Food Hydrocolloids 29 (2012) 100–105

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

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

Seasonal differences in the properties of gelatins extracted from skin of silver carp (*Hypophthalmichthys molitrix*)

Junjie Zhang [a,*], Rui Duan [a], Yiling Wang [a], Binlun Yan [b], Wanli Xue [a]

[a] School of Marine Science and Technology, Huaihai Institute of Technology, Cangwu Road 59, Lianyungang 222005, China
[b] Jiangsu Key Laboratory of Marine Biotechnology, Lianyungang 222005, China

ARTICLE INFO

Article history:
Received 6 September 2011
Accepted 13 February 2012

Keywords:
Silver carp
Gelatin
Viscosity
Melting point
Functional property

ABSTRACT

The gelatins were extracted from skins of silver carp (*Hypophthalmichthys molitrix*) caught in winter and summer, respectively. The physicochemical (molecular weight distribution and melting point) and rheological characteristics (viscosity property), as well as functional properties (emulsifying capacity and stability) of the gelatin from winter silver carp skin were compared with those of the summer equivalent. The results showed the properties of the summer gelatin were superior to those of the winter one, showing higher viscosity, emulsion stability, melting point and lower concentration for gelling. The summer gelatin has slightly denser strands of the gel microstructure which was observed by scanning electron microscopy (SEM). Different properties of gelatins from skin of silver carp may be attributed to the big discrepancy of the environmental temperature in the two seasons.

1. Introduction

Gelatin has been widely used in food, pharmaceutical, cosmetic, and photographic industries for its unique functional and technological properties (Karim & Bhat, 2009). The global demand for gelatin has been increasing over the years, especially in Asia. Most of the world output of gelatin derives from pig skin, bovine hides and bones (GME, 2008). However, the out-breaks of bovine spongiform encephalopathy (BSE) and the foot-and-mouth disease (FMD) have resulted in anxiety among the users of gelatin. Therefore, fish skin, as by-products from fish processing, have been studied as a replacement for mammalian sources (Gudmunsson & Hafsteinsson, 1997).

China is the largest freshwater fish producer in the world. According to the statistical data from the Ministry of Agriculture (2006), the yield of freshwater fish was around 20,093,500 tons in 2005. The production of cultured silver carp was about 3,524,800 tons and has become one of the most important sources of fish supply. A lot of fish skin, scale and bone were produced as fish processing wastes, which are rich in collagens and can be used as the raw materials for gelatin preparation.

Thermal stability is an essential prerequisite for any protein (Miles, Knott, Sumner, & Bailey, 1998). It is reported that freshwater fish such as carp and silver carp expresses muscle protein myosin with different stability seasonally as a consequence of adaptation to water temperature (Nakaya, Watabe, & Ooi, 1995; Yuan et al., 2006). Duan et al. reported the different stability of collagens from scales of carp caught in winter and summer (Duan, Konno, Zhang, Wang, & Yuan, 2010). The quality of gelatin largely depends on its physicochemical properties, which are greatly influenced by both the species and tissue from which it is extracted and the method of extraction (Gilsenan & Ross-Murphy, 2000). Besides, seasonal differences in the properties of gelatins from skin of carp were also reported (Duan, Zhang, Xing, Konno, & Xu., 2011). Therefore, for gelatin from freshwater fish, the properties may be influenced by season. The collagens from scale of silver carp showed seasonal difference in the thermostability (Duan et al., 2010). The gelatins from silver carp skin in the two seasons may have different properties. In the present study, skins of silver carp caught in winter and summer were employed as the raw materials to extract gelatins at a certain temperature. In addition to the physicochemical (molecular weight distribution and melting point) and rheological characteristics (viscosity property), functional properties (emulsifying capacity and stability) of the gelatins from sliver carp skin were also investigated and compared. The better knowledge of the properties of gelatins from silver carp skin could be helpful in rationalizing the use of fish residues.

* Corresponding author. Tel.: +86 13912160158; fax: +86 518 85893890.
E-mail address: zjjdr180@yahoo.com.cn (J. Zhang).

0268-005X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2012.02.005

2. Materials and methods

2.1. Raw materials

Live cultured silver carps (average body weight of 880 g) were obtained from a free market in Lianyungang, Jiangsu province. The skins were removed manually and washed with chilled tap water to get rid of the adhering tissues. The samples were then placed in polyethylene bags and stored at −25 °C until used.

2.2. Chemical reagents

All reagents were of analytical grade.

2.3. Methods

2.3.1. The hydroxyproline content of winter and summer skins

The hydroxyproline content was determined using the colorimetric method recommended by the ISO (Anonymous, 1978) after the material was hydrolyzed in 6 M hydrochloric acid for 8 h at 110 °C. The hydrolyzed samples (50 μl) were mixed with a buffered chloramines-T regent (450 μl, pH 6.5) and the oxidation was allowed to proceed for 25 min at room temperature. 500 μl Ehrlich's aldehyde reagent was added to each sample, mixed gently, and the chromophore was developed by incubating the samples at 65 °C for 20 min. The absorbance of reddish purple complex was measured at 550 nm using a Model 1601 UV–vis spectrophotometer (Shimadzu, Japan). The measurement was done in triplicate.

2.3.2. Gelatin extraction

After thawing, the skins were cut into small pieces by a scissor at the size of 1 × 1 cm and mixed well. The remaining skins were mixed with 0.1 M NaOH for 6 h with continuous stirring at a sample/alkali solution ratio of 1:8(w/v) to remove non-collagenous proteins. The alkali solution was changed every 3 h. Then the samples were washed with cold distilled water, until neutral pH of washing water was obtained. After that, the skins were soaked in 10% (v/v) butyl alcohol with a solid/solvent ration of 1:10 (w/v) overnight to remove fat, and then washed with cold distilled water repeatedly (Nagai et al., 2000). The gelatins were extracted from the pretreated silver carp skin using a solid/distilled water ratio of 1:15 at 50 °C for 3 h and lyophilized (Duan et al., 2011). Winter gelatin was termed as w-k and summer one was termed as s-k. Triplicate extractions were performed.

2.3.3. Molecular weight distribution

The molecular weight distributions of the extracted gelatins were determined by SDS-polyacrylamide gel electrophoresis. The gelatin samples were dissolved in distilled water, and then mixed with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 5% SDS, 20% glycerol) at a 1:2 ratio in the presence of 10% β-ME. The samples were heated at 100 °C for 2 min. SDS-PAGE was performed on 7.5% gels according to the method of Laemmli (1970). After the electrophoresis, the gel was stained with Coomassie brilliant blue R250 dissolved in water, methanol and trichloroacetic acid (5:4:1) and de-stained using a solution containing methanol, distilled water and acetic acid at a ratio of 5:4:1. 15 μg of protein was loaded in each well. High molecular weight markers (Sigma–Aldrich Chemical Co, USA) were used to estimate the molecular weight distribution of the gelatins.

2.3.4. Viscosity properties

The viscosity of gelatin samples was determined by the method described by Kittiphattanabawon, Benjakul, Visessanguan, Nagai, and Tanaka (2005) with some modification. Gelatin solution (1.4%, w/v, 10 ml) was prepared by dissolving lyophilized gelatin in distilled water at 40 °C for 15–20 min. The viscosity studies of gelatin solutions were performed on a circumvolving viscometer (model NDJ-7, Tongji university Labs Inc., Shanghai, China) using rotator No. 1 (annulated-column shaped, outer diameter = 1.6 cm, inner diameter = 1.3 cm). During the testing, gelatin solution was firstly cooled down from 40 to 10 °C, kept at 10 °C for 5 min, then heated from 10 to 40 °C. The cooling and heating rate was 2 °C/min. At the designated temperature, the solution was held for 5 min prior to viscosity determination. The measurements were done in triplicate.

2.3.5. Melting point determination

The melting point was measured by the method described by Choi and Regenstein (2000) with a slight modification. Gelatin solutions (6.67%, w/v) were prepared at 40 °C and a 5-ml aliquot of each sample was transferred to a test tube (12 mm × 75 mm). The samples were degassed in the vacuum desiccators for 5 min. The tubes were then covered with Parafilm (Laboratory Film, Chicago, USA) and immediately cooled in ice-chilled water and matured at 5 °C for 18 h. 20 μl of a mixture of 75% chloroform and 25% (w/v) red dye (Beijing Hengye Zhongyuan Chemical Co. Ltd, Beijing, China) were placed on the surface of the gel. The gels were put in a water bath (circulator CTE 42W, Yamato Scientific Co. Ltd, Yamanashi, Japan) at 10 °C and then heated at the rate of 0.4 °C/min. The temperature at which the drops began to move freely down the gel was taken as the melting point. The measurements were done in triplicate.

2.3.6. Emulsifying properties

Emulsion activity index (EAI) and emulsion stability index (ESI) of gelatin were determined at 20 °C according to the method of Pearce and Kinsella (1978) with a slight modification. Soybean oil (2 ml) and gelatin solution (2%, w/v, 6 ml) were homogenized (Model PRO-250, PRO Scientific Inc. Monroe, CT, USA) at a speed of 20,000 rpm for 1 min. 100 μl Emulsions were pipetted out at 0 and 10 min and diluted with 5 ml 0.1% (w/v) SDS. The mixture was mixed thoroughly for 10 s using a vortex mixer (XW-80A, Huxi Qingpu. Inc., Shanghai, China). A_{500} of the resulting dispersion was measured using a spectrophotometer (UV-160, Shimadzu, Japan). EAI and ESI were calculated by the following formulae:

$$\mathrm{EAI}\left(\mathrm{m^2/g}\right) = 2.303 \times A_0/(0.25 \times \text{protein weight (g)})$$

where A_0 = absorbance at 500 nm

$$\mathrm{ESI\,(min)} = A_0 \times \Delta t/\Delta A$$

$$\Delta A = A_0 - A_{10\mathrm{min}}$$

$$\Delta t = 10\ \mathrm{min}$$

The measurement was done in triplicate.

2.3.7. Scanning electron microscopy (SEM)

Microscopic examination of gelatin was done using scanning electron microscopy by adopting the method as described previously (Jongjareonrak, Benjakul, Visessanguan, Prodpran, & Tanaka, 2006). The 6.67% (w/v) gelatin gel (frozen at −80 °C and cut) having a thickness of 2–3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 1 h. The samples were rinsed with distilled water 3 times and dehydrated in ethanol with a serial concentration of 30, 50, 70, 80, 90 and 100% (v/v). Dried samples were mounted on a bronze stub (Coax Group Corporation Ltd., Bangkok, Thailand) and sputter-coated with