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Thermal gelling properties and mechanism of porcine myofibrillar protein containing flaxseed gum at different NaCl concentrations

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ABSTRACT

This study aimed to investigate the thermal gelling properties of porcine myofibrillar protein (MP) containing flaxseed gum (FG) at different NaCl concentrations. In this work, MP-FG gels with 0.4% FG were prepared with varying NaCl concentrations (0.2–0.6 M). FG enhanced the water holding capacity (WHC) and decreased the gel strength of MP sols at different NaCl concentrations. The particle size of the MP gels increased with FG addition at a low NaCl concentration (0.2 M) but decreased under a high NaCl concentration. Dynamic rheological tests proved that the storage modulus and gelation rates of MP-FG gels were lower than those of MP gels. FG induced the partial transformation of α-helices into β-sheets in the gels under different NaCl concentrations. Scanning electron microscopy images revealed that the MP-FG network became more compact with increasing NaCl concentration. These results provide novel insights into the structural–functional relationships of MP-FG gels at different NaCl concentrations.

1. Introduction

Myofibrillar protein (MP), which mainly consists of myosin and actin, presents important functions in producing a desirable texture and promoting the water holding capacity (WHC) of comminuted meat products, such as sausages. These functions are attributed to the ability of the protein to produce three-dimensional gels upon heating and subsequent cooling (Smith, 1988; Xiong, 1997). Increasing consumer's demand for low-fat products has recently drawn attention to utilizing non-protein ingredients as potential substances to restore product characteristics as fat is removed and water is added (Desmond, Troy, & Buckley, 1998; Mansour & Khalil, 1997).

Hydrocolloids or polysaccharides, such as κ-carrageenan, chitosan, and locust bean gum, which are derived from a variety of plants and microorganisms, have been widely applied as highly effective fat substitutes to develop low-fat ground-meat products with the desirable binding characteristics, texture, and appearance (Pan et al., 2016).

Flaxseed gum (FG) is a type of hydrocolloid used extensively in the food industry. As a heteropolysaccharide, FG contains anionic and neutral polysaccharides (Qian, Cui, Wu, & Goff, 2012) consisting mainly of- xylose, rhamnose, galactose, glucose, arabinose, fucose, and galacturonic acid (Cui, Mazza, & Biliaderis, 1994). Higher relative amounts of neutral polysaccharides, such as xylose, have been observed to enhance the rheological properties of FG by increasing its shear thinning and weak-gel properties (Cui et al., 1994). FG presents great potential applications in meat products because of its favorable hydrophilicity and emulsibility. An understanding of the gelation properties of porcine MPs containing hydrocolloid additives is thus beneficial for the development of comminuted meat products as well as maintaining the quality of meat products.

When FG is applied to a meat system, protein-polysaccharide interactions play a significant role in the three-dimensional gel structures obtained. Related studies in non-meat products have revealed that the major forces in gum-protein binding and gelling processes are electrostatic and hydrogen bonding (Imeson, Ledward, & Mitchell, 1977). These interactions have been proposed to be formed between negatively charged carboxyl groups in

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the FG molecules and positively charged side chains of the amino acids in the protein (Chen, Xu, & Wang, 2007). The results indicated the interactions existed between FG and MP were caused by electrostatic attraction (Sun, Li, Xu, & Zhou, 2011).

Investigating the interactions between meat proteins and other ingredients is crucial to promote the quality and development of new processed meat products. In previous studies, FG showed the ability to enhance the WHC of MPs (Sun et al., 2011) and salt-soluble meat protein gels (Chen et al., 2007), as well as increase the emulsification properties of soybean protein isolate (Pan et al., 2016). Our previous research also suggested that pH plays a key role in MP-FG systems to meet the textural and WHC requirements for meat processing (Pan et al., 2016). Among the factors affecting the gelation properties of MP, ionic strength has been considered to be highly important because it influences the solubility of the protein (Sun & Holley, 2011). Addition of salt improves the solubility of meat batter, resulting in improvements in gelling properties (Guzey & Mcclements, 2006). However, limited reports are available on the mechanism of the interaction between meat proteins and FG under different NaCl concentrations.

The objectives of the present study are to evaluate (i) the effects of NaCl levels on the properties of heat-induced MP-FG gel and (ii) the changes responsible for these effects at the molecular level. We also propose a mechanism of thermal gelation for MP-FG as affected by NaCl.

2. Materials and methods

2.1. Materials

FG (powder, purity 99.8%) was provided by Sinkiang Luqi Biotechnology Ltd. (Sinkiang Province, China). The viscosity of 1% FG solution (w/v) is 17,000 mPa s at 25 °C. Fresh pork *longissimus dorsi* was purchased from Su Shi Meat Corporation through a local supermarket, and MP extraction was done within 60 min of purchase.

2.2. Extraction of MP

MP was extracted from pork *longissimus dorsi* at 4 °C using a modified procedure originally reported by Doerscher, Briggs, and Lonergan (2004). The formulation of cold extracting solution (pH 7.0) is 100 mM KCl, 20 mM potassium phosphate, 2 mM $MgCl_2$ and 1 mM ethylene glycol bis (β-amino-ethyl) ethertetra-acetic acid (EGTA). Homogenized for 1 min at 10,000 rpm by a homogenizer (T25 digital Ultra-Turrax, IKA, Germany) and centrifuged at 1500×*g* for 15 min. The procedure was repeated twice. After that, the sediment was treated with extracting solution and homogenized.1% Triton X-100 was added into the mixture. The final MP pellet was slowly stirred into 50 mL sodium phosphate buffer (0.6 M NaCl, 50 mM sodium phosphate, pH 6.0). The protein concentration was measured using the biuret method (Bowker, Gamble, & Zhuang, 2015), and the MP obtained was kept at 4 °C and tested within 3 days after extraction.

2.3. Preparation of MP-FG sols

FG was added to the MP solutions to produce MP-FG sols (MP concentration, 4%; FG concentration, 0% or 0.4%). NaCl was then added to the sols at a concentration of 0.2, 0.3, 0.4, 0.5, or 0.6 M. All of the samples were stirred, homogenized and kept at 4 °C for 5 h prior to particle size, dynamic rheology measurements and Raman spectroscopic analysis.

2.4. Preparation of MP-FG gels

MP-FG sols prepared as described above were separately added to test tubes, heated in a water bath from 20 °C to 80 °C, and then kept at 80 °C for 20 min. The obtained gels were placed in an ice bath and kept overnight (12 h) at 4 °C prior to WHC, gel strength determination and scanning electron microscopy (SEM).

2.5. WHC

The WHC of the samples was measured using the protocol described by Kocher and Foegeding (1993). Exactly 5 g of gel was placed in a 10 mL centrifuge tube and centrifuged at 10,000×*g* for 10 min at 4 °C. WHC was then calculated as the percentage of a gel's weight retained after centrifugation relative to its initial weight. Each sample was measured in triplicate.

2.6. Gel strength

The strength of the gels was analyzed using a TA-XT Plus texture analyzer (TA.XT Plus, Stable Micro Systems, UK) at ambient temperature (approximately 20 °C). The gels were subjected to a compression test using a cylindrical probe (P/0.5 inch, aluminum) with a trigger type button under a 1.5 mm/s pre-test speed, 1.0 mm/s test speed, 1.0 mm/s post-test speed, 4.0 mm distance, and a 5 g trigger force. The peak load after compression was recorded, and the maximum sustained compression force was considered as the gel strength. At least seven replicates were performed, and the average value of these measurements was recorded as the gel strength.

2.7. Particle size measurement

Particle size measurement was carried out according to Li, Kang, Zhao, and Xu (2014), using a Mastersizer laser light scattering analyzer (Mastersizer 2000, Malvern Instruments Ltd., Worcester shire, UK). A modicum of each sample was dispersed in distilled water, and particle distributions were determined over three successive readings. Particle size was expressed as D_{10}, D_{50}, and D_{90} (accumulated value to achieve particle size volume percentages of 10%, 50%, and 90%, respectively).

2.8. Dynamic rheological measurements

Dynamic oscillatory measurements were performed using a rheometer (Anton Paar, Physica MCR 301, Austria) in oscillatory mode, as described by Westphalen, Briggs, and Lonergan (2006) with slight modifications. A 25 mm parallel steel plate geometry with a 500 μm gap was used. A constant oscillation frequency of 0.1 Hz and a strain of 0.5% were applied to monitor the storage modulus (G′) of the samples, and a tolin was used to prevent water evaporation. The samples were heated from 20 °C to 80 °C at a rate of 2 °C/min and then cooled from 80 °C to 20 °C at a rate of 4 °C/min. Each measurement was performed in triplicate and three replicates were performed for each sample.

2.9. Raman spectroscopic analysis

The Raman spectrum of each sample was measured on an FRA 106/S FT-Raman spectrometer equipped following the modified procedure of Shao, Zou, Xu, Wu, and Zhou (2011). The secondary structures of each sample were determined as percentages of α-helix, β-sheet, β-turn, and unordered conformations (Alix, Pedanou, & Berjot, 1988).