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Applications of high pressure to pre-rigor rabbit muscles affect the water characteristics of myosin gels

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ABSTRACT

Myosin was extracted immediately after high-pressure treatment (HP, 100–300 MPa for 15 or 180 s) to pre-rigor rabbit muscles (PRRMs) for evaluating the influences of HP-treatment on gel properties, using untreated muscles as Controls. Assessment of myosin yields, water-holding capacity (WHC), water mobility and distribution demonstrated that HP modified myosin before its extraction. Myosin gels subjected to HP at 100 MPa 180 s and 200 MPa 15 s had enhanced WHC compared with Controls. Also, the highest proportion of immobile-water was observed in myosin gels treated at 200 MPa for 15 s. HP-treatment of PRRMs affected their physicochemical properties as evidenced by alterations in tertiary, secondary conformations and rheological properties during subsequent heating. These modifications appear to induce various degrees of exposure of hydrophobic and sulfhydryl groups, resulting in different gelation rates. These alterations partly explain the various gel qualities obtained and indicate the potential of HP for pre-rigor muscles.

1. Introduction

High-pressure processing (HPP), one of the most prevalent non-thermal processing technologies in current commercial use has been applied in many sectors of food sterilization and processing (Bajovic, Bolumar, & Heinz, 2012), especially in the meat industry. With HPP, many advantages can be achieved without impairing the functional properties of meat and meat products. The sodium chloride content of beef sausage batter can be reduced to 1% by using HPP at 200 MPa for 2 min at 10 °C (Sikes, Tobin, & Tume, 2009). O'Flynn, Cruz-Romero, Troy, Mullen, and Kerry (2014) reported that HPP (150 MPa, 5 min, 20 °C) of breakfast sausages enabled a reduction in the amount of added phosphate to 0.25% with improved acceptability. Other studies have used high-pressure (HP) technology alone, or in combination with polysaccharide additives, to manufacture low-fat meat products, which meet the requirements of health-conscious consumers (Yang, Han, Bai, et al., 2015). Also, some studies have applied HP to pre-rigor muscles so as to obtain improved textural properties. Macfarlane (1973) reported that the tenderness of sheep and ox muscles was significantly improved by applying appropriate HP to pre-rigor muscles (103 MPa, 2 min, 35 °C) and similar results were also observed in pork (215 MPa, 15 s, 33 °C) (Souza et al., 2011). Apart from an improvement in tenderness, there was a marked increase in the WHC when meat and meat products were treated with HP (Sikes et al., 2009; Souza et al., 2011). Moreover, the intensity of HPP conditions required for manufacture of gel-type meat products having improved functionalities was less for pre-rigor muscles than for post-rigor muscles, based on our previous study (200 MPa for 15 s, 25 °C) with pre-rigor rabbit muscle, which resulted in significant improvements of textural properties and WHC (Xue et al., 2017).

Water holding capacity (WHC) is an important characteristic that plays a crucial role in evaluating the quality of meat and/or meat products. Water mobility and water distribution can be estimated using a non-destructive, non-invasive technique, low-field NMR (Bertram, Whittaker, Shorthose, Andersen, & Karlsson, 2004). Generally, the lower spin-spin relaxation times of water protons (T_2) correspond to the more tightly-bound water molecules, indicating a higher WHC (Bertram et al., 2004). Nevertheless, the proportion of water distributed in various forms should also be considered. Improvement in water binding of gel-type meat products leads to higher production yields and to improved gel qualities (Trout, 1988) through changes in cavity sizes of the gel structure, the extents of protein-solvent interactions, and the manner by which myosin is cross-linked (Puolanne & Halonen, 2010). Although numerous works have reported that application of HP to pre-rigor muscles, post-rigor muscles and meat batters enhances WHC, the results have been varied and inclusive (Campus, 2010). The complexity

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of meat, consisting of various components such as collagen, myofibrillar and sarcoplasmic proteins, might partly explain this variation. Therefore, by focusing on myosin, an understanding of the effects of HPP on water characteristics should be possible.

Myosin is the major component in the meat system, accounting for 50–55% of the total myofibrillar proteins, and is the only protein in meat that can form a gel (Hayakawa et al., 2012). Previous work has shown that myosin begins to unfold with HP treatment at 50 MPa (Hsu & Ko, 2001), and as the pressure intensity increases, the tertiary structures undergo corresponding changes, exposing previously buried amino acid groups (hydrophobic and sulfhydryl groups). It is only at pressures above 700 MPa that the secondary structures of myosin are altered (Cao, Xia, Zhou, & Xu, 2012). Moreover, based on the report of Yamamoto, Hayashi, and Yasui (1993), the head region of myosin molecule is the most sensitive to HPP. Further, myosin in these previous studies had been extracted prior to HPP. Therefore, the effects of other components in the meat or meat batter system were excluded. In addition, the influences of HPP of myosin in pre-rigor muscles were different from the myosin in the post-rigor muscle and/or meat batter system.

To the best of our knowledge, few studies have focused on the HP-induced modifications to myosin in pre-rigor muscles, and how thermal gelation characteristics are affected. Therefore, to unravel the effects of applying HP to pre-rigor rabbit muscles on myosin gelation and its related water characteristics, the purified myosin was extracted after HPP. We also investigated the microstructures of the myosin gels. Finally, we attempted to elucidate the mechanism of HP-modifications to myosin in pre-rigor muscles both from the perspective of tertiary conformational and secondary structural changes, before and during heating, which might be responsible for the changes in water properties.

2. Materials and methods

2.1. Materials

A total of 21 *New Zealand* white rabbits (male), each weighing approximately 3.0 kg and 3 months old, were purchased from the Livestock Institute of Jiangsu Academy of Agricultural Sciences (Nanjing, China). All the rabbits were maintained under similar environmental conditions. Seven rabbits were used for each batch of processing. The rabbits were subjected to jejunitas 12 h before slaughtering; they were electrical stunned and slaughtered by severance of the jugular veins. The *M. psoas* was removed immediately after rabbits were slaughtered. All animal procedures were reviewed and approved by the Animal Care and Use Committee at the Chinese Academy of Agricultural Sciences.

The visible connective tissues and fat were trimmed; approximately 60 g of all the collected muscles from 7 rabbits were vacuum packaged (DC800-FB-E Vacuum package machine, Promarks Inc., Ontario, CA, USA) with polyamide/polyethylene membrane (oxygen permeability < 1 $cm^3/m^2/h$ at 20 °C, Fengyi Company, Hebei, China) in each bag (14 bags in total). Following the collection of muscle and packaging, which took 45 min, muscles were subjected to the selected HP treatments respectively, which needed another 30 min. Then myosin was extracted using the method of Chen et al. (2014) as given below. All the operations were conducted at 4 °C, except for HPP (25 °C).

2.2. Methods

2.2.1. High-pressure processing (HPP)

HPP was carried out in a 0.3 L capacity 850 Mini Food Lab high-pressure vessel (Stansted Fluid Power Ltd., Harlow Essex, UK). A mixture of propylene glycol and distilled water (30:70) was used as the pressure-transfer medium and the medium temperature was controlled at 25 °C by a thermo-stated jacket. Parameters of HPP were selected based on the work of Souza et al. (2011): 100 MPa for 15 s (100-15); 100 MPa for 180 s (100-180); 200 MPa for 15 s (200-15); 200 MPa for 180 s (200-180); 300 MPa for 15 s (300-15); 300 MPa for 180 s (300-180); those untreated were used as Controls. A total of 120 g rabbit muscles (2 bags) were put in the HP vessel each time.

During the processing, the pressurization rate was 20 MPa/s and decreased to ambient at a rate of 12 MPa/s. The maximum temperature during HPP reached 30 °C due to the adiabatic heating. Upon release of pressure and removal from the vessel, the samples were kept at 4 °C.

2.2.2. Extraction of myosin

High-pressure treated muscles (100 g for each treatment) were ground for 12 s (paused at each 3 s to minimize temperature rise) in a chilled cutter at 3000 rpm (Grindomix GM 200, Retsch, Germany). The batters were then subjected to myosin extractions following the protocol of Chen et al. (2014). A series of buffer solutions: A, 0.1 M KCl, 20 mM potassium phosphate, 2 mM magnesium chloride, 1 mM EGTA, 1 mM dithiothreitol, (pH 7.0); B, Guba-Straub solution, consisting of 0.3 M KCl, 0.1 M KH_2PO_4, 50 mM K_2HPO_4, 1 mM EGTA, 4 mM sodium pyrophosphate, 20 mM magnesium chloride, (pH 6.5); C, 1 mM EDTA-2Na; 0.6 M KCl, 20 mM potassium phosphate, 5 mM magnesium chloride and sodium pyrophosphate, (pH 6.5) were used to exclude water-soluble proteins (A), extract salt-proteins (B), and to purify the crude myosin at 4 °C by repeated dissolving, precipitating and centrifugation (C).

Finally, the myosin extraction yield of each HPP rabbit muscle was recorded (percent of obtained myosin weight to original weight of muscle) and the protein concentration in these myosin solutions was measured using the Biuret method. For testing, the final concentration of each solution was adjusted to the appropriate requirement for the specific measurement using 0.6 M KCl containing 20 mM potassium phosphate, pH 6.5.

2.2.3. Preparation of thermal gels

Myosin solutions (10 mg/mL) extracted from HP-treated rabbit muscles and Controls, were placed in 10-mL capped plastic centrifuge tubes (5 mL myosin solution). Three replicates of each treatment were used. The samples were heated from 25 to 85 °C at 1 °C/min in a water bath to form a myosin gel. The tubes were immediately cooled in flowing tap water and then stored overnight at 4 °C for the determination of water-holding capacity, Low-field NMR analysis and for scanning electron microscopy.

2.2.4. Water-holding capacity

The water-holding capacity (WHC, %) was determined using a centrifugal method (Chen et al., 2014) with some modifications. The myosin gels (approximately 5 g) in each centrifuge tube were centrifuged at 8000*g* for 10 min. The WHC was expressed as a percentage of gel weight after centrifugation to the initial gel sample weight.

2.2.5. Low-field NMR (LF-NMR)

The LF-NMR tests were performed according to the method introduced by Han, Wang, Xu, and Zhou (2014) with slight modifications. The gel sample (2.0 ± 0.1 g) was placed in a glass tube (15 mm in diameter) and inserted into the NMR probe of a PQ001 Niumag Pulsed NMR analyzer (Niumag Electric Corporation, Shanghai, China). The analyzer was operated at a resonance frequency of 22.6 MHz at 32 °C. Spin-spin relaxation times of ^1H (T_2) were measured using the Carr–-Purcell–Meiboom–Gill (CPMG) sequence. T_2 was measured made a τ-value of 350 μs. Data from 12,000 echoes were acquired as 32 scan repetitions. The repetition time between subsequent scans was 7000 ms. Post processing of NMR T_2 data distributed exponential fitting of CPMG decay curves were performed by Multi-Exp Inv Analysis software (Niumag Electric Corp., Shanghai, China). Three relaxation times (T_{21}, T_{22} and T_{23}) and their corresponding water populations (P_{21}, P_{22} and P_{23}) were recorded.