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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

protocol of Chen et al. (2016) with slight modifications. The processed MPS were diluted to 10 mg/mL, followed by centrifugation at 20,000*g* for 20 min (4 °C) in a refrigerated centrifuge. The protein concentrations of the supernatants were determined by the Biuret method (Lowry et al., 1951).

2.2.4. Dynamic rheological properties

Measurement of dynamic rheological properties was performed as described by Feng et al. (2017), with some modifications, using a Rheometer (Physica MCR 301, Anton Paar, Austria) under the oscillatory mode. Five-milliliters of each MPS was slowly pipetted onto the 50-mL plate, and the probe was set with the gap of 1.5 mm. Prior to heating (20 °C to 80 °C, 1.5 °C/min), mineral oil was applied to the edges of plates to prevent samples from dehydrating. The storage modulus (G′) and loss modulus (G″) were continuously monitored during heating at 0.1 Hz oscillation condition coupled with a 2% strain. The values of the loss modulus divided by the storage modulus (G″/G′) were defined as the loss tangent (tanδ).

2.2.5. Cooking procedures

Ten-milliliter centrifuge tubes and 10-mL beakers (25 mm in diameter and 35 mm in height) were used to hold the protein suspension (6 mL each) so as to facilitate the subsequent determinations of water-holding capacity (WHC) and gel strength respectively. The beakers were sealed with parafilm prior to heating (25–75 °C, 1 °C/min) in a water bath. The tubes and beakers were kept at 75 °C for 5 min before moving to a cold room (4 °C) overnight prior to subsequent tests.

2.2.6. WHC

2.2.6.1. Cook loss (CL, %). The water that extruded from the gels by cooking was removed using a small wad of cotton. The weight of the extruded water, relative to the initial weight of MPS, was considered as CL (%), based on our previous protocol (Xue et al., 2017).

2.2.6.2. Expressible moisture content (EMC). Cooked gels were subjected to centrifugal treatments (2000 g, 10 min, 4 °C). The expressible water that was extruded by centrifugal force was removed by a small wad of cotton, the weight of which was compared to the weight of gel before centrifugation. The obtained results were considered as EMC (%).

2.2.7. Gel strength

The cooked gels were restored to ambient temperature (25 °C) for 1 h to achieve equilibrium temperature. The extruded water was removed by cotton. According to a modified protocol of Chong and Kang (2007), gels in the beakers, were subjected to TA-XT plus texture analyzer (Stable Micro System Corporation, UK). A P/5 probe was employed for the determinations, in which the parameters were 2.0 mm/s for pre-test speed, 1.0 mm for test speed with the trigger force at 3 g. The penetrative distance was set at 10 mm and the first peak force was recorded as the gel strength.

2.2.8. Surface hydrophobic interactions (H_0)

Determinations of H_0 were carried out based on the method of Xue et al. (2017), using MPS at a concentration of 2 mg/mL. Twenty-microliters of 8-anilino-1-naphthalene sulphonic acid (ANS, 15 mM, pH 7.0) solution was vortexed with 4 mL of MPS (2 mg/mL). The reaction mixture was kept in the dark for 20 min and then subjected to centrifugation (5000 g, 5 min, 4 °C) prior to measurement of fluorescence intensity (SpectraMax M2, Molecular Devices Limited, USA). Using an excitation wavelength of 375 nm, the arbitrary units (a.u.) were recorded at the emission wavelengths ranging from 430 to 610 nm.

2.2.9. Reactive-sulfhydryl contents (R-SH)

Using the method of Ellman (1959), 5 mL of diluted MPS (2 mg/mL) were mixed with 20 μL of 5,5′-dithiobis-2-nitroben-zoic acid (DTNB, 10 mM, pH 7.0). The mixtures were placed in a cold, dark environment (4 °C) for 1 h, after which, they were subjected to centrifugation as above. The absorbance of each supernatant was measured at 412 nm (SpectraMax M2, Molecular Devices Limited, USA). R-SH content of each sample was calculated based on the following equation:

$$\text{R-SH}\ (\mu\text{mol}/100\ \text{mg}) = (A_{412} \times c)/E_M$$

where: A_{412} is the measured absorbance at 412 nm, c represents the protein concentration (2 mg/mL), and E_M is the molar extinction coefficient (13,600 $M^{-1}\ cm^{-1}$).

2.3. Statistical analysis

Three independent trials (replications) were carried out on different occasions, and at least three measurements were performed on each trial ($n = 3$). Results of protein solubility and penetration force were displayed as mean ± standard deviation. One-way ANOVA analysis and Duncan's multiple range test were performed by SPSS version 23.0 (IBM, Armonk, NY, USA). Significance was inferred when the results were within the 95% confidence level ($p < 0.05$).

3. Results and discussion

3.1. Protein solubility

High protein solubility of MPS is essential for the quality of processed meat products. With the extraction method used, the soluble protein content was 4.68 mg/mL (Fig. 1). In contrast, HPP increased the solubility of MPS to 6.43 mg/mL, significantly higher than for the other treatments used ($p < 0.05$). This was in agreement with the previous study of Sikes, Tobin, and Tume (2009) when using low-salt beef batters. On the other hand, the HIU treatment reduced the protein solubility, as found by Li et al. (2014) on PSE-like chicken breast meat. They attributed this to HIU-induced protein conformational changes and to enhancement of protein-protein interactions. Of interest for this work was that the protein solubility of HPH-treated samples was predominantly lower than for the control. Chen and coworkers found that the solubility of MPS in water was significantly improved after HPH treatment (103 MPa, 2 passes) (Chen et al., 2016), however, the solubility of MPS in 0.6 M KCl (20 mM KH_2PO_4/K_2HPO_4, pH 6.5) solution was reduced markedly ($p < 0.05$) compared to the control. Fernández-

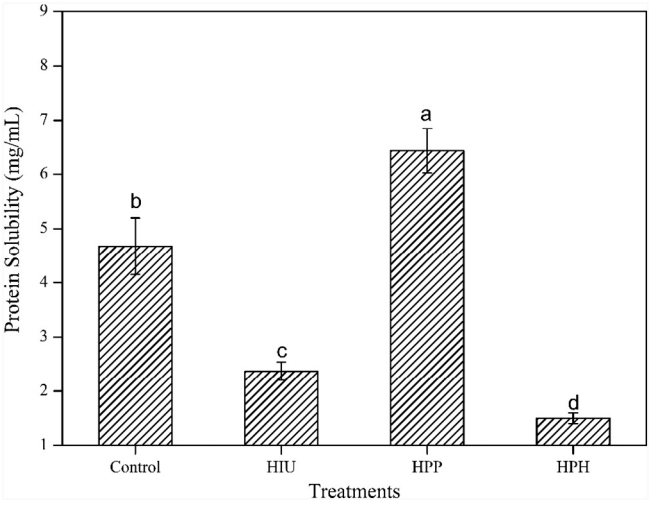

Fig. 1. Effects of HIU, HPP and HPH treatments on the solubility of myofibrillar proteins suspension (MPS). Note: Values are presented as means ± standard deviation (SD); different letters (a–d) above columns indicate significant difference ($p < 0.05$); Control: untreated MPS; HIU: MPS subjected to high-intensity ultrasound treatment; HPP: MPS subjected to high-pressure processing; HPH: MPS subjected to high-pressure homogenization.

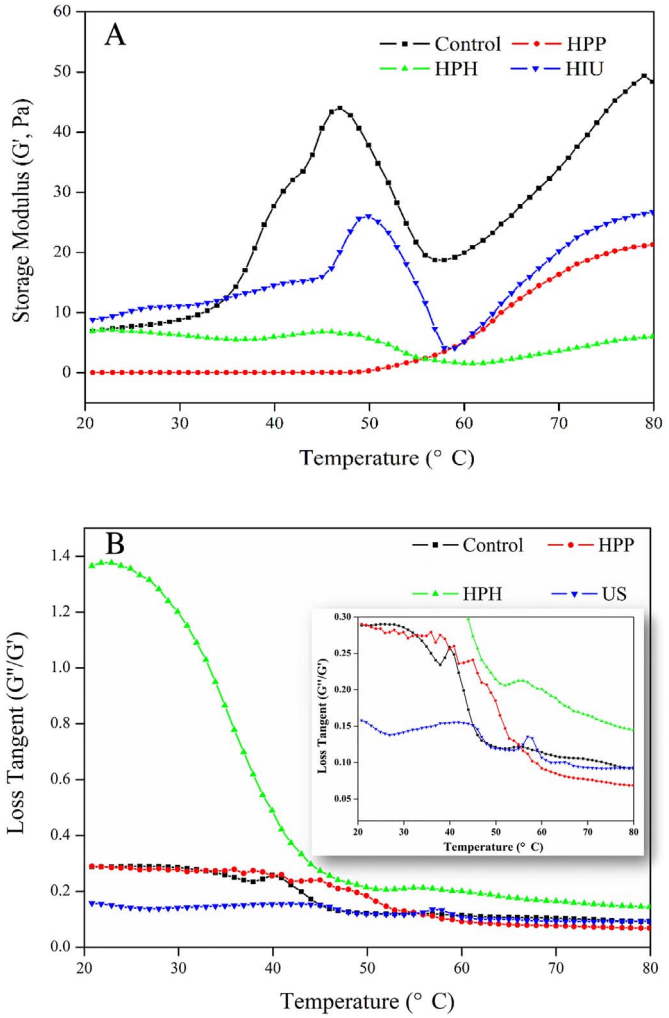

Fig. 2. A. Effects of HIU, HPP and HPH treatments on the dynamic storage modulus (G′) of myofibrillar proteins suspension (MPS) during heating (20–80 °C). B. Effects of HIU, HPP and HPH treatments on the loss tangent (G″/G′, tanδ) of myofibrillar proteins suspension (MPS) during heating (20–80 °C). Note: Control: untreated MPS; HIU: MPS subjected to high-intensity ultrasound treatment; HPP: MPS subjected to high-pressure processing; HPH: MPS subjected to high-pressure homogenization. The inserted figure is the enlarged part of the Control, HPP and HIU treated MPS.

Martín, Cofrades, Carballo, & Jiménez-Colmenero (2002) illustrated that application of sodium chloride (1.5%), tripolyphosphate (0.3%), HPP (400 MPa, 70 °C, 30 min), and their combinations have various impacts on native meat protein status through modifying the ionic strength and/or pH values of the meat batter system. Therefore, it is speculated that the different ionic strengths, pH values and even the different protein extraction methods used by others are likely to have accounted for these differences. Moreover, the modified MPS solubility may have further impacted on the functionalities of the thermally induced MP gels.

3.2. Dynamic rheological properties

Alterations of storage modulus (G′) as a function of temperature (20 °C–80 °C) can be a useful indicator of the gel-forming ability of MP. Moreover, it can provide information regarding the gelation process and the elasticity of gels (Wu, Xiong, & Chen, 2011). As displayed in Fig. 2A, the typical denaturing pattern of MP (up-down-up) was observed in the control and the HIU-treated MPS. However, the initial denaturing temperature (T_i) of HIU samples was around 44 °C, while that of the control was near 32 °C. The G′ of the HIU-treated MPS coincided with that of HIU-treated chicken breast meat batter (Li et al., 2014). It appears that the MP, myosin in particular, has been partly denatured by HIU treatment prior to heating, hence altering the MP's thermal denaturing behavior. This is also applicable to HPP and to HPH-treated MPS, as there were no detectable denaturing peak in the range of 30 °C to 60 °C (Fig. 2A). Many studies ascribed this phenomenon to the impaired sensitivity of the myosin head region to heat (Cao, Xia, Zhou, & Xu, 2012; Chen et al., 2014). Moreover, we assumed that the α-helical conformation of the myosin tail region might be partly uncoiled, since the formation of the G′ peak is mainly attributed to the cross-linking of myosin heads (G′ increment) at the early heating stage, and then the unfolding of myosin tails driven by heating (G′ reduction) before further head-tail cross-linking (Verbeken, Neirinck, Van Der Meeren, & Dewettinck, 2005). Further, unlike the HPP-treated MPS, the G′ of which had a rapid increase when the temperature was higher than 50 °C, the G′ of HPH-treated MPS did not undergo drastic changes in the temperature range applied (20–80 °C).

Regarding the changes of phase angle (δ), which is presented as tanδ in Fig. 2B, further verified that the physicochemical properties of the treated MPS were quite different from the control. Initially, the tanδ of HPH-treated MPS was > 1, denoting a more viscous than elastic nature (Yang et al., 2015). Also, HPH-treated MPS maintained higher tanδ values throughout heating compared with other samples, which is in line with its distinctive G′ trend during heating (Fig. 2A). Regarding the overall transformation trends of all samples, it was shown that the HPH-treated MPS had the most drastic phase transition process during heating, especially in the range of 25 °C to 48 °C. However, except for the HPH-treated MPS, the other samples underwent relatively small phase transitions, from MPS to that of an interconnected 3-D network.

Generally, when MPS were subjected to each of the three technologies, they were denatured in different ways, as indicated by their distinctive gelation patterns observed by the dynamic rheological data (Fig. 2A and 2B). Considering the differing mechanistic actions of the various technologies (HIU, HPP and HPH), we believe that for HPH, the majority of MP in 0.6 M KCl solution (20 mM KH_2PO_4/K_2HPO_4, pH 6.5), were denatured mainly by the simultaneous effects of shearing, cavitation and high-pressure conditions as the MPS passes through the chamber. As a result, chemical interactions such as hydrogen bonding, ionic bonding and disulfide bonds were altered and modified during the subsequent heating process. However, for MPS subjected to HIU and HPP, the modifications were more mild, resulting in unfolding or folding of protein conformations without inducing any major detrimental effects on the MP's gelation properties.

3.3. WHC

The WHC of gel-type meat products is an important attribute as it determines the production yield and quality of these products (Joo, Kauffman, Kim, & Park, 1999). The results of CL (%) and EMC (%) of samples are presented in Fig. 3. However, HPH-treated group was not included since MP were unable to form a gel following HPH processing (Fig. 4). In addition, it is clear that HPP-treated MPS formed thermal gels having significantly lower CL (%), while HIU treatment led to adverse results ($p < 0.05$). It should be noted that the data for EMC (%) did not completely align that of CL (%); the HPP-treated MPS formed thermal gels that gave higher amounts of expressible water, which is in agreement with previous work under the same HPP conditions (Xue et al., 2017). Also, on the basis of the sensory evaluation and statistical analysis of that study, such characteristic denotes better juiciness of gel-type meat products. Nonetheless, the EMC (%) of HIU-treated MP gels was contrary to the findings of Li, Kang, Zou, Xu, and Zhou (2015), who reported that EMC (%) of cooked chicken meat batter was significantly reduced by applying HIU to batters. It is possible that these differences might be attributable to the different sample types used (chicken breast meat batter vs. MPS), hence the effective output energies received by the MP was different. As a result, the extent of

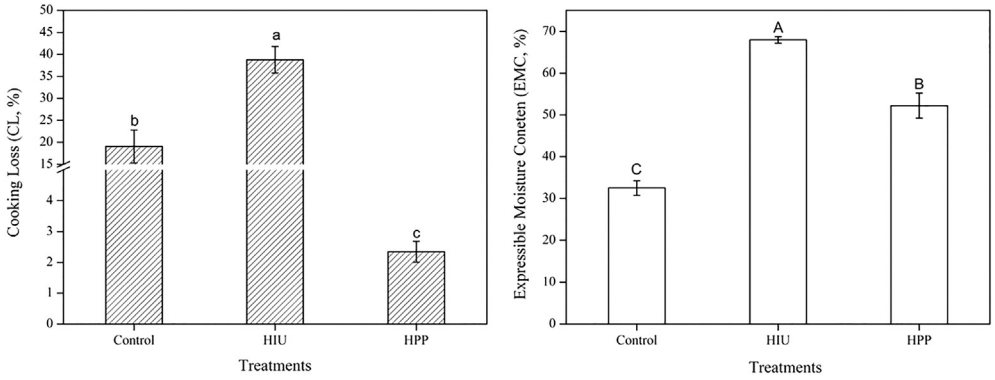

Fig. 3. Effects of HIU, HPP and HPH treatments on the water-holding capacity of thermally-induced myofibrillar protein (MP) gels. Note: Values are presented as means ± SD; different letters (a–c) above columns indicate significant differences ($p < 0.05$) of Cooking loss of samples, and A–C indicated significant differences of EMC of samples. Control: untreated MPS; HIU: MPS subjected to high-intensity ultrasound treatment; HPP: MPS subjected to high-pressure processing.

protein denaturation would likely be different, thus affecting protein aggregation behavior. This is supported by the protein solubility data in Section 3.1. Therefore, HIU-modified proteins were unlikely to undergo adequate unfolding during the early stages of heating (30–40 °C), resulting in a shrunken gel structure, which would limit the entrapment of water (Ferry, 1948). For HPP, where a relatively mild processing conditions were used (200 MPa, 9 min, 25 °C), the MP were modified moderately in a way that exposed more of the originally buried hydrophilic groups of myosin to surface, therefore strengthening the hydration effect of the proteins involved. This led to a reduced CL, yet the relatively larger micro-structural pores were unlikely to retain the water when centrifuged for the EMC (%) determination. Nevertheless, HP-modified meat proteins are likely to form gels that have a lower CL, which might contribute to the particular quality attributes of gel-type meat products desired by processors and consumers, such as juiciness.

3.4. Gel strength

Gel strength is an essential mechanical indicator of the textural properties of gels. As shown in Fig. 5, compared with the control, HPH-treated MPS showed the lowest gel strength value ($p < 0.05$). Moreover, based on the previous results for HPH-treated samples, the gel strength should be represented by the resistance forces of the mixture of aggregated MP and water against the probe, rather than an authentic reflection of gel strength. This would account for the significantly lower "gel strength" of thermally induced protein gels that had previously been subjected to HPH treatment, compared to the other samples ($p < 0.05$). However, the gel strengths of HPP- and HIU-treated MPS were significantly lower than the control ($p < 0.05$). Despite that, many studies have found an improved gel strength following HP pretreatment (e.g. 200 MPa at 10 °C for 2 min) (Sikes et al., 2009; Yang et al., 2015), possibly as a result of differences in parameters used such as HP equipment, HP intensity and/or pressurization/decompression rates (Bajovic, Bolumar, & Heinz, 2012). Also, enhanced WHC should also contribute to weaker gel strength, since more water is entrapped in the network, leading to a less compact gel structure. However, regarding the HIU-treated MPS, which had lower WHC, the inferior gel strength might be associated with the insufficient unfolding before gel network formation and the resulting weak gel structure. Presumably, HIU-modified myosin may be incapable of forming a highly interconnected and homogenous tri-dimensional network, leading to inferior gel properties. In addition, there may be effects of HIU treatment on other components of MP, such as actin or C-protein. Overall, the

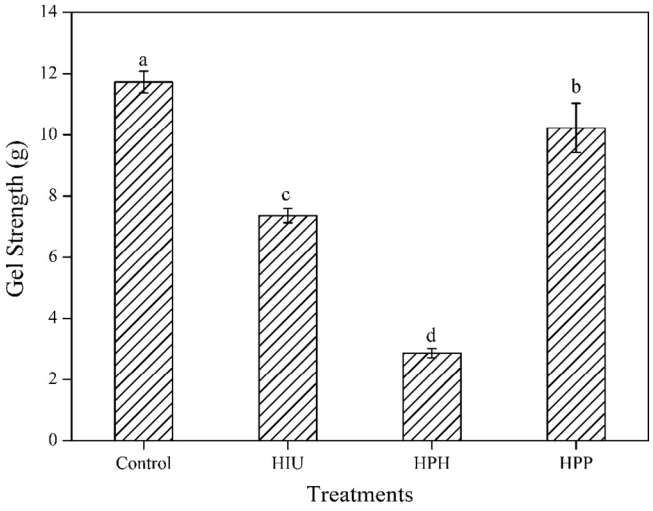

Fig. 5. Effects of HIU, HPP and HPH treatments on gel strength of thermally-induced myofibrillar protein (MP) gels. Note: Values are presented as means ± SD; different letters (a–d) above columns indicate significant differences ($p < 0.05$); Control: untreated MPS; HIU: MPS subjected to high-intensity ultrasound treatment; HPP: MPS subjected to high-pressure processing; HPH: MPS subjected to high-pressure homogenization.

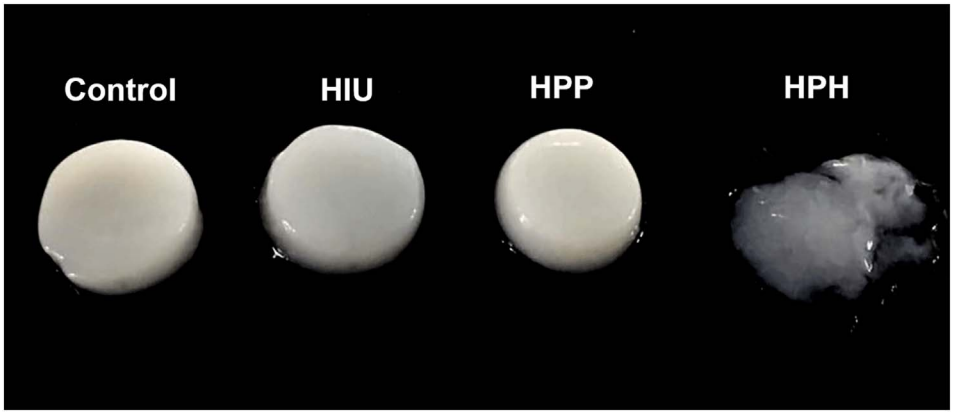

Fig. 4. Effects of HIU, HPP and HPH on the configuration of thermally-induced MP gels. Note: Control: untreated MPS; HIU: MPS subjected to high-intensity ultrasound treatment; HPP: MPS subjected to high-pressure processing; HPH: MPS subjected to high-pressure homogenization.

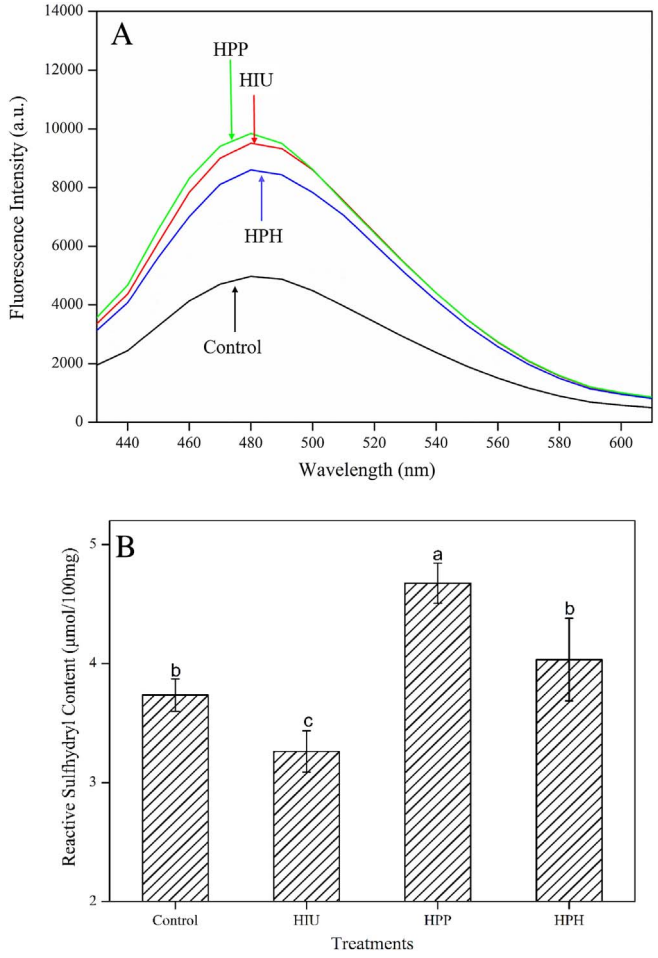

Fig. 6. A. Effects of HIU, HPP and HPH treatments on hydrophobic interactions of myofibrillar proteins suspension (MPS). B. Effects of HIU, HPP and HPH treatments on reactive-sulfhydryl contents of myofibrillar proteins suspension (MPS). Note: Values in Fig. 6A are presented as means; values in Fig. 6B are presented as means ± SD; different letters (a–c) above columns indicate significant differences ($p < 0.05$); Control: untreated MPS; HIU: MPS subjected to high-intensity ultrasound treatment; HPP: MPS subjected to high-pressure processing; HPH: MPS subjected to high-pressure homogenization.

selected three technologies have their own specific effects on MP, of which, the HPP technique appears to be the most effective for modifying meat proteins for the manufacture of quality-enhanced gel-type meat product.

3.5. Tertiary conformational changes

The exposures of hydrophobic (H_0) and reactive sulfhydryl (R-SH) sites are prerequisites for protein gelation (Hu et al., 2013). The extent of their surface exposure is usually associated with the functionalities of the protein gels (Gülseren, Güzey, Bruce, & Weiss, 2007). In addition, alterations of H_0 and R-SH contents are good indicators of tertiary conformational changes of certain proteins, denoting the extents of protein folding/unfolding. The results of H_0 and R-SH contents of MPS following the different treatments are presented in Fig. 6A and 6B. Clearly, applications of HIU, HPP and HPH to MP consistently increased the H_0, indicating that all three treatments favored the surface exposure of hydrophobic amino acid residues, such as tyrosine, tryptophan and/or phenylamine etc. (Howell, Arteaga, Nakai, & Li-Chan, 1999). These findings are in accordance with previous studies using HIU, HPP and HPH technologies (Cao et al., 2012; Chen et al., 2016; Zhang et al., 2017). Therefore, high pressure, cavitation and high shear forces, as well as some other factors such as increasing temperature or cavitation are likely to induce the unfolding of some protein segments that contain these hydrophobic groups.

On the other hand, the overall R-SH contents of the three samples did not follow similar tracks as for H_0 (Fig. 6B). However, these findings do provide important information on the effects of HIU on the folding of certain protein segments under the conditions used; specifically the lower R-SH content ($p < 0.05$). Gülseren and coworkers observed a similar result where HIU-induced a reduction in R-SH. They assumed that hydrogen peroxide was induced by HIU treatment, thus causing oxidation of thiol groups (Gülseren et al., 2007). In contrast, the R-SH content of the HPP-treated MPS was markedly increased ($p < 0.05$) compared to the other samples. However, the content of R-SH in MP following HPH treatment did not differ from the control ($p > 0.05$).

Generally, higher values of H_0 and R-SH contents in proteins are beneficial for the formation of protein aggregations, thereafter affecting the relevant gelation properties (Bryant & McClements, 1998). However, our findings for H_0 and R-SH were not in keeping with changes in protein solubility (Fig. 1), where HPP-treated MPS had the highest contents of dissolved proteins. Since alterations in protein conformations can be very complex, it is possible that inputs in addition to pressure caused modulation of water-protein interactions. Based on this, we postulate that MPS, when subjected to HPP (200 MPa for 9 min at 25 °C), is modified in a manner that improves its hydrophilic ability, which dominates over the simultaneous strengthening of hydrophobicity. Hence gel formation is favorable for WHC. However, regarding the HPH-treated samples, the aforementioned hydrogen peroxide effects should not be ignored since a similar cavitation effect also appears during HPH. It is thus hypothesized that HPH treatment might induce the formation of 'transitional hydrogen peroxide' substances, which could be triggered by changes of molecular movement. Our assumption is that processes such as centrifugation, increase the likelihood of the movement of molecules, which also increases the probability of protein-oxygen contact, thus triggering the status transformation of these 'transitional hydrogen peroxide' matters to be hydrogen peroxide. Therefore, the solubility of HPH-treated MPS, as determined by the centrifugal method, significantly decreased despite having been subjected to similar conditions of high pressure. Also, in comparison to treatments with HIU or HPP, the processing conditions used for HPH were more vigorous, simultaneously involving cavitation, high pressure and severe shear forces (Dumay et al., 2013). Consequently, MPS subjected to HPH treatments were considerably denatured, which essentially destroyed the gelation ability of the MP.

4. Conclusion

Under the processing conditions used, the three non-thermal technologies affected MP through different mechanisms. As displayed in the Supplementary file, for HIU the cavitation effect resulting from treatment, induced a reduction in the reactive-sulfhydryl content and MP solubility (0.6 M KCl, 20 mM KH_2PO_4/K_2HPO_4, pH 6.5); regarding HPP, the static high pressure condition imparted mild effects on MP, exposing some buried groups, which indicated the unfolding of the tertiary conformation of MP; while HPH works in a destructive way, resulting in reduced solubility of MP (0.6 M KCl, 20 mM KH_2PO_4/K_2HPO_4, pH 6.5) as well as altering the structures of MP. With regard to the manufacturing of gel-type meat products, HIU may be a potential technique, however the merits would be compromised by the heterogenous distribution of ultrasonic waves; HPP appears to have the most potential for improving the quality of products, whereas HPH technique is not an appropriate way to modify proteins that used for gel forming. To select the optimal parameter for manufacturing gel-type meat products, additional work needs to be performed.

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