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Physico-chemical properties and fishy odour of gelatin from seabass (*Lates calcarifer*) skin stored in ice

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ABSTRACT

Properties and fishy odour of gelatins from seabass skin stored in ice for different days were investigated. No differences in extraction yields of gelatins were observed when skins stored for up to 18 days were used. With increasing storage time, the gelatin obtained had the increased α-amino group content ($p<0.05$) with coincidental decrease in α-chain band intensity but had the decreased whiteness. Total viable count and psychrophilic bacterial count of skin increased continuously as the storage time increased. Gel strength of gelatin decreased as the skin was stored in ice for a longer time ($p<0.05$). Lipid oxidation took place in skin during the storage as monitored by peroxide value and thiobarbituric acid reactive substances. Fishy odour intensity of gelatin increased with the increases in volatile aldehydes and alcohols when skin stored in ice for longer time was used. Therefore, the delay of skin processing must be avoided to prevent the formation of undesirable fishy odour and the loss in properties of resulting gelatin.

1. Introduction

Gelatin is biopolymer obtained from collagen by partial denaturation or hydrolysis. It has a wide range of applications in food and non-food industries (Regenstein & Zhou, 2007). Generally, gelatin is obtained from mammals, especially pig and cow skins and bones. Outbreaks of mad cow disease (bovine spongiform encephalopathy, BSE) have caused the anxiety for customers. Additionally, porcine gelatin cannot be used in Kosher and Halal foods due to religious constraints (Kittiphattanabawon, Benjakul, Visessanguan, & Shahidi, 2010). As a consequence, fish gelatin, particularly from fish processing byproducts, has gained increasing attention.

Seabass (*Lates calcarifer*) is an economically important fish species in Thailand and other countries, especially tropical and subtropical regions of Asia and Pacific. In general, it is sold as whole fish or fillets. During processing or dressing of seabass, skin is generated and considered as a byproduct. Conversion to value-added products such as collagen, gelatin as well as hydrolysate with bioactivities has been considered as a means to fully exploit aquatic resources (Sinthusamran, Benjakul, & Kishimura, 2013). However, fishy odour associated with gelatin, particularly extracted from unfresh fish, can limit the application, especially as human food ingredients or supplement.

Recently, Sae-leaw, Benjakul, Gokoglu, and Nalinanon (2013) reported that skin from whole tilapia stored in ice for a long time showed the strong fishy odour, mainly due to the enhanced lipid oxidation. Lipid oxidation is associated with the development of undesirable odour, especially fishy odour, in fish stored for an extended time (Maqsood & Benjakul, 2011). Fishy odour in protein hydrolysate from Nile tilapia

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muscle caused by lipid oxidation was reported by Yarnpakdee, Benjakul, Nalinanon, and Kristinsson (2012). The delay in production of gelatin from fish skin might lead to the undesirable fishy odour of obtained gelatin. This might limit the utilisation of gelatin from fish skin. However, there is no information regarding the properties and fishy odour of gelatin extracted from skin stored in ice for an extended time. Thus, the aim of this study was to evaluate the changes in gelling property, colour, lipid oxidation, fishy odour and volatile compounds development in gelatin extracted from seabass skin stored in ice up to 18 days.

2. Materials and methods

2.1. Chemicals

L-Leucine, 1,1,3,3-tetramethoxypropane and 2,4,6-trinitrobenzenesulphonic acid (TNBS) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 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). Ferrous chloride was obtained from Merck (Darmstadt, Germany). 2-Thiobarbituric acid and cumene hydroperoxide were purchased from Fluka (Buchs, Switzerland). Food grade bovine bone gelatin with the bloom strength of 150–250 g was obtained from Halagel (Thailand) Co., Ltd. (Bangkok, Thailand). All chemicals were of analytical grade.

2.2. Collection and preparation of seabass skins

Fresh skins of seabass (*L. calcarifer*) were obtained from Kingfisher Holdings Co., Ltd., Songkhla, Thailand. Skins were kept in a polystyrene box containing ice using a skin/ice ratio 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. Upon arrival, the remaining meat was removed manually. The skins were washed with tap water, drained for 5 min on a screen, placed in polyethylene bag and kept in ice with a skin/ice ratio of 1:2 (w/w). The packed samples were placed and distributed uniformly between the layers of ice in the insulated boxes, which were subsequently left at room temperature (28–30 °C). To maintain the skin/ice ratio, the molten ice was removed and replaced with new ice every 2 days. The temperature of skin was maintained at 0–2 °C throughout the storage of 18 days. The skin samples were taken at day 0, 6, 12 and 18 for microbiological analysis.

2.3. Microbiological analysis

Total viable count (TVC) and psychrophilic bacteria count from fish skins stored in ice were monitored according to the method of Alfaro, Hernández, Baliño-Zuazo, and Barranco (2013) with a slight modification. Each sample (10 g) was mixed with 90 mL of peptone water (0.1%, w/v) in a stomacher bag and homogenised for 2 min. Appropriate 1:10 dilutions of the resultant homogenate were prepared using 0.1% peptone water. From each dilution, 0.1 mL was spread onto Plate Count Agar (PCA) plates in triplicate, using a glass spreader. The plates were incubated at 37 °C for 2 days and at 5 °C for 7 days for determination of TVC and psychrophilic bacterial count, respectively. The plates containing 30–300 colonies were counted and the results were expressed as log of colony forming units per gram (log CFU/g).

2.4. Extraction of gelatin from seabass skins

Fresh skins and skins stored in ice for 6, 12 and 18 days were used for gelatin extraction as described by Benjakul, Oungbho, Visessanguan, Thiansilakul, and Roytrakul (2009) with some modifications. To remove non-collagenous proteins, skins were soaked in 0.05 M NaOH with a skin/solution ratio of 1:10 (w/v). The mixture was stirred for 2 h at room temperature (about 26–28 °C). The alkaline solution was changed every hour. Alkaline-treated skins were then washed with tap water until neutral or faintly basic pH of wash water was obtained. Thereafter, the skins were rinsed with distilled water. Subsequently, the skins were then soaked in 0.05 M acetic acid with a skin/solution ratio of 1:10 (w/v) for 1 h with gentle stirring to swell the collagenous material in the skin matrix. Acid-treated skins were washed thoroughly as previously described. After swelling, the swollen skins were immersed in distilled water (55 °C) with a skin/water ratio of 1:10 (w/v) in a water bath (W350, Memmert, Schwabach, Germany) for 6 h with a continuous stirring using an overhead stirrer (RW 20.n, IKA labortechnik, Germany) at a speed of 150 rpm to extract the gelatin from skin matter. The mixture was then filtered using two layers of cheesecloth. The resultant filtrate was lyophilised using a freeze-dryer (Model Duratop™ lP/Dura Dry™ lP, FTS® System, Inc., Stone Ridge, NY, USA). The lyophilised gelatins produced from fresh skins and skins stored in ice for 6, 12 and 18 days were referred to as GD0, GD6, GD12 and GD18, respectively. The obtained gelatins were subjected to analyses.

2.5. Analyses

2.5.1. Yield

Gelatin yield was calculated by the following equation:

$$\text{Yield } (\%) = \frac{\text{weight of dry gelatin (g)}}{\text{weight of dry skin (g)}} \times 100$$

2.5.2. α-Amino group content

The α-amino group content was determined according to the method of Benjakul and Morrissey (1997). Lyophilised gelatin samples were dissolved in distilled water. Properly diluted samples (125 μL) were mixed thoroughly with 2.0 mL of 0.20 M phosphate buffer, pH 8.2, followed by the addition of 1.0 mL of 0.01% TNBS solution. The mixtures were then placed in a water bath at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 mL of 0.1 M sodium sulphite. The mixtures were cooled down at room temperature for 15 min. The absorbance was measured at 420 nm and α-amino group content was expressed in terms of L-leucine.

2.5.3. Protein patterns

Protein patterns were determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of