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Phase behaviors involved in surimi gel system: Effects of phase separation on gelation of myofibrillar protein and kappa-carrageenan

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ABSTRACT

Phase behaviors of mixtures of myofibrillar protein and κ-carrageenan at different mixing ratios and temperatures were examined by digital images and confocal scanning laser microscopy, showing that that the extent of phase separation was enhanced as the ratio of polysaccharides and temperature increased. The zeta potential of the mixtures became less negative as the protein ratio increased, and the complex became saturated at or above the protein/κ-carrageenan ratio of R_4 (3.2%:0.8%). Gelation process performed by dynamic rheological analysis demonstrated that the presence of carrageenan decreased the gelation temperature but increased the storage modulus. Analysis of the microstructures of the mixed gels showed that the networks were significantly influenced by the concentrations of κ-carrageenan. The present work could be applied to evaluate the mechanism of competition between phase separation and gelation in mixtures of proteins and polysaccharides.

1. Introduction

Co-solubility, complex formation, and thermodynamic incompatibility are the three types of interactions between proteins and polysaccharides (De Kruif & Tuinier, 2001). Many studies on protein–polysaccharide phase behaviors have concentrated on the liquid state, utilizing solutions or emulsions (Jones & McClements, 2011; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003; Turgeon, Schmitt, & Sanchez, 2007) of whey proteins, β-lactoglobulin, sodium caseinate, egg white protein, soy protein and other plant proteins mixed with different kinds of polysaccharides (Evans, Ratcliffe, & Williams, 2013). However, there exist some deficiencies in the studies on protein–polysaccharide phase behaviors in gel systems, especially, with proteins derived from muscle. One of the most typical representatives of this kind of food is surimi products, and many reports have examined the effects of adding polysaccharide hydrocolloids on the textures of surimi products. Moreover, because the physicochemical properties of fish products can be modified with additives such as carbohydrates and proteins, applications of food hydrocolloids in fish products offer opportunities to develop novel products based on surimi or restructured product technology (Martín-Sánchez, Navarro, Pérez-Álvarez, & Kuri, 2009; Ramírez, Uresti, Velazquez, & Vázquez, 2011). The interactions of proteins with polysaccharides, govern the solubility of biopolymers, the ability to form viscoelastic gels, and other physicochemical properties (Tolstoguzov, Damodaran, & Paraf, 1997).

Understanding the phase behavior of food hydrocolloids and proteins may contribute to improving the texture of gelled food and could be helpful for innovative development of new structures for foods (Turgeon et al., 2003). Up to now, few studies have examined phase behaviors during the gelation process in surimi gelling systems. Indeed, phase separation processes have always been applied for structuring processed food, and it could be feasible to generate and control the structural elements of foods at different length scales (Van den Berg, Rosenberg, Van Boekel, Rosenberg, & Van de Velde, 2009). Moreover, the complicated interactions between the phase separation and gelation processes could also be utilized to alter the microscopic network structure of foods (Jin, Xu, Ge, Li, & Li, 2015).

Myofibrillar proteins (salt-soluble proteins) are the main components of surimi obtained from fish muscle, contributing to the three-dimensional, viscoelastic gel matrices of these products. Myofibrillar proteins play an important role in maintaining quality and developing processed meat product during heat-induced gelation with unfolding, aggregation, and formation of interwoven network. These functional properties are the major factors that contribute to the palatability or sensory perception of processed meat products (Khoury, 2004).

κ-Carrageenan is a linear sulfated polysaccharide consisting of repeating units of galactose and 3, 6-anhydrogalactose, extracted from red algae (Trius, Sebranek, & Lanier, 1996) that belongs to the carrageenan family. κ-carrageenan is widely used in the food industry for a broad range of applications owing to its water-binding, thickening, and gelling properties, and the effects of carrageenan addition on the functional properties of meat products have been the subject of

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numerous studies (Verbeken, Neirinck, Van Der Meeren, & Dewettinck, 2005). Pérez-Mateos, Solas, and Montero (2002) found that the addition of κ-carrageenan could significantly influence the texture and microstructure of fish (blue whiting) mince gels. κ-Carrageenan has been shown to interact with salt-soluble meat proteins to increase the compressive force of gels, and generated better texture of salt-soluble meat proteins and κ-carrageenan mixed gels (DeFreitas, Sebranek, Olson, & Carr, 1997). Moreover, κ-carrageenan has been reported to improve the gel strength of Alaska Pollock fish protein gelatin (Hunt & Park, 2013). In muscle protein gels, *l*-carrageenan probably forms an independent network, which supports the principal structure formed by proteins during gelation (Gómez-Guillen, Solas, Borderías, & Montero, 1996). κ-Carrageenan was added to blue whiting mince to study the distribution in gels, and was found mainly inside the cavity interiors and formed a continuous structure (Montero, Hurtado, & Pérez-Mateos, 2000).

Here, we aimed to examine changes in phase behaviors and gelation at different temperatures, using myofibrillar protein and κ-carrageenan as a model in a surimi gelling system. Additionally, the rheological behaviors of myofibrillar protein and κ-carrageenan mixtures were monitored, and the microstructures and secondary structures of the complex gels were examined to determine whether these structures were affected by the addition of polysaccharides.

2. Materials and methods

2.1. Materials

Frozen Alaska Pollock surimi (grade AAA) was purchased from Jincan Foods Co., Ltd. (Qingdao, China). The surimi was maintained at − 20 °C until use. κ-Carrageenan was purchased from Yantai SHELI Hydrocolloids Co., Ltd. (Yantai, China). All of the chemicals used were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of myofibrillar protein

Three-hundred-gram frozen surimi was thawed at 4 °C for 4 h and then cut into small cubes (of about 3 cm) that were chopped at a speed of 1500 rpm for 5 min in a vertical vacuum cutter (Model UM 5, Stephan Machinery Co., Hameln, Germany). The surimi paste obtained was homogenized with 5-fold (w/v) volumes of Tris-maleate buffer (50 mM KCl/20 mM Tris-maleate, pH 7.0, containing 3 mM NaN_3 to prevent bacterial growth), and then was centrifuged at 8000 $\times g$ using Anke GL-20G-II centrifuge (Anting Scientific Instrument Factory, Shanghai, China) at 4 °C for 10 min. The precipitate was immersed in Tris-maleate buffer (0.6 M KCl/20 mM Tris-maleate, pH 7.0, 3 mM NaN_3), and then homogenized at 10,000 $\times g$ for 3 min. The homogenate was kept at 4 °C for 30 min to sufficiently dissolve the proteins, and then was centrifuged at 8000 $\times g$ at 4 °C for 20 min. The supernatant was then precipitated with 10-fold (w/v) volumes of distilled water. The precipitate obtained was myofibrillar protein, and then dissolved in 0.6 M KCl for use. The content of protein was measured as 40.21 ± 0.43 mg/mL (approximately 4 wt%), according to the Biuret method (Gornall, Bardawill, & David, 1949).

2.3. Preparation of myofibrillar protein/κ-carrageenan mixtures

Myofibrillar protein stock solution was homogenized at 4 °C for 10 min. κ-Carrageenan dispersions (4.0 wt%) were obtained by dispersing κ-carrageenan powder in distilled water through mechanical agitation at 4 °C for 2 h and then placed in a refrigerator at 4 °C until use. Mixtures of myofibrillar protein and κ-carrageenan solutions were prepared in different ratios and then homogenized at 4 °C for 10 min. By changing the ratio of the two components, mixtures were prepared as R_1 to R_7, corresponding to ratios of 3.8%:0.2%, 3.6%:0.4%, 3.4%:0.6%, 3.2%:0.8%, 3.0%:1.0%, 2.8%:1.2%, and 2.5%:1.5% (protein: κ-carrageenan). Each of the mixtures was then divided into three parts. The first part was incubated for 24 h at 4 °C, the second part was heated at 45 °C for 15 min above the gelation temperature of κ-carrageenan and then cooled at 4 °C, and the third part was heated at 80 °C for 15 min and then cooled at 4 °C. Digital images of the final state were collected.

2.4. Confocal laser scanning microscopy (CLSM)

The myofibrillar protein/κ-carrageenan mixtures were prepared as described above. According to the method described by Zhang, Li, Wang, Xue, and Xue (2016), a mixture of rhodamine B (0.001%) in distilled water was used for noncovalent labeling. The sample was allowed to rest for 1 h within a closed receiver in the dark, washed with distilled water, and covered with a glass cover slip. A small portion of paste was cut and then spread on a glass slide with a rolling pin, following by immediate addition of the dye solution. CLSM was carried out in fluorescence mode, using a Fluo View FV1000 inverted microscope (Olympus, Japan) equipped with He-Ne lasers. Observations of the proteins were performed by excitation of rhodamine B at 544 nm, with the emission recorded between 550 and 750 nm.

2.5. Zeta potential analysis

Electrophoretic mobility (particle velocity in an electric field) for myofibrillar protein/κ-carrageenan mixtures were investigated using a Zetasizer NanoZS80 (Malvern Instruments, Worcestershire, UK). Mixed systems were tested as a function of the biopolymer mixing ratio and heating temperature. All samples were prepared at a total biopolymer concentration of 0.1% (w/v) and then added into the sample cell; the first part was incubated at 4 °C, the second part was heated at 45 °C for 15 min and then cooled at 25 °C, and the third part was heated at 80 °C for 15 min and then cooled at 25 °C for measurement.

2.6. Rheological measurements of myofibrillar protein/κ-carrageenan mixtures

Dynamic viscoelastic measurements of myofibrillar protein/κ-carrageenan mixtures were carried out on an MCR101 rheometer (Austria Anton Paar Ltd.). A parallel plate with 50 mm diameter and a 1.0-mm gap was used to measure the dynamic viscoelastic parameters. The temperature was controlled by a water bath connected to a Peltier system in the bottom plate. The viscoelastic properties of samples were measured within the linear viscoelastic (LVE) region, which was determined through strain sweep tests carried out at 1 Hz. A thin layer of low-viscosity silicone oil was used to prevent evaporation during measurements. An equilibration time of 2 min was maintained before each measurement.

Mixtures were loaded onto the measuring apparatus at 20 °C and equilibrated at this temperature. Solutions were heated from 20 °C to 80 °C at a rate of 5 °C/min, held at 80 °C for 30 min, cooled to 20 °C at a constant rate of 5 °C/min, and maintained at this temperature for 15 min. The storage modulus (G') was measured continuously during the entire thermal treatment at a frequency of 1 Hz and a strain of 2%. A frequency sweep from 0.1 to 100 Hz was performed at a strain of 2% at ambient temperature (20 °C) before and after gelation at 80 °C for 30 min.

2.7. Scanning electron microscopy (SEM)

Gels obtained by heating at 80 °C for 30 min were cut into 2–3-mm-thick sections and fixed with a 3% glutaraldehyde solution. The samples were then rinsed for 1 h in distilled water before being dehydrated in a gradient ethanol series of 50%, 70%, 80%, 90%, and 100% (*v*/v). The dried samples were mounted on a bronze stub and sputter-coated with

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