J. Fish. Sci. Technol. 12(1), 6-15, 2009

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Characterization of Acid- and Pepsin-soluble Collagens from Rockfish *Sebastes schlegeli* Skin

Hyung Jun Kim, Seong Joon Jee, Min Suck Yoon, Mu-Ho Youn[1], Kyung Tae Kang[2], Dong-Ho Lee[3], Min Soo Heu and Jin-Soo Kim*

Division of Marine Life Science/Institute of Marine Industry, Gyeongsang National University, Tongyeong 650-160, Korea
[1]General Marine Product Processing Factory, Geoje Federation of Fisheries Cooperatives, Geoje 656-881, Korea
[2]Hansung Enterprise Co., LTD, Gimhae 621-914, Korea
[3]The Bureau of Risk Management, Korea Food & Drug Administration, Seoul 122-704, Korea

Biochemical and functional properties of acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) from rockfish skin were characterized. Yield of PSC (90.0%) was higher than that of ASC (63.2%). Both ASC and the PSC consisted of α1 and α2 chains, and α -cross-linked components. According to the results of hydroxylation of proline and lysine, and FT-IR, no difference between the helical structure of ASC and PSC was identified. Thermal denaturation temperature (TDT) of ASC from rockfish skin was 22.8°C, the same as exhibited in PSC. Both ASC and PSC were higher in water absorption capacity (WAC) and oil absorption capacity (OAC) than other vegetable proteins. According to the results of emulsifying activity (EA) and cooking stability (CS), both ASC and PSC from rockfish skin were inferior compared to the commercial emulsifier (Tween-80). The results of FT-IR suggested that the structure of PSC was slightly different when compared to that of ASC. No differences in solubility were established between ASC and PSC from rockfish skin at various pH and NaCl concentrations.

Key words: Collagen, Fish collagen, Rockfish skin, *Sebastes schlegeli*, By-products

Introduction

Collagen is the most abundant protein in animals, comprising approximately 30% of total animal protein (Muyonga et al., 2004a). It is the main structural element of bones, cartilage, skin, tendons, ligaments, blood vessels, teeth, cornea and all other organs of vertebrates. Collagen, commonly manufactured from land animal processing by-products, is widely used in the food (sausage casing, clarification agent, emulsifier, or whipping agent), pharmaceutical (film-forming agent, microencapsulation, or tablet coating) applications because of an important biomaterial. Its usage extends even further to other industrial applications (shampoo and lipstick). Today's health-conscious consumers, however, have a negative attitude toward collagen extracted from land animals because of the recent outbreaks of bovine sponge form encephalopathy (BSE), foot and mouth disease, and chicken flu (Kim and Park, 2004).

Furthermore, religious or cultural reasons also restrict some people from consuming certain animal by-products. Therefore, many scientists have been focusing their experiments to find out alternative sources of collagen. They have found that fish skin (Muyonga et al., 2004a; Park et al., 2007; Nagai and Suzuki, 2000), bone (Nagai and Suzuki, 2000), fin (Nagai and Suzuki, 2000) and scales(Nagai et al., 2004), in addition to squid skin (Nagai and Suzuki, 2002), and refiner discharge (Kim and Park, 2004) from surimi provide the best source of raw material among various collagen alternatives because of its high availability, no risk of disease transmission and problem of religious reasons.

Molecular structure of collagen consists of three α chains wound together in a tight triple helix. Some parts of collagen from skin, bone, scale, fin and muscle of fish can only be extracted using pepsin. Thus, the use of pepsin is considered to be an

*Corresponding author: jinsukim@gnu.ac.kr

effective method for extracting collagen from most seafood by-products (Wang et al., 2007).

Rockfish is among the top aquaculture products in Korea, with an estimated yield of 39,500 M/T in 2007. This provides a unique opportunity for collagen extraction from rockfish skin, which is generated in large quantities (approximately 3.4% based on whole fish) from sliced raw fish production. However, the use of rockfish skin in human foodstuff production has not been widely studied yet. Most of the rockfish skin is conventionally used to produce fish meals and fertilizers or are directly discharged into rivers and estuaries, resulting in environmental pollution (Gildberg, 2004). New challenges must be attempted to find a way to upgrade the processing of waste to food grade ingredients, such as collagen and gelatin.

Collagen has significantly different properties based on the source-organism and its habitat or type of tissue (Park et al., 2007; Kim and Park, 2004). For the effective use of rockfish skin collagen, the physicochemical and functional properties (effect of pH and NaCl on solubility, water and oil absorptions, and emulsion ability and stability) must be investigated.

The objective of this study was to isolate pepsin-soluble and acid-soluble collagens from rockfish skin and to characterize their properties for assessment of potential commercial applications as alternatives to mammalian collagen in foods, cosmetics, and biochemical products.

Materials and Methods

Materials

Rockfish (*Sebastes schlegeli*) skin was obtained from a fisheries manufacturer (Geoje, South Korea) in December 2006. The skin was mechanically separated, and adhering muscle was removed manually. After washing thoroughly with running water, the skin was placed in polyethylene bags and stored at -25°C until used.

Pepsin (EC 3.4.23.1) and protein markers were purchased from Sigma-Aldrich Co. (St. Louis, Mo., USA).

All other reagents were of analytical grade.

Preparation of collagen fractions

All analyses were performed in a cold room (5°C). Native collagen was prepared as described by Park et al. (2007). Frozen rockfish skin was thawed for 20 hrs in cold tap-water and cut into small pieces (2 to 3 cm). Cleaned fish skin was squeezed to rid it off excess water. To the residues, 10 volumes (v/w) of 0.1 N NaOH was added to remove non-collagenous proteins effectively and to exclude the effect of endogenous proteases on collagen. The homogenate was stirred overnight before centrifuging at 10,000×g for 20 min. The alkali-extraction was repeated 3 additional times. Final precipitate was washed thoroughly with cold distilled water. To the residues, 10 volumes (v/w) of 0.5 M acetic acid was added. Suspensions were stirred for 3 days and then centrifuged at 10,000×g for 20 min. The acid-extraction was repeated once more. The precipitate was then washed with cold distilled water at a 1:2 (w/v) ratio. The supernatant from acid-extraction and the filtrate from rinsing were combined and subjected to salting out by adding NaCl to 2.0 M before centrifuging at 20,000×g for 20 min. The precipitate was redissolved in 0.5 M acetic acid and then dialyzed (molecular weight cut-off 10,000) against cold distilled water to remove salt. Salting-out and dialysis were repeated twice more for further purification of collagen. The final dialyzed solution was lyophilized and used as acid-soluble collagen (ASC).

Pepsin-soluble collagen (PSC) was prepared as described by Hwang et al. (2007) with slight modifycations. The alkali-extracted residue was digested in 10 volumes (v/w) of 0.5 M acetic acid containing a porcine pepsin (EC 3.4.23.1, Sigma-Aldrich Co., Mo, USA)/substrate ratio of 1:20 (w/w) for 2 days before centrifuging at 10,000 x g for 20 min. The pepsin-extraction was repeated once more. The precipitate was then washed with cold distilled water at a 1:2 (w/v) ratio. The supernatant from pepsin-extraction and the filtrate from rinsing were combined and subjected to salting out and dialysis by the same method as explained in purification procedures of ASC. The final dialyzed solution was lyophilized and used as PSC.

Insoluble collagen was prepared as described by Kim and Park (2004) with slight modifications. Separately from preparation of ASC and PSC, centrifuge residue obtained after pepsin-extraction was heated with 5 volumes (v/w) of distilled water in an autoclave at 120°C for 1 hr and then centrifuged at 10,000×g for 20 min. The precipitate was rinsed using hot distilled water at a 1:2 (w/v) ratio. The supernatant from centrifugation and the filtrate from rinsing were combined, lyophilized and used as insoluble collagen for calculating total collagen-N concentration.

Acid- and pepsin-soluble fractions were lyophilized and used for further analyses of collagen characteristics. Concentration and yield of collagen were calculated, respectively, as (total collagen-N concentration, % / total-N concentration, %)×100 and

(ASC-N or PSC-N concentrations, % / total collagen-N concentration, %)×100.

Proximate composition, volatile basic nitrogen (VBN) and heavy metal

According to AOAC methods (AOAC, 1995), moisture content was quantified by oven drying at 105°C, total protein by the Kjeldahl procedure, and crude ash by incineration in a muffle furnace at 550°C. In addition, total lipid was extracted into a methanol-chloroform mixture according to the method of Bligh and Dyer (1959). The concentration of VBN was determined by the method of Conway (Ministry of Social Welfare of Japan, 1960). The mercury content was determined by the combustion gold amalgamation method (KFDA, 2006) using a mercury analyzer (SP-3A, Nippon Instrument Co., Tokyo, Japan). Other heavy metals, such as Pb, Cd, and Cr, were determined by the wet ash method (Tsutagawa et al., 1994) using an inductively coupled plasma spectrophotometer (ICP, Atomscan 25, Thermo Electrron Co., Waltham, MA, USA).

Amino acid composition and hydroxylation of proline and lysine

Amino acid composition was determined using an amino acid analyzer (Biochrom 30, Pharmacia Biotech, Cambridge, UK) according to the method of Kimura et al. (1988). The sample was hydrolyzed in 6 N HCl in evacuated/sealed tubes at 110°C for 16 hrs. The hydrolysates was evaporated to dry in a vacuum evaporator at 40°C and then diluted up to 25 mL with Li^+-citrate buffer (pH 2.2) for analyses of amino acids containing hydroxyproline (Hyp) and hydroxylysine (Hyl).

Hydroxylation (%) of proline (Pro) and lysine (Lys) was calculated on the basis of the amino acid composition according to the following equations:

Hydroxylation of Pro (%)=[Hyp/(Pro+Hyp)] ×100
Hydroxylation of Lys (%)=[Hyl/(Pro+Hyl)] ×100

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed by the method of Laemmli (1970) using 7.5% running gel containing 0.1% SDS at pH 8.8, and 5% stacking gel. Protein samples and protein markers (M 4038; Sigma-Aldrich Co., St. Louis., MO., USA) were heated at 100°C for 3 min in 10 mM tris-HCl buffer (pH 6.8) containing 2% SDS, 1% 2-mercaptoethanol, 25% glycerol, and 0.1% bromo-phenol blue. The gels were stained for protein with 0.1% Coomassie brillant blue R-250 and destained in 10% methanol and 10% acetic acid.

Thermal denaturation temperature (TDT)

TDT was performed according to the method of Zhu and Kimura (1991). The Ostwald viscometer was filled with 5 mL of collagen solution (30 mg collagen dissolved in 100 mL of 0.1 M acetic acid solution) or 0.1 M acetic acid as a control sample. The viscometer was immersed in an 8°C water bath for 30 min to allow the collagen solution to equilibrate with the water bath temperature. The temperature was incrementally increased up to 45°C and maintained at each temperature (15°C to 31°C at every 2°C, and at 45°C) for 30 min. Efflux time of the solution incubated in the water bath was checked in the tube of an Ostwald viscometer and then specific viscosity (η_{sp}) was calculated according to the following equation :

Specific viscosity $(\eta_{sp})=(t-t_0)/t_0$
t=efflux time of the collagen solution
t_0=efflux time of the 0.1 M acetic acid

We assumed that collagen helical conformation was undenaturated at 8°C, whereas breakdown was completed at 45°C.

TDT of collagen solution was defined as the temperature at which the change in viscosity reached by 50%. Fraction change was calculated according to the following equation:

Fraction change = $[(\epsilon_2/C)-(\epsilon_3/C)] / [(\epsilon_1/C)-(\epsilon_3/C)]$
C=collagen concentration (mg/mL);
ϵ_1=specific viscosity at 8°C;
ϵ_2=specific viscosity at measured temperature (°C);
ϵ_3=specific viscosity at 45°C

Fourier transform infrared spectroscopy (FT-IR)

FT-IR was determined as described by Muyonga et al. (2004b). FT-IR spectra were obtained from discs containing 2 mg collagen in approximately 100 mg potassium bromide (KBr). All spectra were obtained from 4,000 to 500 cm^{-1} at a data acquisition rate of 2 cm^{-1} by using a FT-IR spectrophotometer (Bruker IFS 88, Bruker, Germany).

Effect of pH and NaCl concentration on collagen solubility

Effect of pH and NaCl, on collagen solubility was determined by the method of Montero et al. (1991). Samples for measuring collagen solubility were dissolved in 0.5 M acetic acid with gentle stirring at 5°C. This was required to obtain final concentrations

of 3 mg/mL for measuring effect of pH and 6 mg/mL for measuring effect of NaCl concentration.

To investigate the effect of pH on collagen solubility, 8 mL of collagen solution was added into 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 solution was made up to 10 mL by distilled water previously adjusted to the same pH as the collagen solution and centrifuged at 20,000×g at 5°C for 30 min. Protein content in the supernatant was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard. Relative solubility was calculated in comparison with that obtained at the pH rendering highest solubility.

To investigate the effect of NaCl concentration on collagen solubility, 5 mL of collagen (6 mg/mL) in 0.5 M acetic acid was mixed with 5 mL of NaCl at various concentrations (0% to 6% at every 1%, w/v) in 0.5 M acetic acid. The mixture was stirred continuously at 5°C for 30 min before centrifuging at 20,000×g at 5°C for 30 min. Protein content and relative solubility in the supernatant were measured and calculated as described by methods above.

Functional properties

Water and oil absorption capacities were determined by the method of Beuchat (1981). Freeze-dried collagen (0.3 g) was mixed with 10 mL distilled water for water absorption measurement and with 10 mL soybean oil for oil absorption measurement. Mixing was conducted at fast speed using a vortex mixer for 30 sec. Samples were then allowed to stand at room temperature (22°C) for 30 min before centrifuging at 1,300×g for 30 min. The volume of supernatant was measured in a graduated cylinder (10 mL), and each capacity was expressed as (10 mL - measured volume)/collagen weight (0.3 g).

Emulsifying activity and cooking stability were determined by the method of Wang and Kinsella (1976). Freeze-dried collagen (0.1 g) and Tween-80 (0.1 g, Fisher Scientific, Pittsburg, PA, USA) were added to 10 mL of 0.1 N acetic acid, respectively, and the mixture was set at room temperature for 2 min using a PT 10/35 polytron homogenizer at setting 3 (Kinematica, Luzern, Switzerland). Ten milliliters of soybean oil (Dongbang Co., Korea) was added before mixing for 3 min using a PT 10/35 polytron homogenizer at high speed. The resulting emulsion was centrifuged at 1,300×g for 15 min. Emulsifying activity was measured according to the following equation:

$$\text{Emulsifying activity (\%)} = \left(\frac{\text{height of emulsified layer}}{\text{height of total contents in the tube}}\right) \times 100$$

Cooking stability was determined similarly to the emulsifying activity except that the emulsion in the centrifuge tube (height×inner dia, 11.5×3.0 cm) was initially heated in a water bath (SWB-10, Jeio Tech., Korea) at 80°C for 30 min and subsequently cooled to 15°C before centrifuging at 1,300×g for 15 min. Cooking stability was measured according to the following equation:

$$\text{Cooking stability (\%)} = \left(\frac{\text{height of emulsified layer after centrifugation}}{\text{height of total content in the tube}}\right) \times 100$$

Results and Discussion

Proximate composition, collagen content and safety of rockfish skin

Proximate composition, collagen and volatile basic nitrogen (VBN) contents of raw rockfish skin are shown in Table 1. The proximate composition of rockfish skin was 71.3% for moisture content, 23.3% for crude protein content, 1.4% for lipid content, and 3.5% for crude ash content. Moisture content of rockfish skin was the same as shown in Alaska pollock skin (71.3%) (Park et al., 2007) and very similar to that of Pacific whiting skin (70.5%) (Kim and Park, 2004). However, it was much higher than those of conger eel skin (58.3%) (Kim and Cho, 1996) and filefish skin (60.3%) (Kim and Cho, 1996). The collagen-included protein content of rockfish skin was lower than those of other fish skins [Alaska pollock skin, 26.1% (Kim and Cho, 1996); Pacific whiting skin, 26.1% (Kim and Park, 2004); conger eel skin, 30.3% (Kim and Cho, 1996); filefish skin, 24.6% (Kim and Cho, 1996)]. The sum (4.9%) of ash and lipid contents, which are foreign components for collagen extraction, of rockfish skin was lower than

Table 1. Proximate composition, collagen and volatile basic nitrogen (VBN) contents of rockfish skin

Proximate composition (%)				Collagen (%)	VBN (mg/100 g)
Moisture	Protein	Lipid	Ash		
71.3±0.5	23.3±0.2	1.4±0.5	3.5±0.8	4.2±0.1	77.4±0.2

that of filefish skin (15.1%) (Kim and Cho, 1996), while higher than that of Alaska pollock skin (1.9%) (Park et al., 2007). The VBN content of rockfish skin was 4.2 mg/100 g, which is believed to be an acceptable limit (less than 20 mg/100 g) for marine products (Kim et al., 2002). Collagen content of rockfish skin was 77.4% based on the dry weight, which was slightly lower than those of filefish skin (81.4%) (Kim and Cho, 1996), and conger eel skin (81.5%) (Kim and Cho, 1996), while much higher than that of Alaska pollock skin (57.1%) (Park et al., 2007). All heavy metals, such as lead, cadmium, and chrome, were not detected in examined rockfish skin (data not shown). According to the Codex Code (2004), the heavy metal safety values were 0.2 to 1.0 mg/kg for chrome, 0.2 to 0.4 mg/kg for lead, and zero for mercury and cadmium. Because the concentration of heavy metals was below the safety value limits, rockfish skin used in this study appeared safe as a raw material for collagen extraction.

Yield

The yields of ASC and PSC from rockfish skin are shown in Fig. 1. The yield of PSC (90.0%) was higher than that of ASC (63.2%). The results were in agreement with the patterns observed for ASC and PSC yields from several other fish species, such as Brownstripe red snapper (Jongjareonrak et al., 2005), deep-sea redfish (Wang et al., 2007), and hake (Ciarlo et al., 1997). The higher PSC yield may be because pepsin cleaved the cross-linked molecules at the telopeptide region without damaging the integrity of the triple helix. Burghagen et al. (1999) reported that collagen molecules in rockfish skin were mostly likely cross-linked by covalent bonds through the condensation of aldehyde groups at the telopeptide region, as well as the inter-molecular cross-linking, leading to a decrease in solubility of collagen.

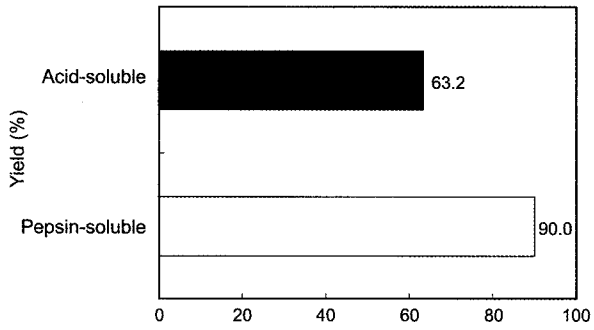

Fig. 1. Yield of acid- (ASC) and pepsin-soluble collagens (PSC) from rockfish skin.

Amino acid composition and hydroxylation ratio of proline and lysine

Amino acid compositions, expressed as residues per 1,000 total amino acid residues and hydroxylation ratios of proline (HDP) and lysine (HDL) of ASC and PSC from rockfish skin are shown in Table 2. Glycine was the most abundant amino acid (319 and 322 residues/1,000 residues, respectvely) in both ASC and PSC, and there were relatively high compositions of proline (128 and 124 residues/1,000 residues, respectively), alanine (103 and 110 residues/1,000 residues, respectively), glutamic acid (78 and 76 residues/1,000 residues, respectively), and hydroxyproline (66 and 68 residues/1,000 residues, respectively), while the compositions of methionine (12 and 10 residues/1,000 residues, respectively), isoleucine (11 and 10 residues/1,000 residues, respecttively), tyrosine (2 and 3 residues/1,000 residues, respectively), hydroxylysine (7 and 8 residues/1,000 residues, respectively), and histidine (7 residues/1,000 residues for both ASC and PSC) were very low. Cysteine was not detected. In general, glycine occurs uniformly at every third residue throughout most collagen molecules, except for the first 14 amino acid residues from the N-terminus and the first 10 amino acid residues from the C-terminus (Jongjareonrak et al., 2005; Burghagen, 1999). The similar patterns of amino acid compositions of ASC and PSC from rockfish skin were also found in skin and muscle of various fishes, such as Pacific whiting (Kim and Park, 2004), deep-sea redfish (Wang et al., 2007), conger eel (Kim and Cho, 2002), mackerel (Kim and Cho., 2002), salmon (Kim and Cho., 2002), Alaska pollock (Park et al., 2007), filefish (Kim and Cho., 2002), brown-stripe red snapper (Jongjareonrak et al., 2005), black drum (Ogawa et al., 2003) and sheepshead (Ogawa et al., 2003).

HDP and HDL from rockfish skin were 30.3% and 17.5%, respectively, for ASC, and 31.8% and 19.8%, respectively, for PSC. The results showed that no differences were found in both HDP and HDL between ASC and PSC from rockfish skin. It was probably because pepsin used for improving extraction ratio of collagen only cleaved some cross-linked molecules at the telopeptide region without damaging the integrity of the triple helix. HDP of ASC from rockfish skin was lower than those of ASC from yellow sea bream muscle (40%) and tiger puffer muscle (39%). It was might be due to the fact that proline and hydroxyproline contents vary with fish species and their living habitat (Jongjareonrak et al., 2005). The low HDP is also significant because it

Table 2. Amino acid composition and hydroxylation ratio of proline (HDP) and lysine (HDL) of acid- (ASC) and pepsin-soluble collagens (PSC) form rockfish skin (residues/1,000 residues)

Amino acids	ASC	PSC	Amino acids	ASC	PSC
Aspartic acid	47.1	41.9	Isoleucine	10.7	9.5
Hydroxyproline	65.6	67.7	Leucine	21.8	20.6
Threonine	27.5	28.1	Tyrocine	2.0	2.5
Serine	41.8	45.8	Phenylalanine	17.4	16.8
Glutamic acid	78.1	75.8	Hydroxylysine	6.7	7.6
Proline	127.7	123.7	Lysine	31.5	30.7
Glycine	318.9	321.8	Histidine	7.1	7.3
Alanine	102.8	109.8	Arginine	55.1	53.7
Valine	26.5	26.9	HDP (%)	30.3	31.8
Methionine	11.7	9.8	HDL (%)	17.5	19.8

affects the functional properties of gelatin that can be derived from collagen (Jongjareonrak et al., 2005). The result suggested that collagen and gelatin from rockfish skin could partially be limited for use in several applications such as sausage casing, film-forming agent, tablet coating and lipstick (Park et al., 2007; Kim and Park, 2004).

SDS-PAGE pattern

Sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) patterns of ASC and PSC from rockfish skin are shown in Fig. 2. According to the SDS-PAGE patterns, 3 distinctive chains, 2 α bands (α1, upper; α2, lower) with their molecular weight at about 116 kDa and their β cross-linked components with a molecular weight 205 kDa, were clearly detected in both ASC and PSC from rockfish skin. The α3 chain, if present, could not be separated under the electrophoretic conditions employed because α3 migrates electrophoretically to the same position as α1 (Kimura, 1992). The results were similar to the patterns observed for several other fish species (Gomez-Guillen et al., 2002). Based on their electrophoresis mobility and subunit composition, it suggested that ASC and PSC from rockfish skin were mainly of type I collagen. This observation was in agreement with the findings reported for deep-sea redfish (*Sebastes mentella*) (Wang et al., 2007), Nile perch (Muyonga et al., 2004a), black drum (Ogawa et al., 2004), sheepshead seabream (Ogawa et al., 2003), bigeye snapper (Kittiphattanabawon et al., 2005), and Brownstripe red snapper (Jongjareonrak et al., 2005). In the electrophoresis mobility, the chains of PSC were a little faster than those of ASC, which indicated that the primary structures of ASC and PSC were slightly different.

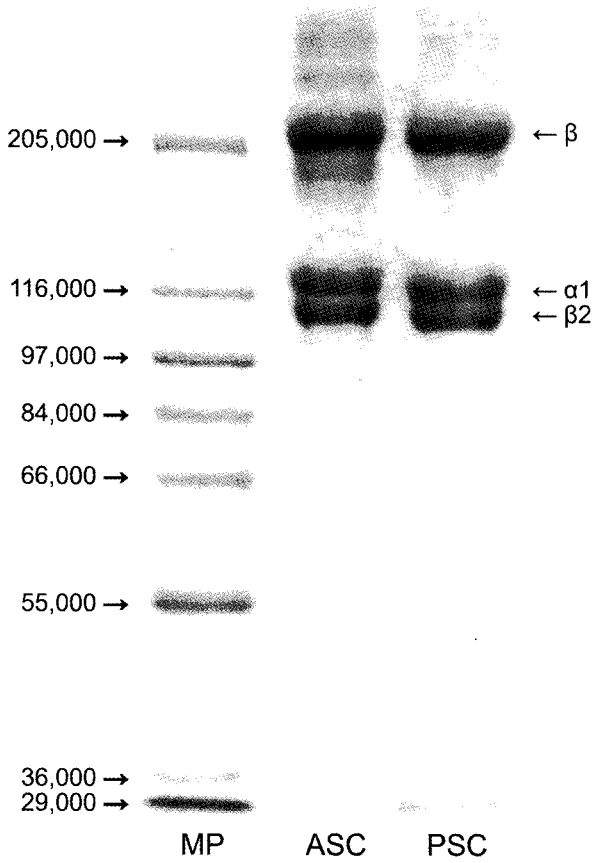

Fig. 2. SDS-PAGE pattern of collagen from rockfish skin (MP=marker protein; ASC=acid soluble collagen; PSC=pepsin soluble collagen).

Thermal denaturation temperature (TDT)

Investigations on the TDT of native collagen molecule deal with processes associated with collagen-gelatin transition (Hawng et al., 2007). The fractional changes in specific viscosity of ASC and PSC from rockfish skin are shown in Fig. 3. Due to the de-

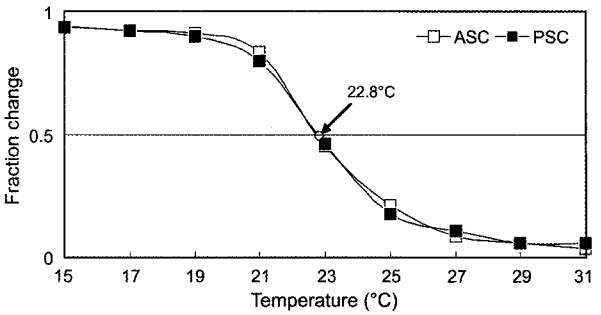

Fig. 3. Thermal denaturation temperature of acid-(ASC) and pepsin-soluble collagens (PSC) from rockfish skin.

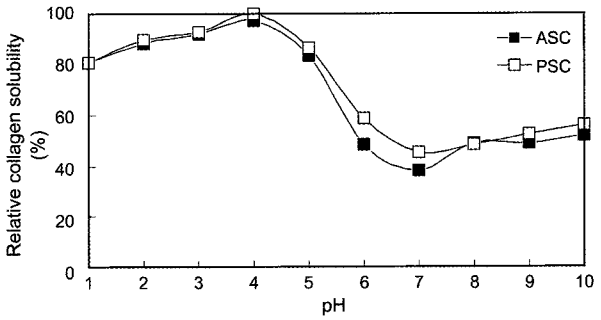

Fig. 4. Fourier transform infrared spectra of acid-soluble (ASC) and pepsin-soluble collagens (PSC) from rockfish skin.

naturation of protein, both ASC and PSC exhibited a similar rapid loss of viscosity with heating from 19°C to 29°C, and remained lower at more than 29°C. No difference in TDT (22.8°C) of both ASC and PSC was found, which indicated that the triple helical structure of PSC was still predominant. The TDTs of ASC and PSC from rockfish skin were higher than those of cold water fishes, such as Alaska pollock (17°C) (Park et al., 2007) and lower than those of temperate fish as well as tropical fish, such as conger eel (29°C) (Kim and Cho, 1996), skipjack tuna (30°C) (Zhu and Kimura, 1991), and Nile perch (37°C) (Muyonga et al., 2004a).

Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of ASC and PSC from rockfish skin are shown in Fig. 4. From the FT-IR spectra, 4 amides (amide A, amide I, amide II and amide III) were identified as major peaks of ASC and PSC from rockfish skin, which exhibited FT-IR spectra similar to that shown by other collagens (Jackson et al., 1995). The regions of amide I, amide II and amide III are known to be directly related with the shape of a polypeptide. Amide A band (3,300-3,340 cm^{-1}) is related to N-H stretching vibrations. Amide I band (1,600-1,660 cm^{-1}) is associated with stretching vibrations of carbonyl groups in peptides, being the most important factor in investigating the secondary structure of a protein. Amide II (-1,550 cm^{-1}) is associated with NH bending and CN stretching. Amide III (1,220-1,320 cm^{-1}) is related to CN stretching and NH, and is involved with the triple helical structure of collagen (Muyonga et al., 2004a). According to the literatures above, the spectra for ASC and PSC from rockfish skin differed slightly, indicating some differences in the secondary structure of the two proteins. The triple helical structure of ASC was confirmed from the absorption ratio between 1,247 cm^{-1} (amide III) and 1,454 cm^{-1} bands, which was approximately to 1.0. However, the ratio of PSC was much higher than that of ASC from rockfish skin, showing some changes of helical structure of PSC. In general, ASC held its special triple helical structure well and possessed a higher extent of intermolecular cross-link (Wang et al., 2007). The structure of PSC was changed slightly due to the loss of N- and C-terminus domains, but the triple helical structure was still predominant a result of the formation of more and/or stronger hydrogen bond.

Effect of pH and NaCl concentration on collagen solubility

The effects of pH and NaCl concentration on solubility of collagen from rockfish skin are shown in Figs. 5 and 6, respectively. The highest solubilities in ASC and PSC, 92.8% and 98.4%, respectively, were found at pH 4. It sharply decreased between pH 4 and pH 8, but then remained relatively stable up to pH 10. Solubility was higher in PSC than in ASC except for pH 1. The results showed that both ASC and PSC exhibited higher solubility in acidic conditions. When pH values are above and below p*I*, a protein has a net negative or positive charge, respectively (Vojdani, 1996). As a consequence, more water interacts with the charged proteins, while charge repulsion contributes to greater protein solubility (Vojdani, 1996). According to the report of Foegeding et al. (1996), the p*I* range in collagen was between pH range of 6 to 9.

Collagen solubility at various NaCl concentrations (0-6%) was measured in 0.5 M acetic acid solution. Solubility in 0.5 M acetic acid of ASC and PSC slowly decreased with increasing NaCl concentrations until reaching 3% (w/v), and rapidly decreased at 4% (w/v), after which it remained relatively stable. The decreasing of collagen solubility at high NaCl concentrations was thought to be mainly due to the

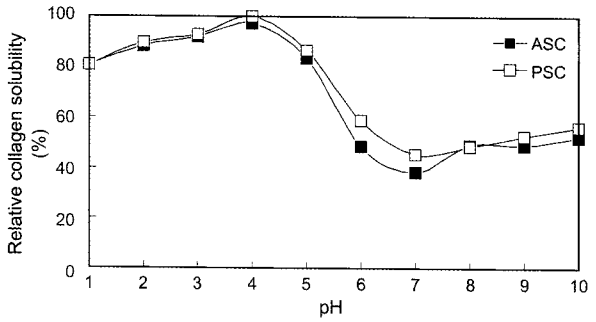

Fig. 5. Solubility of acid- (ASC) and pepsin-soluble collagens (PSC) from rockfish skin as affected by pH.

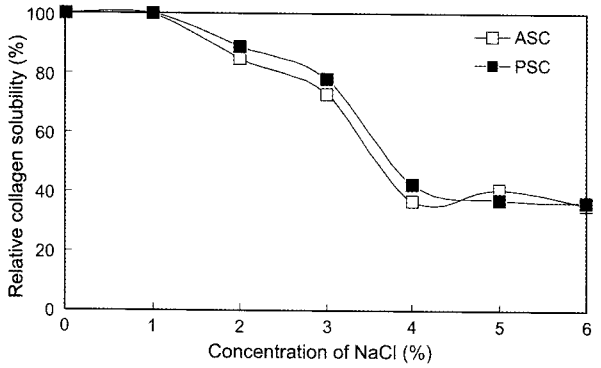

Fig. 6. Solubility of acid- (ASC) and pepsin-soluble collagens (PSC) from rockfish skin as affected by NaCl concentration.

phenomena of salting out. No difference in solubility between ASC and PSC at various NaCl concentrations was found. The results of the solubility of ASC and PSC from rockfish skin at various pH were in accordance with those previously reported from collagens from skins of Brownstrip red snapper (Jongjareonrak et al., 2005), trout (Montero et al., 1991), and bigeye snapper.

Water (WAC) and oil absorption capacities (OAC)

WAC and OAC of ASC and PSC from rockfish skin are shown in Fig. 7. The WAC of ASC (7.0 mL/g) was lower than that of PSC (14.7 mL/g). When compared to vegetable proteins, WAC of both ASC and PSC from rockfish skin were also superior to those of alfalfa leaf protein (1.85-3.58 mL/g) (Wang and Kinsella, 1976), soybean protein isolates (3-8 mL/g) (Yim and Lee, 2000), and bean protein (1.67-5.93 g/g) (Sathe and Salunkhe, 1981). This difference may be due to the high swelling ability of collagen (Sathe and Salunkhe, 1981) along with the differrences of size, shape, hydrophilic-hydrophobic balance of amino acids in the protein molecule and the physicochemical environment such as pH, ionic

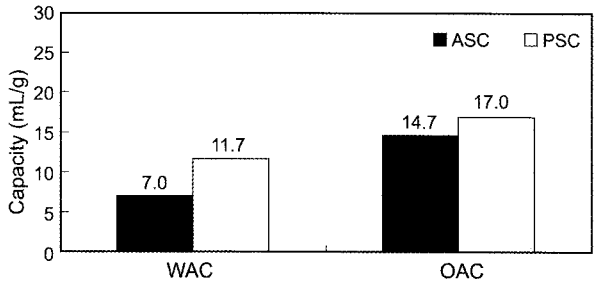

Fig. 7. Water (WAC) and oil absorption capacities (OAC) of acid- (ASC) and pepsin-soluble collagens (PSC) from rockfish skin.

strength, and temperature (Sathe and Salunkhe, 1981).

The OAC of ASC (11.7 mL/g) from rockfish skin was slightly lower than that of PSC (17.0 mL/g). However, OACs of both ASC and PSC from rockfish skin were higher than those of soybean protein isolates (2-10 mL/g), protein from alfalfa leaf (1.85-3.58 mL/g) (Wang and Kinsella, 1976), hydrolysates from salmon muscle (2.86-7.07 mL/g) (Kristinsson and Rasco, 2000), and soybean protein concentrates (1.00-4.12 mL/g) (Sathe and Salunkhe, 1981).

Emulsifying activity and cooking stability

Emulsifying activity (EA) and cooking stability (CS) of ASC and PSC from rockfish skin are shown in Fig. 8. The EA of ASC (40.4%) was higher than that of PSC (22.6%). In comparison to the EA of commercial emulsifier (Tween-80, 35.8%), EA of ASC was higher, while that of PSC was lower. The EA of fish collagen is primarily determined by orientation at the interface between the 2 phases where a monomolecular film is formed around the colloidal particle (Yang, 1997). When compared to CS of commercial emulsifier (Tween-80, 17.8%), those of ASC and PSC (15.7% and 5.4%, respectively) were lower. From the results, it showed that CS of ASC was similar to Tween-80, while PSC was inferior.

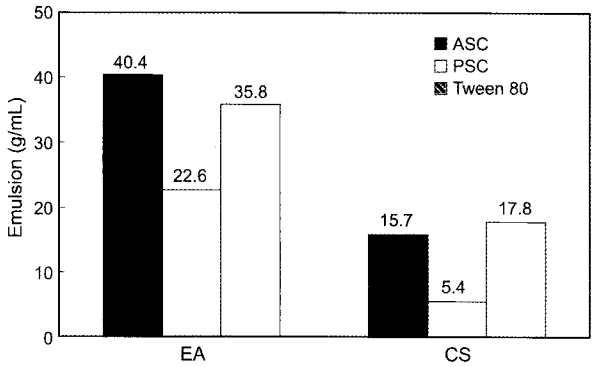

Fig. 8. Emulsion activity and cooking stability of acid- (ASC) and pepsin-soluble collagens (PSC) from rockfish skin.

Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2008-521-F00044).

References

AOAC. 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Washington DC., USA, 69-74.

Beuchat, L.R. 1981. Functional and electrophoretic characteristics of succinylated peanut flour proteins. J. Agric. Food Chem., 46, 71-75.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of lipid extraction and purification. Can. J. Biochem. Physiol., 37, 911-917.

Burghagen, M. 1999. Meat. In: Food Chem. 2nd ed. Belitz, H.D. and W. Grosch. Springer-Verlag. Berlin, Germany, 527-580.

Ciarlo, A.S., M.E. Paredi and A.N. Fraga. 1997. Isolation of soluble collagen from hake skin (*Merluccius hubbsi*). J. Aquatic. Food Product. Technol., 6, 65-77.

Codex Code. 2004. European Community Comments for the Codex Committee on Food Additives and Contaminants-agenda Item 15(a) and 16(f), The Joint FQA/WHO Food Standards Propramme. Food Standards Propramme, Rome, Italy.

Foegeding, E., T.C. Lanier and H.O. Hultin. 1996. Characteristics of edible muscle tissue. In: Food Chemisty. 3rd. ed. Fennema, O.R., ed. Academic Press, New York, USA, 879-942.

Gildberg, A. 2004. Enzymes and bioactive peptide from fish waste related to fish silage, fish feed and fish sauce production. J. Aquatic Food Products Technology. 13, 3-11.

Gómez-Guillén, M.C., J. Turnay, M.D. Fernández-Díaz, N. Ulmo, M.A. Lizarbe and P. Montero. 2002. Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocoll., 16, 25-34.

Hwang, J.H., S. Miuta, Y. Yokoyama and R. Yoshinaka. 2007. Purification and characterization of molecular species of collagen in the skin of skate (*Raja kenojei*). Food Chem., 100, 921-925.

Jackson, M., L. Choo, P.H. Watson, W.C. Halliday and H.H. Mantsch. 1995. Beware of connective tissue proteins: assignment and implications of collagen absorptions in infrared spectra of human tissues. Biochima et Biophysica Acta., 1270, 1-6.

Jongjareonrak, A., S. Benjakul, W. Visessanguan, T. Nagai and M. Tanaka. 2005. Isolation and characterisation of acid and pepsin-solubilised collagens from the skin of Brownstripe red snapper (*Lutjanus vitta*). Food Chem., 93, 475-484.

KFDA (Korean Food and Drug Administration). 2006. Food Code. Moon-Yeoung Publishing Co., Seoul, Korea, 70-72.

Kim, J.S. and J.W. Park. 2004. Characterization of acid-soluble collagen from Pacific whiting surimi processing byproducts. J. Food Sci., 69C, 637-642.

Kim, J.S. and S.Y. Cho. 1996. Screening for raw material of modified gelatin in marine animal skins caught in coastal offshore water in Korea. Agricultural Chemistry and Biotechnology, 39, 134-139.

Kim, J.S., D.M. Yeum, H.G. Kang, I.S. Kim, C.S. Kong, T.G. Lee and M.S. Heu. 2002. Fundamentals and Applications for Canned Foods. Hyoil Publishing Co., Seoul, South Korea, 94-96, 276-277, 351-354.

Kimura, S., X.P. Zhu, R. Matsui, M. Shijoh and S. Takamizawa. 1988. Characterization of fish muscle type I collagen. J. Food Sci., 53, 1315-1318.

Kimura, S. 1992. Wide distribution of the type I collagen α_3 chain in bony fish. Comp. Biochem. Physiol., 102B, 255-260.

Kittiphattanabawon, P., S. Benjakul, W. Visessanguan, T. Nagai and M. Tanaka. 2005. Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*). Food Chem., 89, 363-372.

Kristinsson, H.G. and B.A. Rasco. 2000. Biochemical and functional properties of Atlantic salmon (*Salmo salar*) muscle proteins hydrolyzed with various alkaline protease. J. Agric. Food Chem., 48, 657-666.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriopage T_4. Nature, 227, 680-685.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with Folin phenol reagent. J. Bio. Chem., 193, 256-275.

Ministry of Social Welfare of Japan. 1960. Guide to Experiment of Sanitary Infection. III. Volatile basic nitrogen. Kenpakusha, Tokyo, Japan, 30-32.

Montero, P., F. Jimenez-Colmenero and J. Borderias. 1991. Effect of pH and the presence of NaCl on some hydration properties of collagenous material from trout (*Salmo irideus* Gibb) muscle and skin. J. Agric. Food Chem., 38, 604-609.

Muyonga, J.H., C.G.B. Cole and K.G. Duodu. 2004a. Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food. Chem., 85, 81-89.

Muyonga, J.H., C.G.B. Cole and K.G. Duodu. 2004b. Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (*Lates niloticus*). Food Chem., 86, 325-332.

Nagai, T. and N. Suzuki. 2000. Isolation of collagen from fish waste material-skin, bone and fins. Food Chem., 68, 277-281.

Nagai, T. and N. Suzuki. 2002. Preparation and partial characterization of collagen from paper nautilus (*Argo nauta argo, Linnaeus*) outer skin. Food Chem., 76, 149-153.

Nagai, T., M. Izumi and M. Ishii. 2004. Fish scale collagen preparation and partial characterization. Int. J. Food Sci. Technol., 39, 239-244.

Ogawa, M., J.R. Portier, M.W. Moody, J. Bell, M.A. Schexnayder and J.N. Losso. 2004. Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (*Pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*). Food Chem., 88, 495-501.

Ogawa, M., M.W. Moody, R.J. Portier, J. Bell, M.A. Schexnayder and J.N. Losso. 2003. Biochemical properties of black drum and sheepshead seabream skin collagen. J. Agric. Food Chem., 51, 8088-8092.

Park, C.H., J.H. Lee, K.T. Kang, J.W. Park and J.S. Kim. 2007. Characterization of acid-soluble collagen from Alaska pollock surimi processing by-products (refiner discharge). Food Sci. Biochem., 16, 549-556.

Sathe, S.K. and D.K. Salunkhe. 1981. Functional properties of the great nothern bean (*Phaseolus vulgaris L.*) proteins: emulsioning, foaming, viscosity, and gelation properties. J. Food Sci., 46, 71-74.

Tsutagawa, Y., Y. Hosogai and H. Kawai. 1994. Comparison of mineral and phosphorus contents of muscle and bone in the wild and cultured horse mackerel. J. Food Hyg. Soc. Japan, 34, 315-318.

Vojdani, F. 1996. Solubility. In: Methods of Testing Protein Functionality. 1st ed. Hall, G.M., ed. St Edmundsbury Press, London, UK, 11-60.

Wang, J.C. and J.E. Kinsella. 1976. Functional properties of novel proteins : alfalfa leaf protein. J. Food Sci., 41, 286-292.

Wang, L., X. An, Z. Xin, L. Zaho and Q. Hu. 2007. Isolation and characterization of collagen from the skin of deep-sea redfish (*Sebastes mentella*). J. Food Sci., 72, E450-E455.

Yang, J.S. 1997. Applications of gelatin in food and biotechnology. J. Food Sci. Nutr., 2, 263-268.

Yim, M.H. and J.H. Lee. 2000. Functional properties of fractionated soy protein isolates by proteases from meju. J. Food Sci. Biotechnol., 9, 253-257.

Zhu, X.P. and S. Kimura. 1991. Thermal stability and subunit composition of muscle and skin type I collagens from skipjack. Nippon Suisan Gakkashi, 57, 755-760.

(Received 6 January 2009; Reviced 3 February 2009; Accepted 19 March 2009)