

## Effects of organic acid on the physicochemical properties of gelatine extracted from Fringescale sardinella (*Sardinella fimbriata*) bones

<sup>1</sup>Arshad, N.M., <sup>2</sup>Abd. Ghaffar, M. and <sup>1,\*</sup>Mohtar, N.F.

<sup>1</sup>Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.

<sup>2</sup>Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu.

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### Abstract

Gelatine from marine sources especially from the fish becomes a popular alternative to replace the commercial gelatines from bovine and porcine. Fringescale sardinella (*Sardinella fimbriata*) is commonly used in the fish processing industry and has a high potential to produce gelatine from its by-products. The extraction of gelatine is one of the most important steps that influence its functional properties. Inorganic acid was commonly used in the extraction, however, nowadays it faces some issues due to health safety and its effect on the environment. The aim of this study was to assess the effects of organic acid (fruit vinegar) as a replacement for inorganic acid (hydrochloric acid) during extraction on the physicochemical properties of *S. fimbriata* gelatine. The extracted gelatines were characterized in terms of their physico-chemical properties such as the yield percentage, gel strength, melting point, molecular weight, structural composition, and amino acid analyses. Results showed that gelatine extracted with organic acid demonstrated low yield percentage ( $2.9 \pm 0.01\%$ ) and poor gel strength ( $75.9 \pm 1.06$  g) and melting point ( $22.0 \pm 1.00^\circ\text{C}$ ) values compared to the standard. This gelatine also demonstrated a low amount of proline and hydroxyproline contents, which were  $43.77 \pm 8.21$  g/mg and  $34.99 \pm 6.50$  g/mg, respectively. Overall findings have suggested that different types of acid used in the extraction resulted in different physico-chemical properties of the gelatine. Such findings suggested that *S. fimbriata* bone hold the potential to be an alternative source for vast applications in industries. Further studies may focus on different concentrations of acid used in the extraction process. Such findings will help to improve *S. fimbriata* bone gelatine properties for extended uses in various industries.

## 1. Introduction

*Sardinella fimbriata* is one of the most commercial marine species in Malaysia especially for local consumption and economic development. It is commonly found in the coastal area especially in the East Coast regions such as Terengganu, Kelantan and Pahang. It is locally known as Tamban (Ambak *et al.*, 2010). It has high commercial value due to its high demand from the food industry to produce otoshimi, *keropok lekor* and other fisheries products. The high demand for this marine species has led to high production of by-products such as skin, scales, head and bone, which was estimated at around 22%. Such by-products contain up to 30% of high-value collagen (Gómez-Guillén *et al.*, 2002). However, the discarded by-products are commonly

transformed into a low economic product or commonly discarded into the sea (Khiari *et al.*, 2011). As the fishery by-product industries are developing, it has produced more by-products thus affecting the environment in the long run. The utilisation of fish by-products into high value-added products is important for the environment to reduce the organic contaminant charge (Jespersen *et al.*, 2000). Therefore, this study was proposed to utilize waste into wealth. This indirectly may contribute to reducing the impact on the environment and increase sustainable economic development among locals.

Gelatine is one of the high-value by-products that can be derived from fisheries waste such as skin, scales, head and bone. It can be derived from the partial hydrolysis of collagen. It is a protein collected from

\*Corresponding author.

Email: [fazliyana@umt.edu.my](mailto:fazliyana@umt.edu.my)

animal by-products that can be obtained through partial hydrolysis of collagen found in connective tissue, bones, and some of the intestines of animals (Mohtar *et al.*, 2010). One unique property of the gelatine is its thermoreversibility which is able to turn solid into a liquid when heated and turn back to the solid when cooled. It has low in calories and can easily melt in the mouth. Gelatine can be classified into two types which are Type A (produce from acid-treated collagen) and Type B (produce from alkaline treated collagen) (Ahmad and Benjakul, 2011). There are three major steps in extraction, which are pre-treatment (alkaline or acid), followed by extraction with warm water at certain temperatures. By undergoes this process, it will be converted collagen into gelatine. The last and most crucial step is a clarification that involves filtration, concentration, drying, and milling (Schrieber and Gareis, 2007). Different raw materials used in producing gelatine required a specific extraction process as it will determine the quality of gelatine produced (Khiari *et al.*, 2013). The extraction process designed will influence the functional properties of gelatine. A few processing parameters which are temperature, time, and pH was designed accordingly to every sample to produce high-quality gelatine. pH is one of the parameters that majorly contribute to the successfulness of gelatine extraction.

Normally, gelatine is extracted from the fishbone produced from acid-treated collagen (Type A). Usually, hydrochloric acid (HCl) which is inorganic acid was used to break down the bond during the extraction process. As health and green environment issues arise nowadays, organic acid may be one of the alternatives that can be used to replaced inorganic acid during the extraction process for gelatine production. Besides, gelatine production industries especially in small industries face some problems to get inorganic acid chemicals such as acid and base for producing the gelatine. Organic acids are derived from organic compounds such as animal, plant, and microbial sources as they retain acidic properties (Papagianni, 2019). *Nypa fruticans*, commonly known as the nipa palm (nipa or mangrove palm) is a plant used in producing fruit vinegar of organic acids in Terengganu. It is locally known as nipa palm vinegar and is traditionally produced by fermentation of a nipa palm (Yusuf *et al.*, 2015). The chemical properties of nipa palm were stated as nipa palm lignin mainly consists of guaiacyl and syringyl propane units with a small amount of p-hydroxyphenyl propane, phenolic and many others (Tamunaidu and Saka, 2011; Chatatikun and Kwanhian, 2020). Fermented nipa palm vinegar is one of the organic acids produced by organic sources. Therefore, this study was focused on extracting gelatine from fishbone by replacing inorganic acid with an organic acid, which is

the fruit vinegar (nipa palm vinegar) purchased from local industry and also to determine the effects of acid used during extraction on the physicochemical properties of *S. fimbriata* gelatine.

## 2. Materials and methods

### 2.1 Preparation of raw materials

*Sardinella fimbriata* bones were collected from the local fish processing industry and were cleaned from impurities. The bones were undergone series of boiling and washing processes to remove excessive flesh and unwanted materials. The cleaned and dried bones were then stored at -20°C until further used.

### 2.2 Extraction of gelatine from *Sardinella fimbriata* bones

Treatment 1 was carried out according to the method of Sanaei *et al.* (2013) in which the bones were treated in 1 M of inorganic acid (HCl) at 4°C for 14 hrs. The bones were then neutralized by washing four times under tap water until their pH was 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). They were extracted at 67°C for 5 hrs in a water bath. Gelatine solutions were filtered and centrifuged at 7800×g at 4°C for 20 mins. The solutions were dried in an oven at 60°C for 72 hrs. Dried gelatines were then packed in zip-locked bags and stored in a desiccator.

Treatment 2 was carried out according to the method of Sanaei *et al.* (2013). The bones were treated in organic acid (fruit vinegar) at 4°C for 14 hrs. The bones were then neutralized by washing four times under tap water until their pH was 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). They were then extracted at 67°C for 5 hrs in a water bath. Gelatine solutions were filtered and subsequently centrifuged at 7800×g at 4°C for 20 mins. The solutions were dried in an oven at 60°C for 72 hrs. Dried gelatines were then packed in zip-locked bags and stored in a desiccator.

### 2.3 Yield of gelatine

The percentage of yield was calculated according to Sanaei *et al.* (2013). Gelatine obtained was calculated based on the following formula:

$$\text{Percentage yield of gelatine} = \frac{\text{Weight of dried gelatine (g)} \times 100}{\text{Weight of dried fishbone (g)}}$$

### 2.4 Proximate composition

Protein, ash, moisture and lipid content were analysed according to AOAC (2000) method. The results of the samples were measured and recorded in triplicates.

## 2.5 Physical measurement of gelatine

### 2.5.1 Gel strength

The gel strength of gelatine was determined according to Gómez-Guillén *et al.* (2002) with slight modification. An amount of 6.67% (w/v) of the solution was prepared by adding 0.33 g of gelatine to 5.0 mL of distilled water and dissolved at 60°C for 30 mins. The gelatine solutions were filled in a beaker and allowed to cool for 15 mins at 25°C. The gelatines were kept in a refrigerator at 4°C for 18 hrs. The samples were measured by using a texture analyser (TA.XT. PLUS, Stable Micro Systems, United Kingdom) with 5 kN load cell equipped with 1.27 mm diameter plunger. The maximum force obtained had penetrated at 4 mm into the centre of gelatine gel and a penetration speed of 1 mm/s was used.

### 2.5.2 Melting point

The melting point of gelatine was determined according to Muyonga *et al.* (2004b) with slight modification. A 6.67% (w/v) of gelatine solutions was prepared by adding 0.33 g of gelatine to 5.0 mL of distilled water were prepared and dissolved completely. The gelatine solution was placed in a refrigerator at 4°C for 18 hrs. The cooled gelatines 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 was increased, and the melting point was defined at which the gel starts to move down. All data were recorded.

## 2.6 Chemical measurement of gelatine

### 2.6.1 Molecular weight distribution

The molecular weight distribution of gelatine was analysed according to Laemmli (1970) with slight modification. An amount of 2 mg/mL of the gelatine was dissolved in 5% of SDS solution and the mixture was incubated at 85°C in a water bath for 1 hr. The mixture was centrifuged at 5000×g for 5 mins at room temperature to remove undissolved debris. An amount of 200 µL of samples was mixed with 200 µL of Laemmli sample buffer (4× Laemmli Sample Buffer, Biorad, United States) at a ratio of 1:1 (w/v) and heated for 3 mins in boiling water. Samples were loaded onto the readymade polyacrylamide gel (12% Mini-Protean TGX Precast Gels, Biorad, United States) and subjected to electrophoresis at a constant voltage of 120 V. The gel then was stained for 4 hrs with commassie blue (Bio-Safe Coomassie Stain, Biorad, United States) and destained with 7.5% (v/v) methanol and 5% (v/v) acetic acid overnight. Amide range (10 – 250 kD) molecular weight was used to estimate the molecular weight of gelatine. The gel was photographed using a gel doc.

### 2.6.2 Amino acid content

Amino acid content was determined according to Sanaei *et al.* (2013) with slight modification. The most important amino acid in gelatine was imino acid which is hydroxyproline and proline. The amount of imino acid represents the quality of the extracted gelatine. An amount of 0.1 g gelatine sample was dissolved in 5 mL of 6M HCl, dried and hydrolyse in the test tube at 110°C for 24 hrs. The samples were then dissolved in 400 µl of 50 µmol of Amino Acid Standard. Deionized water was added to make up the solution until 100 µL. A number of 10 µL of the samples were injected. Amino acid content was determined using a High-Performance Liquid Chromatography (HPLC) (1525 Binary HPLC Pump, Waters, United States) equipped with Fluorescence Detector and AccQ Tag Column (3.9×150 mm). AccQ Eluent A and AccQ Eluent B or 60% acetonitrile acid was used as the mobile phase with a flow rate of 1 mL/min.

### 2.6.3 Structural composition

Fourier – Transform Infrared Spectra (FTIR) analysis was carried out according to Sira (2016). Gelatine samples were subjected to FTIR spectrometer (FTIR-iS10, Thermo Scientific, United States) to identify the functional properties of the gelatine. A number of 2 mg of gelatine powder were placed on the crystal cell of the FTIR spectrometer and was clamped in close contact with the crystal by a force arm. The FTIR spectra over an absorbance between 500 cm<sup>-1</sup> to 4000 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup> from 32 scans were recorded. The spectra were analysed using the Omnic 5.0 spectroscopic software.

## 2.7 Statistical analysis

The average means value was 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 significant difference was considered at (P<0.05).

## 3. Results and discussion

### 3.1 Gelatine yield and proximate composition

Treatment 1 represented gelatine extracted using inorganic acid (HCl) while Treatment 2 represented the gelatine extracted using organic acid (fruit vinegar). The yield and the proximate content of the Treatment 1 and Treatment 2 were recorded and compared to each other. All data were shown in Table 1.

Table 1. Yield and proximate composition of gelatine from Treatment 1, Treatment 2 and Control

Analysis (%)	Treatment 1	Treatment 2	Control
Yield	6.0±0.03 <sup>a</sup>	2.9±0.01 <sup>b</sup>	-
Protein	59.1±0.29 <sup>b</sup>	43.9±0.38 <sup>c</sup>	82.2±4.88 <sup>a</sup>
Moisture	14.3±0.21 <sup>b</sup>	16.1±0.68 <sup>c</sup>	13.1±0.55 <sup>a</sup>
Lipid	0.76±0.44 <sup>a</sup>	0.89±0.31 <sup>a</sup>	0.41±0.21 <sup>a</sup>
Ash	4.7±0.21 <sup>b</sup>	5.5±0.13 <sup>c</sup>	2.3±0.11 <sup>a</sup>

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

The results show Treatment 2 has a lower yield percentage (2.9±0.01) compared to Treatment 1 (6.0±0.03). The different amounts of yield may be due to the different types of acid used in the extraction process (Niu *et al.*, 2013). Inorganic acid (HCl) used in Treatment 1 was a strong acid while organic acid (fruit vinegar) used in Treatment 2 was a weak acid. A strong acid has a greater number of reactive hydrogen ions and resulted in a higher yield during the extraction process (Niu *et al.*, 2013). Moreover, strong acid produces more hydrogen ions than weak acid and is easier to facilitate the dissolution of the intra- and intermolecular cross-linked collagen. When these cross-linking are cleaved, the triple helix structure of collagen was changed to random coils and gelatine were produced (Sri *et al.*, 2017).

Treatment 1 indicated higher protein content (59.1±0.29%) compared to Treatment 2 (43.9±0.38%) but lower than control (82.2±4.88%). As stated by Muyonga *et al.* (2004a), the amount of protein content was related to the amount of yield produced. Higher of protein content resulted in higher of production of yield. This is in good alignment with the previous findings on the yield percentage of gelatine that shows Treatment 1 has higher yield production than Treatment 2. This may be due to the different acid used during extraction process (Kusumaningrum *et al.*, 2018).

The highest moisture content was obtained from Treatment 1 (14.3±0.21%). Followed by Treatment 2 (16.1±0.68%) and control (13.1±0.55%), respectively. This result shows that control has lower moisture content compare to Treatment 1 and Treatment 2. A previous study carried out by Kusumaningrum *et al.* (2018) stated that the maximum amount of moisture content for gelatine ranging from 14% to 16%. This means the result of moisture content for both treatments are within the range and can be accepted.

Treatment 2 shows higher lipid content compared to Treatment 1 and control samples with the amount of 0.89±0.31%, 0.76±0.44% and 0.41±0.21%, respectively. The lipid content of Treatment 1, Treatment 2 and

Control were still in an accepted range which is less than 1% (Cho *et al.*, 2004).

The results obtained for ash content for all samples were 4.7±0.21% for Treatment 1, 5.5±0.13% for Treatment 2 and 2.3±0.11% for control. It shows that Control has a much lower ash content compared to Treatment 1 and Treatment 2. Usually, the amount of ash content up to 2.0% can be accepted in food applications (Cho *et al.*, 2004). This means these results can be accepted. Besides, the different amounts of ash found in this study may probably be due to the different amounts of minerals from the fish bones (Khiari *et al.*, 2011).

### 3.2 Physical measurement of gelatine: gel strength, and melting point

Gel strength and melting point are some of the most important physical properties of gelatine (Sanaei *et al.*, 2013). These properties were specifying the most suitable industry gelatine that can be applied. Table 2 shows the gel strength and melting point of gelatine extracted from Treatment 1, Treatment 2, and Control.

Table 2. Gel strength and melting point of gelatine from Treatment 1, Treatment 2 and Control.

Analysis	Treatment 1	Treatment 2	Control
Gel strength (g)	132.4±2.64 <sup>a</sup>	75.9±1.06 <sup>b</sup>	350.5±8.48 <sup>c</sup>
Melting point (°C)	25±1.52 <sup>a</sup>	22.0±1.00 <sup>b</sup>	38±1.53 <sup>c</sup>

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

The gel strength of gelatine for all samples was 132.4±2.64 g, 75.9±1.06 g and 350.5±8.48 g respectively. According to a previous study, the gel strength of fish gelatine ranging from 124 to 426 g compared to 200 to 350 g for commercial gelatine such as bovine and porcine (Khiari *et al.*, 2011). It means that gelatine from this study was still in the range and can be accepted. Results show Treatment 1 has better gel strength compare to Treatment 2 but is poor than the Control. Treatment 1 used inorganic acid (HCl) which is strong acid while Treatment 2 used organic acid (fruit vinegar) which is a weak acid. The different values of gel strength may be affected by the extraction process (Tinrat and Sila-Asna, 2017). Herpandi *et al.* (2011) revealed that Salmon and Bigeye snapper had the gel strength of 108.0 g and 105.7 g, respectively is lower than that of gelatine from Treatment 1 in this study. Unfortunately, the gel strength of Nile tilapia which is 252.0 g was higher than Treatment 1 and Treatment 2. The variation value of gel strength among these species may be due to imino acid content which is proline and hydroxyproline. According to a previous study, the difference in gel strength correlated to amino acid

composition and size of the protein chain (Muyonga *et al.*, 2004b). Low gel strength value may result from low concentration and molecular weight distribution (Ockerman and Hansen, 1988).

Gelatin gel will start to melt at a certain temperature called the gel melting point. The melting point for fish gelatin and bovine range from 11 to 28°C and 28 to 38°C respectively. In this study, Table 2 shows the result of the melting point of gelatin from Treatment 1, Treatment 2 and Control. The results were 25±1.52°C, 22.0±1.00 and 38±1.53°C respectively. Treatment 1 has a higher melting point compared to Treatment 2 but is lower than Control. Fish gelatin usually has a much lower melting point compared to mammalian gelatin such as bovine and porcine (Cho *et al.*, 2005). This is further supported by the previous study conducted at 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. Different melting point values may be due to different amino acid content. It is interesting to find out from the previous study that the lower amino acid contents and less proline and hydroxyproline were affected the characteristics of melting point (Gómez-Guillén *et al.*, 2002). Besides, the origin of the raw material used in the process gives greatly influenced the melting point values (Gilsenan and Ross-Murphy, 2000).

### 3.3 Chemical measurement of gelatin: molecular weight distribution, amino acid, and structure composition

The protein pattern of gelatin was determined by using SDS-PAGE as shown in Figure 1. Gelatin commonly consists of a mixture of a fragment with molecular weights in the range from 80 to 250 kDa (Karim and Bhat, 2009). Treatment 1 and Treatment 2 were characterized as type 1 collagens due to the presence of  $\alpha_1$ ,  $\alpha_2$  and  $\beta$  chains (Benjakul *et al.*, 2009). Each treatment shows that the molecular weight for  $\alpha_1$  and  $\alpha_2$  chains was around 100 kDa, while  $\beta$  chains were around 200 kDa. The vary of molecular weight distribution may be related to the extraction process (Jongjareonrak *et al.*, 2010) Gelatin with the higher content of  $\alpha$  chain has better gel strength (Nagarajan *et al.*, 2012). This is in good alignment with previous findings in gel strength that shows Treatment 1 has a good gelling ability. In addition, the distribution of molecular weight also affected the amount of amino acid composition (Jongjareonrak *et al.*, 2010). The presence of  $\beta$  chains was related to the intermolecular cross-linked (Kim, 2013). A large amount of  $\beta$  chains reported showing a negative effect on the functional properties of gelatin such as lower viscosity and melting point (Muyonga *et al.*, 2014b). It shows that this result can be accepted because each treatment has a lower melting

point as shows in the melting point part.

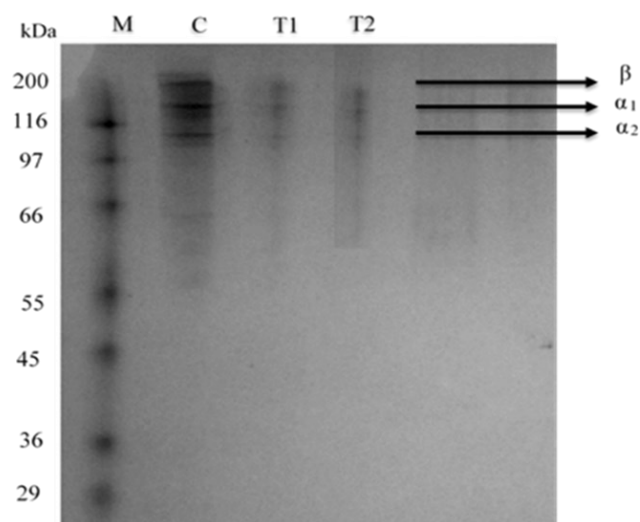


Figure 1. SDS-PAGE pattern of gelatin extracted from Treatment 1 (T1), Treatment 2 (T2) and Control (C) while M as a marker.

Table 3. Amino acid compositions (mg/g) of gelatin from Treatment 1, Treatment 2 and Control.

Amino acids (g/mg)	Samples		
	Treatment 1	Treatment 2	Control
Glutamic acid	88.77±4.6 <sup>a</sup>	46.03±7.20 <sup>b</sup>	156.19±15.75 <sup>c</sup>
Phenylalanine	20.82±0.34 <sup>a</sup>	8.93±1.53 <sup>b</sup>	45.51±7.09 <sup>c</sup>
Tyrosine	6.87±0.11 <sup>a</sup>	3.97±0.49 <sup>b</sup>	17.47±2.98 <sup>c</sup>
Glycine	209.50±6.32 <sup>a</sup>	88.33±16.34 <sup>b</sup>	373.91±6.21 <sup>c</sup>
Proline	107.65±3.25 <sup>a</sup>	43.77±8.21 <sup>b</sup>	225.84±3.93 <sup>c</sup>
Alanine	96.64±3.68 <sup>a</sup>	40.07±8.20 <sup>b</sup>	147.20±6.34 <sup>c</sup>
Methionine	26.55±0.20 <sup>a</sup>	10.53±2.12 <sup>b</sup>	28.13±3.87 <sup>a</sup>
Hydroxyproline	89.05±0.49 <sup>a</sup>	34.99±6.5 <sup>b</sup>	230.66±26.6 <sup>c</sup>
Aspartic acid	51.27±3.53 <sup>a</sup>	24.62±4.32 <sup>b</sup>	86.55±11.82 <sup>c</sup>
Valine	21.81±0.32 <sup>a</sup>	9.82±1.58 <sup>b</sup>	49.43±2.32 <sup>c</sup>
Isoleucine	10.24±0.03 <sup>a</sup>	5.07±0.63 <sup>b</sup>	23.46±1.33 <sup>c</sup>
Leucine	23.53±0.22 <sup>a</sup>	10.00±1.69 <sup>b</sup>	54.75±2.52 <sup>c</sup>
Lysine	32.76±1.81 <sup>a</sup>	15.99±2.69 <sup>b</sup>	61.92±4.63 <sup>c</sup>
Serine	33.70±0.14 <sup>a</sup>	15.13±2.60 <sup>b</sup>	70.68±8.09 <sup>c</sup>
Histidine	9.83±0.22 <sup>a</sup>	3.83±0.69 <sup>b</sup>	20.43±3.95 <sup>c</sup>
Arginine	80.61±0.87 <sup>a</sup>	32.25±5.64 <sup>b</sup>	169.51±25.53 <sup>c</sup>
Threonine	23.95±0.14 <sup>a</sup>	10.14±1.74 <sup>b</sup>	40.39±6.57 <sup>c</sup>

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

The amino acid composition is one of the important elements to characterize the quality of the gelatin. Table 3 shows the amino acid composition of gelatin from Treatment 1, Treatment 2 and Control. There was 17 amino acid that found in these gelatines. The most dominant amino acid in Treatment 1, Treatment 2 and Control was glycine that resulted in 209.50±6.32, 88.33±16.34 and 373.91±6.21 respectively. These results were further supported by a previous study that state glycine was the dominant amino acid found in Carp

gelatine (Duan *et al.*, 2009). Treatment 1 and treatment 2 have lower glycine compare to Control. The result obtained was consistent with the finding by Zhang *et al.* (2011) and Liu *et al.* (2008) stated that fish gelatine had lower quantities of glycine compared to the commercial gelatine.

The most important amino acid in gelatine was proline and hydroxyproline, called imino acid. The stability of the triple helix structure in denatured gelatine is generally affected by the content of the amino acid (Nikoo *et al.*, 2011). Hydroxyproline plays a major role in the stabilization of the triple helix structure. This means gelatine has high imino acid content tend to have a better characteristic such as melting point, gel strength and viscosity. This is further supported by a previous study by Shyni *et al.* (2014) reported that the variation amount of imino acid especially hydroxyproline were affected by the viscosity and gel formation of the gelatine.

Treatment 1 shows higher imino acid content which has  $107.65 \pm 3.25$  g/mg of proline and  $89.05 \pm 0.49$  g/mg of hydroxyproline compare to Treatment 2. Treatment 2 only has  $43.77 \pm 8.21$  g/mg of proline and  $34.99 \pm 6.50$  g/mg of hydroxyproline. The difference in such results may be due to the difference in acid used during the extraction process. It was being proven by the previous study that shows the different amounts of hydroxyproline content was affected by the extraction process (Nikoo *et al.*, 2011). However, imino acid content in Treatment 1 and Treatment 2 was lower compared to the Control. Control gelatine has  $225.84 \pm 3.93$  g/mg of proline and  $230.66 \pm 26.6$  g/mg of hydroxyproline. Generally, mammalian gelatine contains higher imino acids compare to fish gelatine (Shyni *et al.*, 2014). Gelatine with low imino acid content tends to have a low melting point and gel strength (Muyonga *et al.*, 2004b). This is in good alignment with the previous findings which shows Treatment 1 and Treatment 2 have a lower gel strength and melting point than a Control.

Fourier transform infrared spectroscopy (FTIR) analysis was conducted to observe and compare the main structural characteristics of the gelatine from Treatment 1, Treatment 2 and Control. Comparison between this gelatine was based on the comparison absolute peak intensity. The region was divided into several regions which are amide A, amide B, amide I, amide II and amide III (Kong and Yu, 2007). Similar results were shown in this study which has amide A, amide B, amide I, amide II and amide III for all gelatine.

There were five basic regions involved known as amide A, amide B, amide I, amide II and lastly amide III. The characteristic of the amide group was an exhibit by

protein due to the absorption bands in the infrared spectra. This absorption is due to C=O stretching, N-H stretching, C-N stretching and O-C-N bending. All the gelatine shows similar spectra, in which the absorption bands are situated in the amide region. Besides, these gelatine samples had major peaks in the amide region. However, it also shows there were slight differences in spectra.

There were three major peaks regions know as regions 1, 2 and 3. Figure 2 shows three major peak regions for Treatment 1, Treatment 2 and Control. Treatment 1 resulted three major peak regions as 1 ( $3400$  to  $1800$   $\text{cm}^{-1}$ ), 2 ( $1750$  to  $1300$   $\text{cm}^{-1}$ ) and 3 ( $1200$  to  $700$   $\text{cm}^{-1}$ ). Amide A was  $3232.25$   $\text{cm}^{-1}$ , amide B was  $2968.04$   $\text{cm}^{-1}$ , amide I was  $1633.00$   $\text{cm}^{-1}$ , amide II was  $1538.50$   $\text{cm}^{-1}$ , and lastly, amide III was  $1057.33$   $\text{cm}^{-1}$ . While Treatment 2 show region 1 ( $3650$  to  $1800$   $\text{cm}^{-1}$ ), 2 ( $1750$  to  $1300$   $\text{cm}^{-1}$ ), and 3 ( $1250$  to  $550$   $\text{cm}^{-1}$ ). The wavenumber of amide A, amide B, amide I, amide II, and amide III were  $3232.25$   $\text{cm}^{-1}$ ,  $2968.04$   $\text{cm}^{-1}$ ,  $1621.90$   $\text{cm}^{-1}$ ,  $1538.50$   $\text{cm}^{-1}$ , and  $1053.95$   $\text{cm}^{-1}$  respectively. Control shows the wavenumber of regions 1, 2 and 3 where  $3600$  to  $1800$   $\text{cm}^{-1}$ ,  $1750$  to  $1350$   $\text{cm}^{-1}$ , and  $1200$  to  $500$   $\text{cm}^{-1}$  respectively. The regions were divided into amide A ( $3280.62$   $\text{cm}^{-1}$ ), amide B ( $2969.00$   $\text{cm}^{-1}$ ), amide I ( $1634.44$   $\text{cm}^{-1}$ ), amide II ( $1538.98$   $\text{cm}^{-1}$ ), and amide III

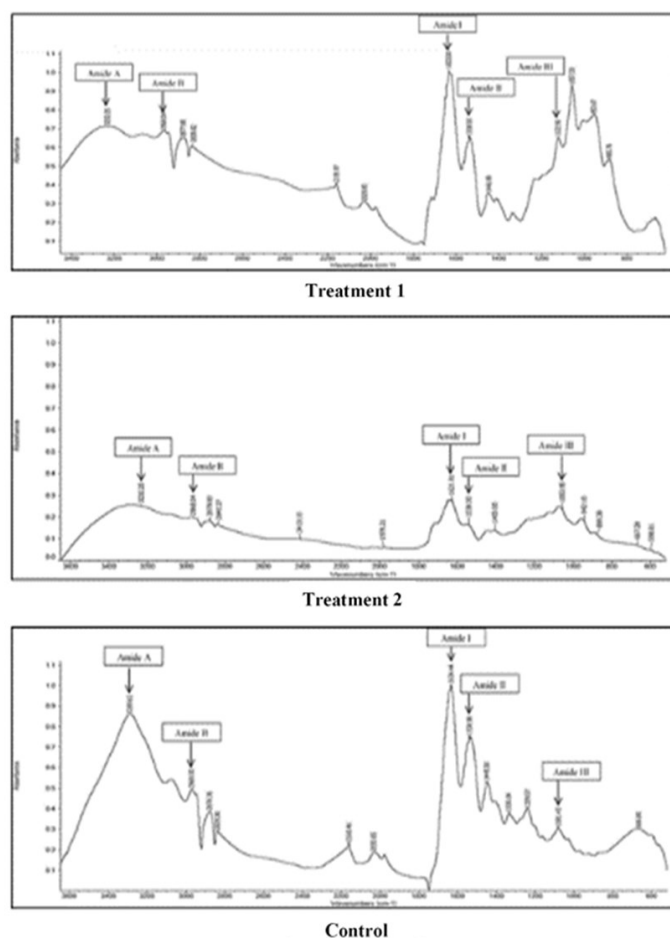


Figure 2. FTIR spectrum of Treatment 1, Treatment 2 and control gelatine demonstrated three major peaks.

(1081.43  $\text{cm}^{-1}$ ).

The intensity of amide I was the result of C=O stretching vibration coupled with C-N stretch and CNN deformation (Liu *et al.*, 2012). While amide II peak was a result of N-H bending and C-N stretching vibration (Cebi *et al.*, 2016). Amide III is generally related to C-N stretching and N-H bending, it also includes vibration of the triple helix structure (Muyonga *et al.*, 2004a). Amide I and amide II for all gelatine in this study were observed was slightly different which ranging from 1700 to 1600  $\text{cm}^{-1}$  and 1560 to 1500  $\text{cm}^{-1}$  respectively. It is in good alignment with the previous study by Muyonga *et al.* (2004a) and Yakimets *et al.* (2005) which have similar results. However, the different spectral values among this gelatine may be due to the different conformation and orientation of polypeptide chains. A previous study also shows that a lower wavenumber was resulted in a decrease in molecular order due to conformational change (Ahmad *et al.*, 2011). Amide I is the most important for the determination of the secondary structure of protein due to the C=O stretch vibration of peptide linkages (Jackson and Mantsch, 1995).

The wavenumber of amide III was 1057.33  $\text{cm}^{-1}$  for Treatment 1, 1053.95  $\text{cm}^{-1}$  for Treatment 2 and 1081.43  $\text{cm}^{-1}$  Control. These results show that both treatments have a much lower wavenumber compare to the Control gelatine. This is maybe due to a greater disorder from the  $\alpha$ -helical to a random coil structure (Friess and Lee, 1996). A previous study also reported that a lower wavenumber may be due to loss of triple helix states and denaturation of collagen to protein (Muyonga *et al.*, 2004a). The wavenumber of amide A for Treatment 1 was 3232.25  $\text{cm}^{-1}$  and Treatment 2 was 3289.62  $\text{cm}^{-1}$ . Generally, amide A mainly associated with the N-H stretching due to the existence of a hydrogen bond. According to a previous study, the position N-H group of peptides was shifted to lower frequencies when the hydrogen bond involved (Jridi *et al.*, 2013).

#### 4. Conclusion

Overall findings have suggested that different types of acid used in the extraction process resulted in different physico-chemical properties of the gelatine. Findings from this study have suggested that *S. fimbriata* bone hold the potential to be an alternative source for vast applications in industries. *S. fimbriata* bone gelatine may demonstrate low properties, however, it is comparable to other species carried out in previous studies. It can be used in the food and beverages industry that require low gel strength value in the formulation ingredients. Further research on the potential properties of *S. fimbriata* bone gelatine should be carried out to better understand its

potential for Halal and Kosher markets.

#### Conflict of interest

The authors declare no conflict of interest.

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