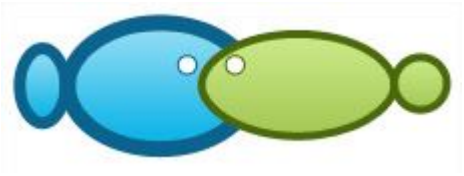

Physico-chemical characterization and skin gelatin rheology of four freshwater fish as alternative gelatin source

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Abstract. This study aimed to assess the characteristics of skin gelatin of different freshwater fish. The subjected species was snakehead murrel *(Channa striata),* pangas catfish *(Pangasius pangasius)*, walking *catfish (Clarias batrachus)*, *red snakehead (Channa micropeltes)* of which skin gelatin was extracted in citric acid. The fish skin gelatin was tested for proximate composition (water, protein, ash and fat content), gel strength, viscosity, gelling temperature, melting temperature, pH, and instrumental color, and the gelatin tissue observed under a *Scanning Electron Microscope* (SEM). The gelatin obtained from the fish skin used showed differences in yield of gelatin and physico-chemical characteristics (proximate composition, color, pH, gelling temperature) and rheological properties (gel strength, viscosity, melting temperature). The skin gelatin of *P. pangasius* had higher values of gel strength, viscosity, gelling temperature, color, water content, protein and ash ($P<0.05$) than that of other studied species in the present study. It had also higher gel strength, viscosity, gelling temperature, melting temperature, water content, protein level, ash, and fat than the other species, but lower than those of commercial gelatin. Micrograph Scanning Electron Microscopy (SEM) indicated that the skin gelatin structure of *P. pangasius* had rather thick strand with small voids and clear and uniform tissue. As conclusion, the skin gelatin of the four freshwater fish species used in this study is potential to be new alternative source of gelatin as gel former, elmusifier, stabilizer and thickener.

Key Words: fish gelatin, alternative gelatin resource, gel strength, viscosity, gelling temperature, melting temperature.

Introduction. Gelatin protein is obtained from collagen hydrolysis of animal origin, either skin or bone. Gelatin is produced through thermal denaturation or collagen partial degradation of animal skin and bone (Hao et al 2009). It is mainly used in food, pharmaceutical, medical, cosmetic and photographic industries, with unique technological and functional characteristics (Karim & Bhat 2009). Global gelatin demand rose in the last few years, particularly in Asia, i.e. skin and bone gelatin of pig and cow (GME 2008).

Gelatin of the world is made of swine skins and cows bone and skins. Its use in food industries increases every year (Montero & Gomez-Guillen 2000). However, mad cow (Bovine Spongiform Enchephalopathy/BSE), mouth, and food disease issues make people worry to use it under health reason. For this, mammal production is limited to functional food, cosmetic, and pharmaceutical products. Hence, gelatin taken from fish skin and bone is studied to replace its source from mammals (Gudmundsson 2002). In the last few years, fish gelatin was actively investigated. Some information was reported on gelatin processing through extraction and gelatin characteristic of skin and bone from black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis niloticus*) (Bakar & Harvinder 2002; Bakar et al 2011), nile perch (*Lates niloticus*) (Muyonga et al 2004), sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma)* (Cheow et al 2007), grouper (*Epinephelus sexfasciatus*), yellow streaked snapper (*Lutjanus lemniscatus*), mackerel (*Rastrelliger kanagurta*), and sand bass (*Morone chrysops*) (Irawandi et al 2009), common carp (*Cyprinus carpio*) (Duan et al 2011).

Fish gelatin is potential alternative to replace mammal gelatin, so that new alternative sources of fish need to be found, one of which is freshwater fish as promising

raw materials for gelatin processing in product application. Freshwater fish *Pangasius pangasius*, *C. batrachus*, and *C. striata* are important warm water fish commodities commonly consumed. These fish can be cultivated and become one of important fish supply sources.

Fish skin is main product of fisheries and agricultural industries. Number of fish from fish producers contributes 36 % of total fish weight to the availability of fish skin (MSC 2009). For this reason, researchers find the possibility of alternative gelatin by increasing the added value of fish processing wastes. There are high number of fish skin, scale and bone produced from fish processing wastes, rich in collagen, and can be used for gelatin processing materials.

Gelatin quality is highly affected by physico-chemical characteristics, not only by species and tissue extract, but also by processing methods (Johnston-Barks 1990). Good rheological characteristic is also needed for some applications, such as thickener, emulsifier, and gel former.

This study aimed the extraction of gelatin from freshwater fish skin and characterizing the physico-chemical features (proximate composition, color, pH, gelling temperature, solubility) and the rhelogical features (gel strength, viscosity, melting temperature) compared with commercial gelatin from bovine in order to find new alternative source for gelatin processing, and physico-chemical and rheological characteristics usable as suitable indicator in processing as product application material.

Material and Method

Materials. This study employed live freshwater fish, *P. pangasius* (approximately 600-700 g body weight), red snakehead (*Channa micropeltes*) (600-700 g), walking catfish, (*Clarias batrachus*), (200-300 g), snakehead murrel (*Channa striata*) (500-600 g). *P. pangasius* was obtained from local fish farmer, while *C. striata*, *C. micropeltes* and *C. batrachus* were collected from local retailer in Palangka Raya, Central Kalimantan. Fish skin was harvested, cleansed, placed into polyethylene plastic bag, and stored in freezer at -20^{o}C until use.

Gelatin extraction. Fish skin was thawed and cleansed. It was then cut to 1x1 cm pieces and cleaned from attached flesh, fat, and other impurities. One-hundred grams of skin were washed and soaked in 1% citric acid (1:3 b/v) for 12 hours. The skin was washed 3 times until the pH was neutral (pH 6–7). Fish skin gelatin was extracted in water at 60^{o}C for 6 hours. Gelatin solution was then filtered through cloth and then Watman no. 1 filter paper, and then cooled up to gelatin gel formation. The gelatin gel was dried in a Cabinet Dryier at 60^{o}C for 24 hours. The dried gelatin was refined and sieved through Watman no.1 filter paper to obtain gelatin powder.

Yield of gelatin. Gelatin production was gained from the following calculation:

$$\% \text{ yield (wet weight basis)} = \frac{\text{Dry weight of gelatin}}{\text{Wet weight of skin}} \times 100$$

Color measurement. Color measurement used a color reader (model Minolta Cr-10 Series, US). The sample was placed into a clean plastic and then the color was read. The reading was done 3 times for each sample. The value was expressed as 'L' – lightness, 'a' – redness, and 'b'- yellowness.

Proximate composition of gelatins. The moisture, ash, protein and fat content of the gelatin extract were determined using the AOAC (2000) methods.

Gel strength determination. Gelatin was dissolved in distilled water at 60^{o}C to obtain 6.67% gelatin concentration (w/v). The solution was then stirred using a magnetic stirrer up to be homogenous and poured into standard bloom jars (3-cm diameter and 2.7 cm high), left for 2 minutes, and cooled in a refrigerator at 10^{o}C for 16-18 hours so that gel

was formed. The gel strength was measured using a tensile strength instrument (Digital Force Gause model Imada/ZP-200N), under a load cell of 5 kN and 1 mm diameter flat-teflon cylindrical surface. A probe of 0.5 mm/s was pressed to 4 mm depth, and the gel strength was expressed in gram force.

Determination of viscosity. Gelatin solution of 6.67% concentration was boiled in a waterbath while continuously stirred up to 60^{o}C. The viscosity was measured using a viscometer brookfield. A spindle was previously heated at 60^{o}C and then installed to the viscometer brookfield. The spindel position in the hot solution was set accurately, then the viscometer was turned on and the solution temperature measured. When the solution temperature reached 60^{o}C, the viscosity value was known through the viscometer reading at scale 1-100. The reading was done after 1 minute of full rotation 2 times for spindel no. 1.

pH

pH was measured with glass electrode (Toledo MPC 227 pH meter, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) after the pH meter had been standardized in pH 4.0–7.0 buffers. The pH value was recorded on the screen.

Determination of gelling temperature and melting temperature. Twenty mL of gelatin extract solution was placed into a test tube, placed in a cool box with a thermometer. The crushed ice cube was added gradually up to gelatin gel formation and the gelling temperature was recorded.

The gelatin gel was moved into a beaker glass, placed into a waterbath, and turned on the waterbath at 40^{o}C, and then the temperature of beaker glass was measured since the waterbath was turned on. When the gelatin gel melted, the temperature measurement was taken as melting temperature.

Gelatin solubility. Gelatin was dissolved in distilled water at 60^{o}C to obtain the final concentration of 2% (w/v) and the solution was stirred at room temperature up to be completely dissolved. The gelatin solution was set in different pHs (1-10) using 6 N NaOH or 6 N HCl. To the solution was added distilled water up to 10 mL after it had been adjusted to the same pH, then centrifuged at 8,500 rotations/min at room temperature for 10 minutes. The determination of protein content in the supernatant applied Biuret method using bovine serum albumin as standard. Relative solubility was calculated by comparing the solubility value at the pH producing high solubility.

Scanning electron microscopy (SEM). Gelatin sample was palced in a ±10 mm holder. The non-conductive samples, such as organic sample, polymers, and others, need to be coated using Au-Pd in order to make it be more conductive. The sample was inserted into SEM chamber and pumped (High Vacuum or Low Vacuum) up to be absolutely vacuum, and SEM/EDX equipment (Merk FEI, Type Inspect S50) is ready to use.

Statistical analysis. ANOVA was used to compare the mean value of 3 measurements, and $P<0.05$ was taken as significance value. Duncan's Multiple Range test was employed for significance test using Microsoft SPSS 17.0 for windows (SPSS Inc, Chicago, II, USA).

Results and Discussion

Gelatin extract. Analysis demonstrated difference in yield of gelatin extract among the mentioned 4 freshwater fish species in this study ($P<0.05$) (Table 1). Differences could result from that each fish species produce different collagen content. It is in agreement with Koli et al (2012) that variations in gelatin production could be caused by difference in collagen content, skin composition in the skin matrix, and extraction method (Gomez-Guillen et al 2002; Bakar & Harvinder 2002; Jongjareonrak et al 2006).

Table 1 shows that the highest gelatin yield was found in *P. pangasius* (21.93 %), followed by *C. batrachus* (20.57 %), *C. straiata* (20.17%), and *C. micropeltes* (20.76%) respectively.

Table 1

The yield and instrumental color of gelatin from four freshwater fish species

Properties	*Pangasius pangasius*	*Clarias batrachus*	*Channa striata*	*Channa micropeltes*	*Commercial (bovine)*
Yield (%)	21.93[d]	20.57[b]	20.17[a]	20.76[c]	-
Appearance color value	White	White	Yellow	Light yellow	Dark yellow
L*	64.67±0.06[e]	62.57±0.06[d]	61.90±0.1[b]	61.67±0.06[c]	61.73±0.06[a]
a*	15.43±0.15[c]	14.63±0.16[a]	15.27±0.23[bc]	15.10±0.15[ab]	17.60±0.61[d]
b*	15.13±0.16[a]	15.45±0.06[a]	15.57±0.06[a]	15.43±0.1[a]	23.33±1.24[b]

*Value color is mean±SD from triplicate determination;
* Duncan 5%;
* P < 0.05 and 0.01 (highly significant).

Fish skin gelatin obtained from *P. pangasius*, *C. batrachus, C. striata*, and *C. micropeltes* is higher than that reported by Bakar & Harvinder (2002) in *O. niloticus* (7.81 %), and *O. mossambicus* (5.39%), Cheow et al (2007) in *J. dussumieri* (14.3%). Other reports indicated that skin gelatin content was 7.5% in humbold squid (*Dosidicus gigas*) (Uriarte-Montoya et al 2011), 7.3% in Dover sole (*Solea solea*), 7.4% in four-spot megrim (*Lepidorhombus boscii*), 7.2% in Atlantic cod (*Gadus morhua*), and 6.5% in European hake (*Merluccius merluccius*) (Gomez-guillen et al 2002), 12.5% in juvenile *L. niloticus*, 16% in adult *L. niloticus* (Muyonga et al 2004), and 10.1% in *G. morhua* (Arnesen & Gildberg 2007), respectively. High skin gelatin of *P. pangasius*, *C. batrachus, C. micropeltes*, and *C. striata* is due to differences in skin type with fish species, acid concentration, pH use, few collagen lost in washing, and good swelling process (crosslinkage separation during the swelling). According to Koli et al (2012), collagen washing and skin washing can yield low gelatin.

Gomez-Guillen et al (2001) stated that different aquatic environment could cause differences in gelatin structure and physical properties. Different gelatin characteristics with species can be determined from intrinsic properties of the skin and collagen molecule, collagen content, number of soluble components in the skin, and loss of collagen extract through crosslinking termination during swelling and washing phases or incomplete collagen hydrolysis (Jamilah & Harvinder 2002; Songchotikunpan et al 2008; Tabarestani et al 2010). Collagen conversion rate to gelatin is dependent upon the processing parameters, such extraction time, temperature and pH, pretreatment condition, raw material characteristics, and initial handling method (Karim & Bhat 2009).

Color determination. Duncan test on L value (lightness) and a value (redness) indicates significant difference (P<0.05) among skin gelatin of the four freshwater fish species, while b value (yellowness) does not show significant difference (P>0.05) (Table 1). These results reflect that the lightness of skin gelatin of *P. pangasius* (64.67) is higher than that of commercial gelatin from bovine (61.73), while the redness (a-value) and the yellowness (b-value) of the commercial gelatin from bovine is higher than that of *P. pangasius*, *C. batrachus*, *C. micropeltes*, and *C. striata*. It could result from different species used and their living environment. According to Jongjareonrak et al (2010), gelatin color is, in general, dependent upon raw material extracted and extraction condition. Mean L, a, and b values of skin gelatin of *P. pangasius*, *C. striata*, *C. micropeltes,* and *C. batrachus* are higher than those reported by Jongjareonrak et al (2010), for Mekong giant catfish (*Pangasianodon gigas*), with L* of 20.32, a* of -0.61, and b* of 1.36, while the gelatin gel color of walking catfish has L* of 15.45), a* of -1.86), and b* of 3.72.

The color of gelatin powder of *C. striata* visually looked nearly like that of commercial gelatin from bovine, rather yellowish, while that of *P. pangasius* and *C.*

batrachus was whitish. The gelatin gel color of the 4 freshwater fish used in this study was also different, in which the gelatin gel of *P. pangasius* and *C. batrachus* had clear white color, and that of *C. micropeltes* and *C. striata* was rather yellowish, while commercial gelatin gel had clear yellow color (Figure 1). According to Ockerman & Hansen (1999), gelatin color is dependent upon the raw material and color, in general, does not influence other functional properties.

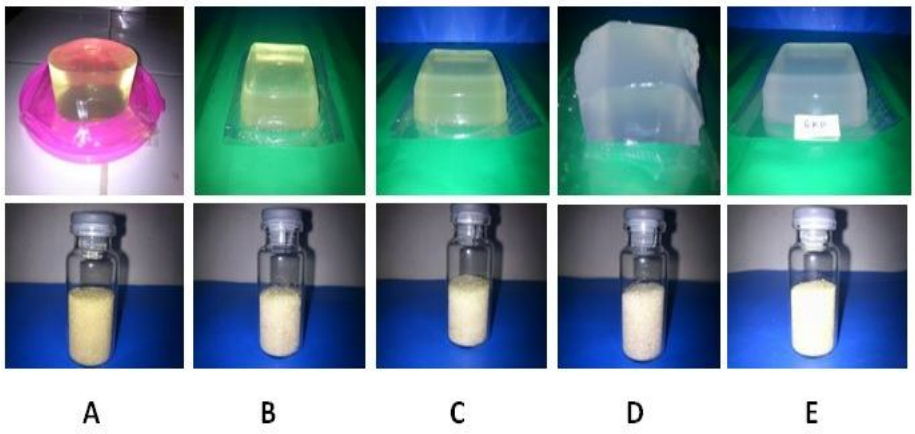

Figure 1. Gelatin extract from 4 freshwater fish species (A. Commercial bovine gelatin; B. *Channa striata* gelatin; C. *Channa micropeltes* gelatin; D. *Clarias batrachus* gelatin; E. *Pangasisus pangasius* gelatin) (original).

Proximate composition of gelatin. Gelatin proximate composition of the freshwater fish used in this study is demonstrated in Table 2. The use of different freshwater fish skin gives significant difference on water, protein and ash content ($P<0.05$), but no significant difference occurs in fat content ($P>0.05$). In general, the fish skin gelatin extract was almost free of fat. It could result from that during extraction in acid solution and washing processing, the skin fat was lost or dissolved, so that the gelatin product had very low fat (nearly free of fat). Similar to Cheow et al (2007), the ash content of the gelatin is less than 0.5 % and almost free of fat.

Table 2

Gelatin proximate composition of four selected freshwater fish

Proximate composition	*Pangasius pangasius*	*Clarias batrachus*	*Channa micropeltes*	*Channa striata*	*Commercial (bovine)*
Water (%)	2.080 ± 0.003^{a}	3.480 ± 0.12^{d}	2.680 ± 0.01^{b}	2.723 ± 0.05^{c}	4.523 ± 0.07^{e}
Protein (%)	87.10 ± 0.99^{d}	85.92 ± 0.09^{d}	82.63 ± 0.53^{b}	87.27 ± 0.78^{b}	78.79 ± 0.85^{a}
Ash (%)	0.055 ± 0.02^{a}	0.210 ± 0.02^{b}	0.180 ± 0.03^{b}	0.189 ± 0.1^{b}	0.377 ± 0.12^{c}
Fat (%)	0.002 ± 0.03^{tn}	0.017 ± 0.03^{tn}	0.000 ± 0.00^{tn}	0.000 ± 0.00^{tn}	0.000 ± 0.00^{tn}

*Values are mean±SD from triplicate determination;
*Duncan 5%;
*$p<0.01$ and 0.05, except fat level $p> 0.05$ and 0.01.

Furthermore, protein content of skin gelatin in *P. pangasius*, *C. batrachus*, *C. micropeltes* and *C. striata* was 87.10%, 85.52%, 82.63%, and 87.27%, respectively and higher than that of commercial gelatin from bovine, 78.9%, but ash, fat, and water content were lower than those in commercial gelatin. This condition is influenced by raw material used and processing process.

The water content of skin gelatin extracted from *P. pangasius*, *C. batrachus*, *C. striata*, and *C. micropeltes* was 2.08%, 3.48%, 2.72%, and 2.68%, respectively, and it was lower than that of commercial gelatin (4.52%). The water content of all samples is below the established standard for edible gelatin, 15% (GME 2008). Gelatin is also very hydroscopic under 6-8% water content, and this condition makes the physico-chemical properties to be difficult to accurately determine (Cole 2000).

The ash content of the skin gelatin was 0.06% for *P. pangasius*, 0.21% for *C. batrachus*, 0.18% for *C. micropeltes*, and 0.19% for *C. striata*, respectively. It is lower than commercial gelatin (0.38%) and that recommended by Jones (1997) that maximum ash content was 2.6%, while it was 2% for edible gelatin (GME 2008). This study also found lower skin gelatin ash content than that of other fish species, such as brownstripe red snapper (*Lutjanus vitta*) (1.9%) (Jongjareonrak et al 2006), *J. dussumieri* (1.49%) and *D. macrosoma* (1.15%) (Cheow et al 2007), and *L. niloticus* (0.4%) (Songchotikunpan et al 2008). According to Benjakul et al (2009), the ash content of high quality gelatin is below 0.5%. It could result from different mineral contained in the fish skin (Jongjareonrak et al 2006).

Determination of Gel Strength

Gel strength has important functional properties in gelatin. This finding showed that each fish species produced gelatin of different gel strength (Table 3), which conclude that the gel strength is significantly different among species ($P<0.05$). *P. pangasius* had the highest gel strength (273.58) g, followed by *C. striata* (257.25) g, *C. batrachus* (223.50 g), and *C. micropeltes* (192.20 g), but lower than that of commercial gelatin of bovine origin (283.79 g). This study also revealed that the gel strength of *P. pangasius* was higher than several previous findings on skin gelatin of *O. niloticus*, 128.1 g (Bakar & Harvinder 2002), Tilapia *spp,* 263 g, (Grossman & Bergman 1992), grass carp *Ctenopharyngodon idella*, 267 g, (Kansakala et al 2007), *J. dussumieri*, 124.94 g, and *D. macrosoma*, 176.92 g, (Cheow et al 2007), *L. Niloticus*, 229 g, (Muyonga et al 2004), tigertooth croaker (*Otolithes ruber*), 170 g, and Japanese threadfin bream (*Nemipterus japonicus*), 140 g, respectively.

Table 3

Physico-chemical and rheological properties of gelatin obtained from the studied freshwater fish species

Properties	*Pangasius pangasius*	*Clarias batrachus*	*Channa micropeltes*	*Channa striata*	*Commercial Gelatin*
Gel strength (g)	273.58 ± 3.54^{d}	223.50 ± 3.54^{b}	192.20 ± 3.54^{a}	257.20 ± 0.0^{c}	283.79 ± 3.54^{e}
Viscosity (cP)	3.43 ± 0.21^{d}	2.37 ± 0.1^{c}	19.3 ± 0.1^{a}	$31.5\ 0.1^{b}$	39.5 ± 0.1^{e}
Gelling temperature (^{o}C)	11.67 ± 0.0^{c}	10.0 ± 0.0^{ab}	9.68 ± 0.0^{a}	10.67 ± 0.0^{c}	16.0 ± 0.0^{d}
Melting temperature (^{o}C)	29.0 ± 0.0^{b}	28.33 ± 0.0^{a}	29.67 ± 0.0^{ab}	29.83 ± 0.0^{c}	33.67 ± 0.0^{d}
pH	5.8 ± 0.0^{ab}	5.9 ± 0.0^{b}	5.7 ± 0.06^{a}	5.8 ± 0.1^{ab}	6.2 ± 0.06^{c}
Solubility (%)	99.40 ± 0.003^{b}	99.40 ± 0.005^{b}	99.21 ± 0.12^{a}	99.21 ± 0.002^{a}	99.60 ± 0.002^{c}

*Values are means±SD from triplicate determination.
*p<0.05 and 0.01 (highly significant).
* Duncan notation 5%.

The gel strength of fish gelatin was reported in a broad range, 124-426 g, while that of commercial gelatin of bovine and swine origin had a gel strength range of 200–300 g (Karim & Bhat 2009). Gomez-Guillen et al (2011) reported that commercial gelatin had the same range of gel strength and the melting temperature higher than 30^{o}C, but the gelatin of cold water species had 100 g gel strength or less and melting temperature less than 17^{o}C, and that of warm water species had >200 g gel strength and melting temperature range of 24-29^{o}C.

Different gel strength with fish species could be explained by extraction process used and variation in collagen intrinsic properties among fish species (Koli et al 2012). According to Minh Thuy le et al (2014), different gelatin gel strength could be caused by

environmental temperature or water temperature where the fish live, while Gudmundsson & Hafsteinsson (1997) claimed that it could be dependent upon the isoelectric point and pH control. According to Arnesen & Gildberg (2002), hydrogen bonding with water molecule and free group of amino acid is essential for gelatin gel strength. High content of hydroxyproline could also yield high gelatin gel strength (Sarabia et al 2000). Several previous findings indicated that the gel strength of gelatin extracted from various fish species was not always the same under different procedures in sample preparation, experimental settings, and equipment used *(*Boran et al 2010*).* In addition, the gel strength and viscosity previously found was positively correlated, in which high gel strength yielded high viscosity as well (Zhou & Regenstein 2004; Boran & Regenstein 2009).

Viscosity. Viscosity is the second important parameter of gelatin after gel strength (Schrieber & Gareis 2007). Table 3 shows different viscosity characteristic with fish species ($P<0.05$), the viscosity values (cP) obtained from the skin gelatin of *P. pangasius*, *C. batrachus*, *C. micropeltes* and *C. striata* are 3.63, 2.37, 1.87, and 3.17 cP, respectively, and they are lower than that of commercial gelatin (3.93 cP). This findings are not quite different from that reported by Yang et al (2007) for *C. batrachus*, 3.2 cP, and Bakar & Harvinder (2002) for *O. niloticus*, 3.2 cp., while the viscosity of skin gelatin of *O. mossambicus* is higher (7.72 cP). Natural variation of the viscosity could result from different freshwater fish species and environment, despite the role of extraction method.

Increase in viscosity is followed by increased gelling temperature and gel strength, and reduced melting temperature. It is in line with that reported by Koli at al (2012) that high gelatin viscosity of *O. ruber* (10.53 cP) yields lower gel strength (170 g) and that of *N. japonicus* (8.47 cP) produces lower gel strength than *O. ruber*, 140 g. Change in pH value could also raise and reduce the gelatin viscosity at the pH range of 6-8 (Stainsby 1987a).

Gelling and melting temperature. Gelling and melting temperatures of 4 freshwater fish skin gelatin are given in Table 3. They are significantly different ($P<0.05$), while the gelatin of walking catfish and red snakehead does not show significantly different gelling and melting temperatures. This difference could result from different fish species used and their living environment.

This study found that gelling and melting temperatures of *P. pangasius* (11.67^{o}C and 29^{o}C) were higher than those of the *C. batrachus* (10^{o}C and 28.33^{o}C), *C. micropeltes* (9.68^{o}C and 29.67^{o}C) and *C. striata* (10.67^{o}C and 29.8^{o}C), respectively, but lower than those of commercial gelatin of bovine (15.67^{o}C and 33.8^{o}C). This finding is also lower than that reported by Karim & Bhat (2009) that fish gelatin gelling and melting temperatures ranged between 8-25^{o}C and 11-28^{o}C. This difference could result from different raw materials used. According to Gudmundsson (2002), environmental temperature affects the gelling and melting temperatures of the gelatin produced. Different types of gelatin also results in different physico-chemical properties influencing thermal and rheological characteristics, including gelling temperature, melting temperature, and gel strength (Norziah et al 2009). Low amino acid (proline and hydroxyproline) could also cause low gelling and melting temperature of the gelatin (Haug et al 2004).

As thermoreversible gel, gelatin gel starts melting when temperature rises over the certain point. Melting temperature of gelatin gel is one of the important properties of the gelatin beside gel strength, viscosity, and gelling temperature. Skin gelatin extracted from *P. pangasius*, *C. batrachus, C. micropeltes*, and *C. striata* had different melting temperature. *P. pangasius* had the highest melting temperature, 29^{0}C, and *C. batrachus* had the lowest one, 28.33^{o}C, but the melting temperature of all freshwater fish gelatin was lower than that of commercial gelatin of bovine (33.8^{o}C). Nevertheless, these findings are higher than that reported by Pranoto et al (2007) for skin gelatin of tilapia (*Oreochromis* sp.), 24.55^{o}C, Muyonga et al (2004) for *L. niloticus*, 26.3^{o}C, Gomez-Guillen et al (2000) for *G. morhua*, 13.8^{o}C, Mohtar et al (2010) for blue grenadier

(*Macruronus novaezelandiae*), 26.9^{o}C, Liu et al (2008) for channel catfish (*Ictalurus punctatus*), 23-27 ^{o}C, and Kansakala et al (2007) for *C. idella*, 26.8 ^{o}C, respectively.

High melting temperature of the skin gelatin from *P. pangasius*, *C. striata, C. micropeltes*, and *C. batrachus* could be affected by fish species used, environmental condition, and difference in amino acid content. This study found that high melting temperature of skin gelatin of *P. pangasius* is followed by increased gel strength, viscosity, and gelling temperature of the gelatin. This finding is in agreement with Choi & Regeistein (2000) that increased gelatin gel strength is followed by increased melting temperature.

Gomez-Guillen et al (2000) and Gilsenan & Ross-Murphy (2000) generally concluded that melting temperature of skin gelatin obtained from cold-water fish was lower than that of collagen and gelatin of mammals skin and warm-water fish, and then low content of amino acids (proline and hydroksiproline). Therefore, gelatin of cold water fish reflects reaction as viscous liquid at room temperature that restricts its utilization in several applications.

pH. The pH values of skin gelatin of *P. pangasius*, *C. batrachus, C. micropeltes, C. striata*, and commercial product of bovine are presented in Table 3. The use of different fish species yielded significant difference ($P<0.05$) of gelatin pH. They were 5.8 for *P. pangasius*, 5.9 for *C. batrachus*, 5.7 for *C. micropeltes*, and 5.8 for *C. striata*, respectively. All these were lower than that of commercial gelatin of bivine, 6.2. Low pH (acidic) found in the gelatin solution is affected by washing treatment. Mean pH of the fish gelatin used ranged from 5.7 to 5.9. This value was higher than the gelatin solution pH in *J. dussumieri*, 3.35, and *D. macrosoma*, 4.87 (Cheow et al 2007). *O. niloticus*, *O. mossambicus* (Bakar & Harvinder 2002), and the commercial gelatin also had lower gelatin solution pH than the present findings, 3.05, 3.91, and 6.2, respectively. Increased gelatin pH is positively correlated with viscosity increment, in which high viscosity will raise the gelatin pH. Stainsby (1987b) reported that pH change could increase and reduce the gelatin viscosity at pH range of 6-8.

Solubility. Table 3 shows that the skin of *C. batrachus, C. micropeltes*, and *C. striata* has significantly different gelatin solubility ($P<0.05$), but there was no significant difference ($P>0.05$) between *P. pangasius* and *C. batrachus* and between *C. micropeltes* and *C. striata* (Table 3). The gelatin solubility of 4 freshwater fish species was observed at broad pH range of 1-10, and it was nearly similar to that of commercial gelatin of cow. High gelatin solubility obtained is correlated with pH of the gelatin solubility obtained. Cow gelatin has very low solubility at pH 5, while skin gelatin solubility of *Priacanthus tayenus and P. macracantus* is very low at pH 8 (Benjakul et al 2009). This difference could be due to dissimilarity of molecular weight and polar and non-polar group concentration in amino acid (Zayas 1997). The solubility of skin gelatin of *P. tayenus* and *P. macracanthus* (>90%) at pH 1-10 can be extensively and effectively used since it is the requirement for food functionality protein (Benjakul et al 2009), and it is higher than the solubility found in 4 freshwater fish species used in this study, about 99%.

Scanning electron microscopy (SEM). The microstructures of commercial gelatin from bovine, *P. pangasius*, *C. striata*, *C. micropeltes*, and *C. batrachus* are presented in Figure 2. In general, the protein molecular structure and combination in gel matrix contribute to gelatin gel strength (Benjakul et al 2009). All gelatins have sponge or coral structures.

The commercial gelatin from cow does not exhibit uniform tissue with unclear strand (Figure 2A), while the gelatin of *P. pangasius* has clear tissue structure with sufficient thickness, smooth, and uniform strand (Figure 2E). In the gelatin of *C. batrachus, C. micropeltes*, and *C. striata* gelatin, the tissue is not homogenous in thickness, with thin strand and unclear and coarse tissue structures (Figure 2B, 2C, and 2D). The tissue with thick and uniform strand is correlated with the extent of gelatin strength of *P. pangasius* skin (Figure 2E). According to Kittiphattanabawon et al (2010), coarse gel tissue has low gel strength and is easily disturbed by strength.

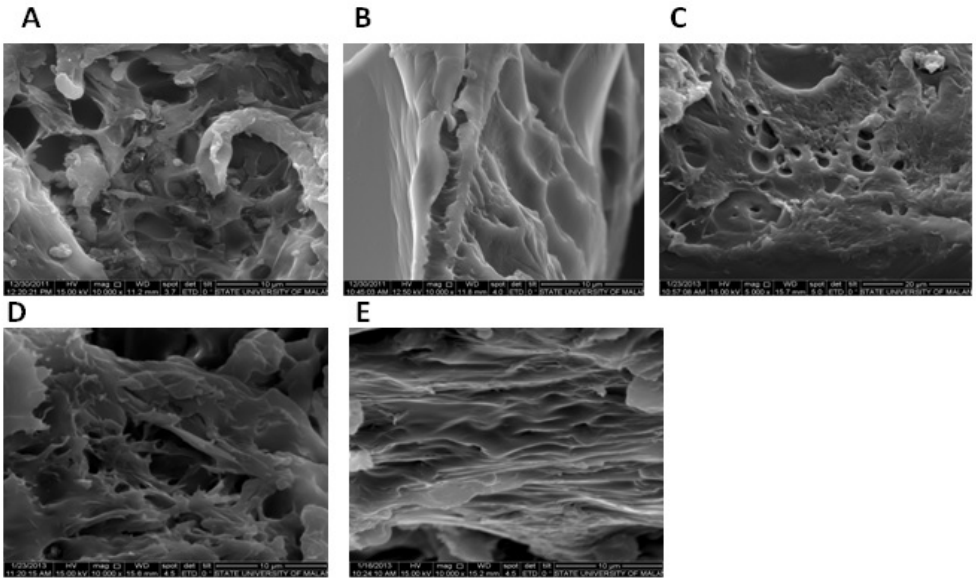

Figure 2. Scanning electron microscopy (SEM) of freshwater skin gelatin. A - commercial gelatin of bovine origin; B - gelatin of *Channa striata*; C - gelatin of *Channa micropeltes*; D - gelatin of *Clarias batrachus*; and E - gelatin of *Pangasius pangasius* (10,000x enlargement).

Conclusions. Gelatin otained from skin of different freshwater fish species (*P. pangasius, C. batrachus, C. micropeltes and C. striata*) showed different yield of gelatin and physico-chemical (proximate composition, color, pH, gelling temperature) and rheological (gel strength, viscosity, melting temperature) characteristics. The gelatin of *P. pangasius* had higher yield of gelatin and physico-chemical and rheological features than that of *C. batrachus*, *C. micropeltes*, and *C. striata*, but lower than that of commercial gelatin. The SEM microcrograph indicated that the gelatin structure of the *P. pangasius* had rather thick strand with small voids and clear and uniform tissue. Based on physico-chemical and rheological features, the skin gelatin of *P. pangasius*, *C. batrachus*, *C. micropeltes and C. striata* can be used as new alternative source of application materials in product processing.

Acknowledgements. We are thankful to the University of Palangka Raya for providing convenience in using the experimental equipments and the ministry of research and technology for the financial support.

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Received: 20 October 2016. Accepted: 21 November 2016. Published online: 23 November 2016.
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How to cite this article:
Ratnasari I., Firlianty, 2016 Physico-chemical characterization and skin gelatin rheology of four freshwater fish as alternative gelatin source. AACL Bioflux 9(6):1196-1207.