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Studies on collagen from the skin of channel catfish (*Ictalurus punctaus*)

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Abstract

Acid-soluble and pepsin-soluble collagens (ASC and PSC) were extracted from the skin of channel catfish (*Ictalurus punctaus*) and partially characterized . The collagen obtained in the experiment contained more than 23% glycine as the most abundant amino acid. The denaturation temperature of acid-soluble collagen was 32.5 °C, about 5 °C lower than that of the porcine skin collagen. SDS–PAGE showed that the collagens were composed of two distinct alpha chains, which is similar to the porcine type I collagen. The contents of the skin ASC and PSC, on a dry weight basis, were 25.8% and 38.4%, respectively. These results suggest that channel catfish skin has potential as a supplement to the sources of vertebrate collagens.

Keywords: Channel catfish; Collagen; Amino acid content; Denaturation temperature

1. Introduction

Collagen is the most primary protein of animal origin and has been extracted from the skins of some vertebrate species, especially pig and calf. Extraction of collagens from fish skin, however, is less common. Collagens of fish studied in recent years were from Baltic cod (*Gadus morhua*) (Sadowska, Kolodziejska, & Niecikowska, 2003); chub mackerel (Nagai Takeshi & Suzuki Nobutaka, 2000); ocellate puffer fish (*Takifugu rubripes*) (Nagai, Araki, & Suzuki, 2002); Indian catfish (*Gariepinus* spp.) (Sivakumar, Arichandran, Suguna, Mariappan, & Chandrakasan, 2000); paper nautilus (*Argonauta argo*, Linnaeus) (Nagai Takeshi & Suzuki Nobutaka, 2002); *Pagrus major* and *Oreochromis niloticas* (Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003); black drum (*Pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*) (Ogawa et al., 2004).

It was found that collagens of many teleosts always had lower contents of hydroxyproline and lower denaturation temperatures compared to the collagens of mammals (Nagai et al., 2002). The teleost collagens contained two (Muyonga, Cole, & Duodu, 2004a, 2004b; Nagai et al., 2002) or three distinct α chains (Kimura, Ohno, Miyauchi, & Uchida, 1987), whereas those of mammals contained only two different α chains. There are some unique characters of fish collagens for their different structures and contents of amino acids. As we know, the bovine and pig skins are the main sources of industrial collagens which have been used in functional foods, biomedical materials and cosmetics especially. Fish collagen can be a useful substitute for bovine and pig collagens for its excellent characteristic in the future.

Channel catfish (*Ictalurus punctaus*) were originally found in North America. Since then channel catfish have been widely introduced throughout the United States and the world. This fish species is the most important aquatic animal commercially cultured in the United States (Thomas L. Wellborn, 1998). Channel catfish have been widely bred in China, especially in Jiangsu and Hubei provinces since 1984. The meat of the fish was always sliced for exporting to America and Europe. Approximately 150,000 t of channel catfish were processed in China and 8000 t were transported to other countries in 2004. Because of the great profit the yield of channel catfish will rise sharply in recent years. At the same time, a great amount of fish skin will be dumped as processing waste. The skin of

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channel fish contains a large amount of collagen; therefore it is suggested that the skin can be a useful source of collagen and gelatin. In this paper, we extracted and partially characterized the collagens of channel catfish skin for potential industrial applications.

2. Materials and methods

2.1. Materials and pretreatment

Skin of Channel catfish (*I. punctaus*) was provided by Ao Food Co. Ltd. (Jiangsu province), and stored at −20 °C. Before use, the skins were cut into 2–5 mm pieces by a scalpel at 0 °C. The pieces of skin were allowed to thaw below 10 °C, were washed with chilled water (4 °C) for 20 min, mixed with 8 volumes (v/w) 0.1 M NaOH (4 °C) to remove noncollagenous proteins and other residues, then washed with distilled water (4 °C) and lyophilized.

2.2. Extraction of collagen

Fat in the skin was extracted for 2 days successively with ether and hexane (4 °C), respectively, washed with distilled water (4 °C). The residues were extracted with 0.5 M acetic acid (1 g of skin per 20 ml of 0.5 M acetic acid) for 24 h. Then the sample was filtered with a double layer of gauze .The viscous solution was centrifuged at 4000*g* for 30 min . The residues of filtration and centrifugation were mixed and re-extracted with 0.5 M acetic acid (1 g of residue per 20 ml acetic acid) for 24 h, then centrifuged under the same conditions. The supernatants were combined and salted out by adding NaCl to a final concentration of 0.9 M. The precipitated collagen was separated by centrifugation at 4000*g*, re-dissolved in 0.5 M acetic acid and precipitated with NaCl again. The resultant precipitate was dialyzed against 0.5 M acetic acid, 0.1 M acetic acid, distilled water and lyophilized. The residues of filtration and centrifugation were suspended in 0.5 M acetic acid and digested with 0.1% pepsin (w/v) for 72 h at 4 °C. The pepsin-soluble collagen was obtained by the same method to acid-soluble collagen.

2.3. Amino acid analysis

Collagen samples were hydrolyzed in 6 M HCl at 110 °C for 22 h, and the hydrolysates were analyzed by an amino acid analyzer (HP1100, Agilent).

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

SDS–PAGE was performed as described by Laemmli (1970) using a 7.5% resolving gel and 4% stacking gel. The samples (4 μl) and standard sample (pig type I collagen) were applied and subjected to electrophoresis. After electrophoresis, the gel was stained with Coomassie Brilliant blue R-250.

2.5. Determination of denaturation temperature

A Ubbelohde Viscometer containing a solution of 0.03% collagen in 0.1 M acetic acid was immersed in a water bath at temperatures of 20–46 °C.Viscosities of collagen solution were measured at temperature intervals of 2 °C. Fractional Viscosities were computed for each temperature as follows:

Relative viscosity $= \eta_r =$ flow time of sample/flow time of control (0.1 M acetic acid)

Specific viscosity $= \eta_{sp} = (\eta - \eta_0)/\eta_0 = \eta_r - 1$

The denaturation temperature was determined as the temperature of which the change in viscosity was half completed.

2.6. Fourier transform infrared spectroscopy

FTIR spectra were obtained by a Nicolet Nexus FT-IR spectrometer (Thermo Electron Corporation).

2.7. UV–Vis spectra

The UV–Vis adsorption spectra of the acid-solubilized collagen were obtained using a Shimadzu spectrophotometer (UV-240).

3. Results and discussion

3.1. Collagen

The skin of channel catfish was only partially solubilized in 0.5 M acetic acid after 24 h. Therefore, the residues were re-extracted with pepsin. and the collagen was easily solubilized by the limited pepsin proteolysis. In this way, the skin of channel catfish was similar to the bovine and porcine skins, cuttlefish outer skin (Nagai, Yamashita, Taniguchi, Kanamori, & Suzuki, 2001) and ocellate puffer fish skin (Nagai et al., 2001). The ASC and PSC were 25.8% and 38.4%, respectively. This indicated that the skin of channel catfish can be a useful source of collagen.

3.2. Electrophoresis

The acid-soluble and pepsin-soluble collagen from the skin of channel catfish were examined by SDS–PAGE using a 7.5% resolving gel (Fig. 1). There were two distinct species for their different positions in mobility in the α region (lane 1, lane 3 and lane 4). This showed that ASC and PSC existed as trimers consisting of two distinct α chains (α_1 and α_2) which is similar to the porcine type I collagen (lane 2) with two distinct α chains. The electrophoretic mobility position of α_1 was different from that of porcine skin collagen, whereas α_2 migration was analogous to the α_2 of porcine skin collagen. This demonstrated that the molecular weight of the α_1 was smaller than the α_1 of porcine skin collagen and the molecular weight of the α_2 was similar to porcine skin collagen's α_2.