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Original research paper

ANTIOXIDATIVE AND ANTIMELANOSIS EFFECTS OF STRAW MUSHROOM EXTRACT AND CHITOSAN ON REFRIGERATED PACIFIC WHITE SHRIMP

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Abstract: The present study evaluated the efficacy of combining chitosan (CS) and straw mushroom extract (ME) in preventing melanosis formation and lipid oxidation in Pacific white shrimp (*Litopenaeus vannamei*) during refrigerated storage. The study found that treatment with either CS, ME, or a combination of CS and ME (CS+ME) significantly reduced both melanosis formation and lipid oxidation when compared to control shrimp without treatment. Shrimp treated with CS+ME had lower melanosis scores and TBARS values than shrimp treated with either CS or ME alone, indicating that the combination treatment (CS+ME) was the most effective for reducing melanosis formation and lipid oxidation. Additionally, there was no significant difference in melanosis or sensory scores between shrimp treated with CS+ME and those treated with sodium metabisulfite (SMS). These results indicate that treating shrimp with CS+ME is an effective way to use natural ingredients instead of synthetic SMS to prevent melanosis formation and lipid oxidation in shrimp during refrigerated storage. The present study's findings offer a more natural approach to the preservation of shrimp.

Key words: *black spots, ergothioneine, lipid oxidation, polyphenol oxidases, post-harvest handling*

INTRODUCTION

Pacific white shrimp (*Litopenaeus vannamei*) is a commercially important shrimp species that is widely reared in Vietnam and has a high-demand community around the world. During postharvest handling and storage of shrimp, controlling the formation of melanosis in shrimp is a challenge for farmers and producers. Melanosis, so-called black spots, is a natural biochemical process that occurs in *post-mortem* crustaceans due to the presence of polyphenol oxidase (PPO). PPO catalyzes the conversion of phenolic substrates into quinones, which are then auto-oxidized and polymerized to form melanin, a dark pigment with a high molecular weight that accumulates largely beneath the carapace of the cephalothorax (Nirmal & Benjakul, 2011a). Although the formation of black spots in shrimp does not affect the health of consumers, it affects the appearance of shrimp, reducing their sensory quality and commercial value, causing a significant monetary loss (Gómez-Guillén, Martínez-Alvarez, Llamas & Montero, 2005). Controlling melanosis in post-harvest shrimp is inevitable in decreasing the financial loss. Several approaches have been used to control

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melanosis in shrimp, including proper handling, cold storage, and chemical treatments. Treatment with sulfite compounds is the most often-used method for controlling melanosis in shrimp (Andrade et al., 2015). However, sulfite compounds have adverse health effects, so their use in food is strictly controlled by regulations (Hardisson, Rubio, Frías, Rodríguez, & Reguera, 2002). In addition, today's consumers tend to prefer to use foods that contain natural bioactive compounds, such as extracts from herbs, spices, mushrooms, and other edible materials. The use of natural bioactive compounds to control melanosis in shrimp is, therefore, necessary to meet these consumers' needs. Previous studies reported that melanosis development in shrimp could be controlled by extracts from certain plants such as pomegranate peel (Basiri, Shekarforoush, Aminlari & Akbari, 2015), acerola fruit (Gonçalves, Oliviera & Abrantes, 2015), grape seed (Sun, Lv, Yuan & Fang, 2014), avocado (Phan, Bui, Doan, Nguyen & Ly, 2021), tea (Nirmal & Benjakul, 2011b), lead seed (Nirmal & Benjakul, 2011a), and mushrooms (Encarnacion, Fagutao, Hirono, Ushio & Ohshima, 2010). Phenolics and amino acids such as ergothioneine (EGT), and mimosine were indicated to have anti-melanosis activity *in vitro* as well as in shrimp. Especially, EGT has long been recognized as a potent radical scavenger and is considered a longevity vitamin (Beelman, Kalaras, Phillips & Richie, 2020). Therefore, the use of mushroom extracts containing EGT for controlling melanosis in post-harvest shrimp has been interesting. Hai et al. (2013) reported that the hydrophilic extract prepared from straw mushroom (*Volvariella volvacea*) containing EGT had antioxidant activity and PPO inhibitory activity *in vitro*, as well as significant inhibition of melanosis development and lipid oxidation in shrimp (Bao, 2014; Bao & Hai, 2022). Since the bioactive compounds found in the hydrophilic mushroom extract are water-soluble, they are easily lost during shrimp storage. Therefore, controlling the release of these bioactive compounds during shrimp storage is necessary to retain the inhibitory effects on melanosis development and lipid oxidation, thereby reducing the storage cost. Chitosan is a natural substance with antioxidant and antimicrobial activities that has been shown to inhibit melanosis and prolong the shelf life of shrimp (Bingöl et al., 2015; Yuan, Zhang, Tang & Sun, 2016; Farajzadeh, Motamedzadegan, Shahidi & Hamzeh, 2016; Chen et al., 2022). In addition, chitosan can be used as a film-forming agent to control the release of water-soluble bioactive compounds (Muñoz-Tebar, Pérez-Álvarez, Fernández-López & Viuda-Martos, 2023). Thus, treating shrimp with a combination of chitosan and straw mushroom extract can retain the water-soluble bioactive compounds in the mushroom extract during shrimp storage. This can boost the inhibitory effects on melanosis formation and lipid oxidation in shrimp, which can improve shrimp quality and reduce storage costs. Therefore, the present study was designed to evaluate the effectiveness of using a combination of chitosan and straw mushroom extract to inhibit melanosis formation and lipid oxidation in Pacific white shrimp under refrigeration.

MATERIALS AND METHODS

In accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purpose, crustaceans, including shrimp, are not considered subjects that require ethical approval or consent.

Materials and chemicals

Pacific white shrimp (*Litopenaeus vannamei*) of 60–70 pcs/kg were purchased from an intensive farm in Khanh Hoa, Vietnam. The shrimp were kept alive during their transport to Nha Trang University's Seafood Processing Laboratory. The fresh fruiting body of straw mushroom (*Volvariella volvacea*) was purchased from a farm in Khanh Hoa, Vietnam, following two months of cultivation. Food-grade low-molecular-weight chitosan produced from shrimp shells (viscosity ≤ 150 cPs, deacetylation ≥ 85%) was supplied by Vietnam Food (VNF) Company, Vietnam. The analytical-grade chemicals were purchased from Sigma-Aldrich (St. Louis, Mo., USA).

Mushroom extract preparation

Straw mushroom extract (ME) was prepared in accordance with our previously established method with a slight modification (Bao et al., 2010). The mushroom fruiting bodies were blended with distilled water in a 1 to 10 (w/v) ratio using a BlueStone blender model BLB-5339 (BlueStone Singapore). Subsequently, the mixture was incubated at 95 ± 2 °C for 1 h in a water bath before being centrifuged at

5000 rpm for 30 min at 5 °C. The supernatant was collected and evaporated under vacuum at 40 °C. The residue was suspended in 70% (v/v) aqueous ethanol, vortexed, and allowed to stand at 4 °C for 2 hours before being centrifuged at 5000 rpm for 30 min at 5 °C. To remove ethanol, the supernatant was collected and evaporated under vacuum at 40 °C. Finally, the ethanol-free residue obtained from 100 g of the mushroom fruiting bodies was dissolved in 10 mL of distilled water. As a consequence, 10 g of the mushroom fruiting bodies yielded 1 mL of ME.

Shrimp treatment

The shrimp were divided into five groups at random. Each group was then separately soaked in the following cold solutions: mushroom extract 1 % (ME), chitosan 1% in acetic acid 1% (CS), a mixture combining 50% volume of chitosan 1 % in acetic acid 1 % and 50% volume of mushroom extract 1% (CS+ME), sodium metabisulfite 1.25% (SMS), and distilled water (Control). The soaking was conducted in a 1 to 2 (w/v) ratio for 10 minutes at 4 ^{o}C. After soaking, ten shrimp were packed in zipper-sealed polyethylene bags 240 × 170 × 0.08 mm, and stored in a refrigerator at 4 ^{o}C or lower for up to 10 days to evaluate melanosis development, lipid oxidation, and sensory quality. All treatments were performed in triplicate.

Determination of PPO inhibitory activity

PPO inhibitory activity was determined following the method of Jang, Sanada, Ushio, Tanaka and Ohshima (2003) with a slight modification. Briefly, a reaction mixture containing 2.9 mL phosphate buffer (50 mM, pH 6.8), 0.1 mL catechol (500 mM), 0.1 mL L-proline (500 mM), and 0.1 mL PPO (50 units/mL). A corresponding volume of the test solution was used to replace the buffer solution in the reaction mixture to determine its PPO inhibitory activity. The absorbance of the reaction mixture at 530 nm (A_{530}) was measured after 5 min at 25 °C using a Biochrom Libra S50 UV/VIS spectrophotometer (Cambridge, UK). The PPO inhibitory activity of the test sample was calculated as follows:

$$\text{PPO inhibitory activity, \%} = \left(\frac{A_0 - A}{A_0}\right) \times 100$$

where A = A_{530} of the test sample and A_0 = A_{530} of the control. The half maximal inhibitory concentration (IC50) was determined by creating a graph that plots various con-centrations of the test solution against the percentage of PPO inhibitory activity.

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined following the method of Binh, Bao, Prinyawiwatkul and Trung (2021) with a slight modification. Briefly, a reaction mixture containing 1 mL DPPH methanol solution (0.1 mM), 1 mL methanol, and 2 mL distilled water. A corresponding volume of the test solution was used to replace distilled water in the reaction mixture to determine its DPPH radical scavenging activity. The absorbance of the reaction mixture at 517 nm (A_{517}) was measured after 30 min at 25 °C using the spectrophotometer. The DPPH radical scavenging activity of the test sample was calculated as follows:

$$\text{DPPH radical scavenging activity, \%} = \left(\frac{A_0 - A}{A_0}\right) \times 100$$

where A = A_{517} of the test sample and A_0 = A_{517} of the control. The half maximal inhibitory concentration (IC50) was determined by creating a graph that plots various concentrations of the test solution against the percentage of DPPH radical scavenging activity.

Determination of lipid oxidation inhibitory activity

Lipid oxidation inhibitory activity was determined following the method of Binh et al. (2021). Briefly, 0.2 mL of phosphate buffer (50 mM, pH 7.4) containing 50 µM metmyoglobin and 100 µM H_2O_2, 0.2 mL of Tween 20 containing 50 µM eicosapentaenoic acid, and 0.1 mL of the test solution were added to a test tube. A corresponding volume of the buffer solution was used to replace the test solution in the control reaction mixture. After incubating at 37 ^{o}C for 30 min, the absorbance at 535 nm of the reaction products in n-butanol (A_{535}) was measured with the spec-trophotometer. The lipid oxidation inhibitory activity of the test sample was calculated as follows:

$$\text{Lipid oxidation inhibitory activity, \%} = \left(\frac{A_0 - A}{A_0}\right) \times 100$$

where A = A_{535} of the test sample and A_0 = A_{535} of the control. The half maximal inhi-

bitory concentration (IC50) was determined by creating a graph that plots various concentrations of the test solution against the percentage of lipid oxidation inhibitory activity.

Evaluation of melanosis development in shrimp

Melanosis development in shrimp was visually evaluated using a panel of five members. Panelists were trained on a scale of 0 to 10, with 0 denoting absence, 2 being slight (affecting up to 20% of the shrimp's surface), 4 being moderate (affecting 20–40% of the shrimp's surface), 6 being notable (affecting 40–60% of the shrimp's surface), 8 being severe (affecting 60–80% of the shrimp's surface), and 10 denoting extremely heavy (affecting 80–100% of the shrimp's surface).

Color measurement

The color (L*a*b*) of both sides of the shrimp was measured by a Konica Minolta CR-400/CR-40 (Japan). The whiteness index (WI) was calculated as follows:

$$WI = 100 - \sqrt{\left(100 - L^*\right)^2 + \left(a^*\right)^2 + \left(b^*\right)^2}$$

The color difference (ΔE) was calculated as follows:

$$\Delta E = \sqrt{\left(L_0^* - L^*\right)^2 + \left(a_0^* - a^*\right)^2 + \left(b_0^* - b^*\right)^2}$$

where L_0^*, a_0^*, b_0^* values were of the initial fresh shrimp prior to treatment; L^*, a^*, b^* values were of the compared shrimp.

Sensory evaluation

The sensory quality of shrimp was evaluated using the sensory scheme described by Luo, Xu and Ye (2015), with scores ranging from 10 (best quality) to 1 (worst quality). A panel consisted of five trained panelists who volunteered to participate in the evaluation. All panelists were fully informed of the study's objective, procedures, potential hazards, and benefits and they were aware of their ability to withdraw at any time. Panelists with shrimp allergies or other dietary restrictions were excluded to avoid potential health hazards. Panelists' personal information is kept secret.

Determination of thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) were determined according to the method described by Uchiyama and Mihara (1978). In brief, the reaction mixture, consisting of 0.5 mL of homogenate from 0.5 g of minced shrimp with 4.5 mL of 1.15% KCl, 0.3 mL of 1% phosphoric acid, and 1 mL of 0.6% 2-thiobarbituric acid, was thoroughly mixed in a screw-capped test tube and incubated at 95 °C for 45 minutes. After cooling, 4 mL of n-butanol was added, and the mixture was vortexed and centrifuged at 3000 rpm for 10 minutes. Absorbances were measured at 535 nm and 520 nm using the spectrophotometer. The TBARS value was calculated as the difference between these absorbances, with a calibration curve established using 1,1,3,3'-tetraethoxypropane. TBARS values are reported as malondialdehyde (MDA) equivalents.

Statistical analysis

Microsoft Excel 2013 was used to calculate the means and standard deviations for all multiple measurements and to generate graphs. R software version 4.2.2 (http://cran.R-project.org) was used to analyze the data. A two-way ANOVA was used to identify significant differences, and Tukey's Multiple Comparisons of Means was used to identify significant differences between samples at $p < 0.05$.

RESULTS AND DISCUSSION

PPO and lipid oxidation inhibitory activities of ME and CS

The antioxidant and PPO enzyme inhibitory activities of extracts from straw mushrooms have been reported in a number of previous studies (Hai, Tri & Bao, 2013; Bao, 2014; Bao & Hai, 2022; Park, Kwon & Lee, 2015). Many mycochemicals, including phenolic compounds, EGT, vitamin C, vitamin E, glutathione, beta-glucans, flavonoid compounds, and carotenoids, have been shown to inhibit antioxidant and/or PPO enzymes (Hai et al., 2013; Bao, Ochiai & Ohshima, 2010; Lo et al., 2012; Kozarski et al., 2015; Acharya, Ghosh & Kundu, 2016; Sangthong, Pintathong, Pongsua, Jirarat & Chaiwut, 2022; Masitah, Candra, Masruhim & Kusumaningtyas, 2023). Among them, EGT is a potent bioactive ingredient with health benefits that is of great interest.

The straw mushroom extract (ME) used in the present study had PPO and lipid oxidation inhibitory activities, with IC50 values of 31.63 µL and 25.10 µL, respectively.

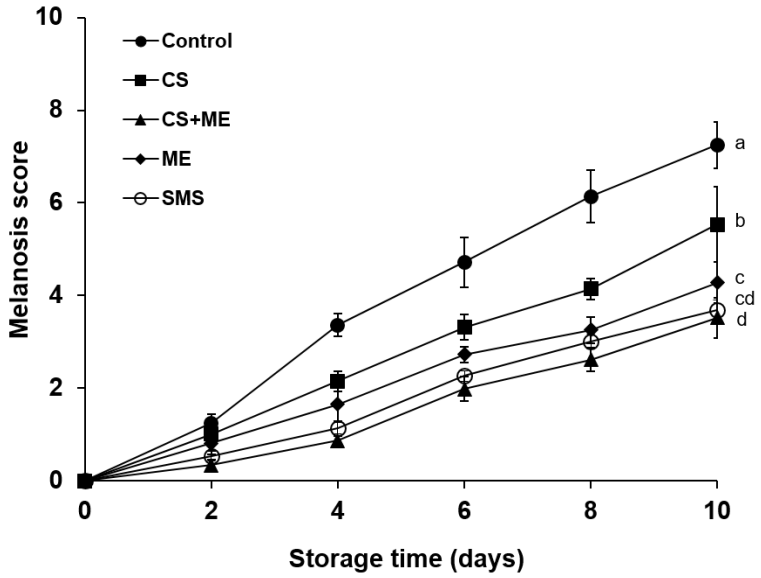

Figure 1. Melanosis score of Pacific white shrimp with treated CS (■), treated CS+ME (▲), treated ME (♦), treated SMS (○), and control without treated (●) during refrigerated storage (SMS, sodium metabisulfite; CS, chitosan; ME, straw mushroom extracts). Data are provided as mean ± SD (n = 3). Curves with different letters indicate a significant difference ($p < 0.05$)

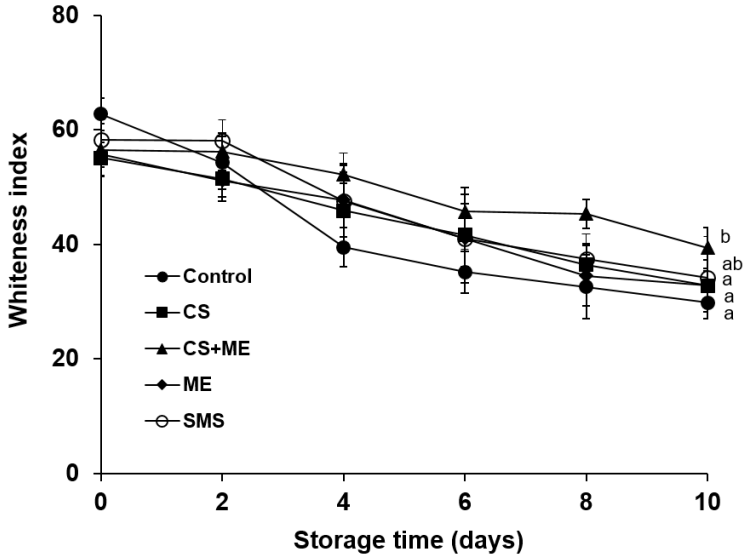

Figure 2. Whiteness index of Pacific white shrimp with treated CS (■), treated CS+ME (▲), treated ME (♦), treated SMS (○), and control without treated (●) during refrigerated storage (SMS, sodium metabisulfite; CS, chitosan; ME, straw mushroom extracts). Data are provided as mean ± SD (n = 3). Curves with different letters indicate a significant difference ($p < 0.05$)

These findings are consistent with earlier investigations (Hai et al., 2013; Bao, 2014; Bao & Hai, 2022). Chitosan has been shown to have antioxidant activity *in vitro* and in foods during storage and processing (Binh et al., 2021; Rinaudo, 2006; Silva, De Souza & Lacerda, 2019). The chitosan used in the present study had DPPH radical scavenging and lipid oxidation inhibitory activities, with IC50 values of 4.4 mg and 0.33 mg, respectively.

Inhibitory effects of the combination of ME and CS treatment on melanosis in shrimp under refrigerated conditions

The effect of the combination of ME and CS on melanosis in Pacific white shrimp during refrigerated storage was evaluated based on

criteria including melanosis score, whiteness index (WI), and color difference (ΔE). The changes in melanosis score of treated Pacific white shrimp (CS, ME, CS+ME, SMS) and the control without treatment during refrigerated storage are shown in Fig. 1.

Figure 1 shows melanosis scores in all samples increased significantly ($p < 0.05$) during 10 days of refrigerated storage. The melanosis development in shrimp treated with CS, ME, CS+ME, or SMS was significantly inhibited ($p < 0.05$) compared with those of the control throughout the storage period. Chitosan reduces the possibility of enzymatic reactions that cause melanosis by forming a film on shrimp surfaces, limiting their exposure to oxygen and moisture, both of which are required for melanosis-related enzymatic reactions (Yuan et al., 2016; Chen et al., 2022). Meanwhile, the anti-melanosis activity of ME was proven to be due to EGT, a unique amino acid found in some mushrooms in nature (Encarnacion et al., 2010; Bao & Hai, 2022).

The present study found that melanosis scores of shrimp treated with CS were significantly higher ($p < 0.05$) than those of shrimp treated with ME, which indicated ME inhibited melanosis formation in shrimp stronger than CS. Although ME showed a stronger effect on inhibiting melanosis in shrimp, it does not last long because EGT is water-soluble and easily lost during refrigerated storage (Bao & Hai, 2022). Contrary to this, CS showed a lower effect on inhibiting melanosis in shrimp, but it has antibacterial, antioxidant, and film-forming properties, which help extend the shelf life of shrimp (Bingöl et al., 2015; Farajzadeh et al., 2016). Shrimp treated with a combination of CS and bioactive compounds such as green tea extract (Yuan et al., 2016) or hypotaurine (Chen et al., 2022) had significantly lower melanosis scores compared to those treated with either CS or bioactive compounds alone. A similar phenomenon was found in the present study: the shrimp treated with a combination of CS and ME (CS+ME) had signifycantly lower ($p < 0.05$) melanosis scores compared to those treated with CS or ME alone, which indicated that the combination treatment was more effective than individual treatments.

Figure 1 also showed that the melanosis scores of shrimp treated with the combination were not significantly different ($p > 0.05$) from those treated with SMS, indicating that the combination treatment was as effective as SMS treatment. This suggests that the combination can be used instead of SMS, a substance that can cause allergic reactions in humans, to inhibit melanosis formation in shrimp.

The more melanosis occurs in shrimp, the larger the area of the black spots on their appearance, resulting in a lower measured whiteness index. The whiteness index of treated Pacific white shrimp (CS, ME, CS+ME, and SMS) and the control without treatment during refrigerated storage is shown in Fig. 2.

Results of the present study showed that the whiteness index of the control shrimp decreased rapidly during storage and was consistently significantly lower than that of the treated shrimp after 4 days of refrigerated storage.

The shrimp treated with the combination (CS+ME) had a significantly higher ($p < 0.05$) whiteness index compared to those treated with either CS or ME alone as well as the control, and there was no significantly different ($p > 0.05$) whiteness index between the shrimp treated with CS, ME, or SMS during 10 days of storage.

The color difference (ΔE) between treated Pacific white shrimp (CS, ME, CS+ME, and SMS) and the control without treatment during refrigerated storage is depicted in Fig. 3.

A standard observer will notice a clear difference in color when $3.5 < \Delta E < 5$, and two different colors when $\Delta E > 5$ (Mokrzycki & Tatol, 2011). Results in Fig. 3 show a clear difference in color of the shrimp treated with CS, CS+ME, or ME at day 0, with corresponding ΔE values of 4.45, 4.66, and 4.27.

This indicates that treatment with CS, CS+ME, or ME has a noticeable effect on shrimp color. The color difference was not clear for shrimp treated with SMS ($\Delta E = 3.08$). However, the regression lines for the color difference of shrimp during storage (Fig. 3) show that the color change rate of control shrimp was faster than that of treated shrimp, and the color change rate of shrimp treated with CS+ME was slower than that of shrimp treated with either CS or ME alone, as well as those treated with SMS.

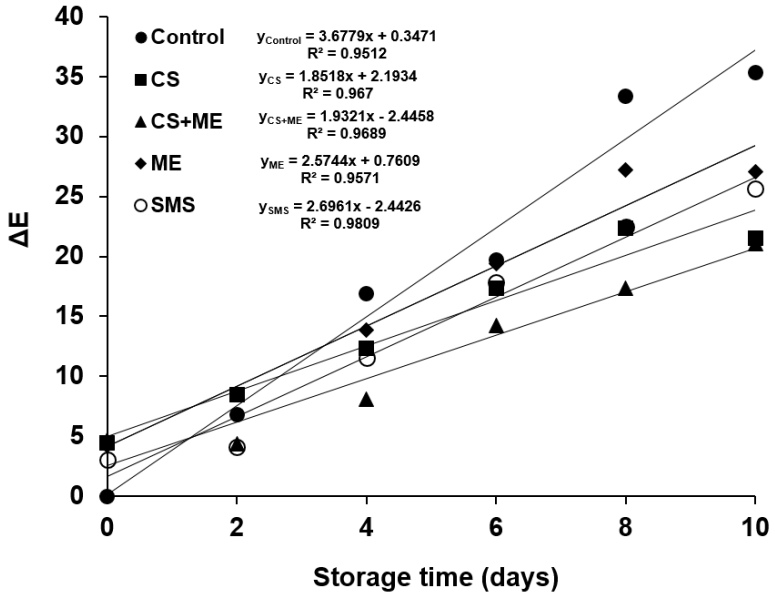

Figure 3. The color difference (ΔE) of Pacific white shrimp with treated CS (■), treated CS+ME (▲), treated ME (♦), treated SMS (○), and control without treated (●) during refrigerated storage (SMS, sodium metabisulfite; CS, chitosan; ME, straw mushroom extracts). Data are provided as mean ± SD (n = 3). Curves with different letters indicate a significant difference ($p < 0.05$)

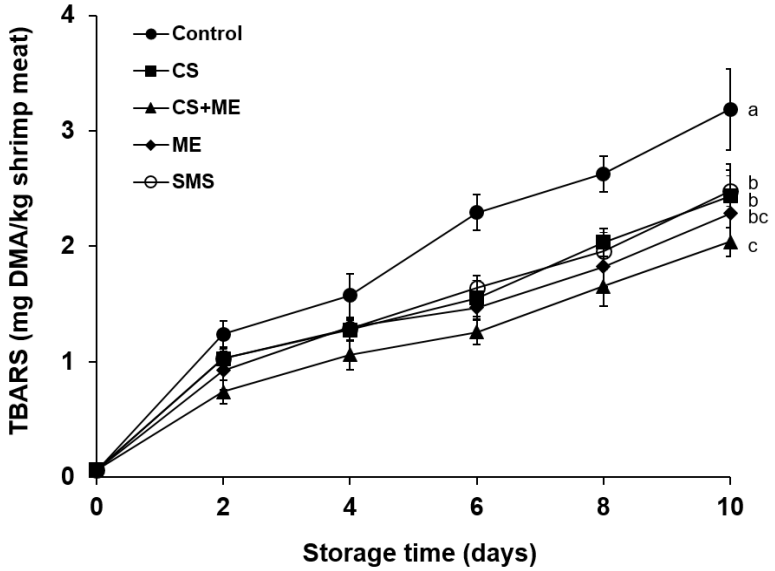

Figure 4. TBARS value of Pacific white shrimp with treated CS (■), treated CS+ME (▲), treated ME (♦), treated SMS (○), and control without treated (●) during refrigerated storage (SMS, sodium metabisulfite; CS, chitosan; ME, straw mushroom extracts). Data are provided as mean ± SD (n = 3). Curves with different letters indicate a significant difference ($p < 0.05$)

To provide a comprehensive understanding of the observed trends, we first refer to Fig. 6 which summarizes the correlations among the observed parameters (Fig. 6 is presented in the last paragraph of the Results and Discussion section). As shown in Fig. 6, the melanosis score is highly negatively correlated with the whiteness index (r = - 0.94) and highly positively correlated with the color difference (r = 0.94), proving that shrimp treated with the combination (CS+ME) were more anti-melanosis effective than those treated with CS or ME alone and as effective as SMS treatment.

Inhibitory effects of the combination of ME and CS treatment on lipid oxidation in shrimp under refrigerated conditions

Previous studies reported that Pacific white shrimp have a low lipid content but a high polyunsaturated fatty acids (PUFA) ratio (An-

drade et al., 2015; Gualda, Dos Santos, Figueiredo, Petenuci & Visentainer, 2018). Therefore, significant lipid oxidation was observed in shrimp during refrigerated storage. TBARS were assessed as a marker of lipid oxidation through secondary products. In the present study, the changes in TBARS value of treated Pacific white shrimp (CS, ME, CS+ME, SMS) and the control without treatment during refrigerated storage are shown in Fig. 4.

Figure 4 shows the TBARS value of all shrimp samples significantly increased during 10 days of refrigerated storage, and the treated shrimp (CS, ME, CS+ME, SMS) had a significantly lower ($p < 0.05$) TBARS value in comparison to the control shrimp. Straw mushrooms have been shown to have *in vitro* antioxidant activity and inhibit lipid oxidation in Pacific white shrimp because they contain antioxidants such as EGT and polyphenols (Hai et al., 2013; Bao & Hai, 2022). Chitosan has been shown to inhibit lipid oxidation in seafood. Chitosan's antioxidant mechanism is explained by its ability to scavenge free radicals and chelate metal ions. Both free radicals and metal ions are agents that mediate lipid oxidation.

Furthermore, chitosan can form a film that helps limit contact between seafood lipids and oxygen in the air, thereby minimizing oxidetion (Farajzadeh et al., 2016; Binh et al., 2021). Pacific white shrimp treated with a combination of CS and bioactive compounds, such as green tea extract (Yuan et al., 2016) or hypotaurine (Chen et al., 2022), showed significantly greater inhibition of lipid oxidation than those treated with either CS or bioactive compounds alone. The present study found the shrimp treated with CS+ME had a lower ($p< 0.05$) TBARS value compared to those treated with either CS or SMS alone, and there was no significant difference ($p > 0.05$) in TBARS values between shrimp treated with CS+ME and shrimp treated with ME during 10 days of refrigerated storage. Shrimp are considered to be of good quality when their TBARS value is less than 3 mg MDA/kg (Abreu et al., 2010).

The present study found that treated shrimp had TBARS values less than 3 mg MDA/kg after 10 days of refrigerated storage (Fig. 4). Meanwhile, the TBARS values of the control shrimp without treatment increased rapidly over the storage period and exceeded 3 mg MDA/kg after 8 days.

Effects of the combination of ME and CS treatment on the sensory quality of shrimp under refrigerated conditions

The sensory scores of treated Pacific white shrimp (CS, ME, CS+ME, and SMS) and the control without treatment during refrigerated storage are shown in Fig. 5.

All shrimp samples were very fresh, with sensory scores of 10 at the start of storage and significantly decreasing over 10 days of refrigerated storage. Although the sensory scores of shrimp treated with CS tended to decrease more slowly than those of control shrimp during the storage period, there were no significant differences ($p > 0.05$) in sensory scores between the shrimp treated with CS and the control shrimp, indicating that CS treatment was not significantly effective in delaying shrimp sensory quality changes in this particular case. The shrimp treated with ME, SME, or CS+ME received higher sensory scores ($p < 0.05$) compared to the control shrimp during storage.

During storage, shrimp treated with the combination (CS+ME) had higher sensory scores than shrimp treated with ME, but there was no significant difference ($p > 0.05$) between shrimp treated with CS+ME and shrimp treated with ME.

Correlation between melanosis development, lipid oxidation, and sensory quality of Pacific white shrimp under refrigerated conditions

Previous studies have reported that melanosis development in shrimp did not significantly affect its nutritional value but had a serious effect on its sensory quality. Therefore, the market value of shrimp with melanosis is drastically reduced, leading to significant economic losses (Gonçalves & de Oliveira, 2016).

The present study found a strong negative correlation ($r = - 0.98$) between melanosis scores and sensory scores of all shrimp samples (Fig. 6), confirming that melanosis development has a negative effect on shrimp sensory quality during refrigerated storage Due to the high ratio of PUFA, the lipids of post-harvest *Litopenaeus vannamei* are easily oxidized (Gualda et al., 2018; Okpala, Choo & Dykes, 2014). Lipid oxidation occurs in shrimp, resulting in a decrease in quality.

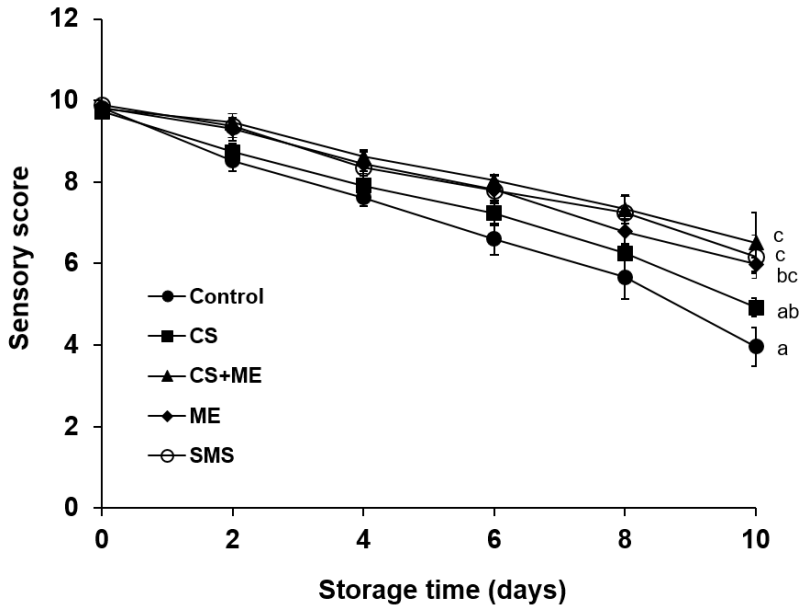

Figure 5. Sensory score of Pacific white shrimp with treated CS (■), treated CS+ME (▲), treated ME (♦), treated SMS (○), and control without treated (●) during refrigerated storage (SMS, sodium metabisulfite; CS, chitosan; ME, straw mushroom extracts). Data are provided as mean ± SD (n = 3). Curves with different letters indicate a significant difference ($p < 0.05$)

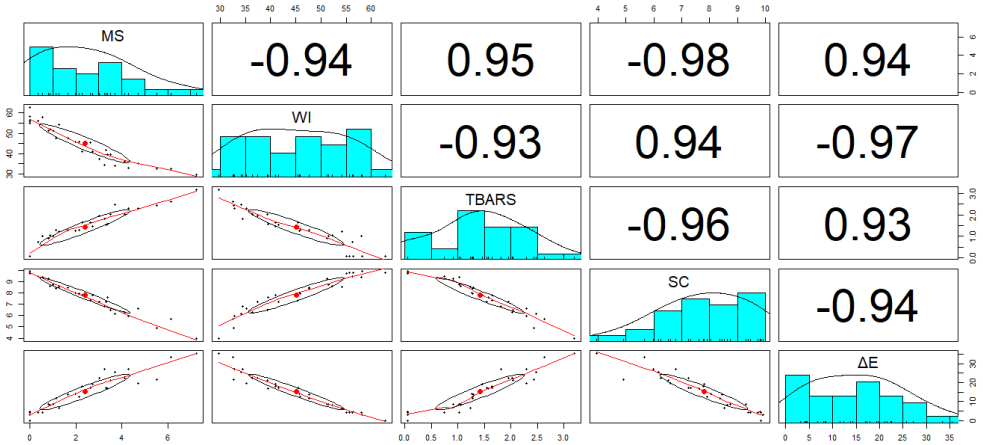

Figure 6. Correlation between melanosis score (MS), whiteness index (WI), color difference (ΔE), sensory score (SC), and TBARS value of all Pacific white shrimp samples (the shrimp with treated SC, SC+ME, ME, SMS, and control shrimp without treatment) during refrigerated storage

The decline in quality becomes most apparent in the later stages of lipid oxidation and is associated with changes in taste, color, texture, and nutritional value (Bao & Ohshima, 2013).

The present study found a strong negative correlation (r = - 0.96) between TBARS values and sensory scores of all shrimp samples (Fig. 6), which shows lipid oxidation has a significant effect on the sensory quality of shrimp during refrigerated storage.

CONCLUSIONS

Melanosis formation and lipid oxidation were significantly reduced, and sensory quality was significantly improved in Pacific white shrimp treated with either CS, ME, or CS+ME compared to the control without treatment. The shrimp treated with CS+ME had significantly greater inhibitory effects on melanosis formation and lipid oxidation than those treated with CS or ME alone, and they were comparable to those treated with 1.25% SMS. These findings

indicate that treating shrimp with CS+ME can be used as an effective natural alternative to synthetic anti-melanosis agents such as SMS in postharvest shrimp preservation.

AUTHOR CONTRIBUTIONS

Conceptualization, H.N.D.B.; Methodology, H.N.D.B.; Investigation, formal analysis, validation, writing-original draft preparation, H.N.D.B.; Writing-review and editing, H.N.D.B.; Supervision, H.N.D.B.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ANTIOKSIDACIONI I ANTIMELANOZNI EFEKTI EKSTRAKTA SLAMNATE GLJIVE I HITOZANA NA OHLAĐENIM BELIM PACIFIČKIM ŠKAMPIMA

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Sažetak: Ova studija je procenila efikasnost kombinovanja hitozana (CS) i ekstrakta slamnate pečurke (ME) u sprečavanju formiranja melanoze i oksidacije lipida kod pacifičkih belih škampa *(Litopenaeus vannamei)* tokom hladnog skladištenja. Studija je pokazala da tretman sa CS, ME ili kombinacijom CS i ME (CS+ME) značajno smanjuje formiranje melanoze i oksidaciju lipida u poređenju sa kontrolnim netretiranim škampima. Škampi tretirani sa CS+ME imali su niže ocene za pojavu melanoze i vrednosti TBARS u poređenju sa škampima tretiranim samo sa CS ili ME, što ukazuje da je kombinacija CS+ME bila najefikasnija u smanjenju formiranja melanoze i oksidacije lipida. Pored toga, nije bilo značajne razlike u ocenama za pojavu melanoze ili senzorskog kvaliteta između škampa tretiranih sa CS+ME i onih tretiranih sa natrijum metabisulfitom (SMS). Ovi rezultati ukazuju da je tretiranje škampa sa CS+ME efikasan način korišćenja prirodnih sastojaka umesto sintetičkog SMS-a za sprečavanje formiranja melanoze i oksidacije lipida kod škampa tokom hladnog skladištenja. Nalazi ove studije nude prirodniji pristup očuvanju roka trajanja škampa u uslovima hladnjaka.

Ključne reči: *crne pege, ergotionein, oksidacija lipida, polifenol oksidaze, rukovanje nakon izlovljavanja*

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