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Screening of factors influencing the extraction of gelatin from the skin of cuttlefish using supersaturated design

Mourad Jridi[a,*], Imen Lassoued[a], Amel Kammoun[b], Rim Nasri[a], Moncef chaâbouni[b], Moncef Nasri[a], Nabil Souissi[c]

[a] Laboratoire de Génie Enzymatique et de Microbiologie, Université de Sfax, Ecole Nationale d'Ingénieurs de Sfax, BP 1173-3038 Sfax, Tunisia

[b] Laboratoire de Chimie Industrielle, Université de Sfax, Ecole Nationale d'Ingénieurs de Sfax, BP 1173-3038 Sfax, Tunisia

[c] Laboratoire de Biodiversité et Biotechnologie Marine, Institut National des Sciences et Technologies de la Mer, Centre de Sfax, BP 1035-3018 Sfax, Tunisia

ABSTRACT

Supersaturated design (SSD) was used for screening the key parameters influencing gelatin extraction yield from cuttlefish (*Sepia officinalis*) skin. Results indicated that among a list of 17 factors only five parameters, namely, alkali (NaOH) concentration, acid reagent (acetic acid), enzyme, thermal treatment temperature and centrifugation time, were factors influencing gelatin yield. The optimal conditions for gelatin extraction were found to be: pretreatment with NaOH 0.03 M for 1 h; treatment with pepsin for 24 h at 4 °C in acetic acid 100 mM; extraction for 14 h at 40 °C. The yield of gelatin extraction was 54.6%. Cuttlefish skin gelatin (CSG) contained protein as the major compound (90.95%) and low fat (0.3%) and ash (0.05%) contents. The physico-chemical properties of the CSG were characterized and compared with those of bovine gelatin (BG). The result of textural properties showed that hardness, elasticity and cohesiveness of CSG were lower than those of BG. Further, the gel strength of CSG (192.01 g) was lower than that of BG (259.65 g), possibly due to lower imino-acid content. The functional properties, including emulsion activity index and foam stability were similar to those of BG. The CSG showed stronger ability of apple juice clarification, than BG without affecting its nutritional values.

Keywords: Supersaturated design; Gelatin extraction process; Cuttlefish skin; Textural properties; Functional properties; Application

1. Introduction

Gelatin is defined as a denatured protein derived from collagen by thermo hydrolysis and has a rheological property of thermo-reversible transformation between solution and gel (Cho et al., 2004). Gelatin has been widely utilized in food, pharmaceutical and photographic industries (Karim and Bhat, 2009). Generally, most of the commercial gelatins are made from the skin or bone of porcine or bovine. However, due to the outbreak of bovine spongiform encephalopathy and the foot-and-mouth disease crisis, the search for alternative gelatin raw materials, especially from aquatic animals, has received increasing attention.

It is estimated that fish-processing waste after filleting accounts for approximately 75% of the total fish weight (Shahidi, 1995) and 25% of the waste is in the form of bones and skins (Gòmez-guillen et al., 2002). Generally, fish gelatin is produced by a mild acid treatment process (type A gelatin; Muyonga et al., 2004). Acid treatment has been used to disrupt acid-labile cross-links with negligible peptide bond

* Corresponding author. Tel.: +216 28 142 818; fax: +216 74 275 595.
E-mail address: jridimourad@gmail.com (M. Jridi).

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hydrolysis and amino acid degradation. Since collagen cross-links are stable to thermal and acid treatment (Benjakul et al., 2009), a low yield of the resulting gelatin is generally obtained with the traditional process. To increase gelatin extraction, some proteases may be carefully employed. Pepsin has been reported to cleave peptides in the telopeptide region of native collagen, thus the yield of partially cleaved collagen can be increased (Jridi et al., 2013; Nalinanon et al., 2008).

Recently, skin gelatins have been isolated from various fish species such as brownbanded bamboo shark and blacktip shark (Kittiphattanabawon et al., 2010), Giant squid (Uriarte-Montoya et al., 2011), Jumbo squid (Uriarte-Montoya et al., 2010), grey triggerfish (Jellouli et al., 2011) and zebra blenny (Ktari et al., 2014).

Cuttlefish 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.

Experimental studies related to screening variables, based on classical factorial designs often used when the number of factors is not very high, guarantee that all the factor effects can be estimated (Rais et al., 2009; Cela et al., 2009). However, when the experiment is expensive and the number of factors is very large, these classical experimental designs are not applicable due to the limitation of the number of experimental runs. A supersaturated designs (SSD), which is a design containing a number of factors greater than the number of experimental runs, is particularly useful in screening experimentation due to its run-size economy.

In this work, SSD was employed to screen variables influencing gelatin extraction from the skin of cuttlefish. The physico-chemical properties as well as functional properties of CSG obtained under optimum conditions, and its application in clarification of apple juice, in comparison with bovine gelatin, were determined.

2. Materials and methods

2.1. *Materials*

Bovine Gelatin (BG), 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). Skin from cuttlefish (*Sepia officinalis*) was obtained from the fish market of Sfax City, Tunisia. Other chemicals and reagents used were of analytical grade.

2.2. *Experimental procedures*

2.2.1. *Preparation of smooth hound crude acid protease*

Smooth hound (*Mustelus mustelus*) was purchased from the local market at Sfax City, Tunisia. After the fish was washed with tap water, internal organs were separated into individual organs and only stomach was collected. The stomach was rinsed with cold distilled water, cut into pieces with a thickness of 1 cm × 1.5 cm and then homogenized for 1 min with 10 mM Tris–HCl buffer, pH 7.5, at a ratio of 1:2 (w/v). Smooth hound crude acid proteases (SHCAP) was prepared and then used for gelatin extraction.

2.2.2. *Gelatin extraction*

Gelatin was prepared according to the method of Nalinanon et al. (2008) with a slight modification. Skin cuttlefish (100 g) was cut into 1 or <1 cm squares, using scissors, washed in cold-water. Non-collagenous proteins were removed by soaking cuttlefish skin (contained 14% of protein) in alkali solution (NaOH or KOH at 0.03 or 0.05 M) at a sample/alkaline solution ratio of 1:10 (w/v) according to the experimental screening design (Tables 1 and 2).

The mixture was stirred for 1 or 2 h at 4 or 25 °C and the alkaline solution was changed every 30 min. The alkaline-treated skins were then washed with cold distilled water until the neutral pH of wash water was obtained.

The alkaline-treated skins were soaked in acetic acid or HCl solution (0.1 or 0.2 M) with a solid/solvent ratio of 1:10 (w/v) and subjected to limited hydrolysis with SHAP or commercial pepsin at 10 or 20 units/g of alkaline treated skin. The mixtures were stirred for 24 or 48 h at 0 or 4 °C. To inactivate the enzyme pH of the mixtures were then raised to 7.0 using 10 M NaOH and stirred gently for 30 or 60 min h at 0 or 4 °C.

The mixtures were then incubated at 40 or 50 °C for 14 or 18 h with continuous stirring to extract the gelatin from the skin. The mixtures were centrifuged at 10,000 or 13,000 × *g* for 20 or 30 min at 4 °C, using a refrigerated centrifuge to remove insoluble material. The supernatant were freeze-dried (Moduloyd Freeze dryer, Thermo Fisher, USA).

The powders obtained referred to as cuttlefish-skin gelatins (CSG) were stored at 4 °C until used. The extraction yields (Hydroxyproline content) were calculated. The optimal conditions giving the highest yield were chosen for further study.

2.3. *Experimental design*

A supersaturated designs (SSD) was applied in this study to screen of key factors influencing gelatin extraction from cuttlefish skin. The construction of two-level supersaturated designs has been studied and described in the literature (Rais et al., 2009). As perfect orthogonality is impossible to achieve when constructing a SSD, statisticians looked for obtaining designs that are as nearly orthogonal as possible (Dejaegher and Vander Heyden, 2008). In this study, SSD is constructed according to Lin's method via a half fraction of the Hadamard matrix. This method uses a column of a Hadamard matrix as a branching column to build two supersaturated designs. The design matrix of the first one is obtained by first selecting only the runs corresponding to the high level (+1) appearing in the branching column and then deleting this column. The design matrix of the second SSD is obtained in the same way replacing the high level by the low one (−1). One of the two SSD matrixes is retained to conduct the screening study.

In this work, as the reaction process includes 17 factors, we choose the half fraction of Hadamard matrix with $N_H = 20$ experiments, to check the effect of these factors by performing 10 runs. We used the 19th column as the branching column and selected the experiments corresponding to the high level (+1) to generate the SSD matrix (Table 1).

For the data analysis, several approaches have been suggested in the literature in recent years for the analysis of supersaturated designs. The literature mentions that the correlation structure inherent in supersaturated designs can