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Structural modification by high-pressure homogenization for improved functional properties of freeze-dried myofibrillar proteins powder

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ABSTRACT

To expand utilization of meat in various products, the structural, physicochemical and functional changes of water soluble myofibrillar protein powder (WSMP-P) were investigated as affected by high-pressure homogenization (HPH) intensities (0–20,000 psi). HPH modified the structure of WSMP-P by random dissociation (myofibril and myosin polymer dissociation), partial unfolding and rearrangement (actin trimer formation), producing an amorphous protein structure with high thermal stability. α-Helix and β-turn conversion to β-sheet structures occurred at pressures above 15,000 psi, suggesting an increase in myosin conformation flexibility with minor aggregation. Moreover, HPH was able to improve the water solubility and emulsifying properties of WSMP-P. This might be resulted from its unfolded flexible structure with submicron size and high surface net charge in aqueous suspensions induced by HPH. The findings regarding the improved functionality evidence potential of applying WSMP-P as protein supplements in formulated food or beverage at low ionic conditions.

1. Introduction

Proteins are being increasingly used to facilitate the engineering for fabrication of novel food products, such as sports protein beverages and therapeutic powder foods. The effectiveness of protein utilization in food production depends on their functional characteristics, which can be tailored to satisfy the various demands of food product manufacturers. These properties are influenced by the intrinsic factors (e.g., protein structure and conformation) and extrinsic elements (e.g., pH, ionic strength, temperature and food processing) (Siddique, Maresca, Pataro, & Ferrari, 2016).

Generally, some native proteins rarely show good functional properties desirable for the food industry. To improve the functional properties for broad applications, protein structural modification is often implemented, and innovative non-thermal processing technologies can serve this purpose. High-pressure homogenization (HPH) is a unit operation where a fluid is forced through a small orifice (valve or nozzle) and several physical factors (shear stress, high hydrostatic pressure, turbulence, cavitation, and impingement) promote dispersion of aggregates, modify the structure of protein and thereby the physicochemical properties of foods (Ye & Harte, 2014). It is, effectively, a continuous process which could be easily scale up. With the development of high pressure technology, HPH has undergone an enormous progress with the change of scale from laboratory devices to the design and assembly of a full pilot-plant scale equipment in the food industry (Chen, Xu, & Zhou, 2016b). Recently, it has been proposed the application of high-pressure homogenization (HPH) to modify the structural and functional properties of dairy (Lee, Lefèvre, Subirade, & Paquin, 2009; Ye & Harte, 2014) and plant proteins (Sun, Dai, Liu, & Gao, 2016), aiming at tailor-making protein substrates having targeted functionality suitable for different formulated foods. However, the impact of HPH treatments on the physicochemical as well as on the functional properties of meat proteins has been scarcely determined yet.

Meat with high-nutritious proteins provides a broad range of peptides and amino acids for humans. Differently from beans and whole wheat proteins, meat proteins comprise all the essential amino acids, with a particularly high content of lysine, and are all highly digestible (Ito et al., 2004; Pereira & Vicente, 2013). However for extraction of component, meat has not been exploited as a supplementary protein ingredient to the same extent as milk or soybean, mainly due to the poor functionality of myofibrillar proteins (MP, approximately 50% of the total meat proteins) in water or at low ionic strength (Krishnamurthy et al., 1996). To enlarge the application scope of meat in various products, we recently proposed the potential application of high-pressure homogenization (HPH) to selectively modify the

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structure of MP for improved solubility in water without obvious hydrolysis of individual protein polypeptides (Chen et al., 2016b). However, the physicochemical and functional properties of HPH induced water-soluble myofibrillar proteins (WSMP) still remained unclear. As these properties constitute the major criteria for food product formulation, processing and storage, the physicochemical properties must be elucidated to utilize WSMP as an effective protein supplement.

To enable the application of protein extracts as functional ingredient in food formulation and prolong their stable storage, it is common to convert them into a dry powder form (Aguilera, Chiralt, & Fito, 2003; Huda, Abdullah, & Babji, 2001; Sun, Senecal, Chinachoti, & Faustman, 2002). Freeze-drying has been reported to be an attractive way to extending the shelf life of high-valued materials without quality deterioration and with minimum damage in structure, texture, appearance and flavor (Vega-Mercado, Góngora-Nieto, & Barbosa-Cánovas, 2001). Therefore, the aim of the present study was to investigate the effects of HPH treatment on the structural, physicochemical and functional properties (solubility and emulsifying properties) of freeze-dried WSMP powder (WSMP-P). So far, there is still limited information available on the structural and functional properties of freeze-dried WSMP-P. Attempts addressed toward physicochemical of WSMP would unlock a promising area of research for development of novel meat products and broaden the application area of HPH technology in food industry.

2. Materials and methods

2.1. Materials

Chicken breast muscle (*Pectoralis major*) was collected at 36 h postmortem from Sushi Food Co., Ltd. (Nanjing, China). Muscle samples were vacuum packaged, stored in a − 30 °C freezer, and used within 4 days after slaughter.

2.2. Preparation of freeze-dried WSMP-P

WSMP were prepared according to our previous report (Chen et al., 2016b). In brief, the minced meat (100 g) was homogenized and washed four times with cold (4 °C) deionized, distilled water (pH 7.0). Then, the washed myofibrils were suspended in water (5 mg/mL protein) and treated by 10,000 psi (69 MPa), 15,000 psi (103 MPa) or 20,000 psi (138 MPa) HPH for two passes. The inlet temperature for the samples should be maintained within 4–6 °C. The HPH was carried out by a high pressure homogenizer (Mini DeBee, Bee International, USA) equipped with a single pressure intensifier and a 75-μm opening Y-type diamond nozzle (Genizer™, Los Angeles, USA) and implemented with a rapid cooling system for maintaining the outlet temperature blow 20 °C. Previous study has shown that no significant change in pH and ionic strength was detected for the treated suspensions (pH 6.92–7.05 and ionic strength 1.3 mM) (Chen et al., 2016b). Finally, the HPH treated dispersions were lyophilized for 48 h using a freeze dryer (Alpha 2–4 LSCplus, Martin Christ, Germany) at − 80 °C compressor temperature and 0.1 mbar vacuum pressure. The freeze-dried powders were milled and sieved using a screen mesh (0.3 mm in aperture). The freeze-dried myofibril sample without HPH treatment was used as the control. All samples contained about 87% of protein (w/w) and no significant differences in protein, moisture, ash and lipid contents were detected (Supplemental Table 1). Each HPH treatment was conducted for four replicates.

2.3. Determination of the structural and physicochemical properties

2.3.1. Fourier transform infrared (FTIR) measurements

FTIR measurements were performed on flakes of KBr (100 mg) containing sample powder (3 mg). All spectra in the region of 4000–500 cm^{-1} were performed with 64 times scanning on a Nicolet 6700 spectrophotometer (Thermal Fisher Scientific, WI, USA) at ambient temperature (20 °C).

Raw absorbance spectra were cut between 1700 and 1600 cm^{-1} for the analysis of Amide I. Fourier self-deconvolution of Amide I spectra was used to analyze the secondary structure of protein by using OMNIC 7.2 software from Thermo Nicolet Corporation (Thermal Fisher Scientific, WI, USA). The secondary structural contents of α-helix, β-sheet, turn and random coil were calculated using integrated areas of each peak according to the method of Kong and Yu (2007).

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

Raw myofibril or WSMP-P were mixed with a sample buffer (20% glycerol, 5% β-mercaptoethanol, 4% SDS, 0.125 M Tris, pH 6.8) to reach a final protein concentration of 2 mg/mL. SDS-PAGE was conducted with a 4% acrylamide stacking gel and a 12% separating gel to observe the myofibrillar profiles following the method previously described (Chen et al., 2016a).

2.3.3. Nano liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry (Nano LC-ESI-MS/MS) analysis

Unknown protein bands in the SDS-PAGE gels were excised manually and were pre-treated as previously reported (Chen et al., 2016a). Treated samples were then analyzed using an ion trap mass spectrometer (Ltq Linear Ion Trap Mass Spectrometer System, Thermo, CA, USA) coupled with a high pressure liquid chromatography (HPLC) system for data collection. All the MS data were searched in the non-redundant protein database (NR database, NCBI). The relative abundance of protein in a sample (excised protein band) was calculated according to a label-free quantitation method (Griffin et al., 2010).

2.3.4. Differential scanning calorimetry (DSC) analysis

Thermal characteristics of WSMP-P were determined using a differential scanning calorimeter (DSC 1 STAR^e System, Mettler Toledo, Switzerland). An amount of 18 mg of WSMP-P sample was accurately weighed and sealed in an aluminium pan for heat scanning (from 30 to 120 °C at a heating rate of 10 °C/min). An aluminium pan without sample was used as the reference. The peak (T_d) denaturation temperatures and enthalpy of denaturation (ΔH) were estimated from the thermograms using the associated software (Mettler Toledo, Switzerland).

2.3.5. X-ray diffraction measurement

X-ray diffraction (XRD) measurement was conducted on a D/MAX2500V diffractometer (Rigaku Corporation, Japan) to analyze the physical structure. Copper Kα was used at a voltage of 40 kV and a current of 40 mA. The XRD scans were set in the range of 2θ from 15° to 45° at an angular speed of 2°/min.

2.3.6. Scanning electron microscopy (SEM) observations

WSMP-P samples were fixed on aluminium stubs by the pieces of double-sided conductive carbon tabs, and sputter-coated (Jeol JFC-1600 Tokyo, Japan) with gold/palladium before the test. The SEM observations were then conducted on a scanning electron microscope (Jeol, JSM 6490LV, Tokyo, Japan) at an accelerating voltage of 20 kV.

2.4. Determination of the functional properties

2.4.1. Solubility

Protein samples were completely dispersed (5 mg/mL) in aqueous solution (10 mM sodium phosphate, pH 7.0). Then, the samples were centrifuged at 8000*g* for 20 min (Liu, Geng, Zhao, Chen, & Kong, 2015). The solubility was defined as the ratio of protein concentration in the supernatant relative to that of protein suspension before centrifugation.