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THE SHELF-LIFE OF BLACK TIGER SHRIMP (*PENAEUS MONODON*) TREATED WITH THE DIFFERENT CONDITIONS

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Abstract. This study examines the chemical and microbiological changes and the sensory attributes of black tiger shrimp *(Penaeus monodon*) when stored at the temperature of 0 ºC. Sodium propionate and sodium lactate were used to treat shrimps before storage. Vacuum packaging was also carefully investigated. The quality indicators including total volatile basic nitrogen (TVB-N), trimethylamine (TMA-N), histamine, quality index (QI) and total viable count (TVC) were used to evaluate the quality changes of shrimp during storage. The results showed that the values of TVB-N, TMA-N, and histamine increased with storage time in two different phases, while QI value increased linearly with preservation time. The process of black spot formation in shrimp almost does not happen under vacuum storage conditions. The quality of shrimps treated with salts of organic acids or those packed in the vacuum bags was significantly higher than that of the control. In particular, the shelf-life of the sample packed in the vacuum packages was twelve days, which was four days longer than that of the control. The QI of control samples, NaP samples, NaL samples and vacuum samples were 14.43, 14.66, 14.85, and 11. 37 at the end of its shelf-life, respectively.

Keywords: *Penaeusmonodon*, sodium propionate, sodium lactate, vacuum packaging.

Classification numbers: 1.1.3, 1.4.4, 1.5.4.

1. INTRODUCTION

The black tiger shrimp (*Penaeus monodon*) is one of the most commercially important aquacultured shrimp species having persistent demand in the world market due to its distinct flavor, texture, and high nutritive value [1]. However, shrimps are quickly spoiled after harvest if not handled appropriately [2]. Shrimps are usually stored in ice after harvest, then frozen and distributed to the markets and the consumers. Therefore, the quality of shrimps may significantly change during storage in ice. Several methods have been studied to extend the shelf-life of shrimps including organic salts treatment [3], ozone treatment [4], modified atmosphere

packaging (MAP) [5], and vacuum packaging [6]. The change in the quality of shrimps undergoes two phases such as autolytic and degradation [7]. The spoilage of shrimps is mainly caused by three factors including enzymes, bacteria, and chemistry [8]. The effects of these factors significantly decrease the sensory, the chemical and the microbiological characteristics of shrimps [9]. Therefore, the methods of quality assessment must be based on those attributes of shrimps [9]. Several scientific studies have used the total volatile basic nitrogen(TVB-N), trimethylamine(TMA-N) indexes for seafood quality assessment [10]. The degradation process of proteins to form the biological amines was studied by Mietz *et al*. [11] and Veciana-Nogues *et al*. [12], who evaluated the quality of shrimps by quality index (QI) and biogenic amines index (BAI) in the following formulas.

$$QI = \frac{histamine + putrescine + cadaverine}{1 + \ spermidine + spermine}$$

$$BAI = (histamine + putrescine + cadaverine + tyramine).$$

Histamine is considered a critical chemical compound due to its toxicity. Many organizations around the world have carefully regulated the threshhold of histamine in seafood [13]. The sensory evaluation using quality index method (QIM) is the most preferable method as of today. The assessment methodology is implemented in the characteristics of each species; therefore, the quality of fishery products can be efficiently assessed compared to other methods [14]. Moreover, the QIM method can also estimate the remaining shelf-life of shrimps [15]. As a consequence, in Europe, this method is highly recommended for evaluation [9]. The spoilage of seafood after death is mainly caused by microorganisms [16]. Total viable count (TVC) is considered an important quality indicator, and is used by scientists to evaluate the quality of seafood [17, 18]

The aim of this study is to evaluate the effects of different treatments on the sensory, the chemical and the microbiological changes of shrimps during storage. Such indexes as TVC, QI (the quality score obtained from the QIM evaluation), TVB-N, TMA-N, and histamine were recorded each day during storage. The results of this experiment have provided a correlation between the quality indexes and the shelf life of the shrimp samples.

2. MATERIALS AND METHODS

2.1. Materials

Chemicals including histamine standard and TMA were obtained from Sigma –Aldrich (Singapore). Methanol, ethanol, toluene, picric acid, and trichlomethanol were provided by Merck Vietnam Ltd.

Black tiger shrimps were collected at three shrimp farms in Ca Mau province in October 2017. Harvested shrimps were intact and alive for this study. 30 kilograms of shrimps with the size of 35-40 pieces per kilogram were used. Shrimps were washed with water and distributed in 300 polyethylene bags. The sample bags were stored in a polystyrene box containing ice in the ratio of shrimps to ice being 1:2 (w/w) and transferred to the laboratory after 8 hours. At the laboratory, the polyethylene bags of shrimps were placed in a polystyrene box and shrimps were divided into 4 groups as followed:

- Control;

- Treated with 2.5 % sodium propionate (NaP);
- Treated with 2.5 % sodium lactate (NaL);
- Vacuum packaging (Vacuum).

Shrimps were immersed in 2.5 % sodium propionate (NaP) or 2.5 % sodium lactate for 10 minutes at 4 °C. All samples were stored at 0 ºC for the evaluation.

HPLC equipment including pump 600 Controller Serial # A9847454617, fluorescent detector Waters 474 Serial # M 996 CE 334T, and Millenniums software were used to evaluate the histamine content.

2.2. Methods

2.2.1. Determination of TVC

TVC was determined based on the previous report of Leboffe and Pierce [19]. 10 g of shrimps stored at different intervals were minced with 90 ml of 0.9 % NaCl solution and then centrifuged. Obtained solution was diluted into three different concentrations including 10^{-3}, 10^{-4} and 10^{-5}. TVC was assessed by the plate count agar method. The experiments were repeated three times and the TVC values were expressed as log CFU/g (colony forming units).

2.2.2. Quality index method (QIM)

The QIM assessment was based on the reported of Le Nhat Tam *et al*. [20]. The panel consisted of 6 experts developing QIM method for black tiger shrimp. The experts observed, described the characteristics of shrimp including color, texture and odor from fresh to natural spoilage. Then, experts will arrange terms into the table according to scale from 0 to 3 score with the decline of freshness. The quality of the shrimp was evaluated by the total score of all attributes.

2.2.3. Determination of TVB-N and TMA-N

The level of TVB-N in shrimps was determined according to Kolita Kamal Jinadasa [21]. After removing the shell and the head of shrimps, 5 grams of flesh was minced with 90 milliliters of perchloric acid using a blender (MX-SM1031S, Panasonic, Japan). Then, the solution was centrifuged and filtered with the Whatman No.1 filter paper (Sigma Aldrich, Germany) and diluted with distilled water to the mark of the 100ml volumetric flask. The distillation process was carried out in an alkaline medium. The composition in TVB was absorbed by 0.1 N NaOH and 0.1 N HCl used for titration.

The TMA-N content was determined according to AOAC 971-14 [22]. 10 grams ± 0.01 of shrimps was extracted with 30ml TCA solution 7.5 % (w / v.). The extraction was conducted three times. The obtained solution was centrifuged by the centrifuge (Hettich-EBA 20S, Sigma-Aldrich, Germany) at 4000 rpm for 10 minutes, then filled with distilled water to the mark of the 100 ml volumetric flask. Next, trimethylamine reacted with picric acid to form yellow pirate salt. This amount of pirate salt was determined by UV-Vis spectrometry, with a maximum absorption wavelength of 410 nm.

2.2.4. Determination of histamine

Histamine in black tiger shrimps was determined according to Gouygou *et al*. [23]. Histamine was extracted with ethanol, then purified and separated by SPE C_{18} column at 40 °C

(Agilent Technologies, USA) with 80 % ethanol mobile phase (flow rate 1 ml / min) as solvent elution. Fluorescent head (Waters 474, USA) wavelength λ_{EX} = 359 nm, λ_{EM} = 445 nm. Histamine formed fluorescent derivatives with *o*-phthalaldehyde (OPA) and 2-mercapto-ethanol.

2.2.5. Statistics

All experiments were conducted in three replicates. Obtained data were preprocessed with Microsoft Excel (version 2010) and analyzed using the Statgraphics centurion software. Linear model was fitted to readings. The significance level was adjusted to $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Total viable count (TVC) in shrimp

The initial number of microorganisms in shrimps was primarily from the breeding environment [24]. Figure 1 presented the change of TVC during storage.

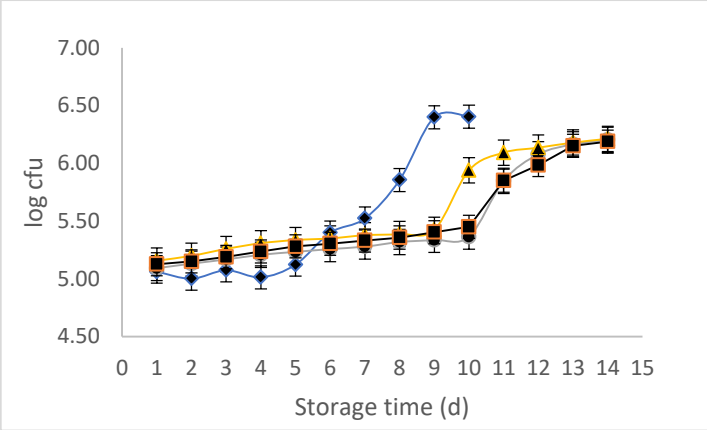

Figure 1. TVC in shrimps during storage.

Results showed that TVC values in the original samples were not significantly different. The TVC levels of samples at day 1 ranged from 4.98 to 5.15 log cfu/g, almost similar to the initial. TVC was stable during the first 5 days of storage for the control, 8 days for shrimps treated with NaP, and 6 days for NaL samples and vacuum packaging samples. Afterwards, the TVC values in the samples increased significantly and at different times, were higher than the threshold. The value of 6 log cfu/g is considered the limit allowed by the International Commission on Microbiological Specifications for Foods (ICMSF) for frozen shrimps. This threshold is also indicated in the agreement with the (Viet Nam) Ministry of Health Decision No. 46/2007 / QD-BYT. Thus, the shelf life of the control, NaP, NaL and vacuumed samples were 8, 10, 11 days, and 12 days, respectively. Two recent studies by Prasad Naik *et al*. [17], and Okpala *et al*. [25] on black tiger shrimp and white shrimp samples have indicated that the shelf life are 8 days, and 7 days. This difference might be due to the different species, seasons, harvest techniques, age and physiological conditions [9]. Dawson *et al*. have successfully extended the shelf-life of white shrimp to 10 days by using sodium sulfite and MAP (36 % CO_2 + 64 % N_2) [26]. Begum *et al*. also achieved 10 days of storage for freshwater prawns

(Macrobrachium rosenbergii) stored in ice by using 5 % formalin [27]. The effect of organic salts on the shelf life of shrimps was reported by Le Nhat Tam *et al*. [28].

3.2. Quality index method - QIM

The results of the sensory evaluation of four groups were shown in Fig. 2.

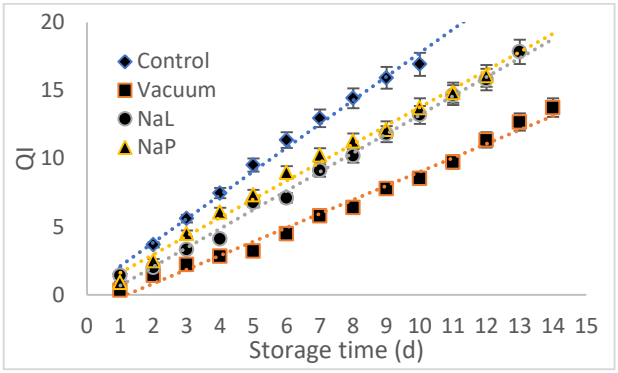

Figure 2. QI score during storage.

The QI scores of all four groups were significantly different between the storage time (Fig. 2). The quality of shrimps in all four groups at day 1 was almost unchanged compared to the initial, the QI of control, vacuum, NaL, and NaP samples were 1.53, 0.33, 1.42, 0.92, respectively. In contrast, the sensory attributes of the samples changed dramatically in the following days. Based on the results of the TVC, shrimps were not acceptable after 8 days. On day 8, the head was almost separated from the body of shrimp; the shell was separated from the flesh; the whole body was black; the meat was pink or yellow. Overall, shrimps had sour taste at this time. These signs of spoilage also appeared on day 12 with NaL samples, on day 13 with vacuumed samples, and on day 11 with NaP samples. The differences between the samples were that at the shelf-life, the score of the vacuumed samples was relatively low (11.37), the control (14.43), the NaL samples (14.66), and the NaP samples (14.85). This might be due to the absence of black spots. Black spots appeared in shrimps caused by PGBP (Peptidoglycan binging protein), LGBP (Lipopolysacharide and β-1,3-glucan binding protein) and BGBP (β-1,3-glucan binding protein) that stimulated the activities of PPO. Later, PPO catalyzed the conversion of phenols to colorless quinones oxidized forming black pigments called melanin [29]. In addition, NaP was better at inhibiting black spots formation than NaL. Black spots were also reported by Pardio *et al*. [30] and Nirmal *et al*. [31]. These authors investigated the effect of feruvicacis, ascorbic acid, citric acid, potassium sorbate, and 4-hexyl resorcinol solutions on shrimps (Panaeusaztecus).The concentration of 2.5% salt has also been used by Salam *et al*. in a study of changes in chemical and sensory properties as well as shelf-life determination of sliced salmon treated with salts of organic acids [32].

3.3. TVB-N and TMA

Figure 3 showed the results of total volatile basic Nitrogen, and trimethylamine during storage.

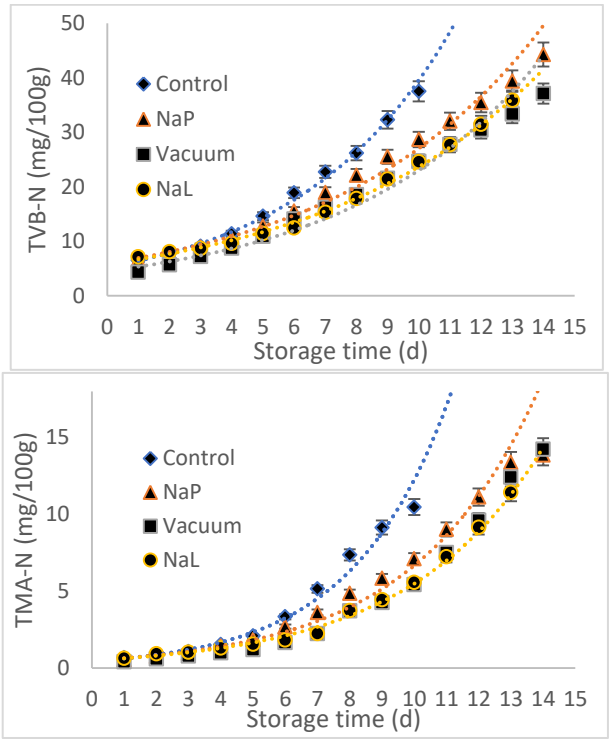

Figure 3. TVB-N and TMA-N content during storage.

TVB-N and TMA-N are important indicators in the assessment of the fishery quality [33]. The initial TVB-N values in all groups were almost the same, with an average value of 6.70 mgN/100 g. The formation of TVB-N could be divided in two different periods. The first stage occurred with slow rate and the next stage with fast rate. The first period for the control samples was from day 1 to 4; for vacuumed, NaL, and NaP samples were from day 1 to 5. Then, the amount of TVB-N increased rapidly in the samples. The results showed that the control had the fastest speed, followed by NaP, NaL and vacuumed samples. At the end of the shelf life, the TVB-N values in all samples were almost the same. The lowest value for the control was 26.17 mgN/100 g and the highest for the vacuumed sample was 30.39 mgN/100 g. The TVB-N values of all samples were less than 35 mgN/100 g, which was considered an acceptable limit for consumers [9].

TMA-N had the similar trend as TVB-N. The rate of TMA-N formation in the samples also occurred in two periods as shown in TVB-N (Fig. 3). The first stage was from day 1 to 4 for the control, from day 1 to 5 for NaP samples, from day 1 to 6 for NaL samples, and from day 1 to day 7 for vacuumed samples. Later, the TMA-N content increased rapidly in the samples. At the end of the shelf life, the TMA-N value was about 7.20 mg/100 g for the control, NaP, NaL. Vacuumed samples had the lowest value (5.42 mg /100 g).

The difference in the rate of TVB-N and TMA-N formation was due to the spoilage process of shrimps. The production of TVB-N and TMA-N was affected by bacteria. The spoilage process occurred in two major phases including autolysis and decomposition corresponding to slow and rapid periods [7]. The second stage was mainly caused by bacteria. Thus, with the exponential growth of bacteria, the content of TVB-N and TMA-N also increased rapidly. The

similar result was observed for TVC. These explanations are also relevant for histamine formation in the following section.

3.4. Histamine

Histamine is an important indicator in quality assessment of fisheries [20]. Histamine is formed from the decarboxylation reaction of acid amine histidine [34]. The histamine content was shown in Fig. 4.

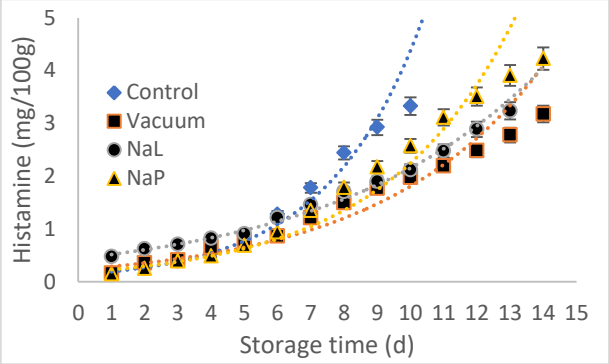

Figure 4. Histamine during storage.

Similar to TVB-N and TMA-N, histamine formation was slow during the first stage (until day 4 for the control, day 6 for NaP samples, day 7 for NaL and vacuumed samples) and fast in the later stage. At the end of shelf-the life (8 days for control samples, 10 days for NaP samples, 12 days for vacuum samples, and 11 days for NaL samples), the histamine level of control, NaP, vacuumed bag and NaL samples were 2.44 mg/100 g, 2.58 mg/100 g, 2.48 mg/100 g and 2.66 mg/100 g, respectively. These values were below the acceptable threshold of 50 mg/kg for histamine toxicity according to the international standards [35].

4. CONCLUSION

This study provides a crucial information for postharvest seafood processing. The effect of organic salts and vacuum packaging on the quality of shrimps at three farms during storage was investigated. Results have showed that vacuum packaging significantly improves the shelf life of postharvest shrimps (12 days), compared to the control (8 days). The changes in sensory characteristics and chemical compositions occurred in accordance with the changes in fisheries after death. The dramatic increase of TVB-N, TMA-N and histamine levels at the second stage followed the theoretical consideration. In particular, the slow increase in QI index of vacuumed samples was due to the inhibition of black spots formation in shrimps.

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