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Cryoprotective and antioxidative effects of gelatin hydrolysate from unicorn leatherjacket skin

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ABSTRACT

Cryoprotective properties of gelatin hydrolysates from autolysed non-swollen and swollen unicorn leatherjacket skin using partially purified glycyl endopeptidase (GE) from papaya latex were examined. Gelatin hydrolysates from autolysed non-swollen skin showed higher cryoprotective property in salt solution system than that from autolysed swollen skin as indicated by lower enthalpy for melting of eutectic and ice crystal. The cryoprotective effect of gelatin hydrolysate (0.5 and 1.0%) was in dose-dependent manner. Gelatin hydrolysate retarded physicochemical changes of natural actomyosin from washed mince system, as evidenced by lower changes in Ca^{2+}-ATPase activity, surface hydrophobicity and disulfide bond formation, compared with the control. Based on DSC analysis, the enthalpy of myosin and actin was also higher in the presence of gelatin hydrolysate. Gelatin hydrolysate could prevent lipid oxidation in washed mince system as shown by lower TBARS value and less abundance of hexanal, heptanal and 1-pentene-3-ol.

Effets cryoprotecteurs et antioxydants d'un hydrolysat de gélatine à partir de peau d'Aluterus monoceros

Mots clés : Aluterus monoceros ; Hydrolysat de gélatine ; Endopéptidase de glycyle ; Propriété cryoprotectrice ; Activité antioxydante

1. Introduction

Freezing and frozen storage have been widely used to retain sensory quality and nutrients of fish. During frozen storage, proteins undergo denaturation associated with quality loss. Furthermore, lipid oxidation in frozen food can be retarded but still occurs at a lower rate (Zarzycki and Swiniarska, 1993). Moreover, temperature fluctuation and abuse during transportation and storage lead to the deterioration of fish muscle quality. This is due to the destabilisation of bondings and interactions between protein molecules (Benjakul and Sutthipan, 2009). To alleviate or retard protein denaturation and lipid oxidation in muscle food caused by the formation of ice crystals as well as the changes in salt concentration in unfrozen phase during the frozen storage, cryoprotectants and antioxidants have been widely used, especially in fish mince or surimi (Benjakul and Visessanguan, 2011; Alvarez

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et al., 2010). As cryoprotectants, sucrose and sorbitol are amongst the common additives to maintain quality of frozen foods, however they may contribute to sweetness in products. Synthetic antioxidants, e.g., butylated hydroxyanisole, bytylated hydroxytoluene, propyl gallate and tertiary butylhydroquinone, etc. have been widely used in foods. Recently, synthetic chemicals and ingredients pose the adverse effects on the product, particularly in term of safety concern (Kim and Wijesekara, 2010). Natural and safe additives without negative effect on quality have therefore been searched.

Gelatin hydrolysate, especially from fish skin, has gained increasing interest as the additives with multi-functions. Protein hydrolysates and peptides have been shown to exhibit cryoprotective effect (Kittiphattanabawon et al., 2012a) and antioxidative activity (Qiu et al., 2014) in fish products during frozen storage. Nikoo et al. (2014) reported that tetrapeptide isolated from Amur sturgeon skin gelatin showed the antioxidative and cryoprotective effects in Japanese sea bass mince subjected to repeated freeze-thawing. Several proteases have been used to produce gelatin hydrolysates (Gómez-Guillén et al., 2011). Autolysis-assisted process mediated by indigenous protease has recently shown the potential for production of gelatin hydrolysates from skin of unicorn leatherjacket with antioxidative activity (Karnjanapratum and Benjakul, 2014a). Kittiphattanabawon et al. (2012b) reported that gelatin hydrolysate from blacktip shark skin prepared using crude enzyme from papaya latex acted as an alternative cryoprotectant with the lower sweetness in fish mince product. Recently, partially purified glycyl endopeptidase (GE) from papaya latex could serve as potential protease and yielded hydrolysates with antioxidative activity (Karnjanapratum and Benjakul, 2014b). Additionally, gelatin hydrolysate does not have the strong bitter taste and can thus be used in a wide range of products (Phillips and Williams, 2011). Since both protein denaturation and lipid oxidation cause the loss in quality and consumer's acceptance of fish, natural additives having both cryoprotective and antioxidative properties, especially gelatin hydrolysate, could be promising to burden such deteriorative processes. Therefore, the objective of this work was to study the cryoprotective and antioxidative effects of gelatin hydrolysate from unicorn leatherjacket skin prepared using partially purified glycyl endopeptidase from papaya latex.

2. Material and methods

2.1. Chemicals

Polyethylene glycol (PEG) 6000 was obtained from Fluka (Buchs, Switzerland). Tris-maleate, adenosine triphosphate (ATP), 1-anilinonaphthalene-8-sulfonic acid (ANS) and 2-nitro-5-thiosulfobenzoate (NTSB) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Sucrose, sorbitol and trichloroacetic acid (TCA) were obtained from Merck (Darmstadt, Germany). 2-thiobarbituric acid was procured from Fluka (Buchs, Switzerland). All chemicals were of analytical grade.

2.2. Preparation of gelatin

2.2.1. Preparation of fish skins

The skins of unicorn leatherjacket (*A. monoceros*) were obtained from a dock, Songkhla, Thailand. Three different lots of skins were collected. For each lot, skins were pooled and used as the composite sample. Skin were stored in ice with a skin/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. Upon arrival, the skins were washed with iced tap water (0–2 °C) and cut into small pieces (0.5 × 0.5 cm^2), placed in polyethylene bags and stored at −20 °C until use. The storage time was less than 2 weeks.

2.2.2. Pretreatment of fish skin

The skins were pretreated to remove non-collagenous proteins using the method of Kaewruang et al. (2013). Fish skins (0.5 × 0.5 cm^2) were soaked in 0.05 M NaOH with a skin/alkaline solution ratio of 1:10 (w/v) to remove non-collagenous proteins. The mixture was stirred continuously at room temperature using an overhead stirrer equipped with a propeller (RW 20.n, IKA Labortechnik, Staufen, Germany) at a speed of 150 rpm. The alkaline solution was changed after 2 h and total pretreatment time was 4 h. Pretreated skins were washed with tap water until neutral or faintly basic pH of wash water was obtained.

2.2.3. Preparation of autolysed fish skin

2.2.3.1. Use of non-swollen skin. Autolysis was conducted using pretreated skin (non-swollen skin) following the method of Karnjanapratum and Benjakul (2014a). The pretreated skins were mixed with deionised water at a ratio of 1:5 (w/v). The autolysis was conducted by incubating the mixture in a water bath (Model W350, Memmert, Schwabach, Germany) at 55 °C for 12 h and terminated by heating at 90 °C for 15 min. The mixture was centrifuged at 5000 ×g at 4 °C using a refrigerated centrifuge model Avanti J-E (Beckman Coulter, Inc., Palo Alto, CA, USA) for 10 min to remove the debris. Autolysed skin was collected and referred to as 'NS'.

2.2.3.2. Use of swollen skin. To prepare swollen-skin, the pretreated skin was soaked in 0.1 M phosphoric acid with a skin/solution ratio of 1:10 (w/v) for 6 h with a gentle stirring at room temperature. The acidic solution was changed every 3 h. Acid-treated skin was washed thoroughly with tap water until wash water became neutral or faintly acidic. To prepare autolysed skin, the swollen skin was mixed with deionised water at a ratio of 1:5 (w/v) and subjected to autolysis as previously described. Autolysed skin obtained was referred to as 'SS'.

2.3. Production of gelatin hydrolysate from autolysed skin using partially purified glycyl endopeptidase (GE)

2.3.1. Preparation of crude extract from papaya (C. papaya) latex

Fresh papaya latex was collected from green papaya fruit cultivated in Songkhla, Thailand. Four to six longitudinal incisions were made on the green papaya fruit using a stainless steel knife. The exuded latex was collected using a receiving