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Physicochemical and structural properties of Asian Swamp Eel *(Monopterus albus)* skin gelatin as compared to bovine gelatin

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Abstract

The aims of this study are to report on the extraction and characterization of Asian swamp eel *(Monopterus albus)* skin gelatin. The characterization conducted were includes chemical composition, pH, gel strength, viscosity, thermal property, color and structure determination of extracted eel skin gelatin. Eel skin contains 70.28% moisture, 11.07% protein, 4.21% fat, and 5.01% ash. The chemical composition of eel skin gelatin (yield of 12.75%) was 18.8% moisture, 67.64% protein, 0.34% fat and 1.08% ash, with a pH of 4.62 and gel strength of 215.96 g (± 9.62 g). Although viscosity (2.8 cPa/min) profile of eel skin gelatin showed lower than that of bovine gelatin, the higher melting temperature (35 °C) of eel skin gelatin indicating its higher stability than bovine gelatin with FTIR spectrum similar to that of typical bovine gelatin. Eel skin gelatin has a 71.4 (± 1.14), a +3.2 (± 0.29), and a +7.52 (± 0.29) for L^*, a^* and b^* value respectively, indicate a darker and less yellow colour. These findings show promising potential for the application of eel skin gelatin as an alternative to commercial gelatin.

Introduction

Gelatin is a polypeptide produced by the partial hydrolysis of collagen derived from animal skin, connective tissue, and bones (Morrison *et al.*, 1999). Gelatin has gelling, foaming and emulsifying properties that contribute to a wide range of applications in food, pharmaceutical, photographic and cosmetic industries. The unique properties of gelatin are solubility in water and its ability to form thermo-reversible gels with a melting temperature close to human body temperature (Zhou *et al*., 2006). Most available gelatins are manufactured from mammalian sources such as pig skin, cattle hide and bones. However, other sources of gelatin are becoming increasingly relevant, such as fish bone, scales and skin (Jongjareonrak *et al*., 2006)).

Gel strength or bloom value, including low (<150), medium (150–220), and high bloom (220–300), determines the quality of gelatin and its viscoelastic properties such as gelling and melting. Rheological data are required for the analysis of flow conditions in different food processing operations and the measurement of texture (Binsi *et al*., 2009). The quality of gelatin depends on its physicochemical properties which are influenced by the species, tissue and processing method(s). The rheological properties of thermo-reversible gelatin gels are primarily a function of temperature and the concentration of gelatin for a given gelatin type. The development of gelatin alternatives has gained importance in recent years due to demands for non-bovine and non-porcine gelatin that have increased due to the BSE (bovine spongiform encephalopathy) crisis, as well as for religious and social reasons. Hence, there has been much concern about gelatin derived from possibly infected animal parts. Pig skin gelatin is not acceptable in Judaism and Islam, and beef gelatin is acceptable only if it has been prepared according to religious requirements (Badii and Howell, 2006). Therefore, food processors highly desire the development of gelatin alternatives for a rapidly growing, certified halal global food market (Karim and Bhat, 2009).

To date however, few alternatives to mammalian gelatin are available. A number of studies on developing gelatin substitutes for mammalian gelatin have been reported, including harp seal skin (Arnesen and Gildberg, 2002); horse mackerel skin (Badii and Howell, 2006); sin croaker and shortfin scad skin (Cheow *et al*., 2007); and black and red tilapia skin (Jamilah and Harvinder, 2002). Similarly, in South Korea, investigations on the feasibility of using chicken feet to replace cowhides for jokpyun (traditional Korean gel-type food) have been undertaken (Jun *et al*., 2000). Additionally, there is growing interest in developing alternative substitute raw materials such as chicken bone and fishery by-products (Lim *et al*., 2001).

The Asian swamp eels, *Monopterus albus* are

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widely distributed in many countries from India to China, Japan, Malaysia, Indonesia, Bangladesh, Thailandand Vietnam (Guan *et al*., 1996; Froese and Pauly, 2008). The fish, which is considered a nutritious and tasty species, is also a valued remedy in medicinal practices (Khanh and Ngan, 2010). As the market of eels getting high demand, many countries such as China, Philippines and Vietnam start the eel culture activities while in Malaysia, the activities are still unsuccessful (Subasinghe and Hasan, 2010; Khanh and Ngan, 2010; Baquiran and Prudencio, 2013). China is by far the largest producer of farmed Japanese eels (e.g. 73% of global production in 2003), but the Taiwan Province of China, as well as Japan, are also major producers. Other countries declaring farm production to FAO standards for this species are the Republic of Korea and Malaysia. Raising this species is easy to do, considered low cost to the farmers and achieves more profit than some other small size fish culture activities (Khanh and Ngan, 2010). In Vietnam, seed production initially succeeded by leaving swamp eel *(Monopterus albus)* to grow naturally in tanks (Khanh and Ngan, 2009). In recent years, the marketable ricefield eel culture has strongly increased in some areas of Vietnam with the farm gate price of 85,000-100,000 VND or RM12-RM15 per kg eel (Khanh and Ngan, 2010). Besides that, eels were sold at 50 pesos or RM3.66 per kilogram in Tuguegarao City, Philippines (Baquiran and Prudencio, 2013). Eels are consumed mainly by domestic market and some by export (Khanh and Ngan, 2010).

To our knowledge, there are no reported studies on the production of gelatin from eel skin. As skin is a waste by-product of fish processing, it may be possible to replace mammalian sources of gelatin with gelatin extracted from eel skin. The production of gelatin from this species which traditionally believe to have high beneficial value which expected from its bioactive properties will expand or broaden the application of gelatin especially in food, pharmaceutical and cosmetic industries.

Therefore, the objectives of the present study were to prepare gelatin extracted from eel skin and then compare the physicochemical and structural properties of the extracted gelatin with available commercial bovine gelatin.

Material and Methods

Materials

Wild Asian swamp eel *(Monopterus albus)* with average size 40-60 cm in length and 370-400 g in weight were purchased fresh from a local market in Kuala Terengganu, Terengganu, Malaysia. It was transported in ice to the laboratory for beheading and gutting. The skins were removed manually after filleting, then cleaned and stored at -80°C until use. Commercial bovine gelatin (Halagel), imported from Pakistan, was used for comparison (Ahmad, 1999). All chemicals used in the process of extraction were of analytical grade.

Sample preparation

The skins were thawed in a chiller at 4–5°C for overnight followed by thorough water rinse in order to remove impurities, cut into small portions after which they were uniformly placed on a tray and dried in a cabinet dryer at 40°C overnight.

Extraction

Gelatin was extracted from Asian swamp Eel *(Monopterus albus)* skin according to the method described by Badii and Howell (2006); Sarbon *et al.* (2013), with some modification. About 20 g of dried eel skin was mixed with 200 ml (0.15% w/v) sodium hydroxide (NaOH). The mixture was shaken well and slowly stirred at room temperature for 40 min before being centrifuged at 4000 x g for 5 min. The alkaline treated pellets were rinsed with distilled water. The resulting pellets were then mixed with 200 ml (0.15 % v/v) of sulphuric acid (H_2SO_4), followed by 200 ml of citric acid solution 0.7 % (w/v). The alkaline solution was changed every 40 min to remove non-collagenous proteins and pigments. Each treatment was repeated three times and each treatment takes about 2 h for completion. The pellets were then subjected to a final wash with distilled water to remove any residual salts followed by centrifuging at 4000 x g for 10 min. The final extraction was carried out in distilled water at a controlled temperature of 45°C overnight without stirring. The resultant mixture was filtered in a Buchner funnel with Whatman filter paper (no.4). The pH was adjusted to 6.0 with 6 N NaOH. The volume was reduced to 1/10 by evaporation under a vacuum at 45°C, and then kept in a freezer at -80°C overnight before being freeze-dried. The dry matter was referred to as 'gelatin powder'. Below is the formula used to calculate the yield of extracted gelatin.

$$\text{Yield (\%)} = \frac{\text{Weight of freeze-dried gelatin X 100}}{\text{Weight of dried eel skin}}$$

Characterization of eel skin gelatin

Chemical composition analysis

The moisture, protein, fat and ash contents of

extracted gelatin were determined according to the AOAC standard (1995). A factor of 5.55 was used to convert the nitrogen value to gelatin protein.

pH determination

This method follows the British Standard Institution method (BSI 757, 1975). The pH of raw eel skin and extracted gelatin was measured using a pH meter with a glass electrode (pH-103 Merthom/ Brinkmann, Brinkmann Instrument Inc., Westbury, NY). The pH meter was calibrated using a buffer (Certified Buffer Solution, pH 4.00 ± 0.01, at 25°C, and pH 7.00 ± 0.01, at 25°C; Fisher Scientific Co., Fail Lawn, NJ, lot 87623-24). Samples were weighed to 1 g and then diluted in 100 ml of distilled water to form a 1% (w/v) eel skin and eel skin gelatin solution. Subsequent readings taken were an average of three determinations.

Determination of gel strength

The gel strength (bloom value) of gelatin gel was determined according to the method described by Wainewright (1977). The gel was formed by dissolving a (6.67% w/v) dry gelatin powder in distilled water at 60°C. The jar was covered and allowed to cool for 15 min at room temperature. Bloom jars with gelatin solution were kept in a refrigerator at 7°C (maturation temperature) for 16–18 hours. Gel strength at 8–9°C was determined by the TA.XT2i Texture Analyzer (Stable Micro System, Godalming, UK) according to British Standard BS 757 (BSI, 1975), with a load cell of 5 kg (cross-head speed 1 mm/s), equipped with a 0.5 in diameter, flat bottomed plunger. The standard glass Bloom jar (capacity 150 mL, overall height 85 mm, inside diameter 59 mm) was centrally placed under the plunger and the penetration test was then performed. The maximum force (in g) was determined when the probe penetrated the gel to a depth of 4 mm. Readings were then averaged from three determinations.

Determination of gelatin viscosity

The viscosity of gelatin samples was determined according to the method of Cho *et al*. (2005). Gelatin solutions (10 g/100 ml) were prepared by dissolving the gelatin powder in distilled water which was then heated to 60°C. Viscosity was determined by using a Brookfield digital viscometer (Model LV-DV-II, Brookfield Engineering; MA, USA), equipped with spindle No. 2 (Vane Spindle Set) at 100 rpm. The viscosity (cPa) of a gelatin solution is determined at 60.00 ± 0.05ºC and the reading was taken in triplicate.

Determination of melting temperature

The gelatin's melting temperature (T_m) was determined by a differential scanning calorimetry (DSC) technique (Perkin Elmer Instruments, Norwalk, USA). Gelatin powder with water as a reference of approximately 10 mg were weighed using the Metler Toledo precision balance (AL 204, Mettler- Toledo Ltd., Beaumont Leys Leicester, UK) and then kept in air-tight aluminum pans. These were analyzed at a heating rate of 10°C/min ranging from 0 – 80°C. The helix coil transition temperature (T_m) was calculated as the melting temperature where the endothermic peak occurred. The temperature at which one-half of the gelatin denatured was taken as the top of the peak. The total energy required for denaturing the protein (the enthalpy change, ΔH) was measured by integrating the area under the peak. The endothermic peak was selected as the melting temperature for gelatin gels and an average reading was taken from three determinations.

Determination of color

Gelatin color was measured by using the Minolta colorimeter (Model CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) based on three color co-ordinates, namely L^*, a^*, b^*. Color values were expressed as follows: L^* (whiteness or brightness/ darkness); a^* (redness/greenness); and b^* (yellowness/ blueness). The instrument, (65°/0° geometry, D25 optical sensor, 10° observer), was calibrated by using white (L = 92.8; a = -0.8, b = 0.1) and black reference tiles. Total color difference (TCD) indicating the magnitude of color change after treatment was then calculated. Color values (L^*, a^* and b^*) were recorded as a mean based on three determinations.

Fourier transforms infrared spectroscopy (FTIR)

In order to determine the structure conformation of extracted eel skin gelatin, the functional group possessed by the gelatin has been investigated by FTIR technique. The FTIR spectra were obtained from discs that contained 1 mg of gelatin powder in approximately 10 mg potassium bromide (KBr). To form a disc, all required equipment was cleaned with acetone. A mixture of a sample and KBr was then ground and well blended, then placed in a palletizer to form a miniature thin disc. The disc was then inserted in the Bruker infrared spectrophotometer (Bruker Instruments, Billerica, MA). Spectra from 4000 to 500 cm^{-1} were obtained at a data acquisition rate of 2 cm^{-1} per point, and background deduction was accomplished with Opus software (Bruker Instruments, Billerica, MA). Triplicate samples of gelatins were analyzed and each sample's reading

was then averaged from three independent readings. Fourier self-de-convolution was conducted on the average spectra for the amide I band by using a resolution enhancement factor of 1.8 and a full height band width of 13 cm^{-1}. The self-de-convolution process provided data on component locations and incidence. Curve fitting was then performed with peak fit software (SPSS Inc., Chicago, IL, USA).

Statistical analysis

Triplicate data collected in this study were analyzed using the MINITAB, version 14.0. All data was subjected to analysis as a mean ± SD from three determinations. The independent t-test was used to determine significant differences between means ($p < 0.05$).

Results and Discussion

Extraction of gelatin

The yield of extracted gelatin (12.75%) obtained in this study was determined by the dry weight of eel skin samples (Table 1).

Table 1. Chemical composition of raw eel skin and eel

Composition (%)	Raw eel skin	Gelatin	
		Eel skin	Bovine
Yield	-	12.75	-
Moisture	70.28 ± 1.841	18.8 ± 50.10^{a}	4.26 ± 0.20^{b}
Protein	11.07 ± 0.05	67.64 ± 0.03^{b}	74.31 ± 0.02^{a}
Fat	4.21 ± 0.02	0.34 ± 0.03^{a}	0.20 ± 0.02^{b}
Ash	5.01 ± 0.06	1.08 ± 0.06^{a}	0.70 ± 0.05^{b}
pH	4.27 ± 0.01	4.62 ± 0.02^{a}	3.12 ± 0.06^{b}

Note: $^{a, b}$ indicates that rows with different letters are significantly different ($p < 0.05$)

Other studies have reported yields for fish skin gelatin as follows: 14.3% and 7.25% for sin croaker and shortfin scad, respectively (Cheow *et al*., 2007); and 7.81% and 5.39% for red and black tilapia skin, respectively (Jamilah and Harvinder, 2002). Our results demonstrated significantly higher yields for eel skin gelatin as compared to red and black tilapia yields. The variance of gelatin yield reported for fish is mainly due to differences in collagen content, fish skin composition, as well as fish skin matrix (Jongjareonrak *et al*., 2006). Variations in yield have also been reported due to diverse extraction methods (Jamilah and Harvinder, 2002; Muyonga *et al*., 2004; Jongjareonrak *et al*., 2006). However, such information is not always available in published data (Songchotikunpan *et al*., 2008). Furthermore, reporting gelatin yield as 'dry gelatin weight' compared to the weight of 'wet skin' is common and reliable. Water content may vary because of different treatments such as freezing, salting, scraping, draining, etc. (Arnesen and Gildberg, 2006).

Gelatin extraction from fish skin is generally achieved by pretreatment with acid or alkali to obtain the desired properties (Arnesen and Gildberg, 2006; Cho *et al*., 2006). The degree of collagen conversion to gelatin is related to the intensity of both the pre-treatment and extraction processes, and also depends upon pH, temperature, and extraction time (Johnston-Banks, 1990). The aim of alkali or acid pre-treatment is to weaken the collagen structure in order to dissolve non-collagen proteins and hydrolyze peptide bonds while maintaining the consistency of collagen fibers (Wainewright, 1977). Hence, during our final step of collagen conversion to gelatin, the extraction temperature was kept at 40–45°C in order to achieve a controlled partial hydrolysis of cross-links and peptide bonds in the original collagen structure. Furthermore, yields at 50°C of extraction have been reported to be better than those at 40°C, even though the quality is lower at 50°C of extraction (Cho *et al*., 2006).

Gelatin Characterization

Chemical composition and pH determination of eel skin gelatin

The chemical compositions of eel skin and bovine gelatin are presented in Table 1. The protein content of freeze-dried eel skin gelatin was 67.64%, while moisture, fat and ash content were 18.8%, 0.34%, and 1.08%, respectively. The protein content of eel skin gelatin was similar to other types of fish skin gelatin as previously reported by Cheow *et al*. (2007), who reported its content at 69.2% and 68.7% for sin crocker and shortfin scad skins, respectively. Although the fat content of eel skin gelatin was significantly higher than bovine gelatin, generally ($p<0.05$), extracted eel skin gelatin is fat free. According to Jones (1977), extracted gelatin with fat content <0.5% was considered as fat free gelatin. In addition, the ash content of eel skin gelatin remained lower than the maximum recommended level (3.0%). The pH (4.62) of the extracted eel skin gelatin also indicated its category (Type B). In the present study, alkali pre-treatment was employed during the extraction process. pH play an important role in determining the stability and mechanical properties of gelatin produced, especially in the production of gelatin films.

Gel strength of eel skin gelatin

The gel strength of eel skin gelatin [6.67% (w/v) in distilled water] which prepared for the present

study showed a significantly higher gel strength of 215.96 g (±9.62 g) ($p < 0.05$) when compared to bovine gelatin (181.28 g ± 9.10 g). This is most likely due to the intrinsic characteristics of its protein chain composition, molecular weight distribution, amino acid content (especially proline and hydroxyproline), as well as its collagenous properties and the extraction method (Sarbon *et al*., 2013). Fish gelatin typically has a gel strength range of 0–270 g (tested under standard Bloom test conditions), as compared to gel strength values for bovine or porcine gelatin which have gel strength of 200–240 g. Gel strengths for various fish skin gelatins were reported at 98 g for Alaska Pollock (Zhou *et al*., 2006); 124.9 g and 176.9 g for sin croaker and shortfin scad, respectively (Cheow *et al*., 2007); 150 g for hake and 260 g for harp seal (Arnesen and Gildberg, 2002). However, a gel strength as high as 426 g has been reported for yellowfin tuna (Cho *et al*., 2005). Some species of warm water fish gelatins have been reported to have relatively high gel strength values, close to the high gel strength value of porcine gelatin (Gudmundsson and Hafsteinsson, 1997). The wide range of gel strength values found for various gelatins arises from differences in proline and hydroxyproline content in the collagen of different species, and is also associated with the temperature of the animal's habitat. Badii and Howell (2006) have shown that hydrophobic amino acids (Ala, Val, Leu, Ile, Pro, Phe, Met) may also contribute to the high gel strength value of tilapia gelatin when they found a lower number of hydrophobic amino acids in the commercial, non-gelling cod gelatin, as compared to tilapia and horse-mackerel gelatin. It is well established that proline and hydroxyproline are responsible for the stability of the triple-helix collagen structure as the result of hydrogen bonding between free water molecules and the hydroxyl group of the hydroxyproline in gelatin (Badii and Howell, 2006). According to Arnesen and Gildberg (2002), the low hydroxyproline content in fish skin gelatin is a major reason for the lower gel strength of these gelatins.

Viscosity of eel skin gelatin

Figure 1 shows a comparison of eel skin gelatin with bovine gelatin based on the gelatin viscosity rate, and demonstrates no relative difference in the change of viscosity rates between eel skin and bovine gelatin during a heating (60.00 ± 0.05ºC) time of 60 minutes. However, the viscosity rate (cPa/min) of eel skin gelatin (2.8 cPa/min) was lower than the viscosity rate of bovine gelatin (3.2 cPa/min). Although eel skin gelatin has a slower viscosity rate compared to that of bovine gelatin, it remains comparable based on

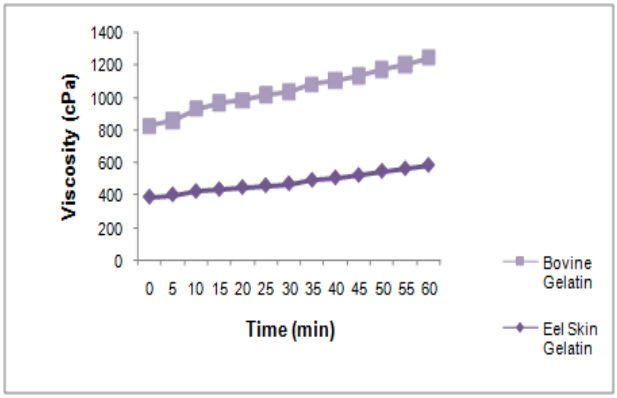

Figure 1. Comparison on viscosity rate between eel skin and bovine gelatin

the mimic pattern of modulus increment. A study by Yang and Wang (2009) found that the viscosity rate of gelatin decreased with cooling time, which indicates that gelatin concentrates when cooling and becomes harder to sweep during this period. Theoretically, the molecular weight distribution appears to play a greater role in the effect on viscosity than gel strength. Some gelatins with higher gel strength may have lower viscosities than gelatins of lower gel strength (GMIA, 2012). The viscosity of gelatin solutions increases with increasing gelatin concentration and decreasing temperature. Furthermore, viscosity is minimized at the isoelectric point (GMIA, 2012). This graph (Figure 1) characterizes gelatin properties during cooling as the mixture become increasingly viscous.

Melting temperature of eel skin gelatin

The melting point of eel skin gelatin at a heating rate of 10°C/min was significantly higher (35°C) than that of bovine gelatin (22.5°C) ($p < 0.05$). The higher melting temperature of eel skin gelatin may due to its higher gel strength as discussed above. (The data were not shown). The higher gel strength value contributed to higher melting and gelling points and also to the shortened gelling time of the final product (Babin and Dickinson, 2001). These melting points are much higher than those reported for sin croaker (17.7°C) and shortfin scad (23.8°C) (Cheow *et al.*, 2007). It is also known that fish gelatin has a lower melting point than mammalian gelatin, and that the amino acid composition may also contribute to melting point characteristics (Norland, 1990). This finding indicates that the structural stability of eel skin gelatin is higher than that of bovine gelatin. As a thermo-reversible, gelatin gels begin melting as the temperature increases above a certain point called the gel melting point, which is generally lower than the human body's temperature. The melt-in-the-mouth property of gelatin gels has become one of its most important characteristics, and is widely used as a

factor in food and pharmaceutical industries.

Color analysis of eel skin gelatin

Table 2 shows the instrumental color measurement comparison of eel skin and bovine gelatin.

Table 2. Comparison based on instrumental color measurements of eel skin and bovine gelatin

	L* value	a* value	b* value
Eel skin gelatin	71.40 ±1.14	+3.2 ±0.29	+7.52 ±0.29
Bovine gelatin	78.19 ±0.81	+1.44 ±0.15	+21.37 ±0.70

Note: L^* = whiteness or brightness/darkness; a^* = redness/ greenness; b^* = yellowness/ blueness.

The 'L' value of bovine gelatin [78.19] was significantly higher ($p < 0.05$) than that of eel skin gelatin [71.4]. Eel gelatin was darker than bovine gelatin, with an 'L' value of 78.19 (±0.81). There was also significant difference ($p < 0.05$) in redness between eel skin [+3.20 (±0.29)] and bovine [+1.44 (±0.15)] gelatin. This could possibly be due to different sources and origins of the gelatin. However, bovine gelatin had a significantly higher 'b^*' value [+21.37 (±0.70)] and was more yellowish ($p < 0.05$) than eel skin gelatin [+7.52 (±0.29)]. The appearance of eel skin gelatin is snowy white. In general, color does not appear to influence gelatin's functional properties. However, color will influence the sensory acceptability of the sample.

Secondary Structure Analysis

The secondary protein structure is the specific geometric shape caused by the intra- and inter-molecular hydrogen bonding of amide groups. The geometry assumed by the protein chain is directly related to the molecular geometry concepts of hybridization theory. Experimental evidence shows that the amide unit is a rigid planar structure derived from the planar triangular geometry of the carbonyl unit (C = O) (Orphardt, 2003). Table 3 shows the FTIR spectra peaks for Amide wavenumbers^{-1} for both eel skin and commercial bovine gelatins. In general, eight peaks were identified by the software program, six of which were identified as amide bonds based on wave numbers presented in the literature. These are amide A, B, I, II, III, and V (Badii and Howell, 2006). Comparisons of gelatins based on conformational changes in structure have been based on comparisons of absolute peak intensity. The amide I band (between 1600 and 1700 cm^{-1}), was the most useful for infrared spectroscopic analysis of protein structure (Kong and Yu, 2007). The intensity of the amide III band has been also associated with a triple helical structure (Muyonga *et al*., 2004). In this study, the ratio between the amide III band (1209-1209 cm^{-1}) and amide I band (1600-1628 cm^{-1}) was used to observe a loss of the collagen's secondary structure and its formation of a random coil structure. This technique was used because of difficulties faced when assessing absolute peak intensity in order to compare different samples. An increase in amide I band intensity is related to an increase in random coil at the expense of the ordered secondary structure (Kong and Yu, 2007). As temperature increases, the random coil structure also increases as the protein becomes denatured and loses its triple structure (Al-Saidi *et al*., 2012). Thus, low temperature/low/ acidity conditions showed little effect on either yield (results not shown) or the power of denaturation.

Muyonga *et al.* (2004) determined the FTIR spectra of Nile perch skin collagen and gelatin at 50°C and 70°C. They showed that in some regions, the spectra were so complex and rugged that the segregation of different extracted gelatins was not possible. In this case, multivariate analysis (RCA) was performed on the spectra for discrimination and classification. Hashim *et al.* (2010) used the deformation of N-H bonds in the ranges of 3290–3440 cm^{-1}, and 1660–1200 cm^{-1} for discriminate analysis. They found that these regions gave data related to the origin of the gelatin. Principal component analysis of spectra in these regions clearly showed different loci for eel skin and bovine gelatin in the biplot. Based on results obtained from FTIR, both gelatin types share the same composition of functional groups in their secondary structure. Both eel skin and bovine gelatin gave off spectrum points that fell into six different functional groups (Table 4). The first; eel skin (3434), bovine (3302), was in the amine range of 3100–3500 cm^{-1}, which contains N-H bonds of medium intensity and stretch vibration mode. The second; eel skin (2249), bovine (2248), was nitriles with a range of 2300–2200 cm^{-1}, containing C≡N of medium intensity and stretch vibration mode. The third; eel skin (2107), bovine (2117), was alkynes with a range of 2100–2260 cm^{-1}, containing C≡C of varied intensity and stretch vibration mode. The forth; eel skin (1608), bovine gelatin (1628), most likely comprises two different functional groups: alkenes and amides of two ranges (1600–1675 cm^{-1}, and 1550–1640 cm^{-1}) containing C=C and N-H with bends and stretch vibration modes, respectively. Alkenes have no intensity while amides have strong intensity. The fifth; eel skin gelatin (1216), bovine (1628), was acidic with a spectral range of 1200–1320 cm^{-1}, containing C-O of strong intensity and stretch vibration mode. The sixth; eel skin (630.4), bovine

Table 3. FTIR spectra peak wavenumbers^{-1} for (Amides) in eel skin and commercial bovine gelatins

Type of gelatin	Amide A	Amide B	Amide I	Amide II	Amide III	Amide IV	Amide V	Amide VI
Eel skin	3434 ±8.41^{a}	2249 ±3.15^{a}	1608 ±0.84^{b}	2249 ±3.15^{a}	1216 ±3.43^{a}	-	630 ±5.80^{b}	-
Bovine	3302 ±40.63^{b}	2248 ±1.70^{a}	1628 ±9.02^{a}	2248 ±1.70^{a}	1209 ±1.97^{b}	-	658 ±12.81^{a}	-

Note: $^{a, b}$ indicates that rows with different letters are significantly different ($p < 0.05$)

Table 4. FTIR spectra peak wavenumber^{-1} with their assignment based on functional group of eel skin gelatin as compared to bovine gelatin

Sample	FTIR spectra peak wavenumber^{-1}	Assignment
Eel skin gelatin	3434 ±8.41	**Amines (N-H), stretch with medium intensity**
	2249 ±3.15	Nitriles (C=N), stretch with medium intensity
	2107 ±10.94	Alkynes (C=C), stretch with vary intensity
	1608 ±0.84	Alkenes (C=C), bend with no intensity / **Amides (N-H), stretch with strong intensity**
	1216 ±3.43	Acid (C-O), stretch with strong intensity
	630.4 ±5.80	Alkyl halide (C-Cl), stretch with strong intensity
Bovine gelatin	3302 ±40.63	**Amines (N-H), stretch with medium intensity**
	2248 ±1.70	Nitriles (C=N), stretch with medium intensity
	2117 ±3.85	Alkynes (C=C), stretch with vary intensity
	1628 ±9.02	Alkenes (C=C), bend with no intensity/ **Amides (N-H), stretch with strong intensity**
	1209 ±1.97	Acid (C-O), stretch with strong intensity
	657.7 ±12.81	Alkyl halide (C-Cl), stretch with strong intensity

Note: Sentences in *Bold* type indicate the importance of that functional group(s) in the structure of gelatin.

(657.7), was alkyl halide with a range of 600–800 cm^{-1}, containing C-Cl of strong intensity and stretch vibration mode.

Conclusion

Eel (*Monopterus* sp.) skin gelatin is a potential alternative to mammalian gelatin. Fish based gelatin has gained importance in recent years as the demand for non-bovine and non-porcine gelatin has increased due to the BSE crisis and growing halal needs. In addition, there has been increasing interest in investigating approaches to more effective use of under-utilized marine resources and related industrial waste. From the above cited yield obtained, we note a 12.75% extracted of edible gelatin from eel (*Monopterus* sp.) skin. The higher gel strength and melting temperature of eel skin gelatin indicates that it has good gelatin properties. These happened probably due to low quality of standard (bovine gelatin). These properties confirm that eel (*Monopterus* sp.) skin has a promisingly high potential for use as an alternative to mammalian gelatin in the future.

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