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Extraction and characterization of gelatin from different fresh water fishes as alternative sources of gelatin

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Abstract

Gelatins from the skin of four different species of fresh water fish, namely pangas catfish (*Pangasius pangasius*), Asian redtail catfish (*Hemibagrus nemurus*), striped snakehead (*Channa Striata*), and Nile tilapia (*Oreochromis niloticus*) have been successfully extracted by citric acid. The gelatin from pangas catfish was found to possessed the highest rheology properties compared to the others. It had the following properties: gel strength of 273.58 g, viscosity of 36.5 cP, melting point at 32°C, gelling temperature at 12°C, melting temperature at 29°C and total amino acid content of 754.47 mg/g. The gelatin from fresh water fish had lower physicochemical and rheological properties compared to the commercial gelatin, though total amino acid were 699.86 mg/g for pangas catfish and 734.94 mg/g for striped snakehead, respectively. The fishes investigated in this study were potential alternative sources of gelatin.

Introduction

Gelatin is obtained through hydrolysis of collagen, which is the principal protein found in skin and bones. It is an ingredient widely used in food industry, pharmaceutical, medical, cosmetic and photographic industries due to its unique functional and technological properties (Karim and Bhat, 2009). Recent reports indicate that the annual world output of gelatin is increasing, especially in Asia, and it is mostly obtained from pig and cow skins and bones (Gomez-Guillen and Montero, 2001; GME, 2008). However, the use of gelatin from those resources is restricted due to the outbreaks of bovine spongiform encephalopathy (BSE) or "mad cow disease" and religious reasons. Therefore, there is an increasing interest in the production of fish gelatin as an alternative for mammalian counterpart (Gudmundsson *et al*., 2002). In recent years, extraction and characteristic of gelatin properties has been reported from various sources such as the skins of black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*), nile pech (*Lates niloticus*) skin and bone gelatin (Muyonga *et al*., 2004), sin croaker dan shortfin scad (Cheow *et al*., 2007), "kerapu" (*Epinephelus sexfasciatus*), "jenahak" (*Lutjianus argentimaculatus*), "kembung" (*Rastrelliger kanagurta*), and "kerisi" (*Pristipomodes typus*) (Irwandi *et al*., 2009), carp (*Cyprinus carpio*) (Duan *et al*., 2011), catfish (Liu *et al*., 2008) and red tilapia (*Oreochromis nilotica*), walking catfish (*Clarias batrachus*) and striped catfish (*Pangasius sutchi fowler*) (Jamilah *et al*., 2011).

The major physical properties of gelatin are gel strength and melting point, which are governed mainly by the amino acid composition (pro + hyp content), molecular weight distribution and also the ratio of α/β chains in the gelatin (Karim and Bhat, 2009). The amino acid content in a gelatin is dependent on the origin of the raw materials. Many studies have indicated that collagen extracted from warm water fish species contains more amino acids than that of cold water fish (Gudmundsson, 2002). However, the later has weaker gelling properties due to the low content of proline and hydroxyl proline compared to the bovine and porcine derived gelatins. There is very limited information of collagen derived from fresh water fish as an alternative gelatin source. The fresh water fish might be more favorably new sources of gelatin.

The aim of the present research was to study the extraction and some physicochemical characteristics

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(proximate, colour, pH, solubility) and rheology properties (gel strength, viscosity, melting point and melting temperature) of gelatin using the skin of various fresh water fish (Pangas catfish, Nile tilapia, Asian redtail catfish, Striped snakehead) as raw materials compared with commercial cow gelatin found in Palangka Raya, Kalimantan Tengah, Indonesia.

Materials and Methods

Raw materials and chemicals

Four different species of fresh water fishes were obtained from local vendors in Palangka Raya, Province of Kalimantan Tengah, Indonesia, namely "pangas catfish" (*Pangasius pangasius*) (600-700 g each), "nile tilapia" (*Oreochromis niloticus*) (300-400 g), "Asian redtail catfish" (*Hemibagrus nemurus*) (600-700 g) and "Striped snakehead" (Channa Striata) (500-600 g). Pangas catfish and nile tilapia were obtained from local fisherman whereas striped snakehead and Asian redtail catfish were obtained from fish collector in Palangka Raya. Residual meat in the skin was removed manually and the cleaned fish skin was washed with tap water. The skin fish was packed in polyethylene plastic bags and stored at -20^{o}C until it was used. Commercial powder gelatin was purchased from E. Merck, D-6100 Darmstadt, Germany.

Gelatin extraction

Fish skins stored at -20^{o}C were thawed and cut it into small size of about 1 cm^{2}. The fish skins were thoroughly rinsed with limewater to remove superfluous materials. The samples (100 g) were rinsed and soaked in 1% (1:3 b/v) citric acid (pH 3) for 12 h. The samples were neutralized by washing several times until the pH of the washing water was faintly at basic pH (pH 6-7).

The fish skins were extracted in distilled water at 60^{o}C for 6 h. The solubilized gelatin was separated from residual skin fragments by filtration through a fabric filter followed by Whatman No. 1. The mixture was cooled until gelatin gel was formed, and then were dried using a cabinet drier at 60^{o}C for 24 h. The dried gelatin was ground and sieved with a 60 mesh screen to produce gelatin powder.

Physical and chemical analysis

Yield of gelatin extracts produced from each fish was determined according to the following equation: % yield (wb) = weight of gelatin/weight of skin x 100%, Yield of gelatin and also proximate analysis (moisture, ash, protein and fat contens) were carried out according to AOAC (2000). Samples were packed in clean plastic bags and the colour of gelatin gel were measured based on the method as described by Jamilah *et al.* (2011) using a Colour Reader (model Minolta Cr-10 Series, US). Samples were read three times and reported as L*, a* and b* parameters indicating lightness, redness/greenness and yellowness/ blueness.

Amino acid profile analysis

The amino acids compositions of the gelatin were determined by Amino Acid Analyzer High Performance Liquid Cromatography (Waters 501 Millipore Corporation, USA), equipped with the amino acid analyzing software (Waters Millenium32 Chromatography Software v. 4.0 on a Pentium 4 PC). The column used was Waters-Pico Tag (3.9 x 150 mm). Each sample was hydrolysed with 6N HCl at 110°C for 24 h prior to measurements.

Determination of gel strength

Gel strength of gelatin was determined according to the method of Benjakul *et al*. (2009). The samples were dissolved in aquadest at 60^{o}C to obtain a.67% (w/v) gelatin solution concentration, stirred to using a magnetic stirrer and the homogenous solution was transferred into and molded in standard bloom jars (3 cm in diameter and (2.7 cm in heigh). The sample in the jar was stored for 2 min and cooled in refrigerator at 10^{o}C for 16-18 h until a gel was formed. The strength of the gel was measured by Tensile Strength Instrument (Digital Force Gause model Imada/ZP-200N), using load cell 5 kg equipped with a 1 mm diameter flat-faced cylindrical Teflon plunger. The speed of the plunger was 0.5 mm/s. The maximum force (in grams) taken was when the penetration distance of 4 mm was obtained.

Determination of Viscosity

Viscosity of gelatin extract was determined by the AOAC (2000) method. Gelatin was dissolved in distilled water (6.67%, w/v) followed by heating in a water bath at 60°C for 30 min. Then the viscosity (mPa.s) of 20 ml of gelatin solutions was determined using a Brookfield LVDV-IIviscometer (Brookfield Engineering Laboratories Ltd., Middleboro, MA) with a small sample adaptor equipped with a No. 1 spindle at 90 rpm.

Determination of gelatin pH

The pH of the liquid solutions of the gelatin were determined according to the method of Choi and Regestein (2009), using a glass electrode (Toledo MPC 227 pH meter, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) based on the British Standards Institution (BSI). The gelatin solutions

were added with buffer solutions (pH 4), until the gelatin were completely precipitated and then the pH of the supernatant was measured.

Detemination melting point

The method for melting point measurement was described by Choi and Regenstein (2000). The sample (1 g) was heated and stirred using a spatula and repeated three times.

Determination Gelling temperature and Melting temperature

The gelatin extracts (20 mL) were transferred into a tube reaction and held in a cold box cooled with crushed ice tubes until the gelatin gelled, transferred it into a glass beaker and soaked in a water bath at 40°C. The melting temperature was measured when the gelatin gel was melting.

Solubility of skin gelatin

The effect of pHs on gelatin solubility was determined by the method of Benjakul *et al*. (2009). Gelatin was 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 6N NaOH or 6N HCl. The volume of solution was made up to 10 ml with distilled water, which was previously adjusted to the same pH of the gelatin solution. The solution was centrifuged at 8,500 *g* at room temperature for 10 min. The determination of protein content in the supernatant was carried out and bovine serum albumin was used as a standard. Relative solubility was calculated in comparison with the result of pH which yielded the high solubility.

Scanning Electron Microscopy (SEM) studies

The microstructure of gelatin extract were analyzed using SEM (Scanning Electron Microscopy) (Merk FEI, Type Inspect S50). Samples were dehydrated at its critical point using a drying equipment, then fastened to stub (samples holder). Samples were left to dry for ±1 day. Samples which were nonconductive (such as organic sample and polymer) were coated with pure gold or carbon for 1 h at a coating evaporator machine prior to observation in microscope.

Statistical analysis

All experiments were run in three times and were analysed with Microsoft Excel 2007. Data were subjected to analysis of variance (ANOVA) followed by Duncan Multiple Range Test at a level of $P < 0.01$ if there was significant differences between samples.

Results and Discussions

Yield of gelatin

The yield of gelatin from the different species of fresh water fish are shown on Table 1. The highest yield was obtained from Pangas catfish (22% wb), followed by Asian redtail catfish, Striped snakehead and Nile tilapia at 21.28, 20.25 and 21.93%, respectively. This result was higher than that reported by Jamilah and Harvinder (2002), with the yield of extracted gelatin of red tilapia and black tilapia of 7.81% and 5.39%, respectively; higher than that of sin croacker (14.3%) as reported by Cheow *et al.* (2007), as well as that of squid (7.5%) as reported by Uriarte *et al*. (2011). Similarly, it was also higher than those reported by Gomez-guillen *et al.* (2002) for Sole (7.3%), megrim (7.4%), cod (7.2%) and megrim (6.5%); Muyonga *et al.* (2004) for young nile perch (12.5%), adult nile perch (16%) and salmon 11.3%; and also for cod (10.1%) as reported by Arnesen and Gildberg (2007). The different kind of skin, acid concentration, pH condition, the rate of collagen break down when washing treatment and swelling process were among the possible reasons for the high of gelatin yield from the three species of fresh water fish.

Gomez-Guillen *et al.* (2001) noted that the different marine species has different structural and physical properties of gelatin. While Jamilah and Harvinder (2002), Songchotikunpan *et al.* (2008), and Tabarestani *et al.* (2010) suggested that the wide diversity among the fish species present intrinsic differences in the collagen molecules present in their skin. Moreover, the higher susceptibility of the collagenous material from fish skin to degradation is due to the lower content in intra- and interchain non-reducible crosslinks. While Karim and Bhat (2009) noted that the yield and quality of gelatin are influenced by the species and age of the fish, extraction process and pretreatment temperature.

Colour measurement of gelatin

The gelatin obtained from the different species of fresh water fish and its appearance visually were shown in Table 1. The lightness (L^*) value of gelatin extracted from pangas catfish skin (64.67) was higher compared to the commercial gelatin (61.73). However, a^* (redness) and b^* (yellowness) value of

Tabel 1. The yields and colour measurement of gelatin extracted from four species of freshwater fish

Properties	*Pangas catfish*	*Asian redtail catfish*	*Striped snakehead*	*Nile tilapia*	Commercial Gelatin
Yield (%)	22%	21.28%	20.25%	21.93%	-
Appearance	White	White	White	Light yellow	Dark yellow
colour value	$64.67^{e} \pm 0.06$	$62.57^{d} \pm 0.06$	$61.90^{b} \pm 0.1$	$62.13^{c} \pm 0.06$	$61.73^{a} \pm 0.06$
L*	$15.43^{c} \pm 0.15$	$14.63^{a} \pm 0.16$	$15.27^{bc} \pm 0.23$	$14.77^{ab} \pm 0.15$	$17.60^{d} \pm 0.61$
a*	$15.13^{a} \pm 0.16$	$15.23^{a} \pm 0.06$	$15.57^{a} \pm 0.06$	$1520^{a} \pm 0.1$	$23.33^{b} \pm 1.24$
b*					

*Results are means ± standard deviation (n = 3). Means within the same column followed by same supercript are not significantly different ($P < 0.05$).

Table 2. The Proximate composition of the four selected freshwater fish gelatins

Proximate composition (%)	*Pangas catfish*	*Asian redtail catfish*	*Nile tilapia*	*Striped snakehead*	Commercial gelatin
Moisture	$2.840^{a}\pm0.003$	$3.514^{d}\pm0.12$	$2.580^{b}\pm0.01$	$2.723^{c}\pm0.05$	$4.543^{e}\pm0.07$
Protein content	$87.10^{d}\pm0.99$	$85.59^{d}\pm0.09$	$82.53^{b}\pm0.53$	$87.27^{b}\pm0.78$	$78.79^{a}\pm0.85$
Ash content	$0.055^{a}\pm0.02$	$0.208^{b}\pm0.02$	$0.166^{b}\pm0.03$	$0.189^{b}\pm0.1$	$0.377^{c}\pm0.12$
Fat content	$0.002^{ns}\pm0.03$	$0.033^{ns}\pm0.03$	$0.000^{ns}\pm0.00$	$0.000^{ns}\pm0.00$	$0.000^{ns}\pm0.00$

*Results are means ± standard deviation (n = 3). Means within the same column followed by same supercript are not significantly different (P < 0.05).
*ns = not significantly.

commercial gelatin was higher compared to the four different fresh water fish gelatin. Ockerman and Hansen (1999) noted that the appearance of gelatin from striped snakehead visually are close to that of commercial one whereas pangas catfish and Asian redtail catfish gelatin are similar to pig gelatin. The color of the gelatin depends on the raw material. However, it does not influence other functional properties.

Proximate composition of gelatin

Table 2 shows the proximate composition of gelatin extracted from four different fresh water fish. Generally, gelatin from fish skin is extracted from fat free skin. Cheow (2007) reports that gelatin extracted from almost-fat free raw material contains <0.5% of ash. Protein content of pangas catfish, Asian redtail catfish, nile tilapia dan striped catfish were 87.10%; 85.59%; 82.53% and 87.27%, respectively, which were higher than that of commercial gelatin (78.9%). Ash content of gelatin from the four different fresh water fishes studied were lower to the one suggested by Jones (1997) that is maximum at 2.6%; for instance brownstripe red snapper is 1.9% (Jongjareonrak *et al.*, 2006), sin croaker and shortfin scad 1.49% and 1.15%, respectively (Cheow *et al.*, 2007) and nile perch 0.4% (Songchoticupan *et al.*, 2008).

Benjakul *et al.* (2009) noted that high quality of gelatin should contain no more than 0.5% ash. Jongjareonrak *et al.* (2006) suggest that the high protein content and the less moisture, ash and fat contents are determined by raw material or may be contributed by the residual of chemicals after processing, or also the possibility of mixing with other ingredients

Amino acid composition

Amino acid composition at of different species of fresh water fish is presented on in Table 3. Since the hardness of gelatin gel was in direct correlation with prolin (pro) and hydroxyproline (hyp) (Holzer, 1996) content, the important amino acid i.e. glycine and proline as part of total amino acid content (~25%) is also shown in Table 3. Glycine and proline found in pangas catfish gelatin were 167.31 mg/g and 117.39 mg/g, respectively. It was slightly lower than commercial gelatin (123.28 mg/g). Gómez-Guillèn

Tabel 3. Amino acid composition (mg/g) of gelatins extracted from four species of fresh water fish

Amino Acid (mg/g gelatin)	*Pangas catfish*	*Asian redtail catfish*	*Nile tilapia*	*Striped snakehead*	Commercial Gelatin
Asparagine	40.77	40.46	39.47	40.82	40.18
Threonine	9.46	8.90	8.42	11.09	9.67
Serine	30.85	32.78	28.12	33.93	35.49
Glutamic	83.81	77.66	79.03	77.66	70.51
Proline	117.39	99.8	98.06	110.38	123.28
Glycine	167.31	151.41	154.80	150.76	147.75
Alanine	68.57	67.52	64.70	80.51	69.54
Valine	16.64	15.40	13.44	14.53	13.77
Methionine	9.04	6.44	8.52	7.144	5.64
Isoleucine	8.64	5.76	7.53	6.61	5.15
Leucine	26.52	24.32	21.17	29.15	23.43
Tyrosine	4.83	4.00	3.58	4.24	3.88
Phenylalanine	18.24	18.08	15.72	19.61	16.85
Histidine	8.98	9.42	7.42	13.05	8.98
Lysine	35.23	22.47	28.06	29.12	24.04
Arginine	70.19	48.37	39.49	67.26	65.13
Tryptophan	38.00	38.31	37.92	39.08	36.57
Total	754.47	671.1	655.45	734.94	699.86

*Results obtained from duplicate readings

Tabel 4. Physico-chemical and rheological properties of gelatin extracted from four fresh water fishes

Properties	*Pangas catfish*	*Asian redtail catfish*	*Nile tilapia*	*Striped snakehead*	Commercial gelatin
Gel strenght (g)	$273.58^{d}\pm3.54$	$222.54^{b}\pm3.54$	$191.20^{a}\pm3.54$	$257.25^{c}\pm0.0$	$283.79^{e}\pm3.54$
Viscosity (cP)	$36.5^{c}\pm0.21$	$23.5^{ab}\pm0.1$	$19.3^{a}\pm0.1$	$31.5^{b}\pm0.1$	$39.5^{c}\pm0.1$
Isoelectric point (Ip)	$5.1^{b}\pm0.06$	$4.8^{a}\pm0.06$	$5.3^{c}\pm0.06$	$4.8^{a}\pm0.1$	$5.0^{b}\pm0.006$
pH	$5.8a^{b}\pm0.0$	$5.9^{b}\pm0.0$	$5.7^{a}\pm0.06$	$5.8^{ab}\pm0.1$	$6.2^{c}\pm0.06$
Melting point (°C)	$32.0^{b}\pm0.0$	$26.0^{a}\pm0.0$	$25.0^{ab}\pm0.0$	$31.0^{c}\pm0.0$	$35.0^{d}\pm0.0$
Gelling Temperature (°C)	$12.0^{c}\pm0.0$	$10.0^{ab}\pm0.0$	$10.0^{a}\pm0.0$	$11.0^{b}\pm0.0$	$16.0^{d}\pm0.0$
Melting temperature (°C)	$29.0^{b}\pm0.0$	$28.0^{a}\pm0.0$	$28.5^{ab}\pm0.0$	$30.0^{c}\pm0.0$	$34.0^{d}\pm0.0$
Solubility (%)	$99.40^{b}\pm0.003$	$99.41^{b}\pm0.005$	$99.14^{a}\pm0.12$	$99.21^{a}\pm0.002$	$99.60^{c}\pm0.002$

*Results are means ± standard deviation (n = 3). Means within the same column followed by same supercript are not significantly different (P < 0.05).

et al. (2002) report that the amino acids composition of gelatin extracted from the skin of sole, megrim, cod, hake and squid had more than 30% Gly and ~17% imino acids. However, Jamilah and Harvinder (2002) report that the proline contents of the gelatins extracted from red and black tilapia is very low and almost undetectable

Glutamic acid is the third order from amino acid after glycine and proline. In this study, the difference between amino acid of pangas catfish (80.31 mg/g) and commercial gelatin (70.51 mg/g) is obvious. On the other hand, alanine in striped snakehead gelatin (80.51 mg/g) was higher compared to that of pangas catfish (68.57 mg/g), Asian redtail catfish (67.52 mg/g), nile tilapia (64.70 mg/g) and commercial gelatin (69.54 mg/g). On the contrary, arginine of pangas catfish gelatin (70.19 mg/g) was higher compared to that of commercial gelatin (65.13 mg/g), Asian redtail catfish (48.37 mg/g), nile tilapia (39.49 mg/g) and striped snakehead (67.26 mg/g).

The amino acid composition plays main roles in the physical properties of gelatin. However, the relative content of β- or γ- components and higher molecular weight aggregates, as well as the presence of lower molecular weight protein fragments are known to contributes significant effects on the physical properties (Johnston-Barks, 1990). Apparently, gelatin with limited imino acid content should result in a less sterically hindered helix and may affect the dynamic properties of the gelatins, and also gives low melting point compared to gelatin with high imino

acid (Gilsenan and Ross-Murphy, 2000).

Determination of gel strength

The gel strength of commercial gelatin (6.67% w/v) observed in the present study showed that each fish species produced gelatins with different gel strength (Table 4). The gel strength of pangas catfish gelatin gel was found to be the strongest with a gel strength of 273.58 g followed by striped snakehead (257.25 g), Asian redtail catfish (222.54 g) and nile tilapia (191.20 g). However, this result was less than commercial gelatin (283.79 g).

The gel strength of pangas catfish (273.58 g) in this study was considerably high when compared to those reported in other studies, such as gelatins from red tilapia (128.1 g) (Jamilah and Harvinder, 2002), tilapia spp. (263 g) (Grossman and Bergman, 1992), grass carp (267 g) (Kansakala *et al*., 2007), and catfish (252 g) (Yang *et al*., 2007).

Gudmonsson and Hafsteinsson (1997) noted that gel strength probably depend on isoelectric point and control of pH. The gel strength of commercial gelatin has a range value of 200-300 g and melting point is >30^{o}C. The gel strength from cold marine species is 100 g or less and melting temperature is <17^{o}C whereas warm water species is higher than 200 g and melting temperature 24-29^{o}C.

Determination of viscosity

Viscosity is the second most important commercial physical property of gelatin (Schrieber and Garies, 2007). Table 4 shows the different viscosity of four species of fresh water fish with gelatin solution treatment (concentration 6.67%) at 60^{o}C. The viscosity obtained from pangas catfish, Asian redtail catfish, nile tilapia and striped snakehead were 36.5 cP, 23.5 cP, 19.3 cP and 31.5 cP, respectively. These results were higher than that of commercial gelatin (39.5cP). The viscosity increases with increasing gelling temperature, melting temperature, melting point and gel strength.

Grossman and Bergman (1992) report that the viscosity of gelatin of tilapia, walking catfish and striped catfish are 7.70 cp, 6.28 cp and 8.21cp, respectively. However, Yang *et al.* (2007) report the less viscosity (<3.0 cp) of channel catfish gelatin.

pH and isoelectric point

pH values of Pangas catfish, Asian redtail catfish, Nile tilapia, Striped snakehead gelatin and commercial gelatin are shown in Table 4. pH value of gelatin solutions extracted from fish skin showed that the four fresh water fishes studied were less than commercial gelatin. Meanwhile, the isoelectric point of pangas catfish gelatin reached 5.1 and Nile tilapia 5.3 higher than that of commercial gelatin (5.0). On the contrary, the results were less than those of Nile tilapia and striped snakehead, ca 4.8. pH acid of the gelatin solution obtained was influenced by washing treatment. Cheow *et al.* (2006) report that pH of gelatin solution extracted from sin croaker and shortfin scad are 3.35 and 4.87, respectively, which is less compared to bovine gelatin (5.48).

Detemination melting point

Melting points of all gelatin obtained are shown in Table 4. The extract of four fresh water fish gelatin showed different melting points, where pangas catfish gelatin (32^{o}C) was higher than Asian redtail catfish (26^{o}C), Nile tilapia (25^{o}C), striped snakehead (31^{o}C), but lower than commercial gelatin (34^{o}C).

Jamilah and Harvinder (2002) reported that melting point of tilapia red and black gelatin were 22.4 and 28.9^{o}C, respectively. Several studies reported variables values for the melting points of gelatins: of *tilapia* spp. gelatin is 25.4^{o}C (Gudmundsson, 2002), red and black tilapia 22.4 and 28.9^{o}C, respectively (Jamilah and Harvinder, 2002), *tilapia* spp. was 25.4^{o}C (Gudmundsson, 2002), young Nile perch and adult Nile perch were 21.4 and 26.3^{o}C, respectively (Muyonga *et al*., 2004), grass carp 26.8^{o}C (Kansakal *et al.*, 2007) and Catfish 23-27^{o}C (Liu *et al.*, 2008).

Determination gelling temperature and melting temperature

Gelling and melting temperatures of the four fresh water fish gelatin are shown on Table 4. The results showed that gelling and melting temperatures of pangas catfish gelatin (12^{o}C and 29^{o}C) were higher compared to that of Asian redtail catfish (10^{o}C and 28^{o}C), Nile tilapia (10^{o}C and 28.5^{o}C) and striped snakehead (11^{o}C and 30^{o}C), but lower than commercial gelatin (15^{o}C and 34^{o}C). These might be caused by its low proline contents. Karim and Bhat (2009) report that gelling and melting temperatures of fish gelatin is about 8-25^{o}C and 11-28^{o}C, respectively. Several studies had reported that melting temperature of tilapia is 24.55^{o}C (Pranoto *et al*., 2007), which is higher than that of Nile perch (26.3^{o}C) (Muyonga *et al.*, 2004) and lower than cod (13.8^{o}C) (Gomez-Guillen *et al*., 2000).

The range of gelling temperatures may be contributed by the residual of chemicals after processing and the from raw material. The melting temperature of gelatin prepared from the skins of warm-blooded animals and warm-water fish species is generally higher than that of gelatin from the skin of fish species living in cold-water (Gilsenan and

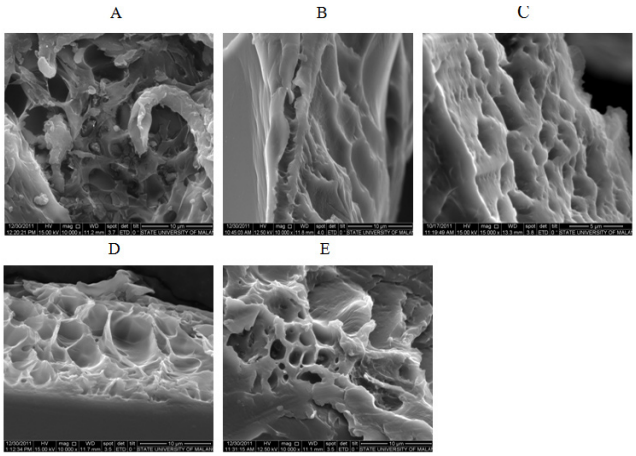

Figure 1. Image of Scanning Electron Microscopy (SEM) of commercial (A) and skin-originated fresh water fish gelatin; B. from Striped snakehead; C. from Pangas catfish; D. from Nile tilapia; and E. from Asian redtail catfish (Magnification at 10,000).

Ross-Murphy, 2000).

Determination of gelatin solubility

The solubility of the four fresh water fish gelatins are shown in Table 4; which were obtained from the solution adjusted to different pHs (1–10). The solubility of the four fresh water fish skin gelatin was more than 99%; similarly to that of commercial gelatin. Bovine gelatin had the lowest solubility at pH 5 (Benjakul *et al*., 2009). During the treatment, several glutamine and asparagines could be acidified, for example to be glutamic acid and aspartic acid (Jamilah and Havinder, 2002). The differences in solubility of different gelatins might be resulted from the differences in molecular weights and the ratios of polar and non-polar groups in amino acids (Zayas, 1997). Similarly, gelatins extracted from both *P. tayenus* and *P. macracanthus* have relative solubility greater than 90% at all pH tested (1-10) (Benjakul *et al.*, 2009).

Scanning electron microscopy (SEM)

Microstructure of commercial and the four fresh water fish gelatins are shown in Figure 1. Benjakul *et al.* (2009) noted that the arrangement and combination of protein molecules in gel matrix contributes to the gel strength. The commercial gelatin showed a non uniform network (Figure 1A) and pangas catfish gelatin had denser strand with small pores (Figure 1C), whilst Nile tilapia gelatin (Figure 1D) slightly stranded compared to asian redtail catfish and striped snakehead gelatin networks which were rough structures. Benjakul *et al*. (2009) noted that the rough gel network can produce a low gel strength and become unstable.

Conclusion

The gelatins extracted from the skin of Pangas catfish, Asian redtail catfish, Nile tilapia and Striped snakehead showed different physico-chemical characteristics. Gelatin extracted from pangas catfish shows the highest physicochemical and rhelogical properties. The physicochemical and rheological properties of the four fresh water fish gelatins had illustrated the potential of high quality of gelatins that could be used in food applications to replace mammalian gelatin.

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