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Isolation and characterisation of collagens from the skin of largefin longbarbel catfish (*Mystus macropterus*)

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ABSTRACT

Acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) were extracted from the skin of largefin longbarbel catfish (*Mystus macropterus*) with yields of 16.8% and 28.0%, respectively, on the basis of dry weight. Both ASC and PSC contained α1 and α2 chains and the amino acid composition of collagen was close to that of calf skin type I collagen. The intrinsic viscosities of ASC and PSC were 14.9 dl/g and 14.5 dl/g, respectively. Similar ultraviolet and FTIR spectra of ASC and PSC were observed. However, peptide maps of ASC and PSC, hydrolysed by trypsin, revealed some differences in primary structures between the two fractions. Denaturation temperatures of ASC and PSC were 32.1 °C and 31.6 °C, respectively. The higher T_m showed that it is possible to use largefin longbarbel catfish skin collagen as an alternative source of vertebrate collagens for industrial purposes.

1. Introduction

Collagen is the predominant protein of animal bodies, constituting approximately 30% of total protein. There are at least 27 different types of collagen, named type I–XXVII (Birk & Bruckner, 2005). Type I collagen is a right-handed triple superhelical rod consisting of three polypeptide chains and is found in connective tissues, including tendons, bones and skins (Muyonga, Cole, & Duodu, 2004).

Collagen can be made into photographic film and sausage casings and also can be used as a food additive. Furthermore, collagen serves in pharmacy, cosmetics and tissue engineering due to its excellent biocompatibility and biodegradability (Bailey & Light, 1989; Hassan & Sherief, 1994; Zhang, Li, & Shi, 2006). So far, collagen has been mainly derived from the skins and bones of bovine or porcine origin which might carry pathogens of bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD). In contrast with mammalian collagens, fish collagens seem to be much safer. During processing of fish into such various kinds of food as surimi and fish floss, a large amount of wastes, including skins, bones, scales and fins, are generated. Thus, fish processing wastes may be alternative collagen sources and these underutilized resources have attracted the increasing attention of scientists all over the world.

Collagens of fish skins studied in recent years were mainly from marine species, such as black drum (*Pogonia cromis*) (Ogawa et al., 2003), brownstripe red snapper (*Lutjanus vitta*) (Jongjareonrak, Benjakul, Visessanguan, Nagai, & Tanaka, 2005), and ocellate puffer fish (*Takifugu rubripes*) (Nagai, Araki, & Suzuki, 2002a). Isolation and characterisation of collagen from freshwater fish, however, was rarely reported, except for the Nile perch (*Lates niloticus*) (Muyonga et al., 2004), grass carp (*Ctenopharyngodon idella*) (Zhang et al., 2007) and channel catfish (*Ictalurus punctaus*) (Liu, Li, & Guo, 2007).

Nile perch, channel catfish and grass carp are all freshwater fishes. However, they are tropical, subtropical and temperate fishes, respectively. Collagen from different species and living environments might have different properties (Foegeding, Lanier, & Hultin, 1996). Denaturation temperatures of collagens from the skins of Nile perch, channel catfish and grass carp were reported to be 36.5 °C, 32.5 °C and 24.6 °C, respectively (Liu et al., 2007; Muyonga et al., 2004; Zhang et al., 2007). Obviously, collagen prepared from grass carp skin with scales has a poor thermal stability, whereas there is no information about the collagen from temperate freshwater fish without scales. Biochemical properties of collagen, such as thermal stability, might be different between the fishes with and without scales.

Largefin longbarbel catfish (*Mystus macropterus*), distributed in the Changjiang river system, is a typical temperate freshwater fish without scales. It is an important commercial fish in the southwest of China, especially in Sichuan and Guizhou Provinces. In this paper, collagens (ASC and PSC) from largefin longbarbel catfish skins were extracted and partly characterised for potential applications.

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2. Materials and methods

2.1. Fish skin preparation

Largefin longbarbel catfish (*M. macropterus*), with weights of about 1.5 kg, was purchased from a market in Chengdu, Sichuan Province. The skins were instantly denuded, washed with distilled water and cut into small pieces (2 mm × 8 mm), then frozen at −20 °C within a week prior to collagen extraction.

2.2. Extraction of collagen

All procedures were carried out at 4 °C with gentle stirring. Skins were soaked in 0.1 M NaOH with a sample/solution ratio of 1:20 (w/v), containing 0.5% non-ionic detergent for 24 h. The solution was changed once to get rid of non-collagenous proteins, partial pigments and fat. The samples were then washed with distilled water until the pH of the water became neutral or faintly basic. Residual fat was removed in 15% (v/v) butyl alcohol with a sample/solution ratio of 1:20 for 24 h with a change of solution each 12 h. Defatted skins were thoroughly washed with distilled water. To remove pigments more effectively, defatted skins were bleached with 3% H_2O_2 solution for 24 h and the solution was changed once.

The matter was thoroughly washed with distilled water and then stirred in 15 volumes of 0.5 M acetic acid for 24 h. The suspensions were centrifuged at 9,000*g* for 15 min at 4 °C. The precipitate was re-extracted in 0.5 M acetic acid with a sample/solution ratio of 1:10 for 12 h, terminated by centrifugation at 9,000*g* for 15 min at 4 °C. The supernatants obtained were combined and salted-out by the addition of NaCl to a final concentration of 0.7 M. The resultant precipitate was collected by centrifugation at 9,000*g* for 15 min at 4 °C and then dissolved in 0.5 M acetic acid. The solution obtained was dialysed against 0.1 M acetic acid for 3 days with change of solution once per day, followed by lyophilisation in a freeze-dryer (Labconco FreeZone 2.5L, USA) and finally acid-soluble collagen (ASC) was obtained.

Undissolved residue, obtained after acid extraction, was suspended in 2 volumes of 0.5 M acetic acid containing 1.5% (w/w) pepsin (EC 3.4.23.1, 1:10,000, Sigma Chemical Co.) for 30 h at 4 °C with continuous stirring. The pepsin-soluble collagen (PSC) was obtained by the same method as ASC. The extraction of collagens was performed three times and the yield value was the average of triplicate determinations.

2.3. Amino acid analysis

The collagen sample was hydrolysed in 6 M HCl at 110 °C for 24 h. The hydrolysate was vapourised and the residue was dissolved in 25 ml citric acid buffer solution. An aliquot of 0.05 ml was applied to an automated amino acid analyser (HITACHI 835-50 Amino Acid Analyzer, Japan).

2.4. Electrophoretic analysis

Electrophoretic patterns were measured according to the method of Laemmli (1970) with a slight modification, using 7.5% resolving gel and 4% stacking gel. The collagen samples were dissolved in a sample buffer, with or without 10% β-ME (β-Mercaptoethanol), to reach a final collagen concentration of 1 mg/ml, and the mixed solution was boiled for 5 min. After electrophoresis, the gel was stained for 45 min using 0.25% Coomassie brilliant blue R250 solution and destained using 7.5% acetic acid and 5% methanol.

2.5. Thermal transition measurement

The thermal transition of collagen was determined by differential scanning calorimetry (DSC) (Netzsch DSC 200PC, Germany). The lyophilised collagen samples were dissolved in 0.05 M acetic acid to reach a concentration of 5 mg/ml. The dissolved samples (approximately 10 mg) were weighed accurately into aluminium pans and sealed, and scanned over the range 18 °C–40 °C at a heating rate of 1.5 K/min in a nitrogen atmosphere. Liquid nitrogen was used as a cooling medium. Pans equipped with 0.05 M acetic acid (approximately 10 mg) were used as the reference. The collagens were applied to DSC three times and T_m values were calculated from three replicates.

2.6. Intrinsic viscosity

Intrinsic viscosity was determined by the method of Ogawa et al. (2004) with slight modification. Ubbelohde viscometers were employed for the intrinsic viscosity measurement. The lyophilised collagen samples were rehydrated in 0.5 M acetic acid to reach collagen concentrations of 0.1–0.5 mg/ml. Approximately 10 ml of the sample solution were incubated for 30 min at 20 °C, and then the efflux time of the solution was measured in the tube. The intrinsic viscosity, $[\eta]$ dl/g, was calculated by the equation $\eta_{sp}/c = [\eta] + kc$, where k dl^2/g^2 is the slope, c was the collagen concentration (g/dl), and η_{sp} (specific viscosity) was calculated by the equation $\eta_{sp} = (t - t_0)/t_0$, where t was efflux time of the collagen solution and t_0 was efflux time of the solvent. The collagens were applied to the viscometer three times. For each selected concentration, nine viscosity measurements were taken.

2.7. Ultraviolet spectra

The ultraviolet absorption spectra of largefin longbarbel catfish skin collagens were recorded by a spectrophotometer (PE Lambda 25, USA). The collagen samples were prepared by dissolution in 0.5 M acetic acid solution with a sample/solution ratio of 1:1000 (w/v).

2.8. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were obtained from tablets containing 2 mg collagen samples in approximately 100 mg potassium bromide (KBr). All spectra were recorded using an FTIR spectrophotometer (PE spectrum GX, USA) from 4000 to 500 cm^{-1} at a data acquisition rate of 2 cm^{-1} per point.

2.9. Peptide mapping of collagen

Peptide mapping of collagen was examined by the method of Zhang et al. (2007) with some modification. The collagen samples were dissolved in 0.5 M acetic acid, pH 2.5, at a concentration of 5 mg/ml. After the addition of trypsin (EC 3.4.21.4, 1:250, Sigma Chemical Co.) with an enzyme/substrate ratio of 1/2 (w/w) to collagen solutions, the reaction mixtures were incubated at 37 °C for 3 h. To terminate the reaction, the SDS-PAGE sample buffer was added to the digestion samples, and the mixture was boiled for 3 min. SDS-PAGE was performed to separate peptides generated by the protease digestion, using 7.5% gel.

Another collagen sample was dissolved in 0.5 M acetic acid to a concentration of 2.5 mg/ml and the pH was adjusted to 4.4 by the addition of 0.2 M sodium phosphate buffer. The digested collagen solution was obtained by the same method as mentioned above.

The other collagen sample was dissolved in 0.2 M sodium phosphate buffer, pH 7.8, at a concentration of 5 mg/ml. Also, the digested collagen solution was obtained by the same method as

mentioned above except that the incubation time was 3 min instead of 3 h. The calf skin type I collagen (EC No. 232-697-4, Sigma Chemical Co.) was also digested by trypsin in the same manner and the peptide maps were compared.

2.10. Statistical analysis

Means for the properties of the collagens from largefin longbarbel catfish skin were analysed using the *t*-test.

3. Results and discussion

3.1. Isolation of ASC and PSC from largefin longbarbel catfish skin

ASC and PSC were isolated from largefin longbarbel catfish skin with yields of 16.8% (±0.8% SD) and 28.0% (±0.9% SD) (dry weight basis), respectively. The skin was not completely solubilised with 0.5 M acetic acid, but further solubilisation of the remaining residues was achieved by limited pepsin proteolysis. This result might suggest that there were lots of cross-links at the telopeptide region, as well as at the inter-molecular cross-links, leading to a low solubility of collagen in acid (Foegeding et al., 1996; Zhang et al., 2007). With further limited pepsin digestion, the cross-links at the telopeptide region were cleaved without damaging the integrity of the triple helix. Therefore, a high solubility of collagen in acid was obtained after adding pepsin. The yields of ASC and PSC from different fish skins have been reported for black drum (2.3% and 15.8%, respectively), for channel catfish (25.8% and 38.4%, respectively), for paper nautlius (5.2% and 50%, respectively) and for ocellate puffer fish (10.7% and 44.7%, respectively) (Liu et al., 2007; Nagai & Suzuki, 2002; Ogawa et al., 2003b; Nagai et al., 2002a). The yields of ASC and PSC from largefin longbarbel catfish skin were similar to those of channel catfish, but were different from those of other fish species. Thus, it could be supposed that the degree of cross-linking at the telopeptide region of collagen from largefin longbarbel catfish skin was in accordance with those of collagen from channel catfish, but was lower than those of collagen from many other fish species.

3.2. Amino acid composition

Considering that ASC and PSC had similar amino acid compositions similar to those previously reported (Jongjareonrak et al., 2005; Liu et al., 2007; Sai & Babu, 2001), only the amino acid composition of ASC was determined. The result is shown in Table 1 and compared with that of calf skin collagen (Giraud-Guille, Besseau, Chopin, Durand, & Herbage, 2000) and with those of other fish collagens, such as grass carp skin collagen (Zhang et al., 2007) and PSC from brownstripe red snapper skin (Jongjareonrak et al., 2005). ASC from largefin longbarbel catfish skin was rich in glycine (31.7%), alanine (11.6%), and proline (13.9%) and was very low in methionine (0.6%), histidine (0.6%), tyrosine (0.4%) and cystine (0.2%) like other collagens (Nagai et al., 2002b; Hwang, Mizuta, Yokoyama, & Yoshinaka, 2007). Generally, glycine occurs as every third residue in collagen molecules, except for the first 14 amino acid residues from the N-terminus and the first 10 from the C-terminus (Foegeding et al., 1996; Wong, 1989). The number of imino acid residues, proline and hydroxyproline, in ASC from largefin longbarbel catfish skin was 213 residues/1000 residues, which was higher than those of most fish collagens reported, such as grass carp skin collagen (186 residues/1000 residues) and bigeye snapper skin collagen (193 residues/1000 residues) (Kittiphattanabawon, Benjakul, Visessanguan, Nagai, & Tanaka, 2005; Zhang et al., 2007), but was similar to those of calf skin collagen (215 residues/1000 residues) (Giraud-Guille et al., 2000) and PSC from brownstripe red snapper skin(221 residues/1000 residues) (Jongjareonrak et al., 2005).

Table 1
Amino acid composition of ASC from skin of largefin longbarbel catfish (residues/1000 residues).

Amino acid	ASC from largefin longbarbel catfish skin	Grass carp skin collagen	Calf skin collagen	PSC from brownstripe red snapper skin
Hyp	74	65	94	86
Asp	50	42	45	49
Thr	23	24	18	30
Ser	36	39	33	39
Glu	68	61	75	79
Pro	139	121	121	135
Gly	317	334	330	235
Ala	116	135	119	142
Cys	2	4	0	0
Val	24	31	21	17
Met	6	10	6	14
Ile	13	10	11	8
Leu	22	22	23	24
Tyr	4	2	3	2
Phe	13	17	3	16
Hyl	11	8	7	15
Lys	27	23	26	34
His	6	5	5	6
Arg	49	57	50	68
Imino acid	213	186	215	221

The higher the imino acid content, the more stable are the helices (Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003). However, the degree of hydroxylation of proline residues will also influence the stability of the helix structure of collagen (Ramachandran, 1988). ASC from largefin longbarbel catfish skin had a hydroxylation of 34.7%, which was similar to that of collagen from grass carp skin collagen (34.9%) (Zhang et al., 2007), but was lower than those of collagens from cuttlefish skin (47%), ocellate puffer fish skin (39%), brownstripe red snapper skin (38.9%), brownbacked toadfish skin (45%), Nile perch skin (40.2%) and calf skin (43.7%) (Nagai, Yamashita, Taniguchi, Kanamori, & Suzuki, 2001; Nagai et al., 2002a; Jongjareonrak et al., 2005; Senaratne, Park, & Kim, 2006; Giraud-Guille et al., 2000; Muyonga et al., 2004). Considering that the imino acid contents were similar, it appeared that the lower degree of hydroxylation of proline residues was the main reason for the lower stability of the helix structure of collagen from largefin longbarbel catfish skin than those of collagens from calf skin and brownstripe red snapper skin.

3.3. SDS-PAGE pattern

The SDS-PAGE patterns of ASC and PSC were analysed under reducing and non-reducing conditions (Fig. 1). It was found that both ASC and PSC consisted of two α-chains (α1 and α2) as the major constituents. High molecular weight components, including β- and γ-components, as well as their cross-linked molecules, were also observed in both ASC and PSC. The patterns were similar to that of the type I collagen from calf skin (lane 2), and also in accordance with those of collagens from most other fish species previously reported (Giraud-Guille et al., 2000; Muyonga et al., 2004; Nagai et al., 2001). It was suggested that both ASC and PSC isolated from largefin longbarbel catfish skin were probably type I collagen, which consisted of two α1 chains and one α2 chain.

No differences in the electrophoretic patterns of ASC or PSC, analysed in the presence or absence of β-ME, were observed, indicating there were no disulfide bonds in ASC or PSC. Although the patterns of ASC and PSC were similar, the band intensity ratio of cross-linked components (β- and γ-components) to total non-cross-linked monomer chains (α1 and α2) of ASC was visibly higher than that of PSC. Thus, it could be concluded that the intra- and inter-molecular cross-links of collagens were richer in ASC than in

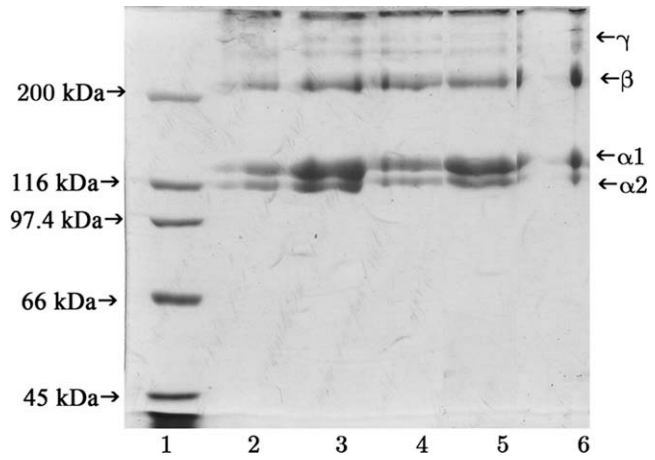

Fig. 1. SDS-PAGE patterns of ASC and PSC from the skin of largefin longbarbel catfish on 7.5% gel. Lane 1: protein markers; lane 2: calf skin collagen; lane 3: PSC; lane 4: ASC; lane 5: PSC (non-reducing); lane 6: ASC (non-reducing).

PSC. This was explained by conversions of some β- and γ-components in the PSC matrix to α-components by the treatment with pepsin. Pepsin cleaves the cross-link containing teleopeptide, and the β-chain is converted to two α-chains (Sato et al., 2000).

However, it can not be determined whether α3-chain exists in the collagens, since α3-chain has a migration similar to that of α1-chain and it can not be separated from α1-chain under the electrophoretic conditions employed.

3.4. Thermal stability

Fig. 2 shows the typical thermal transition curves of ASC and PSC from largefin longbarbel catfish skin in 0.05 M acetic acid. Endothermic peaks, with peak maximum temperatures (T_m) of 32.1 °C (±0.2 °C SD) and 31.6 °C (±0.1 °C SD), were observed for ASC and PSC, respectively ($P < 0.05$). T_m of ASC was relatively higher than that of PSC. Although the secondary structure of PSC was still stable when the fish skin was subjected to limited pepsin proteolysis, it seemed that the cleavage of cross-links at the peptide region influenced the stability of PSC. T_m values of ASC and PSC were much lower than that of calf skin collagen (40.8 °C) and Nile perch skin collagen (36.5 °C) (Komsa-Penkova, Koynava, Kostov, & Tenchov, 1999; Muyonga et al., 2004), but higher than that of collagens from other fish species, such as bigeye snapper (28.68 °C), cuttlefish (27.0 °C), ocellate puffer fish (28.0 °C), brownbacked toadfish (28.0 °C), paper nautilus(28.0 °C) and especially higher than that of the collagen from grass carp (24.6 °C) (Kittiphattanabawon et al., 2005; Nagai et al., 2001; Nagai et al., 2002a; Senaratne et al., 2006; Nagai et al., 2002b; Zhang et al., 2007). Fish species with denaturation temperature of collagens higher than 30 °C are quite limited. The higher T_m of collagens from the skin of largefin longbarbel catfish might be related to the higher content of imino acids than that of the collagen from grass carp skin. However, considering that both of them were temperate freshwater fish and their living environments were similar, it seemed that the denaturation temperatures of collagens were also probably influenced by the presence of scales. Scales of teleosts are generated by the deposition of calcium in dermis. Thus, it was supposed that the content of calcium in the dermis of grass carp with scales might higher than that of largefin longbarbel catfish without scales. The possible combination of collagen with calcium in dermis seemed to have an influence on the properties of collagens from the two fishes.

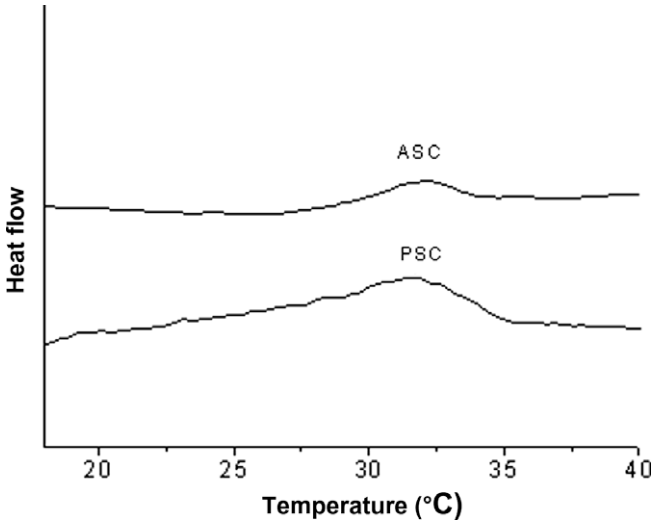

Fig. 2. Typical DSC thermograms of ASC and PSC from the skin of largefin longbarbel catfish.

3.5. Intrinsic viscosity

The intrinsic viscosities of ASC and PSC were 14.9 dl/g (±0.4 dl/g SD) and 14.5 dl/g (±0.2 dl/g SD), respectively ($P < 0.05$), which were higher than those of black drum bone PSC (13.7 dl/g), cod skin ASC (12.8 dl/g), and marine catfish collagen (12.7 dl/g) (Ciarlo, Paredi, & Fraga, 1997; Ogawa et al., 2004; Rose & Mandal, 1996). The high intrinsic viscosity might be attributed to the high proportion of cross-linked components (β- and γ-components), resulting in a higher average molecular weight (Ogawa et al., 2004). Collagen is a kind of amphoteric electrolyte which has different isoelectric points, the higher intrinsic viscosity might also be related to the stronger electrostatic repulsion amongst the collagen molecular chains in solution.

3.6. Ultraviolet spectra

Generally, the maximum absorption wavelength of protein in the near ultraviolet region is 280 nm. However, there is no obvious absorption for collagen in this region. As can be seen from the ultraviolet spectra (Fig. 3), both ASC and PSC have absorptions near 233 nm. This was in agreement with those of collagens from bullfrog skin (236 nm) (Li, Liu, Gao, & Chen, 2004) and channel catfish skin (232 nm) (Liu et al., 2007). Also, this result further proved that a high-purity was achieved for ASC and PSC from largefin longbarbel catfish skin.

3.7. Fourier transform infrared spectroscopy

Fig. 4 shows the FTIR spectra of ASC and PSC from largefin longbarbel catfish skin. Both spectra (ASC and PSC) are similar to those of collagens from other fish species (Liu et al., 2007; Muyonga et al., 2004). Amide A band positions of ASC and PSC were found at 3429 and 3439 cm^{-1}, respectively; amide I band positions of ASC and PSC were found at 1660 and 1659 cm^{-1}, respectively; Both amide II band positions of the two fractions were at 1553 cm^{-1} and the amide III band positions of ASC and PSC were found at 1240 and 1241 cm^{-1}, respectively. It seemed that peaks were obtained at similar wave numbers for the two fractions.

The amide A band is associated with N—H stretching vibrations. Amide I band, amide II band and amide III band, which were known to be related to the degree of molecular order and to be involved with the triple helical structure of collagen, resulted from

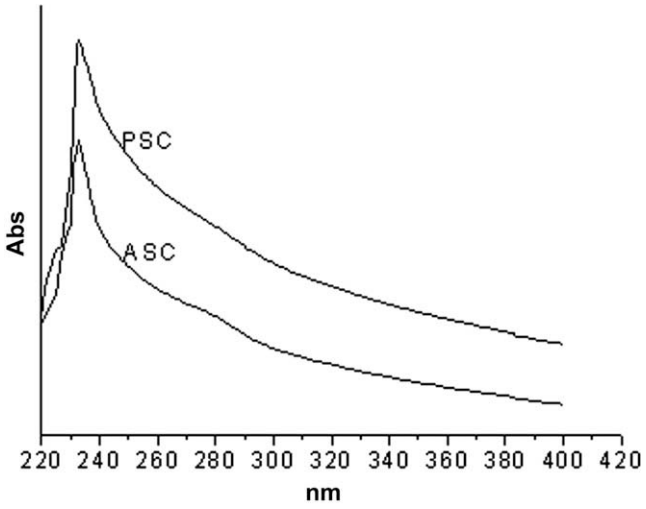

Fig. 3. Ultraviolet spectra of ASC and PSC from the skin of largefin longbarbel catfish.

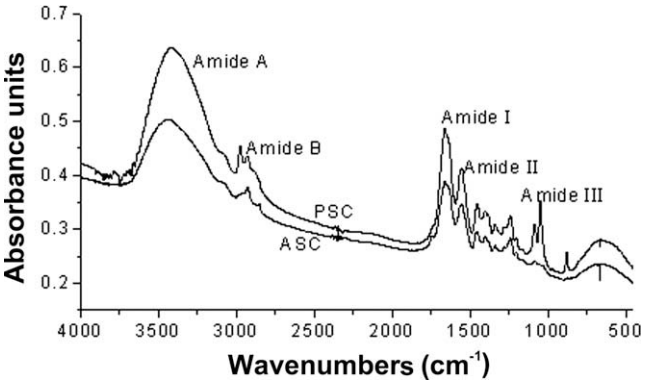

Fig. 4. FTIR spectra of ASC and PSC from the skin of largefin longbarbel catfish.

C=O stretching, N—H bending and C—H stretching, respectively. (Muyonga et al., 2004).

3.8. Peptide mapping

Peptide maps of collagens digested by trypsin at different pH are shown in Fig. 5. For peptide maps of collagens digested at pH 7.8 (lanes 2–4), ASC and PSC underwent a strong hydrolysis and only one peptide fragment of molecular weight (MW) about 25 kDa was obtained, whereas calf skin collagen was hydrolysed to some extent, with the nearly even appearance of peptide fragments of MW ranging from 116 to 23 kDa. This result suggested that collagens from largefin longbarbel catfish skin were less tolerant to hydrolysis by trypsin than was calf skin collagen.

Peptide maps of collagens (ASC and PSC) digested by trypsin at pH 4.4 (lanes 5–6) were similar to those of collagens (ASC and PSC) digested by trypsin at pH 7.8 (lanes 3–4), except that one more peptide fragment of MW around 31 kDa was obtained.

From the peptide maps of collagens (ASC and PSC) digested by trypsin at pH 2.5 (lanes 7–8), it could be found that ASC was more tolerant to digestion by trypsin than was PSC, as high MW components (β- and γ-components) of PSC were entirely digested and peptide fragments with MWs of 106.7, 84.7, 78.5 and 47.5 kDa appeared, whilst those of ASC still remained to some extent and only one peptide fragment with a MW of 47.5 kDa appeared.

Peptide maps of collagens were reported to differ amongst sources and species (Mizuta, Yamasa, Miyagi, & Yoshinaka, 1999).

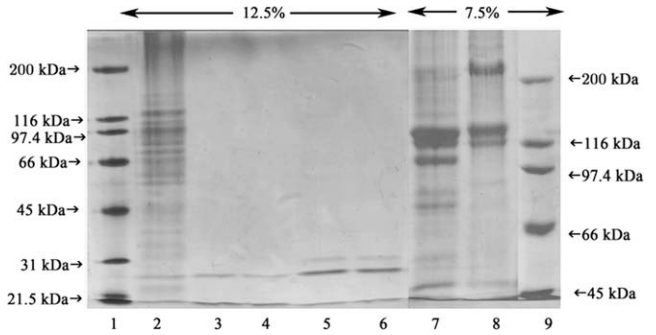

Fig. 5. Peptide maps of ASC and PSC from the skin of largefin longbarbel catfish digested by trypsin. Lanes 1 and 9: protein markers on 12.5% and 7.5% gels, respectively; Lanes 2–4: calf skin collagen type I, ASC, PSC with trypsin digestion at pH 7.8, respectively; Lanes 5 and 6: ASC, PSC with trypsin digestion at pH 4.4, respectively; Lanes 7 and 8: PSC, ASC with trypsin digestion at pH 2.5, respectively.

Thus, ASC and PSC from largefin longbarbel catfish skin might be slightly different in primary structure, and be different from calf skin type I collagen in terms of sequence and the composition of amino acids (Jongjareonrak et al., 2005).

Moreover, both ASC and PSC were hydrolysed to a greater extent when the pH was increased from 2.5 to 7.8. This result was attributed to the activity of trypsin, which was greater for the collagen in neutral or faintly basic medium than in acid medium (Zhang et al., 2007).

4. Conclusion

A great quantity of collagens (ASC and PSC) could be extracted from largefin longbarbel catfish skin without scales. The collagens consisted of two α-chains (α1 and α2) and were characterised as type I collagen. The imino acid content and intrinsic viscosity of collagen were higher than those of most fish collagens reported. Although similar ultraviolet and FTIR spectra of ASC and PSC were observed, there were still some differences in protein patterns and sequence of primary structure between the two fractions. The transition temperatures of collagens were above 30 °C, about 7 °C higher than that of the collagen from the skin of grass carp, which is also a temperate freshwater fish with scales. Therefore, the collagen from largefin longbarbel catfish skin has a considerable potential as a substitute for mammalian collagen. However, further studies should be carried out to demonstrate how properties of collagen from fish skin relate to scales.

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