Purification and Structural Aspects of Type I Collagen from Walleye Pollock (Theragra chalcogramma) Skin

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ABSTRACT

Type I collagen was extracted from walleye pollock (Theragra chalcogramma) skin and purified by DEAE-52 cellulose chromatography and gel filtration chromatography with Sephacryl S-300 HR. Fourier transform infrared spectroscopy spectra and X-ray diffraction pattern showed the existence of a helical arrangement, with the distance between the molecular chains of 1.18 nm and the unit height, typical of the triple helix, of 0.27 nm. Scanning electron microscopy (SEM) revealed the collagen had a filamentary structure. Peptide mapping, obtained by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), indicated that peptides with the molecular weight of 1,470, 1,565, 1,570, 2,150, and 2,470 Da were the major products of trypsin digestion of pollock skin collagen.

Keywords: Purification; structural aspect; collagen; walleye pollock; fish skin

Introduction

Collagen, widely distributed in animal tissues, approximately accounts for 30% of total protein (Zhang et al., 2014). At present, at least 29 variants of collagen have been reported, designated as type I–XXIX (Veeruraj et al., 2015). Type I collagen is the main component of extracellular matrices; it is present in skin, bone, tendon, etc., and gives strength and resistance to the biological tissues (Giraud-Guille et al., 2000). In tissue engineering, collagen scaffold can often be found in the form of gel, sponge, fiber and film, showing good biocompatibility and biodegradable properties (Xiong et al., 2009). Its pharmaceutical uses include production of wound dressings, vitreous implants, and as carriers for drug delivery (Singh et al., 2011). Currently, marine collagen is receiving increased attention for the outbreaks of bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD) and religious objections (Veeruraj et al., 2015).

Walleye pollock is one of the commercially important fish species in China (Yan et al., 2008). Large numbers of pollock were processed annually, mainly in Shandong Province, followed by plenty of by-products generated during processing per year. The amount of by-products produced occupied a very high percentage, reaching 75% of the total weight of fish (Woo et al., 2008). These by-products—including fish skin, bone, scale, and fin—are generally dumped without utilization except being used in food feed, causing environmental pollution and resource waste. Research shows that there is a great deal of collagen in the waste produced from fish processing. It has significant indirect environmental benefits to use the fish waste as a source of collagen (Veeruraj et al., 2015).

For making thorough application of the fish processing by-products, it is beneficial to identify the purification procedure and structural aspects of collagen. In this study, the collagen from walleye pollock skin was prepared by salt fraction, DEAE-52 cellulose, and gel filtration chromatographic procedure, with the aim of yielding large quantities of type I collagen. Furthermore, the FTIR
spectra, X-ray diffraction pattern, morphology, and peptide mapping were evaluated to determine the structural aspects of the purified collagen.

**Materials and methods**

**Materials**

Walleye pollock (*Theragra chalcogramma*) were caught from the Bering Sea by commercial fishing boat, stored at −18°C immediately after gutting, and transported to the dock in Qingdao, P.R. China. 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.

**Extraction of collagen**

The collagen was isolated from walleye pollock skin in the laboratory (College of Food Science and Technology, Ocean University of China, Qingdao, Shandong Province, P.R. China). The extraction procedure was performed as previously described (Yan et al., 2008). The skin was soaked in 0.1 M NaOH to remove noncollagenous materials effectively and then rinsed with distilled water thoroughly until a neutral pH was reached. Minced skins were stirred with 0.5 M acetic acid solution for 48 h, and the mixture was centrifuged at 10,000 × g for 30 min. The supernatants were salted out by adding NaCl to a final concentration of 0.9 M, followed by centrifugation at 8,000 × g for 20 min. The resultant precipitate was dissolved in 0.5 M acetic acid, dialyzed against 0.1 M acetic acid for 1 day, distilled water for 2 days, and then lyophilized.

**SDS-PAGE**

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (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. After electrophoresis, the gel was stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 in 45% (v/v) methanol and 10% (v/v) acetic acid for 20 min and then destained using a solution with 10% (v/v) methanol and 10% (v/v) acetic acid.

**DEAE-52 cellulose chromatography**

The lyophilized collagen (about 15 mg) was dissolved in 5 mL of 50 mM Tris-HCl buffer (pH 7.5) containing 2 M urea and stirred for 2 days at 4°C. Following centrifugation for 30 min at 5,000 × g, 3 mL of supernatant were loaded onto the 2.6 × 50 cm column of DEAE-52 cellulose, previously equilibrated with 50 mM Tris-HCl buffer (pH 7.5). The adsorbed protein was subsequently eluted by 50 mM Tris-HCl buffer (pH 7.5) with a linear gradient of 0 to 0.5 M NaCl at a flow rate of 1 mL/min. The column effluent was monitored and recorded at 220 nm. Appropriate fractions were pooled, dialyzed against distilled water, concentrated with Polyethylene Glycol 20000, and then lyophilized.

**Gel filtration chromatography**

Gel filtration chromatography of fibrillar collagen was performed on a column (1.6 × 60 cm$^2$) of Sephacryl S-300 HR (Pharmacia, Kalamazoo, MI, USA), essentially as described previously (Mizuta et al., 1992). The lyophilized collagen, after DEAE-52 cellulose chromatography, was stirred in 50 mM Tris-HCl buffer (pH 7.5) containing 2 M urea for 2 days at 4°C and then centrifuged for 30 min at 5,000 g. The supernatant was applied to the gel filtration column and eluted at a flow rate of 0.6 mL/min with 50 mM Tris-HCl buffer (pH 7.5) containing 0.1 M NaCl. Fractions of 3 mL were
collected and monitored at 220 nm. Appropriate fractions were pooled, dialyzed against distilled water, concentrated with Polyethylene Glycol 20000, and then lyophilized.

**HPLC**

Prior to high performance liquid chromatography (HPLC) analysis, the collagen after DEAE-52 cellulose chromatography and gel filtration chromatography was dissolved in 0.5 M acetic acid (pH 2.5) at a concentration of 5 mg/mL and filtered through a 0.45 μm membrane filter. Chromatography was performed on an Agilent 1100 HPLC system (Agilent, Palo Alto, CA, USA) using the column of a 4.6 × 250 mm² TSK GEL G3000PWXL (Tosoh, Tokyo, Japan) with an ultraviolet detector. The injection volume of collagen solution was 20 μL. The mobile phase used was 50 m M Tris-HCl buffer (pH 7.5) containing 0.1M NaCl. The sample was eluted at a flow rate of 0.5 mL/min and monitored at 220 nm.

**FTIR**

Fourier transform infrared spectroscopy (FTIR) spectra of collagen were obtained using infrared spectrophotometer (Nicolet 200SXV) from 4,000 to 500 cm⁻¹ at a data acquisition rate of 2 cm⁻¹ per point by preparing a 100 mg KBr disc containing 2 mg of the sample. Analysis of the resulting spectra was carried out using Omnic 6.0 software (Thermo-Nicolet, Madison, WI, USA).

**X-ray diffraction study**

X-ray diffraction pattern of collagen was analyzed using an X-ray diffractometer (Rigaku D/MAX 2500; Tokyo, Japan) in the angular range of 5–40 (2θ) with nickel-filtered Cu Kα radiation (λ = 0.154 nm) at a voltage of 40 kV and current of 40 mA.

**Scanning electron microscopy**

Morphology of collagen was determined using a scanning electron microscope (JSM-840; Jeol Ltd., Tokyo, Japan). Freeze-dried collagen was fixed on stubs using copper-conducting adhesive tape and sputter-coated with gold. The coated sample was observed under SEM at an acceleration voltage of 2 kV.

**Peptide mapping**

A gel fragment with an α subunit was cut after the SDS-PAGE and destained by being soaked in 100 μL of 50% acetonitrile in the presence of 25 mM ammonium bicarbonate (repeated several times). Subsequently, it was dried by vacuum centrifugation, and 10 μL of trypsin solution (0.01 μg/μL) was joined. After the mixture was incubated at 37°C for 15 h, 100 μL of 5% trifluoroacetic acid was introduced, followed by the sample solution placed at 40°C for 1 h. The supernatant was separated and subjected to analysis with matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS; Applied Biosystems 4700 Proteomics Analyzer, Foster City, CA, USA).

**Results and discussion**

**Purification of collagen**

The collagen was extracted from walleye pollock skin and classified as type I collagen with the helical arrangement verified by UV-vis spectra, amino acid composition, SDS-PAGE, and FTIR, as previously described (Yan et al., 2008). The type I collagen was further purified by DEAE-52 cellulose chromatography and gel filtration chromatography with Sephacryl S-300 HR. As shown in Figure 1a, the collagen was resolved into three peaks using DEAE-52
The three fractions were collected, respectively, and detected by SDS-PAGE analysis. The results showed that the first peak contained collagen component, while peaks II and III contained no collagenous protein. In general, the proteoglycan filaments appear to connect neighboring collagen fibrils by transversely and periodically attaching to a specific site of the fibrils (Ushiki, 2002). Scott (1980) reported that very thin filaments could be observed within the bundle of collagen fibrils in specimens stained with a cationic dye, such as Alcian blue and Cupromeronic blue. So, it was inferred that there was a small amount of proteoglycan component in collagen extract, corresponding to the second and third peaks in Figure 1a. It was concluded that DEAE-52 cellulose chromatography could separate type I collagen from contaminants in the pollock skin extract successfully, which was in agreement with the results of Miller (1973) and Cao and Xu (2008).

The type I collagen recovered from DEAE-52 cellulose chromatography was applied to a column of Sephacryl S-300 HR, which carried out separation based on the molecular weights (MW) of components. As shown in Figure 1b, the major peak appeared containing collagen component revealed by SDS-PAGE. The other peaks were very small, which could be due to the components with low molecular weight generated in the extraction process. The collagen fraction was collected,
and a simple HPLC method was employed to determine the homogeneity of type I collagen using a TSK GEL G3000PWXL column. As shown in Figure 2, a single, sharp, and symmetrical peak was observed, which suggested that the purified collagen with homogeneous molecular weight was obtained from walleye pollock skin by the combination of acetic acid extraction, salting out, DEAE-52 cellulose chromatography, and gel filtration chromatography with Sephacryl S-300 HR.

**FTIR**

Fourier transform infrared spectroscopy (FTIR) spectra of the purified type I collagen from walleye pollock skin are presented in Figure 3. The major peaks were similar to those of pollock skin collagen before being purified by DEAE-52 cellulose chromatography and Sephacryl S-300 HR gel filtration chromatography, as shown previously (Yan et al., 2008). The amide A peak is associated with the N–H stretching vibration. It was reported that a free N–H stretching vibration occurs in the range of 3,400–3,440 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 3,300 cm\(^{-1}\) (Doyle et al., 1975). The
amide A peak of the purified collagen was observed at 3,333 cm$^{-1}$, indicating that the NH groups of the collagen were involved in hydrogen bonds. However, the amide A band of purified collagen had a higher wave number than that of crude collagen (3,328 cm$^{-1}$; Yan et al., 2008), implying that the NH groups of purified collagen were less involved in hydrogen bonds. This result suggested that a spot of hydrogen bonds in collagen was destroyed during the purification process, which might be related to the purification temperature.

From Figure 3, the amide I, II, and III bands of the purified collagen were found at wave numbers of 1,648, 1,530, and 1,237 cm$^{-1}$, respectively; all were situated in the amide band regions—such as amide I (1,600–1,700 cm$^{-1}$), amide II (1,500–1,600 cm$^{-1}$), and amide III (1,200–1,300 cm$^{-1}$; Ahmad and Benjakul, 2010). The absorption bands between 1,237 cm$^{-1}$ (amide III) and 1,530 cm$^{-1}$ (amide II) demonstrated the existence of the helical arrangement in collagen (Su et al., 2009), which was reconfirmed by the IR ratio of approximately 1 between amide III and 1,450 cm$^{-1}$ band of collagen (Plepis et al., 1996; Nalinanon et al., 2010). The amide II band of purified collagen (1,530 cm$^{-1}$), resulting from N–H bending vibration coupled with C–N stretching vibration, showed a lower wave number than that of crude collagen (1,542 cm$^{-1}$; Yan et al., 2008), indicating the purified collagen had a lower degree of molecular order, since the shift of the peak to higher frequency was associated with an increase in the molecular order (Wang et al., 2014). FTIR spectra showed that the collagen well maintained the triple helical structure after the purification process.

**X-ray diffraction pattern**

The X-ray diffraction pattern of collagen fibril has been of vital importance in determining fibrillar structure (Cameron et al., 2002). In general, X-ray diffraction pattern of collagen shows three peaks—namely, Peaks C, A$_1$, and A$_2$—with the diffraction angle usually being in 5–10°, near 20°, and 30–35°, respectively. A diffraction pattern obtained for type I collagen from pollock skin is shown in Figure 4. It was found that this diffraction pattern contained three peaks at the position of 7.46, 19.75, and 33.18°, corresponding to Peaks C, A$_1$, and A$_2$, respectively, which was the typical X-ray diffraction pattern of collagen. Peak C is the first peak, displaying the distance between the molecular chains, a second peak (Peak A$_1$) is generated by diffuse scattering, and Peak A$_2$, corresponding to the third peak, represents the unit height, typical of the triple helical structure (Giraud-Guille et al., 2000). Peak A$_2$, present in Figure 4, shows that the purified type I collagen maintained the helical arrangement in good accordance with the result of FTIR spectra. As seen from the peak profile, Peak C was sharp, mainly induced by the structured area of collagen; while Peak A$_1$ was broad, generated

![Figure 4. X-ray diffraction pattern of type I collagen from walleye pollock skin.](Image)
from the amorphous region of collagen. Therefore, it was concluded that two phase constructions were present in the pollock skin collagen, one structured, the other structureless. The core-shell theory (Kronick and Buechler, 1986) could be employed to demonstrate the collagen construction, the structured area being the core, and the amorphous region to be the shell, which well corresponded to the two-phase assembly kinetic process of collagen (Yan et al., 2012).

The Bragg equation \(2d \sin \theta = \lambda\) is obtained in the experiments that X-rays or neutrons diffract off crystal surfaces at certain angles, where \(\lambda\), \(d\) and \(\theta\) correspond to the X-ray wavelength (0.154 nm), the spacing between the planes in the atomic lattice, and the Bragg diffraction angel, respectively (Wang et al., 2009a). By calculating the basis of Peak C, the distance between the molecular chains of pollock skin collagen was 1.18 nm, similar to that of collagen from bovine and flat fish dermis (Giraud-Guille et al., 2000). The \(d\) value, corresponding to Peak A, reflected the distance of adjacent amino acid residues in the triple helical structure. Eyre (1980) reported that it was 0.29 nm in the collagen helix, unlike the 0.15 nm in the normal \(\alpha\)-helix. In this study, the distance of adjacent amino acid residues was 0.27 nm in the helix of pollock skin collagen. This varies slightly from the value given in the literature and can be explained by the molecular structure of purified collagen being a little different from that of native collagen.

**Morphology of collagen**

The morphology of purified type I collagen from walleye pollock skin was observed by scanning electron microscopy. Figure 5 displays the SEM images of collagen with the magnification of 800 and 3,000. They show that the collagen was endowed with a uniform filamentary structure, different from collagens from grass carp skin (Zhang et al., 2007), the coelomic wall of Sipunculida (Su et al., 2009), skin and bone of Spanish mackerel (Li et al., 2013), and skin of sailfish (Tamilmozhi et al., 2013)—all exhibiting the uniform and regular alveolate pores, which are mainly attributed to the species of collagen. The result demonstrates that the purified collagen was still fibrils, although collagen structures were somewhat disrupted during the purification process.

**Peptide mapping**

Peptide mapping is an identity test for proteins. It may be viewed as a fingerprint of a protein and is the end product of several chemical processes that provides a comprehensive understanding of the protein being analyzed (http://www.nihs.go.jp/dbcb/Bio-Topic/peptide.pdf). The ease, simplicity, efficiency, and low cost of the approach offer great promise for rapid and high-throughput protein identification (Wang et al., 2009b). For collagen, peptide mapping was an effective method for identification of the type, and it also provided an approach for collagen identification in particular tissues (Zhang et al., 2006). Previous study on vertebrate collagens has revealed that the native collagen, also known as the triple helical collagens, show good resistance

![Figure 5. SEM images of type I collagen from walleye pollock skin.](image-url)
to the most proteinases, expect specific collagenase (Zhang et al., 2009). In this study, the α subunit of collagen was collected after the SDS-PAGE, and it was susceptible to hydrolysis by trypsin. The molecular weight ranges of type I collagen from walleye pollock skin digested by trypsin were analyzed by MALDI-TOF-MS. As shown in Figure 6, all peptides from type I collagen showed molecular weights less than 4,000 Da, similar to the result obtained from bovine collagen (Zhang et al., 2006). The peptides with the MW of 1,470, 1,565, 1,570, 2,150, and 2,470 Da had the higher intensity in the mass spectra, meaning that they were the major products of trypsin digestion of pollock skin collagen. By indexing in Mascot Database, the sequences of peptides matching to MW 1,470, 1,565, 1,570, 2,150, and 2,470 Da were identified as AREELAALALTGVR, VPISEGLSDEEFDK, QLQTTGGIIDTVGQR, VEESERLFQVENQSAEK, and QRALALDEAVSQSTQITEFHDK, respectively, which could be regarded as characteristic peptides of type I collagen from walleye pollock skin.

**Conclusions**

Type I collagen was extracted from walleye pollock skin and purified by a combination of DEAE-52 cellulose chromatography and gel filtration chromatography with Sephacryl S-300 HR. The helical arrangement of collagen was maintained after the purification process, and the purified collagen could reconstruct the fibril structure. The peptides with the molecular weight of 1,470, 1,565, 1,570, 2,150, and 2,470 Da were the major products of trypsin digestion of pollock skin collagen in peptide mapping, which were helpful to confirm collagen source and type.

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**References**

Ahmad, M., and Benjakul, S. 2010. Extraction and characterisation of pepsin-solubilised collagen from the skin of unicorn leatherjacket (*Aluterus monoceros*). Food Chem. 120: 817–824.

Cameron, G. J., Alberts, I. L., Laing, J. H., and Wess, T. J. 2002. Structure of type I and type III heterotypic collagen fibrils: An X-ray diffraction study. J. Struct. Biol. 137: 15–22.
Cao, H., and Xu, S. Y. 2008. Purification and characterization of type II collagen from chick sternal cartilage. Food Chem. 108: 439–445.

Doyle, B. B., Blout, E. R., and Bendit, E. G. 1975. Infrared spectroscopy of collagen and collagen like polypeptides. Biopolymers 14(5): 937–957.

Eyre, D. R. 1980. Collagen: Molecular diversity in the body’s protein scaffold. Science 207: 1315–1322.

Giraud-Guille, M., Besseau, L., Chopin, C., Durand, P., and Herbage, D. 2000. Structural aspects of fish skin collagen which forms ordered arrays via liquid crystalline states. Biomaterials 21: 899–906.

Kronick, P. L., and Buechler, P. R. 1986. Effect of beaming and tanning on collagen stability studied by differential scanning calorimetry. J. Am. Leather Chem. As. 81(7): 213–220.

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

Li, Z., Wang, B., Chi, C., Zhang, Q., Gong, Y., Tang, J., Luo, H., and Ding, G. 2013. Isolation and characterization of acid soluble collagens and pepsin soluble collagens from the skin and bone of Spanish mackerel (Scomberomorus miphonius). Food Hydrocolloid. 31: 103–113

Miller, E. J. 1973. Isolation and characterization of the cyanogens bromide peptide from the chain of bovine and human cartilage collagen. Biochemistry 17: 3153–3159.

Mizuta, S., Yoshinaka, R., Sato, M., Itoh, Y., and Sakaguchi, M. 1992. Subunit composition of distinct types of collagens in the muscle of the kuruma prawn Penaeus japonicus. Comp. Biochem. Phys B 102(4): 803–811.

Nalinanon, S., Benjakul, S., and Kishimura, H. 2010. Collagens from the skin of arabesque greenling (Pleuragrammus azonus) solubilized with the aid of acetic acid and pepsin from albacore tuna (Thunnus alalunga) stomach. J. Sci. Food Agr. 90: 1492–1500.

Plepis, A. M. D. G., Goissis, G., and Das-Gupta, D. K. 1996. Dielectric and pyroelectric characterization of anionic and native collagen. Polym. Eng. Sci. 36: 2932–2938.

Scott, J. E. 1980. Collagen-proteoglycan interactions. Biochem. J. 187: 887–891.

Singh, P., Benjakul, S., Maqsood, S., and Kishimura, H. 2011. Isolation and characterisation of collagen extracted from the skin of striped catfish (Pangasianodon hypophthalmus). Food Chem. 124: 97–105.

Su, X. R., Sun, B., Li, Y. Y., and Hu, Q. 2009. Characterization of acid-soluble collagen from the coelomic wall of Sipunculida. Food Hydrocolloid. 23: 2190–2194.

Tamilmozi, S., Veeruraj, A., and Arumugam, M. 2013. Isolation and characterization of acid and pepsin-solubilized collagen from the skin of sailfish (Istiophorus platypterus). Food Res. Int. 54: 1499–1505.

Ushiki, T. 2002. Collagen fibers, reticular fibers and elastic fibers. A comprehensive understanding from morphological viewpoint. Arch. Histol. Cytol. 65(2): 109–126.

Veeruraj, A., Arumugam, M., Ajithkumar, T., and Balasubramanian, T. 2015. Isolation and characterization of collagen from the outer skin of squid (Doryteuthis singhalensis). Food Hydrocolloid. 43: 708–716.

Wang, C., Chen, Z., He, Y., Li, L. and Zhang, D. 2009a. Structure, morphology and properties of Fe-doped Zno films prepared by facing-target magnetron sputtering system. Appl. Sur. Sci. 255: 6881–6887.

Wang, L., Liang, Q., Chen, T., Wang, Z., Xu, J., and Ma, H. 2014. Characterization of collagen from the skin of Amur sturgeon (Acipenser schrenckii). Food Hydrocolloid. 38: 104–109.

Wang, S., Liu, T., Zhang, L., Chen, G., and Yang, P. 2009b. Chymotryptic proteolysis accelerated by alternating current for MALDI-TOF-MS peptide mapping. J. Proteomics 72: 640–647.

Woo, J. W., Yu, S. I., Cho, S. M., Lee, Y. B., and Kim, S. B. 2008. Extraction optimization and properties of collagen from yellowfin tuna (Thunnus albacares) dorsal skin. Food Hydrocolloid. 22: 879–887.

Xiong, X., Ghosh, R., Hiller, E., Drepper, F., Knapp, B., Brunner, H., and Rupp, S. 2009. A new procedure for rapid, high yield purification of type I collagen for tissue engineering. Process Biochem. 44: 1200–1212.

Yan, M., Li, B., Zhao, X., and Qin, S. 2012. Effect of concentration, pH and ionic strength on the kinetic self-assembly of acid-soluble collagen from walleye pollock (Theragra chalcogramma) skin. Food Hydrocolloid. 29: 199–204.

Zhang, G., Sun, A., Li, W., Liu, T., and Su, Z. 2006. Mass spectrometric analysis of enzymatic digestion of denatured collagen for identification of collagen type. J. Chromatogr. A 1114: 274–277.

Zhang, J., Duan, R., Huang, L., Song, Y., and Regenstein, J. M. 2014. Characterisation of acid-soluble and pepsin-solubilised collagen from jellyfish (Cyanea nozakii) Kishinouye. Food Chem. 150: 22–26.

Zhang, J., Duan, R., Tian, Y., and Konno, K. 2009. Characterisation of acid-soluble collagen from skin of silver carp (Hypophthalmichthys molitrix). Food Chem. 116: 318–322.

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