Effect of ε-polylysine and ice storage on microbiota composition and quality of Pacific white shrimp (*Litopenaeus vannamei*) stored at 0 °C

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ABSTRACT

This study evaluated the effects of ε-Polylysine and ice storage on microbiota composition and quality attributes of Pacific white shrimp stored at 0 °C. The sensorial shelf-life of control, 0.1% ε-Polylysine treated group, and ice stored group were 5, 8, and 7 days, respectively. Microbiota composition was explored by the Illumina-MiSeq high throughput sequencing targeting of 16S rRNA genes. At the time of sensory rejection, *Pseudoalteromonas*, followed by *Candidatus* Bacilloplama and *Psychromonas*, were the dominant microbiota in spoiled control samples on day 5. However, 0.1% ε-Polylysine inhibited the growth of *Pseudoalteromonas* and *Psychromonas*. Consequently, *Candidatus* Bacilloplama followed by *Aliivibrio* became the dominant microbiota in the ε-Polylysine treated group on day 8. Meanwhile, *Aliivibrio*, followed by *Moritella* and *Pseudoalteromonas* were the dominant microbiota in ice stored samples on day 7. Furthermore, due to the modulating effect of ε-Polylysine and ice storage on microbiota, chemical changes of the treated groups were slower, which was reflected as lower concentrations of total volatile basic nitrogen, putrescine, cadaverine, and hypoxanthine, and higher contents of inosine 5′-monophosphate and hypoxanthine riboside at the end of storage. In conclusion, ε-Polylysine and ice storage altered the microbiota composition and delayed quality deterioration of Pacific white shrimp.

1. Introduction

Pacific white shrimp (*Litopenaeus vannamei*) is a warm-water species native to the eastern Pacific coast from the Gulf of California, Mexico to northern Peru. The world aquaculture production of Pacific white shrimp was about 4.2 million tonnes with a global market value of USD 24.4 billion in 2016 (FAO, 2018). Currently, Pacific white shrimp is the most important species economically, and it is accounted for about 70% of the total shrimp production in the world due to its high nutrient content of amino acids, peptides, polyunsaturated fatty acids, and other useful substances (Li et al., 2018; Qian et al., 2015). However, because shrimp is subject to natural contamination by many bacterial species and contain a large amount of non-protein nitrogenous compounds and autolytic enzymes, it is highly perishable and its post-mortem changes occur rapidly, which result in an obvious off-taste and soft texture (Encarnacion et al., 2012; Mastromatteo et al., 2010). In general, seafood microbiota originate from the skin or intestines of the processed objects, and contamination occurs during the successive steps of food processing (Chaillou et al., 2015). Shrimp spoilage is attributed mainly to the uncontrolled growth and subsequent various metabolic activities of microbiota. It is commonly assumed that only specific spoilage organisms (SSO) participate in the spoilage process, and they produce metabolites that result in off-odors and off-flavors (Gram and Huss, 1996). The identification of SSO that are responsible for spoilage requires sensory, microbiological and chemical studies. The growth of SSO results in the breakdown of macromolecules in shrimp, which causes the tissue of shrimp to lose its elasticity and produce off-odors. The modification of microbiota would lead to the change in pattern and process of spoilage in shrimp.

To date, advances in next-generation sequencing have allowed further research to monitor microbiota of shrimp during storage. Cultivable bacteria represent a minor fraction of the total bacterial population that is present (Giovannoni et al., 1990). Culture-independent analyses, such as high throughput sequencing (HTS), arose

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to overcome the limitations of the classical culture-based approach and has been used extensively to reveal the microbiota in food. Currently, 16S rRNA amplicon HTS has been applied extensively to study food spoilage or food fermentation (Ercolini, 2013). Thousands of sequences available from HTS analysis can be analyzed readily to ensure swift, reliable identification of most microorganisms that occur in food samples. Understanding how microbiota change over time is a priority in studying the microbial ecology of foods. Changes in microbial populations provide useful information to observe the shifts in spoilage-associated populations that are related to food storage conditions. Detection of such changes can be invaluable to plan appropriate storage conditions for food products to inhibit specific microbial populations. Therefore, characterizing the composition of microbiota during shrimp storage, analyzing the pattern and mechanism of spoilage in shrimp, and then targeted screening for effective food preservation techniques are important for quality control.

Recently, various food preservation techniques have been used to improve quality and to extend the shelf-life of shrimp. Frozen storage is one of the most effective preservation methods for shrimp. However, repetition and speed of freezing and thawing, storage temperature, and temperature fluctuations in market, retail stores, and restaurants could affect shrimp quality during frozen storage (Nirmal and Benjakul, 2010). Ice storage is another common preservation method for shrimp, especially for freshly captured shrimp. However, ice occupies a great deal of space in the storage container and has to be changed regularly during ice storage. At present, natural preservatives are used increasingly in the preservation of the Pacific white shrimp due to their strong antibacterial properties and high safety, and they do not change the original flavor and quality of foods (Lee and Paik, 2016). In this case, combining natural preservatives with cold storage may be a good choice to maintain the quality of shrimp and to prolong the shelf-life of shrimp instead of ice storage.

Natural preservatives refer to natural human-safe preservatives that are usually extracted from plants, animals, and microorganisms (Anupama et al., 2010). ε-Polylysine is a typical kind of microbiological food preservative with excellent antiseptic performance. It is a naturally occurring homopolymer composed of 25–35 L-lysine units that is linked by isopeptide bonds between the carboxyl group and the ε-amino groups; and it was isolated originally from the fermentation broth of *Streptomyces albulus* (Zinoviadou et al., 2010). This peptide is water soluble, thermally resistant, and biodegradable (Shih et al., 2006). It has broad-spectrum antibacterial properties and has inhibitory effects on the reproduction of Gram-positive and Gram-negative bacteria such as *E. coli, Bacillus subtilis*, yeast, lactic acid bacteria, and *Staphylococcus aureus* (Ye et al., 2013). In addition, ε-Polylysine is safe for humans, which has been confirmed by absorption, distribution, metabolism and excretion (ADME) studies (Hiraki et al., 2003). Meanwhile, ε-Polylysine is certified as GRAS (Generally Recognized as Safe) by the US Food and Drug Administration (FDA) with US GRAS No.: GRN000135 (FDA, 2004). Currently, ε-Polylysine is approval as a food additive in Korea, Japan, USA and some other countries (Bankar and Singhal, 2013). ε-Polylysine is used widely in aquatic products, however, there is little research on the effect of ε-Polylysine on the microbiota composition of Pacific white shrimp. Therefore, this study aimed to analyze the changes of microbiota composition and indicators of quality (sensory assessment, total volatile basic nitrogen (TVB-N), ATP-related compounds, K-value, biogenic amine, and total viable counts (TVC)) of shrimp that were stored with 0.1% ε-Polylysine and with ice at 0 °C to provide a theoretical basis for the quality control of shrimp during storage. High throughput sequencing of 16S rRNA amplicons was used to explore the composition of microbiota of Pacific white shrimp during storage to deepen our understanding of different spoilage patterns of shrimp due to the changes of microbiota.

2. Materials and methods

2.1. Sample preparation

Fresh live Pacific white shrimp (weight 15.37 ± 2.23 g, length 13.37 ± 0.63 cm) were purchased from the Beijing aquatic market. After being transported to the laboratory, shrimp was slaughtered by immersion in ice-water slurry (the ratio of ice to water was 3:1) for 30 min, then washed and drained. Subsequently, they were divided into three groups: untreated group (control), 0.1% ε-Polylysine treated group (PL), and ice stored group (Ice). Control and Ice samples were immersed in sterile water for 30min, and PL samples were immersed in 0.1% (w/v) ε-Polylysine solution (dissolved with sterile water) for 30 min. Shrimp-to-solution ratio was 2:1 (w/v). After that, shrimp from the three groups were removed from the solution and the water was drained. Samples from the control and PL groups were packed in polyvinyl chloride bags (six shrimp per bag) and stored in a refrigerated room at 0 °C. The Ice group was not packaged with polyvinyl chloride bags and was stored in a refrigerator at 0 °C in the form of a layer of crushed ice - a layer of shrimp - a layer of crushed ice. ε-Polylysine (food grade, purity ≥ 99%) was purchased from Bainafo Biotechnology Co. Ltd (Zhengzhou, China). The polyvinyl chloride bags were purchased from Cleanwrap Co., Ltd. (Korea) and the oxygen permeability of the pouch was 13,960 $cm^3/(m^2 \times d \times atm)$. Three samples (i.e. three different packages) of each group were selected randomly and analyzed for microbiological, chemical, and sensorial quality.

2.2. Sensory assessment

The sensory attributes of shrimp were evaluated by a sensory panel of nine trained and experienced assessors (six females and three males). The panelists were asked to evaluate the quality parameters of shrimp that included color, odor, texture, and overall acceptability during storage. The panelists scored the samples using a 9-point descriptive scale (Amerine et al., 1965). A score of 7–9 indicated "very good" quality, a score of 4.0–6.9 represented "good" quality, a score of 1.0–3.9 represented as spoiled.

2.3. Determination of TVC, TVB-N, and pH

At each sampling date, three samples (i.e., three different packages) of each group were selected randomly and, TVC, TVB-N, and pH were determined. TVC (total viable counts) were performed according to the method provided in Jia et al. (2018a) with some modification. From each packet, 5 g sample was homogenized with 10-fold sterile 0.9% NaCl solution for 30 s in a stomacher, and then diluted serially (1:10, 0.9% NaCl solution). Samples (100 μL) of each dilution were spread on a plate count agar and incubated at 30 °C for 72 h. Microbiological number was expressed as log CFU/g. TVB-N was determined according to the semi-micro, steam distillation method (Hong et al., 2012) and expressed as mg N/100 g flesh. Briefly, 5 g of fish flesh was dispersed in 50 mL distilled water and stirred for 30 min. The mixture was centrifuged at 1610 g for 3 min, and then the supernatant was collected and used for the determination of TVB-N with a Kjeldahl Apparatus (KDY-9820, Beijing, China). Meanwhile, the pH of the supernatant was measured using a digital pH meter (Mettler Toledo FE20/EL20, Shanghai, China).

2.4. Determination of ATP-related compounds and K value

ATP-related compounds (adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine 5′-monophosphate (IMP), hypoxanthine riboside (HxR), and hypoxanthine (Hx)) were extracted according to the method described by Huang et al. (2017). Briefly, 1 g of shrimp flesh was homogenized with 2 mL cold 10% perchloric acid (PCA) solution and centrifuged at 5000

r/min for 3 min at 4 °C. The supernatant was collected. The sediment was washed with 2 mL cold 5% PCA and centrifuged at 5000 r/min for 3 min. This process was repeated twice. Then, all supernatants were collected to adjust the pH to 6.4 ± 0.05 with 1M and 10M KOH solution, which was followed by centrifuging at 5000 r/min for 3 min at 4 °C. The supernatant was made up to 10 mL using neutral PCA (pH 6.4) and stored at −20 °C for further analysis. The extracted ATP-related compounds were analyzed by high-performance liquid chromatography (HPLC), which was equipped with an SPD-10A (V) detector and a COSMOSIL 5C18-PAQ column (4.6ID × 250 mm). K-value was calculated by the following equation: K-value (%) = [(HxR + Hx)/(ATP + ADP + AMP + IMP + HxR + Hx)] × 100.

2.5. Determination of biogenic amines

Biogenic amines were extracted according to the method of Jia et al. (2018b), with minor modification. Briefly, 5 g of minced sample were homogenized with 10 mL of cold 0.6 M perchloric acid solution and centrifuged at 10,000 g for 10 min. This process was repeated two times. All supernatants were combined and made up to 25 mL by 0.6 M perchloric acid and stored at −20 °C for further analysis. Afterwards, the concentration of biogenic amines was determined using HPLC.

2.6. Microbiota composition analysis based on Illumina-MiSeq sequencing

Bacterial DNA was extracted according to the method of Liu et al. (2018). Three DNA samples from the same group were mixed together for PCR amplification. The V3-V4 regions of 16S rRNA genes were amplified by PCR (95 °C for 3 min, followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45s and, a final extension at 72 °C for 10 min) using primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′- GGACTACHVGGGTWTCTAA-3′) with different barcodes.

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, California, USA) according to the manufacturer's instructions and quantified using QuantiFluor™-ST (Promega, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300 bp) on an Illumina-MiSeq platform according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database. Raw fastq files were demultiplexed and quality-filtered using QIIME (version 1.17). Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU115) 16S rRNA database using a confidence threshold of 70%. Principal coordinate analysis (PCoA) on weighted unifrac distance was performed to reflect differences of the bacterial community profiles in samples using R-package vegan.

2.7. Statistical analysis

All the measurements were performed in triplicates (three different packages). The results were expressed as means ± standard deviation and analyzed by SPSS 19.0 software (SPSS Inc., Chicago, IL USA). One-way analysis of variance (ANOVA) and Duncan's multiple range test were carried out to test the significant differences among various treatments. Values of $P < 0.05$ were considered as significantly different.

3. Results

3.1. Microbial analysis

3.1.1. Total viable counts (TVC)

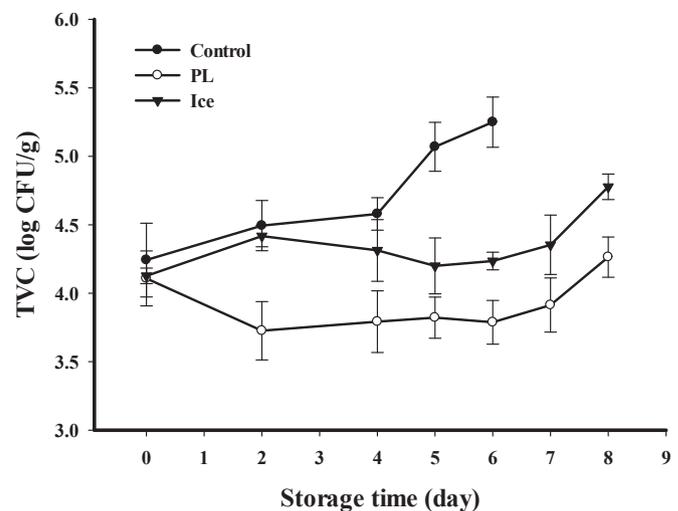The initial TVC of the control, PL and Ice groups were 4.24, 4.11,

Fig. 1. Changes in TVC of Pacific white shrimp in the control, 0.1% ε-Polylysine-treated group (PL), and ice-stored group (Ice) during storage at 0 °C.

and 4.12 log CFU/g, respectively (Fig. 1), and there were no differences among the three groups. TVC of shrimps treated with ε-Polylysine was significantly lower than the other two groups from the 2nd day of storage ($P < 0.05$). In addition, TVC in the Ice group was significantly lower than the control group from the 4th day of storage ($P < 0.05$). With successive increases in TVC of the control, the spoiled control samples had a microbial load of 5.07 log CFU/g on day 5. However, the TVC of spoiled PL and Ice samples were only 4.26 on day 8 and 4.37 log CFU/g on day 7, respectively. The results also showed that ε-Polylysine treatments reduced the TVC by 1.0 log CFU/g compared with the control samples from the 5th day of storage.

3.1.2. Microbiota composition

Illumina-Miseq high throughput sequencing was used to investigate the succession of microbial communities in the three groups at the initial and end of storage. Based on sensory evaluation, the control, PL and Ice samples became spoiled after 5, 8 and 7 days of storage, respectively. The bacterial community barplot showed the relative abundances of the top 19 genera in all samples (Fig. 2a).

On day 0, *Photobacterium* and *Candidatus* Bacilloplama were the dominant bacteria in the control samples, which accounted for 42.8% and 28.8% of the total microbiota, respectively. However, the ε-Polylysine and ice storage treatment decreased the relative abundance of *Photobacterium* and *Candidatus* Bacilloplama at the initial stage of storage. Consequently, *Vibrio* (19.9%) followed by *Candidatus* Bacilloplama (17.7%) became the dominant microbiota in PL samples; *Photobacterium* (26.6%) followed by *Candidatus* Bacilloplama (14.8%) became the dominant microbiota in Ice samples. As storage time increased, the microbiota composition in the three groups underwent significant changes. At the end of the shelf-life, the relative abundances of *Photobacterium* and *Vibrio* were reduced to very low levels in all groups. The relative abundances of *Candidatus* Bacilloplama remained stable at 22.1% in the control group on day 5 and 22.6% in the PL group on day 8. However, it decreased to 3.7% in the Ice group on day 7. Correspondingly, *Pseudoalteromonas* (38.0%) was the dominant bacterial group, followed by *Candidatus* Bacilloplama (22.1%) and *Psychromonas* (14.9%), in the spoiled control samples on day 5. In the meantime, *Candidatus* Bacilloplama (22.7%) comprised the most abundant bacterial group, followed by *Aliivibrio* (12.2%), *Pseudoalteromonas* (10.3%), and *Psychromonas* (9.0%) in the spoiled PL samples on day 8. In addition, on the 7th day, when the samples in the Ice group were completely spoiled, *Aliivibrio* (59.6%) became the main microbial genus, followed by *Moritella* (13.1%) and *Pseudoalteromonas* (11.6%).

(a)

(b)
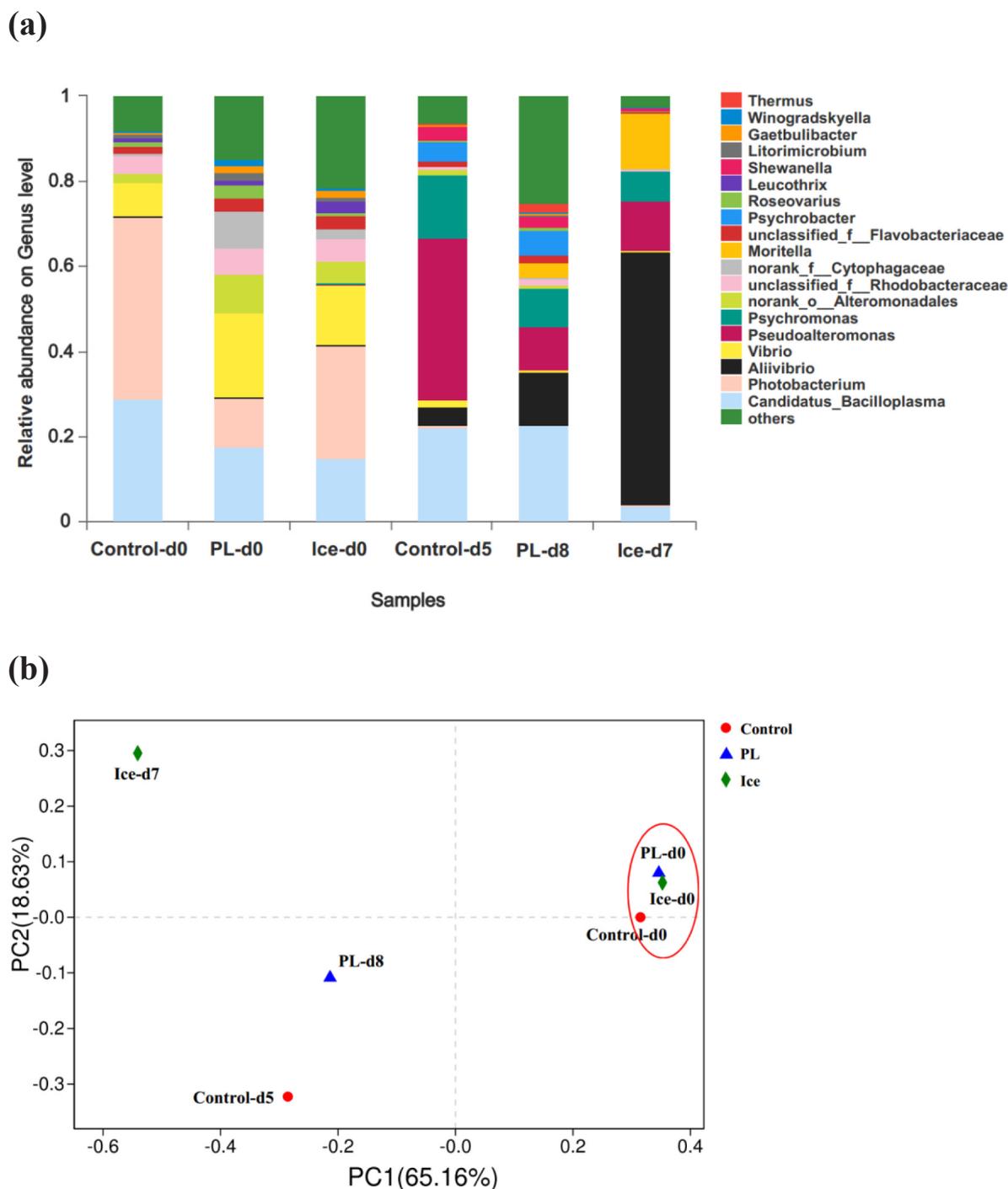
Fig. 2. Relative abundance of the bacteria (a) and principal coordinates analysis (PCoA) (b) of microbiota from the control, 0.1% ε-Polylysine-treated group (PL), and ice-stored group (Ice) during storage at 0 °C. Only the bacteria with relative abundances > 1.5% at the genus level were indicated specifically. The category "others" represents a collection of genera with relative abundance ≤1.5%. The PCoA analysis for each sample was based on weighted unifrac distance. (control-d0: control samples on day 0; control-d5: control samples on day 5; PL-d0: 0.1% ε-Polylysine treated samples on day 0; PL-d8: 0.1% ε-Polylysine treated samples on day 8; Ice-d0: ice stored samples on day 0; Ice-d7: ice stored samples on day 7.)

The similarities and differences of microbiota composition among all samples at different sampling points were analyzed using β-diversity analysis based on principal co-ordinates analysis (PCoA). PCoA similarity analysis of all samples were visualized using weighted unifrac distance, and the longer distance between any two groups in the PCoA plot indicated less similarity between the two groups (Fig. 2b). The results showed that the similarity of the three groups at the initial stage of storage was relatively high, while the microbiota structure changed a lot at the end of storage. The composition of microbiota was totally

changed by the ε-Polylysine and ice storage at the end.

3.2. Chemical analysis

3.2.1. TVB-N and pH

TVB-N values increased continuously in all samples during the entire storage (Fig. 3a). The initial TVB-N values of the control, PL group, and Ice group were 7.89, 8.35 and 7.40 mg N/100 g, respectively. From the 4th day to the end of storage, the PL and Ice samples showed a
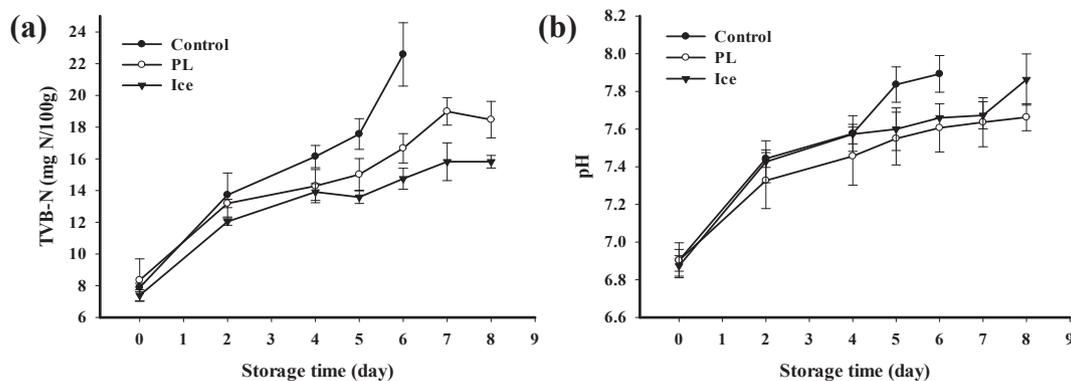
Fig. 3. Changes in TVB-N(a) and pH value (b) of Pacific white shrimp in the control, 0.1% ε-Polylysine-treated group (PL), and ice-stored group (Ice) during storage at 0 °C.

significantly lower level of TVB-N than the control ($P < 0.05$). Furthermore, at the time of sensory rejection (day 5), the TVB-N content in the control samples increased to 17.60 mg N/100 g, which was much higher than that of other groups ($P < 0.05$). Fig. 3b showed the changes in pH of three groups with storage time at 0 °C. The initial pH values were 6.90, 6.90, and 6.87 for the control, PL, and Ice group, respectively. The pH of the three groups tended to increase during the whole storage process, although shrimp in the PL group and Ice group showed a significantly lower pH in comparison with the control group from the 5th day of storage ($P < 0.05$).

3.2.2. ATP-related compounds

As shown in Fig. 4, changes of ATP breakdown products in three groups could be summarized as a loss of IMP and an increase of Hx. Meanwhile, a trend where HxR increased and then decreased was observed. The concentration of IMP in the control, PL, and Ice samples was at a maximum level on day 4, day 4, and day 5, respectively (with a value of 5.34, 5.51, and 5.40 µmol/g, respectively, Fig. 4a). From the 5th day of storage, IMP content in PL and Ice samples was significantly higher than that of control samples ($P < 0.05$), which indicated that ε-Polylysine and ice storage treatment inhibited the further degradation of IMP. As storage time increased, IMP was converted to HxR and Hx. There were no significant differences among the three groups during the first 4 days of storage (Fig. 4b). However, the content of HxR in the control samples increased to its maximum level (1.62 µmol/g) on day 5, which was significantly higher than that of other two groups. HxR

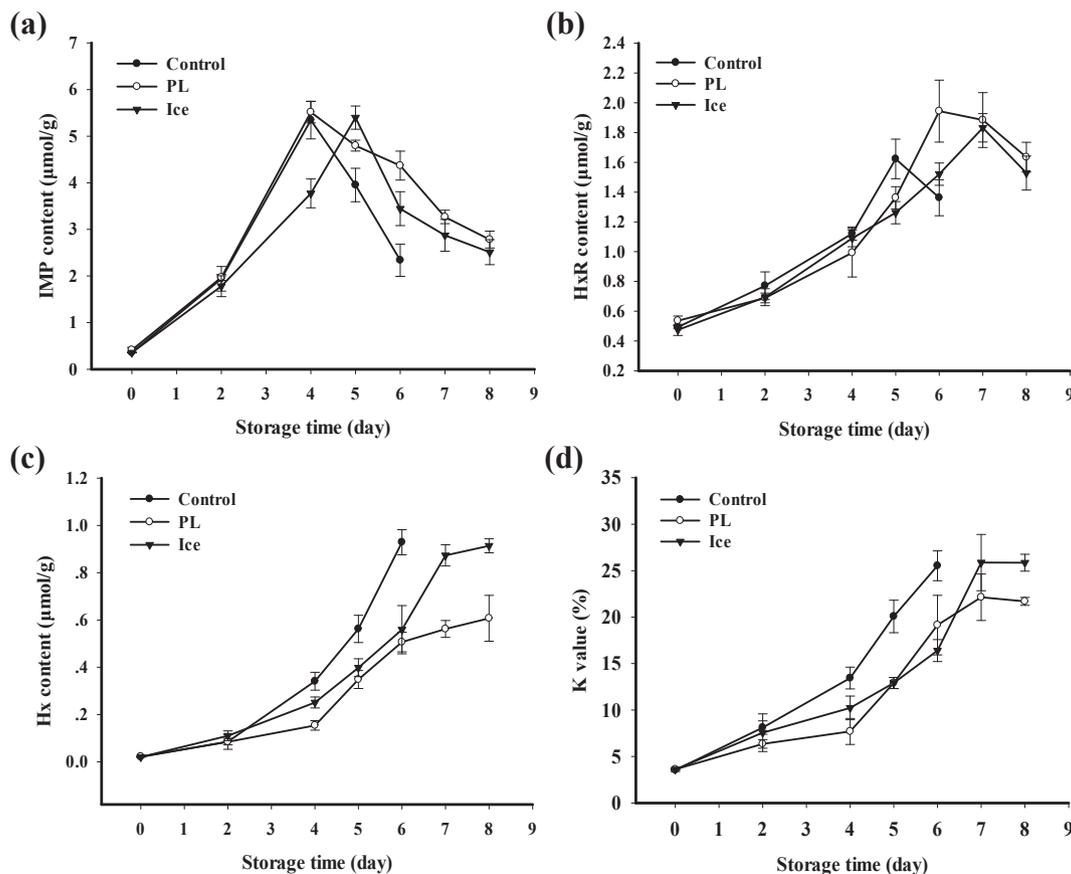**Fig. 4.** Changes in IMP(a), HxR(b), Hx(c), K-value(d) of Pacific white shrimp in the control, 0.1% ε-Polylysine-treated group (PL), and ice-stored group (Ice) during storage at 0 °C.

Table 1
Changes in biogenic amines content of Pacific white shrimp in the control, PL, and Ice groups during storage at 0 °C.

Biogenic amines (mg/kg)	Groups	Storage time (day)						
		0	2	4	5	6	7	8
Putrescine	Control	0.22 ± 0.09[Aa]	1.12 ± 0.08[Bb]	1.41 ± 0.16[Cb]	2.10 ± 0.18[Db]	3.22 ± 0.13[Eb]		
	PL	0.13 ± 0.05[Aa]	0.52 ± 0.13[Ba]	0.72 ± 0.10[BCa]	1.01 ± 0.06[Ca]	1.73 ± 0.28[Da]	1.89 ± 0.22[Da]	2.35 ± 0.42[Ea]
	Ice	0.20 ± 0.03[Aa]	0.52 ± 0.15[Ba]	0.93 ± 0.21[Ca]	1.14 ± 0.14[Ca]	1.85 ± 0.14[Da]	2.21 ± 0.16[Eb]	2.45 ± 0.23[Ea]
Cadaverine	Control	ND	1.19 ± 0.19[Ab]	1.84 ± 0.35[Ab]	5.65 ± 0.20[Bb]	9.56 ± 1.22[Cb]		
	PL	ND	0.20 ± 0.09[Aa]	0.92 ± 0.17[ABa]	1.79 ± 0.28[Ba]	2.55 ± 0.80[Ca]	3.89 ± 0.67[Ca]	4.94 ± 0.80[Da]
	Ice	ND	0.42 ± 0.26[Aa]	1.09 ± 0.26[ABa]	1.69 ± 0.52[Ba]	2.89 ± 0.88[Ca]	4.13 ± 0.65[CDa]	4.88 ± 0.89[Da]

Note: Same lowercase letters in a column indicate no significant differences ($P > 0.05$); same uppercase letters in a raw indicate no significant differences ($P > 0.05$). (Control: untreated samples; PL: 0.1% ε-Polylysine treated samples; Ice: ice stored samples; ND: not detected.)

content in the PL and Ice samples increased to its maximum level on day 6 and day 7, respectively (with a value of 1.94 and 1.83 μmol/g, respectively). As storage time increased, the Hx content of these three groups increased gradually (Fig. 4c). From the 4th day of storage, the Hx content of the PL and Ice samples was significantly less compared with the control samples ($P < 0.05$). K value is used commonly to evaluate the freshness of aquatic products. The results revealed that K value increased gradually with storage time in all groups (Fig. 4d), and significant differences among three groups were observed from the 4th day of storage ($P < 0.05$).

3.2.3. Putrescine and cadaverine

Putrescine and cadaverine were detected in all samples during the entire storage period (Table 1). The initial concentrations of putrescine were low in the three groups. However, when the control samples were rejected by the sensory panelists (day 5), contents of putrescine for the control, PL, and Ice samples increased to 2.10, 1.01 and 1.14 mg/kg, respectively. Cadaverine was not detected in the initial period of storage, but its value was up to 5.65 mg/kg in spoiled control samples on day 5. Notably, cadaverine in spoiled PL and Ice samples was only 3.89 and 4.88 mg/kg, respectively. The results also showed that contents of putrescine and cadaverine in PL and Ice samples were significantly lower than the control samples from the 2nd day of storage ($P < 0.05$).

3.3. Sensory analysis

In our preliminary experiment, all panelists considered a concentration of 0.1% (w/v) ε-Polylysine to be acceptable and not to have negative effects on the organoleptic quality of shrimp. Sensory scores of all parameters for all samples declined significantly as storage time increased (Table 2). However, an apparent difference in color and texture between control and treated samples was observed from the 2nd day of storage ($P < 0.05$). The sensory results also indicated that PL samples maintained better color and texture compared with Ice samples. PL and Ice samples showed significantly higher overall acceptability scores from the 2nd day of storage compared with the control samples ($P < 0.05$). According to the sensory assessment, sensorial shelf-life of Pacific white shrimp was 5 days for the control, 8 days for the PL group, and 7 days for the Ice group. However, to compare the biochemical changes with the PL group, the quality indicators in the control on the 6th day and those in the Ice group on the 8th day were also determined.

4. Discussion

The data presented here provide detailed insight into the changing bacterial profile and quality attributes of Pacific white shrimp treated by ε-Polylysine and ice storage at 0 °C. According to the sensory analysis, the control, PL and Ice samples were perceived as spoiled after being stored for 5, 8, and 7 days, respectively (Table 2). The PL and ice storage treatment enhanced the beneficial effects significantly ($P < 0.05$) on overall acceptability of the samples during storage, and a longer sensory shelf life was observed in the two treated samples. The TVC of the spoiled control samples at day 5 were 5.07 log CFU/g, which was consistent with the results of Nirmal and Benjakul (2009); however, ε-Polylysine treatments reduced the TVC by 1.0 log CFU/g compared with the control samples from the 5th day of storage. At the end of storage, the TVC of spoiled PL and Ice samples at days 8 and 7 were 4.26 and 4.37 log CFU/g, respectively. Both 0.1% ε-polylysine and ice storage had an inhibitory effect on the growth of microorganisms. Furthermore, the antimicrobial effect of 0.1% ε-Polylysine was better than that of using ice. These results indicated that ε-Polylysine was an effective antibacterial chemical in the shrimp flesh system. Previous studies have also revealed the excellent preservation effect of ε-

Table 2
Changes in sensory scores of Pacific white shrimp in the control, PL and Ice groups during storage at 0 °C.

Sensory indicators	Groups	Storage time (day)						
		0	2	4	5	6	7	8
Color	Control	9.00 ± 0.00[Aa]	6.75 ± 0.39[Bc]	4.25 ± 0.19[Cc]	2.05 ± 0.09[Dc]	1.25 ± 0.05[Ec]		
	PL	9.00 ± 0.00[Aa]	8.25 ± 0.35[Ba]	6.75 ± 0.34[Ca]	5.80 ± 0.15[Da]	5.25 ± 0.31[Ea]	3.75 ± 0.29[Fa]	1.50 ± 0.05[Ga]
	Ice	9.00 ± 0.00[Aa]	7.50 ± 0.29[Bb]	4.75 ± 0.23[Cb]	4.50 ± 0.20[Cb]	4.00 ± 0.39[Db]	1.25 ± 0.09[Eb]	1.00 ± 0.05[Fb]
Odor	Control	9.00 ± 0.00[Aa]	7.50 ± 0.35[Bb]	4.63 ± 0.29[Cb]	1.50 ± 0.19[Cb]	0.75 ± 0.09[Eb]		
	PL	9.00 ± 0.00[Aa]	8.25 ± 0.40[Ba]	7.50 ± 0.45[Ca]	7.00 ± 0.35[CDa]	6.55 ± 0.35[Da]	4.75 ± 0.29[Ea]	3.50 ± 0.23[Fa]
	Ice	9.00 ± 0.00[Aa]	8.00 ± 0.29[Ba]	7.25 ± 0.39[Ca]	6.95 ± 0.29[CDa]	6.15 ± 0.33[Ea]	4.25 ± 0.39[Fa]	2.75 ± 0.18[Gb]
Texture	Control	9.00 ± 0.00[Aa]	7.25 ± 0.35[Bb]	5.25 ± 0.19[Cc]	4.00 ± 0.28[Dc]	1.25 ± 0.08[Ec]		
	PL	9.00 ± 0.00[Aa]	8.00 ± 0.29[Ba]	6.75 ± 0.35[Ca]	5.80 ± 0.39[Da]	5.00 ± 0.29[Da]	4.20 ± 0.19[Eb]	1.00 ± 0.25[Fb]
	Ice	9.00 ± 0.00[Aa]	7.30 ± 0.27[Bb]	6.00 ± 0.30[Cb]	5.25 ± 0.29[Db]	4.75 ± 0.20[Eb]	2.10 ± 0.11[Fa]	1.75 ± 0.07[Ga]
Overall acceptability	Control	9.00 ± 0.00[Aa]	7.15 ± 0.45[Bc]	5.05 ± 0.15[Cc]	3.19 ± 0.29[Dc]	1.31 ± 0.15[Eb]		
	PL	9.00 ± 0.00[Aa]	8.15 ± 0.29[Ba]	6.90 ± 0.30[Ca]	6.05 ± 0.35[Da]	5.53 ± 0.23[Ea]	4.25 ± 0.17[Fa]	2.75 ± 0.05[Ga]
	Ice	9.00 ± 0.00[Aa]	7.68 ± 0.35[Bb]	6.15 ± 0.25[Cb]	5.24 ± 0.20[Db]	4.25 ± 0.20[Ea]	2.16 ± 0.25[Fb]	1.56 ± 0.09[Gb]

Note: Same lowercase letters in a column indicate no significant differences ($P > 0.05$); same uppercase letters in a raw indicate no significant differences ($P > 0.05$). (Control: untreated samples; PL: 0.1% ε-Polylysine treated samples; Ice: ice stored samples.)

Polylysine on Japanese sea bass (*Lateolabrax japonicas*) (Cai et al., 2015) and bighead carp (*Aristichthys nobilis*) (Liu et al., 2018).

Shrimp spoilage is mainly attributed to microbial growth. The modification of microbiota would lead to the change in pattern and process of spoilage in shrimp. To reveal the succession of microbes in shrimp under different storage conditions, Illumina-MiSeq high throughput sequencing was used to analyze the microbiota composition of Pacific white shrimp at the initial and spoiled stages of storage. The sequencing results indicated that ε-Polylysine inhibited the growth of *Photobacterium* and *Candidatus* Bacilloplama on day 0. Therefore, *Vibrio*, followed by *Candidatus* Bacilloplama, became the core bacterial communities of PL samples. It is also worth noting that although *Photobacterium* was still the dominant microbiota in Ice samples, the relative abundance of *Photobacterium* was decreased from 42.8% to 26.6% due to the ice storage treatment. *Photobacterium* is a genus of Gram-negative bacteria in the family *Vibrionaceae*. This genus is adapted for optimal growth in the deep cold seas, which enables to grow and to reproduce in cold temperatures. *Photobacterium* is commonly associated with seafood spoilage. Dalgaard et al. (1998) reported that *Photobacterium* was the specific spoilage organism (SSO) responsible for spoilage of modified atmosphere packaged cod fillets, and they claimed that any preservation procedure that was able to reduce growth of *Photobacterium* was likely to extend shelf life of packed cod. *Photobacterium* were also identified as the main spoiler of Norway lobster (*Nephrops norvegicus*) tail meat; this genus could reduce trimethylamineoxide (TMAO) to trimethylamine (TMA) and produce H_2S during storage (Gornik et al., 2011). *Photobacterium* grow rapidly on raw salmon (*Salmo salar*) matrix, and samples inoculated by *Photobacterium* species presented amine and sour off-odors (Macé et al., 2013). These may be the reasons that the odor of control samples in this study became unacceptable quickly. Lakshmanan et al (2002) reported that the genus *Photobacterium* was responsible for the formation of putrescine and cadaverine in fish and shrimp. Meanwhile, *Photobacterium* may contribute to spoilage through activity in the digestive gland, followed by diffusion of volatile compounds and amines to the squid mantle (Paarup et al., 2002). Due to the inhibiting effect of ε-Polylysine and ice storage on *Photobacterium*, the content of putrescine and cadaverine in PL and Ice groups increased slowly. *Candidatus* Bacilloplama is a genus that existed continuously in the control shrimp at the initial and at the end of storage. *Candidatus* Bacilloplama generally exists in the intestine of aquatic products such as lampreys (*Lampetra morii*) and Chinese mitten crab (*Eriocheir sinensis*) (Chen et al., 2015; Li et al., 2016). There has been little research about the spoilage potential of *Candidatus* Bacilloplama in aquatic products because it has not proved possible to maintain this bacterium in culture (Štrus and Avguštin, 2007).

Based on the sensory analysis, the shelf-life of control, PL and Ice samples were 5, 8, and 7 days. At the end of storage, *Pseudoalteromonas* was the dominant bacterial group, followed by *Candidatus* Bacilloplama and *Psychromonas* in the spoiled control samples on day 5. As the most abundant microbiota, *Pseudoalteromonas* were able to produce TMA, H_2S, ammonia, and acetic acid, which are closely related to deterioration of seafood quality (Broekaert et al., 2013). Broekaert et al. (2013) also claimed that *Pseudoalteromonas* dominated the microbiota of brown shrimp, and this genus enhanced shrimp spoilage by degrading small lipids and hydrolyzing amino acids and proteins, which was also verified by Tirloni et al (2016). Additionally, *Pseudoalteromonas* were also the most abundant microbiota in whole and gutted squid during storage (Paarup et al., 2002). Previous study reported that *Pseudoalteromonas* strains generally formed robust biofilms under laboratory condition and produced extracellular proteases in a biofilm-dependent manner (Iijima et al., 2009). *Pseudoalteromonas* were also known to produce a variety of extracellular compounds that inhibit or control adaptive and behavioral responses in many target organisms so that this genus can survive and dominate in the environment (Lee et al., 2000; Mai-Prochnow et al., 2004). ICMSF (1986)suggested that the threshold

for deterioration of aquatic products due to TVC be defined as 7 log CFU/g. However, in this study, the TVC of spoiled control samples was only 5.07 log CFU/g, probably because the second most abundant microbiota in spoiled control samples was identified as the uncultivable genus *Candidatus* Bacilloplama (22.1%). Chaillou et al (2015) reported that the most abundant microbiota recovered from shrimp was assigned to a taxon that contained several uncultured endosymbiotic bacteria, which could be the reason why shrimp have low counts of viable population compared with other meat products. The genus *Psychromonas* includes psychrophilic (low temperature adapted), halophilic (high-salt adapted), and piezophilic (high-pressure adapted) species that are distributed widely in aquatic environments and are an important component of polar and deep-sea microbiota (Lauro et al., 2013). Previous study revealed that *Psychromonas* possessed proteolytic and lipolytic activity during the storage of American lobsters (*Homarus americanus*), and this genus showed higher lipolytic activity than proteolytic activity. The psychrotrophic genus *Psychromonas* were also confirmed through Miseq sequencing as dominant bacteria in the gills of spoiled oysters at 4 °C storage (Chen et al., 2019). According to our results, *Pseudoalteromonas* and *Psychromonas* were not tolerant to ε-Polylysine. Consequently, *Candidatus* Bacilloplama comprised the most abundant bacterial group in the spoiled PL samples. Meanwhile, ice storage treatment decreased the relative abundances of *Pseudoalteromonas*, *Psychromonas*, and *Candidatus* Bacilloplama, which made the microbiota composition of Ice samples show an obvious alteration. However, the specific mechanisms that were involved and the applications of ε-Polylysine and ice storage to inhibit the growth of spoilage bacteria need to be studied more intensively.

The modulating effects of two treatments on microbiota resulted in the change in pattern and process of spoilage in shrimp, which was reflected in the changes of biochemical indicators eventually. TVB-N is produced when protein is decomposed into basic nitrogen-containing compounds such as ammonia, dimethylamine and trimethylamine under the action of microorganisms and enzymes (Huang et al., 2017; Ocaño-Higuera et al., 2011), which is an important bio-chemical indicator of the freshness in the evaluation of seafood quality. The initial values of TVB-N were 7.39–8.35 mg N/100 g, which was consistent with the research reported by Okpala et al (2014). During storage, the TVB-N content of the control group increased slowly during the first 5 days, which was followed by a sharp increase. Similar results were obtained by Qian et al. (2015). From the 4th day to the end of storage, the PL and Ice samples showed a significantly lower level of TVB-N than the control. In addition, Goncalves and Gindri Junior (2009) reported that 20 mg N/100 g was considered as a limiting value of freshness for aquatic products. In our samples, only the TVB-N of control shrimp exceeded this limit on the 6th day. Our study revealed that ε-Polylysine and ice storage treatment inhibited the increase of TVB-N significantly during storage, which was probably due to the inhibitory activity of ε-Polylysine and ice storage treatment towards spoilage bacteria such as *Photobacterium* and *Pseudoalteromonas*. In this case, the degradation of macromolecular components that contained nitrogen may have been inhibited effectively. The initial pH values were about 6.90 for all three groups, which was similar to the results reported for tiger shrimp (Kaur et al., 2013). The pH of the three groups tended to increase during the storage process, while shrimp in PL group and Ice group showed a significantly lower pH compared with the control group from the 5th day of storage. Varlik et al. (2000) reported that, in general, acceptable pH levels for shrimp were 7.0–8.0. Mehmet et al. (2009) found that shrimp retained good quality when the pH did not exceed 7.7. Thus, to some extent, the smaller increase in pH was more likely related to the lower microbial growth in the sample during refrigerated storage. The increase in pH of aquatic products during storage was attributed mainly to the decomposition of proteins and other nitrogen-containing substances into volatile bases such as amines and trimethylamines with the action of microorganisms and endogenous enzymes (Ramezani et al., 2015).

After death of either fish or shrimp, the pathway for ATP degradation therein has been documented as a catabolite sequence: ATP→ADP→AMP→IMP→HxR→Hx. Following the death of aquatic organism, the conversion of ATP to IMP is presumed to be a totally autolytic process. According to the results of ATP-related compounds, ε-Polylysine and ice storage treatment inhibited the further degradation of IMP that made the shrimp maintain better flavor. As storage time increased, IMP was converted to HxR and Hx, which resulted from both autolytic and bacterial enzymes; meanwhile, the accumulation of Hx resulted in the progressive loss of a desirable flavor, and it produced a bitter taste (Hernández-Cázares et al., 2011). In the present study, there was no significant difference in Hx among the three groups during the first 2 days of storage; however, compared with the control samples, higher contents of HxR were observed in two treated samples after 5 days of storage, meanwhile, lower contents of Hx were observed in the two treated samples after the 2nd day of storage. This may have been due to the effect of PL and Ice storage on the composition of microbiota in the samples, which inhibited further degradation of HxR to Hx. Hernández-Cázares et al. (2011) also reported that the formation of Hx was related to the nucleoside phosphorylase that was produced by spoilage bacteria.

Biogenic amines are nonvolatile low-molecular weight nitrogenous compounds that are formed mainly by bacteria through the decarboxylation of free amino acids (Smith, 1981). Generally, the most common biogenic amines in seafood associated with spoilage are putrescine and cadaverine. Putrescine is regarded as the best indicator of decomposition for Penaeid shrimp and Norway lobster (*Nephrops norvegicus*) stored at a wide range of temperatures, meanwhile, the production of cadaverine followed a pattern similar to that of putrescine in several shrimp species (Prester, 2011). We found spoilage-related biogenic amines that included putrescine and cadaverine in shrimp. Putrescine and cadaverine levels in PL and Ice samples were significantly lower than the control samples from the 2nd day of storage. Lakshmanan et al (2002) reported that *Photobacterium* that survived in chilled storage may be responsible for the formation of putrescine and cadaverine in fish and shrimp. ε-Polylysine inhibited the production of putrescine and cadaverine during storage and we speculated that it may be due to a direct modification of the decarboxylating enzyme activity by ε-Polylysine and/or the inhibitory effect of ε-Polylysine on spoilage bacteria such as *Photobacterium* from the initial stage of storage. Previous study showed that Polylysine was able to bind to some proteins, nucleic acids, viruses, or bacteria through electrostatic or hydrophobic interaction and inhibited their functions (Tsujita et al., 2003). Some *in vitro* studies demonstrated that ε-Polylysine as a reversible reaction inhibitor strongly inhibited the activity of lipase and the inhibition capacity increased with the degree of Polylysine polymerization (Tsujita et al., 2003; Tsujita et al., 2006). Biogenic amines are derived through the decarboxylation of corresponding amino acids, and various kinds of amino acid-decarboxylases were involved in the formation of biogenic amines. Screening of the decarboxylase inhibitors is greatly significant to suppress the production of spoilage related biogenic amines. However, there is little research about whether ε-Polylysine can affect the activities of decarboxylase and which kinds of decarboxylases can be affected by ε-Polylysine. Hence, further research is needed to verify this speculation.

Sensory analysis is one of the most important quality indicators during shrimp storage. Microbial spoilage involves growth of bacteria to high numbers and products of their metabolism give rise to the sensory impressions perceived as spoilage. Sensory rejection is generally caused by discoloration, physical changes, textural changes, slime or gas formation, or the development of off-flavors and off-odors. In this study, sensory scores of color, odor, texture, and overall acceptability were reduced significantly in all three groups with increasing time in storage (Table 1). The control samples were rejected by the sensory panelists on day 5. *Pseudoalteromonas* were the core bacterial genus in spoiled control shrimp, and *Pseudoalteromonas* can produce large quantities of sulphides and acetone (Broekaert et al., 2013). Similarly, *Pseudoalteromonas* isolates were also capable of hydrolyzing proteins, which resulted in deterioration of texture. PL and Ice samples maintained higher scores for odor, color, and texture from the 2nd day of storage compared with the control, which may be due to the inhibitory effect of two treatment towards the genus *Pseudoalteromonas*. However, we should note that the texture softening of shrimp muscle was also associated with auto-proteolysis caused by endogenous proteinases including cathepsin B or L, cathepsin D, alkaline proteases and calcium-dependent proteases (Benjakul et al., 1997). Hence, the effects of ε-Polylysine on endogenous proteinases also need to be studied further. Notably, another reason why the control samples were rejected by the sensory panelists was the formation of black spots (melanosis) that is also called enzymatic browning. The brown color is developed by the enzymatic reaction mediated by polyphenoloxidase (Benjakul et al., 2005), which is followed by nonenzymatic polymerization that give rise to pigments of high molecular weight and very dark or black coloring. According to the sensory results, melanosis was observed less frequently in the PL samples. The inhibitory mechanism of ε-Polylysine towards melanosis (e.g., whether less melanosis was related to the modification effect of ε-Polylysine on shrimp microbiota or the inhibitory effect of ε-Polylysine toward polyphenoloxidase) should be investigated in the future. Overall, good sensory quality and longer shelf-life were observed in PL and Ice samples during storage. In addition, sensory scores of color and texture in PL samples were higher than that of Ice samples from the 2nd day of storage.

5. Conclusion

ε-Polylysine and ice storage affected microbiota composition of Pacific white shrimp during storage at 0 °C, which resulted in better quality characteristics and longer shelf-life compared with untreated shrimp. *Pseudoalteromonas*, *Candidatus* Bacilloplama and *Psychromonas* were dominant in spoiled control samples. However, *Pseudoalteromonas* and *Psychromonas* were inhibited by ε-Polylysine and ice storage and, consequently, *Candidatus* Bacilloplama followed by *Aliivibrio* became the dominant microbiota in ε-Polylysine treated group. Meanwhile, *Aliivibrio*, followed by *Moritella* and *Pseudoalteromonas* were the dominant microbiota in ice stored samples. Shrimp treated with ε-Polylysine had better sensory quality and maintained longer shelf-life compared with ice stored samples. Therefore, we recommended that ε-Polylysine treatment be used in shrimp stored at 0 °C. Meanwhile, further research should be conducted to elucidate the underlying mechanism of quality changes caused by specific spoilage organisms during the storage of Pacific white shrimp.

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