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Isolation and characterization of collagen extracted from channel catfish (*Ictalurus punctatus*) skin

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ABSTRACT

Channel catfish skin is a by-product from catfish fillet production. Collagens were extracted from catfish skins by: (1) acid; (2) homogenization-aided; and (3) pepsin-aided extraction methods. Kinetic analysis of extraction was performed. SDS-PAGE was carried out for all collagens extracted under different conditions. Protein solubility, zeta potential, circular dichroism and gel strength of the collagen extracted by three methods were studied to determine optimal extraction conditions. Protein recovery rate from minced skins extracted with pH 2.4 HCl containing 23.6 KU/g pepsin was the highest (64.19%). SDS-PAGE showed that collagens extracted with different methods had different proteins ratio patterns, even though the molecular mass of collagen subunits were similar, 123 and 113 KDa for α_1 and α_2 chains, 226 KDa for β chain and 338.5 KDa for γ chain, respectively. Channel catfish skin collagens were typical type I collagens and could have applications in food, medical and cosmetic industries.

1. Introduction

Collagen is a long cylindrical protein, it is the major fraction and contributes to the unique physiological function of connective tissue in skin. Up to 27 types of collagen have been identified, and type I collagen exists the most widely in connective tissues. Type I collagen is made up of three polypeptide chains, two of the polypeptides are designated as α_1, and α_1 bonded to another chain to form a third chain α_2 through hydrogen bond. Total molecular mass of collagen is about 300 KDa with each chain has a molecular mass of about 100 KDa, and it has a wide range of applications of pharmaceutical, leather, biomedical and film industries (Ogawa et al., 2004). Collagen usually extracted from porcine skins and bones, however, it is not acceptable by Judaism and Islam due to religious restrictions (Nalinanon, Benjakul, Visessanguan, & Kishimura, 2007). In addition, collagen extracted from bovine might be contaminated with bovine spongiform encephalopathy and transmissible spongiform encephalopathy (Choi & Regenstein, 2000). Therefore, aquatic sources for collagen production is a substitution for mammalian sources even though the yield of collagen from aquatic sources is much lower than that from mammalian sources. However, the yield of collagen extracted from fish skin was greatly improved in recent years (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011). Researchers have been reported that around 50% of collagen can be isolated from fish skin, and 45% of collagen from fish bone (Nagai & Suzuki, 2000).

Channel catfish aquaculture is an important aquaculture in the United States, and Mississippi State ranks No. 1 in catfish production with annual production of $185 million in 2015 (MSU extension data). Catfish fillet processing contributes to the vitality of the local economy. The fish skins account for about 10% of the by-products and can be used as a potential collagen source. More and more studies about different alternative sources and new functionalities of collagen had been reported in the last 10–15 years. However, most studies used a single set of extraction conditions. There is a lack of a systematic approach for collagen extraction, particularly for catfish collagen extraction. And the extraction yield of collagen from fish skins still remained low, and the extraction method have less practical application for food industries since most of them required long time, high energy input and dialysis processing. In addition, no systematic kinetic analysis of extraction yield has been performed. Our objectives were to use a systematic approach to optimize the extraction condition and modify the extraction method to recover the maximum yield, and to characterize the properties of collagen extracted with different extraction conditions.

2. Material and method

2.1. Materials

Catfish skin was collected from a local catfish fillet processing plant (Country Select, Isola, MS). The catfish skin was buried in crushed ice

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during transportation to our laboratory and stored at −80 °C until use.

2.2. Chemicals

All chemicals, reagents and porcine gastric pepsin (EC 3.4.23.1) were analytical grade and obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, U.S.A).

2.3. Proximate analysis

Moisture, protein, ash and total fat content were determined by AOAC International methods 934.01, 955.04, 942.05 and 2003.06, respectively (AOAC International, 2012). A conversion factor 5.4 was used for calculating the protein content from nitrogen content. The hydroxyproline contents in skin material and extracted collagen were determined according to the method of Bergman and Loxley (1963). The protein recovery rate (%) was obtained by the following equation:

$$\text{Protein recovery rate (\%)} = \frac{\text{hydroxyproline content in extracted collagen sample}}{\text{hydroxyproline content in skin material}} \times 100\%$$

2.4. Extraction of collagen with acids (ASC)

Catfish skins were pretreated by washing with iced water containing 1% NaCl (1:6 W/V) and ground into small pieces, then passed through 35 mesh sieve. One hundred grams of catfish skins were mixed with 4 different acids (acetic acid, hydrochloric acid, citric acid and lactic acid, W:V = 1:50) with varies of pH (1.8, 2.1, 2.4, 2.7 and 3.0), and shaken for 48 hrs (100 rpm) using an orbital shaker at 4 °C, then centrifuged at 15,000*g* for 20 min at 4 °C to collect the supernatant, the residue was re-extracted with the corresponding acid (W/V = 1:50) for another 12 hrs. After the desire time was achieved, the mixture was centrifuged at the same condition, the supernatant was combined and salted out by adding NaCl to a final concentration of 0.9 M, the precipitated collagen was separated by centrifugation at 15,000*g* for 15 min at 4 °C. The resultant precipitate was washed quickly by type-I water (ultrapure water) (W/W = 1:2) for 3 times to remove NaCl and then lyophilized.

2.5. Extraction of collagen with homogenization-aided (HSC) method

Preliminary experiments showed that homogenization (Model: GLH 580, Spindle: 30 * 195 mm, Omni International, Kennesaw, GA, USA) at solid-to-liquid ratio (1: 50, w/v) for 5 min was the optimal condition for extraction. Therefore, catfish skins were mixed with hydrochloric acid (store in 4 °C for 6 hrs in advance) at pH 2.3, 2.4 and 2.5 with solid-to-liquid ratio varying from 1:15 to 1:50 (W/V). The mixture was then homogenized at 7000 rpm for 5 s and stop 5 s, the cycle was repeated until 5 min was achieved, the mixture was kept in a foam box which filled with ice during homogenization processing. After homogenization, the mixture was stirred for 1 h in 4 °C. After the desired time was achieved, the mixture was centrifuged at the same condition in Section of 2.4, and the following procedure was the same as described in Section of 2.4.

2.6. Extraction of collagen with pepsin and homogenization aided (PHSC) method

Catfish skins and hydrochloric acid (pH 2.4) were mixed with solid to liquid ratio varying from 1:5 to 1:20, and pepsin concentrations from 0.118 to 23.6 KU/g skin. The mixture was homogenized at 7000 rpm for 5 s and stop 5 s, the cycle was repeated until 5 min was achieved. The mixture was centrifuged at the same conditions as described in Section 2.4, the following procedures was the same as described in Section 2.4.

2.7. Kinetic analysis of collagen extraction

All the extraction curves could be described by the model developed by Peleg (1988):

$$C(t) = C(0) + \frac{t}{K_1 + K_2 \cdot t} \quad (1)$$

where C(t) (%) is the protein recovery rate at time t, t is the extraction time (hours), C_0 (%) is the protein recovery rate at time t = 0, K_1 is Peleg's rate constant and K_2 is Peleg's capacity constant. C_0 in all experimental was zero, the above equation was rearranged in the following form:

$$C(t) = \frac{t}{K_1 + K_2 \cdot t} \quad (2)$$

The Peleg rate constant K_1 relates to extraction rate (B_0) at the very beginning :

$$B_0 = \frac{1}{K_1} \quad (3)$$

The Peleg's capacity constant K_2 relates to maximum of protein recovery rate. When t→ ∞, the following equation describes the relations between protein recovery rate and K_2 constant:

$$\text{C| t→ } \infty = \frac{1}{K_2} \quad (4)$$

Therefore, Eq. (1) can be transformed to a linear relationship in the final form:

$$\frac{t}{C(t) - C(0)} = K_1 + K_2 t \quad (5)$$

2.8. Sodium-dodecyl-sulfate gel electrophoresis (SDS-PAGE)

Electrophoresis was carried out according to the method of Laemmli (Laemmli, 1970). Collagen samples were dissolved in 0.02 M sodium phosphate (pH 7.2) containing 0.5 M urea. Electrophoresis was performed on a 6% resolving gel and a 4% stacking gel. Proteins were stained with 0.1% Commassie Brilliant Blue R-250 dissolved in water, methanol and acetic acid (9:9:2, v/v/v) for 30 min, then destained using a solution containing water, methanol and acetic acid (17:1:2, v/v/v). For the quantification of α and β chains, gels were scanned and analyzed by a Molecular Imager (Bio-Rad Chemidoc™ XRS+, Hercules, CA, USA) equipped with Image Lab™ Analysis Software (version 5.2).

2.9. Effect of pH on solubility

Collagen extracted with different conditions were dissolved in 0.5 M acetic acid to obtain a final concentration of 3 mg/mL. The pH of protein solution was adjusted with 2 M HCl or 2 M NaOH to 1–10. The solution was centrifuged at 10,000*g* for 20 min at 4 °C. Protein content in the supernatant was determined by the method of Bradford (1976).

2.10. Effect of NaCl on solubility

Collagen extracted with different conditions were dissolved in 0.5 M acetic acid to obtain a final concentration of 6 mg/mL. Five milliliters of protein solution were mixed with 5 mL of 0.5 M acetic acid containing a serious concentration of NaCl to make a final concentration of 0%, 1%, 2%, 3%, 4%, 5% and 6%. Protein solutions were centrifuged at 10,000*g* for 20 min at 4 °C. The protein concentration in the supernatant was determined by the method of Bradford (1976).