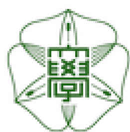
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1 **Characteristics and properties of gelatin from seabass (*Latescalcarifer*)**

2 **swim bladder : Impact of extraction temperatures**

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6 To be submitted to Waste and Biomass Valorization

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24

25 **Abstract**

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

29 *Methods* Gelatin from seabass swim bladderwas extracted at different temperatures (45, 55,
30 65 and 75 °C). The gelatins obtained using various extraction temperatures were
31 characterized.

32 *Results* The yield and recovery of gelatin from swim bladder(44.83-71.95% and 49.08-
33 74.83%, based on dry weight) increased with increasing extraction temperatures.All gelatins
34 contained α-chainsas the predominant components, followed by β-chain. Gelatin from
35 seabass swim bladder showed a high imino acid content (195 residues/1000 residues). FTIR
36 and CD spectra revealed the loss of triple helix during heating via breaking down hydrogen
37 bonds between α-chains. Gel strength generally increased as the extraction temperature
38 increased up to 65 °C ($P<0.05$). Gelatin extracted at 65 °C for 6 h showed a higher gel
39 strength, compared to bovine gelatin ($P<0.05$). Gelling and melting temperatures were 10.4-
40 19.7 and 19.3-28.4 °C, respectively, depending on extraction temperature.

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

44

45 ***Keywords:***Extraction temperature, Swim bladder, Gelatin,Gel strength, Gellingtemperature

46

47 **1. Introduction**

48 Gelatin is a fibrous protein produced by thermal denaturation of collagen. It
49 represents a major biopolymer with wide range of applications in food, pharmaceutical,
50 cosmetic and photographic industries [1]. In general, the main sources of gelatin production
51 are porcine and bovine skin and bones [2]. Nevertheless, religious restriction has led to an
52 increasing interest in alternative sources for gelatin [3].Fish processing by-products have
53 become important for gelatin production. Fish gelatin can be extracted from skin of several
54 aquatic animals including clown featherback [4], Nile tilapia and channel catfish [5], octopus
55 [6], cobia [7] and unicorn leatherjacket [8].Nevertheless, the utilization of fish gelatin is
56 limited,due to its poorgelling property [9]. Moreover, it has been known that extraction
57 conditions, especially extraction temperature and time,determine the properties of gelatin [3].
58 Increasing extraction temperature and time (harsh conditions) generally render the gelatin
59 with the higher yield, butdecreased gelling properties [4]. Thus, extraction condition,
60 including temperature and time, should be optimized.

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

72 gelatin from swim bladder ofseabass (*Latescalcarifer*) at various temperatures and to
73 determine properties of resulting gelatins.
74
75 **2. Materials and methods**
76 2.1 Chemicals
77 All chemicals were of analytical grade. Sodium dodecyl sulphate (SDS), Coomassie
78 blue R-250 and *N*,*N*,*N*',*N*'-tetramethylethylenediamine (TEMED) were procured from Bio-
79 Rad Laboratories (Hercules, CA, USA). High-molecular-weight markers were purchased
80 from GE Healthcare UK Limited (Buckinghamshire, UK). Fish gelatin (FG) produced from
81 tilapia skin (~240 bloom) was obtained from Lapi Gelatine S.p.A (Empoli, Italy). Food grade
82 bovine bone gelatin (BG) with the gel strength of 150–250 g was purchased from Halagel
83 (Thailand) Co., Ltd. (Bangkok, Thailand).
84
85 2.2 Preparation of seabass swim bladder
86 Swim bladdersof seabass (*L. calcarifer*) with a length of 20-25 cm were obtained
87 from Sirikhun Seafood Co., Ltd, Samutsakhon, Thailand. The frozen swim bladders packed
88 in a polyethylene bag, were kept in ice with a skin/ice ratio of 1:3 (w/w) using a polystyrene
89 box as a container and transported to the Department of Food Technology, Prince of Songkla
90 University, within 10 h.Upon arrival, frozen swim bladders were thawed with running water
91 until the core temperature reached 8-10 °C. The samples were washed with tap water andcut
92 into small pieces (1×1 cm^2) using scissors. Prepared swim bladder samples were placed in
93 polyethylene bags and stored at -20 °C until used, but not longer than 2 months. Swim
94 bladder had moisture, protein, fat and ash content of 69.74, 29.67, 0.34 and 0.23%, as
95 determined by AOAC methods with the analytical numbers of 927.05, 984.13, 920.39 B and
96 942.05, respectively [14].The conversion factor of 5.4 was used for calculation of protein

97 content. It was rich in hydroxyproline (24.48 mg/g) as measured by the method of Bergman,
98 Loxley [15].
99
100 2.3 Extraction of gelatin from swim bladder
101 Gelatin was extractedfrom swim bladder according to the method of Sinthusamran et
102 al. [3] with a slight modification. Before gelatin extraction, swim bladder was soaked in 0.1
103 M NaOH, with a sample/solution ratio of 1:10 (w/v), to remove non-collagenous proteins.
104 The mixture was stirred continuously for 3 h at room temperature (28–30°C) using an
105 overhead stirrer with a propeller (W20.n IKA®-Werke GmbH & CO.KG, Staufen, Germany).
106 The alkaline solution was changed every 1 h for totally 3 times. The residues were then
107 washed with tap water until a neutral or faintly basic pH was obtained. The deproteinized
108 matters were then mixed with 0.05 M acetic acid at a sample/solution ratio of 1:10 (w/v) to
109 swell collagenous material. The mixture was stirred at room temperature for 2 h. The swollen
110 swim bladders were washed using tap water until wash water became neutral or faintly acidic
111 in pH.
112 To extract the gelatin, the swollen swim bladders were mixed with distilled water at a
113 ratio of 1:10 (w/v) at 45, 55, 65 and 75 °C for 6 h with continuous stirring. The mixtures were
114 filtered with two layers of cheesecloth. Then, the filtrates were mixed with 1 % (w/v)
115 activated carbon for 1 h with continuous stirring. The mixtures were centrifuged at 17,500x*g*
116 for 15 min at 25 °C using a Beckman model Avanti J-E centrifuge (Beckman Coulter, Inc.,
117 Palo Alto, CA, USA) to remove insoluble material. The supernatants were filtered using a
118 Buchner funnel with Whatman No.4 filter paper (Whatman International, Ltd., Maidstone,
119 England).Finally, the filtrates were freeze-dried using a freeze-dryer (CoolSafe 55, ScanLaf
120 A/S, Lynge, Denmark) at -50 °C for 72 h. The dry gelatinsextracted from swim bladder at 45,

121 55, 65 and 75 °C were referred to as ‘G45’, ‘G55’, ‘G65’ and ‘G75’, respectively. The gelatin
122 samples were subsequently subjected to analyses.

123

124 2.4 Analyses

125 2.4.1 Determination of yield and recovery

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

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

127 Recovery was also determined using the following equation:

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

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

130

131 2.4.2 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

132 SDS–PAGE was performed by the method of Laemmli [16]. Gelatin samples were
133 dissolved in 5% SDS solution. The obtained mixtures were then heated at 85 °C for 1 h using
134 a temperature controlled water bath model W350 (Memmert, Chwabach, Germany).
135 Solubilized samples were mixed at a 1:1 (v/v) ratio with sample buffer (0.5 M Tris–HCl, pH
136 6.8 containing 5% SDS and 20% glycerol). Samples were loaded onto a polyacrylamide gel
137 made of 7.5% separating gel and 4% stacking gel and subjected to electrophoresis at a
138 constant current of 15 mA/gel. After electrophoresis, gels were stained with 0.05% (w/v)
139 Coomassie blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid for 30 min. Finally,
140 they were destained with the mixture of 50% (v/v) methanol and 7.5% (v/v) acetic acid for 30
141 min and destained again with the mixture of 5% (v/v) methanol and 7.5% (v/v) acetic acid for

142 1 h. High-molecular-weight protein markers were used to estimate the molecular weight of
143 proteins.

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

147

148 2.4.3 Fourier transform infrared (FTIR) spectroscopic analysis

149 FTIR spectra of gelatin samples were obtained using a FTIR spectrometer
150 (EQUINOX 55, Bruker, Ettlingen, Germany) equipped with a deuterated l-alanine tri-glycine
151 sulphate (DLATGS) detector. A horizontal attenuated total reflectance accessory (HATR)
152 was mounted into the sample compartment. The internal reflection crystal (Pike Technologies,
153 Madison, WI, USA), made of zinc selenide, had a 45° angle of incidence to the IR beam.
154 Spectra were acquired at room temperature, at a resolution of 4 cm^{-1} and the measurement
155 range was 4000–400 cm^{-1} (mid-IR region). Automatic signals were collected in 32 scans at a
156 resolution of 4 cm^{-1} and were ratioed against a background spectrum recorded from the clean
157 empty cell at 25 °C. Analysis of spectral data was carried out using the OPUS 3.0 data
158 collection software programme (Bruker, Ettlingen, Germany).

159

160 2.4.4 Circular dichroism (CD) spectroscopic analysis

161 Gelatin samples (0.05 mg protein/mL) were dissolved in 10 mM sodium phosphate
162 buffer (pH 7.0) according to the method of Wierenga et al. [17]. CD spectra were measured at
163 20 °C, with a scan speed of 100 nm/min from 190 to 250 nm, a data interval of 0.2 nm, a
164 bandwidth of 1 nm and a response time 0.125 s using a JASCO J-801 spectrometer (Jasco
165 Corp, Tokyo, Japan). The secondary structure was estimated using a CDPro software with the
166 reference spectra as described by Johnson [18].

167 2.4.5 Surface hydrophobicity

168 Surface hydrophobicity of gelatin samples was determined by the method of Benjakul
169 et al. [19]. Gelatin was dissolved in 10 mM phosphate buffer, pH 6.0, containing 0.6 M NaCl
170 to obtain a final protein concentration of 5 g/L. The gelatin solution was diluted to 0.125,
171 0.25, 0.5, and 1 g/L using the same buffer. The diluted gelatin solutions (2 mL) were well
172 mixed with 10 μL of 8 mM 1-anilinonaphthalene-8-sulfonic acid (ANS) in 0.1 M phosphate
173 buffer, pH 7.0. The relative fluorescence intensity of ANS-protein conjugates was measured
174 using a spectrofluorometer (RF-15001, Shimadzu, Kyoto, Japan) at the excitation wavelength
175 of 374 nm and the emission wavelength of 485 nm.Surface hydrophobicity was calculated
176 from initial slopes of plots of relative fluorescence intensity versus protein concentration
177 (g/L) using linear regression analysis. The initial slope was referred to as SoANS.

178

179 2.4.6 Determination of gel strength

180 Gelatin gel was prepared by the method of Kittiphattanabawon et al. [2]. Gelatin was
181 mixed with distilled water (60 °C) to obtain the concentration of 6.67% (w/v). The solution
182 was stirred until gelatin was solubilized completely and transferred to a cylindrical mold with
183 3 cm diameter and2.5 cm height. The solution was incubated at the refrigeratedtemperature
184 for 18 h prior to analysis.

185 Gel strength was determined at 8-10 °C using a texture analyzer (Stable Micro System,
186 Surrey, UK) with a load cell of 5 kg, cross-head speed of 1 mm/s, equipped with a 1.27 cm
187 diameter flat-faced cylindrical Teflon® plunger. The maximum force (gram) was recorded,
188 when the plunger had penetrated 4 mm into the gel samples.

189

190

191

192 2.4.7 Determination of gel color

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

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

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

200

201 2.4.8 Determination of gelling and melting temperatures

202 Gelling and melting temperatures of gelatin samples were measured following the
203 method of Sinthusamran et al. [3]. The measurement was performed using a RheoStress RS 1
204 rheometer (HAAKE, Karlsruhe, Germany) in the oscillatory mode. The measuring geometry
205 used was a stainless steel 60-mm-diameter parallel plate and the gap was set at 1.0 mm.
206 Gelatin solution (6.67%, w/v) was prepared in the same manner as described in the methods.
207 The solution was preheated at 60 °C for 30 min. Then the solution (2.9 mL) was loaded on
208 the Peltier plate and equilibrated at 60 °C for 10 min before measurements. The
209 measurements wereconduct at a constant frequency of 1 Hz, and a constant applied stress of 3
210 Pa.The samples were cooled from 60to 5 °C and subsequently heated to 60 °C at a constant
211 rate of 1.0 °C/min.The gelling and melting temperatures were calculated, where tan δ became
212 1 or δ was 45°.

213

214

215 2.4.9 Microstructure analysis of gelatin gel

216 Microstructure of gelatin gel (6.67%, w/v) prepared as previously described was
217 visualized using a scanning electron microscopy (SEM). Gelatin gels having a thickness of 2-
218 3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 12 h.
219 The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol with
220 serial concentrations of 50, 70, 80, 90 and 100% (v/v). Dried samples were mounted on a
221 bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA,
222 USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800
223 LV, Tokyo, Japan) at an acceleration voltage of 20 kV.
224
225 2.5 Characterization of selected gelatin
226 2.5.1 Proximate analysis
227 Proximate compositions were determined as per AOAC method [14].
228
229 2.5.2 Amino acid analysis
230 Amino acid composition of gelatin samples was analyzed using an amino acid
231 analyzer. Gelatin samples were hydrolyzed under reduced pressure in 4 M methane sulphonic
232 acid containing 0.2% 3-2(2-aminoethyl)indole at 115 °C for 24 h. The hydrolysates were
233 neutralized with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). An aliquot of
234 0.04 mL was applied to an amino acid analyzer (MLC-703; Atto Co., Tokyo, Japan).
235
236 2.6 Statistical analysis
237 All experiments were run in triplicateusing three different lots of samples. Data were
238 subjected to analysis of variance (ANOVA) and mean comparisons were carried out using the

239 Duncan's multiple range test [21]. Statistical analysis was performed using the Statistical
240 Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).
241

242 **3. Results and discussion**

243 3.1 Yield andrecoveryof gelatin

244 Yield and recovery of gelatin from seabass swim bladder extracted at different
245 temperaturesvaried as shown in Table 1. Yields of44.83-71.95% andrecovery of 49.08-
246 74.83% were obtained.The yield was the amount of solid released from pretreated swim
247 bladder matrix, while the recovery represented the percentage of collagen converted to
248 gelatin by heat[22]. Both extraction yield and recoveryincreased as the extraction temperature
249 increased ($P<0.05$). The highest yield (71.95%) was found in gelatin extracted at 75 °C
250 (G75).However, there was no difference in the recoverybetween G65 and G75 ($P>0.05$).
251 Similar results were reported for gelatin from clown featherback skin [4] and seabass skin [3],
252 in which the yield of gelatin increased as extraction temperatures increased. The higher
253 temperature used for extraction more likely provided higher energy, thereby destroying
254 hydrogen bonds stabilizing the triple helix in collagen structure to higher extent. As a
255 consequence, α- and β-chains were more released into medium, resulting in the higher
256 amount of gelatinobtained [3,22]. Collagen from seabass swim bladder showed a T_{max} of
257 35.02 °C [12]. In the present study, extraction temperatures were above $T_{max}$and could induce
258 thedenaturation of collagen to gelatin. By raising the temperature of extraction, higher
259 conversion of collagen to gelatin could be achieved as shown by the increased recovery.
260

261 3.2 Protein pattern

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

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

283

284 3.3 Fourier transform infrared (FTIR) spectra

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

289 cm^{-1}, respectively. This band is related to a C=O stretching vibration coupled to contributions
290 from the CN stretch [23]. Amide I band of all gelatin samples, especially G75 was shifted to
291 higher wavenumber. The result was in agreement with the slight decrease in α- and β-chain of
292 G75. Kittiphattanabawon et al. [2] reported that gelatin extracted from shark skins at the
293 higher temperature with longer time generally contained a higher amount of low molecular
294 weight components, in which the C=O groupwere more exposed. Amide II band of G45, G55,
295 G65 and G75 was observed at wavenumber ranging from 1529 to 1546 cm^{-1}. Amide II band
296 represents the combination between bending vibration of N-H groups and stretching
297 vibrations of C-N groups [24]. Furthermore, the amide III bands of all gelatin samples were
298 observed at wavenumbers of 1233-1238 cm^{-1}, demonstrating disorder from an α-helical to a
299 random coil structure, leading to the loss of triple helix state. The amide III was more likely
300 associated with C-N stretching vibrations and N-H deformation from the amide linkages as
301 well as the absorptions arising from wagging vibration of CH_2 groups in the glycine
302 backbone and proline side-chains [9]. In the present study, amide III peak of G75 had the
303 lower amplitude when compared with that of other gelatin samples.This suggestedthat G75
304 plausibly had more disordered structure due to higher heat applied for extraction.

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

314 2924 cm^{-1} for G45, G55, G65 and G75, respectively. It represents CH stretching vibrations of
315 the –CH_2 groups [24]. Amide B peaks of G55 and G65 were shifted to the lower
316 wavenumber, compared with other samples. The result suggested that CH_2 groups were
317 plausibly interacted with adjacent molecules. This was in accordance with the higher MW
318 cross-links in both G55 and G65. Therefore, the extraction temperature affected the
319 secondary and functional group of gelatins obtained from swim bladder of seabass.
320
321 3.4 CD-spectra
322 CD-spectra of gelatin from swim bladder extracted at different temperatures are
323 depicted in Fig. 2B. Circular dichroic (CD) spectroscopy has been used to characterize the
324 secondary structure of protein [26]. It can also be used to analyze the conformation of
325 peptides [27]. Native collagen generally exhibited a CD spectra with a positive peak at
326 around 220-230 nm and a negative peak at around 200 nm [28]. After complete denaturation
327 of collagen, the positive peak at 220-230 nm, characteristic of the triple-helix, disappears
328 completely and only the negative peak at 200 nm of gelatin remains[28]. The negative peak at
329 200 nm was related to the random conformation ofgelatin [29]. In the present study, CD
330 spectra ofall samples from seabass swim bladder showed the maximum negative peak at 197-
331 200 nm, but the positive peak in the CD spectra almost disappeared, suggesting that the triple
332 helical structure was almost converted to the random coil. The result was in agreement with
333 that reported by Nikoo et al. [30] on the farmed Amur sturgeon skin gelatin. However, small
334 positive peak at 218-220 nm was still found in G55 and G65 samples, suggesting the
335 presence of some α-helix. This was coincidental with higher content of longer chain length
336 (β- and γ-chain) of protein (Fig. 1). In addition, the positive peak as observed in G55 and G65
337 samplesmight be related with the partialrefolding of protein chains. α-, β- and γ-chains of
338 G55 and G65 at high content could enhance refolding inter- or intra-molecularly to some

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

344

345 3.5 Surface hydrophobicity

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

359 3.6Gel strength

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

364 found in G65 sample (P<0.05). The increase in gel strength wascoincidental withhigher
365 proportion of α-, β- and γ-chains (Fig. 1). However, when gelatin was extracted at 75 °C, gel
366 strength of gelatin decreased (P<0.05). This was related with the slight decreases in those
367 aforementioned components.Sinthusamran et al. [3] also reported that gelatin from seabass
368 skin extracted at lower temperature had higher gel strength than those extracted at higher
369 temperature. Among all samples, the lowest gel strength (188.3 g) was observed for gelatin
370 extracted at 45 °C (P<0.05).The low gel strength of G45 was related tothe lower proportion
371 of α-, β- and γ-chains (Fig. 1). FG and BGhad the gel strength of 201.6 and 246.3 g,
372 respectively. G55 and G65 showed higher gel strength thanBG and FG (P<0.05). The results
373 suggested that long chain components (α-, β- and γ-chains) werethe major factor affecting the
374 development of strong gel[3]. Furthermore, the ways those chains interacted each other or the
375 junction zoneswere formed were also crucial for gel formation. In addition, gelatin contained
376 highhydroxyproline content (Table 3). The OH groups of hydroxyproline might be involved
377 in gel formation by hydrogen bonding with adjacent chains[2]. Gelatin with different gel
378 strength was reported for clown featherback skin (225-284 g) [4], *Catlacatla* swim bladder
379 (264.6 g) [13], seabass skin (223-322 g) [10], farmed Amur sturgeon skin (141 g) [30], cobia
380 skin (232 g) and croaker skin (212 g) [7].In the present study, G65 with higher amount of α-,
381 β- and γ-chains showed the highest gel strength and 65 °C was consider as the optimal
382 temperature for extraction of gelatin from seabass swim bladder.
383
384 3.7 Color of gel
385 The color of gelatin gel from seabass swim bladder extracted at different temperatures
386 expressed as L^*, a^*, b^* and ΔE^* is shown in Table 2. FG and BG had the higher lightness (L^*-
387 value), compared with all gelatin from seabass swim bladder, regardless of extraction
388 temperatures (P<0.05). TheL^*-value of gel from swim bladder gelatin decreased with

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

404 3.8 Gelling and melting temperatures

405 Gelling and melting temperatures of gelatin from seabass swim bladder with different
406 extraction temperatures are presented in Table 2. Changes in the phase angle have been used
407 to monitor the thermal transitions of gelatin solution[3]. The gelling temperatures of G45,
408 G55, G65 and G75 were 10.4, 19.6, 19.7 and 18.9 °C, respectively. It was noted that G45 had
409 the lowest gelling temperature (P<0.05).There were no differences in gelling temperature
410 amongG55, G65 and G75 (P>0.05). Low gelling temperature observed in G45might
411 beassociated with lower amount of α-, β- and γ-chains and high content of low MW peptides
412 (Fig 1). Short chains could not undergo network formation effectively[10]. Bovine gelatin
413 had the gelling temperature of ~24.7 °C as reported by Kittiphattanabawon et al. [4].The

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

420 The melting temperatures of gelatin from seabass swim bladder with different
421 extraction temperatures were in the range of 19.3-28.4 °C.G45 showed the lowest melting
422 temperature (~19.3 °C) (P<0.05), indicating that gel could not be maintained at room
423 temperature for a longer time. Higher melting temperatures were found in gelatin extracted at
424 55-75 °C (P<0.05). Nevertheless, there were no difference in melting temperatures among
425 those samples (P>0.05). Gelatin gel with higher melting temperature provides a better mouth
426 feel when consumed, compared to gelatin with lower melting temperature [3]. Varying
427 melting temperatures were reported for gelatin from yellowfin tuna skin (26.9-33.9 °C)[35],
428 *Catlacatla* swim bladder (23.3 °C) [13], cobia skin (26.8 °C), croaker skin (25.7 °C) [7],
429 seabass skin (26.3-27.0 °C) [3] and New Zealand hoki skin (21.4 °C) [36].The present study
430 demonstrated that the high MW components of gelatin more likely contributed to the
431 enhanced junction zone formation, in which strong gel network was developed. Such a gel
432 required higher temperature to disrupt the network formed.

433

434 3.9 Microstructure of gelatin gels

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

439 strength showed the gel network with the thinner strands and larger voids, compared with
440 G55 and G65 gels. The finer structure and denser strands in gel matrix observed in gels from
441 G55 and G65 sampleswere in accordance with higher gel strength (Table 2).Zhang et al. [38]
442 reported that the higher amount of high MW peptides (γ- and β-chains) contributed to denser
443 strands and smaller voids of gelatin gel fromsilver carp skin. However, gel from G75 had the
444 looser strands and very large voids. Such a gel network had the less resistance to the force
445 applied, leading to a lower gel strength. Basically, the gelatin gel matrix was developed via
446 the formation of hydrogen-bonded junction zones[10]. The resultsrevealed thatG55 and
447 G65yielded the gel network, which was stronger than G45 and G75.Thus, components and
448 their chain length directly affected the arrangement of proteins in the gel network.
449
450 3.10 Characteristics of selected gelatin
451 3.10.1 Proximate composition
452 Gelatin (G65) from swim bladder, possessing the highest gel strength, had high
453 protein content (91.98%) with low fat (2.06%) and ash (1.55%) contents. The moisture
454 content was 3.92%. Similar compositions of gelatins from *Catlacatla* swim bladder (5.5%
455 moisture, 92.8% protein and 1.5% ash) [13], cobiaskin (9.4% moisture, 88.6% protein, 1.6%
456 fat and 1.0% ash) and croaker skin (10.2% moisture, 88.2% protein, 0.6% fat and 0.9% ash)
457 [7] have been reported. In general, the recommended moisture and ash content of edible
458 gelatin are less than 15 and 2%, respectively [24]. Therefore, the composition of obtained
459 gelatin was complied with the standard.
460
461 3.10.2 Amino acid profile
462 Amino acid composition of G65 is shown in Table 3. Glycine is a major amino acid in
463 G65 (334 residues/1000 residues), followed by alanine (136 residues/1000 residues), proline

464 (108 residues/1000 residues) and hydroxyproline (87 residues/1000 residues). Glycine is
465 located at every third position of the triple helix of collagen [39]. No cysteine was found in
466 G65. Cysteine is not generally present in the structure of type I collagen [24]. The contents of
467 tyrosine (3 residues/1000 residues), histidine (5 residues/1000 residues) and isoleucine (7
468 residues/1000 residues) were low. The imino acid content (195 residues/1000 residues) of
469 G65 was higher than that of Amur sturgeon skin (~171 residues/1000 residues) [30], skipjack
470 tuna skin(~149 residues/1000 residues), dog shark skin (~165 residues/1000 residues), rohu
471 skin (~154 residues/1000 residues) [40] and croaker skin (188 residues/1000 residues) [7].
472 Nevertheless, it was slightly lower than that found in clown featherback skin (207
473 residues/1000 residues) [4], cobia skin (~205 residues/1000 residues) [7], seabass skin (~198-
474 202 residues/1000 residues) [3] and grass carp skin (~201 residues/1000 residues) [41]. The
475 differences in the species, environment living habitat, body temperature of fish and age of
476 fish are the main factor affecting the contents of proline and hydroxyproline, which
477 contributed to the stability of triple helical structure in collagen molecule [38,12]. High
478 content of hydroxyproline enhanced the viscoelastic and gelling properties of gelatin [37].
479 Hydroxyproline might be involved in hydrogen bondingbetween molecules during gelation of
480 gelatin.

481

482 **4. Conclusion**

483 Characteristics and properties of gel were affected by extraction temperatures.
484 Extraction yield and recovery increased with increasing extraction temperatures. Gelatin
485 extracted at 65 °C, havingα- and β-chainsas major components, exhibited the highest gel
486 strength and also showed higher gel strength than bovine gelatin. Nevertheless, G65 had
487 similar gelling and meltingtemperatures, compared with thoseextracted at 55 and 75 °C.

488 Therefore, gelatin with high gel properties and extraction yield could be extracted from
489 seabass swim bladder at 65 °C for 6 h.
490

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497
498

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617

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

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

Values are presented as mean ± 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

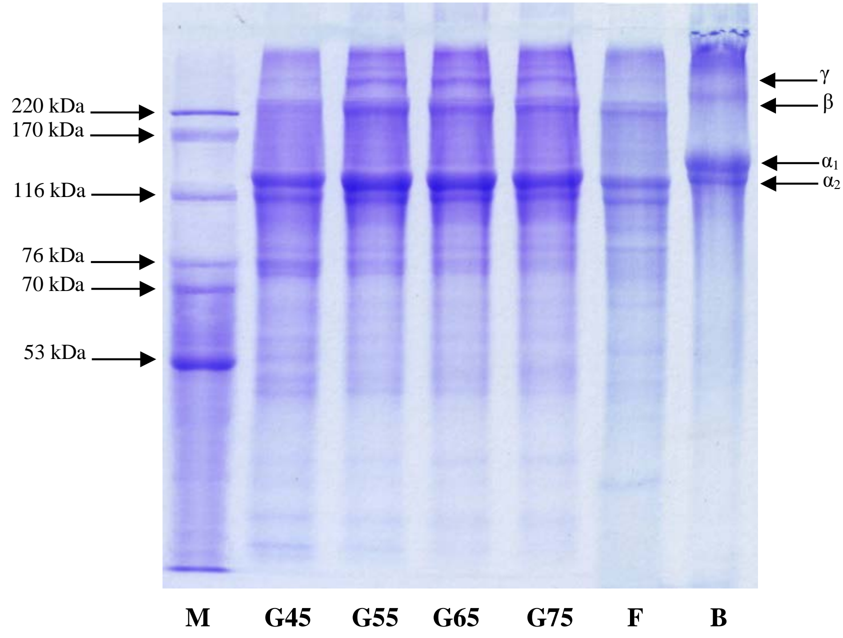


Fig. 1

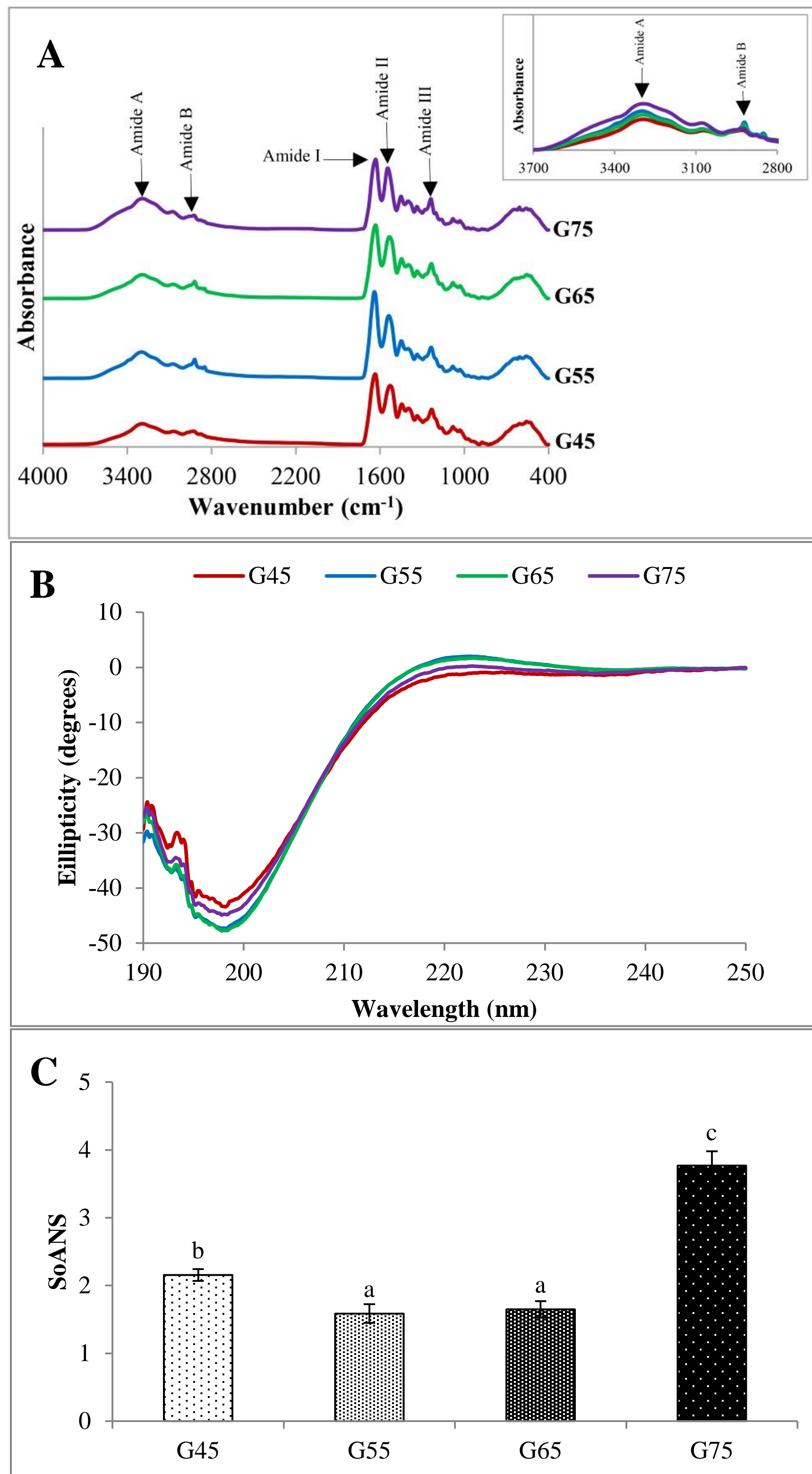


Fig. 2

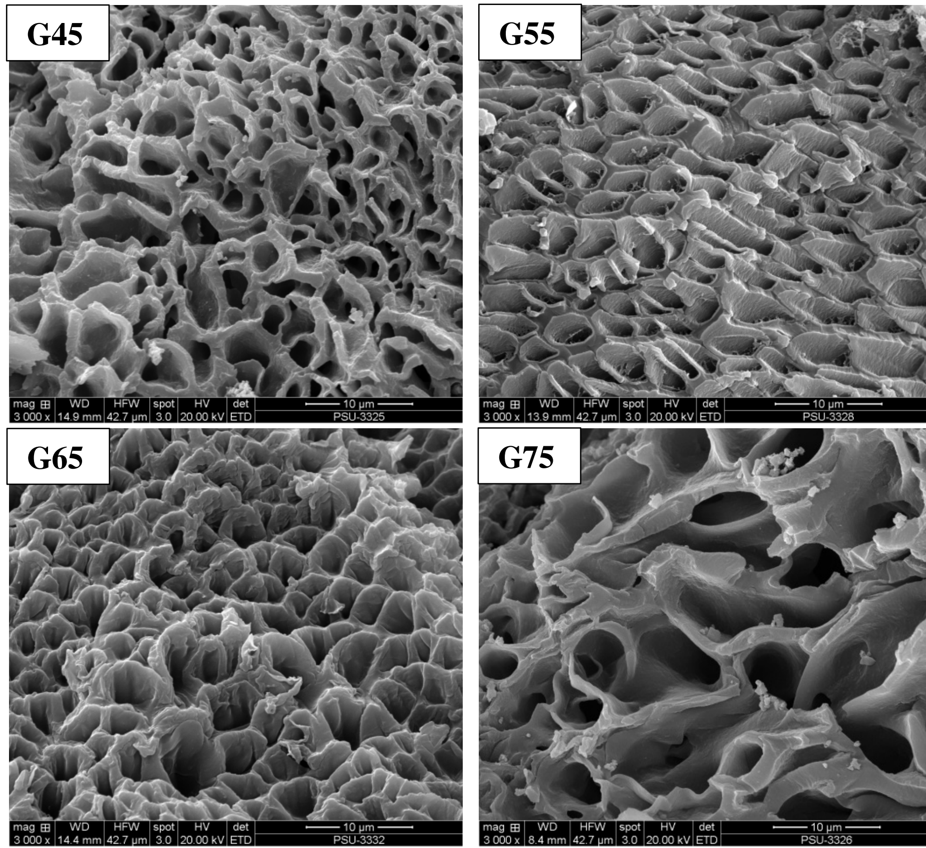


Fig. 3

Figure legend

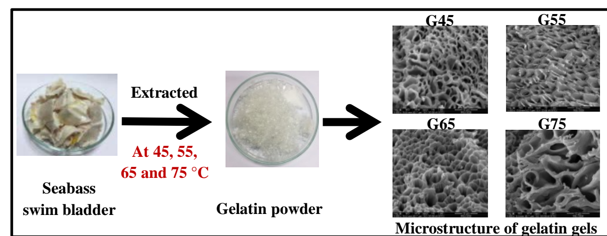
Fig. 1 SDS-PAGE patterns of gelatin from the swim bladder of seabass extracted at different temperatures. G45, G55, G65 and G75 denote gelatin from seabass swim bladder extracted at 45, 55, 65 and 75 °C, respectively, for 6 h. M, F and B denote high molecular weight markers, commercial fish gelatin and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