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Molecular and functional properties of gelatin from the skin of unicorn leatherjacket as affected by extracting temperatures

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ABSTRACT

Gelatins extracted from the skin of unicorn leatherjacket at different temperatures (45, 55, 65 and 75 °C) in the presence and the absence of soybean trypsin inhibitor (SBTI; 100 units/g pretreated skin) for 12 h were characterised. In general, the addition of SBTI resulted in the lower yield, regardless of extraction temperature. Higher yield was obtained when higher extraction temperature was used ($P < 0.05$). Gelatin from skin extracted at 75 °C in the absence of SBTI showed the highest yield (10.66 ± 0.41%) (based on dry weight). The highest α-amino group content was observed in gelatin extracted at 55 °C without SBTI incorporated. The band intensity of β-chain and α-chains increased as the extraction temperature increased, particularly above 55 °C. Gelatin extracted at 65 °C with and without SBTI incorporation exhibited the highest gel strength (178.00 ± 7.50 g and 170.47 ± 1.30 g, respectively). FTIR spectra indicated that a greater loss of molecular order of triple helix with a higher degradation was found in gelatin extracted at 55 °C in the absence SBTI. Gelatin extracted at 65 °C, either with or without SBTI, had the highest EAI and ESI with high foam expansion and stability. Thus, the extraction of gelatin from the skin of unicorn leatherjacket at temperature sufficiently high could render the gelatin with less degradation.

1. Introduction

Gelatin, the denatured form of collagen, has been widely used in the food industry, pharmaceuticals, photography and in other technical applications (Kittiphattanabawon, Benjakul, Visessanguan, Nagai, & Tanaka, 2005). Generally, gelatin is produced from skins and skeletons of land animals (Gilsenan & Ross-Murphy, 2000). However, the occurrence of bovine spongiform encephalopathy (BSE) and foot-and-mouth disease (FMD) has caused major concerns for human health. Furthermore, porcine and bovine gelatins are prohibited for some religions (Sadowska, Kolodziejska, & Niecikowska, 2003). Therefore, fish processing by-products, especially skin, which contains 30% of the total material, have become a potential raw material alternative for gelatin production (Shahidi, 1994).

Unicorn leatherjacket (*Aluterus monoceros*) belongs to the order Tetraodontiformes and is a member of the Monacanthidae family (Ahmad & Benjakul, 2011a). This species has been used for fillet production in Thailand and other countries in South-East Asia. As a consequence, a large amount of skin has been produced as a by-product, which can be further used for gelatin production. This leads to the increase in revenue for the fish processing industry.

Indigenous proteases play a vital role in hydrolysis of polypeptide, contributing to the decrease in functional properties of resulting gelatin (Ahmad, Benjakul, Ovissipour, & Prodpran, 2011c). These enzymes are bound with matrix components such as collagens (Woessner, 1991). Recently, Ahmad and Benjakul (2011b) reported that the use of soybean extract containing trypsin inhibitor during extraction at 50 °C was able to prevent the degradation of gelatin to some degree, suggesting the remaining proteases in the skin. Hence, the inactivation of those proteases using a sufficiently high temperature, without thermal degradation, might be another approach to maintain those chains, leading to the improved functional properties of the resulting gelatin. As a consequence, gelatin from marine sources can be utilised more widely as a food ingredient or for other applications. Nevertheless, there is no information about the impact of extraction temperatures, particularly in conjunction with the use of protease inhibitor, on properties of gelatin from unicorn leatherjacket skin. Therefore, the objective of this investigation was to study the impact of extraction temperature on chemical composition and functional properties of gelatin from the skin of unicorn leatherjacket in the presence and absence of protease inhibitor.

2. Materials and methods

2.1. Chemicals, collagen and gelatin

All chemicals were of analytical grade. Phosphoric acid was obtained from Lab-Scan (Bangkok, Thailand). Sodium dodecyl

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sulphate (SDS) and Coomassie Blue R-250 were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Type I collagen from calf skin was purchased from Elastin Products Co., Inc. (Owensville, MO, USA). Food grade bovine bone gelatin was obtained from Halagel (Thailand) Co., Ltd. (Bangkok, Thailand).

2.2. Collection and preparation of fish skin

The skin of unicorn leatherjacket (*A. monoceros*) was obtained from a dock, Songkhla, Thailand. Three different lots of skin were collected. For each lot, all skins were pooled and used as the composite sample. The sample was stored in ice with a skin/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. Upon arrival, the skin was washed with iced tap water (0–2 °C) and cut into small pieces (0.5×0.5 cm^2), placed in polyethylene bags and stored at −20 °C until use. The storage time was less than 2 months.

2.3. Preparation of soybean extract containing trypsin inhibitor

Soybean was ground using a blender (Model MX-T2GN, National, Taipei, Taiwan). The seed flour was defatted by mixing with hexane at the ratio of 1:5 (w/v) for 10 min. The mixture was filtered through a Whatman No.1 filter paper and the retentate was rinsed with hexane 3 times to remove the residual oil. The defatted sample was air-dried at room temperature (28–30 °C) until dry and free of hexane odour.

To extract trypsin inhibitor, defatted sample was mixed with 0.15 M NaCl with a ratio of 1:10 (w/v). The mixture was shaken at 180 rpm at room temperature for 3 h using a shaker (Heidolph UNIMAX 1010, Schwabach, Germany). The supernatant was recovered by centrifuging the mixture at 5000*g* for 30 min. Thereafter, the supernatant was heated at 90 °C for 10 min and then cooled using iced water. The mixture was then centrifuged at 8000*g* for 15 min. The resulting supernatant was freeze-dried using a Model Coolsafe 55 freeze dryer (Scanvac, Coolsafe, Lynge, Denmark) and the obtained powder was used as crude trypsin inhibitor. Crude trypsin inhibitor was subjected to the measurement of trypsin inhibitor as described by Benjakul, Visessanguan, and Thummaratwasik (2000). One unit of trypsin inhibitor activity was defined as the amount of inhibitor which reduced trypsin activity by one unit. Activity of trypsin was determined using BAPNA as a substrate and the absorbance at 410 nm due to *p*-nitroaniline released was measured. One unit of trypsin was defined as an increase of 0.01 absorbance unit/min at pH 7 and 37 °C.

2.4. Non-collagenous protein removal and swelling of skin

Removal of non-collagenous proteins and swelling were carried out according to the method of Ahmad and Benjakul (2011a) with a slight modification. Fish skin (0.5×0.5 cm^2) was soaked in 0.05 M NaOH with a skin/alkaline solution ratio of 1:10 (w/v) to remove non-collagenous proteins. The mixture was stirred continuously for 4 h at room temperature using an overhead stirrer equipped with a propeller (RW 20.n, IKA Labortechnik, Germany) at a speed of 150 rpm. The alkaline solution was changed every 2 h. Alkaline-treated skin was washed with tap water until neutral or faintly basic pH of wash water was obtained. The alkaline-treated skin was soaked in 0.1 M phosphoric acid with a skin/solution ratio of 1:10 (w/v) for 12 h with a gentle stirring at room temperature. The acidic solution was changed every 6 h. Acid-treated skin was washed thoroughly with tap water until wash water became neutral or faintly basic.

2.5. Extraction of gelatin

The swollen skin was mixed with distilled water at a ratio of 1:5 (w/v) at different temperatures (45, 55, 65 and 75 °C) in the absence and the presence of trypsin inhibitor at a level of 100 units trypsin inhibitor/1 g pretreated skin (Ahmad et al., 2011b). The mixture was incubated in a temperature controlled water bath (Memmert, Schwabach, Germany) and stirred continuously for 12 h. The extract was centrifuged at 5,000*g* for 10 min to remove insoluble material. The supernatant was collected, freeze-dried and subjected to analyses.

2.6. Analyses

2.6.1. Determination of yield and recovery

Gelatin yield was calculated by the following equation:

$$\text{Yield}\% = [\text{weight of dry gelatin(g)}/\text{weight of initial skin(g)}] \times 100$$

Recovery of gelatin was calculated as follows:

$$\text{Recovery}(\%) = [\text{hydroxyproline content of supernatant(g/ml)} \times \text{volume of supernatant(ml)}]/[\text{hydroxyproline content of initial skin(g/g)} \times \text{weight of initial skin(g)}] \times 100$$

2.6.2. Determination of chemical composition

2.6.2.1. Hydroxyproline content. Hydroxyproline content was analysed according to the method of Bergman and Loxley (1963). Hydroxyproline content was calculated and expressed as mg/g sample.

2.6.2.2. α-amino group content. The α-amino group content was determined according to the method of Benjakul and Morrissey (1997). Properly diluted sample (125 μl) was mixed thoroughly with 2.0 ml of 0.2125 M phosphate buffer, pH 8.2, followed by the addition of 1.0 ml of 0.01% TNBS solution. The mixture was then placed in a temperature controlled water bath at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulphite. The mixture was cooled down at room temperature for 15 min. The absorbance was measured at 420 nm using a double beam spectrophotometer (model UV-1800, Shimadzu, Kyoto, Japan) and α-amino group content was expressed in terms of L-leucine.

2.6.2.3. Protein patterns. SDS–PAGE was performed by the method of Laemmli (1970). The samples were dissolved in 5% SDS and the mixtures were incubated at 85 °C for 1 h. Solubilised samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris HCl, pH 6.8, containing 4% SDS and 20% glycerol). The mixtures were boiled in boiling water for 2 min. Samples (15 μg protein) were loaded onto polyacrylamide gels comprising a 7.5% running gel and a 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA/gel using a Mini Protein II unit. After electrophoresis, gel was stained with 0.05% (w/v) Coomassie blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% (v/v) acetic acid. Type I collagen was used as a standard.

2.6.3. Determination of functional properties

2.6.3.1. Gel strength. Gelatin gel was prepared as per the method of Fernández-Díaz, Montero, and Gómez-Guillén (2001) with a slight modification. Gelatin sample was dissolved in distilled water at 60 °C to obtain the final concentration of 6.67% (w/v). The gelatin solution was stirred until the gelatin was solubilised completely and cooled in a refrigerator at 10 °C for 16–18 h for gel maturation.