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PREVENTION OF MELANOSIS AND QUALITY LOSS OF PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*) BY ETHANOL *PERSICARIA ODORATA* EXTRACT DURING FROZEN STORAGE

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ABSTRACT

Ethanolic extract from *Persicaria odorata* leaf was applied to investigate the ability to preserve Pacific white shrimp (*Litopenaeus vannamei*) during frozen storage of 5 days at -21°C in comparison with 1.25% sodium metabisulfite. From evaluating the effects of *P.odorata* extract concentration and immersion time on melanosis formation, shrimps treated with the extract at 1/15 (mg/mL, w/v) in 10 minutes showed the lowest degree of melanosis. Microbiological analyses showed that Pacific white shrimp treated with *P. odorata* extract possessed lower values in total plate count and *Enterobacteriaceae* count compared with the control (p<0.05). pH and total volatile base content saw a lower increase in samples treated with *P. odorata* crude extract (p<0.05). Freshness loss, protein degradation, and melanosis growth in shrimps with crude extract treatments were impeded. The results show that *P. odorata* extract can be used a potential source of melanosis inhibitors, and natural preservatives for shelf-life extension of Pacific white shrimp during frozen storage.

1.Introduction

Pacific white shrimp (*Litopenaeus* vannamei) is a major cultured shrimp species, constituting 80% of the total shrimp production in the world (Sae-leaw & Benjakul 2018). This crustacean is rich in nutrients such as protein, minerals, amino acids, and fatty acids (Gunalan B et al. 2013), but it is highly perishable and has a limited shelf life (Na et al. 2018). The deterioration of shrimp is mainly associated with chemical, physical and microbial changes (Na et al. 2018), including melanosis (discoloration), the production of unpleasant odor and soft texture (Kim et al. 2020, Kustyawati et al. 2021). Melanosis caused by the activity of tyrosinase during storage, significantly diminishes the shrimp's market value and the consumer's acceptability (Sae-leaw & Benjakul 2018). Offflavors are reported to be caused by aldehydes, trimethylamine, ammonia, ketones, and sulfur compounds originating from the degradation of proteins and lipids by microorganisms. The softening of meat is also related to protein degradation by microbial spoilage (Kim et al. 2020).

To retard melanosis and maintain the quality of shrimp, 4-hexyl-1,3-benzenediol, sulfiting - based agents, and phosphates have been widely used (Sun et al. 2014). Nevertheless, due to regulatory attention and increasing consumer awareness of the health risks, the use of synthetic compounds is limited since they can result in allergic reactions in some sensitive individuals. Therefore, it is required that novel and safe alternatives should be discovered (Saeleaw & Benjakul 2018). Many studies on natural

products, especially plant phenolics with antioxidant and antimicrobial activities have been evaluated for that purpose (Nirmal & Benjakul 2009). It is reported that cashew leaf extract (Sae-leaw & Benjakul 2018), grape seed extract (Sun et al. 2014), pomegranate peel extract (Yuan et al. 2016a), green tea extract (Yuan et al. 2016b), Coffea arabica Sediment extract (Phan et al. 2021a) could inhibit melanosis and extend the shelf-life of Pacific white shrimp.

Persicaria odorata, commonly known as Vietnamese coriander, belongs to the family Polygonaceae. It is a tender perennial plant that is traditionally used in traditional Chinese medicine, pharmacy, cosmetics, and Asian cuisine (Ridzuan 2014). This plant is native to Southeast Asia and it grows best in tropical and subtropical areas which are damp and warm. Essential oils obtained from Persicaria odorata contain terpenes, aldehydes, alcohols, and fatty acids. and they possess antimicrobial, antioxidant, antitumor, and anti-inflammatory activities (Rebíčková et al. 2020). It is reported that it inhibits Salmonella choleraesuis (Fujita et 2015). Enterococcus faecium. Staphylococcus epidermidis, Staphylococcus aureus and Enterococcus faecalis (Chansiw et al. 2018).

In a study by Phan (2021b), P. odorata extract possessed potent antioxidant and antityrosinase activities and it proved to have the ability to inhibit the growth of black spots and maintain the quality of Pacific white shrimp during cold storage at 2°C. In this study, we aim to (i) evaluate the effects of plant extract concentration and immersion time on the development of melanosis and (ii) investigate the potentials of *Persicaria odorata* extract to prevent the formation of melanosis (measure the gray values of steamed shrimp), microbiological spoilage, physicochemical deterioration during 5 days of frozen storage at -21°C.

2. Materials and methods

2.1. Materials and preparation extract

2.1.1.Chemicals

Sodium metabisulfite (SMS), Ethanol, were obtained from Merck (Darmstadt, Germany). Other chemicals in the study were of analytical grade.

2.1.2.Shrimp collection and treatment

Alive Pacific white shrimps (*L. vannamei*) (with the size of 30-40 shrimps/kg) were purchased from Thu Duc market in Ho Chi Minh City, Viet Nam. The shrimps were kept alive and immediately transported to the laboratory at HCMC University of Technology Education, Viet Nam within 1 h. The shrimp samples were washed with water. The samples were divided into 3 equal parts, each of which was immersed in the *P. odorata* extract solution, the sodium metabisulfite solution (SMS, 1.25%, w/v), and distilled water (as the control), in the shrimp/solution ratio of 1:2 (w/v) at 4°C for 10 min. After being removed from the solutions, the samples were drained on a sieve at 4°C for 3 min. After that, the shrimps were put in plastic bags and stored at -21 °C for 5 days after, thawing for 30 minutes at room temperature 32 ± 5°C which were analyzed for different assessments: melanosis development, changes, total volatile basic nitrogen (TVB-N), shear force, and microbiological analysis

2.1.3. Preparation extraction of P. odorata

The leaves of *P. odorata* bought from Thu Duc Market, Thu Duc District, Ho Chi Minh City, Viet Nam were thoroughly washed under tap water to remove impurities on the surface. The collected material was dried to constant weight in a hot air-blowing oven (Memmert, Germany), and then ground in a blender to obtain a homogenous fine powder.

100 ml of ethanol was mixed with 50 g of dry powder at room temperature for 24h. The mixture was stirred thoroughly and filtered. The filtrate was collected in a flask. The residue was mixed again with ethanol and filtered again, the filtration was repeated one more time. Subsequently, the extract was concentrated in a rotary evaporator (Yamamoto, Japan) to obtain the *P. odorata* crude extract (CE).

2.2. Survey of shrimp storage conditions

Before performing frozen storage, we served shrimp storage conditions, including immersion time and P. odorata extract concentration. These experiments were evaluated after 5 days of storage at -2°C and thawing for 30 minutes at room temperature (32°C \pm 2°C). Gray values were criteria used to evaluate survey storage conditions.

2.2.1.Effect of extract concentration

The effect of *P. odorata* extract concentration on the formation of melanosis of shrimps after 5 days of frozen storage was carried out to find an optimum concentration at which the growth of black spots was minimal. Briefly, shrimps were immersed in the extract solutions at six concentrations of 0, 1/30, 1/25, 1/20, 1/15, and 1/10 mg/mL (*w/v*). After 5 days of storage, the photos of shrimps were carefully taken to calculate the gray mean values that indicate levels of melanosis growth.

2.2.2.Effect of immersion time

Shrimps were dipped into the *P. odorata* solutions (at 1/15 mg/mL, w/v) for 3, 5, 10, and 15 minutes, after which they evaluated their gray mean values.

2.3. Assessment of shrimp's quality during frozen storage

Shrimp samples were frozen at -21°C for 5 days and thawed at room temperature (32°C \pm 2°C) after 30 minutes. pH values, physicochemical and microbiological properties, and melanosis development at 0, 1, 3, and 5 days were assessed during the frozen storage.

2.3.1.Melanosis assessment

All shrimp samples were defrosted at room temperature ($32^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for 30 min and then steamed at 100 °C for 3 min to make it easier for sensory evaluation before being photographed using a digital camera (Canon Eos M10, Japan). The change of color in the obtained photographs was analyzed by ImageJ software to calculate mean gray values. The lower mean gray value indicates a higher level of melanosis in the carapace of shrimp (Phan et al. 2021a).

2.3.2.pH evaluation

pH values of shrimps were measured according to the method of Chouljenko et al.

(2017). 2 grams of shrimp were homogenized with 20 mL of deionized water in a homogenizer for 1 min (Kinematica AG, CH-6014, Littau/Luzern, Switzerland). Following this, the samples were kept for 5 min at room temperature. The pH values were determined by a pH meter (Sartorious, Edgewood, USA). All measurements were conducted in triplicate.

2.3.3. Microbiological analysis

The microbiological quality of shrimps was evaluated according to the bacterial count method in three aspects: Total plate count (ISO 4833-1:2013), *Enterobacteriaceae* count (ISO 21528-2:2017), and *Pseudomonas aeruginosa* count (3347/2001/QĐ-BYT). The colonies formed were counted and expressed as log CFU/g of weight.

2.3.4.Physicochemical analysis

Protein. The protein contents of shrimps were determined according to AOAC Official Method 992.15. All experiments were conducted at Center of Analytical Services and Experimentation (CASE, Ho Chi Minh City, Viet Nam).

Shear force. The shear force of samples during 5 days of storage was tested using the Rheo Tex SD 700II texture analyzer (Sun Scientific, Japan) at Research Institute for Aquaculture No 2 (RIA2, Ho Chi Minh City, Viet Nam) with a cross head speed of 10 mm/s.

Total volatile basic nitrogen (TVB-N). TVB-N was assessed according to the national standard (TCVN 9215-2012) at the Center of Analytical Services and Experimentation (CASE, Ho Chi Minh City, Viet Nam). The total volatile basic nitrogen values were reported as mg N/100 g of shrimp.

2.3.5.Statistical analysis

All results were expressed as mean \pm SD. Experimental data were analyzed by the analysis of variance (one-way ANOVA) with Tukey's test (p < 0.05). Statistical analyses were conducted using an SPSS package (SPSS 26 for Windows Evaluation Version, IBM Corporation, New York, USA).

3. Results and discussions

3.1. Shrimp storage conditions

3.1.1.Effect of extract concentration

The effect of *P. odorata* extract on the growth of melanosis, as demonstrated by decreased mean gray values of the carapace of shrimps after 5 days of frozen storage is illustrated in Figure 1. Overall, it is clear the mean gray values of shrimp samples rose significantly when the concentrations of extract increased, indicating that the growth of black spots in all shrimp samples was inhibited with the presence of plant extract (p<0.05). As the concentration progressed, the mean gray values

increased to reach a peak of 141.68 at the concentration of 1/15 (mg/mL), after which the figure started to decline at 1/10 (mg/mL) (p<0.05). Therefore, the concentration of crude extract at 1/15 (mg/mL) was chosen in this study to treat all shrimp samples for other experiments. Nirmal et al. (2011) reported that phenolic compounds in the plant extract at a high dose were likely to cross-link the proteinaceous tissues of Pacific white shrimp at which the polyphenoloxidase (PPO) was located, thereby limiting the penetration of these substances to inactivate the activity of PPO.

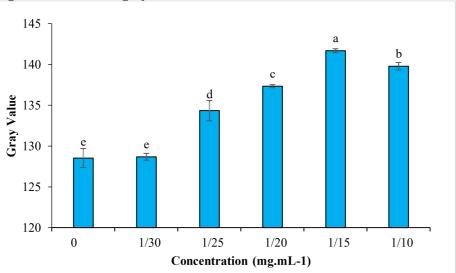


Figure 1. Changes in the mean gray values of *L. vannamei* treated with *P. odorata* extract at different concentrations during frozen storage. Values represent the mean \pm standard deviation (n=3). Different lower-case letters represent statistically significant differences (p<0.05).

3.1.2.Effect of immersion time

Figure 2 represents the gray mean values of shrimp treated with *P. odorata* extract at different immersion times. As can be seen from the chart, in all samples surveyed, the longer the shrimps were immersed, the more effective the retardation of black spots was. However, there

were no statistically significant differences in the gray values among samples treated with the plant extract in 10 and 15 minutes and they showed the highest mean gray values (p>0.05). Therefore, in this study, 10 minutes of immersion was chosen to treat shrimps in succeeding experiments.

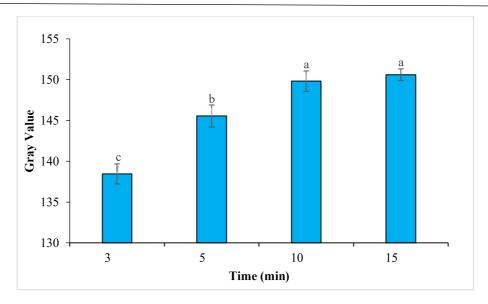


Figure 2. Changes in the mean gray values of *L. vannamei* treated with *P. odorata* extract at storage time during frozen storage. Key: see the caption for Figure 1.

3.2. Shrimp's quality during frozen storage 3.2.1.pH measurement

The changes in pH values of Pacific white shrimp with various treatments: CE, and SMS during frozen storage at -21°C compared with the control (treated with distilled water) are represented in Figure 3. In general, the pH values of all shrimps saw a rise (p<0.05) over the period shown, and the figures for the CE treatment batches increased to a lesser extent. After 5 days of frozen storage, a 15% increase was observed in pH values of shrimps without treatments (the control) whilst the increase in shrimps preserved in SMS and CE was lower at 11.2 and 10%, respectively. At day 0, no significant differences were found among pH values from all groups (p>0.05). After 1 day of storage, the pH level began to grow and the CE

batches had the lowest pH at 6.62, followed by shrimps treated with SMS at 6.71 and the control at 6.89 (p<0.05). This pattern was also the case for day 3 and day 5. At the end of the period, pH of the control reached 7.40 while those of the **SMS** and CE treatment batches were considerably lower at 7.36 and 7.29, respectively. It should be noted that pH change connected with the was accumulative development of basic substances, mainly as a result of either microbial or endogenous enzyme activities (Huang et al. 2012, Nirmal & Benjakul 2009). The lower degree in the pH rise of shrimps immersed in the SMS and CE solutions was consistent with the lower microbial count (Figure 6). In sum, the lower pH changes in the CE treatment batches indicated that the ethanolic extract of P. odorata could inhibit endogenous or microbial enzyme activities.

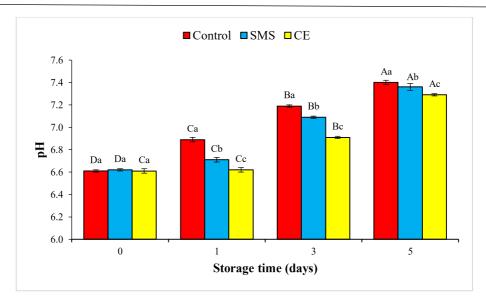


Figure 3. Changes in pH of *L. vannamei* during frozen storage. Values represent the mean \pm standard deviation (n=3). Control: no treatment, SMS: samples treated with sodium metabisulfite (1.25%), CE: samples treated with *P. odorata* extract. Different lower-case letters within the same storage time in the same column indicate statistically significant differences (p < 0.05). Different uppercase letters within the same treatment in the same column indicate statistically significant differences (p<0.05).

3.2.2.Melanosis evaluation

Figure 4 represents the mean gray values and Figure 5 illustrates the photographs of Pacific white shrimp with different treatments during frozen storage. In general, the gray mean values of all samples at all different storage times saw a downward trend, but shrimps preserved in CE and 1.25 wv% SMS solutions decreased to a lesser extent than those treated with distilled water (the control) (p<0.05). At day 0 and day 1, all samples had no statistically significant differences in the gray mean values (p>0.05). As time progressed, the mean gray values started to decline continuously after 1 day of storage, indicating the formation of melanosis, but there were still no differences in the gray values for the SMS and the CE (p>0.05). At day 5, the shrimps treated with CE had the highest mean gray values, followed by samples treated with This result showed that P. SMS (p < 0.05).

odorata extract impeded the growth of black spots in shrimps. This finding is consistent with our previous research that this plant extract had high antioxidant and antityrosinase activities (Phan 2021b). According to Kumar et al. 2011, copper-containing tyrosinase, a enzyme, catalyzes the formation of melanin from tyrosine by oxidative processes and phenolics in the plant extract are likely to inactivate this enzyme by interacting with its active site via hydrogen bonding or hydrophobic interactions. In a previous study, by HPLC-EIS-MS analysis, the P. odorata extract contained 36 compounds, 22 of which were phenolics (Phan 2021b). Therefore, the presence of phenolic compounds, especially flavonoids, may involve in the antioxidant and tyrosinase inhibition activities and the ability of melanosis inhibition.

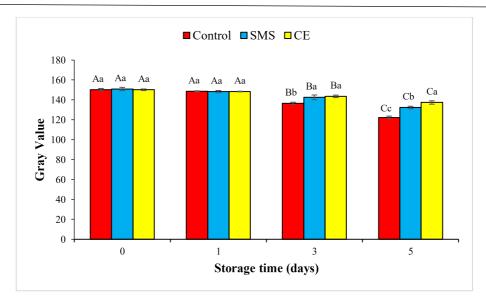


Figure 4. Changes in the mean gray values of the carapace area of *L. vannamei* during frozen storage. Key: see the caption for Figure 3.

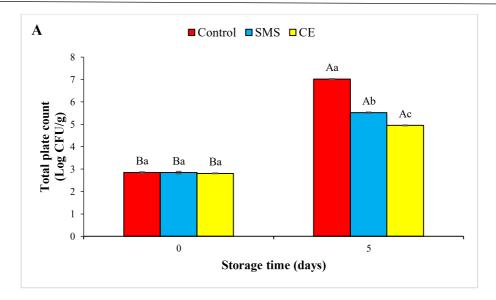


Figure 5. Photos of *L. vannamei* without and with different treatments at day 5 of frozen storage, defrosted at room temperature $(32^{\circ}C \pm 2^{\circ}C)$ for 30 min, and steamed for 3 min.

3.2.3. Microbiological analysis

The microbiological spoilage in shrimp samples after 5 days of frozen storage at -21°C is illustrated in Figure 6. It is obvious that at day 0, no significant difference was found in total (Figure counts (TPC) plate 6A) Enterobacteriaceae counts (EBC) (Figure 6B) in all treatment batches (p>0.05). In general, after 5 days of frozen storage, the TPC and EBC of all samples were greater than those on day 0 (p<0.05). The initial values of TPC in this study ranged from 2.81 to 2.85 Log CFU/g which agreed with the TPC of 2-3 Log CFU/g given by other studies in Pacific white shrimp (L. vannamei) (Arancibia et al. 2015) and in pink

shrimp (*Parapenaeus longirostris*) (López-Caballero et al. 2002). After 5 days of storage, the TPC of SMS, CE treatment groups were 5.52, 4.95 Log CFU/g, respectively which were smaller than those of the control group (7.02) (p<0.05). According to the Viet Nam national standard TCVN 5289: 2006, the acceptable limit of total aerobic microorganisms for frozen aquatic products is 6.0 Log CFU/g. Hence, all microbial counts of shrimps treated with SMS, and CE met the requirements of this standard, whilst the values of the control were higher than this limit.



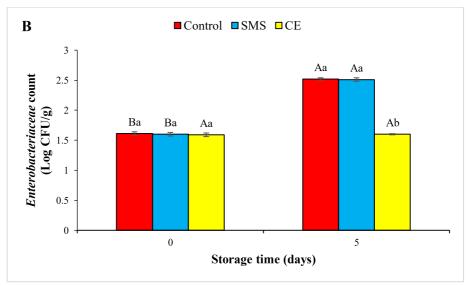


Figure 6. Total plate count (A) and Enterobacteriaceae count (B) of *L. vannamei* during frozen storage. Key: see the caption for Figure 3.

Notably, *Pseudomonas aeruginosa c*ounts of all treatment batches were lower than the detection limit of 1.0 Log CFU/g at both day 0 and day 5 (not illustrated in figures). A study by Shiekh et al. (2019) indicates that *P. aeruginosa* counts of Pacific white shrimp preserved with Chamuang leaf extract were observed in the range of 2.15–2.18 Log CFU/g at day 0 and 5.08-6.82 Log CFU/g at day 10. Nevertheless, counts are dependent on postmortem conditions

and the area in which shrimps lived (Huang et al. 2012).

Another noticeable point worth mentioning is that shrimps treated with CE possessed the lowest EBC of 1.60 Log CFU/g in comparison with other treatment batches (p<0.05) during frozen storage at -21°C. *Enterobacteriaceae* are a family of Gram-negative bacteria, including a large number of pathogens, namely Salmonella, Escherichia coli, Shigella, Klebsiella, and Yersinia pestis (Rai et al. 2020). Besides,

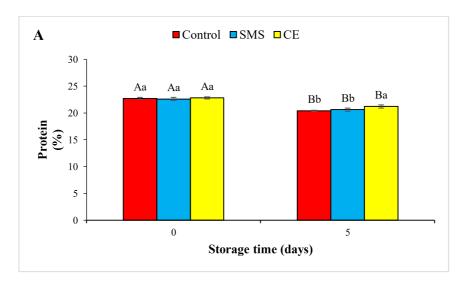
Ibrahim Sallam (2007) noted that the spoilage ability of *Enterobacteriaceae* should be taken into consideration especially for polluted water or lack of frozen post-harvest storage. The results show that the *P. odorata* extract could be used to retard the growth of spoilage microorganisms during frozen storage.

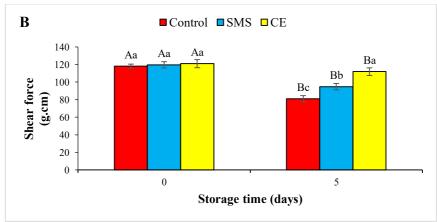
3.2.4. Physicochemical Changes

Physicochemical properties including protein content, shear force, and total volatile basic nitrogen (TVB-N) after 5 days of frozen storage were evaluated and displayed in Figure 7.

According to Figure 7A, the protein content of shrimps immersed in SMS was comparable to that of the control, in the range of 20.4-20.6 (p>0.05) and lower than the value of CE

(p<0.05), suggesting that P. odorata extract lowered protein loss and that it could replace SMS in preventing this deterioration. believed that the weakening of myofibrils and intramuscular tissues leads to protein loss in shrimps when proteases like cathepsins are released (Pan et al 2019). In literature, there is not much information available on the deterioration of shrimp protein during chilled storage. A study by Kamal et al. (2000) reported that the protein contents declined from 18.46 to 17.05% for giant freshwater prawn (Macrobrachium rosenbergii) and 18.06 to 16.85% for tiger shrimp (Penaeus monodon) after 10 days of iced storage.





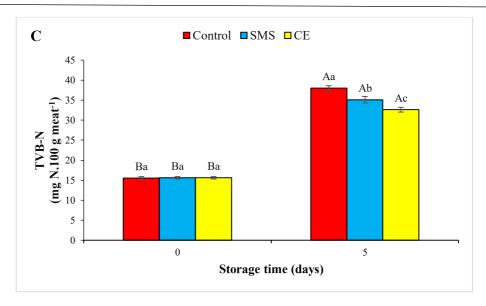


Figure 7. Protein content (A), Shear force (B), and Total volatile base (TVB-N) content (C) of *L. vannamei* during frozen storage. Key: see the caption for Figure 3.

Figure 7B represents that all treatment groups had a similar shear force in the range of 118 - 121 g.cm (p>0.05) at day 0. In addition, a higher shear force of shrimps stored with CE was found, compared to those of the SMS and the control after 5 days of frozen storage (p<0.05). It is noticeable that the higher shear force of the shrimps in CE treatment batches was in agreement with the lower microbial counts, illustrating that CE inhibited the microbial spoilage and limited collagenase and proteinases are mainly accountable deterioration of muscle protein (Nirmal et al. 2009). The changes in shrimp texture can be caused by many factors: pH, the myofibrillar proteins, and degradation of connective tissues (Yuan et al. 2016a). The texture loss in Pacific white shrimps (L. vannamei) was retarded when they were treated with 0.05% catechin and 0.1% catechin (Nirnal et al. 2009) and coated with chitosan and pomegranate peel extract (Yuan et al. 2016a), chitosan-gelatin (Farajzadeh et al. 2016), chitosan-carvacrol (Wang et al. 2018).

Figure 7C shows the values of TVB-N of Pacific white shrimp during frozen storage at -21°C. It is clear that TVB-N contents of all treatment groups were not statistically different at day 0 (p>0.05). After 5 days of frozen storage,

shrimp treated with SMS, CE showed similar TVB-N values in the range of 32.6 to 35.1 mg N/100g shrimp meat (p>0.05) which were smaller than that of the control (38 mg N/100g) (p<0.05). It should be noted that P. odorata extract could lower the formation of TVB-N and that TVB-N contents were consistent with microbial counts. A delay in the growth of TVB-N was reported when Pacific white shrimp was stored with Chamuang (Garcinia cowa Roxb.) leaf extract in combination with a pulsed electric field (Shiekh et al. 2019), 0.05 or 0.1% chitosan solutions (Nirmal et al. 2009), grape seed extracts (Sun et al. 2014), coated with 1-1.5% Ocarboxymethyl chitosan and 1-1.5% chitosan (Huang et al. 2012), chitosan combined with pomegranate peel extract (Yuan et al. 2016a) and green tea extract (Yuan et al. 2016b). Different authors proposed different acceptable limits of TVB-N. To be specific, TVB-N content of 30 mg/100 g shrimp meat was chosen for a quality safety standard by Shiekh et al. (2019) and Huang et al. (2012) while higher limits of 42 mg/100 g of, 40 mg N/100 g was suggested for cephalopods (Altissimi et al. 2018), shrimps (Mendes et al. 2001), respectively; and the European Commission (EC) had a TVB-N limit of 35 mg/100g fish (Asensio et al. 2019). TVB-

N is widely used to evaluate the quality of seafood products and is an indicator of chemical and microbial changes (Huang et al. 2012). The increase of TVB-N content is due to the degradation of nitrogenous substances such as proteins, peptides, amino acids, and nucleotides (Pan et al. 2019).

4. Conclusions

The results of the study indicated that the ethanolic extract of *P. odorata* leaf was effective in preventing melanosis, microbial growth, and physicochemical deterioration of Pacific white shrimp during frozen storage. The samples were soaked at the concentration of 1/15 mg. mL⁻¹ for 10 minutes before they were stored at -21°C for 5 days of storage. Furthermore, the efficacy of *P. odorata* extract was greater than that of sodium metabisulfite solution. Therefore, it has a promising potential to replace sulfite additives, which have a large number of adverse health effects, contributing to maintaining food safety in the shrimp industry, and adding value to the economy.

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