Effect of high intensity ultrasound on gelation properties of silver carp surimi with different salt contents

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ABSTRACT

Surimi from silver carp with different salt contents (0–5%) was obtained treated by high intensity ultrasound (HIU, 100 kHz 91 W·cm−2). The gelation properties of samples were evaluated by puncture properties, microstructures, water-holding capacity, dynamic rheological properties and intermolecular interactions. As the salt content increased from 0 to 5%, gel properties of surimi without HIU significantly improved. For samples with low-salt (0–2% NaCl) content, HIU induced obvious enhancement in breaking force and deformation. HIU promoted the protein aggregation linked by S–S bonds, hydrophobic interactions and non-disulfide covalent bonds in surimi gels with low-salt content. Moreover, microstructures of HIU surimi gels with low-salt content were more compact than those of the corresponding control samples. HIU also improved the gelation properties of surimi with 3% NaCl to an extent. However, for high-salt (4–5% NaCl) samples, HIU decreased the breaking force and deformation of surimi gels due to the degradation of proteins suggested by increased TCA-soluble peptides. In conclusion, HIU effectively improved the gelation properties of surimi with low-salt content (0–2% NaCl), but was harmful for high-salt (4–5% NaCl) surimi. This might provide the theoretical basis for the production of low-salt surimi gels.

1. Introduction

To obtain desirable properties of surimi gels, 2–3% NaCl is usually added during the commercial production [1–3]. For that the main muscle protein - myosin- is able to release from the thick filament and facilitate the aggregation in the heat-induced gel formation [4]. However, excessive intake of salt content is not beneficial to people's health and easily induces diseases, such as high blood pressure, high cholesterol, etc. [5]. Therefore, surimi gels with low-salt content are imperatively taken into consideration. But reduced salt addition would limit the extraction of myosin and affect the formation of well network [6]. To improve gel properties of low-salt products, some sodium substitutes were used, such as potassium chloride, monosodium glutamate [7]. Cando et al. [8] reported that cysteine and lysine could cause the unfolding of myofibrillar proteins and enhance gel properties of Alaska Pollock surimi with 0.3% salt. However, salt alternatives arose new problems as suggested that a high level of KCl led to bitter taste [9]. Phosphate compounds would chelate the Ca2+ and had the detrimental effect on the cross-linking by endogenous transglutaminase which depended on Ca2+ [10]. Taking these into consideration, emerging technology has been investigated to produce low-salt products. High hydrostatic pressure above 100–150 MPa was reported to induce denaturation of protein, resulting in a desirable solubility and enhancing gel properties of surimi with 0.3% salt [11]. Intense heat generated by microwave was proven to break aggregation of proteins and provide dispersed substrate for interactions, improving the gel properties of silver carp surimi with 1% salt [12].

As the important role of solubility and dissociation of proteins prior to heating, our previous study showed that high intensity ultrasound (HIU) could improve the solubility of myosin [13]. In recent years, HIU is widely studied and owns enormous advantages, such as safety, easy-operation and environmental friendliness [14–17]. In addition, the cavitation phenomenon generated by HIU could be beneficial to the structural changes of proteins and expose reactive residues to the surface. These changes facilitated protein–protein interactions and were important for the formation of elastic gels [18–20]. It was reported that HIU improved gel strength of tilapia surimi gels with 2.5% salt [21]. However, the effect of HIU on surimi gels from silver carp with low-salt content is rarely reported.

In this study, surimi from silver carp with different salt contents...
(0–5%) was produced by HIU (300 W for 10 min). Gel properties of surimi were assessed by puncture properties, microstructures, water-holding capacity (WHC) and dynamic rheological properties. The changes in protein structure and interactions were evaluated by sulf-hydryl (SH) content and solubility. TCA-soluble peptides were also determined. The relationship between gel properties and physico-chemical indexes were analyzed. The aim was to illustrate the effect of HIU on the gelation properties of low-salt surimi.

2. Materials and methods

2.1. Materials

Silver carp (Huypophthalmichthys molitrix) (1.0–1.5 kg) was obtained from a local market in Wuhan, Hubei, China, and transported to the laboratory in a plastic bag filled with water, within 15 min. All reagents used were of analytical grade and obtained from Sigma-Aldrich chemical Co. (St. Louis, MO, USA) or Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of surimi

The live fish were immediately killed by blunt force trauma to the head, gutted by hand, washed with tap water, and then filleted manually with a knife. Only white muscle was used, the muscle was minced using a K600 food processor (Braun GmbH, Germany). Firstly, the mince was washed twice with five volumes of cold water, followed by washing using 0.5% (w/v) NaCl solution with a ratio of solution to mince at 5:1. Secondly, the mince was centrifuged at 1089 g for 30 min to remove the water. After draining off, the moisture content of surimi was adjusted to 80%, and the surimi was stored at 4 °C.

2.3. HIU treatment and preparation of surimi gels with different salt contents

Surimi (150 g) was put in a package as thin as possible and was sealed. Then packaged surimi was treated using a KQ-300DE ultrasonic processor (Kun Shan Ultrasonic Instruments Co. Ltd., Jiangsu, China). During the ultrasonic treatment, packaged surimi was immersed in a 20 °C water bath. Parameters of ultrasonic treatment were set as frequency at 100 kHz, power at 300 W and duration at 10 min. The ultrasound power and intensity were calculated calorimetrically according to the method of Jambrak et al. [22]. In this experiment, the corresponding ultrason intensity was 91 Wcm⁻².

To prepare surimi gels, 150 g of surimi was chopped using K600 food processor at 5000 rpm for 3 min. Then, the surimi was mixed with NaCl (0–5%), followed by being chopped at 5000 rpm for 5 min, the temperature of paste should be kept below 10 °C. After vacuum-pumping, the salted surimi paste was stuffed into polyvinylidene chloride casings (2.5 cm diameter) and both ends were sealed tightly before setting at 40 °C for 60 min followed by cooking at 90 °C for 30 min. After cooking for designated time, surimi gels were expected to be cooled quickly with flowing tap water for about 30 min and stored at 4 °C overnight.

2.4. Determination of puncture properties

Surimi gels were balanced at room temperature about 30 min before testing. Gels were cut into cylinder-shaped with a length of 2.0 cm and then were measured using a TA-XTPlus texture analyzer (Stable Micro System Co. Ltd., Godalming, Surrey, England), equipped with a P/0.25S probe attachment. The breaking force and deformation were recorded when surimi gels were penetrated at a constant speed of 1 mm/s to a compression strain of 50%. All determinations were performed in quintuplicate.

2.5. Determination of microstructures and WHC

For observation of microstructures, gel samples were cut into 2 × 2 × 2 mm and fixed in 2.5% glutaraldehyde in 0.1 M KH₂PO₄ buffer overnight. The samples were dehydrated in 50%, 70%, 80% ethanol for 15 min, respectively. Then dehydration was done using a 90% and 100% ethanol for 10 min, respectively. After that, samples were transferred into tertiary butyl alcohol for 30 min. Samples were freeze-dried using a freeze dryer (FreeZone 4.5 Plus, Labconco, Kansas City, MO, USA) and sputter-coated with 10 nm of gold. Samples were observed at an accelerating voltage of 10 kV and a magnification of 10,000 using a scanning electron microscope (SEM, TM 3000, Hitachi Co., Tokyo, Japan).

To determine the WHC of samples, firstly, surimi gels were cut into pieces with a thickness of 5 mm and weighed as W₁. Secondly, the slice was wrapped with filter paper, followed by pressing using the hardness tester (Kiya Seisakushq Ltd., Tokyo, Japan) with 5 kg force for 3 min, the slice was weighed again as W₂.

\[ \text{WHC} = \frac{W_2 - W_1}{W_1} \times 100\% \]  

2.6. Determination of dynamic rheological properties

Dynamic rheological properties were studied on an AR500 dynamic rheometer (TA Co. Ltd., Manchester, England). Measurements were carried out immediately when surimi pastes were subjected to ultrasonic treatment. A 40 mm parallel steel plate geometry with a 1 mm gap was used. Then, samples were covered by liquid paraffin to avoid evaporation. Samples were heated at a rate of 2 °C/min from 4 to 90 °C. The oscillation stress was 0.1 Pa, and the oscillation frequency was 0.1 Hz. Elastic modulus (G') and phase angle (δ) were recorded [23].

2.7. Determination of total SH content

Samples were prepared according to the method of Fu et al. [12] with some modifications. Eight grams of surimi gels were mixed in 22 mL of 0.2 M Tris-HCl buffer (0.6 M NaCl, 10 mM EDTA, 8 M urea, 2% SDS, pH 7.0). The mixture was dispersed at 5000 rpm for 1 min using a high-speed homogenizer (Model FJ-200, Shanghai Specimen and Models Factory, China). Then, homogenates were centrifuged using a high-speed refrigerated centrifuge (Avanti J-26 XP Centrifuge, Beckman Coulter, Fullerton, CA, USA) at 5000 g for 15 min to remove insoluble protein and only supernatant was remained. The protein concentration of supernatant was determined according to Lowry's method [24]. Protein concentration was adjusted to 1 mg/mL⁻¹ for the determination of total SH content.

For determination of total SH content, to 0.5 mL of protein solution (1 mg/mL⁻¹), 5.0 mL of 0.2 M Tris-HCl buffer (containing 8 M urea, 10 mM EDTA and 2% SDS, pH 8.0) were added. Then the mixture was mixed with 100 μL of Ellman's reagent, and incubating at 40 °C for 25 min [25]. The total SH content was measured at 412 nm using a spectrophotometer (UV-721, Shanghai Precision Instrument Co., Ltd., Shanghai, China) and calculated using a molar extinction coefficient of 13,600 M⁻¹·cm⁻¹ [26].

2.8. Determination of chemical interactions

Solutions for the determination of chemical interactions were 0.05 M NaCl (solution A, Sₐ), 0.6 M NaCl (solution B, S₈), 0.6 M NaCl + 1.5 M urea (solution C, S₉) and 0.6 M NaCl + 8 M urea (solution D, S₊), respectively. Two grams of surimi gels were separately dispersed in the four kinds of solutions (10 mL) and were homogenized. The mixture was incubated at 4 °C for 60 min prior to be centrifuged at 9800 g for 15 min. The protein concentration of supernatant was determined according to Lowry's method [24]. The chemical interactions were calculated by the following Eqs. (2)–(4):
Ionic bonds = c(S_B) - c(S_A)  
Hydrogen bonds = c(S_C) - c(S_B)  
Hydrophobic interactions = c(S_D) - c(S_C)  

where c(S_B) - c(S_A) was the difference in the value of the protein concentration of the S_B and S_A. In addition, c(S_C) - c(S_B) and c(S_D) - c(S_C) were calculated in the same way [27]. The unit of protein concentration was g soluble protein/L of homogenates.

2.9. Determination of solubility and TCA-soluble peptides

Determination of solubility was carried out according to the method of Benjakul et al. [28] with slight modifications. A solution of 20 mM Tris-HCl buffer (pH 8.0), containing 1% (w/v) SDS, 8 M urea and 2% (v/v) β-ME was expected to destroy all the chemical bonds except non-disulfide covalent bonds. Surimi gels (1 g) were dispersed in 20 mL of solution at 4000 rpm for 1 min before heating in the boiling water (100 °C) for 2 min. Then, the mixture was stirred at room temperature for 4 h using a magnetic stirrer (HJ-3, Guohua Instrument Inc., China). The resulting homogenate was centrifuged at 2500 g for 30 min and supernatant was needed. Two milliliters of 50% (w/v) cold TCA were added to 10 mL of supernatant followed by keeping at 4 °C for 18 h before centrifuging at 2500 g for 15 min. The precipitate was washed with 10% (w/v) TCA and then solubilized in 0.5 M NaOH. Protein concentration was determined according to Lowry’s method [24]. Solubility was expressed as percent of the total protein.

For determination of TCA-soluble peptides, 3 g of minced surimi gels were mixed with 27 mL of 5% (w/v) TCA and the mixture was homogenized for 1 min. The homogenate was kept at 4 °C for 60 min before centrifuging at 2500 g for 10 min. The content of peptides in the supernatant was determined according to the Lowry’s method [24].

2.10. SDS-PAGE analysis

To prepare the protein sample, 9 mL of 5% (w/v) SDS was added to 1 g surimi gels, and the mixture was homogenized for 1 min. The homogenate was incubated in 85 °C water bath for 60 min to dissolve total proteins, followed by centrifuging at 9800 g for 20 min, and the supernatant was retained. Protein concentration of the supernatant was determined by Lowry’s method [24] and adjusted to 2 mg/mL. 

The system used for experiment was Mini-Protein Electrophoresis system (Bio-Rad Laboratories, Inc., USA). Firstly, the protein solution (2 mg/mL) was mixed with the loading buffer at 4:1 (protein: buffer), then the mixture was heated at 95 °C for 5 min before loading. Ten microliters of sample were added to each lane. The concentration of separating and stacking gel was 8% and 5%, respectively. At the beginning, voltage was set at 80 V, followed by being elevated to 120 V when the entry of samples was into the stacking gel. Furthermore, the gel was dyed at 37 °C for 90 min in the staining solution (containing 0.125% (w/v) Coomassie brilliant blue R-250), and was destained in the solution containing 25% (v/v) ethyl alcohol and 8% (v/v) acetic acid until the appearance of clear protein bands.

2.11. Statistical analysis

All experiments were carried out three times and were presented as mean ± SD. SPSS 22.0 Software (SPSS Institute Inc., Chicago, USA) and Origin 9.0 were used for statistical analysis of data. Significant differences between means were set as P < 0.05 by Duncan’s multiple range testing.

3. Results and discussion

3.1. Effect of HIU on puncture properties of surimi gels

Fig. 1 illustrates the breaking force and deformation of surimi gels with different salt contents. Bars represent the standard deviation from triplicate determination. Different lowercases in the same species indicate significant differences among different salt contents (P < 0.05). Different capitals indicate significant differences between HIU and control samples (P < 0.05).

Fig. 1. Effect of HIU on the breaking force (A) and deformation (B) of surimi gels with different salt contents. Bars represent the standard deviation from triplicate determination. Different lowercases in the same species indicate significant differences among different salt contents (P < 0.05). Different capitals indicate significant differences between HIU and control samples (P < 0.05).

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As shown in Fig. 1, HIU increased the breaking force and deformation of surimi gels with ≤ 3% NaCl. However, a reversible tendency was observed in the samples with > 3% NaCl. At low-salt content, myosin presented in the form of thick filaments [2]. Our previous study showed that HIU could break myosin assemblies into...
smaller aggregates, leading to a good dispersion of proteins [13]. Well dispersion of myosin could facilitate exposure of reactive groups and the intermolecular interactions during heating, furthermore was beneficial for improving the puncture properties of surimi gels [32]. Interestingly, breaking force of HIU surimi gel with 1% salt was even higher than that of the control sample with 2% salt. Hu et al. [33] also demonstrated that HIU improved puncture properties of soy protein isolate gels. However, myosin could be degraded into peptides by HIU with > 150 W [13]. Since myosin dissolved/dispersed well at 4% and 5% NaCl, HIU energy might mainly cause the degradation of myosin. Therefore, the decrease in breaking force and deformation of HIU surimi gels was observed at high-salt content (> 3% NaCl) compared with the corresponding control samples.

3.2. Effect of HIU on the microstructures and WHC of surimi gels

The microstructures of samples were observed by SEM (Fig. 2). Surimi gels with 0% and 1% salt presented non-uniform microstructures with large pores (Fig. 2A and B). This was consistent with its poor puncture properties (Fig. 1). As the salt content increased, the microstructures of surimi gels became more uniform and compact with smaller pores. Myosin, salt-soluble protein, plays a key role in surimi gelling. In low-salt environment, myosin could not fully dissolve and assemble into thick filaments, and thus formed a coarser gel network during heat-induced gelling. As the salt content increased, the compact gel network might be closely related to good dissolution of myosin and formation of more chemical bonds. As shown in Fig. 2, HIU obviously improved the microstructures of surimi gels with < 3% salt, particularly the sample with 1% salt. Microstructures of HIU surimi gel with 1% salt were more compact than those of the control sample with 2% salt. At low-salt content (< 3% NaCl), HIU mainly dissociated the myosin assemblies, leading to good dispersion and the formation of a compact structure. It was also reported that HIU induced casein to form a compact and uniform gel network [34]. However, HIU hardly improved microstructures of surimi gels with > 3% salt.

Fig. 3 shows WHC of surimi gels with different salt contents. For the samples without HIU, the WHC of surimi gels rapidly increased from 85.02 to 93.63% as the salt content increased from 0 to 2%, followed by a flat up to 5% salt. NaCl could shield surface charges of myosin molecules and reduce the electrostatic attractions between proteins, increasing protein-water interactions [35]. However, myosin at > 3% NaCl was most likely to fully dissolve, this might contribute to the

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Fig. 2. SEM micrographs at 10,000× of control and HIU surimi gels with different salt contents. A-F indicate the control samples with the salt contents of 0%, 1%, 2%, 3%, 4%, 5%. A’-F’ indicate the HIU samples with the salt contents of 0%, 1%, 2%, 3%, 4%, 5%.

Fig. 3. Effect of HIU on the WHC of surimi gels with different salt contents. Bars represent the standard deviation from triplicate determination. Different lowercases in the same species indicate significant differences among different salt contents (P < 0.05). Different capitals indicate significant differences between HIU and control samples (P < 0.05).
relative stable WHC. In addition, compact microstructures of surimi gels were beneficial to trapping more water (Fig. 2) and improving WHC of surimi gels. HIU surimi gels showed significantly higher WHC than the corresponding control samples ($P < 0.05$), especially samples with $\leq 1\%$ salt. Moreover, WHC of HIU surimi gel with $1\%$ salt was higher than that of the control sample with $2\%$ salt. Similarly, Rienier et al. [36] reported that HIU could improve the WHC of yoghurt.

3.3. Effect of HIU on the dynamic rheological properties of surimi gels

Fig. 4 shows the thermal gelation profiles of salted surimi in terms of G’ and $\delta$ formed in the range of 4–90 °C. For samples with 2–5% salt, the G’ increased continuously from 4 °C and reached the 1st peak at approximately 30 °C. This could be related to the protein interactions that occurred in low temperature and the formation of preliminary gel network by weak bonds [20]. Thereafter, G’ rapidly decreased and the minimum values were obtained at around 55 °C. This was closely related to modori phenomenon due mainly to the degradation of preliminary gel network by endogenous protease. The enzyme proteolytic occurred in the temperature range of 55–60 °C [37]. Besides, it could also be due to the heat-induced partial rupture of hydrogen bonds and the dissociation of myosin and actin [3,20]. Above 55 °C, the increased G’ and decreased $\delta$ values suggested the further gelation. The $\delta$ value showed the contrary tendency to the G’ value. For the low-salt (0–1%) samples without HIU, the G’ values were obviously higher than those of the samples with 2–5% salt at 4 °C. Myosin usually forms filaments by hydrogen bonds and ionic bonds in low-salt environment [2], which could be supported by the low $\delta$ value. The decreased G’ in the range of 4–43 °C might originate from the destroyed hydrogen bonds and ionic bonds by heating. As the temperature further increased, the G’ profiles were similar to those of the samples with 2–5% salt. In order to state the details clearly in the profiles of G’, the key points are listed in the Table 1. Accordingly, the G’ of low-salt samples (0–1%) started to increase above 43 °C, while this occurred at 4 °C in samples with 2–5% salt. It was presumed that some reactive groups were buried in myosin filaments in low-salt (0–1%) environment, which was not beneficial for the protein interactions. As the temperature increased to above 43 °C, hydrogen bonds and ionic bonds were disrupted, and some exposed reactive groups interacted with each other. Thereafter, the G’ value started to increase above 43 °C. Increasing salt content to above 2%, good dissolution of myosin was beneficial to intermolecular interactions which increased the G’ values. At the end of heating, the highest G’ value was obtained for sample with 5% salt.

For the samples with 0–1% salt, the temperature where G’ initially started to increase shifted to lower point as a function of HIU, which was related to good dissolution of myosin. It was possible that HIU could promote the dispersion of myosin, which was beneficial for myosin unfolding and exposure of reactive groups. Moreover, HIU increased the G’e values (G’ value at the end of heating) of samples with $\geq 3\%$ salt, but decreased the G’e values of samples with 4% and 5% salt. This phenomenon was consistent with the results of the puncture properties (Fig. 1). In low-salt environment, myosin

Table 1

| Salt (%) | $G'_0$/Pa | $T_s$/°C | $T_1$/°C | $G'_{1}$/Pa | $T_2$/°C | $G'_{2}$/Pa | $G'$e/Pa |
|---------|------------|----------|----------|-------------|----------|------------|----------|
| Control 0% | 4497.0 | 45.7 | 47.2 | 1757.0 | 57.0 | 1572.0 | 2967.0 |
| 1% | 3949.0 | 43.4 | 46.4 | 3301.0 | 55.5 | 873.8 | 2226.0 |
| 2% | 3422.0 | 4.0 | 24.4 | 4481.0 | 54.7 | 1003.0 | 2793.0 |
| 3% | 2659.0 | 4.0 | 26.7 | 4490.0 | 55.5 | 854.5 | 2447.0 |
| 4% | 1740.0 | 4.0 | 32.0 | 4723.0 | 55.5 | 1043.0 | 2524.0 |
| 5% | 3312.0 | 4.0 | 25.0 | 4683.0 | 54.7 | 1226.0 | 4263.0 |
| HIU 0% | 5148.0 | 44.1 | 47.2 | 2177.0 | 57.0 | 2111.0 | 4145.0 |
| 1% | 3016.0 | 42.6 | 46.4 | 3007.0 | 54.7 | 989.2 | 2739.0 |
| 2% | 3151.0 | 4.0 | 26.7 | 3887.0 | 53.2 | 1167.0 | 3320.0 |
| 3% | 3337.0 | 4.0 | 25.9 | 4631.0 | 54.0 | 835.6 | 2358.0 |
| 4% | 2954.0 | 4.0 | 27.4 | 4259.0 | 54.7 | 898.8 | 2093.0 |
| 5% | 3568.0 | 4.0 | 27.4 | 8163.0 | 55.5 | 2107.0 | 4123.0 |

Note: $G'_0$ represents the G’ value at the beginning of heating, $T_s$ represents the temperature where G’ started to increase, $T_1$ represents the temperature of the first peak, $G'_{1}$ represents the G’ value of the first peak, $T_2$ represents the temperature at the end of modori, $G'_{2}$ represents the G’ value at the end of modori, $G'$e represents the G’ value at the end of heating.
3.4. Effect of HIU on protein conformation and intermolecular interactions

Spontaneously assembled into thick filaments prior to heating, and HIU mainly disrupted the assemblies, leading to the good dispersion. However, myosin could well disperse and form smaller assemblies in high-salt environment than in low-salt environment [2]. It was presumed that less energy was needed to disrupt myosin assemblies in high-salt environment. Thus, part of HIU energy caused the degradation of myosin molecules before heating, subsequently decreased the $G'_0$ values.

3.4.1. Effect of HIU on total SH content of surimi gels

Fig. 5 exhibits the total SH content of surimi gels with different salt contents. For the samples without HIU, the total SH content attained a minimum at 5% NaCl. The lower SH content indicated the formation of more disulfide bonds. Namely, as the salt content increased, myosin gradually dissolved and more disulfide bonds were formed during heat-induced gelling. This result was in great agreement to the report on cod protein that the protein molecules tended to aggregate linked by disulfide bonds in high-salt environment [38].

It can also be observed that HIU decreased the total SH content ($P < 0.05$) of surimi gel with 1% salt compared with the control sample, this might be ascribed to protein oxidation or aggregation under ultrasound power. This was consistent with the report that HIU promoted oxidation of SH groups to S-S bonds in bovine serum albumin [39]. However, HIU increased the total SH content of samples with 3% and 5% salt. It was speculated that HIU disrupted the disulfide bonds of dissolved proteins at high-salt content, causing the reduction of S-S bonds and the increase in total SH content. But further research was required to support this hypothesis.

3.4.2. Effect of HIU on chemical interactions of surimi gels

Table 2 shows the chemical interactions of surimi gels with different salt contents. For the samples without HIU, ionic bonds of samples with 1% NaCl were significantly higher than those of the samples with 3% or 5% salt ($P < 0.05$), suggesting a decrease of ionic bonds with increasing salt content. Additionally, hydrophobic interactions of surimi gel with 5% salt were higher than those of the samples with 1% or 3% salt ($P < 0.05$). In low-salt environment (1%), myosin spontaneously assembled into filaments through ionic bonds and hydrogen bonds [2]. As the salt content increased, ions (Na$^+$, Cl$^-$) might interact with surface opposite charges (COO$^-$, NH$_2^+$) of myosin molecules and break ionic bonds. Myosin filaments became dissociated and extended, thus more hydrophobic groups were exposed to the surface. Subsequently, hydrophobic interactions were strengthened during heat-induced gelling. Also, $G'_0$ value of surimi with 5% salt was the highest among all samples (Fig. 4A). Disulfide bonds and hydrophobic interactions contributed to high breaking force and deformation of surimi gels as the salt content increased (Fig. 1).

HIU increased ionic bonds and hydrogen bonds of surimi gels with 1% NaCl, which might originate from the high temperature and high pressure induced by cavitation phenomenon. This could be reflected by the similar beginning $G'_0$ values of samples after HIU (Fig. 4A). In addition, HIU increased hydrophobic interactions of surimi gels with 1% and 3% NaCl. Hydrophobic interactions were important in maintaining the gel network. This explained higher puncture properties of low-salt surimi gels after HIU (Fig. 1).

3.4.3. Effect of HIU on solubility and TCA-soluble peptides of surimi gels

Solubility of surimi gels in solution (including 1% (w/v) SDS, 8 M urea and 2% (v/v) β-ME) is shown in Fig. 6A. The solution could destroy all chemical bonds except for non-disulfide covalent bonds. For the samples without HIU, solubility of surimi gels with 3% or 5% NaCl was significantly lower than that of the sample with 1% NaCl ($P < 0.05$). It was suggested that the formation of more non-disulfide covalent bonds in the samples with 3% or 5% NaCl. Non-disulfide covalent bonds (particularly ε-(γ-glutamyl) lysine linkage) were mainly catalyzed by transglutaminase [40]. There was endogenous transglutaminase in silver carp surimi [41]. Meanwhile, the rate of catalyzing largely depended on transglutaminase activity and myosin conformation [42]. More non-disulfide covalent bonds in surimi gels with 3% or 5% salt possibly attributed to the appropriate conformation of myosin molecules under high-salt content. Compared with the control samples, HIU significantly promoted the formation of non-disulfide covalent bonds of surimi gels suggested by the decreased solubility ($P < 0.05$). It was possible that HIU increased endogenous transglutaminase activity or changed myosin structure into appropriate conformation for transglutaminase action. However, further research was required to support this hypothesis.

TCA-soluble peptides of surimi gels with different salt contents are depicted in Fig. 6B. For the samples without HIU, the highest TCA-soluble peptides were observed at 1% NaCl, suggesting the pronounced degradation of proteins. This was one of reasons for the lower breaking force of surimi gels with 1% NaCl (Fig. 1A). There were endogenous

| Salt contents (%) | Samples | Ionic bonds | Hydrogen bonds | Hydrophobic interactions |
|-------------------|---------|-------------|----------------|-------------------------|
| 1                 | Control | 0.10 ± 0.04$^{bc}$ | 1.04 ± 0.04$^{bc}$ | 0.55 ± 0.05$^{bc}$ |
|                   | HIU     | 0.06 ± 0.01$^{bc}$ | 0.84 ± 0.02$^{bc}$ | 0.92 ± 0.03$^{bc}$ |
| 3                 | Control | 0.03 ± 0.00$^{bc}$ | 1.21 ± 0.07$^{bc}$ | 0.56 ± 0.06$^{bc}$ |
|                   | HIU     | 0.03 ± 0.03$^{bc}$ | 0.81 ± 0.03$^{bc}$ | 0.80 ± 0.08$^{bc}$ |
| 5                 | Control | 0.03 ± 0.02$^{bc}$ | 0.78 ± 0.04$^{bc}$ | 1.03 ± 0.03$^{bc}$ |
|                   | HIU     | 0.04 ± 0.01$^{bc}$ | 1.05 ± 0.02$^{bc}$ | 1.03 ± 0.03$^{bc}$ |

Note: Different lowercases in the same column indicate significant differences between different salt contents (1%, 3%, 5%) for the same treatment (control or HIU) ($P < 0.05$). Different capitals indicate significant differences between HIU and control samples for the same salt content (1% or 3% or 5%) ($P < 0.05$). The unit of numbers is g/L.
proteases in surimi which could degrade proteins into peptides during heat-induced gelling, and activity of the proteases was higher in low-salt environment [43]. Lower TCA-soluble peptides of surimi gels with 3% or 5% NaCl were closely related to the low activity of endogenous proteases. Similar finding was reported in white croaker that 3% NaCl suppressed the endogenous protease activity [43]. Furthermore, comparing with the controlsamples, HIU decreased TCA-soluble peptides of surimi gel with 1% NaCl, indicating a depressing degradation of proteins. It was also reported that HIU of 100 W for 30 min passivated the activities of endogenous proteinase in tilapia surimi [21]. However, HIU increased TCA-soluble peptides of surimi gel with 5% salt. According to the report by Liu et al. [13], HIU caused the degradation of myosin.

3.4.4. Effect of HIU on protein patterns of surimi gels

Fig. 7 shows the electrophoresis image of surimi or gels with different treatments. The main components in surimi were myosin heavy chains (MHC) and actin (AC). For all gel samples, the MHC bands were thinner than surimi samples. In addition, there were new larger-molecular bands above MHC bands in gel samples, verifying the protein aggregation by non-disulfide covalent bonds. For the samples without HIU, the larger-molecular bands were gradually thicker with increasing salt content. This was consistent with the solubility. Comparing with the control gel sample, HIU decreased the MHC content of gel sample with 1% salt evidenced by the thinner MHC bands. As mentioned above, it was speculated that HIU might activate the endogenous transglutaminase, however, this hypothesis needed further research. For HIU gel samples with 3% and 5% salt, some bands slightly became thicker between MHC and actin, as compared to the corresponding control gels, indicating the protein degradation by ultrasound intensity.

4. Conclusions

For the samples without HIU, the breaking force and deformation increased as the salt content increased from 0 to 5%. The effect of HIU on gelation properties depended on salt contents. In low-salt (0–2% NaCl) environment, HIU promoted dispersion and unfolding of proteins. These changes facilitated the formation of disulfide bonds, hydrophobic interactions and non-disulfide covalent bonds, characterized by the decreased total SH content and solubility. Therefore, higher puncture properties and WHC were obtained after ultrasound treatment. Meanwhile, more uniform and compact microstructures were observed for low-salt (0–2% NaCl) samples with HIU. HIU also improved gelation properties of surimi with 3% NaCl. For high-salt (4–5% NaCl) samples, HIU also improved the WHC, but decreased the breaking force and deformation. This phenomenon was closely related with protein degradation as a function of HIU, indicated by TCA-soluble peptides analysis of high-salt samples. In conclusion, HIU significantly improved gelation properties of low-salt surimi by promoting the dispersion of proteins and intermolecular interactions. However, HIU was harmful for high-salt surimi gels due to the protein degradation. This could provide data basis for the production of surimi gels with low-salt content.

CRediT authorship contribution statement

Xia Gao: Software, Formal analysis, Writing - original draft, Writing - review & editing. Yaru Xie: Methodology, Data curation, Investigation. Tao Yin: Methodology. Yang Hu: Validation. Juan You: Visualization. Shanbai Xiong: Resources. Ru Liu: Conceptualization, Writing - review & editing, Supervision, Project administration.

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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