Ultrasonic pretreatment improves the gelation properties of low-salt Penaeus vannamei (Litopenaeus vannamei) surimi

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The effects of different ultrasonic pretreatments (120–600 W, 20 min; 360 W, 10–30 min) on the gel properties of shrimp surimi were investigated. Gel properties and protein functional properties were analysed to clarify the mechanism of action of ultrasound. The gel strength, water holding capacity and surface hydrophobicity of shrimp surimi gel increased initially and then decreased with the increase in ultrasound power or time, but the change in total sulphhydryl content showed the opposite trend, which indicated that proper ultrasound pretreatment could improve the gel properties of shrimp surimi, expand the protein to a greater extent and expose more SH groups and hydrophobic groups. According to scanning electron microscopy observation, ultrasound made shrimp surimi gel form a denser gel network. Fourier transform infrared analysis indicated that the α-helix content in shrimp surimi gel decreased initially and then increased with the increase of in ultrasound power or time, whereas the change in β-sheet content showed the opposite trend. And the protein was the most stable in 360 W/20 min pretreatment. SDS-PAGE patterns showed that proper ultrasound inhibited the degradation of actin and troponin C. In addition, dynamic rheology illustrated that the G′ values of the ultrasonic pretreatment group were higher than that of the control group, indicating that ultrasound could improve the elasticity and stability of shrimp surimi gel. The results suggested that the shrimp surimi gel pretreated by 360 W/20 min ultrasound showed the best gel properties. Furthermore, the correlation between the indexes affecting the properties of the gel was analyzed. This study provides a new technical means to improve the gel properties of shrimp surimi.

1. Introduction

Surimi is a concentrated myofibrillar protein (MP) made from fish flesh [1] and is produced by salt chopping and other processes. It is a kind of marine gel product with high nutritional value [2]. The production of shrimp surimi is similar to that of surimi, and can be processed into shrimp balls and other products, which are favoured by consumers [3]. In the process of making surimi, 2%–3% salt is usually added to extract MP to form the desired texture during cooking [4]. The swelling proteins dissolved by salt form a continuous matrix, which forms a good three-dimensional solid network through thermal aggregation and cross-linking, thus forming an elastic gel [5]. However, excessive salt intake can easily lead to high blood pressure and other diseases that are not good for human health [6]. Thus, there is a growing shift toward low-salt shrimp surimi gel products. However, low-salt concentration is not conducive to gelation, because MP cannot fully dissolve, expand and interact with each other under low-salt conditions, resulting in the low strength, poor water holding capacity and rough taste of low-salt shrimp surimi gel. Therefore, it is necessary to find other methods to reduce the salt content of shrimp surimi products in gel processing and maintain their good gel properties, so as to provide reference for enterprises to produce high-quality shrimp surimi products.

The current research mainly added exogenous substances, such as transglutaminase (TGase), polysaccharides and starch, to improve the gel properties of shrimp surimi. Studies have shown that MTGase can induce the polymerisation of MHC to form non-dissulphide bonds [7], and dense-phase carbon dioxide can promote the interaction and cross-linking between proteins to form a dense three-dimensional network structure [8], which improves gel properties. However, soybean oil has an adverse effect on the gel-forming ability of the gel [9]. New physical processing methods such as ultra-high pressure [10,11] and microwave [12], have been applied to low-salt surimi gel with remarkable improvement effect. However, the use of these methods in industrial conditions.
production is limited owing to their high cost and difficult operation [13].

As an advanced non-thermal processing technology, ultrasound has been widely studied in the field of food processing because of its advantages such as non-pollution, safety and simple operation [14–16]. On the one hand, cavitation bubbles are formed under the action of ultrasound, and the violent collapse of these bubbles leads to the accumulation of energy in hot spots and the formation of high-shear energy waves and turbulence. On the other hand, a large number of highly active free radicals produced by the ultrasonically facilitated decomposition of water molecules can react with other active groups (such as SH/molecules) [17]. Ultrasound can induce conformational changes in protein molecules, resulting in high surface hydrophobicity [18]. Ultrasound can remarkably improve the gel properties of low-salt silver carp surimi by promoting protein dispersion and intermolecular interaction [6]. Although ultrasound has been successfully used to improve the gel properties of surimi, its application in shrimp surimi has not been reported.

The main objective of this work was to investigate the effects of ultrasound pretreatment with different power and time on the gel properties of shrimp surimi of Penaeus vannamei. The changes in the gel properties of shrimp surimi after ultrasound pretreatment were determined using scanning electron microscopy (SEM) and analysis of the gel strength and water holding capacity (WHC). Moreover, the changes in the functional properties of shrimp surimi protein after ultrasound pretreatment were investigated by using total SH, surface hydrophobicity (Hₒ), SDS-PAGE, protein secondary structure and dynamic rheology. The mechanism of action of ultrasound on shrimp surimi was expounded to provide theoretical basis on the use of ultrasonic technology to improve low-salt shrimp surimi products.

2. Materials and methods

2.1. Materials

Penaeus vannamei (Litopenaeus vannamei), cultured in the southern coastal area of Tangshan, was purchased from a local market in Baoding, Hebei, China. The selected shrimp samples with a good shape and uniform size (20–25 g). The fresh shrimp was packed in a foam box filled with ice bags, transported to the laboratory within 30 min and stored at –80 °C until use. The edible meat of shrimp contains 76.73 ± 0.33% moisture, 21.17 ± 0.54% protein, 1.05 ± 0.07% fat and 1.19 ± 0.06% ash.

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 chemical reagents were analytical grade and purchased from Beijing Solaibao Technology Co., Ltd. (Beijing, China) or Beijing Reagent Biotechnology Co., Ltd. (Beijing, China).

2.2. Ultrasound pretreatment and preparation of shrimp surimi gel

The shrimp was removed from the –80 °C refrigerator and placed into a 4 °C refrigerator overnight. The next day, the thawed shrimp was decapitated, tailed and shelled. A meat grinder (S3-LA166, Jiuyang Co., Ltd, Jinan, China) was used to grind the shrimp meat into uniform size (20 mm in diameter) in accordance with the method of Chen et al. [20] with some slight modifications. The test conditions were as follows: test speed, 1 mm/s; trigger force, 30 g; displacement, 10 mm.

The WHC of shrimp surimi gel was determined according to the method of Buda et al. [21] with slight modifications. The shrimp surimi gels were sliced (2 g, Wₒ) wrapped in three-layer filter paper, placed in a 50 mL centrifuge tube and centrifuged at 9690 g for 15 min at 4 °C. Then, the gels were removed and weighed (W₂).

\[ WHC (\%) = \left(\frac{W_0 - W_2}{W_0}\right) \times 100 \]

2.5. Determination of total SH content

The total SH content in the proteins was determined by a total mercapto assay kit (Solarbio, product number: BC1375). Shrimp surimi (0.1 g) was mixed with 1 mL of extractive liquid, homogenized and centrifuged at 8000 g for 10 min at 4 °C. The supernatant was collected for testing. The standard was diluted with distilled water into a standard solution with different concentration gradients. The control tube, measuring tube, standard tube and blank tube were prepared according to the kit instruction manual. After mixed homogeneously and balanced for 10 min at room temperature, absorbance values were measured at 412 nm by a full-wavelength microplate reader (MULTISKAN GO, Thermo Fisher Scientific, Madison, USA) and recorded as Aₒ, Aₒ, Aₛ and Aₚ, respectively. The standard curve is drawn by using the standard solution concentration and \( Aₒ = Aₒ \) and the standard equation \( y = kx + b \) was obtained. Then, total SH content was calculated according to the formula:

\[ \text{Total SH content (µmol/g)} = \frac{x}{W} \]

Where \( x \) (µmol/mL) is the result of bringing \( \Delta Aₘ (Aₘ - Aₒ) \) into \( y = kx + b \), and \( W \) (g) is the sample mass.

2.6. Extraction of MPs and determination of surface hydrophobicity (Hₒ)

MP was extracted according to the method of Gullian-Klanian et al. [22] with slight modifications. RIPA Lysis Buffer (Strong) was added with protease inhibitor cocktail in a ratio of 99:1 before use. The sample (0.5 g) was mixed with 1 mL of RIPA Lysis Buffer (Strong) and homogenized in high-throughput tissue grinder (SCIENTZ-48, Ningbo Xinzhi
Fig. 1. Scanning electron microscope photos of shrimp surimi gel with different ultrasound power (20 min) and different ultrasound time (360 W). A ~ F indicate the control samples with the ultrasound of 120 W/20 min, 240 W/20 min, 480 W/20 min, 600 W/20 min, 360 W/10 min, 360 W/15 min, 360 W/20 min, 360 W/25 min and 360 W/30 min.
Biologically, the homogenized mixture was incubated with ice for 20 min, and then centrifuged at 14,000 g for 10 min at 4 °C. The supernatant is the MP solution.

The H₀ of the MP sample from shrimp surimi was determined using bromophenol blue (BPB) according to the method of Cheh et al. [23] with slight modifications. About 1 mL of the protein sample solution was collected and added with 200 μL of 1 mg/mL BPB. The solution was mixed well, shaken for 20 min at room temperature and centrifuged at 8000 g for 15 min. The absorbance of the supernatant was determined under 595 nm and is recorded as A. The blank sample of BPB was made of 1 mL of 20 mmol/mL phosphate buffer (pH 6.0) and 200 μL of BPB. The absorbance was determined under 595 nm and recorded as A₀. The calculation formula is as follows:

$$BPB(\mu g) = \frac{[200 \mu g \times (A_0 - A)]}{A_0}$$

2.7. SDS-PAGE analysis

SDS-PAGE was performed according to the method of Gao et al. [6] with slight modifications. Firstly, the concentration of the extracted MP solution was determined by BCA kit, and the protein concentrations of all samples were adjusted to 2 mg/mL. Then, the protein solution (2 mg/mL) was mixed with sample buffer at a ratio of 4:1 (protein:buffer) and heated in a water bath at 95 °C for 5 min. The supernatant was centrifuged at 14,000 g at 4 °C for 1 min, and the supernatant was retained. The mixtures were loaded onto SDS-PAGE gel (5% (w/v) stacking gel, 10% (w/v) running gel) at a voltage of 120 V to carry out protein separation. After running, proteins were stained with 0.25% Coomassie Blue R-250 and decolourised for 4 h in distilled water until the protein bands were clearly visible.

2.8. Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectroscopy (IRAffinity-1S, Shimadzu Enterprise Management Co., Ltd., Shanghai, China) was used to investigate the secondary structures of shrimp surimi proteins. According to the method of Zhang et al. [24] with slight modifications, a 2 mg freeze-dried sample was mixed with 200 mg KBr and pressed into a diaphanous sheet. The infrared spectrum scan range was 4000–400 cm⁻¹ with a resolution of 4 cm⁻¹. Each sample was scanned 32 times. The PeakFit software (Systat Software, Inc.) was used to analyse the amide I region (1600–1700 cm⁻¹) of all data. Each test was repeated three times.

2.9. Determination of dynamic rheological properties

The gelation properties of shrimp surimi were determined using a rotary rheometer (AR2000ex, TA Co., Ltd., USA) according to the method of Zhou et al. [25] with slight modifications. A cone-and-plate fixture having a cone angle of 1° and a diameter of 40 mm was used. After the sample was put away, it was sealed with silicone oil to prevent water evaporation. Deformation of 1% and frequency of 0.1 Hz were used for temperature sweep test. The storage modulus (G‘) and loss modulus (G″) were measured from 4 °C to 90 °C with a heating rate of 2 °C/min.

2.10. Statistical analysis

All experiments were performed in triplicate, and data were presented as means and standard deviations (mean ± SD). All statistical analyses and Pearson correlation analysis were performed in the SPSS 22.0 Software (SPSS Institute Inc., Chicago, USA), and Origin 9.0 was used in the visualisation of the results. One-way analyses of variance (ANOVA) and Duncan’s test (P < 0.05) were performed to determine the differences between the samples.
structure.

3.2. Gel strength and WHC

Gel strength is a key index used to evaluate the quality of shrimp surimi gel products. The product of the breaking force (g) and the amount of breaking distance (mm) is the gel strength [28]. The greater the breaking force and breaking distance of the gel, the better the strength and quality of the gel [29].

Fig. 2 shows the effect of ultrasound pretreatment on the breaking force, breaking distance and gel strength values of heat-induced gels. The gel strength of shrimp surimi increased initially and then decreased with the increase in ultrasonic power. The gel strength of the 360 W/20 min group was the highest, which was 54.02% higher than that of the non-ultrasonic pretreatment group (Fig. 2A, \( P < 0.05 \)). The change trend of gel strength pretreated with different ultrasonic time is consistent with that of different ultrasonic power, and always higher than that of the control group (Fig. 2B). These results were in accordance with the effect of ultrasound on surimi with 0.5% salt content [30]. Zhang et al. showed that the gel strength of tilapia surimi increased initially and then decreased after treatment with 0–45 kHz ultrasound [31]. These findings show that proper ultrasound pretreatment improved gel properties. The shearing and cavitation effects of ultrasonic wave exposed the hydrophobic groups amongst protein molecules, which promoted the interaction between proteins and formed a dense and uniform gel [32]. However, the decrease of gel strength may be due to the excessive expansion of MP caused by high-power or long-term ultrasound, and the conformation of protein is seriously disturbed, resulting in the formation of a poor gel network [30].

WHC refers to the ability of proteins to maintain the water content in their three-dimensional structure, and it can reflect the spatial structure of shrimp surimi gel [2]. As shown in Fig. 3, the WHC of shrimp surimi gel increased with the increase of ultrasonic power or time. The 360 W/20 min ultrasound pretreatment group had the largest WHC, which increased by 26.69% compared with the control group (\( P < 0.05 \)). However, WHC gradually decreased when the ultrasonic power was higher than 360 W and the ultrasonic time was longer than 20 min. Amiri et al. [33] found that after ultrasound treatment, the WHC of Holstein bull MP increased by 69% compared with the control group. Under the action of ultrasound, the enhancement of protein gel network
3.3. Total SH content

Fig. 4 shows the change in the total SH content of shrimp surimi gels with different ultrasound pretreatments. The SH content in the ultrasound group was always lower than that in the control group, and the total SH content in the 360 W/20 min pretreatment group was the lowest, which was 54.96% lower than that in the control group (P < 0.05). Of note, when the ultrasonic power was 600 W, the total SH content was significantly higher than that at 360 W (P < 0.05).

Moreover, when the ultrasonic time exceeded 20 min, the total SH content gradually increased. Tang et al. [30] showed that the total SH content of threadfin bream surimi gel decreased slightly as the ultrasound intensity increased from 0 W/cm² to 16.45 W/cm². The total SH content of silverfish myosin decreased considerably with the further increase in ultrasonic power and time [37]. The decrease in total SH content is attributed to the fact that the high pressure and shear force produced by ultrasound can lead to more obvious protein unfolding and the exposure of SH groups. With the enhancement of ultrasonic effect, the exposed SH groups will be oxidised to hydrogen peroxide and form more disulphide bonds [38]. Disulphide bonds can promote the formation of the protein three-dimensional network structure, so the 360 W/20 min ultrasound pretreatment group had the densest gel network structure (Fig. 1). High power ultrasound or long ultrasound time may disrupt the intermolecular or intramolecular disulphide bonds, resulting in an increase in total SH content.

3.4. Surface hydrophobicity (H₀)

H₀ is an index of hydrophobic groups on the surface of protein molecules, which can be used to evaluate the conformational changes of proteins and is closely related to the functional properties of proteins [39]. The effect of different ultrasonic pretreatment on the H₀ of shrimp surimi gel is shown in Table 1. When the ultrasonic power was higher than 360 W or the ultrasonic time exceeded 20 min, H₀ increased gradually with the increase in power or time, which indicates that more hydrophobic groups were exposed. Appropriate ultrasound increased H₀, which reached the maximum value at 360 W/20 min ultrasound pretreatment, and H₀ was 1.61 times higher than that of the control group (P < 0.05). An et al. [40] study also showed that the H₀ of silver carp myosin increased considerably after ultrasonic treatment, indicating that the conformation of silver carp myosin changed further. The α-helix structure unfolds under the high pressure and shear force produced by ultrasound, the protein conformation changes, and the myosin aggregates break, which will expose more hydrophobic groups [37].

| Ultrasonic pretreatment conditions | Surface hydrophobicity (H₀) | Ultrasonic pretreatment conditions | Surface hydrophobicity (H₀) |
|-----------------------------------|-----------------------------|-----------------------------------|-----------------------------|
| control                           | 4.08 ± 1.15<sup>a</sup>     | control                           | 4.08 ± 1.15<sup>b</sup>     |
| 120 W/20 min                     | 6.32 ± 2.93<sup>a</sup>     | 360 W/10 min                      | 8.73 ± 2.70<sup>b</sup>     |
| 240 W/20 min                     | 7.13 ± 1.44<sup>b</sup>     | 360 W/15 min                      | 9.56 ± 0.85<sup>a</sup>     |
| 360 W/20 min                     | 10.66 ± 1.06<sup>a</sup>    | 360 W/20 min                      | 10.66 ± 1.06<sup>a</sup>    |
| 480 W/20 min                     | 6.87 ± 0.80<sup>b</sup>     | 360 W/25 min                      | 5.22 ± 0.72<sup>b</sup>     |
| 600 W/20 min                     | 2.48 ± 1.95<sup>a</sup>     | 360 W/30 min                      | 1.06 ± 0.30<sup>b</sup>     |

Note: Different lowercase letters in the same column indicate significant differences between different ultrasound pretreatment (360 W/10 min, 360 W/15 min, 360 W/20 min, 360 W/25 min, 360 W/30 min, 120 W/20 min, 240 W/20 min, 480 W/20 min and 600 W/20 min) (P < 0.05).

Fig. 5. SDS-PAGE patterns of shrimp surimi gels with different ultrasound pretreatments. Samples were applied to 5% stacking and 10% separating gels at 8 μg protein/lane. 1 ~ 10 indicate the control samples with the ultrasound of 120 W/20 min, 240 W/20 min, 360 W/20 min, 480 W/20 min, 600 W/20 min, 360 W/10 min, 360 W/15 min, 360 W/25 min and 360 W/30 min. M = Molecular marker, AC = actin, Tn I = troponin I, Tn C = troponin C.
H0 is also used to characterise the degree of protein oxidation. A higher H0 corresponds to a higher degree of protein oxidation in the shrimp surimi gel. After pretreatment with 360 W for 30 min and 600 W for 20 min ultrasound, the H0 of shrimp surimi gel decreased to 1.06 and 2.48, respectively, which decreased by 74.02% (P < 0.05) and 39.22% compared with the control group, respectively. This result may be attributed to the formation of hydrophobic interactions between the previously exposed hydrophobic groups. It may also be attributed to high-power or long-term ultrasound promoting the formation of large numbers of protein aggregates, thereby reducing H0 [33].

3.5. SDS-PAGE

The MP was analysed by SDS-PAGE to elucidate the effect of ultrasound on the shrimp surimi gel protein. The SDS-PAGE patterns of proteins with different ultrasound pretreatment are shown in Fig. 5. The main bands of gel containing actin (AC), troponin I (Tn I) and troponin C (Tn C) corresponded to the molecular weight of 43, 23 and 21 kDa, respectively. The results showed that with the increase of ultrasonic power, the molecular band intensity of AC and Tn C increases initially and then decreases, whereas Tn I had no obvious change (Fig. 5). After pretreatment at 360 W/20 min, AC and Tn C had the darkest protein molecular bands. Appropriate ultrasound pretreatment can inhibit AC and Tn C degradation. AC has a great influence on the formation of ideal gel properties [41], which is also the reason why the 360 W/20 min pretreatment group had good gel properties. In the electrophoretic patterns of the groups pretreated with different ultrasonic time, the molecular band intensities of the three proteins increased initially and then decreased. When the ultrasound time was over 20 min, the amount of protein degradation increased, and the gel strength decreased (Fig. 2). In addition, protein cross-linking may be limited under high-power or long-term ultrasound pretreatment. Many small molecular bands appeared above and below the AC bands in the gel samples, which may be caused by the degradation of high-molecular-weight protein during heating or the shear action of peptide bonds by free radicals generated by ultrasound [42], which needs to be further confirmed. In conclusion, proper ultrasonic pretreatment can inhibit MP degradation.

3.6. FT-IR spectroscopy

The protein secondary structure refers to the spatial structure of the local peptide in the polypeptide chain skeleton, which determines the functional properties of the protein [24,43]. The amide I band (1600–1700 cm⁻¹) is considered the most sensitive region to the secondary structure composition of the protein [44]. It contains α-helix (center at 1650–1660 cm⁻¹), random coil (center at 1640–1650 cm⁻¹), β-sheet (center at 1610–1640 and 1680–1700 cm⁻¹) and β-turn (center at 1660–1680 cm⁻¹) [38]. The secondary structure of proteins in the shrimp surimi was dominated by β-sheet and β-turn, which accounted for about more than 70%. The α-helix content was relatively low. Compared with the control group, the α-helix content in gel protein decreased, and the β-sheet, β-turn and random coil contents increased after pretreatment with different ultrasonic time at 360 W (Fig. 6B, P < 0.05). Tang et al. [30] also found a similar phenomenon in the study of the ultrasonic treatment of threadfin bream surimi. The content of α-helix in the 360 W/20 min group was the lowest, which decreased by 45.25% compared with the control group, and the content of β-fold was the highest. The change trends of α-helix and β-fold in different ultrasound power pretreatment groups were consistent with those in different ultrasound time groups (Fig. 6A, P < 0.05). This result suggests that higher ultrasound power and longer ultrasound duration induce higher protein defolding [30]. Of note, the α-helix was destroyed during ultrasound pretreatment, and a lower α-helix content characterises a higher gel strength (Fig. 2). This leads to alterations in protein aggregation and crosslinking, resulting in a more stable protein skeleton and a more orderly gel structure. The shrimp surimi gel containing more β-sheet had better mechanical properties [24]. β-Sheet mainly depends on intermolecular hydrogen bond [36]. Therefore, the intermolecular aggregation of proteins may be promoted by proper ultrasound because of the hydrogen bonding between protein molecules. The present study indicates that ultrasound changes the secondary structure of shrimp surimi protein, which is beneficial to the exposure of protein hydrophobic groups (Table 1). However, Liang et al. [43] did not find an effect on the secondary structure of mixed crab meat surimi by ultrasound. This finding was attributed to the low ultrasound power and frequency used in the study.

3.7. Dynamic rheological properties

The rheological properties are one of the important protein functional properties during meat processing [26]. Dynamic rheology characterises the gel properties of shrimp surimi [28]. G′ and G″ represent the elastic and viscose properties of the shrimp surimi gel, respectively. The variations in G′ and G″ with increasing temperature are also the result of protein denaturation [44]. The relationships between the G′ and G″ of the shrimp surimi gel and temperature are shown in Fig. 7.

As shown in Fig. 7A, ultrasonic pretreatment makes the heat-induced
Gel have a higher $G'$ value, and the 360 W/20 min pretreatment group consistently had higher $G'$ value than the other ultrasound pretreatment and control groups. Before heating to 42 °C, a slight decrease in $G'$ with increasing temperature was observed in all samples. This may be due to the disruption of the hydrogen bonds and ionic bonds by heating. Then, $G'$ increased and reached its first peak at 48 °C. At low temperatures, protein interactions occur in the gel and form a preliminary gel network structure through weak bonds [20]. Thereafter, the second low value was reached at about 55 °C and then rose rapidly. This gel-weakening phenomenon may be attributed to the disruption of the initial gel network, with the denaturation of the myosin tail and the increased mobility [3]. It may also be related to the high activity of the endogenous protease at around 55 °C, where protein degradation occurs [6]. However, when the temperature exceeds 55 °C, a dense and stable gel network structure forms due to the hydrophobic interactions between myosin molecules and the generation of numerous disulphide bonds [26]. It may also involve the degradation of actin in forming an irreversible gel network. High intensity ultrasound may destroy the assembly of myosin, cause the degradation of protein molecules and lead to good dispersion, which is consistent with the finding mentioned earlier. This is associated with higher $H_0$ (Table 1) and the production of more disulphide bonds (Fig. 4), which result in the higher elasticity and stability of the formed shrimp surimi gel. The trend of $G''$ was essentially consistent with that of $G'$ (Fig. 7B), except for a slight increase in $G'$ at low temperatures. The same phenomenon was found by Yang et al. [44] and Song et al. [3]. The $G''$ value is always smaller than the $G'$ value, which indicates a higher elastic component in the shrimp surimi gel system. Therefore, ultrasound promotes the formation of stable elastic gels.

3.8. Relativity analysis

Correlation analysis based on heatmap was performed to obtain relationship between the ultrasonic pretreatment indexes of different ultrasonic power or different time, as presented in Fig. S1. It can be seen from Fig. S1A, the gel strength of shrimp surimi was significantly positively correlated with $H_0$ ($P < 0.05$) after pretreatment with different ultrasonic power. WHC was significantly positively correlated with $H_0$ ($P < 0.01$), $\beta$-sheet ($P < 0.05$) and random coil ($P < 0.01$), and significantly negatively correlated with $\alpha$-helix ($P < 0.05$), which indicated that more $\alpha$-helix content would lead to poor WHC of shrimp surimi. Moreover, there is a significant positive correlation between $H_0$ and random coil ($P < 0.01$). After ultrasonic pretreatment, the internal hydrophobic groups of the protein molecules were exposed, and the unfolding of the protein exposed the previously buried residues, changing the protein structure and the hydrophobic interaction of the protein [45]. Fig. S1B showed that the gel strength and WHC of shrimp surimi gel were significantly negatively correlated with total SH content and $\alpha$-helix ($P < 0.05$). It is consistent with previous research results that the decrease of total SH content and $\alpha$-helix will lead to the formation of more disulphide bonds and $\beta$-sheet, so the gel strength of shrimp surimi gel is stronger and the WHC is better [38]. To sum up, gel strength and WHC are closely related to the secondary structure and molecular interaction of protein. Besides, the correlation among the indexes of

Fig. 7. Effect of storage modulus ($G'$) (A) and loss modulus ($G''$) (B) of shrimp surimi gel with different ultrasound pretreatment.
different ultrasonic power pretreatment is stronger than that of different ultrasonic time pretreatment, which the difference of these results need to be further studied.

4. Conclusions

The results showed that the gel properties of shrimp surimi were improved after proper ultrasound pretreatment. This is because ultrasound makes proteins expand more larger and promotes the interaction and aggregation between proteins. These changes expose more SH and hydrophobic groups, promote the formation of disulphide bonds and dense gel networks, promote the interaction between protein and water, and change the secondary structure of proteins. Therefore, the SH content decreased, the H3 and WHC increased, and the protein was more stable. The gel properties of shrimp surimi pretreated by 360 W/20 min ultrasound were improved to the greatest extent. However, high-power or long-term ultrasound can promote protein denaturation and degradation, and destroy the gel network structure, thus reducing the gel properties of shrimp surimi. To sum up, the shearing and cavitation effect of ultrasound promoted the interaction between protein molecules and improved the gel properties of shrimp surimi. However, ultrasound overaction is not conducive to the formation of good gels.

CRediT authorship contribution statement

Tong Zhang: Data curation, Writing – original draft, Investigation.
Jie Wang: Supervision. Jiaqi Feng: Visualization. Yaqiong Liu: Conceptualization, Methodology. Ran Suo: Writing – review & editing.
Jingyu Jin: Formal analysis. Wenxiu Wang: Validation.

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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Appendix A. Supplementary data

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