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Isolation and Characterization of Acid-soluble Collagen and Pepsin-soluble Collagen from the Skin of Hybrid Sturgeon

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Abstract: By using the wastes fish skin of sturgeon processed as a raw material, a macromolecule biomaterial of collagen was extracted. Acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) were successfully isolated from the skin of hybrid sturgeon with two extraction methods. The yields of ASC and PSC based on the wet weight of skin were 5.73 ± 0.11% and 10.26 ± 0.39%, respectively. The denaturation and melting points of ASC (26.83 ℃ and 110.49 ℃) and PSC (26.54 ℃ and 102.99 ℃) were assessed by Circular dichroism (CD) and Differential scanning calorimetry (DSC). ASC and PSC appeared to be dense sheet-like film linked by random-coiled filaments under scanning electron microscopy (SEM). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Fourier transform infrared spectroscopy (FTIR) confirmed that both the ASC and PSC were Type I collagen and maintained a complete triple helix structure. These results indicated that both ASC and PSC possessed good biological activity and could be widely used in medical biomaterials and other fields.

Key words: hybrid sturgeon; acid-soluble collagen (ASC); pepsin-soluble collagen (PSC); isolation; characterization

1 Introduction

Collagen is the most abundant protein in animal origin, approximately accounting for 30% of total protein, and also the major structural protein in connective tissue of animals[1,2]. It is a functional protein, which is fibrous to maintain the elasticity of skin. Until now, at least 29 kinds of collagens have been identified from various animals[3,4]. Collagen, a right-handed triple superhelical structure, is formed by three polypeptide chains folding and winding[1,5]. Traditionally, collagen is extracted from the skins of terrestrial animals[3,6]. And collagen from these sources has a wide range of applications in medical biomaterials, food, cosmetics and other industries because of its unique physical and functional properties[5,7]. But in recent years, the outbreak of terrestrial infectious diseases has resulted in the restrictions on collagen application. As a consequence, a search for alternative safe sources of collagen has become imminent[8].

Among the collagen alternatives, the skin of fish is considered as a kind of desired raw materials, because it is rich in collagen and easy to get without any religious barriers or risks of disease transmission[8]. Several studies have found out that the collagens could be varied obviously by fish species, habits and age[3]. The amino acid compositions of collagens extracted from fishes of different growing environments are widely different[9]. The denaturation temperatures of collagens increase with the increase of imino acid content[10], because imino acid is involved in inter-chain hydrogen bonding[2]. The physical properties of collagen have shown that the higher the collagen content, the firmer the meat[11].

Hybrid sturgeon is a kind of unique excellent artificially breeding sturgeon species in China. It is a cross between a Huso dauricus-female and an Acipenser dabryanus-male. And it is also the heaviest freshwater fish in China that adults can weigh up to 500 kg in maximum. Hybrid sturgeon belongs to chondrostei. In processing hybrid sturgeon, a huge amount of skin from hybrid sturgeon is discarded directly. Because

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the skin is thick and hard to eat, most of it is treated as trash[7]. From environmental protection and bioresource utilization point of view, the practice is a tremendous waste. The skin from the fish can be used as a potential raw material for extracting collagen, which may promote the development of Hybrid sturgeon industries and reduce environmental pollution. However, there have been only a few reports on the collagen from Hybrid sturgeon so far. Thus, the present study involving the extraction and characterization of the collagen biomaterials from the skin of Hybrid sturgeon would be of great practical value in relevant industries.

2 Experimental

2.1 Materials

Hybrid sturgeo (about 400 kg) was provided by Amur Caviar Co. Ltd, Yunnan, China. The skin was removed as a byproduct in fillet processing, and stored at low temperature and transported by air. Upon arrival, the skin of Hybrid sturgeon was sliced into small pieces (0.3 cm × 0.3 cm) and then stored at a temperature of −20 ℃. All reagents were of analytical grade. Pepsin (1:3000 units) was purchased from Shanghai Macklin Biochemical Co., Ltd. Shanghai, China.

2.2 Proximate analysis

The moisture, ash, lipid, sugar, protein contents of skin from Hybrid sturgeon were measured according to AOAC methods. The contents of microelements in the skin were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES).

2.3 Tissue slice of the skin of hybrid sturgeon

The tissue structure and distribution of collagenous fibers were investigated according to hematoxylin-eosin staining[12]. The fresh skin of Hybrid sturgeon without frozen was immersed in 10% (*v/v*) neutral formaldehyde solution over 48 h. The skin was divided into three parts and sliced with frozen section method. The thickness of slices was 18 μm. The tissue slices were visualized by hematoxylin-eosin staining under microscope.

2.4 Extraction of ASC and PSC

The isolation procedure of ASC and PSC from Hybrid sturgeon skin are shown in Fig.1. ASC was extracted as previously described[13] with some modifications. All steps were kept at 4 ℃ and in continuous stirring. The as-prepared skin from this fish was immersed in 0.1 M NaOH to remove non-protein contents at the ratio of skin and alkali solution 1:20 (*w/v*). After that, the treated skin was rinsed with cold distilled water to neutral pH. Then, the pretreated skin was mixed with 10% (*v/v*) *N*-butyl alcohol to remove lipid content at the ratio of skin and solvent 1:20 (*w/v*) for 24 h. And then the defatted skins were washed with cold distilled water. The solvent was changed every 6 h.

After pretreatment, the skin was extracted with 0.5 M four acids (hydrochloric acid, acetic acid, citric acid and lactic acid) at the ratio of skin and acid 1:15 (*w/v*) for 24 h, respectively. The mixtures were filtered through two layers of gauze. The residues were extracted again under the same conditions, and both the filtrates were mixed. The ASC was salted-out by adding sodium chloride until 2.6 M NaCl solutions under acidic conditions. The salted-out precipitate was collected by centrifugation at 15,000 r/min for 30 min (Allegra 64R centrifuge, Beckman, Ltd., USA). The precipitate was redissolved in 0.5 M acetic acid solution, and the solutions were dialyzed against 0.1 M acetic acid and

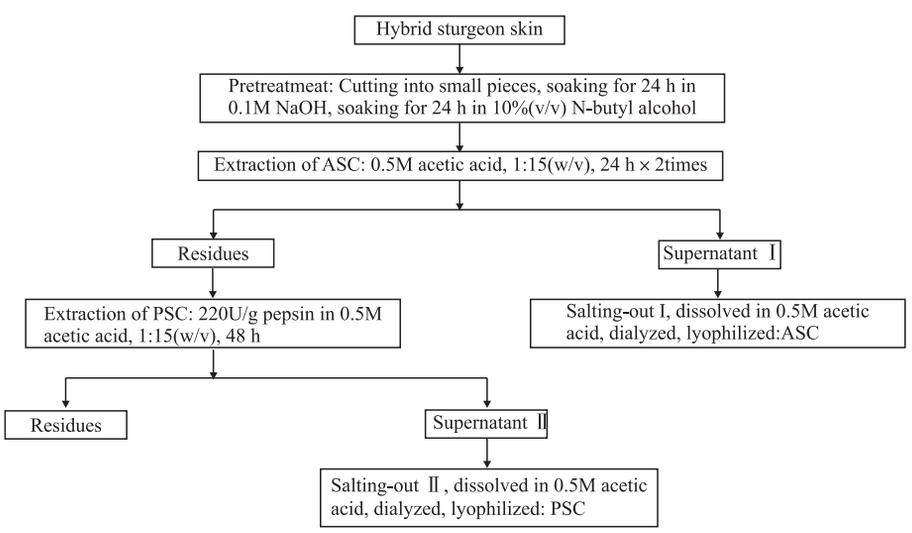

Fig.1 Flow chart for the isolation procedures of ASC and PSC from Hybrid sturgeon skin

distilled water for 24 h, respectively. And dialysis fluid was changed per 6 h. Then, the dialyzate was freezingly dried and referred to ASC. The optimized acid extractant was determined using the yields of ASC as target. ASC was extracted under the condition of optimized acid extractant.

The undissolved residues after extraction of ASC were used for PSC extraction[5]. The residues were added to 0.5 M acetic acid at the ratio of residue and acetic acid 1:15 (*w/v*) while the porcine pepsin (120 U/g residue) was added to mixture. The mixtures were stirred continuously at 4 ℃ for 48 h, after enzyme denaturalixation. The mixtures were filtered through two layers of gauze and the filtrate was collected. The filtrate was salted-out and the precipitate were dialyzed by the same method used in extracting ASC before. The dialyzates were freezingly dried and referred to PSC. The yields calculation methods of ASC and PSC were the same and the dry weight of collagen accounted for the percentage of wet skin of the Hybrid sturgeon initial skin.

2.5 Scanning electron microscopy (SEM)

The surface morphology and structure characteristics of the initial skin, ASC and PSC were studied by SEM (JSM-IT300, Japan). The samples were sputtered with gold and then observed by SEM in different magnification.

2.6 Amino acid analysis

ASC and PSC were mixed with 6 M HCl and hydrolyzed at 110 ℃ for 24 h, respectively. The hydrolyzates were vaporized and the remaining matters were dissolved in 25 mL citric acid buffer solution. An aliquot of 50 μL was applied to an amino acid analyzer (HITACHI, L-8900, Japan).

2.7 SDS-PAGE

SDS-PAGE was performed using a discontinuous Tris-glycine buffer (pH=8.3) system with 7.5% separating gel and 4% stacking gel.

The collagen samples were dissolved in 0.1 M acetic acid to a concentration of 1 mg/mL. Solubilized ASC and PSC samples were mixed with sample buffer (0.5 M Tris-HCl, pH 6.8, containing 2% SDS, 25% glycerol and 10% β-ME) at the ratio of sample and sample buffer 1:1 (*v/v*) and then boiled for 3 min, respectively. Samples (10 μL) and protein markers (10 μL) were loaded onto polyacrylamide gels, respectively. The high molecular weight protein markers were used to estimate the molecular weight of proteins.

After electrophoresis, the gels were stained for 30 min with 0.05% (*w/v*) Coomassie blue R-250 in 15% (*v/v)* methanol and 5% (*v/v*) acetic acid. Finally, the gels were destained for 1 h with 30% (*v/v*) methanol and 10% (*v/v*) acetic acid solutions until the bands were clear.

2.8 Differential scanning calorimetry (DSC)

The melting points of ASC and PSC were determined with DSC (HS-DSC-101, HESON, Shanghai, China). The ASC and PSC were dissolved in 0.05 M acetic acid at the ratio of sample and acetic acid solution 1:40 (*w/v*), respectively. The mixtures were held at 4 ℃ for 48 h. The samples were accurately weighed into an aluminum pan, and then the samples were blotted uniformly and sealed. The sample was heated from 15 ℃ to 150 ℃ at the heating rate of 15 ℃/min. An empty pan as blank control. The melting points of ASC and PSC were then defined, respectively.

2.9 Circular dichroism (CD)

The molecular conformation and denaturation temperature of ASC and PSC were assessed by CD spectrophotometer (CHIRASCAN, Applied Photophysics Limited, Britain). The samples were immersed in 0.1 M acetic acid solution to a concentration of 0.8 mg/ml, and then small aliquots were placed into a quartz cell (1-mm pathway). The spectra from 190 to 250 nm were recorded. The scan speed was 50 nm/min and the temperature was 15 ℃.

The denaturation temperature of sample solution was determined. Detector was set at 220 nm. The $[\theta]_{220}$ was recorded while the sample was heated from 15 ℃ to 45 ℃ at the heating rate of 1 ℃/min. The denaturation temperature was determined as the midpoint temperature between native-folded and completely unfolded forms.

2.10 Fourier transform infrared spectroscopy (FTIR)

The ASC and PSC samples were analyzed using a Fourier transform IR spectrophotometer (Nicolet iS5, Thermo Fisher, USA). The ASC and PSC samples were mixed with dried KBr at a sample/KBr ratio of 1:50 (*w/w*) and grinded evenly. The mixtures were pressed into a disk for spectrum recording.

2.11 Zeta-potential analysis

ASC and PSC were immersed in 0.5 M acetic acid to a concentration of 0.05% (*w/v*), respectively. Then the solution was divided equally into six parts, then the pH of the solutions was adjusted to 2.0-7.0 using 3.0 M nitric acid or 3.0 M potassium hydroxide[14]. The zeta potential was measured by a Nano-ZS 90 zetasizer (Malvern, UK).

2.12 Viscosity of ASC and PSC solution

The viscosity of ASC and PSC solutions was mea-

sured by DNJ-8S viscometer (Fangrui Instruments Co., Ltd., Shanghai, China). The samples were dissolved in distilled water at a concentration of 0.6% (*w/v*). ASC and PSC solutions were heated from 4 ℃ to 40 ℃ by water bath. At the designed temperature, the solutions were held for 30 min to balance the temperature gap between the solution and water bath. The viscosity of samples were determined according to viscometer with appropriate spindles and speed[14,15].

3 Result and discussion

3.1 Proximate analysis

The proximate compositions of skin are shown in Fig.2. The moisture content was 56.22%, which is lower than the balloon fish skin (62.23%)[16]. The total protein, lipid, total sugar and ash contents of the skin from Hybrid sturgeon on the basis of wet weight were 41.38%, 2.93%, 0.75%, and 0.65%, respectively. Additionally, the protein, lipid, ash contents of the of the shark skin were 24.75%, 0.19%, and 12.12%, respectively[15]. The nile perch skin contains 21.6% protein, 6.8% lipid, and 6.0% ash contents[2]. From the above data, we can conclude that the content of protein in Hybrid sturgeon skin was higher than that of shark skin and Nile perch skin. These indicated that the Hybrid sturgeon skin might be the desired collagen source with less non-protein substances.

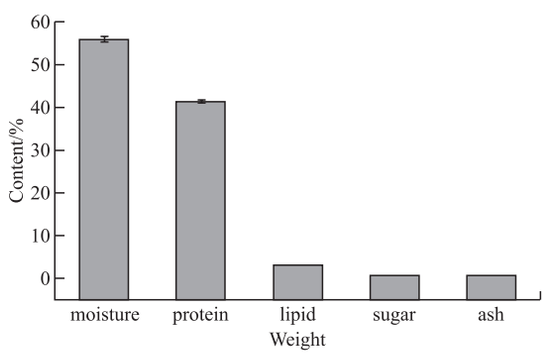

Fig.2 Proximate compositions of skin from Hybrid sturgeon on the basis of wet weight

The contents of microelement in the skin are shown in Table 1. There are rich elements in the skin of Hybrid sturgeon. Na, S, and Ca on the dry weight are 4 730.957, 3 851.652, and 2 007.826 mg/kg, which are the most abundant elements in the skin. In addition, the skins contain not only a large amount of P, Fe, B, K, Mg, Al, and other common elements, but also Zn, Cr, Mn, Se, and other trace elements, which have important effect on human health. This indicates that the skin of Hybrid sturgeon might be the desired collagen source.

Table 1 Microelement compositions in the skin of Hybrid sturgeon (expressed mg element/kg dry skin)

Element	Content	Element	Content	Element	Content
As	41.623	Li	119.246	Ca	2007.826
B	295.404	Mg	242.029	K	255.014
Ba	40.058	Mn	5.797	Na	4730.957
Bi	13.681	Ni	8.348	S	3851.652
Cd	2.493	Pb	21.565	Zr	16.464
Co	1.275	Sr	5.565	P	963.536
Cr	28.290	Ti	7.884	W	1.217
Cu	27.942	V	17.565	Al	147.710
Fe	428.638	Zn	198.493	Se	15.014

3.2 Histological structure

Microstructure of skin tissue is measured by Tissue Slices technique. Tissue slices shown in Fig.3(a) were acquired from the skin of Hybrid section A along the arrow direction. From the outside to inside it can be observed by the order: squamous epithelium (① arrow), melanin layer (② arrow), the longitudinal collagen layer (③ arrow), annular collagen layer (④ arrow) and other connective tissue. Tissue slices shown in Figs.3(b) and 3(c) were taken from the skin of section B and section C, respectively. The tissue slice B and tissue slice C both have annular collagen layer. Compared with tissue slice A, tissue slice B has fewer melanin layer and a non-uniform distribution of melanin layer, and tissue slice C has no melanin layer. Seen from the tissue slices A, B, and C, it can be found that the skin of Hybrid is rich in collagen and a high-quality collagen resource. The tissue slice (Fig.3) indicated that the skin of Hybrid sturgeon has a dense tissue structure and collagen distribution.

According to the special tissue distribution of Hybrid sturgeon skin, acid method and pepsin method were employed to achieve ASC and PSC. At first, the skin treated with acetic acid could make the skin of Hybrid sturgeon tissue swelling, and made a portion of the collagen dissolve in the acetic acid. Then, swollen tissue afforded pepsin easier access into deeper tissue of the skin to cleave the end of helical regions, which led to more collagen dissolved.

3.3 Yield analysis

The effect of different acids on the extraction ratio of ASC with the source of the skin of Hybrid sturgeon is shown in Fig.4. The terminal group and side-chain of collagen peptide contain amino-group. After the skin of Hybrid sturgeon was treated with acid, the amino-group of collagen peptide combined with acid. The ionic bond between collagen molecules and the hydrogen bond be-

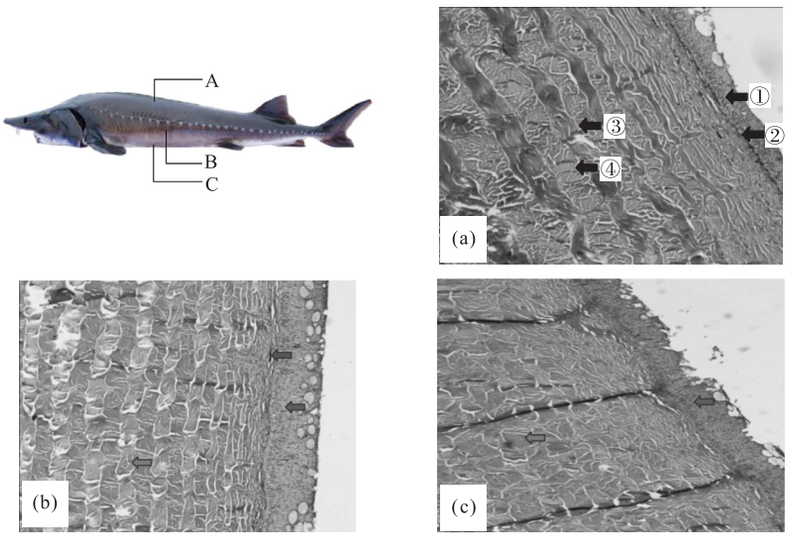

Fig.3 Tissue sections of different sites from the skin of hybrid sturgeon

tween peptide chains would be broken, collagen could swell and dissolved in acid solutions. Different kinds of acids have different effects on the internal structure of collagen molecules due to their different chemical properties. Finally, different acids have different effects on the yield of collagen. Thus, Acetic acid would be an optimized acid extractant as shown in Fig.4.

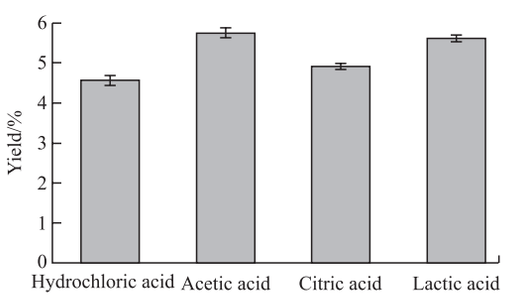

Fig.4 The acids effect on the extraction ratio of ASC from the skin of hybrid sturgeon

The yields of ASC and PSC isolated from the skin of Hybrid sturgeon were 5.73 ± 0.11% and 10.26 ± 0.39% on a wet weight basis, respectively. In addition, the yields of ASC and PSC from the skin of Spanish catfish were 13.68% and 3.49% (based on the wet weight)[1]. However, the yields of ASC and PSC from the skin of Triped catfish were 5.1% and 7.7% (based on the wet weight)[5]. The results suggested that the skin was not completely solubilized in 0.5 M acetic acid. Inter-chain cross-linkages existing in the telopeptide region of collagen made the collagen less soluble under acidic conditions[6]. With further digestion by limited pepsin that the cross-linked molecules were most likely cleaved, which is the reason for increasing the rate of collagen extraction[14].

The differences in fish species led to the difference in yields. From the tissue slice of skin (Fig.3) and SEM micrographs of pretreated skin (Figs.5. 1-1,1-2), it can be found that the skins have very dense tissue structure and full of protein. Due to low solubility of cross-links formed, the yield of ASC is lower. The treatment with 0.5 M acetic acid made the skin of Hybrid sturgeon tissue swelling so that only a portion of the ASC was dissolved in the acetic acid. Swelling tissue made pepsin easier access into deeper region. Pepsin cleaved the end of telopeptide helical region, and then the yield of PSC could be raised[17].

3.4 Surface morphology

The surface images of the pretreated skin, ASC and PSC are presented by SEM in Fig.5. The pretreated skin had non-collagenous substances and a very dense structure (Figs.5. 1-1, 1-2). The lyophilization conditions for ASC and PSC were the same. The ASC from the skin of Hybrid sturgeon was a sponge-like structure with pores (Figs.5. 2-1, 2-2, 2-3). The surface of ASC was partially wrinkled (Figs.5. 2-4), possibly because of water sublimation during cryodesiccation process[18].

The PSC looked like irregular curled filamentous structure in the SEM images (Figs.5. 3-1, 3-2, 3-3). The ultrastructure of ASC and PSC showed great difference because of different extraction process of ASC and PSC. The ASC was partially dissolved in 0.5 M acetic acid, but the pepsin cleaved the telopeptide helical region, which made the PSC free from the skin tissue. The results demonstrated that the primary structures might be different between ASC and PSC[19].

3.5 Amino acid profile

The amino acids component of ASC and PSC ex-

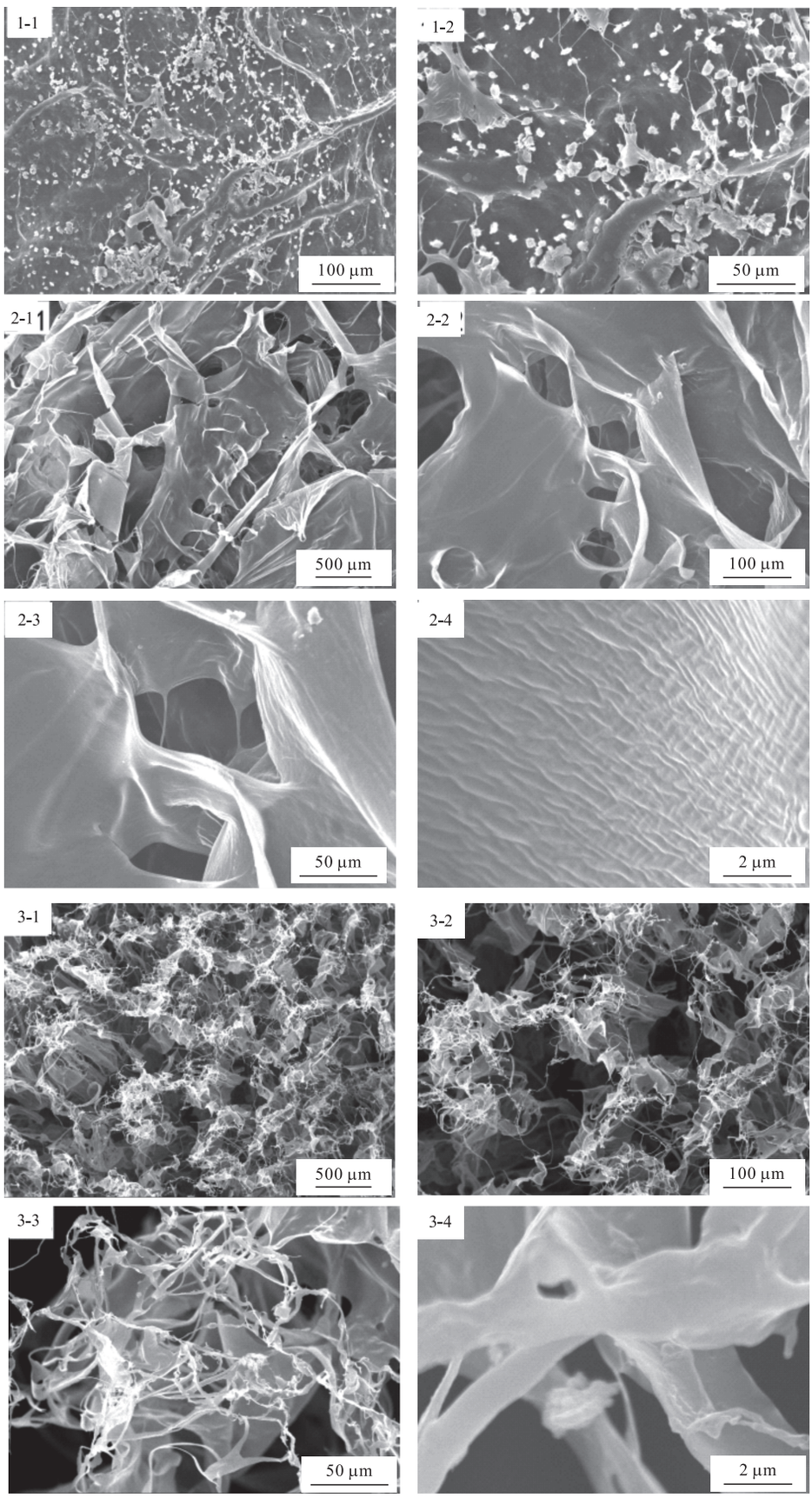

Fig.5 SEM images of pretreated skin (1-1, 1-2), ASC (2-1, 2-2, 2-3, 2-4), PSC (3-1, 3-2, 3-3, 3-4)

tracted from skin of hybrid sturgeon are given in Table 2.

In ASC and PSC products, glycine (Gly) constitutes approximately one third of total amino acid res-

idues (337.5-341.3 residues/1000 residues), followed by alanine (Ala) (116.3-118.6 residues/1000 residues), proline (Pro) (113.9-115.4 residues/1000 residues), glutamic acid (Glu) (80.8-83.5 residues/1000 residues) and hydroxylproline (Hyp) (63.8-65.0 residues/1000 residues). In addition, the contents of histidine (His), hydroxylysine (Hyl), tyrosine (Tyr), methionine (Met) were very less and cystine (Cys) were not detected in ASC but very trace (0.7 residues/1000 residues) in PSC. The data are similar to the products obtained for the skin of ball fish[16] and skin of Spanish mackerel[1]. Among these amino acids, Gly is the major amino acid and plays an important role in reducing steric hindrance[10-20].

Table 2 Amino acid profiles of the ASC and PSC from the skin of Hybrid sturgeon (residues/1000 residues)

Amino acids	ASC	PSC
Hydroxylproline (Hyp)	63.8	65.0
Aspartic acid (Asp)	50.0	50.9
Threonine (Thr)	23.9	24.2
Serine (Ser)	51.5	50.7
Glutamic acid (Glu)	83.5	80.8
Glycine (Gly)	337.5	341.3
Alanine (Ala)	118.6	116.3
Cystine (Cys)	0	0.7
Valine (Val)	16.8	16.7
Methionine (Met)	3.0	2.2
Isoleucine (Ile)	11.8	12.2
Leucine (Leu)	18.6	18.3
Tyrosine (Tyr)	4.4	3.7
Phenylalanine (Phe)	15.4	14.5
Hydroxylysine (Hyl)	4.6	5.2
Lysine (Lys)	26.3	26.4
Histidine (His)	5.4	4.8
Arginine (Arg)	51.3	50.7
Proline (Pro)	113.9	115.4
Total	1000	1000
Imino acid	177.7	180.4

The imino acids, proline and hydroxyproline, were also found in ASC and PSC. The Pro and Hyp contents of ASC and PSC similar to ball fish skin collagen (179 and 174 residues per 1000 amino acid residues)[16] and Spanish mackerel fish collagen (177.1 and 180.8 residues per 1000 amino acid residues)[1]. The data were slightly less than that of amur sturgeon skin collagen (212 and 218 residues per 1000 amino acid residues)[3], striped catfish skin collagen (206 and 211 residues per 1000 amino acid residues)[5] and marine eel-fish collagen (190 and 200 residues per 1000 amino acid residues)[10]. These results support that the different imino acid contents among the animals are due to the different habit, particularly living environment temperature.

3.6 Protein patterns

The protein patterns of ASC and PSC are tested by SDS-PAGE and shown in Fig.6. It can be seen that ASC and PSC are comprised of at least two different α chains (α_1 and α_2) and dimer (β). Therein, α_1 and α_2 chains molecular weight of ASC (approximately 118 kDa and 140 kDa) and PSC (approximately 116 kDa and 138 kDa), respectively. The intensity of α_1 chain is about twice than that of α_2 chain, which is typical electrophoretic profile of Type I collagen and similar to the patterns of many other fish species[21,22]. The results demonstrate that ASC and PSC extracted from the skin of Hybrid sturgeon is Type I collagen.

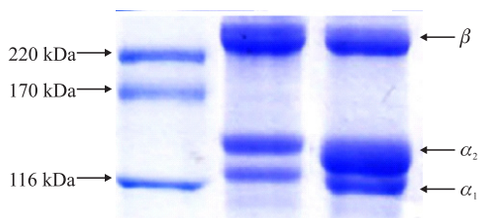

Fig.6 SDS-PAGE patterns of ASC and PSC from the skin of Hybrid sturgeon. Lane 1, high molecular weight marker; lane 2, ASC; lane 3, PSC

The shift speed of PSC chains were a little higher than that of ASC chains. This indicates that both α_1 and α_2 chains of ASC are slightly higher in molecular weight than that of PSC. Because with further digestion by limited pepsin that the cross-linked molecules were most likely cleaved and part of peptide chain was ruined. Besides, β-chains are observed in both ASC and PSC. However, the β-chain of PSC is slightly lower in molecular weight than that of ASC. The result indicates that ASC contains more inter- and intra-crosslink structure than PSC[23], which infers that the primary structures of ASC and PSC are slightly different.

3.7 Thermal behaviors

The thermal behaviors of ASC and PSC were characterized by circular dichroism spectroscopy (CD) and different scanning calorimetry thermograms (DSC). The images of CD are shown in Fig.7(a) at the wavelength of 190-250 nm. The typical characteristics of triple helical conformation of collagen include a rotatory maximum 220 nm, a rotatory minimum 198 nm and a consistent crossover 213 nm[24]. ASC and PSC solutions in a cuvette were heated from 15 to 45 ℃ at 220 nm. In Fig.7(b), the normalized CD spectra sharply decreased when being heated from 23 to 30 ℃ due to the fact

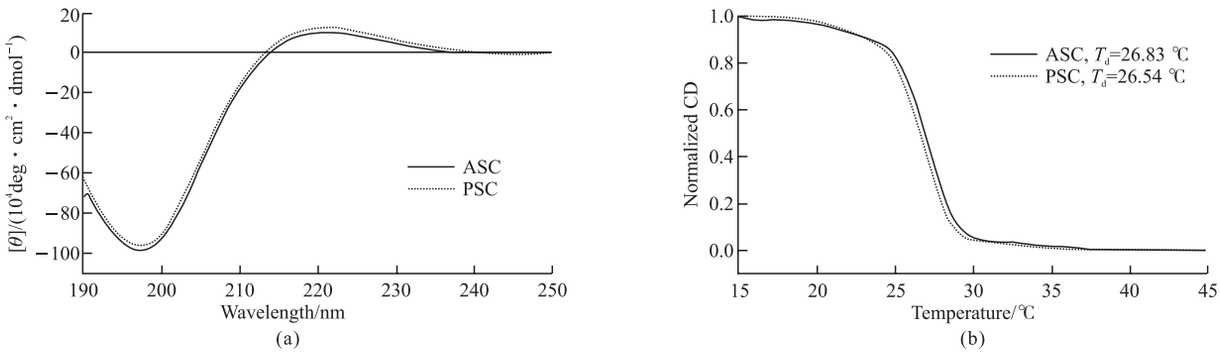

Fig.7 CD spectra (a and b) of ASC and PSC from the skin of hybrid sturgeon

that the helical structure of collagen was destroyed. The denaturation temperatures (T_d) were 26.83 ℃ and 26.54 ℃ for ASC and PSC, respectively. The results enunciated that the T_d of ASC and PSC were 32.78 ℃ and 32.46 ℃, respectively. This result discloses that environmental and body temperature affect the thermal stability of collagen. In general, the denaturation temperature of collagen is related to the content of Pro+Hyp and the degree of Hyp+Hy[25]. The contents of this amino acid profiles are similarly between ASC and PSC. So, the T_d of ASC and PSC are approximate.

The images of DSC are shown in Fig.8. The DSC patterns show a significantly endothermal peak, which may be the destruction of collagen materials[26]. The melting temperature (T_m) of ASC and PSC are 110.49 ℃ and 102.99 ℃, respectively.

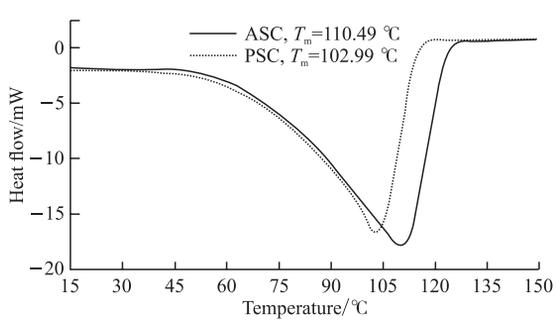

Fig.8 DSC thermograms of ASC and PSC from the skin of hybrid sturgeon

3.8 Fourier transform infrared spectra

The structures of ASC and PSC from the skin of Hybrid sturgeon are characterized by FTIR spectra and shown in Fig.9 and Table 3.

The transmittance characteristics of amide A are commonly in the range of 3 400-3 440 cm^{-1}. When the N-H group is involved in a hydrogen bond of the peptide, the peak is shifted to lower frequency[14]. The transmittance peaks of ASC and PSC were at 3 424 and 3 339 cm^{-1}, respectively. These indicate that less N-H groups in ASC combined with hydrogen bond of peptide than those in PSC, by which the triple helical structure of collagen was twisted together. Amide B peaks, representing the asymmetrical stretch of CH_2 groups at 2 928 and 2 934 cm^{-1} in the FTIR spectra of ASC and PSC, respectively.

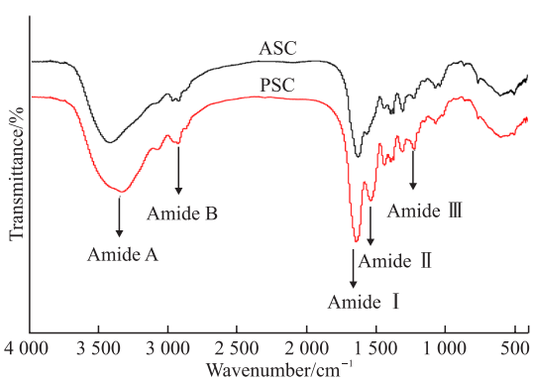

Fig.9 FTIR spectra of ASC and PSC from the skin of hybrid sturgeon

Amide I with a characteristic wave number in the range of 1 600-1 700 cm^{-1} is the sensitive marker of the peptide secondary structure[14]. The Amide I transmittance peaks of ASC and PSC occurred at 1 640 and 1 653 cm^{-1}, respectively. Amide II is primarily associated with the combination of the N-H in-plane bend and the C-N stretching vibration. The Amide II transmittance peaks of ASC and PSC were at 1 579 and 1 549 cm^{-1}, respectively. Amide II of ASC was found at higher wavenumber compared with PSC. These results are in accordance with Amide A band, which suggests a lower hydrogen bonding in ASC than that in PSC. Because of intermolecular interactions in collagen, the Amide III peak is complex[15], including C-N stretching vibrations and N-H deformation from amide linkages as well as wagging vibrations from CH_2 groups of the glycine backbone and proline side-chains[27]. The Amide III transmittance peaks of ASC and PSC were at 1 239 and 1 238 cm^{-1}. FTIR spectra exhibit the peaks of Amide I, Amide II, Amide III as well as Amide A

Table 3 FTIR spectra peak locations and their assignments for ASC and PSC from the skin of Hybrid sturgeon

Region	Peak wavenumber/cm^{-1}		Assignment
	ASC	PSC	
Amide A	3424	3339	NH stretch, coupled with hydrogen bond
Amide B	2928	2934	CH_2 asymmetrical stretch
Amide I	1640	1654	C=O stretch/hydrogen bond coupled with COO-
Amide II	1579	1549	NH bend coupled with CN stretch
-	1450	1451	CH_2 bend
-	1404	1404	COO- symmetrical stretch
-	1319	1319	CH_2 wag
Amide III	1239	1238	NH bend coupled with CN stretch
-	1084	1081	C-O stretch
-	618	612	Skeletal stretch

and Amide B have some differences in the secondary structure between ASC and PSC.

Additionally, the triple helical structures were acknowledged from the intensity ratio of Amide III and 1 450 cm^{-1} bands. The ratio in Fig.8 approached to 1.0[28], confirming that the triple helix structures were well maintained. The removal of telopeptide region by pepsin led to slight difference of the molecular structure of ASC and PSC are slightly. At present, the triple-helical structures were still predominant with stronger bonds. Therefore, both ASC and PSC have specific triple-helical structures[29].

3.9 Zeta potential

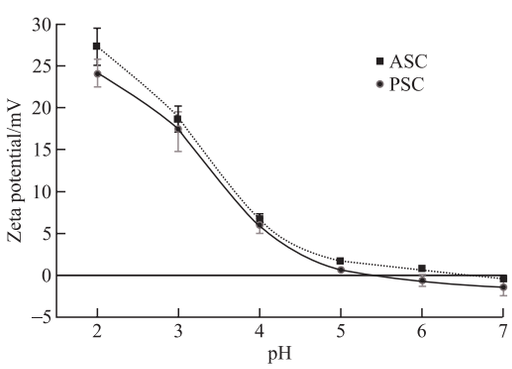

Fig.10 Zeta (ζ) potential of ASC and PSC from the skin of Hybrid sturgeon

The zeta potentials of ASC and PSC solutions are characterized by DLS and shown in Fig.10. Zeta potential of ASC and PSC show that net charge of zero were 6.56 and 5.36, respectively. Previous studies have revealed that protein aqueous solution has a net charge of zero at its isoelectric points (pI)[14,15]. The pI of ASC and PSC were at acidic pH, indicating there might be high content of acidic amino acids (Glu and Asp) in ASC and PSC. And pepsin might cleave the telopeptide region and cause differences in acidic and basic amino acid residues of ASC and PSC. ASC and PSC from the skin of striped catfish exhibited the pI were 4.72 and 5.43, respectively. And the values from the skin of brown-banded bamboo shark were observed at 6.21 and 6.56, respectively. The different net charge of zero may also be caused by the different content of amino acid in ASC and PSC[29].

3.10 Viscosity of ASC and PSC solutions

The viscosity of collagen is one of the important characteristics, and different viscosities of ASC and PSC solutions were subjected for heating treatment at different temperatures, which are recorded by viscometer and shown in Fig.11.

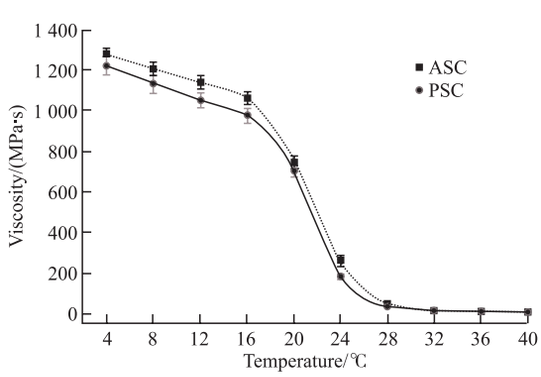

Fig.11 Viscosity of ASC and PSC from the skin of Hybrid sturgeon

ASC and PSC have a high viscosity at low temperature, because their high proportion of polymers results in a higher average molecular weight[29-31]. The viscosities of ASC and PSC decrease with the elevation temperature. When the ASC and PSC solutions increase to a certain temperature, the viscosities would be constant on a low level. In the range of 4-16 ℃, the viscosities of ASC and PSC solutions are relatively high. And viscosity both of ASC and PSC sharply reduces with being heated from 16-28 ℃. These illustrate that the

triple helical structure of ASC and PSC still maintains well at low temperature. With temperature increasing, the triple helical structure begins to break down[32-33]. This result agrees with the denaturation temperature of ASC (26.86 ℃) and PSC (26.54 ℃) assessed by CD (Fig.6). These results demonstrated that heating treatment can break the hydrogen bonds of collagen which stabilizes collagen native helical structure.

4 Conclusions

The ASC and PSC were successfully isolated from the skin of Hybrid sturgeon and tested by SEM, SDS-PAGE, DSC, CD, and FTIR *et al.* ASC and PSC are consisted of two α chains (α_1 and α_2) and classified as collagen type I. The existence of helical arrangements was characterized by FTIR. The ASC and PSC show similar protein patterns, while their differences can be found in yields, thermal stability, surface morphology, FTIR spectra, zeta potentials, and viscosity. Because the structure and amino acid profiles of ASC and PSC have slight difference. The results show that the ASC and PSC from Hybrid sturgeon maintain good biological activity and can serve as an important alternative source of collagens and promise for further application in food or biomaterials.

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