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Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*)

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Abstract

The compositions and some properties of acid-soluble collagens (ASC) of the skin and bone of bigeye snapper, (*Priacanthus tayenus*) were investigated. The collagens were extracted with the yields of 10.94% and 1.59% on the basis of wet weight, from skin and bone, respectively. Similar electrophoretic patterns of collagens from the skin and bone were observed. Both collagens comprised two different α chains, α1 and α2 and were classified as type I collagen. However, peptide maps of collagen from the skin and bone of bigeye snapper, digested by V8 protease and lysyl endopeptidase, revealed differences between collagens from skin and bone, and both were completely different from those of calf skin collagen. Collagen rehydrated in acetic acid had lower T_{max} and enthalpy than those rehydrated in deionised water, suggesting a conformational change caused by acid. Collagens form the skin and bone had the highest solubility at pH 2 and 5, respectively. No changes in solubility were observed in the presence of NaCl up to 3% (w/v). However, a sharp decrease in solubility was found with NaCl above 3% (w/v).

Keywords: Acid-soluble collagen; Skin; Bone; Bigeye snapper; Characterisation

1. Introduction

Thailand is one of the largest surimi producers in Southeast Asia. At present, twelve surimi factories are located in Thailand, with a total production of about 60,000 metric tonne per year (Morrissey & Tan, 2000). The fish used for surimi production are mostly threadfin bream (*Nemipterus* spp.), bigeye snapper (*Priacanthus* spp.), croaker (*Pennahia* and *Johnius* spp.) and lizardfish (*Saurida* spp.) (Benjakul, Chantarasuwan, & Visessanguan, 2003). During surimi processing, numerous wastes, both liquid and solid form, are generated (Morrissey, Park, & Huang, 2000). The solid wastes constitute 50–70% of the original raw material, depending on the processing used. These wastes are a mixture of heads, viscera, skin and bone (Morrissey et al., 2000). Although the nutritional values of these wastes are fairly high, these useful resources have been mainly used as fish meal or fertiliser with low value (Nagai & Suzuki, 2000a). Also, improper disposal of these wastes may cause pollution and emit an offensive odour. Hence, optimal utilisation of surimi processing wastes, especially in the production of value-added products is a promising means to increase revenue for the producer and to decrease the cost of disposal or management of these wastes.

Collagen has a wide range of applications in leather and film industries, pharmaceutical, cosmetic and biomedical materials and food (Bailey & Light, 1989; Cavallaro, Kemp, & Kraus, 1994; Hood, 1987; Hassan & Sherief, 1994; Nimni, 1988; Slade & Levine, 1987; Stainsby, 1987). Generally, pig and cow skins and bones

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are the main sources of collagen isolation. However, the outbreak of mad cow disease has resulted in anxiety among users of cattle gelatin. Additionally, the collagen obtained from pig bones cannot be used, due to religious constraints (Sadowska, Kolodziejska, & Niecikowska, 2003). As a consequence, increasing attention has been paid to alternative collagen sources, especially fish skin and bone from seafood processing wastes. About 30% of these wastes consist of skin and bone, which are very rich in collagen (Gomez-Guillen et al., 2002; Shahidi, 1994). However, fish collagens have lower thermal stability than mammalian collagens because fish collagens contain lower imino acid contents than mammalian collagens (Foegeding, Lanier, & Hultin, 1996). So far, skin and bone collagen from several fish species have been isolated and characterised (Ciarlo, Paredi, & Fraga, 1997; Kimura, Miyauchi, & Uchida, 1991; Kimura, Ohno, Miyauchi, & Uchida, 1987; Nagai, Araki, & Suzuki, 2002; Nagai & Suzuki, 2000a, 2000b; Sadowska et al., 2003; Yata, Yoshida, Mizuta, & Yoshinaka, 2001). However, no information regarding the collagen from tropical fish, particularly from surimi processing waste, has been reported. Bigeye snapper (*Priacanthus tayenus*) is commonly used for surimi production due to its high gel-forming ability (Benjakul, Visessanguan, Ishizaki, & Tanaka, 2001). Therefore, the objective of this investigation was to isolate and characterise acid-soluble collagen from skin and bone of bigeye snapper.

2. Materials and methods

2.1. Fish skin and bone preparation

The skin and bones of bigeye snapper (*Priacanthus tayenus*) were obtained from Man A Frozen Foods Co. Ltd., Songkhla, Thailand. Residual meat was removed manually and cleaned samples were washed with tap water. The skin was descaled, followed by thorough washing. Descaled samples were then cut into small pieces (0.5×0.5 cm^2), placed in polyethylene bags and stored at −20 °C until used. Bone was cut into small pieces (1–2 cm in length) and powdered by mixing the samples in liquid nitrogen for 20 s using a blender (National, Tokyo, Japan). The prepared samples were kept at −20 °C until used.

2.2. Chemical reagents

All reagents were of analytical grade. Type I collagen from calf skin was purchased from Elastin products Co., Inc. (Owensville, MO, USA). Types II, III and V collagens from porcine cartilage, porcine skin and porcine placenta, respectively, were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

2.3. Proximate analyses

Moisture, ash, fat and protein content were determined according to the method of AOAC (1999). Hydroxyproline content was analysed according to the method of Bergman and Loxley (1963) with a slight modification. The samples were hydrolysed with 6 M HCl at 110 °C for 24 h in an oil bath (model B-490, BUCHI, Flawil, Switzerland). The hydrolysate was clarified with activated carbon and filtered through Whatman No. 4 filter paper. The filtrate was neutralised with 10 M and 1 M NaOH to obtain the pH of 6.0–6.5. The neutralised sample (0.1 ml) was transferred into a test tube and isopropanol (0.2 ml) was added and mixed well; 0.1 ml of oxidant solution (mixture of 7% (w/v) chlororamine T and acetate/citrate buffer, pH 6, at a ratio of 1:4 (v/v)) was added and mixed thoroughly; 1.3 ml of Ehrlich's reagent solution (mixture of solution A (2 g of *p*-dimethylamino-benzaldehyde in 3 ml of 60% (v/v) perchloric acid (w/v)) and isopropanol at a ratio of 3:13 (v/v)) were added. The mixture was mixed and heated at 60 °C for 25 min in water bath (Memmert, Schwabach, Germany) and then cooled for 2–3 min in a running water. The solution was diluted to 5 ml with isopropanol. Absorbance was measured against water at 558 nm. A hydroxyproline standard solution, with concentration ranging from 10 to 60 ppm, was also included. Hydroxyproline content was calculated and expressed as mg/g sample.

2.4. Preparation of collagen from skin and bone

The collagens were prepared by the method of Nagai and Suzuki (2000a) with a slight modification. All the preparation procedures were performed at 4 °C with the continuous stirring. To remove non-collagenous proteins, the skin and bone were mixed with 0.1 N NaOH at a sample/alkali solution ratio of 1:10 (w/v). The mixture was stirred for 6 h. The alkali solution was changed every 2 h. Then, the deproteinised samples were washed with cold water until neutral or faintly basic pH of wash water were obtained.

Deproteinised skins were defatted with 10% butyl alcohol with a solid/solvent ratio of 1:10 (w/v) for 18 h and the solvent was changed every 6 h. Defatted skins were washed with cold water, followed by soaking in 0.5 M acetic acid with a solid/solvent ratio of 1:30 (w/v) for 24 h. The mixture was filtered with two layers of cheese cloth. The residue was re-extracted under the same conditions. Both filtrates were combined. The collagen was precipitated by adding NaCl to a final concentration of 2.6 M in the presence of 0.05 M tris(hydroxymethyl)aminomethane, pH 7.0. The resultant precipitate was collected by centrifuging at 20,000*g* for 60 min. The pellet was dissolved in 0.5 M acetic acid,

dialysed against 0.1 M acetic acid and distilled water, respectively, and then freeze-dried.

To extract collagen from bones, deproteinised bones were decalcified with 0.5 M ethylenediaminetetraacetic acid (EDTA)–4Na (pH 7.4) using a solid/solution ratio of 1:10 (w/v) for 40 h. The solution was changed every 8 h. The residue was washed thoroughly with cold water (4 °C). Collagen was further extracted from the bone in the same manner as that used for skin (as previously described).

2.5. Amino acid analysis

Collagens from the skin and bone were hydrolysed under reduced pressure in 4.0 M methanesulfonic acid containing 0.2% (v/v) 3-2(2-aminoethyl)indole at 115 °C for 24 h. The hydrolysates were neutralised with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). An aliquot of 0.4 ml was applied to an amino acid analyser (MLC-703; Atto Co., Tokyo, Japan).

2.6. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed by the method of Laemmli (1970). The collagen samples were dissolved in 0.02 M sodium phosphate containing 1% SDS and 3.5 M urea (pH 7.2) with continuous stirring at room temperature. The mixtures were centrifuged at 8500*g* for 5 min at room temperature to remove undissolved debris. Solubilised samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 4% SDS, 20% glycerol) in the presence or absence of 10% β-ME. Samples were loaded onto the PAGEL®-Compact precast gel (5% gel) and subjected to electrophoresis at a constant current of 20 mA/gel using a Compact-PAGE apparatus (Atto Co., Tokyo, Japan). After electrophoresis, gel was stained with 0.05% (w/v) Coomassie blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% (v/v) acetic acid. High molecular weight markers (Sigma Chemical Co., St. Louis, Mo., USA) were used to estimate the molecular weight of proteins. Type I, II, III and V collagens were used as standards.

2.7. Peptide mapping of collagen from skin and bone

Peptide mappings of collagen samples were performed according to the method of Saito, Kunisaki, Urano, and Kimura (2002) with a slight modification. The freeze-dried samples (0.2 mg) were dissolved in 0.1 ml of 0.1 M sodium phosphate, pH 7.2, containing 0.5% (w/v) SDS. After the addition of 10 μl of the same buffer containing 5 μg of *Staphylococcus aureus* V8 protease (EC 3.4.21.19, Sigma Chemical Co., St. Louis, Mo., USA) or 0.05 μg of lysyl endopeptidase from *Achromobacter lyticus* (EC 3.4.21.50; 4.5 AU/mg protein; Wako Pure Chemical Industries, Ltd., Tokyo, Japan), to collagen solutions, the reaction mixture was incubated at 37 °C for 25 and 5 min for V8 protease and lysyl endopeptidase, respectively. The reaction was terminated by subjecting the reaction mixture to boiling water for 3 min. Peptides generated by the protease digestion were separated by SDS–PAGE using 7.5% gel. Peptide mapping of calf skin collagen acid-soluble type I was conducted in the same manner and the peptide patterns were compared.

2.8. Differential scanning calorimetry

The samples (collagens from the skin and bone) were prepared by the slightly modified method of Rochdi, Foucat, and Renou (2000) and Komsa-Penkova, Koyonava, Kostov, and Tenchov (1999). The samples were rehydrated by adding deionised distilled water or 0.05 M acetic acid to dried samples at a solid/solution ratio of 1:40 (w/v). The mixture was allowed to stand for 2 days at 4 °C.

Differential scanning calorimetry (DSC) was performed using a Model DSC 7 (Norwalk, USA). Temperature calibration was run using the Indium thermogram. The samples (5–10 mg) were accurately weighed into aluminium pans and sealed. The samples were scanned at 1 °C/min over the range of 20–50 °C using ice water as the cooling medium. An empty pan was used as the reference. Total denaturation enthalpy (ΔH) was estimated by measuring the area in the DSC thermogram. The maximum transition temperature (T_{max}) was estimated from the thermogram.

2.9. Viscosity of collagen solution

Five hundred milliliters of 0.03% collagen in 0.1 M acetic acid were subjected to viscosity measurements using a Brookfield Synchorolectic viscometer (model DV II+, Brookfield Eng Labs Inc., Stoughton, MA, USA.) with spindle No. 1 and speed of 100 rpm. Collagen solution was heated from 4 to 50 °C with a heating rate of 4 °C/min. At the designated temperature, the solution was held for 30 min prior to viscosity determination. Measurement was carried out in triplicate. The relative viscosity was calculated in comparison to that obtained at 4 °C.

2.10. Collagen solubility

The collagen solubility was determined by the method of Montero, Jimenez-Colmenero, and Borderias (1991) with a slight modification. The collagens were dissolved in 0.5 M acetic acid to obtain a final concentration of 3 mg/ml and the mixture was stirred at 4 °C until collagen was completely solubilised.

2.10.1. Effect of pH on collagen solubility

Collagen solution (8 ml) was added to a centrifuge tube and the pH was adjusted with either 6 N NaOH or 6 N HCl to obtain a final pH ranging from 1 to 10. The volume of solutions was made up to 10 ml by distilled water previously adjusted to the same pH as the collagen solution. The solution was centrifuged at 20,000*g* at 4 °C for 30 min. Protein content in the supernatant was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin as a standard. Relative solubility was calculated in comparison with that obtained at the pH rendering highest solubility.

2.10.2. Effect of NaCl on collagen solubility

Five milliliters of collagen (6 mg/ml) in 0.5 M acetic acid were mixed with 5 ml of NaCl in 0.5 M acetic acid at various concentrations (0%, 2%, 4%, 6%, 8%, 10% and 12% (w/v)). The mixture was stirred continuously at 4 °C for 30 min, followed by centrifuging at 20,000*g* at 4 °C for 30 min. Protein content in the supernatant was measured by the method of Lowry et al. (1951), using bovine serum albumin as a standard. Relative solubility was calculated as above.

3. Results and discussions

3.1. Proximate analyses of bigeye snapper skin and bone and their collagen

The proximate analyses of bigeye snapper skin, bone and their collagen are shown in Table 1. Skin and bone contained a high moisture content (62.3–64.1%). The ash and fat contents of bone were greater than those of skin. Conversely, a lower protein content was observed in bone than in skin. Skin had a higher content of hydroxyproline than bone. Sadowska et al. (2003) reported that hydroxyproline content of cod skin was 14.6 mg/g sample, which was lower than that of bigeye snapper skin (19.5 mg/g sample). Different hydroxyproline contents between two species might depend upon the species, environment and body temperature of fish (Rigby, 1968; Sikorski, Kolakowska, & Pan, 1989). When collagen was extracted from the skin and bone, it was noted that a higher yield (10.9%) was obtained from skin THAN from bone (1.6). The yield of collagen extracted was coincidental with hydroxyproline content found in the skin or bone. Extracted collagens from skin and bone both had low contents of ash and fat, indicating the efficacy of removal of both inorganic matter and fat. Collagen samples had low moisture contents, with protein content ranging from 84.2% to 94.0%. Increase in hydroxyproline content in collagen samples was generally accompanied with an increased protein content.

3.2. Amino acid composition of collagens from the skin and bone

The amino acid composition, expressed as residues per 1000 total residues is shown in Table 2. Collagens from the skin and bone had glycine as the major amino acid. Relatively high contents of alanine, proline, glutamic acid and hydroxyproline, in descending order, were observed. Amino acid compositions of collagen from skin were slightly different from those of

Table 2
Amino acid composition of skin and bone collagens of bigeye snapper (residues/1000 residues)

Amino acid	Skin	Bone
Asp	51	47
Hyp	77	68
Thr	29	25
Ser	36	34
Glu	78	74
Pro	116	95
Gly	286	361
Ala	136	129
Val	22	17
Met	12	8
Ile	5	5
Leu	24	25
Tyr	4	2
Phe	15	12
Hyl	10	20
Lys	31	25
His	10	6
Arg	60	46
Total	1000	1000

Table 1
Proximate analyses and hydroxyproline content of skin and bone of bigeye snapper and the extracted collagens

Sample	Proximate compositions[a] (% wet wt.)				Hydroxyproline (mg/g sample)
	Moisture	Ash	Fat	Protein	
Skin	64.08 ± 0.05	3.23 ± 1.41	0.98 ± 0.23	32.0 ± 0.19	19.5 ± 0.41
Bone	62.27 ± 0.29	14.40 ± 0.68	8.77 ± 0.46	13.3 ± 0.43	5.71 ± 0.23
Skin collagen	7.06 ± 0.58	0.68 ± 0.09	0.33 ± 0.07	94.0 ± 0.75	58.5 ± 0.85
Bone collagen	11.57 ± 0.43	0.88 ± 0.07	0.48 ± 0.11	84.2 ± 1.63	42.5 ± 0.94

[a] Average ± SD from triplicate determinations.

collagen from bone. Compared to collagen from bone, collagen from skin contained higher amount of hydroxyproline, proline and arginine but lower amount of glycine and hydroxylysine. Glycine content of collagens from the skin and bone was approximately 30% of total amino acids. Generally, glycine represents nearly one-third of the total residues and occurs as every third residue in collagen except for the first 14 or so amino acid residues from the N-terminus and the first 10 or so from the C-terminus (Burghagen, 1999; Foegeding et al., 1996; Pearson & Young, 1989; Wong, 1989). The imino acid contents of collagens from the skin and bone were 193 and 163 residues/1000 residues, which were lower than those of porcine dermis collagen (220 residues/1000 residues) (Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003). Foegeding et al. (1996) reported that fish collagens had lower imino acid contents than mammalian collagens. The imino acid content of animal collagens correlated with their habitat (Foegeding et al., 1996; Rigby, 1968). The degrees of hydroxylation of proline in collagens from the skin and bone were 39.9% and 41.1%, respectively. Collagen from the skin and bone had degrees of lysine hydroxylation of 24.4% and 44.4%, respectively. Oxidation of proline and lysine to their hydroxylated residues is catalysed by proline hydroxylase and lysine hydroxylase, respectively (Burghagen, 1999; Foegeding et al., 1996; Pearson & Young, 1989; Wong, 1989). From the result, hydroxylation of proline and lysine in collagen from bone was slightly greater than that in collagen from skin. Hydroxylated proline plays a role in stabilising the triple helix (Ramachandran, 1988) and hydroxylated lysine contributes to the formation and stabilisation of cross-links, giving rise to complex, non-hydrolysable bonds (Asghar & Henrickson, 1982; Stimler & Tanzer, 1977). The results suggested that collagen from bone might have a slightly more complex structure than that from skin as shown by the higher degree of hydroxylation.

3.3. SDS–polyacrylamide gel electrophoresis patterns of collagens from skin and bone

The electrophoretic patterns of collagens from skin and bone under reducing and non-reducing conditions are shown in Fig. 1. No differences in electrophoretic patterns between collagens from the skin and bone were observed. Both collagens, from the skin and bone, comprised at least two different α chains, α1 and α2. The α3 chain, if present, could not be separated under the electrophoretic conditions employed because α3(I) migrates electrophoretically to the same position as α1(I) (Kimura, 1992; Kimura & Ohno, 1987; Matsui, Ishida, & Kimura, 1991). This result is in agreement with those of skin and bone from other fish species (Ciarlo et al., 1997; Kimura, 1985; Kimura & Ohno, 1987; Matsui et al., 1991; Nagai & Suzuki, 2000a, 2000b; Piez, 1965).

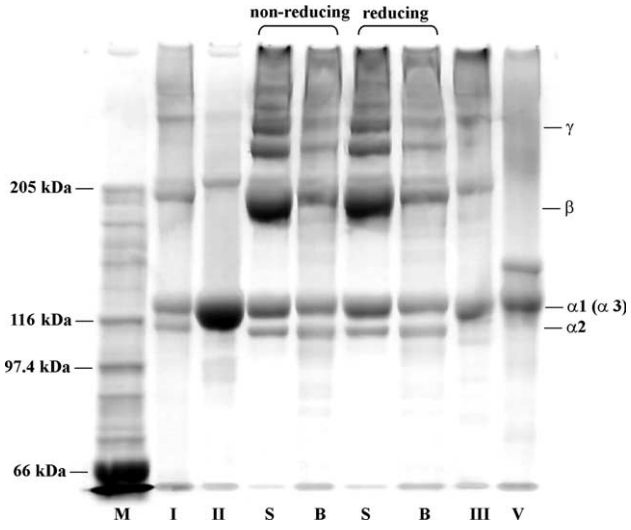

Fig. 1. SDS–PAGE pattern of collagen from the skin (S) and bone (B) of bigeye snapper under reducing and non-reducing conditions. M, I, II, III and V denote high molecular weight protein markers, and collagens type I, II, III and V, respectively.

The electrophoretic patterns of collagens from the skin and bone under non-reducing and reducing conditions were similar, indicating the absence of the disulfide bonds in those collagens. The result was in accordance with that previously reported from hake and trout collagens (Montero, Borderias, Turnay, & Leyzarbe, 1990). Generally, type I collagen consisted of low amounts of cysteine (~0.2%) and methionine (~1.24–1.33%) (Owusu-Apenten, 2002), which play an essential role in disulfide bond formation. However, type III and IV collagens contain oxidisable cysteine residues (Foegeding et al., 1996). Based on electrophoretic mobility and subunit composition, it was suggested that collagens from skin and bone of bigeye snapper were type I collagens. Type I collagen consists of two identical α1(I) chains and one α2 chain (Burghagen, 1999; Foegeding et al., 1996; Pearson & Young, 1989; Wong, 1989). Fish skin and bone have been reported to contain type I collagen as the major collagen (Ciarlo et al., 1997; Kimura & Ohno, 1987; Montero et al., 1990; Nagai & Suzuki, 2000b).

From the result, both collagens were rich in inter- and intra-molecular cross-linked components, β and γ components. Starving fish contain more collagen with a greater degree of cross-linking than do fish that are well fed (Foegeding et al., 1996; Regenstein & Regenstein, 1991). Moreover, Love, Yamaguchi, Creach, and Lavety (1976) reported that myocommata are thickened during starvation with a mechanically stronger collagen than was present when the fish were adequately nourished. Such collagen contains more intermolecular cross-links. The number of cross-links in mammalian animal collagen increases with increasing age. However, most connective tissue in fish is renewed annually and highly

cross-linked protein is not generally found in fish (Foegeding et al., 1996; Zayas, 1997).

3.4. Peptide mapping of collagens from the skin and bone

The peptide maps of collagens from the skin and bone of bigeye snapper, digested by V8 protease and lysyl endopeptidase, in comparison with calf skin collagen type I, are shown in Fig. 2. For peptide maps of collagens digested by V8 protease (Lane 5–7), the band intensities of molecular weight cross-linked components, β- and γ-components, of calf skin collagen slightly decreased with the appearance of a 144.8 kDa peptide fragment. Additionally, α-components, α1 and α2, of calf skin collagen were hydrolysed to some extent. For bigeye snapper collagens, α- and molecular cross-linked components of collagens from the skin and bone of bigeye snapper were markedly degraded after digestion with V8 protease. This result suggested that α- and molecular cross-linked components of calf skin collagen were more tolerant to digestion by V8 protease than collagens from the skin and bone of bigeye snapper. V8 protease exhibits a high degree of specificity for glutamic acid and aspartic acid residues of peptides and proteins (Vercaigne-Marko, Kosciarz, Nedjar-Arroume, & Guillochon, 2000). Thus, calf skin collagen might contain lower glutamic acid and aspartic acid residues than collagens from the skin and bone of bigeye snapper. After digestion, collagen from the skin of bigeye snapper was degraded into three major peptide fragments with MW values of 53.3, 36.9 and 33.8 kDa, while collagen from bone was degraded into seven major peptide fragments of MW values 85.1, 77.9, 65.3, 59.0, 53.3, 36.9 and 33.8 kDa. When comparing peptide maps between collagens from the skin and bone of bigeye snapper, similar peptide fragments of MWs ranging from 53.3 to 33.8 kDa were observed. However, fragments with MW larger than 59.0 kDa disappeared in collagen from the skin of bigeye snapper.

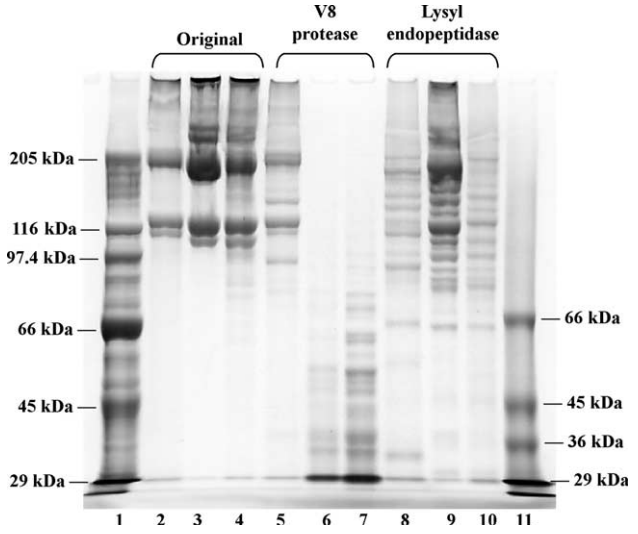

Fig. 2. Peptide mapping of collagens from the skin and bone of bigeye snapper digested by V8 protease and lysyl endopeptidase. Lanes 1 and 11: high and low MW protein markers, respectively; lanes 2–4: type I collagen, collagens from the skin and bone, respectively; lanes 5–7: peptide fragments of type I collagen, collagens from the skin and bone with V8 protease digestion, respectively; lanes 8–10: peptide fragments of type I collagen, collagens from the skin and bone with lysyl endopeptidase digestion, respectively.

For peptide maps of collagens digested by lysyl endopeptidase (Lane 8–10), all components of calf skin collagen were more hydrolysed, compared to the hydrolysates with V8 protease, as shown by the lower original band intensity of each component remaining with a concomitant increase in lower MW peptide fragments. Molecular cross-linked components and – components of calf skin collagen and collagens from the skin and bone of bigeye snapper were susceptible to hydrolysis by lysyl endopeptidase especially calf skin collagen and collagen from the bone of bigeye snapper. This result suggested that those components of collagen from the skin of bigeye snapper were more tolerant to digestion by lysyl endopeptidase than those of calf skin collagen and collagen from bone. After digestion, peptide fragments of collagens from the skin and bone of bigeye snapper were quite similar with MW ranging from 186.5 to 69.5 kDa. Nevertheless, calf skin collagen showed a peptide map distinct from those of collagens from the skin and bone of bigeye snapper. Thus, it was presumed that primary structures of collagens from the skin and bone of bigeye snapper were quite similar in terms of amino acid sequence. However, accessibilities of susceptible bonds to the proteinase might be different, leading to varying degrees of hydrolysis between collagens from bone and skin.

3.5. Thermal stability of collagen from skin and bone

Differential scanning calorimetry thermograms of collagens from the skin and bone of bigeye snapper rehydrated in 0.05 M acetic acid and deionised distilled water are shown in Fig. 3. An endothermic peak, with peak maximum temperature (T_{max}) of 31.0 and 31.5 °C, was observed for collagens from the skin and bone rehydrated in water, respectively. For collagens rehydrated in acetic acid, it was noted that T_{max} shifted to a lower temperature, 28.68 and 30.80 °C, for collagens from skin and bone, respectively. Though T_{max} was generally higher for collagen from bone, either rehydrated in water or acetic acid, a lower enthalpy was observed, compared with collagen from the skin. Calf skin collagen had an endothermic transition with T_{max} of 40.8 °C and enthalpy of 2.83 J/g (Komsa-Penkova et al., 1999). The different T_{max} of transitions among collagen from animal species (fish and calf) seems to be correlated with the content of imino

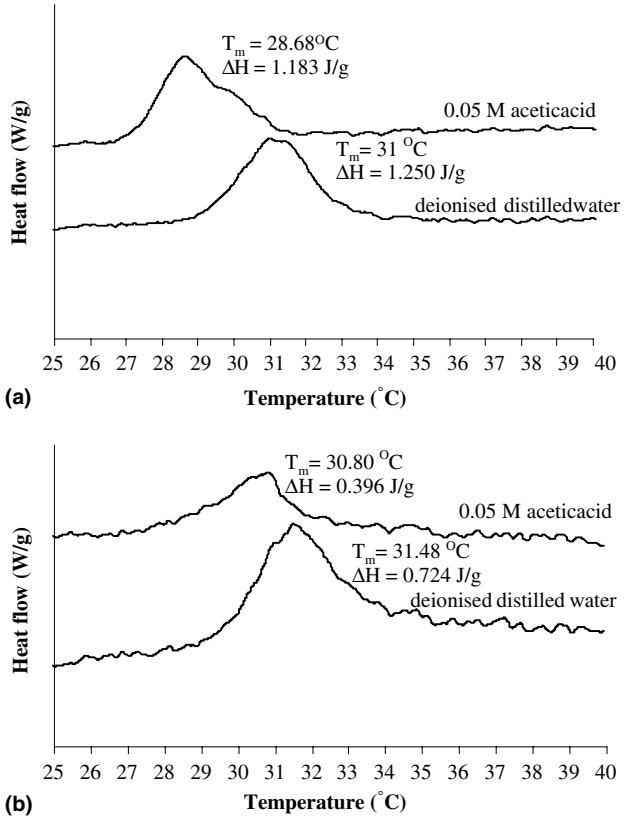

Fig. 3. Thermograms of collagens from the skin (a) and bone (b) of bigeye snapper rehydrated in 0.05 M acetic acid and deionised distilled water.

acids (proline and hydroxyproline). The higher the imino acid content, the more stable are the helices (Wong, 1989). The imino acid content of calf skin collagen is higher than that of fish skin collagen (Foegeding et al., 1996). Moreover, collagen stability is correlated with environmental and body temperature (Rigby, 1968). Collagen from cold water fish has a low imino acid content (Piez & Gross, 1960). The increasing imino acid (proline and hydroxyproline) content resulted in increased denaturation temperature of collagen (Gustavson, 1955; Kimura, 1992; Piez & Gross, 1960; Yamaguchi, Lavety, & Love, 1976).

From this result, transition temperatures of collagen from skin and bone were similar. However, acetic acid played an essential role in the change in thermal properties of collagen, especially that from skin. Collagens from the skin and bone rehydrated in acetic acid had decreases of T_{max} and enthalpy by 2.32 and 0.68 °C, respectively, and decreases of transition enthalpy (ΔH) of about 0.07 and 0.33 J/g, respectively, when compared to those rehydrated in water. Acetic acid can cleave hydrogen bonds (Gustavson, 1956), which stabilises collagen, triple-helical structure (Xiong, 1997). So the collagen structure was broken, leading to decreased thermal stability of collagens, as shown by the lowered T_{max} and enthalpy.

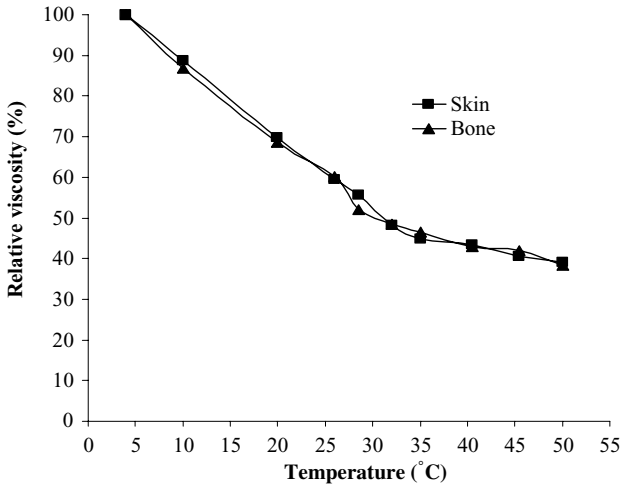

Fig. 4. Relative viscosity of the solution of collagens from the skin and bone of bigeye snapper at different temperatures.

3.6. Viscosity of skin and bone collagen solutions

Relative viscosity of collagen in acetic acid subjected to heat treatment at different temperatures is depicted in Fig. 4. Relative viscosity decreased continuously on heating up to 30 °C. Rate of decrease was retarded in the temperature range of 35–50 °C. Heat-treatment at high temperature can break down the hydrogen bonds, which stabilise collagen structure (Wong, 1989). Collagen denatures at temperatures above 40 °C to a mixture of random-coil single, double and triple strands (Wong, 1989). From the result, similar changes in viscosity of collagens from the skin and bone, caused by heat-treatment, were observed. Denaturation of collagen structure caused by heat-treatment is associated with the changes in viscosity (Nagai et al., 1999; Nagai & Suzuki, 2000a, 2002). Denaturation temperatures of collagens from the skins of Japanese sea-bass, chub mackerel, bullhead shark and ocellate puffer fish ranged from 25.0 to 28.0 °C and those of collagens from the bones of Japanese sea-bass, skipjack tuna, ayu, yellow sea bream and horse mackerel were from 29.5 to 30.0 °C (Nagai & Suzuki, 2000a, 2002).

3.7. Solubility of collagens from the skin and bone

The effects of pH and NaCl concentrations on collagen solubilities are depicted in Figs. 5 and 6, respectively. Highest solubility of collagens from the skin and bone were found at pH 2 and 5, respectively. Generally, both collagens could be more soluble in the acidic pH ranges (Foegeding et al., 1996). Sharp decrease in solubility was observed at neutral pH. However, solubility was slightly decreased at very acidic pH. When pH values are above and below pI, a protein has a net negative or positive charge, respectively (Vojdani, 1996). As a consequence, more water interacts with the charged

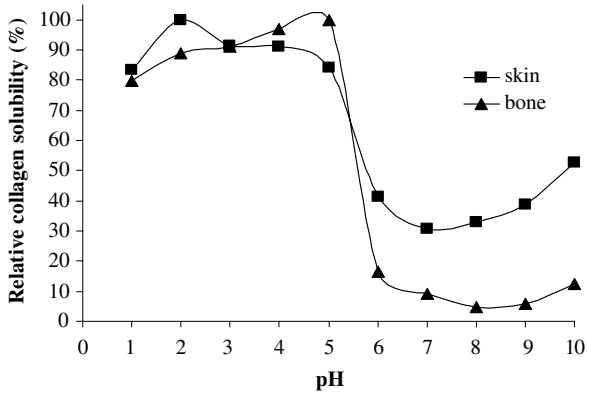

Fig. 5. Relative solubility (%) of collagens from the skin and bone of bigeye snapper at different pH.

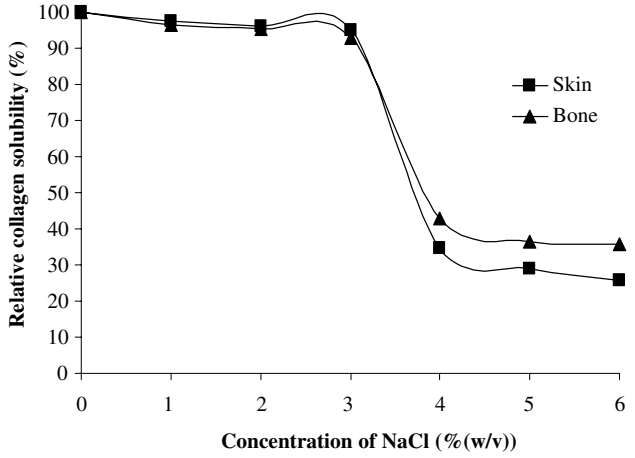

Fig. 6. Relative solubility (%) in 0.5M acetic acid of collagens from the skin and bone of bigeye snapper in the presence of NaCl at different concentrations.

proteins (Vojdani, 1996). Nevertheless, slight increase in solubility was found within the alkali pH ranges. Charge repulsion contributes to greater protein solubility (Vojdani, 1996). The differences in pH maxima for solubility between collagens from skin and bone suggested differences in molecular properties and conformations between the collagens. Collagen from skin showed higher solubility than bone collagen at pH > 6 (Fig. 5). This result indicated that collagen from skin might possess a lower degree of cross-linking. The predominance of weaker bonds in collagen from skin was also presumed in comparison with collagen from bone.

The solubility in 0.5 M acetic acid of collagens from both skin and bone was maintained in the presence of NaCl up to 3%. A marked decrease in solubility was observed with an increasing NaCl concentration, especially, at concentrations above 3%. NaCl, at a higher concentration, might result in decreasing protein solubility (salting out effect) by increasing hydrophobic interaction and aggregation, and competing with the protein for water, thereby causing the protein to precipitate (Vojdani, 1996). From the result, collagen from bone was more tolerant to salt than collagen from skin, as shown by the greater solubility in the presence of NaCl at 4–6% (Fig. 6). Thus, collagen from different sources might have different molecular properties, leading to the varied characteristics both collagens.

4. Conclusion

Collagens extracted from skin and bone of bigeye snapper were classified as type I with slightly different amino acid compositions. Peptide maps of collagens from the skin and bone digested by V8 protease and lysyl endopeptidase were slightly different, indicating some differences in amino acid sequence or conformation. Collagens showed high solubility at acidic pH (2–5) and the solubility markedly decreased in presence of NaCl (above 3%).

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