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Effects of high-intensity ultrasound, high-pressure processing, and high-pressure homogenization on the physicochemical and functional properties of myofibrillar proteins

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ABSTRACT

High-Intensity Ultrasound (HIU), High-Pressure Processing (HPP), and High-Pressure Homogenization (HPH) were applied to myofibrillar protein suspensions (MPS) under selected conditions, with an untreated sample designated as the control. The results revealed that MPS subjected to HPP had increased solubility, but solubility was reduced with HIU and HPH ($p < 0.05$). Furthermore, rheological studies indicated that HPH-treated MPS had poor gel-formation ability, whereas HPP-treated MPS formed gels in a modified manner, which resulted in lower gel strength than the control, however it had the lowest cooking loss ($p < 0.05$). Investigations of reactive-sulfhydryl and hydrophobic contents of the treated MPS indicated that the three techniques each denatured MPS differently. HIU, HPP and HPH all tended to expose hydrophobic residues; however, HIU reduced the reactive-sulfhydryl contents significantly, while HPP and HPH acted in the opposite manner. We concluded that HPP would be the most effective process for the manufacturing of gel-based meat products.

Industrial relevance: The modern meat industry is seeking novel technologies to modify and/or improve the quality of meat and meat products, hence adding value to products as well as meeting demands of consumers. High-intensity ultrasound (HIU), high-pressure processing (HPP) and high-pressure homogenization (HPH), were applied to myofibrillar proteins suspensions. Their effects on myofibrillar proteins (MP), and different outcomes were observed. HIU can be an effective technique to improve the quality of MP gels, but some technical problems, such as heterogeneous distribution of ultrasonic waves, must be improved; HPP was the most effective technique in modifying MP in a manner that improved yield of MP gels when heated, whereas HPH was not effective for gel-type meat product. The information derived from this study provided a direct comparison of the three techniques and their influences on MP and thermally induced gels. This provides a useful reference for meat scientists and processors when choosing innovative technologies for the manufacture of meat products.

1. Introduction

Muscle-based foods are widely consumed due to their desirable flavor, unique textural attributes and high protein content which provide a complete content of essential amino-acids (Jiménez-Colmenero, Carballo, & Cofrades, 2001). Studies have reported that meat proteins, myofibrillar proteins (MP) in particular, determine the quality of meat products (Choi & Kim, 2009). Moreover, the physicochemical properties of MP, such as protein solubility, hydrophobicity, and reactive-sulfhydryl content etc., are a cluster of factors that can impart significant effects on the functional properties of thermally induced MP gels (Li-Chan, Nakai, & Wood, 1985). From a more practical perspective, information relevant to protein's physicochemical properties can be useful in optimizing manufacturing procedures. This is particularly relevant in the era of novel technologies that are emerging, and applications of novel techniques enabling the development of quality-improved and/or value-added meat products. Thus, the effective selection of the optimal processing factors is beneficial for the meat industry.

Non-thermal technologies are considered as replacements for, or adjuncts to, thermal treatments. The characteristics of these technologies on meat processing at low temperatures allows for improved preservation of nutrients and sensory qualities of food (Koubaa, Roselló-Soto, Barba-Orellana, & Barba, 2016). Many of these non-thermal

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treatments, such as high-intensity ultrasound (HIU), high-pressure processing (HPP), and high-pressure homogenization (HPH), have long been studied. With respect to muscle-based products, HIU has been shown to be an effective technique for improvement of water-holding capacity (WHC) and textural properties of cooked meat batter prepared from meat having poor manufacturing properties, such as pale, soft, exudative (PSE-like) chicken breast meat (Li, Kang, Zhao, Xu, & Zhou, 2014). Also, Zhang, Regenstein, Zhou, and Yang (2017) found that appropriate HIU treatment could improve the functionalities of MP gels by affecting their protein conformation via ultrasound-induced cavitation effect and micro-streaming current (Hu, Cheung, Pan, & Li-Chan, 2015), and therefore improve protein interactions in the formulation. On the other hand, HPP, which is distinguished for its instantaneous and homogeneous mode of action, has long been studied due to its moderate effects on proteins governed by the Le Chatelier's principle (Hite, 1899). More recently, a growing number of researchers have demonstrated that the functionality of proteins when subjected to HPP can be affected either positively or negatively depending on the parameters used (Buckow, Sikes, & Tume, 2013). Our previous work has demonstrated that when rabbit meat batter is subjected to 200 MPa HP for 9 min (25 °C) there was an amelioration of taste and texture. Despite the fact that the centrifugal juice loss of the HPP-treated rabbit meat sausages was slightly higher than the untreated samples, the cook loss was significantly reduced, denoting a higher production yield. In contrast to the HIU and HPP techniques, HPH has not been applied to meat or meat proteins for the manufacture of quality-improved or texture-modified products. Nonetheless, Chen and coworkers found that the HPH technique is an effective tool for improving the solubility of MP in water, owing to the multiple effects of shear force, high pressure (103 MPa), and cavitation, when the MP (dispersed in water) is passed through the homogenizing chamber (Chen et al., 2016; Chen, Xu, & Zhou, 2016). Therefore, HPH could be a valuable tool for modifying the quality of gel-based meat products. However, to our knowledge, no studies have addressed this potential. Since the principles of action of HIU, HPP and HPH technologies have some overlap, e.g. high pressure, cavitation effect, temperature rise etc., there is a need for a direct comparison of how the three techniques affect the MP and the gelation properties.

To this end, the functionalities of MP gels after treatments with HIU (frequency of 20 kHz, output power of 450 W, amplitude 60%, 6 min.), HPP (200 MPa, 9 min, 25 °C) and HPH (15,000 psi, 2 passes) were investigated. The parameters applied were selected based on previous studies (Chen et al., 2016; Li et al., 2014; Xue et al., 2017). In addition, we attempted to elucidate the underlying mechanism from the perspectives of protein tertiary conformations and the mechanisms of action of the applied techniques.

2. Materials and methods

2.1. Materials

A total of 5 kg frozen chicken breast meat (*musculus pectoralis major*) was purchased from a local supermarket (Suguo supermarket Co., Ltd., Nanjing, China) and was kept at − 20 °C until required. The frozen chicken breast meat was thawed at 4 °C for 18 h, followed by the determinations of pH value and color L* value of each meat based on the protocols of Li et al. (2014). Chicken breast meat was subjected to the myofibrillar protein suspension (MPS) preparation if pH values and L* values were in the range of 5.7–6.1 and 46–53, respectively (Barbut, 1997). For each batch of experiments, at least 6 chicken breast meat, weighing around 1.2 kg, were required for the sample preparations.

2.2. Methods

2.2.1. Extraction of myofibrillar proteins (MP)

MP was extracted using the protocol of Xiong, Lou, Wang, Moody, and Harmon (2000) with several modifications. Adipose and connective tissues were trimmed from the thawed meat. Approximately 300 g of thawed and well-trimmed meat was weighed and required for each batch of experiments. The rigor buffer, which contained 0.1 M KCl, 2 mM $MgCl_2$, 1 mM Ethylene Glycol Tetraacetic Acid (EGTA), 0.5 mM dithiothreitol, and 10 mM K_2HPO_4 (pH 7.0) was applied to extract MP with the assistance of appropriate homogenization (Ultra Turrax T25 BASIS, IKA, Labortechnik, Germany) and centrifugation (Beckman Avanti J-E, Beckman Coulter, Fullerton, CA, USA). Subsequently, the MP were dispersed in a high-ionic strength buffer solution (0.6 M KCl, 20 mM K_2HPO_4/KH_2PO_4, pH 6.5) and were dialyzed against the same buffer solution for 12 h at 4 °C. Protein concentration was determined by Biuret method (Lowry, Rosebrough, Farr, & Randall, 1951) before further adjustment. And a final concentration of 20 mg/mL of the MPS was achieved by adding buffer solution prior to further treatments. Finally, approximately 1300 mL of MPS was obtained and divided into 300 mL, 300 mL, and 400 mL for HIU, HPP and HPH treatments respectively.

2.2.2. Non-thermal processing treatments

Three non-thermal processing techniques were selected as pre-treatments for MPS, in order to directly compare the effects of these treatments on the thermal gelation properties of MPS.

2.2.2.1. High-intensity ultrasound (HIU). The HIU treatment was performed as described by Li et al. (2014) with some modifications. Thirty milliliters of MPS (20 mg/mL) was placed in a 50-mL glass beaker and subjected to a Vibra-Cell TM Ultrasonic Processor (VC 750, Sonics & Materials, Inc., USA) for ultrasound treatment. Therefore, 10 beakers were required to facilitate each independent trial. The HIU device was equipped with an ultrasonic probe (13 mm in diameter), ten millimeters of which was immersed in the MPS. Samples were treated at a frequency of 20 kHz, output power of 450 W, amplitude 60% for 6 min. The pulse mode (time-on 2 s and time-off 4 s) was as described by Li et al. (2014). The temperature of the samples, which did not exceed 22 °C, was measured with a thermometer immediately after the HIU-treatment. Afterwards, treated samples were mixed, and stored at 4 °C overnight prior to further determinations. The ultrasound energy efficiency is 32.18 ± 1.89 W/cm^{-2}, which was measured referred to a previous protocol (Carcel, Benedito, Bon, & Mulet, 2007).

2.2.2.2. High-pressure processing (HPP). HPP was performed on MPS (30 mL, sealed in a polyamide/polyethylene bag without air bubbles) based on the method of Xue et al. (2017), using a 0.3 L capacity 850 Mini Food Lab high-pressure vessel (Stansted Fluid Power Ltd., UK). A total of 10 bags of MPS was treated for each batch of experiments. The pressure transfer medium was pre-heated to 25 °C, and samples were subjected to 200 MPa HPP for 9 min. During pressurization (20 MPa/s) and decompression (12 MPa/s), the temperature of the transfer medium, monitored by a T-type thermocouple fixed inside the vessel, did not exceed 30 °C. Subsequently, samples were mixed, transferred to a chiller, and kept at 4 °C overnight before next step.

2.2.2.3. High-pressure homogenization (HPH). Using the procedures of Chen et al. (2016), an aliquot of 400 mL of the MPS was subjected to a high-pressure homogenizer (Mini DeBee, Bee International, USA). The single pressure intensifier with a 75-μm opening Y-type diamond nozzle (Genizer™, Los Angeles, USA), was installed inside the homogenizer in a modular homogenization cell. MPS in 0.6 M KCl buffer (20 mM KH_2PO_4/K_2HPO_4, pH 6.5) was treated at 103 MPa for each of two passes. Approximately 300 mL of HPH-treated MPS was obtained, and were transferred to and stored in the chiller (4 °C) overnight before further applications.

2.2.3. Protein solubility

The solubility of various MPS samples was determined based on the

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