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Concentration-dependent suppressive effect of shrimp head protein hydrolysate on dehydration-induced denaturation of lizardfish myofibrils

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Abstract

To utilize fishery waste products as functional food material, the shrimp head protein hydrolysate (SHPH) was produced from three species of shrimp wastes, Northern pink shrimp, Endeavour shrimp and black tiger shrimp, by enzymatic hydrolysis. The SHPH was used as a natural food preservative by adding to lizardfish myofibrils at concentrations ranging from 2.5% to 10%. Their effects on the state of water and the denaturation of myofibrils during dehydration were evaluated. The amount of monolayer and multilayer water in myofibrils containing SHPH were higher than those without SHPH (control). DSC analyses revealed that the amount of unfrozen water increased significantly after addition of SHPH. The Ca-ATPase inactivation rate of myofibrils containing SHPH decreased during dehydration while 5–7.5% concentrations of SHPH exhibited optimum effect regardless of the species. The results implicated that SHPH can be used as an alternative food preservative for suppressive the dehydration-induced denaturation of myofibrils.

Keywords: Shrimp waste; Protein hydrolysate; Ca-ATPase; Myofibrils; Dehydration; Denaturation; DSC; Unfrozen water

1. Introduction

Utilization of waste from food industry, such as fishery by-products, has gained an increased interest over the past 10 years. Generally, these wastes have been utilized for the preparation of fishmeal and silage because of their poor functional properties (Jeon et al., 1999; Benjakul and Morrissey, 1997). Processing techniques for seafood waste are needed to convert the underutilized wastes into more marketable, valuable and acceptable products. Enzymatic hydrolysis of protein is a viable option because it avoids the extremes of chemical and physical treatments and thus minimizes undesirable reactions (Clemente, 2000; Liaset et al., 2003). The enzymatic protein hydrolysates are composed of a certain amount of free amino acids and short chain peptides. Moreover, they exhibit several advantages in functional properties such as improved solubility, heat stability, water binding ability and increased nutritional quality (Petersen, 1981; Rebeca et al., 1991; Liceaga-Gesualdo and Li-Chan, 1999). According to a report by Meyers (1986), 49% on raw material in shrimp industry when

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processed as waste, consisted of 35% head and 14% shell. Previous studies indicated that shrimp head protein hydrolysates are a high source of amino acids, peptides and proteins, which have potential for recovery and utilization as additives in food industry (Mandeville et al., 1992; Ferrer et al., 1996; Synowiecki and Al-Khateeb, 2000).

Several investigators have devoted considerable efforts to develop protein hydrolysate from shrimp waste by enzymatic hydrolysis (Ferrer et al., 1996; Synowiecki and Al-Khateeb, 2000). However, there were only limited reports on functional properties of shrimp protein hydrolysates and their applications to foods. The objective of this study was to evaluate the concentration-dependent (2.5–10%, w/w) effects of shrimp head protein hydrolysate (SHPH) from three species of shrimp on the state of water and denaturation of lizardfish myofibrils during dehydration.

2. Methods

2.1. Materials

Heads of black tiger shrimp (*Penaeus monodon*), Endeavour shrimp (*Metapenaeus endeavouri*) and Northern pink shrimp (*Pandalus eous*), were obtained from Hoko Fishing Co., Ltd. (Samutsakorn, Thailand) and used as raw materials for preparation of protein hydrolysate by enzymatic hydrolysis. Endo-protease and exo-proteases were obtained from Shin-Nihon Chemical Industries Inc. (Anjo, Japan). Fresh lizardfish (*Saurida wanieso*) was purchased from Nagasaki fish market (Nagasaki, Japan) and used for preparation of fish myofibrils.

2.2. Preparation of SHPH

The SHPH was prepared according to the method of Iwamoto et al. (1991) with slight modification. Shrimp head was added with a 2-fold volume of distilled water and heated at 90 °C for 30 min to inactivate endogenous hydrolyzing enzymes. Thereafter, the mixture was homogenized at 10,000 rpm for 1 min and adjusted to pH 8.0 with 1 N NaOH at 60 °C for an optimal enzyme activity. The endo-protease derived from *Bacillus subtilis* was added at 0.1% (w/w) and continuously hydrolyzed with stirring for 2 h. The mixture was heated at 90 °C for 30 min to inactivate the enzyme. The pH of the mixture was adjusted to 6.0 by adding 10% HCl at 60 °C and exo-protease derived from *Aspergillus oryzae* was added at 0.1% (w/w) to the mixture in the same approach as described previously. The solution was removed sludge and heated at 80 °C for 10 min. For quantifying fat, the mixture was centrifuged at 8000*g* for 20 min. The solution was then desalted with Micaro Acilyzer G3 type (Asahi Kasei Inc., Kawasaki, Kanagawa, Japan) and filtered with Pellicon-2 Mini Holder Ultrafiltration system (Millipore Corp., Bedford, MA, USA) in order to achieve a cut-off molecular weight 30,000 Da. Finally, the solution was made into a powder by spray drying with IGA 32 (Yamato Science Inc., Tokyo, Japan) and the powder of SHPH was stored in a plastic container with a desiccating agent at 4 °C for further use. Each species of shrimp head was prepared for SHPH by the same manner.

2.3. Proximate composition, amino acids analysis and molecular weight distribution of SHPH

Each type of SHPH was analyzed for moisture content, crude protein, crude lipid and crude ash by the standard method (AOAC, 1984), and sugar content was analyzed by the phenol–sulfuric acid colorimetric method. Amino acids were determined after digestion of the SHPH in 6 N HCl at 110 °C for 20 h by using high performance liquid chromatography (HPLC; JLC-300; Nihon Electronic Industries Inc., Sagamihara, Kanagawa, Japan). Molecular weight distributions of SHPH were determined by the gel filtration chromatography using Sephadex G-25 column (inner diameter 2.2 cm × 60 cm). Two millilitre sample contained 5 mg protein/ml was eluted with 30 mM phosphate buffer (pH 7.0) containing 0.1 M NaCl at flow rate of 30 ml/h, and the absorbance of each fraction was measured at 220 nm.

2.4. Preparation of fish myofibrils

Fish myofibrils were prepared according to the method of Katoh et al. (1977) with slight modification by Nozaki et al. (1991). Fresh lizardfish mince was washed three times with 5-fold 0.1 M KCl–20 mM Tris–maleate buffer (pH 7.0) and compressed with hydraulic press machine to remove excessive water. After dewatering, the meat was added with 3-fold of the same buffer and homogenized in a foam-preventive blender for 90 s at 10,000 rpm, and subsequently filtered through a nylon net (#16) to remove connective tissue. The minced meat was added with 20% TritonX-100 at the final concentration of the suspension to 1%. After 30 min, the suspension was centrifuged at 1800*g* for 10 min. The residue was washed several times with 5-fold of the same buffer until the supernatant appeared clear, and then the sediment was washed with cold distilled water in order to remove the buffer used. The sediment was centrifuged at 5,000*g* for 10 min and 27,500*g* for 20 min for removal of excessive water. Finally, the sediment was collected and used as myofibrils specimens. The general components of myofibrils consisted of 86.76% moisture, 12.38% crude protein, 0.13% crude fat and 0.49% crude ash.