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Improved gel functionality of myofibrillar proteins incorporation with sugarcane dietary fiber

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ABSTRACT

The effects of sugarcane dietary fiber (SDF) on the gelation properties of porcine myofibrillar proteins (MP) were studied to understand its mechanism of action in improving gel functionality. Rheological tests on all composite gels (MP with SDF) showed the visco-elastic nature of MP, but the G′ significantly increased with contents of SDF and with particle size ($P < 0.05$). However, the δ exhibited the opposite effect. Light microscopy suggested that SDF affected moisture distribution in the gel by drawing water from MP and homogenously embedded in gelation. It is proposed that during the heating the more concentrated MP promoted the unfolding of MP chains and facilitated the formation of β-sheet instead of α-helices, leading to a compact and homogenous three-dimensioned network. These results indicated that the SDF changed the water distribution and resulted in the enhanced gelation which reacted to firmly bind SDF and form a synergistic interaction system.

1. Introduction

As consumers become conscious of the relationship between foods and health, processed meat product industry is constantly striving to develop new palatable, nutritionally-sound products with improved functional properties. Generally, the emphasis has been on reducing fat content and fat type (Jimenez-Colmenero, 1996). One method used by the meat processing industry in order to reduce fat content is the replacement with various types of dietary plant fibers. The addition of dietary fiber, replaced animal fat, is desirable not only for their nutritional properties and health benefits, but also for their ability to improve protein gelling functionality and textural properties (Choi et al., 2011; Debusca & Tahergorabi, 2014; Mehta & Ahlawat, 2015). It is recognized that the gelation process of myofibrillar proteins during cooking is responsible for obtaining high quality, finely comminuted meat products. Dietary fiber, common polysaccharides of plant origin, has been accepted by consumers as functional ingredients for their beneficial health effects (Anderson et al., 2009; Schneeman, 1998).

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 (Fu, Gao, & Li, 2013). 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: increases the faucal bulk, stimulates colonic fermentation (Fernandez & Borroto, 1996). When treated with alkaline hydrogen peroxide (AHP), the whiteness, water-holding capability and oil-binding capability of sugarcane dietary fiber could be increased sharply. Moreover, sugarcane dietary fiber (SDF) was insoluble and neutral without any taste and odor (Sangnark & Noomhorm, 2003, 2004). So sugarcane dietary fiber was an ideal candidate for dietary fiber to add in processed meat product.

In previous study, the researchers have focused on utilizing the dietary fiber extracted from different vegetable or fruit by-product in processed meat products. They found that the suitability of dietary fiber incorporated was able to make the blending system decrease cooking loss, modify textural quality and improve rheological property (Hu & Yu, 2015; Ktari, Smaoui, Trabelsi, Nasri, & Ben Salah, 2014; Selani et al., 2016). Ziegler and Foegeding (1991) presented the model that explained the spatial distribution of gelled protein with gelling and non-gelling additives in the protein gel matrix. When the dispersed phase was unconnected with the gel matrix, known as term ingredients “passive” fillers. While the co-ingredient associated directly via non-specific interactions with the primary gelling component (“active” fillers). The studies above indicated that dietary fiber incorporated with myofibrillar protein as “active filler”. Therefore, it is very interesting to

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study how dietary fiber interacted with the gelled protein matrix. The objective of this study was to explain the mechanism of improved gel functionality with SDF by determining the changes in physicochemical property, microstructure and secondary structure of protein gelation.

2. Materials and methods

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

Sugarcane bagasse was purchased from a local fruit supermarket in Nanjing. The dried sugarcane bagasse was cut into 0.5 cm length pieces before the AHP treatment. A 50-g sugarcane bagasse was added in the 5000 ml of AHP solution (pH = 11.5, 1% H_2O_2 W/V) for 12 h as described by Sangnark and Noomhorm (2003) with slight modification. After neutralization with HCl, it was collected by filtration and washed with water. Repeating the AHP-treated step, then drying the material in a fan-forced air-oven at 55 °C. The SDF (cellulose 58.44%, hemicellulose 27.94%, and lignin 9.13%, AOAC 2000) was ground in a centrifugal mill fitted with 40-mesh (420 μm) and 80-mesh (177 μm) screen. The SDF needed in experiment was under 80-mesh (termed 80-mesh SDF) and that between 40-mesh and 80-mesh (termed 40-mesh SDF). The water holding capability of 40-mesh SDF was 11.32 g, and tristimulus color values (L*, a* and b*) were 92.13, − 0.74 and 9.30. While the water holding capability of 80-mesh SDF was 10.49 g, and tristimulus color values (L*, a* and b*) were 93.87, − 0.65 and 6.35.

2.2. Extraction of myofibrillar proteins (MP)

Fresh pork leg meat (24 h post-mortem, 72.18% moisture, 20.17% protein, 6.75% fat; AOAC 2000) was purchased from market. The meat was trimmed of any excess fat and connective tissue and stored at − 20 °C until required for the MP extraction. The meat ground for 10 s at 2000 r/min (repeated three times) using a Waring Blender (GM 200, Retsch, Germany). Extraction of MP was carried out as described by Han, Xu, and Zhou (2014). The ground muscle was mixed with four volumes of isolation buffer (10 mM Na_2HPO_4/NaH_2PO_4, 0.1 mM NaCl, 2 mM $MgCl_2$, 1 mM EGTA, pH 7.0, 4 °C) and homogenized (T25, IKA, Inc., Germany) three times for 30 s at 6000 r/min. The homogenates were filtered through a 20-mesh sieve (0.9 mm) and centrifuged (Model 225, Beckman Coulter, Inc., California, USA) at 3000 × *g* for 15 min. The supernatant was decanted, and the aforementioned steps were repeated two times. Then the pellet was mixed in four volumes of salt solution (0.1 M NaCl), centrifuged (5000 × *g* for 15 min), and washed three times. The final pellet collected was MP. The biuret method was used to determine the protein concentration of 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). Sugarcane dietary fiber with either 40 or 80 mesh was added at 3 concentrations: 1, 2 and 3 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 SDF. The samples were briefly chilled in and freezer and stored at 4 °C overnight.

2.3. Sodium-dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

Myofibrillar protein (MP) solutions (8 mg/ml) and the soluble protein of MP gelation (4 mg/ml) were diluted 1:1 with loading buffer, then heated in 100 °C water for 10 min. For SDS–PAGE, a separating gel of 10% acrylamide and a stacking gel of 4% acrylamide were used. Molecular weight standards (Thermo Fisher Scientific Co., Ltd., Waltham, USA) were 17–205 kDa. 10 μl of samples was loaded. Electrophoresis was performed in a vertical unit with running buffer (50 mM Tris–HCl, 384 mM glycine, and 0.1% SDS, pH 8.3) and conducted at 80 V for stacking gel and 120 V for separating gel. Protein bands were stained with Coomassie Brilliant Blue for 30 min, and destained with a solution containing 250 ml/l methanol and 75 ml/l acetic acid.

2.4. Dynamic rheological measurements

Dynamic rheological measurements (both visco-elastic properties and viscosity) of SDF-MP solution were performed using a rheometer (Physica MCR301, Anton Paar, Graz, Austria). Viscoelastic properties were measured to examine the dynamic formation of the gel networks, as described by Jiang and Xiong (2013). Samples were loaded between two 50 mm diameter parallel plates with a 1 mm gap. To induce gel formation, the samples were stored at 20 °C for 3 min and heated from 20 °C to 80 °C at a heating rate of 2 °C/min. Prior to heating, the exposed composites were sealed with silicone oil. To ensure that working conditions remained within the linear viscoelastic region, measurements were performed with a sweep strain (0.01–100%) at a frequency of 1 Hz, respectively. The oscillatory test was conducted by applying frequency of 1 Hz and constant strain of 1%, which was within the linear region of the treatments. Changes in the storage modulus (G′), loss modulus (G″), and phase shift angle (δ) were constantly recorded.

2.5. Texture profile analysis (TPA)

Plastic tubes (50 ml) were filled with the blended myofibrillar proteins solution, and then centrifuged (Model 225, Beckman Coulter, Inc., California, USA) to remove any bubbles. The sample solutions were heated at 80 °C for 20 min in a water bath (TW20, Julabo Co., Ltd., Lower Saxony, German). After heating the samples immediately put into ice-water tank to cooled and stored at 4 °C overnight. The cylindrical gel samples (20 mm in height, 40 mm in diameter) were tested with a cylindrical probe (P/50, 50 mm diameter) integrated with a texture analyzer (TA-XT Plus, Stable Micro systems Ltd., Surrey, UK). Each sample was compressed twice to 30% of its original thickness. The analysis was performed using the following conditions: pre-speed, 3.00 mm/s; trigger force, 5 g; test speed, 2.00 mm/s; and post-speed, 5.00 mm/s. There was no delay between the two compression cycles (Savadkoohi, Shamsi, & Farahnaky, 2013). The data acquisition rate was 200 pps. The textural properties of gels were expressed as hardness, springiness, cohesiveness, gumminess, and chewiness (Caceres, Garcia, & Selgas, 2006). Five samples of each replicate were carried out.

2.6. Light microscopy of gel structures

Sections of blended gels (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 Zhou (2009). Slides observed and photographed using a light microscope mounted with a digital camera (Motic China Group Co., Ltd., Fujian, China).

2.7. Scanning electron microscopy (SEM) of gel structures

Protein gels were examined with a Hitachi S-3000 N scanning electron microscope (Tokyo, Japan) at an accelerating voltage of 20 kV. Gel samples were fixed for at least 48 h in 0.1 mol/L phosphate buffer solution (pH 7.0) containing 2.5% glutaraldehyde, and then dehydrated with a series of solutions of increasing ethanol concentration. Each sample was freeze-dried, sputter-coated with 10 nm of gold/palladium. Five images of each replicate were obtained.

2.7.1. Fractal dimension (D_f) of protein gels

The microscopy images were analyzed with ImageJ v1.47 (Rasband, 2013) and the FracLac 2.5v (Karperien, 2013) plugin for ImageJ v1.47. Micrographs were threshold and were transformed into 8-bit binary images of 640 × 432 pixels. The gray level was used for thresholding as described by Pugnaloni, Matia-Merino, and Dickinson (2005). Then, D_f

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