



Insight into the mechanism of myofibrillar protein gel improved by insoluble dietary fiber



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ABSTRACT

The effects of sugarcane insoluble dietary fiber (SIDF) on water holding capacity (WHC), gel strength, microstructure, and secondary structures of myofibrillar protein (MP) gels were studied. The gel strengths and WHC were improved with the increase of SIDF content. Paraffin section showed that SIDF did not have direct contact with the MP and was simply trapped in the gel network. The SEM micrograph indicated that water channels appeared through the pure MP gel network, so the integrity of gel network and the gelation quality would be deteriorated. The SIDF acted as active dehydrating agent and change the water distribution. Hence, the water channels disappeared in the internal of heat-induced gelation, which promote to aggregate compact and well-linked gel structure. The analysis of T_2 relaxation revealed that T_{21} relaxation time of MP gel decreased significantly with SIDF addition, which indirectly suggested that the gel with SIDF had better three-dimension network and could bind the water more firmly. Raman spectroscopic study showed the SIDF addition resulted in the left shift and right shift of characteristic peak in Amide I and Amide III of the gelation, respectively. Moreover, the intensity of characteristic peak in aliphatic residues band also had a significant decrease. The changes of Raman spectroscopy reflected the SIDF addition have the positive effect on forming firm and compact MP gel.

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1. Introduction

Protein is considered essential to optimal human growth and development, especially the brain and intellectual development. Processed meat product was richness in all the 20 basic amino acids without limiting amino acids and mainly resource of dietary protein intakes, while it contains 15–30% animal fat which has a high content of cholesterol and saturated fat, especially frankfurter. Diets with high animal fat contents had been related to an increased incidence of obesity, hypertension, cardiovascular diseases, and coronary heart diseases (Siri-Tarino, Sun, Hu, & Krauss, 2010). However, due to the fat functional properties: improve product quality by reducing cooking loss and providing appropriate flavor substance, the low-fat product which simply using water substitute for animal fat was unacceptable to consumers. Therefore the meat industry faces a new challenge: finding an additive to improve low-

fat healthy meat product having good textural properties and, especially, overall acceptability.

Because of low price and well processing characters, the polysaccharide was the mainly additive to improve the quality and acceptability of low-fat meat products at present (Ramírez, Uresti, & Vázquez, 2011; Talukder, 2015). However, the term polysaccharide refers to a range of substances, including starch, gum, insoluble dietary fiber etc. And the physicochemical properties of polysaccharides were different and various. So the mechanism of polysaccharide improved gel functionality mainly contains: 1) Packing effect: proposed starch improve the physicochemical properties of fish-meat gel through packing effect (Kong, Ogawa, & Iso, 1999). When the temperature reach the gelatinization of starch during heating process, the diameter of starch granules start to grow. Because the surimi had formed gel network, the growth of the starch granules, trapped in the surimi, was limited more than in the free swelled state during heating. The starch gelatinization could exert pressure against the surimi protein network, lead to improve the gel functionality of fish-meat gel. 2) Filling effect: Gum (carrageen, Konjac glucomannan and so on) could self-aggregation

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through hydrogen bonding and hydration and act as good fillers to improve the gel properties. Also same study reported that the kappa carrageen could have interaction with myofibrillar proteins to reinforce the filling effect (Ramírez et al., 2011).

In recent years insoluble dietary fiber has been widely used in low-fat meat product to improve the quality or replace the fat. In addition of the functional characteristics, insoluble dietary fiber is protective against diabetes, obesity, and intestinal disorders (Anderson et al., 2009). Many study reported that the low-fat meat product with insoluble dietary fiber could had similar consumer acceptability as tradition product, like low-fat sausage, low-fat salami, low-fat beef patty (Debusca, Tahergorabi, Beamer, Matak, & Jaczynski, 2014; Gibis, Schuh, & Weiss, 2015; Zhuang, Han, & Zhou, 2016). However, the mechanism of insoluble dietary fiber improved gel functionality could not be explained by the theory above.

China is the third largest nation in producing sugarcane, and sugarcane bagasse is an extremely resource as by-product from sugar production, estimated at 14 million tons per year. Normally, the sugarcane bagasse is used for fertilizer and cattle feed or even abandoned directly. However, sugarcane bagasse contains the large amount of dietary fiber which has beneficial functions on human health. When treated with alkaline hydrogen peroxide (AHP), the whiteness, water-holding capability and oil-binding capability of sugarcane insoluble dietary fiber (SIDF) could be increased sharply. Moreover, SIDF was insoluble and neutral without any taste and odor (Sangnark & Noomhorm, 2003). So SIDF is an ideal dietary fiber to be utilized in meat processed products. Zhuang and Zhou (2016) reported that compare to the pure myofibrillar protein with dense and homogeneous structures, the light microscopy images of gels with SIDF contained large cavities, presumably of SIDF-absorbed water. However, the gel with insoluble dietary fiber had better gel strength and WHC than the pure gel. Therefore, the objective of this study was to investigate the mechanism of SIDF improved gel functionality in MP system.

2. Materials and methods

2.1. Sugarcane insoluble dietary fiber preparation and treatment with alkaline hydrogen peroxide (AHP)

The preparation of sugarcane insoluble dietary fiber with AHP treatment was described as Sangnark and Noomhorm (2003). Sugarcane bagasse (SB) was purchased from a local fruit supermarket in Nanjing. The SB was dried in a fan-forced air-oven (DHG-90338S-III, Shanghai, China) at 65 °C. It was treated with AHP and the final product was ground and separated according to particle size using a sieve shaker. Particles, less than 0.075 mm in diameter, were selected as the sugarcane insoluble dietary fiber (SIDF).

2.2. Extraction of myofibrillar protein (MP)

Fresh pork ham meat (24 h post-mortem, 72.18% moisture, 20.17% protein, 6.75% fat; AOAC 2000) was purchased from Muxuyuan market. The excess fat and connective tissue was cut off, and the meat was stored at -20 °C until required for the MP extraction. The meat ground with blender (GM 200, Retsch, Germany) for 10 s at 2000 rpm/min (repeated three times). Extraction of MP was carried out as described by (Xu, Han, Fei, & Zhou, 2011) with minor modifications. The ground muscle was mixed with four volumes of isolation buffer (10 mM Na₂HPO₄/NaH₂PO₄, 0.1 mM NaCl, 2 mM MgCl₂, 1 mM EGTA, pH 7.0, 4 °C) and homogenized (T25, IKA, Inc., Germany) three times for 30 s at 8000 × g. The homogenates were

filtered through a 20-mesh sieve (0.9 mm) and centrifuged (Model 225, Beckman Coulter, Inc., California, USA) at 5000 × g for 10 min. The precipitant was collected as a crude MP. The aforementioned steps were repeated two times to obtain high-quantity MP. Then the MP pellet was homogenized in four volumes of salt solution (0.1 M NaCl), centrifuged (5000 g for 10 min), and washed three times. The final precipitant was collected as pure MP. The biuret method was used to determine the protein concentration of pure MP using bovine serum albumin as the standard. The MP was diluted to a final protein concentration of 60 mg/mL (0.6 M NaCl, pH 7.0). SIDF was added at 4 concentrations: 0.5, 1, 1.5 and 2 g/100 g of MP, then the blend MP solution were stirred with a power whisk (AHM-P125A, Appliance company Co., LTD, Shanghai, China) to ensure homogeneous distribution of SIDF. The samples were briefly stored at 4 °C overnight.

2.3. Gel texture analysis

The gelling properties of the blending gels were measured according to the method of Liu et al. (2013) with some modifications. The sample solutions were heat-coagulated at 80 °C for 20 min in a water bath (TW20, Julabo Co., Ltd., German). After cooling to room temperature the gel samples were subjected to a compression test by a 0.5-cm-diameter plate probe integrated with a texture analyzer (TA-XT Plus, Stable Micro systems Ltd., Surrey, UK). Typical parameters were as follows: Sequence Title: 1 Return To Start, Test Mode: Compression, Pre-Test Speed: 2.0 mm/s, Test Speed: 1.2 mm/s, Post-Test Speed: 2.0 mm/s; Target Mode: Distance, Distance: 15.0 mm; Trigger Type: Auto (Force), Trigger Force: 10.0 g, Advanced Option: Off, and Point per second: 200. Each sample was analyzed five times.

2.4. WHC

WHC (%) was determined according to the method developed by (Han, Xu, & Zhou, 2014). The gel samples were placed into PVC cylinders with filter paper, which were suspended inside centrifuge tubes and then centrifuged at 10,000 g (Model 225, Fisher Scientific, Pittsburgh, Pa., U.S.A.) for 10 min at 4 °C. The results are reported as percentage (w/w) of water retained after centrifugation. Each sample was analyzed five times.

2.5. Low-field NMR

The sample placed into cylindrical glass tubes (10 mm in diameter) after heating at 80 °C for 20 min in a water bath (TW20, Julabo Co., Ltd., German). The measurements of the transverse relaxation time (T₂) were performed on a Niumag Benchtop Pulsed NMR analyzer (Niumag PQ001; Niumag Electric Corporation, Shanghai, China) operating at 22.6 MHz. The T₂ was measured using a Carr–Purcell–Meiboom–Gill (CPMG) with 32 scans, 12,000 echoes, 6.5 s between scans, and 250 μs between pulses of 90° and 180°. The low-field NMR relaxation curves were analysis with the MultiExp Inv Analysis software (Niumag Electric Corporation, Shanghai, China).

2.6. Light microscopy of gel structures

Sections of samples (8 μm thick) were cut using a microtome (CM1900, Leica, German) and then fixed and stained with hematoxylin–eosin following the procedure outlined by Wu, Xiong, and Chen (2011). Slides observed and photographed using a light microscope mounted with a digital camera (Motic China Group Co., Ltd., Fujian, China).

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