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Prevention of denaturation of freshwater crayfish muscle subjected to different freeze-thaw cycles by gelatin hydrolysate

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ABSTRACT

The cryoprotective effect of gelatin hydrolysates from the skin of beluga sturgeon (*Huso huso*) on freshwater crayfish (*Astacus leptodactylus*) muscle subjected to different freeze-thaw cycles was investigated. Untreated muscle was particularly susceptible to quality loss as indicated by the formation of secondary lipid oxidation products and the loss in sulfhydryl groups and Ca^{2+}-ATPase activity. Hydrolysate produced using flavourzyme which was mainly consisted of oligopeptides as the main fraction as well as small fraction of polypeptides could lower the denaturation of crayfish myosin heavy chain when compared to the control. In addition, lipid oxidation in treated muscle was impeded to some extent. Peptides with smaller or longer chain length than those in flavourzyme hydrolysate although exhibited antioxidant activity, but were less effective in maintaining the muscle quality during storage. Thus, the potential of flavourzyme hydrolysate as the alternative cryoprotectant might be employed during crustacean processing.

1. Introduction

Freezing and frozen storage are widely used methods to preserve seafoods and their products. Chemical reactions responsible for the oxidation or denaturation of seafood lipids and proteins are occurred especially when products are exposed to temperature changes during the extended storage (Benjakul & Visessanguan, 2011). Temperature changes during the storage of seafoods generally destabilize the bondings and interactions that stabilize proteins conformation and maintain their functionalities (Velasco, Dobarganes, & Mártinez-Ruiz, 2010). As a result of denaturation of seafoods, the loss in functional properties of myofibrillar proteins, the main proteins of fish and shellfish, such as the loss in water holding capacity, solubility and essential nutrients take place (Andersen & Jørgensen, 2004; Lund & Baron, 2010). To alleviate such a problem, cryoprotective compounds that decrease the rate of ice crystal growth in muscle or other biological tissues are widely used (MacDonald & Lanier, 1997; Sultanbawa & Li-Chan, 2001; Herrera & Mackie, 2004).

Marine hydrolysates with differences in their composition and structure associated with protein sources or hydrolysis conditions have been produced from seafood processing leftovers and underutilized or by-catch species (Ordóñez-Del Pazo et al., 2014). Besides nutritive potential of marine hydrolysates associated with the presence of essential amino acids, several studies have also shown that peptides present in hydrolysates could maintain the quality of seafood proteins by lowering the modification of proteins structure or by inhibiting the formation of denaturation products such as carbonyls and protein cross-links (Nikoo et al., 2014). Therefore, functional properties of protein such as solubility and water holding capacity associated with quality of products can be maintained (Cheung, Liceaga, & Li-Chan, 2009; Karnjanapratum & Benjakul, 2015; Korzeniowska, Cheung, & Li-Chan, 2013; Nikoo et al., 2014). It is also shown in several studies that some hydrolysates with cryoprotective effect possess antioxidant activity, thus they can serve as the additives with bifunctions to prevent both lipid oxidation and protein denaturation in seafoods during storage (Nikoo & Benjakul, 2015).

Freshwater crayfish (*Astacus leptodactylus*) inhabits in the natural or artificial dam lakes. This aquatic is highly appreciated by consumers and has high export value. Crayfish muscle is highly susceptible to denaturation and quality loss particularly during frozen storage. Nevertheless, no study so far investigated the cryoprotective effect of fish protein hydrolysate in crustacean, especially freshwater crayfish, during frozen storage of freeze-thaw cycles. This study, for the first time, aimed to determine the cryoprotective effects of gelatin hydrolysates from the skin of cultured beluga sturgeon in crayfish muscle subjected to different freeze-thaw cycles.

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2. Materials and methods

2.1. Chemicals

Alcalase (from *Bacillus licheniformis*, 2.4 L, 2.4 AU/g), Flavourzyme (from *Aspergillus oryzae*, ≥500 U/g), Neutrase (from *Bacillus amyloliquefaciens*, ≥0.8 U/g), 5,5-Dithiobis(2-nitrobenzoic acid) (DTNB), 2-diphenyl-1-picrylhydrazyl (DPPH) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 2-thiobarbituric acid (TBA) and bromophenol blue (BPB) was purchased from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China). Other chemicals were purchased from Merck Co. (Germany).

2.2. Extraction of gelatin

Cleaned fish skin was pretreated to remove non-collagenous proteins. Prepared skins were treated with 0.1 M NaOH with a skin/alkali solution ratio of 1:10 (w/v) to remove non collagenous proteins. The mixture was stirred for 6 h at room temperature using and overhead stirrer (FTDS-11, Sci Finetech Co., South Korea) and then washed to neutral pH. The alkaline treated skins were defatted using *n*-butanol with a solid/solvent ratio of 1:10 (w/v) for 18 h and washed to remove alcohol odor. The defatted skins were swollen by mixing the skins with 0.05 M acetic acid at a skin to solution ratio of 1:10 (w/v) for 3 h at room temperature. Gelatin was extracted from the swollen skins at 50 °C for 6 h. The mixtures were continuously stirred at using overhead stirrer. Gelatin solutions were centrifuged for 10 min and the supernatants were freeze dried.

2.3. Preparation of gelatin hydrolysates

Gelatin was hydrolysed using Alcalase (pH 8.0 and 50 °C), Flavourzyme or Neutrase (pH 7.0 and 50 °C) at an enzyme to substrate ratio of 1:20 for 3 h using a temperature-controlled water bath according to previous study (Nikoo et al., 2014). During the hydrolysis, the pH was maintained constant using 1 M NaOH. After the 3 h of hydrolysis, the enzyme was inactivated by heating in boiling water for 10 min and the resulting hydrolysate solutions were centrifuged at 6000*g* for 10 min at 4 °C and then freeze-dried (Model 5005; Dena Vacuum Industry Co., Ltd., Tehran, Iran). The powders were placed in polyethylene bags and stored at −18 °C until further analysis.

2.4. Protein determination

The protein content in gelatin hydrolysates was determined by the Biuret method (Gornall, Bardawill, & David, 1949). Bovine serum albumin (BSA) was used as a protein standard.

2.5. Molecular weight distribution of peptides

Molecular weight distribution of the gelatin hydrolysates prepared from gelatin extracted at different temperatures were determined using a TSK gel 2500 PWXL (7.8 × 300 mm, Tosoh, Tokyo, Japan) column coupled with a HPLC system (Agilent 1100, USA). Water/Acetonitrile/TFA (70:30:0.1, v/v) was used as mobile phase. The absorbance was monitored at 225 nm with flow rate of 0.5 mL/min. Bovine serum albumin (66,000 Da), cytochrome *C* (12,384 Da), bacitracin (1423 Da) and reduced glutathione (307 Da) were used as the molecular weight standards.

2.6. Measurement of antioxidant activities

2.6.1. ABTS radical scavenging activity

ABTS radical scavenging activity was determined according to the method described by Senphan and Benjakul (2013). $ABTS^{\cdot+}$ was generated by reacting 7.4 mM ABTS with 2.6 mM potassium persulphate at a ratio of 1:1 (v/v). The mixture was stored in the dark for 12 h at room temperature. Prior to assay, $ABTS^{\cdot+}$ was diluted with methanol to obtain an absorbance of 1.1 (±0.02) at 734 nm. Peptide (0.15 mL) with different concentrations was mixed with 2.85 mL of $ABTS^{\cdot+}$ and the mixture was left at room temperature for 2 h in the dark. The absorbance was then read at 734 nm. The ABTS radical scavenging activity was calculated as follows: A734 of control − A734 of sample - /A734 of control × 100.

2.6.2. Metal chelating activity

Metal chelating activity was determined according to Nikoo et al. (2014). Briefly, 1 mL of solution was mixed with 0.1 mL of FeCl2 (2 mM) and 0.2 mL of ferrozine (5 mM). Distilled water was added to the solution to adjust the final volume 139–5 mL. The reaction mixture was incubated at room temperature for 20 min before measuring the absorbance at 562 nm using a spectrophotometer.

2.7. Cryoprotective effects of gelatin hydrolysates in freshwater crayfish

Freshwater crayfish was purchased from local fishermen and transported to the laboratory in ice (1:2; w/w). Crayfish tail edible muscles were removed from tail shell and immersed in gelatin hydrolysate solution (8 g protein/100 mL distilled water) at a muscle /solution ratio of 1:2 (w/mL) at 4 °C for 15 min. This concentration of gelatin was according to the concentration of hydrolysates used in seafoods as cryoprotective compounds. After immersion, the treated samples were drained for 2 min. The crayfish without any treatment served as control. Treated muscles were divided into 18 portions, vacuum packaged and subjected to 6 freeze-thaw cycles at −18 °C. The samples were randomly taken for analyses after 0 (fresh, unfrozen) and 6 freeze-thaw cycles.

2.8. Preparation of natural actomyosin (NAM)

NAM was prepared according to the method of MacDonald and Lanier (1997). NAM was extracted by homogenizing 3 g surimi in 30 mL chilled 0.6 M KC1 (pH 7.0) in 50 mL falcon tubes. To avoid heating during extraction, tubes were placed in ice and homogenized for 20 s followed by a 20 s rest for a total extraction time of 4 min. The extract was then centrifuged at 5000*g* for 30 min (4 °C). To precipitate NAM, three volumes of chilled distilled water was added and NMA was collected by centrifuging at 5000*g* for 20 min (4 °C). The AM was then dissolved by gentle stirring with an equal volume of chilled 1.2 M KC1 (pH 7.0) for 30 min. The solution was centrifuged again to remove insoluble protein.

2.9. Thiobarbituric acid-reactive substances (TBARS)

The secondary lipid oxidation products was measured using the method described by Buege and Aust (1978) and expressed as mg malonaldehyde (MDA) equivalents kg^{-1} sample. Based on this method, 1 g of sample was homogenized with 5 mL of thiobarbituric acid reagent (15% TCA (w/v), 0.375% TBA (w/v) in 0.25 M HCl). The mixture was heated in a boiling water bath for 10 min until a pink color developed. The sample was then cooled with iced water and centrifuged at 5000*g* at 25 °C for 10 min. The absorbance of the supernatant was measured at 532 nm. TBARS was expressed