaccharides and positively charged subunits of proteins can still occur, and as a result polysaccharides can adsorb to the casein particles. Moreover at pHs below 5.1 (the pI of the whey proteins), GT and whey protein carry opposite net charges resulting in a maximum electrostatic attraction. In addition, the contribution of hydrophobic interaction in attraction between these three biopolymers should not be ignored. Thus, the presence of gum during acidification hinders the formation of a continuous casein network. Even when the milk proteins were heated separately (sample B), the values for the rheological parameters were less than the individual milk-protein gel (sample A). In contrast, sample B showed higher structural strength than sample A, indicating the negative effect of the presence of gum on the interaction between milk proteins during heat treatment. Sample B had the highest strain sweep parameter values. The large clusters observed in the sample B micrograph confirmed the appropriate complex formation between milk proteins in the heating stage (in the absence of GT). However, the particulate clusters were separated from each other, forming a fairly open structure. Yokoyama et al. (1988) reported that the strong interaction of GT attached to the different particles presumably made the conformational change, producing tails and loops. Also, the formation of such loops on casein micelles was previously reported for pectin by Tromp, de Kruif, van Eijk, and Rolin (2004). It is likely that the presence of these trains, loops and tails on the protein surfaces causes a steric repulsion between protein aggregates and leads to the formation of a less-dense network.

When the whey proteins were preheated separately at 85 °C and added to the caseinate–GT mixture before acidification (sample C), they denatured and self-aggregated to form particles mainly linked by disulphide bonds. These aggregated particles are unable to covalently attach to the k-casein; however, during heating, electrostatic interactions between anionic carboxylic groups of GT and positively charged subunits of caseinate can occur. Such conditions result in strong interactions between positively charged protein groups and negatively charged GT sites during acidification. These interactions probably formed preferentially between the GT and the WPC aggregates after reaching a pH value lower than 5.1 (pI of whey proteins). Therefore caseinate and whey protein aggregates can easily link by polysaccharide bridges through electrostatic interaction as an alternative for covalent bonds that decrease the partly steric stabilization induced by GT. These hypotheses were corroborated by scanning electron microscopy. For sample C, that network is much more dense (Fig. 4c) than for the other samples. It is likely that the binding of denatured whey to the micelle surface through polysaccharide chains favors the formation of bridges between the casein particles, leading to a narrow-pored casein network. Another possible reason for the stronger gel obtained in sample C is more disulfide interactions occurring between whey proteins in comparison to other sample on cold-set gelation. Cold-set gelation is a two-step gelation process. In the first step reactive protein aggregates are obtained by heating a protein solution at relatively low concentrations. In the second step the gelation is induced by reducing the electrostatic repulsion between the aggregate by lowering the pH (de Jong et al., 2009). The whey proteins only in sample C were separately preheated at 85 °C. This may help in providing a better condition for forming the disulfide interactions between whey proteins, and for cold-set gelation the whey proteins, which eventually improved the strength of the mixed gel for sample C.

As in sample D, when the whey–GT mixture is co-heated, the presence of polysaccharides likely influences the aggregation process of whey during heating. Croguennoc, Nicolai, Durand, and Clark (2001) summarized thermo-gelation of β-Lg: at room temperature, native β-Lg is present mainly in the form of dimers in equilibrium, with a small fraction of monomers. As temperature increases, the equilibrium between the two states shifts toward the monomer, which is less resistant to denaturation. Aggregation occurs in two stages. In the first stage, small, well-defined clusters of ~100 denatured monomer units are formed by disulphide bridging. In the second stage, these clusters associate into larger aggregates, leading to gel formation at sufficiently high concentrations of polymer. Introducing of polysaccharide into cold-set gelation process can affect the mechanical properties of mixed gels through the more developed micro-phase separation in the mixed gels (de Jong et al., 2009). In the current study's sample D, macroscopic phase separation was not observed, but the addition of the micro-phase separated mixture of whey–GT to caseinate dispersion during acidification seemed to cause steric interference with the formation of casein-gel network connections. This hypothesis was corroborated by the lowest density and homogeneous microstructure of sample D compared to the other samples.

5. Conclusion

This study has shown that the microstructure and the rheological properties of mixed biopolymer gels are greatly influenced by heat-treatment sequence. When whey proteins are heated separately and subsequently added to a sodium caseinate–GT mixture, acid-induced gelation leads to gel with a more dense microstructure and higher structure strength than gels made from heated systems through other heat-treatment sequences. The gels resulting from co-heating all three biopolymer showed a stranded microstructure and the lowest structure strength of all the samples. This paper highlights the importance of controlling formulation parameters such as the heat-treatment sequence of biopolymer mixtures. Of course, more information and studies are needed in this area (especially with use of running any reducing and non-reducing SDS-PAGE) before the results of this study can be generalized.

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