LWT - Food Science and Technology 66 (2016) 186–192

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Gelatin from clown featherback skin: Extraction conditions

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ARTICLE INFO

Article history:
Received 30 June 2015
Received in revised form
8 October 2015
Accepted 9 October 2015
Available online 23 October 2015

Keywords:
Gelatin
Extraction time
Extraction temperature
Clown featherback
Chitala ornata

ABSTRACT

Gel properties of gelatin from clown featherback skin as affected by different extraction temperatures (45, 65 and 85 °C) and times (6 and 12 h) were determined. The gelatin recovery was found in the range of 73.99–95.85 g/100 g. Gelatin had glycine as major amino acid, followed by alanine and proline. Gel strength generally decreased as extraction temperature and time increased. With increasing temperature and time, band intensity of α-, β- and γ-chains decreased. Gelling temperature (15.53–24.71 °C) and gelling time (11.62–49.27 min) varied, depending on extraction condition. All gelatin could set at 25 °C, except those extracted at 65 °C for 12 or at 85 °C for 6 and 12 h. Gelatin extracted at 45 °C for 6 and 12 h showed higher gel strength, compared bovine gelatin ($P < 0.05$). Therefore, properties of gelatin from clown featherback skin could be influenced by extraction condition.

1. Introduction

Clown featherback (*Chitala ornata*) is freshwater fish, which is commonly found in Thailand. It has been generally used for fish ball and fish cake production since its meat is white and has good gel forming ability. During dressing, skins (17–22 g/100 g of total weight) are removed. Currently, the skin has been processed as the crispy fried fish skin. However, the market value is still low. The production of high-value products could pave the way for gaining the higher benefit or revenue. The skins are rich in collagen can serve as raw material for production of gelatin (Foegeding, Lanier, & Hultin, 1996; Wong, 1989), Gelatin is biopolymer obtained from partial denaturation of collagen. It has a wide range of applications in food and non-food (photographic, cosmetic and pharmaceutical) industries (Regenstein & Zhou, 2007). Generally, the skin and bone of bovine and porcine are the major sources for gelatin production. Due to the religious restrictions of Muslim, Jew and Hindu, an alternative source for gelatin production, especially from skin, bone and scale from fish processing by-products, have gained attention (Benjakul, Kittiphattanabawon, & Regenstein, 2012). However, fish gelatin has limited applications owing to some limitations. Fish gelatin has been known to show poorer gel properties than mammalian counterparts. Additionally, fish gelatin is not able to set at room temperature. The gel properties are mainly governed by imino acid content (proline and hydroxyproline contents) and molecular weight distribution, chain length of α-, β- and γ-chains (Muyonga, Cole, & Duodu, 2004a). When harsh extraction condition was implemented, gelatin with higher content of imino acid content might show the poor gel properties (Cho, Gu, & Kim, 2005). However, gelatin extracted with milder condition with the superior gel properties mostly shows the low yield (Kittiphattanabawon, Benjakul, Visessanguan, & Shahidi, 2010). As a consequence, the extraction conditions for gelatin production should be optimised. So far, gelatin from the skin of freshwater fish has been extracted at varying temperatures and times from African catfish (Alfaro, Biluca, Marquetti, Tonial, & de Souza, 2014), seabass (Sinthusamran, Benjakul, & Kishimura, 2014), wami tilapia (da Trindade Alfaro, Fonseca, Balbinot, de Souza, & Prentice, 2014), farmed giant catfish (Jongjareonrak et al., 2010) and channel catfish (Yang et al., 2007). Nevertheless, there is no information regarding gelatin from the skin of clown featherback, a freshwater fish widely used for fish ball production in Thailand, as influenced by extraction conditions. Therefore, the aim of this study was to extract and characterise gelatin from skin of clown featherback using different extraction temperatures and times.

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http://dx.doi.org/10.1016/j.lwt.2015.10.029
0023-6438/

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Sodium dodecyl sulphate (SDS), Coomassie Blue R-250, and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). High-molecular-weight marker was purchased from GE Healthcare UK Limited (Buckinghamshire, UK). Food grade bovine bone gelatin with the gel strength of 150–250 g was obtained from Halagel (Thailand) Co., Ltd., (Bangkok, Thailand).

2.2. Preparation of clown featherback skin

Skin of clown featherback (*C. ornata*) with the weight of 0.7–1.5 kg was obtained from a local fish ball and fish cake processing plant at Talaadthai in Pathumthani province, Thailand. The skin was prepared following the method of Kittiphattanabawon et al. (2010). The prepared skin was placed in polyethylene bags (50–100 g/bag) and stored at −20 °C until used but not longer than 3 months. The moisture content of prepared skin was 65.72 g/100 g as determined by AOAC method (AOAC, 2000). Prior to collagen extraction, the frozen skin was thawed with running water until the core temperature of the skin reached 8–10 °C.

2.3. Extraction of gelatin from clown featherback skin

Gelatin from the skin was extracted following the method of Kittiphattanabawon et al. (2010). Firstly, the skin was mixed with 0.1 mol/L NaOH at a sample to solution ratio of 1:10 (mass/volume ratio) and the mixture was stirred for 2 h at 15–20 °C to remove non-collagenous proteins. The alkali solution was changed every 40 min. The pretreated skin was washed with tap water until the neutral pH of wash water was obtained. The skin was subsequently swollen by mixing with 0.05 mol/L acetic acid at a sample to solution ratio of 1:10 (mass/volume ratio) and stirred for 30 min at room temperature (26–28 °C). Stirring was carried out using an overhead stirrer (model W20.n, IKA®-Werke GmbH & CO.KG, Stanfen, Germany) at a speed of 250 rpm. After swelling process, the residue was washed thoroughly with tap water until pH of wash water became neutral.

To extract the gelatin, the swollen skin was mixed with distilled water at different temperatures (45, 65 and 85 °C) using a sample to water ratio of 1:2 (mass/volume ratio). The mixtures was continuously stirred using an overhead stirrer at a speed of 150 rpm for 6 h and 12 h under designated temperatures, followed by filtration with two layers of cheesecloth. Then, 100 mL of filtrates were mixed with 1 g of activated carbon. The mixtures were then filtered using a Buchner funnel with Whatman No.4 filter paper (Whatman International, Ltd., Maidstone, England), followed by freeze-drying using a freeze-dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark). The obtained gelatin samples were calculated for recovery and subjected to analysis.

2.4. Recovery of gelatin

Recovery of gelatin obtained was calculated based on the weight of starting raw material using the following equation:

$$\text{Recovery (g/100 g)} = \frac{\text{Hyp content in skin (mg/g skin)} \times \text{skin used for extraction (g)}}{\text{Hyp content in gelatin (mg/g gelatin)} \times \text{gelatin obtained (g)}} \times 100$$

Hydroxyproline (Hyp) contents in the skin and gelatin were determined according to the method of Bergman and Loxley (1963).

2.5. Characterisation of gelatin

2.5.1. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed by the method of Laemmli (1970). The sample preparation and electrophoretic conditions were performed according the method of Kittiphattanabawon et al. (2010). High-molecular-weight protein markers (GE Healthcare UK Limited, Buckinghamshire, UK) were used to estimate the molecular weight of proteins.

2.5.2. Fourier transform infrared (FTIR) spectroscopic analysis

FTIR spectra of samples were obtained using a Bruker model EQUINOX 55 FTIR spectrometer (Bruker, Ettlingen, Germany). The measuring condition and analysis of data were performed by the method of Kittiphattanabawon et al. (2010).

2.5.3. Amino acid analysis

Gelatin samples were hydrolysed under reduced pressure in 4.0 mol/L methane sulphonic acid containing 2 g/L 3-2(2-aminoethyl)indole at 115 °C for 24 h. The hydrolysates were neutralised with 3.5 mol/L NaOH and diluted with 0.2 mol/L citrate buffer (pH 2.2). An aliquot of 0.4 mL was applied to an amino acid analyser (MLC-703; Atto Co., Tokyo, Japan).

2.6. Determination of gel properties

2.6.1. Colour of gel

The colour of gelatin gels (66.7 g/L) was measured with a Hunter lab colourimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA) in term of L^*, a^* and b^* values.

2.6.2. Turbidity

Gelatin solution (66.7 g/L) was poured into 1 cm cuvette at room temperature. The turbidity of gelatin solution was measured using spectrophotometer (model UV-1800, Shimadzu, Kyoto, Japan) at 600 nm against distilled water.

2.6.3. Gel strength

Gel strength was determined according to the British Standard 757: 1975 method (BSI, 1975).

2.6.4. Gelling temperature and time

Gelatin solution (66.7 g/L) was prepared in the same manner as described in the method of Sinthusamran et al. (2014). The gelling time and temperature were measured using a controlled stress rheometer (HAAKE RheoStress 1, Karlsruhe, Germany) equipped with a cone/plate geometry (diameter of 35 mm, 1°).

The gelling temperatures of the gelatin samples were measured following the method of Sinthusamran et al. (2014) with a slight modification. For gelling time measurement, the temperature of gelatin solution was fixed at 25 °C and the same measurement conditions for gelling temperature were used.

Plot of phase angle as a function of temperature and time was prepared and used for determination gelling temperature and time,