

Isolation of Collagen from Sailfin Sandfish Skins as By-product for Use in Some Industrial Purposes

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ABSTRACT

The objective of this study was to isolate and characterize collagen from sailfin sandfish skins as an useful resource for potential in commercial applications. The fats and pigments of the skins could easily be removed by cold acetone treatment. High-purity, pure-white, and odorless collagen, with a chain composition of $\alpha 1\alpha 2\alpha 3$, was successfully obtained with high-yield approximately 31.0 % on dry skin's weight basis. Denaturation temperature was relatively high, approximately 30 °C as well as that of bovine Achilles tendon collagen, although sailfin sandfish lived in the Japan Sea where the water temperature was low. ATR-FTIR analysis showed that the secondary structure was different from that of bovine Achilles tendon collagen. The finding suggests that collagen from sailfin sandfish waste skins may be applied not only to food products and nutraceuticals as materials of edible sausage casings and gelatin but also to cosmetics, pharmaceuticals, and advanced biomedical applications as peptides.

Keywords: Marine collagen, Acetone treatment, Characterization, Effective utilization, High-yield, Waste material

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INTRODUCTION

Collagen is present in almost all tissues of animals, such as blood vessels, bones, cartilages, ligaments, skins, and tendons and is the most abundant protein in mammals (Arumugam et al., 2018; Liu et al., 2012). It has been widely used as foods, cosmetics, pharmaceutical, and biomedical fields due to its excellent biocompatibility, high tensile strength and water holding property, and weaker antigenicity (Fu et al., 2018; Hu et al., 2017; Venkatesan et al., 2017). Traditionally, collagen, gelatin or these hydrolysates for industrial use have been prepared from skins and bones of land-based animals, mainly porcine and bovine animals. However, because

of risk of diseases such as transmissible spongiform encephalopathy, bovine spongiform encephalopathy, swine influenzas, foot-and-mouth disease (Bhagwat and Dandge, 2016), or dietary restriction for religious reasons as the Hindu, Jews, and Muslims who made up 38.4 % of global population (Hackett and Grim, 2012), there has been a strong desire and acquisition of collagens from safer alternative sources.

The aquatic organisms are one of the important food resources for human consumption. However, a large quantity of non-edible portions is generated at home and in fish shops and processing factories. These marine by-

products account for approximately 20-80 % of the body weight depending on the difference in processing process and the type of fish (Ghaly et al., 2013). These by-products may be wasted except for the production of animal feeds, fertilizers, and pet foods.

Sailfin sandfish (*Arctoscopus japonicus*) is a deep-sea fish that mainly lives in the Northwest Pacific ocean, particularly, in the Japan Sea, the Sea of Okhotsk, the Kuril Islands, and the Kamchatka Peninsula. According to Standard Tables of Food Composition in Japan 2020 (Kagawa, 2020), the rate of disposal parts on sailfin sandfish, such as skins and bones, is fairly high at approximately 60 %. Interestingly, sailfin sandfish have no scales among fish species. Therefore, collagen can be extracted from the skins without the removal of scales. Until now, there is no literature on sailfin sandfish collagen. The present study aims to isolate collagen from sailfin sandfish waste skins as a potential by-product to elucidate the physicochemical properties for industrial purposes.

MATERIALS AND METHODS

Materials

Fresh sailfin sandfish was obtained from Yamagata Fisheries Cooperative Association Yura Branch (Yamagata, Japan). All chemicals were of analytical grade.

Proximate Composition of Raw Skins

The moisture content was measured using a Moisture Determination Balance (FD-600; Kett Electric Laboratory, Tokyo, Japan). The crude proteins were analyzed by the Kjeldahl method using a conversion factor of 6.25. The measurements of crude lipids were performed by chloroform-methanol extraction. The crude ashes were investigated using a furnace (AMI-II; Nitto Kagaku Co., Ltd., Aichi, Japan). Carbohydrates were calculated by difference. Each assay was repeated 3 times independently, and the results were reported as means.

Collagen isolated from the Skins of Fresh sailfin sandfish

All preparative procedures were performed at 4 °C. The skins were removed from the fish, cut into small pieces (0.5 x 0.5 cm), and extracted with 10 volumes of 0.1 M NaOH solution for 2 days by changing the solution twice a day to remove the non-collagenous proteins. These were squeezed using the cheesecloth and were then washed with distilled water for 2 days by changing the solution. These were squeezed, and the residue was gently stirred with 5 volumes of cold acetone for 2 days by changing the solution twice a day to remove fats and pigments. The dried matter was gently stirred with 10

volumes of 0.5 M acetic acid for 2 days to extract the collagen. The viscous solution obtained was centrifuged at 50,000 x g for 1 h. To purify the collagen, the supernatants were pooled, and the solution was added NaCl to a final concentration of 0.9 M NaCl, followed by precipitation with 2.2 M NaCl in 0.05 M Tris-HCl buffer (pH 7.5). These were centrifuged at 23,000 x g for 30 min, and the precipitates were dissolved in a minimum volume of 0.5 M acetic acid solution. These were dialyzed using a dialysis membrane for Biochem (Wako Pure Chemical Industries, Ltd., Osaka, Japan) to remove acetic acid against 100 volumes of distilled water for 2 days by changing the solution twice a day and then lyophilized.

Color Analysis

The color of collagen was analyzed using a colorimeter (NR-11A; Nippon Denshoku Industries Co., Ltd., Tokyo, Japan) with illuminant D65 calibrated to black and white standards. The CIE $L^*a^*b^*$ system was used. The analysis was repeated 10 times independently, and the results were reported as means.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed using 7.5 % gel as described previously (Nagai et al., 2015). Molecular weight markers from Nacalai tesque Inc. (Kyoto, Japan) were used as marker proteins. These consists of myosin from rabbit muscle (molecular weight of 200 kDa), β -galactosidase from *Escherichia coli* (116.25 kDa), albumin from bovine serum (66.2 kDa), ovalbumin from chicken egg white (45 kDa), carbonic anhydrase from bovine erythrocytes (31 kDa), trypsin inhibitor from soybean (21.5 kDa), lysozyme from chicken egg white (14.4 kDa), and aprotinin from bovine lung (6.5 kDa).

Peptide Mapping

The peptide mapping was performed as described previously (Nagai et al., 2015). Molecular weight markers were used as mentioned above.

Ultraviolet Absorption Spectrum

Collagen was dissolved in 5 volumes of 0.5 M acetic acid for a day at 4 °C, and then the solution was centrifuged at 50,000 x g for 1 h at 4 °C. The supernatants were used for analysis of ultraviolet absorption spectrum. Before the analysis, baseline correction was performed using 0.5 M acetic acid.

Amino Acid Composition

Collagen was hydrolyzed in 1000 volumes of 6 M HCl for 24 h at 110 °C, and then the hydrolysates were analyzed on a JASCO liquid-chromatography system by on-line



Figure 1. Lyophilized collagen from sailfin sandfish skins.

precolumn derivatization with *o*-phthalaldehyde.

Subunit Composition

The subunit components of collagen were separated as described previously (Nagai et al., 2015). The absorbance of the components was measured at 230 nm, and the fractions indicated by the numbers were applied to SDS-PAGE.

Denaturation Temperature (Td)

The viscosity of 0.03 % (w/v) collagen solution in 0.1 M acetic acid was measured using a Canon-Fenske type viscometer with an average shear gradient of 400 sec⁻¹. The solution in a viscometer was incubated for 30 min at 10 °C in a water bath, and then the viscosity was determined. The temperature was raised stepwise to 55 °C. Each point is the mean of six determinations. The Tds were calculated as described previously (Nagai et al., 2015).

Attenuated Total Reflectance (ATR) -Fourier Transform Infrared (FTIR) Spectroscopy

ATR-FTIR collagen spectra were obtained over the range of 4,000-650 cm⁻¹ at 4 cm⁻¹ resolution by coupling ATR accessory (ATR PRO410-S: JASCO Co., Tokyo, Japan) to a JASCO FT/IR-4100 spectrometer (temperature: 20 °C, relative humidity: 40 %). The spectra were also analyzed using a JASCO IR Protein Secondary Structure Analysis Program.

RESULTS AND DISCUSSION

The proximate composition of raw skins was determined

as follows: water (65.1 %), crude proteins (27.8 %), crude lipids (6.1 %), carbohydrates (0 %), and crude ashes (1.0 %), respectively.

Collagen extraction

The skins were treated for removal of the non-collagenous proteins, and the fats and pigments were easily removed by cold acetone treatment. Collagen was completely solubilized with acetic acid, and the yield was 31.0 % on dry skin's weight basis (3.8 % on raw skin's weight basis). Lyophilized collagen (Figure 1) was pure-white ($L^* = 96.28$, $a^* = -0.03$, $b^* = 1.14$) and odorless. Nagai and co-workers isolated collagens from marine organisms. The yields of acid-soluble collagens (ASCs) from bullhead shark, chub mackerel, and Japanese sea bass skins were in the range of 44.7-51.4 % (Nagai and Suzuki, 2000a). In contrast, the yields of pepsin-solubilized collagens (PSCs) on dry weight basis were as follows: diamondback squid outer skins, 35.6 % (Nagai, 2004); ocellate pufferfish skins, 44.7 % (Nagai and Suzuki, 2002b), paper nautilus outer skins, 50 % (Nagai and Suzuki, 2002a), and *Sepia lycidas* outer skins, 35.0 % (Nagai et al., 2001), respectively. Moreover, Nagai et al. (1999, 2000, 2008) prepared PSCs from edible jellyfish exumbrella, rhizostomous jellyfish mesogloea, and common minke whale *unesu* by limited pepsin digestion. These yields were 46.4 % and 35.2 % on dry weight basis, and 28.4 % on raw weight basis, respectively. Kittiphattanabawon et al. (2015) prepared ASCs and PSCs from clown featherback skins. These yields were 27.64 % and 44.63 %, respectively, on dry weight basis. Saveboworn et al. (2017) reported the yield (64.2 % on dry weight basis) of ASC from rohu skin. Thus, collagens were obtained with high yields from aquatic organisms, including sailfin sandfish as reported in the present study. Recently, Song et al. (2018)

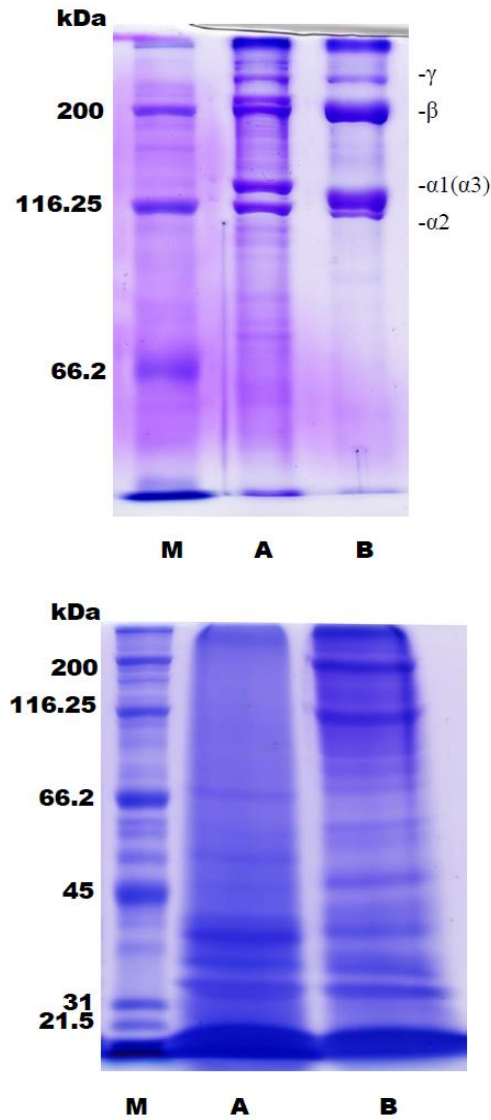


Figure 2. SDS-PAGE profile (upper figure) of collagens and peptide mapping (lower figure) of collagen hydrolysates digested by lysyl endopeptidase. (M) Molecular marker; (A) bovine Achilles tendon collagen; (B) sailfin sandfish skin collagen.

developed a mass-production system of collagen from flatfish skins using ultrasound extraction equipment. The yields (31.3 % and 46.2 %, respectively) of collagens after 1.5 h and 3 h ultrasound treatments were 1.5 and 2 times higher in comparison with the conventional method. These methods could shorten the extraction time, reduce the amount of acid, and increase the yield of collagen. Khong et al. (2018) tried to extract collagen from jellyfish bell and oral arms by the sonication for 15 min followed by rigorous mixing for 1 h. The yields increased 2-7 folds in comparison with the conventional methods such as acetic acid extraction and pepsin digestion. Moreover, collagen with significantly high protein contents, hydroxyproline contents, and L^* values

and also with markedly low ash contents and b^* values were obtained. Thus, the ultrasound extraction method or the sonication method may possibly increase the efficiency, reduction of time and cost (reduction of the amount of acid) for collagen extraction.

SDS-PAGE Analysis

Sailfin sandfish skin collagen was analyzed by SDS-PAGE. Collagen was highly purified, and two distinct protein bands, $\alpha1$ chain with molecular weight (MW) of 118 kDa and $\alpha2$ chain with MW of 112 kDa, were detected (Figure 2). The existence of $\alpha3$ chain could not identify. A large amount of β chain and γ chain as cross-

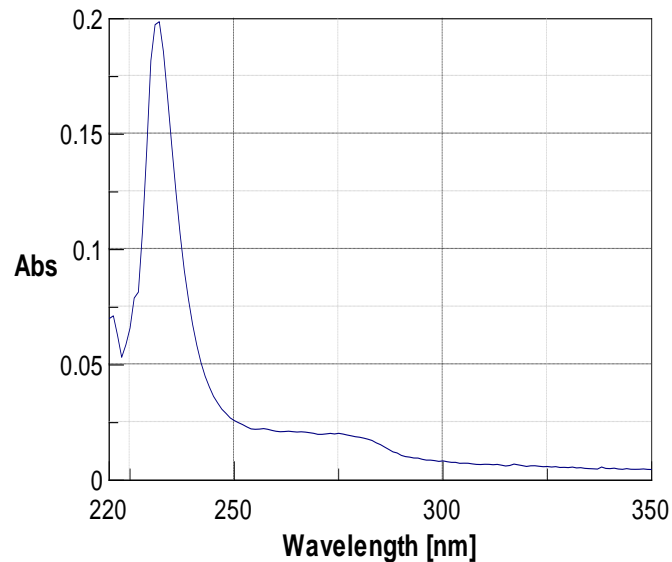


Figure 3. Ultraviolet spectrum of sailfin sandfish skin collagen.

linked α chains with MW of above 200 kDa were detected. The results suggested that sailfin sandfish skin collagen was typical type I collagen with a chain composition of $(\alpha 1)_2\alpha 2$ heterotrimer or of $\alpha 1\alpha 2\alpha 3$ heterotrimer. In contrast, the position of α chains on bovine Achilles tendon collagen (Nacalai tesque Inc., Kyoto, Japan) was different from that on sailfin sandfish skin collagen (Figure 2). However, β chain on bovine Achilles tendon collagen showed the same MW as that on sailfin sandfish skin collagen.

Arumugam et al. (2018) prepared collagen with the subunit composition of $(\alpha 1)_2\alpha 2$ from marine waste, *Aseraggodes umbratilis* skins. In contrast, Cheng et al. (2017) isolated ASCs and PSCs from jellyfish (*Rhopilema esculentum*) mesogloea. $\alpha 2$ chains of these collagens showed high MW than $\alpha 1$ chains. Generally, $\alpha 1$ chain of type I collagen is detected in position with high MW than $\alpha 2$ chain.

Peptide Mapping

The collagens after lysyl endopeptidase digestion were applied to SDS-PAGE using 10 % gel to compare the patterns of peptide fragments on sailfin sandfish skin collagen and bovine Achilles tendon collagen. The same patterns were observed in the protein bands under MWs of 39 kDa (39, 35, and 32.5 kDa) on these collagens (Figure 2). In contrast, different fragment patterns were shown in protein bands of 47-190 kDa: MWs of dominant products from these collagens were 47, 61, 73, 78, 116, and 190 kDa (sailfin sandfish skins) and 54 and 72 kDa (bovine Achilles tendon), respectively. It suggested that sailfin sandfish skin collagen was less affected by lysyl endopeptidase digestion than bovine Achilles tendon collagen.

Ultraviolet Absorption Spectrum

The ultraviolet absorption spectrum of collagen was investigated. The distinct absorption was observed near 231 nm (λ_{max}) and 223 nm (λ_{min}), respectively (Figure 3). The UV maximum absorbance of collagen was in agreement with those of collagens as follows: channel catfish skins; 232 nm (Liu et al., 2007), largemouth longbarbel catfish skins; 233 nm (Zhang et al., 2009), and red drum fish scales; 230 nm (Chen et al., 2016). The peak around 230 nm on type I collagen was attributed to the peptide bond absorptions by $n \rightarrow \pi^*$ transitions of groups of C=O, -COOH, and CONH₂ in the polypeptide chains (Veeruraj et al., 2013). The obvious absorption at 280 nm was not detected due to the absence of tryptophan residue, as discussed below (Table 1). In addition, it was not detected in the absorption between 250 and 290 nm because of the low content of aromatic amino acids, such as phenylalanine and tyrosine (Table 1).

Amino Acid Composition

Amino acid composition of collagen as residues per 1000 total residues is shown in Table 1. Glycine (349 residues) accounted for more than one-third of total amino acids. Alanine (120 residues), proline (104 residues), hydroxyproline (75 residues), and glutamic acid (72 residues) were the typical amino acids. In contrast, the contents of tyrosine, hydroxylysine, histidine, and isoleucine were low. Cysteine and tryptophan were not detected at all. The composition on sailfin sandfish skin collagen was in agreement with those of collagens from other aquatic organisms (Ali et al., 2018; Chuaychan et al., 2015; Huang et al., 2016; Kittiphattanabawon et al., 2019; Nagai, 2004; Nagai et al., 2001, 2004, 2008; Nagai

Table 1. Amino acid composition of sailfin sandfish skin collagen (per 1000 residues).

Amino acid	Residues
Hydroxyproline	75
Hydroxylysine	5
Aspartic acid	48
Threonine	24
Serine	38
Glutamic acid	72
Proline	104
Glycine	349
Alanine	120
Valine	17
Methionine	15
Isoleucine	11
Leucine	23
Tyrosine	3
Phenylalanine	15
Lysine	25
Histidine	5
Arginine	51
Degree of hydroxylation (%)	
Proline	41.9
Lysine	16.7

and Suzuki, 2000b, 2002b; Savedboworn et al., 2017). Imino acid (proline and hydroxyproline) content is related to the stability of collagen helices: the collagens with higher imino acid contents have greater stability of the helices. The structure of collagen is maintained by limited changes of polypeptide chains on the secondary structure, by pyrrolidine rings on hydroxyproline and proline and by hydrogen bonding ability of hydroxyl groups on hydroxyproline residues. The total imino acid content in sailfin sandfish skin collagen was calculated as 179 residues (Table 1). Other researchers reported the contents on skin collagens as follows: cuttlefish (186-188 residues) (Nagai, 2004, Nagai et al., 2001), ocellate puffer (170 residues) (Nagai and Suzuki, 2002b), golden pompano (187 residues) (Weng and Wang, 2018), clown featherback (201-202 residues) (Kittiphattanabawon et al., 2015), largefin longbarbel catfish (213 residues) (Zhang et al., 2009), and walleye Pollack (184 residues) (Yan et al., 2008). The imino acid content is also associated with the inhabitation condition (Arai, 1994). In addition, the imino acids play an important role in the rheological properties as gel strength.

The hydroxylation degree of proline residues is strongly related to the thermal stabilities of collagens. The degree on sailfin sandfish skin collagen was approximately 41.9 %, which was similar to the degree of clown featherback collagen (41.3-42.1 %) (Kittiphattanabawon et al., 2015). However, it was higher than those on collagens from ocellate puffer (39.4 %) (Nagai and Suzuki, 2002b), golden pompano (38.5 %) (Weng and Wang, 2018), largefin longbarbel catfish (34.7 %) (Zhang et al., 2009), and walleye pollack (37.5 %) (Yan et al., 2008). In contrast, the degree on sailfin sandfish skin collagen was lower than those on skin collagens from cuttlefish (47.8-

47.9 %) (Nagai, 2004, Nagai et al., 2001), channel catfish (42.9 %) (Liu et al., 2007), and brown-backed toadfish (45.3 %) (Senaratne et al., 2006).

Subunit Composition

The subunit components of collagen were separated, and these fractions indicated by numbers were applied to SDS-PAGE. Two protein fractions, consisted α chain, were investigated, and these fractions were α_1 chain (number 1), α_2 chain (numbers 3 and 4), and α_3 chain (numbers 3 and 4), respectively (Figure 4). Thus, sailfin sandfish skin collagen formed heterotrimer with a chain composition of $\alpha_1\alpha_2\alpha_3$. Kittiphattanabawon et al. (2015) separated the subunit components of clown featherback skin collagen. They elucidated that this was type I collagen comprising $(\alpha_1)_2\alpha_2$ heterotrimer. In contrast, Senaratne et al (2006) investigated the chain composition of brown-backed toadfish skin collagen. It consisted of a $\alpha_1\alpha_2\alpha_3$ heterotrimer. In our previous study, the existence of α_3 chain in aquatic organisms collagens was reported in ayu bone (Nagai and Suzuki, 2000a), diamondback squid outer skin (Nagai, 2004), edible jellyfish exumbrella (Nagai et al., 1999), and paper nautilus outer skin (Nagai and Suzuki, 2002a). On the contrary, α_3 chain did not exist in collagens from cuttlefish outer skin [$(\alpha_1)_2\alpha_2$ heterotrimer] (Nagai et al., 2001) and ocellate puffer skin [$(\alpha_1)_2\alpha_2$ heterotrimer] (Nagai and Suzuki, 2002b).

Td

Td of sailfin sandfish skin collagen was calculated at 30

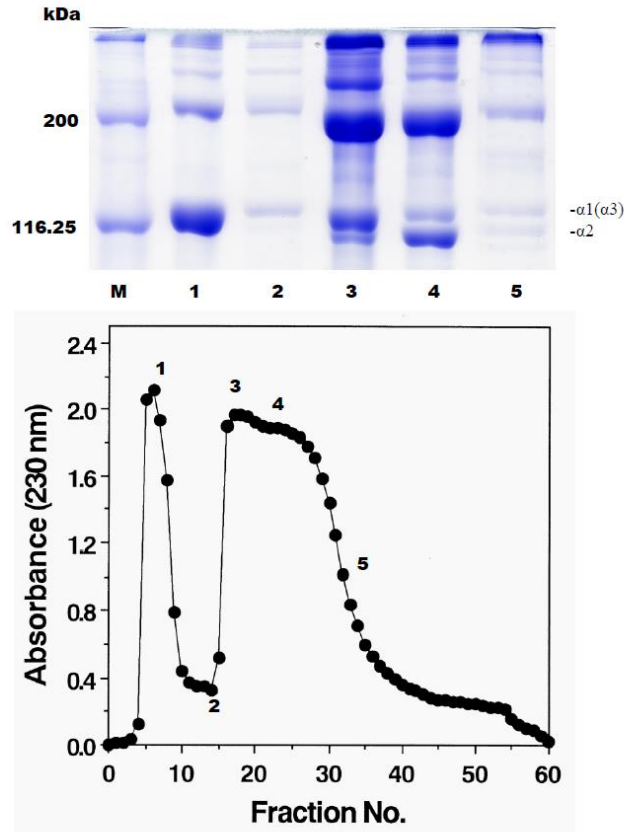


Figure 4. CM-Toyopearl 650M column chromatography of denatured sailfin sandfish skin collagen. The fractions indicated by the numbers were examined by SDS-PAGE using 7.5% gel.

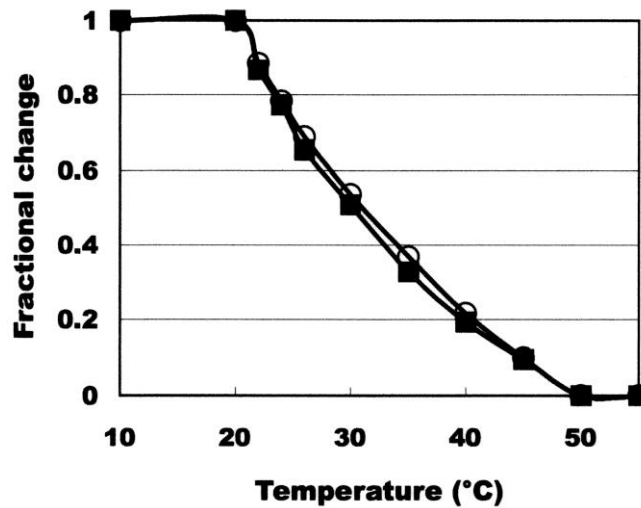


Figure 5. Thermal denaturation curves of collagens from sailfin sandfish skins and bovine Achilles tendon. The denaturation temperatures of collagens were measured by viscosity in 0.1 M acetic acid. The incubation time at each temperature was 30 min. (■) sailfin sandfish skin collagen; (○) bovine Achilles tendon collagen.

°C (Figure 5). In contrast, bovine Achilles tendon collagen showed slightly higher Td at 31 °C. In general,

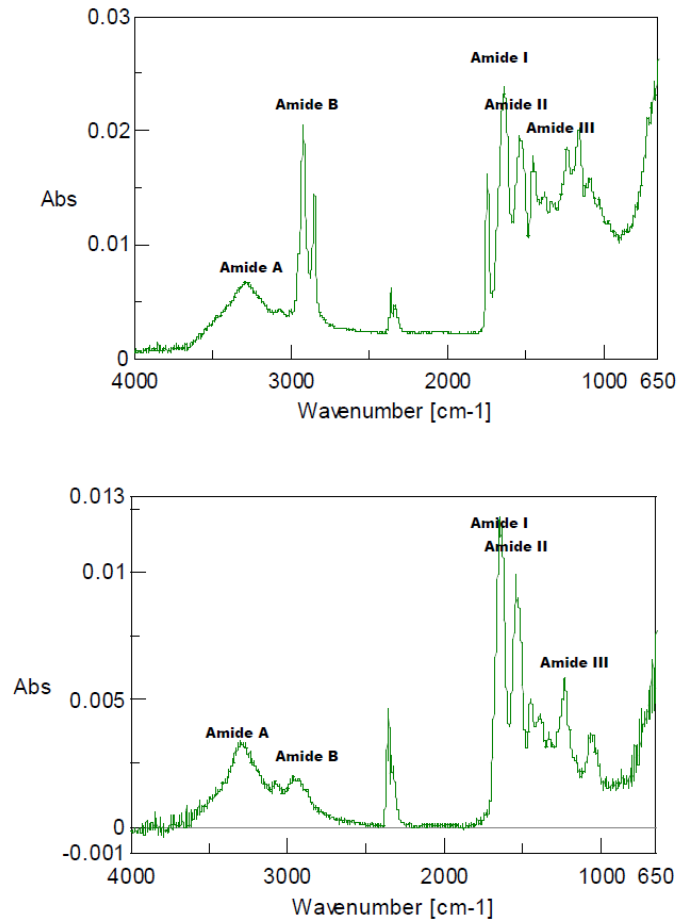


Figure 6. Fourier transform infrared spectra of collagens from bovine Achilles tendon and sailfin sandfish skins. Bovine Achilles tendon collagen (upper graph); sailfin sandfish skin collagen (lower graph).

Tds of collagens are related to the body and the environmental temperatures: Tds of aquatic organisms collagens are lower than those of land animals. Moreover, collagens from aquatic organisms live in cold water show lower Tds than those from organisms in warm water (Kimura et al., 1988). Surprisingly, sailfin sandfish skin collagen showed relatively high Td, although it lived in the Japan Sea where the water temperature was low. Other researchers elucidated Tds of collagens from aquatic organisms by viscosity measurements as follows: brown backed toadfish skin (28 °C) (Senaratne et al., 2006), deep-sea redfish skin (15.7-16.1 °C) (Wang et al., 2007), walleye pollack skin (26.4 °C) (Yan et al., 2008), and largefin longbarbel catfish skin (31.6-32.1 °C) (Zhang et al., 2009), respectively. In contrast, Nagai and co-researchers reported Tds of collagens from aquatic organisms as follows: cuttlefish outer skin (27.0-27.5 °C) (Nagai, 2004; Nagai et al., 2001), and jellyfish exumbrella and mesogloea (26.0-28.8 °C) (Nagai et al., 1999, 2000), ocellate puffer skin (28.0 °C) (Nagai and Suzuki, 2002b), octopus outer skin (27.0 °C) (Nagai and Suzuki, 2002a), respectively. Collagens with higher imino acid contents

have greater stability of the helical structures: Tds of collagens are related with the imino acid contents. Fishes adjust the hydroxyproline contents by control of the hydroxylation degree of proline residues and add the thermal stabilities according to the changes of body temperatures to its type I collagens (Kimura, 1997). However, it was not associated with Tds and imino acid contents in collagens from sailfin sandfish skins, cuttlefish outer skin (Nagai, 2004, Nagai et al., 2001), and ocellate puffer skin (Nagai and Suzuki, 2002b).

ATR-FTIR Spectroscopy Analysis

ATR-FTIR spectrum of sailfin sandfish skin collagen is shown in Figure 6. Amide A band is related to NH-stretching frequency, and free NH-stretching vibration is observed at 3400-3300 cm^{-1} . The position is shifted to low frequencies around 3300 cm^{-1} , when NH groups of peptides are coupled with hydrogen bonds. Amide A band of sailfin sandfish skin collagen was observed at 3297.68 cm^{-1} , indicating the existence of hydrogen bonds. Amide B band is associated with CH_2 -asymmetrical stretch. Sailfin sandfish skin collagen

showed an amide B band at 2962.13 cm^{-1} . Amide I band, which is a marker of peptide secondary structure, is related to stretching vibrations of C=O bond, and the vibration occurs in 1600-1700 cm^{-1} . Amide I band was observed at 1646.91 cm^{-1} , indicating C=O stretching vibration or the existence of hydrogen bonds coupled with COO⁻. Amide II band is associated with NH-bending vibration coupled with CN-stretching. It was detected at 1540.85 cm^{-1} . Moreover, amide III band was observed at 1238.08 cm^{-1} , indicating NH-bending vibration coupled with CN-stretching. These results suggested the existence of helical arrangements in sailfin sandfish skin collagen using the acetone and acetic acid extraction. In addition, C-H stretching vibration was investigated at 2364.25 cm^{-1} as well as sole fish skin collagen (Arumugan et al., 2018). Generally, the vibration occurs in between 2854 cm^{-1} and 1745 cm^{-1} . In contrast, the bands of bovine Achilles tendon collagen were detected as follows: amide A (3294.79 cm^{-1}), amide B (2926.45 cm^{-1}), amide I (1633.41 cm^{-1}), amide II (1542.77 cm^{-1}), and amide III (1238.08 cm^{-1}), respectively. Moreover, C-H stretching vibration was observed.

Next, the rate of secondary structural components was calculated. The percentage of these components on sailfin sandfish skin collagen was 20 % (α -helix), 29 % (β -sheet), 23 % (β -turn), and 25 % (others as random coil structure), respectively. On the contrary, the rate on bovine Achilles tendon collagen was as follows: 9 % (α -helix), 35 % (β -sheet), 20 % (β -turn), and 22 % (others), respectively. These results indicated that sailfin sandfish skin collagen had α -helix-rich structures in comparison with bovine Achilles tendon collagen, as sailfin sandfish skin collagens had N-terminal and C-terminal telopeptides. In contrast, the rate of β -sheet structures on bovine Achilles tendon collagen was lower than that on sailfin sandfish skin collagen. Thus, it was suggested the difference in sheet structures among these collagens.

An increase in demand for marine products is in prospect more and more with the growing of the world's population. Accordingly, an abundance of wastes or by-products is generated in fish processing factories. Effective utilization of these resources is a critical issue of the world, as the transformation of wastes or by-products to valuable materials is emerging as a strong trend to build sustainable societies. The wastes or by-products produced from fishes and shellfishes have high nutritional values due to high protein contents. Particularly, the skins contain large amounts of collagens. Therefore, the skins as wastes or by-products can reprocess into sustainable products for industrial uses. For example, due to excellent bioactivity against skin repair and regeneration, high absorbing capacity, and low odor, fish collagens can be used for the development of cosmeceuticals with high market value than mammalian collagens. Recently, it became clear that sardine scale collagen possessed anti-larval activity using *Aedes aegypti* larvae, indicating potential as a biomedical agent against yellow fever, dengue fever, and

Zika fever (Muthumari et al. 2016). In addition, fish collagens can be utilized as a resource of gelatin, which has been widely used to improve emulsifying, foaming, gelation, and water binding properties, elasticity, and viscosity of food products in food industries. Moreover, it is known that the peptides derived from marine collagen have unique functions, such as antibacterial, antihypertensive, antioxidant, anti-skin aging, and neuroprotective effects (Karayannakidis and Zotos, 2016). Thus, collagen from sailfin sandfish skins as a useful resource could be applied not only to food products or nutraceuticals as materials of edible sausage casings and gelatin but also to cosmetics, pharmaceuticals, and advanced biomedical applications as peptides in the future.

CONCLUSION

It could be concluded that cold acetone treatment was a superior method to efficiently remove the fats and pigments from sailfin sandfish skins as by-product. Pure-white and odorless collagen with relative high denaturation temperature prepared with high-yield will be a potential alternative to land-based animal collagens for further application on foods, cosmetics, pharmaceuticals, biomedical, and its related industries.

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