Synergistic effect of preheating and different power output high-intensity ultrasound on the physicochemical, structural, and gelling properties of myofibrillar protein from chicken wooden breast

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ABSTRACT

The effects of preheating to 50 °C and the subsequent application of high-intensity ultrasound (HIU, 20 kHz) at 200, 400, 600, and 800 W on the physicochemical, structural, and gelling properties of wooden breast myofibrillar protein (WBMP) were studied. Results suggested that the WBMP structure expanded to the balanced state at 600 W, and rheological properties exhibited that 600 W HIU (P < 0.05) significantly improved the storage modulus (G') of WBMP. Notably, the WBMP gel (600 W) had the best hardness (65.428 ± 0.33 g), springiness (0.582 ± 0.01), and water-holding capacity (86.11 ± 0.83%). Raman spectra and low-field NMR indicated that 600 W HIU increased the β-sheet content (37.94 ± 0.04%) and enlarged the immobilized-water proportion (93.87 ± 0.46%). Scanning electron micrographs confirmed that the gel was uniform and dense at 600 W. Therefore, preheating to 50 °C followed by HIU (600 W) helped form a superior WBMP gel.

1. Introduction

Chicken is widely favored by people owing to its cheap price and comprehensive nutrition profile [1]. The rapid growth of chickens has become a primary selection criterion in the modern broiler industry. This has caused numerous meat-quality problems, such as abnormal meat. Wooden breast (WB) is a type of abnormal meat that has negatively impacted the poultry industry [2]. WB meat exhibits distinct hardening on the upper part of the pectoralis major muscles, accompanied by stripes and bleeding spots [3]. According to the existing survey, the incidence of WB is more than 60% worldwide, and serious cases also account for a large proportion (>42% were severe or very severe) [4]. WB has adverse effects on the function of chicken meat [5]. Myofibrillar protein (MP) is the main component of muscle proteins, accounting for 55%-65% of total muscle protein [6]. MP is closely related to the functional characteristics of meat products, including gelation, emulsification, and water-binding properties [7]. The impaired functional properties of the MP in muscles are the main reason for the decline in meat quality, such as solubility and emulsification [8]. The strength of WBMP gels is low, which results in large agglomerations or cavity in the gel network [4]. Overall, WB leads to a decrease in the processing properties of chicken meat [9]. It is necessary to improve the functional properties of WBMP.

There are many physical and chemical methods that can be applied to improve the functional properties of WBMP and subsequently enhance the texture and water-holding capacity (WHC) of the resulting WBMP gel. These strategies include high-intensity ultrasound (HIU) [10], pulsed electric fields [11], pH shifting and low-speed shearing [12], glycosylation [13], and isoelectric solubilization/precipitation [14]. Among these, HIU is convenient for use in actual production. HIU has been widely used in the meat-processing industry to improve the quality of meat products [15]. With the continuous circulation of ultrasound, countless cavities and bubbles form and then collapse in the medium. This implosion releases powerful micro-jet-like energy and
changes the microenvironment, resulting in the modification of muscle proteins. However, simple HIU is unable to effectively improve the quality of protein gels [16]. Many studies have reported that preheating can promote the formation of regular clusters of proteins, which can contribute to the formation of protein gels [17–19]. Moreover, the pre-agglomeration of protein caused by preheating can influence HIU effects. Therefore, combining preheating with HIU can be considered for the quality improvement of the WBMP gel.

In this study, HIU was performed under uniform frequency (20 kHz) conditions after preheating the WBMP solution to 50 °C (in the pre-experiment, we found that preheating to 50 °C is the most suitable parameter for HIU synergy), and the quality improvement effect of different power outputs (200, 400, 600, and 800 W) on the WBMP gel was explored. Preheated samples without HIU were used as the control group. The physicochemical, structural, and gelling properties of WBMP and its gel were studied. This study could provide a theoretical basis for combining preheating with HIU technology to enhance the functional properties of MP and contribute to the utilization of poor-quality proteins like WBMP.

2. Material and methods

2.1. Materials

WB meat was procured from Zhucheng Waimao Co. Ltd. (Zhucheng, China) and selected using visual and pressing tests based on the method reported by Cai, Shao, Chen, Campbell, Nair, Suman, Beach, Guyton and Schilling [20]. Samples with medium/severe degrees of WB were selected for this study. Other chemicals were of at least analytical reagent grade.

2.2. Extraction of WBMP

Protein extraction was conducted following the method reported by Han, Wang, Xu and Zhou [21]. Muscles were ground and homogenized at 10,000 × g for 30 s in a four-buffer system (100 mM KCl, 2 mM MgCl₂, 1 mM EGTA, and 10 mM K₃HPO₄; pH 7.0; chicken: buffer = 1:4 [w/v]). This process was repeated three times. Bovine serum albumin was used as a standard to evaluate the protein concentration according to the Biuret method. Fresh proteins were stored at 4 °C for subsequent dissolution using a phosphate buffer (0.6 mol/L KCl, 0.01 mol/L KH₂PO₄, pH 6.0) and concentration adjustment.

2.3. Preheating combined with HIU treatment, and the preparation of WBMP gels

WBMP was diluted to 40 mg/mL in a phosphate buffer (0.6 M KCl, 0.01 M KH₂PO₄, pH 6.0). Samples were preheated to 50 °C and then subjected to HIU treatment at 200, 400, 600, and 800 W. Samples were treated at different powers for 15 min (20 kHz, pulse durations of 5 s ON and 1 s OFF). The Vibra-Cell TM Ultrasonic Processor model VC 750 (Sonic & Materials, Inc., USA) was equipped with a 6 mm diameter probe, and HIU was conducted under ice-bath conditions; the samples without HIU treatment served as the control group. Thereafter, the treated WBMP gel was placed in a water bath and heated from 20 to 80 °C at a heating rate of 1 °C/min, followed by incubation at 80 °C for 30 min. After cooling, the sample was measured.

2.4. Particle size distribution

Dynamic light scattering (DLS) measurements were performed following the method reported by Chen, Zou, Han, Pan, Xing, Xu and Zhou [22]. The concentration of each WBMP particle solution was reduced to 0.5 mg/mL. The sample was transferred to a 1 cm path-length quartz cuvette subjected to DLS measurement, and the detection angle was 90° (25.0 ± 0.1 °C). Each sample was measured using a DLS instrument (ZEN 3690, Malvern Instruments Inc., Malvern, UK).

2.5. Solubility

Slight modifications were made following the method reported by Anion, de Lamballerie and Speroni [23]. Samples were diluted to 3 mg/mL with 5 mM sodium phosphate (5 mM EDTA, pH 7.0) and then centrifuged at 10,000 × g for 15 min. The protein content in the supernatant was the solubility value, expressed as a percentage relative to the total protein content. The protein content was determined using the biret method.

2.6. Surface hydrophobicity

The fluorescent probe, 8-anilinonaphthalene-1-sulfonate (ANS), was used to determine surface hydrophobicity [24]. A RF-5301PC fluorescence spectrophotometer (Shimadzu Co., Kyoto, Japan) was used to measure the fluorescence spectra from 410 to 760 nm with an excitation wavelength of 390 nm, as well as to record the highest fluorescence intensity.

2.7. Intrinsic tryptophan fluorescence-emission spectroscopy

A slight modification was made to the method reported by Xu, Zhao, Wei, Zhang, Dong, Huang, Han, Xu and Zhou [25]. Intrinsic tryptophan fluorescence-emission spectra were obtained using an RF-5301PC fluorescence spectrophotometer (Shimadzu). Emission spectra were recorded using the samples with a concentration of 0.2 mg/mL at an excitation wavelength of 290 nm, a spectral wavelength range of 320–420 nm, and a constant slit of 5 nm.

2.8. Rheological properties

The rheological properties of the samples were determined using a DHR-1 hybrid rheometer (MCR302, Anton Paar, Austria) equipped with a parallel plate (60 mm diameter, 1 mm thickness) and connected to a cooling system (Thermo Cube, New York, NY, USA). The sample concentration was 40 mg/mL. The flow behavior of the samples was determined using tests based on a previously reported method [26]. Apparent viscosity was determined in the shear-rate range of 0.1–1000 s⁻¹ at 25 °C with a gap width of 1000 μm. Additionally, the viscoelastic properties of the samples were analyzed to determine the storage modulus (G’), and the loss modulus (G’’), as functions of the angular frequency (ω) in the range of 0.1–1000 rad/s. Frequency scanning was conducted when the amplitude strain in the linear viscoelastic region was 1%. The storage modulus (G’) profile of WBMP was measured as the temperature increased from 20 to 80 °C at a heating rate of 1 °C/min (thermal gelation), and G’ values were continuously recorded.

2.9. Texture and WHC of WBMP gels

A texture analyzer (Texan 200, Lamy Inc., France) was used to measure the texture of WBMP gels [27]. WBMP gels were cut into small cubes (2 cm × 2 cm × 2 cm). The probe was P 0.5, and measurement parameters were set as follows: strain of 50%; trigger force of 5 g; and pre-test, test, and post-test speeds of 0.2, 0.8, and 0.8 mm/s, respectively. Each sample was measured three times. The WHC (%) of WBMP gels was determined using a formula based on the centrifugal method [16]:

\[
WHC(\%) = \frac{W_2 - W}{W_1 - W} \times 100\%
\]

where \(W_2\) is the total weight (g) of the centrifuge tube and the WBMP gel (wipe dry the water) after centrifugation, \(W_1\) is the total weight (g) of the centrifuge tube and the WBMP gel before centrifugation, and \(W\) is...
the weight (g) of the centrifuge tube. Each measurement was performed three times.

2.10. Raman spectroscopy

The Raman spectra of WBMP gels were collected at room temperature (25 °C) using a laser Raman spectrometer (INVIA REFLEX 12-80000, Renishaw Inc., UK). Detection parameters were set as follows: power of 100 mW, aperture of 600 μ, raster of 600 g/mm, scanning area in the range of 400–3500 cm⁻¹, resolution of 4 cm⁻¹, and scanning speed of 120 cm⁻¹/min. The content of each protein structure (α-helix, β-fold, β-turn, and random coil) was calculated using the method reported by Alix, Pedanou and Berjot [28]. Each measurement was performed three times.

2.11. Low-field NMR

The mobility and distribution of the water in WBMP gels were evaluated using the spectra obtained at 32 °C under a resonant frequency of 22.6 MHz and a scanning frequency of 32 MHz using a low-field nuclear magnetic resonance (LF-NMR) spectrometer (MicroMR20-025, Niumag Inc., Shanghai, China) following the method of Chen, Li, Zhou, Liu, Lu, Lin, Xu and Zhou [29].

2.12. Scanning electron microscopy

The WBMP gel was cut into small pieces of 1 cm³. The samples were treated with a 2.5% (V/V) glutaraldehyde solution (GR degree) for 24 h, 0.1 M cold phosphoric acid buffer solution for 15 min, 1% (V/V) osmium tetroxide (GR degree) at 4 °C for 2 h, graded ethanol series (30%, 50%, 70%, 90%, and 100%) for 15 min, and 100% isoamyl acetate for 15 min. Samples were freeze-dried under vacuum. Scanning electron microscopy (SEM) (7500F, JEOL Inc., Japan) profiles were obtained at an accelerating voltage of 5 kV following the method of Li, Sun, Han, Chen and Tang [30]. The micrographs of the gels were taken at a magnification of ×5000.

2.13. Statistical analysis

One-way analysis of variance was conducted to determine statistical differences. Multiple comparisons were performed according to Duncan’s multiple range test using the SPSS19.0 software. All values were expressed as mean ± standard deviation with a significant difference of P < 0.05.

3. Results and discussion

3.1. WBMP properties after preheating and HIU treatment

3.1.1. Particle size

The particle size of protein reflected the effect of HIU on the microstructure of WBMP. Fig. 1 shows the particle size of the WBMP solution by the preheating synergy of HIU with different power outputs. After preheating to 50 °C, the particle size of WBMP was mainly distributed in the range of 4150–6440 nm, which was significantly larger (P < 0.05) than that of HIU-treated groups. It may be that the protein agglutination caused by preheating increased the WBMP particle size. The self-assembly of the thick and thin filaments in muscle owing to electrostatic interaction is the main reason for the increase in the MP particle size [22]. Under the action of the mechanical force generated during HIU, the myofilament structure may be randomly oriented, decomposed, and separated. The release of some monomers, such as myosin or actin, leads to the bimodal transfer of distribution and particle size reduction [31]. The particle size of WBMP constantly decreased as HIU power increased. This showed that HIU could inhibit myofilament assembly and hindered the WBMP aggregation caused by preheating [31]. At 400 W, the particle size of WBMP was mainly in the range of 1480–3580 nm. By further increasing the HIU power up to 800 W, the particle size distribution of WBMP reduced to 615–3580 nm (P < 0.05). Therefore, the HIU treatment at 600 W more significantly suppresses protein aggregation.

3.1.2. Solubility

The solubility of the MPs treated with the HIU of different power outputs is shown in Fig. 2. The solubility of non-treated WBMP was significantly lower (P < 0.05) than those of HIU-treated groups (57.23 ± 0.33%). This may be due to the protein denaturation caused by preheating [32]. When HIU was introduced, the solubility of WBMP was markedly improved (P < 0.05) at 200 W (67.44 ± 1.06%). WBMP solubility increased with increasing HIU power output, which was similar to the trend observed for the particle size (Fig. 1). When the HIU power reached 800 W, the solubility of WBMP was significantly higher (P < 0.05) than those of other groups (88.11 ± 0.87%). The aggregation of myofibrils is a process of myofilament assembly, including thick and thin filaments. Liu, Zhang, Liu, Chen and Kong [31] verified that HIU inhibits the assembly of filaments. Moreover, Li, Li, Zhu, Ning, Cai and
Zhou [33] reported that the increase in MP solubility correlates with the change in the protein structure and particle size. Considering the particle size distribution shown in Fig. 1, the increased HIU output power led to an increase in solubility, which was evident by the formation of soluble protein aggregates or monomers [34]. HIU may impart strong physical forces through cavitation (i.e., mechanical shear and impact forces) to destroy the highly ordered filamentous myosin structure and thus reduce the particle size of MPs. Increasing the specific area of particles may enhance the solubility of MPs [35].

3.1.3. Surface hydrophobicity and intrinsic tryptophan fluorescence emission spectroscopy

The exposure of the hydrophobic group is strongly related to the change in the protein structure. Preheating and HIU treatment have corresponding effects on the surface hydrophobicity of proteins. As HIU power was increased, the surface hydrophobicity of WBMP also exhibited an increasing trend (Fig. 3). However, an HIU power of 200 W did not significantly affect hydrophobicity. This could be because the energy of 200 W is insufficient to significantly influence the structure of WBMP; thus, the hydrophobic group was not sufficiently exposed. When HIU power reached 400 W, surface hydrophobicity significantly increased from $319.2 \pm 2.36$ to $543.5 \pm 10.45$ ($P < 0.05$). At 600 W, the hydrophobic group became significantly exposed; thus, WBMP hydrophobicity significantly increased. When HIU power reached 800 W, hydrophobicity began to slightly decrease, which was also reflected in the endogenous tryptophan spectrum. Fig. 4.

The fluorescence intensity was the highest at 330 nm, and the trend exhibited by the curves in the endogenous tryptophan spectrum was like that of surface hydrophobicity. At different HIU powers, fluorescence curves indicated changes in the tertiary structure of WBMP (Fig. 3B). It is noteworthy that the surface hydrophobicity of protein is closely related to the tertiary structure [36]. To intuitively analyze the changes in WBMP tertiary structures, the maximum fluorescence intensities were plotted. As HIU power increased from 200 to 600 W, the maximum fluorescence intensity of WBMP significantly increased ($P < 0.05$) from $3.01 \pm 0.11$ to $4.79 \pm 0.10$. As HIU power increased to 600 W, the maximum fluorescence intensity increased to its maximum value (Fig. 3C), which was consistent with the changes in surface hydrophobicity. This indicated the damage degree of the protein structure by HIU, which reached a maximum at 600 W and promoted the exposure of tryptophan groups. However, when HIU power was increased to 800 W, the maximum fluorescence intensity decreased. The formation of protein aggregates caused by excessive denaturation may be related to the phenomenon [37]. In other words, the excessive unfolding of proteins would release a large amount of hydrophobic residues. Therefore, the hydrophobic interaction between protein molecules would be promoted, resulting in the reduction in fluorescence intensity and surface hydrophobicity [38].

3.1.4. Rheological properties

Myosin comprises a rod-like structure with a head and a tail that can be oriented under shearing action. This determines the rheology and gel properties of MP [39]. The measurement of apparent viscosity could be identified by protein–protein interaction. At the beginning, the WBMP

Fig. 3. (A) WBMP surface hydrophobicity, (B) intrinsic tryptophan fluorescence spectra, and (C) maximum fluorescence intensity after preheating to 50 °C and applying HIU of different power outputs. The different lowercase letters (a–c) indicate the significant differences between the groups ($P < 0.05$).
apparent viscosity of the control group without HIU was significantly higher \((P < 0.05)\) than those of other groups. As HIU power was increased, apparent viscosity exhibited a gradual decline in the same shear-rate range \((10–1000 \text{ s}^{-1})\). This may be because the rapid movement of molecules caused by HIU cavitation was sufficient to break the protein particles \([40]\), thereby destroying weak protein binding and preventing protein aggregation \([41]\). With the increase in HIU power \((200–800 \text{ W})\), the shear stress gradually decreased due to HIU induced protein denaturation.

In the diluted solution, the following trends occur: \(G''\) is always higher than \(G'\); in the entangled network, the \(G'\) and \(G''\) curves intersect in the entire frequency range; in the weak gel, \(G'\) is higher than \(G''\), and it tends to be parallel throughout the range; and in a strong gel, \(G'\) is typically significantly higher than \(G''\). The \(G'\) of WBMP processed with 800 W HIU was almost parallel to \(G''\), which was intended in the late stage of high-frequency testing. Therefore, it could only be classified in weak gels. This may be because high-power HIU leads to protein denaturation and less natural protein remaining in the WBMP solution. When preheating, there would be less natural protein denaturation to enhance \(G'\) and \(G''\). Therefore, more protein denaturation results in weaker gels \([16]\). The \(G'\) for the remaining sample was significantly larger than \(G''\); thus, the sample could be classified as a strong gel. Among them, the \(G'\) of 600 W samples is significantly higher \((P < 0.05)\) than \(G''\). A trend of intersection was observed in the later-stage test, indicating strong gel properties.

HIU could increase the thermal sensitivity of WBMP myosin \([4]\). The first peak of WBMP is typically representative of the crosslinking of myosin heads, which are dominated by internal molecular strength \([42]\). Therefore, the first peak of the non-treated WBMP represents the lower crosslinking of the myosin head owing to the decrease in MP performance. As HIU power increased from 200 to 600 W, the amplitude of the peak fluctuated. The crosslinking of the myosin head was enhanced to a maximum when 600 W HIU was applied. Increasing the temperature from 65 to 70 °C in the pre-modification step inhibited the fluidity of fibers \([25]\); however, when HIU power reached 800 W, the fluidity of myosin reduced owing to the excessive effect of HIU. Therefore, this also proved that the power of HIU should not be excessively high. The fluctuations in the range of 70–80 °C represent the crosslinking effect of the myosin tail, and the \(G'\) curve tends to step from 70 °C. This indicated that preheating synergistic HIU inhibited the role of the WBMP tail. When HIU power reached 800 W, the crosslinking of the myosin tail was increased, and its strength was significantly far from that of the preheated control group \((P < 0.05)\).

3.2. WBMP gel quality after the synergistic preheating with HIU

3.2.1. Textural characteristics and WHC

The texture of protein-based gels influences the perception and quality of the final products, and it is determined by the composition and organizational structure \([43]\). HIU could significantly improve the texture and WHC of the WBMP gel after preheating. The hardness, springiness, and WHC of the non-treated WBMP gel were distinctly lower \((P < 0.05)\) than those of the HIU treatment group \((42.544 \pm 1.12 \text{ g, } 0.277 \pm 0.04, 49.78 \pm 0.63\% )\). The WBMP gel was processed at 200 W to yield the hardness, springiness, and WHC values of 55.136 \pm 0.65 g, 0.339 \pm 0.03, and 75.23 \pm 0.25\%, respectively. With increased HIU power, the hardness, springiness, and WHC of the WBMP gel reached the highest values at 600 W \((65.428 \pm 0.33 \text{ g, } 0.526 \pm 0.02, 86.11 \pm 0.27\% )\).
0.83%, respectively). According to Kao, Su and Lee [44], uniform and delicate proteins can form more solid gels. More water molecules could be retained owing to the rich crosslinking structure, thus improving the WHC of WBMP gels. Increased HIU reduced the protein particle size, thus promoting the formation of a more dense and elastic gel. However, as HIU power increased from 600 to 800 W, the hardness, springiness, and WHC of the WBMP gel decreased to 57.02 ± 0.03, 49.78 ± 0.63, and 69.25 ± 1.13%, respectively. This may be because excessive HIU power weakened the WBMP molecules, blocking the protein–protein and protein–water interactions [16]. Therefore, the structure became loose and the WHC decreased.

3.2.2. Raman spectroscopy

The Raman spectra obtained in the range of 500–2500 cm⁻¹ for WBMP gels are shown in Fig. 5. The reduced area of the amide I band at 1650 cm⁻¹ indicated a decrease in the total α-helix content [45]. The non-treated group has a higher peak area at 1650 cm⁻¹, indicating that its α-helix content was sufficient. Similarly, a decline in the intensity of the amide III band at 1250–1300 cm⁻¹ signifies a decrease in the content of all conformations. The trend of the amide III band was essentially identical to that of the amide I band. HIU treatment reduced the total α-helix content. As HIU power increased, the α-helix content of the WBMP gel significantly decreased at 600 W (P < 0.05). Results show that preheated protein tends to form a stable cross-linked structure with the addition of HIU. However, excessive HIU power may lead to an increase in the thermal effect [46], flocculation of insoluble proteins, and reaggregation of protein particles. Thus, the gel-formation promoting effect of preheating on WBMP was counteracted. Table 1

Table 1

| Ultrasonic power (W) | Hardness (g) | Springiness | WHC (%) |
|----------------------|--------------|-------------|---------|
| Non-treated          | 42.54 ± 1.12  | 277 ± 0.04  | 49.78 ± 0.63 |
| 200                  | 55.136 ± 0.55 | 339 ± 0.05  | 75.23 ± 0.25 |
| 400                  | 61.696 ± 0.58 | 525 ± 0.02  | 80.09 ± 0.61 |
| 600                  | 65.428 ± 0.33 | 582 ± 0.01  | 86.11 ± 0.83 |
| 800                  | 75.620 ± 1.09 | 471 ± 0.01  | 69.25 ± 1.13 |

Means with different letters (a-e) within the same column differ significantly (P < 0.05).

Fig. 5. Effect of different HIU powers on the Raman spectra of WBMP gels at 500–2500 cm⁻¹.

Table 2

| Strength of disulfide bonds*(a.u.) | I₁₇₀/I₁₀₀₀ | I₂₅₀/I₃₀₀ | I₃₂₀/I₃₃₀ | α-helix (%) | β-fold (%) | β-turn (%) | Random coil (%) |
|-----------------------------------|------------|-----------|-----------|-------------|------------|------------|-----------------|
| Non-treated                       | 0.49 ± 0.08 | 0.08 ± 0.01 | 0.08 ± 0.01 | 50.19 ± 0.08 | 28.55 ± 0.04 | 45.67 ± 0.12 | 9.38 ± 0.08 |
| 200                               | 0.54 ± 0.04 | 0.09 ± 0.01 | 0.09 ± 0.01 | 56.75 ± 0.04 | 32.68 ± 0.02 | 40.64 ± 0.04 | 7.19 ± 0.08 |
| 400                               | 0.61 ± 0.02 | 0.10 ± 0.01 | 0.10 ± 0.01 | 69.25 ± 0.02 | 37.15 ± 0.02 | 46.64 ± 0.04 | 8.59 ± 0.08 |
| 600                               | 0.89 ± 0.02 | 0.13 ± 0.01 | 0.13 ± 0.01 | 75.23 ± 0.02 | 37.94 ± 0.02 | 53.77 ± 0.04 | 7.06 ± 0.08 |
| 800                               | 1.09 ± 0.02 | 0.16 ± 0.01 | 0.16 ± 0.01 | 83.71 ± 0.02 | 37.16 ± 0.02 | 65.77 ± 0.04 | 10.11 ± 0.08 |

The letters a, b, c, d, and e in the same row indicate significant differences (P < 0.05). *represents the normalized intensities for the vibration of disulfide bonds in Raman spectra, and the maximal peaking position of the disulfide bonds of WBMP gels is assigned to the corresponding bracket. # denotes the percentage composition of conformation in the protein secondary structure.
3.2.3. Low-field NMR

The spin relaxation times (T2) of bound (1–10 ms), immobilized (10–200 ms), and free water (1000–5000 ms) are denoted by T21, T22, and T23, respectively [51]. The corresponding area fractions are denoted by P21, P22, and P23, respectively [52,53]. As shown in Fig. 6, WBMP gels contained bound (T21), immobilized (T22), and free water (T23). Immobilized water is the major contributor to the water content of the WBMP gel. With a longer T2 relaxation time binds more loosely to macromolecules, while water with a shorter relaxation time has stronger fluidity [16]. The addition of HIU significantly reduced the relaxation time of T2. Table 3 summarizes the peak areas and T2 relaxation times of the water populations in WBMP gels. With increasing HIU power, the ratio of bound water (P21) to immobilized water (P22) increased as follows: 1.54 ± 0.13% to 4.57 ± 0.02% and 75.79 ± 1.25% to 93.44 ± 1.78%. The trend for the proportion of free water (P23) was opposite to that of the other two types of water, exhibiting a decreasing trend (20.67 ± 0.46% to 1.99 ± 0.28%). When the HIU treatment with 200 W was applied, the P22 of the WBMP gel increased from 75.79 ± 1.25% to 82.71 ± 1.33%, indicating that the addition of ultrasound significantly improved the immobilized-water content of the gel. When HIU power was further increased to 400 W, the immobilized water of the WBMP gel was stable. At 600 W, the T22 relaxation time was significantly shortened (P < 0.05, 24.77–305.39 ms). This showed that bound water has lower mobility and is more closely related to protein. Moreover, HIU increased the distribution of free water, further indicating the positive effect of HIU on the WBMP gel. It was evident that more free water was attracted by the negative charge and became trapped in the microstructure of the gel after moderate HIU treatment (600 W). With the increase in HIU power, free water transforms into bound water and immobilized water [54]. After proper preheating (50 °C), WBMP formed more regular aggregates. Subsequently, HIU could develop the WHC of the WBMP gel. Gelatin blocks contained more water molecules and increased the proportion of immobilized water. However, it is noteworthy that when HIU power reached 800 W, HIU could no longer assist in water molecule storage and water population rearrangement. This was due to the excessive expansion of the protein structure caused by the higher HIU power, which made the expansion speed higher than the agglomeration speed of protein. This phenomenon caused more water molecule loss and destabilized the WBMP gel structure. Therefore, 600 W may be the most effective HIU power after preheating.

3.2.4. Scanning electron microscopy

The microstructure of the WBMP gel after synergistic preheating and HIU treatment is shown in Fig. 7. The WBMP gel not subjected to HIU contained an uneven distribution of large clumps and huge pores because the disulfide bonds of protein were not completely converted, and protein only irregularly aggregated without network crosslinking [4]. When HIU power reached 200 W, larger gaps and caking persisted, and the surface of the gel was coarse. When HIU power was increased to 600 W, the WBMP gel exhibited a dense and uniform surface state. The disulfide bond strength of this group was 0.83 ± 0.02, which was significantly (P < 0.05) higher than other groups. This may be because the exposure of hydrophobic groups impels the molecules to interact with one another through disulfide bonds or hydrophobic interactions to form a uniform network gel [55]. However, when power was increased to 800 W, ultrasonic physical energy resulted in a poor gel structure. It may be that the formation of heat-induced protein microstructures mainly depends on the relative speed of protein unfolding and aggregation. When the aggregation velocity is higher than the starting velocity, the microstructure of the gel is denser and more uniform. When the aggregation velocity is lower than the starting velocity, the microstructure of the gel is coarser and more uneven [16]. Moreover, after HIU treatment, WBMP was severely denatured, and many hydrophobic and sulfhydryl groups in the protein molecules were exposed. When the MP solution is heated to gelation, the natural MP molecule unfolds more slowly. The aggregation rate is much slower, resulting in a coarser and more uneven gel microstructure [18]. Therefore, we confirmed that the HIU treatment at medium power (600 W) after preheating to 50 °C was helpful for the formation of a robust WBMP gel network.

### Table 3

| Ultrasonic power (W) | P21 (%) | P22 (%) | P23 (%) | T21 (ms) | T22 (ms) | T23 (ms) |
|----------------------|--------|--------|--------|---------|---------|---------|
| Non-treated          |        |        |        |         |         |         |
| 200                  | ± 1.25d| ± 0.46a| ±       | ± 1.19a | ± 36.43a| ± 81.13a|
| 400                  | ± 0.85b| 0.02c  | ±       | ± 2.47f | ± 11.14f| ± 5.99f |
| 600                  | ± 0.46a| 0.47c  | ±       | ± 3.44b | ± 5.99d | ± 18.16d|
| 800                  | ± 1.78b| 0.28d  | ±       | ± 1.76b | ± 4.04c | ± 4.04c |

T21, T22, and T23 are the T2 relaxation peak times of each water population. The corresponding area fractions are indicated as P21, P22, and P23. The letters a, b, c, d, and e in the same column indicate the significant differences (P < 0.05) for different treatment groups.
intercepted by the protein, enhancing protein–water interactions and forming a superior WBMP gel. However, when power was further increased to 800 W, the agglutination effect of WBMP was damaged owing to the destructive effect of ultrasonic energy, and the tissue structure loosened. This phenomenon showed that under the action of moderate HIU (600 W) after preheating, the aggregation and expansion rate of WBMP could be adjusted to an equilibrium state, thus forming a uniform and dense gel structure.

4. Conclusions

The improvement in the gelation properties of synergistically preheated WBMP with HIU was confirmed by analyzing its texture, WHC, secondary structure, and water migration. At 600 W HIU, the WBMP solution contained particles of smaller sizes and exhibited excellent solubility. The sample solution formed before gelation revealed that 600 W HIU could expand the structure of WBMP and improve protein function. The WBMP gel exhibited the best hardness, springiness, and WHC, as well as remarkable gelation ability. The proportion of immobile water was highest using an HIU power of 600 W. The gel structure became uniform and dense. Under 600 W HIU, the combination of preheating and HIU enabled the expansion of the protein structure and the speed of thermal condensation to reach a balanced state, which helped the WBMP form a stable gel structure. This discovery further expands the application of HIU in remedying inferior meat protein (e.g., WBMP). In the future, we can further improve the gel quality of WBMP by combining HIU and preheating with other technologies.
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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