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Isolation of collagen from fish waste material — skin, bone and fins

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Abstract

In an investigation into making more effective use of under-utilized resources, type I collagen was prepared from fish skin, bone and fin, respectively. As a result, the yields of these collagens were as follows: (1) skin collagen, 51.4% (Japanese sea-bass), 49.8% (chub mackerel) and 50.1% (bullhead shark), respectively; (2) bone collagen, 42.3% (skipjack tuna), 40.7% (Japanese sea-bass), 53.6% (ayu), 40.1% (yellow sea bream) and 43.5% (horse mackerel), respectively; (3) fin collagen, 5.2% (Japanese sea-bass acid-soluble collagen) and 36.4% (Japanese sea-bass acid-insoluble collagen), on the basis of lyophilized dry weight. The denaturation temperatures of these collagens were as follows: skin collagen (25.0–26.5°C), bone collagen (29.5–30.0°C) and fin collagen (28.0–29.1°C), respectively. These values were lower about 7–12°C than that of porcine skin collagen. This report indicates that these fish waste materials have potential in supplementing the skin of land vertebrates as a source of collagen.

Keywords: Collagen; Under-utilized fish waste; Yield; Denaturation temperature

1. Introduction

A great amount of food has been dumped as commercial and domestic waste. Although there is an attempt to decrease the waste in the world, the quantity of the waste produced is increasing annually. Recently, there has been much interest in investigating possible means of making more effective use of under-utilized resources and industrial wastes.

The Japanese consume a wide range of fish species daily. Fish is widely eaten as sliced raw fresh *sashimi*, which, for its preparation, requires the removal of skin, bones and fins. Moreover, great quantities of these wastes are also produced in fish shops and fish-processing factories. Although fish fins are eaten as a fried food, *karaage*, at times all of these may be dumped as waste. If these wastes are dumped they may cause pollutions and emit an offensive odor. Although the nutritional values of fish skin, bone and fin are fairly high, these useful resources may be wasted except for some used in fish-meal manufacture.

Although world fish stocks are suffering from over-fishing, three-fourths of the earth is sea, so there is a great deal of fish.

As a first stage of the study of unutilized resources, we have reported the preparation and characterization of edible jellyfish exumbrella collagen (Nagai et al., 1999). Particularly, however, little information is available on collagen in calcified tissues such as fin, scale and bone (Kimura, Miyauchi & Uchida, 1991; Kimura, Omura, Ishida & Shirai, 1993; Nomura, Sakai, Ishii & Shirai, 1996; Omura, Urano & Kimura, 1996). In this paper, we report the preparation and thermal properties of collagens obtained from fish skin, bones and fins. These have potential use as alternatives to mammilian collagen in foods, cosmetics and biomedical materials.

2. Materials and methods

2.1. Fish species

Skipjack tuna *Katsuwonus pelamis*, Japanese sea-bass *Lateolabrax japonicus*, ayu *Plecoglossus altivelis*, yellow

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sea bream *Dentex tumifrons*, chub mackerel *Scomber japonicus*, bullhead shark *Heterodontus japonicus* and horse mackerel *Trachurus japonicus* were obtained from a wholesale market in Shimonoseki. These skins, bones and fins were removed, cut into small pieces, and stored at −25°C until used.

2.2. Preparation of collagen from skin, bone and fin

All the preparative procedures were performed at 4°C. The skin, bones and fins were extracted with 0.1 N NaOH to remove non-collagenous proteins, then washed with distilled water and lyophilized.

2.2.1. Skin collagen

The skin was extracted fat with 10% butyl alcohol for 1 day, washed with distilled water and lyophilized. The insoluble matter was extracted with 0.5 M acetic acid for 3 days, and the extract centrifuged at 20,000×*g* for 1 h. The residue was re-extracted with the same solution for 2 days, and the extract was centrifuged in the same conditions. Each viscous solution was mixed and salted out by adding NaCl to a final concentration of 0.9 M, followed by precipitation of the collagen by addition of NaCl (final concentration of 2.6 M) at neutral pH (in 0.05 M Tris–HCl, pH 7.5). The resultant precipitate was obtained by centrifugation at 20,000×*g* for 1 h and dissolved in 0.5 M acetic acid, dialyzed against 0.1 M acetic acid, distilled water, and then lyophilized.

2.2.2. Bone collagen

The insoluble bone was decalcified with 0.5 M ethylenediaminetetraacetic acid (EDTA) (pH 7.4) for 5 days by changing the EDTA solution once a day. After washing the residue with distilled water, fat was removed with 10% butyl alcohol. The residue was then washed with distilled water and lyophilized. Following procedures were performed essentially as described for skin.

2.2.3. Fin collagen

The insoluble matter was extracted with 0.5 M acetic acid for 3 days, and the extract was centrifuged at 20,000×*g* for 1 h. The matter was separated into two fractions, acid-soluble and acid-insoluble. The collagen in the acid-soluble fraction was salted out by adding NaCl to a final concentration of 0.8 M, followed by a further precipitation of the collagen by addition of NaCl (final concentration of 2.5 M) at neutral pH (in 0.05 M Tris–HCl, pH 7.5). The resultant precipitate was obtained by centrifugation at 20,000×*g* for 1 h and dissolved in 0.5 M acetic acid, dialyzed against 0.1 M acetic acid, and then lyophilized (acid-soluble collagen; ASC). On the other hand, the acid-insoluble fraction was washed with distilled water and decalcified with 0.5 M EDTA (pH 7.4) for 5 days by changing the EDTA solution once a day. After the resultant matter was washed with distilled water, fat was removed with 10% butyl alcohol for 1 day, washed with distilled water and lyophilized. Following procedures were performed essentially as described for skin. The resultant precipitate was obtained by centrifugation at 20,000×*g* for 1 h and dissolved in 0.5 M acetic acid, dialyzed against 0.1 M acetic acid, and lyophilized (acid-insoluble collagen; AIC).

2.3. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed by the method of Weber and Osborn (1969). The collagen sample was dissolved in 0.02 M sodium phosphate (pH 7.2) containing 1% SDS and 3.5 M urea. Electrophoresis was performed on 3.5% gels in 0.1 M phosphate buffer (pH 7.2) containing 0.1% SDS.

2.4. Determination of denaturation temperature

The denaturation temperature was measured by the method of Nagai et al. (1999). The thermal denaturation curve was obtained by measuring its viscosity at several temperatures. Five milliliters of 0.03% collagen solution in 0.1 M acetic acid were used for viscosity measurements. The denaturation temperature, T_d, was determined as the temperature that the change in viscosity was half completed.

3. Results and discussion

3.1. Skin collagen

Skin collagen was prepared from Japanese sea-bass, chub mackerel and bullhead shark. The skins of these fishes were not completely solubilized with 0.5 M acetic acid for 3 days. So the residues were re-extracted with the same solution for a further 2 days. All residues were then solubilized and highly viscous solutions were obtained. The collagen was precipitated by the addition of solid NaCl to a final concentration of 0.9 M in 0.5 M acetic acid and of 2.6 M in 0.05 M Tris–HCl (pH 7.5). The yields of these collagens were very high and these values were about 51.4% (Japanese sea-bass), 49.8% (chub mackerel), and 50.1% (bullhead shark), respectively, on the basis of lyophilized dry weight. When the subunit compositions of these collagens were examined by SDS-PAGE, Japanese sea-bass and bullhead shark collagens were shown to comprise at least two different α chains, α1 and α2 (Fig. 1). Although these collagens contained an α2 chain, the amount was very small. Even if an α3 chain was present in these collagens, it was not separated from the corresponding α1 chain under these

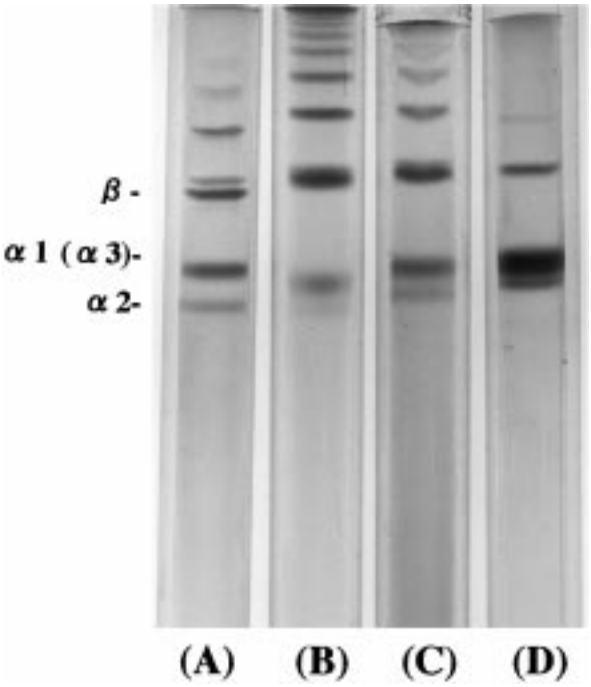

Fig. 1. SDS-polyacrylamide gel electrophoresis of porcine skin type I collagen and fish skin collagen on 3.5% gels containing 3.5 M urea: (A) porcine; (B) Japanese sea-bass; (C) bullhead shark; (D) chub mackerel.

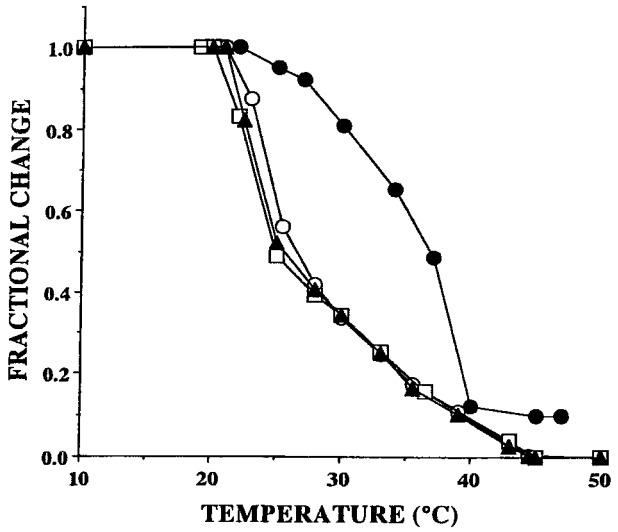

Fig. 2. Thermal denaturation curve of some fish skin collagen solution as measured by viscosity in 0.1 M acetic acid. The incubation time at each temperature was 30 min. Collagen concentration: 0.03%. ●: porcine; ○: Japanese sea-bass; □: bullhead shark; ▲: chub mackerel.

electrophoretic conditions. On the other hand, chub mackerel collagen showed only a single α band, α1. A great amount of the β chain was obtained in all these collagens.

The denaturation temperature was determined by viscosity measurement. In comparison, T_d of porcine skin collagen was measured under the same conditions. As a result, T_d of porcine collagen was about 37°C. On the other hand, T_d*s* of these skin collagens were fairly low and these were about 26.5°C (Japanese sea-bass), 25.6°C (chub mackerel), and 25.0°C (bullhead shark), respectively (Fig. 2). These values were lower about 10.0°C than that of porcine skin collagen. It is known that collagen stability is correlated with environmental and body temperature (Rigby, 1968). Moreover, T_d of collagen from cold-water fish is lower than that of warm-water fish (Takahashi & Yokoyama, 1954). The habitable temperatures of fishes used in this study were relatively similar. So differences of T_d among these fishes were not observed.

3.2. Bone collagen

Bone collagen was prepared from skipjack, Japanese sea-bass, ayu, yellow sea bream and horse mackerel. These bones were decalcified with 0.5 M EDTA and were solubilized easily with 0.5 M acetic acid. The collagen was extracted as a highly viscous solution. The collagen in 0.5 M acetic acid was precipitated by addition of solid NaCl to a final concentration of 0.9 M and the collagen was precipitated by addition of NaCl (final concentration of 2.5 M) in 0.05 M Tris–HCl (pH 7.5). The yields of collagens were very high and were as follows: skipjack tuna, 42.3%, Japanese sea bass, 40.7%, ayu, 53.6%, yellow sea bream, 40.1%, and horse mackerel, 43.5%, respectively, on the basis of lyophilized dry weight. When these collagen samples were examined by SDS-PAGE, Japanese sea-bass, skipjack tuna, and ayu collagens were shown to comprise at least two different α chains, α1 and α2 (Fig. 3), and Japanese sea bass was rich in inter- and intra-molecular crosslinked components. In electrophoretic mobility, these α chains were closely comparable with porcine skin α chains. That is, these α chains are closely related to one another in this primary structure. Under these experimental conditions, in bone collagen, an α3 chain was not observed. On the other hand, Fig. 4 showed the electrophoretic patterns of yellow sea bream and horse mackerel collagens. These collagens showed only a single α band and it seemed that this band was α1. These collagens, also, were rich in inter- and intra-molecular crosslinked components. Although these collagens contained an α2 chain, its content was very low.

Thermal denaturation curves of fish bone and porcine skin collagens are compared in Fig. 5. The Japanese sea-bass collagen had a T_d of 30°C, considerably lower (about 7°C) than that of porcine skin collagen (T_d, 37°C). T_d of skipjack tuna and ayu was 29.7°C. Moreover, yellow sea bream and horse mackerel collagens had a T_d of 29.5°C. The Japanese sea-bass collagen had the highest T_d among these fish. It is known that about 30% of

bone components is collagen and the remainder is inorganic substances such as calcium, phosphorus, sulfur, and nitrogen. It was found that these bones contained a large amount of collagen.

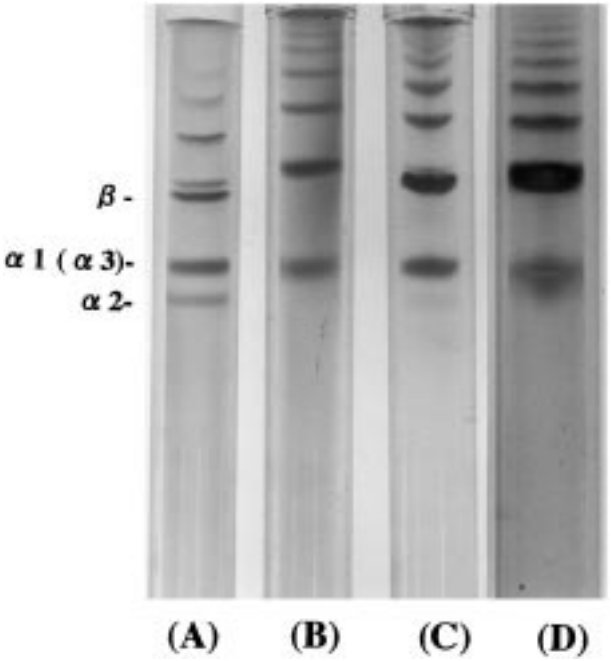

Fig. 3. SDS-polyacrylamide gel electrophoresis of porcine skin type I collagen and some fish bone collagen on 3.5% gels containing 3.5 M urea: (A) porcine skin; (B) Japanese sea-bass; (C) skipjack tuna; (D) ayu.

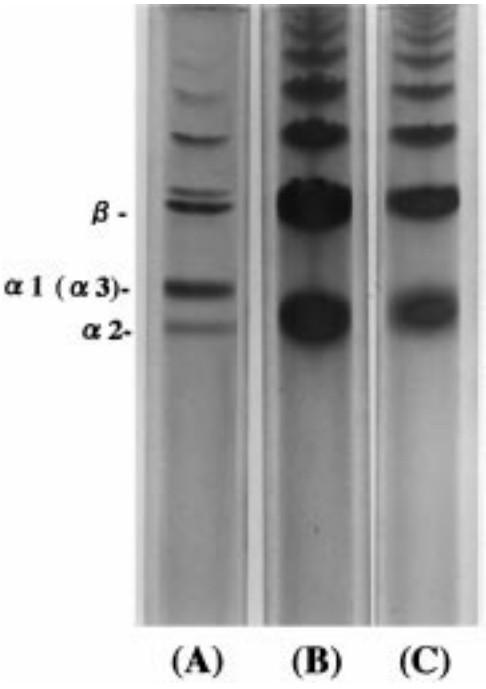

Fig. 4. SDS-polyacrylamide gel electrophoresis of porcine skin type I collagen and some fish bone collagen on 3.5% gels containing 3.5 M urea: (A) porcine skin; (B) horse mackerel; (C) yellow sea bream.

3.3. Fin collagen

ASC and AIC were extracted from Japanese sea-bass caudal fin. The yield of ASC was very low, and its value was about 5.2%. During the extraction with 0.5 M acetic acid, the collagen was hardly solubilized. However, after decalcification with 0.5 M EDTA, it was easily solubilized with 0.5 M acetic acid. The collagen

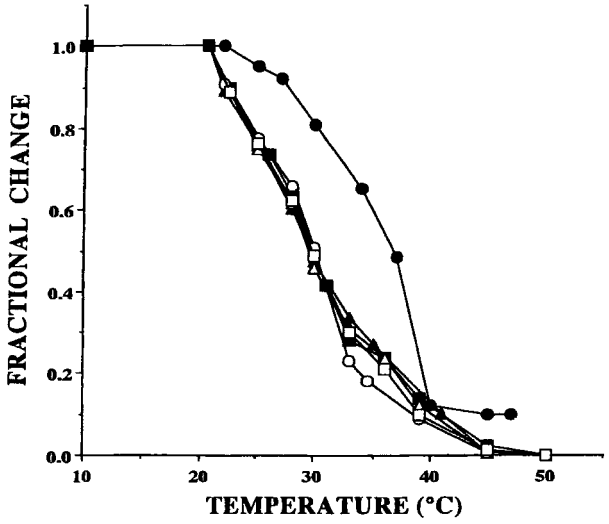

Fig. 5. Thermal denaturation curve of fish bone collagen solution as measured by viscosity in 0.1 M acetic acid. The incubation time at each temperature was 30 min. Collagen concentration: 0.03%; ●: porcine skin, ▲: Japanese sea-bass, ■: skipjack tuna, ○: ayu, △: yellow sea bream, □: horse mackerel.

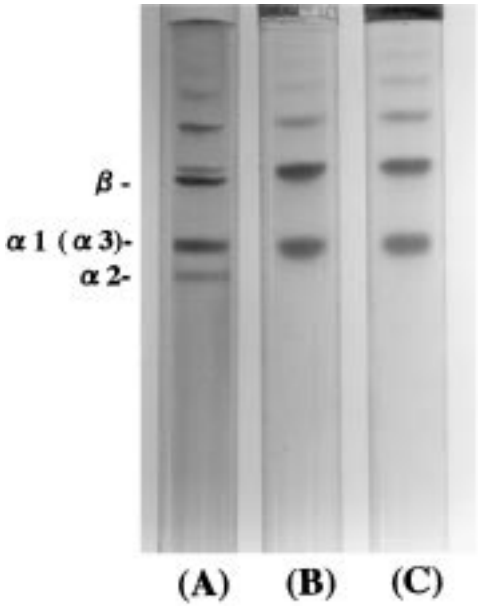

Fig. 6. SDS-polyacrylamide gel electrophoresis of porcine skin type I collagen and Japanese sea-bass caudal fin collagen on 3.5% gels containing 3.5 M urea: (A) porcine skin; (B) Japanese sea-bass caudal fin acid-soluble collagen; (C) Japanese sea-bass caudal fin acid-insoluble collagen.

was extracted as a highly viscous solution. AIC was precipitated by addition of solid NaCl to a final concentration of 0.8 M in 0.5 M acetic acid and of 2.5 M in 0.05 M Tris–HCl (pH 7.5). The yield was fairly high and about 36.4%. When examined by SDS-PAGE, these collagens showed different chains such as α, β, and γ, and these were poor in inter- and intra-molecular crosslinked components (Fig. 6). These collagens showed only a single α band, and it seemed that this band was α1. It seemed that, although these collagens contained an α2 chain, there was little of it.

The T_ds of these collagens were determined. ASC had a T_d of 28.0°C that was lower by about 9°C than that of porcine collagen (data not shown). AIC had a T_d of 29.1°C. This value was lower by about 8°C than that of porcine collagen (data not shown).

In this study, we have described the preparation of skin, bone and fin collagens and the thermal properties of these collagens. It was found that a great amount of collagen could be obtained from fish skin, bone and fin. Although fish skin, bone and fin are dumped as waste, the yield of collagen from them is very high (about 36–54%). Thus far, the industrial use of collagen has been limited to vertebrate collagen, but fish skin, bone and fin clearly have potential as alternative sources of collagen. If we can improve the thermal stability of fish collagen, the industrial use of fish skin, bone and fin collagens could have potential in various fields. This report is only one of many studies into making better use of under-utilized resources.

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