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Characterization of acid-soluble collagen from the skin of walleye pollock (*Theragra chalcogramma*)

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Abstract

Acid-soluble collagen (ASC) was extracted from the skin of walleye pollock (*Theragra chalcogramma*) and partially characterized. It exhibited a maximum absorbance at 220 nm, but little absorbance near to 280 nm. Amino acid composition and SDS-PAGE suggested that the collagen might be classified as type I collagen. Moreover, FTIR investigations showed the existence of helical arrangements of collagen. The denaturation temperature (T_d) and shrinkage temperature (T_s) were 24.6 °C and 47 °C, respectively, both lower than those of mammalian collagens. However, T_d of walleye pollock skin collagen was higher than that of cod skin collagen reported previously. These results indicate that walleye pollock skin is a potential source of collagen and provide the theoretical basis for further research.

Keywords: Characterization; Collagen; Thermal stability; Walleye pollock

1. Introduction

Collagen is an abundant protein in animal tissues and constitutes approximately 30% of total animal protein (Muyonga, Cole, & Duodu, 2004). It is widely distributed in skin, bones, cartilage, tendons, ligaments, blood vessels, teeth, cornea and all other organs of vertebrates (Senaratne, Park, & Kim, 2006). Collagen has a wide range of applications in leather and film industries, pharmaceutical, cosmetic and biomedical materials, and food (Kittiphattanabawon, Benjakul, Visessanguan, Nagai, & Tanaka, 2005). The highest utilization of collagen is in pharmaceutical applications including production of wound dressings, vitreous implants and as carriers for drug delivery. Moreover, collagen is used for the production of cosmetics because it has a good moisturizing property (Swatschek, Schatton, Kellermann, Muller, & Kreuter, 2002). In addition, collagen has been utilized to produce edible casings, which are needed in the meat processing industries (sausages/salami/snack sticks), and heat-denatured collagen, called gelatin, is important in food manufacturing (Senaratne et al., 2006).

For industrial purposes, the main sources of collagen are limited to those of land-based animals, such as bovine or porcine skin and bone. However, the outbreak of bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE) and the foot-and-mouth disease (FMD) crisis have resulted in anxiety among users of collagen and collagen-derived products of land animal origin (Jongjareonrak, Benjakul, Visessanguan, Nagai, & Tanaka, 2005). In addition, the collagen extracted from porcine sources cannot be used as a component of some foods, due to religious barriers. Therefore, alternative sources of collagen should be sought. Scientists have found that skin, bone, scale, fin and cartilage of freshwater and marine fish, scallop mantle (Shen, Kurihara, & Takahashi, 2007), adductor of pearl oyster (Mizuta, Miyagi, Nishimiya, & Yoshinaka, 2002) and the muscle layer of the ascidian (Mizuta, Isobe, & Yoshinaka, 2002) can be used as new sources of collagen.

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Pollock is one of the commercially important fish species in China. Approximately 400–500 thousand of tons pollock were processed annually, mainly in Shandong Province. Seafood processors generate more than 30 thousand of tons fish skins through their processing of pollock for food service per year (data were provided by Bureau of Fisheries, Ministry of Agriculture, People's Republic of China). The skin is dumped without utilization, which would cause environmental pollution and resources waste. However, 70% of the pollock skin dry matter is collagen (Zhang, Luo, Zhang, Song, & Jiang, 2003). For making effective use of the dumped skin as a collagen resource, it is necessary to obtain fundamental information about the pollock skin collagen. Therefore, the present paper describes the isolation and physicochemical properties of the collagen from walleye pollock skin.

2. Materials and methods

2.1. Fish skin preparation

Walleye pollock (*Theragra chalcogramma*) were caught from the Bering Sea by commercial fishing boat in August, 2006, stored at −18 °C, immediately after gutting, and transported to the dock in Qingdao. After arrival at a local fish processing factory, frozen fish were thawed using running water, and skins were removed and descaled manually. These skins were transported to the laboratory and stored at −20 °C until used. All other reagents used were of analytical grade.

2.2. Extraction of collagen

All procedures were performed at 4 °C, as previously described (Nagai & Suzuki, 2000) with a slight modification. The skin was extracted with 0.1 M NaOH to remove non-collagenous materials effectively and to exclude the effect of endogenous proteases on collagen (Sato, Yoshinaka, Sato, & Shimizu, 1987), then thoroughly rinsed with distilled water until a neutral pH was reached. Minced skins were slowly stirred with 0.5 M acetic acid solution for 48 h, and the extract was centrifuged at 10,000*g* for 30 min. Then the acid-soluble collagen (ASC) in the supernatant was salted out by adding NaCl to a final concentration of 0.9 M. The solution was left overnight, and the resultant precipitate, collected by centrifugation at 8000*g* for 20 min, was dissolved in 0.5 M acetic acid, dialyzed against 0.1 M acetic acid for 1 d (1:15, v/v, changed every 4 h), distilled water for 2 d (1:15, v/v, changed every 4 h), and then lyophilized.

2.3. UV–vis spectra

A collagen sample was dissolved in 0.5 M acetic acid to obtain a concentration of 2 g l^{-1}. The UV–vis adsorption spectra were recorded by a Shimadzu spectrophotometer UV-2550 (Shimadzu, Tokyo, Japan) from 190 to 400 nm at a scanning rate of 210 nm min^{-1}.

2.4. Amino acid composition

ASC samples were hydrolyzed under reduced pressure with 6 M HCl at 110 °C for 24 h, and the hydrolysates were analyzed on a Hitachi 835-50 amino acid analyzer (Hitachi, Tokyo, Japan).

2.5. Sodium dodecyl sulphate polyacylamide-gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed by the method of Laemmli (1970), using the discontinuous tris-HCl/glycine buffer system with 7.5% resolving gel and 5% stacking gel. Proteins were stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 dissolved in water, methanol and acetic acid (9:9:2, v/v/v) for 20 min, then destained using a solution containing water, methanol and acetic acid (8:1:1, v/v/v).

2.6. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were obtained from discs containing 0.2 mg lyophilized collagen and about 10 mg potassium bromide (KBr) ground together under dry conditions. The spectra were recorded using infrared spectrophotometer (Nicolet 200SXV) from 4000 to 500 cm^{-1} at a data acquisition rate of 2 cm^{-1} per point. The resulting spectra were analyzed using Omnic 6.0 software (Thermo-Nicolet, Madison, Wisconsin).

2.7. Determination of denaturation temperature

The denaturation temperature was measured from the viscosity changes, using an Ubbelohde viscometer, according to the method of Zhang et al. (2007) with some modification. Ten millilitres of 0.03% collagen solution in 0.1 M acetic acid + 0.2 M sodium acetate buffer (pH 5.0) were used for viscosity measurements. The thermal determination curve was obtained by measuring solution viscosity at several temperatures from 16 to 42 °C, and the temperature was raised stepwise and maintained for 30 min. Fractional viscosity at the given temperature was calculated by the equation:

$$\text{Fractional viscosity} = (\eta_{sp(T)} - \eta_{sp(42\,^\circ C)})/(\eta_{sp(16\,^\circ C)} - \eta_{sp(42\,^\circ C)}),$$

where η_{sp} is the specific viscosity. These fractional viscosities were plotted against the temperatures and the denaturation temperature was taken to be the temperature where fractional viscosity was 0.5.

2.8. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed on a Netzsch DSC 200PC (Netzsch, Bavaria, Germany) instrument fitted with an air cooling compressor and a liquid nitrogen cooler at ambient temperature (Cui et al.,

2007). The temperature was effectively calibrated using indium as standard. Collagen fibre was weighed (3 mg) accurately and sealed in aluminium pans (BO 6.239.2–64.502). At least triplicate samples were heated from 20 to 100 °C at a scanning rate of 2 K min^{-1}, with an empty sealed pan as a reference. The shrinkage temperature was measured at the top of the transition peak.

3. Results and discussion

3.1. UV–vis spectra

As can be seen from the UV–vis spectra (Fig. 1), the distinct absorbance of the collagen was obtained near 220 nm, which is contributed by $n \rightarrow \pi^*$ transition of C=O in the peptide bond. Generally, tyrosine and phenylalanine are sensitive chromophores and absorb UV light at 283 nm and 251 nm (Liu & Liu, 2006), where ASC has no evident absorbance. Therefore, acid-soluble collagen from walleye pollock skin well supports the property of collagen that there is absorbance at 220–230 nm, with little or no absorbance near 280 nm. Thus the protein is collagen.

3.2. Amino acid composition

The amino acid composition of the acid-soluble collagen from walleye pollock skin is shown in Table 1. Glycine was the major amino acid although it was only 19.7%, significantly lower than that of brown backed toadfish (Senaratne et al., 2006), but similar to that of Nile perch (Muyonga et al., 2004) and channel catfish (Liu, Li, & Guo, 2007). The reason that the glycine content was lower may be related to contamination by other proteins. The collagen was found to be very low in tyrosine, histidine and isoleucine, and no cystine was detected. It also consisted of imino acids (proline + hydroxyproline), which were unique amino acids found in collagen. The imino acid content was 18.4%, similar to the 18.6% of grass carp collagen (Zhang et al., 2007), but lower than the 22% of porcine skin collagen (Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003). Hydroxyproline is derived from proline by post-translational hydroxylation mediated by prolylhydroxylase (Li, Liu, Gao, & Chen, 2004). The degree of hydroxylation of proline residues in collagen from walleye pollock skin was 37.5%, similar to the 39% of ocellate puffer fish (Nagai, Araki, & Suzuki, 2002), but lower than the 43% of channel catfish (Liu et al., 2007) and 48% of cuttlefish (Nagai, Yamashita, Taniguchi, Kanamori, & Suzuki, 2001). The distribution patterns of amino acid composition, similar to that of acid-soluble collagen from the skin of channel catfish (Liu et al., 2007), indicate that ASC might be classified as type I collagen.

Table 1
Amino acid composition of acid-soluble collagen from walleye pollock skin

Amino acid	Amino acid residues/1000 total amino acid residues
Hydroxyproline	69
Aspartic acid	54
Threonine	23
Serine	66
Glutamic acid	116
Glycine	197
Alanine	104
Cystine	0
Valine	21
Methionine	24
Isoleucine	13
Leucine	27
Tyrosine	4
Phenylalanine	21
Lysine	35
Histidine	13
Arginine	98
Proline	115

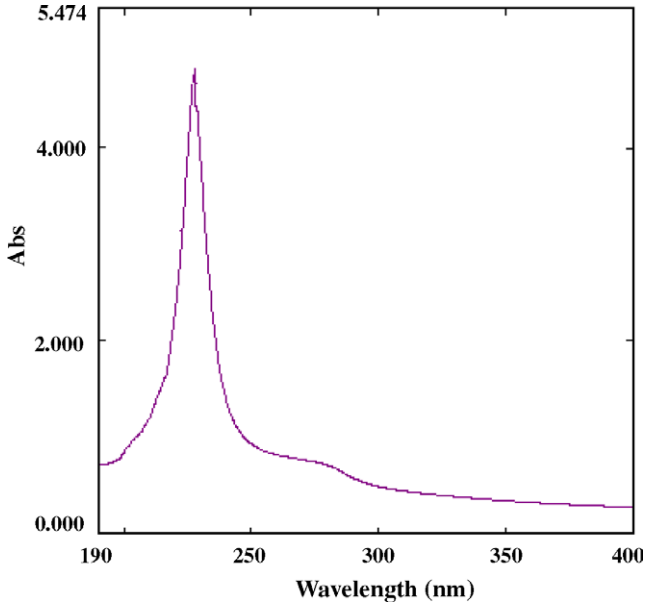

Fig. 1. UV–vis spectra of acid-soluble collagen from walleye pollock skin.

3.3. SDS-PAGE

The acid-soluble collagen from walleye pollock skin was examined by SDS-PAGE, using a 7.5% resolving gel (Fig. 2). This collagen consisted of α chains (α1 and α2 chain), which showed two distinct species varying in their mobility, and their dimer (β chain), and small amounts of γ components were also found. The existence of at least two different subunits shows that a major collagen from walleye pollock skin might be type I collagen. There was no clear difference between the electrophoretic patterns, with or without β-mercaptoethanol, suggesting absence of disulphide bonds. This is consistent with the observation that the collagen was almost devoid of sulphur-containing amino acids (Muyonga et al., 2004). From the electrophoretic patterns of collagen, we could not determine whether the collagen contained an α3 chain or not. If present, the α3 chain could not be separated under the condition employed

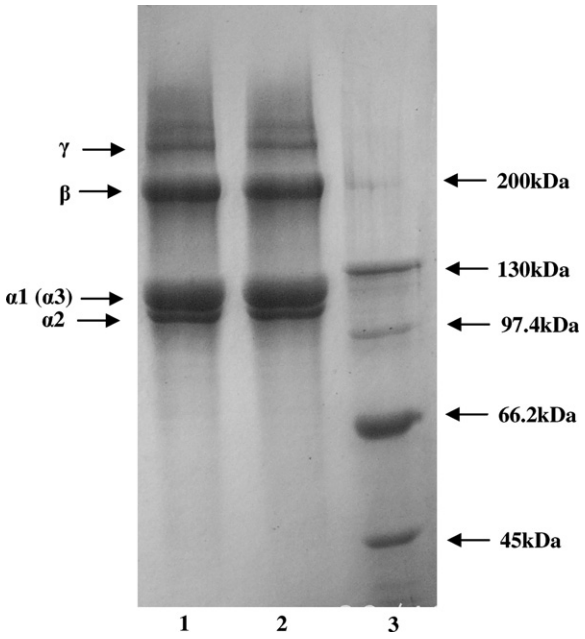

Fig. 2. SDS-PAGE patterns of acid-soluble collagen from walleye pollock skin on 7.5% gel. Lane 1: ASC (the sample solution without β-mercaptoethanol); lane 2: ASC (the sample solution with β-mercaptoethanol); lane 3: protein markers.

because α3(I) migrates electrophoretically to the same position as α1(I) (Kimura, 1992).

3.4. Fourier transform infrared spectroscopy

Fig. 3 shows the FTIR spectra of the acid-soluble collagen from walleye pollock skin, similar to that exhibited by other collagens (Muyonga et al., 2004; Liu et al., 2007). The amide A band is associated with the N–H stretching frequency. A free N–H stretching vibration occurs in the range of 3400–3440 cm^{-1}, and when the NH group of a peptide is involved in a hydrogen bond, the position is shifted to lower frequency, usually near 3300 cm^{-1} (Li et al., 2004). The amide A band of walleye pollock skin collagen was found at 3328 cm^{-1}, which shows that there were NH groups involved in hydrogen bonds. The amide B band of collagen was found at 3080 cm^{-1}, which is related to asymmetrical stretch of CH_2 (Muyonga et al., 2004).

The amide I band position was observed at 1648 cm^{-1}, which is the absorption band of C=O stretching. It is associated with the secondary structure of the protein. The absorption between the 1236 cm^{-1} (amide III) and 1452 cm^{-1} bands demonstrated the existence of helical structure (Liu et al., 2007). Therefore, the FTIR investigations show the existence of helical arrangements of walleye pollock skin collagen.

3.5. Thermal stability

Thermal stability of collagen is usually described by the denaturation temperature (T_d) in solution and the shrinkage temperature (T_s) of fibre. The temperature at which the triple helix structure of collagen in solution is disintegrated into random coils is taken as T_d (Hao & Li, 1999). Fig. 4 shows the thermal denaturation curve of acid-soluble collagen from walleye pollock skin. T_d of collagen was 24.6 °C, lower by about 12 °C than that of collagen from porcine skin (Nagai et al., 1999). The shrinkage temperature refers to the temperature at which fibre shrinks to one third of its length (Fathima, Madhan, Rao, Nair, & Ramasami, 2004). In the shrinkage process, a phase transition involving the conversion of a crystalline triple helical collagen structure to an amorphous random coil form (Fathima, Balaraman, Rao, & Nair, 2003) occurs. T_s of collagen from walleye pollock skin was 47 °C (Fig. 5), lower by about 15 °C than that of type I collagen from bovine skin (Cui et al., 2007). These results proved that the helices

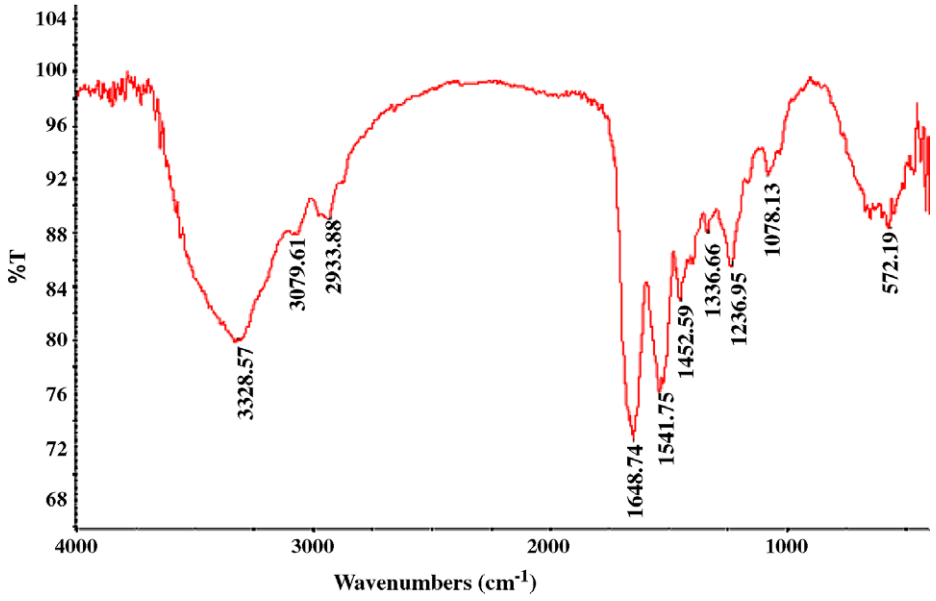

Fig. 3. Fourier transform infrared spectra of acid-soluble collagen from walleye pollock skin.

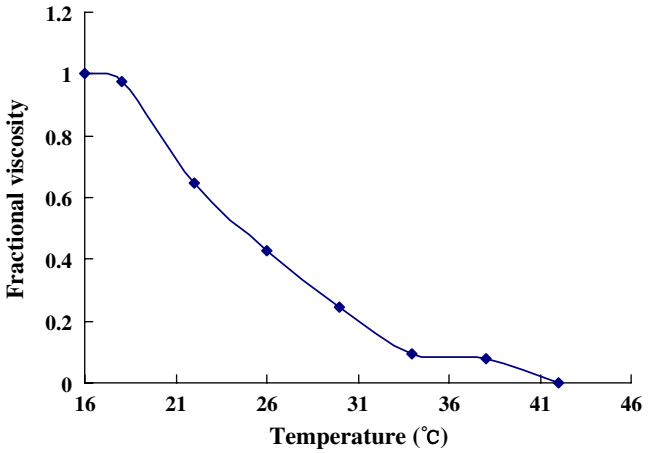

Fig. 4. Thermal denaturation curve of acid-soluble collagen from walleye pollock skin.

of collagen from walleye pollock skin were less stable than those of mammalian collagens. We also found that the DSC thermogram was broader, which reveals smaller cooperatives among the participating subunits (Usha & Ramasami, 2004). There were still two small peaks within the temperature range 20–35 °C. The former could be observed in the DSC thermogram of collagen from calf and sea cucumber (*Stichopus japonicus*) (Cui et al., 2007). The latter is a pre-denaturational transition, which has been recorded in previous studies, but its nature has not been ultimately identified (Komsa-Penkova, Koynova, Kostov, & Tenchov, 1996). Some studies ascribe it to shortened or nicked collagen fragments (Condell, Sakai, Mercado, & Larenas, 1988) or protein oxidation (Komsa-Penkova et al., 1996). The difference between T_s and T_d of walleye pollock skin collagen was about 22 °C, which is obviously consistent with the conclusion that the difference between T_s and T_d of marine collagen is not influenced by species, about (20–25)°C (Hao & Li, 1999).

The thermal stability is influenced by the imino acid content. The higher the imino acid content, the more stable are the helices, because the molecular structure of collagen is maintained mainly by restrictions on changes in the secondary structure of the polypeptide chain, imposed by the pyrrolidine rings of proline and hydroxyproline, and also partially maintained by the hydrogen bond ability through the hydroxy group of hydroxyproline (Zhang et al., 2007). Therefore, porcine and bovine skin collagens, having higher imino acid contents (220 and 215 residues per 1000 residues, respectively) (Ikoma et al., 2003; Cui et al., 2007) than walleye pollock skin collagen (184 residues per 1000 residues), denature at higher temperatures. It is known that the stability of collagen is also correlated with environmental and body temperature. Bigeye snapper and Brownstripe red snapper are tropical fish, so the collagens are more stable (Kittiphattanabawon et al., 2005; Jongjareonrak et al., 2005). T_d of walleye pollock skin collagen in this study was higher than that of cod skin collagen, determined by Rigby (1968), probably due to the environmental and body temperature.

4. Conclusion

ASC extracted from walleye pollock skin was classified as type I collagen. It has a distinct amino acid composition and thermal stability, different from those of bovine or porcine skin collagens. FTIR investigations show the existence of helical arrangements of collagen. Thus, walleye pollock skin is a good source of collagen. This study provides a theoretical basis for collagen modification and utilization.

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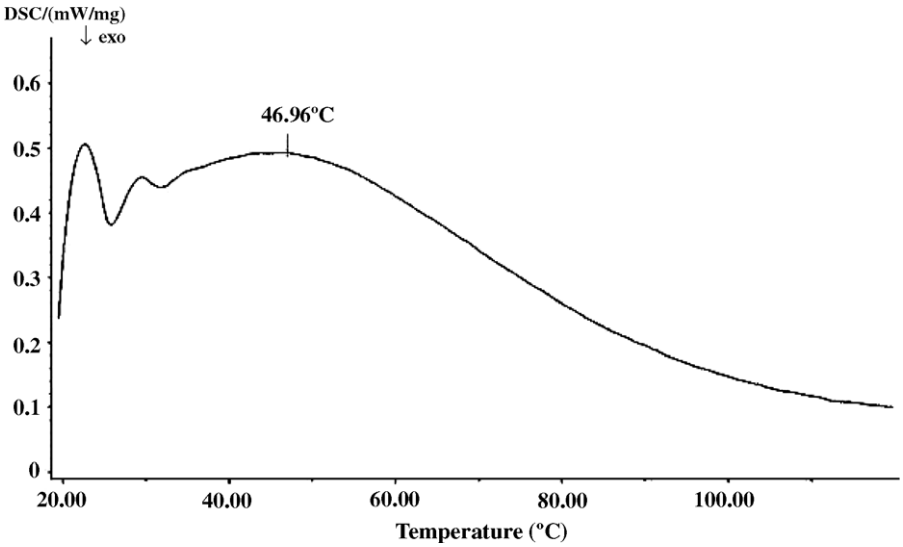

Fig. 5. Thermal transition curve of acid-soluble collagen from walleye pollock skin, as shown by DSC.

References

Condell, R. A., Sakai, N., Mercado, R. T., & Larenas, E. (1988). Quantitation of collagen fragments and gelatin by deconvolution of polarimetry denaturation curves. *Collagen and Related Research, 8*, 407–418.

Cui, F. X., Xue, C. H., Li, Z. J., Zhang, Y. Q., Dong, P., Fu, X. Y., et al. (2007). Characterization and subunit composition of collagen from the body wall of sea cucumber Stichopus japonicus. *Food Chemistry, 100*, 1120–1125.

Fathima, N. N., Balaraman, M., Rao, J. R., & Nair, B. U. (2003). Effect of zirconium (IV) complexes on the thermal and enzymatic stability of type I collagen. *Journal of Inorganic Biochemistry, 95*, 47–54.

Fathima, N. N., Madhan, B., Rao, J. R., Nair, B. U., & Ramasami, T. (2004). Interaction of aldehydes with collagen: effect on thermal, enzymatic and conformational stability. *International Journal of Biological Macromolecules, 34*, 241–247.

Hao, L. H., & Li, B. F. (1999). Extraction of collagen from body wall of *Asterias amurensis*. *Journal of Fishery Sciences of China, 6*(2), 18–21.

Ikoma, T., Kobayashi, H., Tanaka, J., Walsh, D., & Mann, S. (2003). Physical properties of type I collagen extracted from fish scales of *Pagrus major* and *Oreochromis niloticas*. *International Journal of Biological Macromolecules, 32*, 199–204.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., Nagai, T., & Tanaka, M. (2005). Isolation and characterization of acid and pepsin-solubilised collagens from the skin of Brownstripe red snapper (*Lutjanus vitta*). *Food Chemistry, 93*, 475–484.

Kimura, S. (1992). Wide distribution of the skin type I collagen α3 chain in bony fish. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 102*, 255–260.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Nagai, T., & Tanaka, M. (2005). Characterization of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*). *Food Chemistry, 89*, 363–372.

Komsa-Penkova, R., Koynova, R., Kostov, G., & Tenchov, B. G. (1996). Thermal stability of calf skin collagen type I in salt solution. *Biochimica et Biophysica Acta, 1297*, 171–181.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

Li, H., Liu, B. L., Gao, L. Z., & Chen, H. L. (2004). Studies on bullfrog skin collagen. *Food Chemistry, 84*, 65–69.

Liu, H. Y., Li, D., & Guo, S. D. (2007). Studies on collagen from the skin of channel catfish (*Ictalurus punctaus*). *Food Chemistry, 101*, 621–625.

Liu, Y. K., & Liu, D. C. (2006). Comparison of physical-chemical properties of type I collagen from different species. *Food Chemistry, 99*, 244–251.

Mizuta, S., Isobe, S., & Yoshinaka, R. (2002). Existence of two molecular species of collagen in the muscle layer of the ascidian (*Halocynthia roretzi*). *Food Chemistry, 79*, 9–13.

Mizuta, S., Miyagi, T., Nishimiya, T., & Yoshinaka, R. (2002). Partial characterization of collagen in mantle and adductor of pearl oyster (*Pinctada fucata*). *Food Chemistry, 79*, 319–325.

Muyonga, J. H., Cole, C. G. B., & Duodu, K. G. (2004). Characterization of acid soluble collagen from skins of young and adult Nile perch (*Lates nilotics*). *Food Chemistry, 85*, 81–89.

Nagai, T., Araki, Y., & Suzuki, N. (2002). Collagen of the skin of ocellate puffer fish (*Takifugu rubripes*). *Food Chemistry, 78*, 173–177.

Nagai, T., Ogawa, T., Nakamura, T., Ito, T., Nakagawa, H., Fujiki, K., et al. (1999). Collagen of edible jellyfish exumbrella. *Journal of the Science of Food and Agriculture, 79*, 855–858.

Nagai, T., & Suzuki, N. (2000). Isolation of collagen from fish waste-skin, bone and fins. *Food Chemistry, 68*, 277–281.

Nagai, T., Yamashita, E., Taniguchi, K., Kanamori, N., & Suzuki, N. (2001). Isolation and characterization of collagen from the outer skin waste material of cuttlefish (*Sepia lycidas*). *Food Chemistry, 72*, 425–429.

Rigby, B. J. (1968). Amino-acid composition and thermal stability of the skin collagen of the Antarctic ice-fish. *Nature, 219*(1), 166–167.

Sato, K., Yoshinaka, R., Sato, M., & Shimizu, Y. (1987). Isolation of native acid-soluble collagen from fish muscle. *Nippon Suisan Gakkaishi, 53*, 1431–1436.

Senaratne, L. S., Park, P., & Kim, S. (2006). Isolation and characterization of collagen from brown backed toadfish (*Lagocephalus gloveri*) skin. *Bioresource Technology, 97*, 191–197.

Shen, X. R., Kurihara, H., & Takahashi, K. (2007). Characterization of molecular species of collagen in scallop mantle. *Food Chemistry, 102*, 1187–1191.

Swatschek, D., Schatton, W., Kellermann, J., Muller, W. E. G., & Kreuter, J. (2002). Marine sponge collagen: isolation, characterization and effects on the skin parameters surface-pH, moisture and sebum. *European Journal of Pharmaceutics and Biopharmaceutics, 53*, 107–113.

Usha, R., & Ramasami, T. (2004). The effects of urea and *n*-propanol on collagen denaturation: using DSC, circular dicroism and viscosity. *Thermochimica Acta, 409*, 201–206.

Zhang, Y., Liu, W. T., Li, G. Y., Shi, B., Miao, Y. Q., & Wu, X. H. (2007). Isolation and partial characterization of pepsin-soluble collagen from the skin of grass carp (*Ctenopharyngodon idella*). *Food Chemistry, 103*, 906–912.

Zhang, H. J., Luo, C. X., Zhang, X. S., Song, M. Z., & Jiang, X. P. (2003). Application of collagen protein. *Leather Science and Engineering, 13*(6), 37–46.