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Characterization of tilapia (*Oreochromis niloticus*) skin gelatin extracted with alkaline and different acid pretreatments

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ABSTRACT

Tilapia production is growing worldwide and to better utilize wastes from the processing industry, one important application is production of high quality fish gelatin to meet the needs of markets that are not amenable to beef or porcine gelatin. The extraction process from tilapia skin gelatin was optimized through the use of a combination of alkali (0.3 M NaOH) with different types and concentrations of acids before thermal hydrolysis. The effects of acid pretreatments on the protein yields and the physico-chemical properties of tilapia gelatin were investigated. Acid concentrations (0.01–0.20 M) influenced gelatin protein recovery: 10.52%–22.40% for citric acid, 1.92%–21.55% for acetic acid, and 4.47%–24.35% for HCl. It was possible to increase gelatin yield for each of the tested acids by adjusting the acid concentration. Gelatin viscosity and the molecular weight distribution of gelatin proteins were related to the acid concentration used. Gelatin prepared using too low a concentration (e.g. 0.01 M acetic acid or HCl) or too high a concentration (e.g. >0.05 M HCl or citric acid) yielded an extract with a smaller ratio of large molecule components, such as β-chains, and exhibited lower viscosity. The film forming properties of gelatins extracted from three acid-optimized pretreatments showed no significant difference in transparency, tensile strength and elongation at break; though the gelatin film made from 0.03 M citric acid pretreated gelatin had somewhat better water barrier property than those made with HCl or acetic acid.

1. Introduction

Gelatin, a polypeptide derived from collagen in mammal or fish skins and bones, provides a diversity of functional properties, such as water binding capacity, film-forming properties, foaming and emulsifying abilities, making it a versatile ingredient in food, pharmaceutical and cosmetic industries (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011). Gelatins are traditionally produced from porcine and bovine hide, and often do not meet the requirements for kosher and halal foods due to religious dietary restrictions (Zhou, Mulvaney, & Regenstein, 2006), while fish skin gelatin provides an ideal alternative.

Worldwide, tilapia is the second most cultured fish after carps with an annual production reaching 3.1 million tons in 2009, and China accounts for about 41% of the global tilapia production (FAO, 2011, 2012). In addition, the tilapia production in China has continuously expanded at an annual growth rate of approximately 6% since 2000 (FAO, 2011). In southeast China, the tilapia industry has been prosperous for more than a decade, providing an abundant source of fish products, such as tilapia fillets, for consumers around the world. At the same time, a large amount of byproducts (such as skins and bones) from processing these tilapia are discarded, which poses environmental problems and wastes fish resources.

There are reports on extracting skin gelatin from a wide range of fish species, including rainbow trout (Tabarestani, Maghsoudlou, Motamedzadegan, & Mahoonak, 2010; Tammineni et al., 2012), catfish (Yang et al., 2007; Yang, Wang, Zhou, & Regenstein, 2008), croaker and shortfin scad (Cheow, Norizah, Kyaw, & Howell, 2007), snapper (Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka, 2006), Alaska pollock (Zhou et al., 2006), Nile perch (Muyonga, Cole, & Duodu, 2004), and tilapia (Jamilah & Harvinder, 2002; Zeng et al., 2010). Gelatin can be extracted from fish skin using alkaline or acid pretreatment (Giménez, Turnay, Lizarbe, Montero, & Gómez-Guillén, 2005; Jamilah, Tan, UmiHartina, & Azizah, 2011), or a combination of both (Yang et al., 2008; Zeng et al., 2010; Zhou & Regenstein, 2004), followed by thermal

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hydrolysis. Since the combination of alkali and acid pretreatment could provide higher yield of gelatin with better qualities than alkali or acid pretreatment alone, it has become a widely accepted method for fish gelatin extraction over the past decade (Jamilah & Harvinder, 2002; Yang et al., 2007; Zhou & Regenstein, 2004). For example, Gómez-Guillén and Montero (2001) compared the viscoelastic and gelling properties of megrim skin gelatin extracted with alkali and seven different organic acids (0.2 M NaOH, 0.05 M for each acid), though the extraction yields for the treatments were not reported. Zhou and Regenstein (2005) investigated the extraction yield and gel strength of pollock skin gelatin as affected by pretreatment involving 0.1 M of $Ca(OH)_2$ and three acids (acetic, citric, and sulfuric with 0.01–0.1 M of hydrogen ions), and the results were analyzed mainly based upon the pH of gelatin solutions. Zeng et al. (2010) applied response surface methodology to optimize the alkali-acid pretreatment conditions involving the use of various concentrations of NaOH (1–5% w/v) and HCl (0.01–0.09% w/v) for extraction of Nile tilapia skin gelatin, and the highest gelatin extraction rate achieved was 19.3%. Although there are some publications on alkali-acid pretreatments for fish gelatin extraction, due to the diversity in composition of different fish species and the disparity of the gelatin extraction methods (such as acid types and concentration), there is still a lack of systematic study on how the protein yield and physicochemical of gelatin from tilapia skin is affected by different acid pretreatments during extraction; this might be the key to explain the huge difference in extraction yields (5.4–19.3%) of tilapia skin gelatin previously reported (Jamilah & Harvinder, 2002; Jamilah et al., 2011; Zeng et al., 2010).

This study aimed to optimize the extraction process of gelatin from tilapia skin through the use of alkali with different types and concentrations of acids (including citric acid, acetic acid and HCl) before thermal hydrolysis, and to characterize the gelatin by electrophoresis along with the viscosity and film forming properties analyses (including film thickness, mechanical properties, water vapor permeability and transparency). The study could help understand the effects of acid pretreatments on tilapia gelatin extraction efficiency and provide a systematic basis for how extraction affects gelatin properties. This will allow for better utilization of skin and potentially other collagen containing byproducts from tilapia processing.

2. Materials and methods

2.1. Extraction of tilapia skin gelatin

Tilapia skins purchased from Hengfa Aquatic Products Ltd (Guangdong, China) were stored at −50 °C until further use. Frozen skins were thawed at 4 °C for 20 h, scaled and cut into small pieces (about 1×1 cm^2) immediately before gelatin extraction.

The tilapia skin gelatin was extracted based on modified method of Grossman, Gan, Bergman, and Holon (1992). First, tilapia skins (30 g) were soaked in tap water (ratio of skins to water is 1:6 w/v) for 10 min, drained on cheesecloth, and then washed twice (1:6 w/v) to remove the suspended residues. The washing step included shaking (IS-RDD3, Incushaker, Crystal Technology & Industries Inc, USA) the water-skin mixture for 4 min at 180 rpm, and then draining the skins on cheesecloth for 5 min. After the washing, excess water was removed by manually squeezing the skins, then the skins were immersed into 0.3 M NaOH solution (1:6 w/v) for 1 h. Next, the skins were drained for 5 min and washed five times using the procedure described above. After this, fish skins were soaked in a selected acid (1:6 w/v) for 1 h. The acids used included: citric acid (0.01, 0.02, 0.03, 0.04, 0.05, 0.07, 0.10 and 0.20 M), acetic acid (0.01, 0.03, 0.05, 0.10, 0.13, 0.15, 0.18 and 0.20 M) and HCl (0.01, 0.02, 0.03, 0.04, 0.05, 0.07, 0.10 and 0.20 M). The skins were drained and washed five times as described above. Following the acid treatment, the skins were soaked in deionized water (1:4 w/v) at 50 °C in a water bath (HH-6, digital thermostatic water bath, Jinfen Equipment Ltd, Jintan, Jiangsu, China) for 3 h, and then filtered to recover the gelatin-containing solution. The volume of the solution was recorded, and 2 ml solution was used to determine protein yield. The remaining solution was dried (GZX-9146 MBE, digital drying oven, Boxun, Ltd, Shanghai, China) at 50 °C until a constant weight was obtained. For each pretreatment, the processes were repeated twice with duplicate samples each time ($n = 4$).

2.2. Determination of protein yield

The Biuret method of Gornall, Bardawill, and David (1949) was used to determine the protein yield for gelatin extracted from each of the 24 acid pretreatments. Bovine serum albumin purchased from Sigma (catalog No. A8020, St. Louis, MO, USA) was used as the protein standard. The mixture of gelatin solution and Biuret reagent was left to stand at room temperature for 30 min before the absorbance of the mixture at 540 nm was recorded with a UV-3000PC spectrophotometer (Mapada Instrument Ltd, Shanghai, China). Tilapia gelatin was extracted from two different batches of fish skin and run in duplicate.

2.3. Molecular weight distribution in gelatin by gel electrophoresis

Tilapia gelatin solutions (2 mg/ml) were prepared by dissolving dried gelatin into deionized water. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted based upon a modified method of Zhou et al. (2006). Protein molecular weight (MW) marker with 66.4–200 kDa (TAKARA Biotechnology Ltd, Dalian, China) was used as the standard. The loading volume for both gelatin solution and protein marker was 10 μl. Separating gel and stacking gel used were 6% and 5%, respectively, and a constant voltage of 120 V was applied with an electrophoresis system (JY-SCZ2+, JY300C, Beijing Junyi-Dongfang Electrophoresis Equipment Ltd, China). Following the separation, the separating gel was stained with Coomassie brilliant blue R250 (Sinopharm Chemical Reagent Ltd, Shanghai, China) and de-stained (Nalinanon, Benjakul, Visessanguan, and Kishimura, 2008) with results recorded using a BioSpectrum™ 500 Imaging System (Ultra-Violet Products Ltd, Cambridge, UK).

2.4. Viscosity

A 6.67% (w/v) gelatin solution was obtained by dissolving dried gelatin in deionized water at 60 °C. The viscosity of this gelatin solution was determined with a Brookfield LVDV-II+P viscometer (Brookfield Engineering Laboratories Ltd, Middleboro, MA) equipped with an ultra low viscosity adapter (16 ml solution, 20 rpm) (Tabarestani et al., 2010).

2.5. Film preparation

Tilapia gelatin films were prepared by following the method of Jiang, Liu, Du, and Wang (2010) with some modifications. Dried fish gelatin (1% w/v) was dissolved in deionized water and shaken for 1 h at 50 °C. Then, 50 ml of the solution was cast onto a Petri dish (dia, 150 mm) and placed in an environmental chamber (SH-241, Bench-top Type, ESPEC Corp, Japan) for 48 h at 23 °C and 50% relative humidity. Following this, the film was carefully peeled off the dishes, and placed into the environmental chamber for 24 h before further testing.