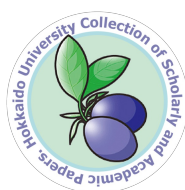




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Characteristics and properties of gelatin from seabass (*Latescalcarifer*)
swim bladder : Impact of extraction temperatures

To be submitted to Waste and Biomass Valorization

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Abstract

Purpose This study aimed to investigate the impact of various extracting temperatures on yield and properties of gelatin from swim bladder of seabass (*Latescalcarifer*), a byproduct from processing.

Methods Gelatin from seabass swim bladder was extracted at different temperatures (45, 55, 65 and 75 °C). The gelatins obtained using various extraction temperatures were characterized.

Results The yield and recovery of gelatin from swim bladder (44.83-71.95% and 49.08-74.83%, based on dry weight) increased with increasing extraction temperatures. All gelatins contained α -chains as the predominant components, followed by β -chain. Gelatin from seabass swim bladder showed a high imino acid content (195 residues/1000 residues). FTIR and CD spectra revealed the loss of triple helix during heating via breaking down hydrogen bonds between α -chains. Gel strength generally increased as the extraction temperature increased up to 65 °C ($P < 0.05$). Gelatin extracted at 65 °C for 6 h showed a higher gel strength, compared to bovine gelatin ($P < 0.05$). Gelling and melting temperatures were 10.4-19.7 and 19.3-28.4 °C, respectively, depending on extraction temperature.

Conclusion Properties of gelatin from swim bladder were affected by extraction temperature. Therefore, seabass swim bladder could serve as an alternative collagenous material for gelatin production, when the appropriate extraction condition was implemented.

Keywords: Extraction temperature, Swim bladder, Gelatin, Gel strength, Gelling temperature

1. Introduction

Gelatin is a fibrous protein produced by thermal denaturation of collagen. It represents a major biopolymer with wide range of applications in food, pharmaceutical, cosmetic and photographic industries [1]. In general, the main sources of gelatin production are porcine and bovine skin and bones [2]. Nevertheless, religious restriction has led to an increasing interest in alternative sources for gelatin [3]. Fish processing by-products have become important for gelatin production. Fish gelatin can be extracted from skin of several aquatic animals including clown featherback [4], Nile tilapia and channel catfish [5], octopus [6], cobia [7] and unicorn leatherjacket [8]. Nevertheless, the utilization of fish gelatin is limited, due to its poor gelling property [9]. Moreover, it has been known that extraction conditions, especially extraction temperature and time, determine the properties of gelatin [3]. Increasing extraction temperature and time (harsh conditions) generally render the gelatin with the higher yield, but decreased gelling properties [4]. Thus, extraction condition, including temperature and time, should be optimized.

Seabass (*Lateolabrax japonicus*) is an economically important fish in Thailand. A large amount of seabass has been exported as well as domestically consumed [10]. In general, by-products including skins, bones, scales and swim bladder, constituting around 30%, are generated during dressing [11,12]. Skin, bone and scales have been widely used for gelatin production to increase the value. However, seabass swim bladder rich in collagen has been rarely used for gelatin extraction. Swim bladders from a few marine fish species were used for the production of isinglass (fining agents) [13]. Swim bladder of fresh water carp was used for gelatin extraction with high yield (13.5%) and gel strength (264.6 g) [13]. Thus, the seabass swim bladder can be the potential source for production of gelatin with prime quality. Nevertheless, no information regarding the characteristics and properties of gelatin from seabass swim bladder has been reported. Therefore, the aims of the study were to extract

gelatin from swim bladder of seabass (*Latescalcarifer*) at various temperatures and to determine properties of resulting gelatins.

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 (FG) produced from tilapia skin (~240 bloom) was obtained from Lapi Gelatine S.p.A (Empoli, Italy). Food grade bovine bone gelatin (BG) with the gel strength of 150–250 g was purchased from Halagel (Thailand) Co., Ltd. (Bangkok, Thailand).

2.2 Preparation of seabass swim bladder

Swim bladders of seabass (*L. calcarifer*) with a length of 20–25 cm were obtained from Sirikhun Seafood Co., Ltd, Samutsakhon, Thailand. The frozen swim bladders packed in a polyethylene bag, were kept in ice with a skin/ice ratio of 1:3 (w/w) using a polystyrene box as a container and transported to the Department of Food Technology, Prince of Songkla University, within 10 h. Upon arrival, frozen swim bladders were thawed with running water until the core temperature reached 8–10 °C. The samples were washed with tap water and cut into small pieces (1×1 cm²) using scissors. Prepared swim bladder samples were placed in polyethylene bags and stored at -20 °C until used, but not longer than 2 months. Swim bladder had moisture, protein, fat and ash content of 69.74, 29.67, 0.34 and 0.23%, as determined by AOAC methods with the analytical numbers of 927.05, 984.13, 920.39 B and 942.05, respectively [14]. The conversion factor of 5.4 was used for calculation of protein

content. It was rich in hydroxyproline (24.48 mg/g) as measured by the method of Bergman, Loxley [15].

2.3 Extraction of gelatin from swim bladder

Gelatin was extracted from swim bladder according to the method of Sinthusamran et al. [3] with a slight modification. Before gelatin extraction, swim bladder was soaked in 0.1 M NaOH, with a sample/solution ratio of 1:10 (w/v), to remove non-collagenous proteins. The mixture was stirred continuously for 3 h at room temperature (28–30°C) using an overhead stirrer with a propeller (W20.n IKA[®]-Werke GmbH & CO.KG, Staufen, Germany). The alkaline solution was changed every 1 h for totally 3 times. The residues were then washed with tap water until a neutral or faintly basic pH was obtained. The deproteinized matters were then mixed with 0.05 M acetic acid at a sample/solution ratio of 1:10 (w/v) to swell collagenous material. The mixture was stirred at room temperature for 2 h. The swollen swim bladders were washed using tap water until wash water became neutral or faintly acidic in pH.

To extract the gelatin, the swollen swim bladders were mixed with distilled water at a ratio of 1:10 (w/v) at 45, 55, 65 and 75 °C for 6 h with continuous stirring. The mixtures were filtered with two layers of cheesecloth. Then, the filtrates were mixed with 1 % (w/v) activated carbon for 1 h with continuous stirring. The mixtures were centrifuged at 17,500xg for 15 min at 25 °C using a Beckman model Avanti J-E centrifuge (Beckman Coulter, Inc., Palo Alto, CA, USA) to remove insoluble material. The supernatants were filtered using a Buchner funnel with Whatman No.4 filter paper (Whatman International, Ltd., Maidstone, England). Finally, the filtrates were freeze-dried using a freeze-dryer (CoolSafe 55, ScanLaf A/S, Lyngø, Denmark) at -50 °C for 72 h. The dry gelatin extracted from swim bladder at 45,

55, 65 and 75 °C were referred to as ‘G45’, ‘G55’, ‘G65’ and ‘G75’, respectively. The gelatin samples were subsequently subjected to analyses.

2.4 Analyses

2.4.1 Determination of yield and recovery

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

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

Recovery was also determined using the following equation:

$$\text{Recovery (\%)} = \frac{[\text{HYP of gelatin (g/g)} \times \text{weight of gelatin (g)}]}{[\text{HYP of initial swim bladder (g/g)} \times \text{weight of initial swim bladder (g)}]} \times 100$$

Hydroxyproline (HYP) content in both gelatin and initial swim bladder was determined according to the method of Bergman, Loxley [15].

2.4.2 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS–PAGE was performed by the method of Laemmli [16]. Gelatin samples were dissolved in 5% SDS solution. The obtained mixtures were then heated at 85 °C for 1 h using a temperature controlled water bath model W350 (Mettler, Chwabach, Germany). Solubilized 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.

Quantitative analysis of protein band intensity was performed using a Model GS-700 Imaging Densitometer (Bio-Red Laboratories, Hercules, CA, USA) with Molecular Analyst Software version 1.4 (image analysis systems).

2.4.3 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. A 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 room temperature, at a resolution of 4 cm⁻¹ and the measurement range was 4000–400 cm⁻¹ (mid-IR region). 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).

2.4.4 Circular dichroism (CD) spectroscopic analysis

Gelatin samples (0.05 mg protein/mL) were dissolved in 10 mM sodium phosphate buffer (pH 7.0) according to the method of Wierenga et al. [17]. CD spectra were measured at 20 °C, with a scan speed of 100 nm/min from 190 to 250 nm, a data interval of 0.2 nm, a bandwidth of 1 nm and a response time 0.125 s using a JASCO J-801 spectrometer (Jasco Corp, Tokyo, Japan). The secondary structure was estimated using a CDPro software with the reference spectra as described by Johnson [18].

2.4.5 Surface hydrophobicity

Surface hydrophobicity of gelatin samples was determined by the method of Benjakul et al. [19]. Gelatin was dissolved in 10 mM phosphate buffer, pH 6.0, containing 0.6 M NaCl to obtain a final protein concentration of 5 g/L. The gelatin solution was diluted to 0.125, 0.25, 0.5, and 1 g/L using the same buffer. The diluted gelatin solutions (2 mL) were well mixed with 10 μ L of 8 mM 1-anilinonaphthalene-8-sulfonic acid (ANS) in 0.1 M phosphate buffer, pH 7.0. The relative fluorescence intensity of ANS-protein conjugates was measured using a spectrofluorometer (RF-15001, Shimadzu, Kyoto, Japan) at the excitation wavelength of 374 nm and the emission wavelength of 485 nm. Surface hydrophobicity was calculated from initial slopes of plots of relative fluorescence intensity versus protein concentration (g/L) using linear regression analysis. The initial slope was referred to as SoANS.

2.4.6 Determination of gel strength

Gelatin gel was prepared by the method of Kittiphattanabawon et al. [2]. Gelatin was mixed with distilled water (60 °C) to obtain the concentration of 6.67% (w/v). The solution was stirred until gelatin was solubilized completely and transferred to a cylindrical mold with 3 cm diameter and 2.5 cm height. The solution was incubated at the refrigerated temperature for 18 h prior to analysis.

Gel strength was determined at 8-10 °C using a texture analyzer (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 (gram) was recorded, when the plunger had penetrated 4 mm into the gel samples.

2.4.7 Determination of gel color

The color of gelatin gels (6.67% w/v) was measured by a Hunter lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). L^* , a^* and b^* values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. The colorimeter was warmed up for 10 min and calibrated with a white standard. Total difference in color (ΔE^*) was calculated according to the following equation [20]:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^* , Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of white standard ($L^* = 90.77$, $a^* = -1.27$ and $b^* = 0.50$).

2.4.8 Determination of gelling and melting temperatures

Gelling and melting temperatures of gelatin samples were measured following the method of Sinthusamran et al. [3]. The measurement was performed using a RheoStress RS 1 rheometer (HAAKE, Karlsruhe, Germany) in the oscillatory mode. The measuring geometry used was a stainless steel 60-mm-diameter parallel plate and the gap was set at 1.0 mm. Gelatin solution (6.67%, w/v) was prepared in the same manner as described in the methods. The solution was preheated at 60 °C for 30 min. Then the solution (2.9 mL) was loaded on the Peltier plate and equilibrated at 60 °C for 10 min before measurements. The measurements were conducted at a constant frequency of 1 Hz, and a constant applied stress of 3 Pa. The samples were cooled from 60 to 5 °C and subsequently heated to 60 °C at a constant rate of 1.0 °C/min. The gelling and melting temperatures were calculated, where $\tan \delta$ became 1 or δ was 45°.

2.4.9 Microstructure analysis of gelatin gel

Microstructure of gelatin gel (6.67%, w/v) prepared as previously described was visualized using a scanning electron microscopy (SEM). Gelatin gels 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 12 h. The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol with serial concentrations of 50, 70, 80, 90 and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 20 kV.

2.5 Characterization of selected gelatin

2.5.1 Proximate analysis

Proximate compositions were determined as per AOAC method [14].

2.5.2 Amino acid analysis

Amino acid composition of gelatin samples was analyzed using an amino acid analyzer. Gelatin samples were hydrolyzed under reduced pressure in 4 M methane sulphonic acid containing 0.2% 3-2(2-aminoethyl)indole at 115 °C for 24 h. The hydrolysates were neutralized 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 analyzer (MLC-703; Atto Co., Tokyo, Japan).

2.6 Statistical analysis

All experiments were run in triplicate using three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out using the

Duncan's multiple range test [21]. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 Yield and recovery of gelatin

Yield and recovery of gelatin from seabass swim bladder extracted at different temperatures varied as shown in Table 1. Yields of 44.83-71.95% and recovery of 49.08-74.83% were obtained. The yield was the amount of solid released from pretreated swim bladder matrix, while the recovery represented the percentage of collagen converted to gelatin by heat [22]. Both extraction yield and recovery increased as the extraction temperature increased ($P < 0.05$). The highest yield (71.95%) was found in gelatin extracted at 75 °C (G75). However, there was no difference in the recovery between G65 and G75 ($P > 0.05$). Similar results were reported for gelatin from clown featherback skin [4] and seabass skin [3], in which the yield of gelatin increased as extraction temperatures increased. The higher temperature used for extraction more likely provided higher energy, thereby destroying hydrogen bonds stabilizing the triple helix in collagen structure to higher extent. As a consequence, α - and β -chains were more released into medium, resulting in the higher amount of gelatin obtained [3,22]. Collagen from seabass swim bladder showed a $T_{\max}$ of 35.02 °C [12]. In the present study, extraction temperatures were above $T_{\max}$ and could induce the denaturation of collagen to gelatin. By raising the temperature of extraction, higher conversion of collagen to gelatin could be achieved as shown by the increased recovery.

3.2 Protein pattern

Protein patterns of gelatin from seabass swim bladder extracted at different temperatures are illustrated in Fig. 1. All gelatin samples consisted of α_1 - and α_2 -chains with

MW of 130 and 115 kDa, respectively, as the major constituents. FG and BG also contained α -chains as major components. MW of α_1 -chain(150kDa) and α_2 -chain (133kDa) of BG was higher than that from other gelatin samples. Among gelatin from swim bladders, that extracted at 45 °C had the lowest band intensity of α -chains as well as β - and γ -chains. With lower heat, energy was not sufficient to destroy the bondings stabilizing triple helix of mother collagen. As a consequence, all components were released to lower degree as evidenced by lower yield and recovery (Table 1). Additionally, some heat labile proteins might be co-extracted at 45 °C as shown by the higher band intensity of protein with MW lower than α -chains. However, the band intensity of protein with MW lower than α -chain decreased with increasing the extraction temperature. This might be associated with precipitation and aggregation of those low MW contaminating proteins at high temperature. During centrifugation or filtration, those precipitated proteins could be removed. Nevertheless, slight decrease in band intensity of both γ - and β -chains was found in G75. This might be caused by degradation induced by the thermal process [3]. Kittiphattanabawon et al. [4] reported that bands of α -, β -, and γ -chains were more degraded with increasing extraction temperature and time. Sinthusamran et al. [3] also found that the band intensity of protein components in gelatin from seabass skin slightly decreased with increasing extraction temperatures. The result reconfirmed that extraction temperatures played a paramount role in components of gelatin extracted from collagenous matrix of seabass swim bladder.

3.3 Fourier transform infrared (FTIR) spectra

FTIR spectra of gelatin from the swim bladder of seabass extracted at various temperatures are presented in Fig. 2A. Generally, all gelatin samples showed similar spectra, in which amide A, amide B, amide I, amide II and amide III bands were detected. The amide I band of G45, G55, G65 and G75 was found at wavenumbers of 1636, 1632, 1632 and 1639

cm⁻¹, respectively. This band is related to a C=O stretching vibration coupled to contributions from the CN stretch [23]. Amide I band of all gelatin samples, especially G75 was shifted to higher wavenumber. The result was in agreement with the slight decrease in α - and β -chain of G75. Kittiphattanabawon et al. [2] reported that gelatin extracted from shark skins at the higher temperature with longer time generally contained a higher amount of low molecular weight components, in which the C=O group were more exposed. Amide II band of G45, G55, G65 and G75 was observed at wavenumber ranging from 1529 to 1546 cm⁻¹. Amide II band represents the combination between bending vibration of N-H groups and stretching vibrations of C-N groups [24]. Furthermore, the amide III bands of all gelatin samples were observed at wavenumbers of 1233-1238 cm⁻¹, demonstrating disorder from an α -helical to a random coil structure, leading to the loss of triple helix state. The amide III was more likely associated with C-N stretching vibrations and N-H deformation from the amide linkages as well as the absorptions arising from wagging vibration of CH₂ groups in the glycine backbone and proline side-chains [9]. In the present study, amide III peak of G75 had the lower amplitude when compared with that of other gelatin samples. This suggested that G75 plausibly had more disordered structure due to higher heat applied for extraction.

Amide A band of G45, G55, G65 and G75 samples was located at 3294, 3298, 3296 and 3294 cm⁻¹, respectively. Amide A arises from the stretching vibrations of the NH group coupled with hydrogen bonding [24]. Normally, a free NH stretching vibration is found in the range of 3400-3440 cm⁻¹. When the NH group of a peptide is involved in a H-bond, the position is shifted to lower frequency, usually 3300 cm⁻¹ [25]. Additionally, the higher amplitude of amide A was found in G75, which was associated with the degradation of gelatin, leading to higher free amino groups. However, lower wavenumber of G75 also implied that NH groups of this sample more likely interacted with adjacent chains via H-bond, resulting in the decreased wavenumber. Amide B band was observed at 2932, 2920, 2922 and

2924 cm⁻¹ for G45, G55, G65 and G75, respectively. It represents CH stretching vibrations of the -CH₂ groups [24]. Amide B peaks of G55 and G65 were shifted to the lower wavenumber, compared with other samples. The result suggested that CH₂ groups were plausibly interacted with adjacent molecules. This was in accordance with the higher MW cross-links in both G55 and G65. Therefore, the extraction temperature affected the secondary and functional group of gelatins obtained from swim bladder of seabass.

3.4 CD-spectra

CD-spectra of gelatin from swim bladder extracted at different temperatures are depicted in Fig. 2B. Circular dichroic (CD) spectroscopy has been used to characterize the secondary structure of protein [26]. It can also be used to analyze the conformation of peptides [27]. Native collagen generally exhibited a CD spectra with a positive peak at around 220-230 nm and a negative peak at around 200 nm [28]. After complete denaturation of collagen, the positive peak at 220-230 nm, characteristic of the triple-helix, disappears completely and only the negative peak at 200 nm of gelatin remains[28]. The negative peak at 200 nm was related to the random conformation of gelatin [29]. In the present study, CD spectra of all samples from seabass swim bladder showed the maximum negative peak at 197-200 nm, but the positive peak in the CD spectra almost disappeared, suggesting that the triple helical structure was almost converted to the random coil. The result was in agreement with that reported by Nikoo et al. [30] on the farmed Amur sturgeon skin gelatin. However, small positive peak at 218-220 nm was still found in G55 and G65 samples, suggesting the presence of some α -helix. This was coincidental with higher content of longer chain length (β - and γ -chain) of protein (Fig. 1). In addition, the positive peak as observed in G55 and G65 samples might be related with the partial refolding of protein chains. α -, β - and γ -chains of G55 and G65 at high content could enhance refolding inter- or intra-molecularly to some

degree during measurement at 20 °C. G75 had more random coil in structure as evidenced by the decrease in positive ellipticity at 220 nm [28]. This indicated that higher extraction temperature more likely caused unfolding and degradation of collagen, leading to higher random structure. Thus, extraction conditions mainly caused the changes in the secondary structure of resulting gelatin.

3.5 Surface hydrophobicity

The surface hydrophobicity (S_o ANS) of gelatin from the swim bladder extracted at different temperatures is shown in Fig. 2C. ANS probe can bind to hydrophobic amino acids (tyrosine, phenylalanine and tryptophan) and has been used to indicate the surface hydrophobicity of proteins [19]. G45 showed higher S_o ANS than G55 and G65 ($P<0.05$). However, no difference in S_o ANS between G55 and G65 was observed ($P>0.05$). As extraction temperature increased, drastic increase in S_o ANS was noticeable, particularly for G75. An increase in S_o ANS of G75 was more likely associated with an exposure of the interior of molecule [31]. Kittiphattanabawon et al. [32] also reported that S_o ANS of gelatin extracted from shark skin increased as the extraction temperature increased. The hydrophobic domains might be more exposed or released, when the peptide molecules were cleaved by heat[32]. The difference in S_o ANS from gelatin extracted at different temperatures might be governed by the variation in protein conformation, related with protein degradation and precipitation induced by heat.

3.6 Gel strength

Gel strength of gelatin from seabass swim bladder extracted at different temperatures in comparison with FG and BG is shown in Table 2. Gelatin gel is a thermally reversible gel network stabilized mainly by hydrogen bond[33]. Gel strength of gelatin increased as extraction temperature increased up to 65 °C ($P<0.05$). The highest gel strength (280.9 g) was

found in G65 sample ($P<0.05$). The increase in gel strength was coincidental with higher proportion of α -, β - and γ -chains (Fig. 1). However, when gelatin was extracted at 75 °C, gel strength of gelatin decreased ($P<0.05$). This was related with the slight decreases in those aforementioned components. Sinthusamran et al. [3] also reported that gelatin from seabass skin extracted at lower temperature had higher gel strength than those extracted at higher temperature. Among all samples, the lowest gel strength (188.3 g) was observed for gelatin extracted at 45 °C ($P<0.05$). The low gel strength of G45 was related to the lower proportion of α -, β - and γ -chains (Fig. 1). FG and BG had the gel strength of 201.6 and 246.3 g, respectively. G55 and G65 showed higher gel strength than BG and FG ($P<0.05$). The results suggested that long chain components (α -, β - and γ -chains) were the major factor affecting the development of strong gel [3]. Furthermore, the ways those chains interacted each other or the junction zones were formed were also crucial for gel formation. In addition, gelatin contained high hydroxyproline content (Table 3). The OH groups of hydroxyproline might be involved in gel formation by hydrogen bonding with adjacent chains [2]. Gelatin with different gel strength was reported for clown featherback skin (225-284 g) [4], *Catlacatla* swim bladder (264.6 g) [13], seabass skin (223-322 g) [10], farmed Amur sturgeon skin (141 g) [30], cobia skin (232 g) and croaker skin (212 g) [7]. In the present study, G65 with higher amount of α -, β - and γ -chains showed the highest gel strength and 65 °C was considered as the optimal temperature for extraction of gelatin from seabass swim bladder.

3.7 Color of gel

The color of gelatin gel from seabass swim bladder extracted at different temperatures expressed as L^* , a^* , b^* and ΔE^* is shown in Table 2. FG and BG had the higher lightness (L^* -value), compared with all gelatin from seabass swim bladder, regardless of extraction temperatures ($P<0.05$). The L^* -value of gel from swim bladder gelatin decreased with

increasing extraction temperatures ($P < 0.05$). BG showed the higher a^* -value ($P < 0.05$). No differences in a^* -value were found among gelatins from swim bladder with different extraction temperatures ($P > 0.05$). Furthermore, yellowness (b^* -value) increased as extraction temperature increased up to 65 °C ($P < 0.05$). However, b^* -value of gelatin gel decreased when extraction temperature was higher than 65 °C ($P < 0.05$). The increase in b^* -value might be associated with non-enzymatic browning reaction during extraction at higher temperature, leading to higher yellowness [3]. The decrease in b^* -value found in G75 might be due to the denaturation of indigenous pigments. The higher b^* -value (28.76) was found in gel from BG ($P < 0.05$). Kittiphattanabawon et al. [4] reported that the higher yellowness in gelatin gel from bovine bone might be affected by the harsher extraction process as required for bone with complex structure, leading to formation of coloring components. For total difference in color value (ΔE^* -value), FG exhibited the lowest ΔE^* -value (26.48) ($P < 0.05$). ΔE^* -value of gel from swim bladder gelatin increased with increasing extraction temperatures ($P < 0.05$). This coincided with the decrease in L^* -value. The result indicated that extraction temperature had the impact on color of gel from gelatin extracted from seabass swim bladder.

3.8 Gelling and melting temperatures

Gelling and melting temperatures of gelatin from seabass swim bladder with different extraction temperatures are presented in Table 2. Changes in the phase angle have been used to monitor the thermal transitions of gelatin solution [3]. The gelling temperatures of G45, G55, G65 and G75 were 10.4, 19.6, 19.7 and 18.9 °C, respectively. It was noted that G45 had the lowest gelling temperature ($P < 0.05$). There were no differences in gelling temperature among G55, G65 and G75 ($P > 0.05$). Low gelling temperature observed in G45 might be associated with lower amount of α -, β - and γ -chains and high content of low MW peptides (Fig 1). Short chains could not undergo network formation effectively [10]. Bovine gelatin had the gelling temperature of ~24.7 °C as reported by Kittiphattanabawon et al. [4]. The

gelling temperature of gelatin from seabass swim bladder was lower than that of bovine gelatin. Gelation process is related to formation of junction zones in the three-dimensional network of gelatin gel, which was governed by amino acid compositions as well as molecular weight distribution [9,3]. The imino acid plays the importance role in formation of nucleation zones via hydrogen bonds with water [34,2]. The result indicated that extraction temperature affected the gelling temperature of gelatin from seabass swim bladder.

The melting temperatures of gelatin from seabass swim bladder with different extraction temperatures were in the range of 19.3-28.4 °C. G45 showed the lowest melting temperature (~19.3 °C) ($P < 0.05$), indicating that gel could not be maintained at room temperature for a longer time. Higher melting temperatures were found in gelatin extracted at 55-75 °C ($P < 0.05$). Nevertheless, there were no difference in melting temperatures among those samples ($P > 0.05$). Gelatin gel with higher melting temperature provides a better mouth feel when consumed, compared to gelatin with lower melting temperature [3]. Varying melting temperatures were reported for gelatin from yellowfin tuna skin (26.9-33.9 °C) [35], *Catlacatla* swim bladder (23.3 °C) [13], cobia skin (26.8 °C), croaker skin (25.7 °C) [7], seabass skin (26.3-27.0 °C) [3] and New Zealand hoki skin (21.4 °C) [36]. The present study demonstrated that the high MW components of gelatin more likely contributed to the enhanced junction zone formation, in which strong gel network was developed. Such a gel required higher temperature to disrupt the network formed.

3.9 Microstructure of gelatin gels

Microstructures of gel from gelatin with different extraction temperatures are illustrated in Fig. 3. The microstructure of gel is directly related to the gel strength, which is governed by the conformation and chain length of gelatin [37]. In general, all gelatin gels from seabass swim bladder showed a sponge or coral-like in structure. G45 gel with lower gel

strength showed the gel network with the thinner strands and larger voids, compared with G55 and G65 gels. The finer structure and denser strands in gel matrix observed in gels from G55 and G65 samples were in accordance with higher gel strength (Table 2). Zhang et al. [38] reported that the higher amount of high MW peptides (γ - and β -chains) contributed to denser strands and smaller voids of gelatin gel from silver carp skin. However, gel from G75 had the looser strands and very large voids. Such a gel network had the less resistance to the force applied, leading to a lower gel strength. Basically, the gelatin gel matrix was developed via the formation of hydrogen-bonded junction zones [10]. The results revealed that G55 and G65 yielded the gel network, which was stronger than G45 and G75. Thus, components and their chain length directly affected the arrangement of proteins in the gel network.

3.10 Characteristics of selected gelatin

3.10.1 Proximate composition

Gelatin (G65) from swim bladder, possessing the highest gel strength, had high protein content (91.98%) with low fat (2.06%) and ash (1.55%) contents. The moisture content was 3.92%. Similar compositions of gelatins from *Catlacatla* swim bladder (5.5% moisture, 92.8% protein and 1.5% ash) [13], cobiaskin (9.4% moisture, 88.6% protein, 1.6% fat and 1.0% ash) and croaker skin (10.2% moisture, 88.2% protein, 0.6% fat and 0.9% ash) [7] have been reported. In general, the recommended moisture and ash content of edible gelatin are less than 15 and 2%, respectively [24]. Therefore, the composition of obtained gelatin was complied with the standard.

3.10.2 Amino acid profile

Amino acid composition of G65 is shown in Table 3. Glycine is a major amino acid in G65 (334 residues/1000 residues), followed by alanine (136 residues/1000 residues), proline

(108 residues/1000 residues) and hydroxyproline (87 residues/1000 residues). Glycine is located at every third position of the triple helix of collagen [39]. No cysteine was found in G65. Cysteine is not generally present in the structure of type I collagen [24]. The contents of tyrosine (3 residues/1000 residues), histidine (5 residues/1000 residues) and isoleucine (7 residues/1000 residues) were low. The imino acid content (195 residues/1000 residues) of G65 was higher than that of Amur sturgeon skin (~171 residues/1000 residues) [30], skipjack tuna skin (~149 residues/1000 residues), dog shark skin (~165 residues/1000 residues), rohu skin (~154 residues/1000 residues) [40] and croaker skin (188 residues/1000 residues) [7]. Nevertheless, it was slightly lower than that found in clown featherback skin (207 residues/1000 residues) [4], cobia skin (~205 residues/1000 residues) [7], seabass skin (~198-202 residues/1000 residues) [3] and grass carp skin (~201 residues/1000 residues) [41]. The differences in the species, environment living habitat, body temperature of fish and age of fish are the main factor affecting the contents of proline and hydroxyproline, which contributed to the stability of triple helical structure in collagen molecule [38,12]. High content of hydroxyproline enhanced the viscoelastic and gelling properties of gelatin [37]. Hydroxyproline might be involved in hydrogen bonding between molecules during gelation of gelatin.

4. Conclusion

Characteristics and properties of gel were affected by extraction temperatures. Extraction yield and recovery increased with increasing extraction temperatures. Gelatin extracted at 65 °C, having α - and β -chains as major components, exhibited the highest gel strength and also showed higher gel strength than bovine gelatin. Nevertheless, G65 had similar gelling and melting temperatures, compared with those extracted at 55 and 75 °C.

Therefore, gelatin with high gel properties and extraction yield could be extracted from seabass swim bladder at 65 °C for 6 h.

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Table 1 Extraction yield and recovery of gelatin from the swim bladder of seabass extracted at different temperatures

Samples	Yield (%)	Recovery (%)
G45	44.83 $\pm$ 1.33a	49.08 $\pm$ 1.45a
G55	55.35 $\pm$ 1.33b	63.77 $\pm$ 1.49b
G65	64.69 $\pm$ 1.67c	73.27 $\pm$ 1.91c
G75	71.95 $\pm$ 2.31d	74.83 $\pm$ 2.40c

Values are presented as mean $\pm$ SD (n = 3).

Different lowercase letters within the same column indicate significant differences ($P < 0.05$).

Table 2 Gel strength gelling and melting temperatures as well as gel color of gelatin from the swim bladder of seabass extracted at different temperatures

Sample	Gel strength (g)	Gelling temperature (°C)	Melting temperature (°C)	L^*	a^*	b^*	ΔE^*
FG	201.6±5.0b	ND	ND	71.16±0.14f	-0.97±1.42a	8.09±0.31e	26.48±0.39a
BG	246.3±10.8c	ND	ND	62.21±0.79e	0.05±0.02b	28.76±0.61f	40.06±0.55b
G45	188.3±8.3a	10.4±3.1a	19.3±3.2a	52.59±0.55d	-1.59±0.07a	-2.63±0.05a	43.79±0.43c
G55	263.5±8.3d	19.6±1.4b	28.3±1.4b	49.77±0.20c	-1.55±0.04a	1.52±0.26b	47.54±0.18d
G65	280.9±4.6e	19.7±0.6b	28.4±0.7b	48.14±0.18b	-1.58±0.08a	7.38±0.22d	50.10±0.16e
G75	204.3±8.19b	18.9±0.5b	27.4±0.4b	42.52±0.23a	-1.41±0.02a	4.46±0.17c	55.10±0.20f

Values are presented as mean ± SD (n = 3). ND: Not determined

Different lowercase letters within the same column indicate significant differences ($P < 0.05$).

Table 3 Amino acid compositions of gelatin from the swim bladder of seabass extracted at 65 °C for 6 h (residues/1000 residues)

Amino acid	Content (residues/1000 residues)
Alanine	136
Arginine	53
Aspartic acid/asparagine	44
Cysteine	0
Glutamine/glutamic acid	71
Glycine	334
Histidine	5
Isoleucine	7
Leucine	21
Lysine	25
Hydroxylysine	8
Methionine	14
Phenylalanine	14
Hydroxyproline	87
Proline	108
Serine	25
Threonine	24
Tyrosine	3
Valine	20
Total	1000
Imino acid	195

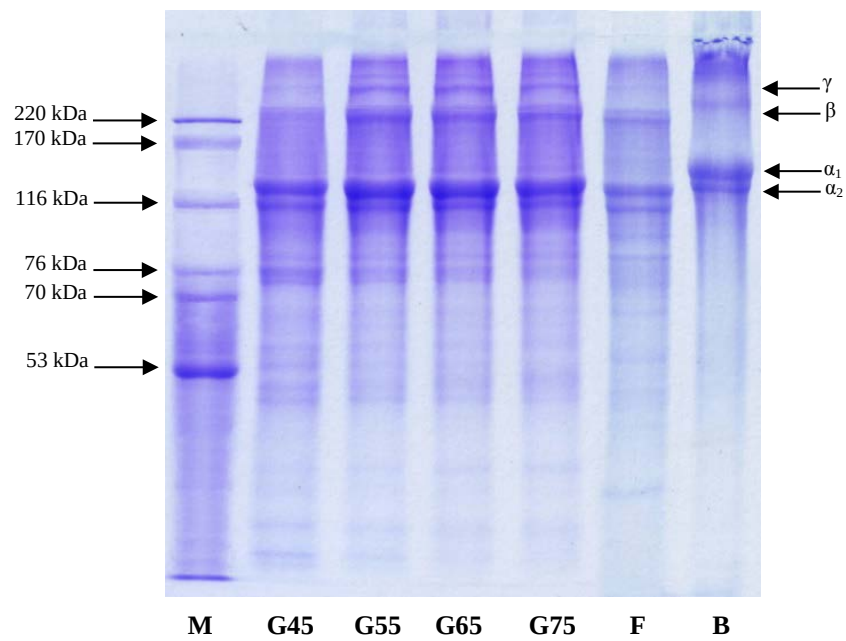


Fig. 1

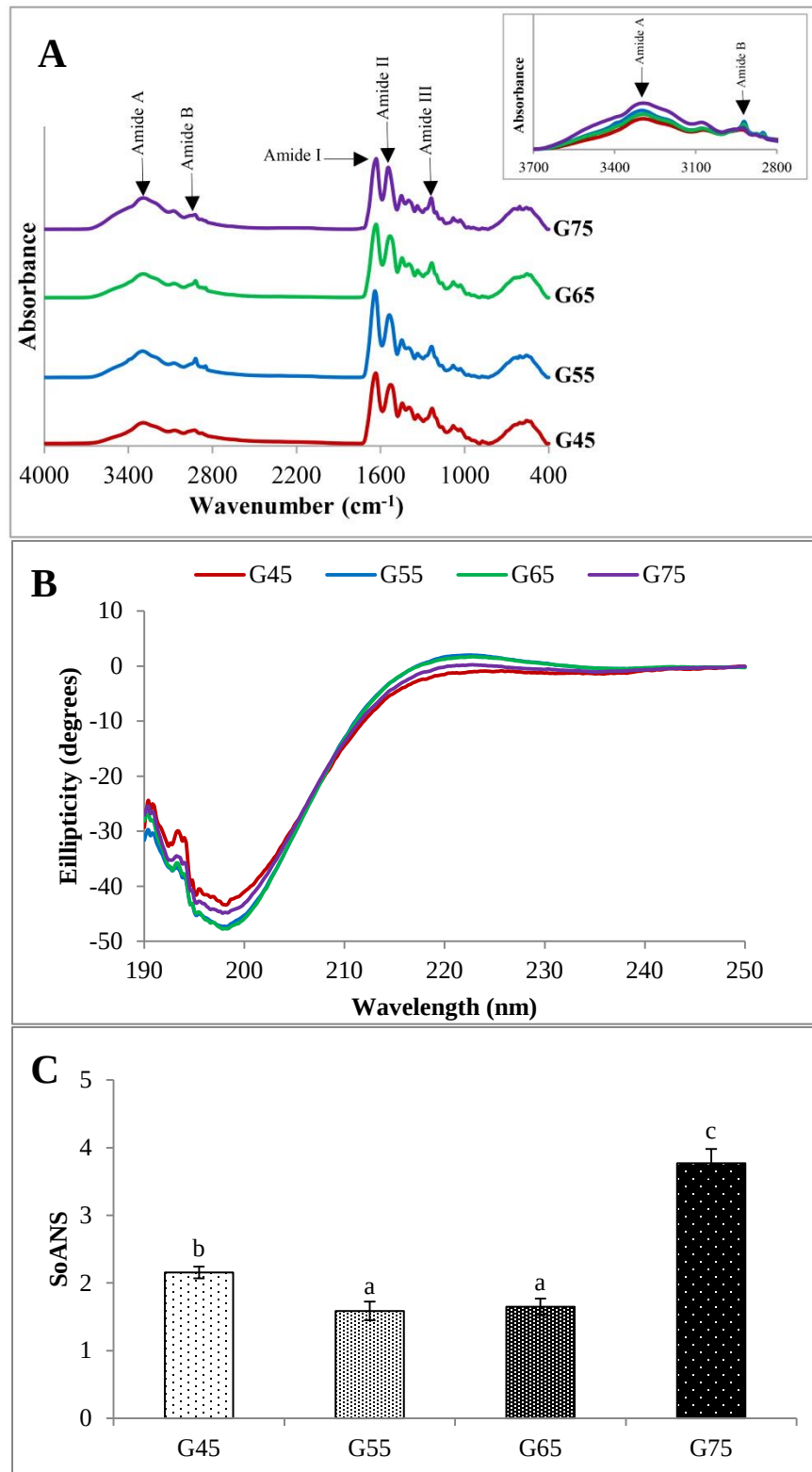


Fig. 2

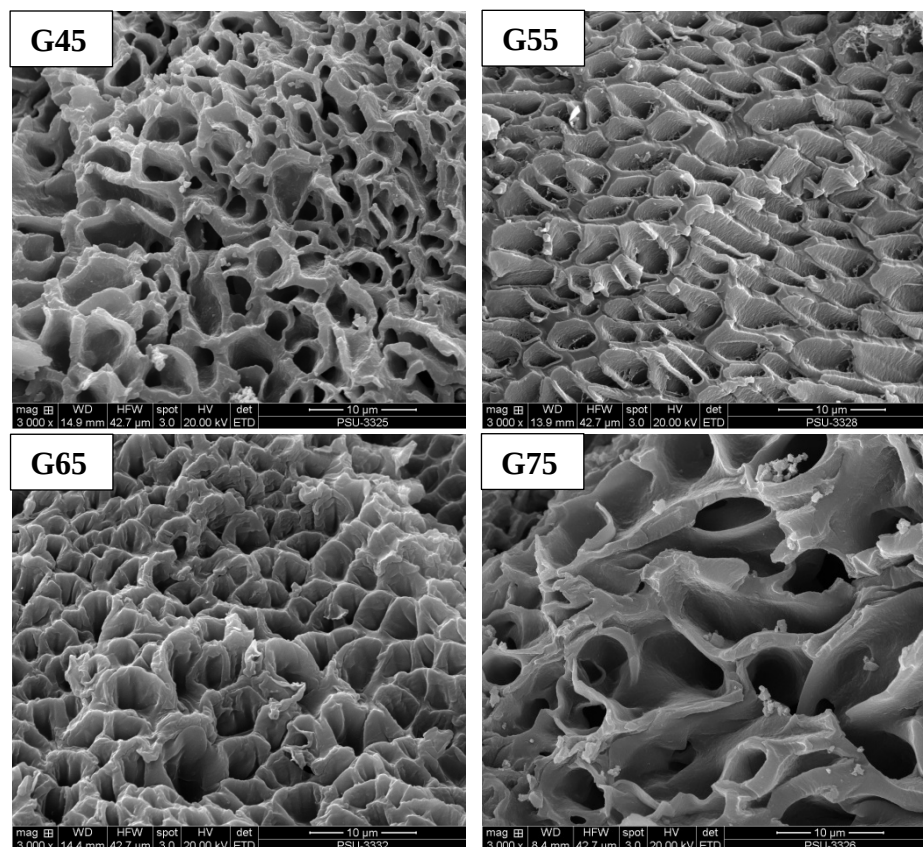


Fig. 3

Figure legend

Fig. 1 SDS-PAGE patterns of gelatin from the swim bladder of seabass extracted at different temperatures. G45, G55, G65 and G75 denote gelatin from seabass swim bladder extracted at 45, 55, 65 and 75 °C, respectively, for 6 h. M, F and B denote high molecular weight markers, commercial fish gelatin and bovine gelatin, respectively.

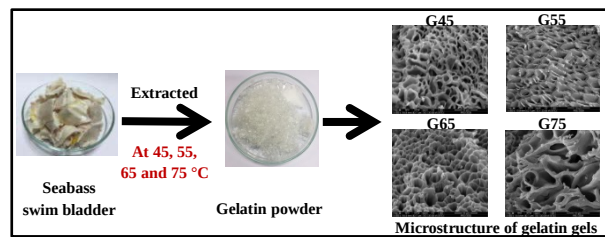
Fig. 2 FTIR spectra (A), CD spectra (B) and surface hydrophobicity (C) of gelatins from the seabass swim bladder extracted at different extraction temperatures. G45, G55, G65 and G75 denote gelatin from seabass swim bladder extracted at 45, 55, 65 and 75 °C, respectively, for 6 h. Bars represent the standard deviation (n=3).

Fig. 3 Microstructures of gelatin gel from the swim bladder of seabass extracted at different temperatures. G45, G55, G65 and G75 denote gelatin from seabass swim bladder extracted at 45, 55, 65 and 75 °C, respectively, for 6 h. Magnification: 3000x.

Characteristics and properties of gelatin from seabass (*Lates calcarifer*)

swim bladder : Impact of extraction temperatures

Sittichoke Sinthusamran, Soottawat Benjakul, Yacine Hemar and Hideki Kishimura



Research highlights

- ♦ Gelatin from seabass swim bladder was extracted at different temperatures.
- ♦ Extraction temperatures affected the characteristics and gel properties of gelatin.
- ♦ Gelatin extracted at 65 °C for 6 h showed the high gel strength and extraction yield.
- ♦ Gelatin from seabass swim bladder had higher gel strength than bovine gelatin.