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Synergistic effects of ultrasound and soluble soybean polysaccharide on frozen surimi from grass carp

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ABSTRACT

Ultrasound and water soluble soybean polysaccharide (SSPS) were applied during the freezing of grass carp surimi. Ultrasound-assisted immersion freezing (UF) process was observed from 0 °C to −15 °C. Based on characteristic freezing time, tempering-stage freezing rate and the quality change of surimi after a 14-day storage period, the optimal sonication was performed 5 times at 300 W, for 10 s, with 40 s intervals. The cryoprotective effects of SSPS content on surimi myofibillar protein were subsequently investigated during 28-day frozen storage at −18 °C. The Ca^{2+}-ATPase activity, total sulphydryl content, active sulphydryl content, salt extractable protein content, whiteness and water-holding capacity of frozen surimi were determined after adding SSPS (0%, 1%, 3% and 5%). The results showed that synergism of SSPS and UF occurred. Consequently 3% SSPS was best for mitigating the protein denaturation during processing.

1. Introduction

Freezing is a well-known preservation method widely used in the food industry. Conventional methods include air blast freezing, immersion freezing, plate contact freezing, fluidised-bed freezing, circulating brine freezing and liquid nitrogen freezing (Heldman et al., 2006). These methods have been criticised for their high energy consumption, slow freezing rate and uneven distribution of ice crystals. However, immersion freezing possesses some advantages such as a high-heat transfer coefficient, resulting in, good freezing quality and energy savings. In recent years new freezing methods have been developed such as the ultrasound-assisted technologies for product or process improvement (Hu et al., 2013; Zheng and Sun, 2005). Ultrasonication is generally considered safe, non-toxic and environmentally sound (Awad et al., 2012; Kentish & Ashokkumar, 2011).

Surimi is a wet concentrate of myofibrillar protein that can be produced from the muscles of deboned fish after removing blood, lipids, sarcoplasmic proteins and other impurities with cold water (Alakhrash et al., 2016). The production of frozen surimi has been growing due to an increase in consumer demand. However, freezing of surimi causes denaturation and aggregation which may result in a loss of functional properties (Bueno et al., 2013; Leygonie et al., 2012; Shenouda, 1980). Many researchers have reported that surimi protein denaturation can be inhibited by cryo-protectants such as low-molecular-weight sugars, polyols, protein hydrolysates, starch hydrolysates, polyols, and oligosaccharides (Chen et al., 2013; Wang et al., 2014; Xie et al., 2017). Water soluble soybean polysaccharide (SSPS) may be used for surimi products due to its low sweetness and low calorific value (Gao et al., 2017a, 2017b). The aim of this work was to investigate the effects of ultrasound-assisted immersion freezing (UF) with SSPS on freezing process and protein denaturation during the freezing of grass carp (*Ctenopharyngodon idellus*) surimi.

2. Materials and method

2.1. Materials and chemicals

Fresh grass carp were purchased from a local supermarket (Guangzhou, China). SSPS was obtained from Guangzhou Huahui Bioindustrial Co., Ltd. (Guangzhou, China). Ca^{2+}-ATPase test kit and protein quantitative test kit, were purchased from Institute of Nanjing Jiancheng Bioengineering (Nanjing, China). The 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) was purchased from Shanghai Aladdin biochemical technology Co., Ltd. (Shanghai, China). The bovine serum albumin and sodium dodecyl sulphate (SDS) were purchased from Shanghai Boao Biotechnology Co., Ltd. (Shanghai, China). The disodium ethylenediaminetetraacetate (EDTA-2Na) was purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Trihydroxymethylaminomethane (Tris), maleic acid and sodium dihydrogen phosphate were purchased from Sinopharm Group Chemical

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Reagent Co., Ltd. (Guangzhou, China). Urea and disodium hydrogen phosphate were purchased from Guangzhou Jinhua Chemical Reagent Co., Ltd. (Guangzhou, China). Other chemical reagents were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China).

2.2. Surimi preparation

Fresh grass carp were purchased and transported in an ice box to the laboratory within 1 h. Grass carp were washed thoroughly with chilled water. The head, scale and viscera of grass carp were removed, and the meat was picked out manually. Only white meat was picked, it was then carefully washed with ice water (1:2 w/w) twice. The meat was cut into fillets, minced and washed with water below 10 °C at the minced meat-solution ratio of 0.2 kg/L three times (twice with distilled water, then once with 1.5 g/L NaCl aqueous solution, stirred for 5 min each time and left to stand for 3 min). The surimi was obtained after dewatering with gauze. Every piece of sample was 15 g and the thickness was 1 cm. Samples were prepared by adding SSPS at 1%, 3% and 5% of surimi (mass weight), respectively. Surimi without SSPS was used as a control. Samples were separately packaged in polyethylene bags and kept in a refrigerator at 4 ± 1 °C for 8 h to achieve uniform initial temperature. The samples were frozen using a coolant at −18 °C. Freezing was considered to be complete when the centre temperature of surimi samples reached −15 °C. The samples were transferred to a freezer at −18 °C and kept there until analysis at 0, 7, 14, 21 and 28 days.

2.3. Ultrasonic freezing process

The ultrasonic equipment comprised of an ultrasonic treatment device, ultrasonic refrigeration cycle system and temperature measurement system (Fig. 1). The ultrasonic tank was filled with a mixture of ethylene glycol and water in the proportion of 2:1 (v:v), which was cooled to the desirable temperature by circulating the fluid from a refrigerated circulator. Surimi samples were immersed into the circulating coolant and the centre temperatures were probed by T-type thermocouples connected to a data acquisition system. Temperature readings were recorded at 5 s intervals with a data logger. The circulator temperature was set to reach an average solution temperature of −18 ± 0.5 °C.

To characterise freezing efficiency, characteristic freezing time (t_{cf}) and tempering-stage freezing rate were used to evaluate the freezing process. Characteristic freezing time represented the time at which the centre temperature of the sample had fallen to a temperature at which 80% of the total water content was converted to ice (Zaritzky, 2006). In this research the temperature for determining characteristic freezing time ranged from 0 °C to −5 °C. Most of the water inside the food matrix was converted into ice crystals by phase change. Tempering stage, is when the small amount of remaining water inside the matrix continues to freeze. In this study the freezing rate of tempering-stage is expressed as the temperature dropping rate from −5 °C to −15 °C in the centre of surimi sample.

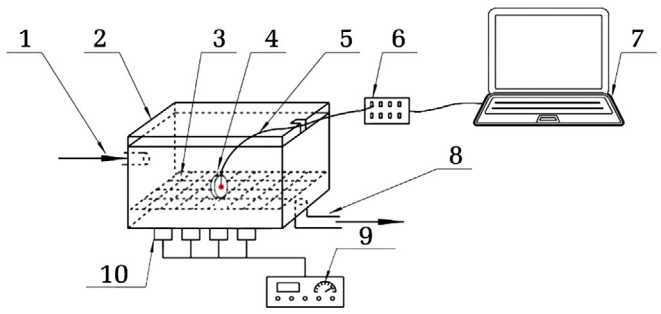

Fig. 1. Schematic diagram of ultrasound-assisted freezing equipment 1: Circulating freezing liquid inlet; 2: Ultrasonic processor; 3: Special rack bar; 4: Experimental sample placement; 5: T-type thermocouple; 6: Multichannel data recorder; 7: Computer; 8: Circulating cryogenic liquid outlet; 9: Ultrasonic control panel; 10: Ultrasonic transducer.

2.4. Application of power ultrasound

Ultrasound was applied at a fixed frequency of 28 kHz. Ultrasound application during the phase-transition stage of the freezing process has been shown to significantly increase the freezing rate (Li and Sun, 2002; Hu et al., 2013). Thus ultrasound was applied when the centre temperature of grass carp surimi reached −0.5 °C. The ultrasonic treatment was carried out at power levels of 180, 300, 420 and 540 W. Each treatment was conducted for 10 s, samples were then rested for 40 s, and the treatment repeated 5 times. The influence of ultrasonic exposure time on the surimi freezing process was studied when ultrasonic treatment was carried out for 5, 10, 15 and 20 s at the optimal power level. Each treatment was conducted for specific time, samples were then rested for 40 s, and the treatment repeated 5 times. The influence of 5% SSPS on the temperature of surimi during freezing process was investigated by immersion freezing and ultrasound-assisted immersion freezing. Ultrasound-assisted immersion freezing was carried out under the optimal condition obtained as above. Ultrasonic exposure time was referred to every treatment time (Li and Sun, 2002).

2.5. Determination of Ca^{2+}-ATPase activity

Myofibrillar proteins were prepared and analysed as described previously (Ma et al., 2015) with minor modifications. Myofibrillar proteins were diluted ten times with 0.6 M KCl. The Ca^{2+}-ATPase activity of myofibrillar protein was determined according to the instruction of Ca^{2+}-ATPase kit. Briefly, 100 μL of the diluted sample were mixed with the chemical reagent of Ca^{2+}-ATPase kit and incubated at 37 °C for 10 min. The reaction mixture was centrifuged at 3500 rpm for 10 min and the supernatant was obtained. Then 150 μL of the supernatant were mixed with the chemical reagent of Ca^{2+}-ATPase kit and incubated at 25 °C for 5 min. The absorbance value was recorded at 636 nm. The Ca^{2+}-ATPase activity is expressed as the mole number of inorganic phosphorus which is produced by a milligram of protein for 1 min at 25 °C (μmol Pi/mg pro·min).

2.6. Determination of total sulphydryl content and active sulphydryl content

Total sulphydryl content of myofibrillar protein solution was determined using 5′,5-dithiio-bis (2-nitrobenzoic acid) (DTNB) according to the method of Turgut et al. (2016) with a slight modification. Firstly 0.5 mL of myofibrillar solution (4 mg/mL) was mixed with 4.5 mL of 0.2 M Tris–HCl buffer (pH 6.8, containing 8 M urea, 2% SDS and 10 mM EDTA). Then 4 mL of the mixture was mixed with 0.4 mL of 0.1% DTNB in 0.2 M Tris-HCl (pH 8.0) and incubated at 40 °C for 25 min. The absorbance of total SH at 412 nm was measured using a UV-1800 Spectrophotometer (Shimadzu, Japan). A blank was conducted by replacing the sample with 0.6 M KCl. Total SH content is calculated from the absorbance using the molar extinction of 13,600 $mol^{-1}cm^{-1}$ and expressed as 10^{-5} mol/g protein. Total SH content is calculated by equation (1):

$$\text{Total SH content (nmol/mg)} = A \times D/\xi \times C. \quad (1)$$

Where A is the absorbance at 412 nm, C is the concentration of myofibrillar protein, ξ is the molar extinction coefficient of 13,600/mol·cm/L, and D is the dilution factor.

Active sulphydryl content of myofibrillar protein solution was determined using 5′,5-dithiio-bis (2-nitrobenzoic acid) (DTNB) according to the method of Turgut et al. (2016) with a modification. Firstly 0.5 mL of myfibrillar solution (4 mg/mL) was mixed with 4.5 mL of 0.2 M Tris–HCl buffer (pH 6.8, containing 2% SDS and 10 mM EDTA). Then

4 mL of the mixture was mixed with 0.4 mL of 0.1% DTNB in 0.2 M Tris-HCl (pH 8.0) and kept in a refrigerator at 4 °C for 1 h. The absorbance of active SH at 412 nm was measured and a blank was conducted by replacing the sample with 0.6 M KCl. Active SH content is calculated from the absorbance using the molar extinction of 13,600 $mol^{-1}cm^{-1}$ and expressed as 10^{-5} mol/g protein. Active SH content is calculated by equation (2):

$$Active\ SH\ content\ (nmol/mg) = A \times D/\xi \times C. \quad (2)$$

Where A is the absorbance at 412 nm, C is the concentration of myofibrillar protein, ξ is the molar extinction coefficient of 13,600/mol·cm/L, and D is the dilution factor.

2.7. Determination of salt extractable protein (SEP) content

The SEP content of surimi was determined according to the method of Mi et al. (2013). Surimi (2 g) was homogenised with 20 mL of phosphate buffer (5 mmol/L NaH_2PO_4, 15 mmol/L Na_2HPO_4, pH = 7.5). After standing for 5 min, the slurry was centrifuged at 4000 rpm for 10 min. The supernatant, which contained sarcoplasmic proteins, was poured away. The entire procedure was repeated a second time. The washed muscle residue was homogenised with 20 mL of Weber-Edsall solution (0.6 mol/L KCl, 0.04 mol/L $NaHCO_3$ and 0.01 mol/L Na_2CO_3). Extraction was performed at 4 °C for 20 h and then centrifuged at 6000 rpm for 10 min. SEP in the supernatant was determined by the Biuret method (Sadouki et al., 2006).

2.8. Determination of whiteness

The colour of surimi was determined according to the method of Xie et al. (2017) by measuring L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness) values. The colour of surimi was determined by automatic measurement spectrophotometer (Model CR-400, Konica Minolta Co. Ltd). The L^*, a^* and b^* values of surimi samples were directly determined, and three replications were carried out for each test. The *whiteness* is calculated as equation (3) (Fujii et al., 1973):

$$Whiteness = 100 - [(100 - L^*)2 + a^*2 + b^*2]1/2 \quad (3)$$

2.9. Determination of water-holding capacity

The water-holding capacity (WHC) of surimi was determined according to the method of Vega-Warner et al. (1999) with a modification. Briefly, the WHC of surimi was evaluated using a centrifuge method. A 2 g sample of surimi with 2 layers of Whatman filter paper were placed in a centrifuge tube and centrifuged at 4000 rpm for 10 min at 4 °C. The WHC is calculated as the percentage of the surimi weight after centrifugation divided by the surimi weight before centrifugation.

2.10. Statistical analysis

In this study three replications were carried out for each test. SPSS software (version 16.0) was used to analyse the experimental data. Significant differences in the treatments were assessed by one-way analysis of variance (ANOVA, 95% significance level) and Duncan's test. All experiment data were calculated as mean ± standard deviation (SD). Differences between means of data were compared by Duncan's test and least significant difference (LSD) ($p < 0.05$). Origin 9.1 software was used for drawing.

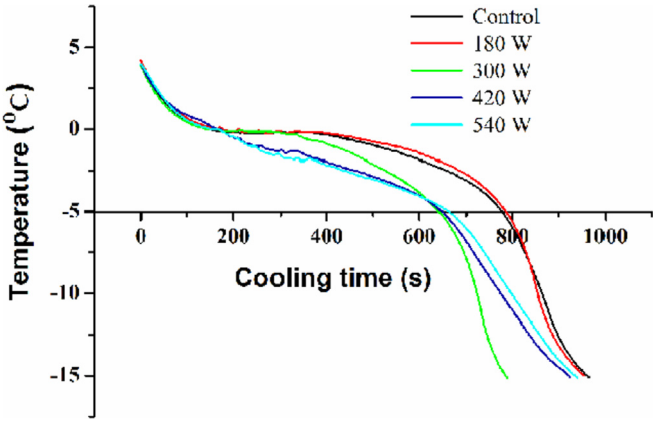

Fig. 2. Influence of ultrasonic power levels on temperature of surimi by ultrasound-assisted immersion freezing. Exposure time 10 s, interval time 40 s, ultrasound frequency 5 times, and coolant temperature of −18 °C.

3. Result and discussion

3.1. Ultrasonic freezing

3.1.1. Influence of ultrasonic power on surimi freezing process

3.1.1.1. Freezing curves. Fig. 2 illustrates that the freezing curve for power of 180 W was close to the immersion freezing curve. In addition, the freezing curve for power of 420 W was close to that for power of 540 W. The temperature of surimi dropped fastest from 0 °C to −15 °C using 300 W of power.

3.1.1.2. Freezing parameters. Compared with the immersion freezing in Table 1, the freezing process of the surimi samples was significantly improved ($p < 0.05$) at the power levels of 300, 420 and 540 W. The characteristic freezing time of surimi was increased by 22.04%, 21.79% and 19.77%, respectively. However there was no significant difference in the characteristic freezing time between 300, 420 and 540 W. Fig. 2 illustrates that at about 610s the curves of 300, 420 and 540 W intersect. Such observations indicate that during the previous phase of characteristic freezing the higher power of ultrasound could promote nucleation and heat conduction, leading to high efficient heat transfer to refrigerant. Then the temperature of surimi treated with 300 W dropped faster than other surimi samples. During the tempering stage the freezing rates of surimi for the power levels of 420 and 540 W were reduced by 30.19% and 32.08%, as compared with the immersion freezing. Due to the high power of 420 and 540 W, the more residual heat which was accumulated in the internal matrix of surimi could not be transferred to refrigerant although ultrasound was not performed during the tempering stage. The heat resulted in crushing effect on the

Table 1
Effects of ultrasound power on freezing parameters of surimi during freezing process and myofibrillar protein content of surimi after 14-day frozen storage.

Ultrasonic power (W)	Characteristic freezing time (s)	Tempering-stage freezing rate (°C/s)	14d Myofibrillar protein content (mg/mL)
Control	642.50 ± 17.08^a	0.053 ± 0.002^b	3.41 ± 0.15^c
180	644.70 ± 12.79^a	0.059 ± 0.002^a	3.69 ± 0.12^b
300	500.89 ± 21.77^b	0.067 ± 0.001^a	4.34 ± 0.21^a
420	502.50 ± 14.43^b	0.037 ± 0.001^c	3.96 ± 0.14^b
540	515.50 ± 13.54^b	0.036 ± 0.001^c	3.84 ± 0.15^b

Data were expressed as the mean ± standard deviation. The different lowercase letters (a–c) indicated a significant difference ($p < 0.05$) based on the Duncan's test and least significant difference (LSD). Ultrasonic conditions: Exposure time 10 s, interval time 40 s, ultrasound frequency 5 times, and coolant temperature of −18 °C.

formed ice crystals and even the large ice crystals formed during the late phase of freezing process, which could also be distributed unevenly in the internal matrix of surimi. In addition, the thermal conductivity of ice ($2.25\ W\ m^{-1}K^{-1}$) (Evans, 2008) is higher than that of fish ($< 0.549\ W\ m^{-1}K^{-1}$) (Rahman, 1993). A large number of uniform and fine ice crystals were formed inside the surimi treated with 300 W, leading to high efficiency of heat conduction. Therefore the tempering-stage freezing rate of surimi at 300 W was faster than those at 420 and 540 W.

3.1.1.3. Myofibrillar protein. Table 1 shows that the application of ultrasonic power had a significant effect ($p < 0.05$) on the myofibrillar protein content for surimi samples after 14 days of frozen storage. The myofibrillar protein content of surimi was 4.34 mg/mL at 300 W. It was significantly higher ($p < 0.05$) than those of other samples.

The quality of frozen foods theoretically depends on the size of ice crystals. Large ice crystals can result in mechanical damage and drip loss, reducing product quality. The size and location of ice crystals are closely related to freezing rate. Rapid freezing generally produces small intracellular ice, while slow freezing produces large ice crystals (Comandini et al., 2013; Hu et al., 2013; Islam et al., 2014; Jambrak et al., 2014). Therefore, according to the characteristic freezing time, the tempering-stage freezing rate and the quality change of frozen product, the optimal ultrasonic power was 300 W in this experiment.

3.1.2. Influence of exposure time on surimi freezing process by power ultrasound

3.1.2.1. Freezing curves. Fig. 3 illustrates the freezing process and indicates that freezing was improved when the exposure time of ultrasound was 10, 15 and 20 s. However the curve which represents exposure time of 5 s was very similar to that of the control. The temperature of surimi was seen to drop fastest from 0 °C to −15 °C with an exposure time of 10 s.

3.1.2.2. Freezing parameters. As shown in Table 2, the characteristic freezing time was significantly reduced by 22.14%, 18.03% and 15.18% ($p < 0.05$) when surimi was exposed to ultrasound for 10, 15 and 20 s, respectively. Surimi required the shortest characteristic time to reach the final temperature with an exposure time of 10 s. The shortest characteristic freezing time indicated that the maximum ice crystal formation belt was quickly passed, thus the ice crystals were small and distributed more evenly in the tissue. Compared with immersion freezing, the tempering-stage freezing rate of surimi samples was significantly increased by 26.41%, 9.43%, 54.72% ($p < 0.05$) when exposure time was 10, 15 and 20 s, respectively.

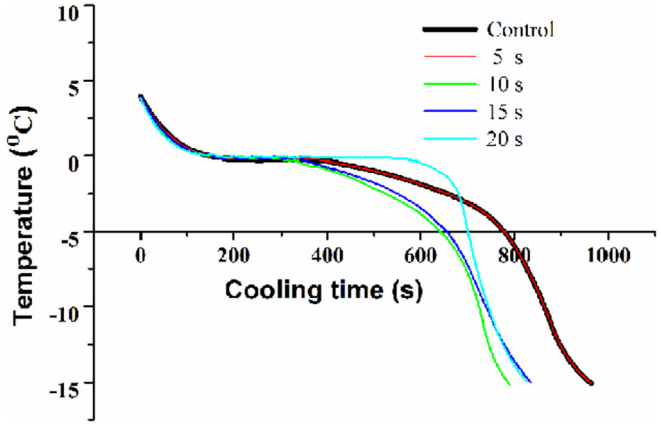

Fig. 3. Influence of exposure time on temperature of surimi by ultrasound-assisted immersion freezing. Ultrasonic power 300 W, interval time 40 s, frequency 5 times, and coolant temperature −18 °C.

Table 2
Effects of exposure time on freezing parameters of surimi during freezing process and myofibrillar protein content of surimi after 14-day frozen storage.

Ultrasonic exposure time (s)	Characteristic freezing time (s)	Tempering-stage freezing rate (°C/ s)	14d Myofibrillar protein content (mg/mL)
Control	642.50 ± 17.08^{a}	0.053 ± 0.002^{c}	3.41 ± 0.14^{c}
5	641.25 ± 20.56^{a}	0.054 ± 0.002^{c}	3.63 ± 0.13^{c}
10	500.25 ± 20.11^{c}	0.067 ± 0.001^{b}	4.32 ± 0.20^{a}
15	526.67 ± 24.03^{bc}	0.058 ± 0.002^{bc}	3.85 ± 0.15^{b}
20	545.00 ± 23.42^{b}	0.082 ± 0.004^{a}	3.73 ± 0.11^{b}

Data are expressed as the mean ± standard deviation. Different lowercase letters (a–c) indicated a significant difference ($p < 0.05$) based on the Duncan's test and least significant difference (LSD). Ultrasonic conditions: Ultrasonic power 300 W, interval time 40 s, frequency 5 times, and coolant temperature −18 °C.

3.1.2.3. Myofibrillar protein. Table 2 shows that the ultrasonic exposure time had a significant effect ($p < 0.05$) on the myofibrillar protein content for surimi samples after 14 days of frozen storage. The myofibrillar protein content of surimi was 4.32 mg/mL for exposure time of 10 s. This was significantly higher ($p < 0.05$) than those of other samples. From a practical point of view, exposure time should be chosen in consideration of the characteristic freezing time, the tempering-stage freezing rate and the quality change of the frozen product. Therefore exposure time 10 s was optimal for the ultrasonic experiment.

3.1.3. Influence of SSPS on surimi temperature during freezing process

3.1.3.1. Freezing curves. Fig. 4 illustrates that the surimi freezing curve for immersion freezing with 5% SSPS was close to that of the control samples. At 300 W the freezing processes of surimi samples with or without 5% SSPS were significantly improved ($p < 0.05$), as compared with those of immersion freezing. The temperature of surimi with 5% SSPS dropped fastest from 0 °C to −5 °C at 300 W.

3.1.3.2. Freezing parameters. Compared with the immersion freezing (Table 3), the characteristic freezing time of surimi samples with or without 5% SSPS was respectively reduced by 33.00% and 22.10% ($p < 0.05$) when the ultrasonic power of 300 W was carried out. The surimi with 5% SSPS required the shortest characteristic time to reach the final temperature at 300 W. Compared with the immersion freezing, the tempering-stage freezing rate of surimi with 5% SSPS was reduced by 13.21% and 11.30% when freezing was carried out with or without ultrasound at 300 W, respectively.

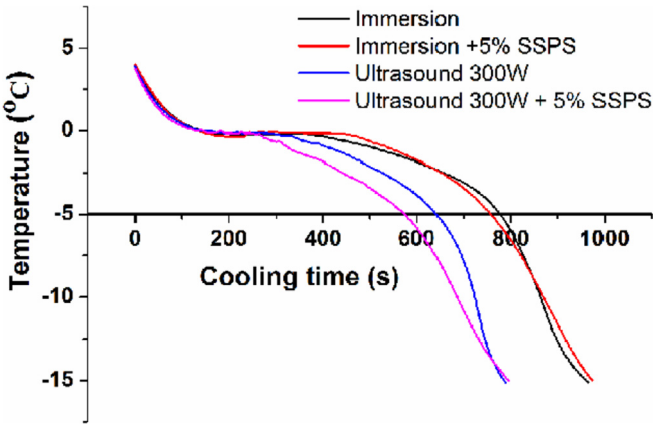

Fig. 4. Influences of SSPS on temperature of surimi during freezing process. Immersion freezing: coolant temperature of −18 °C. Ultrasound-assisted immersion freezing: ultrasonic power 300 W, exposure time 10 s, interval time 40 s, ultrasound frequency 5 times, and coolant temperature of −18 °C.

Table 3
Effects of SSPS on freezing parameters of surimi during freezing process and myofibrillar protein content of surimi after 14-day frozen storage.

Freezing process	Characteristic freezing time (s)	Tempering-stage freezing rate (°C/s)	14d Myofibrillar protein content (mg/mL)
Immersion	642.50 ± 17.08^{a}	0.053 ± 0.002^{b}	3.41 ± 0.15^{d}
Immersion + 5%SSPS	630.77 ± 16.42^{a}	0.047 ± 0.002^{c}	3.89 ± 0.12^{c}
Ultrasound 300 W	500.46 ± 20.03^{b}	0.067 ± 0.001^{a}	4.33 ± 0.19^{b}
Ultrasound 300 W + 5%SSPS	430.50 ± 15.79^{c}	0.046 ± 0.001^{c}	4.77 ± 0.14^{a}

Data were expressed as the mean ± standard deviation. The different lowercase letters (a–d) indicated a significant difference ($p < 0.05$) based on the Duncan's test and least significant difference (LSD). Immersion freezing: coolant temperature of −18 °C. Ultrasound-assisted immersion freezing: ultrasonic power 300 W, exposure time 10 s, interval time 40 s, ultrasound frequency 5 times, and coolant temperature of −18 °C.

3.1.3.3. Myofibrillar protein. Table 3 shows that ultrasound processing and 5% SSPS addition, had a significant effect ($p < 0.05$) on the myofibrillar protein content of surimi after a 14-day frozen storage period. The myofibrillar protein content of surimi with 5% SSPS was 4.77 mg/mL at 300 W. It was significantly higher ($p < 0.05$) than those of other samples. Based on the results of characteristic freezing time, tempering-stage freezing rate and myofibrillar protein content, the synergistic effect of ultrasound and SSPS on frozen surimi occurred.

3.2. Effect of SSPS on the Ca^{2+}-ATPase activity of myofibrillar protein from grass carp surimi during frozen storage

In general, surimi protein denaturation during frozen storage can be determined by the following indicators: Ca^{2+}-ATPase activity, total sulphydryl content, active sulphydryl content, salt extractable protein content, whiteness and water-holding capacity. The corresponding values of these indicators are significantly reduced when frozen-storage time is prolonged.

Compared to the initial value of 1.60 μmol (pi). mg^{-1} (protein). min^{-1}, the Ca^{2+}-ATPase activity of all surimi myofibrillar protein decreased over storage time (Fig. 5). After a 14-day storage period, the Ca^{2+}-ATPase activity was 0.84, 1.03, 1.33 and 1.06 μmol (pi).mg^{-1} (protein).min^{-1} for the control, 1% SSPS-added surimi, 3% SSPS-added surimi and 5% SSPS-added surimi, decreasing by 47.50%, 35.63%, 20.30% and 33.75%, respectively. The Ca^{2+}-ATPase activity of 3% SSPS-added surimi was significantly higher than those of other samples. After a 28-day storage period, the Ca^{2+}-ATPase activity was 0.11, 0.22, 0.39 and 0.26 μmol (pi).mg^{-1} (protein).min^{-1} for the control, 1% SSPS-added surimi, 3% SSPS-added surimi and 5% SSPS-added surimi, decreasing by 93.13%, 86.25%, 75.62% and 83.75%, respectively. These results indicate that 3% SSPS had better cryopropective effect on myofibrillar proteins from grass carp surimi, which were stored for 28 days after ultrasonic-assisted freezing.

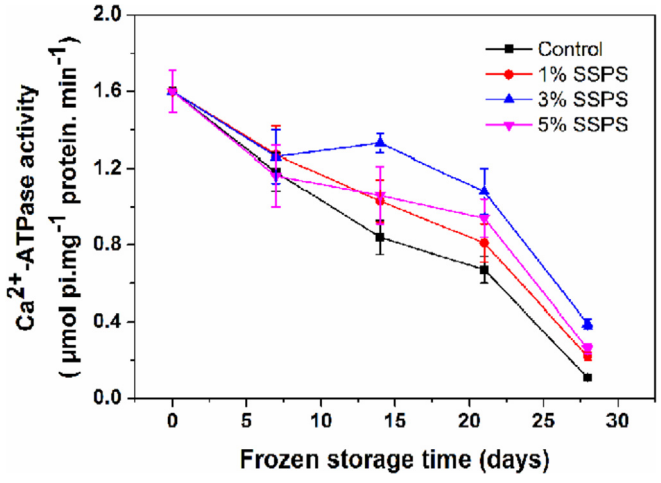

Fig. 5. Influences of SSPS content on Ca2+-ATPase activity of grass carp myofibrillar protein by ultrasound-assisted immersion freezing during the frozen storage at −18 °C. Ultrasound-assisted immersion freezing: ultrasonic power 300 W, exposure time 10 s, interval time 40 s, ultrasound frequency 5 times, and coolant temperature of −18 °C. Error bars represent the standard deviation of the mean of triplicate experiments.

The 3-dimensional structure of proteins is formed and stabilised by hydrogen bonds, hydrophobic interactions and hydration of polar residues. However, proteins might be denatured when those bonds are disturbed, resulting in the loss of physiological activities (Wu et al., 2014). During frozen storage protein denaturation normally causes the Ca^{2+}-ATPase activity decrease of myofibrillar protein from grass carp. Therefore, Ca^{2+}-ATPase activity could be used as a primary indicator for the integrity of myosin molecules and indicates myosin denaturation, especially in head region (Benjakul et al., 1997). Ca^{2+}-ATPase activity has also been commonly used as a measure of actomyosin integrity to monitor post-mortem changes in marine species during ice shipment or frozen storage (Zhang et al., 2017b). The globular heads of myosin are responsible for Ca^{2+}-ATPase activity, and a decrease in activity indicates the denaturation of myosin during frozen storage. Xiong et al. (2009) found that Ca^{2+}-ATPase activity for grass carp myofibrillar protein without konjac glucomannan was significantly reduced during frozen storage. It has been suggested that konjac glucomannan could mitigate the decrease in Ca^{2+}-ATPase activity. Zhang et al. (2017a, b) found that saccharide treatment significantly mitigated the decrease in myofibrillar protein during storage. The result might be due to the considerable reduction in the size of ice crystals in frozen samples, thereby avoiding the irreversible destruction of myofibrils. Similar results were found with other cryoprotectants, such as different konjac glucomannan hydrolysates, sodium lactate, trehalose and alginate oligosaccharides (Chen et al., 2013; Ma et al., 2015; Ramirez et al., 2010; Wang et al., 2014; Xiong et al., 2009; Zhou et al., 2006). The protein rearrangement via protein-protein interactions caused by low water-retention ability might contribute to low Ca^{2+}-ATPase activity. The SSPS-treated samples which maintained relatively high Ca^{2+}-ATPase activity were possibly caused by stabilising the myofibrillar protein fraction. The results showed that SSPS and ultrasonic-assisted freezing could be simultaneously used to prevent the decrease in Ca^{2+}-ATPase activity of surimi.

3.3. Effect of SSPS on the total sulphydryl content and active sulphydryl content of myofibrillar protein from grass carp surimi during frozen storage

3.3.1. Total sulphydryl content

Compared to the initial value of 5.89×10^{-5} mol/g protein, the total sulphydryl content of all surimi myofibrillar protein decreased over storage time (Fig. 6). After a 28-day storage period, the total sulphydryl content of myofibrillar protein was 2.86×10^{-5}, 3.89×10^{-5}, 4.00×10^{-5} and 3.75×10^{-5} mol/g protein for the control, 1% SSPS-added surimi, 3% SSPS-added surimi and 5% SSPS-added surimi, decreasing by 51.44%, 33.96%, 32.09% and 36.33%, respectively. The total sulphydryl content of the control decreased sharply while those of the SSPS-added surimi decreased to a lower extent. Among all samples 3% SSPS-added surimi showed the maximum total sulphydryl content during 28-day storage.

3.3.2. Active sulphydryl content

The decreasing tendencies of active sulphydryl content of myofibrillar proteins were similar to those of total sulphydryl content (Fig. 7). Compared to the initial value of 4.42×10^{-5} mol/g protein, the total sulphydryl content of all surimi myofibrillar protein decreased

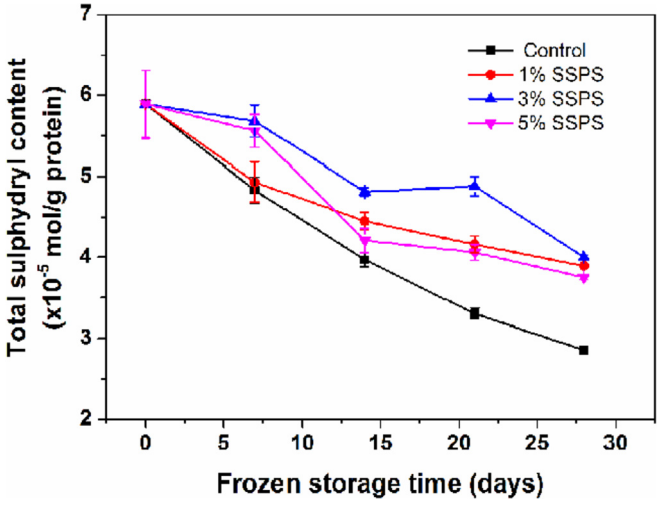

Fig. 6. Influences of SSPS content on total sulphydryl content of grass carp myofibrillar protein by ultrasound-assisted immersion freezing during the frozen storage at −18 °C. Ultrasound-assisted immersion freezing: ultrasonic power 300 W, exposure time 10 s, interval time 40 s, ultrasound frequency 5 times, and coolant temperature of −18 °C. Error bars represent the standard deviation of the mean of triplicate experiments.

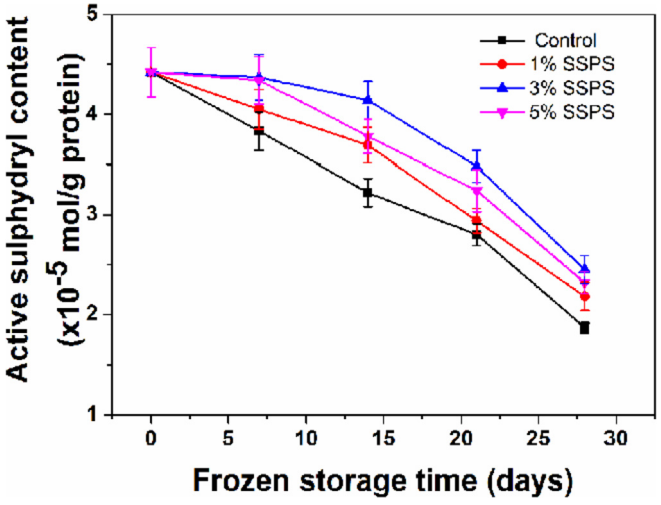

Fig. 7. Influences of SSPS content on active sulphydryl content of grass carp myofibrillar protein by ultrasound-assisted immersion freezing during the frozen storage at −18 °C. Ultrasound-assisted immersion freezing: ultrasonic power 300 W, exposure time 10 s, interval time 40 s, ultrasound frequency 5 times, and coolant temperature of −18 °C. Error bars represent the standard deviation of the mean of triplicate experiments.

over storage time. After a 28-day storage period, the active sulphydryl content was 1.87×10^{-5}, 2.18×10^{-5}, 2.45×10^{-5} and 2.32×10^{-5} mol/g protein for the control, 1% SSPS-added surimi, 3% SSPS-added surimi and 5% SSPS-added surimi, decreasing by 57.69%, 50.68%, 44.57% and 47.51%, respectively. The active sulphydryl content of the control decreased more rapidly than other samples. This suggests that SSPS could mitigate the decrease in the active sulphydryl content of myofibrillar protein from grass carp during frozen storage.

The conformational changes of myosin molecules may cause active sulphydryl groups to be exposed, resulting in the decrease of active sulphydryl content. Sulphydryl groups which are considered to be the most reactive functional group in myofibrillar proteins (Sultanbawa and Li-Chan, 2001), can be easily oxidised to form disulphide bonds during frozen storage and the freeze-thaw process, hence resulting in the obvious decreases in total and active sulphydryl contents (And and Lichan, 2001; Benjakul et al., 1997, 2003; Ramirez et al., 2010). The decrease of total sulphydryl content implicated that more S-S crosslinks were formed during 28-day frozen storage, while the significant decrease in active sulphydryl content was coincidental with that in Ca^{2+}-ATPase activity. Myosin (SH_1 and SH_2) played an important role in ATPase activity because the oxidation of these groups caused the decrease in Ca^{2+}-ATPase activity (Benjakul et al., 1997). The decrease of total sulphydryl content was coincidently ascribed to oxidation and exposure of natively buried SH groups. Zhang et al. (2017b) confirmed that carrageenan oligosaccharide-treated samples maintained relatively high Ca^{2+}-ATPase activity possibly by stabilising the myofibrillar protein fraction. Zhang et al. (2018) found that the oxidation of SH group and the decrease in Ca^{2+}-ATPase activity could be retarded by the incorporation of carrageenan oligosaccharides due to the antioxidant activity, which in turn preserved the quality of shrimp during extended frozen storage. These results suggest that SSPS could mitigate the decrease in the total sulphydryl content and active sulphydryl content of myofibrillar protein from grass carp during frozen storage.

3.4. Effect of SSPS on the salt extractable protein (SEP) content of myofibrillar protein from grass carp surimi during frozen storage

The salt extractable protein content from grass carp surimi decreased over time (Fig. 8). There were significant differences in the SEP content of myofibrillar protein between the control and those surimi with SSPS. Compared to the initial value of 106.81 mg/g, after a 7-day storage period the SEP content of the control decreased most quickly to 54.64 mg/g, decreasing by 48.84%. After 28 days of storage, the SEP content of the control decreased to 31.91 mg/g, while that of 1%, 3% and 5% SSPS-added surimi was 44.34, 60.83 and 47.01 mg/g, respectively. Furthermore, 3% SSPS had the best cryoprotective effect on protein denaturation for grass carp surimi. This result suggests that SSPS and ultrasonic-assisted freezing presented in a synergistic effect for frozen surimi.

The SEP content is a primary indicator of protein denaturation during frozen storage because the formation of hydrogen or hydrophobic bonds and disulphide bonds, as well as ionic interactions results in a decrease of SEP content (Auh et al., 2010; Benjakul and Bauer, 2000; Chen et al., 1988). The significant decrease in SEP content of the control showed that protein denaturation was induced by frozen storage. Zhou et al. (2006) found a significant decrease in the SEP content

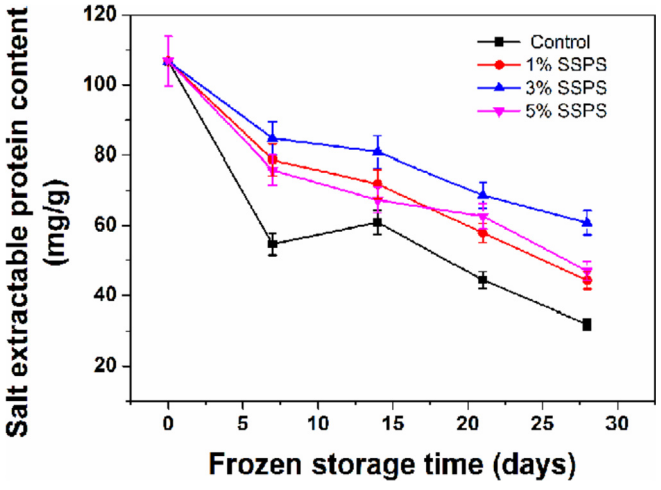

Fig. 8. Influences of SSPS content on salt extractable protein content of grass carp surimi by ultrasound-assisted immersion freezing during the frozen storage at −18 °C. Ultrasound-assisted immersion freezing: ultrasonic power 300 W, exposure time 10 s, interval time 40 s, ultrasound frequency 5 times, and coolant temperature of −18 °C. Error bars represent the standard deviation of the mean of triplicate experiments.

Table 4
Effects of SSPS content on the colour parameters and water-holding capacity of 28-day-storage surimi by ultrasound-assisted immersion freezing.

SSPS content (%)	L*	a*	b*	Water-holding capacity (%)	Whiteness
0	70.77 ± 0.79[a]	−1.82 ± 0.04[a]	−0.83 ± 0.16[d]	57.25 ± 0.45[b]	70.71 ± 1.16[a]
1	68.57 ± 1.82[a]	−1.79 ± 0.07[a]	0.11 ± 0.21[c]	59.25 ± 0.05[b]	68.52 ± 1.01[b]
3	67.63 ± 1.30[a]	−1.75 ± 0.06[a]	2.94 ± 0.33[b]	62.75 ± 0.02[a]	67.45 ± 0.87[b]
5	66.89 ± 2.16[b]	−1.56 ± 0.03[b]	4.79 ± 0.49[a]	61.50 ± 0.01[a]	66.51 ± 0.95[c]

Data were expressed as the mean ± standard deviation. Within a column, different lowercase letters (a–d) indicated a significant difference ($p < 0.05$) based on the Duncan's test and least significant difference (LSD). Ultrasound-assisted immersion freezing: ultrasonic power 300 W, exposure time 10 s, interval time 40 s, ultrasound frequency 5 times, and coolant temperature of −18 °C.

for grass carp myofibrillar protein without trehalose and sodium lactate during extended frozen storage. This suggests that trehalose and sodium lactate could mitigate the decrease in SEP content. Similar results have been found using other cryoprotectants, such as konjac glucomannan and different konjac glucomannan hydrolysates (Chou and Lin, 2010; Laura et al., 2010; Wang et al., 2014; Xiong et al., 2009). Mitigative decrease in the SEP content of SSPS-added surimi suggests that SSPS could be a valuable cryoprotectant in the food industry.

3.5. Effect of SSPS contents on colour, whiteness and water-holding capacity of grass carp surimi

Different levels of SSPS affected the colour values (L*, a* and b*) of grass carp surimi during 28-day storage (Table 4). Along with increasing SSPS content, the L* values decreased while the values of a* and b* presented in increasing tendency. Whiteness is one of the most important quality indices for surimi (Park, 2005). As storage time was prolonged, the whiteness of surimi decreased gradually. The whiteness of surimi decreased appreciably as SSPS content increased. Compared to the initial value of 75.97, after 28 days of storage the whiteness of the control, 1% SSPS-added surimi, 3% SSPS-added surimi and 5% SSPS-added surimi decreased to 70.71, 68.52, 67.45 and 66.51, respectively. The whiteness of SSPS-added surimi was slightly lower than that of control and was probably related to the yellowish colour of SSPS itself.

Water-holding capacity (WHC) is a physical parameter of surimi. WHC is also an important index to measure the degeneration of grass carp surimi. Compared to the initial value of 69.00%, after 28 days of storage the WHC for the control, 1% SSPS-added surimi, 3% SSPS-added surimi and 5% SSPS-added surimi was 57.25%, 59.25%, 62.75% and 61.50%, respectively (Table 4). The WHC of 3% SSPS-added surimi and 5% SSPS-added surimi were significantly higher than that of the control ($p < 0.05$). However, the WHC of surimi decreased slightly when adding more than 3% SSPS. Therefore 3% SSPS should be optimally added to grass carp surimi.

3.6. Synergistic effect analysis of ultrasound and SSPS on the protein denaturation of grass carp surimi

Ultrasound can play a significant role in accelerating freezing processes. Ultrasound cavitation and microstreaming cause strong oscillation in liquids, thus increasing heat and mass transfer. Concurrently cavitation bubbles which can act as nuclei may further promote nucleation occurrence and increase nucleation rate. In addition, the disruption of ice crystals facilitated the formation of the small-and-evenly distributed ice crystals, causing less damage to food microstructure and maintaining a high quality (Li and Sun, 2002; Hu et al., 2013). Therefore, the appropriate ultrasound conditions could significantly improve the quality of frozen surimi while increasing freezing efficiency.

Based on the analytical results of six evaluation indicators (Ca^{2+}-ATPase activity, total sulphydryl content, active sulphydryl content, salt extractable protein content, whiteness and water-holding capacity), SSPS can reduce the degeneration of surimi protein during frozen storage. The antifreeze effect of SSPS may be closely related to its molecular structure. SSPS which is approximate to a spherical shape has a pectin-like structure. The main chain comprises galacturonan (GN) consisting of −4)-α-D-GalA-(1- and rhamno galacturonan (RG) composed of the diglycosyl repeating unit, −4)-α-D-GalA-(1 → 2)-α-L-Rha-(1-, which is branched by β-1,4-galactans, α-1,3- or α-1,5-arabinan chains, and homogalacturonan (Gao et al., 2018; Nakamura et al., 2001; Nakamura, Furuta, Maeda, Takao and Nagamatsu, 2002a, b; Liu2017). In acidic or slightly acidic systems negatively charged galacturonan in the SSPS backbone can adsorb positively charged protein. In addition several layers of SSPS are attached around the surface, and the electrostatic repulsion between molecules causes the particles to be dispersed. To a certain extent, it leads to the suppression of denaturation caused by the aggregation of surimi protein. Some groups of SSPS can bind to proteins and form specific structures, reducing polypeptide chain expansion. Thereby the unfolding of protein molecules is also suppressed to a certain extent. Therefore SSPS addition can effectively alleviate the decline of surimi quality during frozen storage.

In summary, using a combination of SSPS and UF, the water molecules could be well anchored in the protein network structure of the surimi, thus forming tiny ice crystals, and therefore will cause less damage to the spatial structure of the myofibrillar protein.

4. Conclusion

The use of ultrasound in the immersion freezing process of grass carp surimi was evaluated. Results showed that ultrasound could significantly increase the freezing rates of surimi in the power range (300–540 W). Based on characteristic freezing time, tempering-stage freezing rate and the quality change of surimi after a 14-day storage period, the optimal ultrasound treatment was found to be 300 W, for 10 s, rest for 40 s and repeat 5 times. Subsequently the storage of frozen surimi indicated that adding SSPS could alleviate the decline in Ca^{2+}-ATPase activity, total sulphydryl content, active sulphydryl content, salt extractable protein content and water-holding capacity of grass carp surimi. Using the above indicators, 3% SSPS effectively mitigated the protein denaturation of grass carp surimi during storage at −18 °C. The research suggests that as a novel cryoprotectant, SSPS can significantly enhance the quality of surimi products while avoiding an excessively sweet taste and high calorie value. It will be a promising advance in technology to apply ultrasonic-assisted freezing in conjunction with SSPS in frozen surimi products.

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