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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.

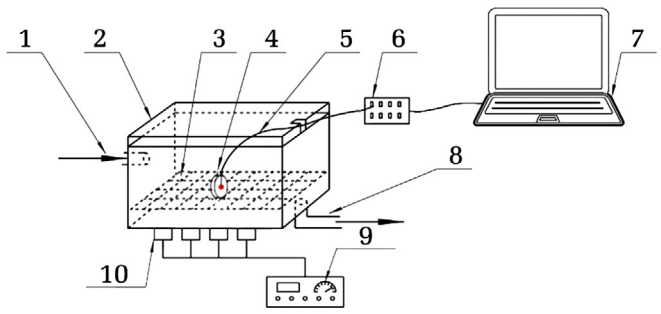


**Fig. 1.** Schematic diagram of ultrasound-assisted freezing equipment 1: Circulating freezing liquid inlet; 2: Ultrasonic processor; 3: Special rack bar; 4: Experimental sample placement; 5: T-type thermocouple; 6: Multichannel data recorder; 7: Computer; 8: Circulating cryogenic liquid outlet; 9: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

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