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Dynamic rheological and structural characterization of fish gelatin – Gum arabic coacervate gels cross-linked by tannic acid

Mohammad Anvari [a, b], Donghwa Chung [c, d, *]

[a] Department of Marine Food Science and Technology, Gangneung-Wonju National University, Gangneung, Gangwon 210-702, Republic of Korea
[b] School of Food Science, University of Idaho, 606 Rayburn St., MS 2312, Moscow, ID 83844, United States
[c] Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang 232-916, Republic of Korea
[d] Institute of Food Industrialization, Institutes of Green Bio Science and Technology, Seoul National University, Pyeongchang 232-916, Republic of Korea

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ABSTRACT

The propose of this study was to investigate the rheological and structural properties of fish gelatin (FG)–gum arabic (GA) complex coacervate gels treated with oxidized or non-oxidized tannic acid (OX-TA or NO-TA, respectively) at different concentrations ranged from 0.0 to 0.3% (v/v), using compositional, dynamic oscillatory rheological, and Fourier transform infrared (FTIR) analyses. The results revealed that the degree of NO-TA incorporation for cross-linking with FG via hydrogen bindings into the system was more pronounced than affinity of OX-TA for reaction through covalent bindings as evidenced by greater loss in volume fraction, moisture content, and biopolymer content values into the FG–GA coacervate phase. FTIR analysis showed addition of NO-TA led to more molecular disorder into the system, so that higher concentrations (0.2 and 0.3%) of the cross-linker disrupted electrostatic interactions through formation of stronger hydrogen bindings with proteins. Rheological results mentioned that addition and enhancement of (NO- or OX-) TA concentration improved gelling ability and mechanical properties of the FG–GA coacervate gels. However, frequency sweep test results implied that all the gels obtained can be classified as weak gels with shear-thinning behavior. Based on weak gel model, the gels obtained by treated FG–GA coacervates with NO-TA had more developed network structures and stronger intermolecular connectivities than those of obtained by the coacervates modified by OX-TA. The results of the current study provide basic knowledge necessary for the use of reinforced FG–GA complex coacervate gels in many useful applications, such as microencapsulation and hydrogel formation, in food and pharmaceutical industries.

1. Introduction

During last decade, interactions between proteins and polysaccharides have received increasing attention because of their important role in produce or develop novel products, such as bioactive delivery devices, fat replacers, meat analogues, gels, emulsions, and edible films in food and pharmaceutical industries (Anvari, Pan, Yoon, & Chung, 2015). When the two biopolymers are mixed together in an aqueous environment, they may undergo either attractive or repulsive interactions, depending mainly on biopolymer characteristics, solvent properties, or mixing conditions (Yang, Anvari, Pan, & Chung, 2012). 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 form a two-phase system consisting of complex-rich and solvent-rich phases. The formation of two-phase system by attractive interactions is called associative separation, which is also called complex coacervation or precipitation for the separation of liquid- or solid-state insoluble complexes, respectively (McClements, 2006; Yang et al., 2012).

Protein–Polysaccharide complex coacervations have been considered extensively for the development of food-grade delivery systems to encapsulate, protect, and deliver bioactive compounds (Chen, Li, Ding, & Suo, 2012). For these applications, in order to obtain a suitable bioactive compounds release profile and controlled delivery in the human gastrointestinal tract, the parameters to form desired complex coacervates by biopolymers have

* Corresponding author. Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang 232-916, Republic of Korea.
E-mail address: dchung@snu.ac.kr (D. Chung).

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been optimized (Thimma & Tammishetti, 2003). The main problem of the technique is that complex coacervates are highly unstable under different conditions and that chemical cross-linking is necessary to stabilize them (Sanchez & Renard, 2002). Cross-linking by chemical agents is a sound process that can be applied to stabilize and modify microstructure and functional properties of complex coacervate for the specific utilizations, such as release refraining or promoting of bioactive components (Chen et al., 2012). In light of reinforcing the complex coacervates, chemical cross-linking agents, such as formaldehyde and glutaraldehyde have been successfully used through linking hydroxyl residues on polysaccharides or amine residues on the proteins (Farris, Song, & Huang, 2010; Farris et al., 2011). However, genotoxic effects on use such chemical cross-linkers in food systems is of great concern (Speit, Neuss, Schutz, Frohler-Keller, & Schmid, 2008). Furthermore, previously, it has been shown that the strength of polysaccharide-protein complex coacervate can be improved by cross-linking of polysaccharides using an enzyme (Chen et al., 2012; Selinheimo, Lampila, Mattinen, & Buchert, 2008), but, the high cost of the enzymes has limited their further applications (Cao, Fu, & He, 2007). Thus, polyphenols that are abundant in plants have been received attention as natural cross-linkers. The interactions between phenolic compounds and proteins play an important role in the processing of certain food products (Balange & Benjakul, 2010). For instance, tannin, which is used as a food additive, contains sufficient hydroxyls and other groups such as carboxyls to form strong complexes with proteins and other macromolecules (Kroll, Rawel, & Rohn, 2003). Combination of tannins with protein solutions (e.g., gelatin and soy protein isolate) under different conditions has been widely investigated in order to improvement of mechanical and gel properties of the proteins (Aewsiri, Benjakul, Visessanguan, Wierenga, & Gruppen, 2010; Balange & Benjakul, 2010; Cao et al., 2007; Kroll et al., 2003). Moreover, previous studies have shown that different forms (oxidized or non-oxidized) of the tannins can modify functional properties (e.g., emulsifying and gelling abilities) of the proteins, where quinone (oxidized form of tannic acid) through formation of covalent bonds between protein molecules improved gel strength and emulsifying properties of fish gelatin (Aewsiri et al., 2010; Balange & Benjakul, 2010). Although, the gelatin treated with non-oxidized tannic acid (NO-TA) showed greater *in vitro* antioxidant activity, which as associated with higher degree of incorporation of cross-linker in gelatin modified with NO-TA (Aewsiri et al., 2010). To the best of our knowledge, however, less information are available about using of the tannins as a cross-linker into protein-polysaccharide mixtures.

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 (Yang et al., 2012). The formation of soluble or 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 showed greater thermo-stability (higher gelling and melting temperatures) and gel strength under gel state by decreasing the phase separation temperature from 40 to 10 °C (Anvari et al., 2015). 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, the objective of this study was to obtain better fundamental understanding about influence of tannic acid (TA), as a natural cross-linker of proteins, on rheological and structural characteristics of FG–GA coacervate gels. Consequently, specific applications of the FG–GA complex coacervate for food-grade delivery systems can be achieved without the addition of toxic chemicals in the future.

2. Materials and methods

2.1. Materials

Fish gelatin (FG, from cold water fish skin) was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and gum arabic (GA, from Acacia tree) and tannic acid were purchased from Carl Roth GmbH (Karlsruhe, Germany). The viscosity average molecular weights of FG and GA, determined by viscometry using Ostwald's viscometer and Mark–Houwink equation in our preliminary experiments, were 58 and 382 kDa, respectively (Nishihara & Doty, 1958). Sodium azide (NaN_3) was obtained from Daehung Chemicals & Metals (Siheung, Korea).

2.2. Preparation of FG–GA complex coacervate mixture

The aqueous FG–GA coacervate mixtures were prepared according to a method published by Yang et al. (2012) at FG:GA ratio of 1:1 (w/w), total concentration of 2% (w/v), pH 3.55, and 40 °C. Sodium azide was added as a preservative to prevent bacterial growth at a concentration of 0.02% (w/v). The resulting mixtures were incubated at 40 °C in a shaking water bath at 100 rpm for 24 h and then, treated with different forms (oxidized and non-oxidized) of TA.

2.3. Treatment of FG–GA complex coacervate mixture by TA

The TA was dissolved in distilled water at given concentrations and the pH of the solutions was adjusted to 9 and 3.55 for preparation of oxidized TA (OX-TA) and non-oxidized TA (NO-TA), respectively. The prepared TA solutions at pH 9 were placed in a water bath at 40 °C and oxygenated for 2 h to convert TA to quinone. The solutions were then adjusted to pH 3.55 with acetic acid (50%) and referred to as OX-TA.

The OX-TA or NO-TA aqueous solutions were added to the FG–GA coacervate mixtures at final concentrations of 0.0, 0.05, 0.1, 0.2, and 0.3% (v/v), which were then incubated at 40 °C for 2 h, followed by phase separation at 10 °C (as the best phase separation temperature) for 24 h.

2.4. Volume fraction and composition of treated FG–GA coacervate phase

The changes in the volume fraction ($\varnothing_c$, %) of coacervate phase was determined as a function of TA concentrations by the following equation:

$$\varnothing c(\%) = \frac{V_c}{V_t} \times 100 \quad (1)$$

where V_c and V_t were the volumes of coacervate phase and total biopolymer mixture, respectively. The moisture content and amounts of FG and GA in the coacervate phase were measured by drying in an oven at 105 °C for 24 h and by using the Lowry method (Lowry, Rosebrough, Farr, & Randal, 1951) and phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), respectively. All measurements were performed at least in triplicate.