

## Effect of Extraction Temperature on the Physicochemical Properties of Gelatine from the Skin of Black Tilapia (*Oreochromis mossambicus*)

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**ABSTRACT:** *The physicochemical properties of gelatine from the skin of black tilapia (*Oreochromis mossambicus*) as affected by different extraction temperatures (45°C, 55°C, 65°C and 75°C) were investigated. The yield of gelatine increased significantly as the extraction temperature increased. Electrophoretic protein pattern and free amino acid content of tilapia gelatine showed that high extraction temperature (75°C) contributed to the decrease in major protein components ( $\alpha$ - and  $\beta$ -chains) of the extracted gelatine. Fourier transform infrared (FTIR) spectroscopy showed a significant loss of triple helical structure in all samples. The gel strength, gelling temperature and melting temperature of tilapia gelatine decreased as the extraction temperature increased. The gelling and melting temperature of gelatine extracted at 45°C (G45) was 18.9°C and 26.2°C, respectively, which was about 2°C lower than those of the bovine gelatine tested. This suggested that G45 and bovine gelatine gel possessed similar thermal stability. In addition, the rheological test revealed that, as the extraction temperature increased, the storage modulus ( $G'$ ) of tilapia gelatine was frequency dependent and took a relatively long time to achieve maximum  $G'$  at 10°C. Black tilapia gelatine showed higher emulsion ability and emulsion stability when lower extraction temperatures were used. The foaming properties of tilapia gelatine was similar among gelatine extracted from 45°C to 65°C. A significant decrease in foamability and stability was observed when extraction temperature further increased to 75°C. Therefore, black tilapia gelatine extracted at 45°C has the functional properties that are comparable to bovine gelatine.*

**Keywords:** Black tilapia, gelatine, extraction temperature, rheology, emulsion

## 1. INTRODUCTION

Gelatine is a food biopolymer produced from collagen through partial hydrolysis. It has been widely used in food, pharmaceutical and cosmetic industries attributed to its gelling, emulsifying and film forming properties. In general, commercial gelatine is usually extracted from bovine and porcine connective tissue such as skin and bone.<sup>1</sup> However, porcine and bovine gelatine are not acceptable in some religions. For example, porcine gelatine is prohibited for Muslim community and bovine gelatine only can be accepted if the production of gelatine meets the halal requirement.<sup>2</sup> Therefore, as the increasing market for certified halal food product, fish gelatine has received considerable attention as the alternative to bovine and porcine gelatine.

Tilapia is the second most cultured fish in the world, making it a sustainable source for fish products such as tilapia fish fillet.<sup>3</sup> However, the huge amount of waste (such as skin and bone) produced during the industrial processing of tilapia fillet has contributed to the environmental issue. Therefore, the extraction of gelatine from tilapia provides a sustainable alternative to mammalian gelatine and help to reduce the waste. Tilapia gelatine has been successfully extracted by several researchers.<sup>2,3</sup> Apart from species and type of tissue, the extraction process of gelatine is an important factor that could affect the physicochemical properties of gelatine.<sup>4,5</sup> Niu et al. report that the type and concentration of acid during of pretreatment tilapia skin significantly affect the yield, viscosity and molecular weight distribution of the extracted tilapia fish gelatine.<sup>2</sup> In addition, the optimum type of acid and its concentration for tilapia skin pretreatment during gelatine extraction were 0.03 M for citric acid, 0.18 M for acetic acid, and 0.05 M for HCl.<sup>2</sup> Another parameter that affects the physicochemical properties of gelatine is the extraction temperature during the thermal hydrolysis of collagen. Studies show that the yield of gelatine from chum salmon, seabass and clown featherback increased as the extraction temperature increased.<sup>6-8</sup> However, those gelatines had lower functional properties such as gel strength when extraction temperature increased. There is no report regarding how the extraction temperature affect the physicochemical properties of tilapia skin gelatine especially gelling properties. Therefore, the objective of this study is to investigate the effect of extraction temperature on the physicochemical properties of gelatine from black tilapia skin.

## 2. EXPERIMENTAL

### 2.1 Chemicals

All chemicals used in present study were of analytical grade. Coomassie Blue R-250 were purchased from Bio-Rad Laboratories (Hercules, CA, United States). Citric acid, 2,4,6-trinitrobenzenesulfonic acid (TNBS), sodium sulfite and L-leucine were bought from Sigma Aldrich (M) Sdn. Bhd. (Selangor, Malaysia). EZ-Run™ Pre-stained Rec Protein Ladder was purchased from Fisher Scientific (M) Sdn. Bhd. (Penang, Malaysia). Bovine gelatine with bloom strength of 240 g was purchased from Sim Company (Penang, Malaysia). Palm oil was purchased from local supermarket (Penang, Malaysia).

### 2.2 Preparation of Black Tilapia Skin

Black tilapia fishes were purchased from local market in Penang, Malaysia. Upon arrival at the laboratory, the scales and skin were then removed from the fish manually. The removed skin was washed with tap water thoroughly to remove the adherent muscle and tissue before it was cut into smaller pieces (1.5 cm × 1.5 cm). The black tilapia skin was then stored at –20°C prior to extraction.

### 2.3 Extraction of Gelatine from Black Tilapia Skin

Gelatine from black tilapia skin was extracted according to the method described by Kittiphattanabawon et al. with some modifications.<sup>8</sup> The prepared black tilapia skin was soaked in 0.05 M NaOH at skin-to-NaOH ratio of 1:10 (w/v). The mixture was then stirred at 250 rpm using an overhead stirrer at room temperature for 4 h. The NaOH solution was changed every 2 h. The NaOH treated tilapia skin was then washed with tap water until the pH of washed water become neutral. Then, the skin was soaked in 0.03 M citric acid at skin-to-citric acid ratio of 1:10 (w/v). The mixture was stirred at 250 rpm using an overhead stirrer at room temperature for 1 h. The acid treated tilapia skin was washed with tap water until the pH of washed water was neutral. The gelatine was extracted by soaking the acid treated skin in distilled water at skin-to-water ratio of 1:4 (w/v). The mixture was then incubated in a temperature-controlled water bath at different temperatures (45°C, 55°C, 65°C and 75°C) and stirred at 200 rpm using overhead stirrer for 12 h. The extracted gelatine was then filtered by using cheese cloth. This filtrate was then further filtered with Whatman filter paper No. 3 using a Buchner funnel. The filtrate was then freeze-dried using freeze drier. The gelatine samples extracted at 45°C, 55°C, 65°C and 75°C were referred to as G45, G55, G65 and G75, respectively. All the gelatine samples were stored in desiccator containing silica gel at room temperature prior to analysis.

## 2.4 Characterisation of Gelatine

### 2.4.1 Yield

The dry weight of black tilapia skin was determined by drying the tilapia skin at 105°C in a hot-air oven for 24 h. The skin was then weighed every hour until the weight of the skin was constant. The constant weight then was recorded as the weight of initial dry skin. The yield of tilapia skin gelatine was calculated according to the following equation:

$$\text{Yield} = \frac{\text{weight of freeze dried gelatine}}{\text{weight of initial dry skin}} \times 100\% \quad (1)$$

### 2.4.2 SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method by Kittiphattanabawon et al.<sup>8</sup> A total of 30 µg of gelatine sample was loaded on the polyacrylamide gel made of 10% resolving gel. SDS-PAGE was then performed using Mini-PROTEAN® Tetra Vertical Electrophoresis Cell (Bio-Rad Laboratories, Hercules, CA, United States). All the gelatine samples were run at 80 V for the first 10 min and then run at 120 V until the solvent front reach about 1 cm before the end of the gel. After SDS-PAGE, the gel was stained overnight with staining solution containing 0.1% (w/v) Coomassie blue R-250 in 40% (v/v) methanol and 10% (v/v) acetic acid. EZ-Run™ Pre-stained Rec Protein Ladder (broad range) was used to estimate the molecular weight of gelatine samples.

### 2.4.3 Determination of free amino group content

Free amino group content was determined according to the method described by Benjakul et al.<sup>9</sup> A total of 125 µl of gelatine sample with a concentration of 10 mg ml<sup>-1</sup> was added to 2 ml of phosphate buffer (pH 8) and 1 ml of 0.01% 2,4,6-trinitrobenzenesulfonic acid (TNBS). Then, the mixture was then incubated at 50°C using a water bath for 30 min in dark. After incubation, 2 ml of sodium sulfite (Na<sub>2</sub>SO<sub>3</sub>) was added to the mixture to terminate the reaction. The mixture was left at room temperature for 15 min to let it cool down. The absorbance of the sample was measured at 420 nm using UV Mini-1240 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). L-leucine with concentration ranging from 0.5 mM to 5.0 mM was used as standard to plot the standard curve.

#### 2.4.4 FTIR spectroscopy

FTIR spectra of tilapia gelatine samples were examined using IR Prestige-21 FTIR spectrometer (Shimadzu, Kyoto, Japan) with MIRacle™ Single Reflection ATR (Pike Technologies, Madison, WI, United States) mounted in the sample compartment. The spectra of gelatine samples were acquired in attenuated total reflection (ATR) mode within the IR range of  $4000\text{ cm}^{-1}$  to  $400\text{ cm}^{-1}$  with a resolution of  $4\text{ cm}^{-1}$ . Automatic signals were collected in 32 scans against the background spectrum. IR solution software (Shimadzu, Kyoto, Japan) was used to analyse the FTIR spectra obtained.

#### 2.4.5 Determination of gel strength

The gel strength of gelatine was determined according to the method as described by Kittiphattanabawon et al. with some modifications.<sup>8</sup> Gelatine samples with a concentration of 6.67% (w/v) were prepared and transferred to a cylindrical container with an internal diameter of 3 cm and height of 3.6 cm. The gelatine solution was left to cool down to room temperature ( $25^{\circ}\text{C}$ ). The solution was then incubated at  $10^{\circ}\text{C}$  for 18 h prior to gel strength determination. The dimensions of all gelatine gels were 3 cm in diameter and 1.5 cm in height. The gel strength of the sample was determined using TA XT Plus texture analyser (Stable Micro Systems, Surrey, United Kingdom) with 5 kg load cell. The maximum force (in g) was recorded when the standard radius cylinder (P/0.5R) probe penetrated the gel to the depth of 4 mm at  $0.5\text{ mm s}^{-1}$ . It should be noted that the determination of gel strength in the present study was performed using a container that smaller than standard bloom jar (internal diameter is 5.9 cm). The results obtained were expected higher than the gel strength that was measured using a standard bloom jar. The results obtained were intended for comparison among tilapia gelatine extracted at different temperatures and commercial bovine gelatine.

#### 2.4.6 Rheological properties of gelatine

Dynamic viscoelastic properties of gelatine samples were investigated using AR 1000-N stress-controlled rheometer (TA Instruments, New Castle, DE, United States) equipped with a steel cone-plate geometry (diameter: 40 mm; cone angle:  $2^{\circ}$ ; transition gap:  $51\text{ }\mu\text{m}$ ). Gelatine solution (6.67% (w/v)) was prepared. Prior to rheological test, stress sweep was performed at the frequency of 1 Hz to determine the linear viscoelastic region (LVR) of gelatine samples. The outer edge of the sample was covered with a thin layer of silicone oil to prevent evaporation during the experiment. Change in storage modulus ( $G'$ ) and loss modulus ( $G''$ ) were measured in the rheological test.

#### **2.4.6.1 Temperature sweeps**

Temperature sweep was performed to determine the gelling and melting temperature of the gelatine samples. The samples were equilibrated at 40°C for 2 min before the temperature sweep test. The gelatine samples (except G75) were cooled on a Peltier plate from 40°C to 10°C and heated back from 10°C to 40°C. The scanning rate for both cooling and heating was 1°C min<sup>-1</sup>. Temperature sweep test for G75 was performed in the same manner as other gelatine samples except it was cooled from 40°C to 4°C and then it was heated back from 4°C to 40°C. This is because the preliminary test showed that the gelling temperature of G75 was lower than 10°C. Generally, the temperature at which  $G'$  equals to  $G''$  ( $G'/G''$  cross over) is considered as the point where the phase transition of gelatine takes place.<sup>10</sup> Therefore, in the present study, the temperature at which  $G'/G''$  cross over occurred during cooling was considered as gelling temperature whereas the temperature at which  $G'/G''$  cross over occurred during heating was considered as melting temperature. All samples were measured in triplicate.

#### **2.4.6.2 Frequency sweeps**

Frequency sweep test was performed to investigate the changes in the storage ( $G'$ ) and loss ( $G''$ ) modulus of gelatine sample as a function of frequency (0.1–100.0 rad s<sup>-1</sup>) at 10°C. The sample was equilibrated at 10°C for 2 min before frequency sweep. The oscillating stress applied to all samples were within their LVR.

#### **2.4.6.3 Time sweeps**

Time sweep was conducted for 3 h at 10°C. The test was carried out at a frequency of 1 Hz. The oscillating stress applied to all samples were within their LVR.

#### **2.4.7 Determination of emulsion activity and emulsion stability of gelatine**

Emulsion activity index (EAI) and emulsion stability index (ESI) of gelatine samples were determined according to the method described by Liu et al.<sup>6</sup> Gelatine solution with a protein concentration of 10 mg ml<sup>-1</sup> was prepared. An appropriate amount of palm oil (20 wt%) was added to gelatine solution (80 wt%). The mixture was then homogenised using T25 digital Ultra-Turrax homogenizer (IKA, Selangor, Malaysia) at the speed of 16,200 rpm for 1 min. A total of 30 µl of the emulsion was pipetted into a test tube at 0 min (immediately after emulsion was prepared) and 10 min. The pipetted emulsion was then diluted 250 times with 0.1% (w/v) SDS and the mixture was mixed thoroughly using a vortex mixer.

The absorbance of the diluted emulsion was measured at 500 nm using UV Mini-1240 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). ESA and ESI were calculated according to the following equations:

$$\text{EAI}(\text{m}^2/\text{g}) = \frac{2 \times 2.303 \times A_0 \times \text{DF}}{l\phi C} \quad (2)$$

where  $A_0$  = absorbance of the diluted emulsion at 0 min, DF = dilution factor,  $l$  = path length of the cuvette (in meter),  $\phi$  = oil fraction of emulsion and  $C$  = protein concentration ( $\text{g m}^{-3}$ ) in aqueous phase before emulsion formation.

$$\text{ESI}(\text{min}) = \frac{A_0}{A_0 - A_{10}} \times \Delta t \quad (3)$$

In Equation 3,  $A_0$  = absorbance of the diluted emulsion at 0 min,  $A_{10}$  = absorbance of the diluted emulsion at 10 min and  $\Delta t$  = time interval (10 min).

#### 2.4.8 Determination of foaming ability and stability of gelatine

Foaming expansion (FE) and foam stability (FS) were carried out according to the method of Duan et al.<sup>11</sup> Gelatine solution with a protein concentration of  $10 \text{ mg ml}^{-1}$  was prepared. A total of 10 ml gelatine sample was poured into a centrifuge tube and the sample was homogenised using T25 digital Ultra-Turrax homogeniser at the speed of 13,400 rpm for 1 min. The foam was then poured into a measuring cylinder immediately after homogenisation and it was left at room temperature for 30 min. The volume of foam was recorded at 0 min (right after homogenisation) and 30 min. FE and FS were calculated according to the following equations:

$$\text{FE} = \left( V_0 / V_T \right) \times 100\% \quad (4)$$

$$\text{FS} = \left( V_{30} / V_T \right) \times 100\% \quad (5)$$

where  $V_T$  = total volume of gelatine solution before homogenisation,  $V_0$  = volume of the gelatine foam at 0 min and  $V_{30}$  = volume of foam at 30 min.

## 2.5 Statistical Analysis

Statistical analysis of data was performed using SPSS statistical program (Version 20) (SPSS Inc., Chicago, IL). Experiments were carried out in triplicate. The data presented were analysed by one-way analysis of variance (ANOVA) and Duncan's multiple range test (for mean comparison). The significant level in the present study was set at  $p < 0.05$ .

### 3. RESULTS AND DISCUSSION

#### 3.1 Yield

In general, the gelatine yield increased significantly as the extraction temperature increased (Table 1). This result was in agreement with Kittiphattanabawon et al. who reported that the yield and functional properties of gelatine were depended on the pretreatment of raw material and the processing parameters (pH, temperature and time) during gelatine extraction.<sup>8</sup> As the extraction temperature increased, more energy was applied to the extraction system and enhanced the disruption of triple helical structure of collagen into random coil structure.<sup>7</sup> Therefore, more collagen could be extracted into the aqueous medium, leading to the increase of yield.

Table 1: Extraction yield, emulsifying properties and foaming properties of gelatine from black tilapia skin extracted at different temperatures.

Samples	Yield (%)	Emulsion activity index ( $\text{m}^2\text{g}^{-1}$ )	Emulsion stability index (min)	Foam expansion (%)	Foam stability (%)
G45	11.52 ± 1.92 <sup>c</sup>	81.05 ± 4.81 <sup>a</sup>	62.16 ± 2.69 <sup>a</sup>	146.7 ± 6.7 <sup>c</sup>	42.3 ± 4.67 <sup>b</sup>
G55	10.79 ± 0.91 <sup>c</sup>	55.06 ± 0.74 <sup>b</sup>	39.30 ± 14.69 <sup>b</sup>	144.5 ± 3.9 <sup>c</sup>	41.5 ± 3.71 <sup>b</sup>
G65	14.91 ± 0.89 <sup>b</sup>	36.64 ± 2.06 <sup>d</sup>	18.43 ± 1.81 <sup>c</sup>	157.8 ± 3.9 <sup>b</sup>	40.0 ± 1.26 <sup>b</sup>
G75	18.27 ± 0.63 <sup>a</sup>	30.27 ± 0.18 <sup>c</sup>	16.68 ± 0.39 <sup>c</sup>	131.1 ± 7.7 <sup>d</sup>	19.4 ± 1.47 <sup>c</sup>
Bovine gelatine	–	43.53 ± 0.10 <sup>c</sup>	47.32 ± 2.25 <sup>b</sup>	195.6 ± 3.9 <sup>a</sup>	49.1 ± 1.22 <sup>a</sup>

<sup>a</sup>Values are presented as mean ± standard deviation ( $n = 2$  for yield;  $n = 3$  for emulsifying and foaming properties);

<sup>b</sup>Different lowercase letters in the same column indicate significant difference ( $p < 0.05$ ). G45, G55, G65 and G75 denoted tilapia gelatine extracted at 45°C, 55°C, 65°C and 75°C, respectively.

#### 3.2 Protein Pattern and Free Amino Group Content of Gelatine

The protein patterns of tilapia skin gelatine extracted at different temperatures are shown in Figure 1(a). The SDS-PAGE result showed that G45 contained  $\alpha$ -,  $\beta$ - and  $\gamma$ -chain as the major protein components and had a low portion of low molecular weight peptides. The decreasing band intensity of  $\alpha$ - and  $\beta$ -chain and concomitant increasing band intensity of low molecular weight peptides were observed in G55 and G65. Furthermore, in sample G75, the  $\alpha$ -,  $\beta$ - and  $\gamma$ -chain completely disappeared and have the highest portion of low molecular weight peptides. This result suggested that collagen in G75 was greatly degraded during gelatine extraction compared to other gelatine samples. During gelatine extraction, cleavage of the inter-chain cross-links of collagen occurred and resulted in the formation of gelatine with varying molecular weight peptides.<sup>6</sup>

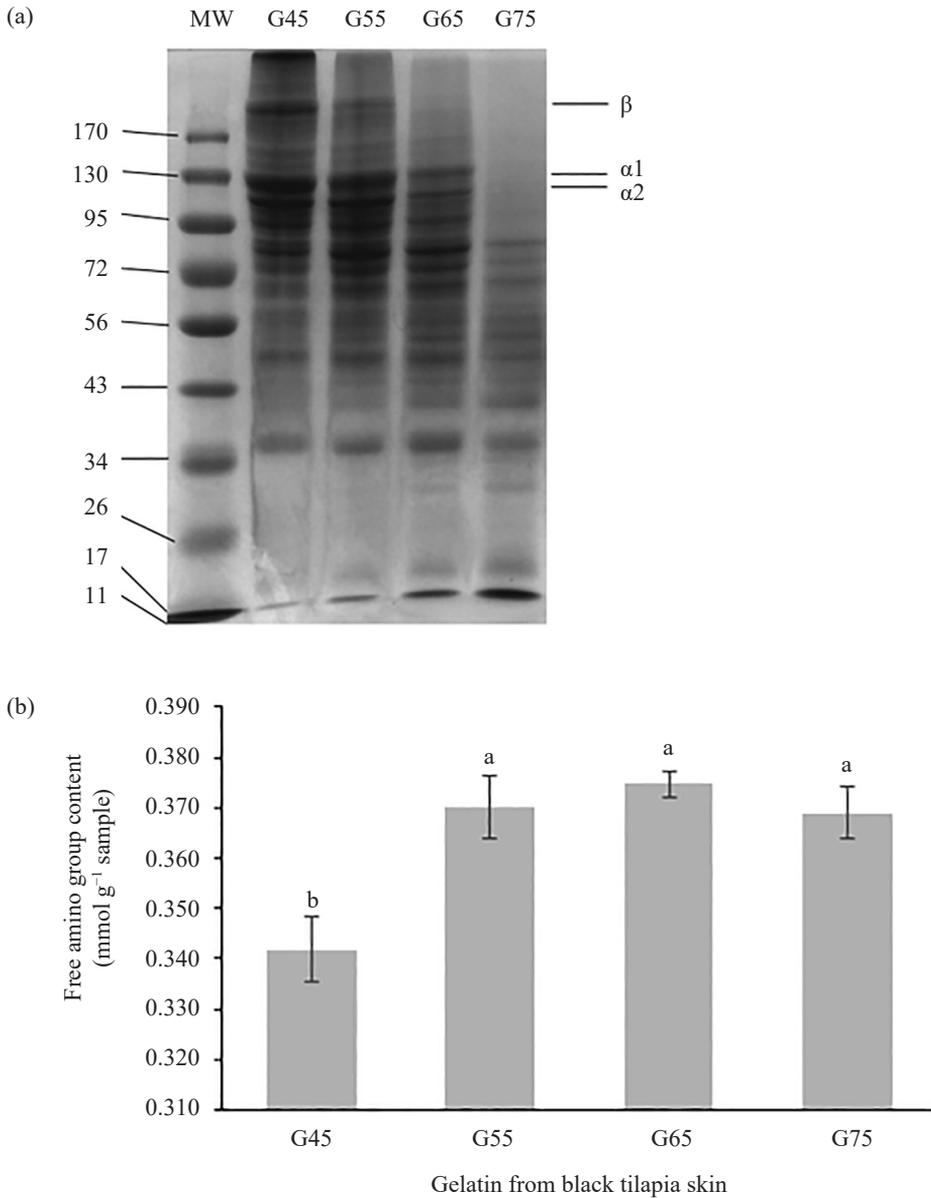


Figure 1: Protein pattern (a) and free amino group content (b) of gelatine from black tilapia skin gelatine extracted at different temperatures. MW: molecular weight marker. G45, G55, G65 and G75 denoted tilapia gelatine extracted at 45°C, 55°C, 65°C and 75°C, respectively. Bars represent the standard deviation ( $n = 3$ ).

In addition, the degree of thermal degradation of gelatine as affected by extraction temperatures was also expressed as free amino group. When the peptide bonds of a protein cleaved during hydrolysis, the amino groups of protein were exposed and detected as free amino groups. The result showed that the content of free amino group was increased ( $p < 0.05$ ) as the extraction temperature increased from 45°C to 55°C, but no significant difference was found with further increase of extraction temperature, shown in Figure 1(b). This suggests that the degree of degradation of gelatine was significantly higher when the extraction temperature was above 45°C. The result was in agreement with the SDS-PAGE result as in Figure 1(a) that shows the major components of collagen degrading at high extraction temperature. Therefore, it can be confirmed that high extraction temperature enhanced the hydrolysis of tilapia collagen and result in the decreased of major protein components in the extracted gelatine.

### 3.3 FTIR Spectra of Gelatine

FTIR spectra of black tilapia gelatine extracted at different extraction temperatures (45°C–75°C) are shown in Figure 2. The FTIR spectra of gelatine from black tilapia skin was similar to those found in gelatines from other fish species.<sup>6–8</sup>

The amide I of tilapia gelatine extracted at 45°C, 55°C, 65°C and 75°C were recorded at wavenumbers 1630.76 cm<sup>-1</sup>, 1631.85 cm<sup>-1</sup>, 1632.14 cm<sup>-1</sup> and 1631.57 cm<sup>-1</sup>, respectively. The location of amide I was shifted to a higher wavenumber as the extraction temperature increased. This result suggested that an increase in the random coil of gelatine due to the enhanced hydrolysis of inter-chain cross-links of collagen which led to the loss of triple helical structure.<sup>6,8</sup> In the amide II region, the absorption bands were found at 1545.11 cm<sup>-1</sup>, 1543.78 cm<sup>-1</sup>, 1542.10 cm<sup>-1</sup> and 1542.69 cm<sup>-1</sup> for G45, G55, G65 and G75, respectively. The amide II was contributed by an out-of-phase combination of a CN stretch and in-plane NH deformation modes of the peptide group.<sup>7</sup> Amide III absorption bands for G45, G55, G65 and G75 were detected at wavenumber of 1235.22 cm<sup>-1</sup>, 1233.74 cm<sup>-1</sup>, 1234.38 cm<sup>-1</sup> and 1233.71 cm<sup>-1</sup>, respectively. Amide III band represents the combination of peaks between C-N stretching vibrations and N-H deformation from the amide bonds.<sup>10</sup> In addition, it also associates with the absorptions contributed by wagging vibrations of CH<sub>2</sub> groups in the glycine backbone and proline side-chains of gelatine.<sup>10</sup> It is noted that the amplitude of amide III band was decreased when the extraction temperature increased from 45°C to 75°C. This suggested that the  $\alpha$ -helix of gelatine was experienced greater disruption, leading to the loss of triple helical structure as the extraction temperature increased.<sup>8,10</sup>

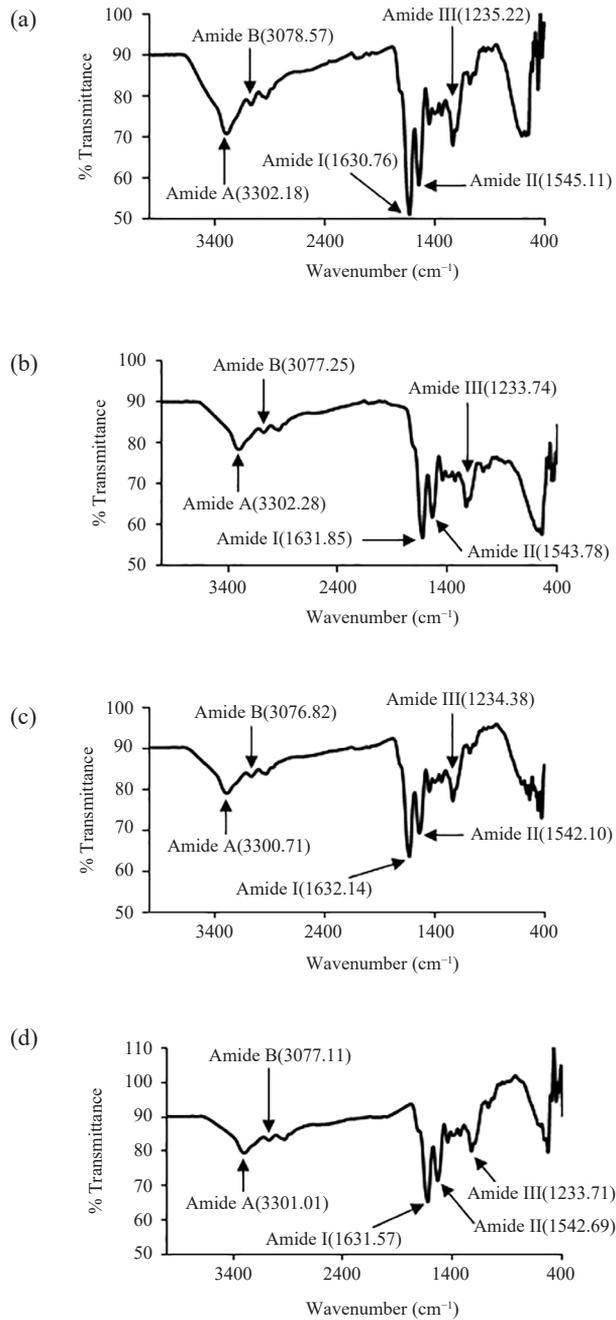


Figure 2: FTIR spectra of gelatines from black tilapia skin extracted at (a) 45°C, (b) 55°C, (c) 65°C and (d) 75°C. Arrows show the major amide bands (amide A, B, I, II, III) and their corresponding peak wavenumbers.

Amide A band was recorded at 3302.18  $\text{cm}^{-1}$ , 3302.28  $\text{cm}^{-1}$ , 3300.71  $\text{cm}^{-1}$  and 3301.01  $\text{cm}^{-1}$  for sample G45, G55, G65 and G75, respectively. A free N-H stretching is usually found in the wavenumber range of 3400–3440  $\text{cm}^{-1}$ .<sup>7</sup> The position of amide A in all gelatine samples were shifted to a lower wavenumber because N-H group of gelatine peptide was involved in hydrogen bonding.<sup>8,12</sup> Furthermore, a lower wavenumber of amide A was observed in gelatine that was extracted at a higher temperature. This result might be due to pronounced degradation of collagen at higher extraction temperature which contributed to the exposure of free amino groups of gelatines. These free amino groups released from the gelatine might interact with other reactive groups and resulted in the location of amide A shifted to lower wavenumber.<sup>7</sup> In addition, the adsorption bands of amide B for G45, G55, G65 and G75 were 3078.57  $\text{cm}^{-1}$ , 3077.25  $\text{cm}^{-1}$ , 3076.82  $\text{cm}^{-1}$  and 3077.11  $\text{cm}^{-1}$ , respectively. These wavenumbers corresponded to the asymmetric stretching vibration of =C-H and -NH<sub>3</sub>.<sup>7</sup> Among all tilapia gelatine samples, G65 and G75 showed lower wavenumber for amide B band compared to G45. This result indicated that the interaction of -NH<sub>3</sub> group between peptide chains might occur in sample which was extracted at high temperature.<sup>7</sup> Therefore, the difference in wavenumber and amplitude of the major peaks in amide regions suggested the secondary structure and function groups of tilapia gelatine were affected by the extraction temperature.

### 3.4 Gel Strength

The gel strength of gelatine from black tilapia skin extracted at different temperatures is shown in Figure 3. In the present study, the gel strength of gelatine decreased as the extraction temperature increased from 45°C to 75°C ( $p < 0.05$ ). This result was in accordance with the study on gelatine from Wami tilapia, clown featherback and chum salmon where lower gel strength was reported when gelatine was extracted at a higher temperature.<sup>6,8,13</sup> G45 had a higher gel strength compared to commercial bovine gelatine whereas the gel strength of G55 had no significant different with bovine gelatine ( $p < 0.05$ ). On the other, the gel strength of G65 and G75 were lower than bovine gelatine ( $p < 0.05$ ).

The molecular weight distribution of gelatine molecules plays an important role in gelation process.<sup>6</sup> During gelation, gelatine with long peptide chains (containing a high amount of  $\alpha$ -chains and  $\beta$ -chains) are able align or self-aggregate more effectively to form strong junction zone, which is a three-dimensional network that contains solvent in the interstices between polymer chains.<sup>8</sup> On the other hand, gelatine with the shorter peptide chains (low molecular weight peptides) are not able to form the strong inter-chain gel network during gelation, leading to low gel strength.<sup>8</sup> Therefore, G45 and G55 containing a higher amount of  $\alpha$ -chains and

$\beta$ -chains could form a stronger gel network than G65 and G75. In addition, it was noted that the gel strength of G75 was much lower than other gelatine samples. This could attribute to the absence of  $\alpha$ -chains and  $\beta$ -chains contributed by high extraction temperature, as shown in Figure 1(a). Thus, extraction temperature plays an important role in determining the gel strength of the resulting tilapia gelatine.

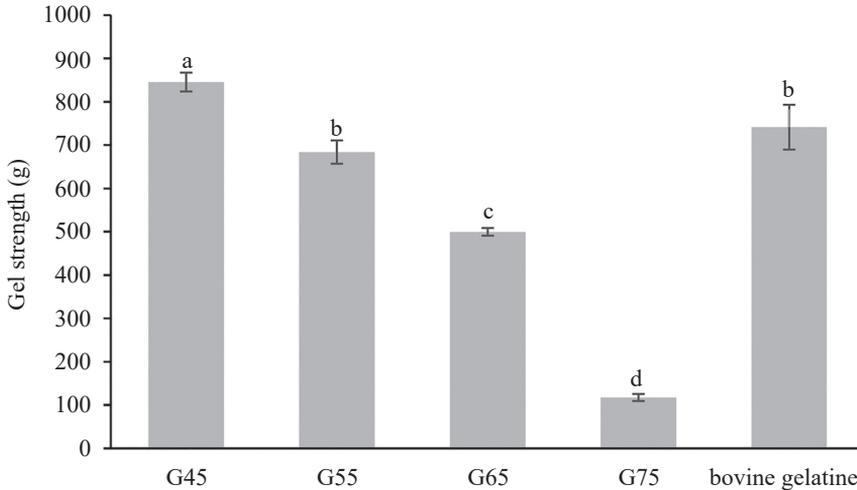


Figure 3: Gel strength of gelatine from black tilapia skin extracted from different temperatures. G45, G55, G65 and G75 denote the tilapia gelatines extracted at 45°C, 55°C, 65°C and 75°C, respectively. Bars represent standard deviation ( $n = 3$ ). Different lowercase letters represent significant difference ( $p < 0.05$ ).

### 3.5 Rheological Properties of Gelatine Gel

#### 3.5.1 Effect of extraction temperature on the melting and gelling temperature of gelatine

Table 2 presents the maximum moduli as well as the gelling temperature and melting temperature of gelatine from black tilapia with a concentration of 6.67% (w/v). The maximum storage modulus ( $G'$ ) of all gelatine samples was higher than its corresponding loss modulus ( $G''$ ) at 10°C, except sample G75. This suggests that all gelatine samples, except G75, had reached their gelling point and were in gel state. During cooling scan, all gelatine samples showed similar viscoelastic behaviour where the  $G'$  and  $G''$  of gelatine was first increased linearly followed a steep increase in the range of 24°C–13°C and 24°C–14°C, respectively. This sharp change in  $G'$  and  $G''$  indicated the thermal transition of gelatine from solution to gel, where the rapid formation of a gel network occurred.<sup>10</sup> Similarly, during heating,

the  $G'$  and  $G''$  of all gelatine samples decrease linearly at the beginning and then sharply decreased in the range of 19°C–30°C and 31°C–20°C, respectively. The result suggested that gelatine underwent a thermal transition from gel to solution in these range of temperatures.

Table 2: Gelling temperature, melting temperature, storage modulus ( $G'$ ) and loss modulus ( $G''$ ) of tilapia gelatine and bovine gelatine.

Sample	Gelling temp. (°C)	Melting temp. (°C)
G45	18.9 ± 0.1 <sup>b</sup>	26.2 ± 0 <sup>b</sup>
G55	16.6 ± 0.1 <sup>c</sup>	22.9 ± 0.1 <sup>c</sup>
G65	14.6 ± 0.1 <sup>d</sup>	21.6 ± 0.1 <sup>d</sup>
G75	8.7 ± 0.1 <sup>e</sup>	17.1 ± 0.1 <sup>e</sup>
Bovine gelatine	21.4 ± 0.1 <sup>a</sup>	28.2 ± 0.1 <sup>a</sup>

Sample	Maximum moduli value after cooling at 10°C	
	$G'$ (Pa)	$G''$ (Pa)
G45	1525 ± 18 <sup>b</sup>	36.16 ± 13.27 <sup>ab</sup>
G55	1307 ± 97 <sup>c</sup>	41.57 ± 4.40 <sup>a</sup>
G65	527 ± 27 <sup>d</sup>	19.20 ± 0.76 <sup>c</sup>
G75	0.19 ± 0.02 <sup>e</sup>	0.77 ± 0.01 <sup>d</sup>
Bovine gelatine	2486 ± 79 <sup>a</sup>	27.01 ± 4.06 <sup>bc</sup>

<sup>a</sup>Values are mean ± standard deviation ( $n = 3$ ). Different lowercase letters in the same column represent significant difference ( $p < 0.05$ ). G45, G55, G65 and G75 denoted tilapia gelatine extracted at 45°C, 55°C, 65°C and 75°C, respectively.

In addition, the thermal stability of tilapia gelatine decreased as the extraction temperature increased from 45°C to 75°C, evidenced by the decrease of thermal transition temperature during both cooling and heating scan (Figure 4). Degradation of gelatine during extraction resulted in the decrease or loss of high molecular weight of peptides and a concomitant increase of low molecular weight of protein fragments. This contributed to gelatine not able to form a strong gel network, thereby decreasing in thermal stability.<sup>10</sup> Therefore, it was noted that the gelling and melting temperature of tilapia gelatine were decreased as the extraction temperature increased (Table 2). When compared to commercial bovine gelatine, the gelling and melting temperature of all tilapia gelatine samples were significantly lower than those of bovine gelatine. However, G45 showed slightly lower (approximately 2°C) gelling and melting temperature than those of bovine gelatine, suggesting that tilapia gelatine extracted at low temperature (45°C) could be the potential alternative gelling agent to bovine gelatine.

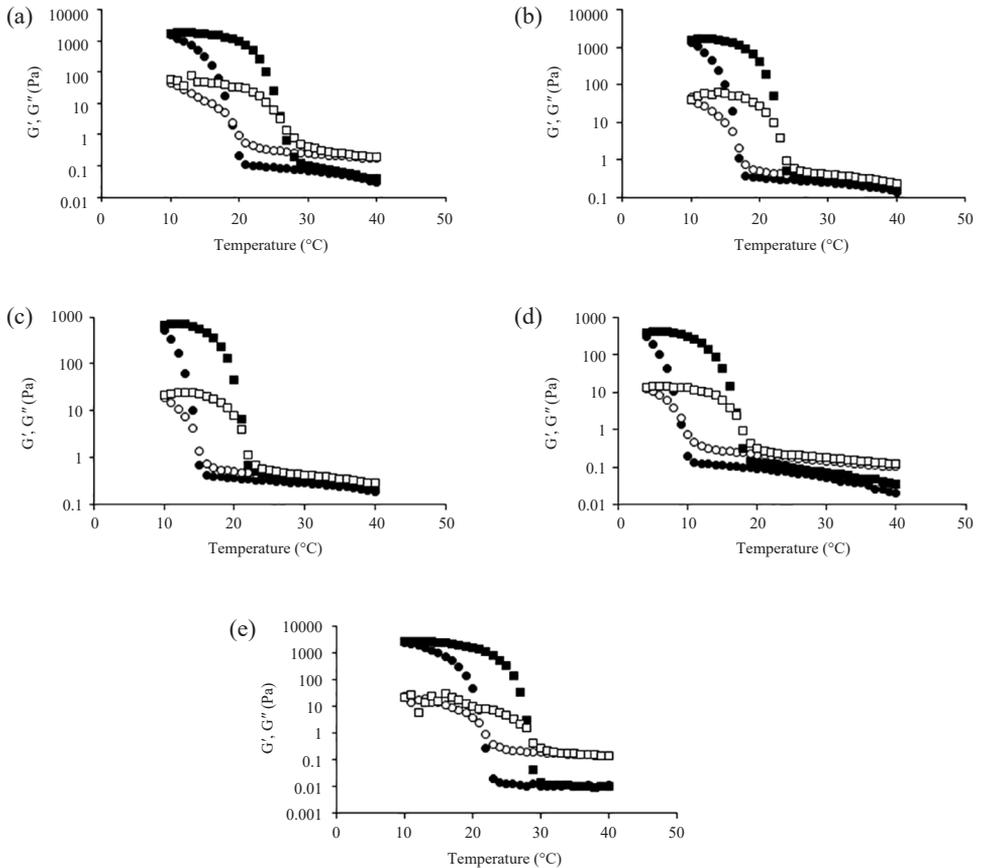


Figure 4: Storage modulus ( $G'$ ) (filled symbols) and loss modulus ( $G''$ ) (open symbols) as a function of temperature during a temperature sweep at cooling (circle symbols) and followed by heating (square symbols) (both at  $1^{\circ}\text{C min}^{-1}$ ) for black tilapia skin gelatine extracted at (a)  $45^{\circ}\text{C}$ , (b)  $55^{\circ}\text{C}$ , (c)  $65^{\circ}\text{C}$  and (d)  $75^{\circ}\text{C}$  and (e) bovine gelatine.

### 3.5.2 Effect of extraction temperature on frequency sweep

The study on storage modulus ( $G'$ ) and loss modulus ( $G''$ ) of a gel over a range frequency provides insights on the strength of gel structure and the behaviour of the gel during storage and application.<sup>10</sup> Figure 5 shows  $G'$  and  $G''$  of tilapia gelatine (extracted at different temperatures) as a function of frequency over  $0\text{--}100\text{ rad s}^{-1}$  at  $10^{\circ}\text{C}$ . All gelatine samples reached their gelling point in the given frequency range evidenced by the  $G'$  value was higher than  $G''$ . In general, all samples tested exhibited a similar viscoelastic behaviour where  $G'$  was increased with frequency in the range of  $0.01\text{--}1.00\text{ rad s}^{-1}$  followed by an almost constant value from 1 to

100 rad s<sup>-1</sup>. When the extraction temperature increased, the G' of tilapia gelatine became more frequency dependent, indicated by the increase of the slope of G' curve. This trend is more prominent for G75 where the G' and G'' increase with frequency at a much higher rate compared with the corresponding moduli of other tilapia gelatine samples (G45, G55 and G65).

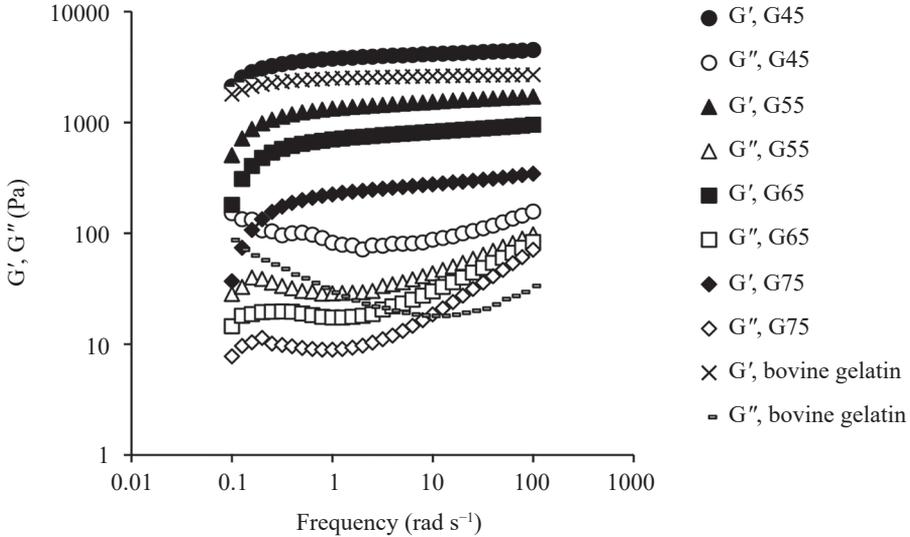


Figure 5: Frequency-dependent changes in storage modulus ( $G'$ ) (filled symbols) and loss modulus ( $G''$ ) (open symbols) at 10°C for black tilapia skin gelatine extracted at different temperatures and bovine gelatine. G45, G55, G65 and G75 denote tilapia gelatine extracted at 45°C, 55°C, 65°C and 75°C, respectively.

In addition, among the tilapia gelatine samples, the  $G'$  and  $G''$  values decreased with an increase in extraction temperature. This suggests that tilapia gelatine exhibited weak gel structure as the extraction temperature increased, especially at high temperature (75°C). This could be contributed by the decrease of high molecular weight protein components in gelatine. On the other hand, the  $G'$  and  $G''$  of G45 and bovine gelatine were nearly independent of frequency, with the  $G'$  value of G45 was slightly higher than that of bovine gelatine. This suggested that G45 and bovine gelatine formed a strong gel network over the range of frequency tested. A similar result was also reported for duck feet gelatine, which suggested the strong gel network formed by the avian gelatine was due to a strong intermolecular interaction between gelatine protein components.<sup>10</sup>

### 3.5.3 Effect of extraction temperature on time sweep

Figure 6(a) shows the storage modulus ( $G'$ ) of bovine gelatine and tilapia gelatine extracted at different temperatures as a function of time at  $10^\circ\text{C}$ . The  $G'$  value of all samples, except G75, were higher than their corresponding  $G''$  value over the length of time tested, suggesting that the samples had reached their gelling point at  $10^\circ\text{C}$  (data not shown). Interestingly, the  $G'$  value of G75 was lower than  $G''$  before the  $G'/G''$  crossover at 112 s, shown in Figure 6(b). This indicated that G75 reached its gelling state after equilibrated at  $10^\circ\text{C}$  for 112 s. The  $G'$  of bovine gelatine was found being time-independent whereas  $G'$  of tilapia gelatine samples increased with time before reached their respective maximum  $G'$  value. Furthermore, duck feet gelatine took approximately 1 h to reach the almost constant  $G'$  value.<sup>14</sup> This could be attributed to the difference in gelation kinetic of among the gelatines, which was influenced by their molecular weight and amino acid composition. During gelation of gelatine, junction zones are formed and separated by flexible chains that are stabilised by non-covalent interactions.<sup>14</sup> This involves the transformation from random coil to helical structure during cooling (i.e., formation of junction zones from the renaturation process).<sup>14</sup>

Furthermore, it was noted that tilapia gelatine took a longer time to reach maximum  $G'$  as the extraction temperature increased, especially for G75. As extraction temperature increased, the resulting low molecular weight protein fragments may not able to form the strong junction zone, especially via hydrogen bond or other non-covalent bonds thereby reducing the ability of the peptide chains to anneal correctly to form gel network.<sup>8</sup> In addition, the  $G'$  value of tilapia gelatine samples decreased as the extraction temperature increased. This trend was in good agreement with the result of gel strength (Figure 3).

### 3.6 Emulsifying Properties of Gelatine

Table 1 shows the emulsifying activity index (EAI) and emulsifying stability index (ESI) of tilapia skin gelatine extracted at different temperatures. The EAI of all gelatine samples decreased ( $p < 0.05$ ) as the extraction temperature increased. This result suggested that the low molecular weight peptides of tilapia gelatine (contributed by the excessive hydrolysis of collagen during extraction) were not able to unfold and reorient at the interfacial region to form a strong film around the newly formed oil droplet during emulsification.<sup>6,15</sup>

Furthermore, the ESI of gelatine also decreased ( $p < 0.05$ ) when the extraction temperature increased (Table 1). This result suggested that gelatine extracted at low temperature, which contains high molecular weight peptides, was able to form stronger and stiffer interfacial film surrounding the oil droplet.<sup>6,16</sup> This provides

stability to the oil droplets against coalescence and creaming. Gelatine samples extracted at a low temperature which had higher viscosity were able to restrict the movement of the droplet and reduce the chance of droplet aggregation.<sup>17,18</sup> Therefore, this led to the increase of emulsion stability of gelatine. In addition, the EAI and ESI of G45 were significantly higher than bovine gelatine. This suggested that tilapia gelatine could offer better emulsifying properties than bovine gelatine when low extraction temperature was employed.

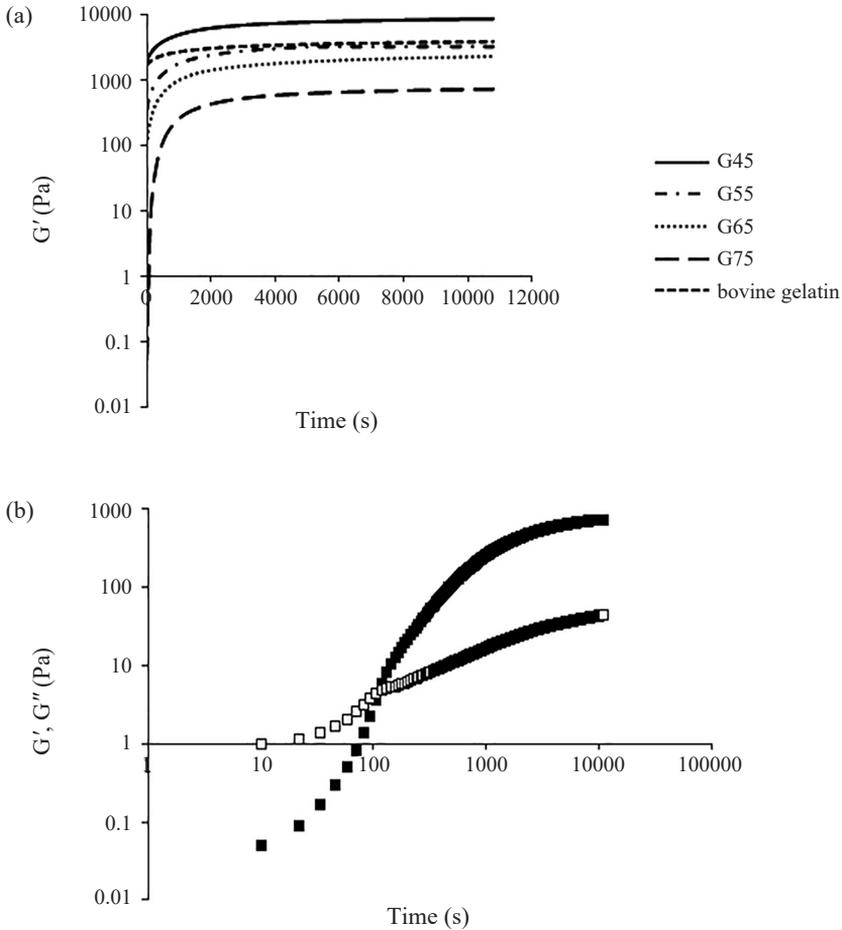


Figure 6: Plots of (a) storage modulus ( $G'$ ) as a function of time during a time sweep for 3 h at 10°C for black tilapia skin gelatine extracted at different temperatures and bovine gelatine. G45, G55, G65 and G75 denote tilapia gelatine extracted at 45°C, 55°C, 65°C and 75°C, respectively. Whereas (b) illustrates storage modulus ( $G'$ ) (filled symbols) and loss modulus ( $G''$ ) (open symbols) change during a time sweep for 3 h at 10°C for black tilapia skin gelatine extracted at 75°C.

### 3.7 Foaming Properties of Gelatine

The results showed that foam expansion (FE) of gelatine samples increased when the extraction temperature increased from 45°C to 65°C (Table 1). As the extraction temperature increased, the hydrophobic domains of gelatine were more exposed, leading to the increase of surface hydrophobicity.<sup>11</sup> Therefore, tilapia gelatine extracted at a higher temperature (65°C) might be able to adsorb to the air-water interface then unfold and rearrange at the interface more effectively during bubbles formation. This enhanced the foaming ability of gelatine. However, the FE of gelatine decreased ( $p < 0.05$ ) when the extraction temperature was further increased from 65°C to 75°C. This was contributed by the presence of a high portion of small peptides in gelatine sample extracted at 75°C (G75), which have the poor foaming ability. The small peptides might induce protein aggregation through hydrophobic interaction and reduced the protein adsorption to air-water interface, leading to the decrease of foamability.<sup>19</sup>

The foam stability (FS) of gelatine samples have no significant difference when extraction temperature increased from 45°C to 65°C but the FS of gelatine decreased significantly when extraction temperature was further increased to 75°C. This result suggests that G75 which has a higher portion of low molecular weight peptides not able to form a cohesive and viscoelastic film through intermolecular interaction, resulting in low foam stability.<sup>19</sup> In addition, bovine gelatine exhibited significantly higher foam expansion and foam stability compared to all tilapia gelatine samples. This might be due to the difference in the intrinsic properties of bovine and tilapia gelatine such as surface activity and structure of protein in solution and at the air-water interface.

## 4. CONCLUSION

The yield of gelatine from black tilapia skin increased as the extraction temperature increased. However, high extraction temperature contributed to poor functional properties of the extraction gelatine. This was mostly associated with the degradation of high molecular weight peptides of gelatine at high extraction temperature, especially at 75°C. On the other hand, gelatine extracted at 45°C possessed gelling properties that were similar to bovine gelatine. Therefore, tilapia skin gelatine could be the alternative to bovine gelatine when low extraction temperature was employed during gelatine extraction.

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