Food Chemistry 200 (2016) 283–292

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Fish gelatin combined with chitosan coating inhibits myofibril degradation of golden pomfret (*Trachinotus blochii*) fillet during cold storage

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ARTICLE INFO

*Article history:*
Received 26 June 2015
Received in revised form 17 December 2015
Accepted 8 January 2016
Available online 11 January 2016

*Keywords:*
Edible coating
Gelatin
Chitosan
Matrix assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF-MS)
Atomic force microscopy (AFM)
Myofibril
Myosin light chain
Myoglobin

ABSTRACT

Coating of gelatin and chitosan can improve fish fillet's quality, but the mechanism is not clear. Chitosan/gelatin coatings significantly prevented deterioration of golden pomfret fillet at 4 °C. Chitosan with 7.2% gelatin group showed the best effect on preserving the length of myofibril, which remained greater than 15 μm at day 17 of storage, while for control, chitosan and chitosan combined with 3.6% gelatin group, it was 5.03, 10.04 and 9.02 μm, respectively. The MALDI-TOF MS result revealed that the coatings slowed down the protein deterioration of fillet. On days 13 and 17, the myosin light chain and myoglobin in control group degraded, while the two proteins still existed in chitosan/gelatin coated groups. Overall, the chitosan with 7.2% gelatin coating had the best effect on preserving fillet's quality during storage. The coating may exert its protective effect via inhibiting myofibril degradation within fillet.



## 1. Introduction

Edible coating was developed to preserve the quality of seafood and extend its shelf life. For example, acetylated monoglyceride and whey protein isolate coatings prevented the moisture loss and lipid oxidation in frozen salmon (Stuchell & Krochta, 1995). Chitosan was applied as edible and invisible coating on herring and Atlantic cod to preserve its quality (Jeon, Kamil, & Shahidi, 2002). Cat fish gelatin was developed as antimicrobial coating to extend the shelf life of fresh white shrimp (*Penaeus vannamei*) (Jiang, Liu, Du, & Wang, 2010). Recently, edible coating has been applied to fish in various studies to preserve the quality (Nowzari, Shábanpour, & Ojagh, 2013; Ojagh, Rezaei, Razavi, & Hosseini, 2010; Qiu, Chen, Liu, & Yang, 2014; Zhao et al., 2013). Edible coatings such as alginate calcium coating incorporated with cinnamon, EDTA (Ethylenediaminetetraacetic acid) and nisin were developed to preserve the quality of fresh northern snakehead fish fillets (Lu, Ding, Ye, & Liu, 2010). Other edible coatings that have been developed included whey protein isolate incorporated with oregano essential oil (Atarés, De Jesús, Talens, & Chiralt, 2010) and soy protein isolate incorporated with cinnamon and ginger essential oil (Atarés et al., 2010). The antimicrobial effect of these edible coatings and their effects on fish physicochemical properties were studied (Nowzari et al., 2013; Ojagh et al., 2010; Qiu, Chen, Liu, & Yang, 2014; Zhao et al., 2013). The parameters including pH, Total Volatile Basic Nitrogen (TVB-N), microbiological analysis and sensory evaluation were investigated (Qiu et al., 2014). Different properties of edible film were also investigated, such as mechanical properties, water vapour permeability, water solubility, colour and transmittance, thermal properties (Jiang et al., 2010). However, certain aspects especially the underlying mechanism of the protective effect of coating remain unclear.

Most commonly used materials for edible films are biopolymers such as carbohydrates and proteins. Both derived from marine sources, gelatin and chitosan are hydrophilic biopolymers with good affinity and compatibility, which are suitable to protect

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http://dx.doi.org/10.1016/j.foodchem.2016.01.030
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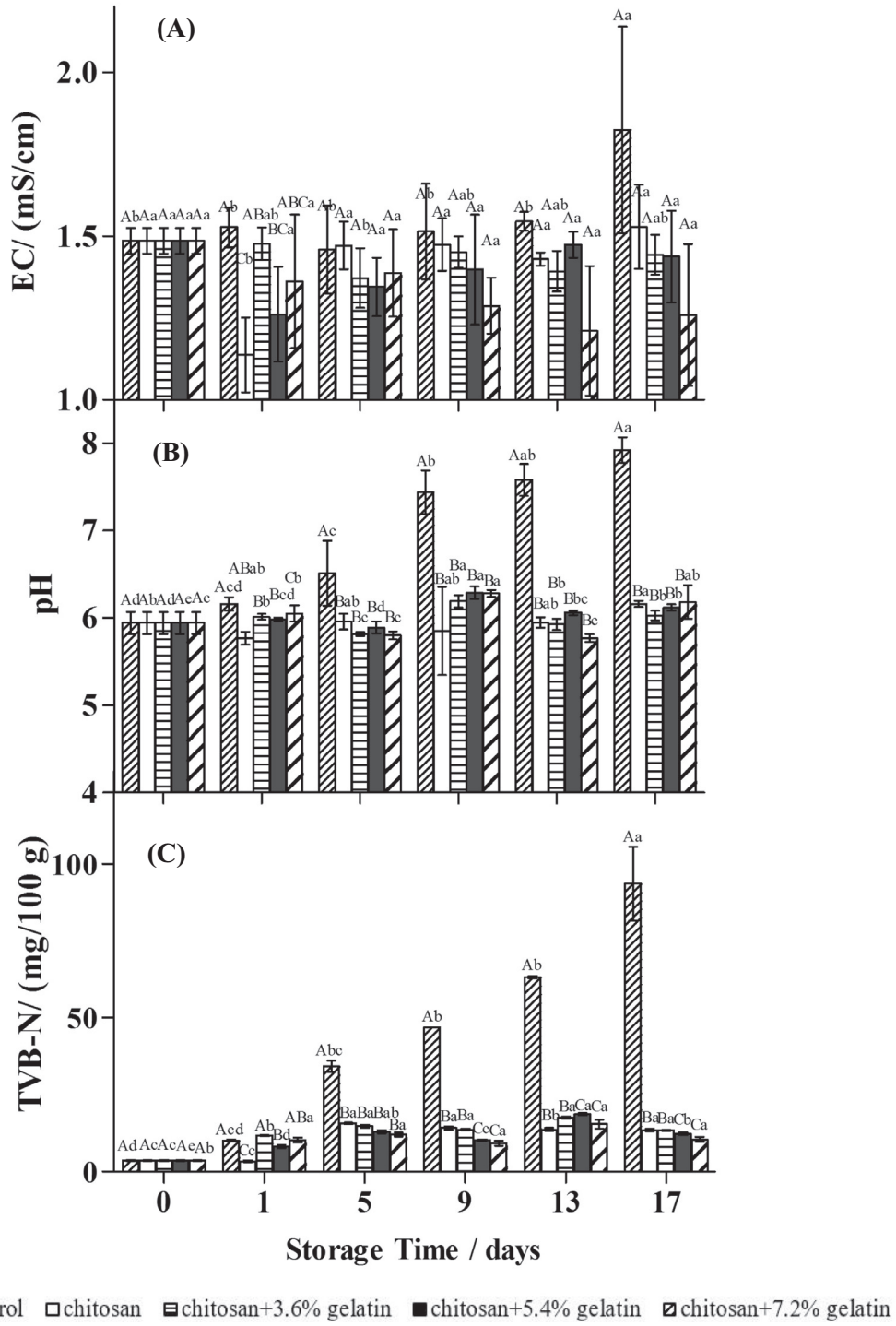


**Fig. 1.** Electrical Conductivity (EC), pH and Total Volatile Basic Nitrogen (TVB-N) of different groups of fish fillet (1) control; (2) chitosan; (3) chitosan + 3.6% gelatin; (4) chitosan + 5.4% gelatin; (5) chitosan + 7.2% gelatin during 17 day cold storage at 4 °C: (A) EC; (B) pH; (C) TVB-N. *Values with different capital superscript letters at the same day and lower case superscript letters of the same group indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively.

seafood products (López, Gómez, Pérez, & Montero, 2005). Gelatin, extracted from fishery processing byproducts like skin or bones, can be applied as edible coating to form a barrier preventing oxygen, drying and light (Yang & Wang, 2009). Extracting and applying gelatin from fishery byproducts improves seafood sustainability (Endo & Yamao, 2007; Yang et al., 2007) and protects seafood quality (Feng, Lai, & Yang, 2014).

Chitosan is a natural polysaccharide obtained from deacetylation of chitin from shrimp and other crustacean shells (Nowzari et al., 2013). Due to its antimicrobial effect and film forming ability, chitosan has been applied as coating to preserve the quality of fish, fruit and other food products (Chong, Lai, & Yang, 2015; Nowzari et al., 2013). Utilisation of chitosan also helps to improve food sustainability.

**Table 1**
Dimensions of myofibril extracted from different groups of fish fillet (1) control; (2) chitosan; (3) chitosan + 3.6% gelatin; (4) chitosan + 5.4% gelatin; (5) chitosan + 7.2% gelatin during 17 day cold storage at 4 °C*.

| Dimension | Storage time/days | Treatment groups | | | |
|---|---|---|---|---|---|
| | | Control | Chitosan | Chitosan+3.6% gelatin | Chitosan + 7.2% gelatin |
| Length/μm | 0 | >15 | >15 | >15 | >15 |
| | 9 | $10.77 \pm 1.03^{b}$ | $12.01 \pm 1.17^{b}$ | >15 | >15 |
| | 17 | $5.03 \pm 4.36^{a}$ | $10.04 \pm 2.31^{b}$ | $9.02 \pm 0.80^{b}$ | >15 |
| Width/nm | 0 | $2.98 \pm 0.31^{a}$ | $2.98 \pm 0.31^{a}$ | $2.98 \pm 0.31^{a}$ | $2.98 \pm 0.31^{a}$ |
| | 9 | $1.85 \pm 0.09^{bc}$ | $1.48 \pm 0.18^{c}$ | $1.89 \pm 0.78^{bc}$ | $2.51 \pm 0.19^{ab}$ |
| | 17 | $2.03 \pm 1.11^{bc}$ | $2.05 \pm 0.32^{bc}$ | $2.04 \pm 0.17^{bc}$ | $2.09 \pm 0.13^{bc}$ |
| Height/nm | 0 | $512.8 \pm 4.9^{a}$ | $512.8 \pm 24.9^{a}$ | $512.8 \pm 24.9^{a}$ | $512.8 \pm 24.9^{a}$ |
| | 9 | $279.1 \pm 35.0^{b}$ | $288.1 \pm 33.6^{b}$ | $191.8 \pm 13.1^{d}$ | $207.0 \pm 27.6^{cd}$ |
| | 17 | $264.6 \pm 86.7^{bc}$ | $269.9 \pm 14.0^{bc}$ | $163.6 \pm 33.3^{d}$ | $159.5 \pm 27.7^{d}$ |

* Values with different lower case superscript letters indicate significant differences by the Duncan's multiple range test ($P < 0.05$) for each parameter (length, width and height, respectively).

Though gelatin, chitosan and their combination were applied as coating before, the mechanism of how the components exert their protective effects remains largely unknown (López et al., 2005; Nowzari et al., 2013). Matrix-Assisted Laser Desorption/Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS) is powerful in analysing protein and peptide profile in fillet muscle (Bauchart et al., 2007). Atomic force microscopy (AFM) could be applied to investigate single molecular morphological and structural changes (Sow & Yang, 2015).

The objectives of this study are to develop edible coating based on chitosan and gelatin to preserve fish fillet quality and microbial safety. The coating's protective effect and its underlying mechanism were analysed from the aspects of physicochemical properties, microbial safety, nanostructure and protein/peptide composition.

## 2. Materials and methods

### 2.1. Edible coating solution preparation

Commercial tilapia fish gelatin (200 Bloom) was purchased from Jiangxi Cosen Biology Co., Ltd (Yingtan, Jiangxi, China), which contained 83.14% protein, 0.68% ash, 9.12% moisture and 7.06% of others according to the product information. Fish gelatin solution (6%, 9%, 12%, w/w) was prepared following the method with some modifications (Yang & Wang, 2009). Gelatin was soaked in distilled water at 4 °C overnight. The gelatin solution was placed in 55 °C water bath for 15 min till it was totally dissolved and homogenised.

Chitosan was purchased from Zhengzhou Yanlord Chemical Products Co., Ltd (Zhengzhou, Henan, China), and the deacelylation (DAC) of chitosan was 90.0%. Chitosan solution of 1% was prepared. chitosan solution of 120 ml and gelatin solution of 180 ml were mixed and homogenised with a magnetic stirrer for 5 min. Five edible coatings including Control (deionised water), 0.4% (w/w) chitosan, 0.4% chitosan with 3.6% gelatin (w/w), 0.4% chitosan with 5.4% gelatin, and 0.4% chitosan with 7.2% gelatin were made. Gelatin only group was not used for making coating solution since gelatin itself didn't have antimicrobial effect from our test.

### 2.2. Fillet preparation

Golden pomfret (*Trachinotus blochii*) from a local supermarket was purchased, and the head, bone and skin were removed. The fillet was cut into 10 g pieces and immersed in different coating solutions for 3 min. Then the fillets were dried at room temperature of 25 °C for 1 h and packed in polyethylene bags with zip lock (S.C. Johnson & Son Pte. Ltd, Singapore, Republic of Singapore). Packed fillets with different coatings were stored at 4 °C for 17 days.

### 2.3. Physicochemical parameters

#### 2.3.1. Weight loss, cooking loss and colour

The weight of fish fillet before storage ($W_1$) and after storage ($W_2$) was measured and used to calculate the percentage of weight loss according to the equation as shown in the following (Chong et al. 2015).

$$\text{Weight loss}/\% = (W_1 - W_2)/W_1 * 100$$

Fish fillets coated with different antimicrobial coatings were immersed in a water bath at 85 °C for 15 min (Fan, Luo, Yin, Bao, & Feng, 2014), and the original weight was recorded as $W_b$. After heating, each sample was weighed and the weight was recorded as $W_a$. The cooking loss was calculated according to the formulation below.

$$\text{Cooking loss}/\% = (W_b - W_a)/W_b * 100$$

Colour of the fillet was measured through Minolta colourimeter CM-3500d (Konica Minolta, Inc., Japan). The $a^*$, $b^*$ and $L^*$ value was recorded, and $\Delta E$ was calculated through the equation below, which indicated the overall difference of colour between the tested sample and the original sample at day 0 (Chong et al., 2015).

$$\Delta E^* = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

All the experiments were performed in triplicate.

#### 2.3.2. Electrical Conductivity (EC), pH and Total Volatile Basic Nitrogen (TVB-N)

Fillet sample of 10 g was minced and dissolved in 100 ml water. The mixture was homogenised via magnetic stirrer for 15 min. After that, sample was centrifuged at 1000 *g* for 5 min. The supernatant was measured with a digital EC metre and then stored in the 4 °C fridge for further analysis.

pH was measured after EC with a digital pH metre, followed by TVB-N analysis with method modified from Santos et al. (2013). Sample supernatant of 5 ml from pH and EC test and 5 ml 1% MgO solution were transferred into a Kjeldahl tube. Boric acid solution (10 ml, 2%) with 5–6 drops of indicator was transferred to a flask which was placed under the condensation tube of the Kjeldahl instrument. The sample was distilled by the Kjeldahl machine to evaporate the volatile nitrogen which was absorbed by the boric acid solution. Distillation was lasted for 5 min, and the solution in the flask was titrated with 0.001 M HCl solution until the solution

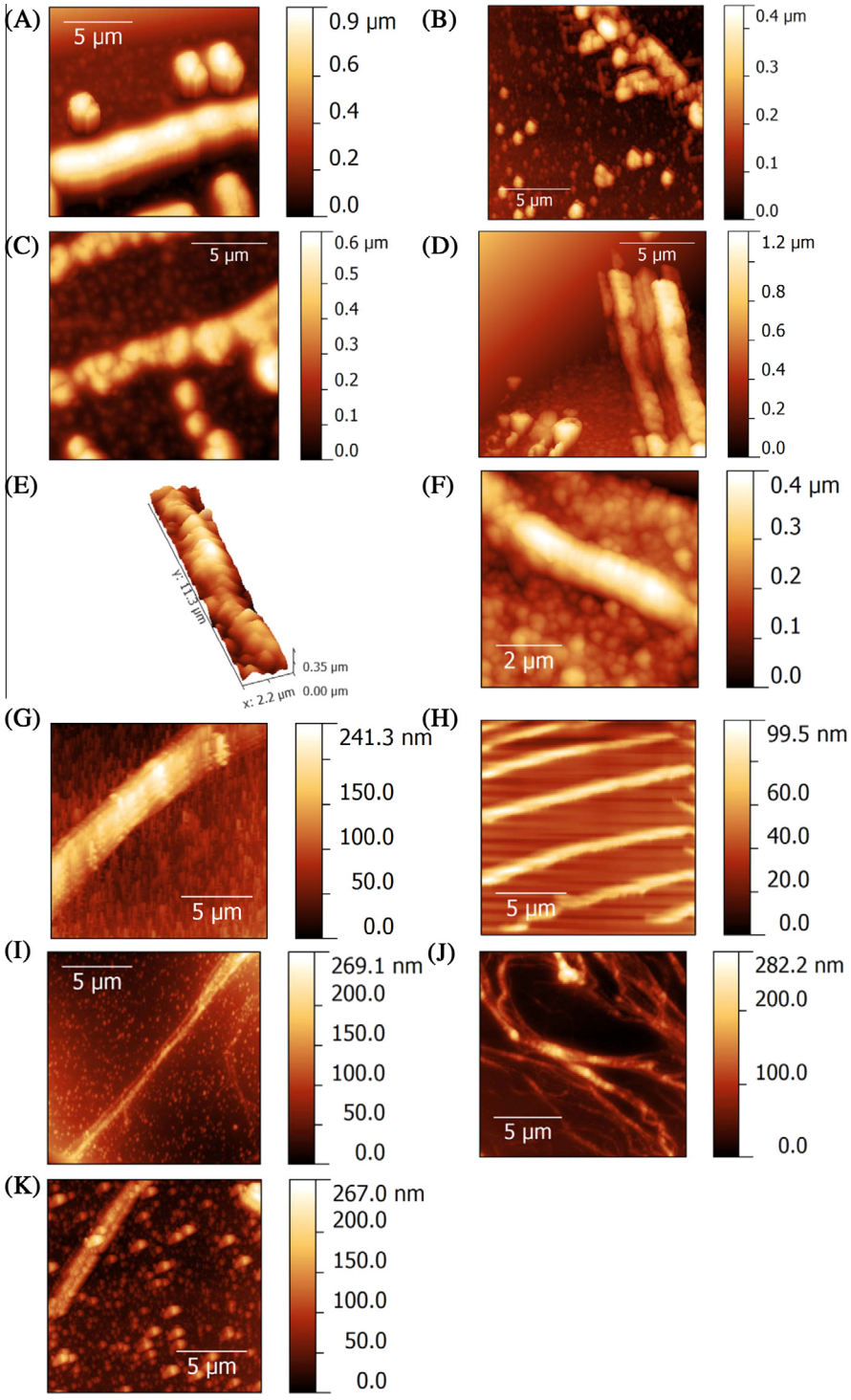


**Fig. 2.** Nanostructures of myofibril extracted from fish fillet. (A) day 0; (B) day 9 control; (C) & (D) day 17 control (E) day 9 chitosan; (F) day 17 chitosan; (G) day 9 chitosan + 3.6% gelatin (H) day 17 chitosan + 3.6% gelatin; (I) & (J) day 9 chitosan + 7.2% gelatin (K) day 17 chitosan + 7.2% gelatin.

turned to blue purple. Blank experiment was carried out at the same time. TVB-N value was expressed as mg N/100 g sample.

$$\text{TVB-N} = (V_1 - V_2)N \times 100 \times 14 \times 50/W \times 5$$

In the formula, $V_1$ and $V_2$ represent the volume (ml) of HCl solution used for the sample and the blank, respectively, while $N$ represents the normality of HCl solution, and $W$ is the weight of the sample (g) (Santos et al., 2013).

### 2.4. Atomic force microscopy

Myofibril was extracted from each fish sample according to the method described by Martone (Martone, Busconi, Folco, Trucco, & Sanchez, 1986). Solution A was freshly prepared before the start of each extraction, which included 0.10 M KCl, 1 mM phenylmethylsulfonylfluoride (PMSF), 0.02% $NaN_3$, 20 mM Tris–HCl buffer pH 7.5. Solution B was comprised of 0.45 M KCl, 5 mM β-mercaptoethanol (β-MCE), 0.2 M $Mg(CH_3COO)_2$, 1 mM ethylene

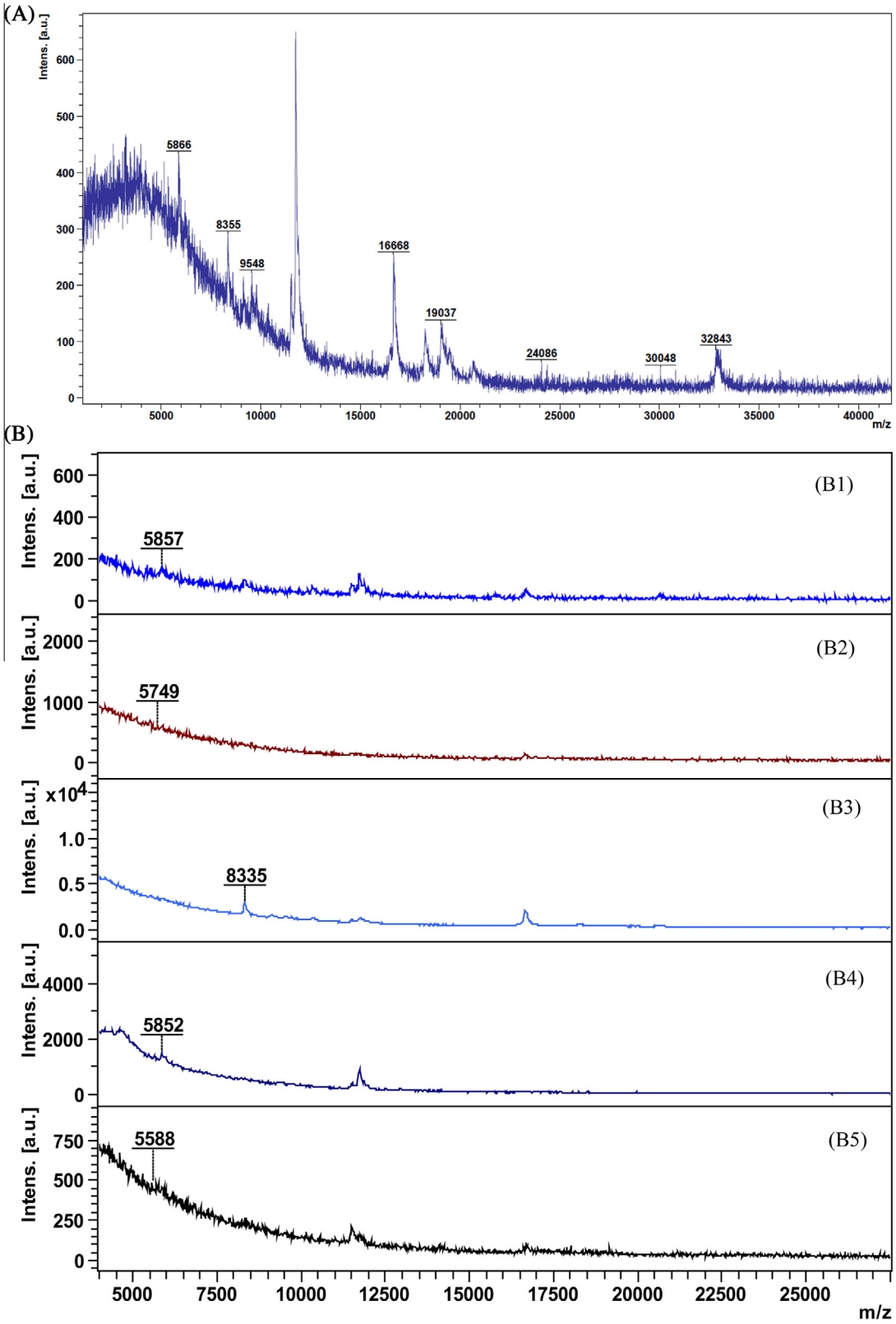


**Fig. 3.** Effect of edible coating on the preservation of small protein and peptide from fish fillet by MALDI-TOF-MS. (A) day 0. (B1) day 5 chitosan + 7.2% gelatin (B2) day 13 control. (B3) day 13 chitosan + 7.2% gelatin. (B4) day 17 control. (B5) day 17 chitosan + 7.2% gelatin.

glycol-bis (β-aminoethyl ether) N,N,N′N′-tetraacetic acid (EGTA), and 20 mM Tris–maleate buffer pH 6.8. Fish fillets were homogenised with blender and 5 g sample was added to 25 ml solution A. The mixture was slightly mixed with magnetic stirrer, kept at 0 °C for 15 min and centrifuged at 1000 *g* for 10 min at 4 °C. The supernatant was discarded. The pellet was resuspended in 50 ml

**Table 2**
MALDI-TOF-MS result of small molecules from different groups of fish fillet (1) control; (2) chitosan; (3) chitosan + 3.6% gelatin; (4) chitosan + 5.4% gelatin; (5) chitosan + 7.2% gelatin during 17 day cold storage at 4 °C.

| Storage time/days | Treatment groups | | | | |
|---|---|---|---|---|---|
| | Control | Chitosan | Chitosan + 3.6% gelatin | Chitosan + 5.4% gelatin | Chitosan + 7.2% gelatin |
| 0 | 5866, 8355, 9548, 11,735, 16,668, 19,037, 24,086, 30,048, 32,843 | 5866, 8355, 9548, 11,735, 16,668, 19,037, 24,086, 30,048, 32,843 | 5866, 8355, 9548, 11,735, 16,668, 19,037, 24,086, 30,048, 32,843 | 5866, 8355, 9548, 11,735, 16,668, 19,037, 24,086, 30,048, 32,843 | 5866, 8355, 9548, 11,735, 16,668, 19,037, 24,086, 30,048, 32,843 |
| 5 | 5824, 8305, 10,332, 11,735, 14813, 16,675, 20,610 | 5824, 8305, 10,332, 11,735, 14,813, 16,675, 20,610 | 5824, 8305, 10,332, 11,735, 14,813, 16,675, 20,610 | 5824, 8305, 103,32, 11,735, 14,813, 16,675, 20610 | 5824, 8305, 10,332, 11,735, 14,813,16,675, 20,610 |
| 9 | 8330, 9601, 10,300, 11,500, 16,621, 18,206, 19,183, 20,641 | 8310, 11,723, 16662, 18,221, 19039, 20,543 | 5815, 8330, 11,500, 16,706, 18,237, 19,043, 20,610 | 5815, 8330, 11,500, 16,706, 18,237, 19043, 20,610 | 5815, 8330, 11,500, 16,706, 18,237, 19,043, 20,610 |
| 13 | 11500, 16670, 17343 | 5815, 8325, 10326, 11762, 16651, 18329, 20698 | 8333, 10325, 11737, 16702, 18229, 19060, 20640 | 5863, 8333, 9124, 10319, 11056, 11743, 16667, 18229, 19052, 20640 | 8335, 9124, 10202, 11736, 16667, 18229, 19141, 20640 |
| 17 | 4700, 11735, 16670 | 8333, 11475, 16723 | 11506, 14115, 16715 | 11506, 14115, 16612 | 11511, 14115, 16612 |

solution B, and added with 10 mM ATP. After incubation for 1 h at 0 °C, the mixture was centrifuged at 10,000 *g* for 15 min. The supernatant contained myofibril.

The myofibril supernatant of 1 ml was diluted with distilled water 20 times and vortexed before AFM analysis. Sample of 20 μl was pipetted onto a newly cleaved mica sheet, which was attached to a magnetic disc. The sample was dried at room temperature before AFM analysis. TT-AFM (AFM workshop, Signal Hill, CA, USA) equipped with a Sensaprobe TM190-A-15 tip (Applied Nanostructures, Mountain View, CA, USA) was used to analyse the morphology of myofibril extracted from fish fillet. 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 dimensions of myofibril in the image were analysed through the Gwyddion software (Sow & Yang, 2015).

### 2.5. Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS)

The Bruker Autoflex III TOF/TOF with smart beam laser was used for fillet protein and peptide analysis. The MALDI-TOF-MS method was modified according to Luccia et al. (2005). The supernatant for AFM analysis was dialysed to eliminate existing salts, since salts would affect the MALDI-TOF-MS operation and result. After dialysis, the sample of 1 ml was diluted with 50% acetonitrile of 1.5 ml, which was used for the MALDI-TOF MS analysis. Sample of 2 μl was mixed with 2 μl DHB (2, 5-dihydroxybenzoic acid) and pipetted on the MALDI target followed with air drying. The signal $m/z$ was measured. The conversion of $m/z$ into Dalton was performed, and compounds from 0 to 40,000 Da were listed (Luccia et al., 2005).

### 2.6. Microbiological analyses

Fish fillet sample (10 g) was homogenised with 0.1% peptone water (90 ml) using stomacher for 90 s. Serial dilutions (1:10 v/v each time) were made. The mesophilic aerobics count was determined using spread plate technique on standard plate count agar (PCA) after 48 h incubation at 37 °C. Sample was also spread on potato dextrose agar (PDA) and incubated at 25 °C for 96 h for enumerating yeasts and moulds count. The microbial analyses were performed on the exact day of sample preparation as well as day 5, day 9, day 13 and day 17 during cold storage. Results were expressed as log CFU/g sample (Chong et al., 2015).

### 2.7. Statistical analysis

All the experiments were conducted at least triplicate. The results were reported as mean ± standard deviation. The differences of results among different groups were determined by ANOVA ($P < 0.05$) and Duncan's multiple range test with SAS software (SAS Institute Inc., Cary, NC, USA). For AFM analyses, dozens of parallel images were obtained for achieving statistically valid results.

## 3. Results and discussion

### 3.1. Physicochemical properties

#### 3.1.1. Weight loss, cooking loss and colour

To investigate the effect of combined chitosan and gelatin coating on fillet quality, the weight loss, cooking loss and colour changes of fillet were examined. Gelatin coating significantly inhibited the weight loss of fish fillet during the 17 day cold storage. As a comparison to control and chitosan coated group of which the weight loss increased dramatically from 0.5% at day 0 to almost 5% at day 17, the weight loss of gelatin coated groups remained below 1.5% throughout the storage period of 17 days (Fig. S1). The less weight loss by gelatin coating might be caused by the water holding capacity and water barrier properties of gelatin (Limpisophon, Tanaka, Weng, Abe, & Osako, 2009). The effectively suppressed weight loss by edible gelatin coating was beneficial to the coated products because it decreased the economic loss and preserved the nutrient, especially the water-soluble protein. This result was consistent with the result from Kaale, Eikevik, Rustad, and Nordtvedt (2014).

Interestingly, the developed coating was not effective in suppressing the cooking loss of fillet (Fig. S1). A previous report

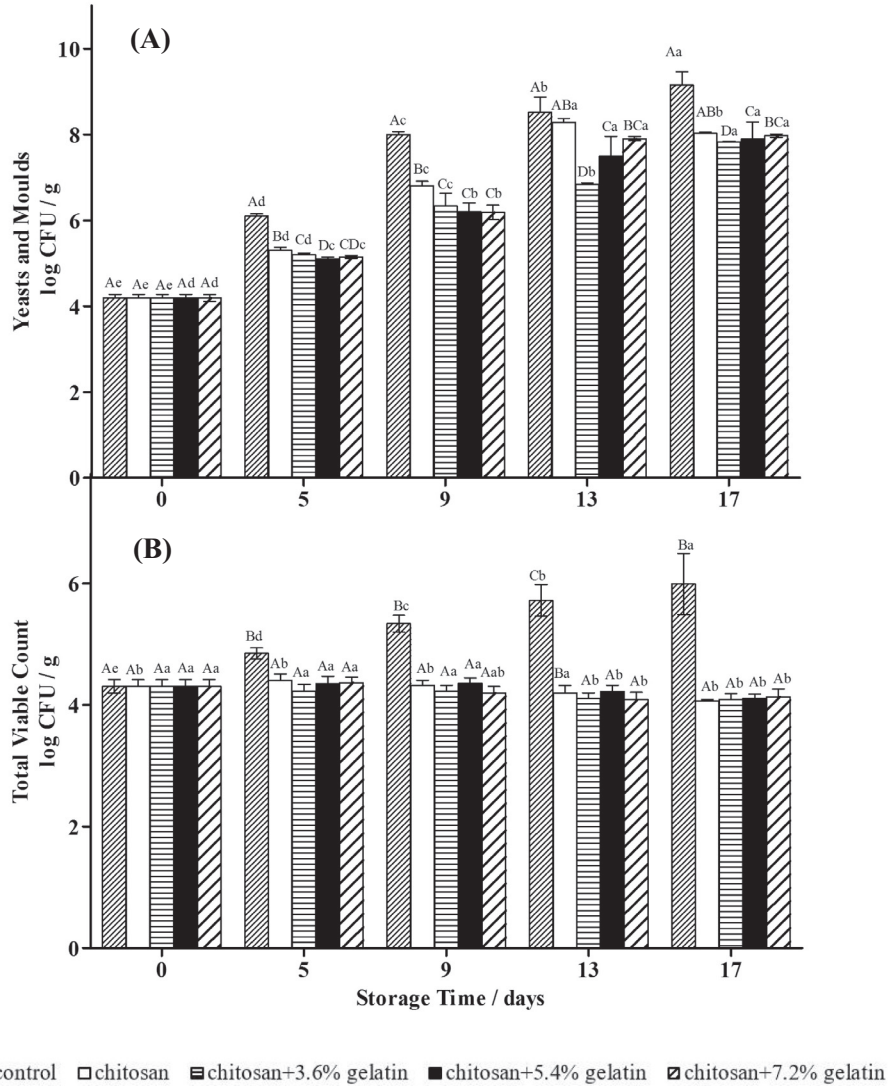


**Fig. 4.** Effect of edible coating on the microbial survival of fish fillet: (A) yeasts and molds; (B) total viable count. *Values with different capital superscript letters at the same day and lower case superscript letters of the same group indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively.

indicated that water and water soluble substances contributed to the cooking loss (Fan et al., 2014). Salted fish fillet had less cooking loss than control due to the changes in muscle proteins, especially salt-soluble protein, leading to increased water holding capacity of muscle (Fan et al., 2014). In the current study, the edible coating was developed as a coating film protecting the fish fillet from oxygen, light or bacteria. The coating may not be able to interact with the muscle protein inside the fillet and change the water holding capacity of the muscle protein, which might explain why the current edible coating didn't affect the cooking loss of fillet.

Fig. S1 shows that all the coatings helped to maintain the colour of fish fillet during cold storage. $\Delta E$ of coated fillet remained around 7% from day 5 to day 17, while $\Delta E$ of control group increased from 7.9% at day 9 to around 14.5% at days 13 and 17. Although the effects of gelatin with different concentrations on colour didn't differ during cold storage, the colour maintaining effects of the developed edible coating agreed well with a previous study on fruits (Chong et al., 2015). $L^*$ for the coated group was significantly greater than the control group, demonstrating the developed edible coating can effectively improve the lightness of fish fillet during cold storage. However, there was no significant change of $a^*$ and $b^*$ value between control and coated groups during storage.

### 3.1.2. Electrical Conductivity (EC), pH and Total Volatile Base Nitrogen (TVB-N)

Next, whether the coating affected the electrolytes of fish fillet was examined. EC was not significantly different among different coating groups during cold storage (Fig. 1). Since EC is an index of the concentration of electrolytes in the muscle tissue (Fan et al., 2014), the current result suggests that the chitosan and chitosan/gelatin combined coating did not affect the electrolytes in the fish muscle tissue.

Fig. 1 shows that the pH for control group increased dramatically from 5.94 at day 0 to 7.92 at day 17. The result was consistent with a previous report that the pH increased dramatically from around 6.5 at day 1 to about 7.8 at day 10 of storage (Lu et al., 2010). On the contrast, pH for the other four treatment groups remained around 6 from day 0 to day 17. During cold storage, the basic amine produced from degradation of amino acid contributed to the increased pH. For the coating treated groups, the degradation of amino acid caused by microbes was significantly

inhibited, leading to less production of biogenic amines. Therefore, the pH of the coated fish was more stable than the control group, showing that the coating helped to maintain the quality of fish fillet during cold storage.

TVB-N is an indicator of the freshness of meat product (Santos et al., 2013). Fig. 1 shows that the TVB-N for Control group increased dramatically from 3.59 mg/100 mg at day 0 to 93.52 mg/100 g at day 17, suggesting the freshness of control fillet decreased quickly and became unacceptable during cold storage. The result is consistent with previous study, which showed that the TVB-N increased from 11.1 mg/100 g at day 0 to 100.2 mg/100 g at day 12 (Qiu et al., 2014). The amino acids in control fish muscle may be metabolized by bacteria or degraded by endogenous enzyme, leading to the production of ammonia, monoethylamine, dimethylamine, trimethylamine, and other volatile bases, and unpleasant smell of spoilt fish (Duan, Jiang, Cherian, & Zhao, 2010). On the contrary, the fillet with edible coating had a much lower TVB-N during storage. For example, the TVB-N value for control group was 34.22 mg/100 g at day 5; however, for treatment group it was around 14 mg/100 g. Specifically, at day 17 the TVB-N was 13.69 mg/100 g for chitosan group, 13.48 mg/100 g for chitosan with 3.6% gelatin group, 12.31 mg/100 g for chitosan with 5.4% gelatin group and 10.51 mg/100 g for chitosan with 7.2% gelatin group. These results showed that fillet coated with either chitosan only or chitosan/gelatin combination maintained fresh and acceptable even after 17 day storage at 4 °C. Chitosan and chitosan/gelatin combined coating effectively extended the shelf life of golden pomfret fillet.

It should be noted that the added gelatin itself didn't show significant preservative effect on fish fillet's microbial quality. The reason may be that gelatin itself didn't possess effective antimicrobial effect. However, the positively charged amino group on chitosan may interact with negatively charged protein on cell membrane of microbes so as to change the permeability of cell membrane, which may lead to autolysis and inhibit the reproduction and metabolism of microorganisms. Moreover, the combined chitosan and gelatin may inhibit the oxygen permeability thus preserving the fish fillet quality (Nowzari et al., 2013).

### 3.2. Atomic force microscopy

To understand the coating effect of gelatin and chitosan from the structural and molecular level, the nanostructural changes of myofibril, the major component of fillet muscle, were analysed. AFM is a powerful tool for imaging the morphology of biomolecules. It has been successfully applied to image the nanostructure of fish gelatin and polysaccharides (Chong et al., 2015; Sow & Yang, 2015). Applying AFM may reveal the degradation pathway of myofibril and the effect of edible coating on the preservation of fillet muscle during cold storage.

The myofibril component extracted from golden pomfret fillet showed rod like structure by AFM (Fig. 2), which was comparable to myofibril from *Drosophila* and rabbit skeletal muscle (Yoshikawa, Yasuike, Yagi, & Yamada, 1999; Nyland & Maughan, 2000). The quantitative analysis was carried out to demonstrate how myofibril degraded during cold storage. The myofibril extracted from fish fillet at day 0 was intact with the length greater than 15 μm, the width of 2.98 μm, and the height of 512.8 nm. Length greater than 15 μm was not measured because the maximum scan size of the equipment was 15 μm × 15 μm. During cold storage, the length for the control group decreased from day 0 level to 10.77 μm at day 9 and 5.03 μm at day 17 (Table 1). In general, chitosan and chitosan/gelatin combined coating effectively prevented the degradation of myofibril during 17 day cold storage. More specifically, the chitosan coated group had a myofibril length of 12.01 μm at day 9, which was slightly longer than the control group at the same day. The gelatin coated groups maintained the myofibril length more than 15 μm even at day 9 of storage. At day 17, the myofibril length of the chitosan only coated group and chitosan combined with 3.6% gelatin group was 10.04 μm and 9.02 μm, respectively. Both lengths were significantly longer than 5.03 μm of the control group at the same day. However, chitosan combined with 7.2% gelatin coating best preserved the length of myofibril, keeping the length greater than 15 μm at the end of the storage. These results suggest that the chitosan/gelatin combined coating effectively prevented the degradation of myofibril during cold storage, which might be caused by the fact that gelatin coated in the edible coating decreased the weight loss and stabilized the hydrated water around myofibrils (Ruttanapornvareesakul et al., 2005).

The width of myofibril was not significantly different among different coating groups both at day 9 and day 17 of storage. Overall, the width of myofibril decreased significantly from 2.98 μm at day 0 to around 1.89 at day 9, but there wasn't further decrease from day 9 to day 17 for all 5 groups. For the height of myofibril, it can be seen from Table 1 that similar to the width. The height decreased significantly from 512.8 nm at day 0 to around 280 nm for control and chitosan coated group and around 200 nm for chitosan/gelatin combined group at day 9. The height of myofibril from chitosan/gelatin coated group was slightly less than that of myofibril of control and chitosan only coated group. Similar to the width of myofibril, the height of myofibril didn't further decrease from day 9 to day 17 for each group.

The degradation of myofibril may be caused by endogenous enzyme and bacterial activity. Endogenous enzymes such as calpain, Caspase-3 and myofibril-bound serine proteinase (MBSP) may exert proteolytic effect on the myofibril, leading to its fragmentation, fibre rupture and detachment (Zhang, Pan, Cao, & Wu, 2013; Zhong et al., 2012). The fragmentation contributes to the decrease of myofibril length, while the rupture and detachment may lead to the decrease of height and width of myofibril.

### 3.3. Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI-TOF-MS)

To understand the coating effects from protein and peptide level, MALDI-TOF-MS was applied to analyse fish fillet sample. MALDI-TOF-MS can analyse the precise molecular weight of protein and peptide which are easily to be destroyed by other ionisation methods (Chaurand, Luetzenkirchen, & Spengler, 1999).

As shown in Fig. 3, many peaks representing molecules with different molecular weights ($M_w$) were present at day 0. The largest molecule in the spectrum, which may be tropomyosin, had $M_w$ of 32,946 Da (Pazos, Méndez, Vázquez, & Aubourg, 2015). As a comparison, the largest protein or peptide for trout muscle extract in MALDI-TOF-MS was only 4820 Da. The difference may be due to the different methods of sample preparation (Bauchart et al., 2007). Even though there were still many peaks in the spectrum at day 5, the largest molecule with $M_w$ of 32,946 Da disappeared in all the five coating groups (Table 2), suggesting that tropomyosin may deteriorate during cold storage. This result was consistent with previous study showing the 32 kDa bands in electrophoresis spectrum of goose muscle sample degraded into smaller fragments (Zhang et al., 2013). The loss of tropomyosin may contribute to the disintegration of myofibril, since it is a component of myofibril and has the function of regulating the interaction between actin and myosin, and muscle contraction in muscle cells (Gunning, Ghoshdastider, Whitaker, Popp, & Robinson, 2015).

At day 9, the spectrum was similar to day 5 for the five groups and the largest molecule was 20,641 Da. However, at day 13, the 20,641 Da molecule in the control group disappeared, although this molecule still existed in the spectrum of the coated group,

further confirming that the developed edible coating can prevent the protein component of fillet muscle from degradation.

At day 13, for control and chitosan coated group, the molecule with $M_w$ of 19,043 Da, which may be myosin light chain, degraded (Zhao et al., 2013), although it still remained in the chitosan/gelatin coated groups. However, this 19,043 Da band/peak in chitosan/gelatin coated fish fillet sample disappeared at day 17. The result showed that the added gelatin helped to delay the degradation of myosin light chain during cold storage. At day 17, the signal for 16,670 Da molecule, likely the myoglobin, was so weak in the control group that it was hard to be identified in the spectrum (Di Luccia et al., 2005). On the contrary, the 16,670 Da molecule remained strong in the coated group. These results further demonstrated that the chitosan and chitosan/gelatin coatings significantly protected the proteins including myoglobin in fish muscle and slowed down protein deterioration during cold storage. Interestingly, there was no significant difference among the four coated groups.

However, it should be noticed that MALDI-TOF-MS has disadvantages when detecting myofibril. The signal for molecules with large molecular weight is much weaker than that for low molecular weight peptide or protein. The major components of myofibril are actin, myosin heavy chain (MHC) and troponin, which are 45,000, 200,000 and 35,000 Da, respectively (Pazos et al., 2015). Therefore, the peaks representing actin, MHC and troponin cannot be easily distinguished in the spectrogram. In the future, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) can be applied to measure myofibril and aid to demonstrate the myofibril degradation process during cold storage and illustrate the effect of coating on the fish fillet.

### 3.4. Microbiological analyses

To investigate the coating's effect on inhibiting microbial growth, microbial analyses were carried out. A gradual increase in yeast and mold count was observed from day 0 to day 17 for all the five groups (Fig. 4). During storage, there was around one log reduction of yeast and mold count in the four coated groups compared with the control group, suggesting that the developed edible coating inhibited the growth of yeasts and molds. Moreover, the edible coating exhibited stronger effect on inhibiting the growth of bacteria. The total viable count (TVC) of bacteria is a key indicator of the quality of fish fillet. Fresh fish with TVC lower than 6 log CFU/g is acceptable for consumers (Qiu et al., 2014). The TVC of control group increased dramatically from 4.31 log CFU/g on day 0 to 5.99 log CFU/g on day 17. On the contrary, the TVC for the four coating treated groups remained around 4 log CFU/g during the 17 day storage, demonstrating the freshness and consumer acceptance of fish fillet.

Coating has been shown to inhibit the growth of microbes. A previous report showed that chitosan/gelatin coating inhibited the growth of TVC on the trout fillet during cold storage at 4 °C (Nowzari et al., 2013). In the study, the gelatin and chitosan coated samples at day 8 had bacterial count around 4 log CFU/g, which was similar to the current result at day 9. However, in the previous study, the TVC for the coated group increased gradually till around 6 log CFU/g at day 16, while in the current study, the TVC of chitosan only and chitosan/gelatin coated groups remained around 4 log CFU/g during the duration of 17 day storage. The discrepancy might be due to the different coating methods that were applied to the fillets. For the previous study, the fillets were immersed in the solution for first 30 s and allowed to stand for 2 min followed by a second immersion for 30 s, while in the current research the fillets were immersed in the coating solution for 3 min. The longer immersion time may lead to a better coating effect, and more significant effects on suppressing the growth of various bacteria.

## 4. Conclusions

The chitosan only and chitosan/gelatin combined coating significantly prevented the deterioration of golden pomfret fillet during 17 days cold storage at 4 °C. The weight loss, pH, TVB-N, and TVC of the control group increased dramatically during 17 day cold storage, indicating the fillet's quality, freshness and acceptability decreased quickly during storage. However, for the chitosan only and chitosan/gelatin coated group, the pH, TVB-N and TVC remained stable at 6.1, 13.56 mg/100 g and 4 log CFU/100 g, respectively during cold storage. Furthermore, gelatin coated groups showed lower weight loss and longer myofibril compared with the chitosan only coated group. Among all the coated groups, the weight loss of gelatin coated group remained around 1.1% at day 17, and for the group coated with 7.2% gelatin combined with 0.4% chitosan the length of myofibril remained greater than 15 μm, demonstrating better preservative ability than other coatings. Based on MALDI-TOF-MS analysis, the edible coatings also helped slow down the deterioration rate of myoglobin, tropomyosin and myosin light chain within fish muscle. Moreover, the edible coating had significant antimicrobial effect on inhibiting microbial growth on fish fillet during cold storage. Overall, the edible coating of 0.4% chitosan combined with 7.2% gelatin had the best effect on preserving the quality of fish fillet during cold storage.

## Acknowledgements

We acknowledge the financial support by Singapore Ministry of Education Academic Research Fund Tier 1 (R-143-000-583-112). Projects 31371851, 31471605 and 31200801 supported by NSFC and Natural Science Foundation of Jiangsu Province (BK20141220), and Applied Basic Research Project (Agricultural) Suzhou Science and Technology Planning Programme (SYN201522) also contributed to this work.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.01.030.

## References

Atarés, L., De Jesús, C., Talens, P., & Chiralt, A. (2010). Characterization of SPI-based edible films incorporated with cinnamon or ginger essential oils. *Journal of Food Engineering, 99*, 384–391.

Bauchart, C., Chambon, C., Mirand, P. P., Savary-Auzeloux, I., Rémond, D., & Morzel, M. (2007). Peptides in rainbow trout (*Oncorhynchus Mykiss*) muscle subjected to ice storage and cooking. *Food Chemistry, 100*, 1566–1572.

Chaurand, P., Luetzenkirchen, F., & Spengler, B. (1999). Peptide and protein identification by matrix assisted laser desorption ionization (MALDI) and MALDI-post-source decay time-of-flight mass spectrometry. *Journal of the American Society for Mass Spectrometry, 10*, 91–103.

Chong, J. X., Lai, S., & Yang, H. (2015). Chitosan combined with calcium chloride impacts fresh-cut honeydew melon by stabilising nanostructures of sodium-carbonate-soluble pectin. *Food Control, 53*, 195–205.

Di Luccia, A., Picariello, G., Cacace, G., Scaloni, A., Faccia, M., Liuzzi, V., ... Musso, S. S. (2005). Proteomic analysis of water soluble and myofibrillar protein changes occurring in dry-cured hams. *Meat Science, 69*, 479–491.

Duan, J., Jiang, Y., Cherian, G., & Zhao, Y. (2010). Effect of combined chitosan-krill oil coating and modified atmosphere packaging on the storability of cold-stored lingcod fillets. *Food Chemistry, 122*, 1035–1042.

Endo, A., & Yamao, M. (2007). Policies governing the distribution of by-products from scientific and small-scale coastal whaling in Japan. *Marine Policy, 31*, 169–181.

Fan, H., Luo, Y., Yin, X., Bao, Y., & Feng, L. (2014). Biogenic amine and quality changes in lightly salt-and sugar-salted black carp fillets stored at 4 °C. *Food Chemistry, 159*, 20–28.

Feng, X., Lai, S., & Yang, H. (2014). Sustainable seafood processing: Utilisation of fish gelatin. *Austin Journal of Nutrition and Food Sciences, 2*, 2.

Gunning, P. W., Ghoshdastider, U., Whitaker, S., Popp, D., & Robinson, R. C. (2015). The evolution of compositionally and functionally distinct actin filaments. *Journal of Cell Science* [jcs-165563].

Jeon, Y. J., Kamil, J. Y., & Shahidi, F. (2002). Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. *Journal of Agricultural and Food Chemistry, 50*, 5167–5178.

Jiang, M., Liu, S., Du, X., & Wang, Y. (2010). Physical properties and internal microstructures of films made from catfish skin gelatin and triacetin mixtures. *Food Hydrocolloids, 24*, 105–110.

Kaale, L. D., Eikevik, T. M., Rustad, T., & Nordtvedt, T. S. (2014). Changes in water holding capacity and drip loss of Atlantic salmon (*Salmo salar*) muscle during superchilled storage. *LWT-Food Science and Technology, 55*, 528–535.

Limpisophon, K., Tanaka, M., Weng, W., Abe, S., & Osako, K. (2009). Characterization of gelatin films prepared from under-utilized blue shark (*Prionace glauca*) skin. *Food Hydrocolloids, 23*, 1993–2000.

López, C. M. E., Gómez, G. M. C., Pérez, M. M., & Montero, P. (2005). A chitosan–gelatin blend as a coating for fish patties. *Food Hydrocolloids, 19*, 303–311.

Lu, F., Ding, Y., Ye, X., & Liu, D. (2010). Cinnamon and nisin in alginate–calcium coating maintain quality of fresh northern snakehead fish fillets. *LWT-Food Science and Technology, 43*, 1331–1335.

Luccia, A. D., Picariello, G., Cacace, G., Scaloni, A., Faccia, M., Liuzzi, V., & Musso, S. S. (2005). Proteomic analysis of water soluble and myofibrillar protein changes occurring in dry-cured hams. *Meat Science, 69*, 479–491.

Martone, C. B., Busconi, L., Folco, E. J., Trucco, R. E., & Sanchez, J. J. (1986). A simplified myosin preparation from marine fish species. *Journal of Food Science, 51*, 1554–1555.

Nowzari, F., Shábanpour, B., & Ojagh, S. M. (2013). Comparison of chitosan–gelatin composite and bilayer coating and film effect on the quality of refrigerated rainbow trout. *Food Chemistry, 141*, 1667–1672.

Nyland, L. R., & Maughan, D. W. (2000). Morphology and transverse stiffness of d*rosophila m*yofibrils measured by atomic force microscopy. *Biophysical Journal, 78*, 1490–1497.

Ojagh, S. M., Rezaei, M., Razavi, S. H., & Hosseini, S. M. H. (2010). Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chemistry, 120*, 193–198.

Pazos, M., Méndez, L., Vázquez, M., & Aubourg, S. P. (2015). Proteomics analysis in frozen horse mackerel previously high-pressure processed. *Food Chemistry, 185*, 495–502.

Qiu, X., Chen, S., Liu, G., & Yang, Q. (2014). Quality enhancement in the Japanese sea bass (*Lateolabrax japonicas*) fillets stored at 4 °C by chitosan coating incorporated with citric acid or licorice extract. *Food Chemistry, 162*, 156–160.

Ruttanapornvareesakul, Y., Ikeda, M., Hara, K., Osako, K., Kongpun, O., & Nozaki, Y. (2005). Effect of shrimp head protein hydrolysates on the state of water and denaturation of fish myofibrils during dehydration. *Fisheries Science, 71*, 220–228.

Santos, J., Lisboa, F., Pestana, N., Casal, S., Alves, M. R., & Oliveira, M. B. P. (2013). Shelf life assessment of modified atmosphere packaged turbot (*Psetta maxima*) fillets: Evaluation of microbial, physical and chemical quality parameters. *Food and Bioprocess Technology, 6*, 2630–2639.

Sow, L. C., & Yang, H. (2015). Effects of salt and sugar addition on the physicochemical properties and nanostructure of fish gelatin. *Food Hydrocolloids, 45*, 72–82.

Stuchell, Y. M., & Krochta, J. M. (1995). Edible coatings on frozen king salmon: effect of whey protein isolate and acetylated monoglycerides on moisture loss and lipid oxidation. *Journal of Food Science, 60*, 28–31.

Yang, H., & Wang, Y. (2009). Effects of concentration on nanostructural images and physical properties of gelatin from channel catfish skins. *Food Hydrocolloids, 23*, 577–584.

Yang, H., Wang, Y., Jiang, M., Oh, J. H., Herring, J., & Zhou, P. (2007). 2-step optimization of the extraction and subsequent physical properties of channel catfish (Ictalurus Punctatus) skin gelatin. *Journal of Food Science, 72*, C188–C195.

Yoshikawa, Y., Yasuike, T., Yagi, A., & Yamada, T. (1999). Transverse elasticity of myofibrils of rabbit skeletal muscle studied by atomic force microscopy. *Biochemical and Biophysical Research Communications, 256*, 13–19.

Zhang, X., Pan, D., Cao, J., & Wu, Z. (2013). Changes in the major caspases involved in cytoskeletal degradation of goose muscle during prolonged aging. *Food Research International, 51*, 603–610.

Zhao, J., Lv, W., Wang, J., Li, J., Liu, X., & Zhu, J. (2013). Effects of tea polyphenols on the post-mortem integrity of large yellow croaker (*Pseudosciaena Crocea*) fillet proteins. *Food Chemistry, 141*, 2666–2674.

Zhong, C., Cai, Q. F., Liu, G. M., Sun, L. C., Hara, K., Su, W. J., & Cao, M. J. (2012). Purification and characterisation of cathepsin L from the skeletal muscle of blue scad and comparison of its role with myofibril-bound serine proteinase in the degradation of myofibrillar proteins. *Food Chemistry, 133*, 1560–1568.