Rheology and microstructure of cold-set mixed soybean glycinin-locust bean gum gels

Abstract

The relationship between the rheological behavior and microstructure of glucono-δ-lactone (GDL) induced cold-set soybean glycinin (Gly)–locust bean gum (LBG) mixed gels was studied. In the presence of 0.05%, 0.10% and 0.15% w/v LBG, the onset of Gly network formation (4.0%, w/v) occurred progressively earlier and the viscoelastic modulus was enhanced significantly as the LBG concentration increased. However, when the LBG concentration was increased to 0.25% and 0.35% w/v, phase reversion occurred. Higher acidification rate the formation of mixed gel microstructures with smaller polysaccharide inclusion. These results could facilitate preparation of novel cold-set gels for practical use in food processing.

Keywords: soybean glycinin, locust bean gum, rheological properties, microstructure, gelation

1 Introduction

Recently considerable interest has been given to the study of protein–polysaccharide mixtures in both the industrial and academic sectors. Research has focused on the dynamics of microstructure formation/breakdown and functional properties such as texture appreciation and nutrient release during food product manufacture, storage, consumption, and digestion [1]. These functional properties are mainly controlled by the microstructure of protein-polysaccharide mixtures. In the case of gelation of these mixtures, the final microstructure is determined by the relative kinetics between phase separation and gel formation.

It has been reported that mixtures consisting of various food proteins and polysaccharides can be used to prepare a broad spectrum of mixed gels with significantly different structural and physical properties [2]. Recently, several studies have examined the properties of cold-set protein-polysaccharide mixed gels, mainly those obtained by applying whey protein as the gelation agent [3]. These studies provide valuable insight regarding the relationship between microstructure and macroscopic mechanical properties during processing or storage. However, to the best of our knowledge, there have been few studies on the relationship between gel rheological properties and microstructure of cold-set mixed soy protein and polysaccharide system.

The favorable functional and nutritional properties of soybean protein are well known. It is incorporated as a gelling agent in many food products, often in combination with other biopolymers, such as proteins and polysaccharides. The two major components of soybean protein, β-conglycinin and glycinin (Gly), have different structures and functional properties, and constitute ~70% of the total seed storage protein [4]. Soybean proteins in combination with other biopolymers have potential applications in the food industry. Locust bean gum (LBG) is a galactomannan extracted from the seed of the legume plants Ceratonia siliqua. Galactomannans are linear polysaccharides based on a β-(1-4)-mannose backbone to which single D-galactopyranosyl residues are attached via α-(1-6) linkages [5]. In LBG the side branches are not uniformly spaced and the mannose/galactose (M:G) ratio is about 3.5. Locust bean gum is widely used both in the food industry and in nonfood applications, such as the paper, textile, pharmaceutical and cosmetic industries.

In this study, our objective was to evaluate the rheological behavior and microstructure of glucono-δ-lactone (GDL) induced cold-set Gly–LBG mixed gels at different biopolymer concentration and acidification rate. We also attempted to elucidate the possible relationship between rheological properties and microstructure of GDL-induced cold-set Gly–LBG mixed gels.

2 Materials and methods

2.1 Materials

Soybean defatted flakes were purchased from Shandong Xinjiahua Co. (Shan Dong, China). The flakes contained 55.0% protein (N × 6.25, dry basis). Refined grade locust bean gum and glucono-δ-lactone were purchased from Sigma Chemical Co. (USA) and were used without further purification. All other reagents and chemicals were of analytical grade.
2.2 Preparation of soybean glycinin

Soybean Gly was prepared according to the procedure specified earlier [6]. Gly protein content of this lyophilized powder was determined by the Kjeldahl method (N × 6.25), which was found to be 95.0±1 %.

2.3 Preparation of solutions

Lyophilized Gly powder was dissolved in deionized water containing 0.032 % (w/v) sodium azide, stirred for 2 hours at room temperature and the pH was adjusted to 7.6 with 2 M NaOH. This was stored overnight at 4 °C in order to prepare Gly solution 8% (w/v). The supernatant of Gly solution was carefully moved into a sealed bottle and incubated in a water bath at 95 °C for 30 min. Then the denatured GLY solution was cooled with running tap water and subsequently centrifuged at 4 °C (9000 g ×10 min) to eliminate the remaining suspended protein particles from solutions. The supernatant was used as the pre-denatured Gly stock solution and was diluted to prepare samples of appropriate protein concentration. Prior to use the denatured proteins solutions were diluted to the appropriate protein concentration with water and stored at 4 °C. LBG stock solutions (0.8%, w/v) was prepared by dissolution of the powder in the same buffer using a strong mechanical agitation for 30 min at 85°C in order to ensure the dissolution of the polysaccharide. Remaining suspended particles were eliminated from solutions by centrifugation at 40°C (38,000 g ×1h) and the final concentrations of LBG stock solutions was determined from their dry matter contents.

Blends of Gly and LBG solutions were prepared by mixing equal amounts of their stock solutions at 25 °C with moderate agitation for 5 min. Gelation of the blends was induced by the addition of GDL. The total amount of GDL added depended on the protein concentration. An amount of 0.25 - 0.55 % (w/v) GDL was added to 2 – 6 % (w/v) protein and incubated at different temperatures.

2.4 Low amplitude dynamic oscillatory measurements

Rheological measurements were conducted on a controlled-strain rheometer (Haake RS600 Rheometer HAAKE Co., Karlsruhe, Germany) using parallel plates (dD 27.83 mm, 1 mm gap). Samples were covered with a silicone oil to prevent evaporation. Small-deformation oscillatory measurements were carried out at a frequency of 1 Hz and a strain of 0.5 %. A sequence of measurements was programmed as follows: (1) blends were placed on the plate immediately after addition of GDL and incubated at a set temperature for 5 hours. During this period, the storage modulus (G'), the loss modulus (G'') and the phase angle tan δ (G''/G') were recorded. (2) A frequency sweep between 0.1 rad/s and 100 rad/s; and (3) a strain sweep from 0.0001 to 1 at the corresponding temperature. Three replications were made for each measurement.

2.5 Confocal laser scanning microscopy

Microstructure observations were made using a LEICA TCS SP confocal scanning laser microscope (Leica Microsystems, Heidelberg, Germany). The CLSM was equipped with an inverted microscope and operated in the single photon mode with an Ar/Kr visible light laser. The wavelength of 568 nm was used to excite the Rhodamine-staining proteins and a Leica objective lens of 40x was used (40x/UV/1.25NA/water immersion/PL APO). Digital image files were acquired in 2048×2048 pixel resolution. Unless otherwise stated, the images reported in this paper were recorded at a penetration depth of 15 µm to avoid possible artefacts that occur close to the glass of the cuvette.

2.6 Statistics

Analysis of variance (ANOVA) of the data was performed, and a least significant difference (LSD) test with a confidence interval of 95% was used to compare the means.

3 Results and discussion

3.1 Structural evolution of GDL cold-set mixed Gly–LBG mixtures

The structural dynamic evolution of mixed Gly–LBG gels during acidification at 25°C to a final pH of 5.78±0.21 after 5 hours’ incubation was investigated. Fig.1 shows microstructure evolution synchronized with rheological properties of mixed Gly–LBG mixtures coupled with decreasing pH. At ~2000 s in the initial part of the gelation curve (Stage I), a sharp increase in the value of G’ and G” occurred. G’ exceeded 10 Pa and tanδ reached a value below 1, suggesting the onset of gelation. These are typical characteristics of a network formation [7]. After the onset of a gel network, a stage (II) with moderate increases in viscoelastic moduli and continuous evolution in microstructure of the mixed gel was observed at pH of 6.2-6.4. In this stage, both G’ and G” increased moderately, reflecting incorporation of more protein into the network structure. Microstructure determinations showed that LBG
occupied more space as pH decreased, suggesting that phase separation appeared and gradually progressed (Fig. 1 b to d). In the last stage (III) of the gelation process (Fig. 1 d to f) viscoelastic moduli of mixed gels continually increased, but at a much slower rate. Meanwhile, the pores in the gel microstructure matrix remained almost unchanged, and no changes occurred during further decreases of the pH as a function of time in one CLSM cuvette. These findings suggest that once phase separation was settled by gelation, the microstructure remained almost constant. This is in accordance with the results observed for GDL induced cold-set mixed whey protein isolated/gellan gums gels.

![Viscoelastic Moduli and Microstructure](image)

**Fig. 1** Microstructure evolution and $G'(\Delta)$, $G''(\triangle)$ and $\tan(\delta)$ profiles for mixtures of LBG (0.1%) –Gly (4.0%) system at 25°C and 0.25% w/v GDL as a function of time during decrease of its pH(○). Images (a-f) represent microstructure determined at incubation times of 0, 20, 30, 60, 120 and 180 min, respectively. Each image width is 385 μm.

### 3.2 Effects of polysaccharide (LBG) concentrations

Fig. 2 shows the effect of locust bean gum concentration on rheological properties and microstructure of mixed biopolymer gels with a fixed Gly concentration of 4% (w/v). The storage modulus $G'$ of Gly gels without polysaccharide was continuously increased and reached ~ 1300 Pa at the end of the acidification process with a homogeneous microstructure (Fig. 2 a). Compared with samples without LBG, in the presence of 0.05%, 0.10% and 0.15% (w/v) LBG, the onset of network formation occurred progressively earlier, and initially gelation time decreased as LBG concentration increased. Gel microstructure determinations showed morphology with LBG dispersed into the protein continuous network as polysaccharide inclusions. The discontinuous serum phase with round analogy phase boundaries is consistent with the findings on microstructure of GDL induced cold-set whey protein/polysaccharides mixed gels by [8]. We observed that gels with 0.05% (w/v) LBG had a round, porous, randomly distributed microstructure (Fig. 2 b). Samples containing 0.15% (w/v) LBG showed a trend for the dispersed LBG phase to almost overflow the phase boundary, suggesting that this polysaccharide concentration is near the threshold concentration for retaining dominance over the protein as a continuous phase. Also, with 0.15% (w/v) LBG concentration, a maximum viscoelastic moduli was obtained for the mixed gel (Fig. 2 d). This was attributed to an increased protein concentration in the continuous phase which was caused by microphase separation resulting from the thermodynamic incompatibility between proteins and polysaccharide. The increased polysaccharide space arising from incorporated LBG forces Gly to assemble in local areas and leads to an apparent increase in local protein concentrations. Consequently, gelation occurs earlier than in gels with protein alone.

Gel strength was weakened from the peak value and decreased markedly as the polysaccharide concentration increased from 0.15% to 0.35% (w/v). Interestingly, with addition of 0.25 and 0.35 % LBG, phase reversion on mixed biopolymer mixtures was observed (see CLSM images, Fig. 2 e and Fig. 2 f). This result is in agreement with the previous work on heat-induced protein–polysaccharide mixed gels [9]. As the polysaccharide concentration increased beyond the threshold value, phase separation became dominant, resulting in a significant decrease in the protein network until it was no longer able to retain an integrated dimensional network structure. Therefore, the viscoelastic modulus of the systems containing 0.25 % and 0.35 % LBG showed dramatic decreases (Fig. 2).

Compared with Gly gel alone, observed as a flat modulus profile, samples with low LBG concentrations
Zhu, Min, Yang, Qi

(0.05%, 0.10%, 0.15%) exhibited larger G’ and more frequency dependence over the entire frequency range (Fig.3). This result is in agreement with the findings of Foegeding et al., (2002), who pointed out that a flat slope in frequency sweep could be an indication of a strong, elastic gel [10]. Strain sweep spectrum of mixed Gly-LBG gels performed at the end of the experiment showed that samples with no polysaccharide appeared to have the longest viscoelastic linearity (Fig.4). Addition of increasing concentrations of LBG cause a gradual decrease of linearity, which was shortest at 0.25% and 0.35% LBG. These results strongly suggest the disruption of network formation in the presence of higher LBG concentrations (0.25%, 0.35%, w/v), which coincides with protein gel network weakening in microstructure observations.

![Figure 2](image1.png)

**Fig. 2** Variation of microstructure, G’ and G” for mixtures of Gly (4.0%) and LBG system. (a–f) Represent without LBG, and with 0.05%, 0.1%, 0.15%, 0.25%, 0.35% w/v LBG, respectively. The filled points represent elastic modulus, the empty represent loss modulus. Each image width is 385 μm.

![Figure 3](image2.png)

**Fig. 3.** Frequency sweep for mixtures of Gly (4.0%) and LBG at 25°C, 0.25% w/v GDL. The values and symbols are as described in Fig. 2.

![Figure 4](image3.png)

**Fig. 4.** Strain sweep for mixtures of Gly (4.0%) and LBG at 25°C, 0.25% GDL. The values and symbols are as described in Fig. 2.

### 3.3 Effect of Gly concentration

To investigate the influence of Gly concentration on the gelation process, rheological measurements and CLSM were made at three different protein concentrations (2%, 4%, 6%, w/v), containing 0.1% (w/v) LBG and acidified-induced cold-set gel with 0.25% GDL at 25°C. Fig.5 shows that the elastic modulus and microstructure changed significantly with increasing concentrations of denatured Gly. The initial gelation point of Gly-LBG mixtures occurred later with increasing concentrations of protein, which agrees with the previous findings [11]. Mixed Gly-LBG gels with 4% (w/v) Gly showed the highest G’.
Zhu, Min, Yang, Qi

CLSM observations showed that a mixed gel with 2% (w/v) Gly has a relatively smooth and homogenous gel microstructure, with no clear LBG domain (Fig. 5). At a Gly concentration of 4% (w/v), the microstructure of mixed gels became coarse, spherical LBG domains pores appeared, and the protein network remained continuous. Increasing Gly concentration to 6% (w/v), significantly decreased G’ from the peak value (samples with 4% Gly, w/v), and the shape and size of LBG inclusion distributed in the continuous gel matrix became more nonhomogeneous. In this case, the different effect of various Gly concentrations on rheological properties and microstructures were a result of the concomitant process of phase separation and protein network formation between Gly and LBG, which could be attributed to two significant factors: 1) at increased Gly concentrations, mixtures could require a much longer incubation time to fully develop the gel dimensional network, evidenced by the onset of gelation delay of mixed gels at higher protein concentrations; 2) with increasing protein concentrations, the relative ratios of GDL and LBG to protein become smaller, which could slow the acidification rate resulting in a longer incubation time before phase separation was arrested by gelation.

Fig. 5 Variation of microstructure, G’ and G” for mixtures of LBG (0.1%) and Gly system. (a–c) Represent 2.0%, 4.0% and 6.0% Gly, respectively. The filled points represent elastic modulus, the empty represent loss modulus. Each image width is 385 μm.

Table 1 Effect of temperature and glucono-δ-lactone concentration on the time (t_g) and pH value at initial gel point, rheological parameters G’_f, G”_f, tanδ_f, and pH (pH_f) at the end of time sweep procedure for the Gly/LBG mixed systems.

<table>
<thead>
<tr>
<th>GDL (%w/v)</th>
<th>T(℃)</th>
<th>t_g(h)</th>
<th>pH at t_g</th>
<th>G’_f(Pa)</th>
<th>G”_f(Pa)</th>
<th>tanδ_f</th>
<th>dpH/dt**</th>
<th>pH_f</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>15</td>
<td>1.8 ±0.21^a</td>
<td>6.34</td>
<td>433.0±34^a</td>
<td>77.9 ±4.2^a</td>
<td>0.18±0.00^a</td>
<td>0.27±0.02^a</td>
<td>6.26</td>
</tr>
<tr>
<td>0.25</td>
<td>25</td>
<td>1.0 ±0.10^b</td>
<td>6.36</td>
<td>2662±150^b</td>
<td>439.9 ±35^b</td>
<td>0.17±0.01^b</td>
<td>0.36±0.01^b</td>
<td>5.78</td>
</tr>
<tr>
<td>0.25</td>
<td>35</td>
<td>0.4 ±0.07^c</td>
<td>6.37</td>
<td>3637±230^c</td>
<td>557.3 ±52^c</td>
<td>0.14±0.00^c</td>
<td>0.43±0.01^c</td>
<td>5.46</td>
</tr>
<tr>
<td>0.25</td>
<td>45</td>
<td>0.2 ±0.05^c</td>
<td>6.35</td>
<td>3664±450^c</td>
<td>485.2 ±48^b</td>
<td>0.13±0.02^b</td>
<td>0.45±0.01^c</td>
<td>5.34</td>
</tr>
<tr>
<td>0.4</td>
<td>25</td>
<td>0.6 ±0.05^c</td>
<td>6.34</td>
<td>3997±510^c</td>
<td>623.4 ±64^d</td>
<td>0.16±0.01^d</td>
<td>0.57±0.03^d</td>
<td>4.76</td>
</tr>
<tr>
<td>0.55</td>
<td>25</td>
<td>0.4 ±0.06^c</td>
<td>6.32</td>
<td>2505±131^b</td>
<td>370.9 ±32^b</td>
<td>0.15±0.02^a</td>
<td>0.60±0.01^d</td>
<td>4.63</td>
</tr>
</tbody>
</table>

*Values of G’_f, G”_f and tanδ_f were determined at the end of 5h time sweep.
**dpH/dt at t_f means the pH derivative in relation to whole acidification incubation time (5h).
Mean with different superscript letters in the same column are significantly different (P< 0.05). The standard deviation values represent triplicate measurements.

3.4 Effects of acidification rate

The effect of changes in acidification rate induced by changes in incubation temperature and GDL concentration on the Gly-LBG systems are shown in Table 1. The pH values at t_g for different incubation temperatures all were close to the Gly isoelectric point (pI=6.4), strongly indicating that gel network formation was dominated by denatured Gly. G’ values increased and tan delta values decreased with increasing incubation.
temperature, indicating the formation of stiffer gels with increasing elastic-like behaviour. The $dpH/dt$ was defined as the average acidification rate over the 5 hours incubation time. Results showed that the sample with addition of 0.55% (w/v) GDL had the highest acidification rate and the lowest final pH value. The final pH values (pHf) were decreased as the acidification rate increased ($dpH/dt$), dependent of increasing GDL concentration or incubation temperature. These results coincide with the critical effect of acidification rate on microstructure and rheological properties. In terms of rheological and microstructure changes, incubation of Gly-LBG gels at various temperatures (15°C, 25°C, 35°C and 45°C) with incorporation of 0.25% (w/v) GDL concentration and at various GDL concentration (0.25%, 0.4% and 0.55% w/v) with incubation temperature at 25°C resulted in different patterns of viscoelastic modulus development. A moderate acidification rate, obtained by modulating incubation temperature or GDL concentration, is beneficial for the formation of mixed biopolymer cold-set gels with a higher elastic modulus and a homogenous smooth microstructure (data not shown).

4 Conclusions

Variation in the biopolymer concentration and rate of acidification at acidified-induced gelation resulted in the formation of cold-set Gly-LBG mixed gels with various rheological properties and microstructure, mainly influenced by the final balance between phase separation and gelation. With incorporation of low concentrations of polysaccharide, microstructures of mixed gels showed protein phase continuous characteristics with a dispersed polysaccharide phase as inclusion. However, with an increase in polysaccharide concentration beyond the threshold value, phase inversion occurred in the biopolymer system with polysaccharide turned into the continuous phase, protein were the dispersed phase, markedly decreasing the elastic modulus.

Acknowledgements

This work was supported by the research projects of Chinese National Natural Science Fund (Grant No. 31101215) sponsored by the NNSF of China, the Foundation for Distinguished Young Talents in Higher Education of Guangdong, China (Grant No. LYM10120), and the Open Project Program of Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety (Grant No. 201204).

References