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Effect of cooling–heating rate on sol-gel transformation of fish gelatin–gum arabic complex coacervate phase

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ABSTRACT

The objective of this study was to characterize influence of different cooling and heating rates on gelation of fish gelatin (FG)-gum arabic (GA) complex coacervate phase using rheological measurements. For the coacervate phase prepared at 10 °C, the gelling temperature, melting temperature, gel strength, and stress relaxation decreased with increasing cooling or heating rate, however, no gelation was observed at the highest cooling rate of 0.05 °C/min. Similar trends were obtained for the coacervates phase prepared at 30 °C, but the gelation did not occur at a cooling rate of 0.033 or 0.05 °C/min. The results indicated that rheological properties of FG-GA coacervate gels were highly dependent to the cooling process, where more thermos-stable and stronger gels formed at slower cooling. This was probably because of higher degree of molecular rearrangements, more hydrogen bindings, and formation of greater junction zones into the gel network at slower cooling rates. However, all of the FG-GA coacervate gels obtained at different cooling rates were classified as a weak physical gel.

1. Introduction

Gelatin, as a gelling agent, has found a variety of applications in the food and pharmaceutical industries. The physical properties of gelatin, such as the melting close to the physiological temperature of humans, give the biopolymer the special 'melt-in-mouth' perception; a behavior which is hard to mimic in other polymeric systems [1]. Bone and skin from bovine and porcine sources have usually been used in gelatin production [1]. The outbreak of the mad cow disease in the 1980s accelerated the search for a gelatin alternative. Another reason for finding an alternative to mammalian gelatin is that Muslims, Jews, and Hindus do not accept gelatin produced from bovine and porcine sources [2]. Fish gelatin (FG), as an alternative to the mammalian gelatin, is accepted as a food additive by these religious groups. However, direct substitution of mammalian gelatin with FG is not desirable, because FG, especially from cold-water fish species, possesses weaker gel strength and thermal characteristics (i.e. lower gelling and melting temperatures) due to its lower contents of proline and hydroxyproline, which are known to be involved in the formation and stabilization of collagen-like triple helical structures [3]. Therefore, characterization of different approaches to improvement of thermal and rheological properties of FG may lead to exploring its new applications.

Presence of polysaccharides in many food systems plays an important role in the structure and stabilization of proteins [4]. Hence, mixtures of proteins and polysaccharides are widely used in the food industries for development of bioactive delivery devices, fat replacers, gels, emulsions, and coatings. Interactions between proteins and polysaccharides in an aqueous environment can be either repulsive or attractive depending on the nature of the interacting biopolymers and the medium conditions [4]. Repulsive interactions occur mainly due to the steric exclusion effect between uncharged or similarly charged biopolymers and cause incompatibility between the biopolymers [5,6]. Attractive interactions, mostly caused by electrostatic attractions between oppositely charged biopolymers, induce the formation of biopolymer complexes, which can be either soluble in a single phase or insoluble to build a two-phase system where one phase is enriched in insoluble complexes and the other depleted. The formation of two-phase system through attractive interactions is called associative phase separation, which is also called complex coacervation or precipitation for the separation of liquid- or solid-state insoluble complexes, respectively [3].

In our previous studies, firstly, we found that the FG from cold water fish skin underwent complex coacervation with gum arabic (GA), one of the most widely used anionic polysaccharides, in aqueous solutions at 40 °C [3]. The formation of soluble or

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insoluble FG-GA complexes was strongly influenced by pH, FG to GA weight ratio, and total concentration of biopolymers in the initial mixture. Secondly, it was gained by our group that the rheological and microstructural characteristics of FG-GA complex coacervate phase can be influenced by phase separation temperature, where the phase with higher viscous behavior and more condensed microstructure under the sol state followed by greater thermo-stability (higher gelling and melting temperatures) and gel strength under gel state was obtained by decreasing the phase separation temperature from 40 to 10 °C [7]. Notwithstanding the fact that the complex coacervation could enhance gelling properties of the FG, the rheological properties of the FG-GA coacervate gel is not suitable, at least for design of foods with desired texture or food-grade delivery systems for encapsulation of bioactive ingredients. Therefore, it is necessary to seek efficient methods in order to more improvement of rheological and thermal properties of the FG-GA coacervate gel.

For many gel systems, the gelling temperature is a function of thermal history of the material. Cooling or heating rate is important processing parameter which can be controlled to control gel formation in biopolymeric solutions by affecting on thermal history of the system [8]. The effect of cooling and heating rate on gelation of different proteins have been reported: β-lactoglobulin [9,4], whey protein [10], salt-extracted pea protein isolate [8], pea legumin [11]. It has demonstrated that at slower heating rate there is more time for protein denaturation and then, aggregation of the unfolded protein molecules through intermolecular hydrophobic interactions which eventually, caused to onset of gelation at a lower temperature and improvement of rheological properties of the gel [4,8,10]. Sun and Arntfield [8] and O'Kane et al. [11] considered effect of cooling rate on network development and strength of the gel proteins. They found that stronger gels with more developed network structure can be formed at slower cooling rates because there is a longer time for optimal arrangement of unfolded proteins and formation of hydrogen bindings, as favorable bindings at low temperatures. Beside of pure proteins, the effect of cooling or heating rate on thermal and rheological properties of polysaccharide-protein mixtures have been reported; β-lactoglobulin-basil seed gum [4], whey protein-xanthan gum [10], and gelatin-low acyl gellan gum and sucrose [12]. According to the previous studies, slower heating and cooling rates allow protein molecules more time to rearrange in a certain molecular conformation (e.g. unfolded or helical structure) for compatible or incompatible interactions with polysaccharides, which eventually, strengthens the gel. In general, by changing and controlling cooling or heating rates, it would be possible to create a variety of gel structures with different rheological, thermal, and structural properties. To the best of our knowledge, however, less information is available about effects of different cooling or heating rates on gel formation and rheological properties of protein-polysaccharide complex coacervates. Accordingly, the objective of this study was to investigate the impact of cooling and heating rates on gelation bahvior of FG-GA complex coacervate using dynamic rheological measurements.

2. Materials and methods

2.1. Materials

Fish gelatin (FG, from cold water fish skin) and gum arabic (GA, from Acacia tree) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Carl Roth GmbH (Karlsruhe, Germany), respectively. The average molecular weights of FG and GA, determined by viscometry using an Ostwald viscometer and Mark-Houwink equation in our preliminary experiments, were 58 and 382 kDa, respectively. Sodium azide (NaN_3) was obtained from Daehung Chemicals & Metals (Siheung, Korea).

2.2. Preparation of FG-GA complex coacervate phase

Accurately weighed amounts of FG and GA were separately dissolved in 100 mL of distilled water at 40 °C and mixed together at a weight ratio of FG to GA (FG:GA) of 1:1 and a total biopolymer concentration of 2% (w/v), followed by adjusting the pH to 3.5. Sodium azide was added as a preservative at a concentration of 0.02% (w/v). The FG–GA mixture was incubated at 40 °C in a shaking water bath at 100 rpm for 24 h for sufficient electrostatic interactions between FG and GA molecules. Since it was found that the coacervates phase separated at lower temperatures had better rheological and structural properties [7], in the current study, the incubated mixtures placed statically at 10 and 30 °C for another 24 h for the macroscopic phase separation of FG–GA insoluble complexes to reach equilibrium state.

2.3. Rheological measurements

All of rheological measurements were carried out using a rheometer (TA-AR 2000, TA Instruments Inc., New Castle, DE, USA). During all measurements, the samples were placed into the rheometer equipped with cone–plate geometry (40 mm diameter, angle 2°) at a gap of 53 μm and covered by moisture trap to prevent sample drying during measurements. All the rheological tests were performed in triplicate.

2.3.1. Dynamic oscillatory analysis

To characterize dynamic oscillatory behavior of the coacervate gels, first, the linear viscoelastic region, where G′ and G″ are independent of strain (γ), was determined by strain sweep tests in the γ range of 0.01–100% and at 1 Hz frequency (ω). For the coacervate phases separated at 10 and 30 °C, the linear viscoelastic region was found to be up to 4.1% and 3.8%, respectively (data not shown), and therefore, a γ value of 1% was used for the frequency sweep and temperature sweep tests.

The sol–gel transformation of the coacervate phases was investigated by temperature sweep test, where Gí and Gíí were monitored at ω = 1 Hz and γ = 1% with decreasing temperature from each phase separation temperature to 3 °C and then back to the phase separation temperature at controlled cooling or heating rates of 0.012, 0.016, 0.025, 0.033, and 0.05 °C/min, and the gelling and melting points (T_g and T_m, respectively) of each coacervate phase were determined. Complex modulus (G^*) was obtained at 3 °C and used to compare the overall rigidity of the gels transformed from the coacervate phases.

$$G^* = \sqrt{(G')^2 + (G'')^2} \quad (1)$$

The dynamic oscillatory behavior of the gels obtained by cooling the coacervate phases to 3 °C was also examined by frequency sweep tests at γ = 1% in the range of 0.1–10 Hz.

2.3.2. Stress relaxation analysis

Stress relaxation test was conducted to investigate viscoelastic behavior of FG-GA coacervate gels. 1% strain was applied to all of the gels and held constant with time. Then, stress (τ) was monitored as a function of time for 300 s at 3 °C and ω = 1 Hz.