International Journal of Biological Macromolecules 51 (2012) 477–483

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Functional, antioxidant and film-forming properties of tuna-skin gelatin with a brown algae extract

Anissa Haddar[a], Sabrine Sellimi[a], Raouia Ghannouchi[a], Oscar Martinez Alvarez[b], Moncef Nasri[a], Ali Bougatef[a,*]

[a] Laboratoire de Génie Enzymatique et de Microbiologie – Ecole Nationale d'Ingénieurs de Sfax, Université de Sfax, B.P "1173", 3038 Sfax, Tunisia
[b] Instituto del Frío (CSIC), José Antonio Novais 10, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 7 May 2012
Received in revised form 4 June 2012
Accepted 12 June 2012
Available online 18 June 2012

Keywords:
Gelatin
Gelatin film
Pepsin
Tuna
Physicochemical composition
Functional properties

ABSTRACT

Characteristics and functional properties of gelatin from skin of Atlantic Bluefin tuna (*Thunnus thynnus*) were investigated. The gelatin was extracted by an acid-swelling process in the presence of different concentrations of commercial pepsin, followed by subsequent heating. The extraction yield was higher when increasing concentrations of pepsin were used during the swelling process. Emulsion activity index, foam formation ability and foam stability of gelatin increased with the increase of gelatin concentration. Antioxidant properties (ferric-reducing ability and DPPH-radical-scavenging capacity) of gelatin-based edible films containing aqueous or methanolic extracts of brown algae (*Cystoseira barbata*) were also assessed. For comparative purposes, tuna-skin gelatin edible film with BHA was studied. Antioxidant properties of the films were increased significantly when natural extracts were added.

Extracts of brown algae could be useful additives to obtain edible films from tuna-skin gelatin with interesting functional and antioxidant properties.

1. Introduction

Gelatin is an important functional biopolymer with broad applications in many industrial fields as food, materials, pharmacy and photography [1]. Gelatin is obtained by partial hydrolysis of collagen. Under specific conditions of temperature, solvent and pH, gelatin macromolecules can exhibit flexibility sufficient to achieve a wide variety of conformations. Nevertheless, the characteristics and functionalities of gelatin depend on its molecular structure. Gelatin exhibits interesting amphoteric nature, specific triple-stranded helical structure (not observed in synthetic polymers) and also interaction with water different to that of synthetic hydrophilic polymers. These properties have become gelatin indispensable for many industrial processes [2].

The most abundant sources of gelatin are pig skin, bovine hide and pork and cattle bones. However, the sanitary and socio-cultural restrictions in the use of gelatin from land animals have made that the use of gelatin from alternative non-mammalian species had grown in importance. In this sense, gelatin from marine sources is gaining increasing attention as a new material for edible films [3,4]. However, the films based on marine gelatins shows poorer functional properties than those of the films based on gelatin from mammals [5]. Chemical and physical treatments could in this sense be applied to modify the polymer network through cross-linking of the polymer chains [6]. Different authors have reported the use of different chemical cross-linking agents as gossypol, formaldehyde, glutaraldehyde [7–9], as well as the use of transglutaminase [10]. Nevertheless, the use of some cross-linking agents is limited by their toxicity and also by their high cost [6,11]. Therefore, the use of a safe natural cross-linking agent is of great interest for improving the properties of films, especially protein based film.

In many food products, lipids are commonly found in emulsion forms either as water-in-oil (e.g., butter and margarine), or oil-in-water (e.g., mayonnaise, milk and cream). Lipid oxidation negatively affects the quality of foods, especially emulsion type products by altering appearance, odor, flavor, shelf-life and nutritional value. This results in unacceptability by consumers [12]. To retard or prevent such changes, synthetic antioxidants are normally used. The possible toxicity of synthetic antioxidants makes the use of natural antioxidants positive for consumers. In this sense, the use of polyphenolic compounds as antioxidant agents in food products has recently received considerable attention. The antioxidant properties of phenolic compounds are based on their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers [13]. Additionally, these phenolic compounds can bind metal ions and scavenge the free radicals [14]. The phenolic compounds with the highest antioxidative activity generally include hydroxyl groups in their structure [15].

* Corresponding author. Tel.: +216 74 274 088; fax: +216 74 275 595.
E-mail address: ali.bougatef79@gmail.com (A. Bougatef).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2012.06.016

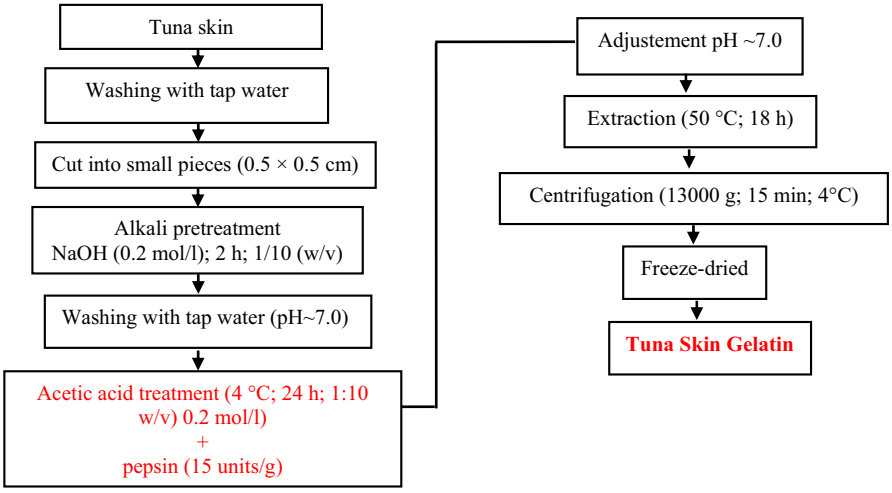

Fig. 1. Procedures for extraction of gelatin from the tuna skin.

Phenolic compounds with interesting applications as antioxidants could be found in seaweeds. In fact, during the last decades seaweeds or their extracts have been intensively studied as potential natural antioxidants [16,17]. The algae from the genus Cystoseira (Phaeophyta) are widespread around the world, and could be a good source of natural antioxidants. In the Mediterranean Sea, the brown algae *Cystoseira barbata* provide a large amount of biomass. For this reason, their chemical composition is of practical and ecological interest. However, the secondary metabolites of Cystoseira species from Mediterranean Sea have not been investigated until now. In contrast, different metabolites have been identified in Cystoseira algae from other seas. For instance, Francisco and Combault [18] reported the occurrence of sterols (mainly fucosterol) in Cystoseira algae from other seas, analogously to other brown algae. Terpenoids, phenols, carotenoids, tetraprenyltoluquinols, naphthoquinones, diterpenoids and acetogenins have also been found and have been used in the taxonomy of this genus [19–21]. Some of these compounds could be excellent natural antioxidants with promising applications in food industry. Bluefin tuna is neither a warm blooded nor a cold-blooded fish which can live for up to 40 years, and grow to over 4 m in length and 600 kg in weight. It was around 2.5 m long and weighed around 350 kg.

The main aim of this work was to obtain edible films with antioxidant capacity based on tuna-fish gelatin with *C. barbata* extracts. Gelatin extraction from the skin of Atlantic Bluefin tuna (*Thunnus thynnus*) was optimized using pepsin. Functional properties and antioxidant activity were evaluated.

2. Materials and methods

2.1. Preparation of skin from tuna

Skin of tuna (*T. thynnus*) was obtained from the processing plant "El AMANI" located in Gabes City, Tunisia. This discard was collected immediately after fish processing and packed in polyethylene bags, kept in ice with a sample/ice ratio of approximately 1/3 (w/w) and immediately transported to the laboratory. Upon arrival, residual meat was removed manually from the skin and cleaned samples were washed in tap water. The skin was then cut into small pieces (0.5 cm × 0.5 cm) and stored in polyethylene bags at −20 °C until used.

2.2. Extraction of gelatin from tuna skin using pepsin

The extraction procedure is shown in Fig. 1. Tuna skins (100 g) were incubated in 0.2 M NaOH at the rate of 1/10, (w/v) for 2 h at room temperature (22 ± 1 °C). The mixture was stirred continuously and the alkaline solution was changed every 30 min.

The alkaline-treated skins were then washed with tap water until neutral pH of wash water was obtained. The alkaline-treated skins were soaked in 0.2 M acetic acid at the rate of 1/10 (w/v), and subsequently hydrolysed with different concentrations of pepsin from bovine stomach mucosa (0–15 AU/g alkaline-treated skin) with continuous stirring for 24 h at 4 °C. The pH was then raised to 7 using 10 M NaOH. The mixtures were stirred gently for 1 h at 4 °C to terminate protease activity. Pepsin-treated skin mixtures were then incubated with continuous stirring at 50 °C for 18 h for gelatin extraction. The mixtures were centrifuged at 10,000 × *g* for 30 min at 4 °C (MPW-350R/RS centrifuge, MPW Med. Instruments, Warsaw, Poland) to remove insoluble material. The gelatin solution was demineralized by adding Purolite cation- and anion exchange resins. After 16 h, the resins were removed by filtration through a 250 mesh nylon filter. The gelatin extract was then concentrated in a rotavapor (40 °C, 30 min) before freeze-drying (Bioblock Scientific Christ ALPHA 1-2, IllKrich-Cedex, France). The powder obtained (tuna skin gelatin, TSG) was stored at 4 °C until used.

The extraction yield was calculated as follows.

$$\text{Yield (g/100 g)} = \frac{\text{weight of freeze dried gelatin (g)}}{\text{wet weight of fresh skin (g)}} \times 100$$

2.3. Seaweed material

Fresh *C. barbata* samples were collected from Kerkennah Island (Sfax, Tunisia) on March 2010 at a depth of 2–8 m. The raw material was washed with distilled water and then dried at room temperature for at least 1 week. The dried samples were ground using a mortar and pestle to obtain a fine powder and then stored in glass bottles at room temperature.

2.4. Preparation of algae extracts

The extraction of bioactive phytochemicals in *C. barbata* seaweeds was carried out separately with methanol and water. The finely powdered algal materials (20 g) were stirred in a shaker incubator with 200 ml of solvent at 37 °C for 24 h. Samples were filtered using Whatman Number 1 filter papers and centrifuged at 10,000 rpm for 15 min. The organic extracts were concentrated to solvent free by evaporation in a rotary vacuum evaporator (Stuart, RE 300B) at 45 °C. The aqueous extract was freeze-dried. The residues obtained were stored at −20 °C until further analyses.