Food Chemistry 217 (2017) 678–686

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of high pressure modification on conformation and gelation properties of myofibrillar protein

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ARTICLE INFO

Article history:
Received 21 June 2016
Received in revised form 5 September 2016
Accepted 6 September 2016
Available online 7 September 2016

Keywords:
Myofibrillar protein
High pressure
Conformation
Gelation property
SEM
DSC

ABSTRACT

The effects of high pressure (HP) treatment (100–500 MPa) on conformation and gelation properties of myofibrillar protein (MP) were investigated. As pressure increased (0.1–500 MPa), α-helix and β-sheet changed into random coil and β-turn, proteins unfolded to expose interior hydrophobic and sulfhydryl groups, therefore surface hydrophobicity and formation of disulfide bonds were strengthened. At 200 MPa, protein solubility and gel hardness reached their maximum value, particle size had minimum value, and gel microstructure was dense and uniform. DSC data showed that actin and myosin completely denatured at 300 MPa and 400 MPa, respectively. Rheological modulus (G′ and G″) of HP-treated MP decreased as pressure increased during thermal gelation. Moderate HP treatment (≦200 MPa) strengthened gelation properties of MP, while stronger HP treatment (⩾300 MPa) weakened the gelation properties. 200 MPa was the optimum pressure level for modifying MP conformation to improve its gelation properties.

1. Introduction

It is known that the gelation properties of myofibrillar protein (MP) are very important for muscle products. The high pressure (HP) has been demonstrated to be able to cause changes of protein conformation, structure, and hence improving the gelation properties of muscle proteins (Hsu, Hwang, Yu, & Jao, 2007).

HP treatment could cause variable alterations on protein conformational structures depended on the pressure level used. Under pressurization, the quaternary structure dissociates at moderate pressures (100–200 MPa), the tertiary structure is significantly affected at pressure level above 200 MPa and secondary structure changes take place at stronger high pressures (300–700 MPa) (Ahmed, Ramaswamy, Kasapis, & Boye, 2010). Acero-Lopez, Ullah, Offengenden, Jung, and Wu (2012) studied the effect of HP treatment on ovotransferrin, and found the secondary structure changed from helices, sheets, turns, and aggregated strand to mostly intermolecular β-sheets or aggregated strands at 200 MPa, but switched back to original structure at higher pressures. Maria, Ferrari, and Maresca (2016) pointed out that HP treatment on bovine serum albumin (BSA) would induce α-helix unfolding into disordered structure and turns. Cao, Xia, Zhou, and Xu (2012) reported that the microstructure of myosin gels below 200 MPa were filament structure with many small cavities, while gels upon 300 MPa were globular aggregates with big cavities. These changes in protein conformation and gel microstructure have profound effects on a protein's gelation properties and its possible food applications.

Gelation properties of proteins have a direct relationship with its conformational structure (Ramaswamy, Singh, & Sharma, 2015). HP treatment can cause modification of the quaternary and tertiary structure of a protein that can lead to denaturation, aggregation and gelation, and improvement of the functional properties by enhancing moisture-protein and protein-protein interactions (Singh, Sharma, & Ramaswamy, 2015). HP modification can lead to the enhancement or weakness of protein gelation properties depended on the pressure level used. Moderate HP treatment (≦200 MPa) would induce the increase of protein solubility, while stronger HP treatment (⩾300 MPa) would cause reduction of solubility (Bravo, Felipe, López-Fandiño, & Molina, 2015; Marcos & Mullen, 2014; Van der Plancken, VanLoey, & Hendrickx, 2005). HP-induced protein unfolding could lead to the reversible or irreversible gelation of the proteins, with repercussions on the viscoelastic characteristics of the protein solution (Ahmed, Ramaswamy, Alli, & Ngadi, 2003). It has been reported that HP

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http://dx.doi.org/10.1016/j.foodchem.2016.09.040
0308-8146/

treatment prior to heating enhanced thermal gelation of muscle proteins. Pressurization (150 MPa, 10 min) enhanced heat-induced (70 °C, 10 min) gelation of myosin from ovine muscle at low ionic-strength (Suzuki & Macfarlane, 1984). The hardness of heat-induced peanut protein isolate gel increased by 50% after HP treatment at 0.1–100 MPa, while gradually decreased with further increased pressures (He et al., 2014). Previous literature pointed out the mechanism of HP-induced myosin gelation was that under pressurization, myosin unfolded, following the exposure of interior hydrophobic and sulfhydryl groups, protein denatured and associated to form gels (Cao et al., 2012).

The purpose of this work was to assess changes in the conformation structure and gelation properties of myofibrillar proteins for use in new food systems with good textures. A deeper understanding of the mechanisms of governing the conformation and gelation properties by HP treatment would be very useful to design novel processes addressed to the improving functionality of MP in meat products.

2. Materials and methods

2.1. Materials

Six-week-old commercial broilers (Arbor Acres Plus, 2.6 ± 0.1 kg in weight, female) were selected and slaughtered according to the National Standard of the People's Republic of China for Operating Procedure of Chicken Slaughtering (GB/T 19478-2004, 2004) on a chicken farm in Nanjing, China. The breast meats (36–40 h post-mortem) were purchased, then frozen at −18 °C and brought to the laboratory. Meats were used within one month. ANS (8-anilino-1-naphthalenesulphonic acid, CAS: 82-76-8), DTNB (5,5-dithiobis [2-nitrobenzoic acid], CAS: 69-78-3), EDTA (ethylenediaminetetraacetic acid, CAS: 60-00-4), EGTA (ethylene-bis (oxyethylenenitrilo) tetraacetic acid, CAS: 67-42-5) and urea (CAS: 57-13-6) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). BSA (bovine serum albumin, CAS: 9048-46-8) was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All other chemicals were analytical grade or better.

2.2. Extraction of myofibrillar protein (MP)

MP was extracted from chicken breast meat carried out as described by Zhang, Yang, Tang, Chen, and You (2016) with some modifications. Before extraction, meat was thawed at 4 °C for 12 h in a refrigerator. Trimmed muscle (40 g) was cut into small pieces (about 0.5 × 0.5 × 0.5 cm^3) and homogenized in 8 volumes of a buffer (0.1 M NaCl, 2 mM $MgCl_2$, 1 mM EGTA, 0.5 mM dithiothreitol, and 10 mM Na_2HPO_4, pH 7.0) in a homogenizer (DS-1, Shanghai Specimen Model Factory, Shanghai, China). The homogenates were centrifuged (4 °C) at 2000*g* for 20 min (Avanti J-26XP centrifuge, Beckman Coulter, Brea, CA, USA). After decanting the supernatant, pellets were re-suspended using homogenizer and centrifuged using the same conditions twice more. After that, the pellet was re-suspended in 8 volumes of another buffer (0.1 M NaCl, 1 mM NaN_3, pH 6.0), filtered with clean and dry gauzes, and centrifuged (2000*g* for 20 min). After decanting the supernatant, MP pellets were re-suspended, filtered and centrifuged twice more. The protein content of MP was determined by the Biuret method using BSA as standard and used in 48 h.

2.3. HP treatment and preparation of MP gel

HP treatment was carried out in a high pressure unit (UHPF-750 MPa, Baotou Kefa, China). An oil (bis (2-ethylhexyl) sebacate, Li-Dong precision machinery company, Shenzhen, China) was used as the pressure-transfer medium. Prior to pressurization, MP samples were sealed in polyethylene bags without trapping air bubbles, and treated by pressures at 100, 200, 300, 400, 500 MPa (±10 MPa). HP increased at a speed of 3.5 MPa/s to designed pressures and held for 10 min, then released within 5 s. The control was MP sample without pressurization (0.1 MPa). After HP treatment, MP were diluted to 40 mg/mL for Raman spectrum testing, 30 mg/mL for preparing MP gel, 5 mg/mL for solubility measurement and 1 mg/mL for sulfhydryl group content, surface hydrophobicity and particle size measurement.

MP gel was prepared as follows: 2 mL of MP solutions (30 mg/mL) were placed in 7 mL capped plastic centrifuge tubes. The tubes were heated in a water bath at a rate of 1 °C/min from 20 to 65 °C and kept at 65 °C for 20 min. Then, the tubes were cooled to room temperature (25 °C) and then kept overnight at 4 °C for SEM and textural tests.

2.4. Amino acid analysis

The determination of amino acid content was according to Park and Xiong (2007) with some modifications. HP-treated MP samples were mixed with 6 M HCl in 10 mL capped glass tubes, hydrolyzed at 110 °C for 24 h; after cooled to room temperature (25 °C), opened the tubes and concentrated the hydrolyzate to dryness with a nitrogen gas purge equipment, then dissolved the dried substance with 0.02 M HCl and moved into a 50 mL volumetric flask, set to 50 mL with deionized water (Milli-Q, Millipore, Boston, MA, USA); part of the solution was transferred to a centrifuge tube, centrifuged at 10,000*g* for 5 min; filtered the supernatant with 0.22 μm membrane, then transferred into a 2 mL vial, using an automatic amino acid analyzer (L-8900, Hitach Corporation, Tokyo, Japan) for amino acid analysis. The calibration standards were L-amino acids (Sigma-Aldrich Inc., St. Louis, MO, USA) in nano pure water.

2.5. Secondary structure

The secondary structure proportions of MP samples were measured by Raman spectroscopy using a Jobin Yvon Labram HR800 spectrometer (Horiba/Jobin. Yvon, Longjumeau, France). MP samples were spread on a glass slide during measurement. Raman spectra were recorded under the following conditions: laser power, 100 mW; laser spot diameter reaching the sample, 1 μm; spectral resolution, 2.0 cm^{-1}; number of sample scans, 3. The time required for the acquisition of 1 spectrum was about 1 min. Spectra were smoothed, baseline corrected, normalized against the phenylalanine band at 1003 cm^{-1}, as it was insensitive to the microenvironment, and Amide I was analyzed using Labspec version 3.01c (Horiba/Jobin. Yvon, Longjumeau, France). Protein secondary structures were determined as percentages of α-helix, β-sheet, β-turn, and random coil using Alix's method (Alix, Pedanou, & Berjot, 1988).

2.6. Surface hydrophobicity

Surface hydrophobicity (S_0-ANS) was determined using ANS as a fluorescence probe. 25 μL of ANS solution (8 mM ANS in 0.1 M NaH_2PO_4 buffer, pH 6.0) solution was added to 5 mL of MP solution (1 mg/mL) and mixed well. Samples were kept under dark conditions at 25 °C for 20 min. Fluorescence intensity was measured by a fluorescence spectrophotometer (F-7000, Hitachi Corp., Japan) at an excitation wavelength of 374 nm and an emission wavelength of 485 nm. The initial slope of the plot of fluorescence intensity versus protein concentration was referred to as S_0-ANS.