



# Gelatin addition improves the nutrient retention, texture and mass transfer of fish balls without altering their nanostructure during boiling



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## ABSTRACT

The effect of fish gelatin addition on mass transfer, nutrient loss, texture and nanostructure of fish balls was investigated. Mass transfer models were built and the root-mean-square-errors were 0.1432, 0.3178 and 0.1000 for exponential, power-law and linear models, respectively. After gelatin addition, the mass transfer coefficient/model parameter and moisture content increased, and the hardness and chewiness of fish balls decreased. Myofibrils were imaged using atomic force microscope (AFM). The length of the myofibrils was greater than 15  $\mu\text{m}$  before and after boiling for 10 min; however, they decreased to around 14 and 11  $\mu\text{m}$  after 20 and 30 min boiling, indicating degradation of myofibrils. Meanwhile, there was no significant difference among different groups, suggesting that the added gelatin did not affect the nanostructure of the fish balls. Furthermore, increasing gelatin addition resulted in fewer water-soluble proteins and peptides in the boiling water. The results suggest that added gelatin increased the mass transfer coefficient/model parameter by increasing the moisture content and decreasing the nutrient loss. It also improved the texture by decreasing the hardness and chewiness, and did not affect the nanostructure of fish ball myofibrils.

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## 1. Introduction

Fish balls are popular in Australia, Germany, Japan, Southeast Asia and China (Tee & Siow, 2014). In Singapore, the fish processing industry produces 30,000 tons of fish balls annually, with a value of S\$80 million, mainly for local consumption. Furthermore, around 90% of the food consumed in Singapore is imported from other countries (AVA, 2015). To improve food security, Golden Pomfret (*Trachinotus blochii*, hereafter GP) has been spawned in local farms. However, the hard texture of GP makes it unsuitable for manufacturing fish balls. Gelatin can be extracted from fishery processing byproducts, such as skin and bones (Jiang, Liu, Du, & Wang, 2010; Kittiphattanabawon, Benjakul, Sinthusamran, & Kishimura, 2016; Mohtar, Perera, & Quek, 2010). Utilising this

byproduct-derived gelatin might improve the texture of the fish balls and affect their mass transfer and nutrient loss. Meanwhile, adding fish gelatin into GP fish balls could also improve food security and address the environment pollution caused by fish processing waste, which is 1500 tons annually in Singapore (Feng, Bansal, & Yang, 2016).

Boiling is the most common method of processing fish balls. During boiling, the proteins in the fish become denatured, gelatinised and form a network, which is accompanied by absorption of water. Meanwhile, water-soluble proteins and peptides leach into the boiling solution, resulting in nutrient loss. The mass transfers of frying and salting meat products (Amirousefi, Mohebbi, Khodaiyan, & Asadi, 2011; Du, Zhou, Xu, & Li, 2010) have been studied; however, reports about mass transfer of boiling meat with added gelatin are limited.

Previous research used pork collagen (PC) in porcine myofibrillar protein (MP) gels to evaluate the changes in viscoelastic and thermal properties (Doerscher, Briggs, & Lonergan, 2004). Collagen has been added into frankfurters to determine the effect of collagen on frankfurter texture (Calhoun, Eilert, & Mandigo, 1996).

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Rheological properties of chicken balls were optimised by adding  $\kappa$ -carrageenan, fish gelatin and chicken meat (Yasin, Babji, & Ismail, 2016). There have also been reports of extending the shelf life of fish balls (Yi et al., 2011), developing different flavours and investigating the composition and physicochemical properties of fish balls (Kolekar & Pagarkar, 2014).

The objective of this research was to establish mass transfer models of fish balls during boiling and investigate the effect of gelatin on the mass transfer. The nanostructure of myofibrils, major component of fish muscle (Li et al., 2016; Pazos, Méndez, Vázquez, & Aubourg, 2015), was investigated to determine fish protein degradation and the effect of gelatin on the nanostructural changes in fish balls. Measuring the texture of fish ball allowed us to correlate the nanostructure and texture. Moreover, proteins and peptides in the boiling solution were analysed by Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS), which improved the understanding of mass transfer process and nutrient loss of fish balls during boiling.

## 2. Materials and methods

### 2.1. Materials

The local farmed GP (*Trachinotus blochii*), salt and potato starch were purchased from a local market. Commercial tilapia fish gelatin (200 Bloom) was bought from Jiangxi Cosen Biology Co., Ltd (Yingtian, Jiangxi, China). The gelatin contained 83.14% protein, 0.68% ash, 9.12% moisture and 7.06% other materials, according to the product information.

### 2.2. Preparation of fish gelatin solution and fish balls

Fish gelatin solutions (6%, 9%, 12%, w/w) were prepared following the method of Yang and Wang (2009), with some modifications. Gelatin was soaked in distilled water at 4 °C overnight. The gelatin solution was then placed in a 55 °C water bath for 15 min until it was totally dissolved and homogeneous.

The purchased fish were transferred to the laboratory in cold storage bags with ice inside within 30 min (Singh, Benjakul, Maqsood, & Kishimura, 2011). To make the fish balls, the fish head was removed, followed by deboning and removal of the skin. Two large fillets were obtained and minced in a homogeniser. The minced fillet (100 g), 7.5 g of potato starch, 1.25 g of salt and 12.5 ml of gelatin solution were mixed and homogenised well. The concentrations of the gelatin solution were 0, 0.06, 0.12 and 0.24 g/ml, respectively. Samples of 20 g of the mixture were taken out and rolled into a ball. The fish balls were boiled for 1, 2, 3, 4, 5, 10, 15, 20, 25, 30 and 60 min respectively, in triplicate, and the fish balls were weighed after boiling.

### 2.3. Mass transfer models and the mass transfer coefficient/model parameter

The mass of fish balls before and after boiling for various time was recorded, and used to fit into three mass transfer models to obtain the formula and mass transfer coefficients/model parameters. Three mass transfer models, exponential, power-law and linear, were built to predict the weight of a fish ball after boiling, which are shown below.

#### 1 Exponential model

$$\frac{M - M_0}{M_\infty - M_0} = 1 - e^{(-kt)} \quad (1)$$

#### 2 Power-law model

$$M - M_0 = at^{0.5} \quad (2)$$

#### 3 Linear model

$$\Delta M = qt + b \quad (3)$$

where,  $M_0$  is the mass of the fish ball before boiling;  $M$  and  $M_\infty$  are the masses of the fish ball after boiling for time  $t$  and for an infinite time, respectively. In this model,  $M_\infty$  is assumed to be mass of fish ball after 60 min of boiling. Letters  $k$ ,  $a$  and  $q$  represent the mass transfer coefficient/model parameter in exponential, power-law and linear models, respectively while  $b$  is a constant of each group.

$$\Delta M = \frac{(M - M_0)}{M_0} \times 100\% \quad (4)$$

The root-mean-square-error (RMSE) and percentage difference (PD) of the three models were calculated according to the equation below.

$$RMSE = \sqrt{\frac{(m_{10} - m'_{10})^2 + (m_{20} - m'_{20})^2}{2}} \quad (5)$$

$$PD = \left[ \frac{m_{10} - m'_{10}}{m_{10} + m'_{10}} + \frac{m_{20} - m'_{20}}{m_{20} + m'_{20}} \right] \times 100\% \quad (6)$$

where,  $m_{10}$  is the average experimental mass value of a fish ball boiled for 10 min, and  $m'_{10}$  is the predicted mass value calculated from the mass transfer models.  $m_{20}$  is the average experimental mass value of a fish ball boiled for 20 min, while  $m'_{20}$  is the predicted mass value calculated from the mass transfer models.

### 2.4. Weight gain ratio and moisture

The weight gain ratio was determined by the formula below.

$$\text{Weight Gain Ratio} = \frac{M - M_0}{M_0} \times 100\% \quad (7)$$

where,  $M_0$  and  $M$  represent the mass of the fish ball before and after boiling.

The middle part of the fish ball was cut into small pieces, and 2 g from each sample was placed onto an aluminium plate, which was put into a 105 °C oven for 24 h for moisture determination. The sample was then taken out and weighed. The moisture was calculated according to the formula below.

$$\text{Moisture} = (M_1 - M_2)/M_1 \times 100\% \quad (8)$$

where,  $M_1$  and  $M_2$  represent the mass before and after drying, respectively.

### 2.5. Texture

After boiling, the fish ball was cooled to room temperature and cut into cylinders of 15 mm height and 17 mm diameter from the centre for texture measurement using a TA.XT2-i Texture Analyser (Stable Micro System, Goldaming, Surrey, UK) (Purohit, Reed, &

Mohan, 2016). The fish ball cylinder was compressed twice to 6 mm using a flat cylindrical probe (47 mm). The testing parameters were selected according to a previous study (Sow & Yang, 2015). Texture parameters were calculated from Texture Profile Analysis (TPA) curves according to the definition provided by Sow and Yang (2015).

## 2.6. Nanostructural changes

Myofibrils were extracted from each fish ball sample (Li et al., 2016; Martone, Busconi, Folco, Trucco, & Sanchez, 1986). Solution A was prepared immediately before each extraction, which comprised 1 mM phenylmethylsulfonylfluoride (PMSF), 0.10 M KCl, 0.02% Na<sub>3</sub>N, and 20 mM Tris-HCl buffer at pH 7.5. Solution B contained 0.2 M Mg(CH<sub>3</sub>COO)<sub>2</sub>, 5 mM β-mercaptoethanol (β-MCE), 0.45 M KCl, 1 mM ethylene glycol-bis (β-aminoethyl ether) N,N,N'-tetraacetic acid (EGTA), and 20 mM Tris-maleate buffer at pH 6.8. Fish balls were minced by a blender and a 5 g sample was added to 25 ml of solution A. The mixture was stirred gently with a magnetic stirrer at 0 °C for 15 min, and then centrifuged at 1000 × g for 10 min at 4 °C. The supernatant was discarded. The pellet was added to 50 ml of solution B, to which ATP was added to a final concentration of 10 mM. The mixture was incubated for 60 min at 0 °C, and then centrifuged at 10,000 × g for 15 min. The supernatant contained the myofibrils.

A 1 ml sample of the supernatant was diluted 40 times with distilled water so that the myofibrils may not overlap during atomic force microscopy (AFM) analysis. For the AFM analysis, a sample of 20 μl was pipetted onto a flat mica sheet attached to a magnetic disc. The sample was dried at room temperature before AFM analysis. A TT-AFM (AFM workshop, Signal Hill, CA, USA) equipped with a Sensaprobe TM190-A-15 tip (Applied Nanostructures, Mountain View, CA, USA) was applied to analyse the morphology of the myofibrils extracted from the fish balls. The conditions were: the resonance frequency at 145–230 KHz; the force constant of 25–95 N/m, and the Z scanner around 0.2–0.4 Hz. A vibration mode was selected. The height, width and length of the myofibrils were analysed using the Gwyddion software (Sow & Yang, 2015; Yang, 2014).

## 2.7. Molecular weights of proteins and peptides in the boiling water

MALDI-TOF-MS was performed using a modified version of the method detailed by Luccia et al. (2005). Firstly, the supernatant containing myofibrils for AFM analysis was dialysed to eliminate salts (Feng, Ng, Mikš-Krajnik, & Yang, 2017). After dialysis, a 1 ml sample was diluted with 1.5 ml of 50% acetonitrile. Two microliters of this mixture were homogenised with 2 μl of 2, 5-dihydroxybenzoic acid (DHB) and pipetted on the MALDI target, followed by air drying. The signal was measured in m/z and transformed into Daltons. The proteins and peptides with molecular weights ranging from 0 to 40,000 Da were shown in the spectrum (Luccia et al., 2005).

## 2.8. Statistical analysis

All the experiments were performed at least in triplicate. The results were reported as means ± standard deviation (SD). The differences in the results between different groups were determined by analysis of variance (ANOVA) and Duncan's multiple range test using SAS software.  $P < 0.05$  was considered statistically significant. For the AFM analyses, dozens of parallel images were obtained to get statistically valid results.

## 3. Results and discussion

### 3.1. Mass transfer models and the mass transfer coefficient/model parameter

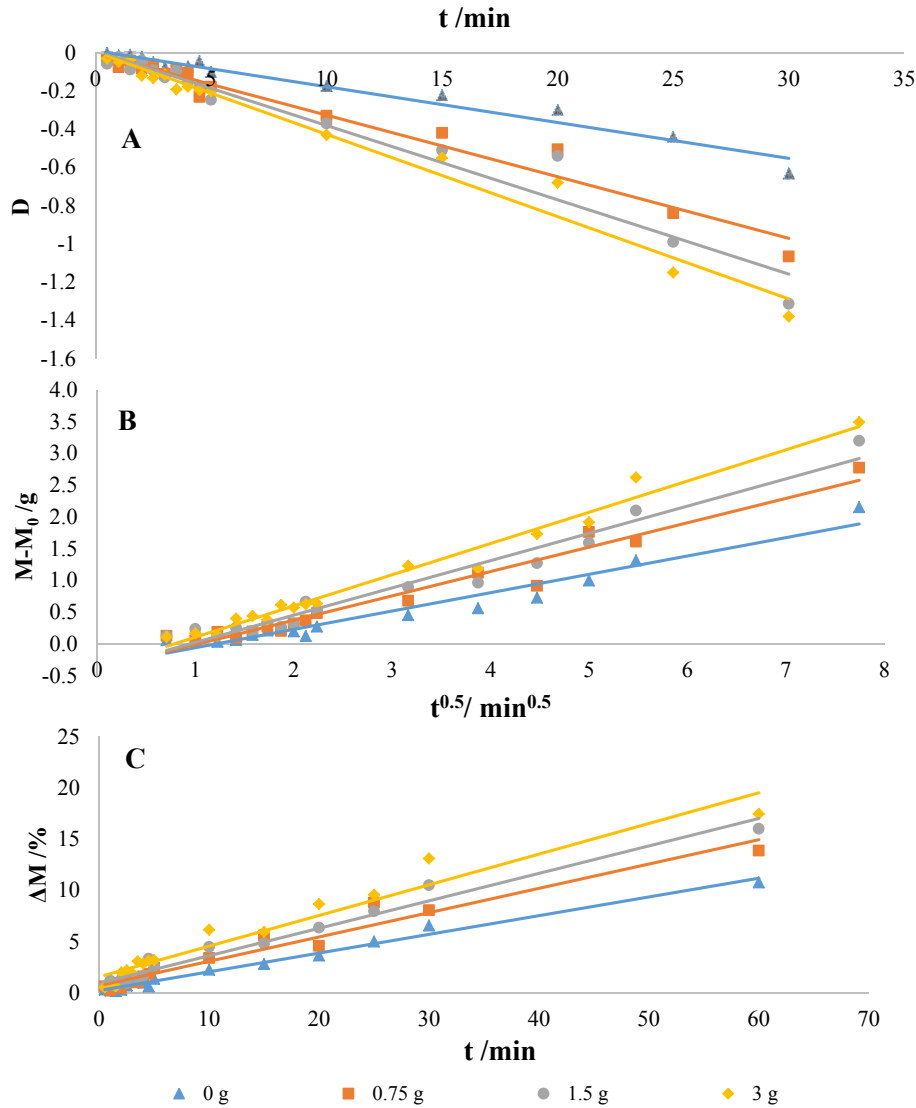
The exponential model was used for the mass transfer process of chicken fillet cooking (Raffray, Goli, Rivier, Sebastian, & Collignan, 2014), while the power-law model was used to predict the mass transfer of button mushrooms, which assumed that weight changes were dependent on the square root of time (Kar & Gupta, 2001). The linear model was used to describe the oil uptake of French fries during pre-frying (Van Koerten, Schutyser, Somsen, & Boom, 2015).

Fig. 1 shows the fitting of the three models, and Table 1 shows the PD, RMSE, R<sup>2</sup> and mass transfer coefficients/model parameters of each model. The RMSEs between the experimental and predicted weight of the fish balls after boiling were 0.1432 for the exponential model and 0.10 for linear model, demonstrating very small deviations between the experimental and predicted values. However, the RMSE of the power-law model was 0.3178, which was higher than the exponential and linear models. The R<sup>2</sup> values were 0.9818 and 0.9937 for the exponential and linear models, respectively; while power-law model had lower R<sup>2</sup> of 0.7735, indicating a larger variation between the predicted and experimental values. The power-law model also showed higher PD values compared with the exponential and linear models. Therefore, the exponential and linear models are more suitable to describe mass transfer of fish balls during boiling (Table 1).

The mass transfer coefficient/model parameter, which represents the mass transfer efficiency and rate (Raffray et al., 2014), increased when gelatin was added during boiling. For the exponential model, the mass transfer model parameter increased dramatically from 0.019 to 0.032 min<sup>-1</sup>, as the amount of gelatin added increased from 0 to 0.75 g/100 g fish (Table 1). A continuous increase was observed from 0.039 to 0.043 min<sup>-1</sup>, as the added gelatin increased from 1.5 to 3 g/100 g fish. For the power-law model, the mass transfer coefficient increased from 0.32 to 0.51 g min<sup>-0.5</sup> as the amount of added gelatin increased from 0 to 3 g/100 g fish. In addition, the mass transfer coefficient gradually increased from 0.18 g min<sup>-1</sup> in the control group to 0.30 g min<sup>-1</sup> in the 3 g gelatin/100 g fish group in the linear model. Thus, the addition of gelatin accelerated the mass transfer process of fish balls during boiling, which might be caused by the hydrophilicity and water holding capacity of fish gelatin (Stavinskaya, Laguta, & Kuzema, 2011). A decrease in the amount of nutrients lost might also contribute to the increased mass transfer coefficients/model parameters of gelatin added fish balls, which is further discussed in section 3.4.

### 3.2. Weight gain ratio, moisture and texture

The effect of fish gelatin addition on the weight gain ratio, moisture and texture of fish balls is shown in Fig. 2. In general, the weight gain ratio increased as the boiling time increased, which might be due to the hydrophilicity and water holding capacity of fish gelatin and fish protein, which can keep absorbing water as the fish ball gels until the water level reaches saturation (Stavinskaya et al., 2011). Fig. 2 shows that the 3 g of added gelatin group had a significantly higher weight gain ratio compared with the other three groups at 5 and 10 min of boiling. At 20 and 30 min, the 3 g of added gelatin group had a higher weight gain ratio than the control and the 1.5 g of added gelatin group. The increased weight gain ratio of fish ball might be caused by the hydrophilicity and water holding capacity of the added fish gelatin (Stavinskaya et al., 2011); therefore the gelatin added fish ball could absorb more water and become juicier. For the moisture result, the 3 g of added gelatin



**Fig. 1.** Mass transfer models of fish ball with different gelatin additions in 100 g fish during boiling: (A) Exponential model; (B) Power-law model; (C) Linear model.\*  
 \*  $D = \ln \frac{M_{\infty} - M}{M_{\infty} - M_0}$ ;  $\Delta M = (M - M_0)/M_0 \cdot 100\%$ ; M is the weight of fish ball after certain time of boiling;  $M_0$  is the weight of fish ball before boiling.

**Table 1**  
 Comparison of exponential, power-law and linear models.<sup>a</sup>

Model	Equation	PD <sup>1</sup> /%	RMSE <sup>2</sup>	R <sup>2</sup>	Mass transfer coefficient/model parameter	Mass transfer coefficient/model parameter (10 <sup>-2</sup> ) with different gelatin additions in 100 g of fish/g			
						0	0.75	1.5	3
exponential model	$(M - M_0)/(M_{\infty} - M_0) = 1 - \exp(-kt)$	0.99	0.14	0.98	k/min <sup>-1</sup>	1.9 ± 0.2 <sup>d</sup>	3.2 ± 0.3 <sup>c</sup>	3.9 ± 0.3 <sup>b</sup>	4.3 ± 0.3 <sup>a</sup>
power-law model	$M - M_0 = at^{0.5}$	1.88	0.32	0.77	a/g · min <sup>-0.5</sup>	32.0 ± 2.5 <sup>d</sup>	38.0 ± 3.2 <sup>c</sup>	46.0 ± 3.1 <sup>b</sup>	51.0 ± 2.2 <sup>a</sup>
linear model	$\Delta M = qt + b$	1.45	0.10	0.99	q/g · min <sup>-1</sup>	18.0 ± 1.9 <sup>d</sup>	23.0 ± 1.7 <sup>c</sup>	27.0 ± 2.1 <sup>b</sup>	30.0 ± 2.5 <sup>a</sup>

PD<sup>1</sup>, percentage difference, RMSE<sup>2</sup>, root-mean-square-error.

<sup>a</sup> Values with different lower case superscript letters indicate significant differences in each row, as assessed by Duncan's multiple range test ( $P < 0.05$ ). The mass transfer coefficient/model parameter is expressed as the mean ± SD (n = 3).

group had a significantly higher moisture content than the 0, 0.75 and 1.5 g of added gelatin groups, which agreed well with the weight gain ratio result. The moisture result also demonstrated the hydrophilicity and water holding capacity of the fish gelatin, which might be the reason for the increased mass transfer coefficients/model parameters of the fish balls during boiling (Stavinskaya et al., 2011).

As shown in Fig. 3, the hardness of the fish balls decreased

gradually as the boiling time increased. The hardness was around 18 N after 5 min of boiling, and fell to below 15 N after 10 min of boiling for all four groups. The general decrease in hardness might relate to the denaturation of proteins in the fish balls and the increased moisture content during boiling (Benjakul, Visessanguan, & Tueksuban, 2003). Fish balls with 3 g of added gelatin showed significant decrease in hardness compared with that in the other groups after 10 min of boiling. All gelatin added groups were softer



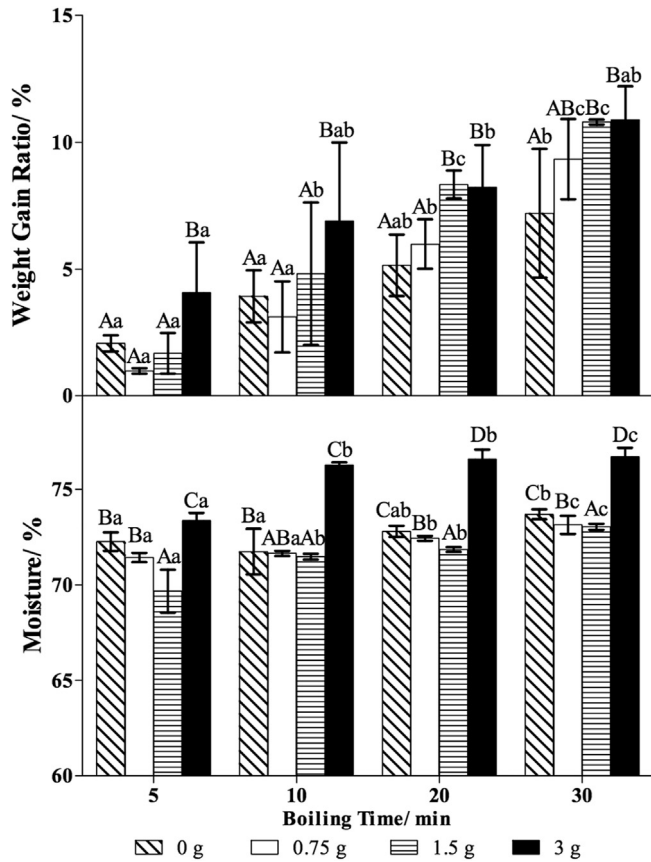


Fig. 2. Weight gain ratio and moisture of fish balls with different gelatin additions in 100 g of fish for different boiling time.\*  
\*Values with different capital letters at the same boiling time and lower case letters of the same group indicate significant differences, as assessed by Duncan's multiple range test ( $P < 0.05$ ), respectively. Data are expressed as the mean  $\pm$  SD ( $n = 3$ ).

than the control group after 30 min of boiling, indicating the gel-weakening effect of gelatin in the fish balls (Brewer, Peterson, Carr, Mccusker, & Novakofski, 2005). A similar result was observed in frankfurters with added connective tissue collagen (Calhoun et al., 1996). The gel weakening effect reflects the fact that fish gelatin lacks of cysteine and cystine, which are amino acids with sulfhydryl and disulfide groups. Without sufficient amount of cysteine and cystine, gelatin cannot form disulfide bonds during gelling, which leads to shorter polypeptide chain lengths and weaker gel strength (Sun & Holley, 2011).

Fig. 3 also shows that there was no significant difference in springiness between the different groups during boiling. For cohesiveness, the value ranged from 0.75 to 0.81 for the different groups of fish balls after various boiling time. This was consistent with a previous study, which showed that the cohesiveness ranged from 0.66 to 0.80 (Tee & Siow, 2014). However, the cohesiveness was not significantly different among the different groups at different boiling time, indicating that the added gelatin did not affect the cohesiveness and springiness of the boiled fish balls. No significant effect on the springiness and cohesiveness of fish balls after gelatin addition indicated that the fish balls were strong and able to reform their structure after compression (Sow & Yang, 2015), showing the good quality of the fish balls after gelatin addition.

The chewiness result was consistent with the hardness result. A significant fall from 12.89 to 6.90 N from 5 to 10 min for 3 g of added gelatin group was observed, which indicated the gel weakening

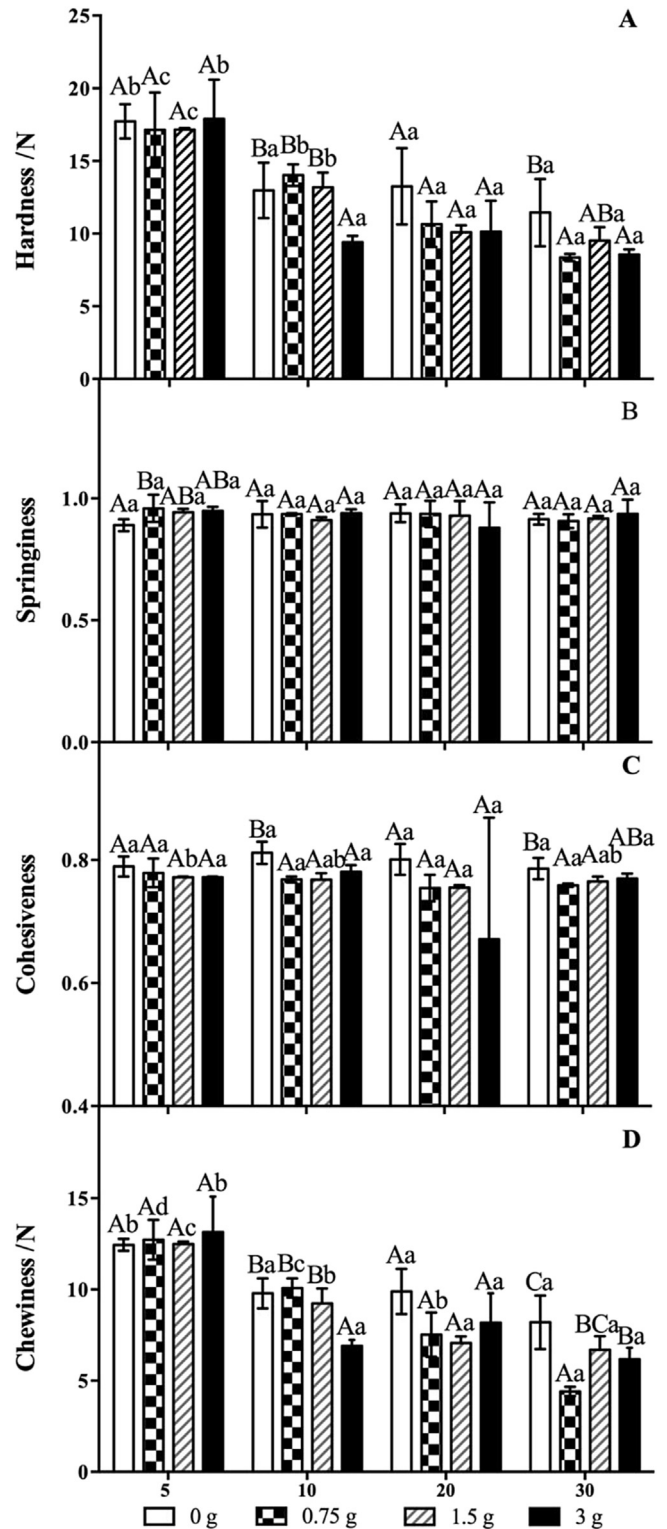
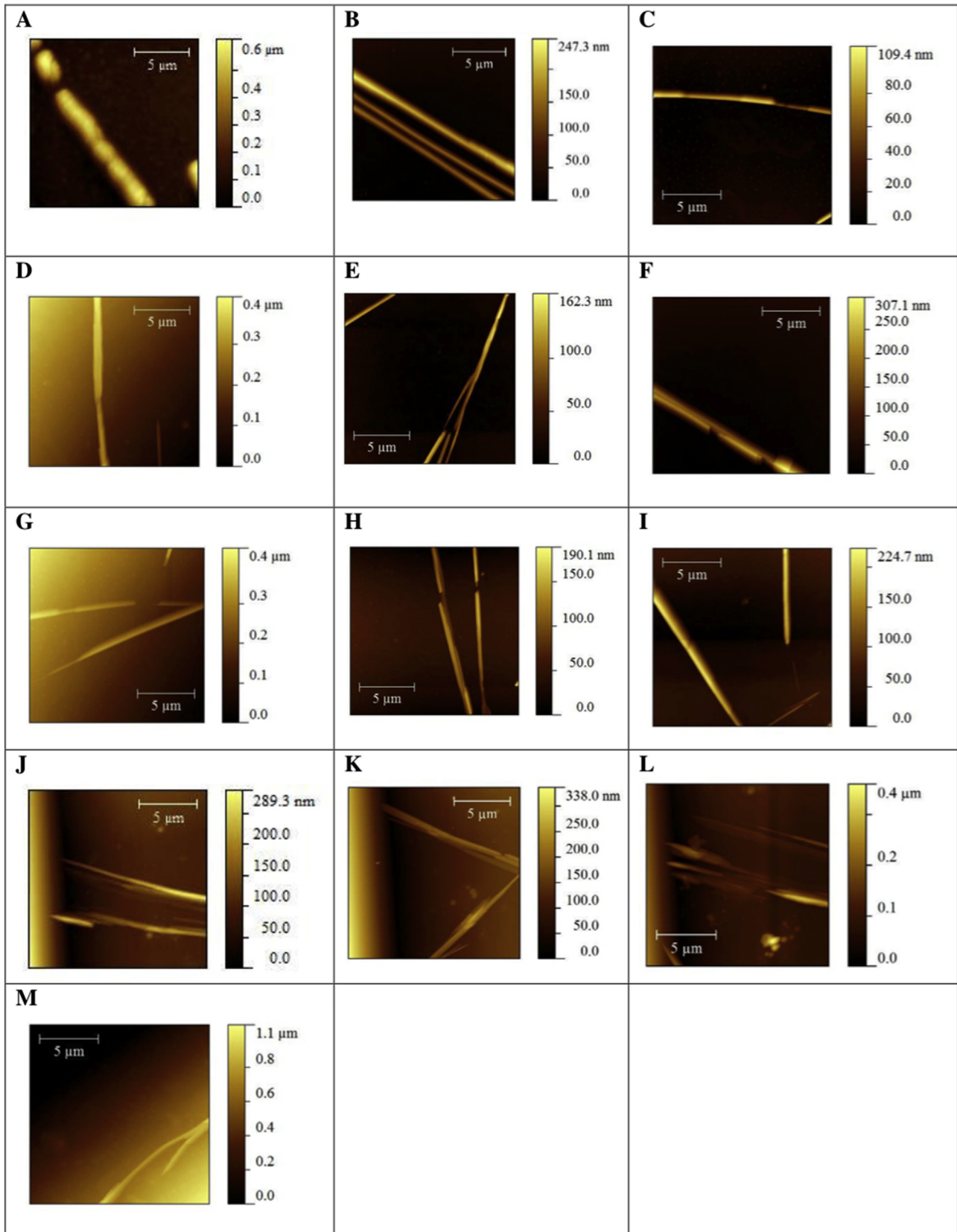


Fig. 3. Effects of gelatin addition and boiling time on (A) Hardness, (B) Springiness, (C) Cohesiveness and (D) Chewiness of fish balls.\*

\*Values with different capital letters at the same boiling time and lower case letters of the same group indicate significant differences, as assessed by Duncan's multiple range test ( $P < 0.05$ ), respectively. Data are expressed as the mean  $\pm$  SD ( $n = 3$ ).

effect of fish gelatin. The chewiness and hardness of all groups of gelatin added fish balls were significantly lower than those of the control group, especially for the 3 g of added gelatin group, which



**Fig. 4.** Nanostructures of myofibrils extracted from fish balls (A) before boiling; fish balls boiled for 10 min with 0 g (B), 0.75 g (C), 1.5 g (D) and 3 g (E) of added gelatin in 100 g of fish; fish balls boiled for 20 min with 0 g (F), 0.75 g (G), 1.5 g (H) and 3 g (I) of added gelatin in 100 g of fish; fish ball boiled for 30 min with 0 g (J), 0.75 g (K), 1.5 g (L) and 3 g (M) of added gelatin in 100 g of fish.

may improve the consumer acceptance of GP fish ball, as the hard texture, high protein and low fat content in GP can lead to excessive hardness and a rubbery texture (Sun & Holley, 2011). The combination of gelatin and myofibril protein is promising to improve the texture and sensory properties of GP fish balls. Sensory evaluation should be performed in a future study.

### 3.3. Nanostructural changes

AFM, an efficient tool to image the morphology of biomolecules, has already been applied to test the morphology and nanostructure of fish gelatin and polysaccharides (Chong, Lai, & Yang, 2015; Sow & Yang, 2015; Yang, 2014). In Fig. 4, the myofibrils extracted from the boiled fish balls showed rod-like structures, which was consistent with the myofibril morphology from rabbit skeletal muscle and *Drosophila* (Nyland & Maughan, 2000; Yoshikawa, Yasuie, Yagi, & Yamada, 1999). Quantitative analysis was done to illustrate the myofibril morphological changes during boiling, which is shown in Table 2. The length of the myofibrils before boiling was greater than 15  $\mu\text{m}$ . A more precise value cannot be given because the maximum scan size of this AFM facility is 15  $\mu\text{m} \times 15 \mu\text{m}$ . The width of myofibril before boiling was 2.98  $\mu\text{m}$  (data not shown). After boiling for 10 min, the length of myofibril remained longer than 15  $\mu\text{m}$  for the control and gelatin addition groups. However, the width decreased from around 2.7  $\mu\text{m}$  to around 0.97  $\mu\text{m}$  for all four groups, and there was no significant difference among the four groups. The decreased myofibril width suggested degradation and aggregation of myofibrils, which may be caused by the thermal process and heat activated proteases, for example, myofibril associated serine proteinases (Benjakul et al., 2003; Deng et al., 2014).

After boiling for 20 min, the length of myofibrils decreased from greater than 15  $\mu\text{m}$  to around 14  $\mu\text{m}$  for all four groups, and there was no significant difference among the different groups. Interestingly, there was no further decrease of the myofibrils width after 10 min of boiling, which might indicate that the proteases hydrolysing the myofibrils by width were inactivated after 10 min of boiling. By contrast, further decreases of the length of the myofibrils were observed to around 11  $\mu\text{m}$  when the fish ball was boiled for 30 min, indicating that the proteases that hydrolyse myofibrils by length might be heat stable, for example, heat-stable alkaline proteinases (Benjakul et al., 2003). Furthermore, the gradual degradation of myofibrils explained the decrease in hardness and chewiness of the fish balls during boiling. These observations were consistent with the finding that the degradation of myosin heavy chain by heat activated proteases disrupted gel microstructure in featherback (*Chitala ornata*) muscle, leading to decreased gel strength (Tachasirinukun, Chaijan, & Riebroy, 2016).

The AFM result showed the denaturation process of myofibril during boiling, and the consistency of myofibril dimensions and

rod-like morphology among all four groups demonstrated that there was no interaction between gelatin and the myofibrils in the fish balls. Thus, the addition of gelatin did not affect the morphology of the myofibrils, nor did it alter the nanostructure of the fish balls. The lack of interaction between the fish gelatin and the myofibrils explained why the addition of fish gelatin did not change the cohesiveness of the fish balls after boiling. A previous study of the interaction of pork collagen (PC) and pork myofibril protein (MP) also found out that there were no specific interactions between PC and MP in mixed gels during thermal gelling (Doerscher et al., 2004). In contrast, the addition of polysaccharide/starch to myofibril proteins affected the microstructure of the gel matrix upon heating and increased the hardness (García-García & Totosaus, 2008).

### 3.4. Molecular weights of proteins and peptides in the boiling water

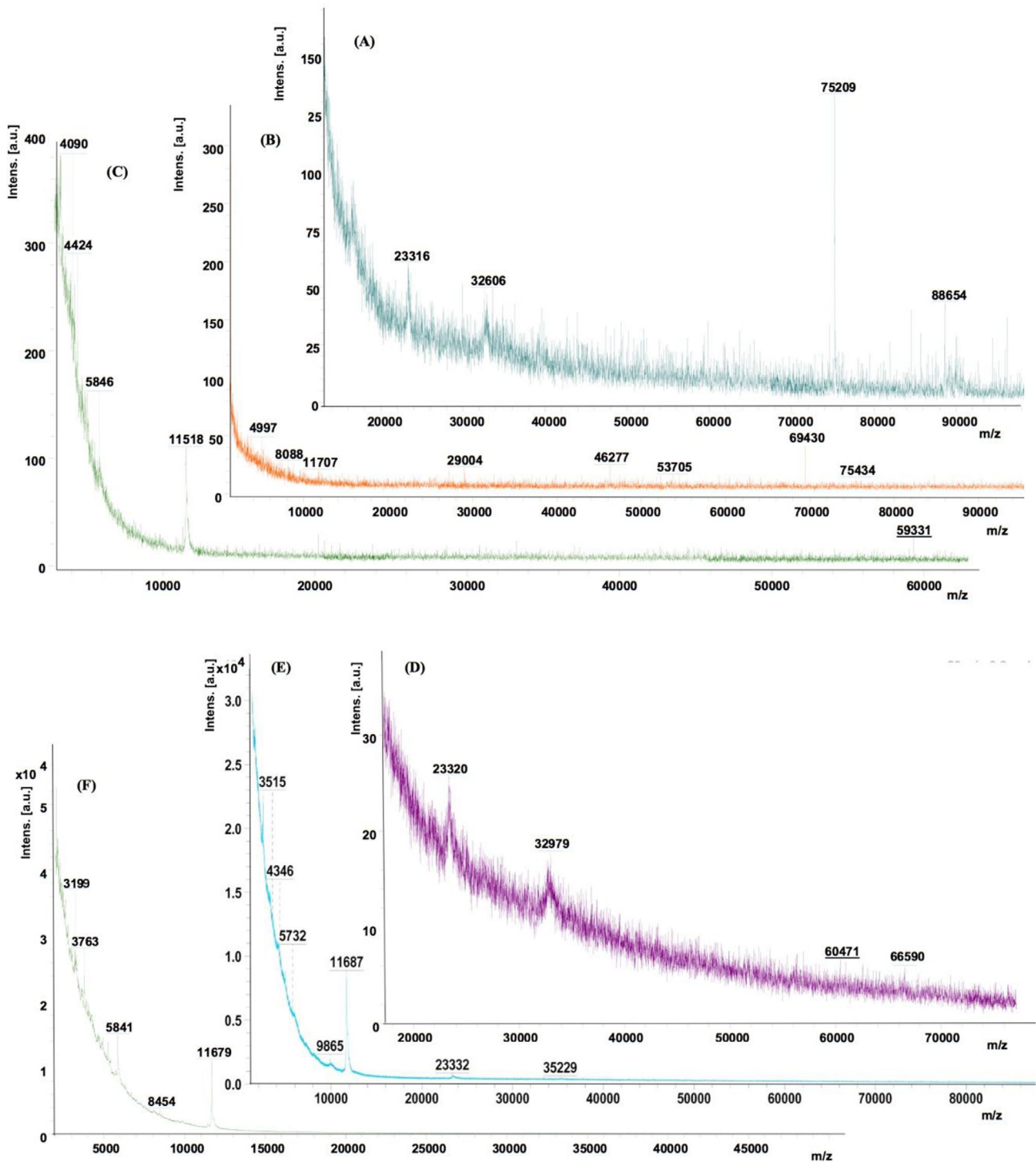
Boiling may cause water-soluble proteins and peptides to diffuse into the boiling solution, resulting in nutrient loss. Fig. 5 shows the MALDI-TOF Mass Spectrum of the peptides and proteins in the boiling solution, and Table 3 summarises the protein and peptide profile of each sample. Twelve proteins and peptides were detected in the mass spectrum for the control group, which ranged from 3199 Da to 96,214 Da, while there were only seven proteins detected for the 0.75 g and 1.5 g of added gelatin groups and five proteins detected for the 3 g of added gelatin group after 5 min of boiling. Significantly fewer proteins and peptides with lower  $M_w$  were observed in the gelatin addition fish ball solution after 10 and 20 min of boiling. These results indicated that the addition of gelatin prevented proteins and peptides with large  $M_w$  from permeating into the boiling solution and effectively decreased the nutrient loss. As gelatin absorbed water and melted during boiling process, the water-soluble proteins and peptides migrated to the melted gelatin instead of leaching into the boiling water. Therefore, the nutrients were preserved in the fish ball as the gelatin gelled after boiling. To further exemplify the mass transfer discussion, the increased moisture content and decreased nutrient caused by gelatin addition contributed to the increased mass transfer coefficients/model parameters of the fish balls during boiling. The increased gelatin concentration further increased the moisture content and decreased the nutrient loss from the fish balls; therefore, there was an increase in the mass transfer coefficients/model parameters as the concentration of gelatin increased.

The results also showed that there was an increase in protein and peptide diversity from 20 to 30 min for the 0.75 g group. The same phenomenon was observed for the 1.5 g of added gelatin group, with molecules of a  $M_w$  of 41,239 Da being generated after 30 min of boiling. However, for the 3 g of added gelatin group, no larger molecules were produced after boiling for 20 and 30 min,

**Table 2**  
Quantitative analysis of myofibrils extracted from different groups of fish balls.<sup>a</sup>

Dimension/ $\mu\text{m}$	Boiling time/min	Fish balls with different gelatin additions in 100 g of fish/g			
		0	0.75	1.5	3
Length	5	>15 <sup>a</sup>	>15 <sup>a</sup>	>15 <sup>a</sup>	>15 <sup>a</sup>
	10	>15 <sup>a</sup>	>15 <sup>a</sup>	>15 <sup>a</sup>	>15 <sup>a</sup>
	20	13.96 $\pm$ 3.12 <sup>b</sup>	14.23 $\pm$ 3.45 <sup>b</sup>	13.76 $\pm$ 3.51 <sup>b</sup>	14.33 $\pm$ 2.89 <sup>b</sup>
	30	11.26 $\pm$ 2.31 <sup>c</sup>	11.55 $\pm$ 1.46 <sup>c</sup>	10.89 $\pm$ 1.53 <sup>c</sup>	11.74 $\pm$ 2.21 <sup>c</sup>
Width	5	2.76 $\pm$ 0.51 <sup>a</sup>	2.78 $\pm$ 0.39 <sup>a</sup>	2.74 $\pm$ 0.44 <sup>a</sup>	2.74 $\pm$ 0.58 <sup>a</sup>
	10	0.98 $\pm$ 0.27 <sup>b</sup>	0.98 $\pm$ 0.20 <sup>b</sup>	0.97 $\pm$ 0.15 <sup>b</sup>	0.95 $\pm$ 0.14 <sup>b</sup>
	20	0.88 $\pm$ 0.17 <sup>b</sup>	0.91 $\pm$ 0.11 <sup>b</sup>	0.94 $\pm$ 0.13 <sup>b</sup>	0.89 $\pm$ 0.15 <sup>b</sup>
	30	0.95 $\pm$ 0.12 <sup>b</sup>	0.94 $\pm$ 0.16 <sup>b</sup>	0.94 $\pm$ 0.18 <sup>b</sup>	0.94 $\pm$ 0.23 <sup>b</sup>

<sup>a</sup> Values with different lower case superscript letters indicate significant differences, as assessed by Duncan's multiple range test ( $P < 0.05$ ) for each parameter (length and width). Data are expressed as the mean  $\pm$  SD ( $n = 15$ ).



**Fig. 5.** MALDI-TOF-MS of proteins and peptides from boiling solution of fish balls: control group boiled for 5 min (A), 10 min (B), 20 min (C) and 30 min (D); boiled for 30 min with 0.75 g (E) and 3 g (F) of added gelatin in 100 g of fish.

which showed that the addition of 3 g of gelatin had the best effect to preserve the nutrients in the fish balls. A molecule with a  $M_w$  of 96,214 Da in the control group after boiling for 5 min disappeared after 10 and 20 min boiling, and further degradation of 75,434 and 85,364 Da molecules in the control group were observed after 30 min of boiling, which might have been caused by the heat treatment. In a future study, the proteins and peptides in the boiling water can be separated using a C18 column or sodium dodecyl

sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then sequenced and subject to fingerprinting to further identify them and to illustrate the mass transfer and nutrient loss from fish balls in detail.

#### 4. Conclusions

The effects of gelatin addition on fish ball's nanostructure,



**Table 3**  
Molecular weights (Da) of proteins and peptides from boiling water of different groups of fish balls determined by MALDI-TOF-MS.

Fish ball with different gelatin additions in 100 g of fish/g	Boiling time/min			
	5	10	20	30
0	3199, 3763, 5841, 8454, 11679, 23316, 32699, 41230, 59832, 75210, 90134, 96214	4925, 5819, 11528, 22041, 30282, 47277, 53705, 69430, 75434, 85364	2934, 4424, 5846, 11518, 17262, 20351, 3489, 46796, 59331, 62606, 75686, 85364	23316, 32651, 56995, 68081
0.75	2327, 4178, 5617, 7209, 9861, 11686, 23381	2327, 4178, 5617, 7209, 9861, 11686, 23381	2516, 4076, 5716, 7950, 10005, 10175, 11673, 23228	2515, 3515, 4346, 5732, 9865, 11687, 23332, 34132, 35229
1.5	2515, 4648, 11685, 5410, 25004	22107, 23588	2515, 2978, 4938, 11702, 32994	11833, 23753, 41239
3	3199, 3763, 5841, 8454, 11679	3199, 3763, 5841, 8454, 11679, 23381	3199, 3763, 5841, 8454, 11679	2515, 3199, 3763, 5841, 8454, 11679

texture and mass transfer were evaluated. Three mass transfer models were proposed to describe the mass transfer of fish balls during boiling. The exponential and linear models were found with very low RMSE, PD value and high  $R^2$ . The mass transfer coefficient/model parameter increased as the amount of gelatin added to the fish balls increased. The hardness and chewiness decreased as the boiling time increased, and the added gelatin reduced the hardness and chewiness of fish balls through a gel weakening effect to improve the fish balls' texture properties. The added gelatin had no significant effect on the springiness and cohesiveness of the fish balls. The added gelatin and long boiling time significantly increased the weight gain ratio and moisture content of the fish balls. The AFM results revealed that the myofibrils degraded gradually after boiling; however, the gelatin did not interact with the myofibrils nor did it affect their morphology. The added gelatin also retained large  $M_w$  proteins and peptides within the fish balls, stopping from leaching into the boiling water and resulting in nutrient retention in fish balls. This study suggests that gelatin addition to fish balls increased the mass transfer coefficient by increasing the moisture content and preserving nutrients; improved the texture through gel weakening effect without altering its nanostructure; and would enhance the sustainability of fishery product processing.

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