High-intensity ultrasound improved the physicochemical and gelling properties of *Litopenaeus vannamei* myofibrillar protein

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**A R T I C L E   I N F O**

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**A B S T R A C T**

The effects of high-intensity ultrasound on the physicochemical and gelling properties of *Litopenaeus vannamei* (L. vannamei) myofibrillar protein (MP) were investigated. MP solutions were subjected to ultrasound treatment (power 100 W, 300 W, and 500 W). It was found that the carbonyl and free amino contents of MP increased significantly with increasing ultrasound power, accompanied by enhanced emulsification properties. The increase of free radical and carbonyl content indicated that ultrasound induced the oxidation of MP. With the increase of ultrasound power, it was found that the total sulphydryl content of the shrimp MP decreased, but the surface hydrophobicity increased significantly, which might be closely related to the conformational changes of MP. Meanwhile, a significant increase of β-sheet but a decrease of α-helix in the secondary structure of MP was observed with increasing ultrasound power, indicating that ultrasound treatment induced the stretching and flexibility of MP molecules. SDS-PAGE showed that L. vannamei MP consisted of myosin heavy chain, actin, myosin light chain, paramyosin and tropomyosin. Ultrasound treatment could lead to some degree of oxidative aggregation of MP. The results of rheological properties indicated that ultrasound treatment enhanced the viscoelasticity of MP and further improved the gel strength of MP gel. This study can provide a theoretical basis for the functional modification of shrimp MP and the processing of its surimi products.

1. Introduction

Surimi is refined myofibrillar protein (MP, also known as a ‘salt-soluble’ protein) produced from aquatic muscle. Surimi-based products are popular among consumers for their ease of portability, consumption, storage, and high protein content. Currently, the raw material for surimi-based products is mainly fish. It is necessary to develop other surimi ingredients with the development of product diversification [9]. A recent review has reported that the meat of shrimps and cephalopods (e.g., squid) can also be used to make surimi [25].

Shrimp is one of the most popular seafood due to its desirable flavor and nutritional value. More than 50 % of the total protein in shrimp meat is MP, which has a good gel-forming ability and can also be used as a raw material for surimi-based products. Changes in the spatial structure of MP can induce changes in the functional properties of the protein to some extent, which in turn affect the quality of surimi products [16]. Therefore, the modification technique of modifying the structure of MP by specific methods to improve its functional properties has attracted much attention. At present, the commonly used protein modification methods include physical modification, chemical modification and enzymatic modification. Chemical modification can effectively improve the functional properties of proteins but often cause environmental and safety problems. Enzyme modification can target the structural changes of proteins through enzymatic hydrolysis, but the difficulty in controlling the degree of hydrolysis has limited its application [14]. In recent years, emerging physical processing technologies have shown great potential in protein modification, product quality improvement, and new product development with their advantages of safety, efficiency, low energy consumption, and low nutritional destructiveness [30].

In recent years, ultrasound treatment as a green non thermal processing technology has been proved to enhance the functional properties of various proteins [18,31]. Ultrasonic cavitation could change the structure of protein, and it was found that pulsed ultrasound could improve the emulsification performance of squid (Dosidicus gigas)
protein [21]. Zhang, Regenstein, Zhou, and Yang [34] reported that the water holding capacity of MP gel was improved after using high-intensity sonication at <600 W. Li et al. [18] demonstrated that ultrasound treatment had the effect of unraveling the self-assembly of myofibrils and enhanced the emulsification properties of chicken MP. Amiri, Sharifian, and Sohlzadeh [1] found that ultrasound treatment (20 kHz, 300 W, 30 min) improved the rheology, gelation, and emulsification properties of beef MP. To the best of our knowledge, few studies have been conducted on the effect of ultrasound on the physicochemical properties of MP of Litopenaeus vannamei (L. vannamei), and the processing of its surimi products, mainly focusing on the MP of fish, squid and poultry meat.

In this study, the main aim was to investigate the effects of different ultrasound treatments (20 kHz, power 100 W, 300 W, and 500 W) on the physicochemical and gelling properties of L. vannamei MP. The oxidation of MP was characterized by free amino, carbonyl, the, and free radical content. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was used to analyze the aggregation and individual subunits of MP. In addition, the emulsification properties, secondary structure, and tertiary structure were also determined. Finally, the rheological properties of MP and the gel strength of MP gel (MPG) were determined. This study can provide theoretical guidance for the ultrasound to improve the functional properties of shrimp MP and the processing characteristics of shrimp-based surimi products.

2. Materials and methods

2.1. Materials

Frozen L. vannamei (average length: 10.2 ± 0.6 cm, average weight: 17.8 ± 1.2 g) were purchased from MEILINYUAN Market (Stall Number E109, Dalian, China) in March 2022. 2,4,6-trinitrobenzenesulfonic acid (TNBS) and 5,5′-dithiobis (2-nitrobenzoic acid, DTNB) were purchased from BIOCITY Co., ltd. (Beijing, China). Dithiothreitol (DTT) was purchased from Aladdin Reagent Co., ltd. (Shanghai, China). Frozen shrimps were decapitated, peeled, and deveined manually after being thawed in water at room temperature. The obtained shrimp tail meat (STM) was stored at −30 °C for further analysis.

2.2. Extraction of shrimp MP

The MP extraction of L. vannamei was carried out according to the method of Chen et al. [5]. Briefly, the STM was mixed with phosphate buffer (0.001 M EDTA, 100 mM NaCl, 100 mM Na2HPO4/NaH2PO4, 2 mM MgCl2, pH 7.0) at a ratio of 1: 4 (w/v), and then mixed using a homogenizer (T25, IKA Corporation, Germany) at 10,000 rpm for 1 min. The resulting homogenate was centrifuged at 10,000 g at 4 °C for 10 min and the supernatant was discarded. The pellet was washed twice with phosphate buffer. The supernatant was discarded and the obtained pellets were the crude MP of L. vannamei. The MP was stored on ice and utilized within 24 h.

2.3. Ultrasound treatment of MP solution

First, the MP solution (20 mg/mL) was prepared and mixed in a high-speed blender (T25, IKA Corporation, Germany) at 12,000 rpm for 1 min. Then, an ultrasound processor (SCIENZT-II D, Ningbo, China) with a 6 mm diameter titanium probe was inserted into the MP solution with a distance of 2.0 cm and a depth of 2.0 cm to the bottom. Circulating ice-cold water was placed around the glass double-walled beaker to maintain the suspension temperature below 10 °C. The MP solution was subsequently sonicated (power 100 W, 300 W, and 500 W; amplitude 60 %; frequency 20 kHz) for 60 min. The intensities of ultrasound in this study were 25–29 W/cm², 74–80 W/cm², and 123–130 W/cm², respectively, according to the calculation method of Hu et al. [11]. An untreated MP solution was used as a control. The obtained samples were stored at 4 °C for further determination.

2.4. Carbonyl

The determination of carbonyl content of MP was carried out using a reagent test kit (No. A087, JIANCHENG Co., ltd., Nanjing, China) based on the contents of 2,4-dinitrophenylhydrazones produced by the reaction between protein carbonyls and 2,4-dinitrophenylhydrazine. The operation was completed in accordance with the manufacturer’s instructions. The carbonyl content was expressed as nmol/mg protein. This experiment was conducted in triplicate.

2.5. Free amines

The determination of free amine content of MP solution (4 mg/mL) was carried out referring to Guo, Jiang, True, and Xiong [9]. Briefly, the MP was mixed with 0.2 M phosphate buffer (pH 8.2, containing 1 % SDS), then reacted with 2 mL of 0.1 % TNBS in a 50 °C water bath for 30 min. The reaction was terminated by the addition of 4 mL of 0.1 M Na2SO3. The absorbance was measured at 420 nm and the content of free amine was calculated using the standard curve generated from L-leucine.

2.6. Emulsifying activity index (EAI) and emulsion stability index (ESI)

The determination of EAI and ESI of MP were carried out according to our previous method [18]. Briefly, the soybean oil and MP solutions (10 mg/mL) were mixed (1: 4, w/v) and homogenized twice for 1 min at 10,000 rpm using a blender (T25, IKA Corporation, Germany). Then, a SDS solution (1 mg/mL) was used to dilute 50 μL of the emulsion 100-fold. The absorbance at 500 nm of the diluted emulsion was recorded immediately (0 min) and 10 min later, respectively, using a microplate reader (Infiniti M200, TECAN, Switzerland). The ESI and EAI were calculated by equation:

\[
\text{EAI} \left( \text{m}^2/\text{g} \right) = 115.15 \times A_0 (1).
\]

\[
\text{ESI} \% = 10 \times A_0 / (A_0-A_1) (2).
\]

\(A_0\) and \(A_1\) represent the absorbance value of the mixture after 0 min and 10 min, respectively. This experiment was conducted in triplicate.

2.7. Free radicals

The determination of free radical content of MP was carried out referring our previous method [17] using an electron spin resonance (ESR) spectroscopy (A200, Bruker BioSpin, German). Briefly, 100 mg of freeze-dried MP powder were transferred into a 5 mm nuclear magnetism tube (Wilmad, NJ, USA). The instrument parameters used were as follows: microwave power, 5.32 mW; center field, 3453.00 G; sweep width, 100.00 G; modulation frequency, 100.00 kHz; modulation amplitude, 1.00 G; conversion time, 480 ms; time constant, 5242.88 ms. The signal intensity was obtained by calculating the average of the absolute values of the high and low peak signals. This experiment was conducted in triplicate.

2.8. Tertiary structure

2.8.1. Total sulfhydryl groups

The determination of total sulfhydryl groups was carried out according to the method of Li, et al. [16]. Briefly, 1.5 mL of MP solution (5 mg/mL) was first mixed with 10 mL of Tris-glycine buffer (4 mM EDTA, 8 mM urea, 90 mM glycine, 86 mM Tris, 0.5 mL of DTNB, pH 8.0). Subsequently, the obtained mixture was left at 25 °C for 1 h and centrifuged (4 °C) for 10 min. The absorbance of the supernatant was measured at 412 nm (A1). The buffer was used as blank (A2). Finally, referred to the following formula to complete the calculation of total sulfhydryl groups.
sulfhydryl groups:

Total sulfhydryl groups (µmol/g prot) = \((A_1 - A_2) \times 14.706\) (3).

2.8.2. Surface hydrophobicity

The determination of surface hydrophobicity of MP solution (5 mg/mL) was carried out using a 1 mg/mL bromophenol blue (BPB) [16]. The absorbance at 595 nm was recorded using a microplate reader. The phosphate buffer without MP was used as blank. This experiment was conducted in triplicate.

2.8.3. Intrinsic fluorescence spectroscopy

The analysis of intrinsic fluorescence emission spectroscopy of MP solution (0.2 mg/mL) was carried out using a RF-6000 fluorescence spectrophotometer (SHIMADZU Co., Japan) [16]. The excitation and emission wavelength were set at 280 nm and 300–400 nm, respectively. This experiment was conducted in triplicate.

2.9. Secondary structure

The analysis of secondary structures of MP solution (2.5 mg/mL) was carried out using a J-1500 circular dichroism (CD) spectrometer (JASCO, Japan). The data acquisition range was set at 200–260 nm. This experiment was performed in triplicate.

2.10. SDS-PAGE

A slab gel system (5% stacking gel, 10% separating gel) was used for SDS-PAGE analysis of MP samples. Both non-reductive and reductive (containing DTT) electrophoresis were performed to clarify the possible involvement of disulfide bonds in ultrasound-induced protein aggregation. The gel was stained by Coomassie brilliant blue (G-250) for 5 h and destained with three times with distilled water. Images of the gels were obtained using a densitometer (MF-ChemiBIS 2.0, DNR Bio-Imaging Systems ltd. Israel).

2.11. Rheological properties

2.11.1. Viscosity

The viscosity of MP solutions (45 mg/mL) was characterized using a TA HR-1 Discovery rheometer (TA Instrument, New Castle, UK) with a flat plate geometry (40 mm diameter, 1 mm gap) at 25 °C. The shear rate was set at 1 to 200 s⁻¹ to conduct viscosity properties at a steady-rate flow mode [21]. This experiment was performed in triplicate.

2.11.2. Temperature sweep analysis

The dynamic viscoelastic of the MP solution (45 mg/mL) was measured at 1% strain and a fixed frequency of 10 rad/s. Thermally induced gelation was achieved by heating the MP solution from 20 °C to 72 °C at 1 °C/min. The phase angle (Tan), storage modulus (G'), and loss modulus (G'') were recorded during dynamic rheological measurements [3]. This experiment was conducted in triplicate.

2.12. Gel strength

The MP gel was performed according to the method of Li et al. [16]. Briefly, 7 mL of MP solution (45 mg/mL) was put into a glass vial with a diameter of 20 cm and a height of 30 cm. The thermally induced gel process was carried out in a two-stage heating water bath: stage 1, 40 °C for 30 min; stage 2, 70 °C for 20 min. Finally, the gel was immediately cooled in an ice-water for 30 min and subsequently kept at 4 °C overnight.

A texture analyzer (TA. XT. Plus, Stable Micro Systems, UK) with a P/0.5 probe was used to determine the strength of MPG [32]. The parameters were set as follows: mid-measurement speed 1 mm/s, post-measurement speed 5 mm/s, and compression distance 20 mm. The initial force (N) required to break the gels was expressed as the gel strength.

2.13. Statistical analysis

The SPSS software 19.0 (IBM Inc., Chicago, USA) was used to analyze the one-way variance (Student-Newman-Keuls model). The significance level of use was p < 0.05. The original 2018 (OriginLab Inc., Northampton, USA) software was used to complete the drawing of paper data charts.

3. Results and discussion

3.1. Carbonyl

The functional groups of side chain amino acids in proteins are easily oxidized to produce carbonyl derivatives, thus carbonyl content is usually considered one of the indicators to characterize protein oxidation. Generally, the higher the carbonyl content, the greater the degree of oxidation [29]. As shown in Fig. 1A, the content of carbonyl groups of MP increased significantly from 0.62 nmol/mg to 1.26 nmol/mg (an increase of approximately 1.03-fold) with increasing ultrasound power from 0 to 500 W. The results showed that ultrasound treatment could lead to the oxidation of MP to a certain extent, and the degree of oxidation also increased with the increase of ultrasound power, which might be due to the fact that MP contained more randomly coiled structures, and the cavitation effect of ultrasound generated local high temperature, which subsequently led to the oxidation of reduced amino acids in MP to form carbonyl groups [8]. In addition, the amino side chains buried inside the MP molecule were exposed to the free radical environment generated by the high-frequency vibrations of ultrasound. The –NH or –NH₂ groups carried on the MP amino acid side chains were easily converted to carbonyl groups by the free radical-mediated oxidative deamidation reactions [7,16].

3.2. Free amines

The free amines content can be used to characterize the degree of the unfolding of the protein structure to some extent [9]. Fig. 1B showed the content of free amines of MP increased from 14.30 to 16.62 nmol/mg (an increase of approximately 16.22 %) with increasing ultrasound power from 0 to 500 W. The results showed that the ultrasound treatment led to the release of free amino acids from MP, and similar results were also found by Mu, Zhao, Yang, Zhao, Cui, and Zhao [22]. The bubble cavitation effect generated during the sonication process increased the local temperature and pressure in the region around the obliquely collapsed bubbles, which led to protein stretching and hydrolysis, peptide bond disruption, and exposure of a large number of amino acid residues. Meanwhile, it was worth noting that the generated carbonyl groups could further react with NH₂ groups to form Schiff base adducts, which subsequently reduced the content of free amines [19]. Thus, the change in amino content was not significant with the increase of ultrasound power.

3.3. EAI and ESI

EAI is the interfacial stability of protein per unit mass and represents the interfacial properties of protein solution and oil/water emulsions. ESI is the ability of a protein to maintain emulsion stability for a period of time [18]. With increasing ultrasound power from 0 to 500 W, the EAI of MP significantly increased (p < 0.05) from 35.48 m²/g to 62.01 m²/g (an increase of approximately 74.77 %) (Fig. 1C). These results showed that ultrasound treatment promoted protein-oil or protein-protein interactions. Fig. 1D showed the ESI of MP increased from 71.40 min to
166.75 min (an increase of approximately 1.34-fold) with increasing ultrasound power from 0 to 500 W, which further demonstrated that ultrasound could improve the emulsification performance and especially the emulsion stability of MP. These results were similar to the observations of Amiri, Sharifian, and Soltanizadeh [1], who found that the ESI and EAI of beef MP both increased after 30 min of treatment with different ultrasound powers. Therefore, the emulsification performance of MP was closely related to the ultrasonic conditions.

3.4. Free radicals

ESR is a relatively new method for oxidative evaluation. It has been widely known that protein oxidation is a chain reaction process mediated by free radicals [23,26]. Fig. 2 showed the intensity of free radicals of MP significantly increased from 6368.50 to 12116.33 (an increase of approximately 90.25 %) with increasing ultrasound power from 0 to 500 W, which indicated that ultrasound treatment could lead to the formation of oxidative free radicals of MP. As mentioned earlier, localized high-energy and high-heat cavitation bubbles were generated in ultrasonic cavitation and thermal effects, thereby promoting the formation of free radicals and eventually inducing protein oxidation [19]. At the same time, the increase of ultrasound power could be accompanied by a rise of thermal effects. Bragagnolo, Danielsen, and Skibsted [2] previously reported that the heat treatment process promoted the generation of free radicals in chicken meat. The excess of free radicals led to the oxidation of amino groups to carbonyl groups, which was consistent with the results of the present study.

3.5. Tertiary conformations

The sulfhydryl group is an essential functional group of protein, and the change of its content reflects the degree of protein unfolding and the formation of disulfide bonds [10,16]. Fig. 3A showed the effect of ultrasound treatment on the content of total sulfhydryl group of shrimp MP, which decreased significantly from 18.49 to 12.79 µmol/g (a decrease of approximately 30.83 %) with increasing ultrasound power.
Similar results were observed by Ma et al. [21], who found that the ultrasound treatments (200 W-950 W) resulted in significantly lower total sulfhydryl group content of cod protein, which might be attributed to the formation of disulfide bonds. Meanwhile, this study showed that ultrasound treatment led to an increase in the free radical content of MP. Previous literature reported that hydrogen peroxide could be formed by generating highly reactive radicals (H− and OH−) during ultrasound treatment, which led to the oxidation of surface-exposed sulfhydryl groups to form intermolecular or intramolecular disulfide bonds [24].

Surface hydrophobicity is an essential indicator of protein tertiary conformation, which reflects the changes of hydrophobic amino acids or the expansion of protein structure. These changes are closely related to the functional properties of proteins, such as the ability to form a gel [13]. As shown in Fig. 3B, the surface hydrophobicity of the MP increased significantly from 6.33 to 30.76 μg (an increase of approximately 3.86-fold) with increasing ultrasound power from 0 to 500 W, which indicated that ultrasound treatment caused the protein to refold, resulting in an exposure of hydrophobic residues hidden in the internal region of the protein [11]. Surface hydrophobicity is a key factor controlling the emulsification performance of proteins. Higher surface hydrophobicity enables proteins to aggregate around oil droplets to form a continuous viscous layer, thereby improving emulsification activity and emulsification stability. Therefore, consistent trends in surface hydrophobicity and emulsifying properties were found in this study.

The exposure of interior side chains and refolding of protein molecules could be reflected by changing of the intrinsic fluorescence of tryptophan residues. As shown in Fig. 3C, the fluorescence intensity increased from 55.25 × 10^3 (control) to 61.34 × 10^3 (500 W ultrasound treated sample), indicating the exposure of more tryptophan residues, which led to an increase in the non-polarity of the microenvironment [21]. These results might be caused by the conformational changes of shrimp MP after ultrasound treatment, and were consistent with the finding in surface hydrophobicity. In the present study, ultrasound unfolded the tertiary structure, exposing hydrophobic amino acids and tryptophan residues inside the protein, which generated more hydrophobic sites, ultimately leading to an increase in the nonpolar microenvironment of MP.

3.6. Secondary structure

The polypeptide chain within the protein molecule is composed of α-helix, β-sheet, β-turn, and random coil, which reflect the changes of secondary structures of protein. The secondary structure of a protein is closely related to its emulsifying properties [18,33]. Fig. 4A illustrated the changes of secondary structures of MP with (100 W, 300 W, and 500 W) and without (the control) ultrasound treatments. With the increase of ultrasound power from 0 to 500 W, the α-helix in the secondary structures of MP significantly decreased by 29.90 %, and the β-sheet significantly increased by 4.36-fold, however, β-turn and random coil had no obvious change trend. These results suggested that moderate ultrasound treatment induced more stretch and flexibility of protein molecules, contributing to the interactions between proteins. The α-helix is mainly stabilized by hydrogen bonds between the carbonyl oxygen (−CO), and amino hydrogen (−NH) of the polypeptide chain, and the increase in the β-sheet structure might be based on the depletion of the α-helix [28]. The β-sheet is usually buried in the polypeptide chain, and as the ultrasound power was increased, the structure of the protein was further unfolded. Similar results were obtained by Chanarat and Benjakul [4], who found that with the increase of ultrasound power, the content of α-helix decreased and the protein molecular structure unfolded. In this study, ultrasound induced protein molecular stretching and flexibility, with a decrease in α-helix and an increase in β-sheet, leading to the unfolding of protein secondary structures and favoring the formation of disulfide bonds. Therefore, it was believed that ultrasound treatment played an essential role in the aggregation behavior of MP.
3.7. Sds-page

Ultrasound promotes protein cross-linking through disulfide and non-disulfide bonds, and this intermolecular interaction plays an essential role in protein gelation. The molecular weight distribution of MP was determined by reducing (−DTT) and non-reducing electrophoresis (−DTT), and then the effects of different ultrasound power treatments on the molecular properties and protein cross-linking of MP were investigated. As shown in Fig. 4B, aggregates were observed in the top lane of non-reducing electrophoresis, which indicated that ultrasound led to the oxidative aggregation of MP. In addition, MP of *L. vannamei* presented a typical SDS-PAGE profile of individual subunits of myosin heavy chain (MHC), paramyosin, actin, tropomyosin, and myosin light chain (MLC) [20]. It could be seen that the overall trend of the electrophoretic bands of MP samples under reducing and non-reducing conditions was consistent. The bands of MHC and actin were more pronounced and wider in the reduced electrophoresis compared to the non-reduced electrophoresis, which further indicated that ultrasound treatment resulted in a certain degree of oxidation and aggregation of MP. Comparing ultrasound treated and untreated MP, ultrasound treatment did not induce major changes in the protein electrophoretic patterns, suggesting that ultrasound treatment did not modify the protein profiles of MP regardless of the sonication conditions in this study. Similar results were reported by Chen et al. [6], who found that 400 W sonication did not cause significant changes in the electrophoretic bands of soy protein isolate. In the present study, SDS-PAGE showed that the presence of aggregates was observed at the top of the non-reducing electrophoresis lanes, suggesting that ultrasound induced oxidative cross-linking of MP and that large aggregates formed by disulfide bond cross-linking might be present in MP.

3.8. Rheological properties

The flow behavior of MP solution is closely related to viscosity. Fig. 5A illustrated the shear viscosity of MP solutions with different ultrasound treatments. The viscosity of all samples decreased rapidly with increasing shear rate, suggesting that MP solutions showed shear thinning and behaved as pseudoplastic fluid [21]. Compared with the control group, a significant decrease in viscosity occurred in the ultrasound treated group, indicating that the viscosity of MP gradually

![Graph showing effect of different ultrasound power on secondary structures and protein composition](image-url)
decreased and the mobility increased gradually with the increase of ultrasound power. It was previously reported that the liquid medium was prone to extreme forces such as shear and oscillation during ultrasonic cavitation, which eventually resulted in a combination of Brownian motion between proteins and the destruction of hydrogen bonds, leading to a decrease in viscosity [1,27]. Ma et al. [21] found that the increase in surface hydrophobicity of cod proteins after ultrasound treatment, which led to increased adsorption of proteins on the surface of oil droplets and disrupted the structural integrity of cod proteins, and finally led to the decrease of viscosity. This was consistent with the results of the present study.

The \( G' \) and \( G'' \) are closely related to the mechanical properties of MP gel, mainly reflected in the impact on elasticity and viscosity. Fig. 5B-C showed the variation of \( G' \) and \( G'' \) MP solutions under different ultrasound treatments with temperature scan measurements. Overall, \( G' \) and \( G'' \) increased after ultrasound treatment compared to the control group, indicating that ultrasound treatment could improve the viscoelasticity of MP solutions and contribute to the formation of gels. Fig. 5B showed that the \( G' \) of the MP solutions with different treatments have a typical rheological transition. From 20 °C to 42 °C, a gradual formation of the gel network accompanied by an increase in \( G' \) could be obtained, mainly due to the degradation of the S1 subunit of the myosin head [1]. As the temperature continued to increase (42 °C to 51 °C), \( G' \) dropped sharply, which was the cleavage phase of the gel, probably due to the shift of the myosin tail helix to irregular coiling, which disrupted the already formed protein meshwork [12]. The increase in % after 51 °C was attributed to the formation of disulfides and hydrophobic bonds in the structure of myosin and actin [18]. Fig. 5C showed the changes of \( G'' \) of MP solutions with different treatments. The trend in \( G'' \) change was observed to be similar to \( G' \). However, the \( G'' \) of all samples was lower than \( G' \), indicating that the specimens were more elastic than dense, i.e., the elastic component of the specimens was more prominent and showed the nature of viscoelastic solids. At temperatures above 51 °C, continuous intermolecular cross-linking and interaction dominated the system, resulting in the conversion of the viscous sol into a highly elastic gel network [18].

Generally, the overall viscoelasticity of the sample is characterized by \( \delta \), which is the ratio of \( G'' \) to \( G' \) (\( \tan \delta = G''/G' \)), for pure solids, where \( \tan \delta \) is zero, and for pure fluids, where \( \tan \delta \) is infinite [35]. It could be seen from Fig. 5D, \( \delta \) showed a decreasing trend from 20 °C to 45 °C, an increasing trend from 40 °C to 50 °C, and a decreasing trend after 50 °C. The lower \( \delta \) and higher \( G' \) presented at the end of sweep temperature indicated the formation of good gel viscoelasticity. The viscoelasticity of the MP solution rose with increasing ultrasound intensity, a phenomenon that suggested that ultrasound treatment increased the cross-linking rate of myosin molecules, which might be due to ultrasound pretreatment exposing more reactive groups of myosin molecules. It was consistent with the previous results for protein carbonyl groups and free radicals [9,18].

3.9. Gel strength

Gel strength is also an important parameter reflecting the mechanical properties of the MP gel. As shown in Fig. 6, the gel strength of MP gel significantly increased from 0.25 N to 0.38 N (an increase of...
approximately 52.00 %) with increasing ultrasonic power from 0 to 500 W. Thermal induction promotes the formation of protein gels involving intermolecular covalent and non-covalent interactions (disulfide bonds and hydrophobic interactions) [12]. Zhao et al. [35] reported that the ultrasonic cavitation effect caused rapid molecular motion, which led to the formation of increased cross-linking of MP molecules, resulting in the formation of rigid and homogeneous gel networks and increased gel strength. Kohyama, Sano, and Doi [15] found that hydrophobic interactions between protein molecules played a dominant role in the formation of protein gel network structures and that gel strength was closely related to hydrogen bonds. Thus, the exposed hydrophobic residues, after sonication favor protein–protein interactions and increased the gel strength. The results of the present study showed that the gel strength and rheological properties followed the same trend, further indicating that ultrasound could improve the mechanical properties of MP gel.

4. Conclusion

This study demonstrated that ultrasound treatment altered the physicochemical properties and conformation of the MP of L. vannamei, thus improving the subsequent rheological properties and gel strength. Ultrasound led to the oxidation of MP, as shown by the increase of carbonyl, free amino, and free radical contents. Moreover, ultrasound significantly decreased total sulphydryl group content, but increased carbonyl, free amino, and free radical contents. Moreover, ultrasound thus improving the subsequent rheological properties and gel strength.

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 authors do not have permission to share data.

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[1] A. Amir, P. Sharifian, N. Soltaniazadeh, Application of ultrasound treatment for improving the physicochemical, functional and rheological properties of myofibrillar proteins, Int. J. Biol. Macromol. 111 (2018) 139-147. [2] N. Braggason, B. Danilensen, L.H. Skibsted, Rosemary as antioxidant in pressure processed chicken during subsequent cooking as evaluated by electron spin resonance spectroscopy, Innovative Food Sci. Emerg. Technol. 8 (1) (2007) 24–29. [3] Y. Cao, Y.L. Xiong, Chlorogenic acid-mediated gel formation of oxidatively stressed myofibrillar protein, Food Chem. 180 (2015) 235–243. [4] S. Chanarat, S. Benjakul, Effect of formaldehyde on protein cross-linking and gel forming ability of surimi from lizardfish induced by microbial transglutaminase, Food Hydrocolloids 30 (2) (2015) 704-711.

[5] J. Chen, Y. Ren, K. Zhang, Y.L. Xiong, Q. Wang, K. Shang, D. Zhang, Site-specific incorporation of sodium tripolyphosphate into myofibrillar protein from mantis shrimp (Ommastrea curacaoensis) by physical cross-linking and gel network formation, Food Chem. 312 (2020) 126113. [6] L. Chen, J. Chen, J. Ren, M. Zhao, Effects of ultrasound pretreatment on the enzymatic hydrolysis of soy protein isolates and on the emulsifying properties of soy hydrolysates, J. Agric. Food. Chem. 59 (6) (2011) 2600-2609. [7] A.J. Cichocki, M.S. Silva, Y.S.V. Leaes, C.C.B. Brasil, C.R. de Menezes, J.S. Barin, R. Wagner, P.C.B. Campagnol, Ultrason: A promising technology to improve the technological quality of meat emulsions, Meat Sci. 148 (2019) 150-155. [8] T.K. Dahlgard, D. Orten, J.H. Nielsen, L.B. Larsen, Changes in structures of myeloid proteins upon photo-oxidation, J. Agric. Food. Chem. 55 (26) (2007) 10968-10976. [9] L. Fu, J. Jiang, A.D. True, Y.L. Xiong, Myofibrillar protein cross-linking and gelling behavior modified by structurally relevant phenolic compounds, J. Agric. Food. Chem. 69 (4) (2021) 1308-1317. [10] O. Figueroa-Barraza, W. Torres-Arreola, J. Esquerra-Brauer, F. Cinco-Moroyoqui, J. R. Figueroa, E. Marquez-Rios, Effect of pulsed ultrasound on the physicochemical characteristics and emulsifying properties of squid (Dosidicus gigas) mantle proteins, Ultrason. Sonochemistry. 38 (2017) 829-834. [11] H. Hu, X. Fan, Z. Zhu, X. Xu, G. Fan, L. Wang, X. Huang, S. Pan, L. Zhu, Acid-induced gelation behavior of soybean protein isolate with high intensity ultrasonic pre-treatments, Ultrason. Sonochemistry. 20 (1) (2013) 187–195. [12] N. Jia, L. Wang, J. Shao, D. Liu, B. Kong, Changes in the structural and gel properties of pork myofibrillar protein induced by catechin modification, Meat Sci. 127 (2017) 45–50. [13] L. Jiang, J. Wang, Y.L. Li, Z. Wang, J. Liang, R. Wang, Y. Chen, W. Ma, B. Qi, M. Zhang, Effects of ultrasound on the structure and physical properties of black bean protein isolates, Food Res. Int. 62 (2014) 595-601. [14] S. Jiang, B. Lamsal, V. Stepyen, L. Johnson, P. Murphy, Functionality of soy protein produced by enzyme-assisted extraction, J. Am. Oil. Chem. Soc. 83 (1) (2006) 71-78. [15] K. Kohyama, Y. Sano, E. Doi, Rheological characteristics and gelation mechanism of myofibrillar protein (soybean curd), J. Agric. Food. Chem. 43 (7) (1995) 1806-1812. [16] D.Y. Li, Z.F. Tan, Z.Q. Liu, C. Wu, H.L. Liu, C. Guo, D.Y. Zhou, Effect of hydroxyl radical induced oxidation on the physicochemical and gelling properties of shrimp myofibrillar protein and its mechanism, Food Chem. 351 (2021) 129344. [17] D.Y. Li, D. Li, X.H. Dong, Z.F. Tan, X.K. Xu, Y.H. Bai, Use of high-intensity ultrasound to improve emulsifying properties of chicken myofibrillar protein and enhance the rheological properties and stability of the emulsion, Food Hydrocolloids 98 (2020) 105275. [18] G. Liu, Y. Xiong, D. Butterfield, Chemical, physical, and gel-forming properties of oxidized myofibrils and whey-and soy-protein isolates, J. Food Sci. 65 (5) (2000) 811-818. [19] L.K. Ma, B. Zhang, S.G. Deng, C. Xie, Comparison of the cryoprotective effects of trehalose, alginate, and its oligosaccharides on peeled shrimp (Lupenanus vannamei) during frozen storage, J. Food Sci. 80 (3) (2015) 540-546. [20] W. Ma, J. Wang, X. Wu, L. Qin, C. Wu, M. Du, Ultrasound treatment improved the physicochemical characteristics of cod protein and enhanced the stability of oil-in-water emulsions, Food Res. Int. (2019) 247-256. [21] L. Mu, M. Zhao, B. Yang, H. Zhao, C. Cui, Q. Zhao, Effect of ultrasonic treatment on the graft reaction between soy protein isolate and gum acacia and on the physicochemical properties of conjugates, J. Agric. Chem. Food. 58 (7) (2010) 4498-4499. [22] L.R. Nissen, L. Mansson, G. Bertelsen, T. Huyh-Ba, L.H. Skildst, Protection of dehydrated chicken meat by natural antioxidants as evaluated by electron spin resonance spectrometry, J. Agric. Food. Chem. 48 (11) (2000) 5548-5556. [23] P. Riesz, T. Kondo, Free radical formation induced by ultrasound and its biological implications, Free Radical Biol. Med. 13 (3) (1992) 247–270. [24] A. Singh, A. Mittal, S. Benjakul, Full utilization of squid meat and its processing by-products: Review, Food Res. Int. 38 (4) (2005) 455-479. [25] O. Solodaye, M. Juarez, J. Alahb, P. Mestres, Oxidative protein oxidation in processed meat: Mechanisms and potential implications on human health, Compr. Rev. Food Sci. Food Saf. 14 (2) (2015) 106–122. [26] C. Sun, S. Gunasekaran, M.P. Richards, Effect of xanthan gum on physicochemical properties of whey proteins stabilized in oil-in-water emulsions, Food Hydrocolloids 21 (4) (2007) 555-564. [27] W. Sun, M. Zhao, B. Yang, H. Zhao, C. Cui, Oxidation of sarcoplasmic proteins during processing of Cantonese sausage in relation to their aggregation behaviour and muscle toxicity, Meat Sci. 88 (3) (2011) 462-467. [28] S.S. Turgut, A. Soyer, F. Isikci, Effect of pomegranate peel extract on lipid and protein oxidation in beef meatballs during refrigerated storage, Meat Sci. 116 (2019) 150-156. [29] O. Higuera-Barraza, W. Torres-Arreola, J. Ezquerra-Brauer, F. Cinco-Moroyoqui, J. A. Aalhus, P. Shand, M. Estevez, Protein oxidation in muscle meats: Revisit, Food Rev. Int. 38 (4) (2020) 455-470. [30] Y. Xiong, Q. Li, S. Miao, H. Zhao, B. Zhang, L. Zhang, Effect of ultrasound on physicochemical properties of emulsion stabilized by fish myofibrillar protein and xanthan gum, Innovative Food Sci. Emerg. Technol. 54 (2019) 225-234. [31] Q.D. Xu, Z.L. Yu, W.C. Zeng, Structural and functional modifications of myofibrillar protein by natural phenolic compounds and their application in pork meatball, Food Res. Int. 148 (2021) 110592.
S. Xue, X. Yu, X. Li, X. Zhao, M. Han, X. Xu, G. Zhou, Structural changes and emulsion properties of goose liver proteins obtained by isoelectric solubilisation/precipitation processes, LWT - Food Sci. Technol. 102 (2019) 190–196.

Z. Zhang, J.M. Regenstein, P. Zhou, Y. Yang, Effects of high intensity ultrasound modification on physicochemical property and water in myofibrillar protein gel, Ultrason. Sonochem. 34 (2017) 960–967.

Y.Y. Zhao, P. Wang, Y.F. Zou, K. Li, Z.L. Kang, X.L. Xu, G.H. Zhou, Effect of pre-emulsification of plant lipid treated by pulsed ultrasound on the functional properties of chicken breast myofibrillar protein composite gel, Food Res. Int. 58 (2014) 98–104.