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Isolation and characterisation of acid and pepsin-solubilised collagens from the skin of Brownstripe red snapper (*Lutjanus vitta*)

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Abstract

Acid-solubilised collagen (ASC) and pepsin-solubilised collagen (PSC) were successfully extracted from the skin of Brownstripe red snapper (*Lutjanus vitta*) with yields of 9% and 4.7%, respectively, on the basis of wet weight. Both ASC and PSC consisted of two different α chains (α1 and α2), and were characterised to be type I with no disulfide bond. PSC had a lower content of high molecular weight cross-links, than did ASC. Peptide maps of ASC and PSC hydrolysed by V8 protease and lysyl endopeptidase showed some differences in peptide patterns between the two fractions and were totally different from those of calf skin collagen type I, suggesting differences in amino acid sequences and collagen conformation. Maximum solubility in 0.5 M acetic acid was observed at pH 3 and pH 4 for ASC and PSC, respectively. A sharp decrease in solubility was observed in the presence of NaCl, above 2% and 3%, (w/v) for ASC and PSC, respectively. T_{max} values of both collagen fractions were similar and shifted to a lower value in the presence of acetic acid, suggesting some changes in the collagen structure caused by acid induction.

Keywords: Collagen; Brownstripe red snapper; Skin; ASC; PSC; Solubility

1. Introduction

Collagen is an abundant protein in vertebrates and constitutes ≈30% of total animal protein (Muyonga, Cole, & Duodu, 2004). Tendon, skin, bone, vascular system and connective tissue sheaths surrounding muscle are the organs of animals that are mainly structured by collagen (Foegeding, Lanier, & Hultin, 1996). Furthermore, collagen has been found in fish skin, bone and scale (Ikoma, Kobayashi, Tanaka, Walash, & Mann, 2003; Kimura, 1992; Nagai & Suzuki, 2000; Nomura, Sakai, Ishi, & Shirai, 1996). Nineteen variants of collagen have been reported, named type I–XIX (Bailey, Paul, & Knott, 1998). So far, the main sources of collagen are limited to those of land-based animals, such as bovine or porcine skin and bone. However, the outbreaks of bovine spongiform encephalopathy (BSE) and the foot-and-mouth disease (FMD) crisis have resulted in anxiety among users of collagen and collagen-derived products of land animal origin (Helcke, 2000). Additionally, collagen obtained from porcine skin or bone cannot be used as a component of some foods due to aesthetic and religious objections (Sadowska, Kolodziejska, & Niecikowska, 2003). Therefore, alternative sources, such as fish processing waste,

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including skin, bone or scale, have received increasing attention for collagen extraction.

The fish processing industry is an important income generator for Thailand. During processing, a large amount of wastes is generated. Fish solid wastes constitute 50–70% of the original raw material, depending on the processes used and types of products. Those wastes have been of interest as high-protein human foods, instead of employing them as pet foods (Montero, Jimenez-Colmenero, & Borderias, 1991; Shahidi, 1994). Among the value-added products derived from those wastes, collagen from skin, scale and bone has increasingly been of interest owing to its abundance. Collagen contents (acid-solubilised collagen; ASC) in fish skins of young and adult Nile perch were 63.1% and 58.7%, respectively (Muyonga et al., 2004), whereas those of Japanese sea-bass, chub mackerel and bullhead shark were 51.4%, 49.8% and 50.1%, respectively (Nagai & Suzuki, 2000). Collagen contents generally vary with fish species, age and season (Ciarlo, Paredi, & Fraga, 1997; Foegeding et al., 1996; Montero et al., 1991; Nagai, Araki, & Suzuki, 2002). Type I collagen is a fibrous collagen and is the major type in fish waste materials, including skin, bone, scale and fins of various fish species (Ikoma et al., 2003; Kimura, 1983; Kimura, 1992; Nagai & Suzuki, 2000; Nagai et al., 2002; Sato, Yoshinaka, Itoh, & Sato, 1989). Recently, biochemical properties of black drum and sheapshead seabream skin collagen, (subtropical fish) have been characterised (Ogawa et al., 2003). Nevertheless, collagen from different species and habitats might be different in terms of molecular compositions and properties (Foegeding et al., 1996). So far, little information regarding the characteristics of marine fish skin collagen, especially from commercially important species involving those used for surimi production, has been reported. Therefore, this investigation aimed to isolate and characterise acid- and pepsin-solubilised collagens (PSC) from the skin of Brownstripe red snapper (*Lutjanus vitta*) which is one of the main fish species used for surimi production in Thailand.

2. Materials and methods

2.1. Chemicals

β-Mercaptoethanol (βME), pepsin (EC 3.4.23.1) powdered; 750 U/mg dry matter, *Staphylococcus aureus* V8 protease (EC 3.4.21.19) and protein markers were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Sodium dodecyl sulfate (SDS), acetic acid, and Tris(hydroxymethyl)aminomethane were obtained from Merck (Darmstadt, Germany). *Achromobacter lyticus* Lysyl endopeptidase (EC 3.4.21.50) was from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

2.2. Fish skin preparation

Brownstripe red snapper (*Lutjanus vitta*), with an average length of 22–25 cm, were caught from the Songkhla coast along the Gulf of Thailand, stored in ice and off-loaded 24–36 h after capture. Upon arrival at the dock in Songkhla, fish were stored in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai. Fish were washed using tap water. Skins were then removed, descaled, and cut into small pieces (0.5×0.5 cm). Skins were kept on ice prior to collagen extraction.

2.3. Skin collagen preparation

The collagen was extracted according to the method of Nagai & Suzuki (2000) with a slight modification. All processes were carried out at 4 °C. Skin was soaked in 0.1 M NaOH with a sample/solution ratio of 1:30 (w/v) for 24 h with gentle stirring. The solution was changed every 8 h to remove noncollagenous proteins and pigments. Alkali-treated skins were then washed with distilled water until neutral or faintly basic pHs of wash water were obtained. Fat was removed in 10% (v/v) butyl alcohol with a sample/solution ratio of 1:30 (w/v) for 24 h with gentle stirring and a change of solution every 8 h. Defatted skins were thoroughly washed with distilled water. The matter was soaked in 0.5 M acetic acid with a sample/solution ratio of 1:30 (w/v) for 24 h with gentle stirring. The mixture was then centrifuged at 20,000*g* for 1 h at 4 °C. The supernatants were collected and kept at 4 °C. The precipitate was re-extracted in 0.5 M acetic acid with a sample/solution ratio of 1:30 (w/v) for 16 h with gentle stirring, followed by centrifugation at 20,000*g* for 1 h at 4 °C. The supernatants obtained were combined with the first extract. The combined extracts were precipitated by the addition of NaCl to a final concentration of 2.6 M in 0.05 M Tris–HCl (pH 7.5). The resultant precipitate was collected by centrifugation at 20,000*g* for 1 h at 4 °C and then dissolved in 10 volumes of 0.5 M acetic acid. The solution obtained was dialysed with 10 volumes of 0.1 M acetic acid in a dialysis membrane with molecular weight cut-off of 30 kDa for 12 h at 4 °C with change of solution every 4 h. Subsequently, the solution was dialysed with 10 volumes of distilled water with changes of water until neutral pH was obtained. The dialysate was freeze-dried and referred to as ASC. Undissolved residue, obtained after acid extraction, was thoroughly rinsed with distilled water, suspended in 2 volumes of 0.5 M acetic acid and subjected to limit hydrolysis with 10% (w/v) pepsin (EC 3.4.23.1; powderised; 750 U/mg dry matter, Sigma Chemical Co. (St. Louis, Mo, USA)) for 48 h at 4 °C with gentle stirring. The viscous solution was centrifuged at 20,000*g* for 1 h at 4 °C. To terminate the pepsin reaction, the supernatant obtained was dialysed against