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# Characteristics and functional properties of gelatin from thornback ray skin obtained by pepsin-aided process in comparison with commercial halal bovine gelatin

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ABSTRACT

Potential utilization of skin by-product from thornback ray (*Raja clavata*) was investigated. Gelatin from alkali-pretreated skin was extracted without or with pepsin at pH 2.0 in 0.2 M acetic acid or 0.1 M glycine-HCl buffer. The addition of pepsin increased the yields of gelatin. Indeed, the yields of gelatin from thornbach ray obtained with pepsin treatment was about 30%, while those obtained in acetic acid or glycine-HCl buffer were 18.32% and 23.01%, respectively. The characteristics and functional properties of thornback ray gelatin (TRGP), obtained by treatment with 5 units of pepsin/g of skin in glycine-HCl buffer, were investigated and compared with halal bovine gelatin (HBG).

TRGP had high protein content (90 g/100 g) similar to halal bovine gelatin (HBG). TRGP had a molecular weight (170 kDa) lower than HBG (226 kDa) and imino-acids content lower than HBG (10 and 22 residues per 100 residues, respectively). Its gel strength (140 g) was lower than that of HBG (260 g), possibly due to lower hydroxyproline content. Textural properties showed that the values hardness, elasticity, cohesiveness and chewiness of TRGP were lower than those of HBG. The functional properties of TRGP were examined in comparison to HBG. TRGP showed stronger ability to clarify apple juice than bovine gelatin. The nutritional component of clarified apple juice was practically not changed.

As expected, the different origin and extraction process of HBG and TRGP influenced the properties of the corresponding gelatin solutions.



## 1. Introduction

Gelatin is a denatured protein derived from collagen by thermal treatment hydrolysis (Bailey & Light, 1989). The most abundant sources of gelatin are pig skin, bovine hide and pork and cattle bones. However, the industrial use of collagen or gelatin obtained from non-mammalian species is growing in importance. The classical food, photographic, cosmetic and pharmaceutical application of gelatin is based mainly on its gel-forming properties (Karim & Bhat, 2009). Recently, and especially in the food industry, an increasing number of new applications have been found for gelatin in products such as emulsifiers, foaming agents, colloid stabilizers, biodegradable film-forming materials and microencapsulating agents, in line with the growing trend to replace synthetic agents with more natural ones (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011).

Raw materials from fish and poultry have received considerable attention in recent years, but their limited production makes them less competitive in price than mammalian gelatins. As far as fish gelatin is concerned, the huge number of species having very different intrinsic characteristics, has aroused the interest of the scientific community in optimizing the extracting conditions as well as characterizing the physico-chemical and functional properties of the resulting gelatins, obtained mainly from skin and bone residues (Gómez-Guillén et al., 2011). Recently, skin gelatins from several marine species such as grey triggerfish (Jellouli et al., 2011), unicorn leatherjacket (Ahmad & Benjakul, 2011) and cuttlefish (Jridi et al., 2013) have been extracted and characterized.

An alternative approach to acid treatment is the use of proteolytic enzymes. Nalinanon, Benjakul, Visessanguan, and Kishimura (2008) demonstrated that enzymatic extraction increases the yield of gelatin and the extraction process should be optimized to

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obtain gelatin with desired functional and rheological properties. Pepsin has been reported to cleave peptides in the telopeptide region of native collagen, which contains the intramolecular and intermolecular covalent crosslinks. Therefore, pepsin might solubilize the collagen in the skin matrix during the acid-swelling process, by cleaving some peptide bonds, resulting in a higher efficacy in gelatin extraction. The quality of fish gelatin depends on its physicochemical and rheological properties. In addition, the overall properties of fish gelatins are also influenced by the method employed.

Generally, fish gelatin is produced by a mild acid treatment process (Giménez, Gomez-Guillén, & Montero, 2005), but no buffer extraction was tested yet like alternative to acid treatment.

Thornback ray, is a widely distributed skate (Rajiformes: Rajidae) in the eastern Atlantic, ranging from Norway and Iceland to Northwest Africa, including the Mediterranean and Black Seas. They may also occur in the Atlantic and Indian Oceans of southern Africa (Stehmann & Bürkel, 1994). During processing *Raja clavata* generates a significant amount of by-products (80 g/100 g fresh weight) (Port Authority of Vigo, 2008). Therefore, the aims of this work were to extract gelatin from the skin of thornback ray and to study its physicochemical properties as well as its functional properties in comparison with halal bovine gelatin (HBG).

## 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). Acetonitrile was of HPLC grade. Pepsin was from MP Biomedicals (France). Food grade halal bovine gelatin (HBG) was obtained from Groupe EURALIS (Brive la Gaillarde, France). AccQ·Tag Reagent Kit was purchased from Waters (France). Other chemicals and reagents used were of analytical grade.

### 2.2. Preparation of thornback ray skin

Fresh thornback ray by-products were purchased from the fish market of Sfax City, Tunisia. The samples were packed in polyethylene bags, placed in ice with a sample/ice ratio of approximately 1:3 (w/w) and transported to the research laboratory within 30 min. Upon arrival, thornback ray skin was collected and scrapped to remove the adhering fat and foreign matter and cut into small pieces (0.5 cm × 0.5 cm). Prepared skin was then stored in sealed plastic bags at −20 °C until used for gelatin extraction and analysis.

### 2.3. Extraction of gelatin from thornback ray skin using the pepsin-aided process

Skin thornback ray was firstly treated with NaOH in order to remove non-collagenous proteins. Thornback ray skins were soaked in 0.05 mol/l NaOH (1:10 w/v) for 1 h at 4 °C. The alkaline solution was changed every 30 min. The alkaline-treated skins were then washed with distilled water until neutral pH was obtained.

Gelatin was extracted according to the method of Nalinanon et al. (2008), with a slight modification. The alkaline-treated skins were soaked in 0.2 M acetic acid or 100 mmol/l glycine-HCl buffer pH 2.0 with a solid/solvent ratio of 1:10 (w/v) and subjected to limited hydrolysis with commercial pepsin at a level of 5 units/g of skin. The mixtures were stirred for 18 h at 4 °C. The pH of the mixture was then raised to 7.0 using 10 mol/l NaOH solution.

Pepsin-treated skins were then incubated at 50 °C for 5 h in distilled water with continuous stirring to extract the gelatin fractions. The mixture was centrifuged at 10,000 × *g* for 30 min using a refrigerated centrifuge to remove insoluble material. The supernatant was collected and freeze dried (Bioblock Scientific, France). The obtained powder referred to as thornback ray skin gelatin (TRGP) was stored at 4 °C until used. Gelatins, extracted in acetic acid (0.2 M) or glycine-HCl 100 mM (pH 2.0) without enzyme, were used as control.

### 2.4. Proximate analysis

The moisture, ash and fat contents of the skin and gelatin powders were determined according to the AOAC methods number 927.05, 942.05 and 920.39 B, respectively (AOAC, 2000). The protein content was determined by estimating total nitrogen content by Kjeldahl method according to the AOAC method number 984.13 (AOAC, 2000). A factor of 6.25 and 5.55 was used to convert the nitrogen value to protein in the case of skin and gelatin respectively. All measurements were performed in triplicate.

The gelatin yield was calculated based on wet weight of fresh skin.

$$\text{Yield}(\%) = \frac{\text{Weight of freeze dried gelatin(g)}}{\text{Wet weight of fresh skin(g)}} \times 100 \quad (1)$$

### 2.5. Determination of gelatin gel colour

The CieLab coordinates ($L^*$, $a^*$, $b^*$) of the extracted gelatin and the halal bovine gelatin (6.67 g/100 ml dissolved at 60 °C) were directly measured using a spectrophotocolorimeter (Tintometre, Lovibond PFX 195 V 3.2, Amesbury, UK). In this coordinate system, the $L^*$ value is a measure of lightness, ranging from 0 (black) to 100 (white); the $a^*$ value ranges from −100 (greenness) to +100 (redness) and the $b^*$ value ranges from −100 (blueness) to +100 (yellowness).

### 2.6. Electrophoretic analysis

SDS-PAGE was performed as described by Laemmli (1970), using 12.5 g/ml resolving gel and 4 g/ml stacking gel. The samples (15 μg protein) were applied and subjected to electrophoresis. Bovine gelatin was used as a standard.

### 2.7. Molecular weight analysis by filtration gel technique

The molecular weight analysis of gelatin was carried out by filtration gel technique using a Silica gel packed in a TSKgel G2000SW$_{xL}$ column (7.8 mm I.D × 30 cm L). The eluant used was phosphate buffer (0.1 mol/l) containing 0.2 mol/l 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.

The liquid chromatographic system consisted of a Waters 600E automated gradient controller pump module, a Waters Wisp 717 automatic sampling device and a Waters 2996 photodiode array detector. Spectral and chromatographic data were stored on a NEC Image 466 computer. Millennium software was used to plot,