Effects of high-pressure, ultrasound and high-pressure homogenizing treatments on myofibrillar protein solubility

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Abstract – Three non-thermal technologies, high-pressure processing (HPP), high-intensity ultrasound (US) and high-pressure homogenization (HPH), were applied to chicken myofibrillar proteins (MPs) in 0.6 M KCl phosphate buffer (pH 6.5). Compared with the Control, the HPP-treated MPs had an increased solubility while both US and HPH had reduced solubilities in this high-ionic strength solution. The sulfhydryl contents of MPs were significantly increased with HPP and HPH treatments ($P < 0.05$), whereas all three technologies enhanced the hydrophobicity of samples significantly. However, regarding the HPP-treated samples, the simultaneous increases in sulfhydryl and hydrophobic groups did not lead to reduction of MPs' solubility in saline solution, thus indicating that the HPP conditions may be conducive to bring more hydrophilic groups to the surface, which dominated over the effects of the enhanced hydrophobicity.

Key Words – Non-thermal treatments; Hydrophobic interactions; Reactive sulfhydryl contents.

I. INTRODUCTION

Myofibrillar proteins (MPs), and their solubility in particular, are instrumental for the quality of meat and meat products [1]. Impairment in quality of meat products is generally related to the decreased amount of extracted salt-soluble proteins [2]. Processes such as tumbling and marination are aimed to increase the solubility of MPs [3]. However, there are reports where superior texture was observed in meat products with reduced solubility of proteins [4, 5], particularly with some additional treatments.

Non-thermal technologies are of interest to meat scientists since MPs are heat-sensitive and inappropriate thermal treatment before formulation and cooking may cause detrimental effects to meat or meat products. Therefore, technologies such as high-pressure processing (HPP) and high-intensity ultrasound (US) are considered as potential tools to modify MPs so as to improve the acceptability of meat or meat products [6]. Li and coworkers have demonstrated that application of US treatment (frequency 20 kHz, 450 W, amplitude 60% for 6 min) can improve the functional properties of PSE-like chicken breast meat batter [7]. Many researchers found that HPP technology can improve the quality of gel-type meat products when meat batters were subjected to appropriate HPP conditions. Our previous work revealed that 200 MPa for 9 min at 25 °C reduced the cooking loss and improved the juiciness of rabbit meat sausages [8]. Recently, scientists have attempted to take advantage of high-pressure homogenization (HPH) to alter the physicochemical properties of meat proteins. Chen *et al.*[9] reported that HPH (two passes at 103 MPa) can improve the solubility MPs' in water (5 mg/mL).

However, few studies have attempted to compare the effects of these three technologies on the physicochemical properties of MPs. Therefore, the main objective of this study was to directly compare the effects of US, HPP and HPH on the solubility of MPs as well as exploring the underlying mechanism from the perspective of tertiary conformational changes.

MATERIALS AND METHODS

Myofibrillar proteins (MPs) were extracted from chicken breast meat, (purchased from a Suguo supermarket, Nanjing, China). Based on the protocol of Xiong *et al*. [10], the rigor buffer, which contained 0.1 M KCl, 2 mM $MgCl_2$, 1 mM EGTA, 0.5 mM dithiothreitol, and 10 mM K_2HPO_4 (pH 7.0) was used to extract myofibrillar proteins. Subsequently, the MPs were dispersed in a high-ionic strength saline solution (0.6 M KCl, 20 mM potassium phosphate, pH 6.5). The concentration of MPs was adjusted to 20 mg/mL prior to different treatments: high-pressure processing (HPP, 200 MPa, 9 min at 25°C), ultrasound (US, frequency 20 kHz, 450 W, amplitude 60% for 6 min) and high-pressure homogenization (HPH, 103 MPa, two passes). A sample without any additional treatments was set

as the Control. Following treatments, the four different samples were placed in a chiller (4°C) overnight prior to further determinations. When required, the protein concentrations of each sample were adjusted to either 10 mg/mL or 2 mg/mL, for determination of protein solubility [11], reactive sulfhydryl content and surface hydrophobic interactions (H_0) using 5, 5′-dithiobis-2-nitrobenzoic acid (DTNB) and 8-anilino-1-naphthalene sulphonic acid (ANS), respectively [12]. A total of three individual experiments were carried out on different days (n = 3). The obtained data were subjected to a one-way ANOVA analysis and Duncan's multiple range test using SPSS version 16.0 (IBM, Armonk, NY, USA) and were presented as mean ± standard deviation (SD). Significance was inferred when the differences were within the 95% confidence level ($P < 0.05$).

II. RESULTS AND DISCUSSION

With the extraction method used, the soluble protein content of the untreated Control was 4.68 mg/mL. The solubilities of MPs' after US, HPP and HPH treatments are displayed in Figure 1. The highest solubility was found for the HPP-treated samples, which was 6.43 mg/mL, significantly higher than for other treatments ($P < 0.05$). This improved solubility of meat proteins, following application of defined HPP conditions was also observed by Sikes *et al*.[13] when using low-salt beef batters. The US treatment reduced the protein solubility, which was in line with previous work conducted by Li *et al*.[7] on PSE-like chicken breast meat. They attributed this to US-induced protein conformational changes and to enhanced protein-protein interactions. It is of interest that the protein solubility of HPH-treated samples was predominately lower than for the Control. Chen and coworkers found that the solubility of myofibrillar proteins in water was significantly improved after HPH treatment (103 MPa, 2 passes) [14], however, the solubility of myofibrillar proteins in saline solution was reduced markedly ($P < 0.05$) compared to the Control. The different ionic strengths, pH values and the different protein extraction methods used by others are likely to have accounted for these differences [15].

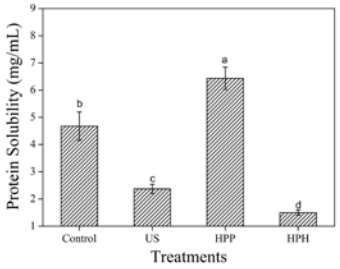

Figure 1. Effects of various non-thermal treatments on protein solubility

To tentatively explore the underpinning mechanisms, the reactive sulfhydryl content and the hydrophobicity of MPs after various treatments were estimated, since their exposure is a prerequisite for protein aggregation [16]. As shown in Figure 2, it can be seen that MPs treated with either HHP or HPH displayed higher reactive sulfhydryl contents than the Control and US ($P < 0.05$). It appears that the US-treated MPs had less exposed sulfhydryl sites, which might be attributed to the US-induced cavitation effect, generating hydrogen peroxide causing oxidation of the sulfhydryl groups [17]. In contrast, the HPP and HPH treatments favored the exposure of the buried groups (Figure 2). Although the HPH technology may also generate cavitation, the high-pressure conditions might protect proteins from being oxidized as the proteins pass through the channel.

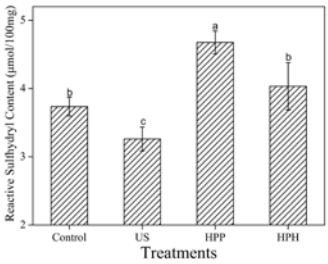

Figure 2. Effects of various non-thermal treatments on the reactive sulfhydryl contents of myofibrillar proteins.

Changes in hydrophobic groups on the surface of proteins indicate the stability, conformation and potential function of proteins [16]. Applications of US, HPP and HPH to MPs consistently improved the H_0 (Figure 3). Generally, the higher level of H_0 of proteins is beneficial for the formation of protein aggregations [18], however, the obtained results were not in line with the results of protein solubility (Figure 1). The modifications of protein conformations can be very complex; therefore, some other changes induced by additional treatments might occur, modulating the water-protein interactions. Therefore, we postulated that MP subjected to HPP (200 MPa for 9 min at 25 °C) was modified in a manner that improved its hydrophilic ability, which dominated over the simultaneously strengthening of hydrophobicity. However, regarding the HPH-treated samples, the aforementioned hydrogen peroxide effects should not be neglected. It is thus hypothesized that HPH treatment induced certain 'transitional hydrogen peroxide' substances, which could be triggered by environmental changes, such as the process of centrifugation, which increases the probability of a molecules' movement. 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 processing.

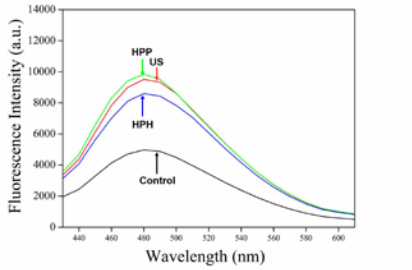

Figure 3. Effects of various non-thermal treatments on the hydrophobicity of myofibrillar proteins

III. CONCLUSION

The solubility of MPs in a high ionic strength solution was increased by HPP, whereas applications of HPH and US led to reduced solubilities. Changes in the sulfhydryl contents and H_0 suggested that the tertiary conformations of MPs were modified by the applied non-thermal treatments, which were highly conducive to expose the hydrophobic groups on the protein surface. However, the enhanced H_0 did not result in lower protein solubility, especially for those samples subjected to HPP. Further work is required to determine the actual changes in the physicochemical properties induced by each of the US, HPH and HPP technologies.

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