Effects of pH on functional, rheological and structural properties of eel *(Monopterus* sp.*)* skin gelatin compared to bovine gelatin

Nurul, A. G. and *Sarbon, N. M.

School of Food Science and Technology, Universiti Malaysia Terengganu
21030, Kuala Terengganu, Terengganu, Malaysia

Abstract

This study examines and compares the influence of pH on the functional, rheological and structural properties of eel skin *(Monopterus* sp.*)* and bovine gelatins. Functional properties studied and compared were emulsifying capacity and stability; water holding capacity; fat binding capacity; foaming capacity; and foaming stability. The rheological properties studied include gel strength and dynamic oscillatory measurements. The structural properties of eel skin and bovine gelatin were determined by Fourier transform infrared spectroscopy (FTIR). Results obtained showed that eel skin gelatin treated at pH 8 (compared to pH 5) exhibited the higher emulsifying, fat binding, foaming and viscoelasticity properties. The FTIR spectrum assay showed that eel skin gelatin presented a similar structure to that of bovine gelatin. This study demonstrated that pH levels influence functional, rheological and structural properties of eel skin gelatin and that these properties were enhanced to either equal or surpass those of bovine gelatin. Hence, this study indicates that eel skin gelatin has immense potential for use as an alternative to bovine gelatin.

Introduction

Gelatin comprises denatured proteins extracted from collagen by the partial hydrolysis of native collagen. The principal raw material for gelatin production includes constituents of animal tissue such as skin, bones and connective tissue (Schrieber and Gareis, 2007). Gelatin is one of the most popular ingredients of processed food and has numerous applications in the food industry as additives to improved elasticity, consistency and stability of food products (Gimenez, Gormez-Guillen and Montero, 2005). In addition, gelatins are used in medical, pharmaceutical and photographic industries. Most commercial gelatin is currently sourced from bovine bone, bovine hide, porcine skin and more recently, porcine bone. It has been reported that 41% of gelatin produced globally is sourced from porcine skin, 28.5% from bovine hides, and 29.5% from bovine bones (Hayatudin, 2005). However, porcine skin and bone gelatin are considered ritually impure (non-Halal) for both Judaism and Islam, which then only allows bovine gelatin as acceptable for foods prepared according to religious requirements (Badii and Howell, 2006). However, bovine spongiform encephalopathy (BSE) or transmissible spongiform encephalopathy (TSE) have presented a serious health crisis for the production of bovine gelatin, and this was in addition to foot-and-mouth disease (FMD), both of which have restricted the gelatin trade (Helcke, 2000). As a result, many researchers are exceedingly interested in finding alternative gelatin sources.

The quality of gelatin depends on functional characteristics such as physical, chemical and structural properties and this hinge on protein origin, collagen type, and processing methods (Montero and Go´mez-Guille´n, 2000). Functional properties of gelatin are related to basic hydration properties such as swelling and solubility. The most significant properties have been divided into two categories: gelling and surface behavior (Schrieber and Gareis, 2007). These functional properties are most important for the food industry as they affect the elasticity, consistency and stability of food products which also includes use as an outer film to protect foods against light and oxygen (Montero and Gomez-Guillen, 2000). Killekar *et al*. (2012) investigated functional properties of gelatin extracted from the skin of the black kingfish *(Rachycentron Canadus)*, including emulsifying capacity, emulsifying stability, water holding capacity, melting point and viscosity. The functional properties of shark *(Isurus oxyrinchus)* cartilage gelatin, including turbidity;

*Corresponding author.
Email: *norizah@umt.edu.my*
Tel: +609 668 4968; Fax: +609 668 4949

foam formation capacity; foam stability; water retention and fat binding were studied by Cho *et al.* (2003). Rheological properties such as gel strength and viscosity are related to flow behavior and are considered the most important physical properties of gelatin. These properties have been studied and include gel strength, dynamic viscoelastic behavior, frequency sweep of gelatin, and gelatin flow behavior (shear rate sweep) (Binsi *et al.*, 2009; Sarbon, Badii and Howell, 2013; Cheow *et al.*, 2007).

Gelatin structure is also related to physical properties that influence quality and potential application (Yang and Wang, 2009). Gelatin structure can be observed by Fourier transform infrared (FTIR) spectroscopy to indentify functional groups of amino acids and secondary protein structures. Sarbon, Badii and Howell (2013) compare the structural properties of chicken skin gelatin with bovine gelatin by using Raman spectroscope and found that chicken skin gelatin possessed a similar structure to that of commercial bovine gelatin. Al-Saidi *et al.* (2012) also used Fourier transform infrared (FTIR) spectroscopy to study the effects of extraction conditions on the structure of gelatin extracted from shaari *(Lithrinus microdon)* skin.

Having both acidic (carboxyl) and basic (amino and guanidine) amino acid groups, gelatin displays amphoteric characteristics due to amino acid functional groups and the terminal amino and carboxyl groups created during hydrolysis.In a strongly acidic solution, gelatin is positively charged and migrates as a cation in an electric field. In strongly alkaline solution, it becomes negatively charged and migrates as an anion. No migration occurs at the intermediate point when the net charge is zero. This is called the isoelectric point and is designated in pH units (Aoyagi, 1999). Gel strength value is maximal while viscosity is minimal at pH 5 as reported by Cole (2000), which signifies the importance of pH for rheological properties. In this study, pH 5 and pH 8 was selected because melting and gelling temperatures of gelatin gels are more stable within a pH range of 5–9 due to stronger structure. According to Fennema (2000), gel should present a stronger structure when the pH is far from the isoelectric, whereas when it is near to the isoelectric point its structure is weaker. However, salts such as sodium chloride greatly reduce this effect.

In addition, the ability of gelatin to retain water at all pH levels and to form gels that do not synergize is another unique property that can be suitably applied in the gelatin industry where other gelling and stabilizing agents have failed. However, no studies have thus far been reported regarding the effect of pH

on functional, rheological and structural properties of gelatin extracted from eel *(M.*sp.*)* skin. Thus, it appeared that properties of alternative gelatins such as eel skin gelatin, which is generally similar to fish gelatin, could be improved by pH treatment to obtain similar or even superior properties compared to bovine gelatin.

The eel *(Monopterus* sp.*)* is a bony fish from the *Synbranchidae* family (Order: *Synbranchiformes*; Class: *Actinopterygii* and *(Monopterus* sp*. or M.*sp.*)* is found throughout India, southern China, Malaysia and Indonesia. Its habitat includes muddy ponds, swamps, canals and rice fields (Rainboth, 1996). The Terengganu Fishery Department in Malaysia reported that the total eel *(M.sp.)* production for 2004 was 0.2 tones, which increased to 4.0 tons by 2012. As reported in *Utusan* Online (2010), eel has been used as a source of nutrition and traditional medicines which include eel lotion, eel jelly, eel coffee and eel capsules. But further commercialization of eel products has remained unexplored, especially in food industry applications. Presently, eel skin is also used as a natural powdered food supplement for which health benefits are known and accepted in Japan and in some parts of Europe. According to Nik Mohd Ikram and Ridzwan (2013), eel *(M.*sp.*)* skin mucous is used traditionally for skincare and wound healing. It is also believed that the eel contains high nutritional value and holds great potential for commercialization. Therefore, the production of eel skin gelatin as a by-product and value added item for commercial use has generated interest in the development of new knowledge for the potential of eel skin gelatin in numerous applications.

Therefore, our objectives with this study were to extract gelatin from eel *(M. sp.)* skin and investigate effects from different pH treatments on functional and rheological properties, including emulsifying capacity, emulsifying stability, water retention, fat binding capacity, foaming capacity, foaming stability, gel strength; oscillatory measurements and structural properties (via FTIR)—and then compare these results with those of commercial bovine gelatin.

Material and methods

Raw materials

Eel *(M.*sp.*)* was purchased from a local supplier in Kuala Terengganu, Malaysia and transported live to the Universiti Malaysia Terengganu. Upon arrival at the laboratory, they were soaked in an ice solution to stop activity and then processed by removing internal organs followed by a tap water wash to remove residual blood and then filleted. The eel was cut,

beheaded, filleted and the skins were cut into small pieces, washed and weighed (wet weight) before storage at -18°C. Bovine gelatin was purchase from Sigma Aldrich as a comparison. Bovine gelatin used in this research was not undergoing any treatment. Palm oil (Vesawit) was used in emulsifying property determination. All chemicals and reagents used in this study were of analytical grade.

Sample preparation

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

Gelatin extraction

The gelatin was prepared by following the procedure described by Sarbon, Badii and Howell (2013) with slight modification. About 15 g of dried eel skin was added to 400 ml of sodium hydroxide (NaOH) (0.15% w/v) and slowly stirred at room temperature for 30 min. This mixture was centrifuged (Cyrozen 1580R, Gyrozen Co. Ltm, Yuseong, Daejon, Korea) at 3500 x g for 10 min and the supernatant was discarded. This same step was repeated in sulphuric acid [0.15% (v/v)] followed by citric acid [0.7% (w/v)], consecutively. The sediment was washed in distilled water in order to remove residual salts and then centrifuged at 3500 x g for 15 min. The gelatin was finally extracted with distilled water at a controlled temperature (45°C) overnight in a water bath shaker. The mixture was filtered in a Buchner funnel with Whatman filter paper (no. 4). The volume of the gelatin solution was then reduced to a tenth by evaporation under a vacuum in a rotary evaporator at 45°C, before freeze-dried. The yield of dry matter (gelatin powder) was calculated based on the dry weight of fresh eel skin using the following formula:

$$\text{Yield of gelatin (\%)} = \frac{\text{Weight of gelatin powder} \times 100}{\text{Weight of dried skin}}$$

Emulsifying capacity (EC) and emulsifying stability (ES) of eel skin gelatin

Emulsifying capacity (EC) and emulsifying stability (ES) properties were determined according to the method described by Neto *et al.* (2001). An emulsion was prepared with 5 ml of gelatin solution (10 mg/ml) in distilled water. The pH of the gelatin solution was adjusted either to pH 5 or 8 with 1.0 N HCl or 1.0 N NaOH, respectively. The solution was homogenized with 5 ml of palm oil and centrifuged

(Gyrozen 1580R, Korea) at 1100 x g for 5 min. The height of the emulsified layer and that of the tube's total content were measured and the EC was calculated. ES was determined by heating each emulsion at 55°C before being centrifuged at 2000 rpm for 5 min after which ES was calculated. Each analysis (EC and ES) was done in triplicate, averaged, and then compared with bovine gelatin EC and ES. Calculations used the following formulae:

$$\text{Emulsifying Capacity (EC) (\%)} = \frac{\text{Height of emulsion layer} \times 100}{\text{Height of the total content}}$$

$$\text{Emulsifying stability (ES) (\%)} = \frac{\text{Height of the emulsion layer after heating} \times 100}{\text{Height of the emulsified layer before heating}}$$

Water holding capacity (WHC) of eel skin gelatin

Water holding capacity (WHC) was determined using the centrifugation method described by Diniz and Martin (1997) with slight modification. About 0.5 g of sample gelatin was dissolved in 10 ml of distilled water in centrifuge tubes and mixed using a vortex mixer for 30 min. The pH was then adjusted to pH 5 and pH 8 with 1.0 N HCl or 1.0 N NaOH respectively. Samples (in triplicate) were then centrifuged at 2800 × g for 25 min. The supernatant was filtered with Whatman No.1 filter paper and the retrieved volume was accurately measured. The difference between the initial volume of distilled water added to the protein sample and the volume of the supernatant was determined and reported as ml of water absorbed per gram of gelatin sample. The analysis was done in triplicate and the WHC was then compared to that of bovine gelatin. WHC was calculated by using the following formula:

$$\text{Water holding capacity (WHC)} = \frac{V_0 - V_1 \text{ (ml)}}{\text{Weight of gelatin (g)}}$$

Where:
V_0 = initial volume of gelatin after adjusted pH
V_1 = volume of supernatant

Fat binding capacity (FBC) of eel skin gelatin

Fat-binding capacity (FBC) was measured by following the method of Shahidi *et al.* (1995). About 0.5 g of gelatin was added to 10 ml of palm oil in a 50 ml centrifuge tube and vortexed for 30 s. The pH of sample solutions were adjusted to pH 5 and pH 8 with 1.0 N HCl and 1.0 N NaOH, respectively. The oil dispersion was centrifuged at 2800 x g for 25 min. Free oil was decanted and the fat binding

capacity was determined by the difference in weight. The analysis was undertaken in triplicate, averaged, and the fat binding capacity was compared with that of bovine gelatin. FBC was calculated by using the following formula:

$$\text{Fat binding capacity (FBC)} = \frac{V_0 - V_1 \text{ (ml)}}{\text{Weight of gelatin (g)}}$$

Where:

V_0 =   initial volume of gelatin after adjusted pH
V_1 =   volume of the supernatant

Foaming capacity (FC) and foaming stability (FS) of eel skin gelatin

Foaming Capacity (FC) and Foaming Stability (FS) were measured by a partially modified method taken from Sathe, Deshpande and Salunkhe (1982). About 1 g sample was weighed and added to 50 ml of distilled water and allowed to swell before dissolution at 60oC. The pH was adjusted to pH 5 and 8 with 1.0 N HCl and 1.0 N NaOH, respectively. The foam was prepared by homogenization at 10,000 x g for 5 min. The homogenized solution was then poured into a 250 ml measuring cylinder. FC and FS were calculated after 30 min in triplicate for each assay, averaged and compared to bovine gelatin FC and FS. FC and FS were calculated using the following formulas:

$$\text{Foaming capacity (FC)} = \frac{\text{Volume of foam + volume of liquid (ml)}}{\text{Initial volume of solution (ml)}}$$

$$\text{Foaming stability (FS)} = \frac{\text{Initial volume of foam + volume of liquid (ml)}}{\text{Volume of foam + volume of liquid (30 min) (ml)}}$$

Gel strength (bloom value)

Gelatin gel strength was determined according to the method described by Sarbon, Badii and Howell (2013) with slight modification. Gelatin solutions [6.67% (w/w)] were prepared in a Bloom jar (SCHOTTGLASS Mainz, Bloom test vessel, product no. 2112501). The mixture was swirled, covered and let stand for 3 h at room temperature to allow the gelatin to absorb water and swell before complete dissolution at 60°C. The pH of sample solutions were adjusted to pH 5 and 8 with 1.0 N HCl or 1.0 N NaOH, respectively. The jar was covered and cooled for 15 min at room temperature to allow equilibration and then kept chilled at 4–7oC overnight (16–18 h) for gel maturation. Samples were tested by the TA-XT2 texture analyzer (Stable Microsystem, Godalming, UK) by gel penetration in a standard glass

Bloom jar centrally placed under the probe (standard radius cylinder, P/0.5R). Maximum force (in g) was determined as the probe penetrated the gel to a depth of 4 mm at 0.5 mm/s. The analysis was undertaken in triplicate, averaged, and then compared with bovine bloom value.

Determination of small deformation oscillatory measurement

Eel skin and bovine gelatins were prepared by dissolving 6.67% (w/w) gelatin powder in distilled water and allowed to swell. Solutions were then heated to 45°C for 15 min and pH was adjusted to pH 5 and 8 with 1.0 N HCl and 1.0 N NaOH, respectively, to produce the experimental samples. Small deformation oscillatory measurements were performed using a rheometer (Rheometer DHR-2, USA). Peltier steel plate 100254 with a controlled strain of 5% utilizing 40 mm parallel plate geometry with a 1000µm gap and 1 rad/s of applied frequency was used. The major parameters determined by dynamic oscillatory testing are (1) the storage or elastic modulus (G'), which is expressed as the amount of energy stored elastically within the tested structure; and (2) the viscous or loss modulus (G") which indicates the amount of energy loss (viscous response). Initially, gelatin samples were kept at 40°C for 10 min to allow equilibration. They were then cooled and reheated (at steps of 5°C) on a Peltier plate: cooled down from 40 to 10°C and then warmed again from 10 to 40°C. When melting occured, values for the elastic modulus (G') began to decrease and those for the loss modulus (G") began to increase. Gelling temperatures were obtained from temperatures at which the elastic modulus began to dramatically increase in value. The function of temperature was therefore determined for both the elastic or 'storage' modulus (G') and loss modulus (G") when changes were recorded. The sol-gel transition (gel formation point) is close to that temperature at which G'/G" crossover occurs during cooling (Gudmundsson, 2002).

Fourier transforms infrared (FTIR) spectroscopy

The gelatin's structural properties were measured using FTIR (Nicole, Thermo Electrin, USA) with a Deuterated triglycine sulfate (DTGS) detector. The sample holder [Multi-bounce horizontal attenuated total reflectance unit (HATR)] with a plate of zinc selenite (ZnSe) crystal was thoroughly cleaned with acetone and the background spectrum (without sample) was subsequently collected within a resolution range of 4000–650 cm^{-1} after thirty-two scans. The gelatin sample was then placed on the

plate for analysis. NB: a single-beam spectrum for each sample was rationed against a single-beam from the ambient air background spectrum before conversion to absorbance units. The analysis was done in triplicate and results compared with those from bovine gelatin.

Data analysis

In this study, triplicate data was collected and analyzed for each tested sample using MINITAB (version 14.0). Data were subjected to analysis as means from triplicate determinations ± standard deviation. One-Way ANOVA was used to determine significant differences between means (p < 0.05).

Results and Discussion

Yield of extraction

The yield of extracted eel skin gelatin obtained, expressed as grams of dry gelatin per 100 g of clean skin, was 11.98%. It has been reported that the average yield of extracted fish gelatin was lower than that of mammalian gelatin which approximately ranged from 6–19% (Karim and Bhat, 2009). Previously, gelatin yielded from eel *(Anguilla Japonica)* skin was reported at 12.75% (Rosli, 2013); higher than the eel (M. sp.) skin gelatin presented in this study. The lower yield may due to a loss of collagen through leaching during the series of washing steps taken and also to eel species. Another possible explanation could be incomplete extraction due to incomplete collagen hydrolysis since lower temperatures yield less gel while higher temperatures tend to degrade the extracted gelatin and thus, affect its quality (Alfaro, Fonseca and Prentice, 2012).

Several yields of extracted gelatins from other fish skins have been reported as follows: black (5.4%) and red tilapia (7.8%) (Jamilah and Harvinder, 2002); megrim (7.4%), Dover sole (8.3%), cod (7.2%) and hake (6.5%) (Gómez-Guillén *et al.*, 2002); short-fin scad (7.3%) and sin croaker (14.3%) (Cheow *et al.*, 2007); big-eye snapper (6.5%) and brown-striped red snapper (9.4%) skins (Jongjareonrak *et al.*, 2006). These difference percentages may be due to variations in extraction methods (Songchotikunpan *et al.*, 2008); or to differences in chemical composition of skins, or in collagen content and the amount of soluble components in the skins, as all of these properties vary according to species and age of the fish.

Emulsifying capacity (EC) and emulsifying stability (ES)

There were significant difference (p <0.05)

between eel skin gelatin (pH 5 and pH 8) and bovine gelatin (without any treatment) for EC as shown in Figure 1. The EC of eel skin gelatin at pH 8 was slightly higher than both eel skin gelatin at pH 5 and bovine gelatin (63.97%, 56.34% and 52.59%), respectively. Similarly, we found significant difference (p <0.05) between eel skin gelatin (pH 5 and pH 8) and bovine gelatin for ES (Figure 1). The ES of eel skin gelatin at pH 8 (54.51%) was slightly higher than that of both eel skin gelatin at pH 5 (45.61%) and bovine gelatin (51.62%). This may have been due to greater hydrophilic and hydrophobic regions which act as emulsifiers in a mixture of oil and water. Our results showed a higher value for the ES of eel skin gelatin treated at different pH concentrations than that of the extracted gelatin reported by Koli *et al.* (2013) where the emulsifying stabilities for tiger-toothed croaker skin and bone were 35.70% and 32.50%, respectively.
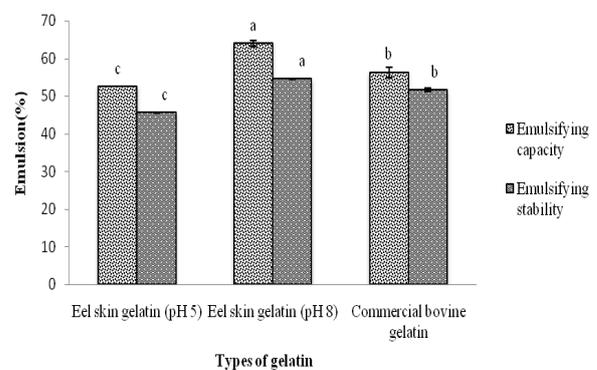

Figure 1. Effect of pH on emulsifying capacity (EC) and stability (ES) of eel skin and commercial bovine gelatins. Values are given as means ± SD taken from three trials; different letters (a, b, c) indicate significant differences (p <0.05)

In addition, the solubility, conformation and surface properties of the gelatin's protein affect the EC while protein charges affect the ES property (Zayad, 1997). Rapid migration of protein molecules to fat droplets is due to high solubility and hydrophobic proteins during the dispersal phase which, in turn, increase emulsifying efficiency (Rawdkuen *et al.*, 2013). Gómez-Guillén et al. (2009) also reported that a higher content of hydrophobic amino acid residue results in effective distribution of hydrophilic/hydrophobic amino acids that improve gelatin emulsifying properties. Thus, in an alkaline condition, eel skin gelatin showed better emulsification, most likely due to the higher content of hydrophobic amino acids. In addition to the different source of raw material, pH levels apparently influenced the emulsifying properties obtained due to amphoteric and hydrophobic regions on peptide chains within the gel.

Water holding capacity (WHC)

The functional property of water holding capacity is approximately related to interactions between water and other gelatin components (Rawdkuen *et al.*, 2013). A significant differences (p <0.05) between the water holding capacity of bovine and eel skin gelatins at different pH levels (pH 5 and 8) were presented. However, there was no significant difference in WHC (p >0.05) between extracted eel skin gelatin at pH 5 and pH 8 (Figure 2), although it was higher at pH 5 (4.65 ml/g) than at pH 8 (3.86 ml/g). The WHC for bovine gelatin was the highest observed (9.81 ml/g). Gelatin's WHC is mostly affected by the amount of hydrophilic amino acid content. This result agrees with a study conducted by Koli *et al.* (2013) which showed similar results due to decreased hydrophilic amino acid and hydroxyproline content. Jellouli *et al.* (2011) presented similar results with grey triggerfish skin gelatin, which showed lower water holding capacity and hydroxyproline (74 per 1000 residue parts) than that of bovine gelatin (hydroxyproline 96 per 1000 residue parts). However, Koli *et al.* (2013) reported that fish gelatin had better WHC than mammalian bone gelatin, especially in an acidic environment. Hence, fish gelatins were suggested to present a plausible alternative for use in acidic food systems requiring high WHC.

Fat binding capacity (FBC)

Fat binding capacity (FBC) is a closely related functional property of gelatin texture due to interactions between oil and other components. Of the three gelatins (eel skin gelatin at pH 5, pH 8 and bovine gelatin), FBCs were significantly different (p <0.05) as shown in Figure 3. FBC for eel skin gelatin at pH 8 was (6.51 ml/g) but for pH 5 it was (4.81 ml/g) while bovine gelatin was 5.73 ml/g. As the degree of hydrophobic residue exposure in gelatin influences FBC, increased pH levels increase hydrophobic residue content in the gelatin, and thus contribute to a higher FBC value.

The variation in the presence of non-polar side chains which bind the hydrocarbon side chain of oil accounts for the differences in FBC between eel skin gelatin and bovine gelatin as demonstrated in Figure 3. Rawdkuen *et al.* (2013) and Cho *et al.* (2003) reported similar results where the FBC of fish gelatin was higher than that of bovine gelatin. The degree of exposure of hydrophobic residue and higher numbers of non-polar side chains in amino acids such as tyrosine, leucine, valine and isoleucine all contributed to an increase in FBC (Ninan *et al.*, 2011). Thus, the results clearly showed that under alkaline conditions, eel skin gelatin had superior FBC compared to eel
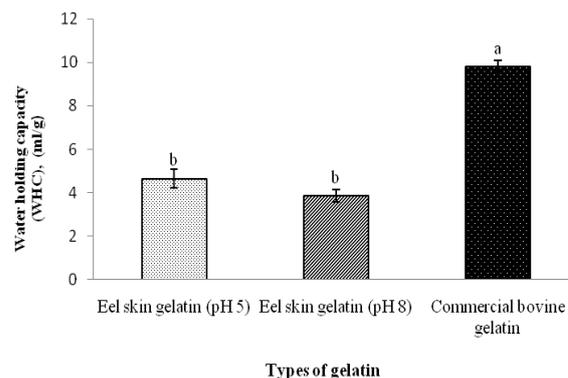

Figure 2. Effect of pH on water holding capacity (WHC) of eel skin and commercial bovine gelatins. Values are given as means ± SD taken from three trials; different letters (a, b) indicate significant differences (p <0.05)
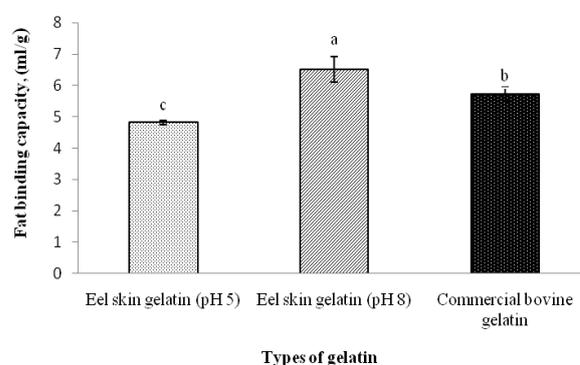

Figure 3. Effect of pH on fat binding capacity (FBC) of eel skin and commercial bovine gelatins. Values are given as means ± SD taken from three trials; different letters (a, b, c) indicate significant differences (p <0.05)
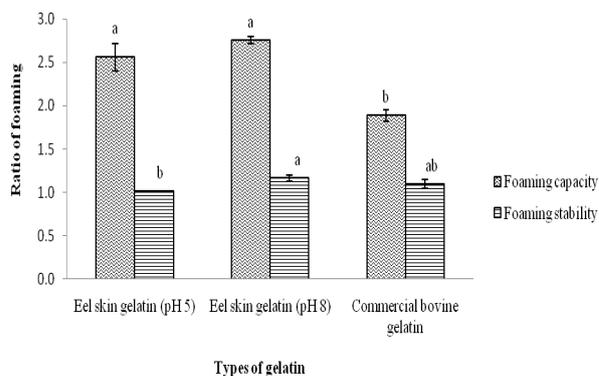

Figure 4. Effect of pH on foam capacity (FC) and foam stability (FS) of eel skin and commercial bovine gelatins. Values are given as means ± SD taken from three trials; different letters (a, b) indicate significant differences (p <0.05)

skin gelatin under acidic conditions as well as bovine gelatin.

Foaming capacity (FC) and foaming stability (FS)

Figure 4 presents ratios for the foaming capacity (FC) and foaming stability (FS) of eel skin gelatin at pH 5 and 8 compared to bovine gelatin. There were significant differences (p <0.05) between the

FC of eel skin gelatin at pH 5 and pH 8 and the FC of bovine gelatin. Ratios of eel skin gelatin at pH 5 (2.56) and pH 8 (2.76) were better than that of bovine gelatin FC (1.89). However, no significant difference (p >0.05) in FC between eel skin gelatin at pH 5 and pH 8 was observed. The difference in FC between eel and bovine gelatins was likely due to the higher hydrophobic amino acid content in eel skin gelatin, which, at pH 8, was also higher than eel skin gelatin at pH 5. Again, this was most likely due to a greater amount of hydrophobic amino acid residue, as suggested by Townsend and Nakai (1983). The latter researchers stated that for 'adsorption at the air–water interface, molecules should contain hydrophobic regions that are more exposed on protein unfolding, thus, facilitating foam formation and stabilization'. However, foaming properties are highly dependent on the characteristics of the raw material utilized. This phenomenon reduces surface tension and allows for the formation of foam (Liceaga-Gesualdo and Li-Chan, 1999).

According to Jellouli *et al.* (2011), the FC of grey triggerfish skin gelatin (123% at 1 g/100 ml) was also slightly higher (p <0.05) than that of bovine gelatin (119%), indicating a difference in foaming ability between both gelatins due to higher hydrophobic amino acid content (alanine, valine, isoleucine, leucine, proline, methionine, phenylalanine and tyrosine) of the grey triggerfish skin gelatin (319 per 1000 residue parts) than that of halal bovine gelatin (313 per 1000 residue parts). Koli *et al.* (2012) also found that tiger-toothed croaker skin gelatin exhibited better characteristics compared to pink perch skin at all pH levels tested (pH 2–10). In addition, Grass carp skin gelatin showed better foam formation ability than mammalian gelatins (Ninan *et al.*, 2011).

FS ratios were 1.02 for eel skin gelatin at pH 5; 1.16 for eel skin gelatin at pH 8; and 1.10 for bovine gelatin (Figure 4). Eel skin gelatin at pH 8 showed a higher FS compared to gelatin formed at pH 5. The lower FS of eel skin gelatin at pH 5 may be due to a lower percentage of negatively charged amino acids. A higher content of negatively charged amino acids in eel skin gelatin at pH 8 may have prevented charge neutralization in gelatin molecules and further increased the FS. In addition, the FS of protein solutions is generally positively charged which correlates with the molecular weights of peptides (van der Ven, Gruppen, de Bont and Voragen, 2002). A study by Jellouli *et al*. (2011) showed that the FS of grey triggerfish skin gelatin was higher than that of halal bovine gelatin at the same concentration for 30 and 60 min, respectively which suggested that grey triggerfish skin gelatin might form a film of stronger

and greater elasticity, leading to stabilized foam. Furthermore, reduced foam formation and stability may result from an aggregation of proteins that interfere with interactions between proteins and the water needed for foam formation (Kinsella, 1977).

The properties of different foams also determine their industrial applications. In the food industry, the determination of foaming properties has a significant impact on the processing and quality of some products (Exerowa and Kruglyakov, 1998). Hence, in this study, eel skin gelatin under the alkaline condition possessed better foaming properties due to a higher content of negatively charged amino acid residue.

Gel strength

The most important functional property of gelatin is gel strength. Fish gelatin typically has lower gel strength than mammalian gelatin (Gilsenan and Ross-Murphy, 2000). The gel strength of commercial gelatins, called the 'bloom value', ranges from 100–300. However, gelatin with bloom values of 250-300 are those that are most desired (Holzer, 1996). Figure 5 presents this study's results on the effects of pH on gel strength (p <0.05). Gel strengths for eel skin gelatin treated at pH 5 and pH 8, and for bovine gelatin were 213.15g, 214.72g, and 273.2g, respectively.
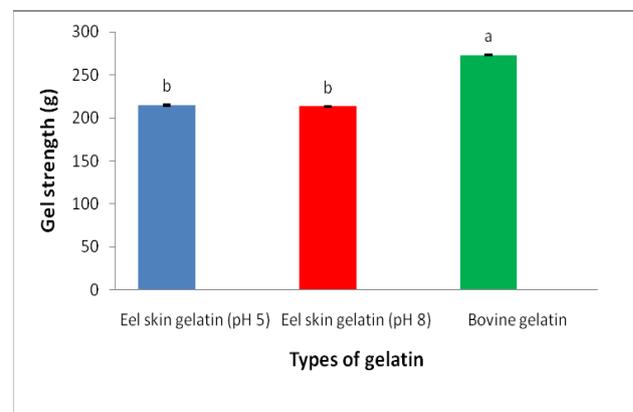

Figure 5. Effect of pH on the gel strength of eel skin and commercial bovine gelatins. Values are given as means ± SD from three trials; different letters (a, b, c) indicate significant differences (p <0.05)

There was no significant difference (p >0.05) between eel skin gelatin at pH 5 and pH 8. As expected, bovine gelatin had the highest gel strength, which is likely due to different intrinsic characteristics such as protein chain composition, molecular weight distribution, amino acid content, and mode of extraction. Moreover, gelatin with a lower pH value is capable of breaking both hydrophobic and hydrogen bonds, and thus, presumably prevents the stabilization of gel junction sites either directly by preventing hydrogen bond formation and/or by
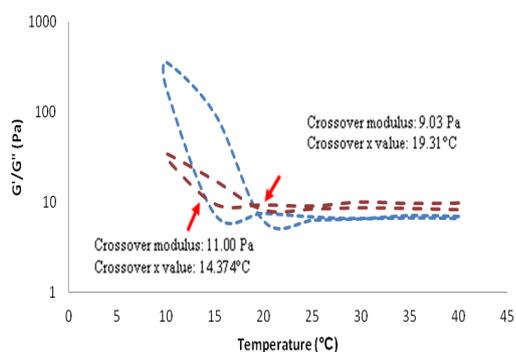
Figure 6. Effect of pH on viscoelastic properties (G' and G" values) for 6.67% (w/w) of eel skin gelatin at pH 5 on cooling from 40 to 10 °C and re-heating from 10 to 40 °C
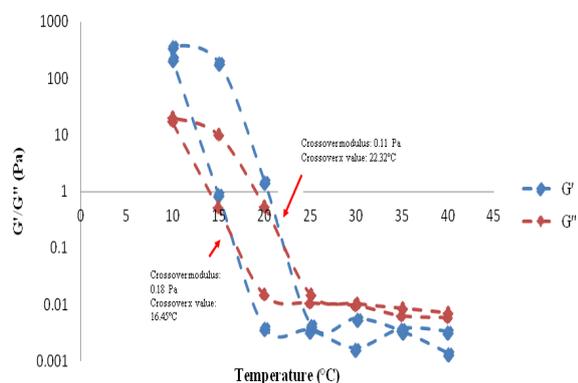

Figure 7. Effect of pH on viscoelastic properties (G' and G" values) for 6.67% (w/w) of eel skin gelatin at pH 8 after cooling from 40 to 10 °C and re-heating from 10 to 40°C

modifying the structure of the liquid (water) in the vicinity of these sites (Finch *et al.*, 1974).

Hydrogen bonds between water molecules and free hydroxyl groups of amino acid also influence gelatin strength (Arnesen and Gildberg, 2007). Previously, Rosli (2013) reported that the gel strength of eel *(Anguilla japonica)* skin gelatin (215.96 g) was higher than that of this study's focus. This may due to differences in amounts of compositional proteins such as hydroxyproline, serine and tyrosine (which have free hydroxyl groups), as well as their molecular weights and species. Similarly, Choi and Regenstein (2000) reported that gelatin from tilapia skin had lower gel strength than that of porcine skin gelatin due to the higher content of free hydroxyl groups in serine and tyrosine in the porcine product. Choi and Regenstein (2000) showed that the gel strength of gelatin extracted from both tilapia and porcine skin and bone markedly decreased at pH levels less than 4 and greater than 8. Crumper and Alexander (1952) found that porcine gelatin was maximally rigid at pH 9 but markedly less rigid when pH was less than 4 or greater than 10.

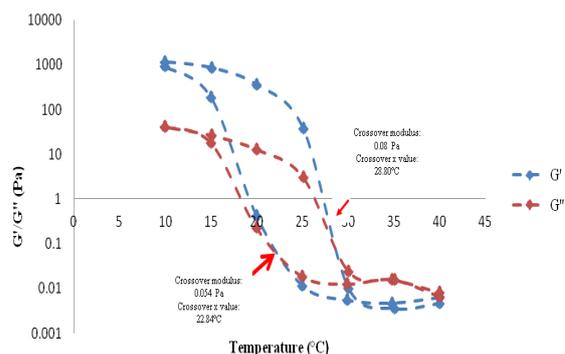In addition, amino acids also contribute to the

Figure 8. Effect of pH on viscoelastic properties (G' and G" values) for 6.67% (w/w) of commercial bovine gelatin after cooling from 40 to 10 °C and re-heating from 10 to 40°C

gel strength property of gelatin. Wong (1989) stated that amino acids play a role in gel formation with hydroxyl groups of hydroxyproline contributing to the stability of the helix through inter-chain hydrogen bonding with the water molecule as a bridge, and also by direct hydrogen bonding to the carbonyl group. Fish skin gelatins of different gel strengths have been reported for Atlantic salmon (108 g) and cod (71 g) (Arnesen and Gildberg, 2007); for skin croaker (125 g) and short-fin scad (177 g) (Cheow *et al.*, 2007); for big-eye snapper (105.7 g), brown-stripe red snapper (218.6 g) (Jongjareonrak *et al.*, 2006); and for cuttlefish (181 g) (Balti *et al.*, 2011).

Viscoelasticity properties of gelatin

The viscoelasticity properties of eel skin gelatin in this study showed that the crossover modulus (G'/G") during heating and cooling at pH 5 (9.03, 11.0) was higher than at pH 8 (0.10, 0.18) and commercial bovine gelatin (0.06, 0.05), respectively (See Figures 6–8). This may be attributed to differences in protein conformation which depend on the isoelectric point as well as amino acid composition (Sarabia *et al.*, 2000). However, the gelling points of eel skin gelatin at pH 5 and pH 8 were 14.37°C and 16.45°C compared to 22.84°C for bovine gelatin, respectively. Both eel skin gelatin samples (pH 5 and pH 8) showed lower gelling points compared to commercial bovine gelatin. The gelling point of eel skin gelatin at pH 8 was higher than at pH 5 due to an increase in charge density that discourage the formation and stabilization of the gelatin network at a lower pH. Fish gelatin generally has a lower gelling point compared to mammalian counterparts (Boran *et al.*, 2010). Prior reports on the gelling point of fish gelatin recorded ranges of 4–12°C and 18–19°C for cold- and warm-water fish species, respectively (Gómez-Guilllen *et al.*, 2011).

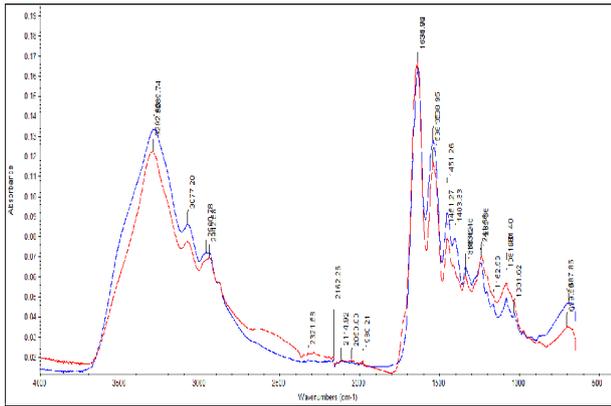The melting points for eel skin gelatin at 19.31°C

Figure 9. Structural properties of untreated eel skin and bovine gelatins using Fourier transform infrared (FTIR)

(pH 5) and 22.32°C (pH 8) and for bovine gelatin at 28.80°C were determined (See: Figures 6–8). A study by Ninan *et al*. (2011) also showed higher melting points (32.2–32.6 °C) for mammalian gelatins than for grass carp skin (29.1°C), rohu (28.1°C), and common carp (28.2°C). The slightly higher melting point for eel skin gelatin at pH 8, compared to pH 5, may due to a higher net negative charge and higher gel strength demonstrated at pH 8 (214.72 g) as reported before. Nevertheless, the melting points for eel skin gelatin were higher than those reported for cold water fish such as cod (13.8°C) and hake (14.0°C) as reported by Gomez-Guillen *et al*. (2002). Generally, fish gelatins have lower hydroxyproline content (~ 7–10% of total amino acids) compared to mammalian gelatins which have higher amounts of hydroxyproline and proline (14%) which contribute to lower melting and gelling points (Nalinanon *et al*., 2008; Benjakul *et al*., 2009).

Structural properties of gelatin

Fourier transform infrared (FTIR) spectrum analysis provided information on both structure and environment of the gelatin backbone and acidic amino side chains. Figure 9 depicts FTIR results for eel skin (blue spectrum) and bovine gelatins (red spectrum). Generally, both showed similar spectra with some peak shifting in the amide region. Table 1 shows both wave number (cm^{-1}) and types of amides found in eel skin and bovine gelatins. Peaks for eel skin gelatin's amides I and II were found at 1634.72 cm-1 and 1538.65 cm^{-1}, while those for bovine gelatin were found at 1633.94 cm^{-1} and 1538.95 cm^{-1}. According to Yakimets *et al*. (2005), the appearance of peaks from amides I and II (eel) were at 1700–1600 cm^{-1} and 1560–1500 cm^{-1} (bovine).

Amine peaks with aromatic C–N stretching for bovine gelatin were at 1241.66 cm^{-1}; those for eel skin gelatin were at 1235.66 cm^{-1} (Figure 9). Peaks at 1081.40 cm^{-1} for both eel skin and bovine gelatin

Table 1. Wave number (cm$^{-1)}$ value and types of amides in eel skin gelatin and commercial bovine gelatin

Wave number (cm^{-1})		Types of amide
Eel skin gelatin	Bovine gelatin	
3292.66	3289.74	Amide A
2942.58	3077.20, 2960.78	Amide B
1634.72	1633.94	Amide I
1538.65, 1451.27	1538.95, 1451.26, 1403.33	Amide II

indicated the presence of C–O vibrations from the amino acid threonine. Peaks at 1162.99 cm^{-1} and 1031.62 cm^{-1} found only in commercial bovine gelatin represented the –OH stretching of tyrosine and the C–O of serine, respectively (Barth, 2000). Amides A and B were less prominent for both bovine and eel skin gelatins with amide A peaks at 3289.74 cm^{-1} and 3292.66 cm^{-1}, respectively, and amide B peaks at 2960.78 cm-1, 3077.20 cm-1 and 2942.58 cm-1, respectively. Ranges for amides A and B were 3300–3610cm^{-1} and 2924–3166 cm^{-1}, respectively. Amide A indicates NH-stretching coupled with hydrogen bonding, whereas amide B indicates weak N-H stretching (Barbara, 2004).

The amide I vibration primarily indicates C=O stretching coupled to contributions from C-N stretching, C-C-N deformation and in-plane N-H bending modes (Bandekar, 1992). Absorption in the amide I region is most useful for the analysis of secondary protein structures (Surewicz and Mantsch, 1988). On the other hand, Barth (2000) explained that strong peaks at 1633.94 cm^{-1} and 1634.72 cm^{-1} represent arginine's C-N stretching. Yakimets *et al*. (2005) also reported that an absorption peak at 1633 cm^{-1} characterized the gelatin's coil structure. Rahmelow *et al*. (1998) stated that peaks at 1633–1636 cm^{-1} (300–340) represented the presence of arginine. Amide II vibrations were attributed to the out-of-phase combination of CN stretching and in-plane NH deformation of the peptide group (Bandekar, 1992). Amide II peaks are generally considered far more sensitive to hydration than to secondary structural changes (Wellner, Belton and Tatham, 1996). The appearance of peaks at 1451.26 and 1451.27 cm^{-1} indicated the presence of C-N stretching from proline, whereas peaks at 1335.45 cm^{-1} and 1336.42 cm^{-1} for both bovine and eel skin gelatins may be ascribed to the C-N vibration of tryptophan (Barth, 2000).

Conclusion

Different origins and treatments of/for eel skin

gelatins influence their functional and thermal properties. Although we demonstrated that eel skin gelatin has lower thermal properties compared to bovine gelatin but processing at different pH concentrations improved both functional and thermal properties. At a pH of 8, emulsifying capacity, and stability, fat binding capacity, foaming capacity, foaming stability, gel strength, gelling temperature and melting temperature of eel skin gelatin were enhanced compared to processing at pH 5. FTIR spectrum analysis showed that both eel skin and bovine gelatins presented with a similar structure. From the results obtained, therefore posit that the eel skin of *Monopterus sp.* shows remarkable potential for the industrial production as an alternative gelatin.

References

Alfaro, A. T., Fonseca, G. G. and Prentice, C. 2012. Enhancement of functional properties of wami tilapia *(Oreochromis urolepis hornorum)* skin gelatin at different pH values. Food and Bioprocess Technology 5: 1-10.

Al-Saidi, G. S., Al-Alawi, A., Rahman, M. S. and Guizani, N. 2012. Fourier transform infrared (FTIR) spectroscopic study of extracted gelatin from shaari *(Lithrinus microdon)* skin: effects of extraction conditions. International Food Research Journal 19: 1167–1173.

Aoyagi, S. 1999. Photographic Gelatin. In M. DeClercq, (ed.), Proceedings of the Seventh IAG Conference, Louvain-la-Neuve: The International Working Group for Photographic Gelatin (pp. 45–53). Fribourg, Switzerland.

Arnesen, J. A. and Gildberg, A. 2007. Extraction and characterization of gelatin from Atlantic salmon *(Salmo salar)* skin. Bioresource Technology 98: 53-57.

Badii, F. and Howell, N K. 2006. Fish gelatin: structure, gelling properties and interaction with egg albumen proteins. Food Hydrocolloids 20: 630–640.

Balti, R., Jridi, M., Sila, A., Souissi, N., Nedjar-Arroume, N. and Guillochon, D. 2011. Extraction and functional properties of gelatin from the skin of cuttlefish *(Sepia officinalis)* using smooth hound crude acid protease-aided process. Food Hydrocolloids 25: 943–950.

Bandekar, J. 1992. Amide modes and protein conformation. Biochim. Biophys. Acta 1120: 123–143.

Barbara, S. 2004. Infrared Spectroscopy Fundamentals and Applications. John Wiley & Sons, Canada.

Barth, A. 2000. Fine-structure enhancement-Assessment of a simple method to resolve overlapping bands in spectra. Spectrochim. Acta A 56: 1223–1232.

Benjakul, S., Oungbho, K., Visessanguan, W., Thiansilakul, Y. and Roytrakul, S. 2009. Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and *Priacanthus macracanthus*. Food Chemistry 116: 445–451.

Binsi, P. K., Shamasundar, B. A., Dileep, A. O., Badii, F. and Howell, N. K. 2009. Rheological and functional properties of gelatin from the skin of Bigeye snapper *(Priacanthus hamrur)* fish: influence of gelatin on the gel forming ability of fish mince. Food Hydrocolloid 2: 132–145.

Boran, G., Mulvaney, S. J. and Regenstein, J. M. 2010. Rheological properties of gelatin from silver carp skin compared to commercially available gelatins from different sources. Journal Food Science 75: 565–571.

Cheow, C. S., Norizah, M. S., Kyaw, Z. Y. and Howell, N. K. 2007. Preparation and characterization of gelatins from the skins of sin croaker *(Johnius dussumieri)* and shortfin scad *(Decapterus macrosoma)*. Food Chemistry 101: 386–391.

Cho, S. M., Kwak, K. S., Park, D. C., Gu, Y. S., Ji, C. I. and Jang, D. H. 2003. Processing optimization and functional properties of gelatin from shark *(Isurus oxyrinchus)* cartilage. Food Hydrocolloids 18: 573–579.

Choi, S. S. and Regenstein, J. M. 2000. Physicochemical and sensory characteristics of fish gelatin. Journal of Food Science 65: 194–199.

Cole, C. G. B. 2000. Gelatin. In F. J. Francis (Ed.), Encyclopedia of food science and technology. (2nd ed.) New York, Wiley, pp. 1183–1188).

Crumper, C. W. N. and Alexander, A. E. 1952. Viscosity and rigidity of gelatin in concentrated aqueous systems. Australian J. Sci. Res. 5: 146–159.

Diniz, F. M., Martin, A. M. 1997. Effects of the extent of enzymatic hydrolysis on the functional properties of shark protein hydrolysate. Lebensmittel-Wissenschaft und-Techologie 30: 266–272.

Exerowa, D. and Kruglyakov, P. 1998. Foam and foam films: theory, experiment, application (Studies in interface science). Elsevier, Amsterdam, pp. 656–737.

Fennema, O. (2000). Quimica de los Alimentos. 2d Edicion. Acribia, S.A. (ed). Zarago, Espana, pp. 155, 220, 466.

Finch, A., Gardner, P. J., Ledward, D. A. and Menashi, S. 1974. The thermal denaturation of collagen fibers swollen in aqueous solution of urea, heaxmethylenetramine, p benzoquione and tetra – alkyl ammonium salts. Biochem. Biophy. Acta 365: 400-07.

Gilsenan, P. M. and Ross-Murphy, S. B. 2000. Rheological characterization of gelatins from mammalian and marine sources. Food Hydrocolloids 14: 191–195.

Gime´nez, B., Go´ mez-Guille´ n, M. C. and Montero, P. 2005. The role of salt washing of fish skins in chemical and rheological properties of gelatin extracted. Food Hydrocolloids 19: 951–957.

Gómez-Guillén, M. C., Giménez, B., López-Caballero, M. E. and Montero, M. P. 2011. Gelatin from alternative sources: A review. Food Hydrocolloids 25: 1813–1827.

Gómez-Guillén, M. C., Pérez-Mateos, M., Gómez-Estaca, J., López-Caballero, E., Giménez, B. and Montero, P. 2009. Fish gelatin: a renewable material for developing active biodegradable films. Trends in Food Science

and Technology 20: 3–16.

Go´mez-Guille´n, M. C., Turnay, J., Ferna´ndez-Dı´az, M. D., Ulmo, N., Lizaebe, M. A. and Montero, P. 2002. Structural and physical properties of gelatin extracted from different marine species. Food Hydrocolloids 16: 25–34.

Gudmundsson, M. (2002). Rheological properties of fish gelatins. Journal of Food Science 67: 2172–2176.

Hayatudin, H. 2005. More Effort Needed to Produce Halal Medicinal Products. The Halal Journal – the online journal of the global halal market. Web. 2 Jan. 2006.

Helcke T. 2000. Gelatin, the food technologist's friend or foe. International Food Ingredients 1: 6-8.

Holzer, D. 1996. Gelatin Production. US Patent #5,484,888.

Jamilah, B. and Harvinder, K. G. 2002. Properties of gelatins from skins of black tilapia and red tilapia fish. Food Chemistry 77: 81–84.

Jellouli, K., Balti, R., Bougatef, A., Hmidet, N., Barkia, A. and Nasri, M. 2011. Chemical composition and characteristics of skin gelatin from grey triggerfish *(Balistes capriscus)*. LWT – Food Science and Technology 44: 1965–1970.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2006. Skin gelatin from bigeye snapper and brownstripe red snapper: chemical compositions and effect of microbial transglutaminase on gel properties. Food Hydrocolloids 20: 1216–1222.

Karim, A. A. and Bhat, R. 2009. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatin. Food Hydrocolloids 23: 563–576.

Killekar, V. C., Koli, J. M., Sharangdhar, S. T. and Metar, S. Y. 2012. Functional properties of gelatin extracted from skin of black kingfish *(Ranchycentron canadus)*. Indian journal of fundamental and applied life sciences 2: 106–116.

Kinsella, J. E. 1977. Functional properties of protein: possible relationships between structure and function in foams. Journal Food Chemistry 7: 273–288.

Koli, J. M., Basu, S., Nayak, B. B., Patange, S. B., Pagarkar, A. U. and Gudipati, V. 2012. Functional characteristics of gelatin extracted from skin and bone of tiger toothed croaker *(Otolithes ruber)* and pink perch *(Nemipterus japonicus)*. Food Bioprod. Process. 90: 555–562.

Koli, J. M., Basu, S., Nayak, B. B., Patange, S. B., Pagarkar, A. U. and Gudipati, V. 2013. Effect of pH and ionic strength on functional properties of fish gelatin in comparison to mammalian gelatin. Fishery Technology 50: 126–132.

Liceaga-Gesualdo, A. M. and Li-Chan, E. C. Y. 1999. Functional properties of fish protein hydrolysate. Journal of Food Science 64: 1000–1004.

Nalinanon, S., Benjakul, S., Visessanguan, W. and Kishimura, H. 2008. Improvement of gelatin extraction from bigeye snapper skin using pepsin-aided process in combination with protease inhibitor. Food Hydrocolloids 2: 615–622.

Neto, V. Q., Narain, N., Silva, J. B. and Bora, P. S. 2001. Functional properties of raw and heat processed cashew nut *(Anarcarduim occidentale* L.*)* kernel protein isolates. Nahrung/Food 45: 258–262.

Ninan, G., Jose, J. and Abubacker, Z. 2011. Preparation and characterization of gelatin extracted from the skins of rohu *(Labeo rohita)* and common carp *(Cyprinus carpio)*. Journal of Food Processing and Preservation 35: 143–162.

Nik Mohd Ikram, M. N. and Ridzwan, B. H. 2013. A preliminary screening of antifungal activities from skin mucus extract of the Malaysian local swamp eel *(Monopterus albus)*. International Research Journal of Pharmacy and Pharmacology, 1–8.

Montero, P. and Gomez-Guillen, M. C. 2000. Extracting conditions for Megrim *(Lepidorhombus boscii)* skin collagen affect functional properties of the resulting collagen. Journal of Food Science 65: 434–438.

Rahmelow, K., Hu¨bner, W. and Ackermann, T. 1998. Infrared absorbance of protein side chains. Anal. Biochem 257: 1–11.

Rainboth, W. J. 1996. Fish of the Cambodian Mekong. FAO species identification field guide for fishery purposes. FAO, Rome. p. 265.

Rawdkuen, S., Thitipramote, N. and Benjakul, S. 2013. Preparation and functional characterization of fish skin gelatin and comparison with commercial gelatin. International Journal of Food Science and Technology 48: 1093–1102.

Rosli, N. A. 2013. Preparation and characterization of eel *(Anguilla Japonica)* skin gelatin as compared to bovine gelatin. Thesis, Universiti Malaysia Terengganu, Malaysia.

Sarabia, A. I., Go´ mez-Guille´n, M. C. and Montero, P. 2000. The effect of added salts on the viscoelastic properties of fish skin gelatin. Food Chemistry 70: 71–76.

Sarbon, N. M., Badii, F. and Howell, N. K. 2013. Preparation and characterization of chicken skin gelatin as an alternative to mammalian gelatin. Food Hydrocolloids 30: 143–151.

Sathe, S. K., Deshpande, S. S. and Salunkhe, D. K. 1982. Functional properties of lupin seed *(Supinus mutabilis)* proteins and protein concentrates. Journal of Food Science 7: 191–197.

Shahidi, F., Han, X. Q. and Syanoiocki, J. 1995. Production and characteristics of protein hydrolysates from cpelin *(Mallotus villosus)*. Food Chemistry 53: 285–293.

Schrieber, R. and Gareis, H. 2007. Gelatine Handbook. Wiley-VCH, GmbH and Co. Weinheim.

Songchotikunpan, P., Tattiyakul, J. and Supaphol, P. 2008. Extraction and electrospinning of gelatin from fish skin. International Journal of Biological Macromolecules 42: 247–255.

Surewicz, W. K. and Mantsch, H. H. 1988. New insight into protein secondary structure from resolution enhanced infrared spectra. Biochim. Biophys. Acra 952: 115–130.

Townsend, A. A. and Nakai, S. 1983. Relationships between hydrophobicity and foaming characteristics of food proteins. Journal of Food Science 48: 588–594.

Utusan Online (2010). Khasiat Belut Melalui Produk Shirah. Web. 3 Mar. 2013.

van der Ven, C., Gruppen, H., de Bont, D. B. A. and Voragen, A. G. J. 2002. Correlations between biochemical characteristics and foam-forming and -stabilizing ability of whey and casein hydrolysates. Journal of Agricultural and Food Chemistry 50: 2938–2946.

Wellner, N., Belton, P. S. and Tatham, A. S. 1996. Fourier transform IR spectroscopic study of hydration-induced structure changes in the solid state of x-gliadins. The Biochemical Journal 319: 741–747.

Wong, W. S. 1989. Proteins. In W. S. Wong (Ed.), Mechanism and theory in food chemistry (pp. 48–104). New York, USA: An AVI Book.

Yakimets, I., Wellner, N., Smith, A. C., Wilson, R. H., Farhat, I. and Michell, J. 2005. Mechanical properties with respect to water content of gelatin films in glassy state. Polymer 46 (12): 577–12,585.

Yang, H. and Wang, Y. 2009. Effects of concentration on nano structural images and physical properties of gelatin from channel catfish skins. Food Hydrocolloids 23: 577-584.

Zayad, J. F. (1997). Solubility of proteins. In Functionality of Proteins in Food. Berlin, Springer-Verlag; 6–22.