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Antioxidant and cryoprotective effects of Amur sturgeon skin gelatin hydrolysate in unwashed fish mince

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ABSTRACT

Antioxidant and cryoprotective effects of Amur sturgeon skin gelatin hydrolysates prepared using different commercial proteases in unwashed fish mince were investigated. Gelatin hydrolysates prepared using either Alcalase or Flavourzyme, were effective in preventing lipid oxidation as evidenced by the lower thiobarbituric acid-reactive substances formation. Gelatin hydrolysates were able to retard protein oxidation as indicated by the retarded protein carbonyl formation and lower loss in sulfhydryl content. In the presence of gelatin hydrolysates, unwashed mince had higher transition temperature of myosin and higher enthalpy of myosin and actin as determined by differential scanning calorimetry. Based on low field proton nuclear magnetic resonance analysis, gelatin hydrolysates prevented the displacement of water molecules between the different compartments, thus stabilizing the water associated with myofibrils in unwashed mince induced by repeated freeze–thawing. Oligopeptides in gelatin hydrolysates more likely contributed to the cryoprotective effect. Thus, gelatin hydrolysate could act as both antioxidant and cryoprotectant in unwashed fish mince.

1. Introduction

In recent years there has been an increasing interest in utilizing waste from seafood industry as functional ingredients to reduce quality deterioration of fish and fish products, especially during frozen storage (Samaranayaka & Li-Chan, 2011). Although freezing is the most widely method used for preservation of seafood, protein denaturation still occurs and is associated with quality loss (Benjakul & Visessanguan, 2011). The formation of ice crystals and the destruction of the hydrate layers surrounding polar residues followed by hydrophobic interactions are considered to be the prime causes of freeze-induced denaturation of fish protein (Hanafusa, 1973; Némethy & Scheraga, 1962). Physical and biochemical changes occurring in seafood during frozen storage or temperature fluctuation determine the distribution and binding of the water in the muscle (Shenouda, 1980). Changes in muscle water distribution are mainly a result of damage to proteins leading to denaturation and aggregation. This results in a decreased protein solubility and loss of functional properties such as water binding capacity (Andersen & Jørgensen, 2004).

Carbohydrate-based cryoprotectants such as sucrose and sorbitol are commonly used to maintain the quality of seafood during frozen storage but impart undesirable sweet taste (Cheung, Liceaga, & Li-Chan, 2009). Several studies have shown that fish protein hydrolysate and peptides could be used as alternative antioxidants and cryoprotectants in seafoods (Harnedy & FitzGerald, 2012; Karnjanapratum & Benjakul, 2015; Nikoo et al., 2014). Hossain et al. (2004) and Damodaran (2007) suggested that peptides in protein hydrolysate with high proportion of hydrophilic amino acids could bind water, thereby lowering the migration of water to form ice crystals. This leads to structural stabilization of proteins during frozen storage. The tetrapeptide isolated from Amur sturgeon skin gelatin could decrease the loss of intra-myofibrillar water and prevent the denaturation of myosin and actin induced by repeated freeze–thawing (Nikoo et al., 2015).

Lipid oxidation is another chemical reaction, which contributes to the deterioration of frozen seafoods. Due to high content of PUFA, iron and haemoglobin, unwashed fish mince is generally susceptible to oxidation, especially during the extended frozen storage (Jacobsen et al., 2008). To minimize the deteriorative reaction, antioxidants have been widely used. Several protein hydrolysates have been demonstrated to exhibit the antioxidant activity. Gelatin hydrolysate from blacktip shark skin and unicorn leatherjacket skin exhibited antioxidant and cryoprotective effects in washed fish mince (Karnjanapratum & Benjakul, 2015;

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Kittiphattanabawon, Benjakul, Visessanguan, & Shahidi, 2012). Nevertheless, antioxidant and cryoprotective effects of gelatin hydrolysate in unwashed fish mince, containing pro-oxidants and other soluble substances, have not been studied. Moreover, scanty information is available on effect of gelatin hydrolysate or commercial cryoprotectants (such as a mixture of sucrose and sorbitol) on the water distribution in unwashed fish mince as influenced by temperature fluctuation. Therefore, the objective of this work was to investigate the antioxidant and cryoprotective effects of Amur sturgeon skin gelatin hydrolysate in unwashed fish mince subjected to the repeated freeze–thawing process.

2. Materials and methods

2.1. Chemicals

Alcalase, Flavourzyme, 5,5-Dithiobis(2-nitrobenzoic acid) (DTNB) and sodium acetate were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). 2,4-dinitrophenylhydrazine (DNPH), 2-thiobarbituric acid, sucrose, D-sorbitol, and guanidine chloride were obtained from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China) and were of analytical grade.

2.2. Preparation of fish skins

The skins of farmed Amur sturgeon (*Acipenser schrenckii*) were obtained from a local sturgeon farm (Zhenjiang, Jiangsu, China). At the laboratory, the skins were washed with iced water to remove surface slime, cut into small pieces (0.5 × 0.5 cm), placed in polyethylene bags and stored at −30 °C until used (<1 month).

2.3. Extraction of gelatin

Prior to gelatin extraction, the skins were 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 a C-MAG HS7 magnetic stirrer (IKA Werke GmbH & Co. KG, Staufen, Germany). Thereafter, the alkaline-treated skins were defatted using n-butanol with a solid/-solvent ratio of 1:10 (w/v) for 18 h at 4 °C. 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 using a skin to water ratio of 1:5 (w/v). The mixtures were continuously stirred at 200 rpm using an overhead stirrer equipped with a propeller (IKA® RW 20 D CHN, Staufen, Germany). Thereafter, skin residues were removed using two layers of cheesecloth and the solution was filtered using a Buchner funnel with filter paper. The resultant filtrate was lyophilized and blended to obtain a fine gelatin powder. The powder was placed in polyethylene bag and stored at −30 °C until use.

2.4. Enzymatic hydrolysis of gelatin

Gelatin was hydrolyzed using Alcalase (pH 8.0 and 50 °C) or Flavourzyme (pH 7.0 and 50 °C) at an enzyme to substrate ratio of 1:20 for 3 h (Nikoo et al., 2014). The pH was maintained constant using 1 M NaOH, while the temperature was maintained using a temperature-controlled water bath. After the 3 h reaction period, the enzyme was inactivated by heating in boiling water for 10 min and the resulting gelatin hydrolysate was centrifuged at 8000×*g* for 10 min at 4 °C and then lyophilised. The gelatin hydrolysate powders prepared using Alcalase or Flavourzyme were referred to as PH-A and PH-F, respectively. The powders were placed in polyethylene bag and stored at −30 °C until use. The protein content of hydrolysates prepared using Alcalase or Flavourzyme were 87% and 91.2%, respectively.

2.5. Antioxidant and cryoprotective effects of gelatin hydrolysate in unwashed mince

2.5.1. Preparation of unwashed fish mince

Japanese sea bass (*Lateolabrax japonicas*) were purchased from a supermarket (Wuxi, Jiangsu, China). All fish were in the *rigor* state at the time of purchase as evidenced by a stiff body. The fish were transported on ice to the laboratory within 1 h using a fish/ice ratio of 1:3, w/w. At the laboratory, the fish were stored on ice to let them pass through *rigor mortis* and molten water was removed every 6 h. After 24–30 h of iced storage, *rigor mortis* was resolved. The *post-rigor* fish were eviscerated, beheaded, and white muscle was minced through a 3 mm plate using a grinder in a cold room (3 °C).

Mince was then divided into 100 g portions, to which gelatin hydrolysates (8 g protein/100 g mince) or a sucrose–sorbitol blend (1:1 w/w) (8 g/100 g mince) was added. The mixture was mixed well using a fork for 1 min to ensure the uniformity. Mince without the addition of a gelatin hydrolysate or the sucrose–sorbitol blend was used as the control. Each mince sample was further divided into 10 portions (10 g each), placed in polyethylene bags and covered using aluminum foil to protect the mince from light. Unfrozen samples were analyzed on the same day, while the other samples were subjected to freeze–thaw cycles (20 h freezing at −18 °C and 4 h thawing at 4 °C for each cycle). At freeze–thaw cycle of 3 and 6, the thawed minces were mixed well using a fork for 1 min to obtain the homogenous sample and then analyzed.

2.5.2. Determination of thiobarbituric acid-reactive substances (TBARS)

Thiobarbituric acid-reactive substances (TBARS) were determined using the method described by Buege and Aust (1978). One g of sample was mixed with 5 mL of thiobarbituric acid (TBA) reagent (15% TCA (w/v), 0.375% TBA (w/v) in 0.25 M HCl). The mixture was heated in a boiling water bath (95–99 °C) for 10 min until a pink color developed. The mixture was cooled with iced water and centrifuged at 5000*g* at 25 °C for 10 min. The absorbance of the supernatant was read at 532 nm. Results were expressed as mg malonaldehyde (MDA) equivalents kg^{-1} sample.

2.5.3. Determination of sulfhydryl groups

Total sulfhydryl group contents were determined using 5,5′-Di thiobis(2-nitrobenzoic acid) (DTNB) according to Ellman's method (1959) with some modifications as described by Emyard, Baron, and Jacobsen (2009). A 0.5 g sample of mince was homogenized in 10 mL of 0.05 M sodium phosphate buffer (pH 7.2) using an Ultra Turax homogenizer (IKA® T25 digital Ultra Turax®, Staufen, Germany) for 30 s at 6000 rpm. To 1 mL of the homogenate, 9 mL of 0.05 M phosphate buffer (pH 7.2) containing 0.6 M NaCl, 6 mM EDTA, and 8 M urea were added and the mixture was centrifuged at 14,000×*g* for 15 min at 4 °C. After centrifugation, 3 mL of the supernatant was mixed with 0.04 mL of 0.01 M DTNB solution in 0.05 M sodium acetate and incubated at 40 °C for 15 min. A blank was prepared by replacing the homogenate with 0.05 M sodium phosphate buffer (pH 7.2) containing 0.6 M NaCl, 6 mM EDTA and 8 M urea. The absorbance was measured at 412 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) and the sulfhydryl group content was calculated using a molar extinction coefficient of 13,600 $M^{-1}cm^{-1}$.