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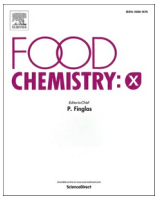

Study on the mechanism of soy protein isolate to improve quality of reduced-salt *Hypophthalmichthys molitrix* surimi gel: Focus on gel quality, protein structure, and in vitro digestibility

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ABSTRACT

Excessive intake of sodium chloride may bring a series of diseases; as a result, reduced-salt surimi gels have gained growing popularity for sodium reduction. This paper studied soy protein isolate (SPI, 2.0%, 4.0%, and 6.0%, w/w) as a gel enhancer for reduced-salt silver carp surimi. Compared with the control (2.0% NaCl), the addition of SPI significantly increased ($P < 0.05$) the total SH content, hydrophobic interaction force, disulfide bond, hardness, gel strength, and water-holding capacity of the gels. During the thermal denaturation process, SPI and myofibrillar protein jointly participated in the formation of the gel network, resulting in a G' value increase at 90 °C, forming a denser/more stable gel network structure. In vitro pepsin digestion results showed the digestibility of the reduced-salt gel with SPI was higher than that of the control. Therefore, appropriate SPI addition can improve the gel performance of reduced-salt surimi gel without affecting digestion and absorption.

1. Introduction

Freshwater fish are made into surimi by meat picking, fine filtering, rinsing, and dehydration, which effectively solves the problem of many spines between the muscles of freshwater fish and improves the processing and utilization rate. On account of its high-quality color, low price, and high protein content (15 ~ 18 g protein/100 g surimi), this product is popular with the processing industry and consumers and has a good development prospect (Mi, Li, Wang, Yi, Li, & Li, 2021). However, its inherently poor gel quality and protein degeneration in the process of processing and transportation results in poor gel strength and easy deterioration (Wang, Wang, Liu, & Yu, 2023). With a global production of 4.8 million tons of silver carp in 2019, it is one of the most important freshwater farmed fish species in the world (FAO, 2021), which is mainly used in the production of surimi-based food in China. Thus, efforts to improve silver carp surimi gel quality are necessary and one of the hot spots of current research.

Myofibrillar protein is the core component of surimi, which is salt-soluble (Xiong, Shi, Zhang, Kong, Yuan, & Gao, 2021), and 2%–3% salt is usually added to the surimi gel products to promote the lysis and swelling of myofibrillar proteins and create a dense three-dimensional network structure during the heating processing, which aids in obtaining the favorable texture of surimi and gel performance (Fu, Hayat, Li, Lin, Xu, & Wang, 2012). Unfortunately, the primary source of sodium consists of salt, which is related to hypertension and increased risks of heart disease and stroke. If global salt consumption were reduced to the recommended levels (Adults should not exceed 5 g per day), it is estimated that 2.5 million deaths per year could be prevented (WHO, 2020). Previous studies have shown about 30% of the daily salt intake in humans comes from meat and processed meat products (Partearroyo et al., 2019). If the addition of sodium chloride is directly reduced, the quality of the gel formation will be reduced. Therefore, some food manufacturers are looking for ways to reformulate recipes to reduce the salt content of their products.

Soy protein isolate (SPI) is a kind of plant protein extracted from soybean meal, which is the by-product of soybean oil pressed at low temperatures. SPI is widely used in meat products due to its effective emulsifying and gelling properties (Kang, Chen, & Ma, 2016). Its excellent functional properties can effectively improve the texture, moisture retention, and gel quality of livestock and poultry products (Niu, Li, Han, Liu, & Kong, 2017) and can even be considered as a substitute for animal fat in emulsified pork gel products (Kang et al., 2016). As Park et al. (1994) observed, adding 1% of SPI to surimi led to a more rigid structure, thereby increasing shear stress and decreasing

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shear strain during gelation. Li et al. (2021) found the combination action of SPI and ultra-high pressure improved the water retention capacity, texture, and thermal stability of low-salt (1% NaCl) pork myofibrillar protein.

However, there are few reports of the effects of SPI in surimi gel products, especially on the techno-functional and digestive and mechanism analysis of reduced-salt surimi products. Based on this background, our study was conducted to investigate the effects of replacing sodium chloride with SPI on the textural characteristics, water distribution, microstructure, and in vitro pepsin digestibility of reduced-salt silver carp surimi gels, and this investigation was combined with protein structure, conformation, and major chemical force changes for comprehensive analysis. This provides a positive effect for the improvement of the mode of action of surimi gel and the application and product development of SPI in reduced-salt surimi gel.

2. Materials and methods

2.1. Materials

Silver carp (*Hypophthalmichthys molitrix)* surimi (75% moisture, 14.37% protein, w/w) was supplied from Hubei Jingli Aquatic Food Co., Ltd. (China) and stored at − 20 ± 2 °C. SPI (10 ~ 200 kDa) was purchased from Shanghai Maclin Biochemical Co. (China). All other chemical reagents were analytical grade.

2.2. Pretreatment of silver carp surimi gel

Frozen silver carp surimi was thawed at 4 °C for 6 h. For each treatment, surimi (200 g) was mixed for 1 min using a JRJ-B04Q1 meat grinder (Bear Electric Appliance Co. Ltd., Guangdong, China) equipped with a slotted plate with 6 mm diameter holes. After that, 1.0% NaCl and 2.0%, 4.0%, and 6.0% (w/w) SPI were added to different treatment groups (named T2, T3, and T4, respectively). After being chopped for 2 min below 4 °C, the moisture content was adjusted to 79.69 ± 0.13%. Surimi with only 2.0% NaCl was added as the control group (T1).

Surimi gel was prepared according to our previous method (Zhang et al., 2023), and 100 ± 5 g of surimi samples were heated at 40 °C for 40 min and 90 °C for 30 min. After the end of the heating, we quickly placed the samples in ice water to cool them and then refrigerated them overnight at 4 ± 2 °C.

2.3. Determination of pH

According to the method of Kang et al. (2021), the prepared surimi was mixed with deionized water at a volume ratio of 1:4 using an FM-200 homogenizer (Shanghai Fokker Equipment Co., China) at a speed of 5000 rpm. A digital pH meter (FE-28, Mettler Toledo, Switzerland) was used to measure the pH of each treatment group.

2.4. Color of surimi

The cylinder (about 2 cm in height) was taken from each gel and determined immediately. The center color was measured by a CR-400 colorimeter (Minolta Camera Co, Japan) after being calibrated with a standard plate. The whiteness value is calculated according to Formula (1).

$$\text{Whiteness} = \left[100 - (100 - L^*)^2 + (a^*)^2 + (b^*)^2\right]^{1/2} \quad (1)$$

2.5. Texture profile analysis (TPA)

Textural properties of cylinder surimi gels with a diameter of 25 mm and a height of 25 mm were measured using a texture analyzer (TA-XT. plus, Stable Micro Systems Ltd., UK) equipped with a P/50 probe. The pretest speed, test speed, and posttest speed were all set to 2 mm/s, and the compression deformation was set to 50%. Numerical changes in hardness, springiness, and chewiness were recorded for different treatment groups.

2.6. Gel strength

Following the previous method (He et al., 2022) and making minor modifications, the gel strength of the surimi gel was determined using a texture analyzer equipped with a P/5S spherical plunger. The parameters were as follows: test speed was set to 1 mm/s, compression distance to 10 mm, and trigger force to 5 g.

2.7. Low field nuclear magnetic resonance (LF-NMR) of surimi gel

Referring to our previous study (Zhang, Guo, & Shi, 2023), the surimi gels tempered at room temperature for 30 min were analyzed by the MesoMR23-060H-I LF-NMR resonance apparatus (Suzhou Niumag Instrument Analysis Co., Ltd., China), and the water distribution and false-color map of ^{1}H proton density were obtained.

2.8. Water-holding capacity (WHC) of surimi gel

Referring to the method of Zhuang et al. (2017) and modifying it slightly, the WHC with different added amounts of SPI was determined. Thin slices of surimi gel (approximately 3.0 g) were placed in a plastic centrifuge tube containing three layers of filter paper and centrifuged for 10 min at 12,000 g using a H-2050R freezing centrifuge (Xiangyi Instruments Co., China). The mass of gel before centrifugation (W_1) and the mass after centrifugation (W_2) were calculated according to Equation (2) to obtain the WHC of gels.

$$\text{WHC}\ (\%) = W_2/\ W_1 \times 100\% \quad (2)$$

2.9. Rheological properties

Raw surimi was heated from 24 °C to 90 °C at a speed of 2 °C /min using a rheometer (KNX2000, Malvern Instruments Ltd., UK) and equipped with a plate gripper (PU40). The sample gap was set at 1.00 mm and sealed with silicone oil to reduce moisture evaporation. The change of storage modulus G′ with temperature was monitored under the condition of a frequency of 1 Hz and a strain of 1%.

2.10. Scanning electron microscope (SEM)

Microstructures analysis of surimi gels was carried out using a Hitachi SU5000 thermal field emission SEM (Japan) according to the method of Guo et al. (2019) with slight modifications. In brief, gels were sliced into 3 × 3 × 1 mm and fixed overnight in a 2.5% phosphate glutaraldehyde buffer. The slices were rinsed with 0.1 M PBS (pH = 7.0) for 10 min and repeated 3 times, and then gradient dehydration was carried out in ethanol at 60%, 70%, 80%, 90%, and 100% (v/v) concentration for 10 min and repeated 3 times. Before being vacuum dried, the samples were replaced with a mixture of anhydrous ethanol and tertiary butyl alcohol (1:1 v/v) for 15 min. Finally, the sample was sprayed with gold and then scanned.

2.11. Total sulfhydryl (SH) content of surimi

Referring to the method of Zhang et al. (2023), the total SH content in raw surimi was determined by using the thiol determination kit (Beijing Solarbio Science & Technology Co., Ltd., China, product code: BC1375). Briefly, the samples and reagents were mixed in sequence as required and the absorbance was measured at 412 nm using a UV-1800pc spectrophotometer (Shanghai Mapada Instrument Co., Ltd.,

China). The sulfhydryl concentration (x) was calculated from the standard curve, and the total SH content per unit mass was obtained from the ratio between total sulfhydryl concentration (x) and sample mass (W) according to Equation (3).

$$\text{Total SH content } (\mu\text{mol/g}) = x/W \quad (3)$$

2.12. Chemical interaction of surimi gels

The chemical interaction was measured using the method described by Li et al. (2017) with slight modifications. Briefly, five mixed solutions—SA (50 mM NaCl), SB (0.6 M NaCl), SC (0.6 M NaCl, 1.5 M urea), SD (0.6 M NaCl, 8 M urea), and SE (0.6 M NaCl, 8 M Urea, 0.5 M β-ME) were prepared first, and then 2 g of minced surimi gel were mixed with 10 mL of the above solution respectively and reacted at 4 °C for 1 h. After that, centrifugation at 8,000 r/min was carried out for 10 min, and the protein content in the supernatant was determined by the biuret method. Finally, the content of ionic bond (mg/mL), hydrogen bond (mg/mL), hydrophobic interaction force (mg/mL), and disulfide bond (mg/mL) of the gels was calculated according to Formulas (4) to (7).

$$\text{Ionic bond} = \rho\text{SB-}\rho\text{SA} \quad (4)$$

$$\text{Hydrogen bond} = \rho\text{SC-}\rho\text{SB} \quad (5)$$

$$\text{Hydrophobic interaction} = \rho\text{SD-}\rho\text{SC} \quad (6)$$

$$\text{Disulfide bond} = \rho\text{SE-}\rho\text{SD} \quad (7)$$

where ρSA, ρSB, ρSC, ρSD, and ρSE represent the mass concentration of dissolved protein (mg/mL) in the SA solution, SB solution, SC solution, SD solution, and SE solution, respectively.

2.13. SDS-PAGE

According to the study of Laemmli (1970), the protein profile of surimi gel was analyzed by SDS-PAGE. First, 27 mL of SDS solution (85 °C) was added to the minced gel (3.0 g) to dissolve the total protein from the surimi gel. After incubation at 85 °C for 1 h, the mixture was centrifuged at 3,500 g for 20 min to remove undissolved material. The extracted protein was loaded onto the 4%–20% mini precast PAGE gel (Beyotime Biotechnology, China) in a volume of 12 μL and then run at 15 mA, 120 V for about 1 h using a vertical electrophoresis system (Bio-Rad Laboratories Inc., USA). Finally, coomassie bright blue R-250 staining was used for 1 h before decolorizing with ethanol and acetic acid.

2.14. Flourier transform infrared spectroscopy (FT-IR)

The secondary structure of surimi was explored with our previous research methods (Zhang et al., 2023). The surimi was lyophilized under vacuum for 48 h, crushed and passed through a 60-mesh sieve, and scanned in the 600–4000 cm^{-1} spectral range using a Nicolet iS10 flourier transform midinfrared micro chemical imaging system (Thermo Fisher Scientific Inc., Canada). The relative contents of α-helix, β-sheet, β-turn, and random coil in the secondary structure of surimi were calculated by deconvolution and Gaussian fitting of the amide band (1600–1700 cm^{-1}) spectra using Peakfit v 4.12 software.

2.15. In vitro pepsin digestibility of surimi gels

In vitro trials using human or animal models are costly, laborious, and prone to cause ethical problems, so in vitro models are mostly used for digestion-related studies (Bhat, Morton, Bekhit, Kumar, & Bhat, 2021). The method of in vitro pepsin digestion referenced the study of Minekus et al. (Minekus et al., 2014) with slight modification. First, the surimi gel was ground, and the total nitrogen content in the sample was determined using a Kjeldahl apparatus (KJELTEC 8400, FOSS, Denmark). After that, simulated gastric fluid (6.9 mM KCl, 47.2 mM NaCl, 0.9 mM KH_2PO_4, 25 mM $NaHCO_3$, 0.2 mM $MgCl_2(H_2O)_6$, 0.5 mM $(NH_4)_2CO_3$, pH 2.0) was prepared for simulated in vitro gastric fluid digestion. For each treatment group, 10 g of minced surimi gel was added to 10 mL distilled water and then homogenized with 20 mL simulated gastric juice (containing a 19 mL stimulated gastric fluid original solution, 0.3 mg $CaCl_2$, and 60 mg porcine trypsin) at 5, 000 r/min for 1 min. After adjusting the pH to 2.0 (with 1 M HCl), the complex solution was shaken and reacted in a water bath at 37 °C for 2 h. Finally, after adjusting pH to 7.0 with 0.5 M $NaHCO_3$, in vitro protein digestibility was calculated as follows:

$$\text{Digestibility } (\%) = \frac{V \times c}{M \times n} \times 100 \quad (8)$$

where V is the total volume of the digestion solution (mL), c is the protein content in the digestion solution (mg/mL), M is the mass of the gel used for digestion (mg), and n is the protein content in the gel (%).

2.16. Statistical analysis

All of the above experiments were performed in three replications, and the final results were expressed as the mean ± standard error (SE). The data were analyzed using analysis of variance (SPSS 19.0, Chicago, IL, USA), and significant differences between groups were determined by Duncan's multiple comparisons, which was accepted at $P < 0.05$.

3. Results and discussion

3.1. pH

The results are shown in Table 1. Compared with the normal-salt group (T1), the pH of reduced-salt silver carp surimi significantly increased ($P < 0.05$) with the addition of SPI. However, with the supplemental increase of SPI, there was no significant difference in each treatment group ($P > 0.05$). This may be due to the pH of SPI itself (7.20 ± 0.07). A similar phenomenon was found in the research of Li, Sukmanov, Kang, & Ma, 2020.

3.2. Color

The effect of added SPI on the color of surimi gels is shown in Table 1. Compared to the T1, the L^* values of surimi gel had no significant effect with the increase of SPI ($P > 0.05$). On the contrary, the values of a^* and b^* showed a significant decrease and increase, respectively ($P < 0.05$), and the maximum was reached with the SPI addition of 6.0% (T4). In addition, there was no significant effect on the whiteness value;

Table 1
The effects of soy protein isolate on pH, L^*, a^*, b^* and whiteness of reduced-salt surimi gel.

Sample	pH	L^*	a^*	b^*	*Whiteness*
T1	6.82 ± 0.04^{b}	75.22 ± 0.31^{a}	1.13 ± 0.08^{c}	6.26 ± 0.10^{d}	74.41 ± 0.28^{a}
T2	6.91 ± 0.02^{a}	75.43 ± 0.11^{a}	0.80 ± 0.07^{b}	7.46 ± 0.18^{c}	74.31 ± 0.08^{a}
T3	6.93 ± 0.01^{a}	75.39 ± 0.14^{a}	0.43 ± 0.04^{a}	8.61 ± 0.16^{b}	73.92 ± 0.14^{a}
T4	6.96 ± 0.01^{a}	74.94 ± 0.12^{a}	0.38 ± 0.08^{a}	9.66 ± 0.05^{a}	73.14 ± 0.10^{b}

T1, 2.0% sodium chloride; T2, 1.0% sodium chloride, 2.0% soy protein isolate; T3, 1.0% sodium chloride, 4.0% soy protein isolate; T4, 1.0% sodium chloride, 6.0% soy protein isolate.
$^{a-d}$Different superscripts in the same column indicate significant differences ($P < 0.05$).

however, it decreased when 6% SPI was added (T4). This was probably linked to the nature color of SPI being predominantly yellow (Li et al., 2021). Li, Sukmanov, Kang, & Ma, 2020 also found the a^* value decreased and the b^* value increased with the increase of the supplemental level of SPI in their study of coprocessing pork meat batters with high pressure.

3.3. TPA

The results of TPA measurements showed SPI had a significant ($P < 0.05$) effect on the texture of reduced-salt silver carp surimi gels (Table 2). The hardness and chewiness of reduced-salt silver carp gel with the addition of SPI (T2, T3, and T4) were significantly higher ($P < 0.05$) than the control (T1), whereas the effect on springiness was not significant. On the one hand, the undenatured SPI aggregates rigidly filled the gel matrix due to the electrostatic screening effect of NaCl and formed a double network structure with myofibrillar proteins, resulting in increased gel hardness (Lv et al., 2021). Furthermore, the addition of SPI provides bonds that can bind to myofibrillar protein and form a stable network structure (Peng, Dayton, Quass, & Allen, 1982). The same result was found in Park (1994)—adding SPI (1.0%) helped the surimi to form a more rigid gel structure. Lee et al. (2017) reported the addition of nonmeat protein to reduced-salt pork myofibrillar protein can effectively improve the texture properties of the gel.

3.4. Gel strength

Gel strength to a large extent represents the aggregation ability of proteins during the heat-induced gelling (He et al., 2022). As described in Table 2, the addition of SPI significantly enhanced ($P < 0.05$) the gel strength of reduced-salt silver surimi, except for the component with 2.0%, and reached a maximum in the addition of 6.0%. In the 1.0% sodium chloride group (T2, T3, and T4), the SPI aggregates due to the presence of NaCl, and the undenatured SPI aggregates provide rigid filling of the myogenic fibrin gel matrix, thus enhancing the gel strength of the reduced-salt surimi (Lv et al., 2021). Furthermore, the interaction between the free soybean 11S component and myosin has been demonstrated (Peng et al., 1982), which enhances the formation of rigid gel structures (Feng & Xiong, 2002).

3.5. LF-NMR and MRI

There are three distinct peaks in Fig. 1A, representing bound water (T_{2b}), immobilized water (T_{21}), and free water (T_{22}) in the surimi gel. The highest peak among them is T_{21}, which represented the immobile water in the gel network, reflecting the WHC of the gels (He et al., 2022). In this study, the relaxation time of T_{21} and T_{22} accelerated significantly ($P < 0.05$) with increasing the SPI, indicating the bound water in the surimi gels with the addition of SPI was tighter (Kang et al., 2021). Typically, immobile water is defined as the water trapped in the myofibrillar protein network, whereas bound water is defined as those water molecules that are retained in the gel through hydrogen bonds (Wang et al., 2023). As shown in Fig. 1B, compared with the control group (T1), the increase of SPI could promote the proportion of bound water in the surimi gel, which was related to the fact the added SPI could provide additional functional groups to form hydrogen bonds with water molecules. The addition of SPI resulted in the increase of P_{21} and decrease of P_{22}, which reduced the effect of the decrease of sodium chloride on the water holding capacity of surimi gel, except for the T2 group. This is closely related to the excellent water holding capacity and structural properties of SPI (Niu, Xia, Wang, Kong, & Liu, 2018).

The water proton density of gels can be considered complementary to the LF-NMR, which provided an additional visual insight into the mobility state according to the depth of gels color on the pseudo-color map (Ye et al., 2022). As shown in Fig. 1C, the water distribution of reduced-salt silver carp gel was different from the amount of SPI. Compared with the control (T1), the hydrogen proton density of the salt reduction group (T2) with SPI addition amount was the lowest, whereas the hydrogen proton density of the treatment group with 4.0% (T3) and 6.0% (T4) addition was relatively high. This may be related to the addition of SPI, which itself has a certain ability to absorb moisture and moisturize, in addition to crosslinking with myofibrillar protein to participate in the formation of the gel network, thereby forming a stable gel structure and binding more water (Niu, Li, Han, Liu, & Kong, 2017). However, compensating for the effect of reduced sodium chloride content on surimi gel may require an increase in the addition of SPI, so the hydrogen proton density at T2 (2.0% SPI) was lower and the binding water capacity of the gel was reduced. This result was also consistent with the WHC.

3.6. WHC

The WHC of surimi gels is presented in Fig. 1D. SPI addition significantly influenced ($P < 0.05$) the WHC of the reduced-salt silver carp gel. Compared with the control group (T1), the centrifugal loss in the T2 group was significantly increased ($P < 0.05$). When the sodium chloride content is lower than 0.3 M and the addition of SPI is lacking at this time, the salt-soluble proteins cannot be fully solubilized and fewer proteins participate in the gel network formation during heating, resulting in a loose gel structure and a decreased WHC (Chin, Go, & Xiong, 2009). Ramírez et al. (2007) found a small amount of whey protein concentrate supplementation is not enough to compensate for the impact of the decrease in salt content (2% to 1%), which then results in the increase of expressible water. Interestingly, with the increase of SPI, its excellent water absorption ability was exerted, and the WHC of the reduced-salt surimi gel was significantly improved ($P < 0.05$), which effectively reduced the influence of the decrease of sodium chloride content on the water holding capacity of surimi gel (Li et al., 2021). This result was again consistent with the results of low-field NMR.

3.7. Rheological properties

Temperature sweep can observe the formation of a gel network during the gelation transition of surimi samples. In general, G′ presents three stages during heating denaturation (Kang et al., 2021). As shown in Fig. 2A, samples exhibited typical dynamic rheological curves during heating from 24 °C to 90 °C. First, there is a slow downward trend around 24 °C–40 °C, which might be due to protein unfolding. Second, the G′ value drops rapidly and to a minimum of around 55 °C as the temperature gradually rises, which may be related to the breaking of hydrogen bonds and the aggregation of myofibrillar protein (Shi, Wang, Chang, Wang, Yang, & Cui, 2014). In addition, endogenous proteolytic enzymes may also induce protein degradation and disrupt the gel

Table 2
The effect of soy protein isolate on TPA and gel strength of reduced-salt surimi gels.

Sample	Hardness (g)	Springiness	Chewiness (g)	Gel strength (g·mm)
T1	4840.68 ± 63.96^{d}	0.921 ± 0.01^{a}	3507.57 ± 45.51^{d}	2943.89 ± 94.79^{c}
T2	5777.69 ± 94.31^{c}	0.922 ± 0.01^{a}	4017.27 ± 83.32^{c}	3119.21 ± 58.53^{c}
T3	6187.39 ± 68.95^{b}	0.922 ± 0.01^{a}	4248.03 ± 39.63^{b}	3581.05 ± 74.49^{b}
T4	6677.17 ± 84.52^{a}	0.920 ± 0.01^{a}	4446.61 ± 36.17^{a}	3846.31 ± 67.52^{a}

T1, 2.0% sodium chloride; T2, 1.0% sodium chloride, 2.0% soy protein isolate; T3, 1.0% sodium chloride, 4.0% soy protein isolate; T4, 1.0% sodium chloride, 6.0% soy protein isolate.
$^{a-d}$Different superscripts in the same column indicate significant differences ($P < 0.05$).

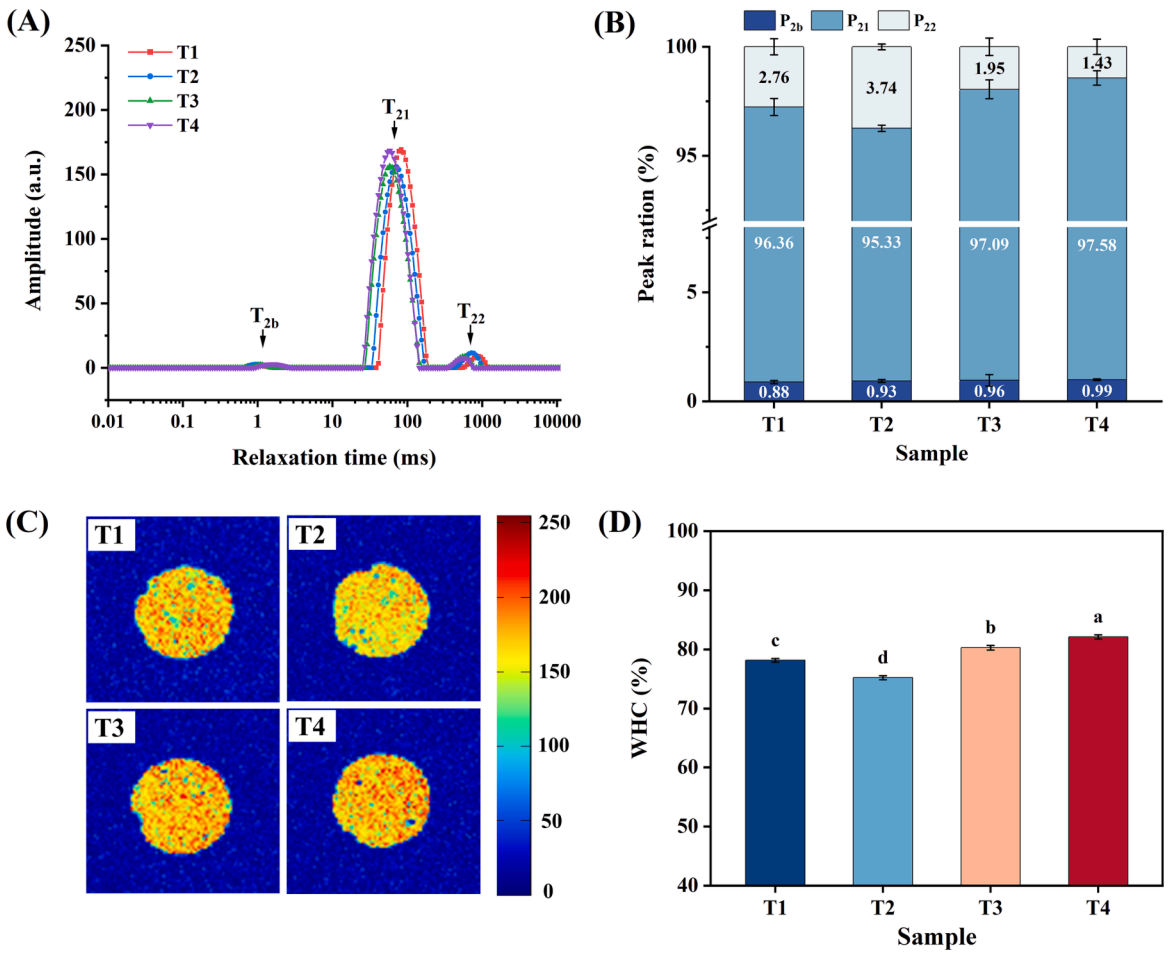

Fig. 1. The curves of relaxation time (A), moisture distribution (B), pseudo color map of the water proton density (C) and WHC (D) of cooked reduced-salt silver carp surimi with various amounts of soy protein isolate. T1, 2.0% sodium chloride; T2, 1.0% sodium chloride, 2.0% soy protein isolate; T3, 1.0% sodium chloride, 4.0% soy protein isolate; T4, 1.0% sodium chloride, 6.0% soy protein isolate.

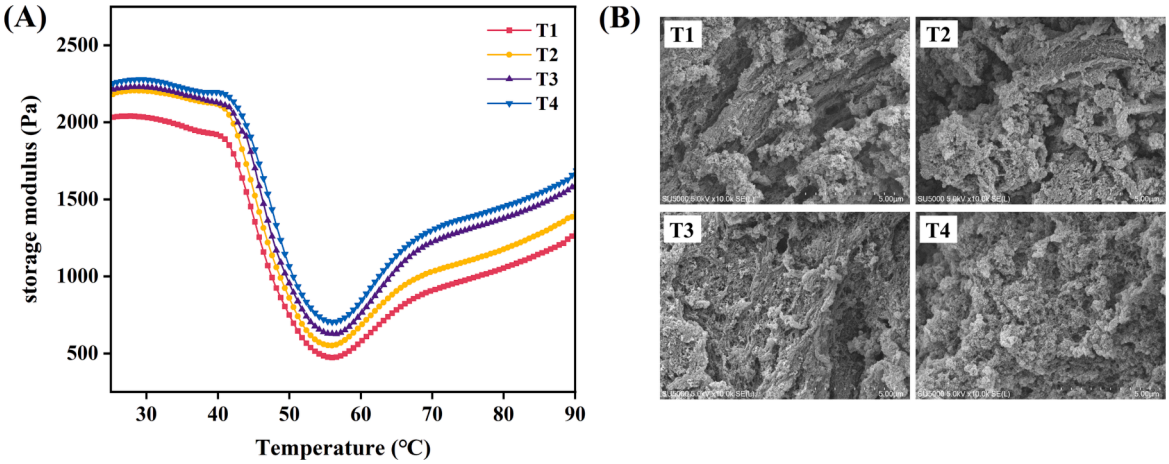

Fig. 2. Storage modulus of surimi (A) and microstructure of surimi gel (B) with different amounts of soy protein isolate. T1, 2.0% sodium chloride; T2, 1.0% sodium chloride, 2.0% soy protein isolate; T3, 1.0% sodium chloride, 4.0% soy protein isolate; T4, 1.0% sodium chloride, 6.0% soy protein isolate.

network structure, resulting in a decrease in G′ (Fang, Shi, Ren, Hao, Chen, & Weng, 2021). Finally, when the sample was further heated, G′ increased again, and the protein formed a stable gel structure after aggregation and crosslinking under heat induction (Zhao, Han, Sun, Liu, Liu, & Kong). Studies have shown the hardness of cooked gels is positively correlated with the terminal G′ value (Zhu, Kang, Ma, Xu, & Zhou, 2018), and at 90 °C the G′ values of T1, T2, T3, and T4 were 1281 Pa, 1416 Pa, 1598 Pa, and 1687 Pa, respectively, indicating a gradual increase with the addition of SPI, which is consistent with the results for hardness (Table 2).

3.8. SEM

Microstructures of surimi gels with and without SPI are presented in Fig. 2B. Results showed the addition of SPI promoted the formation of a tighter structure of surimi gel, but the T2 exhibited a disordered gel network and high porosity. An appropriate SPI facilitated the construction of more compact gel networks with significantly reduced porosity and pore size. However, the SPI addition of 2.0% was insufficient to compensate for a 1.0% sodium chloride reduction to form a stable and ordered gel structure, and the SPI was irregularly present in the gel structure as a physical filling. Previous studies have found it is necessary to add more than 0.3 M sodium chloride to dissolve the myofibrillar protein (Xiong, 2012). If the addition amount of sodium chloride is reduced, the solubility and functional properties of myofibrillar protein will be affected by the decrease of ionic strength, resulting in deterioration of the quality of the gel formed (Gao, Wang, Mu, Shi, & Yuan, 2018). SPI can make the gel network structure denser by promoting the α-helix to β-sheet and random coil transformation in low-sodium pork meat batters (Li, Sukmanov, Kang, & Ma, 2020). Lv et al. (2021) also found there were many pore structures in the myofibrillar protein gel formed by 1.2% SPI addition, resulting in loose structure and decrease quality. Therefore, the formation of myofibrillar gel requires the addition of a sufficient amount of SPI to have a stable network structure (Chin et al., 2009).

3.9. Total SH content

The change of total SH group reflects the change of protein structure. As shown in Fig. 3A, the total SH content in reduced-salt surimi was positively correlated with the increase of the supplemental level of SPI, which was associated with decreased salt content and the addition of SPI. This may be caused by reduced sodium chloride content. A high concentration of salt content can promote the expansion of the myofibrillar protein structure, causing internal SH groups to be exposed to the molecular surface, resulting in the exposure of internal SH groups on the molecular surface and providing more possibilities for the formation of disulfide bonds, which in turn results in a decrease in the total SH content in the control group (Wang et al., 2023). In addition, the main substances constituting SPI are β-glycine (7S) and glycine (11S), which contain a small number of SH groups, especially the six subunits of the glycine molecule that are connected by acid subunits and basic subunits with disulfide bonds, and it is worth adding that each subunit also contains two or three cysteine and cysteine side-chain residues. As a result, as the SPI content increased, the total SH content in the surimi also increased (Li et al., 2021). In the research of Li et al. (2021), it was also found that, with the increase of SPI concentration, the total sulfhydryl content increased.

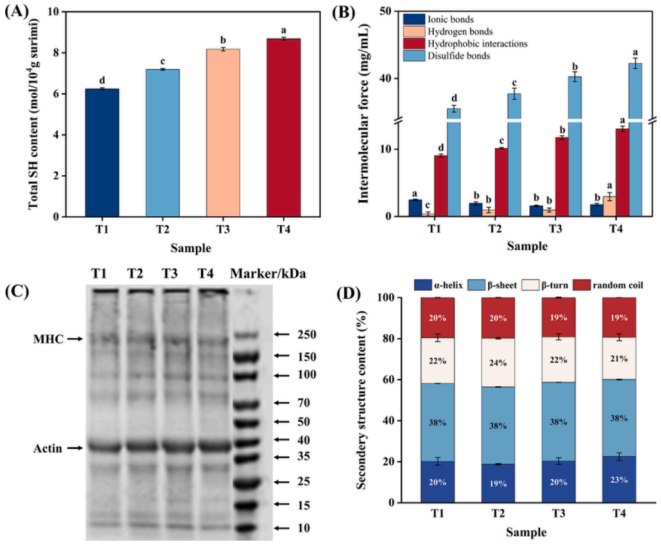

Fig. 3. The total SH content (A), intermolecular force (B), SDS-PAGE pattern (C) of surimi/surimi gel and secondary structure content (D) of surimi with different contents of soy protein isolate. T1, 2.0% sodium chloride; T2, 1.0% sodium chloride, 2.0% soy protein isolate; T3, 1.0% sodium chloride, 4.0% soy protein isolate; T4, 1.0% sodium chloride, 6.0% soy protein isolate.

3.10. Chemical interaction

The chemical forces in the formation of surimi gels mainly include hydrogen bonds, ionic bonds, hydrophobic interactions, and disulfide bonds (Reed & Park, 2011). The formation of the gel network was the result of the balance of mutual attraction and repulsion between proteins (Xiong et al., 2021). Fig. 3B shows the hydrophobic interactions and disulfide bonds were the main components of the chemical forces, indicating they were the main forces for the formation of the surimi gel. This is because, during the heating process of the protein, the hydrophobic groups are exposed, resulting in enhanced hydrophobic interactions where more sulfhydryl groups are converted into disulfide bonds (Li et al., 2019). Compared with the normal-salt group, the ionic bond content significantly decreased ($P < 0.05$) in the reduced-salt group, whereas the hydrogen bond content, hydrophobic interaction force, and disulfide bond content increased significantly ($P < 0.05$) with the addition of SPI. The main proteins in the soy protein isolate—soybean β-glycine (7S) and glycine (11S)—were thermally denatured at 75 °C and 90 °C, respectively (Niu et al., 2018). Thus, the increase in hydrophobic interactions may be caused by the addition of exogenous proteins, whereas the increase in disulfide bonds also indicated the addition of SPI enhances chemical crosslinking in reduced-salt surimi gels (Lv et al., 2021). This may be due to the addition of SPI resulting in increased protein content in surimi, and then the molecular interaction between SPI and SPI and between SPI and myofibrillar protein were increased. Previous research results also indicated thermal aggregation of soybean protein is mainly carried out through hydrophobic interaction and disulfide bond formation and becomes the main force for maintaining the gel structure (Liu & Hsieh, 2007).

3.11. SDS-PAGE

SDS-PAGE as affected by the addition of SPI (2.0%, 4.0%, and 6.0%) to surimi gels is shown in Fig. 3C. As can be seen from the results in the figure, compared with T1, the bands of myosin heavy chain (MHC) and actin were deeper and thicker and the polymer bands at the top also gradually became shallower when the sodium chloride was reduced from 2.0% to 1.0%. This indicates a decrease in crosslinking between myofibrillar proteins. Hamm (1986) found that, when sodium chloride is added in<0.3 M, the solubility of myofibrillar protein decreases, and MHC plays an important role in the gel properties of myofibrillar protein. Therefore, this also explained why the poor quality of surimi gel formed in T2. However, in T4, the MHC band continues to become shallower, and the polymer band at the top deepens significantly. This may be due to crosslinking between SPI and the myofibrillar protein, in addition to increased crosslinking between myofibrillar proteins due to increased pH and more protein being involved in the formation of the gel network. In addition, the addition of soy protein isolate deepened the bands at 70 kDa and 100 kDa, which may be related to the fact that the α′, α, and β subunits of SPI were not involved in the construction of the gel network (Zhang et al., 2023).

3.12. FT-IR

The relative content of the myofibrillar protein secondary structure is the key to the formation of a gel network structure (Wang et al., 2023). As shown in Fig. 3D, compared with the control (T1), the decrease of sodium chloride resulted in the decrease of α-helix content and increase

of random coil content. However, with the increase of SPI, the protein changed from a disordered random coil and β-turn to an ordered α-helix, which may be related to the growth of pH in surimi (6.82–6.96). An increase in pH indicates an increase in the net negative charge in the system, which provides more opportunities for proteins to unfold and an increase in electrostatic interactions between proteins (Li, Zhang, Lu, & Kang, 2021). Therefore, during thermal denaturalization, due to the increase of hydrogen bond content, more α-helix structures were transformed into β-sheet, and the degree of crosslinking between proteins increased, forming a stable spatial network structure (Kang et al., 2016). Although this effect may not be predominant, it can still improve the quality of the reduced-salt surimi gel in conjunction with the physical filling of SPI.

3.13. In vitro pepsin digestibility

The bioavailability of food is usually assessed by the degree of pepsin digestion (Fang, Xiong, Hu, Yin, & You, 2019). The effect of SPI on the digestibility of reduced-salt silver carp gel is shown in Fig. 3B. Except for the T2, the addition of SPI has no significant effect ($P > 0.05$) on the digestibility of reduced-salt silver carp gel, but it was still higher than that of the control (T1). Therefore, the digestibility of the T1, T3, and T4 was lower than the T2 due to increased crosslinking with myofibrillar protein. Previous studies have found foods with a high degree of crosslinking may limit the diffusion of enzymes in the food matrix due to the presence of dense internal structures and polymers and reduce the accessibility of enzymes to digestible sites, leading in turn to reduced digestibility (Fang et al., 2019; Li & Damodaran, 2017).

3.14. Mechanism analysis of SPI to improve the gel quality of low-salt silver carp surimi

Based on the relevant research reports and results of this experiment, we present a schematic model to explain the mechanism of SPI addition to improve the quality of low-salt surimi gel (Fig. 4B). Previous studies have shown myofibrillar protein is a salt-soluble protein, and when the salt content in the system reaches more than 0.3 M, the protein gradually expands, preferably around 0.6 M (Hamm, 1986). Therefore, in the process of meat processing, it is usually necessary to add more than 2.5% sodium chloride to form a good gel structure. In this experiment, when the sodium chloride content in the surimi gel system decreases, myofibrillar protein cannot be completely stretched and expanded, the protein exists in a folded state, and the internal reaction group is tightly wrapped and cannot be opened, and because the increase of temperature during thermal processing cannot occur with complete unfolding, the interaction between proteins becomes weaker, the ability to participate in the formation of three-dimensional network gel structure becomes worse, and fewer proteins participate in the formation of the gel matrix. Interestingly, this "bad" gel structure improved when soy protein isolate was added. First, this is because SPI itself has good functional properties, with a certain ability to absorb, expand, and heat denaturation to form a gel, so with the addition of SPI to the gel matrix, SPI exists in the gel network in a physically filled way to enhance the firmness of the gel structure and enhance its hardness (Kang, Chen, & Ma, 2016). Second, SPI is mainly composed of 7S globulin and 11S globulin, of which 7S globulin has a certain emulsifying ability, whereas 11S has good gel ability (Bainy, Tosh, Corredig, Woodrow, & Poysa, 2008; Manion, & Corredig, 2006). Previous studies have shown soy 11S globulin can bind to myofibrillar proteins to form a stable gel network structure, bind more free water, and increase product yield (Candogan, & Kolsarici, 2003). In this study, SDS-PAGE results, chemical force changes, and secondary structure changes all confirm this hypothesis. Third, when the amount of SPI increased, the pH value in surimi gradually increased, and myofibrillar protein deviated from the isoelectric point, causing the expansion of the myofibrillar protein structure, and even dissociation to form small molecules (Li, Zhang, Lu, & Kang, 2021), which is conducive to the formation of a stable gel network structure.

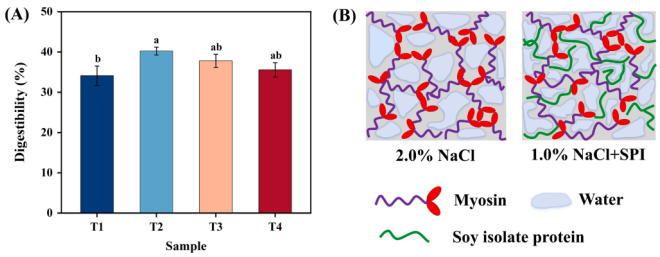

Fig. 4. In vitro pepsin digestibility (A) and schematic mechanism for the interaction (B) of surimi gel with different contents of soy protein isolate. T1, 2.0% sodium chloride; T2, 1.0% sodium chloride, 2.0% soy protein isolate; T3, 1.0% sodium chloride, 4.0% soy protein isolate; T4, 1.0% sodium chloride, 6.0% soy protein isolate.

4. Conclusion

The results showed SPI improved the gel quality degradation problem of reduced salt chub gel mainly through physical action. Compare with the control, the addition of SPI significantly improved the b^* value, hardness, chewiness, and gel strength of the gels and the G′ value at 90 °C. This may be related to the increase of hydrophobic force and disulfide bond content in the gel. Furthermore, the SPI enhanced the three-dimensional network structure of the myofibrillar protein gel under low salt conditions through physical and chemical means. As a result, the percentage of P_{21} in the surimi gel increased and the percentage of P_{22} decreased. Combined with the results of in vitro pepsin digestion, the addition of SPI improved the in vitro digestibility of pepsin in salt-reduced surimi gel, but the effect was not significant except in T2 (2.0% SPI). In conclusion, the replacement of sodium chloride with SPI can reduce the salt content in surimi products while maintaining good texture properties, gel quality, and in vitro digestibility.

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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