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Chemical and biophysical properties of gelatins extracted from the skin of octopus (*Octopus vulgaris*)

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ABSTRACT

Gelatins from alkali-pre-treated skin of Octopus (*Octopus vulgaris*) were extracted with different concentrations of pepsin at pH 2.0. The resulting octopus skin gelatins OSG0, OSG5, OSG10 and OSG15, extracted, respectively, without enzyme treatment or with 5, 10 and 15 U of pepsin/g alkaline treated skin were evaluated for gel strength, textural parameters, thermal and gelling properties. The yield of gelatin extracted without enzymatic pretreatment was only 3.21% and the addition of pepsin (15 U/g) increased the yield of gelatin extraction to 7.78%. Molecular weight distribution of gelatins indicates that OSG10 and OSG15 contain peptides with molecular weights less than 10 kDa (>40%). In addition, Fourier transform infrared (FTIR) spectra of extracted gelatins were slightly different, indicating that the triple helical structure of gelatins was affected by pepsin treatment. Compared to OSG0, pepsin gelatins exhibited lower gel strength, hardness, adhesiveness, transition, gelling and melting temperatures and all of the values decreased with increasing enzyme concentration. In addition foam expansion (FE) was affected by enzyme treatment, and the values decreased as pepsin increased. However, the emulsifying activity index (EAI) values of all gelatins were the same. Further, FE and EAI increased with increasing concentrations (1–3%, w/v). The results showed that octopus skin can be a good source for gelatin.

1. Introduction

Gelatin is an important functional biopolymer widely used in food, pharmaceutical and cosmetic industries as stabilizing, thickening and gelling agent (Boran, Mulvaney, & Regenstein, 2010; Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011; Kittiphattanabawon, Benjakul, Visessanguan, & Shahidi, 2010). Gelatin is commercially made from the skin or bone of porcine and bovine by alkaline or acidic treatment (Gómez-Guillén et al. 2002).

Waste from fish processing are generally considered as low-value resources with negligible market value. It has been reported that about 30% of such wastes comprise of bone and skin are very rich in collagen, the precursor of gelatin (Gómez-Guillén et al. 2002). In this context, fish gelatin has gained increasing interest as the potential alternative of animal gelatins (Kittiphattanabawon et al. 2010). Several studies reported the extraction and characterization of skin gelatins from several fish species such as silver carp (Boran et al., 2010), grey triggerfish (Jellouli et al., 2011), giant squid (Uriarte-Montoya et al., 2011), splendid squid (Nagarajan, Benjakul, Prodpran, Songtipya, & Kishimura, 2012), unicorn leatherjacket (Ahmad & Benjakul, 2011; Kaewruang, Benjakul, & Prodpran, 2013), cuttlefish (Jridi et al., 2013), zebra blenny (Ktari et al., 2014) and thornback ray (Lassoued et al., 2014).

The quality of fish gelatin is largely determined by its gelling strength, rheological properties and thermal stability. The amino acid composition of gelatin is indeed species-specific, but the molecular weight distribution mainly depends on the extraction process (Aewsiri, Benjakul, & Visessanguan, 2009; Boran et al., 2010; Gómez-Guillén et al. 2002; Nagarajan et al., 2012). Nalinanon, Benjakul, Visessanguan, and Kishimura (2008) and Jridi et al. (2013) demonstrated that enzymatic extraction increases the yield of gelatin extraction. Furthermore, pepsin has been reported to solubilize the native collagen in the skin matrix during the acid-swelling process, by cleaving some peptide bonds, resulting in a higher content of peptides with lower molecular weight.

Octopus (*Octopus vulgaris*) has been used for industry transformation in Tunisia and the skin, generated as by-products with a low market value, could be a source of many value-added products, especially gelatin. So far no gelatin has been extracted from the skin of this species and no information on its properties is available.

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Therefore, the objective of this work was to extract gelatin from skin of Octopus (*O. vulgaris*) using different concentrations of pepsin, and to study their physicochemical characteristics and functional properties.

2. Materials and methods

2.1. Chemicals

Bovine hemoglobin, trichloroacetic acid (TCA), glycine and ammonium sulphate were purchased from Sigma Chemical Co. (St. Louis MO, USA). Sodium dodecyl sulphate (SDS), acrylamide, ammonium persulphate, *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED), Coomassie Brilliant Blue R-250 were from Bio-Rad Laboratories (Hercules, CA, USA). Porcine pepsin (Lyophilized powder, 3000 units/mg protein) was purchased from MP Biomedicals (France). Other chemicals and reagents used were of analytical grade.

2.2. Preparation of octopus skin

Skin from Octopus (*O. vulgaris*) was obtained from CALEMBO Company, Sfax City Tunisia. The samples were placed in ice with a skin/ice ratio of approximately 1:3 (w/w) and transported to the laboratory within 20 min. Upon arrival, skins were immediately washed with cold tap water. Cleaned skins were then placed in polyethylene bags and stored at −20 °C until use. The storage time was less than one week.

2.3. Extraction of gelatin

Gelatins were extracted from the prepared Octopus skin according to the method of Jridi et al. (2013). Frozen skins were cut into small pieces (1 cm × 1 cm) and then mixed with 0.05 M NaOH for 1 h at 4 °C with continuous stirring at a sample/alkaline solution ratio of 1:5 (w/v), to remove non-collagenous proteins, solution was changed every 20 min. The alkaline-treated skins were then washed with cold distilled water until the neutral pH of wash water was obtained. The alkaline-treated and washed skins were then soaked in 100 mM glycine-HCl buffer (pH 2), with a solid/solvent ratio of 1:5 (w/v) for 10 h at 4 °C with continuous stirring in the absence or presence of pepsin at different levels 0, 5, 10 and 15 units/g of alkaline treated skin. The pH of the mixtures was then raised to 7.0 using 10 M NaOH, and stirred gently for 1 h at 4 °C. To extract the gelatin, treated skin mixtures were then incubated at 40 °C for 4 h with continuous stirring. The mixtures were centrifuged at 10,000 × g for 30 min at 4 °C, using a refrigerated centrifuge to remove insoluble material. Finally, the supernatants were freeze-dried (Moduloyd Freeze dryer, Thermo Fisher, USA). Gelatins obtained using different levels of pepsin 0, 5, 10, and 15 U/g of skin were referred to as OSG0, OSG5, OSG10 and OSG15, respectively.

2.4. Analysis of gelatin

2.4.1. Calculation of gelatin yield and proximate analysis

The gelatin yield was calculated as follows:

$$\text{Yield}(\%) = \text{weight of dried gelatin(g)} \times 100/\text{weight of dry matter of Octopus skin(g)}.$$

Gelatins were subjected to proximate analysis, including moisture, protein, lipid and ash contents, according to the AOAC (2000) method No: 950.64, 928.08, 963.39 and 920.153, respectively.

2.4.2. Color evaluation

The color of gelatin samples was determined using a ColorFlex spectrocolorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA). The instrument was standardized using standard white plates. CIE lightness (L^*), redness (a^*) and yellowness (b^*) were recorded. The sample was filled in a 64 mm glass sample cup with three readings in the same place and triplicate determinations were taken per sample.

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

Gelatins were analyzed by SDS-PAGE according to the method of Laemmli (1970). Gelatin solutions were prepared by dissolving the dried gelatins in distilled water for 30 min at 60 °C at final concentration of 1 mg/ml, and then mixed with the loading buffer (2% SDS, 5% β-mercaptoethanol, and 0.002% bromophenol blue) in a proportion of 1:4 (v/v). Sample (30 μg of gelatin) was heat-denatured for 5 min at 90 °C and loaded onto an SDS-PAGE using 4% (w/v) stacking and 7% (w/v) resolving gels. After electrophoresis, the gel was stained with 0.1% Coomassie brilliant blue R-250 dissolved in (15%) methanol and (5%) trichloroacetic acid, and finally destained with methanol: distilled water: acetic acid, solution at a ratio of 5:4:1. High molecular weight markers (GE Healthcare UK Limited, Buckinghamshire, UK) were used to estimate the molecular weight of gelatin samples.

2.4.4. Molecular weight analysis by FPLC

The molecular weight analysis of gelatin was carried out by Fast Protein Liquid Chromatography (FPLC), using a Silica gel packed in a TSKgel G2000SWXL column (7.8 mm I.D × 30 cm L). The eluant used was phosphate buffer (0.1 M) containing 0.2 M sodium chloride filtered through whatman cellulose acetate membrane (0.2 μm). The flow rate was adjusted to 0.6 ml/min. Gelatin was loaded to the column at a concentration of 6 mg/ml. Standard molecular weight markers supplied by Sigma Chemicals (Bovine serum albumin (66 kDa), β-amylase (200 kDa), apoferritin (443 kDa) and Blue Dextran (2000 kDa)) were loaded separately at a concentration of 4 mg/ml. A calibration curve was obtained by plotting log molecular weight vs peak elution time. The average molecular weight of gelatin was determined from the standard curve.

2.4.5. Determination of amino acid composition

Gelatins were totally hydrolyzed with 6 N HCl (at final concentration of 5 mg/ml) at 110 °C for 24 h on a heating block, and then filtered through a 0.45 μm membrane filter prior to analysis. 10 μL of the treated sample was derivatized using 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate Waters AccQ Fluor Reagent Kit (according to Waters AccQ Tag Chemistry Package Instruction Manual).

The amino acid composition of the gelatins was analysed on a Waters 2996 Separation Module (Photodiode Array Detector) equipped with a Waters 2475 multi-wavelength fluorescence detector and amino acids were separated on a Waters AccQ Tag amino acid analyzing Column (Nova-Pak C_{18}, 150 × 3.9 mm). The amount of amino acids was calculated, based on the peak area in comparison with that of amino acids standard (Accq tag chemistry kits (WAT088122)). The amino acid content was expressed as amino acid/1000 amino acids in the sample. All analyses were performed in duplicate.

2.4.6. Determination of gel strength and textural parameter analysis (TPA)

Gel strengths of Octopus skin gelatin gels were determined according to the method of Gómez-Guillén et al. (2002), using 6.67% gels (w/v) prepared by dissolving the dried gelatin in distilled water