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Short communication

Coupling process of phase separation and gelation in konjac glucomannan and gelatin system

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ABSTRACT

The influence of konjac glucomannan (KGM) on the cool-induced gelation of gelatin was investigated using dynamic oscillatory rheological measurements at low strain amplitude and microstructural analysis by fluorescence microscopy. Rheological tests were measured during gel formation, including isothermal cooling and heating/cooling at a constant rate. The transition of phase compatibility to segregative phase separation with increase of KGM concentration was observed on KGM/gelatin mixture systems at 40 °C. The structural and mechanical of these hydrogels are dominated by the interplay between phase separation and gelation. In compatible phase status, the presence of KGM prolonged the gelation to occur for disordering the gelatin coil-helix transition, decreased the gel strength. The consequence of phase separation was dependent on KGM concentrations, resulting in bicontinuous microstructures. In the case of phase separation, the gelation was accelerated. It was demonstrated that by using different protein-polysaccharide mixtures at phase compatibility or separation concentrations could be feasible ways to control micro-networks and apply structural design in food formulation.

1. Introduction

Understanding the phase behavior between food hydrocolloids not only mainly contribute to the texture of food, but also are helpful to develop the new structure food (Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). So phase separation processes have been always used for structuring processed food. It could feasible generate and control the structural elements at different length scales (Van den Berg, Rosenberg, Van Boekel, Rosenberg, & Van de Velde, 2009).

Gelatin, used as thickener and emulsifier in food processing, is one kind of natural protein with well gelling property (Young, Wong, Tabata, & Mikos, 2005). The phase separation of gelatin with non-adsorption polysaccharides above the gelation temperature has been extensively studied since 2000, such as gelatin/starch (Firoozmand, Murray, & Dickinson, 2007, 2009), gelatin/dextran systems (Butler, 2002; Butler & Heppenstall-Butler, 2001). Generally, at this point, phase separation takes place when the total concentration of the macromolecules exceeds a certain critical value, suggesting entropy-driven liquid–liquid phase separation (Firoozmand et al., 2007).

However, the complicated interplay between such phase separation and gelation was still an interesting point, which could be used for controlling microscopic network structure. On the one hand, gelation could hinder phase separation process. When gelation occurs induced by lowering the temperature, thermodynamic driving force for phase separation becomes stronger because gelatin molecules entangled with each other to decrease partially osmatic pressure (σ) and system's free energy (△G) (De Kruif & Tuinier, 2001). The microstructures are trapped at the early stages of phase separation and then coarsening changes are blocked by gelation. But it did not prevent drop coalescence of microstructure even gelation hindered the phase separation (Fransson, Lorén, Altskär, & Hermansson, 2009).

Another possible is that gelation may trigger phase separation via the formation of enhanced concentration in one region. In that case, phase separation promoted gelation strength by concentrated effect and each of phases in the demixed system affected the phase separation kinetics. As the former study of gelatin–dextran shown, an increase of viscosity difference between the gelling gelatin domains and the non-gelling dextran domains significant affected the

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phase separation kinetics (Edelman, van der Linden, de Hoog, & Tromp, 2001).

However, KGM possesses high viscous property (30,000 mPa s, 1% w/w) (Crosby, 2002). So, viscosity effect would not be fitted for explanation of gelatin–KGM phase separation and gelation network interplay. The work reported in this paper was aimed to figure out that coupling process of phase separation and gelation during quenching temperature. Even thermodynamic phase separation between gelatin and konjac glucomannan (KGM) above the gelation temperature has been reported (Harrington & Morris, 2009a), the effect of phase separation on gelation by quenching temperature is still not be published. Firstly, the phase separation network transit from polysaccharide-continuous to protein-continuous phase in micro length was investigated. Besides, in order to build up the relationship between phase separation and gelatin network formed upon heating and cooling process, the rheological behaviors of KGM and gelatin mixtures were monitored by rheological temperature sweep. The gelation progress was recorded by time sweep test. The worthy endeavor is to reveal the relationship between the phase behavior and structural properties during the multiple coupling processes of phase transition and gelation.

2. Experimental section

2.1. Materials

Konjac glucomannan (>90% glucomannan) with molecular parameters: Mw, 8.965 × 10^5 Da; Mw/Mn, 1.126; Rg, 95.1 nm, as determined by GPC-MALLS in 0.2 M NaCl at 25 °C was supplied by QiangSen company (Wuhan, China). Gelatin (type B, bloom~300) was purchased from Aladdin chemicals (Shanghai, China). All the other reagents and chemicals used were analytical grade.

2.2. Preparation of mixtures

Gelatin stock solution (8wt%) was prepared by dispersing powder in deionized water at 40 °C for 1 h. KGM solutions, a series concentration of 0.2–1.0 wt%, were obtained by dissolving powder through mechanical agitation at 25 °C for 2 h. Mixtures of gelatin and KGM solutions were prepared with the equal amount, under moderate agitation at 40 °C for 30 min. Gelatin content in mixtures fixed at 4wt%, and then mixtures are remarked as B_1 to B_5 corresponding to KGM concentrations range from 0, 0.1, 0.2, 0.4 and 0.5wt% responsively. All mixtures were sit for 24 h at 40 °C, above the gelation temperature of gelatin, and the digital pictures of final state were taken.

2.3. Fluorescence microscopy

The micro-network microscopic observations of mixtures were visualized using a fluorescence microscopy (Nikon 80i Eclipse, Japan) and the microphotographs were captured. Gelatin was stained by Rhodamine B (0.2wt%), which was non-covalently labeled to the protein network. The light source was a multiline argon laser with an excitation wavelength of 543 nm.

2.4. Rheological measurements

The rheological measurements were analyzed under small deformation amplitude using a controlled-stress rheometer (AR2000ex, TA), fitted with a parallel plate (40 mm diameter, 1 mm gap). In all oscillatory rheological measurements, the frequency and the strain were applied at 1 Hz and 2% respectively, which was chosen within the linear viscoelastic region (LVR).

In the temperature test, the temperature was ramped from 20 °C to 60 °C at 1 °C/min, and maintained at 60 °C for 1 min. Then the temperature decreased back to 10 °C at a rate of 1 °C/min. For isothermal curing experiments, the samples were quickly placed on the rheometer plate preheated at 40 °C and then cooled to 4 °C about 2 min. Time sweep tests were carried on 25 °C lasted for 4 h. The storage modulus (G′) and loss modulus (G″) were recorded continuously during the testing. Exposed edges of sample were covered with a thin layer of mineral oil to minimize water evaporation.

3. Results and discussions

3.1. The network changes during phase transition

Fig. 1A, a visual photograph, showed the appearance of the tube containing mixtures of gelatin (4.0% w/w) and KGM (concentrations from 0 to 0.5 wt%) after 24 h at ~40 °C. The samples are remarked as B_1 to B_5, which corresponded to 0, 0.1, 0.2, 0.4 and 0.5 wt% content of KGM respectively. Photograph B_5 contained an upper transparent gelatin-rich phase and a lower turbid konjac-rich phase. The phase separation can be attributed to thermodynamic incompatibility between KGM molecules and disordered gelatin molecules. Fluorescent microscopy was performed to observe the microstructure network of B_2, B_3 and B_5 solutions. The polysaccharide and protein networks were illustrated in Fig. 1B–D. The light areas are gelatin-rich phase features, which stained by Rhodamine B excited by red fluorescent emission. The dark areas are corresponded to KGM-rich without fluorescent light. The B_2 solution exhibits a uniform fluorescence revealing homogeneous microstructure, some polysaccharides dispersed in gelatin evenly. When the concentration of KGM reaches 0.2 wt% (B_3), the partial polysaccharide network was observed like the evident arborization structure, representing that KGM gathered and formed the micro-network leading to phase separation. The black aggregations continued to accumulate in B_5 solution, which formed the bicontinuous microstructures. In general, this morphology coarsens by self-similar growth, coalescence and ultimately results in two phases (Turgeon et al., 2003). Tobin, Fitzsimons, Chaurin, Kelly, and Fenelon (2012) reported the similar results of KGM-denatured whey protein mixed systems. They also found that increase of biopolymers' concentrations induced phase separation and then separation transformed from micro-phase to macro-phase scale (Tobin et al., 2012). Abhyankar et al. also studied the microstructure of β-lactoglobulin and galactomannan mixtures after phase separation (Abhyankar, Mulvihill, Fenelon, & Auty, 2010). These results showed that phase separation of the systems arises from local fluctuations of the biopolymers' concentration.

3.2. Effect of phase separation on gelatin gelation process during heating/cooling

Fig. 2 presented the changes of G′ and G″ of B_1 to B_5 during a thermal cycle, which includes the heating (Fig. 2A,B, from 20 °C to 60 °C) and cooling process (Fig. 2C,D, back to 10 °C). Upon heating, the both viscoelastic moduli (G′, G″) decreased as the temperature increase, showing "viscosity–temperature correlation" (Fairclough et al., 2012). Initially, the value of G′ was much larger than that of G″, revealing the gel properties dominated to form a gelation network in Fig. 2A,B. The mixtures displayed no significant differences in G′ until the temperature reaches around 30 °C. Below 30 °C, gelatin molecules entangled together to form triple-helix network. The gelation network limited movements of KGM molecules, resulting in hindering phase separation happened. But after reaching the melting point of gelatin, gelatin molecules