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Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*

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ABSTRACT

Gelatins extracted from the skins containing fine scales of two species of bigeye snapper, *Priacanthus tayenus* (GT) and *Priacanthus macracanthus* (GM), were characterised. Both gelatins had the protein as the major component with high content of imino acids (proline & hydroxyproline) (186.29–187.42 mg/g). GT and GM contained calcium at levels of 6.53 and 2.92 g/kg, respectively. Both gelatins contained α1 and α2 chains as the predominant components and some degradation peptides. The absorption bands of both gelatins in Fourier transform infrared (FTIR) spectra were mainly situated in the amide band region (amide I and amide II). GT and GM had a relative solubility greater than 90% in the wide pH ranges (1–10). The bloom strength of GM (254.10 g) was higher than that of GT (227.73 g) ($P < 0.05$), but was slightly lower than that of commercial bovine gelatin (293.22 g) ($P < 0.05$). Finer gel structure with smaller strands and voids was observed in GM gel, in comparison with that observed in GT counterpart.

1. Introduction

Gelatin is an irreversibly hydrolysed form of collagen by thermal hydrolysis (Foegeding, Lanier, & Hultin, 1996). In the food industry, gelatin has been widely applied as an ingredient to improve the elasticity, consistency and stability of foods (OMRI, 2002). Depending on the method in which collagens are pre-treated, two different types of gelatin with different characteristics including type-A, acid-treated collagen, and type-B, an alkaline treated counterpart, can be produced (Johnston-Banks, 1990). Acid treatment is the most suitable treatment for less fully cross-linked collagens commonly found in pig or fish skins, whereas alkaline treatment is appropriate for the more complex collagens found in bovine hides (Foegeding et al., 1996; Gómez-Guillén et al., 2002).

Bovine and porcine skin and bone have usually been utilised commercially for gelatin production (Veis, 1964; Ward & Courts, 1977). However, in some countries, the use of gelatin from warm-blooded animals is restricted owing to the transmission of bovine spongiform encephalopathy and religious reasons (Gilsenan & Ross-Murphy, 2001). Therefore, there is increasing interest in the production of fish gelatin, taking advantage of the byproducts from the fish processing industry. (Gómez-Guillén et al., 2002; Muyonga, Cole, & Duodu, 2004a). Fish gelatin has been produced from black tilapia and red tilapia skins (Jamilah & Harvinder, 2002), Nile perch skin and bone (Muyonga et al., 2004a) and sin croaker and shortfin scad skins (Cheow, Norizah, Kyaw, & Howell, 2007). The quality of gelatin for a particular application is greatly influenced by the sources of raw material and species, and also by the severity of the manufacturing method (Johnston-Banks, 1990).

Bigeye snapper (*Priacanthus* spp.) has become an important fish for surimi production in Thailand due to its good gel forming ability (Benjakul, Visessanguan, Riebroy, Ishizaki, & Tanaka, 2002). During surimi processing, a large amount of the skin of these species is produced as the low value byproduct, commonly used as animal feeds. Conversion of those remainders into a value added product can pave the way for full utilisation of limited fishery resources. Jongjareonrak, Benjakul, Visessanguan, and Tanaka (2006) reported that skins of two species of bigeye snapper (*Priacanthus tayenus* and *Priacanthus macracanthus*) were an excellent source of type I collagen. Thus, skin of those two species can be a promising raw material for gelatin production. Genetic differences may be associated with the differences in properties of gelatin extracted from both species. Therefore, the aims of this investigation were to extract and characterise the gelatins from skin of two species of bigeye snapper, *P. tayenus* and *P. macracanthus*.

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2. Materials and methods

2.1. Chemicals

ß-mercaptoethanol (ßME), bovine serum albumin and protein markers were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium dodecyl sulphate (SDS), Coomassie Blue R-250 and *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). Type I collagen from calf skin was purchased from Elastin Products Co., Inc. (Owensville, MO, USA). Food grade bovine gelatin was obtained from Halagel Co., Ltd. (Bangkok, Thailand).

2.2. Fish skin preparation

Bigeye snappers (*P. tayenus* and *P. macracanthus*) were caught from the Songkhla coast along the Gulf of Thailand, stored in ice and off-loaded after 24–36 h of capture. Upon arrival to the dock in Songkhla, fish were stored in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai. Fish were washed with tap water. Skins containing fine scale were then removed and cut into small pieces (0.5 × 0.5 cm). Prepared skins were kept on ice prior to gelatin extraction.

2.3. Extraction of fish skin gelatin

To extract gelatin, skins were soaked in 0.025 M NaOH with a skin/solution ratio of 1:10 (w/v). The mixture was stirred for 2 h at room temperature (about 25–28 °C). The alkaline solution was changed every hour to remove non-collagenous proteins and pigments. Alkaline-treated skins were then washed with tap water until neutral or faintly basic pHs of wash water were obtained. The skins were then soaked in 0.2 M acetic acid with a skin/solution ratio of 1:10 (w/v) for 2 h with gentle stirring. The acid solution was changed every 40 min to swell the collagenous material in the fish skin matrix. Acid-treated skins were washed thoroughly as previously described. After swelling, the swollen fish skins were soaked in distilled water (45 °C) with a skin/water ratio of 1:10 (w/v) in a water bath (W350, Memmert, Schwabach, Germany) for 12 h with a continuous stirring using an overhead stirrer (RW 20.n, IKA labortechnik, Germany) at a speed of 150 rpm to extract the gelatin from skin matter. The mixture was then filtered using two layers of cheesecloth. The resultant filtrate was freeze-dried and the dry matter was referred to as "gelatin powder".

2.4. Analyses

2.4.1. Proximate analysis

Moisture, protein, fat and ash contents of gelatin powder from both skins were determined according to the methods of (AOAC, 2000) with the method numbers of 950.46, 928.08, 960.39 and 920.153, respectively.

2.4.2. Determination of minerals

Calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K) and sodium (Na) contents were determined by an inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Perkin–Elmer, Model 4300 DV, Norwalk, CT) according to the AOAC (2000) method. Gelatin samples were mixed well with 4 ml of nitric acid. The mixture was heated on a hot plate until digestion was complete. The digested sample was transferred to a volumetric flask and the volume was made up to 10 ml with deionised water. The solution was subjected to ICP-OES analysis. Flow rates of argon to plasma, auxiliary and nebuliser were kept at 15, 0.2, and 0.8 l/min, respectively. Sample flow rate was set at 1.5 ml/min. The wavelengths for analysis of Ca, Mg, Na, P and K were 317.933, 285.213, 588.995, 213.617 and 766.490 nm, respectively.

2.4.3. Determination of amino acid composition

Gelatin samples were hydrolysed under reduced pressure in 4 M methanesulfonic acid containing 0.2% (v/v) 3-2(2-aminoethyl) indole at 115 °C for 24 h and the hydrolysates were neutralised with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). An aliquot of 0.4 ml was applied to an amino acid analyser (MLC-703; Atto Co., Tokyo, Japan).

2.4.4. Electrophoretic analysis

SDS-polyacrylamide gel electrophoresis (SDS–PAGE) was performed by the method of Laemmli (1970). Gelatin and swollen skin samples were dissolved in 0.02 M sodium phosphate containing 1% SDS and 3.5 M urea (pH 7.2). The mixtures were centrifuged at 8500*g* for 5 min at room temperature using microcentrifuge (MIKRO20, Hettich Zentrifugan, Germany) to remove undissolved debris. Solubilised samples were mixed at a 1:1 (v/v) ratio with the sample buffer (0.5 M Tris-HCl, pH 6.8, containing 4% SDS and 20% glycerol) in the presence or absence of 10% ßME, representing reducing or non-reducing conditions, respectively. Samples (15 μg protein) were loaded onto polyacrylamide gels comprising a 7.5% running gel and a 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA/gel using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After electrophoresis, the gel was stained with 0.05% (w/v) Coomassie Blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% (v/v) acetic acid. High molecular weight markers (Sigma Chemical Co., St. Louis, MO, USA) were used to estimate the molecular weight of the proteins. Type I collagen from calf skin was used as a standard collagen.

2.4.5. Fourier transform infrared (FTIR) spectra analysis

FTIR spectra of gelatin samples were recorded using a horizontal ATR Trough plate crystal cell (45° ZnSe; 80 mm long, 10 mm wide and 4 mm thick) (PIKE Technology Inc., Madison, WI) equipped with a Bruker Model Equinox 55 FTIR spectrometer (Bruker Co., Ettlingen, Germany) at room temperature. For spectra analysis, gelatin samples were placed onto the crystal cell and the cell was clamped into the mount of the FTIR spectrometer. The spectra in the range of 400–4000 cm^{-1} with automatic signal gain were collected in 16 scans at a resolution of 4 cm^{-1} and were rationed against a background spectrum recorded from the clean empty cell at 25 °C.

2.4.6. Determination of solubility of skin gelatin

The effect of pHs on gelatin solubility was determined by the method of Montero, Jimenez-Colmenero, and Borderias (1991) with a slight modification. The gelatins were dissolved in distilled water at 60 °C to obtain a final concentration of 2% (w/v) and the mixture was stirred at room temperature until the gelatin was completely solubilised. The gelatin solution was adjusted to different pHs (1–10) with either 6 N NaOH or 6 N HCl. The volume of solution was made up to 10 ml with distilled water, previously adjusted to the same pH of gelatin solution. The solution was centrifuged at 8500*g* at room temperature for 10 min. Protein content in the supernatant was determined by the Biuret method (Robinson & Hodgen, 1940) using bovine serum albumin as a standard. Relative solubility was calculated in comparison with that obtained at the pH giving the highest solubility.

2.4.7. Determination of bloom strength

Gelatin gels were prepared by the method of Fernández-Díaz, Montero, and Gómez-Guillén (2001) with a slight modification.

Gelatin was dissolved with distilled water at 60 °C to obtain the final concentration of 6.67% (w/v). The solution was stirred until the gelatin was solubilised completely and cooled in a refrigerator at 10 °C for 16–18 h for gel maturation. The dimensions of the sample were 3 cm in diameter and 2.5 cm in height. The bloom strength of the gelatin gels at 10 °C was determined using a Model TA-XT2 Texture Analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kN and equipped with a 1.27 cm diameter flat-faced cylindrical Teflon® plunger. The maximum force (in grams), when the penetration distance of 4 mm was obtained, was recorded. The speed of the plunger was 0.5 mm/s.

2.4.8. Scanning electron microscopy

The microstructure of gelatin gels was determined using scanning electron microscopy (SEM). Gelatin gels from bigeye snapper skins and from bovine bone having a thickness of 2–3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol with a serial concentration 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, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 10 kV.

2.4.9. Colour measurement

The colour of gelatin gels (6.67% w/v) were measured by a Hunter lab colour metre (ColorFlex, HunterLab Reston, USA) and reported by the CIE system. L^*, a^* and b^* parameters indicate lightness, redness/greenness and yellowness/blueness, respectively.

2.5. Statistical analysis

The experiments were run in triplicate using three different batches of skins. The analyses were performed in triplicate ($n = 3$), except for the determination of minerals and amino acid composition, in which 2 batches for each gelatin sample were randomly selected and analysed ($n = 2$). For a pair comparison, *t*-test was used (Steel & 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. Proximate composition

Proximate compositions of gelatins extracted from the skins of *P. tayenus* and *P. macracanthus* with the yields of 7.93% and 6.41%, respectively, are shown in Table 1. Gelatin from the skin of *P. tayenus* (GT) and *P. macracanthus* (GM) had the different composition. Both GT and GM contained protein as the major component (93.66% and 97.34%). Both gelatins had low fat content (0.61–0.64%). Although the fat content in the skins used for gelatin extraction was quite low (0.42–0.43%), it could be leached out during extraction, thereby being contaminated in the resulting gelatin.

Ash contents of GT and GM (6.16% and 2.50%) were higher than those of gelatin from other species including brownstripe red snapper skin gelatin (1.9%) (Jongjareonrak et al., 2006), sin croaker skin gelatin (1.49%) and shortfin scad skin gelatin (1.15%) (Cheow et al., 2007). In this study, skins of bigeye snapper without descaling were used for gelatin extraction. Those fine scales, which attached with skin tightly and could not be removed with ease manually, most likely served as the major source of soluble inorganic substances. Ash content in skin was in the range of 15.32–15.84%. It was noted that GT had a higher ash content than did GM ($P < 0.05$). This might be due to the differences in complexity of minerals in the scale, in which the minerals could be leached out during gelatin extraction to varying degrees. To obtain the gelatin with the lower ash content, the appropriate demineralisation of bigeye snapper skin should be accomplished prior to gelatin extraction. However, those minerals in gelatin could be of health benefit or probably had an influence on the functional properties of gelatin, especially gelation.

Both GT and GM contained calcium as the major mineral with contents of 653 and 292 mg/100 g, respectively (Table 1). Moreover, GT had the higher contents of potassium, sodium, magnesium and phosphorus. Those minerals were most likely from the scale attached to the skin, which could be co-solublised with gelatin. Fish scale contains calcium-deficient hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) (Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003). The high calcium, potassium, sodium and phosphorus contents in both GT and GM were in agreement with the high ash content in both gelatins.

3.2. Amino acid composition of skin gelatin

Amino acid compositions of both skin gelatins are presented in Table 2. Slight differences in the amino acid composition between skin gelatins of two species were observed. GT and GM were rich in glycine (246.57 and 259.38 mg/g sample). Glycine is located at every third position of the triple helix of collagen (Wong, 1989). Slightly lower glycine content was observed in comparison with

Table 1
Chemical composition of gelatin from skins of bigeye snapper.

Compositions	Source of gelatin	
	P. tayenus skin	*P. macracanthus* skin
Moisture*	9.62 ± 0.48[a]	8.34 ± 1.19[b]
Protein*	93.66 ± 1.25[b]	97.34 ± 0.62[a]
Fat*	0.64 ± 0.12[a]	0.61 ± 0.09[a]
Ash*	6.16 ± 0.46[a]	2.50 ± 0.61[b]
Ca**	653	292
Mg**	57	17
K**	256	86
Na**	375	124
P**	308	167

Different superscripts in the same row indicate the significant differences ($P < 0.05$).
* Mean ± SD ($n = 3$), expressed as% (wet weight).
** $n = 2$, expressed as mg/100 g.

Table 2
Amino acid composition of gelatins from the skins of bigeye snapper.

Amino acid (mg/g sample)*	Source of gelatin	
	P. tayenus skin	*P. macracanthus* skin
Thr	25.03	26.08
Ser	29.80	31.85
Glu	92.94	95.34
Gly	246.57	259.38
Ala	49.04	108.31
Val	17.82	19.06
Met	20.04	20.55
Ile	10.30	7.16
Leu	22.32	23.60
Tyr	6.07	6.11
Phe	20.01	20.62
Hylys	7.37	6.52
His	4.46	4.36
Lys	35.02	36.40
Arg	87.36	91.76
Hypro	87.75	90.86
Pro	98.54	96.56

* $n = 2$.

the theoretical value, possibly due to the contamination of other proteins from skins during the extraction. GT and GM contained the high content of imino acids, including proline (98.54 and 96.56 mg/g sample) and hydroxyproline (87.75 and 90.86 mg/g sample). The stability of the triple helical structure in renatured gelatins was associated with the total content of pyrrolidine amino acids (proline and hydroxyproline) (Ledward, 1986). Hydroxyproline also plays an essential role in the stabilisation of the triple helix strands of mother collagen *via* its hydrogen bonding ability through its —OH group (Burjandze, 1979; Ledward, 1986). Gelatin, with a higher content of hydroxyproline, is believed to have higher viscoelastic properties and an ability to develop the strong gel structure (Gómez-Guillén et al., 2002). Gelatins of both species also comprised a high content of glutamic acid, glutamine and arginine. Lysine was also found in the range of 35.02–36.40 mg/g sample. However, a low content of hydroxylysine was noticeable. Hydroxylysine could undergo cross-linking, contributing to the tough skin of these species by responsibility for the triple helix stabilisation (Burjandze, 1979; Ledward, 1986). In addition, hydroxylysine-linked carbohydrates may have an impact on the structure of the fibrils in the invertebrate collagen (Sikorski, KolaKoska, & Pan, 1990). Low contents of histidine, tyrosine and isoleucine were obtained in both gelatins.

3.3. Protein patterns of skin gelatins

SDS–PAGE patterns of GT and GM under reducing and non-reducing conditions are shown in Fig. 1. Both gelatins contained α1 and α2-chains as the major constituents and were characterised to be type I without a disulfide bond. Different protein patterns were noticeable between swollen skin and extracted gelatins. The β-component was dominant in swollen skin. The larger band intensity of the ß-chain was found in the swollen skin of *P. macracanthus*, compared with that of *P. tayenus*. Both swollen skins also contained α1 and α2-chains. Due to the higher molecular weight of the ß-chain, dimer of α-chain in skin, it could not be extracted effectively as evidenced by the lower band intensity in the resulting gelatin. Apart from the ß-chain, the proteins with larger molecular weights were also observed in swollen skin. Nevertheless, a low content of those components was found in the gelatin. The proteins or peptides with a molecular weight lower than the α-chain were also found in GT and GM, while they were not present in the swollen skin from both species. This might be caused by the degradation induced by the thermal process or by endogenous proteinases during gelatin preparation. Intarasirisawat et al. (2007) reported that skin of *P. macracanthus* contained endogenous serine and metallo proteinases, which caused autolysis of skin at 50–60 °C. In gelatin manufacture, the conversion of collagen to gelatin yields the molecules of varying mass, due to the cleavage of inter-chain chemical cross-links and some unfavourable breakages of inter-chain peptide linkage (Zhou, Mulvaney, & Regenstein, 2006). Muyonga et al. (2004a) revealed that Nile perch skin gelatins contained low molecular weight peptides, especially, gelatins extracted with higher temperature. Apart from ß-chain and α-chains, other protein bands might represent the contaminating proteins in gelatin, suggesting incomplete removal of non-collagenous proteins prior to extraction. The presence of non-collagenous proteins more likely coincided with the lower glycine content found in both gelatins, GT and GM (Table 2).

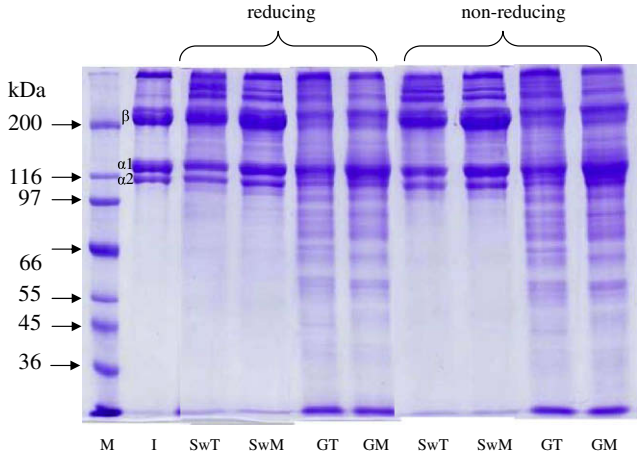

Fig. 1. SDS–PAGE patterns of gelatin from *P. tayenus* skin (GT) and *P. macracanthus* skin (GM) under reducing and non-reducing conditions. M, I, SwT and SwM denote high molecular weight protein markers, type I collagen, swollen skin from *P. tayenus* and swollen skin from *P. macracanthus*, respectively.

3.4. Fourier transform infrared (FTIR) spectra of skin gelatins

The FTIR spectra of GT (A) and GM (B) are depicted in Fig. 2. Generally, both gelatins showed similar spectra, in which absorption bands were situated in the amide band region. Amide I and amide II bands of gelatins from both bigeye snapper skins appeared at around 1630 and 1545 cm^{-1}, respectively. Amide I and amide II bands of gelatins at 1700–1600 and 1560–1500 cm^{-1} were reported by Muyonga, Cole, and Duodu (2004b) and Yakimets et al. (2005). The amide I vibration mode is primarily a C=O stretching vibration coupled to contributions from the CN stretch, CCN deformation and in-plane NH bending modes (Bandekar, 1992; Lavialle, Adams, & Levin, 1982). The absorption in the amide I region is the most useful for infrared spectroscopic analysis of the secondary structure of proteins (Surewicz & Mantsch, 1988). Yakimets et al. (2005) reported that the absorption peak at 1633 cm^{-1} was characteristic for the coil structure of gelatin. The amide II vibration mode is attributed to an out-of-phase combination of CN stretch and in-plane NH deformation modes of the peptide group (Bandekar, 1992; Lavialle et al., 1982). The amide II band is generally considered to be much more sensitive to hydration than to secondary structure change (Wellner, Belton, & Tatham, 1996). Small amide III bands of GT and GM were observed at 1237 cm^{-1} which indicated the disorder in gelatin molecules and were associated with loss of triple helix state (Friess & Lee, 1996). The band at 1079 cm^{-1} in GM was lower than that of GT, indicating that both gelatins showed slight differences in their carbonyl group, which can be attributed to the CO vibration between 1000 and 1100 cm^{-1} (Jackson, Choo, Watson, Halliday, & Mantsch, 1995).

3.5. Solubility

The solubility of GT was similar to that of GM and a high solubility of both gelatins was observed in the wide pH range (1–10). Generally, relative solubility of both gelatins was greater than 90% at all pHs tested (data not shown). GT and GM showed the lowest solubility at pH 8.0. Normally, gelatin type-A and acid-processed gelatin have isoelectric points varying from 6.5 to 9.0 (Foegeding et al., 1996; Johnston-Banks, 1990). During acid pretreatment of skin, some glutamine and asparagine can be converted to their acidic forms, i.e. glutamic acid and aspartic acid, respectively (Jamilah & Harvinder, 2002). Bovine gelatin (GB) had the lowest solubility at pH 5. The difference in solubility of different gelatins might result from the differences in molecular weight and the content of polar and non-polar groups in amino acids (Zayas, 1997). Due to high solubility of both gelatins at pH 1–10, they can be used widely and effectively since solubility is a prerequisite for most functionalities of food proteins.

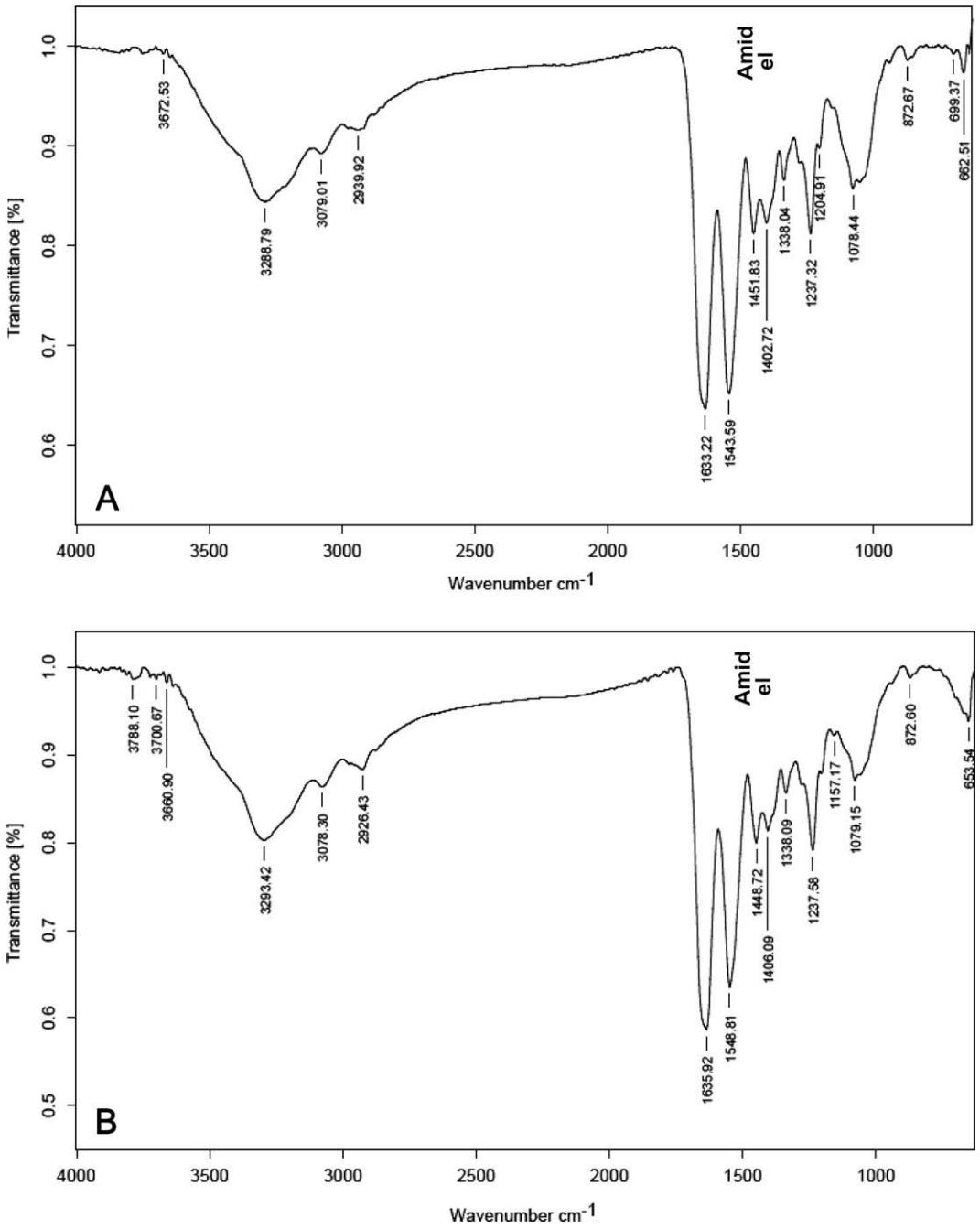

Fig. 2. Fourier transform infrared (FTIR) spectra of gelatins from *P. tayenus* skin (A) and *P. macracanthus* skin (B).

3.6. Properties of gel

GM showed the higher bloom strength (254.10 g) than did GT (227.73 g) ($P < 0.05$). However, both GM and GT had the lower bloom strength than GB did (293.22 g) ($P < 0.05$) (Table 3). This was most likely governed by the differences in the imino acid content. Hydroxyproline contents of GT and GM were about 9–10% of the total amino acids (Table 2), while that of GB was 14% (Nalinanon, Benjakul, Visessanguan, & Kishimura, 2008). The gelling properties of gelatin were influenced by the source of raw materials, which vary in proline and hydroxyproline contents (Jongjareonrak et al., 2006). The main structural difference between fish and mammalian gelatins is the imino acid content, where the mammalian gelatins have the highest amount (Gudmundsson, 2002). Imino acids played a role in gel formation. Hydroxyl groups of hydroxyproline play a part in the stability of the helix by inter-chain hydrogen bonding *via* a bridging water molecule as well as direct hydrogen bonding to the carbonyl group (Wong, 1989). Additionally, the lower bloom strength of GT was associated with the greater degradation of GT, compared with GM (Fig. 1). The result suggested that skin of *P. tayenus* possibly contained a greater amount of endogenous proteases, which were active during the gelatin extraction at 60 °C. Gelatin, with the lower chain length, could not form the strong gel due to the lower inter-junction zones. Intarasirisawat et al. (2007) reported that the autolysis of skin from *P. macracanthus* and the highest activity was found at 60 °C. Bloom strength of gelatin gels from two species of bigeye snapper was higher than that of gels from other fish skin

Table 3
Bloom strength and colour of gels from the skins of bigeye snapper in comparison with commercial bovine skin.

		Source of gelatin		
		Bovine bone	*P. tayenus* skin	*P. macracanthus* skin
Bloom strength (g)		293.22 ± 5.71*,a	227.73 ± 6.20c	254.10 ± 11.13b
Colour	L*	35.85 ± 2.44a	29.71 ± 0.81b	28.55 ± 0.88b
	a*	−0.77 ± 0.14a	−1.61 ± 0.10b	−1.33 ± 0.21b
	b*	18.29 ± 1.96a	−3.22 ± 0.25b	−3.90 ± 0.32b

Different letters in the same row indicate the significant differences ($P < 0.05$).
* Mean ± SD ($n = 3$).

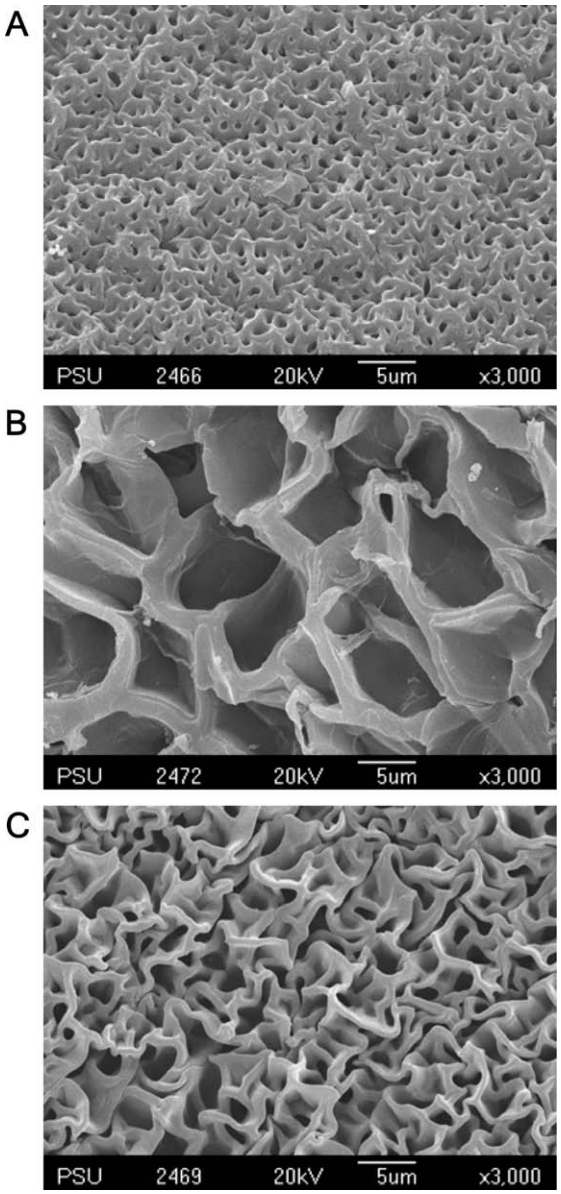

Fig. 3. Microstructure of gelatin gels from bovine bone (A), *P. tayenus* skin (B) and *P. macracanthus* skin (C). Magnification: 3000×.

gelatins previously reported including Alaska pollock gelatin (98 g) (Zhou et al., 2006) and sin croaker gelatin (124.94 g) (Cheow et al., 2007). It was noted that divalent cations including calcium and magnesium ions in gelatins might contribute to the stronger gel *via* the enhancement of cross-linking by divalent bridges between gelatin molecules.

Higher L*-values of GB gel were observed, compared with GT and GM ($P < 0.05$) (Table 3). Nevertheless, no differences in L*-value were found between GT and GM ($P > 0.05$). Gelatin manufacture generally has a good process to clarify the impurities from the gelatin solution, such as chemical clarification and filtration processes (Ward & Courts, 1977). Higher redness (a*-value) and yellowness (b*-value) were found in GB, when compared with both GT and GM ($P < 0.05$). The result indicated that the gelatin gel from bigeye snapper skins had lower yellowness and redness than the commercial gelatin. Pigments from bigeye snapper skin could be removed effectively during pretreatment prior to gelatin extraction. However, the colour did not affect functional properties of gelatin (Ockerman & Hansen, 1988).

3.7. Microstructures of gel

Microstructures of all gels including GM, GT and GB are illustrated in Fig. 3. In general, GB exhibited the finest gel network with very small voids. When comparing between GM and GT, the former showed the finer structure with smaller protein strands and voids. The finer structure of GM was in accordance with the higher bloom strength (Table 3), compared with GT, which possessed the coarser gel structure. The coarser gel network most likely had the lower bloom strength and was easy to disrupt by the force applied. Therefore, the arrangement and association of protein molecules in the gel matrix directly contributed to gel strength of gelatin extracted from bigeye snapper skin.

4. Conclusion

Gelatin extracted from the skin with attached scales of two species of bigeye snapper, *P. tayenus* and *P. macracanthus* contained α1 and α2-chains as the major component. Both gelatins contained protein as the major component with different mineral compositions. It was solubilised in wide pH ranges. The bloom strength of both gelatins from bigeye snapper skins was slightly lower than that of food grade gelatin from bovine bone, but was superior to gelatin from skin of other fish species previously reported. Gelatins from bigeye snapper were less yellowish and reddish in colour. Thus gelatin can be successfully extracted from bigeye snapper skin containing scales with an acceptable property.

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