Effects of ultrasonic–microwave combination treatment on the physicochemical, structure and gel properties of myofibrillar protein in Penaeus vannamei (*Litopenaeus vannamei*) surimi

Tong Zhang , Jie Wang , Jiaqi Feng , Yaqiong Liu [*] , Ran Suo , Qianyun Ma , Jianfeng Sun

College of Food Science and Technology, Hebei Agricultural University, Baoding 071000, Hebei, China

ABSTRACT

The objective of this study was to evaluate the effects of single ultrasound (360 W, 20 min), single microwave (10 W/g, 120 s) and ultrasonic–microwave combination treatment on shrimp surimi gel properties. The structure and physicochemical properties of myofibrillar protein (MP) were also determined. Low-field nuclear magnetic resonance showed that the fluidity of water molecules and the moisture content decreased, the stability and water holding capacity (WHC) increased after single ultrasound, single microwave and ultrasonic–microwave combination treatment. Compared with the traditional water bath treatment, ultrasound and microwave treatment reduced the total sulfhydryl content and promoted the formation of intermolecular disulfide bonds and hydrophobic interactions, which improved the compactness of the network structure of shrimp surimi gel. Moreover, Fourier transform infrared spectroscopy and sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis revealed that these treatments not only inhibited the degradation of MP, but also decreased the α-helix content and increased the β-sheet content. The three treatments also significantly reduced the particle size and decreased the solubility of MP. Overall, the effect of ultrasonic–microwave combination treatment was superior to that of either single treatment.

1. Introduction

Penaeus vannamei is widely favored by people owing to their rich myofibrillar protein (MP) which has unique gelation ability [1]. In recent years, the processing method of shrimp surimi is similar to that of surimi, which is mashed by salt and heated to form an elastic gel-like product. Traditional heating involves a two-step water bath heat treatment, which is to quickly avoid the gel-cracking zone at 50–80 °C. 40 °C was used for protein expansion and cross-linking, and 90 °C for gel network formation. However, water bath heating cannot avoid gel deterioration and serious protein degradation caused by remaining in the gel-cracking zone for a long time, which adversely affects the functional properties of shrimp surimi protein [2]. MP is an important factor influencing the functional properties of shrimp surimi gel. The impairment of the functional properties of MP in muscle leads to low gel strength and large condensation or voids in the protein gel network [3]. Therefore, a novel processing method should be established to obtain meat products with high quality. Understanding the physicochemical, structure and gel properties of MP in heat-induced gelation is crucial in

this process.

Ultrasound and microwave have been widely used as new physical processing methods in the protein modification of surimi gel [4–6]. Ultrasonic pretreatment of protein before chemical reaction can improve the effect of protein modification [4]. Ultrasound destroys the intermolecular or intramolecular interactions of proteins through cavitation, exposing some polar groups and target regions [7]. Moreover, ultrasound can induce a high degree of protein expansion and promote the transformation of α-helix to β-sheet region, thereby increasing the gel strength of surimi [8]. Microwave heating is a heat treatment method with fast heating speed, short time and high thermal efficiency [9]. However, surimi gel treated only by microwave has rough surface and poor gel performance. In recent years, microwave heating combined with water bath treatment can quickly pass through the gel-cracking zone and reduce the effect of endogenous enzymes on surimi gel [2,9]. Compared with water bath heating, surimi gel absorbs the same or less energy during microwave heating, while greatly increasing the breaking force and gel strength of surimi gel. Microwave can also inhibit the hydrolysis of MP and improve the quality of surimi gel [10]. However,

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the excessive heating rate of microwave will lead to uneven heating of the gel, which will lead to structural defects of the gel [11]. Therefore, some studies have applied ultrasonic–microwave combination technology to surimi gel. Although the action mechanisms of ultrasound and microwave are completely different, the continuous and stable cavitation process will reduce the inhomogeneity of gel heating under microwave action to a certain extent, and the microwave effect also helps to accelerate the structural changes of ultrasonic proteins [12]. Ye et al. [11] found that single ultrasound or microwave treatment can significantly improve the gel properties of low-salt surimi, and the combination of the two exerts had a synergistic effect. Although the positive effect of ultrasonic–microwave combination on surimi gel, whether or not this combination treatment exerts the same effect on shrimp surimi gel remains unknown.

In the present study, the effects of single ultrasound, single microwave and ultrasonic–microwave combination on the physicochemical, structure and gel properties of shrimp surimi gel protein were investigated. The water distribution, protein conformation, secondary structure and microstructure of shrimp surimi gel prepared by different heating methods were characterised using low-field nuclear magnetic resonance (LF-NMR), sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), Fourier transform infrared spectroscopy (FTIR) and confocal laser scanning microscopy (CLSM). This study will provide a theoretical support for ultrasonic–microwave combination preparation of low-salt shrimp surimi gel and an efficient strategy for the production of high-quality shrimp surimi gel.

2. Materials and methods

2.1. Materials and chemicals

Penaeus vannamei (*Litopenaeus vannamei*) samples with a good shape and uniform size (20–25 g) were purchased from the Agricultural University Market in Baoding, China. They were packed in a foam box filled with ice bags, transported to the laboratory and then stored at –80 °C until use. Plastic casings were purchased from Dalian Zongbaiwei Food Ingredients Co., Ltd. (Dalian, Liaoning, China). Sodium chloride was purchased from Hebei Zhongyan Longxiang Salt Chemical Co., Ltd. (Xingtai, Hebei, China). Chromatographic grade potassium bromide was purchased from Shanghai McLean Biochemical Technology Co., Ltd. (Shanghai, China). All other chemicals used in this research were of analytical grade and purchased from Beijing Reagan Biotechnology Co., Ltd. (Beijing, China), Beijing Solaibao Technology Co., Ltd. (Beijing, China) or Shanghai Epizyme Biomedical Technology Co., Ltd. (Shanghai, China).

2.2. Preparation of shrimp surimi

The preparation of shrimp surimi refers to the method of Liang et al. [2] with slight modifications. The frozen shrimp was thawed overnight at 4 °C. The head, tail and shell of the shrimp were removed, leaving only the edible part. Subsequently, the shrimp meat was evenly ground for 2 min in a meat grinder (S3-LA166, Jiuyang Co., Ltd, Jinan, China). Shredding and stopping were alternately performed for 30 s each time to prevent overheating. Add 1 % sodium chloride and stir evenly, then manually filled it into the plastic casings with a folding diameter of 50 mm and tied its ends tightly with a rope.

2.3. Application of different heating methods

The different heating methods of shrimp surimi gels are shown in Table 1. "WB + WB'' refers to the traditional two-step water bath heating. "US + WB + WB'' refers to the traditional two-step water bath heating after ultrasonic treatment. "WB + MW'' refers to the use of microwave heating instead of the second step in the traditional two-step water bath heating. "US + WB + MW'' refers to the second step in

Table 1
Different heating methods to prepare shrimp surimi gels.

Heating methods	Description
WB + WB	Water bath heated for 20 min at 40 °C and then water bath heated for 30 min at 90 °C.
US + WB + WB	Ultrasonically treated (frequency was 20 kHz, power was 360 W) for 20 min, water bath heated for 20 min at 40 °C, and then water bath heated for 30 min at 90 °C.
WB + MW	Water bath heated for 20 min at 40 °C and then microwave heated for 120 s (24 s on and 24 s off) under 10 W/g power (frequency was 2450 MHz).
US + WB + MW	Ultrasonically treated (frequency was 20 kHz, power was 360 W) for 20 min, water bath heated for 20 min at 40 °C and then microwave heated for 120 s (24 s on and 24 s off) under 10 W/g power (frequency was 2450 MHz).

replacing the traditional two-step water bath heating with microwave heating after ultrasonic treatment. Ultrasound treatment was carried out using an ultrasound processor (KQ-1018, Keqiao Ultrasonic Equipment Co., ltd, Dongguan, China). Microwave heating was carried out with a microwave chemical reactor (FCMCR-3C, Gongyi Kerui instrument Co., Ltd., Gongyi, China). The heated shrimp surimi gel was cooled rapidly in ice water so that the central temperature was below 10 °C, placed in a refrigerator at 4 °C and used within 5 days.

2.4. LF-NMR and magnetic resonance imaging (MRI)

The water distribution in shrimp surimi gels was evaluated using LF-NMR (MesoMR23-060H-I, Shanghai Newmai Electronic Technology Co., Ltd., Shanghai, China) following the method described by Ye et al. [11]. Each measurement was carried out three times and a false colour image of water proton density in shrimp surimi gel was generated. The water holding capacity (WHC) was determined according to previous study [13]. The *National Standards of China (GB 5009.3-2016)* was used to calculate the moisture content of the shrimp surimi gel samples.

2.5. Chemical interactions

The chemical interactions of the shrimp surimi gel samples were determined following the method described by Chen et al. [14] with slight modifications. The gel sample (2 g) was subjected to the action of different chemical reagents (10 mL), which were distinguished from each other according to their ability to cleave intermolecular interactions or bonds: S_A (0.05 M NaCl), S_B (0.6 M NaCl), S_C (0.6 M NaCl, 1.5 M urea), S_D (0.6 M NaCl, 8 M urea) and S_E (0.6 M NaCl, 8 M urea, 0.5 M β-mercaptoethanol). Then a high-speed disperser (XHF-DY, Ningbo Xinzhi Biotechnology Co., ltd, Ningbo, China) was used to homogenize the samples for 3 min at 5000 rpm. The homogenate was incubated at 4 °C for 1 h and then centrifuged at 9690 g for 15 min at 4 °C. The protein concentration in the supernatant was determined by Lowry's method. The results of chemical interactions were expressed in g protein/L solution. The difference in protein concentration in the supernatant of different solutions showed that.

Ionic bonds = c (S_B − S_A)
Hydrogen bounds = c (S_C − S_B)
Hydrophobic interactions = c (S_D − S_C)
Disulfide bonds = c (S_E − S_D)

2.6. Extraction and processing of MP

As previously described, MP was extracted from fresh shrimp surimi using RIPA Lysis Buffer (Strong) and protease inhibitor cocktail (100 ×) [13]. The extracted MP solution was adjusted to 2 mg/mL in a buffer containing 0.5 M NaCl and 20 mM Tris-HCl. The whole extraction process was carried out below 10 °C. Then, the MP solution was heated differently according to the processing method in Table 1. The treated

MP solution should be immediately put into ice water, stored at –80 °C after cooling, and used within three days.

2.7. Total sulfhydryl and active sulfhydryl content

Total sulfhydryl content was determined using the total mercapto assay kit (Solarbio, product number: BC1375). Active sulfhydryl content was determined following the method described by Yongsawatdigul et al. [15] with slight modifications. The MP solution was adjusted to 1 mg/mL in a buffer containing 0.5 M NaCl and 20 mM Tris-HCl. The MP solution adjusted by 0.5 mL was mixed with 4 mL 0.05 M PBS buffer (containing 0.6 M NaCl, 10 mM EDTA, pH 7.0), then mixed with 0.5 mL 0.2 mM DTNB (prepared with 0.1 M pH 7.0 Tris-HCl buffer) and then incubated at 4 °C for 1 h. Absorbance was determined at 412 nm. The active sulfhydryl content was calculated using the following formula:

Active sulfhydryl contents $= A \times D/\varepsilon \times c$

where A is the absorbance at 412 nm, D is the MP dilution multiple, c is the protein concentration, mg/ml; ε is the molar extinction coefficient, which is 13,600 L/(mol·cm).

2.8. Solubility

Solubility was determined following the method described by Cao et al. [16] with slight modifications. The extracted MP solution was centrifuged at 5000 g and 4 °C for 10 min. The concentration of the MP solution before and after centrifugation was determined using Lowry's method. The solubility of protein was calculated as follows:

Protein solubility (%) $= S_{ac}/S_{bc} \times 100$

where S_{ac} and S_{bc} represent the protein concentration in the supernatant after and before centrifugation, respectively.

2.9. Particle size distribution

The particle size distribution of MP was determined using a nano-particle size analyzer (Nano-ZS/ZEN-3600, British Malvern Corp., UK) as previously described by Cao et al. [16] with slight modifications. The MP solution was adjusted to 0.5 mg/mL in a buffer containing 0.5 M NaCl and 20 mM Tris-HCl. The diluted protein solution was placed in a colorimetric plate, transferred into a measuring cell, and determination after balanced 10 min at room temperature. The scattering angle was 90° and the wavelength was 659 nm.

2.10. SDS-PAGE analysis

The protein map of the shrimp surimi gel was produced by SDS-PAGE. The protein solution was mixed with the sample buffer at the ratio of 4:1 (protein: buffer) and then heated in a water bath at 95 °C for 5 min. Mark (5 μL) was added, and 8 μL protein samples were added to each hole, and the whole process was carried out [13].

2.11. FTIR spectroscopy

All samples were subjected to FTIR spectroscopy (IRAffinity-1S, Shimadzu Enterprise Management Co., ltd., Shanghai, China). The infrared spectrum scan was between 4000 and 400 cm^{-1}. The resolution was 4 cm^{-1} and each sample was scanned 32 times. The amide I region (1600–1700 cm^{-1}) was analyzed by PeakFit software (Systat Software, Inc.) [17].

2.12. CLSM

The microstructure of shrimp surimi gel was studied under a confocal laser scanning microscope (LSM 880, Henan Yingnalide Electronic

Technology Co., ltd., Germany) following the methods described by Huang et al. [18] and Ji et al. [19]. A 0.2 mL Rhodamine B staining solution of 0.01 % (w/w) was dripped on the surface of the sample (5 × 5 × 1 mm) and then incubated for 30 min at 37 °C without light. Excess dyes and stains were washed with sterile saline. The dyed samples were immediately poured into a confocal dish and observed under a 100 × laser confocal microscope. The excitation wavelength of laser was 559 nm, and the emission wavelength of Rhodamine B was between 531 and 703 nm.

2.13. Statistical analysis

All experiments were carried out at least by three duplicate, and the results were presented as the mean ± standard deviation (SD). Statistical difference of the experimental data was calculated using one-way ANOVA and Duncan's test ($P < 0.05$) in SPSS 22.0. Origin 2021 was used for plotting.

3. Results and discussion

3.1. Gel water distribution

The water distribution and fluidity in food are usually characterized by LF-NMR. As shown in Fig. 1, the T_2 relaxation time distribution consisted of three characteristic peaks, which can be divided into T_{21} (0–1 ms), T_{22} (1–100 ms) and T_{23} (>100 ms). T_{21} represents bound water, which binds closely to protein and has the shortest relaxation time. T_{22} represents immobilized water in the MP gel network, and it fluctuates with the change in structure of the protein gel network. Moreover, T_{22}, with a content of more than 75 %, makes the greatest contribution to the WHC of shrimp surimi gel. T_{23} represents free water, which is outside the gel network and can move freely. P_{21}, P_{22} and P_{23} are the area fractions of bound, immobilized and free water, respectively, representing their relative contents.

As shown in Table 2, compared with the traditional water bath treatment, single ultrasound, single microwave and ultrasonic–microwave combination treatments shortened the relaxation time of T_{22} and T_{23} to varying degrees ($P < 0.05$). This result indicated that the fluidity of water molecules decreased and the stability increased. The relaxation time (T_2) reflects the chemical environment of hydrogen protons, which is related to the binding force and degree of freedom of hydrogen protons; that is, the greater the binding force of hydrogen protons or the smaller the degree of freedom, the shorter the relaxation
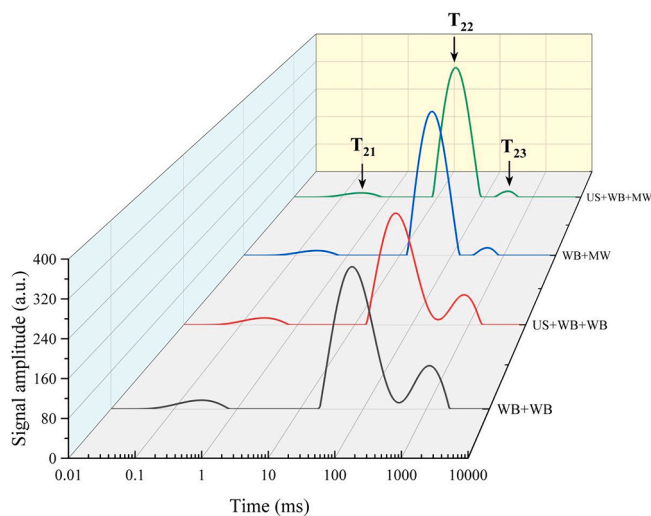

Fig. 1. Effect of the different heating methods on the water distribution in shrimp surimi gels. T_{21}, T_{22}, and T_{23} were the relaxation times of each water population.

Table 2
Effects of the different heating methods on LF NMR spin–spin relaxation time (T_2), peak proportion, WHC and moisture content in shrimp surimi gels.

Heating methods	T_2 relaxation time distribution (ms)			Proportion of T_2 relaxation time peak area (%)			WHC (%)	Moisture content (g/100 g)
	T_{21}	T_{22}	T_{23}	P_{21}	P_{22}	P_{23}		
WB + WB	0.280 ± 0.007^a	67.475 ± 0.098^a	1162.322 ± 1.576^a	5.256 ± 0.007^a	77.784 ± 0.224^c	16.961 ± 0.230^a	65.97 ± 1.23^d	80.66 ± 2.03^a
US + WB + WB	0.261 ± 0.081^a	51.114 ± 0.090^b	821.434 ± 0.400^b	5.388 ± 0.105^a	80.095 ± 0.149^b	14.518 ± 0.071^b	83.58 ± 1.09^b	75.80 ± 3.10^a
WB + MW	0.261 ± 0.037^a	41.504 ± 0.024^c	505.263 ± 0.294^c	3.971 ± 0.142^b	93.528 ± 0.092^a	2.501 ± 0.051^c	77.97 ± 0.82^c	67.69 ± 0.15^b
US + WB + MW	0.261 ± 0.082^a	23.817 ± 0.016^d	289.942 ± 0.015^d	4.077 ± 0.210^b	93.649 ± 0.216^a	2.274 ± 0.007^c	86.32 ± 0.55^a	66.90 ± 4.50^b

Note: Different lowercase letters indicate significant differences among different heating methods ($P < 0.05$).

time of T_2, and vice versa [20]. The decrease in relaxation time of free water (T_{23}) has previously been related to the increase in WHC [21]. The migration of free water (T_{23}) to immobilised water (T_{22}) indicates that the single ultrasound, single microwave and ultrasonic–microwave combination treatments improved the gelation ability of shrimp surimi. However, T_{21} did not change significantly under the different heating methods ($P > 0.05$), which may be attributed to the close binding of bound water to the protein side chain, independent of mechanical stress or alternating electric field, and the ability to withstand heat [22]. The area fraction of water molecules in the shrimp surimi gel showed that compared with the traditional water bath heat treatment, single ultrasound treatment did not significantly affect the proportion of bound water (P_{21}) ($P > 0.05$). Similarly, a previous study found that ultrasonic treatment does not significantly affect the bound water in the gel of low-salt chicken breast meat batter [23]. However, single microwave and ultrasonic–microwave combination treatments slightly decreased the proportion of bound water ($P < 0.05$). The single and combined effects of ultrasound and microwave significantly increased the immobilized water (P_{22}) content and reduced the free water (P_{23}) content of the gel ($P < 0.05$), explaining the high WHC of the gel (Table 2). The slow heating of traditional water bath converted immobilized water into free water, whereas the rapid heating of microwave possibly hindered this transformation to some extent. In addition, the dense gel network formed by microwave heating possibly strengthened the restraint to water. Under ultrasonic treatment, more free water is attracted by the negative charge and trapped in the microstructure of the gel, thus reducing the loss of immobilized water [3]. Remarkably, the ultrasonic–microwave combination treatment was the most prominent group among all the methods, and it exerted the most positive effect on water distribution.

After different heating methods, the MRI images of shrimp surimi are shown in Fig. 2. High signal strength usually represents free water, whereas low signal strength reflects bound water or immobilized water related to macromolecules [24]. Compared with the traditional water bath treatment, single ultrasound treatment, single microwave and ultrasonic–microwave combination treatment decreased the free water content while increased the total content of bound water and immobilized water. Notably, the effect of and ultrasonic–microwave

combination treatment was the most significant, this showed that the distribution of water molecules in the gel was more uniform and orderly. However, the decrease in moisture content after microwave treatment was possibly caused by partial water volatilization (Table 2). Therefore, MRI can not only reflect the water distribution and content, but also provide more intuitive information in the process of gel food processing.

3.2. Chemical interactions

Table 3 shows the changes in chemical interactions in shrimp surimi gel under different heating methods. Compared with the traditional water bath treatment, single ultrasound, single microwave and ultrasonic–microwave combination treatments significantly decreased the ion bonds and hydrogen bonds while significantly increased the disulfide bonds and hydrophobic interactions ($P < 0.05$). In the process of heating, the hydrogen bonds between protein molecules and water are very easy to be destroyed, and the number is relatively small. The breakage of hydrogen bonds and ion bonds may be due to the effect of

Table 3
Effect of different heating methods on chemical interactions (g/L) and solubility (%) of shrimp surimi gels.

Heating methods	Ionic bonds (g/L)	Hydrogen bonds (g/L)	Hydrophobic interactions (g/L)	Disulfide bonds (g/L)	Solubility (%)
WB + WB	0.144 ± 0.046^a	0.097 ± 0.029^a	0.031 ± 0.006^c	0.028 ± 0.007^d	84.92 ± 0.69^a
US + WB + WB	0.021 ± 0.002^b	0.055 ± 0.012^b	0.118 ± 0.055^b	0.163 ± 0.087^c	80.45 ± 0.46^b
WB + MW	0.050 ± 0.008^b	0.030 ± 0.006^{bc}	0.121 ± 0.003^b	1.378 ± 0.018^b	65.97 ± 0.91^c
US + WB + MW	0.013 ± 0.003^b	0.009 ± 0.003^c	0.285 ± 0.018^a	1.516 ± 0.036^a	79.35 ± 2.02^b

Note: Different lowercase letters indicate significant differences among different heating methods ($P < 0.05$).
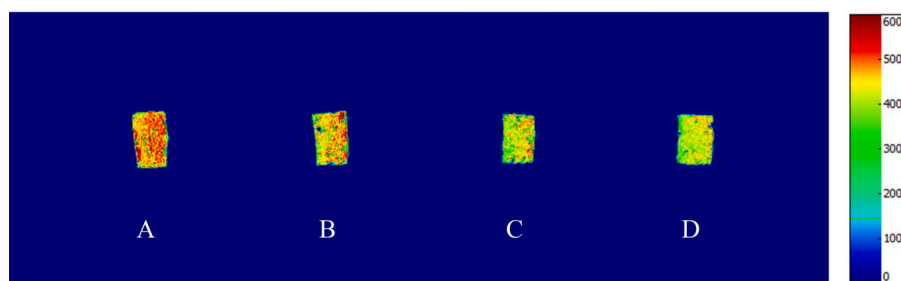
Fig. 2. MRI images of shrimp surimi in different heating methods. A ~ D were WB + WB, US + WB + WB, WB + MW and US + WB + MW.

microwave field on protein hydration, or ultrasonic cavitation and mechanical effects promoting the dissociation of protein aggregates. This finding was consistent with the results obtained from the high-intensity ultrasonic treatment of silver carp surimi with 1 % salt content [25]. Thus, ionic bonds and hydrogen bonds were rarely involved in gel formation, and were not the main forces influencing shrimp surimi gel protein.

Hydrophobic interactions and disulfide bonds are the main forces affecting the gel properties and maintaining the gel network structure [26], and play a dominant role in the gel matrix. He et al. [27] found that ultrasound significantly enhances the hydrophobic interactions of low-salt silver carp surimi possibly by promoting the exposure of hydrophobic groups. This phenomenon leads to the hydrophobic aggregation of proteins, resulting in a dense protein structure and easy cross-linking between sulfhydryl groups to form disulfide bonds. The improvement of hydrophobic interactions after microwave treatment may be due to the dominance of hydrophobic domains after microwave replaces the second step of water bath heating. Yan et al. [28] found that the hydrophobic interactions and disulfide bonds in surimi gel increased significantly after microwave treatment, indicating that protein unfolding intensified and more hydrophobic side chains were exposed. Microwave treatment causes the conformational change of protein and the shift of -SH position, which can easily promote the formation of disulfide bonds and affect the structure of gel network [29,30]. Meanwhile, the hydrophobic interactions and disulfide bond content were the highest under the ultrasonic–microwave combination treatment, which resulted in the greatest improvement in the gel properties of low-salt shrimp surimi.

3.3. The changes of total sulfhydryl and active sulfhydryl content

The total sulfhydryl content refers to the sum of the sulfhydryl content hidden in the protein molecule and exposed on the surface of the protein network, which can reflect the unfolding state of the protein. The active sulfhydryl group on the surface of the natural protein network is one of the most active functional group in proteins that plays an important role in maintaining and stabilizing the spatial structure of proteins [31]. Compared with the traditional water bath heating, single ultrasound, single microwave and ultrasonic–microwave combination

treatments decreased the total sulfhydryl content and increased the active sulfhydryl content (Fig. 3). Myosin in MP is mostly in the form of fine filament polymers, and most of the -SH groups are buried in these filaments; thus, it is not easy to be found by H_2O_2. However, the mechanical action of ultrasound can induce the dissociation of myosin filaments and the expansion of protein structure, resulting in the release of -SH groups [32]. Meanwhile, water molecules can produce many $\bullet$ H and $\bullet$ OH, which recombine to form H_2O_2. H_2O_2 can be rapidly degraded into O_2 and H_2O, which facilitate the oxidation of sulfhydryl groups to disulfide bonds [8]. Ultrasonic treatment can enhance MP expansion, decrease total sulfhydryl content and increase active sulfhydryl content. Similar results were obtained by Tang et al. [8] on threadfin bream surimi with 1 % salt content after different gradients of ultrasound. Under the alternating electric field generated by microwave, the force of polar groups between proteins exposes the sulfhydryl groups buried in protein molecules, which will be reorganized to form disulfide bonds or form new disulfide bonds through H_2O_2, resulting in a gradual reduction of total sulfhydryl groups [33]. However, Feng et al. [34] found different research results, which may be due to different microwave treatment conditions. The increase in the content of active sulfhydryl groups indicated the expansion of MP, which resulted in the exposure of sulfhydryl groups previously buried in natural proteins to the surface. The content of total sulfhydryl group was the lowest and the content of active sulfhydryl group was the highest after ultrasonic–microwave combination treatment, indicating the synergistic effect of ultrasonic and microwave in this combination treatment.

3.4. The changes of solubility

Solubility is not only an index to measure protein aggregation, but also an effective index of protein functional properties. The solubility of MP treated using different heating methods is shown in Table 3. The solubility of MP treated by ultrasound was significantly lower than that of MP treated by traditional water bath ($P < 0.05$). First, ultrasound-induced cavitation and mechanical action may destroy intramolecular or intermolecular interactions and expose more target regions, thus promoting hydrophobic interactions between protein molecules, resulting in reduced solubility. Second, the remodelling of macromolecular aggregates in the ultrasonic process leads to the exposure of more
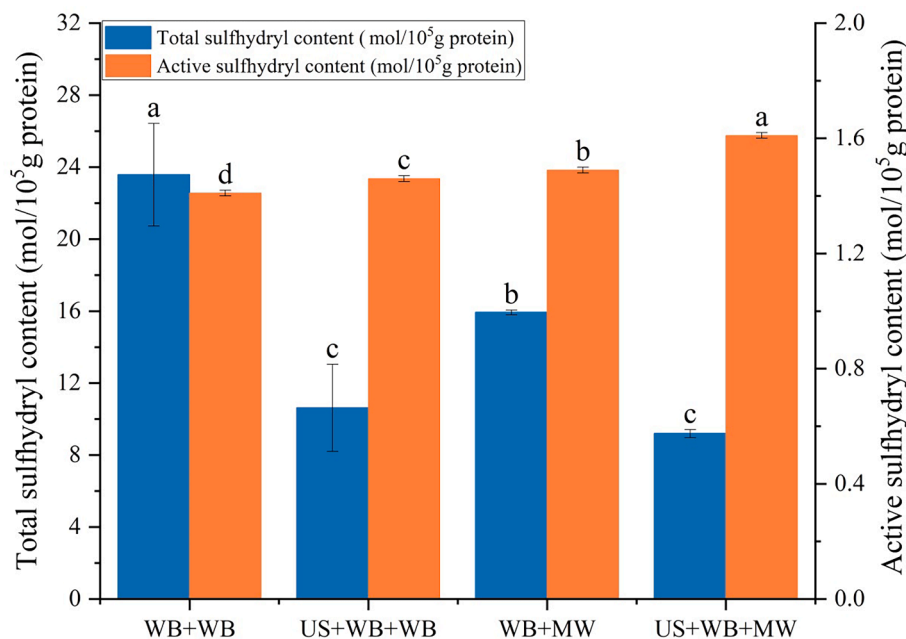

Fig. 3. Effects of different heating methods on total sulfhydryl content and active sulfhydryl content of shrimp surimi. Bars represent the standard deviation from triplicate determination. Different lowercase letters indicate significant differences among different heating methods ($P < 0.05$).

hydrophobic groups and sulfhydryl groups [35]. In the present study, the solubility of MP decreased most greatly after microwave treatment ($P < 0.05$). The protein solubility of silver carp treated by microwave for 60 and 80 s was significantly lower than that of silver carp treated by water bath [10]. And under 100 W and 300 W microwave irradiation, the solubility of silver carp actin solution decreased by 50.09 % and 55.94 %, respectively [34]. Microwave heating causes MP denaturation, which changes the molecular structure and conformation, makes the hydrophobic groups more exposed, and reduces the solubility of proteins. Notably, there was no significant difference between ultrasonic–microwave combination treatment and single ultrasound treatment ($P > 0.05$), but its value was lower than that of water bath treatment and higher than that of microwave treatment ($P < 0.05$). This result may be caused by the combination of the high shear force of ultrasonic wave and the thermal field effect of microwave, which loosens the structure of the protein and causes the polar part to tilt to the water phase. In general, compared with the traditional water bath treatment, the three different heating methods significantly reduced the solubility of MP.

3.5. Particle size distribution

The particle size of protein can reflect the structure and aggregation degree of protein, thus affecting its functional properties [4]. Fig. 4 shows the particle size distribution (A) and average particle size (B) of myofibril dispersions treated by different heating methods. The aggregate size of MP under traditional water bath, single ultrasound and single microwave treatments exhibited three peaks distribution, and the average particle sizes were 438.90, 309.60 and 369.20 nm, respectively. Under these three treatments, the particle size distribution moved to the direction of small particles, and the movement amplitude was gradually increasing. This result indicated that the single ultrasound and single microwave treatments can reduce the average particle size of MP ($P < 0.05$). The cavitation force, turbulence force and micro-streaming generated by ultrasound can exert powerful forces to dissociate protein aggregates by destroying the non-covalent bonds (hydrogen bonds and ion bonds), thereby decreasing particle size [36,37]. Similar results were previously reported for tuna MP [4]. In the traditional water bath heating process, protein molecules expand to form a certain type of cross-linking structure [38]. Microwave heating instead of the second step of water bath heating may inhibit this cross-linking aggregation and thus decrease the average particle size of MP. Li et al. [6] found that the average particle size of myosin in golden threadfin bream treated by 100 W microwave at 40 °C was decreased, which can be attributed to the inhibition of myosin aggregation by electrostatic repulsion induced by microwave. Interestingly, the aggregate size of MP treated by ultrasonic–microwave combination showed bimodal distribution, this may be attributed to the synergistic effect of cavitation force and shear force produced by ultrasonic wave and electrostatic repulsion force caused by microwave, which remains to be further confirmed. However, there was no significant difference in the average particle size between ultrasonic–microwave combination treatment and single ultrasonic treatment ($P > 0.05$), but the former shortened the preparation time of shrimp surimi gel, and exhibited more uniform particle size distribution (Fig. 4A).

3.6. SDS-PAGE analysis

The SDS-PAGE patterns of MP subjected to different heating methods are presented in Fig. 5. Three distinct bands appeared: actin (AC, 43 kDa), troponin I (Tn I, 23 kDa) and troponin C (Tn C, 21 kDa). Protein density analysis showed that the three proteins treated by the traditional water bath teratment had the lowest protein density, indicating that MP was degraded to a certain extent. The degradation of MP lead to the decrease in its molecular weight and the loss of its domain, resulting in the decrease of gel properties. In the traditional water bath heating, the
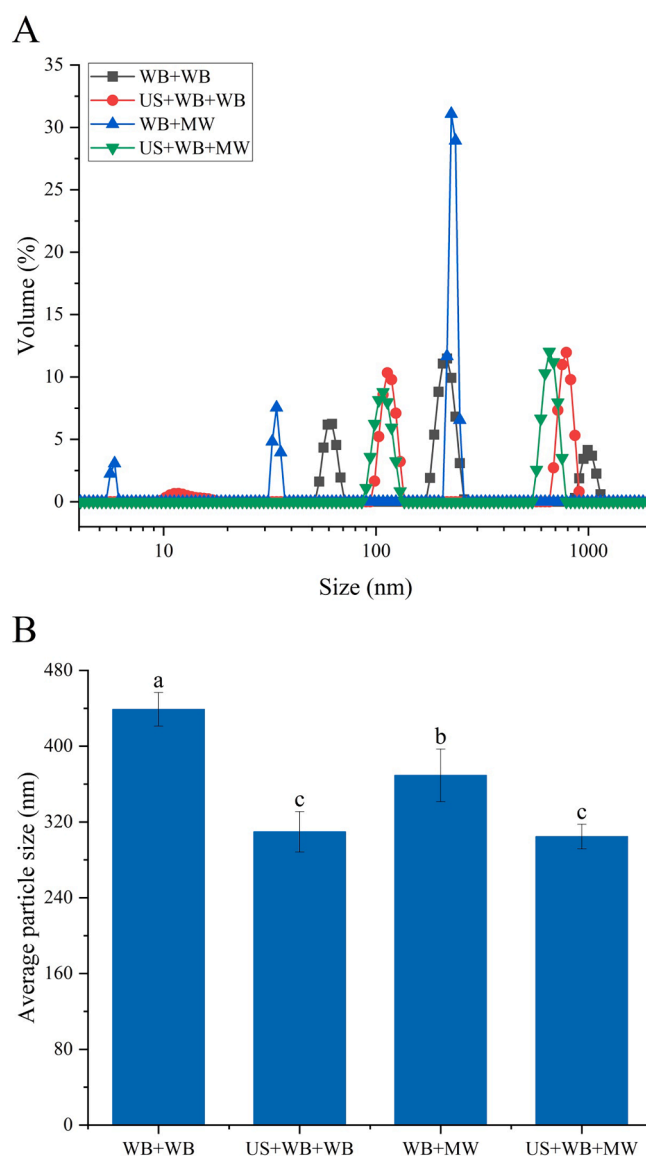
Fig. 4. Effects of different heating methods on particle size distribution (A) and average particle size (B) of myofibrillar proteins in shrimp surimi. Bars represent the standard deviation from triplicate determination. Different lowercase letters indicate significant differences among different heating methods ($P < 0.05$).

shrimp surimi gel remained in the gel-cracking zone for a long time, and the cathepsin in the shrimp surimi was highly stable, which led to the degradation of MP. Single ultrasound, single microwave and ultrasonic–microwave combination treatments decreased the protein degradation, and increased the protein density. The rapid heating of microwave can shorten the action time of protease and inactivate it in a short time, thus inhibiting the hydrolysis of MP. This result was similar to the change of chicken meat proteins during microwave cooking [39]. In addition, the change in molecular weight of MP was a consequence of altered MP structure after ultrasonic treatment. Hu et al. [40] found that ultrasonic treatment for 30 min can significantly degrade proteins in the mantle of jumbo squid, which may be due to the long ultrasonic time. The above results showed that ultrasonic–microwave combined treatment induced the weakest degradation of MP among the different treatments possibly because of the synergistic effect between ultrasonic cavitation affect and microwave rapidly heating. This result was consistent with the effects of ultrasonic–microwave combination treatment on the MP of small yellow croaker [41]. AC is important in the
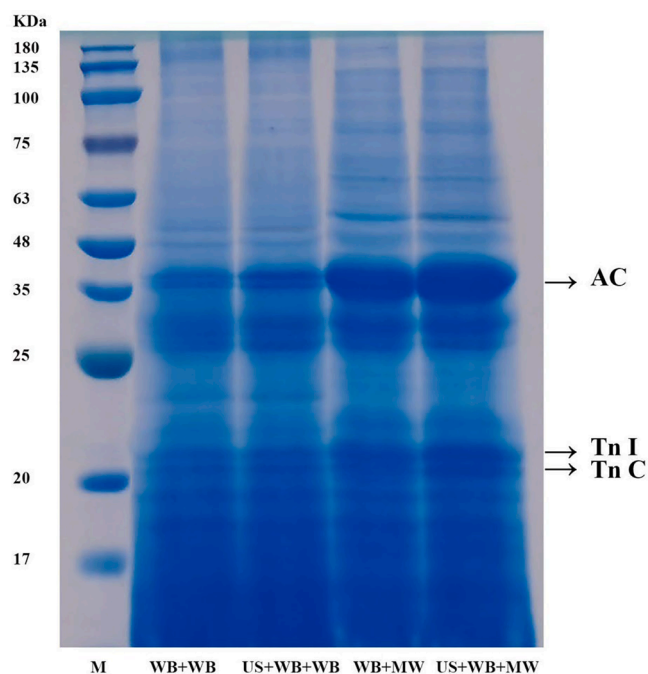
Fig. 5. Protein pattern by SDS-PAGE of shrimp surimi gels as affected by different heating methods. M = Molecular marker, AC = actin, Tn I = troponin I, Tn C = troponin C.

Fig. 6. Effect of different heating methods on the protein secondary structures of shrimp surimi.

formation of good gel properties [13]. Thus, the gel performance of shrimp surimi treated by ultrasonic–microwave combination was the best. The protein with high relative molecular weight was obviously degraded, and the protein was divided into small peptides. This result may be ascribed to the degradation of high-molecular-weight proteins in water bath heating and microwave heating, or the splitting of higher molecular bands caused by the shear stress turbulence effect of ultrasound [42].

3.7. Changes of secondary structure

The amide I (1700–1600 cm^{-1}) region was considered to be one of the most valuable and sensitive region for the study of protein secondary structure [43]. The conformational changes in proteins treated by single ultrasound, single microwave and ultrasonic–microwave combination are shown Fig. 6. The secondary structure of protein was mainly composed of α-helix, random coil, β-sheet and β-turn. The distribution of the peak position was as follows: 1650–1660 cm^{-1} for α-helix, 1640–1650 cm^{-1} for random coil, 1610–1640, 1680–1700 cm^{-1} for β-sheet and 1660–1680 cm^{-1} for β-turn [44]. The gel protein structure of shrimp surimi treated by different heating methods was mainly β-sheet and β-turn. Compared with the traditional water bath heating, single ultrasound, single microwave and ultrasonic–microwave combination treatments significantly decreased the α-helix content and significantly increased the β-sheet content ($P < 0.05$). Among them, the lowest α-helix content and highest β-sheet content of shrimp surimi gel protein were obtained under ultrasonic–microwave combination treatment. However, the β-turn and random coil content only slightly changed ($P > 0.05$).

The effect of high temperature and high pressure caused by cavitation in the ultrasonic process destroys the intramolecular hydrogen bonds and reduces the α-helix, so that the structure of MP can be extended. Fan et al. [45] obtained the same result in silver carp surimi under ultrasonic treatment. Similarly, Dong et al. [46] found that microwave treatment for 5–15 min at 75–125 °C increases β-sheet content in shrimp protein, decreases the α-helix structure and fluctuates the β-turn and random coil structure. Electromagnetic heating of microwave
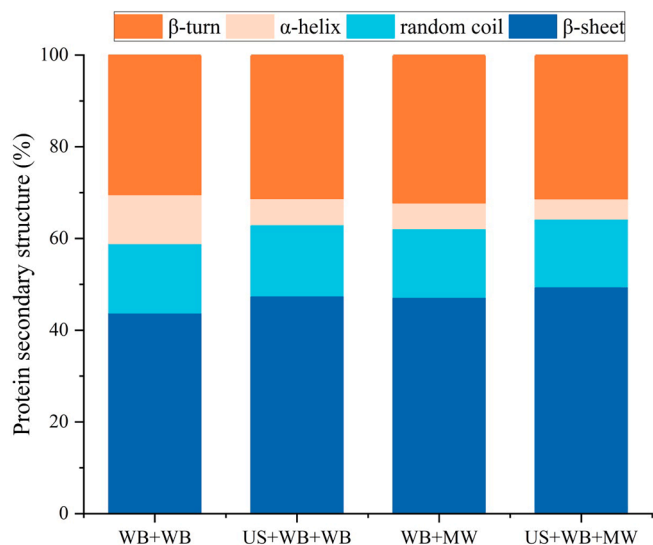
can improve the unfolding rate of protein. The transformation from α-helix to β-sheet showed the spatial rearrangement of protein polypeptide chains. More α-helix hinders the gelation of proteins, and more β-sheet promotes the formation of protein networks. The increase in β-sheet structure promotes the exposure of hydrophobic groups and changes the interaction between proteins, thereby improving the physicochemical properties of the gel network [47]. Therefore, the shrimp surimi gel under ultrasonic–microwave combination treatment had the best gel properties [2].

3.8. Microstructure

The CLSM image in Fig. 7 shows the microstructure of MP under different heating methods. The red areas were protein-rich phase features stained by Rhodamine B, whereas the dark area corresponded to the protein-free aqueous phase. The microstructure of shrimp surimi gel protein heated using the traditional water bath method had large pores and a loose network structure. The deterioration of this structure may be due to the slow heating rate and long heating time, which resulted in the serious degradation of MP. Thus, the number of proteins involved in the surimi gel network structure decreased. However, the shrimp surimi gel formed a more continuous and uniform three-dimensional network structure after single ultrasound, single microwave and ultrasound–microwave combination treatments. Molecules can interact through disulfide bonds or hydrophobic interactions to form a unified gel network structure because ultrasound promotes the exposure of hydrophobic groups [3]. Microwave may inhibit a large number of MP aggregation and the formation of larger aggregates because of its fast heating speed and short heating time, thus maintaining the uniform structure of the gel network. In general, the ultrasound–microwave combination treatment produced the best microstructure among the different treatments used. The network structure of shrimp surimi gel formed under complex physical field was more uniform and dense, which has also been found in previous studies of tilapia surimi [11].

4. Conclusions

Shrimp surimi was treated with the combination of ultrasound and microwave, and the physicochemical properties, structure, and gel properties of the MP were characterised. Ultrasound and microwave increased the proportion of immobilized water, destroyed intermolecular hydrogen bonds and ion bonds, and increased hydrophobic
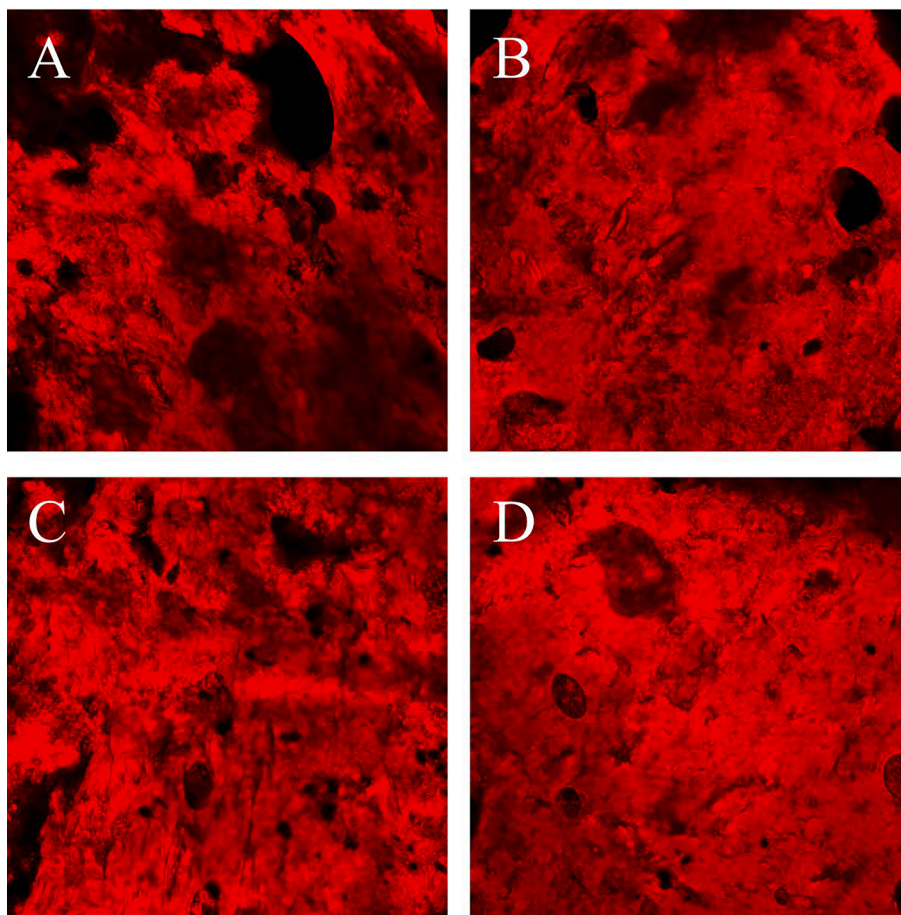
Fig. 7. CLSM photographs of shrimp surimi gel MP under different heating methods. A ~ D were WB + WB, US + WB + WB, WB + MW and US + WB + MW.

interactions and disulfide bonds. The MP particles in shrimp surimi gel were smaller under ultrasound and microwave treatments than under the traditional water bath treatment. In addition, the protein expansion intensified and the exposure of hydrophobic and polar groups increased, which changed the molecular structure and relative molecular weight of protein, decreased the solubility and promoted the formation of a dense gel network. In general, single ultrasound and single microwave treatments can significantly change the structure of shrimp surimi protein and improve the gel properties; moreover, the combination of the two exerts a synergistic effect. Thus, ultrasound–microwave combination could be used as an effective method to improve the quality of shrimp surimi gel products.

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.

Data availability

The data that has been used is confidential.

Acknowledgments

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