เจลาตินจากปลานิล : การกำจัดกลิ่นคาว
Tilapia Gelatin : Elimination of Fishy Odor

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Received: 4 October 2018, Revised: 24 December 2018, Accepted: 26 December 2018

บทคัดย่อ

ปลานิลแล่เป็นผลิตภัณฑ์แปรรูปจากปลาน้ำจืดที่ประเทศไทยส่งออกมากที่สุดจึงมีเศษเหลือที่เกิดจากกระบวนการผลิตจำนวนมาก ได้แก่ หัวปลา ก้างปลา เลือด ไส้ปลา หนังและเกล็ดปลา ซึ่งสามารถนำมาใช้เป็นวัตถุดิบในการผลิตผลิตภัณฑ์พลอยได้จากการแปรรูปสัตว์น้ำ เช่น คอลลาเจนและเจลาตินเป็นต้น หนังปลานิลที่มีปริมาณมากเพียงพอจะใช้เป็นวัตถุดิบในการผลิตเจลาตินเชิงอุตสาหกรรมได้ แต่เนื่องจากหนังปลานิลมีกลิ่นคาวมากจึงเป็นอุปสรรคในการผลิตเชิงการค้า วัตถุประสงค์ของการศึกษาในครั้งนี้เพื่อลดกลิ่นคาวออกจากหนังปลานิลที่ใช้ในการสกัดเจลาติน โดยการเตรียมตัวอย่างหนังปลานิลจะถูกล้างด้วยสารเคมี 5 การทดลอง ได้แก่ โซเดียมคลอไรด์ โซเดียมไฮดรอกไซด์ กรดซัลฟูริกและกรดซิตริก ที่ความเข้มข้นแตกต่างกัน แล้วล้างหนังปลาจนน้ำล้างมี pH เป็นกลาง จึงนำมาสกัดเจลาตินด้วยน้ำร้อนอุณหภูมิ 50 °C เป็นเวลา 3 ชั่วโมง จากนั้นนำมากรองและนำส่วนใสไประเหยน้ำออกที่อุณหภูมิ 50 °C ก่อนนำไปอบที่อุณหภูมิ 50 °C เป็นเวลา 16 ชั่วโมง จากนั้นนำไปวิเคราะห์ผลผลิตสุทธิที่ได้ ค่าความแข็งแรงของเจล วิเคราะห์ปริมาณสารระเหยที่ได้ด้วยเครื่อง GC/MS และประเมินคุณภาพทางประสาทสัมผัส พบว่าการใช้โซเดียมคลอไรด์ที่ความเข้มข้น 1.5% โซเดียมไฮดรอกไซด์ที่ความเข้มข้น 0.2% กรดซัลฟูริกที่ความเข้มข้น 0.2% และกรดซิตริกที่ความเข้มข้น 1% ทำให้เจลาตินที่สกัดได้มีปริมาณผลผลิตสูงสุด (20.37±0.64%) มีค่าความแข็งแรงของเจลสูงสุด (1,811.73±8.80 g) เจลาตินที่ได้ใส ไม่มีสี ไม่มีกลิ่นคาวและได้รับการยอมรับจากผู้ทดสอบจึงทำให้เจลาตินจากหนังปลานิลที่ได้สามารถนำไปประยุกต์ใช้ในอุตสาหกรรมอาหาร เครื่องสำอางและเภสัชกรรมได้

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ABSTRACT

Tilapia fillets are the main processed freshwater fish export from Thailand. Many by-products are created during processing, including head, bone, blood, intestine, skin and scales. These can be used as raw materials for the production of fish oil, fish meal, protein concentrate, calcium, collagen and gelatin. Nile tilapia skin can be used for industrial gelatin production, and it is available in large quantities. However, because of its strong fishy odor, it is not acceptable for this application. The objective of this study was to remove fishy odor from Nile tilapia skin for use in gelatin extraction. Nile tilapia skin was treated by soaking in five different combinations of NaCl, NaOH, sulfuric acid and citric acid, and then rinsed with tap water until neutral pH of the wash water was obtained. Gelatin from Nile tilapia skin was extracted with water at 50 ºC for 3 h, then the filtered solution was evaporated at 50 ºC before oven drying at 50 ºC for 16 h. The product was analyzed for yield, gel strength and volatile compounds with GC/MS and evaluated for sensory characteristics. The pretreatment of 1.5% NaCl, 0.2% NaOH, 0.2% sulfuric acid and 1% citric acid gave high yield (20.37±0.64%) and gel strength (1,811.73±8.80 g) of clear, colorless and odorless gelatin. Nile tilapia skin gelatin was accepted by panelists. Then gelatin from Nile tilapia skin would be suitable for use by food, cosmetics and pharmaceutical industries.

Key words: fishy odor, fish gelatin, Nile tilapia skin

INTRODUCTION

Thailand exports tilapia fillets, as the main freshwater fish species, to the Middle East and USA. Tilapia has white flesh which contains high protein. In 2016, Thailand's tilapia production of 164,630 tons was mainly used for domestic consumption, with 3,108.7 tons exported as frozen whole fish (73.7%), frozen fish fillets (25.7%) and other products (0.6%). Tilapia fillet processing creates 800 tons of by-product per year; 33% head, 16% bone, 8% intestine, and 7% skin and scale of fish. These remains consist of protein, minerals and other nutrients (FAO, 2017). They can be processed into many kinds of products such as gelatin, calcium, chitin, chitosan and protein hydrolysate. Tilapia skin can be used to produce collagen (Waswa *et al*., 2007; Chen *et al*., 2016; Li *et al.*, 2018; Liao *et al*., 2018; Yan and Wang, 2018) or gelatin (Jayathilakan *et al*., 2012; Zhang *et al*., 2016; Pang *et al*., 2017; Santos *et al*., 2018). However, gelatin extraction from tilapia skin is not done commercially because of fishy odors of the raw materials. This remains a major problem in commercial production waiting for a solution. Reduction of fishy odor from salmon skin is possible by soaking in 1% NaCl for 5 minutes, followed by washing with tap water and rinsing in hot water at 50 ºC for 1 minute. The treated fish skin has a slight fishy odor (Tiwtha and Usawakesmanee, 2012). The objective of this study was to remove fishy odor from tilapia skin for use in gelatin extraction.

MATERIALS AND METHODS

Materials

Nile tilapia (*Oreochromis niloticus*) skins were obtained from Grobest Marine Co., Ltd., Bangkok, Thailand. The skins (10 kg) were packed in polyethylene bags and kept in ice with a fish skin to ice ratio of 1:2 (w/w). The material was transported to the Department of Fishery Products, Kasetsart University, Bangkok within 2 h. Skins were prepared by trimming off remaining meat. The skin was then cut into small pieces (1.0 × 1.0 cm^2) and placed in polyethylene bags (1 kg skin/bag) and stored at -20 °C until use.

Gelatin Extraction

Nile tilapia skin was soaked in five different solutions prior to gelatin extraction, at a skin: solution ratio of 1:4 (w/v) and shaken at a speed of 350 rpm at

room temperature. Nile tilapia skin was then rinsed with tap water until a neutral pH of wash water was obtained before extraction of gelatin with 50 ºC water for 3 h. Finally, filtered solution was evaporated at 50 ºC, followed by oven drying at 50 ºC for 16 h. A control (treatment 1) was not soaked in any chemicals, but was extracted in water in the same way as the other treatments.

For the remaining five treatments, fish skin was soaked in the following solutions for the time indicated in parentheses: treatment 2-1.5% NaCl (1 h); treatment 3-0.2% NaOH, 0.2% H_2SO_4 and 1% citric acid (2 h) (according to Grossman and Ramat, 1992); treatment 4-1.5% NaCl, 0.2% NaOH, 0.2% H_2SO_4 and 1% citric acid (2 h); treatment 5-1.5% NaCl, 0.2% NaOH and 0.2% H_2SO_4 (2 h); treatment 6-1.5% NaCl, 0.2% NaOH and 1% citric acid (2 h). For treatments 3-6, solutions were changed after the first hour.

Yield of extracted gelatin

Yield of extracted gelatin was calculated from the formula:

Yield (%) = (dried weight of gelatin/wet weight of skin) × 100

Determination of gel strength

Gel strength was determined using a Texture analyzer (TA.XT Plus, Stable Micro Systems Ltd., Surrey, England). Dried gelatin (6.67%, w/v) was mixed with distilled water at 65 °C for 15 min until completely dissolved. The gelatin solution was added to glass measuring bottles and then kept at 4 °C for 12 h. The dimensions of the sample were 6 cm in diameter and 3.5 cm in height. The maximum force (gram) was recorded when the penetration distance reached 4 mm. The speed of the plunger was 0.5 mm/s. Gel strength of tilapia skin gelatin was compared with commercial fish gelatin.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of gelatin were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), with 7.5% separating gel and 4% stacking gel according to the method described by Laemmli (1970). A 50% (w/v) gelatin solution was mixed with the buffer (0.5 M Tris-HCl, pH 6.8 containing 10% SDS (w/v), glycerol, 0.5% bromophenol blue, 2-mercaptoethanol) at a ratio of 1:1 (v/v). The mixtures were incubated at 90 °C for 30 minutes and centrifuged at 6,000xg to remove undissolved debris. The loading volume of each sample was 10 µl per well. Electrophoresis was performed at a constant voltage of 180 V by using Mini-Protein®II Electrophoresis cell (Bio-Rad Laboratories Ltd, Thailand). After electrophoresis, the gel was stained with 0.1% (w/v) Coomassie blue R-250 in 40% (v/v) methanol, 10% (v/v) acetic acid and 50% (v/v) water, and then de-stained with 40% (v/v) methanol, 10% (v/v) acetic acid and 50% (v/v) water. Precision plus protein standard (New England BioLabs Inc., USA) was used to estimate the molecular weight of protein.

Gas chromatography-mass spectrometry (GC-MS) analysis

Gelatin was mixed (6.67%, w/v) with distilled water at 65 °C for 15 min until completely dissolved. The gelatin solution was added to 5 ml cap vials (head space screw-tap 20 ml clear vials). GC-MS analysis was performed using an Agilent Technologies 7890B coupled with Agilent Technologies 5977A mass-selective detector equipped with a splitless injector and coupled with a quadrupole mass detector (Stable Micro Systems Ltd., Surrey, England). Compounds were separated on a HP-Innowax capillary column (Stable Micro Systems Ltd., Surrey, England) (30 m × 0.25 mm ID, with film thickness of 0.25 mm). The GC oven temperature program was 40 ºC for 3 min followed by an increase of 10 ºC/min to a final temperature of 230 ºC and holding for 3 min. Helium was employed as a carrier gas, with a constant flow of 1 mL/min. The injector was operated in the splitless mode and its temperature was set at 250 ºC. Transfer line temperature was maintained at 270 ºC. The quadrupole mass spectrometer was operated in the electron

ionization (EI) mode and source temperature was set at 250 ºC. Initially, full scan mode data was acquired to determine appropriate masses for the later acquisition in scan mode under the following conditions: mass range 20-450 amu and scan rate 0.220 s/scan. All the analyses were performed with ionization energy of 70 eV, filament emission current at 150 mA, and the electron multiplier voltage at 450 V (Sukkwai *et al*., 2010).

Sensory evaluation

Gelatin gel was prepared as above (6.67%, w/v). Sensory evaluation of fishy odor intensity of the gelatin gel was carried out according to Sae-leaw and Benjakul (2015) using 50 trained panelists from the Department of Fishery products, Kasetsart University with the ages of 23-30. The panelists were asked to open the sealable cup and sniff the head space above the samples in order to determine the intensity of fishy odor, using a just about right scale, in which a score of 3 is ideal, meaning no fishy odors or other odors. A score of 1 indicates no fishy odor, but other odors (such as chlorinated water) are present, while 5 indicates extremely strong fishy odor. Panelists judged the intensity of color using a just about right scale from 1 (opaque) to 5 (strong yellow), with 3 being ideal.

Statistical analysis

All experiments were run in triplicate with completely randomized design (CRD). Sensory evaluation used randomized complete block design (RCBD). Data were subjected to one-way analysis of variance (ANOVA) and mean comparisons were carried out by using Duncan's multiple range test. Statistical tests were done using the SPSS computer program (SPSS Statistical Software Inc., version 23, Chicago, IL, USA). Differences between means were tested by the Duncan's multiple range test. The data were presented as mean ± standard deviation. A probability value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Yield of extracted gelatin

Gelatin preparations were performed under identical conditions with the exception of the treatment solutions. The extracted yields were 1.95±0.19 to 21.03±0.55% and the lowest yield was obtained from treatment 1 (control), while the highest yield was obtained from treatment 4 (1.5% NaCl, 0.2% NaOH, 0.2% H_2SO_4 and 1% citric acid). The gelatin yields from each treatment were different, as shown in Table 1. The treatment of tilapia skin with alkaline/acid removes soluble proteins, lipids and other undesired components, and disrupts some cross links of collagen molecules. The yields of tilapia gelatin in this study are similar to other reports which ranged from 17.63-21.93% (Grossman and Ramat, 1992; Zeng *et al*., 2010; Nui *et al*., 2013), but are higher than Jamilah *et al*. (2011) who used different treatment conditions (chemicals, time, temperature) from this study. The use of NaCl and alkaline/acid increased the yield of extracted gelatin compared to the control. The NaCl helped to remove blood and mucus (Barve and Gardre, 2012), while the alkaline/acid removed non-collagen substances, such as elastin, albumin, mucopoly saccharide and affected the arrangement of new molecular structure.

Table 1 Characteristics of gelatin produced from tilapia skin, following treatment with various solutions.

Treatment	Soaking solution	Yield (%)*	Gel strength (g)*	Methoxy phenyl oxime (%)*	Remarks
1	None	1.95±0.19^{c}	0.00±0.00	22.17^{a}	Control
2	NaCl	18.79±0.23^{b}	541.87±6.74^{d}	20.64^{a}	
3	NaOH+H_2SO_4 +citric acid	21.03±0.55^{a}	856.07±5.30^{c}	23.32^{a}	Grossman and Ramat (1992)
4	NaCl+NaOH+ H_2SO_4+citric acid	20.37±0.64^{a}	1811.73±8.80^{a}	8.41^{c}	
5	NaCl+NaOH+ H_2SO_4	20.02±0.64^{a}	1482.61±2.13^{b}	15.72^{b}	
6	NaCl+NaOH +citric acid	19.96±0.64^{ab}	1504.61±1.63^{b}	14.11^{b}	
commercial gelatin	None	-	964.50±7.92^{c}	-	

* Values presented as mean ± SD from triplicate determinations.
[a-d] Different letters in the same column indicate significant difference ($p < 0.05$).

Determination of gel strength

Gel strength is one of the most important functional properties of gelatin. Gel strength is a function of complex interactions determined by amino acid composition and the ratio of α-chains to β-components. In this experiment, gel strength of commercial fish gelatin was 964.50±7.92 g, which is higher than Grossman and Ramat (1992), although it is not known if the commercial gelatin used in that study was identical to the commercial gelatin in this study, or if the gelatins were prepared in the same way. Control treatment gelatin remained in solution, and therefore gel strength could not be determined. Gel strength of treatment 4 was highest among the experimental treatments and higher than commercial fish gelatin. There were significant differences in gel strengths among all treatments, except for treatments 5 and 6 (Table 1). Differences could be due to intrinsic characteristics, such as molecular weight distribution and amino acid composition, as well as the type of extraction treatment. High gel strength of extracted gelatin may be due to the possible high content of the amino acids proline and hydroxyproline, which could result in massy organized triple helical structures. Proline and hydroxyproline are thought to be responsible for the stability of the triple helix of collagen structure through hydrogen bonding between free water molecules and hydroxyl groups of the hydroxyproline in gelatin (Fernandez-Diaz *et al*., 2001).

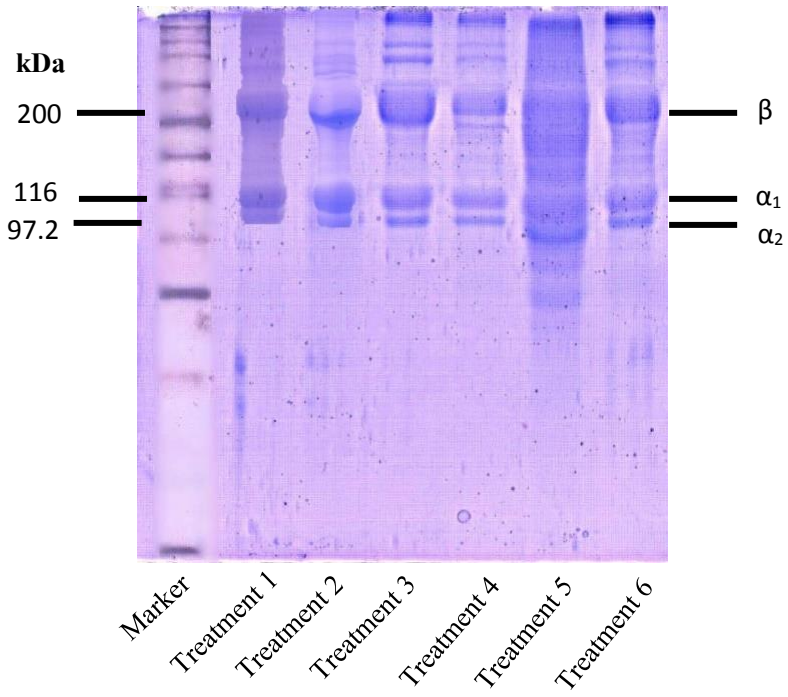

Figure 1 Protein MW distributions of gelatin from tilapia skin extracted following treatment with different solutions, and commercial gelatin.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of tilapia skin gelatin are shown in Fig. 1. All gelatins contained α-chains as the major components. β-component (α-chain dimers) were also noticeable. Tilapia skin gelatin contained α1 and α2 chains and was characterized as type I protein (Benjakul *et al*., 2010; Sukkwai *et al*., 2010). During gelatin extraction, the conversion of collagen to gelatin with varying molecular mass takes place, due to the cleavage of inter-chain cross-links (Zhou *et al*., 2006). Degradation of major components into lower molecular weight fragments or shorter chains might result in lowering gelatin's gel strength and viscoelastic properties.

Fishy odor analysis

The volume of methoxy phenyl oxime remaining in the gelatin after treatments of the tilapia skin (Area normalized (%)) was analyzed by GC-MS, with results shown in Table 1. A high level of methoxy phenyl oxime produces a strong fishy odor. Treatment 2 (1.5% NaCl) had lower levels of this compound compared with the control (treatment 1), although the difference was not significant. Methoxy phenyl oxime in gelatin also decreased when acid was incorporated during gelatin extraction (treatments 4, 5 and 6). The formation of secondary lipid oxidation products is the main contributor to undesirable offensive fishy odor in fish skin (Sae-leaw *et al*., 2013; Sae-leaw and Benjakul, 2014). The fishy odor in skin, which contained high levels of poly unsaturated fatty acids, was mediated by lipid autoxidation and lipoxygenase induced oxidation. The results suggested that the use of NaCl in combination with acid prior to gelatin extraction was a promising means to minimize the formation of fishy odor in the gelatin. Meanwhile, the treatment that did not include any NaCl (treatment 3) actually had higher levels of methoxy phenyl oxime than the control. In the treatment 4, methoxy phenyl oxime was reduced by more than 2 times compared to the control and was lower than all other treatments (p<0.05). Thus, treatment with NaCl or alkaline/acid could not reduce fishy

odor, but a combination of NaCl and alkaline/acid were more effective. Washing fish skin with salt solution has been reported to be an important process to remove lipids and undesirable materials such as blood, pigment and odorous substances (Kristinsson *et al*., 2005). NaOH removes non-collagen substances, such as elastin, albumin and mucopoly saccharide, and plays a role in the arrangement of new structure. Citric acid was reported to help in removal of membrane lipids (Liang and Hultin, 2005) and could act as a metal chelator (Choe and Min, 2009). Citric acid disconnects the linkages between cytoskeletal proteins and phospholipids, linked together via electrostatic interaction (Liang and Hultin, 2005). Moreover, citric acid plays a role as a binding agent for the basic amino acid residues of cytoskeletal proteins, thereby competing with the acidic phospholipids of membranes (Hrynets *et al*., 2011).

Sensory evaluation

Results of the sensory evaluation of gelatin from tilapia skin were similar to the results from GC-MS (Table 2). The panelists detected significant differences in fishy odor and color of gelatin among treatments. Treatment 1 (control) could not be tested, as the gelatin remained in solution. Commercial gelatin and treatment 3 received significantly higher scores than the other treatments, and were found to have strong fishy odor to extremely strong odor. The remaining treatments received lower scores, indicating light odor to moderate odor. Acceptability scores for treatment 4 were higher than commercial fish gelatin. These results are in accordance with Kawahara and Tanihata (2005), who showed that washing Atlantic cod fillets with 1% w/v of sodium chloride solution reduced fishy odor, and that treating with a combination of sodium chloride and sodium bicarbonate could improve flavor and texture, and reduced formation of volatile lipid compounds (Magnus and Turid, 2012).

Table 2 Sensory evaluation scores for gelatin extracted from tilapia skin.

Characteristic	Treatment*						
	1	2	3	4	5	6	Commercial gelatin
Color[1]	-	4.37±0.21^{b}	5.00±0.04^{c}	3.31±0.07^{a}	3.20±0.07^{a}	3.26±0.07^{a}	5.00±0.07^{c}
Odor[2]	-	4.04±0.08^{ab}	5.00±0.17^{c}	3.30±0.11^{a}	4.16±0.11^{b}	3.70±0.11^{a}	5.00±0.11^{c}
Acceptability[3]	-	1.00±0.00^{c}	1.00±0.00^{c}	2.00±0.00^{a}	1.60±0.07^{b}	1.84±0.04^{b}	1.00±0.00^{c}

* Values presented as mean ± SD from triplicate determinations.
a-c Different letters in the same row indicate significant difference (p<0.05).
[1]JAR scale: 1 = opaque, 5 = strong yellow.
[2]JAR scale: 1 = non-fishy odors, 3 = no odors, 5 = strong fishy odors.
[3]A score of 1 indicates rejection, 2 indicates acceptance.

CONCLUSION

The best treatment of tilapia skin prior to gelatin extraction was using a solution of 1.5% NaCl, 0.2% NaOH, 0.2% H_2SO_4 and 1% citric acid. This produced a gelatin with a yield of 20.37±0.64%, high gel strength (1811.73±8.80 g) and was characterized to be type I protein. The treatment also reduced the level of volatile compounds in the extracted gelatin and improved gel strength. The properties of this gelatin produced from tilapia skin

should be suitable for applications in the cosmetic, biomedical, pharmaceutical and food industries.

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