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Interaction between carrageenan/soy protein isolates and salt-soluble meat protein

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ABSTRACT

The effects of combined κ-carrageenan (CAR) and soy protein isolate (SPI) on gel strength, water loss, the rheological properties, particle size, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and Raman spectroscopy of salt soluble meat protein were evaluated. Combination of CAR/SPI increased gel strength and water retention of SSMP (salt-soluble meat protein), suggesting that molecular interaction may have occurred. However, no indications of specific molecular interactions were observed upon addition of stabilizing/destabilizing reagents. The results showed that hydrophobic force affected the water retention of salt soluble protein gel, and hydrogen bond significantly affected the formation of gel. The analysis of the Raman spectra showed that adding of combined CAR/SPI in SSMP decreased the content of α-helix and increased the content of β-sheet. SDS-PAGE did not show significant interactions between SSMP and CAR/SPI indicating that the property improvement of combined SSMP/SPI/CAR gel was not related to chemical reaction. Instead, physical rearrangement process and electrostatic attraction resulted from SPI, CAR and SSMP molecule could contribute to the gel quality improvement.

1. Introduction

Salt-soluble meat proteins (SSMP) play a significant role in emulsification stability, rheological behaviors and microstructure characteristics of chopped meat products with better water-fat binding, desirable texture and unique taste profile (Sun et al., 2012). These characteristics are vital to assure product quality and develop new chopped meat products. However, the functions of SSMP in meat products could be influenced by many factors including salt concentration, pH, heating conditions, processing technology and application of non-meat ingredients. Because of relatively reasonable cost compared to lean meat and their better functional properties, non-meat ingredients and additives have become more and more popular (Kurt and Kilincceker, 2012; Youssef and Barbut, 2011). The thermal properties improving of SSMP by addition of non-meat ingredients may indicate interaction between meat proteins and non-meat ingredient (DeFreitas et al., 1997).

Non-meat ingredients and SSMP interactions occur widely in biological systems. Their interaction plays a role in

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determining the functional properties of food systems (Stainsby, 1980). Interactions between one kind of ingredient (CAR, flaxseed gum, SPI and glutinous rice flour) and meat protein have been well documented (Chen et al., 2007; DeFreitas et al., 1997; Vittadini et al., 2005; Youssef and Barbut, 2011). They suggested that the ingredients increased water holding capacity of meat emulsions by holding the water in interstitial spaces of the gel network rather than by chemical interactions with meat protein and improved qualities of chopped meat products. CAR is a polysaccharide extracted from red seaweeds. Previous study has shown that CAR appeared to be the best fat substitute among 13 different edible gum-hydrates (Hsu and Chung, 2000). Soy proteins have also been widely used in processed meat products for many years due to their higher protein content and better functional properties (Das et al., 2008; Kurt and Kilincceker, 2012; Liu, 2000; Youssef and Barbut, 2011). Previous studies also have suggested that the combination of CAR/SPI improved ground pork patty quality compared with separate supplementation of CAR or SPI (Gao et al., 2015). However, almost no studies have reported about the mechanism of the interaction between SSMP and the combination of CAR/SPI. Understanding the mechanisms involved in the interaction between SSMP and CAR/SPI is key to better further develop their functional properties and apply these components into food products. Therefore, the objectives of this study was to evaluate the effects of combination of CAR/SPI on SSMP using dynamic rheological properties, particle size, hardness, water retention and molecular forces and determine the nature of the interactions occurring in mixed gels formed by combing SSMP and CAR/SPI.

2. Materials and methods

2.1. Materials

Fresh pork thigh meat was purchased from a local market (Sushi Food Co., Ltd., Nanjing, China). The meat was trimmed of excess fat and connective tissue, ground through a 4 mm plate with the addition of 1.5% salt and 20% ice water. Meat samples were stored at 4 °C for 1 d after slaughter. CAR was provided by Cargill Asia Pacific Food System Co., Ltd. (Beijing, China). SPI was obtained commercially from Linyi Biological Products Co., Ltd. (Linyi, Shandong, China).

2.2. SSMP extraction

SSMP was extracted essentially according to DeFreitas et al. (1997) with slight modification. The meat samples were homogenized in a Blender in three volumes isolation buffer (0.5 M NaCl and 17.8 mM $Na_5P_3O_{10}$, pH 8.3) for 60 s at medium speed. The slurry was kept at 4 °C for 2 h and then centrifuge (12,000 × *g* at 4 °C) using a freezing centrifuge for 60 min (Beckman Coulter model Avanti J-26SXP, Beckman Instruments Inc., St. Louis, MO). The protein extract was strained through three layers of cheesecloth. Protein concentrations of meat and supernatant were determined with the BCA Protein Assay Kit (Sigma–Aldrich, USA).

2.3. Combined gel preparation

The final SSMP were standardized to an 8 μg/μl by diluting with the same isolation buffer as the protein extraction. The control treatment consisted of SSMP without CAR/SPI. All treatments were treated as the followings: A1 (control, no reagent), A2 (0.05 M sodium thiocyanate (NaSCN) to neutralize charges), A3 (10% propyl glycol (PG) to disrupt hydrophobic forces), A4 (0.02 M 2-mercapto ethanol (2-MeSH) to reduce disulfide bonds), and A5 (0.05 M urea to disrupt hydrogen bonds). On the basis of the control groups, adding CAR/SPI made combined sols D1, D2, D3, D4 and D5. According to previous paper, the concentration of CAR and SPI were 0.7% and 5% respectively (Gao et al., 2015). Reagents were solubilized in SSMP extracts firstly, followed by the addition of CAR/SPI if required.

All treatments were stirred and homogenized 60 s at medium speed (speed setting 6) by a blender. The samples (30 mL) were added to 50 mL containers. The containers were allowed to equilibrate at room temperature for 2 h and then divided into two groups. One group was for further analysis. The other one was placed in a water bath at 80 °C and heated until the internal temperature of the gel reached 80 °C. The gels were removed, and placed in an ice bath and held overnight at 2 °C.

2.4. Combined gel evaluation

Combined gel evaluation contained water losses and hardness parameter.

Water losses, revealing the water-holding ability, were measured as the percentage of supernatant liquid after centrifugation. The gels were placed in tubes, weighed and centrifuged at low speed (500 × *g* for 15 min, 4 °C) to prevent disruption of the gel by centrifugal force.

Texture parameters of heated combined gels were carried out using a texture analyzer XT Plus at room temperature (Stable Micro Systems Ltd., Godalming, Surrey, UK). The gels (each 10 mm high and 25 mm diameter) were compressed twice to 50% of original height at a constant speed of 60.0 mm/min. A P50 probe (50 mm stainless cylinder) was used to compress the gels. Other parameters were set as described by Gao et al. (2014) with a slight modification: pre-test and post-test speed, 5.0 mm/s; withdrawal speed, 1.0 mm/s; testing interval between two compressions, 5.0 s; trigger type, auto-20 g; and data acquisition rate, 200 points/s. Samples were placed on the center of TPA plate and subjected to two-cycle compression test. Peak load after compressing were recorded. The maximum force making the gel rupture was described as hardness. The average of at least six replicates was recorded.

The attribute of hardness was determined and computed from the curve adopting the method of Brady et al. (1985). The results of TPA were processed by Texture Expert Exceed 2.64a inner macro TPA.MAC. The mean value of six readings for each TPA was reported.

2.5. Dynamic measurement

The thermo-rheological properties of raw sols were assessed using a dynamic rheometer (MCR301, Anton Paar Ltd., Graz, Austria) with an internal heating/cooling circulating water bath and internal measuring devices that transferred the temperature and oscillation readings directly to a linked computer. A parallel stainless steel plate geometry (50 mm) was used with gap size of 1.0 mm. A 10 g sample of raw sol was placed between the two flat plates with the perimeter treated with thin layer of liquid silicone oil to prevent dehydration of sample edge and moisture evaporation from the sample. Then the entire gelling unit was enclosed in an insulated shell to further minimize heat loss. After initial equilibration at 20 °C for 5 min, the sample was heated continuously from 20 °C to