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Molecular characteristics and properties of gelatin from skin of seabass with different sizes

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ABSTRACT

Gelatin was extracted from the skin of seabass (*Lates calcarifer*) with different average sizes (2, 4 and 6 kg/fish), termed G2, G4 and G6, respectively and their characteristics and functional properties were determined. Yields of G2, G4 and G6 were 38.22, 40.50 and 43.48% (based on dry weight), respectively. G2 contained α-chains as dominant component, whilst G4 and G6 comprised α-, β- and γ-chains with a larger content of high MW cross-links. All gelatins had the similar imino acid (hydroxyproline and proline) content. Net charge of G2, G4 and G6 became zero at pH of 6.73, 6.41 and 7.12, respectively. Amongst all gelatin samples, G6 exhibited the highest gel strength (321.5 g) ($p < 0.05$), but had the lowest turbidity ($p < 0.05$). Gels of G6 sample had the lower L^*-value but higher a^*-, b^*- and ΔE^*-value, compared with others. Gelling and melting temperatures of all gelatins were 17.09–19.01 and 26.92–28.85 °C, respectively. Furthermore, all gelatins were able to set at room temperature, regardless of size of seabass used. G6 had the shorter setting time at room temperature than others. Therefore, size of seabass, in which skin was used for gelatin extraction, had the impact on yield, composition and properties of resulting gelatin.

1. Introduction

Gelatin is the denatured or partially hydrolysed form of collagen [1]. It represents a major biopolymer with several applications in food, materials, pharmacy and photography industries [2]. Due to its gelling property and surface behaviour (e.g., formation and stabilisations of foams and emulsions), gelatin has been widely used to enhance the elasticity, consistency and stability of food products [3–5].

In general, the main sources of gelatin are skins and bones of pig and cow obtained from processing by-products. Gelatin from those sources can be a problem for certain consumers, e.g., Muslims and Jews, in which porcine gelatin is prohibited. Occurrence of bovine spongiform encephalopathy (BSE) has led to awareness for consumption of bovine gelatin [3]. Nowadays, an increasing interest has been paid to alternative sources of gelatin, especially from the skins and bones from fish processing by-products [6,7]. Fish gelatin can be extracted from skin of several fish species including skipjack tuna, dog shark [8], cobia [9], farmed Amur sturgeon [10], seabass [6], and brownbanded bamboo and blacktip shark [7]. It has been known that the extraction conditions including temperature, time as well as pretreatment affect the functional properties of gelatin from fish processing by-products [6,11,12]. Additionally, characteristics and properties of gelatin vary with species and age of raw material [13,14]. Muyonga, Cole and Duodu [15] reported that gelatin from adult Nile perch skin exhibited better gel properties than young Nile perch skin when the same extraction condition was used.

Seabass is one of economically important fish in Thailand. A number of seabass farms are located in the south of Thailand, especially in the lake of Songkhla. Due to its delicacy, a large amount of seabass has been exported as well as domestically consumed. During processing, particularly fillet production, skin is generated as by-product. Skins from seabass have been used as raw material for collagen and gelatin extraction with higher yield [16]. Gelatin from seabass skin had higher gel strength than bovine gelatin and could be set at room temperature within 30 min [6]. Size or age of seabass, in which skin is used for gelatin extraction, can have the impact on composition and properties of gelatin. Nevertheless, no information regarding gelatin extracted from skin of seabass with different sizes has been reported. Therefore, the aims of this investigation were to extract and determine the chemical characteristics and functional properties of gelatin from the seabass skin as affected by sizes of fish.

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2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Sodium dodecyl sulphate (SDS), Coomassie blue R-250 and N,N,N′,N′-tetramethylethylenediamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). High-molecular-weight markers were purchased from GE Healthcare UK Limited (Buckinghamshire, UK). Fish gelatin produced from tilapia skin (~240 bloom) was obtained from Lapi Gelatine S.p.A (Empoli, Italy).

2.2. Fish skin preparation

Fresh seabass (*Lates calcarifer*) with different sizes of 1.7–2.3, 3.7–4.3 and 5.7–6.2 kg/fish, equivalent to average size of 2, 4 and 6 kg/fish, respectively, were obtained from a farm in Koyo Island, Songkhla, Thailand. The fish were kept in ice with a fish/ice ratio of 1/3 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h after capture. Fish were washed using cold tap water. Skins were then removed, descaled, and cut into small pieces (0.5 cm × 0.5 cm) using a scissor. The skin was placed in polyethylene bags and stored at −20 °C until used, but not longer than 2 months.

2.3. Extraction of gelatin from the skin of seabass

Gelatin was extracted from seabass skin according to the method of Sinthusamran, Benjakul and Kishimura [6]. Before gelatin extraction, skin was soaked in 0.1 M NaOH with a skin/solution ratio of 1/10 (w/v) to remove non-collagenous proteins. The mixture was stirred for 3 h at room temperature (28–30 °C) using an overhead stirrer model W20.n (IKA®-Werke GmbH & CO.KG, Stanfen, Germany). The alkaline solution was changed every 1 h for totally three times. The pretreated skin was washed with tap water until neutral or faintly basic pH was obtained. Then, the washed skin was mixed with 0.05 M acetic acid at a skin/solution ratio of 1/10 (w/v) to swell collagenous material in the fish skin matrix. The mixture was stirred at room temperature for 2 h. The skin was washed using tap water until neutral or faintly acidic pH of wash water was obtained. Finally, the swollen skin was mixed with distilled water at a ratio of 1/10 (w/v) at 45 °C for 3 h with continuous stirring. The mixtures were filtered using a Buchner funnel with Whatman No.4 filter paper (Whatman International, Ltd., Maidstone, England). Then, the filtrates were freeze-dried using a freeze-dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark). The freeze-dried gelatins extracted from seabass skin with an average size of 2, 4 and 6 kg/fish were referred to as 'G2', 'G4' and 'G6', respectively. Gelatin samples were subsequently subjected to analyses.

2.4. Analyses

2.4.1. Yield

The yield of gelatin was calculated based on dry weight of starting material.

$$\text{Yield}(\%) = \frac{\text{Weight of freeze-dried gelatin(g)}}{\text{Weight of initial dry skin(g)}} \times 100$$

2.4.2. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed by the method of Laemmli [17]. Gelatin samples were dissolved in 5% SDS solution. The mixtures were then heated at 85 °C for 1 h using a temperature controlled water bath model W350 (Memmert, Schwabach, Germany). Solubilised samples were mixed at a 1/1 (v/v) ratio with sample buffer (0.5 M Tris–HCl, pH 6.8 containing 5% SDS and 20% glycerol). Samples were loaded onto a polyacrylamide gel made of 7.5% separating gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA/gel. After electrophoresis, gels were stained with 0.05% (w/v) Coomassie blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid for 30 min. Finally, they were destained with the mixture of 50% (v/v) methanol and 7.5% (v/v) acetic acid for 30 min and destained again with the mixture of 5% (v/v) methanol and 7.5% (v/v) acetic acid for 1 h. High-molecular-weight protein markers were used to estimate the molecular weight of proteins.

2.4.3. Amino acid analysis

Amino acid composition of gelatin samples was analysed using an amino acid analyser. The samples were hydrolysed under reduced pressure in 4 M methanesulphonic acid containing 0.2% (v/v) 3-2(2-aminoethyl)indole at 115 °C for 24 h. The hydrolysates were neutralised with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). An aliquot of 0.04 ml was applied to an amino acid analyser (MLC-703; Atto Co., Tokyo, Japan).

2.4.4. Fourier transform infrared (FTIR) spectroscopic analysis

FTIR spectra of gelatin samples were obtained using a FTIR spectrometer (EQUINOX 55, Bruker, Ettlingen, Germany) equipped with a deuterated L-alanine tri-glycine sulphate (DLATGS) detector. The horizontal attenuated total reflectance accessory (HATR) was mounted into the sample compartment. The internal reflection crystal (Pike Technologies, Madison, WI, USA), made of zinc selenide, had a 45° angle of incidence to the IR beam. Spectra were acquired at a resolution of 4 cm^{-1} and the measurement range was 4000–650 cm^{-1} (mid-IR region) at room temperature. Automatic signals were collected in 32 scans at a resolution of 4 cm^{-1} and were ratioed against a background spectrum recorded from the clean empty cell at 25 °C. Analysis of spectral data was carried out using the OPUS 3.0 data collection software programme (Bruker, Ettlingen, Germany).

2.4.5. Measurement of ζ-potential

Gelatin samples were dissolved in distilled water at a concentration of 0.5 mg/ml. The mixture was stirred at room temperature for 6 h. The Zeta (ζ) potential of each sample (20 ml) was measured using a zeta potential analyser (ZetaPALS, Brookhaven Instruments Co., Holtsville, NY, USA). ζ-Potential of samples adjusted to different pHs with 1.0 M nitric acid or 1.0 M KOH using an autotitrator (BIZTU, Brookhaven Instruments Co., Holtsville, New York, USA) was determined. The p*I* was estimated from pH rendering ζ-potential of zero.

2.4.6. Determination of gel strength

Gelatin gel was prepared by the method of Kittiphattanabawon, Benjakul, Visessanguan and Shahidi [7]. Gelatin was dissolved in distilled water (60 °C) to obtain a final concentration of 6.67% (w/v). The solution was stirred until gelatin was solubilised completely and transferred to a cylindrical mould with 3 cm diameter and 2.5 cm height. The solution was incubated at the refrigerated temperature (4 °C) for 18 h prior to analysis.

Gel strength was determined at 8–10 °C using a texture analyser (Stable Micro System, Surrey, UK) with a load cell of 5 kg, cross-head speed of 1 mm/s, equipped with a 1.27 cm diameter flat-faced cylindrical Teflon® plunger. The maximum force (grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded.

2.4.7. Determination of gel colour

The colour of gelatin gels (6.67%, w/v) was measured by a Hunter lab colourimeter (Colour Flex, Hunter Lab Inc., Reston, VA, USA). L^*, a^* and b^* values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were