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ORIGINAL PAPER

Effects of Fish Gelatin and Tea Polyphenol Coating on the Spoilage and Degradation of Myofibril in Fish Fillet During Cold Storage

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Abstract Fish fillet is easily spoiled during storage. Antimicrobial edible coating of gelatin extracted from fish skins and bones and tea polyphenol (TP) was developed to inhibit the spoilage of fish fillet during cold storage. For coating containing 0.4 % TP and 1.2 % gelatin, the pH only slightly increased from 6.17 at day 0 to 6.32 at day 17 of cold storage, while the pH of control coating increased to 6.87 at day 17. Atomic force spectrometry was used to analyse the nanostructure of myofibril, which is the major component of fish muscle. The results showed that the length of myofibril from 0.4 % TP and 1.2 % gelatin group was greater than 15 μm, while the diameter and height were 3.38 and 0.59 μm, respectively, which exhibited the most intact nanostructure after 17 days of cold storage. Meanwhile, matrix-assisted laser desorption–ionisation–time-of-flight mass spectrometry result showed that TP delayed the degradation of myosin light chain 3 and troponin T in myofibril. Gas chromatography–mass spectrometry of volatile organic compounds (VOCs) also showed that 0.4 % TP and 1.2 % gelatin coating group had minimal production of spoilage markers, such as 1-octen-3-ol, 2-methyl propanoic acid and dimethyl sulfide. The microbial analysis showed that the aerobic mesophilic/psychrotrophic count, yeasts and moulds of 0.4 % TP and 1.2 % gelatin group were significantly lower than the control group. Therefore, 0.4 % TP and 1.2 % gelatin coating showed the best antimicrobial effect and can be used to maintain the nanostructure of fish fillet and prevent the spoilage during cold storage.

Keywords Gelatin coating · Tea polyphenol · Matrix-assisted laser desorption–ionisation–time-of-flight mass spectrometry (MALDI-TOF-MS) · Atomic force spectrometry (AFM) · Headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS/SPME/GC/MS)

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Introduction

Fish is commonly consumed all over the world and has beneficial effects on the human body. However, fish is perishable during cold storage due to enzymatic and microbiological activities, and hence, innovative preservation techniques have to be applied to maintain its quality and supply for human consumption (Broekaert et al. 2011). Irradiation, edible coating, vacuum packaging and modified atmosphere packaging have been developed as innovative technologies to preserve fish fillet and seafood product without usage of chemicals (Ayala et al. 2011; Feng et al. 2016; Mahto et al. 2015), among which edible coating is the most cost effective without requirement of advanced machines. To date, edible zein coating incorporated with iron chelator was developed to preserve the quality of fish ball, and chia by-products incorporated with clove essential oil (CEO) showed significant antimicrobial activity through inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus* (Capitani et al. 2016; Chen et al. 2016). Edible coatings can serve as barriers of water vapour,

volatile compounds, gases and additive carrier to prevent the food surface from being contaminated (Chen et al. 2016).

In light of a greater emphasis on health and nutrition, natural food preservatives have been gaining much attention to extend the shelf life of food products. For instance, chitosan, soy isoflavones and various plant essential oils have been found to display antimicrobial effects and applied in the edible coatings (Dhayakaran et al. 2015; Feng et al. 2016). Tea polyphenols are promising natural food preservatives due to their antimicrobial and antioxidant effects, and they are generally recognised as safe (GRAS) materials (Yi et al. 2011). To date, tea polyphenols have been incorporated in the dried-seasoned squid and fish balls to prolong the shelf life (Dong et al. 2013; Yi et al. 2011). The effects of TP on the post mortem integrity of large yellow croaker (*Pseudosciaena crocea*) fillet proteins were also studied (Zhao et al. 2013), and TP preserved the integrity of muscle proteins to maintain the fish quality.

Fish gelatin has become more and more popular as a coating component due to its excellent film-forming property and its resistance against drying, light and oxygen, which can be applied in food industry (Gómez-Guillén et al. 2009; Mohtar et al. 2014). The biodegradability and abundant source of fish gelatin make it to be environmentally friendly and cost-effective. It has become more and more important to replace mammalian gelatin, since bovine and beef gelatin cannot be consumed by many religious customers. Applying gelatin extracted from fishery by-products also helps to improve seafood sustainability, quality and food security (Feng et al. 2014). Furthermore, incorporation of tea polyphenols into gelatin films has been reported to improve the mechanical property and antioxidant activity of the edible coating (Gómez-Guillén et al. 2009). However, the application of the TP/gelatin coating on food has not been investigated, and the mechanism of the coating preservation effect remains unknown.

The effect of TP on myofibril denature and degradation has been investigated (Zhao et al. 2013). However, the nanostructural changes of myofibril from fish fillet were not known. Light microscopy, transmission electron microscopy (TEM) and scanning electron microscope (SEM) have been applied to investigate the ultrastructure of fish muscle, shrimp muscle and fish gel (Ayala et al. 2011; Mahto et al. 2015; Qian et al. 2015). However, the light microscopy has low resolution, and the sample treatment before TEM and SEM causes significant damages to the molecules detected (Yang et al. 2007). Atomic force microscopy (AFM) is an effective method for characterising nanoscale structure via Van der Waals' force without altering the structure of detected molecules, which has been applied to analyse the nanostructure of pectin in honeydew, fish gelatin and edible film surface morphology (Capitani et al. 2016; Chong et al. 2015; Shiroodi et al. 2016; Sow and Yang 2015).

The objective of this work was to investigate the effects and mechanism of TP/gelatin films in slowing down the rate of fish spoilage. AFM and matrix-assisted laser desorption–ionisation–time-of-flight mass spectrometry (MALDI-TOF-MS) were used to examine nanostructural changes of myofibrillar proteins. Headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS/SPME/GC/MS) was applied to investigate the volatile organic profile changes of fish fillet during cold storage. Physicochemical properties including texture, colour, weight loss, pH and electrical conductivity (EC) were analysed. The effectiveness and the mechanism of edible TP/gelatin coating on fish fillet were comprehensively assessed and elucidated.

Materials and Methods

Edible Coating Solution Preparation

Commercial tilapia (*Oreochromis niloticus*) fish gelatin (200 bloom) was purchased from Jiangxi Cosen Biology Co., Ltd (Yingtan, Jiangxi, China). The gelatin contained 83.14 % protein, 0.68 % ash, 9.12 % moisture and 7.06 % of others according to its product information. Fish gelatin solution (6, 9, 12 %, *w*/*w*) was prepared following the method with some modifications (Sow and Yang 2015). Gelatin was soaked in deionised water produced from Sartorius Arium 611 UV system at 4 °C overnight. The gelatin solution was placed in 55 °C water bath for 15 min till it was totally dissolved and homogenised. TP tablets were purchased from Zhejiang University Tea Scientific Co., Ltd. (99 % purity) (Hangzhou, Zhejiang, China) and grinded with a blender followed by dissolving in deionised water using an Elmasonic S 60H sonicator (Elma Schmidbauer GmbH, Singen, Germany). The tea polyphenol solution of 80 mL and gelatin solution of 120 mL were homogenised by magnetic stirring. The tea polyphenol concentration was 0.4 %, and the concentration of gelatin was 3.6, 5.4 and 7.2 %, respectively.

Preparation of Fish Fillet Samples

Golden Pomfret was purchased from a local supermarket and transferred to the laboratory within 30 min in a cold storage bag with ice inside. The skin and bones were removed manually, and the dorsal part of the fish was cut into 10-g fillets. The fillets were randomly divided into five groups, and each group had six fillets. The five treatment groups were namely the control group in which the fillet was coated with deionised water, the second group was coated with 0.4 % (*w*/*v*) tea polyphenol, and the other three treatment groups were coated with 0.4 % (*w*/*v*) tea polyphenol and 1.2, 2.4 and 3.6 % (*w*/*v*) gelatin, respectively.

The fillets were coated by immersing in the coating solutions for 30 min and dried for 60 min in a well-ventilated fume hood at room temperature. After drying, they were

individually packed in high-quality polyethylene ziplock bags (Glad Product Company, Oakland, CA, USA) and stored at 4 ± 1 °C before subsequent analysis. Colour, weight loss, pH, EC, myofibril, volatile organic compounds and microbial analysis were tested during 17 days of cold storage to evaluate the effects of coating on the shelf life and quality of fish fillets. Cold storage of 17 days was selected since the fillet became unacceptable after 17-day cold storage according to the microbial result (Feng et al. 2016).

Physiochemical Analysis

The colour of the fillet was analysed using a Minolta Colorimeter CM-3500d (Konica Minolta Inc., Japan). The colorimeter expressed colour of the fillet in the CIE $L^*a^*b^*$ space, in which values of L^*, a^* and b^* represented the lightness, greenness–redness and blueness–yellowness, respectively. Using values of L^*, a^* and b^*, values of ΔE were calculated based on the following equation:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (1)$$

Instrument calibration was conducted using standard black and white plates, and all the experiments were performed in triplicate (Capitani et al. 2016).

Texture analysis was carried out by a TA-XT2i texture analyser (Stable Micro Systems Co., Ltd., Godalming, Surrey, UK) using a flat cylindrical probe (SMSP/20). Analysis was carried out using the following conditions: test speed of 1.0 mm s^{-1}, penetrating distance of 4.0 mm and trigger force of 0.05 N (Sow and Yang 2015).

The weight loss was measured and calculated by the following equation:

$$\text{Weight loss } /\% = \frac{W_0 - W_1}{W_0} * 100 \quad (2)$$

W_0 is the mass of the fish on day 0, and W_1 is the mass of the fish at the day of analysis (Souza et al. 2015).

Fish sample of 10 g was minced and dissolved in 100 mL of water by stirring for 15 min. The mixture was centrifuged (Eppendorf Centrifuge 5810 R, Eppendorf, Hamburg, Germany) at 15,000 *g* for 5 min, and the supernatant was collected. The pH and EC analysis was carried out using a Thermo Orion pH meter (Thermo Fisher Scientific, Waltham, MA, USA) and a Horiba ES-14 digital EC meter (Horiba, Ltd. Kyoto, Japan), respectively (Shokri et al. 2015). Analysis was conducted after 0, 5, 9, 13 and 17 days of cold storage.

Myofibril Analysis: AFM and MALDI-TOF-MS

Myofibrillar proteins were extracted using the method with slight modifications (Martone et al. 1986), and chemicals used were purchased from Sigma. Fish muscle (5 g) was minced and stirred in 50-mL solution containing 0.10 M KCl, 0.02 % sodium azide and 1 mM phenylmethylsulfonyl fluoride (PMSF) and 20 mM Tris–HCl buffer at pH 7.5 for 30 min. The pellet was collected after centrifugation at 1000 *g* and was washed with 25 mL of solution containing 0.45 M KCl, 5 mM β-mercaptoethanol (β-MCE), 0.2 M magnesium acetate, 10 mM adenosine triphosphate (ATP), 1 mM ethylene glycol-bis (β-aminoethyl ether) *N*, *N*, *N*′, *N*′-tetraacetic acid (EGTA) and 20 mM Tris–maleate buffer at pH 6.8 for 1 h. The mixture was then centrifuged at 10,000*g*, and the myofibril was in the collected supernatant (Martone et al. 1986).

The extracted myofibrillar proteins were then analysed using atomic force microscope (TT-AFM, AFM Workshop, Signal Hill, CA, USA). The myofibril was diluted 20 times with deionised water, and 20 μL was pipetted onto a freshly cleaved mica sheet for analysis. The sample was dried at room temperature before analysis. The samples were scanned in the vibrating mode with a scan rate of 0.4 Hz. The probe used was an AppNano ACLA-10 Si, N-type probe (Applied NanoStructures, Inc., Mountain, CA, USA). The probe was Al coated with a resonance frequency of 160–225 kHz and force constant of 36–90 N m^{-1}. Images obtained were analysed using the Gwyddion software (AFM Workshop, Signal Hill, CA, USA) (Sow and Yang 2015). Lengths, diameters and heights of the nanostructure obtained were measured for quantitative analysis.

Mass spectrum of myofibril proteins extracted from fillet was analysed using a Bruker Autoflex III Smartbeam MALDI-TOF/TOF (Bruker Corporation, Billerica, MA, USA). The myofibril supernatant was dialysed before dilution with acetonitrile (Sigma). Dialysis was carried out by pipetting 1 mL of myofibril supernatant into a dialysis bag and stirring it in deionised water for 12 h in ice water bath. Dialysed sample was mixed with same volume of 50 % acetonitrile for MALDI-TOF-MS analysis. The mixture of 2 μL was homogenised with 2 μL of 2,5-dihydroxybenzoic acid (Sigma) and was deposited onto a MALDI MTP384 target plate (Bruker Corporation, Billerica, MA, USA) and dried under ambient conditions. Mass spectra were acquired in the linear, positive-ion mode. Analysis of myofibrillar proteins was carried out using the software flexAnalysis (Bruker Corporation, Billerica, MA, USA).

Volatile Organic Compound Analysis

Volatile compounds in fish samples were measured using headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS/SPME/GC/MS). Fillet sample of 5 g was cut and weighed in an empty 10 mL flat-bottomed headspace vial and capped. Analysis was carried out using a Shimadzu GCMS-QP2010 Ultra Gas Chromatography–Mass Spectrometer coupled with a

Shimadzu AOC-5000 Autosampler (Shimadzu Corporation, Kyoto, Japan). A DB-WAX column (Agilent Technologies, Santa Clara, CA, USA) was used, and the SPME fibre used was Supelco 50/30 μm, Dimethylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) StableFlex™ Fibre (Sigma-Aldrich Corporation, St. Louis, MO, USA). The chromatographic conditions used were as follows: column oven temperature was initially held at 35 °C for 3 min, followed by an increase to 70 °C at 3 °C min^{-1} and an increase to 200 °C at 5 °C min^{-1}. Then, it was held at 200 °C for 3 min, and finally, it was increased to 250 °C at 20 °C min^{-1} and held for 5 min. The injection temperature was set at 220 °C using a splitless injection mode.

Optimisation of headspace sampling (extraction time, incubation time and temperature) was carried out on fish fillet which underwent 5 days of cold storage. The optimum conditions for headspace sampling were as follows: sample vial was incubated for 45 min at 50 °C in an agitator speed of 250 rpm. The needle penetration was 12 mm and fibre penetration was 10 mm. The SPME fibre was exposed for 30 min and desorbed into the injector for 10 min. The fibre was conditioned for 20 min before extraction. The electron impact (EI) ionisation MS conditions were as follows: interface temperature, 265 °C; ionisation voltage, 70 eV; mass range *m*/*z*, 35–350; and scan speed, 1666 amu s^{-1}. Identities of the volatile compounds were matched with the National Institute of Standards and Technology (NIST) mass spectral database (NIST07). Semiquantitative analysis was carried out using normalised total ion count (TIC) peak area counts via multiplying peak area counts by 10^{-5}. Volatile compounds of fish muscle were analysed on days 0, 9 and 17 of cold storage.

Microbial Analysis

Aerobic mesophilic count (AMC), aerobic psychrotrophic count (APC) and yeasts and moulds were investigated using the spread plate method with slight modification (Chong et al. 2015). Fish of 10 g was homogenised in 90 mL of 0.1 % peptone water (Peptone Water, Oxoid Ltd., England, UK) using a stomacher. Serial dilution was carried out, and 0.1 mL of sample was inoculated on the agar. To obtain the AMC, the sample was inoculated on Plate Count Agar (PCA, Trypton Glucose Yeast Agar, Oxoid Ltd., England, UK) and incubated at 37 °C for 48 h. Similarly, APC was obtained by inoculating on PCA and incubated for 8 days at 4 °C, and yeast and mould count was obtained by inoculating on Potato Dextrose Agar (PDA, Oxoid Ltd., England, UK) and incubated at 25 °C for 72 h. Microbial analysis was carried out after 0, 5, 9, 13 and 17 days of cold storage.

Statistical Analysis

All the experiments were performed in triplicate, and the data was reported as mean value ± standard deviation. Data analysis was performed using IBM SPSS Statistics Ver. 20 (International Business Machines Co. Armonk, NY, USA). One-way analysis of variance (ANOVA) accompanied by Duncan multiple comparison test was applied at 5 % significance level.

Results and Discussion

Physicochemical Analysis

Figure 1 shows the colour and texture properties of fish fillet, which are considered the most important quality attributes of foodstuff and determine consumer acceptance and marketability of the product (Cheret et al. 2007). Figure 1 reveals that L^* had no significant changes among different groups of fillet over the 17-day period. It could also be seen that for the control group, the value of a^* became more negative over the period, and the value of b^* increased over the period, which was consistent with the discoloration of channel catfish (*Ictalurus punctatus*) and minced hake during storage (Li et al. 2013). Meanwhile, there was no significant difference in ΔE for 0.4 % TP + 1.2 % gelatin and 0.4 % TP + 3.6 % gelatin from day 5 to day 17, showing the effect of 0.4 % TP + 1.2 % gelatin and 0.4 % TP + 3.6 % gelatin coating on maintaining the colour of fish fillet during cold storage.

Figure 1 shows that the hardness of the fish fillet generally decreased over 17 days, from 1475.8 g on day 0 to around 1000 g on day 17. It fluctuated on day 9, which may be affected by external factors such as the age and size of fish, the feeding ingredients and heterogeneity amongst the batches of fish (Cheng et al. 2014). Adhesiveness increased from 2.21 g s on day 0 to around 12.16 g s on day 9, and it continued to increase to around 51.31 g s on day 17. Cohesiveness decreased over time, from 0.545 on day 0 to around 0.468 on day 17. The decrease of hardness and cohesiveness may be associated with the disintegration and degradation of myofibrillar proteins, which make up 70–80 % of the total protein content in fish muscle (Pazos et al. 2013; Mahto et al. 2015). The decrease of hardness and cohesiveness and increase of adhesiveness indicate change of quality, leading to unacceptability of consumers. However, there were no significant changes of hardness, adhesiveness and cohesiveness among the different groups. Interestingly, the other texture parameters tested including springiness, chewiness and resilience did not change significantly during 17-day cold storage.

Figure 2 shows that there was a gradual increase in weight loss of the control group over 17 days. With decreasing freshness of the fish, water held within the myofibril released due to

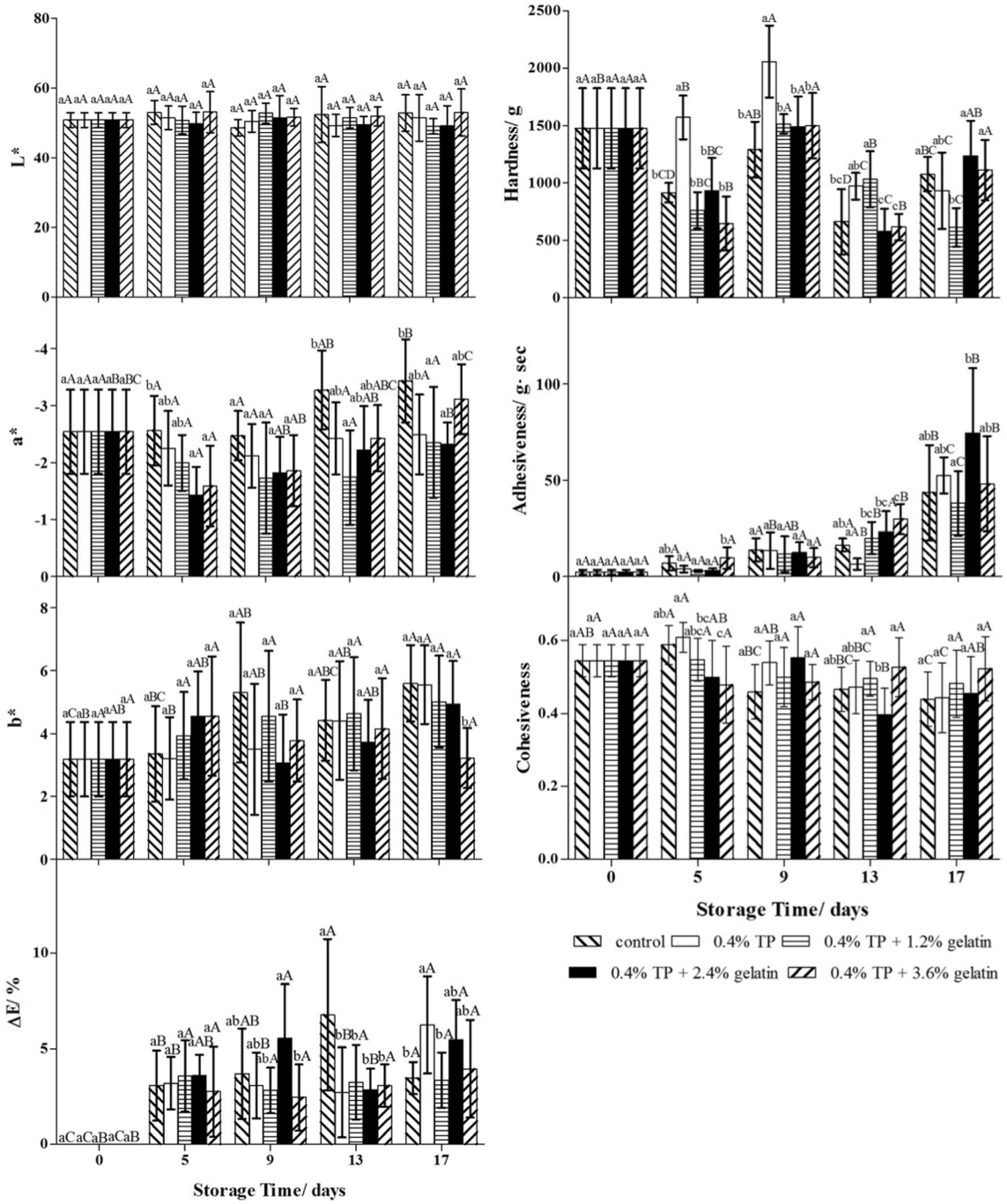

Fig. 1 Effect of tea polyphenol (TP) coating on the colour and texture of fish fillet: **L***, **a***, **b***, ΔE, hardness, adhesiveness and cohesiveness. Values with *different lowercase letters* at the same day and *capital letters* of the same group indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively

myofibrillar protein disintegration, which caused an increase of weight loss (Ocaño-Higuera et al. 2009). It was observed that fish fillet which had a coating that incorporated gelatin had a significantly ($P < 0.05$) lower weight loss than those without gelatin. The weight loss of treatment groups which had incorporated gelatin was less than 1 % on day 9, whereas that of the control group and 0.4 % TP group had a weight loss of more than 2 %. After 17 days of cold storage, it could be observed that the treatment groups incorporated with gelatin still had significantly lower weight loss compared with the other groups. This may be due to that the fish gelatin added into the edible film could act as water vapour barrier to decrease the weight loss of fish fillet (Tongnuanchan et al. 2016).

Changes in pH of the fish fillet are shown in Fig. 2. It could be seen that from day 13 onwards, the pH of the control group was significantly ($P < 0.05$) higher than the treatment groups.

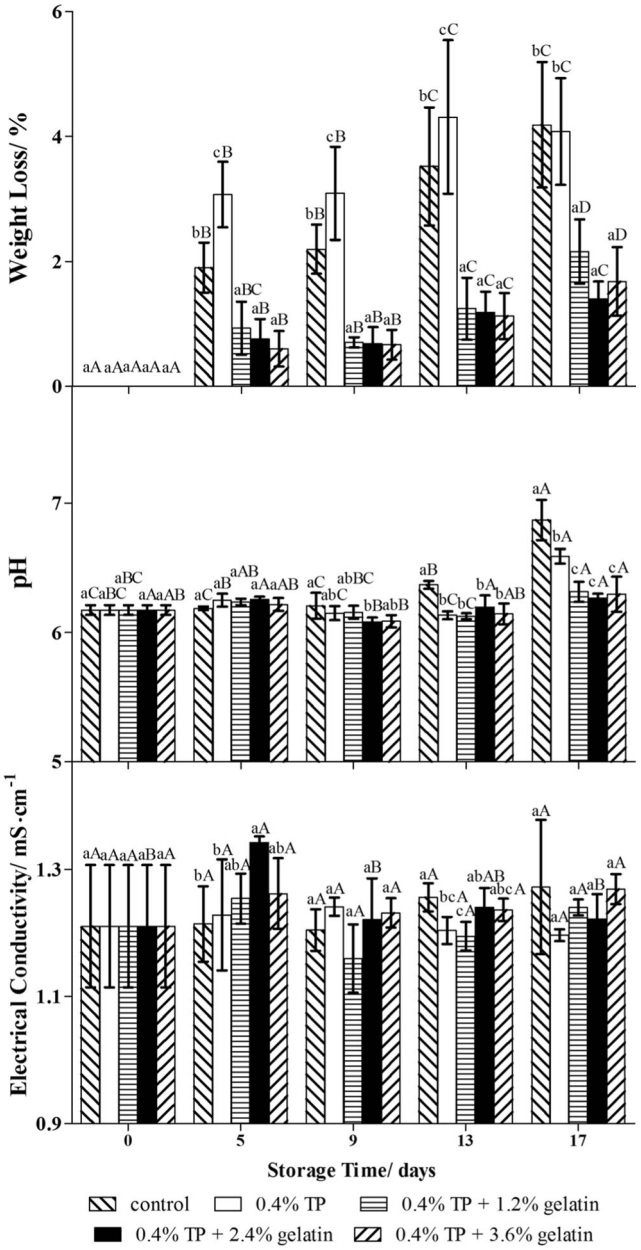

Fig. 2 Effect of TP coating on fish fillet: weight loss, pH and electrical conductivity (EC). Values with *different lowercase letters* at the same day and *capital letters* of the same group indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively

The pH of the treatment groups which had a coating that incorporated gelatin remained relatively constant at approximately 6.1–6.3, whereas the pH of the control group increased drastically to pH 6.4 and 6.9 on days 13 and 17, respectively. The general increase in pH of the fish muscle was attributed to the release of biogenic amines, such as histamine and trimethylamine, which were due to the microbial growth and spoilage (Feng et al. 2016). It could also be observed that there was a slight decrease in pH from day 5 to day 9 for all treatment groups. The decrease was due to the decomposition of glycogen, ATP and creatine phosphate as well as the growth of lactic acid bacteria (Fan et al. 2014). From the results of the pH analysis, it can be deduced that the TP and TP/gelatin coating delayed the rate of spoilage.

In Fig. 2, there was no significant difference of EC value among different groups during cold storage. Since EC is an index of the concentration of electrolytes in the muscle tissue (Fan et al. 2014), the current result suggested that the tea polyphenol and TP/gelatin coating did not affect the electrolytes in the muscle tissue.

Myofibrillar Protein Analysis

Nanostructural Analysis

Figure 3 shows the nanostructure changes of myofibrils. Myofibrils are made up of a series of sarcomeres, which contain thick and thin filaments. The thick and thin filament regions are known as the A bands and I bands, respectively, as shown in Fig. 3. The A band is made up of thick myosin filaments as well as structural proteins such as titin (Soltanizadeh and Kadivar 2014). Each sarcomere in the myofibril is separated by a Z-line, and it is essential in maintaining the myofilament structure as it bundles the thick and thin filaments in the myofibrils (Wakayama et al. 2000).

As seen from Fig. 3, myofibrils have a rod-like structure, which is similar to the myofibril from chicken and rabbit muscle (Iwasaki et al. 2009; Yoshikawa et al. 1999). On day 0, the sarcomere, accompanied by the A band, I band and Z-line, could be evidently observed (Fig. 3 (a1)). The length of the sarcomere was measured between two consecutive Z-lines and was 2.86 μm. This was consistent with studies carried out by Ogneva et al. (2010). After 9 days of cold storage, it could be observed that the myofibrils of the 0.4 % TP (Fig. 3 (c1)) and 0.4 % TP + 3.6 % gelatin (Fig. 3 (f1)) treatment groups had wrinkled surfaces, while the control group (Fig. 3 (b1)) appeared to be slightly wrinkled. For all groups of fish fillet, the rod-like structure was observed, but the Z-line became indistinct on day 9. On day 17 of cold storage, it was evident that whilst the control (Fig. 3 (b2)) and 0.4 % TP (Fig. 3 (c2)) groups were present as a single rod-like structure, the other treatment groups were present as bundles. Meanwhile, the 0.4 % TP + 3.6 % gelatin treatment group had clearly undergone myofibrillar fragmentation to form individual sarcomeres. Similarly, the control group showed fragmentation at the Z-line. This is known as the Z-disk weakening (Cheret et al. 2007). On the contrary, myofibrils in the 0.4 % TP + 1.2 % gelatin and 0.4 % TP + 2.4 % gelatin treatment groups appeared to be intact.

It was obvious that 0.4 % TP and 0.4 % TP + 3.6 % gelatin coating showed less effect on preserving the myofibril in fillet. TP and gelatin interacted and formed firmer coating to act as barrier of oxygen, light and microbes, thus preserving the

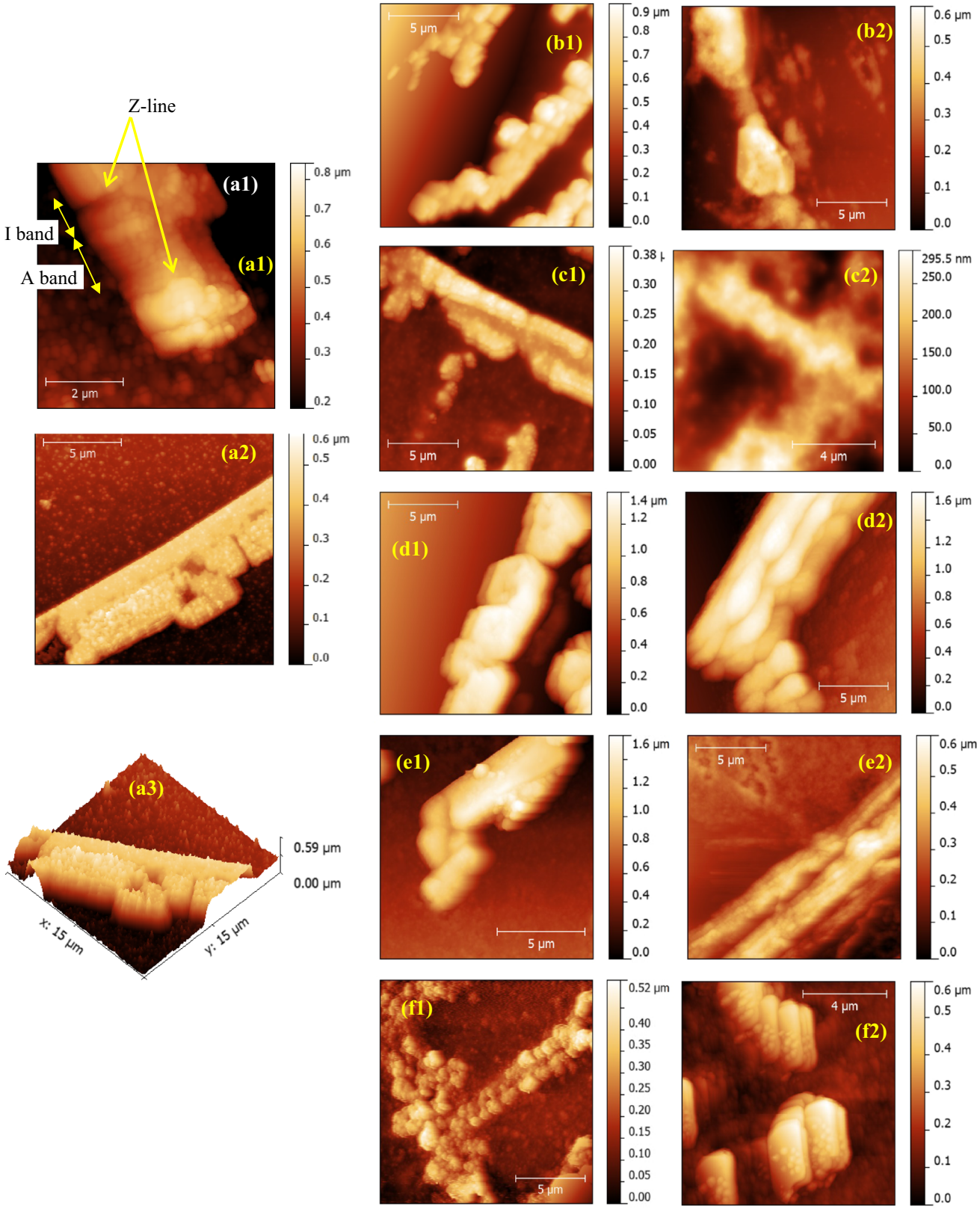

Fig. 3 AFM images of myofibril in fish muscle: control group on day 0 (*a1*, *a2*, *a3*); control group on day 9 (*b1*) and day 17 (*b2*); 0.4 % TP group on day 9 (*c1*) and day 17 (*c2*); 0.4 % TP + 1.2 % gelatin group on day 9 (*d1*) and day 17 (*d2*); 0.4 % TP + 2.4 % gelatin group on day 9 (*e1*) and day 17 (*e2*); and 0.4 % TP + 3.6 % gelatin group on day 9 (*f1*) and day 17 (*f2*)

integrity of myofibril in the fillet (Gómez-Guillén et al. 2009). Therefore, without addition of gelatin and the formation of the firm network, the higher weight loss of fillet may contribute to the growth of microbes. Since TP alone did not have film-forming capacity according to the weight loss result, 0.4 % TP coating cannot act as antimicrobial coating on fish fillet. However, higher concentration of gelatin in 0.4 % TP + 3.6 % gelatin coating might serve as a matrix for the microbes to grow on the coating (Skandamis et al. 2000). Thus, the 0.4 % TP + 3.6 % gelatin group showed relatively high AMC during the 17-day cold storage, leading to significant degradation of myofibril from this group.

During post mortem aging of muscle, proteolytic degradation of myofibrils occurred due to the action of cathepsins and calpains. This resulted in a loss of smoothness of the surface as well as a deterioration of the Z-line in the myofibril (Iwasaki et al. 2009). Calpains readily cleaved titin just near the Z-line, thus breaking their bonds to the proteins in the Z-line. In addition, calpains also eliminated the Z-line by removing the intermediate filament protein, desmin, which attached the Z-line to the sarcomere. As a result, proteins which constituted the Z-line were released and a space formed in the myofibril (Soltanizadeh and Kadivar 2014).

Table 1 shows the quantitative analysis of the myofibril structures. It was evident that after 17 days of cold storage, there was a decrease in the length, diameter and height of the myofibril. This was due to the deterioration and degradation of myofibrillar proteins (Qian et al. 2015). It can be seen that the length of myofibril was greater than 15 μm on day 0 and day 9, and it decreased significantly to 5.18, 9.32 and 2.99 μm on day 17 for control, 0.4 % TP and 0.4 % TP + 3.6 % gelatin treatment groups, respectively. However, myofibril from the 0.4 % TP + 1.2 % gelatin and 0.4 % TP + 2.4 % gelatin treatment groups remained greater than 15 μm on day 17, revealing the preservation effect of these two coatings. Furthermore, the length of the 0.4 % TP + 3.6 % gelatin treatment group on day 17 was 2.99 μm, which was similar to the length of sarcomere, indicating the formation of sarcomeres from myofibril degradation.

The diameter of myofibril was 5.05 μm on day 0, and there was a significant decrease for control, 0.4 % TP + 2.4 % gelatin and 0.4 % TP + 3.6 % gelatin groups on day 9. Meanwhile, the control group had the smallest diameter of 3.21 μm on day 9, suggesting the detachment of myofibril fibres and the fastest degradation, which may explain the decrease of hardness (Ayala et al. 2011). Further decrease of diameter can be seen on day 17, but the diameter of the 0.4 % TP + 1.2 % gelatin group was 3.38 μm, which was significantly greater ($P < 0.05$) than the other four groups, exhibiting best preservation effect on the diameter of myofibril.

From Table 1, it could be seen that 0.4 % TP + 1.2 % gelatin and 0.4 % TP + 2.4 % gelatin groups showed a significant increase of height on day 9, and there was a general decrease of height for all groups on day 17. However, there was no significant difference between the height of the 0.4 % TP + 1.2 % gelatin group and day 0 sample after 17 days of cold storage. Therefore, based on the AFM analysis, it can be deduced that the 0.4 % TP + 1.2 % gelatin coating exerted the best effect on maintaining the nanostructure of fish fillet during cold storage.

Meanwhile, the degradation and fragmentation of myofibril explain the decrease of hardness and cohesiveness of fish fillet in Fig. 1 (Ayala et al. 2011). However, the different nanostructure of fillet is not revealed in the texture result, since there are no significant changes of texture among different groups and fluctuations in the textural parameters could be observed. This is due to that the texture of the fillet is affected by other external factors such as the age and size of the fish, its feeding ingredients and heterogeneity amongst the batches of fish (Cheng et al. 2014).

Table 1 Effect of TP coating on myofibril extracted from different groups of fish fillet during cold storage: length, diameter and height

Dimension (μm)	Storage time (days)	Control	0.4 % TP	0.4 % TP + 1.2 % gelatin	0.4 % TP + 2.4 % gelatin	0.4 % TP + 3.6 % gelatin
Length	0	>15	>15	>15	>15	>15
	9	>15	>15	>15	>15	>15
	17	5.18 ± 1.27 b	9.32 ± 1.36 a	>15	>15	2.99 ± 0.18 b
Diameter	0	5.05 ± 0.11 aA	5.05 ± 0.11 aA	5.05 ± 0.11 aA	5.05 ± 0.11 aA	5.05 ± 0.11 aA
	9	3.21 ± 0.62 cB	4.63 ± 0.41 aA	4.95 ± 0.36 aA	4.40 ± 0.09 bB	4.25 ± 0.39 bB
	17	2.53 ± 0.09 bB	2.51 ± 0.04 bB	3.38 ± 0.20 aB	2.50 ± 0.08 bC	2.23 ± 0.30 bC
Height	0	0.45 ± 0.04 aAB	0.45 ± 0.04 aA	0.45 ± 0.04 aB	0.45 ± 0.04 aB	0.45 ± 0.04 aA
	9	0.57 ± 0.09 cA	0.30 ± 0.05 dB	1.00 ± 0.10 aA	0.81 ± 0.04 bA	0.33 ± 0.02 dB
	17	0.36 ± 0.07 bB	0.20 ± 0.04 bC	0.59 ± 0.15 aB	0.36 ± 0.14 bB	0.39 ± 0.03 bAB

Values are presented as mean ± SD (n = 10). Values with different lower case letters at the same day and capital letters of the same group indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively

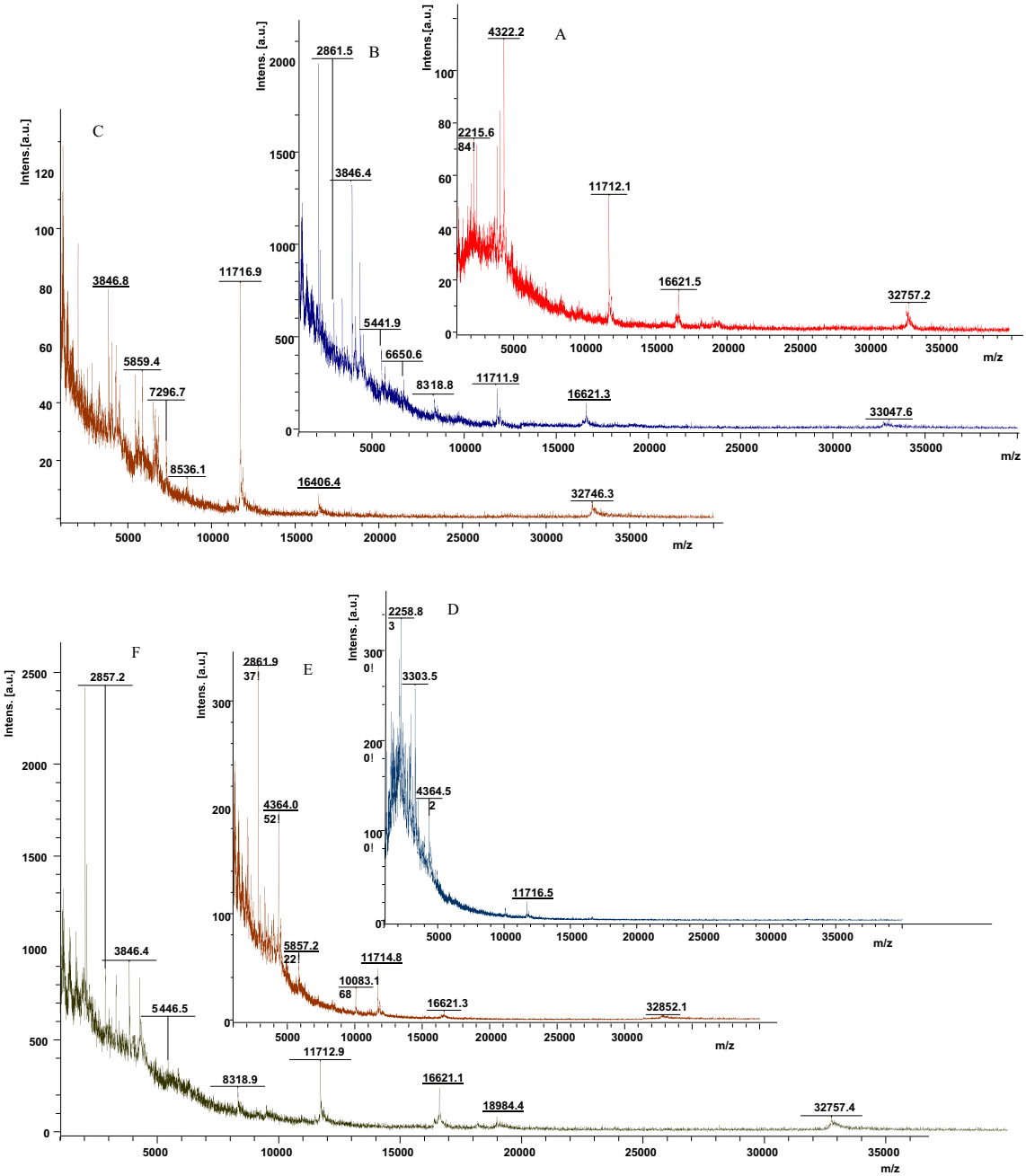

Fig. 4 MALDI-TOF mass spectrum of myofibril in fish muscle: control group on day 0 (**a**) and day 9 (**b**); (**c**) 0.4 % TP + 1.2 % gelatin group on day 9; and control group (**d**), 0.4 % TP + 1.2 % gelatin group (**e**) and 0.4 % TP + 3.6 % gelatin group (**f**) on day 17

MALDI-TOF-MS Analysis

Figure 4 reveals the mass spectrum of myofibril extracted from fillet, and proteins with different molecular weights were presented. Table 2 summarises the protein profile of different groups during cold storage. It could be observed that there was a loss of proteins with molecular weight (M_w) of 3.8, 5.8, 7.3, 8.5, 10.9, 16.4 and 32.8 kDa after 17 days of cold storage, which explains the decrease of hardness and cohesiveness of fillet, disintegration and fragmentation of myofibril in the AFM results. In particular, protein with M_w of 10.9 kDa was found in the day 0 fish fillet as well as 0.4 % TP + 1.2 % gelatin and 0.4 % TP + 3.6 % gelatin groups of fillet on day 9. Meanwhile, the 16.4-kDa fragment, which was likely the myosin light chain 3, was found on day 0, all groups on day 9 except control group and even on the 0.4 % TP + 1.2 % gelatin treatment group on day 17 (Delbarre-Ladrat et al. 2004). This revealed that tea polyphenols could delay degradation of myosin light chain 3, and 0.4 % TP + 1.2 % gelatin showed better preservation effect than other edible coatings, which was

Table 2 Analysis of mass spectra after 17 days of cold storage

Selected peaks (kDa)	Day 0	Day 9					Day 17				
		Control	0.4 % TP	0.4 % TP + 1.2 % gelatin	0.4 % TP + 2.4 % gelatin	0.4 % TP + 3.6 % gelatin	Control	0.4 % TP	0.4 % TP + 1.2 % gelatin	0.4 % TP + 2.4 % gelatin	0.4 % TP + 3.6 % gelatin
3.8	√	√	√	√	√	√				√	√
5.8	√		√	√	√	√			√		
7.3	√			√	√					√	
8.5	√			√							
10.9	√			√		√					
16.4	√		√	√	√	√			√		
18.2			√			√					√
32.8	√	√	√	√	√	√		√	√	√	√

Presence of peaks (*m*/*z*), analysed by MALDI-TOF-MS, is indicated by a "√"

consistent with AFM statistical result showing the highest length, diameter and height of myofibril from 0.4 % TP + 1.2 % gelatine-coated group.

While all the groups of fillet displayed the 32.8-kDa protein fragment, this fragment was not found in the control group on day 17. The 32.8-kDa fragment was hypothesised to be troponin T. Studies conducted by Delbarre-Ladrat et al. (2004) using sea bass have showed that calpains resulted in the degradation of troponin T (Soltanizadeh and Kadivar 2014). Troponin T is a regulatory protein which binds to myofibril thin filaments (Soltanizadeh and Kadivar 2014). The degradation of troponin T of control group on day 17 explains its higher level of deterioration with lower length in the AFM images. In addition to the above observations, the protein with M_w of 18.2 kDa was found on day 9 of both the 0.4 % TP and 0.4 % TP + 3.6 % gelatin treatment groups and on day 17 of the 0.4 % TP + 3.6 % gelatin treatment group. As this fragment was not found on day 0, it was deduced to be a degradation product of a larger protein. As such, these groups were observed to undergo faster protein degradation than the 0.4 % TP + 1.2 % gelatin and 0.4 % TP + 2.4 % gelatin groups. These results were consistent with the AFM result in which myofibrils of these treatment groups were observed to be less intact and incomplete than the 0.4 % TP + 1.2 % gelatin and 0.4 % TP + 2.4 % gelatin groups. Therefore, it is concluded that the 0.4 % TP + 1.2 % gelatin coating was the most effective in slowing down protein degradation in fish fillet, as it preserved most of the components of myofibril during cold storage.

One limitation of the MALDI-TOF-MS protein analysis is that some components in myofibril with big molecular weight cannot be detected. For example, actin and myosin heavy chain (MHC) with molecular weights of 41.5 and 221.4 kDa (Pazos et al. 2013) cannot be found in the spectrum, since the signal for molecules with large molecular weight is much weaker than that for low-molecular-weight peptide or protein. Therefore, for future work, two-dimensional gel electrophoresis will be performed to provide information on high-molecular-weight proteins up to 200 kDa (Pazos et al. 2013).

Volatile Organic Compound Analysis

Volatile organic compounds (VOCs) can be formed by degradation of fish lipids by auto-oxidation, enzymatic reactions and microbial proliferation (Iglesias et al. 2009). Headspace GC-MS is widely used to characterise and analyse VOCs present in fish fillet, and peak areas are used to indicate the amount of VOCs present for semiquantitative analysis. The VOCs identified in this experiment include five kinds of aldehydes, ten kinds of ketones, ten kinds of alcohols, seven kinds of organic acids, ten kinds of esters and nine kinds of miscellaneous compounds. Compounds which were found to have increased after 17 days of storage were trimethylamine, ethyl-2-methyl butanoate, 2-heptanone, 3-methyl-1-butanol, dimethyl disulfide, dimethyl trisulfide, 2-nonanone, acetic acid, 2-methyl propanoic acid, hexanoic acid and phenylethyl alcohol. Due to their increase over storage time, they can be potential spoilage markers for the Golden Pomfret.

Table 3 shows identification and semiquantification of common VOCs in fish fillet during cold storage. Compounds that are classified as oxidative spoilage markers include alcohols, aldehydes and ketones, which are responsible for rancidity of fish fillet. It can be observed from Table 3 that there was an increase in the amount of 2-methyl propanoic acid during cold storage. 2-Methyl propanoic acid is a free fatty acid (FFA) and is thought to be a product of lipid oxidation (Hsieh and Kinsella 1989). Another oxidative spoilage marker identified was 1-octen-3-ol. From Table 3, the amount of 1-octen-3-ol of the control group was observed to be the highest throughout the entire storage time, whereas that of the 0.4 % TP, 0.4 % TP + 1.2 % gelatin and 0.4 % TP + 2.4 % gelatin treatment groups remained consistently lower from day 9 to 17. 1-Octen-3-ol was derived from the oxidation

Table 3 Identification and semiquantification of common VOCs identified in fish fillet after 0, 9 and 17 days of cold storage

Compounds	Day 0	Day 9					Day 17				
		Control	0.4 % TP	0.4 % TP + 1.2 % gelatin	0.4 % TP + 2.4 % gelatin	0.4 % TP + 3.6 % gelatin	control	0.4 % TP	0.4 % TP + 1.2 % gelatin	0.4 % TP + 2.4 % gelatin	0.4 % TP + 3.6 % gelatin
Trimethylamine	ND	5.66 (6.00) d	2.90 (1.7) d	4.82 (0.44) d	5.06 (2.76) d	1.92 (1.90) d	36.2 (35.7) d	9.5 (2.05) d	12.7 (9.1) d	17.0 (2.2) d	7.75 (2.00) d
Ethyl-2-methyl butanoate	ND	ND	ND	ND	ND	ND	3.15 (1.21) de	2.47 (0.72) def	4.15 (0.97) d	0.792 (0.054) f	1.53 (0.47) ef
Ethyl-3-methyl butanoate	ND	ND	ND	ND	ND	ND	6.69 (2.06) d	6.10 (1.82) d	4.59 (0.61) de	2.91 (1.31) e	2.49 (0.60) e
1-Undecene	ND	6.79 (1.73) d	8.52 (1.5) d	ND	7.00 (0.97) d	1.85 (0.84) e	11.7 (1.6) d	11.0 (0.85) d	8.72 (0.64) de	10.1 (0.8) de	7.51 (1.89) e
D-Limonene	41.4 (12.4)	1.45 (5.40) d	16.4 (0.6) de	7.50 (0.32) f	15.7 (4.6) def	8.44(0.02) ef	21.1 (2.4) d	11.8 (1.7) f	13.9 (1.7) ef	9.16 (0.47) f	18.9 (4.4) de
2-Heptanone	ND	2.74 (1.03) d	1.89 (0.02) de	0.852 (0.276) e	2.12 (0.22) d	0.901 (0.371) e	2.82 (0.90) d	1.58 (0.21) e	1.47 (0.34) e	1.63 (0.10) e	1.65 (0.40) e
m-Cymene	5.75 (1.60)	1.91 (0.64) d	1.45 (0.8) d	2.62 (0.58) d	1.60 (0.04) d	1.55(0.36) d	5.00 (0.83) d	2.24 (1.00) e	1.95 (0.02) e	1.78 (0.55) e	2.33 (0.20) e
3-Methyl-1-butanol	ND	56.7 (17.3) d	24.9 (8.3) e	11.2 (1.3) e	9.31 (1.81) e	6.58 (1.30) e	24.3 (2.8) e	15.6 (3.9) e	44.7 (7.6) d	23.7 (9.4) e	17.5 (3.4) e
Dimethyl disulfide	0.879 (0.42)	1.35 (1.18) d	0.338 (0.029) e	ND	ND	ND	3.63 (1.36) d	0.876 (0.578) e	1.42 (0.96) de	1.42 (0.92) de	0.942 (0.249) e
Dimethyl trisulfide	ND	1.52 (0.11) d	1.22 (0.97) d	ND	0.919 (0.521) de	ND	6.73 (3.38) d	ND	2.83 (0.90) e	ND	1.51 (0.69) e
2-Nonanone	ND	7.36 (0.84) d	5.98 (0.86) e	ND	7.29 (0.04) d	ND	24.4 (6.6) d	19.7 (1.5) de	12.4 (4.5) e	18.7 (4.3) de	20.2 (3.1) de
Acetic acid	86.2 (76.6)	61.9 (33.7) d	79/8 (63.3) d	71.3 (22.2) d	116 (25) d	64.8 (8.4) d	203 (62) d	129 (22) d	179 (62) d	196 (30) d	137(45) d
Benzaldehyde	5.56 (1.67)	1.59 (0.42) f	1.24 (0.02) f	2.36 (0.26) e	1.72 (0.00) f	3.20 (0.10) d	1.61 (0.37) d	ND	1.31 (0.29) d	1.31 (0.25) d	1.82 (0.12) d
2-Methyl propanoic acid	ND	1.24 (0.04) d	3.55 (2.91) d	1.49 (0.47) d	1.83 (0.18) d	0.904 (0.095) d	27.2 (14.1) d	2.64 (0.98) e	6.36 (0.82) e	1.58 (0.84) e	2.63 (0.32) e
2,2-Dimethyl propanoic acid	14.8 (2.5)	9.66 (12.00) d	1.84 (1.37) d	5.08 (3.70) d	3.66 (3.68) d	10.9 (3.3) d	9.22 (6.00) d	1.09 (0.91) d	3.67 (3.76) d	2.90 (2.87) d	1.30 (0.53) d
Hexanoic acid	6.01 (2.33)	7.20 (6.60) d	2.63 (0.83) d	6.31 (1.72) d	3.05 (1.72) d	8.82 (4.23) d	21.5 (2.0) d	47.7 (21.2) d	21.2 (7.5) d	38.3 (46.0) d	11.8 (1.8) d
Phenylethyl alcohol	ND	3.20 (1.70) d	3.32 (0.2) d	ND	ND	ND	29.5 (1.4) d	5.84 (0.38) f	17.9 (5.1) e	4.37 (2.44) f	6.39 (1.10) f
1-Octen-3-ol	6.62 (0.00)	13.1 (0.71) d	ND	1.83 (0.87) f	ND	8.72 (0.27) e	8.19 (1.23) d	1.52 (0.62) e	1.15 (0.59) e	1.34 (0.15) e	1.81 (0.07) e

Compounds were identified by comparison with reference substances from the MS NIST library and present in at least three replicates. Semiquantification was expressed in arbitrary unit area (absolute area multiplied by 10^{-5}) and indicated in terms of mean (SD). Means within the same storage time with different lowercase letters are significantly different

ND not detected

of the polyunsaturated fatty acid (PUFA), which was 12-hydroperoxy arachidonic acid. This oxidation resulted in the formation of a 1-octene radical, which subsequently accepted a hydrogen or hydroxyl radical to form 1-octen-3-ol (Hsieh and Kinsella 1989). Based on the amount of 1-octen-3-ol and 2-methyl propanoic acid in Table 3, it can be deduced that the presence of tea polyphenols can slow down the rate of PUFA oxidation and reduce oxidative spoilage.

This can be explained through the antioxidant activity of catechin compounds in tea polyphenols, and the phenolic hydroxyl groups make catechin compounds effective free radical scavengers. In addition, hydroxyl groups facilitated the interaction with the lipid membrane in fish muscle. This decreased the membrane fluidity and reduces the mobility of lipid radicals. As such, lipid oxidation was delayed (Pazos et al. 2013).

Besides lipid oxidation, VOCs can also be produced due to microbial actions. Table 3 reveals the increase of trimethylamine, dimethyl disulfide, dimethyl trisulfide and phenylethyl alcohol over 17 days. Particularly, the control group had the largest amount. Trimethylamine (TMA) is responsible for the fishy odour and originates from trimethylamine oxide (TMAO). TMAO acts as an electron acceptor for Gram-negative bacteria such as *Shewanella putrefaciens* and *Aeromonas* spp., thus enabling the bacteria to exhibit rapid growth and producing TMA in the reaction (Leisner and Gram 2000). Henceforth, TMA is associated with bacterial spoilage of fish. Similarly, production of phenylethyl alcohol was caused by the Gram-negative bacteria of the genus *Achromobacter*, which is dominant in fish spoilage (Chen and Levin 1974). In addition, formation of dimethyl disulfide and dimethyl trisulfide was due to the microbial decomposition of methionine. Both compounds are considered fish spoilage markers and are responsible for putrid odours (Leisner and Gram 2000). On day 9 of storage, both dimethyl sulfide and dimethyl trisulfide were not detected in the 0.4 % TP + 1.2 % gelatin treatment group (Table 3), indicating reduced microbial spoilage of 0.4 % TP + 1.2 % gelatin group. This result was consistent with the microbial result (Table 4) which showed that 0.4 % TP + 1.2 % gelatin coating led to the least AMC, APC, yeasts and moulds throughout the cold storage.

Microbial Analysis

From Table 4, it could be observed that the mesophilic bacteria plate count was much lower than that of psychrotrophic bacteria. This was due to that the fillets were stored at 4 ± 1 °C, which was more optimal for psychrotrophic bacteria to survive (Pothakos et al. 2012). Henceforth, it would be unlikely for mesophilic bacteria to proliferate under cold storage.

Table 4 Effect of TP coating on microbial survivals of fish fillet: aerobic mesophilic count, aerobic psychrotrophic count, yeasts and moulds

Storage time(days)	Control	0.4 % TP	0.4 % TP + 1.2 % gelatin	0.4 % TP + 2.4 % gelatin	0.4 % TP + 3.6 % gelatin
Aerobic mesophilic count					
0	3.64 ± 0.22 aB	3.64 ± 0.22 aD	3.64 ± 0.22 aD	3.64 ± 0.22 aC	3.64 ± 0.22 aC
5	4.18 ± 0.86 aB	4.44 ± 0.43 aC	3.31 ± 0.13 bD	3.46 ± 0.13 bC	4.65 ± 0.60 aB
9	6.61 ± 0.13 abA	6.33 ± 0.00 bB	5.59 ± 0.43 cC	5.54 ± 0.22 cB	7.11 ± 0.15 aA
13	7.14 ± 0.47 abA	7.26 ± 0.45 abA	6.70 ± 0.42 bB	7.07 ± 0.62 abA	7.63 ± 0.26 aA
17	7.37 ± 0.26 abA	7.56 ± 0.10 aA	7.53 ± 0.14 aA	7.25 ± 0.13 bA	7.53 ± 0.35 aA
Aerobic psychrotrophic count					
0	3.09 ± 0.57 aD	3.09 ± 0.57 aD	3.09 ± 0.57 aD	3.09 ± 0.57 aD	3.09 ± 0.57 aD
5	7.20 ± 0.06 aC	6.96 ± 0.15 aC	4.93 ± 0.71 cC	5.81 ± 0.14 bC	6.87 ± 0.11 aC
9	9.00 ± 0.00 aB	8.89 ± 0.03 bB	8.89 ± 0.06 bB	8.95 ± 0.04 abB	8.69 ± 0.02 cB
13	9.47 ± 0.05 bA	9.60 ± 0.06 aA	8.99 ± 0.18 eB	9.17 ± 0.12 dAB	9.31 ± 0.07 cA
17	9.61 ± 0.05 aA	9.63 ± 0.03 aA	9.56 ± 0.01 abA	9.57 ± 0.13 abA	9.51 ± 0.09 bA
Yeasts and moulds					
0	3.59 ± 0.27 aC	3.59 ± 0.27 aD	3.59 ± 0.27 aD	3.59 ± 0.27 aD	3.59 ± 0.27 aD
5	6.42 ± 1.11 aB	7.22 ± 0.11 aC	5.20 ± 0.60 bC	5.35 ± 0.70 bC	6.86 ± 0.03 aC
9	8.99 ± 0.27 aA	9.01 ± 0.16 aB	7.17 ± 0.99 cB	7.98 ± 0.26 bB	8.87 ± 0.04 aB
13	9.58 ± 0.12 abA	9.86 ± 0.22 aA	9.18 ± 0.37 cA	9.45 ± 0.10 bcA	9.42 ± 0.14 bcA
17	9.66 ± 0.08 bcA	9.81 ± 0.03 aA	9.66 ± 0.06 bcA	9.70 ± 0.07 bA	9.56 ± 0.13 cA

Values are presented as mean ± SD ($n = 3$). Values with different lowercase letters at the same day and capital letters of the same group indicate significant differences by the Duncan's multiple range test ($P < 0.05$), respectively

Meanwhile, using the total mesophilic viable counts would be an inaccurate measurement to determine microbial spoilage. Studies showed that the total AMC was frequently underestimated for various food products under cold storage, thus underlining its potential misconception as a reference for shelf life (Pothakos et al. 2012). Furthermore, the spoilage of fish is mainly due to Gram-negative, psychrotrophic bacteria (Sallam 2007). Therefore, psychrotrophic bacteria count was analysed in this experiment.

Table 4 shows that the initial plate counts for the three types of microorganisms were lower than 4 log colony-forming units (CFU) g^{-1}, indicating that the fish purchased was of good quality (Wu et al. 2016). The results show that psychrotrophic bacteria count of the control group was 7.20 log CFU g^{-1} on day 5, which was higher than 7 log CFU g^{-1}, indicating the unacceptability of fish fillet. Since the maximum limit for consumption of total viable bacterial count (TVBC) is 7 log CFU g^{-1} according to the International Commission on Microbiological Specifications for Foods (ICMSF) (Sallam 2007), the fillet was considered to be spoilt on day 5. This was in contrast with the 0.4 % TP + 1.2 % gelatin and 0.4 % TP + 2.4 % gelatin treatment groups, which had a plate count of 4.93 log CFU g^{-1} and 5.81 log CFU g^{-1} on day 5, which was considerably lower than 7 log CFU g^{-1}. Similarly, in the case of yeast and mould count, both 0.4 % TP + 1.2 % gelatin and 0.4 % TP + 2.4 % gelatin groups had significantly lower plate counts on day 9 and day 13. However, for the 0.4 % TP and 0.4 % TP + 3.6 % gelatin groups, there were no significant differences in the microbial counts with the control group over the 17 days of cold storage. Based on the result of the microbial analysis, it can be determined that tea polyphenols had antimicrobial activities and extended the shelf life of fish fillet, especially for the 0.4 % TP + 1.2 % gelatin treatment group.

Conclusion

The 0.4 % TP + 1.2 % gelatin treatment group showed the most potential in slowing down the rate of fish spoilage. In comparison with the control group, this treatment group exhibited the best effect on reducing the weight loss, lowering pH and microbial growth, slowing down the myofibril degradation and preventing production of spoilage markers. The weight loss, pH and mesophilic count of 0.4 % TP + 1.2 % gelatin treatment group were 1.39 %, 6.25 and 7.25 log CFU g^{-1} on day 17, respectively, which showed better quality than the other groups. The myofibril length of 0.4 % TP + 1.2 % gelatin treatment group remained greater than 15 μm on day 17, while the myofibril length of the control group decreased to 5.18 μm at the end of the storage. During cold storage, the tea polyphenol showed significant effectiveness to slow down the rate of PUFA oxidation and reduce oxidative spoilage. As various analyses accounted for different spoilage mechanisms of fish fillet, the 0.4 % TP + 1.2 % gelatin treatment group has shown to be most effective in exhibiting antimicrobial and antioxidant activities by reducing enzymatic, microbial and oxidative spoilage. Therefore, it is concluded that the 0.4 % TP + 1.2 % gelatin coating has the best effect on preserving the quality and extending the shelf life of fish fillet during cold storage.

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