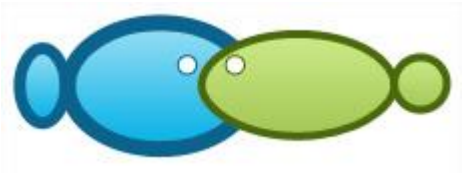

Optimization of the extraction procedures and the characterization of fish gelatin from fringescale sardinella (*Sardinella fimbriata*) bones

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Abstract. *Sardinella fimbriata* is a common species widely used in the fish processing industry, which contributed to the abundance of waste production. Such wastes can be potentially converted into high value-added products such as gelatin. Gelatin is commonly derived from mammalian sources especially from pig skin and bovine bone. Due to certain restrictions among Muslim and Jews, an alternative to find Halal and Kosher gelatins was sought, in order to replace the commercial mammalian gelatins. Therefore, the present study was carried out to determine the best method used to extract gelatin from *S. fimbriata* bones. The gelatins from *S. fimbriata* bones were extracted with four different methods. The best extraction method was found with 1 M of HCl, at 67°C for 14 hours. The gelatin yield was at 6.0±0.03 % and the protein content was 59.1±0.29%. Different extraction methods used resulted in different gelatin yields and protein contents. The physico-chemical properties of the gelatin were further characterized based on the proximate analysis, color value, gel strength, melting point, microstructure and amino acid composition. Gelatin from *S. fimbriata* bones demonstrated better properties in terms of its gel strength (132.4±2.64 g) and melting point (25±1.52°C). *S. fimbriata* bones gelatin demonstrated high proline and hydroxyproline contents, which were 107.65±3.25 mg g^{-1} and 89.05±0.49 mg g^{-1}, respectively. Overall findings have suggested that different methods used during the extraction process will contribute to the difference of its physico-chemical properties.

Key Words: physico-chemical, by-products, gel strength, melting point, amino acid.

Introduction. Fish processing industries usually dispose a huge amount of fish by-product. Such by-products can reach up to 25% of the total production, which is around 20 million tons in Malaysia (FAO 2012). The majority of this waste is in the form of bones, skin, scales, and fins, which constitutes over 70% of the fish (Nurdiyana et al 2008). However, such by-products contain high protein and lipid, as well as high valuable micronutrients such as vitamin and mineral (FAO 2016).

Gelatin is commonly derived from mammalian sources and mostly from skin, bones, hides and connective tissue of bovine and porcine (Gilsenan & Ross-Murphy 2000). Recent reports have demonstrated that gelatin production is nearly 3,326.000 tons, produced from pig skin (44%), bovine hides (28%), bovine bones (27%) and other sources (1%) (Ahmad et al 2011). Compared to the superior properties, gelatin from mammalian sources is more popular than those from marine sources (Cho et al 2005). Quality of the gelatin may be affected by the extraction process. The extraction process is the one of important steps in producing high quality of gelatin. Such processes are different among species and among extracted tissues. The extraction process can influence the functional properties of gelatin; depending on the processing parameter such as the extraction temperature, time and pH. However, even at a low quality, the gelatin still can be used in several industries, such as pharmaceutical, food and cosmetic, due to the different requirements of the functional properties.

The demand of porcine and bovine gelatins are limited due to several issues and the consumer demand decreased year by year, due to the outbreak of diseases such as the mad cow disease or Bovine Spongiform Encephalopathy (BSE) (Karim & Bhat 2009),

Foot and Mouth Disease (FMD) (Jongjareonrak et al 2005), avian influenza (Nagai et al 2010) and Transmissible Spongiform Encephalopathy (TSE) (Wangtueai & Noomhorm 2009). Besides, gelatin consumption was restricted in certain populations, due to the religious objections. Muslim and Jew could not consume porcine gelatin, meanwhile Hindus could not consume bovine gelatins (Mohtar et al 2010).

Therefore, researchers have shifted the attention to extract gelatin from marine sources, given the fact that they are not associated with any disease outbreaks (Karim & Bhat 2009). Fish gelatin can be one of the best alternative sources to replace the mammalian gelatins. However, the production of fish gelatin was quite low, with only 1 % of annual world production (Karim & Bhat 2009). Besides, the production of fish gelatin in Malaysia still in the research phase and need more attention from the researchers.

To overcome such problems, *Sardinella fimbriata* bone can be one of the alternative sources for gelatin. They are also free from any diseases and safe to consume. Several studies have been carried out for skin gelatin from different fish species, however only a few studies were conducted on the bone gelatin. In fact, no studies have been carried out on the extraction of gelatin from *S. fimbriata*. Therefore, this study could potentially provide new insights on the extraction method and on the physico-chemical properties of *S. fimbriata*. The objectives of this study were to determine the optimal method for gelatin extraction from *S. fimbriata* bones and to characterize its physico-chemical properties.

Material and Method

Preparation of raw materials. *S. fimbriata* bones were collected from fish processing industry and transported to the lab for sample preparation. The bones underwent series of washings to remove the flesh and other unwanted materials. The cleaned and dried bones were then stored at -20°C, until further use.

Extraction of gelatin. Gelatin extractions were carried out with four different methods selected from the available literature. The best selected method was further carried out for the characterization part.

Method A. Method A was carried out based on Sanaei et al (2013), where the bones were treated in 1 M of HCl at 4°C for 14 hours. The bones were then neutralized by four washings under tap water, until the pH reached 7. The bones were washed again with distilled water to remove any residuals and were mixed with distilled water at a ratio of 1:8 (g:mL). The gelatins were extracted at 67°C for 5 hours. Gelatin solutions were filtered and centrifuged at 7,800 g at 4°C for 20 minutes. The solutions were then dried in an oven at 60°C for 72 hours. The dried gelatins were then packed in zip locked bag and stored in a desiccator.

Method B. Method B was carried out based on Chandra et al (2013), where the bones were treated in 1 M of HCl at 27°C for 9 days. Acid was changed once in three days. The bones were then neutralized by washing for 9 times under tap water until their pH reached 5. The bones were then washed again with distilled water to remove any residuals and were mixed with distilled water at ratio of 1:3 (g:mL). They were then extracted at 60°C for 12 hours. Gelatin solutions were filtered and centrifuged at 7,800 g at 4°C for 20 minutes. The solutions were dried in an oven at 60°C for 72 hours. The dried gelatins were packed in zip locked bag and stored in a desiccator.

Method C. Method C was carried out based on Shakila et al (2012), where the bones were treated 2 times in 0.01 M of NaOH, 0.04 M of H_2SO_4 and 0.17 M of $C_2H_4O_2$, at 27°C for 45 minutes each. The bones were washed again with distilled water to remove any residuals and were mixed with distilled water at ratio of 1:1 (g:mL). They were then extracted at 45°C for 24 hours. The solutions were dried in an oven at 60°C for 72 hours. The dried gelatins were then packed in zip locked bag and stored in a desiccator.

Method D. Method D was carried out based on Cho et al (2004); where the bones were treated in 2 M of NaOH and 2 M of HCl at 8°C for 3 days. The bones were washed again with distilled water to remove any residuals and were mixed with distilled water at ratio of 1:7 (g:mL). They were then extracted at 65°C for 3 hours. Gelatin solutions were filtered and centrifuged at 7,800 g at 4°C for 20 minutes. The solutions were dried in an oven at 60°C for 72 hours. The dried gelatins were then packed in zip locked bag and stored in a desiccator.

Yield of gelatin. The percentage of yield was calculated based on the method of Sanaei et al (2013), according to the following formula:

$$Yield\ of\ gelatine\ (\%) = \frac{Weight\ of\ dried\ gelatine\ (g) \times 100}{Weight\ of\ dried\ fish\ bones\ (g)}$$

Proximate analysis of gelatin. Protein, ash, moisture and lipid content were analyzed following the method of AOAC (2000). The results of the samples were measured in triplicate and were recorded.

Color measurement of gelatin. The color of gelatin samples were determined according to Cheow et al (2007) using a color spectrophotometer (Chroma Meter CR-400, Konica Minolta, Japan). L*, a* and b* parameters indicate lightness/brightness, redness/greeness and yellowness/blueness, respectively. The reading of the color value was taken three times for an average value. The color changes (ΔE) of the L*, a* and b* parameters were further calculated to determine the whiteness of gelatins based on the reference color and was calculated according to the following formula:

$$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2}$$

Parameter L* shows the lightness of the sample. It differs from black (L=0) to white (L=100). The negative value of parameter a* represents a green color and a positive value signifies a red-purple color. The positive value of parameter b* demonstrates a yellow color and a negative value signifies a blue color. L0, a0 and b0 indicate color parameters of the reference color. White color parameter (L*=100, a0=0, b0=0) was used as a reference to control the whiteness color of the samples.

Gel strength analysis of gelatin. Gel strength was determined according to Gómez-Guillén et al (2002) with slight of modification. An amount of 6.67% (w v^{-1}) of solution was prepared by adding 0.33 g of gelatin to 5.0 mL of distilled water and dissolved at 60°C for 30 minutes. The gelatin solutions were filled in beakers and allowed to cool for 15 minutes at 25°C. The gelatins were kept in a refrigerator at 4°C for 18 hours. The gel strength of samples was measured by using texture analyzer (TA.XT. PLUS, Stable Micro Systems, United Kingdom) with a 5 kN load cell equipped with 1.27 mm diameter plunger. The maximum force determined a penetration depth of 4 mm into the center of gelatin gel, at a speed of 1 mm s^{-1}.

Melting point analysis of gelatin. The melting point of gelatin was determined according to Muyonga et al (2004), with slight modification. A number of 6.67% (w v^{-1}) of gelatin solutions were prepared by adding 0.33 g of gelatin to 5.0 ml of distilled water. The gelatin solutions were placed in a refrigerator at 4°C for 18 hours. The cooled gelatins were immediately transferred into the water at 10°C. The water was heated by using a hot plate magnetic stirrer. The temperature of the water bath was increased at 1°C for every 5 minutes at the rate of 0.2°C $minute^{-1}$. The temperature at which the gel drops to the bottom was recorded as melting point. All the data were recorded.

Amino acid analysis of gelatin. The amino acids composition was determined according to Sanaei et al (2013), with some modification. 0.1 g gelatin sample was dissolved in 5 mL of solution containing 6 M HCl, dried and hydrolyzed in the test tube at

110°C for 24 hours. The samples were then dissolved in 400 µl of solution containing 50 µmol of Amino Acid Standard. Deionized water was added to make up the solution until 100 µL. An amount of 10 µL of the sample was injected. Amino acid composition was examined by a High-Performance Liquid Chromatography (HPLC) (1525 Binary HPLC Pump, Waters, United States) equipped with Fluorescence Detector and AccQ Tag Column (3.9 x 150 mm). AccQ Eluent A and AccQ Eluent B or 60% acetonitrile acid were used as the mobile phase, with a flow rate at 1 mL min^{-1}.

Microstructure analysis of gelatin. The microstructure of gelatin gels were determined using Scanning Electron Microscopy (SEM) following the method of Jongjareonrak et al (2005) with a slight modification. Gel samples were freezed by liquid nitrogen, cut into small pieces and fixed with 2.5% of glutaraldehyde in 0.1 M of sodium cacodylate buffer for 1 hour. The fixed samples were rinsed with 0.1 M of sodium cacodylate buffer for 15 minutes and continued to be rinsed with 1% osmium tetraoxide in 0.1 M sodium cacodylate for 1 hour. The samples were then rinsed with 0.1 M sodium cacodylate buffer for 15 minutes. They were then dehydrated in alcohol with serial concentrations of 35, 50, 70, 80, 90, 95 and 100% (v v^{-1}). The samples were soaked in hexamethyldisilazane and dried in a dry cabinet for 24 hours. The dried samples were mounted on a specimen stub and coated with gold using Auto Fine Coater (JFC-1600, JOEL, Japan). The specimens were then examined on a Scanning Electron Microscopy (SEM) (Analytical Scanning Electron Microscope JSM-6360LA, JOEL, Japan).

Statistical analysis. The average mean values were calculated from three replicate of each experiment and expressed with standard deviations. All data were subjected to Analysis of Variance (ANOVA) and the differences between means were evaluated by post hoc test. The SPSS statistic program (IBM SPSS Statistics version 22.0) was used for data analysis. The level of significance difference was considered at $P<0.05$.

Results and Discussion

Best extraction method for fish gelatin. The percentage of yield and protein content of the *S. fimbriata* gelatin extracted with 4 different extraction procedures were shown in Table 1. The results obtained in the yield percentage of gelatin for all methods were 6.0±0.03% (Method A), 3.4±0.49% (Method B), 1.3±0.12% (Method C) and 0.5±0.19% (Method D). It has shown that the yield of gelatin extracted from Method A was significantly higher than in Methods B, C and D. While Method D has a lower percentage of yield compared to the other methods. The results show that Method A can be potentially accepted as it is in line with previous studies in which the yield percentage of fish gelatin ranged from 6 to 19% (Karim & Bhat 2009).

Table 1

Percentage of yield and protein content of *Sardinella fimbriata* gelatin extracted with four different methods of extractions

Analysis (%)	*Method A*	*Method B*	*Method C*	*Method D*
Yield	6.0 ± 0.03^{a}	3.4 ± 0.49^{b}	1.3 ± 0.12^{c}	0.5 ± 0.19^{d}
Protein	59.1 ± 0.29^{a}	51.1 ± 1.19^{b}	56.5 ± 1.05^{c}	14.2 ± 0.84^{d}

Values are given as mean ± standard deviation of triplicate. Means in the same row with different superscript letter are significantly different ($P<0.05$).

Furthermore, yield of *S. fimbriata* gelatin extracted from Method A was significantly higher than the other methods. This could be due to different extraction procedures that involved different extraction times and temperatures (Jamilah & Harvinder 2002). Besides, previous studies showed that low extraction temperature, especially at room temperature, resulted in low yield (Sanaei et al 2013), which may be due to incomplete hydrolysis of the collagen and to its loss during the washing process (Jamilah & Harvinder 2002). Besides, acid treatment that was carried out in Method A contributed to the

increase of the production of gelatin. This process may have removed some acid-soluble proteins, lipid and unnecessary compounds, disrupting the cross-links of collagen molecules in the bone, which facilitates the gelatin extraction (Ahmad et al 2011).

It is interesting to find out that *S. fimbriata* bone gelatin (Method A) demonstrated higher yield compared to other species tested in previous studies such as from black tilapia (5.4%) and catfish (5.8%). However, the yield of *S. fimbriata* bone gelatin (Method A) was much lower than in red tilapia (7.8%) and yellowfin (18.0%) (Alexandre et al 2014). This could be due to the difference of collagen content in each species. This finding was also supported by previous studies conducted on threadfin bream (43.6%) and grouper (68.5%) which have demonstrated different collagen content in different fish species (Irwandi et al 2009).

In protein analysis, Method A exhibited higher protein content than the other methods, in which the protein content was 59.1±0.29%. This is in line with the previous findings on the yield percentage of *S. fimbriata* bone gelatin obtained from Method A, in which the protein content was 59.1±0.29%. The second highest protein content was from Method C which demonstrated 56.5±1.05%, followed by Method B that has 51.1±1.19% and the least was found by the method D that demonstrated 14.2±0.84% of protein content. According to a previous study, the protein content of the collagenous material represented the maximum possible yield of gelatin (Muyonga et al 2004). It is interesting to find out that *S. fimbriata* bone gelatin obtained by the Method A possessed higher protein content compared to sin croaker fish (24.8%) and Nile perch (20.0%). However, the protein content of *S. fimbriata* bone gelatin obtained by the Method A was much lower than in pangasius catfish (87.1%) and Nile tilapia (82.5%) (Ratnasari et al 2013). The difference of protein content in this study could be related to the process involved during the extraction (Kusumaningrum et al 2018).

Physico-chemical properties of extracted fish gelatin

Proximate content. The proximate composition of *S. fimbriata* bone and bovine gelatin is shown in Table 2. Four analyses were conducted for the proximate analysis: protein, moisture, lipid and ash.

Table 2

The proximate composition of gelatins from *Sardinella fimbriata* bone and bovine gelatin

Analysis (%)	*S. fimbriata bone*	*Bovine*
Protein	59.1±0.29[b]	82.2±4.88[a]
Moisture	14.3±0.21[b]	13.1±0.55[a]
Lipid	0.76±0.44[a]	0.41±0.21[a]
Ash	4.7±0.21[b]	2.3±0.11[a]

Values are given as mean ±standard deviation of triplicate. Means in the same row with different superscript letter are significantly different (P<0.05).

S. fimbriata bone gelatin exhibited a lower protein content (59.1±0.29%) compared to bovine gelatin (82.2±4.88%). The different amount of protein content may be influenced by the process of extraction (Kusumaningrum et al 2018). Besides, the maximum possible amount of gelatin was related to the amount of protein content of the collagenous materials (Muyonga et al 2004). The higher the protein content, the higher the gelatin yield.

A high moisture content was obtained from *S. fimbriata* bone gelatin which has 14.3±0.21%, while bovine gelatin only has 13.1±0.55%. A previous study by Kusumaningrum et al (2018) stated that the maximum amount of moisture content for the fish gelatin ranges from 14 % to 16 %, which is within the acceptable limits.

In lipid analysis, *S. fimbriata* bone gelatin showed a higher lipid content compared to the bovine gelatin, with an amount of lipid of 0.76±0.44% and 0.41±0.21%, respectively. Lipid content of *S. fimbriata* bone and bovine gelatin were below the threshold of 1% (Cho et al 2004).

The results obtained for the ash content for all samples were 4.7±0.21% for the *S. fimbriata* bone gelatin and 2.3±0.11% for the bovine gelatin. It shows that the bovine gelatin has a much lower ash content compared to the *S. fimbriata* bone gelatin. Usually, an amount of ash content up to 2.0% can be accepted in food applications (Cho et al 2004), which are in the acceptable limits. Besides, the different amounts of ash found in this study may probably be due to different amounts of minerals in the fish bones (Zied et al 2011).

Color. The color measurement of the gelatin was carried out to determine its potential to be commercialized in the industry. The color values in this study were expressed as L*, a* and b*, and color changes (ΔE) were calculated. ΔE determines the whiteness of gelatin samples based on the reference color. The ΔE with the lowest value indicates that the color is closer to white color, which shows high purity. The color values in this study are shown in Table 3.

Table 3

Analysis of color value for *Sardinella fimbriata* bone and bovine gelatin

Color value	*S. fimbriata bone*	*Bovine*
L* (Lightness)	70.5±2.87[a]	82.9±1.56[c]
a* (Redness)	-1.9±0.03[a]	-1.9±0.07[c]
b* (Yellowness)	24.4±0.49[a]	14.1±0.43[c]
Color changes (ΔE)	38.3±0.27[a]	22.2±0.11[c]

*Values are given as mean ± standard deviation of triplicate. Means in the same row with different superscript letter are significantly different (P<0.05).

In comparison with the bovine gelatin, the results indicated that *S. fimbriata* bone gelatin had a higher color changes (ΔE) than the bovine, which shows some yellowness. This may be due to the ineffectiveness of the impurities removal during the extraction process, as stated also by Chun et al (2015), who mentioned that the commercial gelatin has an improved color due to processes of chemical clarification and impurities filtration from the gelatin solution. However, the color of the gelatin is not affecting the properties of gelatin (Shahiri et al 2013).

Gel strength. One of the most important physical properties in gelatin is the gel strength (Sanaei et al 2013). Table 4 shows the gel strength of gelatin extracted from *S. fimbriata* and bovine gelatin. The results obtained were 132.4±2.64 g and 350.5±8.48 g, respectively. According to a previous study, the gel strength of fish gelatin ranges from 124 to 426 g, compared 200 to 350 g for commercial gelatin, such as bovine and porcine (Zied et al 2012). It means that gelatin obtained in this study is within the acceptable range.

Table 4

Gel strength and melting point of gelatin from *Sardinella fimbriata* bone and bovine gelatin

Analysis	*S. fimbriata* bone	Bovine
Gel strength (g)	132.4±2.64[a]	350.5±8.48[c]
Melting point (°C)	25±1.52[a]	38±1.53[c]

*Values are given as mean ± standard deviation of triplicate. Means in the same row with different superscript letter are significantly different (P<0.05).

Fish gelatin demonstrated a poor gel strength as compared to bovine gelatin. This may be due to the extraction process that might affect the strength of the gel (Tinrat & Sila-Asna 2017). Herpandi et al (2011) revealed that salmon and bigeye snapper had the gel strength values of 108.0 g and 105.7 g, respectively, lower than in this study, but the gel strength of Nile tilapia, which is of 252.0 g, was higher. The gel strength's value

variations among these species may be due to the proline and hydroxyproline imino acids content. According to a previous study, the difference of gel strength is correlated to the amino acids composition and to the size of the protein chain (Muyonga et al 2004). Lower gel strength values may be related to low concentrations and to the molecular weight distribution (Ockerman & Hansen 1988).

Melting point. As a thermo-reversible gel, gelatin gel will start to melt at a certain temperature, called the gel melting point. Previous study stated that melting point for fish and bovine gelatin range from 11 to 28°C and from 28 to 38°C, respectively. In this study, Table 4 shows the melting points of gelatin from *S. fimbriata* bone and bovine gelatin. The results were 25±1.52°C and 38±1.53°C, respectively. Fish gelatin usually has much lower melting points, compared to the mammalian gelatin, such as bovine and porcine (Cho et al 2005), which is also supported by previous studies conducted on other species, such as sin croaker, cod, yellow tuna and tilapia, that have 18.5°C, 13.5°C, 24.5°C and 25.4°C, respectively. Differences in melting point values may be due to different amino acid contents. It is interesting to find out from previous studies that the lower concentrations of proline and hydroxyproline affected the gelatin characteristics, including the melting point (Gómez-Guillén et al 2002). Besides, the origin of the raw material used in the process greatly influenced the melting point values (Gilsenan & Ross-Murphy 2000).

Amino acid. Amino acids composition characterizes the quality of the gelatin. Table 5 shows the amino acid composition of gelatin from *S. fimbriata* bone and bovine. There were seventeen amino acids found in these gelatins. The dominant amino acid in *S. fimbriata* bone and bovine gelatin was the glycine, with 209.50±6.32 mg g^{-1} and 373.91±6.21 mg g^{-1}, respectively. These results were further supported by a previous study that states that glycine was the dominant amino acid in carp gelatin (Duan et al 2009). *S. fimbriata* bone gelatin has a lower glycine concentration, compared to the bovine gelatin. The result obtained were consistent with the findings of Zhang et al (2010) and Liu et al (2008), stating that fish gelatin demonstrated lower quantities of glycine compared to the commercial gelatin.

Table 5

Amino acid compositions (mg g^{-1}) of gelatin from *Sardinella fimbriata* bone and bovine gelatin

Amino acids (mg g^{-1})	*Gelatins*	
	S. fimbriata bone	*Bovine*
Glutamic acid	88.77±4.6	156.19±15.75
Phenylalanine	20.82±0.34	45.51±7.09
Tyrosine	6.87±0.11	17.47±2.98
Glycine	209.50±6.32	373.91±6.21
Proline	107.65±3.25	225.84±3.93
Alanine	96.64±3.68	147.20±6.34
Methionine	26.55±0.20	28.13±3.87
Hydroxyproline	89.05±0.49	230.66±26.6
Aspartic acid	51.27±3.53	86.55±11.82
Valine	21.81±0.32	49.43±2.32
Isoleucine	10.24±0.03	23.46±1.33
Leucine	23.53±0.22	54.75±2.52
Lysine	32.76±1.81	61.92±4.63
Serine	33.70±0.14	70.68±8.09
Histidine	9.83±0.22	20.43±3.95
Arginine	80.61±0.87	169.51±25.53
Threonine	23.95±0.14	40.39±6.57

*Values are given as mean ± standard deviation of triplicate. Means in the same row with different superscript letter are significantly different (P<0.05).

The most important amino acids in gelatin were proline and hydroxyproline, calledimino acids. *S. fimbriata* bone gelatin shows a lower imino acid content: 107.65±3.25 mg g^{-1} of proline and 89.05±0.49 mg g^{-1} of hydroxyproline compare bovine gelatin. Bovine gelatin has 225.84±3.93 mg g^{-1} of proline and 230.66±26.6 mg g^{-1} of hydroxyproline. The results' variability may be caused by the differences in the methods used during the extraction process. It has been proven by a previous study that different amounts of hydroxyproline contents affected the extraction process (Nikoo et al 2011). Besides, generally, mammalian gelatin contains higher imino acids, compared to the fish gelatin (Shyni et al 2014). Gelatin with a low imino acid content tends to have a low melting point and gel strength (Muyonga et al 2004). This is in line with the previous findings which show that *S. fimbriata* bone gelatin have a lower gel strength and melting point than bovine gelatin.

Microstructure. Figure 1 shows the microstructure of gelatin from *S. fimbriata* bone and bovine gelatin. Generally, all the microstructure of the gelatin gels demonstrated a sponge shape (Phanat & Soottawat 2017). The conformation and protein chain length in gel matrix and the presence of α-, β- and γ-chains directly related with the gel strength (Benjakul et al 2012). Gelatin shows a network of interconnected pores with various uniformity degrees of the strands and pore sizes. *S. fimbriata* bone gelatin has a non-uniform network, looser strands and big pores, while the bovine gelatin has a uniform network, denser strands and small pores. According to a previous study, the arrangement and combination of protein molecules in the gel matrix affected the gel strength of the gelatin (Benjakul et al 2009). Besides, microstructure could be related to the gel strength values, the denser strands causing a higher gel strength than the looser strands (Sutee & Athapol 2009). This is in line with the previous findings on the gel strength analysis, in which *S. fimbriata* bone gelatin has lower gel strength and looser strands.

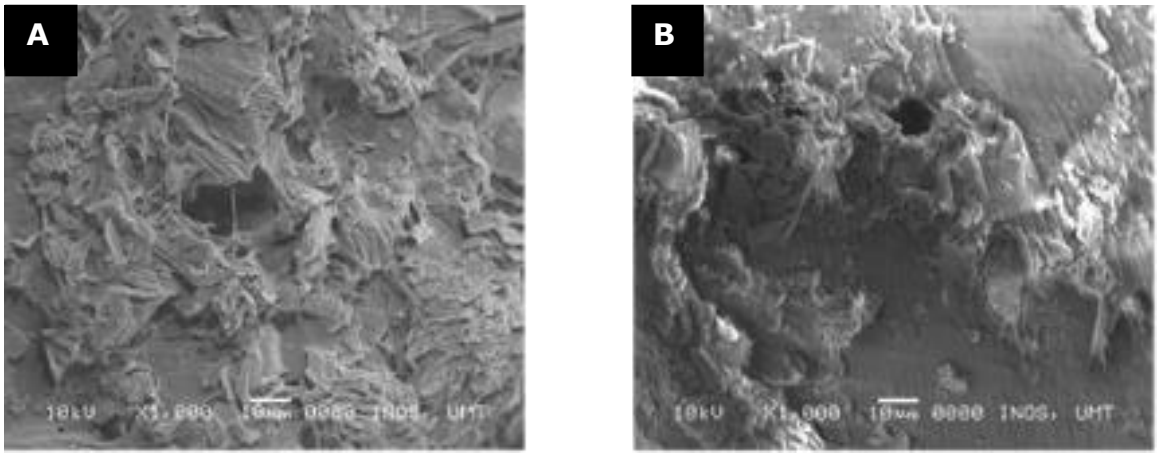

Figure 1. Image of Scanning Electron Microscopy (SEM) of gelatin from *Sardinella fimbriata* bone gelatin (A); and bovine gelatin (B) with 10,000x of magnification.

Conclusions. The best extraction method for *S. fimbriata* bone gelatin was the extraction with 1 M of HCl at 67°C for 5 hours. *S. fimbriata* bone gelatin demonstrated a high quality; therefore it could replace the commercial mammalian gelatins in food applications. However, further studies should be conducted to characterize the properties of *S. fimbriata* bone gelatin. Research is also needed for the development of product that takes advantage from the properties of *S. fimbriata* bone gelatin. Overall findings have suggested the potential of gelatin from *S. fimbriata* bone to meet the requirements for Halal and Kosher markets.

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Conflict of interest. The authors declare no conflict of interest.

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