

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Effect of extraction pH and temperature on yield and physicochemical properties of gelatin from Atlantic salmon (Salmo salar) skin

Gerry Renaldia, Nualpun Sirinupongb,c, Rajnibhas Sukeaw Samakradhamrongthaia,c,

- ^a Food Science and Technology Program, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
- ^b Functional Food and Nutrition Program, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand
- ^c Division of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand

Article Info

Article history:

Received 11 September 2021 Revised 17 January 2022 Accepted 23 May 2022 Available online 26 August 2022

Keywords:

Chemical properties, Extraction, Gelatin, Physical properties, Salmo salar skin

Abstract

<u>Importance of the work</u>: The use of different conditions in the gelatin extraction affected the yield and physicochemical properties of the resultant gelatin.

<u>**Objectives**</u>: To investigate the effects of different extraction levels of pH and temperature on the properties of gelatin from Atlantic salmon (*Salmo salar*) skin.

<u>Materials & Methods</u>: Different extraction levels for pH (4, 6, 8) and temperature (50 °C, 60 °C, 70 °C) were investigated to extract the gelatin and the results were analyzed regarding the yield and physicochemical properties.

Results: The extraction pH and temperature significantly affected the yield and physicochemical properties of the gelatin extracted from Atlantic salmon skin. Extraction at pH 4 and 50°C provided the highest yield (6.20±0.09%), while the extraction at pH 6 and 50 °C resulted in the gelatin containing 9.20±0.37% protein and 73.60±0.46% hydroxyproline content, with the highest gel strength (198.67±4.51 g). The gelatin having values of L*, a* and b* of 81.89±1.09, -0.47±0.01 and 3.07±0.11, respectively with a gelling temperature of 12.54±0.07 °C and a melting temperature of 23.82±0.14 °C. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein pattern analysis showed that the extraction with basic conditions hydrolyzed the protein more than with acidic conditions.

<u>Main finding</u>: The use of different levels of pH and temperatures should be taken into consideration prior to gelatin extraction, especially for gelatin extraction from fish skin, to obtain gelatin with the preferred yield and properties for further application.

[†] Equal contribution.

^{*} Corresponding author.

E-mail address: rajnibhas.s@cmu.ac.th (R.S. Samakradhamrongthai)

Introduction

Atlantic salmon (*Salmo salar*) is a salmonid fish native to North Atlantic temperate and subarctic regions that can be found in landlocked and anadromous environments throughout its range in the North Atlantic (Webb et al., 2007). Since 2013, salmon has the largest single fish commodity by value, reaching 18.1% of the overall share in the world trade of fish and fish products (Food and Agriculture Organization of the United Nations, 2018). High production of fish has resulted in high amounts of by-products, such as the frame and skin that can be further utilized as sources of valuable products, including gelatin, a well-known hydrocolloid in the food industry, with the gelatin obtained from the fish skin being a major, highly valued by-product of the fish-processing industry (Badii and Howell, 2006).

Hydrocolloids are a heterogeneous group of long-chain polymers that are characterized by their property of forming gels or viscous dispersions in water or both (Saha and Bhattacharya, 2010). Gelatin is one of the hydrocolloids obtained from a partial denaturation of collagen that has extensive use in the food, cosmetic and pharmaceutical industries (Benjakul et al., 2012). In food industries, gelatin is commonly used as a thickener, stabilizer and texturizer and as a gelling agent (Badii and Howell, 2006; Saha and Bhattacharya, 2010) in diverse types of products, including confectionery, dairy and meat products (Stevens, 2010). However, due to outbreaks of foot-and-mouth disease and bovine spongiform encephalopathy, the main sources of gelatin are limited to porcine or bovine bone or dermis. Gelatin can be extracted from animal parts, especially skin and bone. Gelatin sources are pork and cattle bones (23.1%), bovine hide (29.4%) and pig skin (46%), according to Gómez-Guillén et al. (2011). Gelatin from marine sources is a possible alternative to porcine and bovine gelatin and is also acceptable to Muslims and Jews. Thus, the study and research of gelatin from fish, and specifically fish skin, has gained more attention.

Existing methods used to obtain fish gelatin are extraction for 3–18 hr at an average temperature of 45–75 °C and a neutral pH (Benjakul et al., 2012; Mahmood et al., 2016). However, the current study was needed, taking into account the scarcity of studies and the potential for the extraction conditions to affect the properties of the resulting gelatin,by investigating the effects of different levels of pH and temperature on the yield and physicochemical properties of gelatin extracted from Atlantic salmon skin.

Materials and Methods

Material and sample preparation

Frozen Atlantic salmon skins were obtained from a fish industry in Hat Yai district, Songkhla, Thailand. After arrival at the Division of Food Science and Technology, Prince of Songkla University, the skins were stored at -20 °C. The frozen skins were thawed using tap water and cut in pieces (approximately 2 cm × 2 cm) prior to the analysis and gelatin extraction.

Chemicals

High molecular weight protein (10–225 kDa) was obtained from Merck (Darmstadt, Germany). Coomassie Blue R-250, *N,N,N'*, *N'*-tetramethylethylenediamine (TEMED) and sodium dodecyl sulfate (SDS) were procured from Bio-Rad Laboratories (Hercules, CA, USA). Glutaraldehyde (PubChem CID 3485) was secured from Sigma-Aldrich Chemie GmbH; Germany. HCl (PubChem CID 313), NaOH (PubChem CID 14798), Chloramine T (PubChem CID 517414) and p-dimethylaminobenzaldehyde (p-DMAB) (PubChem CID 7479) were acquired from Loba Chemie Pvt. Ltd (Mumbai, India). CH3COOH (PubChem CID 176) was obtained from QReC (Auckland New Zealand. Ethanol (PubChem CID 702) and isopropanol (PubChem CID 3776) were purchased from RCI Labscan (Bangkok Thailand). All chemicals were analytical grade.

Gelatin preparation

Removal of non-collagenous protein and defatting

The pretreatment of the salmon skin followed the method of Ali et al. (2018), with slight modification. The skins were immersed in 0.1 M NaOH (1:10 weight per volume, w/v) and stirred for 6 hr at room temperature using a magnetic stirrer (RT 10 power IKAMAG, IKA–Werke; Staufen, Germany). The alkaline solution was replaced every 2 h. The pretreated salmon skin was washed with tap water until the pH of the wash water was slightly basic or neutral (pH 7.0–7.5) that was measured using a digital pH meter (OHAUS, OHAUS Corp.; NJ, USA). The pretreated skin was subjected to a defatting process using 30% isopropanol for 1 h at room temperature (1:10 w/v) with a magnetic stirrer (Sae-Leaw et al., 2016). The solvent was removed and the defatted skins were washed with tap water.

Acid and ultrasound-assisted pretreatment

After the removal of non-collagenous protein and the fat, the skin was subjected to the acid and ultrasound-assisted pretreatment, following the method of Ali et al. (2018), with minor modification. The defatted skin was pretreated with acid and ultrasound consecutively. The salmon skin was immersed in 0.05 M acetic acid (1:10 w/v). The mixture was subjected to continuous stirring using the magnetic stirrer for 2 h at room temperature. Then, the skin was cleaned with running tap water until the pH of the wash water was slightly acidic or neutral (pH 6.5–7.0) and drained on a screen.

The acid-pretreated skin was immersed in distilled water at a skin-to-water ratio of 1:10 (w/v). Ultrasonication of the skin was carried out for 30 min using an ultrasonic bath (Sonorex RK 100 H, Bandelin Electronic GmbH and Co. KG; Berlin, Germany) with power and frequency of 80 W and 35 kHz, respectively. The temperature was maintained at 25±2 °C using an iced bath and the temperature was observed using a digital thermometer.

Gelatin extraction

The skin samples were immersed in distilled water with different levels of pH (4, 6, or 8; 1:10 w/v) that were adjusted using 1 M citric acid and 1 M sodium hydroxide, monitored using the digital pH meter and was incubated using a water bath (Memmert; Schwabach, Germany) at different temperatures (50 °C, 60 °C, or 70 °C) for 6 h with occasional stirring. After 6 h, the extract was filtered through cheesecloth. Then, 1% activated carbon was mixed with the filtrate and subjected to vacuum-assisted filtration using a Buchner funnel with Whatman filter paper No. 4 (Sinthusamran et al., 2014, with slight modification). The obtained gelatin sample was dried using a freeze-dryer (CoolSafe 55, ScanLaf A/S; Lynge, Denmark). The calculation of the yield was based on Equation 1:

Yield (%) = (Freeze-dried gelatin /
Initial skin wet weight))
$$\times$$
 100 (1)

where all weights were measured in grams.

Gelatin characterization

Protein patterns

The protein patterns of the gelatin were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Ali et al. (2017)), with slight modification. Each

gelatin sample was dissolved in 5% SDS and heated at 85 °C for 1 h using a heating block. The polyacrylamide gel used to load of the sample consisted of 7.5% running gel and 4% stacking gel and the electrophoresis was carried out at a constant current of 15 mA/plate using a Mini Protein III unit (Bio-Rad Lab. Inc.; Richmond, CA, USA). The molecular weight of the gelatin sample was estimated using a high molecular weight protein marker.

Protein and hydroxyproline content

The Biuret method (Zhou and Regenstein, 2006) was used to determine the protein content of the gelatin from Atlantic salmon skin, expressed as milligrams per milliliter of sample. The hydroxyproline content was measured based on the method described by Bergman and Loxley (1963), with slight modification. The absorbance of the sample was measured at 558 nm, calculated based on the standard curve on the absorbance of the hydroxyproline solution at different concentrations (0–10 mg/mL). The absorbance of the sample was used in the equation generated from the standard curve and expressed as milligrams per gram gelatin.

Measurement of physical properties

The gelatin samples were measured for their physical properties (color, gelling and melting temperatures and gel strength). The gelling and melting temperatures were determined using the methods described by Elavarasan et al. (2017). For the gelling temperature measurement, the gelatin solutions (6.67% w/v) were heated using a water bath at 40 °C. A digital thermometer was inserted and lifted out every 30 s. The gelling temperature was recorded when the thermometer was not dislodged from the gelatin gel. For the measurement of the melting temperatures, a 5 mL aliquot of each gelatin solution (6.67% weight per weight) was prepared in a test tube. The tubes were covered with parafilm and heated in the water bath at 60 °C for 15 min, cooled and stored immediately in a refrigerator (4 °C) for 16-18 h. After storage, the gels were put in a water bath. Then, every 30 s, the test tubes with gels were lifted and observed visually for melting. The melting point was recorded as the temperature at which the solution began to move freely upon tilting.

The color of the gelatin gel samples (6.67%, w/v) was measured using a color meter (ColorFlex, Hunter Lab Inc.; VA, USA), where the L* color parameter represents the darklight spectrum with a range of black (0) to white (100), the a* color parameter represents red or green color and the b* color parameter represents the yellow or blue color (Mutlu et al., 2018).

The gel strength measurement was done following the method of British Standards Institution (1975) that had been modified by Ali et al. (2018). Gelatin solution (6.67% w/v) was made by dissolving the freeze-dried gelatin in distilled water, heating to 60 °C and stirring until the gelatin was solubilized. The solution (approximately 20 mL) was poured into a mold (3 cm in diameter and 2.5 cm in height) and cooled in the refrigerator at 4 °C for 16–18 h. The gel strength was determined using a texture analyzer model TA–XT2 (Stable Micro System; Godalming, UK) by forcing through a plunger (1.27 cm in diameter). The maximum force (measured in grams) was recorded when the penetration distance reached 4 mm.

Microstructure

The microstructures of the gelatin gels were visualized using scanning electron microscopy (SEM; JEOL JSM-5800 LV; Tokyo, Japan) at an acceleration voltage of 10 kV. The samples were prepared according to the method described by Ali et al. (2018), with slight modification. Gelatin gel samples were cut (3–4 mm thick and 2–3 mm wide) and fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h, rinsed using distilled water for 1 h, dehydrated using ethanol (50%, 70%, 80%, 90% and 100% consecutively, each for 15 min), and subjected to critical point drying using a CO₂ critical point dryer (Tousimis Automatic; city, country). The dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module; West Chester, PA, USA) prior to the observation.

Statistical analysis

The data were presented as mean \pm SD. Two-way analysis of variance was carried out using the SPSS software, version 17 (SPSS Inc.; Chicago, IL, USA). Differences between means were tested using Duncan's multiple range test at a significance level of p < 0.05.

Results and Discussion

Yield, total protein content and hydroxyproline content

The yield, total protein content and hydroxyproline content of the gelatin extracted from Atlantic salmon skin were significantly affected by the pH and temperature used in the extraction process (Table 1, Fig. 1). The gelatin yields extracted under different extraction condition were in the range 2.06–6.20%. The gelatin values showed similar results with the yield from blue whiting (1.5–2.4%), according to Khiari et al. (2015), while being lower than from black and red tilapia (5.4% and 7.8%), megrim (7.4%), Dover sole (8.3%), cod (7.2%) and hake (6.5%), according to Gómez-Guillén et al. (2002) and Jamilah and Harvinder (2002). The statistical analysis showed that the interaction between pH and temperature affected the gelatin yield (*p*-value = 0.012).

Table 1 Yield, protein content and hydroxyproline content of gelatin extracted from Atlantic salmon skin under different levels of pH and temperature

рН	Temperature (°C)	Yield (%)	Protein content	Hydroxyproline content	
			(mg/mL sample)	(mg/g gelatin)	
	50	6.20 ± 0.09^{a}	9.39±0.21	73.60±0.20	
4	60	4.85 ± 0.80^{ab}	9.30±0.15	73.50 ± 0.10	
	70	2.58 ± 1.05^{cd}	8.44±0.43	73.13±0.25	
	50	5.15 ± 1.28^{ab}	9.20 ± 0.37	73.60 ± 0.46	
6	60	4.04 ± 0.63^{abcd}	8.93±0.17	73.53±0.35	
	70	2.20 ± 0.54^{d}	7.58 ± 0.52	73.13±0.25	
	50	4.45 ± 1.46^{abc}	9.09 ± 0.11	73.13±0.25	
8	60	3.44 ± 0.83 bc	8.76 ± 0.09	72.87±0.15	
	70	2.06 ± 0.42^{d}	7.44 ± 0.44	72.80 ± 0.36	
p-value					
Main effect (pH)		< 0.001	0.007	0.034	
Main effect (Temperature)		< 0.001	0.021	0.011	
Interaction (pH×Temperature)		0.012	0.331	0.749	

Mean \pm SD in each column superscripted with lowercase letters indicated significant (p < 0.05) differences between treatments.

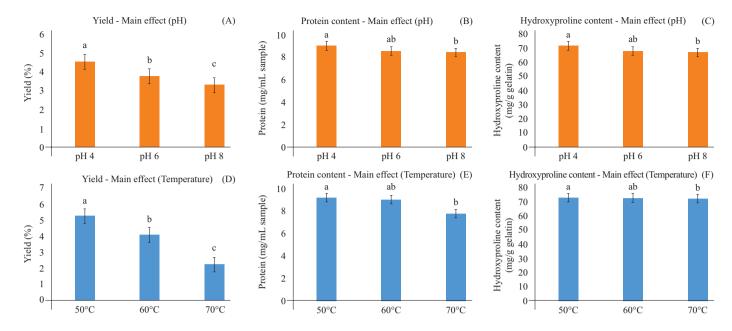


Fig. 1 Yield, total protein content, and hydroxyproline content of gelatin extracted from Atlantic salmon skin under different levels of: pH (A–C) and temperature (D–F) as the main effect, where different lowercase letters above columns indicate significant (p < 0.05) differences and error bars indicate \pm SD.

The differences in gelatin yields were due to the extraction condition and the content of gelatin in the raw materials, along with the impurities (Hue et al., 2017). Processing parameters, such as duration of extraction, the temperature used, pH, pretreatment conditions, characteristics and the handling methods of the raw materials affected the collagen conversion rate into gelatin. Acidic pretreatment aids in increasing the vield of gelatin by disrupting and destabilizing hydrogen bonds in the triple helix of collagen (Zeng et al., 2010). However, further exposure of acids can also affect the yield due to undesirable acid hydrolysis that leads to the formation of low molecular weight peptides that could be lost during the washing steps (Zeng et al., 2010). Incomplete hydrolysis of collagen or the series of washing steps also contributes to a low gelatin yield from fish skin (Jamilah and Harvinder, 2002). Similarly, the gelatin extraction from Atlantic cod (Gadus morhua) skin using different pH levels produced different yields and properties (Derkach et al., 2019). Furthermore, the use of higher temperature in the gelatin extraction further degrades the protein into low molecular weight molecules that might be leached away during different processing steps, thus decreasing the gelatin yield (Jamilah and Harvinder, 2002). Thermal degradation caused by increasing the temperature during gelatin extraction was reported for bamboo and blacktip shark skin (Kittiphattanabawon et al., 2010).

The protein and hydroxyproline contents of the gelatin extracted under different extraction levels of the pH and temperature were in the range 7.44-9.39 mg/mL sample and 72.80–73.60 mg/g gelatin, respectively, being significantly affected by the extraction conditions, while there was no interaction observed between parameters (p-values = 0.331 and 0.749, respectively). Hydroxyproline, along with other hydroxyl groups of amino acids, plays a part in the direct hydrogen bonding to the carbonyl group and inter-chain hydrogen bonding through bridging water molecules (Wong, 1989). Compared to the hydroxyproline content of bovine gelatin (14%), fish gelatins contained lower hydroxyproline content (7-10%), according to Nalinanon et al. (2008), which suggested that the gelatin from the current study contained lower hydroxyproline content. The differences in the amounts of hydroxyproline were due to the preparation method of the gelatin (Gómez-Guillén et al., 2011). The protein and hydroxyproline contents were affected using acid and alkali solutions. During the use of which noncollagenous components such as mucopolysaccharide, globulins, elastin, mucins and albumins were transformed to a more soluble product by the alkali solution, and some fat was turned into polar form so that it could be washed away afterwards. A small quantity of fish collagen was also removed by an acid solution, which was related to the acid lability of crosslinking in the less completely crosslinked skin of catfish (Sukkwai et al., 2011).

Protein patterns

The SDS-PAGE protein patterns (Fig. 2) showed that the gelatin extracted under different levels of pH and temperature exhibited similar patterns. Gelatin consists of different molecular weight molecules in the ranges 70–125 kDa (α-chain), 125–230 kDa (β-chain) and 230–340 (γ-chain). Fig. 2 shows that the gelatin extracted from Atlantic salmon skin consisted of these chains. The inter- and intra-molecular bonds of the collagen and peptides are hydrolyzed during the conversion of gelatin from collagen (Silva et al., 2014). As shown in Fig. 2, extraction at pH 8 hydrolyzed the protein further than extraction at pH 4 and 6. Mohtar and Perera (2019) stated that alkaline treatment was more suitable to bovine skins, hides and bones, due to the collagen contained in the material being more complex, while the collagen found

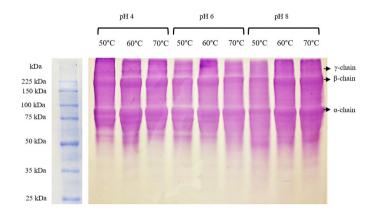


Fig. 2 The SDS-PAGE protein patterns of gelatin extracted from Atlantic salmon under different levels of pH and temperature

in the fish skins was less covalently cross-linked, making it suitable for acidic treatment. Different extraction temperatures affected the molecular weight of the gelatin. Extraction at higher temperature negatively affected the molecular weight, producing gelatin consisting of a higher amount of lower molecular weight. As shown by the decreased bloom strength, the shorter chain fragments of gelatin were unable to establish a junction zone in which a strong network might emerge (Kittiphattanabawon et al., 2010).

Physical properties of gelatin extracted under different extraction conditions

The properties (color, gelling and melting temperatures and gel strength) of gelatin extracted from Atlantic salmon skin were significantly affected by different extraction conditions, while the statistical analysis showed there was no interaction between parameters to the responses (p-value = 0.050–0.508; Table 2, Figs. 3-4). The extracted gelatin from Atlantic salmon had L*, a*, and b* values in the ranges 72.30-81.89, -0.47 to -0.21, and 2.07-10.21, respectively. The color value results corresponded with Liu et al. (2017), who reported that increasing the temperature for the gelatin extraction from chum salmon skin affected L* negatively, while the a* and b* values were affected positively. The lower L* value was due to the gelatin having a lower chain length due to degradation occurring, suggesting reduced aggregation of -NH₃⁺ group during freeze-drying. Higher –NH₃⁺ grouping also contributed to the Maillard reaction to a higher extent, affecting the a* and b* values (Ahmad and Benjakul, 2011). The use of different

Table 2 Physical properties of gelatin extracted from Atlantic salmon skin under different levels of pH and temperature

pН	Temperature	Color		Gelling	Melting	Gel strength	
	(°C)	L*	a*	b*	temperature	temperature	(g)
					(°C)	(°C)	
	50	79.55±1.61	-0.38±0.05	3.90±0.06	12.00±0.26	22.80±0.49	177.33±2.52
4	60	76.82 ± 2.00	-0.24 ± 0.02	4.08 ± 0.14	11.57±0.06	21.98 ± 0.11	158.00±3.61
	70	75.66 ± 1.73	-0.18 ± 0.02	4.89 ± 0.08	11.34±0.06	21.55±0.12	135.33±9.71
	50	81.89±1.09	-0.47±0.01	3.07±0.11	12.54±0.07	23.82±0.14	198.67±4.51
6	60	80.96±1.14	-0.41 ± 0.02	3.35 ± 0.05	12.39 ± 0.09	23.54 ± 0.17	186.67±6.51
	70	79.09 ± 2.05	-0.36 ± 0.02	4.86 ± 0.12	12.07 ± 0.07	22.93 ± 0.14	161.33±6.66
	50	79.55±1.61	-0.35±0.03	4.93±0.05	11.32±0.02	21.50±0.05	129.67±12.42
8	60	73.85 ± 1.35	-0.24 ± 0.02	5.21±0.06	11.09 ± 0.06	21.06±0.11	118.67±14.74
	70	73.30 ± 1.26	-0.21±0.03	5.21 ± 0.07	10.97 ± 0.09	20.84 ± 0.17	103.67±4.73
<i>p</i> -value							
Main effec	et (pH)	0.014	0.038	0.007	0.028	0.041	< 0.001
Main effec	ct (Temperature)	< 0.001	0.025	0.019	< 0.001	< 0.001	< 0.001
Interaction	n (pH×Temperature)	0.050	0.290	0.347	0.153	0.093	0.508

Mean \pm SD in each column superscripted with lowercase letters indicated significant (p < 0.05) differences between treatments.

levels of pH was more than likely to produce some free amino groups in the resultant gelatin and those free amino groups might be involved in a non-enzymatic browning interaction with the carbonyl compounds found in skin, influencing the

gelatin color values (Ali et al., 2018). The differences in the color values were due to the presence of pigment in the material but these did not affect the functional properties of the gelatin (Ahmad and Benjakul, 2011).

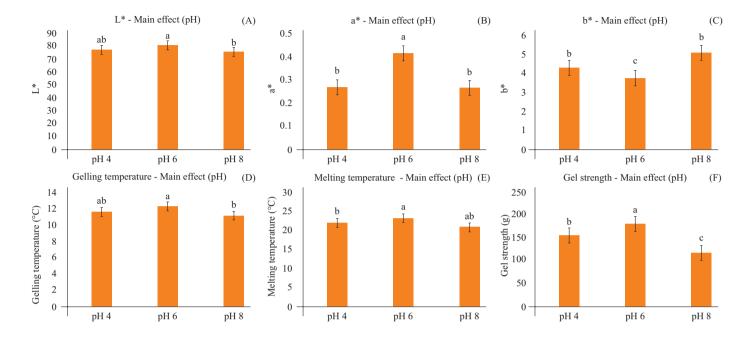


Fig. 3 Physical properties of the gelatin extracted from Atlantic salmon skin under different levels of pH as the main effect: (A) L*; (B) a*; (C) b*; (D) gelling temperature; (E) melting temperature; (F) gel strength, where different lowercase letters above columns indicate significant (p < 0.05) differences of the main effect and error bars indicate \pm SD.

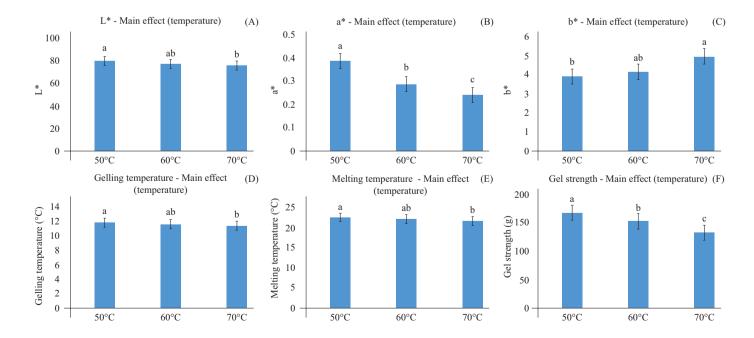


Fig. 4 Physical properties of gelatin extracted from Atlantic salmon skin under different levels of temperature as the main effect: (A) L*; (B) a*; (C) b*; (D) gelling temperature; (E) melting temperature; (F) gel strength, where different lowercase letters above columns indicate significant (p < 0.05) differences of the main effect and error bars indicate \pm SD.

The gelling and melting temperatures are important indicators of gelatin quality. The results showed similarities with gelatin from red tilapia, with a gelling temperature of 22.5 °C (Jamilah and Harvinder, 2002) and from shark (25.8 °C), and tuna (24.2 °C), according to Shyni et al. (2014). In the current study, the melting points observed were higher than for other cold-water fish species, such as hake (14 °C), sole (19.4 °C) and megrim (18.8 °C), according to Gómez-Guillén et al. (2002). The reported gelling and melting temperatures for fish gelatin were in the ranges 8-25 °C and 11-28 °C, respectively (Elavarasan et al., 2017). The species of the fish and the amino acid composition, along with the effect of the preparation of the fish, also contributed to the differences in the gelling and melting points of the gelatin (Hue et al., 2017). Gelling and melting temperatures were influenced by the relative concentrations and molecular weights of the α -, β -, and γ -chains, with lower values shown by gelatin with lower molecular weight (Ali et al., 2018).

The gel strength values of gelatin extracted from Atlantic salmon skin using different level of pH and extraction temperatures were in the range 103.67–198.67 g. The highest gel strength (198.67 \pm 4.51 g) was for gelatin extracted at pH 6 and 50 °C, while the lowest gel strength (103.67 \pm 4.73 g) was for the gelatin extracted at pH 8 and 70 °C. Extraction pH and temperature significantly affected the gel strength of the extracted gelatin, affecting the concentration of α -, β -,

and γ -chains that finally affecting the gel strength of the gelatin, which was also supported by the results on the protein patterns of the gelatins. Gelatin with shorter chain lengths cannot form a strong gel due to the lower number of inter-junction zones (Intarasirisawat et al., 2007). Generally, the quality of gelatin is determined by the gel strength or Bloom value, with values of low (< 150 g), medium (150–220 g) and high Bloom (> 200 g), according to Elavarasan et al. (2017), who suggested that the gelatins extracted from Atlantic salmon skin under different extraction levels of pH and temperature had low-tomedium gel strength. In addition, a study on salmon gelatin by Díaz-Caldéron et al. (2017) showed that the decrease in the pH values used for gelatin extraction significantly decreased the gel strength of the gelatin. The properties of gelatin are affected by various conditions, including the extraction process, with the extraction temperature being one of the important factors in gelatin extraction. As the extraction temperature increased, the gel strength of the gelatin decreased due to the lower molecular weight of the peptide contained in the gelatin (Benjakul et al., 2012; Silva et al., 2014).

Microstructure

The gelatins extracted under different extraction conditions that produced significantly high yields were examined to investigate their microstructures. Fig. 5 shows there was a similar texture among the gelatin samples. The gel strength

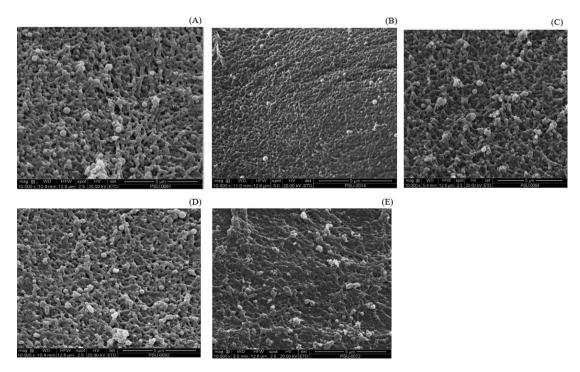


Fig. 5 Microstructure of gelatin extracted from Atlantic salmon skin under different levels of pH and temperature: (A) pH 4 at 50 °C; (B) pH 6 at 50 °C; (C) pH 8 at 50 °C; (D) pH 4 at 60 °C; (E) pH 6 at 60 °C, where magnification is 10,000× and scale bar is 5 μm

of gelatin was commonly influenced by the association and arrangement of protein molecules in the gel matrix (Ratnasari et al., 2013). A rough gel network might produce a gelatin gel with low gel strength (Kittiphattanabawon et al., 2010). The Atlantic salmon skin was likely impacted by the acid ionization constant and ionized H concentration during pretreatment and extraction was possible, resulting in the differences in the component release (Ali et al., 2018). Gelatin with greater α -, β -, and γ -chain concentrations can form stronger networks than if hydrolyzation or fragmentation has occurred, which is also related to the gel strength (Khiari et al., 2017; Ali et al., 2018).

Conclusion

Different extraction levels of pH and temperature significantly affected the yield and physicochemical properties of the gelatin extracted from Atlantic salmon skin. A higher gelatin yield was achieved using gelatin extraction at pH 4 and 50 °C. Gelatin extracted at pH 6 and 50 °C contained higher values of physicochemical properties. The protein pattern analysis showed that protein hydrolysis occurred more under basic conditions, which further affected the yield and physicochemical properties of the resulting gelatin. Investigation of extraction conditions should be conducted, as it was shown by the current study that these affected the yield and physicochemical properties of the gelatin. The use of different levels of pH and temperature should be taken into consideration prior to gelatin extraction, especially extraction from fish skin, to obtain gelatin with a preferred yield and physicochemical properties for further applications.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This research was financially supported by Halal Institute Prince of Songkla University (AGR611060S) and the Thailand Education Hub for Southern Region of ASEAN Countries (TEH–AC) Project Office of the Higher Education Commission. The Faculty of Agro-Industry, Prince of Songkla University, Thailand provide laboratory support for this research.

References

- Ahmad, M., Benjakul, S. 2011. Characteristics of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as influenced by acid pretreatment and extraction time. Food Hydrocoll. 25: 381–388. doi. org/10.1016/j.foodhyd.2010.07.004
- Ali, A.M.M., Benjakul, S., Kishimura, H. 2017. Molecular characteristics of acid and pepsin soluble collagens from scales of golden carp (*Probarbus jullieni*). Emir. J. Food Agric. 29: 450–457. doi. org/10.9755/ejfa.2016-09-1316
- Ali, A.M.M., Kishimura, H., Benjakul, S. 2018. Physicochemical and molecular properties of gelatin from skin of golden carp (*Probarbus jullieni*) as influenced by acid pretreatment and prior-ultrasonication. Food Hydrocoll. 82: 164–172. doi.org/10.1016/j.foodhyd.2018.03.052
- Badii, F., Howell, N.K. 2006. Fish gelatin: Structure, gelling properties and interaction with egg albumen proteins. Food Hydrocoll. 20: 630–640. doi.org/10.1016/j.foodhyd.2005.06.006
- Benjakul, S., Kittiphattanabawon, P., Regenstein, J.M. 2012. Fish gelatin. In: Simpson, B.K. (Ed.). Food Biochemistry and Food Processing, 2nd ed. John Wiley and Sons, Inc. Oxford, UK, pp. 388–405.
- Bergman, I., Loxley, R. 1963. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. Anal. Chem. 35: 1961–1965. doi.org/10.1021/ac60205a053
- British Standards Institution. 1975. Methods for Sampling and Testing Gelatin (Physical and Chemical Methods). British Standards Institution. London, UK.
- Derkach, S.R., Kuchina, Y.A., Baryshnikov, A.V., Kolotova, D.S., Voron'ko, N.G. 2019. Tailoring cod gelatin structure and physical properties with acid and alkaline extraction. Polymers 11: 1724. doi.org/10.3390/polym11101724
- Díaz-Caldéron, P., Flores, E., González-Muñoz, A., Pepczynska, M., Quero, F., Enrione, J. 2017. Influence of extraction variables on the structure and physical properties of salmon gelatin. Food Hydrocoll. 71: 118–128. doi.org/10.1016/j.foodhyd.2017.05.004
- Elavarasan, K., Kumar, A., Uchoi, D., Tejpai, C.S., Ninan, G., Zynudheen, A.A. 2017. Extraction and characterization of gelatin from the head waste of tiger tooth croaker (*Otolithes ruber*). Waste Biomass Valor. 8: 851–858. doi.org/10.1007/s12649-016-9639-5
- Food and Agriculture Organization of the United Nations. 2018. The State of World Fisheries and Aquaculture Meeting the sustainable development goals. Rome, Italy.
- Gómez-Guillén, M.C., Gimenez, B., Lopez-Caballero, M.E., Montero, P. 2011. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocoll. 25: 1813–1827. doi. org/10.1016/j.foodhyd.2011.02.007
- Gómez-Guillén, M.C., Turmay, J., Fernández-Díaz, M.D., Ulmo, N., Lizarbe, M.A., Montero, P. 2002. Structural and physical properties of gelatin extracted from different marine species: A comparative study. Food Hydrocoll. 16: 25–34. doi.org/10.1016/S0268-005X(01) 00035-2
- Hue, C.T., Hang, N.T.M., Razumovskaya, R.G. 2017. Physicochemical characterization of gelatin extracted from European perch (*Perca fluviatilis*) and Volga pikeperch (*Sander volgensis*) Skins. Turk. J. Fish Aquat. Sci. 17: 1117–1125.

- Intarasirisawat, R., Benjakul, S., Visessanguan, W., Prodpran, T., Tanaka, M., Howell, N.K. 2007. Autolysis study of bigeyesnapper (*Priacanthus macracanthus*) skin and its effect on gelatin. Food Hydrocoll. 21: 537–544. doi.org/10.1016/j.foodhyd.2006.05.012
- Jamilah, B., Harvinder, K. 2002. Properties of gelatins from skins of fish-black tilapia (*Orechromis mossambicus*) and red tilapia (*Oreochromis nilotica*). Food Chem. 77: 81-84. doi.org/10.1016/ S0308-8146(01)00328-4
- Khiari, Z., Rico, D., Martin-Diana, A.B., Barry-Ryan, C. 2015. Characterization of blue whiting skin gelatins extracted after pretreatment with different organic acids. J. Aquat. Food Prod. T. 24: 546–555. doi.org/10.1080/10498850.2013.791904
- Khiari, Z., Rico, D., Martin-Diana, A.B., Barry-Ryan, C. 2017. Valorization of fish by-products: Rheological, textural and microstructural properties of mackerel skin gelatins. J. Mater. Cycles Waste Manag. 19: 180–191. doi.org/10.1007/s10163-015-0399-2
- Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Shahidi, F. 2010. Comparative study on characteristics of gelatin from the skins of brownbanded bamboo shark and blacktip shark as affected by extraction conditions. Food Hydrocoll. 24: 164–171. doi.org/10.1016/j. foodhyd.2009.09.001
- Liu, Y., Xia, L., Jia, H., Li, Q., Jin, W., Dong, X., Pan, J. 2017. Physicochemical and functional properties of chum salmon (*Oncorhynchus keta*) skin gelatin extracted at different temperatures. J. Sci. Food Agric. 97: 5406–5413. doi.org/10.1002/jsfa.8431
- Mahmood, K., Muhammad, L., Ariffin, F., Kamilah, H., Razak, A., Sulaiman, S. 2016. Review of fish gelatin extraction, properties and packaging applications. FSQM. 56: 47–59.
- Mohtar, N.F., Perera, C.O. 2019. Gelatin from cold water fish species and their functional properties. In: Rajendran, R., George, A., Kalarikkal, N., Thomas, S. (Eds.). Innovative Food Science and Emerging Technology: The Science Behind Health. Apple Academic Press Inc. Palm Bay, FL, USA, p. 40.
- Mutlu, C., Tontula, S.A., Erbaşa, M. 2018. Production of a minimally processed jelly candy for children using honey instead of sugar. LWT. 93: 499–505. doi.org/10.1016/j.lwt.2018.03.064
- Nalinanon, S., Benjakul, S., Visessanguan, W., Kishimura, H. 2008. Tuna pepsin: Characteristics and its use for collagen extraction from the skin of threadfin bream (*Nemipterus* spp.). J. Food Sci. 73: C413–C419. doi.org/10.1111/j.1750-3841.2008.00777.x

- Ratnasari, I., Yuwono, S.S., Nusyam, H., Widjanarko, S.B. 2013.
 Extraction and characterization of gelatin from different fresh water fishes as alternative sources of gelatin. IFRJ. 20: 3085–3091.
- Sae-Leaw, T., Benjakul, S., O'Brien, N. 2016. Effect of pretreatments and defatting of seabass skins on properties and fishy odor of gelatin. J. Food Biochem. 40: 741–753. doi.org/10.1111/jfbc.12267
- Saha, D., Bhattacharya, S. 2010. Hydrocolloids as thickening and gelling agents in food: A critical review. J. Food Sci. Technol. 47: 587–597. doi.org/10.1007/s13197-010-0162-6
- Shyni, K., Hem, G.S., Ninan, G., Mathew, S., Joshy, C.G., Lakshmanan, P.T. 2014. Isolation and characterization of gelatin from the skins of skipjack tuna (*Katsuwonus pelamis*), dog shark (*Scoliodon sorrakowah*), and rohu (*Labeo rohita*). Food Hydrocoll. 39: 68–76. doi.org/10.1016/j.foodhyd.2013.12.008
- Silva, R.S.G., Bandeira, S.F., Pinto, A.A. 2014. Characteristics and chemical composition of skins gelatin from cobia (*Rachycentron canadum*). LWT. 57: 580–585. doi.org/10.1016/j.lwt.2014.02.026
- Sinthusamran, S., Benjakul, S., Kishimura, H. 2014. Characteristics and gel properties of gelatin from skin of seabass (*Lates calcarifer*) as influenced by extraction conditions. Food Chem. 152: 276–284. doi.org/10.1016/j.foodchem.2013.11.109
- Stevens, P. 2010. Gelatine. In: Imeson, A. (Ed.). Food Stabilisers, Thickeners, and Gelling Agents. Wiley-Blackwell. Oxford, UK, pp. 237–265.
- Sukkwai, S., Kijroongrojana, K., Benjakul, S. 2011. Extraction of gelatin from bigeye snapper (*Priacanthus tayenus*) skin for gelatin hydrolysate production. IFRJ. 18: 1129–1134.
- Webb, J., Verspoor, E., Aubin-Horth, N., Romakkaniemi, A., Amiro,
 P. 2007. The Atlantic salmon. In: Verspoor, E., Stradmeyer, L.,
 Nielsen, J.L. (Eds.). The Atlantic Salmon: Genetics, Conservation and
 Management. Blackwell Publishing. Oxford. UK, pp. 17–56.
- Wong, D.W.S. 1989. Mechanism and Theory in Food Chemistry. Van Nostrand Reinhold Company Inc. New York, NY, USA.
- Zeng, S., Yan, X., Cao, W., Hong, P., Zhang, C., Li, L. 2010. Optimisation of extraction conditions and characteristics of skin gelatin from Nile tilapia (*Oreochromis niloticus*). Int. J. Food Sci. Tech. 45: 1807–1813. doi.org/10.1111/j.1365-2621.2010.02332.x
- Zhou, P., Regenstein, J.M. 2006. Determination of total protein content in gelatin solutions with the Lowry or Biuret assay. J. Food Sci. 71: C474–C479. doi.org/10.1111/j.1750-3841.2006.00151.x