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Isolation and characterisation of collagens from the skin, scale and bone of deep-sea redfish (*Sebastes mentella*)

Lin Wang, Xinxin An, Fangmei Yang, Zhihong Xin, Liyan Zhao, Qiuhui Hu *

Key Laboratory of Food Processing and Quality Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China

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Abstract

To make more effective use of underutilised resources, collagens from skin, scale and bone (SKC, SCC and BOC) of deep-sea redfish were isolated with acetic acid and characterised for their potential in commercial applications. The abundant ash and fat in the materials could be removed effectively by EDTA and hexane treatment in 24 h, with high recoveries of protein. The yield of SKC (47.5%) was significantly higher than that of SCC and BOC (6.8% and 10.3%, respectively). The denaturation temperatures of SKC, SCC and BOC were 16.1 °C, 17.7 °C and 17.5 °C, respectively, which were lower than those of most other fish species. The amino acid profiles of these collagens were similar with a low imino acid content, which might be the reason for the low denaturation temperature. All the collagens were type I mainly and maintained their triple helical structures well with slight molecular structure differences. SKC possessed a higher degree of intermolecular cross-linking and molecular order, but the extent of peptide chain unwinding was also higher, due to the existence of fewer hydrogen bonds, compared to SCC and BOC.

Keywords: Deep-sea redfish (*Sebastes mentella*); Collagen; Isolation; Characterisation

1. Introduction

Collagen has been, traditionally, isolated from the skins of land-based animals, such as cow and pig, and widely used in food, cosmetic, biomedical and pharmaceutical industries (Ogawa et al., 2004). However, the outbreak 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 from land-based animals in recent years (Jongjareonrak, Benjakul, Visessanguan, Nagai, & Tanaka, 2005). Additionally, collagen obtained from pig cannot be used as a component of some foods for religious reasons (Sadowska, Koodziejska, & Niecikowska, 2003). Therefore, there is a strong need to develop alternative collagen sources.

During fish processing, a large amount of waste, 50–70% of the original raw materials is generated, such as skin, bone, scale, viscera and head (Kittiphattanabawon, Benjakul, Visessanguan, Nagai, & Tanaka, 2005). Although the value of this waste is fairly high, these useful resources have been mainly used as feedstuff or fertiliser with low value (Nagai & Suzukib, 2000). Also, improper disposal of the waste may cause serious environment pollution with offensive odour. Therefore, optimised utilisation of this waste, especially the production of value-added products, is a promising means to reduce the cost of disposal and to increase the revenue of producers.

About 30% of fish processing waste consists of skin, scale and bone, which are very rich in collagen and have received increasing attention as collagen sources (Kittiphattanabawon et al., 2005). Recently, collagen from several fish species has been isolated and characterised (Jongjareonrak et al., 2005; Montero, Gómez-Guillén, & Borderías, 1999; Morimura et al., 2002; Yoshimura, Terashima,

* Corresponding author. Tel./fax: +86 25 8439 9086.
E-mail address: qiuhuihu@njau.edu.cn (Q. Hu).

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Hozan, & Shirai, 2000). Collagens from different fish species, habitats and tissues were significantly different in terms of properties.

At the present time, there has been no report on the isolation and characterisation of collagen from deep-sea redfish (*Sebastes mentella*), which is very common and widely distributed in the North Atlantic with an annual catch of more than 100,000 tonnes and processing waste of over 50,000 tonnes (Joensen & Grahl-Nielsen, 2000; Johansen, Danielsdottir, Meland, & Naevdal, 2000; Kittiphattanabawon et al., 2005). To make more effective use of so much waste, collagens were isolated from the skin, scale and bone of deep-sea redfish (*Sebastes mentella*) and characterised for their potential in commercial applications, as alternatives to mammalian collagen in foods, cosmetics and biomedical materials.

2. Materials and methods

2.1. Reagents and materials

Deep-sea redfish (*Sebastes mentella*), with an average length of about 35 cm, was caught from Icelandic waters in the summer of 2004 and provided by Sanxing Food Co. Ltd., Lianyungang, China. The skin, scale and bone, about 15% (w/w) of the fish, were mechanically separated, and the residue of adhering tissues was removed manually. After washing thoroughly with running water, the skin and scale were cut into small slices (about 1.5 × 1.5 mm) with scissors and the bone was broken with a hammer and sieved with a 20 mesh (0.85 mm) sieve, then placed in polyethylene bags and stored at minus 20 °C until used. Collagen type I from bovine Achilles tendon was purchased from Sigma Chemical Co. (St. Louis, MO). PageRuler prestained protein ladder was obtained from MBI Fermentas (St. Leon-Rot, Germany). All other reagents were of analytical grade.

2.2. Proximate analysis

The hydroxyproline content was determined using a colorimetric method recommended by ISO (Anon., 1978). Briefly, the samples were hydrolysed for 16 h at 100 °C in 6 M HCl containing 0.75% (w/v) of $SnCl_2$; then the pH was adjusted to 8.0 with 10 M and 1 M NaOH and filtered. Four millilitres of the filtrate were mixed with 2 ml of isopropanol in citrate-acetate-buffered Chloramine T, and the oxidation was allowed to proceed for 20 min at room temperature. Chromophore was then developed with the addition of 2 ml Ehrlich's reagent and incubation for 20 min at 60 °C in a water-bath, and the absorbance of the complex was measured at 558 nm, using a spectrophotometer (UV/Vis 2802PC; Unico Instruments Co. Ltd., Shanghai, China).

The ash, nitrogen, moisture and total fat content were determined by ISO recommended methods 936, 937, 1442 and 1443, respectively. A conversion factor of 5.95 was used for calculating the protein content from nitrogen content.

2.3. Pretreatment

To remove the noncollagenous substances, the skin was soaked in 20 volumes of 1.0 M NaCl (0.05 M Tris–HCl, pH 7.5) for 24 h after homogenising for 2 min in the same solution (Philips HR2864; Koninklijke Philips Electronics N.V., Eindhoven, Netherlands). The scale and bone were selected to conduct a preliminary optimisation of pretreatment time, due to their high content of ash or fat. The scale and bone were demineralised with 20 volumes of 0.5 M EDTA (pH 7.4), and the fat in bone was removed by soaking in 10 volumes of hexane. The demineralising and defatting solutions were stirred continuously with a magnetic stirrer and changed every 12 h, and proximate analysis of samples was performed every 24 h. After pretreatment, the samples were washed with distilled water 3 times and then lyophilised (FreeZone 4.5L; Labconco Corp., Kansas City, MO). The lyophilised scale and bone were ground to a powder using a personal mill (SCM-40A; Shibata Scientific Technology Ltd., Tokyo, Japan) and sieved with a 100 mesh (0.15 mm) sieve.

2.4. Isolation of collagens

The collagens in skin, scale and bone (SKC, SCC and BOC) were extracted with 0.5 M acetic acid at a sample/solution ratio of 1:100 (w/v) for 24 h with continuous stirring after pretreatment. The extracts were centrifuged at 20,000*g* for 1 h with a refrigerated centrifuge (SIGMA 3-18K; Sigma Laborzentrifugen GmbH, Osterode, Germany), and the residue was re-extracted and centrifuged under the same conditions. The supernatants of the two extracts were combined, and precipitated by the addition of NaCl to a final concentration of 0.9 M. The precipitate was obtained by centrifugation at 2500*g* for 0.5 h and dissolved in 0.5 M acetic acid, dialysed for 48 h against 10 volumes of 0.1 M acetic acid and distilled water, respectively, which were changed every 8 h, before being lyophilized. All pretreating and extracting procedures were carried out at 4 °C. Estimated by measurement of hydroxyproline content in raw materials and prepared collagens, the yield of collagens from skin, scale and bone were 47.5%, 6.8% and 10.3%, respectively (Table 1).

2.5. Analysis of thermal behaviours

The thermal behaviours of the collagens were determined from viscosity changes, using an Ubbelohde viscometer, according to Kimura's method with slight modification (Kimura, Zhu, Matsui, Shijoh, & Takamizawa, 1988). The viscometer was filled with 0.1% (w/v) of collagen in 0.1 M acetic acid, incubated at 5 °C for 30 min. Then the temperature was raised stepwise to 30 °C at intervals of 1 °C and a heating rate of about 0.5 °C/min, and

Table 1
Yield and denaturation temperature of collagens from the skin, scale and bone of deep-sea redfish

	Collagen from		
	Skin	Scale	Bone
Yield[*] (%)	47.5 ± 4.7 a	6.8 ± 0.8 c	10.3 ± 1.5 b
Denaturation temperature[A] (°C)	16.1	17.7	17.5

[*] Mean value ± standard deviation from three separate samples. Values followed by different letters are significantly different at $p < 0.05$. Estimated by measurement of hydroxyproline content in raw materials and prepared collagens.
[A] Determined from viscosity changes of 0.1% (w/v) of collagen in 0.1 M acetic acid.

maintained for 10 min at each temperature. Fractional viscosity was computed for each temperature as follows:

Fractional viscosity
= (measured viscosity − minimum viscosity)
/(maximum viscosity − minimum viscosity),

where the viscosity was specific viscosity (η_{sp}) and was calculated by the equation:

$$\eta_{sp} = (t - t_0)/t_0,$$

assuming the densities of the solution and solvent were the same (t = efflux time of the collagen solution and t_0 = efflux time of the solvent). The denaturation temperature (T_d) was taken to be the temperature at which fractional viscosity was 0.5.

2.6. Amino acid analysis

The collagen samples were hydrolysed under vacuum with 6 M HCl at 110 °C for 24 h in the presence of 1% phenol (v/v), and the hydrolysates were analysed with an amino acid analyser (MLC-703; Atto Corp., Tokyo, Japan).

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

Electrophoresis (SDS–PAGE) was conducted using the discontinuous Tris-glycine buffer (pH 8.3) system, with 3% stacking gel and 5% resolving gel containing 3 M urea. Collagen samples were dissolved in 0.1 M Tris–HCl buffer (pH 6.8, containing 1% SDS (w/v) and 3 M urea) to a final concentration of 0.1% (w/v). The dissolved collagen samples were mixed with sample buffer (0.5 M Tris–HCl, pH 6.8, containing 5% (w/v) SDS, 30% (v/v) glycerol, 0.04% (w/v) bromophenol blue and 10% (v/v) β-mercaptoethanol) at the ratio of 1:1 (v/v), and then 10 μl was loaded per well. The electrophoresis was conducted using a vertical cell (EPS604; Nanjing Kebao Apparatus Institute, Nanjing, China) at discontinuous voltages of 60 V and 90 V and 20 °C. After electrophoresis, the gel was stained for 1 h with 0.05% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 10% (v/v) acetic acid, and destained for 12 h with 5% (v/v) methanol and 7.5% (v/v) acetic acid. The electrophoresis pattern was analysed using a digital gel image analysis system (FR-980; Furi Science and Technology Co. Ltd., Shanghai, China).

2.8. Fourier transform infrared spectra (FTIR)

Infrared spectra from 4000 to 400 cm^{-1} were obtained with KBr disc method using an infrared spectrophotometer (170-SX; Nicolet Instrumental Co., Madison, WI). The number and location of sub-bands of amide I band were provided by Fourier self-deconvolution, which was conducted using a resolution enhancement factor of 2.1 and half-height bandwidth of 13.5 cm^{-1}. Gaussian curve fitting was then performed with Origin 6.0 (OriginLab Corp., Northampton, MA).

2.9. Statistical analysis

The data were presented as means ± standard deviation of three determinations. Statistical analyses were performed using Student's t-test and one-way analysis of variance. Multiple comparisons of means were done by LSD test. A probability value of <0.05 was considered significant. All computations were made by SAS 8.0 (SAS Institute Inc., Cary, NC).

3. Results and discussion

3.1. Pretreatment of skin, scale and bone

Fig. 1a shows the compositions of skin, scale and bone of deep-sea redfish on the basis of dry weight. The ash contents of scale and bone (39.4% and 42.0%, respectively) were significantly higher than that of skin (0.9%), and the fat content of bone (26.8%) was also higher than that of skin (2.9%). Conversely, the content of protein in skin (90.6%) was significantly higher, compared to scale and bone (56.9% and 28.0%, respectively).

The ash in scale and bone could be removed effectively with 0.5 M EDTA (pH 7.4), and reduced to 1.9% and 5.7% in 24 h. Unfortunately, the residues could not be reduced further even after increasing pretreatment time to 72 h. The fat content in bone was reduced to 3.0% by hexane treatment for 24 h, and no further reduction was possible. The residue might be complex or polar lipids, such as phospholipids, glycolipids or lipoproteins, which could

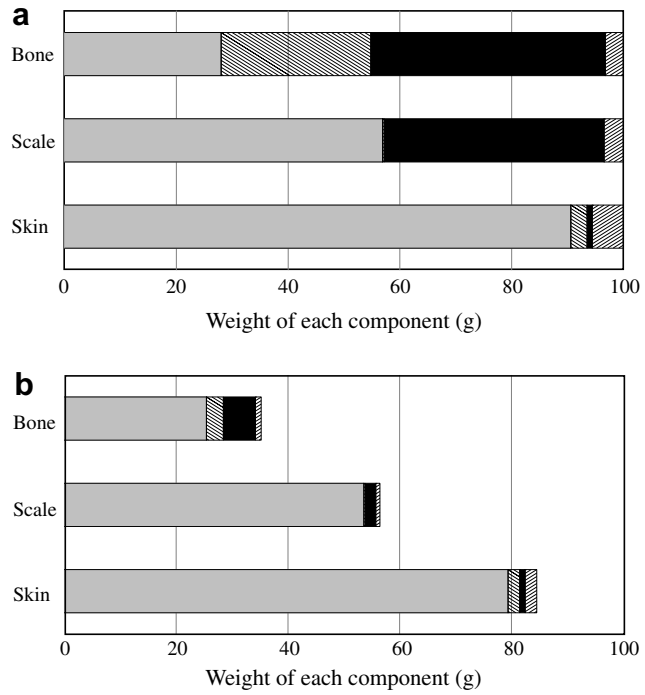

Fig. 1. Proximate compositions of skin, scale and bone of deep-sea redfish before (a) and after (b) pretreatment, on the basis of dry weight. ▭, Protein; ▨, fat; ■, ash; and ▨, other organic matter.

not be extracted by this method (Toschi, Bendini, Ricci, & Lercker, 2003). After pretreatment, the compositions of skin, scale and bone are shown in Fig. 1b, where the values are the amounts remaining following pretreatment of 100 g (dry wt.) raw material. The amounts of protein recovered from 100 g (dry wt.) of skin, scale and bone were 79.4 g, 53.6 g and 25.4 g, respectively, which were significantly higher than amounts obtained with demineralized with hydrochloric acid (Morimura et al., 2002).

3.2. Thermal behaviours

With increasing temperature, the hydrogen bonds of collagen were gradually broken. Consequently, the triple helix structure of collagen organized by hydrogen bonds was converted into the random coil configuration of gelatin by the process of thermal depolymerisation, which was accompanied by a change in physical properties, such as viscosity, sedimentation, diffusion, light scattering and optical activity (Gurdak, Booth, Roberts, Rouxhet, & Dupont-Gillain, 2006; Sai & Babu, 2001; Usha & Ramasami, 2004). All of the collagens exhibited a similar rapid loss of viscosity with heating from 9 °C to 22 °C, and remained low above 22 °C (Fig. 2). The thermal denaturation temperatures (T_d) of SKC, SCC and BOC were 16.1 °C, 17.7 °C and 17.5 °C, respectively (Table 1). The denaturation temperatures of collagens from deep-sea redfish, which inhabits an ocean temperature of 3–8 °C, were similar to those of cold-water fish, like Argentine hake (10.0 °C), Baltic cod (15.0 °C), Alaska Pollack (16.8 °C),

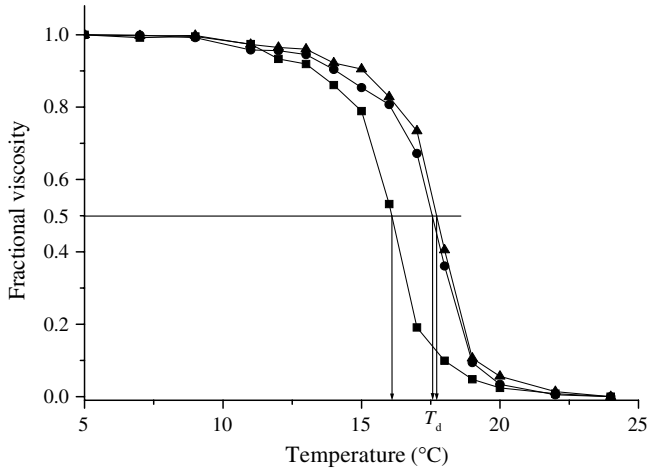

Fig. 2. Thermal behaviours of collagens from deep-sea redfish measured by viscosity change in 0.1 M acetic acid. T_d was the denaturation temperature. —■—, Collagen from skin (SKC); —▲—, collagen from scale (SCC); —●—, collagen from bone (BOC).

chum salmon (19.4 °C), and was much lower compared to temperate and tropical fish species, such as common mackerel (26.1 °C), eel (29.3 °C), Japanese seabass (26.5 °C), skipjack tuna (29.7 °C), ayu (29.7 °C), Nile perch (36.5 °C) (Jongjareonrak et al., 2005; Muyonga, Cole, & Duodu, 2004; Nagai, Araki, & Suzuki, 2002; Nagai, Yamashita, Taniguchi, Kanamori, & Suzuki, 2001). This result was in agreement with Rigby's report that the thermal stability of collagen was correlated with environmental and body temperature (Rigby, 1968).

3.3. Amino acid composition

The amino acid composition of collagens from skin, scale and bone of deep-sea redfish, expressed as residues

Table 2
Amino acid profile of collagens from the skin, scale and bone of deep-sea redfish

	Composition (residues/1000 residues)		
	Skin	Scale	Bone
Hydroxyproline	64	65	61
Aspartic acid	50	54	56
Threonine	23	26	23
Serine	61	56	58
Glutamic acid	78	81	75
Proline	101	95	102
Glycine	335	328	341
Alanine	105	103	98
Valine	25	18	15
Methionine	14	14	12
Isoleucine	9	11	10
Leucine	21	25	25
Tyrosine	6	5	5
Phenylalanine	20	22	25
Hydroxylysine	6	9	9
Lysine	28	33	32
Histidine	4	6	8
Arginine	50	49	46

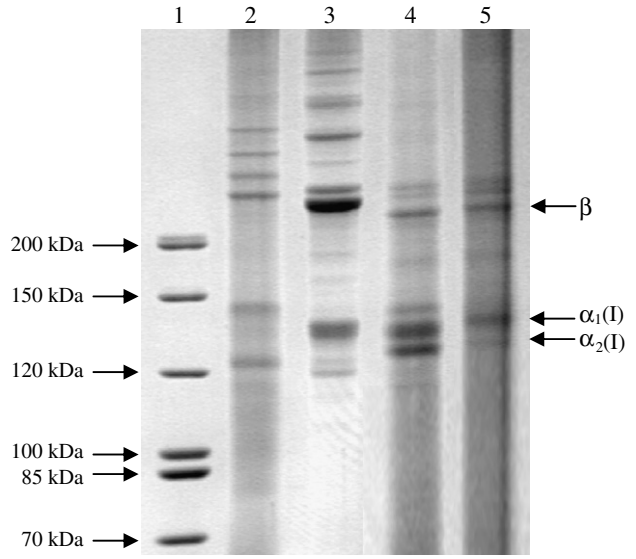

Fig. 3. SDS–polyacrylamide gel electrophoresis pattern of collagens from deep-sea redfish. Lane 1: molecular weight marker; lane 2: type I collagen from bovine tendon; lane 3: collagen from skin (SKC); lane 4: collagen from scale (SCC); lane 5: collagen from bone (BOC).

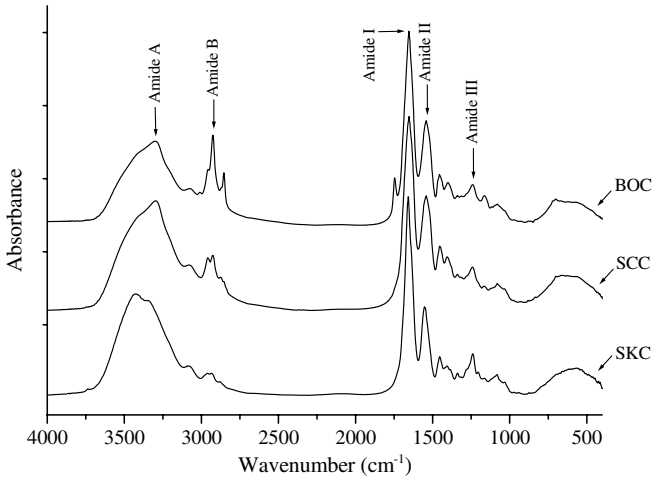

Fig. 4. Fourier-transform infrared spectra of collagens from deep-sea redfish. SKC: collagen from skin; SCC: collagen from scale; BOC: collagen from bone.

per 1000 total residues, is shown in Table 2. Generally, these collagens had similar amino acid profiles. Glycine was the most abundant amino acid in these collagens (about 1/3), and there were relatively high contents of alanine, proline, glutamic acid, hydroxyproline and serine, while the contents of tyrosine, histidine and hydroxylysine were very low, and tryptophan and cysteine was not detected, like other collagens (Jongjareonrak et al., 2005; Muyonga et al., 2004; Yata, Yoshida, Fujisawa, Mizuta, & Yoshinaka, 2001).

The total contents of imino acid (proline and hydroxyproline) of SKC, SCC and BOC were 16.5%, 16.0% and 16.3%, respectively, which were significantly lower than those of collagens from temperate and tropical fish species, and were similar to those of collagens from cold-water fish species (16–18%) (Gilsenan & Ross-Murphy, 2000; Gudmundsson & Hafsteinsson, 1997; Jongjareonrak et al., 2005; Muyonga et al., 2004). The lower imino acid content and lower denaturation temperature of collagens from deep-sea redfish, in comparison with temperate and tropical fish species, were in agreement with the report that the thermal stability of protein was correlated with the content of imino acid (Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003; Privalov, 1982).

The degree of hydroxylation of proline and lysine influences the thermal stability of collagen (Kimura et al., 1988). A higher degree of hydroxylation is associated with higher denaturation temperature, for collagens with similar amino acid profiles. The values for the total degree of hydroxylation of proline and lysine for SKC, SCC and BOC were 35.2%, 36.6% and 34.3%, respectively, which were similar to those reported for Nile perch, Brownstripe red snapper and bigeye snapper (Jongjareonrak et al., 2005; Kittiphattanabawon et al., 2005; Muyonga et al., 2004). It appeared that it was the lower imino acid content, rather than the extent of hydroxylation, which seemed to be the reason for the lower denaturation temperature observed for deep-sea redfish collagens.

Table 3
Fourier-transform infrared spectra peak locations and assignment for collagens from deep-sea redfish

Region	Peak wavenumber (cm^{-1})			Assignment	Reference
	SKC	SCC	BOC		
Amide A	3425	3296	3300	NH stretch coupled with hydrogen bond	Sai and Babu (2001)
Amide B	2935	2926	2926	CH_2 asymmetrical stretch	Abe and Krimm (1972)
–	/	/	2854	CH_2 symmetrical stretch	Abe and Krimm (1972)
Amide I	1658	1653	1654	C=O stretch/hydrogen bond coupled with COO-	Payne and Veis (1988)
Amide II	1552	1541	1541	NH bend coupled with CN stretch	Krimm and Bandekar (1986)
–	1454	1454	1456	CH_2 bend	Jackson et al. (1995)
–	1406	1406	1400	COO- symmetrical stretch	Jackson et al. (1995)
–	1338	1338	1338	CH_2 wag	Jackson et al. (1995)
Amide III	1240	1242	1240	NH bend coupled with CN stretch	Payne and Veis (1988)
–	1083	1080	1082	C–O stretch	Jackson et al. (1995)
–	568	660	702	Skeletal stretch	Abe and Krimm (1972)

–, No common name for the spectral region. /, No peak. SKC: collagen from skin; SCC: collagen from scale; BOC: collagen from bone.

3.4. SDS polyacrylamide gel electrophoresis (SDS–PAGE)

The electrophoresis patterns of collagens of deep-sea redfish and type I collagen from bovine Achilles tendon are shown in Fig. 3. As observed by Hayashi and Nagai (1979), the mobility of α-chains of these collagens was lower than would be expected, based on the molecular weight markers. When globular proteins were used as molecular weight markers, the molecular weight of collagen would be overestimated, as a result of the high content of the smaller amino acid residues, glycine, proline and alanine (Noelken, Wisdom, & Hudson, 1981). The estimated molecular weight for α chains of these collagens, using globular proteins as standards was approximately 120–150 kDa.

As in type I collagen from bovine Achilles tendon, the collagens from deep-sea redfish comprised at least two different α-chains (α1 and α2) with slightly different mobilities, which indicated that the collagens from the skin, scale and bone of deep-sea redfish might be type I collagen mainly, with slight differences in amino acid composition of α-chains. This observation was similar to the findings reported for Nile perch, black drum, sheepshead seabream, bigeye snapper, Brownstripe red snapper, etc. (Jongjareonrak et al., 2005; Kittiphattanabawon et al., 2005; Muyonga et al., 2004; Ogawa et al., 2004).

It seemed that the ratio of α1 to α2 chains of collagens from the three different sources was slightly different. This result might be due to the presence of different quantity of α3 chain, since α3 chain has an indistinguishable molecular mass from α1 chain and cannot be separated from the corresponding α1 chain, even if an α3 chain is present (Jongjareonrak et al., 2005; Kittiphattanabawon et al., 2005; Nagai & Suzukib, 2000).

3.5. Fourier transform infrared spectroscopy (FTIR)

The infrared spectra of collagens from deep-sea redfish and the major peaks with their assignments are shown in Fig. 4 and Table 3. (Abe & Krimm, 1972; Jackson, Choo, Watson, Halliday, & Mantsch, 1995; Krimm & Bandekar, 1986; Payne & Veis, 1988; Sai & Babu, 2001). The IR spectra for collagens from skin, scale and bone of deep-sea redfish differed slightly, indicating some differences in the secondary structure of the three collagens.

The triple helical structures of SKC, SCC and BOC were confirmed from the absorption ratio between 1240 cm^{-1} (amide III) and 1454 cm^{-1} bands, which was approximately equal to 1.0 (Plepis, Goissis, & Das, 1996).

The amide A band is associated with the N–H stretching frequency. According to Doyle, Bendit, and Blout (1975), a free N–H stretching vibration occurs in the range 3400–3440 cm^{-1}, and when the NH group of a peptide is involved in a hydrogen bond, the position is shifted to lower frequencies. The amide A band of SKC was at 3425 cm^{-1}, while those of SCC and BOC were at 3296 cm^{-1} and 3300 cm^{-1}, respectively (Table 3). These indicated more NH groups of SCC and BOC were involved in hydrogen bonding than in SKC.

The peaks of amide I and amide II of SKC (1658 cm^{-1} and 1552 cm^{-1}, respectively) were at a higher frequency than those of SCC (1653 cm^{-1} and 1541 cm^{-1}, respectively) and

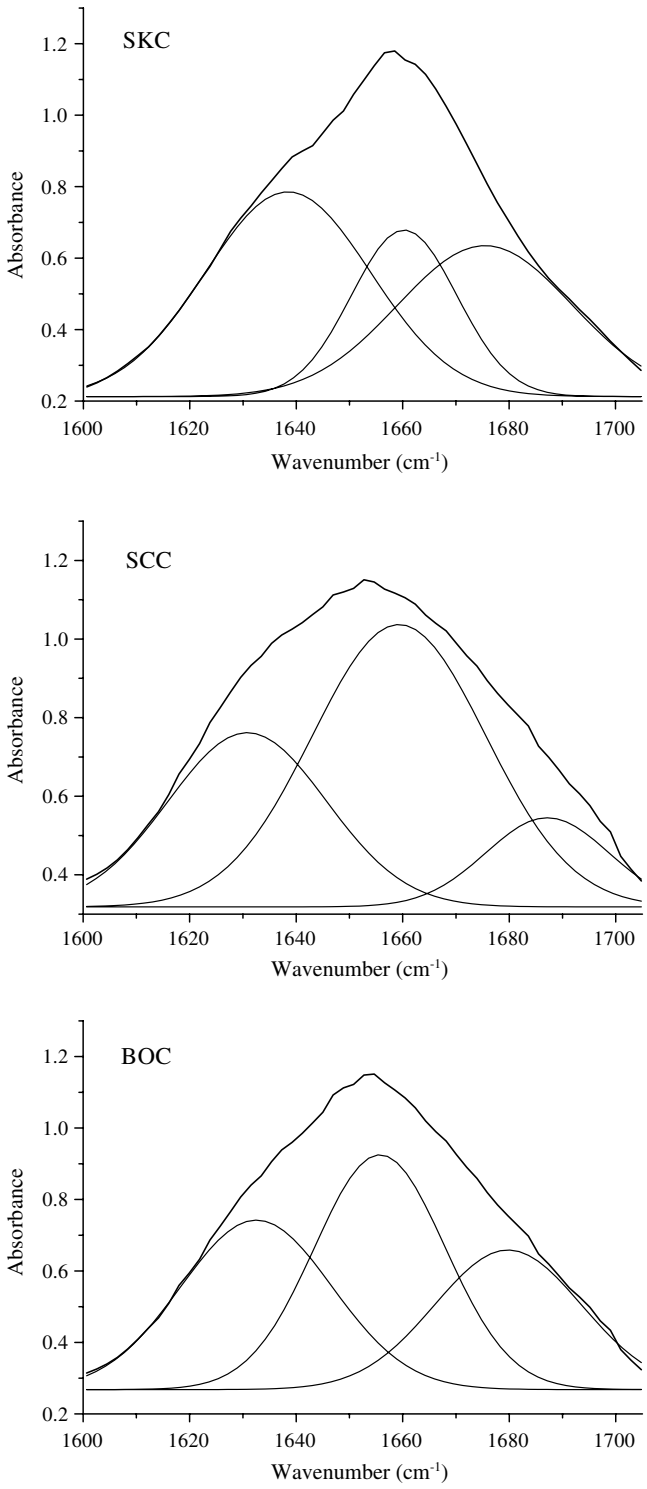

Fig. 5. Amide I band of collagens from deep-sea redfish with fitted band components. SKC: collagen from skin; SCC: collagen from scale; BOC: collagen from bone.

Table 4
Peak location and area percentage of fitted components of amide I band

	Collagen from								
	Skin			Scale			Bone		
Location (cm^{-1})	1696	1660	1633	1694	1654	1632	1694	1654	1631
Area (%)	33.9	22.6	43.5	12.5	55.9	31.5	26.7	39.9	33.4

BOC (1654 cm^{-1} and 1541 cm^{-1}, respectively) (Table 3). These indicated SKC had a higher degree of molecular order than SCC and BOC, since the shift of these peaks to higher frequencies was associated with an increase in the molecular order (Payne & Veis, 1988).

The amide I band, with characteristic frequencies in the range from 1600 to 1700 cm^{-1}, was mainly associated with the stretching vibrations of the carbonyl groups (C=O bond) along the polypeptide backbone (Payne & Veis, 1988), and was a sensitive marker of the peptide secondary structure (Surewicz & Mantsch, 1988). Deconvolution of the amide I band showed the band consisted of three components. The component peaks, their location and area percentage are shown in Fig. 5 and Table 4.

The intensity of the band at 1633 cm^{-1} of SKC (43.5%), attributed to the unwinding of peptide chain (Prystupa & Donald, 1996), was much higher than those of SCC (31.5%) and BOC (33.4%), which indicated the extents of peptide chain unwinding of SCC and BOC were low compared to SKC, which might be due to the formation of more hydrogen bond in SCC and BOC evidenced by the amide A band. The intensity of band at 1660 cm^{-1} of SKC (22.6%) was much lower than SCC (55.9%) and BOC (39.9%). This band has been attributed to hydrogen bond (Payne & Veis, 1988), suggesting there was more and/or stronger hydrogen bond in SCC and BOC. This result was in accordance with the amide A band. Another considerable difference was the higher intensity of the band at 1696 cm^{-1} in SKC. This band was sensitively dependent upon the extent of intermolecular cross-link, which could be analyzed from the intensity ratio of 1696/1660 cm^{-1} (Paschalis et al., 2001). From Fig. 5 and Table 4, it can be easily seen that the intensity ratio of 1696/1660 cm^{-1} in SKC was much higher, compared to SCC and BOC, indicating the extent of intermolecular cross-link was much greater in SKC.

Generally, the triple helical structures of SKC, SCC and BOC isolated by acetic acid after pretreatment were well maintained, but the molecular structure of collagens from different tissues had slight differences. SKC possessed a higher degree of intermolecular cross-link and molecular order, and the extent of peptide chain unwinding was also higher, due to the existence of less hydrogen bond, compared to SCC and BOC.

4. Conclusion

Collagen was successfully isolated from the skin, scale and bone of deep-sea redfish with acetic acid after pretreatment. These collagens were type I mainly, with similar amino acid composition, and maintained their triple helical structures well, with slight differences in terms of thermal stability and molecular structure. These results suggest that collagen could be obtained effectively from the processing waste of deep-sea redfish, and has potential in commercial applications as an alternative to mammalian collagen, about which there are some concerns from consumers and manufacturers. However, deep-sea redfish collagen may need further modification or purification in some fields.

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