PAPER

CHARACTERISTICS OF GELATIN FROM SWIM BLADDER OF YELLOWFIN TUNA (*THUNNUS ALBACORES*) AS INFLUENCED BY EXTRACTING TEMPERATURES

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ABSTRACT

Gelatin was extracted from the swim bladder of yellowfin tuna (*Thunnus albacores*) at different temperatures (60, 70 and 80°C) with the extraction yields of 35.6%, 41.1% and 47.3% (dry weight basis), respectively. The α-chains of gelatin decreased with increasing extraction temperatures. Similar amino acid compositions were noticeable among all gelatins, in which glycine constituted the major amino acid. Imino acids ranged from 169 to 172 residues/1,000 residues. The gel strength of gelatin extracted at lower temperature was higher than that of gelatins extracted at higher temperatures. Gelling and melting temperatures for swim bladder gelatin were 11.07-15.24 and 20.36-22.33°C, respectively. Higher gelling and melting points were observed for gelatin extracted at lower temperatures. Microstructure of gel of gelatin extracted at 60°C was finer with smaller voids, compared with others. FTIR spectra of obtained gelatins revealed the significant loss of molecular order of the triple-helix. Thus, extraction temperatures showed the direct impact on characteristics of gelatin from swim bladder.

- Keywords: gelatin, gel strength, extraction, temperature, swim bladder, yellowfin tuna -

INTRODUCTION

Gelatin is a fibrous protein obtained by partial denaturation or hydrolysis of collagen. Gelatin represents biopolymer with many applications in food, materials (for edible and biodegradable packaging), cosmetic, pharmaceutical and photographic industries (JELLOULI *et al.*, 2011). The source, type of collagen and processing conditions have the influence on the properties of the resulting gelatin (KITTIPHATTANABAWON *et al.*, 2010). Different types of gelatins have varying thermal and rheological properties such as gel strength, melting and gelling temperatures (BENJAKUL *et al.*, 2012). These properties are governed by several factors such as chain length or molecular weight distribution, amino acid composition and hydrophobicity, etc. (GÓMEZ-GUILLÉN *et al.*, 2002; NORZIAH *et al.*, 2009).

Commercial gelatins are produced mainly from porcine and bovine skins and bones by alkaline or acidic extraction (BENJAKUL *et al.*, 2009). However, both Judaism and Islam forbid the consumption of any pork-related products, while Hindus do not consume cow-related products. Additionally, bovine gelatin has a high risk for bovine spongiform encephalopathy (NAGARAJAN *et al.*, 2012). Furthermore, the need to exploit the fish processing byproducts has led to the production of gelatin as an alternative to mammalian counterpart (GÓMEZ-GUILLÉN *et al.*, 2011). Fish gelatin has been extracted mainly from fish skin such as seabass (SINTHUSAMRAN *et al.*, 2014), cobia (SILVA *et al.*, 2014) skipjack tuna, dog shark and rohu (SHYNI *et al.*, 2014) and unicorn leatherjacket (KAEWRUANG *et al.*, 2013), etc.

Among fish processing industries, canned tuna industry is economically important. Tuna including yellowfin, skipjack and tongol have been the important species for canning in Thailand with a large volume of raw materials used. Approximately two-thirds of the whole fish are utilized and the remainings involving the viscera, head, bone and swim bladder become the byproducts (KLOMKLAO *et al.*, 2004). Fish swim bladders can be used for production of "isinglass" (WEBER *et al.*, 2009). Recently, KAEWDANG *et al.*, (2014) reported that alkaline pretreatment was essential for gelatin extraction from yellowfin tuna swim bladder. However, no information on the effect of extracting temperature on characteristics and properties of gelatin has been reported. Therefore, the objectives of this investigation were to extract and characterize gelatin from the swim bladder of yellowfin tuna using different extraction temperatures.

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). Food grade bovine bone gelatin with the bloom strength of 150-250 g was obtained from Halagel (Thailand) Co., Ltd., (Bangkok, Thailand).

2.2. Collection and preparation of swim bladder

Swim bladders of yellowfin tuna (*Thunnus albacares*) were obtained from Tropical Canning Public Co., Ltd., Songkhla, Thailand. Swim bladders with the length of 8-12 cm were placed in polyethylene bags, inserted in ice using a sample/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Songkhla. Upon arrival, swim bladders were washed with distilled water and cut into pieces with the length of approximately 2 cm. The prepared samples were then placed in polyethylene bag and frozen at -20°C. The samples were stored at -20°C until used. The storage time was not longer than 3 months. Prior to extraction, frozen swim bladders were thawed using running water until the temperature was 0-2°C

2.3. Extraction of gelatin from swim bladder

Prior to gelatin extraction, swim bladders were pretreated with alkaline solution as per the method of KAEWDANG *et al.* (2014). Prepared swim bladders were added with the mixed alkaline solution (Na_2CO_3:NaOH; 7:3) having the concentration of 4% (w/v) at a ratio of 1:10 (w/v). The mixture was stirred for 12 h at room temperature (28-30°C) using an overhead stirrer model W20.n (IKA®-Werke GmbH & CO.KG, Stanfen, Germany). The alkaline solution was changed every 6 h. The residues were washed with tap water until a neutral or faintly basic pH was obtained.

To extract gelatin, alkali pretreated samples were soaked in distilled water with different temperatures (60, 70 and 80°C) using a swim bladder/water ratio of 1:5 (w/v) in a temperature-controlled water bath (W350, Memmert, Schwabach, Germany) for 24 h with a continuous stirring at a speed of 150 rpm. The mixtures were then filtered using a Buchner funnel with a Whatman No. 4 filter paper (Whatman International, Ltd., Maidstone, England). The filtrates were freeze-dried using a freeze-dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark). The dry gelatin extracted from swim bladder from yellowfin tuna at 60, 70 and 80 °C was referred to as 'G60', 'G70' and 'G80', respectively. All gelatin samples were weighed, calculated for extraction yield and subjected to analyses.

2.4. Analyses

2.4.1. Yield

Gelatin yield was calculated by the following equation.

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

where the weight of dry swim bladder was calculated by subtracting moisture content determined by AOAC (2000) from the initial wet weight.

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

SDS–PAGE was performed by the method of LAEMMLI (1970). Samples were dissolved in 5% SDS solution. The mixtures were then heated at 85°C for 1 h using a temperature controlled water bath model W350 (Memmert, Schwabach, Germany). The mixtures were centrifuged at 8,500 g for 5 min using a microcentrifuge (MIKRO20, Hettich Zentrifugan, 170 Germany) to remove undissolved debris. Solubilized samples were mixed at 1:1 (v/v) ratio with the 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 20 mA/gel. After electrophoresis, the 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 a mixture of 50% (v/v) methanol and 7.5% (v/v) acetic acid for 30 min and destained again with a 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.

2.4.3. Amino acid analysis

Amino acid composition of gelatin samples was analyzed according to the method of NAGARAJAN *et al.* (2012) with a slight modification. The samples were hydrolyzed under reduced pressure in 4 M methanesulphonic acid containing 0.2% (v/v) 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).

2.4.4. Fourier transform infrared (FTIR) spectroscopic analysis

FTIR spectra of the 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 a resolution of 4 cm^{-1} and the measurement range was between 400 and 4,000 cm^{-1} (mid-IR region) at room temperature. 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).

2.4.5. Determination of gel strength

Gelatin gel was prepared by the method of KITTIPHATTANABAWON *et al.* (2010). Gelatin was dissolved in distilled water (60 °C) to obtain a final concentration of 6.67% (w/v). The solution was stirred until the gelatin was completely solubilized and then transferred to a cylindrical mold with 3 cm diameter and 2.5 cm height. The solution was incubated at the refrigerated temperature (4°C) for 18 h prior to analysis.

The gel strength was determined at 8-10°C using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kg and crosshead speed of 1 mm/s. A 1.27 cm diameter flat-faced cylindrical Teflon® plunger was used. The maximum force (grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded.

2.4.6. Determination of gelling and melting temperatures

Gelling and melting temperatures of gelatin samples were measured following the method of BORAN *et al.* (2010) using a controlled stress rheometer (RheoStress RS 75, HAAKE, Karlsruhe, Germany). The gelatin solution (6.67%, w/v) was prepared in the same manner as described previously. The solution was preheated at 35°C for 30 min. The measuring geometry included a 3.5 cm parallel plate and the gap was set at 1.0 mm. The measurement was performed at a scan rate of 0.5°C/min, frequency of 1 Hz, oscillating applied stress of 3 Pa during cooling from 35 to 5°C and heating from 5 to 35°C. The gelling and melting temperatures were calculated, where tan δ became 1 or δ was 45°.

2.4.7. Microstructure analysis of gelatin gel

The microstructure of gelatin gel 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 a serial concentration of 25%, 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.

2.4.8. Determination of color of gelatin gel

The color of gelatin gels (6.67% w/v) was measured with 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 for 10 min and calibrated with a white standard. The total difference in color (ΔE^*) was calculated according to the following equation. (GENNADIOS *et al.*, 1996):

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

where ΔL^*, Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of the white standard ($L^* = 93.6$, $a^* = -0.94$ and $b^* = 0.40$).

2.5. 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 a Duncan's multiple range test (STEEL and TORRIE, 1980). 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. Extraction yield

Yield of gelatin from the swim bladder of yellowfin tuna extracted at various temperatures was different. Increasing yield was obtained when the extraction temperatures increased ($P < 0.05$). Yield of 35.6%, 41.1% and 47.3% (on dry weight basis) was found for G60, G70, and G80, respectively. This result was in agreement with KAEWRUANG *et al.* (2013), DUAN *et al.* (2011) and KITTIPHATTANABAWON *et al.* (2010) who reported the increasing yield of gelatin as the extraction temperature increased with higher temperatures, the bondings stabilizing α-chains in the native mother collagen were destroyed to a higher extant. As a consequence, the triple helix structure became amorphous and could be extracted into the medium with ease, leading to the higher yield (SINTHUSAMRAN *et al.*, 2014). In addition, the higher energy applied could induce thermal hydrolysis of peptide chains, resulting in the formation of shorter peptides. As a result, those small peptides could be easily extracted into water. The yield and characteristics of gelatin are associated with the type of raw material and gelatin extraction process, including the pretreatment process and extraction temperatures. (NAGARAJAN *et al.*, 2012; KITTIPHATTANABAWON *et al.*, 2010; MONTERO and GÓMEZ-GUILLÉN, 2000).

3.2. Protein patterns

Protein patterns of gelatin from the swim bladder of yellowfin tuna extracted at different temperatures are shown in Fig. 1. The band intensity of α_1-chain and α_2-chain decreased with increasing extraction temperature. The decreases in α_1-chain band intensity were observed in G70 and G80, in comparison with that found in G60. Among all gelatin samples, G80 possessed the lowest α-chain band intensity. This might be caused by the degradation induced by the thermal process. Therefore, the extraction temperatures played a major role in protein components of resulting gelatin. KITTIPHATTANABAWON *et al.* (2010) reported that the gelatins extracted from the skins of brownbanded bamboo shark and blacktip shark with higher extraction temperature contained more peptides with the MW less than α-chain and the lower proportion of high MW (greater than β-chain) fractions, compared with those obtained from lower temperature extraction. Gelatins from splendid squid skin with higher extraction temperatures contained a lower band intensity of the α-chains than those obtained with lower extraction temperature (NAGA-

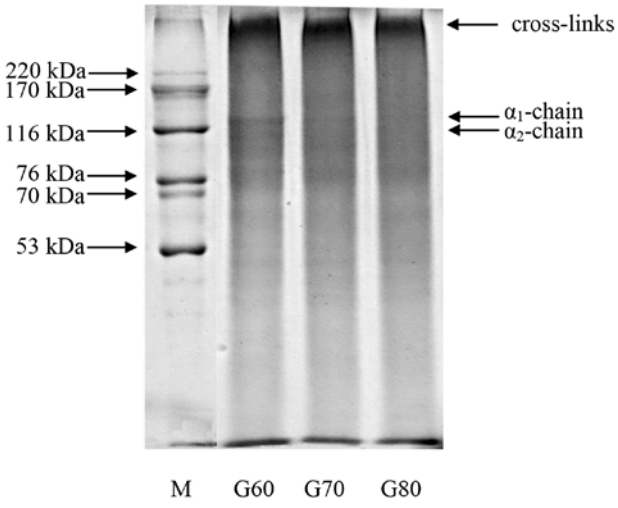

Fig. 1 - Protein patterns of gelatins from the swim bladder of yellowfin tuna extracted at different temperatures. M: high molecular weight markers. G60, G70 and G80 represent gelatin extracted from swim bladder at 60, 70 and 80°C, respectively.

RAJAN *et al.*, 2012). On the other hand, gelatin from skin of unicorn leatherjacket extracted at higher temperature (65-75°C) had α-chain retained at higher level than that extracted at lower temperature (KAEWRUANG *et al.*, 2013). This was due to the thermal inactivation of indigenous proteases in the skin of unicorn leatherjacket at high temperature. Generally, gelatins with a higher content of α-chains showed better functional properties including gelling, emulsifying and foaming properties (GÓMEZ-GUILLÉN *et al.*, 2002). In general, the formation of peptide fragments is associated with lower viscosity, low melting point, low setting point, high setting time, as well as decreased bloom strength of gelatin (MUYONGA *et al.*, 2004a). The results suggested that G70 and G80, which were extracted at higher temperatures, had the shorter chains as indicated by lower content of α-chain.

3.3. Amino acid composition

Amino acid compositions of gelatins from the swim bladder of yellowfin tuna extracted at different temperatures are shown in Table 1. Glycine was the predominant amino acid in all gelatin samples, ranging from 305 to 314 residues/1000 residues. This implied that gelatin obtained was derived from its mother collagen. Collagen consists of one-third glycine in its molecule (BALTI *et al.*, 2011). It was noted that G80 had the higher glycine content than G60 and G70. The higher glycine in G80 might be caused by free glycine, which was released to a high extent during extraction at high temperature. Alanine (121-122 residues/1000 residues) was found at high content. Alanine plays a role in viscoelastic property of gelatin (GIMÉNEZ *et al.*, 2005). Low contents of cysteine (1 residues/1000 residues), tyrosine (5-6 residues/1000 residues), histidine (7-8 residues/1000 residues) and hydroxylysine (10 residues/1000 residues) were observed in all gelatin samples. For imino acids, all gelatins contained proline and hydroxyproline contents of 95–99 and 72–74 residues/1000 residues, respectively. REGENSTEIN and ZHOU (2007) reported that glycine, alanine, proline and hydroxyproline are four of the most abundant amino acids in gelatin. The properties of gelatin are largely influenced by the amino acid composition and their molecular weight distribution (GÓMEZ-GUILLÉN *et al.*, 2009). When comparing the content of imino acids (proline and hydroxyproline), gelatin from swim bladder had the lower imino acid content than those from seabass skin (198-202 residues/1000 residues) (SINTHUSAMRAN *et al.*, 2014) and from carp skin (188-190 residues/1000 residues) (DUAN *et al.*, 2011). The imino acid content of fish collagens and gelatins correlates with the water temperature of their normal habitat (FOEGEDING *et al.*, 1996; REGENSTEIN and ZHOU, 2007). It has been known that imino acid content, especially hydroxyproline content, affected functional properties of gelatin, especially gelling property (AEWSIRI *et al.*, 2008; BENJAKUL *et al.*, 2009). Therefore, amino acid composition of gelatin from swim bladder was governed by extraction temperature.

Table 1 - Amino acid compositions of gelatins from the swim bladder of yellowfin tuna extracted at different temperatures.

Amino acids	Number of residues/1000 residues		
	G60	G70	G80
Alanine	121	121	122
Arginine	52	52	53
Aspartic acid/asparagine	49	48	46
Cysteine	1	1	1
Glutamic acid /glutamine	80	80	78
Glycine	307	305	314
Histidine	7	8	7
Isoleucine	14	14	13
Leucine	29	30	28
Lysine	26	26	26
Hydroxylysine	10	10	10
Methionine	17	16	16
Phenylalanine	16	16	16
Hydroxyproline	74	72	73
Proline	95	99	99
Serine	41	41	40
Threonine	30	30	30
Tyrosine	6	6	5
Total	1000	1000	1000
Imino acids	169	171	172

3.4. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of gelatins extracted using different temperatures are shown in Fig. 2. FTIR spectroscopy has been used as a well-established technique to monitor the functional groups and secondary structure of gelatin (KONG and YU,

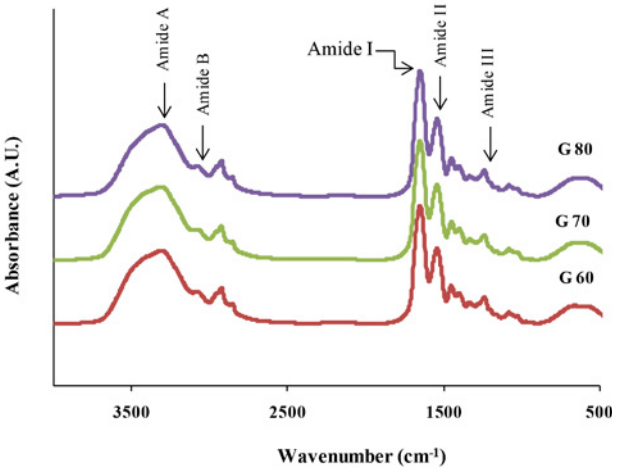

Fig. 2 - ATR-FTIR spectra of gelatins from the swim bladder of yellowfin tuna extracted at different temperatures (see Fig. 1 caption).

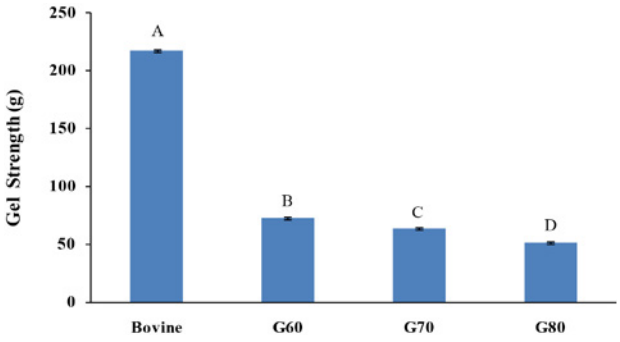

Fig. 3 - Gel strength of gelatin from the swim bladder of yellowfin tuna with different temperatures. Different uppercase letters on the bars denote significant differences (P< 0.05). Bars represent the standard deviations (n = 3). (see Fig. 1 caption).

2007). The absorption bands were situated in the amide region. The absorption in the amide-I region, owing to C=O stretching vibration, is probably the most useful for infrared spectroscopic analysis of the secondary structure of proteins (BENJAKUL *et al.*, 2009). It depends on the hydrogen bonding and the conformation of the protein structure (BENJAKUL *et al.*, 2009; URIARTE-MONTOYAETAL *et al.*, 2011). G60, G70 and G80 exhibited the amide-I bands at the wavenumbers of 1652.8, 1653.7 and 1652.9 cm^{-1}, respectively. The characteristic absorption bands of G60, G70 and G80 in amide-II region were noticeable at the wavenumbers of 1544.6, and 1545.5 and 1543.5 cm^{-1}, respectively. Amide-II arises from bending vibration of N-H groups and stretching vibrations of C-N groups. In addition, amide-III was detected at the wavenumbers of 1241.9, 1241.3 and 1240.8 cm^{-1} for G60, G70 and G80, respectively. The amide-III represents the combination peaks between C-N stretching vibrations and N-H deformation from amide linkages as well as absorptions arising from wagging vibrations from CH_2 groups from the glycine backbone and proline side-chains (JACKSON *et al.*, 1995). G80 had the lowest amplitude, whereas G60 exhibited the highest amplitude at amide-III region. This indicated that the greater disorder of molecular structure due to transformation of an α-helical to a random coil structure occurred at higher temperature. These changes were associated with loss of triple-helix state as a result of denaturation of collagen to gelatin (MUYONGA *et al.*, 2004b). The result reconfirmed the higher degradation of gelatin extracted at higher temperatures.

Amide-A band, arising from the stretching vibrations of the N-H group, appeared at 3338.3, 3339.1 and 3339.3 cm^{-1} for G60, G70 and G80, respectively. Amide-A represents NH-stretching coupled with hydrogen bonding. Normally, a free N-H stretching vibration is found in the range of 3400-3440 cm^{-1} (MUYONGA *et al.*, 2004b). When the N-H of a peptide is involved in a hydrogen bond, the position shifts to lower frequencies (DOYLE *et al.* 1975). In amide-A region, the lower wavenumber was found in G60, suggesting the hydrogen bonding involvement of N-H in α-chain. On the other hand, the lower wavenumber with the concomitantly higher amplitude of amide-A observed in G80 could be associated with the higher degradation of gelatin and higher free amino groups. The amide B was observed at 3082.1, 3080.9 and 3081.8 cm^{-1} for G60, G70 and G80, respectively. Amide B corresponds to asymmetric stretch vibration of =C-H as well as $-NH_3^+$. Thus, the secondary structure of gelatins obtained from the swim bladder of yellowfin tuna was affected to some degree by extraction temperature.

3.5. Gel strength

Gel strength of gelatin from the swim bladder of yellowfin tuna extracted at different temperatures is presented in Fig 3. G60, G70 and G80 had the gel strength of 72, 64 and 51 g, respectively. The difference in gel strength between the samples could be due to the differences in intrinsic characteristics, especially molecular weight distribution. Protein degradation resulted in the formation of peptides with shorter chain length, which might show the lower ability to from the junction zone or anneal each other. The longer chains in G60 could undergo aggregation to form gel network more effectively than G70 and G80. As a result, a stronger gel network could be formed as indicated by the higher gel strength. Bloom strength of commercial gelatins ranges from 100 to 300, but gelatins with bloom values of 250-260 are

Table 2 - Gelling and melting temperatures and gel color of gelatin from the swim bladder of yellowfin tuna extracted at different temperatures.

Samples	Melting point	Gelling point	Colour			
	(C°)	(C°)	*L**	*a**	*b**	Δ*E**
G60	22.33±0.42^{A}	15.24±0.27^{A}	27.98±0.57^{C}	-2.07±0.02^{C}	8.21±0.11^{C}	66.09±0.57^{A}
G70	22.05±0.45^{A}	14.86±0.24^{A}	42.79±0.47^{B}	-0.76±0.10^{B}	16.79±0.24^{B}	53.39±0.42^{B}
G80	20.36±0.27^{B}	11.07±0.58^{B}	45.79±0.78^{A}	-0.34±0.05^{A}	19.03±0.20^{A}	51.32±0.80^{C}

Mean ±SD (*n* = 3).
Different uppercase superscripts in the same column indicate significant differences (P < 0.05).

the most desirable (HOLZER, 1996). Different gel strength was reported for gelatin from skin of different species including splendid squid (85–132 g) (NAGARAJAN *et al.*, 2012), brownbanded bamboo shark and blacktip shark (206–214 g) (KITTIPHATTANABAWON *et al.*, 2010) and bigeye snapper (108 g) (BINSIA *et al.*, 2009).

3.6. Gelling and melting temperatures

The gelling temperatures of all the gelatin samples were in the range of 11.07-15.24°C (Table 2). Thermal transitions were monitored by changes in the phase angle (δ) of dissolved gelatins during cooling (35-5°C) and subsequent heating (5-35°C). It was found that G80 had the lowest gelling point (11.07°C) ($P < 0.05$), while no difference in gelling point were observed between G60 and G70 ($P > 0.05$). In general, fish gelatin is not able to form gel at room temperature (NORLAND, 1990). It has been known that imino acid content is an essential factor governing gelation of getatin (GILSENAN and ROSS-MURPHY, 2000). However, the similar amino acid content was observed among all samples (169-172 residues/1000 residues). The result indicated that the gelling temperature was affected by the extraction temperature, more likely related with varying chain length.

As a thermoreversible gel, gelatin gel starts melting when the temperature increases above a certain point, which is called the gel melting point (KARIM and BHAT, 2009). The melting temperatures of gelatin gel from swim bladder were in the range of 20.36-22.33°C. G80 had the lowest melting point (20.36°C) ($P < 0.05$). Nevertheless, G60 and G70 showed similar melting points ($P > 0.05$). Typical melting points for fish gelatins ranged from 11 to 28°C (KARIM and BHAT, 2009). GÓMEZ-GUILLÉN *et al.* (2002) reported that melting points of cod, hake, sole and megrim were 13.8, 14, 19.4 and 18.8°C, respectively. Melting points of red and black tilapia skin gelatins were 22.4 and 28.9°C, respectively (JAMILAH and HARVINDER, 2002). There was a relationship between melting point and molecular weight of gelatin. Low molecular weight gelatins melt at lower temperature than high molecular weight counterparts (GILSENAN and ROSS-MURPHY, 2000). The results suggested that lower melting point of G80 was attributed to the lower molecular weight of peptide chains. Temperature of the environment also affects the gelling and melting temperatures of gelatin (GUDMUNDSSON, 2002). Poorer gel strength of G80 (Fig. 3) was in accordance with lower gelling and melting points.

3.7. Microstructures of gelatin gels

The microstructures of gelatin gels from swim bladder with different extraction temperatures are illustrated in Fig. 4. In general, the conformation

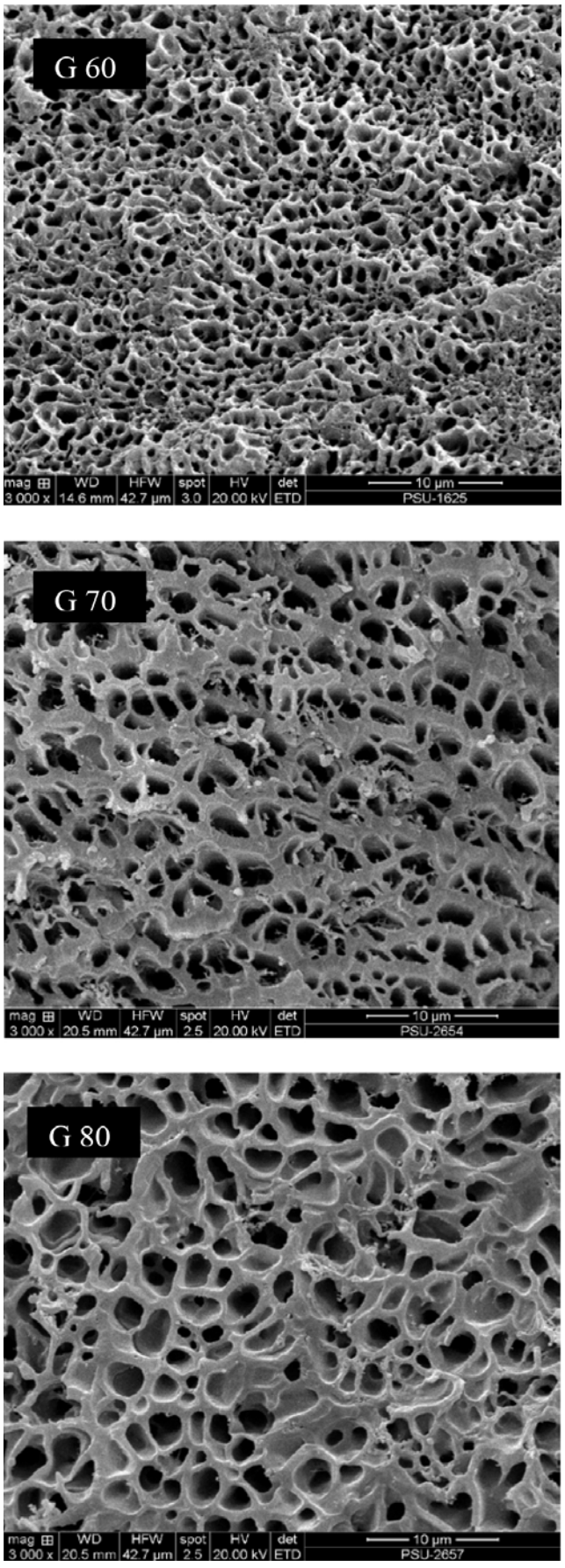

Fig. 4 - Microstructures of gelatin gel from the swim bladder of yellowfin tuna extracted at different temperatures. Magnification: 3000 (see Fig. 1 caption).

and chain length of the proteins in gel matrix directly contributed to the gel strength of gelatin (BENJAKUL *et al.*, 2009). Gelatin extracted at 60°C showed the finest gel network with small voids. Conversely, the coarser networks with the larger voids were found in gel of the gelatin extracted at higher temperatures. The fine gel structure of gelatin extracted at lower temperature was in accordance with the higher gel strength (Fig. 3). It has been known that the microstructure of the gel is related to the physical properties. The gelatin gel network was governed by the pretreatment conditions (YANG *et al.*, 2008) and gelatin concentration (YANG and WANG, 2009). Gelatin extracted at lower temperatures had the lower degradation, in which proteins with higher chain length were present. As a result, junction zones could be formed to a greater extent. This led to the high aggregation with a strong and ordered network. In the first stage of gel network formation, there is competition between intramolecular folding and intermolecular aggregate formation (YANG and WANG, 2009). For gelatin extracted at lower temperature, longer chains might undergo aggregation to a higher extent. Thus, the arrangement of peptides in the network during gelation as determined by chain length directly affected gel properties of gelatin.

3.8. Color

Color of the gelatin gel from swim bladder with different extraction temperatures expressed as *L**, *a** and *b** is shown in Table 2. Gel of gelatin extracted at lower temperatures (G60) showed the lower *L**-value (lightness) than others (G70 and G80) ($P < 0.05$). The higher redness (*a**-value) and yellowness (*b**-value) were found in the latters ($P < 0.05$). Generally, the increases in *L**, *a** and *b**-value of gelatin increased with increasing extraction temperatures. For yellowness (*b**-value), an increase was observed in all gelatin gels when the extraction temperatures increased ($P < 0.05$). This might be due to a non-enzymatic browning reaction taken place at the higher temperature, especially when the extraction time increased (AJANDOUZ and PUIGSERVER, 1999). Among all the gelatin samples, those extracted at a lower temperature (60°C) showed the highest total difference in the color value (ΔE^*) (66.09) with the lowest lightness (*L**-values). These results showed that the extraction temperatures had the impact on color of gelatin extracted from the swim bladder of yellowfin tuna.

4. CONCLUSION

Swim bladder from yellowfin tuna could be an alternative source of gelatin. Gelatin extracted at a higher temperature had the highest extraction yield, but possessed the poorer gel properties. Extraction conditions also affected the color of resulting gelatin. The appropriate extraction temperature for gelatin from swim bladder was 60 °C, providing the highest gel strength.

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