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Characteristics and functional properties of gelatin from splendid squid (*Loligo formosana*) skin as affected by extraction temperatures

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ABSTRACT

Gelatin was extracted from the skin of splendid squid (*Loligo formosana*) at different temperatures (50, 60, 70 and 80 °C) with extraction yield of 8.8%, 21.8%, 28.2%, and 45.3% (dry weight basis) for G50, G60, G70 and G80, respectively. Gelatin from the skin of splendid squid had a high protein content (~90%) with low moisture (8.63–11.09%), fat (0.22–0.31%) and ash contents (0.17–0.68%). Gelatin extracted at higher temperature (G80) had a relatively higher free amino group content than gelatin extracted at lower temperatures (G50, G60 and G70) ($P < 0.05$). All gelatins contained α- and β-chains as the predominant components. Amino acid analysis of gelatin revealed the high proline and hydroxyproline contents for G50 and G60. FTIR spectra of obtained gelatins revealed the significant loss of molecular order of the triple-helix. The gel strength of gelatin extracted at lower temperature (G50) was higher than that of gelatins extracted at higher temperatures including G60, G70 and G80, respectively. The net charge of G50, G60, G70 and G80 became zero at pHs of 6.84, 5.94, 5.49, and 4.86, respectively, as determined by zeta potential titration. Gelatin extracted at higher temperature (G80) had the lower L^* value but higher a^* and b^* values, compared with those extracted at lower temperatures ($P < 0.05$). Emulsion activity index decreased, whilst emulsion stability index, foam expansion and stability increased as the concentration (1–3%) increased ($P < 0.05$). Those properties were governed by extraction temperatures of gelatin. Thus gelatin can be successfully extracted from splendid squid skin using the appropriate extraction temperature.

1. Introduction

Fish gelatin, partially hydrolysed form of collagen, has been given increasing attention as the alternative of land animal gelatin due to religious constraint. Both Judaism and Islam forbid the consumption of any pork-related products, while Hindus do not consume cow-related products (Karim & Bhat, 2009). In addition, there is increasing concern whether land animal tissue-derived collagens and gelatins are capable of transmitting pathogenic vectors such as prions (Wilesmith, Ryan, & Atkinson, 1991). As a consequence, fish gelatin has gained increasing interest as the potential alternative for land animal counterpart. Due to the abundance of skin, fin, scale and bones, etc., which are the by-product from the fish processing industry, it would be of full benefit to fully utilize those resources as promising raw material for gelatin production (Ahmad & Benjakul, 2011; Gomez-Guillen et al., 2002; Muyonga, Cole, & Duodu, 2004a).

Squid and cuttlefish have become an important fishery product in Thailand as well as other Southeast Asian countries, and are mainly exported worldwide (Hoque, Benjakul, & Prodpran, 2010). During processing, skin is generated as a by-product with the low market value and it can create serious ecological problems and environmental pollution without inappropriate management. Squid wastes and by-products are rich in collagen (Brinckmann, 2005). Cuttlefish skin was used for gelatin extraction (Aewsiri, Benjakul, Visessanguan, Wierenga, & Gruppen, 2011). Additionally, gelatin was extracted from skin of giant squid, *Dosidicus gigas* (Gimenez, Aleman, Montero, & Gomez-Guillen, 2009; Uriarte-Montoya et al., 2011).

Gelatin can be used as a foaming, emulsifying and wetting agent in food, pharmaceutical, medical and technical applications due to its surface-active properties (Balti et al., 2011). Gelatins from fish

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and cuttlefish skin have been demonstrated to render the biodegradable films with the superior UV barrier properties (Houqe, Benjakul, & Prodpran, 2011; Rattaya, Benjakul, & Prodpran, 2009). Gelatin from cuttlefish skin had the improved emulsifying property when modified by oxidized fatty acids (Aewsiri et al., 2011). Functional properties of gelatin and other food proteins are governed by many factors such as chain length or molecular weight, amino acid composition and hydrophobicity, etc (Gomez-Guillen et al., 2002).

Owing to the abundance of squid skin in Thailand, it can be used for gelatin extraction. However, very low yield of gelatin (2.6%) was obtained from skin of giant squid, *D. gigas* (Gomez-Guillen et al., 2002). Increasing extraction temperatures might be an effective means to increase the yield; however, the functional properties can be affected differently. Therefore, this study aimed to investigate the impact of extraction temperatures on yield, characteristics and functional properties of gelatin extracted from the skin of splendid squid (*Loligo formosana*) caught in Thailand.

2. Materials and methods

2.1. Chemicals

β-Mercaptoethanol (β-ME) and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). High molecular weight markers were purchased from GE Healthcare UK (Buckinghamshire, UK). Folin Ciocalteu's phenol reagent, acetic acid and phosphoric acid were purchased from Merck (Darmstadt, Germany). Sodium dodecyl sulphate (SDS), Coomassie Blue R-250 and *N,N,N′,N′*-tetramethyl ethylene diamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA).

2.2. Collection and preparation of squid skin

The outer skin of fresh squid (*L. formosana*) was obtained from Sea Wealth Frozen Food Co., Ltd., Songkhla, Thailand and stored in ice using a skin/ice ratio of 1:2 (w/w). Upon arrival to the Department of Food Technology, Prince of Songkla University, Hat Yai, Thailand, the skin was cleaned and washed with iced tap water (0–2 °C). Skin contained 83% of moisture, 14% of protein, 1.1% of ash and 1.5% of fat as determined by the method of AOAC (2000). The skin was then 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 not more than 2 months.

2.3. Extraction of gelatin from squid skin

Gelatin extraction was performed following the method of Ahmad and Benjakul (2011) with slight modification. Before gelatin extraction, the prepared skin was soaked in 0.05 M NaOH with a skin/solution ratio of 1:10 (w/v). The mixture was stirred continuously for 6 h at room temperature at a speed of 150 rpm using an overhead stirrer equipped with a propeller (RW 20.n, IKA-Werke GmbH & CO.KG, Staufen, Germany). The alkaline solution was changed every 90 min to remove non collagenous proteins and pigments. Alkaline-treated skin was then washed with tap water until the neutral or faintly basic pH of wash water was obtained. The skin was then soaked in 0.05 M phosphoric acid with a skin/solution ratio of 1:10 (w/v) for 24 h with gentle stirring at 4 °C. The acidic solution was changed every 12 h to swell the collagenous material in the skin matrix. Acid-pretreated skin was washed thoroughly with tap water until wash water became neutral or faintly basic. To extract gelatin, the swollen skin was soaked in distilled water with different temperatures (50, 60, 70 and 80 °C) using a skin/water ratio of 1:10 (w/v) in a temperature-controlled water bath (W350, Memmert, Schwabach, Germany) for 12 h with a continuous stirring at a speed of 150 rpm. The mixture was then filtered using two layers of cheese cloth. The filtrate was further filtered using a Whatman No. 4 filter paper (Whatman International, Ltd., Maidstone, England) with the aid of JEIO Model VE-11 electric aspirator (JEIO TECH, Seoul, Korea). The resultant filtrate was freeze-dried using a Scanvac Model Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark). The dry matter extracted from skin at different temperatures was referred to as 'G50', 'G60', 'G70' and 'G80', respectively. All gelatin samples were weighed, calculated for extraction yield and subjected to analyses. The yield of gelatin was calculated based on dry weight of skin.

$$\text{Yield}\left(\%\right) = \frac{\text{Weight of freeze dried gelatin (g)}}{\text{Weight of dry skin (g)}} \times 100$$

2.4. Analyses

2.4.1. Determination of proximate composition

Moisture, protein, ash and fat contents were determined following the methods of AOAC (2000) with the analytical Nos. of 950.46, 928.08, 920.153 and 960.39, respectively.

2.4.2. Electrophoretic analysis and free amino group content

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (1970). Gelatin samples were dissolved in 5% SDS and the mixtures were incubated at 85 °C for 1 h. The mixtures were centrifuged at 3500 × g for 5 min at room temperature using a microcentrifuge (MIK-RO20, Hettich Zentrifugan, Tuttlingen, Germany) to remove undissolved debris. Gelatin samples were mixed at a 1:1 (v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 4% SDS and 20% glycerol). Samples (l5 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 (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After electrophoresis, the 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. High molecular weight markers at the range of 53 kDa–220 kDa (GE Healthcare UK, Buckinghamshire, UK) were used. Relative mobility (R_f) of protein band was calculated and the molecular weight of the protein was calculated from the plot between R_f and log (MW) of standards.

Free amino group content was determined following the method of Benjakul and Morrissey (1997). Properly diluted samples (125 μl) were 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% 2,4,6-trinitrobenzenesulfonic acid (TNBS) solution. The mixtures were 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 mixtures were 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 the free amino group content was expressed in terms of L-leucine.

2.4.3. Amino acid analysis

Amino acid composition of gelatin samples was analysed according to the method of Ganno, Hamano, and Kobayashi (1985) with a slight modification. Gelatin samples were hydrolysed under reduced pressure in 4 M methanesulphonic acid containing 0.2% (v/v) 3-2(2-aminoethyl) indole at 115 °C for 24 h. For analyzing the tryptophan content, gelatin samples were hydrolysed by 3 N mercaptoethanesulphonic acid to avoid the decomposition of