Food Hydrocolloids 39 (2014) 68–76

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Isolation and characterization of gelatin from the skins of skipjack tuna (*Katsuwonus pelamis*), dog shark (*Scoliodon sorrakowah*), and rohu (*Labeo rohita*)

K. Shyni*, G.S. Hema, G. Ninan, S. Mathew, C.G. Joshy, P.T. Lakshmanan

Biochemistry & Nutrition Division, Central Institute of Fisheries Technology, Cochin, India

ARTICLE INFO

Article history:
Received 27 January 2013
Accepted 4 December 2013

Keywords:
Gelatin
Skipjack tuna
Dog shark
Rohu
Gel strength
Fish gelatin

ABSTRACT

Gelatin was extracted from the skins of dog shark (*Scoliodon sorrakowah*), skipjack tuna (*Katsuwonus pelamis*) and rohu (*Labeo rohita*) and their physico-chemical properties were measured. The skins of shark, tuna and rohu yielded 19.7, 17.2 and 11.3% gelatin, respectively. The gel strength of dog shark gelatin (6.67%, 10 °C) was found to be higher (206 g) than tuna and rohu skin gelatins (177 g and 124 g, respectively). Similarly, molecular weight, viscosity, melting point, foaming properties, water holding capacity, odour, colour and clarity of dog shark gelatin were in general better than the tuna and rohu skin gelatins. The amino acid analysis showed that hydroxyproline content in dog shark skin gelatin was the highest when compared to tuna and rohu skin gelatins.

1. Introduction

Gelatin is a denatured fibrous protein derived from collagen by partial thermal hydrolysis. It is an important functional biopolymer that has broad applications in the food, pharmacy and photography industries (Hao et al., 2009). The source, type of collagen and the processing conditions will influence the properties of the resulting gelatin. Different types of gelatins have varying thermal and rheological properties such as Bloom strength, melting and gelling temperatures. These properties are governed by factors such as chain length or molecular weight distribution, amino acid composition and hydrophobicity, etc. (Gomez-Guillen et al., 2002; Norziah, Al-Hassan, Khairulnizam, Mordi, & Norita, 2009).

The global demand for gelatin has shown an increasing trend in recent years. Recent reports indicate that the annual world production of gelatin is nearly 326,000 tonnes, with pig skin derived gelatin accounting for the highest (44%) output, followed by bovine hides (28%), bovine bones (27%), and other sources (1%) (Ahmad & Benjakul, 2011). Other sources, which include fish gelatin, accounted for around 1.5% of total gelatin production in 2007, but this percentage was double that in 2002, indicating that gelatin production from alternative non-mammalian species had grown in importance (Gomez-Guillen et al., 2009). This may be due to the shortage of the primary raw materials mostly cattle hides, bones and pigskins (Spend Matters, 2012).

Gelatins from land animal sources are preferred over marine sources due to their superior gel strength, melting point and viscosity (Cho, Gu, & Kim, 2005). However, fish gelatin received increasing attention as an alternative to land animal gelatin due to religious constraints and health issues associated with the latter. Both Judaism and Islam forbid the consumption of any pork-related products and non-religiously slaughtered beef, while Hindus refrain from consuming cow-related products (Karim & Bhat, 2009). In addition, gelatin from aquatic sources has been shown to be free of infectious materials such as bovine spongiform encephalopathy (Sadowska, Kolodziejska, & Niecikowska, 2003).

The fish skins and bones contribute almost 30% of the total weight of the fish (Gomez-Guillen et al., 2002). Fish skins are a major by-product of the fisheries and aquaculture industry. The skin yield is highly variable according to species, fish size and processing styles. Conversion of these wastes into value-added products such as gelatin to yield additional income has both economic and waste management benefits for the fish industry (Choi & Regenstein, 2000).

A number of studies have addressed properties of fish skin gelatins (Arnesen & Gildberg, 2007; Choi & Regenstein, 2000; Fernandez-Diaz, Montero, & Gomez-Guillen, 2001; Gomez-Guillen & Montero, 2001; Grossman & Bergman, 1992;

* Corresponding author. Tel.: +91 4842666845; fax: +91 4842668212.
E-mail address: shynicof@yahoo.co.in (K. Shyni).

0268-005X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodhyd.2013.12.008

Gudmundsson, 2002; Gudmundsson & Hafsteinsson, 1997; Holzer, 1996; Jamilah & Harvinder, 2002; Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006; Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka, 2006; Yang et al., 2007; Zhou & Regenstein, 2005) showing that their properties differ from those of mammalian gelatins. The gelatin properties were found to vary with the method of production and between species.

Around the world, sharks are mainly used for producing shark fins and fillets. As a result, skins are discarded along with the rest of the carcass or treated as a by-product with low market value. Studies have shown that cartilaginous fishes including sharks have a higher content of collagen, the precursor of gelatin, when compared to bony fishes (Nair, 2002). Cho et al. (2005) reported that gelatin extracted from yellowfin tuna skin showed better functional properties than those from other fish sources. Some studies have ascertained that freshwater fish can have a high gelatin yield (Grossman & Bergman, 1992; Jamilah & Harvinder, 2002; Muyonga, Cole, & Duodu, 2004a). Only a few studies have been conducted on warm-water fish gelatin and these showed that gelatin from these species had better functional properties than those from cold-water fish species (Gilsenan & Ross-Murphy, 2000; Grossman & Bergman, 1992; Leuenberger, 1991).

Dog shark and skipjack tuna are available along the south west coast of India. Rohu is one of the major carp species, a natural inhabitant of the freshwater sections of the rivers of India and contributes to the inland catch. At present, the fishery is sustainable for all the above species.

The present study was undertaken to extract and characterize gelatin from the skins of skipjack tuna (*Katsuwonus pelamis*) from the family scombridae, dog shark (*Scoliodon sorrakowah*) from the family of carcharhinidae, a cartilaginous fish and rohu (*Labeo rohita*) from the family of cyprinidae, a freshwater fish.

2. Materials and methods

2.1. Raw material

The species used for the study were skipjack tuna (*K. pelamis*), dog shark (*S. sorrakowah*), and rohu (*L. rohita*). The marine fishes were landed from long-line day boats that iced the fish on board, at the local fish landing centre in Cochin during May 2011, from the Arabian sea. The length of the fishes were measured and recorded, i.e., tuna (92 ± 2.4 cm, 17 in number) shark (73 ± 2.9 cm, 23 in number). The skinning of the fish was carried out by the fisherman at the market under supervision. The iced skin in prime quality was brought to the laboratory. Rohu (39 ± 6.6 cm, 55 in number) freshly caught and dead, in iced condition, was procured from a local fish farm and skinning was carried out at laboratory in the iced condition.

The skins were cut into 2–3 cm^2 pieces using a scalpel and washed with ice cold tap water. They were then placed in polyethylene bag with added glaze water (10%) and stored at −20 °C until use. The storage time was less than 2 months. All chemicals used unless otherwise noted were of analytical grade (Merck KGaA Chemical & Pharmaceutical Company, Darmstadt, Germany; Sigma–Aldrich Corporation, MO, USA).

2.2. Gelatin extraction

Based on preliminary extraction trials, it was decided to follow the gelatin extraction method of Gudmundsson and Hafsteinsson (1997). Thawed skins were thoroughly washed and treated with warm water (38–40 °C) for 10 min to remove superfluous material and reduce the fat content. Before gelatin extraction, skins were soaked in 0.1 M NaOH at ambient temperature (~27 °C) with a skin/solution ratio of 1:10 (w/v) for 2 h. The alkaline solution was changed every 1 h to remove non-collagenous proteins and pigments. Alkaline-treated skins were washed with tap water until the wash water was neutral or faintly basic. The pH of wash water was monitored using Cyberscan 510 pH meter (Eutech Instruments Pte Ltd, Singapore). The skins were then soaked in 0.2 M acetic 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 fish skin matrix. Acid-pretreated skins were washed thoroughly with tap water until the wash water became neutral. The skins were then subjected to a final wash with distilled water to remove any residual matter. The final extraction was carried out in distilled water at 45 °C for 12 h with a skin/water ratio of 1:10 (w/v). The clear extract obtained was filtered through a Buchner funnel with Whatman filter paper No. 4 (Sunshine Instruments, Coimbatore, Tamil Nadu, India). Fat separation was done using a simple fat separating funnel (India-MART, Maharashtra, India). The extract was poured in to the funnel and was allowed to settle for few seconds. Within few seconds, suspended fat formed a layer over the gelatin extract. Then the gelatin was allowed to flow slowly through the outlet valve of the funnel. The valve was carefully closed on reaching the level of suspended fat layer. The clear extract obtained was concentrated by evaporation under vacuum at 5 °C, with a flash evaporator (Buchi rotavapor R215, Buchi Labortechnik, Flawil, Switzerland). The concentrated viscous solution was frozen in an air blast freezer (Icematic T10, CastelMAC SpA, Castelfranco Veneto (TV), Italy) at −40 °C and then freeze-dried with freeze drier (Gamma 1–16 LSC, Osterode am Harz, Germany) in two steps. The solution was subjected to main drying for 8 h at set shelf temperature of 20 °C and set pressure of 0.01 mbar. The final drying was done for 2 h at set shelf temperature of 25 °C and set pressure of 0.01 mbar at a condenser temperature of −55 °C.

2.3. Yield of gelatin

The yields of the gelatins obtained were calculated as:

$$\%\ \text{Yield (wet wt. basis)} = \frac{\text{Dry wt. of gelatin}}{\text{Wet wt. of skins}} \times 100$$

2.4. Determination of proximate composition

Moisture, lipid, ash and protein were determined by AOAC (1995) methods 950.46, 960.39, 900.2A and 928.08, respectively. Protein digestion was done as described by Eastoe and Eastoe (1952) to ensure complete hydrolysis of collagen. A conversion factor of 5.4 was used for calculating the protein content from the Kjeldahl nitrogen content since collagen, the main protein in skin, contains approximately 18.7% nitrogen (Eastoe & Eastoe, 1952). This is an estimate and is based on mammalian gelatin.

2.5. Determination of pH

The pH values of raw fish skins and gelatin solutions were measured using the British Standard Institution method (BSI, 1975). For determining the pH of the skins, samples were chopped and blended for 5 min at ambient temperature (37 °C) by vigorous shaking (ICS-BLENDER, Hyderabad, Andhra Pradesh, India) in distilled water to form a 1% (w/v) skin suspension. For the gelatin solution, a 1.0% (w/v) gelatin solution was prepared by adding 1 g of gelatin in 99 ml of distilled water. The mixture was heated to 45 °C