



# Ultrasound-assisted processing: Changes in gel properties, water-holding capacity, and protein aggregation of low-salt *Hypophthalmichthys molitrix* surimi by soy protein isolate

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## ABSTRACT

The effects of ultrasound combined (25 kHz,  $400 \pm 20$  W/L, ultrasonic time of 5, 10 and 15 min) with soy protein isolate processing on gelling properties of low-salt silver carp surimi, aggregation and conformation of myofibrillar protein were investigated. The results revealed that, compared with only adding soy protein isolate components, ultrasound-assisted soy protein isolate had a more obvious effect on the protein structure in low-salt surimi, leading to the decrease in  $\alpha$ -helix and total sulfhydryl contents, and the increase in  $\beta$ -sheet content and protein solubility. As a result, more proteins participated in the formation of the gel network, and significant improvements in hardness, gel strength and water-holding capacity of the low-salt surimi gel were observed, while the myosin heavy chain in SDS-PAGE was weakened. The low-field NMR results showed that the initial relaxation time of  $T_2$  was apparently shorter, the free water content decreased and the bound water content increased under the action of ultrasound. Scanning electron microscope observation found that the surimi gel treated by ultrasound exhibited smaller holes, and had a more stable and denser network structure. In conclusion, the results of our work demonstrated that ultrasound combined with soy protein isolate can significantly improve the gel quality properties of low-salt silver carp.

## 1. Introduction

With the advantages of rich nutritional value, void of bone spurs, and convenience to the process, surimi has gradually become an essential part of the aquatic processing field and is popular in the processing industry [1–3]. However, the temperature changes of surimi in the process of production, processing, and transportation cause physical damage to surimi, resulting in the poor gelling performance of surimi and the inability to form a dense gel network structure [4]. Therefore, the gel formation usually requires the addition of 2 %–3% sodium chloride during processing to promote the dissolution and swelling of myofibrillar proteins for good texture and flavor [5,6]. Excessive sodium chloride intake may increase the incidence of hypertension and cardiovascular disease [7,8], while direct reduction of sodium chloride also reduces gel product quality [9]. In addition, as plummet marine catches and calls for “low carbon” living growth, the surimi industry is struggling to find more efficient surimi processing techniques to increase

surimi yields and reduce fisheries catches [10,11]. Therefore, minimizing the added amount of sodium chloride in the product and maintain the product quality to meet the growing demand of consumers for a healthy diet has become one of the difficulties in the research of the meat product processing industry.

In recent years, some strategies have been adopted to enhance the quality characteristics of salt-reduced surimi gels, mainly including salt substitutes (such as *L*-arginine, *L*-lysine, etc.), non-thermal processing technologies (microwave, ultrasonic, ultra-high pressure, high-density  $\text{CO}_2$ , etc.) and exogenous additives (hydrophilic colloid, plant proteins, enzymes, etc.) [12–17]. Among these methods, the exogenous addition is popular among food processors because of its simplicity and effectiveness. However, unpleasant flavors might be developed in correspondence with the addition of salt substitutes or textural modifiers [16]. Therefore, the combination of two or more modification methods has attracted more and more attention from researchers.

SPI as the ingredient is widely used in meat products due to it has

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**Table 1**

The formulas (%) of silver carp surimi using different treatments.

Content/ Sample	NaCl	SPI	Ultrasonic treatment time (min)	Ultrasonic power (W)	Ultrasonic frequency (kHz)
Control	2.0	0	/	/	/
L-2.0 %	1.0	2.0	/	/	/
L-2.0 %-U <sub>5</sub>	1.0	2.0	5	400	25
L-2.0 %-U <sub>10</sub>	1.0	2.0	10	400	25
L-2.0 %-U <sub>15</sub>	1.0	2.0	15	400	25
L-4.0 %	1.0	4.0	/	/	/
L-4.0 %-U <sub>5</sub>	1.0	4.0	5	400	25
L-4.0 %-U <sub>10</sub>	1.0	4.0	10	400	25
L-4.0 %-U <sub>15</sub>	1.0	4.0	15	400	25

moisture adsorption and machining process of moisture keeping ability [18]. At present, physical and chemical methods are used to modify most SPI to improve their functional properties. At present, physical and chemical methods are used to modify most SPI to improve their functional properties and application in food. As a non-thermal, green technique, the application of ultrasound is attracting more and more attention recently. Hu et al. [19] found the release of more hydrophobic residues and the prolonged exposure time of binding sites after ultrasound pretreatment provided more chance for self-assembly of SPI. Wang et al. [20] found that suitable HIU treatment can unfold the protein structure in the SPI-PC complex and improve its solubility and stability. At the same time, ultrasound is also used as an important way to improve the quality of meat products. Xing et al. [21] reported that high-intensity ultrasonic treatment promoted the transformation of  $\alpha$ -helical to random coil when the salt addition amount was 1 %, and effectively improved the water holding capacity and thermal stability of chicken wood breast. Zhang et al. [22] and Gao et al. [23] found that ultrasound-based treatments can promote protein cross-linking by activating endogenous glutamine transaminase and protease in surimi, forming more non-covalent and disulfide bonds, which significantly improving the water holding capacity, gel strength, and the microstructure of surimi gels. However, there is little information on the effect of combined treatment of ultrasound processing and other substance to improve the gel texture and quality of low-salt surimi gel products.

Therefore, this study took low-salt silver carp surimi as the research object to explore the effect of ultrasound and SPI addition on physicochemical, structural, and protein structure of low-salt surimi gels from *Hypophthalmichthys molitrix*, in order to identify an appropriate ultrasonication treatment time and SPI addition that can facilitate applications of high-intensity ultrasonication and SPI in low-salt food systems.

## 2. Materials and methods

### 2.1. Materials

Silver carp surimi (*Hypophthalmichthys molitrix*, moisture content was 75.10 % (w/w), crude protein contents of 14.37 %), supplied by Jingli Aquatic Food Co., Ltd. (Hubei, China) and frozen at  $-18^{\circ}\text{C}$  and used within two months. SPI was bought from Shanghai Maclin Biochemical Co., Ltd (Shanghai, China). All chemical reagents were analytical grade, purchased from Shanghai Yuan Ye Bio-Technology Co., Ltd (Shanghai, China).

### 2.2. Pretreatment of surimi

Frozen surimi was thawed overnight in a  $4^{\circ}\text{C}$  refrigerator before the experiment. The surimi was cut into small pieces and placed in a meat grinder equipped with a 6 mm orifice plate for 1 min under an ice-water

bath at a speed of 4,000 r/min. Subsequently, the different contents of sodium chloride and SPI were added to the surimi according to the formula in Table 1, and adjusted the moisture content to 78 % w/w with pre-cooled ice water. The mixture was chopped at 4,000 rpm (ran for 30 s and rested for 10 s) for 2 min. The obtained surimi was placed in a 25 mm diameter Nylon casing (Changzhiyuan Electronic Commerce Co. Ltd., Dalian, China) with a standard of 60 g/bag and maximum thickness of 25 mm for subsequent use.

### 2.3. Ultrasonic treatment and preparation of low-salt surimi gels

For samples that received ultrasound treatment, the prepared surimi samples were placed in the bottom of a 2 L double-layer glass beaker, filled to 1 L with water. The SX-2008 ultrasound processor (Shanghai SX Instruments Co., Ltd) equipped with a 1.0 cm (diameter) titanium probe was immersed in the solution to a depth of 2 cm from the bottom, followed by treatment with ultrasound at 25 kHz,  $400 \pm 20$  W/L, for 5, 10 and 15 min respectively. For maintaining the temperature of the sample was always below  $10^{\circ}\text{C}$ , a cryostat (SX-1006, Shanghai SX Instrument Co., Ltd) was connected to a double-layer beaker with circulating ice water during the ultrasound process.

The surimi gel was formed by two-stage heating with heating conditions set at  $40^{\circ}\text{C}$  for 40 min and  $90^{\circ}\text{C}$  for 30 min. After heat treatment, the gels were immediately placed in ice water to cool and then in a refrigerator at  $4^{\circ}\text{C}$  overnight.

### 2.4. Determination of color

The overnight surimi gel was cut into cylinders with a height of 2.5 cm, and white calibration was performed before measuring the color using a portable colorimeter (CR-400, Konica Minolta, Japan). The values of  $L^*$ ,  $a^*$ , and  $b^*$  for fresh-cut surimi gel were obtained, the whiteness and total color difference (TCD) were calculated based on the following Eqs. (1) and (2).

$$\text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + (a^*)^2 + (b^*)^2} \quad (1)$$

$$\text{TCD} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

In the formula:  $\Delta L^* = L^* - L_{\text{control}}$ ,  $\Delta a^* = a^* - a_{\text{control}}$ ,  $\Delta b^* = b^* - b_{\text{control}}$ .

### 2.5. Texture profile analysis (TPA)

The surimi gels stored overnight were kept at room temperature ( $25 \pm 1^{\circ}\text{C}$ ) for 1 h before the test. And then, the TPA of gels (diameter 2.5 cm and height 2.5 cm) was measured by a TA-XT.plus texture analyzer with a P/50 probe (Stable Microsystem Ltd., Surry, UK). The parameters were as follows: pre-test speed,  $2.0 \text{ mm}\cdot\text{s}^{-1}$ ; test speed,  $2.0 \text{ mm}\cdot\text{s}^{-1}$ ; post-test speed,  $2.0 \text{ mm}\cdot\text{s}^{-1}$ ; trigger type 5 g, and the compression ratio was 50 %.

### 2.6. Gel strength

The gel strength of the surimi gels was determined using the protocol outlined in He et al. [24] with some modifications. The breaking force and distance of gels were performed by using a TA-XT. Plus texture analyzer (Stable Microsystem Ltd., Surry, UK) with a P/5S spherical plunger. The specific parameters were set as follows: the descending distance is 12 mm, and the speeds before and after the test are 2 mm/min, 1 mm/min, and 2 mm/min, respectively. The gel strength of surimi gels was calculated by the following Formula (3):

$$\text{Gel strength}(\text{g}\cdot\text{cm}) = \text{Breaking force (g)} \times \text{Deformation (cm)} \quad (3)$$

## 2.7. Water-holding capacity

Water-holding capacity (WHC) was measured based on Zhuang et al. [25] with slight modifications. Samples (approximately 3.0 g) were transferred into a 50 mL centrifuge tube and centrifuged at  $12,000 \times g$  for 10 min at  $4^\circ\text{C}$ . Three layers of filter paper were inserted underneath for water absorption. WHC (%) was expressed as the weight after centrifugation divided by the weight before centrifugation as Eq. (4):

$$\text{WHC (\%)} = m_2 / m_1 \times 100\% \quad (4)$$

In the formula:  $m_1$  is the weight of the gel sample before centrifugation, g;  $m_2$  is the weight of the gel sample after centrifugation, g.

## 2.8. Low field NMR (LF-NMR)

Water distribution of surimi gels was determined by MesoMR23-060H-I low-field nuclear magnetic resonance apparatus (Niumag Co., Ltd., Shanghai, China) as the previous method [17] with minor modifications. The surimi gel with a fixed size (2.5 cm diameter, 2.5 cm height) was placed in a nuclear magnetic tube to measure the water distribution state and relaxation time. A proton resonance frequency of 21 MHz at  $32^\circ\text{C}$  and the data from 8,000 echoes were acquired as 8 scan repetitions.

## 2.9. Magnetic resonance imaging (MRI) measurements

The MRI of surimi gels was obtained according to the previous method of Traffano-Schiffo et al. [26] with a slight modification. The gels were cut into cylinders (diameter 2.5 cm, height 2.0 cm) and put into an MRI tube. The false-color map of water proton density was obtained using a false color processing image software. The main parameters are echo time (TE) and repetition time (TR) of 18.2 ms and 500 ms, respectively.

## 2.10. Microstructure of surimi gels

The microstructure of the surimi gels was observed using a SU5000 thermal field emission scanning electron microscope (SEM, Hitachi High-tech, Inc., Japan). The surimi gel was cut into small pieces ( $5 \times 5 \times 1 \text{ mm}^3$ ), fixed with 2.5 % (v/v) glutaraldehyde in 0.1 M SPB buffer (pH 7.0) for 12 h, and followed by rinsing three times with 0.1 M SPB buffer (pH 7.0). Fixed specimens were dehydrated with different

concentrations of ethanol (60 %, 70 %, 80 %, 90 %, 100 %, v/v), tert-butanol was used for degreasing, and finally, the samples were obtained by vacuum drying. The freeze-dried surimi samples were transferred to a scanning electron microscope and sputtered with gold to observe the microstructure. The magnification level was set to  $\times 10,000$ , and the corresponding figures were captured using the software equipped with SEM.

## 2.11. Fourier transform infrared spectrum (FT-IR)

The raw surimi samples were lyophilized under a vacuum for 48 h, taken out and crushed, and used a Fourier transform mid-infrared microchemical imaging system. Attenuated total reflection mode for secondary structure analysis. The spectral scanning range is  $600 \text{ cm}^{-1}$ – $4,000 \text{ cm}^{-1}$ , the scanning frequency is 16, and the resolution is  $4 \text{ cm}^{-1}$ . According to the method of Zhang et al. [27] with slight modification, the amide I band ( $1,600$ – $1,700 \text{ cm}^{-1}$ ) is deconvolution and Gaussian fitting calculation using Peakfit v 4.12 software. As a result, the relative contents of  $\beta$ -sheets ( $1,610$ – $1,640 \text{ cm}^{-1}$  and  $1,670$ – $1,680 \text{ cm}^{-1}$ ), random coils ( $1,640$ – $1,650 \text{ cm}^{-1}$ ),  $\alpha$ -helices ( $1,650$ – $1,660 \text{ cm}^{-1}$ ) and  $\beta$ -turns ( $1,680$ – $1,700 \text{ cm}^{-1}$ ) of the surimi protein secondary structure were calculated.

## 2.12. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

The protein content of surimi gels eluted in 10 % (w/w) SDS was determined by SDS-PAGE using precast polyacrylamide gels (5 % to 20 %, Sobaco) according to the method reported by Laemmli et al. [28] and Petcharat et al. [5]. The supernatant extracted from each treatment group was adjusted to 2 mg/mL, then an 8  $\mu\text{L}$  sample was loaded onto a polypropylene gel. In brief, about 3.0 g gel sample was evenly distributed in 27 mL 10 % (w/w) SDS solution, and then heated at  $85^\circ\text{C}$  for 30 min. The mixture was centrifuged at  $10,000 \times g$  for 20 min, and the supernatant was retained. The protein composition was performed on a precast polyacrylamide gel (5 % ~ 20 %, Sobaco), and the protein sample was loaded into 10  $\mu\text{L}$ . Separating gels were run at 80 V and stacking gels were run at 120 V using a Mini-Protein Electrophoresis System (Bio-Rad Laboratories Inc., Hercules, CA, USA). Imaging was performed after overnight destaining with Coomassie brilliant blue R-250.

## 2.13. Salt-soluble proteins

The solubility of surimi salt-soluble proteins was determined following a method previously published by Cofrades et al. [29] with slight modifications. The surimi samples were homogenized in five volumes (w/v) of pre-chilled sodium phosphate buffer (20 mM, pH 7.0) using a homogenizer (AD200L-P homogenizer, Angni Instrument Co., Ltd., Shanghai, China) for 90 s, and then centrifuged (H-2050R, Xiangyi Laboratory Instrument Development Co., Ltd., China.) at  $12,000 \times g$  for 30 min. The protein solubility in the supernatant of each group was determined according to Lowry et al. [30] using the standard curve of bovine serum albumin and expressed as the percentage.

## 2.14. Total sulfhydryl (SH) content

The total SH content was determined using a mercapto assay kit (Solarbio, Beijing, China, product number: BC1375) according to the method of Zhang et al. [27]. The raw surimi (0.5 g) was mixed with 5 mL of the extract and then mixed with the reaction reagents as required and reacted for 10 min. Then, the absorbance was measured by UV spectrophotometer at 412 nm (UV-1800PC Mapada Instrument Co., Ltd., Shanghai, China.). The total SH content was calculated according to the Formula:

**Table 2**

Effect of ultrasonic treatments on gel color of surimi with different salt and soy protein isolate content.

Sample	$L^*$	$a^*$	$b^*$	Whiteness	TCD
Control	$75.48 \pm 1.03^{ab}$	$-1.18 \pm 0.24^c$	$5.77 \pm 0.8^c$	$74.76 \pm 0.86^a$	–
L-2.0 %SPI	$75.33 \pm 0.38^{Aab}$	$-0.83 \pm 0.17^{Ab}$	$7.43 \pm 0.56^{Ab}$	$74.22 \pm 0.26^{Abc}$	$1.71 \pm 0.45^{Ab}$
L-2.0 % SPI-U <sub>5</sub>	$75.58 \pm 0.25^{Aab}$	$-0.88 \pm 0.09^{Ab}$	$7.46 \pm 0.25^{Ab}$	$74.52 \pm 0.18^{Aab}$	$1.75 \pm 0.22^{Ab}$
L-2.0 % SPI-U <sub>10</sub>	$75.72 \pm 0.62^{Aab}$	$-0.87 \pm 0.07^{Ab}$	$7.63 \pm 0.14^{Ab}$	$74.53 \pm 0.59^{Aab}$	$2.02 \pm 0.14^{Ab}$
L-2.0 % SPI-U <sub>15</sub>	$75.73 \pm 0.42^{Aa}$	$-0.84 \pm 0.11^{Ab}$	$7.46 \pm 0.27^{Ab}$	$74.60 \pm 0.35^{Aab}$	$1.72 \pm 0.35^{Ab}$
L-4.0 %SPI	$75.54 \pm 0.36^{Aab}$	$-0.40 \pm 0.04^{Aa}$	$8.92 \pm 0.47^{Aa}$	$73.96 \pm 0.33^{Acd}$	$3.32 \pm 0.48^{Aa}$
L-4.0 % SPI-U <sub>5</sub>	$75.16 \pm 0.37^{Ab}$	$-0.41 \pm 0.08^{Aa}$	$9.04 \pm 0.30^{Aa}$	$73.56 \pm 0.42^{Ad}$	$3.44 \pm 0.33^{Aa}$
L-4.0 % SPI-U <sub>10</sub>	$75.27 \pm 0.45^{Aab}$	$-0.41 \pm 0.14^{Aa}$	$8.99 \pm 0.36^{Aa}$	$73.68 \pm 0.43^{Ad}$	$3.39 \pm 0.37^{Aa}$
L-4.0 % SPI-U <sub>15</sub>	$75.17 \pm 0.42^{Aab}$	$-0.43 \pm 0.16^{Aa}$	$8.98 \pm 0.39^{Aa}$	$73.59 \pm 0.52^{Ad}$	$3.41 \pm 0.49^{Aa}$

Different lower case letters in the same column mean significant difference ( $p < 0.05$ ). Different capital letters indicate significant differences in the duration of the high-intensity ultrasound ( $p < 0.05$ ).

**Table 3**

Effects of ultrasonic treatment combined with soy protein isolate on gel texture characteristics of low-salt surimi.

Sample	Hardness (g)	Springiness	Cohesiveness	Chewiness (g·mm)
Control	4840.68 ± 156.68 <sup>f</sup>	0.92 ± 0.01 <sup>a</sup>	0.79 ± 0.01 <sup>a</sup>	3507.98 ± 111.79 <sup>f</sup>
L-2.0 %SPI	5786.35 ± 229.85 <sup>ce</sup>	0.93 ± 0.01 <sup>Ca</sup>	0.76 ± 0.01 <sup>Ab</sup>	4048.00 ± 165.28 <sup>ce</sup>
L-2.0 % SPI-U <sub>5</sub>	5990.50 ± 270.13 <sup>BCde</sup>	0.92 ± 0.01 <sup>BCa</sup>	0.76 ± 0.01 <sup>Ab</sup>	4191.07 ± 161.16 <sup>Bd</sup>
L-2.0 % SPI-U <sub>10</sub>	6279.04 ± 251.76 <sup>ABcd</sup>	0.93 ± 0.01 <sup>ABa</sup>	0.76 ± 0.01 <sup>Ab</sup>	4400.75 ± 148.16 <sup>ABcd</sup>
L-2.0 % SPI-U <sub>15</sub>	6403.62 ± 328.19 <sup>Bbc</sup>	0.93 ± 0.01 <sup>Aa</sup>	0.76 ± 0.01 <sup>Ab</sup>	4500.50 ± 267.62 <sup>Abc</sup>
L-4.0 %SPI	5981.33 ± 474.60 <sup>Bde</sup>	0.92 ± 0.02 <sup>Aa</sup>	0.75 ± 0.01 <sup>Ab</sup>	4138.33 ± 308.37 <sup>Bde</sup>
L-4.0 % SPI-U <sub>5</sub>	6676.89 ± 374.29 <sup>Aab</sup>	0.92 ± 0.01 <sup>Aa</sup>	0.76 ± 0.01 <sup>Ab</sup>	4676.21 ± 308.09 <sup>Abc</sup>
L-4.0 % SPI-U <sub>10</sub>	6824.73 ± 265.58 <sup>Aa</sup>	0.93 ± 0.01 <sup>Aa</sup>	0.76 ± 0.01 <sup>Ab</sup>	4801.45 ± 239.22 <sup>Aab</sup>
L-4.0 % SPI-U <sub>15</sub>	6857.63 ± 302.85 <sup>Aa</sup>	0.93 ± 0.01 <sup>Aa</sup>	0.76 ± 0.01 <sup>Ab</sup>	4841.58 ± 187.73 <sup>Aa</sup>

Different lower case letters in the same column mean a significant difference ( $p < 0.05$ ). Different capital letters indicate significant differences in the duration of the high-intensity ultrasound ( $p < 0.05$ ).

$$\text{Total SH content } (\mu\text{mol/g}) = x/W \quad (5)$$

Where  $x$  is the concentration calculated by bringing it into the standard curve,  $\mu\text{mol/L}$ ,  $W$  is the sample mass, g.

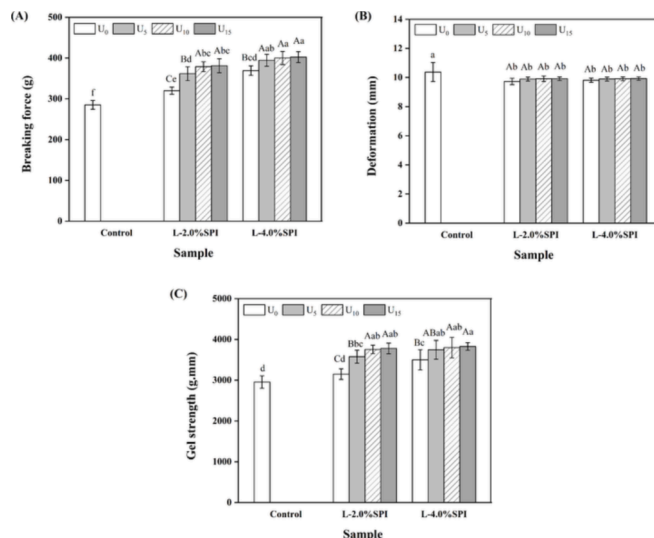
### 2.15. Statistical analysis

All experiments were performed in triplicate. Statistical analysis was performed using SPSS v.18.0 (SPSS Inc., Chicago, IL, USA) and LSD (Least Significant Difference) to analyze the means of tests on different dates and significant calculations. The significant difference was considered when  $p$ -value  $< 0.05$ .

## 3. Results and discussion

### 3.1. Color

The addition of SPI has a significant effect ( $p < 0.05$ ) on the color of silver carp gel (Table 2). The value of  $a^*$  and  $b^*$  of the low-salt group increased significantly with the augmented SPI ( $p < 0.05$ ), while the addition of SPI had no significant effect ( $p > 0.05$ ) on the  $L^*$  value. This was probably linked to the fact that the natural color of SPI is predominantly yellow [31], and the effect is more pronounced with the increase of the additional amount. The study of Li et al. [32] also found that the  $a^*$  and  $b^*$  values of the myofibrillar protein gel increased significantly with the growth of the addition amount of SPI. However, there was no significant difference ( $p > 0.05$ ) in the  $L^*$ ,  $a^*$ ,  $b^*$  under the treatment of high-intensity ultrasound. Whiteness is one of the indicators for measuring the physical properties of food and the consumer's chosen products. The change shown in Table 2 that the whiteness value of low-salt surimi gel caused by the addition of SPI decreased, and with the content of SPI increased from 2.0 % to 4.0 %, the whiteness value decreased significantly ( $p < 0.05$ ). In the low-salt silver carp gel group with the addition of SPI of 2.0 %, with the growth of ultrasonic treatment time, the whiteness value increased slightly, but there had a mild effect on the improvement of the whiteness. The results of TCD also showed that with the increase of soy protein isolate content, it gradually increased, and when the soy protein isolate content was 6.0 %, it exceeded the color difference value that could be recognized by human eyes (TCD  $> 3$ ). The result is consistent with Lu et al. [33] investigating the effect of ultrasound and olive oil treatment on composite surimi gels and found that the whiteness value after ultrasound



**Fig. 1.** Effects of ultrasound and soy protein isolate on gel strength of low-salt surimi gels. Different lower case letters indicate significant difference ( $p < 0.05$ ). Different capital letters indicate significant differences in the duration of the high-intensity ultrasound ( $p < 0.05$ ).

treatment increased slightly. Still, the difference was not significant ( $p > 0.05$ ).

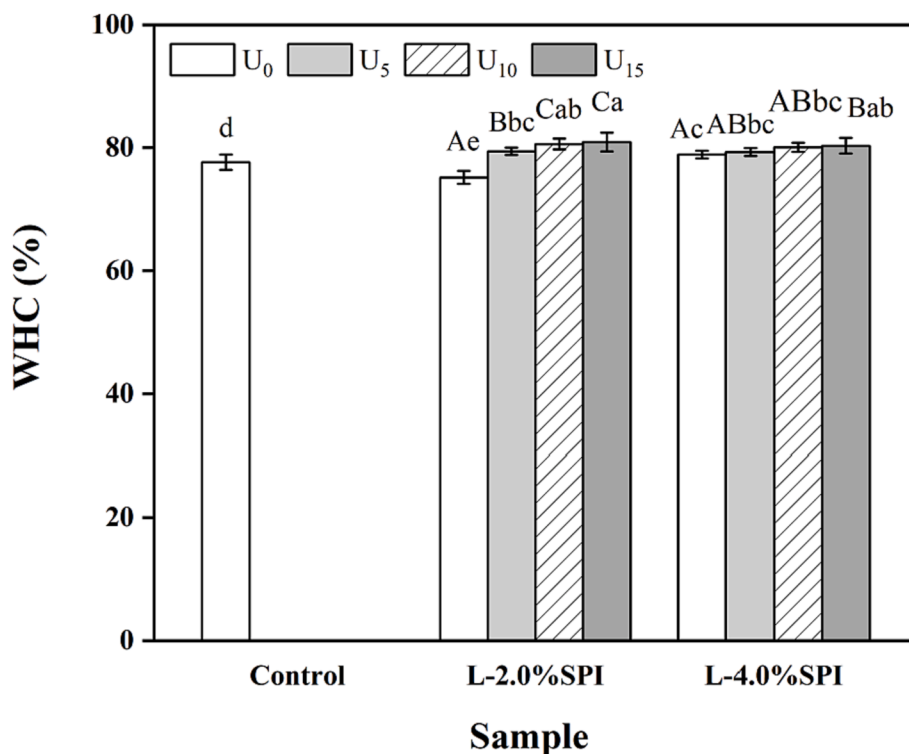
### 3.2. TPA

The effects of different treatments on the texture properties of low-salt silver carp gel are shown in Table 3. Compared with the control, adding SPI and the ultrasonic-assisted SPI processing significantly ( $p < 0.05$ ) improved the hardness and chewiness of the low-salt surimi gel. The springiness and cohesiveness showed that the addition of SPI and ultrasonic treatment had no significant effect ( $p > 0.05$ ). On the one hand, the SPI may cause swelling and denaturation during heating, forming a thermally irreversible gel [34], physically filling the network of the surimi gel or covalently bindings with myofibrillar protein and enhancing the surimi strength of the gel [35]. On the other hand, the mechanical effects (shear force and shock wave) produced by sonication could significantly increase the solubility of proteins and promote the unfolding of protein structure [24], which may enhance the interaction between myofibrillar protein and myofibrillar protein, SPI and SPI, and myofibrillar protein between SPI. Ye et al. [17] also reported that ultrasonic and microwave treatment could synergize through the thermal effect of microwaves and the mechanical effect of ultrasonic waves to compensate for the quality impact caused by the decreased salt content of surimi products.

### 3.3. Gel strength

Gel strength is a crucial indicator in evaluating the quality of surimi and its products, which reflects the aggregation ability of proteins during heat-induced gelation [2,24]. The gel strength is calculated by multiplying the breaking force and the deformation distance. In general, breaking force is a critical parameter in determining the quality of fish gelatin products, which is mainly determined by the packing tightness of protein molecules [36]. As shown in Fig. 1, compared with the control group, the addition of 4.0 % SPI can significantly increase ( $p < 0.05$ ) the breaking force and gel strength of the low-salt surimi gels, which indicates that the SPI promotes the formation of a dense structure in the low-salt gel group. In comparison, the 2.0 % SPI group had no significant effect, because myofibrillar proteins were more likely to dissolve and swell in a high-salt environment, exposing more sulfhydryl groups, promoting cross-linking between proteins, thereby forming a dense





**Fig. 2.** Effect of ultrasound-assisted soy protein isolate on the water-holding capacity of low-salt silver carp gel. Different lower case letters indicate significant difference ( $p < 0.05$ ). Different capital letters indicate significant differences in the duration of the high-intensity ultrasound ( $p < 0.05$ ).

network structure [37]. However, there was no significant difference ( $p > 0.05$ ) in gel strength with the addition of 2.0 % SPI. Ultrasonic-assisted processing significantly increased the breaking force and gel strength in the low-salt surimi gel. The breaking force gradually increased with ultrasonic time, indicating that ultrasonic treatment enhanced protein unfolding and catalyzed the covalent cross-linking of protein molecules and aggregation, thereby accelerating the gelation process under heating, forming surimi gels with high gel strength [24]. The study by Tang et al. [38] also found that the breaking force of the gel increased significantly with the addition of sodium chloride (0.5–2.0 %). In comparison, ultrasonic treatment had no significant effect on the group with the addition of 1.0 % sodium chloride.

### 3.4. WHC

The WHC of the gel indicates the interaction of the myofibrillar protein with water molecules, which reflects the quality of surimi [39]. As shown in Fig. 2, irrespective of the treatment conditions, the WHC of low-salt surimi gels was significantly improved ( $p < 0.05$ ), except for the group of 2.0 % SPI. It may be related to the additional amount of SPI. When the sodium chloride content is lower than 0.3 M, the salt-soluble protein cannot be fully dissolved to form a stable network structure. The addition of a small amount of SPI is not enough to make up for this loss, resulting in a loose gel structure, and a decreased in water holding capacity [35,40]. Consistently, the WHC of the gels was significantly enhanced after high-intensity sonication, and there was no significant ( $p > 0.05$ ) difference between 10 and 15 min. The increased WHC of high-intensity ultrasound gels is associated with a denser protein mechanism, which favors the capture of more water [39]. The ultrasonic treatment promotes the dissolution of myofibrillar protein through cavitation effect and intensifies the intermolecular interactions, thereby improving the WHC of the gel [23].

### 3.5. LF-NMR

LF-NMR has been one of the essential indicators for identifying the water components in gelatinous meat products [32]. The relaxation time ( $T_2$ ) of the gel usually has three peaks between 0 ~ 1000 ms, which represent bound water (0–10 ms), bound water (10–100 ms), and free water (100–1000 ms). Fig. 3(A) depicts the continuous distribution of the  $T_2$  relaxation time for gel samples at different salt contents and under other treatment conditions. The initial relaxation times of  $T_{2b}$ ,  $T_{21}$ , and  $T_{22}$  were shortened when the high-intensity ultrasound time increased and SPI added, indicating that the binding between protein and water became tighter and reduced the fluidity of water [32]. As shown in Fig. 3 (B), compared with the control group, the area of  $P_{21}$  was increased and  $P_{22}$  was decreased in the ultrasound-assisted SPI treatment group, indicating that the gel structure formed at this time can bind more water in the myofibrillar protein in the gel network, the free water molecules in the gel mechanism are immobilized [41], except for the group of 2.0 % SPI added. Previous studies have shown that the denser and more ordered the gel network, the better the confinement of water flow [42,43]. The combination of SPI and ultrasound may significantly improve the solubility of the protein in low-salt surimi by reducing the size of the protein and increasing the contact surface of the protein with water (Fig. 8). In addition, the structural changes of the protein induced by ultrasound may lead to more exposure of charged groups ( $NH_4^+$ ,  $COO^-$ ) [44–46]. These factors can promote myofibrillar protein to have a better gel structure during heating. Therefore, added SPI could improve the water-holding capacity of low-salt surimi gels under high-intensity ultrasound. This was consistent with the result in WHC of surimi gel (Fig. 2). The study of Li et al. [32] the initial relaxation time of  $T_2$  was shortened when SPI added under high pressure processing, with a significant decrease in  $P_{22}$  and a significant increase in  $P_{21}$ . Xing et al. [21] also found that high-intensity ultrasound treatment could improve the retention ability of low-salt gel to free water.

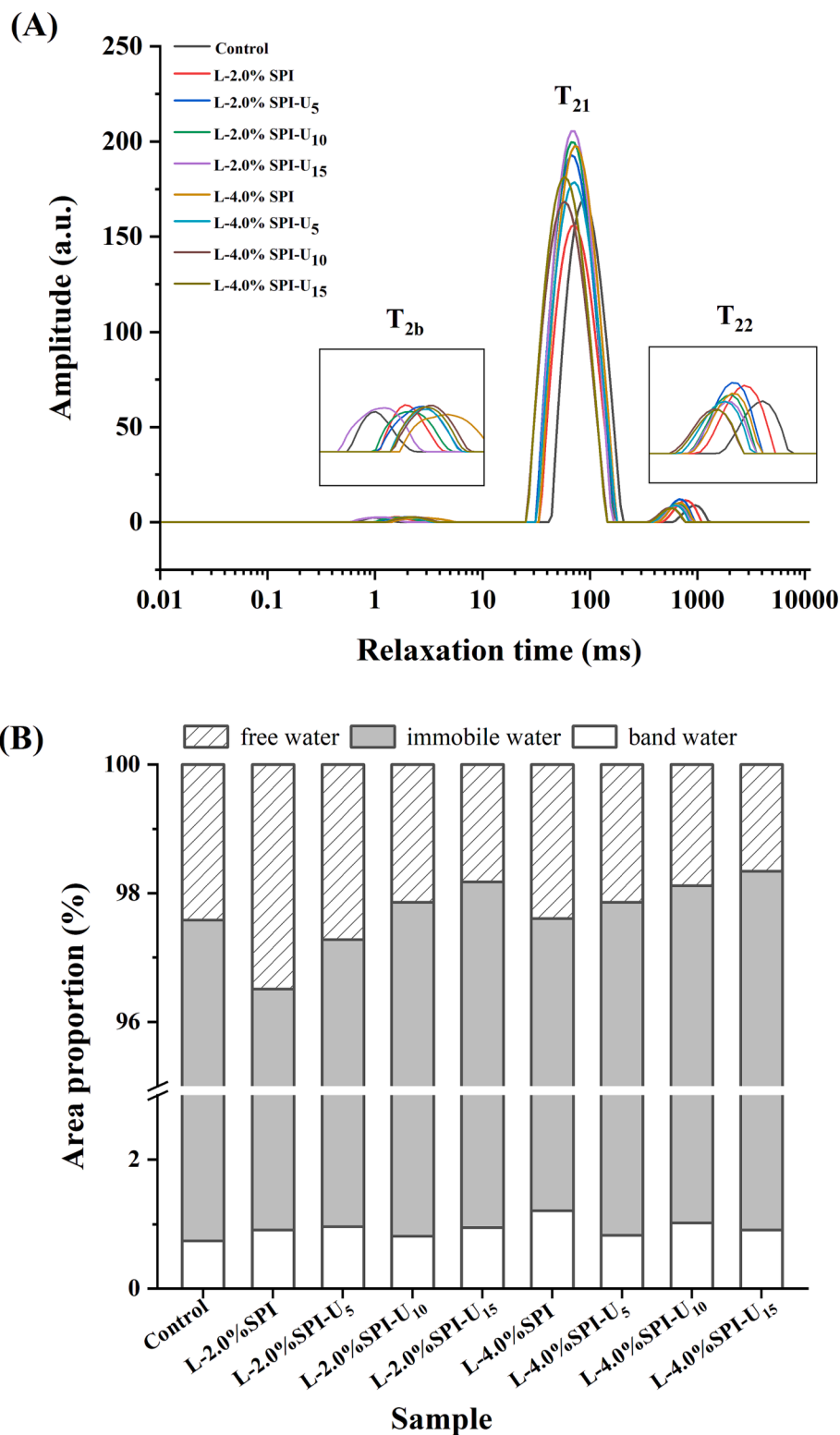
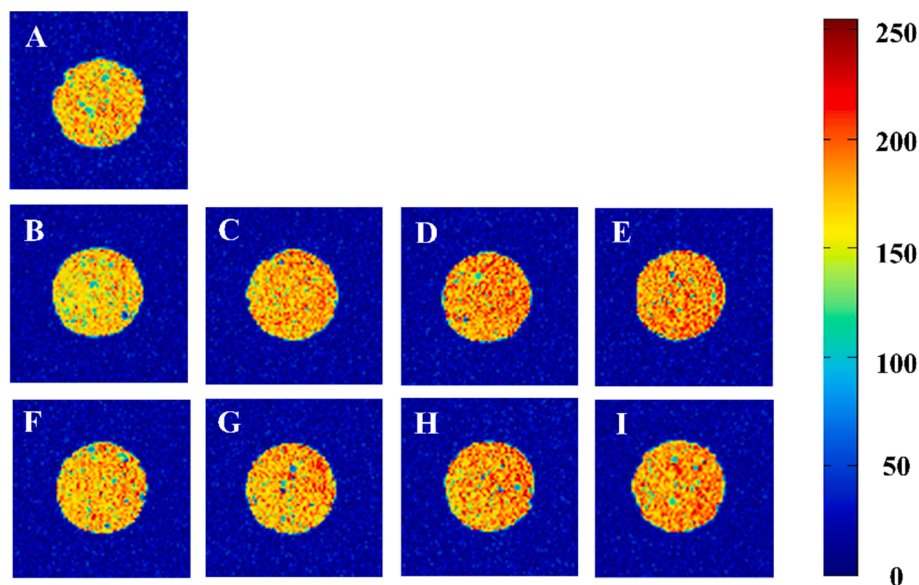


Fig. 3. Effects of ultrasonic treatment on gel relaxation time (A) and areas proportion (B) of low-salt surimi with different soy protein isolate additions.

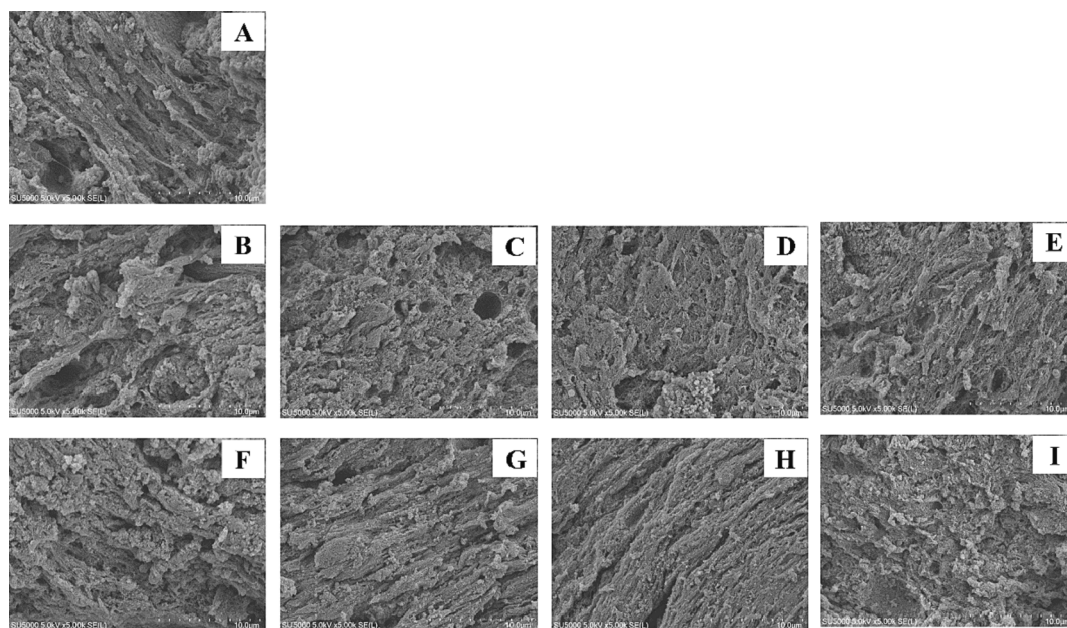
### 3.6. MRI

As a rapid and non-destructive method, MRI can be considered as a complement to LF-NMR, which can provided an intuitive monitoring water migration status in the gel on a pseudo-color map based on the depth of gel color, thereby determining the distribution of water in food and visualizing internal structural changes [17,33,47]. Hydrogen

proton-weighted images of low-salt surimi gels with various SPI and ultrasonic treatments are shown in Fig. 4. The red color indicates a higher H-proton density in the gel, and the blue indicates a lower H-proton density. Compared with the control, the low-salt group with 2.0 % SPI had the lowest hydrogen proton density, while the hydrogen proton density of 4.0 % SPI had relatively high hydrogen proton density; the red color of the map deepened as the distribution became wider. The



**Fig. 4.** LF NMR (A) and hydrogen proton density map (B) of low-salt surimi gel under ultrasound and soy protein isolate. A: Control, B: L-2.0%, C: L-2.0%-U<sub>5</sub>, D: L-2.0%-U<sub>10</sub>, E: L-2.0%-U<sub>15</sub>, F: L-4.0%, G: L-4.0%-U<sub>5</sub>, H: L-4.0%-U<sub>10</sub>, I: L-4.0%-U<sub>15</sub>.



**Fig. 5.** Microstructures of low-salt surimi gel processed by ultrasonic-assisted soy protein isolate. A: Control, B: L-2.0%, C: L-2.0%-U<sub>5</sub>, D: L-2.0%-U<sub>10</sub>, E: L-2.0%-U<sub>15</sub>, F: L-4.0%, G: L-4.0%-U<sub>5</sub>, H: L-4.0%-U<sub>10</sub>, I: L-4.0%-U<sub>15</sub>.

distribution of red areas in the false-color images of the low-salt group treated with ultrasound became more extensive and more uniform compared to the group of SPI. With the increase of sonication time, the yellow and blue regions became less, and the red areas became more prominent and more profound. The sonication may promote the unfolding of myofibrillar proteins and expose the internal hydrophobic sulfhydryl groups, thus forming a more stable three-dimensional network structure in the gel formation process, and enhancing protein-water binding capacity. The study of Ye et al. [17] also showed that the water particle density of low-salt surimi gel assisted by ultrasound treatment was significantly increased, and showed the best performance with the deepest red color and the most widespread distribution. Lu et al. [33] also showed that ultrasonic treatment was more conducive to promoting the water-holding ability of surimi gel.

### 3.7. SEM

The microstructure of the gel is an integral part of evaluating its performance of the gel [27]. The microstructures of low-salt surimi gel with different ultrasonic time assisted SPI are shown in Fig. 5. Compared with the control group, SPI may exist in the gel structure as physically large particle embedding, which plays a role in myofibrillar protein gel formation in a binding/intercalating manner. It was still not enough to make up for the reduction of sodium chloride in the addition of 2.0 % SPI. On the contrary, the microstructure structure of the gel was denser in the expansion of 4.0 %. Then there's an even more apparent result, high-intensity ultrasound treatment significantly improved the structure of low-salt surimi gel, working with SPI to form a denser, firmer, and stronger gel structure. This effect was greatly enhanced with increasing

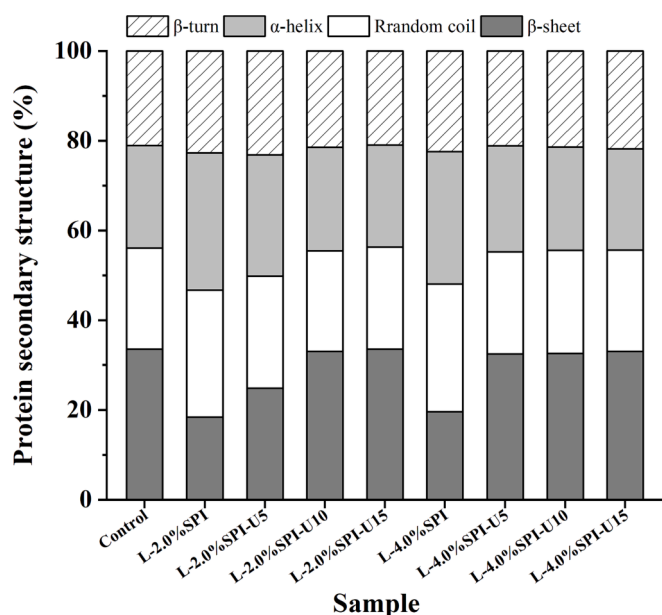


Fig. 6. Effect of ultrasound treatments time on the protein secondary structure of low-salt surimi gels with different soy isolate protein contents.

sonication time. High-intensity ultrasound promotes the expansion of the myofibrillar protein structure [27], and exposed more hydrophobic groups existing in the protein molecules, thereby strengthening the relationship between protein and protein, protein and water, and forming a stable and dense gel network structure under low-salt content [37]. Sun et al. [48] found that the three-dimensional network of low-salt group protein gel under ultrasound treatment was denser and the black gap became uniform, the three-dimensional network of gel was more solid and the water distribution was more uniform. Gao et al. [23] also found that HIU pre-treatment before salt-chopping resulted in the formation of gels with small pore size and homogeneous and dense microstructure.

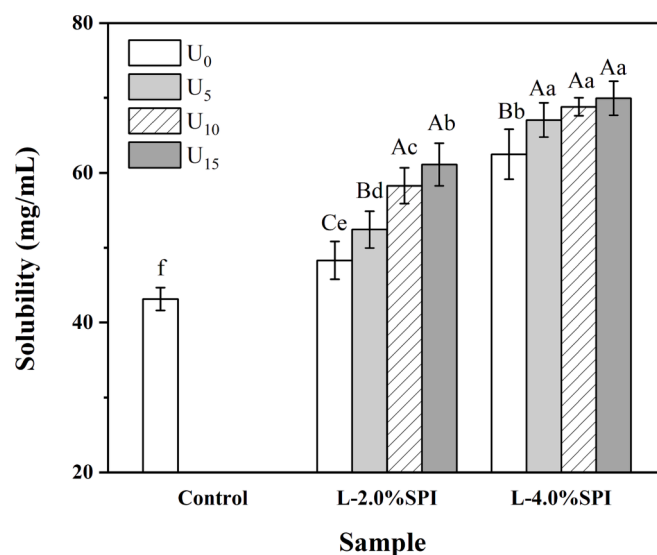


Fig. 8. Effect of ultrasound-assisted soy protein isolate on solubility of salt-soluble protein in low-salt silver carp surimi. Different lower case letters indicate significant difference ( $p < 0.05$ ). Different capital letters indicate significant differences in the duration of the high-intensity ultrasound ( $p < 0.05$ ).

### 3.8. Protein secondary structure

The secondary structure of a protein refers to the spatial structure of the local peptides in the backbone of the polypeptide chain, which determines the functional properties of the protein. The amide I band is dominated by stretching vibrations of C=O and a lesser extent C—N and is considered the most sensitive region for protein secondary structure composition [17,49]. As shown in Fig. 6, compared with the control group, the decrease of salt content will cause the content of  $\alpha$ -helix to increase and the content of  $\beta$ -sheet to decrease. High sodium chloride levels can unfold the myofibrillar protein structure, resulting in reduced  $\alpha$ -helix content and increased  $\beta$ -sheet content in the resulting surimi gel [8]. However, the  $\alpha$ -helix content decreased slightly (30.60 %–29.49 %) and the  $\beta$ -sheet content (18.43 %–19.62 %) increased slightly with the increase of soybean protein isolate content, but the effect was almost

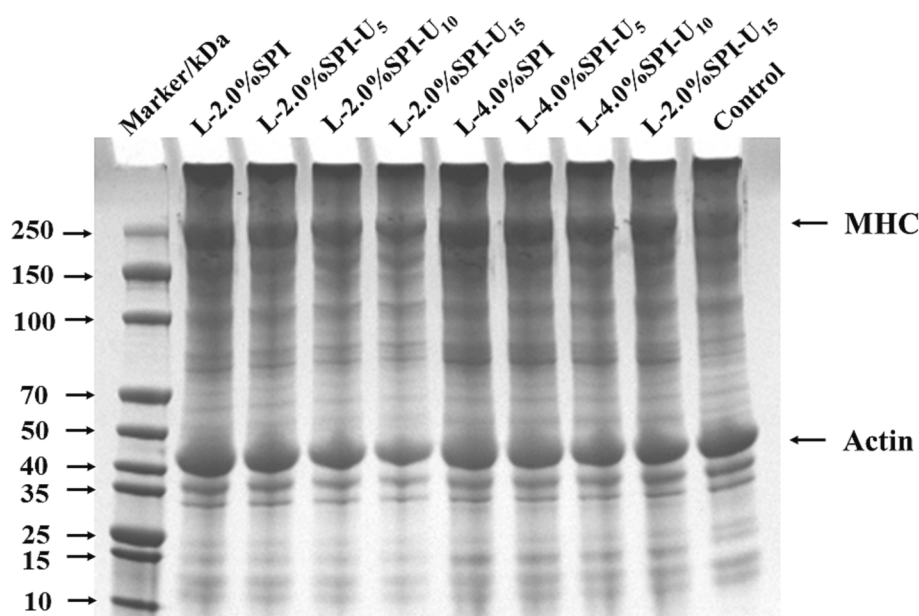
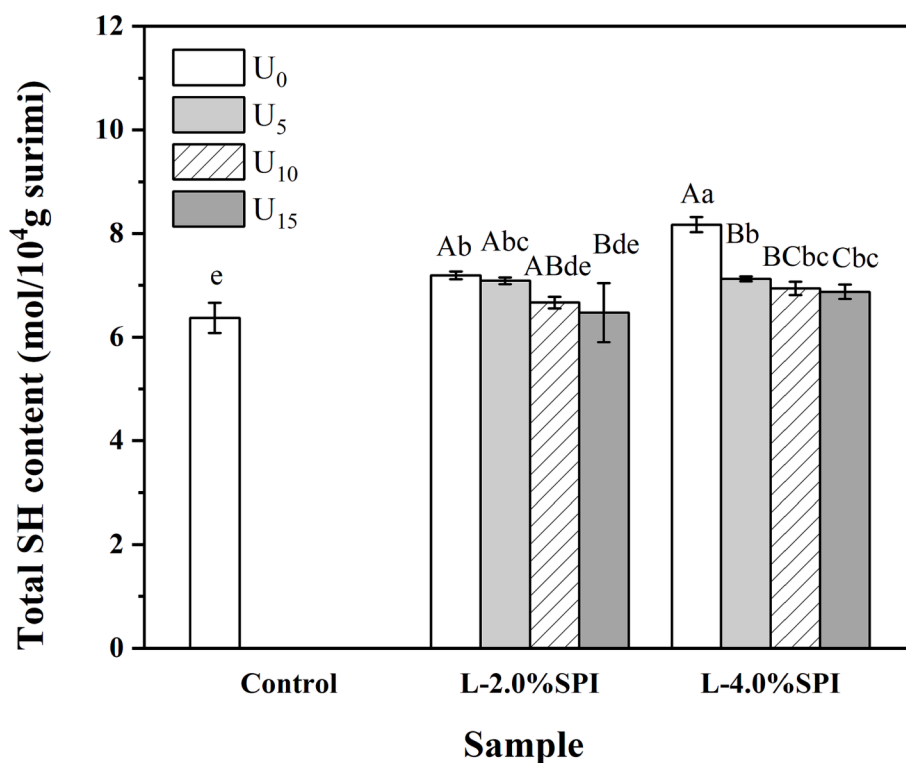


Fig. 7. Effects of ultrasonic-assisted soy protein isolate on SDS-PAGE of low-salt silver carp gel.





**Fig. 9.** Changes of total SH content in the myofibrillar protein of low-salt surimi under ultrasound-assisted soy protein isolate. Different lower case letters indicate significant difference ( $p < 0.05$ ). Different capital letters indicate significant differences in the duration of the high-intensity ultrasound ( $p < 0.05$ ).

negligible.

Ultrasound-assisted processing significantly affected the content of surimi protein secondary structure. Compared with the high-salt control group, with the increase in sonication time, the  $\alpha$ -helix content of the protein under low-salt conditions decreased significantly, the random coil and  $\beta$ -turn contents showed a downward trend, and the  $\beta$ -sheet content increased significantly. In fact, the increase in  $\beta$ -sheet reflect the ultrasonic treatment promoting the increase of the number of protein hydrogen bonds, and the effect is more pronounced with the growth of ultrasonic time, which enhancement of the interaction force between protein molecules and conducive to the formation of an ordered myofibrillar protein gel network later [21]. A similar result was also found in the study by He et al. [24]. In addition, the study by Tian et al. [50] also found that sonication helped soybean protein  $\alpha$ -helix to  $\beta$ -sheet transition, and the solubility of the protein was significantly increased.

### 3.9. SDS-PAGE

Fig. 7 is a gel electropherogram of surimi with different treatments showing typical myofibrillar protein bands. The main components in surimi are myosin heavy chain (MHC) and actin. Compared with the control group, the addition of SPI in the low-salt state deepened the actin (about 220 kDa) and myosin (about 45 kDa) bands, which may be caused by adding SPI and decreasing salt content. The result after ultrasound shows that high-intensity ultrasound narrows and becomes lighter in the heavy chain band of myosin in low-salt surimi. This phenomenon indicated that MHC was degraded into smaller molecules, and the degree of degradation increased with ultrasonic power and time [46,51]. Sonication may cause the unfolding of the protein system and exposed more internal hydrophobic groups, resulting in forming a more stable structure to interact with more proteins and water (Fig. 5). In addition, compared with control group, the acquisition of SPI appeared bands at 70 kDa and 100 kDa, and gradually deepened with the increase of SPI addition, which could be due to the fact that the subunits of SPI ( $\alpha'$ ,  $\alpha$ , and  $\beta$ ) did not involve in the construction of myofibrillar protein

gels [52], and gradually weakened with the increase of ultrasonic times, which may be involved gels formation or decomposition/aggregation under the influence of ultrasonic to from small/large molecules.

### 3.10. Salt-soluble protein solubility

Solubility is mainly determined by pH value and ionic strength in the system [53], and is a crucial indicator for measuring protein aggregation and denaturation [54]. Improving the solubility of protein is profitable to enhance its processing characteristics and broaden the application of protein in food [55,56]. As shown in Fig. 8, adding different SPI improved the protein solubility, which may be due to the solubility of soybean protein isolates themselves. Nevertheless, with the increase in sonication time, the solubility of 2.0 % SPI increased significantly ( $p < 0.05$ ). In comparison, the 4.0 % SPI addition increased significantly ( $p < 0.05$ ) after sonication, but there was no significant ( $p > 0.05$ ) change between different sonication times. It may be due to the fact that the high-intensity ultrasound destroys the bonds among protein molecules through acoustic cavitation, weakens the interaction between protein, reduces the size of protein particles, increases the specific surface area of protein particles, and promotes the interaction between protein molecules and water, thus increasing the solubility of proteins [57]. The study by Liu et al. [56] also found that under low-salt conditions (1.3 mM), the solubility of myofibrillar protein significantly improved due to the cavitation effect and mechanical force generated by the high-intensity ultrasound. Still, the modification of myofibrillar protein has a specific upper limit [58]. Arzeni et al. [54] and Jambrak et al. [59] also showed that ultrasonic treatment could increase the solubility of soybean protein isolate.

### 3.11. Total SH content

Fig. 9 shows the effect of ultrasound-assisted SPI on the total SH content of low-salt silver carp surimi. The total SH group content in the low-salt silver carp gel increased significantly with addition of SPI ( $p <$

0.05). On the one hand, this may be due to the decrease in salt content [60]. On the other hand, the addition of SPI also may bring more sulfhydryl groups into the system. However, ultrasonic treatment significantly reduced the total sulfhydryl content of different SPI additions ( $p < 0.05$ ). The decreased SH content and the more disulfide bond formation indicated that ultrasonic treatment resulted in more disulfide bond formation during thermally induced gel formation. The high-intensity sonication can unfold the molecular structure of the myofibrillar protein. By exposing more tryptophan residues to the polar environment, the thiol groups encapsulated in the protein molecules are exposed to the surface during the unfolding of the protein structure, and part of the disulfide bonds are broken, eventually, leading to the increase in the content of thiol group [61]. This result was consistent with that of Gao et al. [37] explore the effects of high-intensity sonication on silver carp gels with different salt contents.

#### 4. Conclusions

In conclusion, ultrasonic-assisted soy protein isolate processing can improve the gel quality of low-salt silver carp surimi. Compared with the control group (2.0 % sodium chloride), adding soy protein isolate alone was not enough to compensate for the negative effect of reduced sodium chloride on gel quality. High-intensity ultrasound-assisted soybean protein isolate swells and expands myofibrillar protein through the cavitation effect, exposing more reactive groups inside the proteins, and promoting the interaction between proteins and between proteins and water. As a result,  $\alpha$ -helix and the random coil content decreased,  $\beta$ -sheet content increased, the solubility enhanced, and the total thiol content decreased. The protein being transformed into a more stable structure, resulting in the gel pores are significantly reduced, the network is more compact and durable. Meanwhile, the gel hardness and strength, and water-holding capacity were significantly improved. And this change is enhanced with the increase of ultrasonic time. When the processing conditions were at L-2.0% SPI-U<sub>15</sub> (ultrasonic at 400 W/L for 15 min, the sodium chloride content is 1.0 %, and the soy protein isolate content of 2.0 %), the quality of the gel can reach or even surpass the control group (2.0 % sodium chloride).

#### CRediT authorship contribution statement

**Xuehua Zhang:** Data curation, Investigation, Writing – original draft. **Quanyou Guo:** Resources, Project administration. **Wenzheng Shi:** Conceptualization, Writing – review & editing.

#### 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

Data will be made available on request.

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