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Effect of pomegranate peel extract on the melanosis of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage

Xu-Bo Fang [1, 2], Hai-Yan Sun [1, 2], Bing-Yu Huang [1] and Gao-Feng Yuan [1, 2]*

*[1] College of Food and Medicine, Zhejiang Ocean University, 51 Qixiangtai Road, Zhoushan 316000, China. [2] Joint Key Laboratory of Aquatic Products Processing Technology of Zhejiang Province, Zhejiang Ocean University, 51 Qixiangtai Road, Zhoushan 316000, China. *e-mail: gaofengyuan@gmail.com*

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Abstract

Shrimp is a highly perishable product with limited shelf life due to melanosis and biological composition. The effect of pomegranate peel extract (PPE) at different concentrations (0, 7.5 and 15 g L^{-1}) on the melanosis formation and quality of Pacific white shrimp (*Litopenaeus vannamei*) was investigated in the present study. The melanosis formation was significantly inhibited and sensory quality was significantly improved in Pacific white shrimp treated with various concentrations of PPE, compared with the control. The increase of pH, total volatile basic nitrogen content and thiobarbituric acid reactive substances was also significantly inhibited in Pacific white shrimp treated with various concentrations of PPE, compared with the control. The melanosis score, total volatile basic nitrogen, thiobarbituric acid reactive substances and pH values of Pacific white shrimp treated by 15 g L^{-1} of PPE was lower than that treated by 7.5 g L^{-1} of PPE during 10 days of ice storage. However, the effect of PPE treatment on inhibition of melanosis formation and the improvement of quality of shrimp was less than that of 12.5 g L^{-1} sodium metabisulfite. These results suggested that PPE could be used as an effective natural alternative to synthetic antimelanosic agents to inhibit postmortem melanosis in shrimp.

Key words: Pacific white shrimp, melanosis, quality, pomegranate peel extract.

Introduction

Shrimp is a very important fisher resource all over the world because of high market value and nutrition value. However, shrimp is a highly perishable product with limited shelf life due to melanosis and biological composition. Melanosis or blackening, the formation of black spots in shrimp and crabs during postmortem storage, severely damages the market value and usually causes economical loss of this seafood [1]. Melanosis is the result of the polymerization of phenols by polyphenoloxidase (PPO), also known as phenoloxidase, tyrosinase (EC 1.14.18.1), and cathecoloxidase (EC 1.10.3.1) [2]. Usually, iced storage is routinely used to preserve the quality of the shrimp. However, during refrigerated or iced storage, melanosis still takes place since PPO remains active under these conditions [3, 4].

To retard the melanosis and ensure perishables have a longer shelf life, antimelanosic agents, such as 4-hexyl-1,3-benzenediol (4-hexylresorcinol), sulphite-based compounds, and phosphates, have been intensively studied and proved to be effective to inhibit melanosis [5-7]. However, the use of synthetic compounds to inhibit melanosis in seafood is limited due to increasing regulatory attention and food safety concerns [8]. For example, the use of metabisulphite to inhibit melanosis of shrimp could cause the sulfur dioxide residue exceeding problems [9]. The concern with seafood safety associated with chemical additives has led the great interest in natural products to antimelanosis of shrimps and crabs [10]. Recently, a series of studies conducted on the utilization of natural extracts to delay melanosis formation and extend the shelf life of seafood [11-13].

Pomegranate, a small tree originating in the Orient, is grown mainly in Iran, India and the USA, but also in most Near and Far East countries. For centuries, various parts of the pomegranate plant have been widely used for the treatment of different types of human disease [14]. The extracts from pomegranate leaves, barks, roots, peels, juice and seeds have high antimicrobial and antioxidant activity [15]. The pomegranate extract also can control enzymatic browning and inhibit the melanosis in foods such as artichokes, mushrooms and pear juice [16]. However, there is no literature on the influence of pomegranate extract on the melanosis formation and quality of seafood such as shrimps and crabs. The objective of the present study was to investigate the effect of pomegranate peel extract (PPE) at different concentrations on the melanosis formation and quality of Pacific white shrimp (*Litopenaeus vannamei*) during ice storage.

Materials and Methods

Preparation of pomegranate peel extracts: The dried pomegranate peels were powdered using a mixer grinder and one-hundred gram portions of finely-powdered peels were blended with 800 ml L^{-1} methanol for 4 h at 40°C in a shaking water bath.

The ratio peel:solvent was 1:10 (w/v). The extracts were through filter paper (Waterman No. 1) and concentrated under vacuum with a rotary evaporator (Eyela, Rikakikai, Tokio, Japan). The concentrate was dried overnight in an oven at 40°C to form powder which was stored at 4°C until further use. The dipping solutions with different concentrations (0, 7.5 and 15 g L^{-1}) were prepared by dissolving PPE in distilled water.

Shrimp collection and treatments: Pacific white shrimp with the size of 50-55 shrimps/kg were purchased from a local market in Zhoushan, China. The Pacific white shrimp were kept alive and transported to the College of Food and Medicine, Zhejiang Ocean University, Zhoushan. Pacific white shrimp were dipped into the PPE solutions at different concentrations (0, 7.5 and 15 g L^{-1}) at a shrimp/solution ratio of 1:2 (w/v) at 4°C for 30 min. After dipping, the Pacific white shrimp were drained at ambient temperature for 3 min. Another portion of shrimp was treated in 12.5 g L^{-1} sodium metabisulfite (SMS) dissolved in distilled water at a ratio of 1:2 (w/v) for 1 min at 4°C. Ten shrimps from each treatment were covered in plastic bags and stored in ice using a shrimp/ice ratio of 1:2 (w/w). To maintain the shrimp/ice ratio, molten ice was removed and replaced with an equal amount of ice every 2 d.

Melanosis assessment: Melanosis assessment of Pacific white shrimp was conducted through visual inspection by six panelists using ten-point scoring according to the method of Montero *et al.* [17]. Panelists were asked to give the melanosis score (0 to 10) for shrimp, where 0 = absent; 2 = slight (up to 20% of shrimps' surface affected); 4 = moderate (20% to 40% of shrimps' surface affected); 6 = notable (40% to 60% of shrimps' surface affected); 8 = severe (60% to 80% of shrimps' surface affected); 10 = extremely heavy (80% to 100% of shrimps' surface affected). Samples were taken for each treatment every 2 d up to 10 d for melanosis assessment.

Sensory evaluation: Sensory evaluation was carried out according to the Chinese National Standard (GB2741-94) at day 0 and day 10[18].

pH determination: The pH values were measured in shrimp according to the method of Lopez-Caballero *et al.* [19].

Determination of thiobarbituric acid reactive substances: Thiobarbituric acid reactive substances (TBARS) in the shrimp were determined as described by modied by Nirmal and Benjakul [11]. Ground sample (1 g) was mixed with 9 mL of a solution containing 3.75 g L^{-1} TBA, 150 g L^{-1} TCA and 0.25 M HCl. The mixture was heated at 100°C for 10 min and centrifuged at 4000 g for 20 min after the mixture was cooled down to room temperature. The supernatants were collected and used for measurements. The absorbance was read at 532 nm. The malonaldehyde was used as a standard. TBARS value was expressed as mg malonaldehyde/kg shrimp meat.

Determination of total volatile basic nitrogen: Total volatile basic nitrogen (TVB-N) was determined on steam distillation and TVB-N contents were expressed as mg N/kg shrimp meat.

Statistical analyses: Statistical analysis was performed using the SPSS package program version 11.5 (SPSS inc. Chicago, IL, USA). Data was analyzed by one-way ANOVA, followed by Tukey's HSD multiple comparison test. The values are reported as means with the standard error for all results. Differences were considered significant at $p < 0.05$.

Results and Discussion

Effect of PPE treatment on melanosis and quality of Pacific white shrimp during iced storage: The effect of pomegranate peel extract at different concentrations on the melanosis formation and quality of Pacific white shrimp during ice storage was investigated in the present study. The score of melanosis of Pacific white shrimp is shown in Fig. 1. Melanosis scores in all treatments significantly increased during 10 days of iced storage. There was melanogenesis and rapid loss in visual quality occurred in control shrimp during ice storage. The highest scores for melanosis were found in the control group for all the sampling days. PPE and SMS treatment significantly inhibited the formation of melanosis of Pacific white shrimp, compared with the control. A significant difference in score of melanosis between PPE treatment and control was observed during the 10 day storage (Fig. 1). The score of melanosis of Pacific white shrimp treated by 15 g L^{-1} of PPE was less than that treated by 7.5 g L^{-1} of PPE. However, the lowest scores for melanosis were obtained in treatment with SMS.

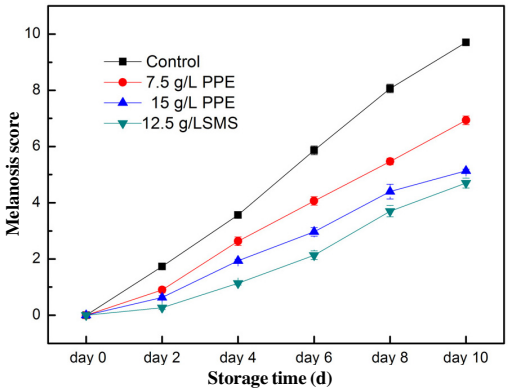

Figure 1. Effect of pomegranate peel extract on melanosis score of Pacific white shrimp during iced storage. Each data is the mean of three replicates per treatment and time point (mean ± standard error). SMS, sodium metabisulfite; PPE, pomegranate peel extract.

The results showed that melanosis formation was significantly inhibited and quality was significantly improved in Pacific white shrimp treated with various concentration of PPE. Similar results were found that natural extracts or natural compounds could inhibit the melanosis formation of Pacific white shrimp. Gokoglu *et al.* [3] found that grape seed extract showed inhibition effect on the formation of melanosis of Pacific white shrimp (*Parapenaeus longirostris*) . Nirmal and Benjakul [11] found that catechin and ferulic acid could delay the melanosis formation and extend the shelf life of Pacific white shrimp when stored in ice [20, 21]. The same group also found that green tea extracts alone or in combination with ascorbic acid could retard the melanosis and the quality changes of Pacific White Shrimp during iced storage [12, 22]. Similar results were obtained from the extracts from *Leucaena leucocephala* which showed the effect of inhibition of melanosis formation in Pacific white shrimp [13] and the extract from the edible mushroom *Flammulina velutipes* which significantly inhibited the formation of melanosis of shrimp and crab [10, 23-25].

It has generally been thought development of melanosis or black spot formation during postharvest of crustaceans attributed to the polymerization of phenol into an insoluble black pigment, the melanin. Phenol polymerization is mainly initiated by the action of an enzymatic complex-PPO [26]. Many studies have focused on PPO inhibition and various techniques and inhibitors, such as heat treatments, high pressure treatments, 4-hexylresorcinol, sulphites and phosphates, have been developed and used [27]. Natural plant extracts, such as ergothioneine from mushrooms, mimosine from lead (*Leucaena leucocephala*), catechin and ferulic acid, were shown to inhibit the PPO of shrimp and crab [13, 20, 27, 28]. On the other hand, the PPE was shown to control enzymatic browning of artichokes, mushrooms and pear juice and inhibit the tyrosinase and polyphenoloxidase activity [16]. It is possible that PPE could inhibit the melanosis formation of Pacific white shrimp by inhibition of the PPO activity of Pacific white shrimp.

Effect of PPE treatment on sensory quality of Pacific white shrimp during iced storage: The changes in sensory quality of Pacific white shrimp treated by PPE in comparison with the control and those treated by SMS are shown in Fig. 2. At day 0, all samples had the score higher than 9 and no differences in likeness were found between all treatments. In general, the sensory scores of Pacific white shrimp showed a tendency to decrease during ice storage. However, the decrease of sensory scores was fastest in the control group for all the sampling days. The decrease of sensory scores of Pacific white shrimp PPE and SMS treatment were significantly inhibited, compared with the control, and the decrease of sensory scores of Pacific white shrimp treated by 15 g L^{-1} of PPE was less than that treated by 7.5 g L^{-1} of PPE.

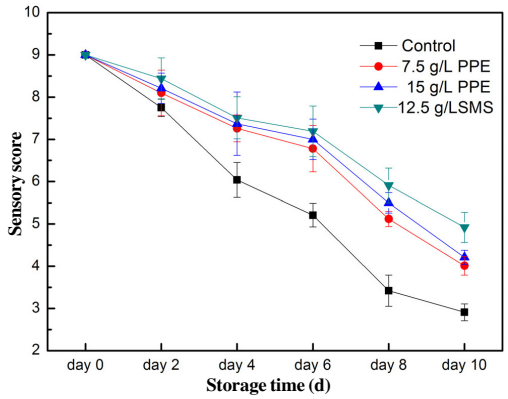

Figure 2. Effect of pomegranate peel extract on sensory score of Pacific white shrimp during iced storage. Each data is the mean of three replicates per treatment and time point (mean ± standard error). SMS, sodium metabisulfite; PPE, pomegranate peel extract.

The sensory score of Pacific white shrimp in the control group was 5.2 on day 6 during ice storage, which was unacceptable level according to the Chinese National Standard (GB2741-94) [18]. On the other hand, the sensory score of Pacific white shrimp treated by 15 g L^{-1} of PPE reached to 5.5 on the 8 during ice storage. These results suggested that the treatment of Pacific white shrimp with PPE could improve the sensory properties of treated shrimps.

Effect of PPE treatment on the changes in pH of Pacific white shrimp during iced storage: The changes in pH of Pacific white shrimp treated by PPE in comparison with the control and those treated by SMS are shown in Fig. 3. The increase of pH of shrimp in all treatments was observed during the iced storage. The increase in pH was highest in the control group for all the sampling days. PPE and SMS treatment significantly inhibited the increase of pH of shrimp, compared with the control. The rise of pH of shrimp treated by 15 g L^{-1} of PPE was less than that treated by 7.5 g L^{-1} of PPE.

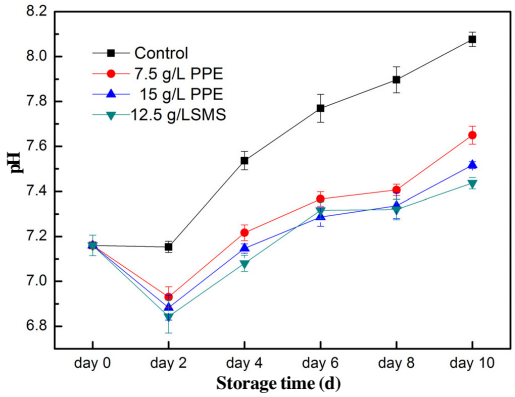

Figure 3. Effect of pomegranate peel extract on pH of Pacific white shrimp during iced storage. Each data is the mean of three replicates per treatment and time point (mean ± standard error). SMS, sodium metabisulfite; PPE, pomegranate peel extract.

The increase in pH of shrimp was due to the accumulation of basic compounds because of activity of bacteria or enzymatic actions [19]. The increase of pH of shrimp was significantly inhibited by different concentration of PPE treatment in the present study, similar with the effect of green tea extract and cinnamaldehyde [29]. However, the inhibition in rise of pH of shrimp was less effective than SMS in our study.

Effect of PPE treatment on the changes in TBARS of Pacific white shrimp during iced storage: The changes in TBARS of shrimp treated by PPE in comparison with the control and those treated by SMS are shown in Fig. 4. In general, TBARS value in all treatments significantly increased during 10 days of iced storage. However, TBARS value of shrimp in the control group was significantly higher than that in the 15 g L^{-1} PPE group, the 7.5 g L^{-1} PPE group and SMS group during 10 days of iced storage. On the other hand, the inhibition in rise of TBARS value in shrimp

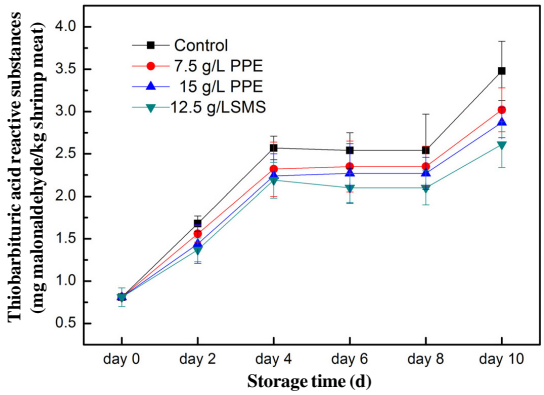

Figure 4. Effect of pomegranate peel extract on thiobarbituric acid reactive substances of Pacific white shrimp during iced storage. Each data is the mean of three replicates per treatment and time point (mean ± standard error). SMS, sodium metabisulfite; PPE, pomegranate peel extract.

was dependent on the concentration. However, the increase of TBARS was lowest in shrimp treated by SMS.

TBARS were measured as an indicator of secondary lipid oxidation products. Tissue membrane of crustacean contains highly polyunsaturated fatty acid and the damage of tissues during processing can induce lipid oxidation [30]. Lipid oxidation produces off-flavours in cold water shrimp during peeling and is an important parameter affecting the shelf-life of shrimp [31]. The results obtained in the present study showed the PPE was effective in retarding the increase of TBARS levels of shrimp during ice storage. Similar results were obtained from previous studies with green tea extract[22].

Effect of PPE treatment on the changes in TVB-N of Pacific white shrimp during iced storage: TVB-N is a common and important indicator of the quality of Pacific white shrimp because the increased TVB-N value is related to microbial spoilage and the activity of endogenous enzymes [32]. TVB-N content of Pacific white shrimp treated by PPE in comparison with the control and those treated by SMS are shown in Fig. 5. The TVB-N content of Pacific white shrimp in both control and treatment was markedly increased during 10 days of ice storage. The increase in TVB-N content was highest in the control group for all the sampling days. PPE and SMS treatment significantly inhibited the increase of TVB-N of Pacific white shrimp, compared with the control. The rise of TVB-N of Pacific white shrimp treated by 15 g L^{-1} of PPE was less than that treated by 7.5 g L^{-1} of PPE. The lower TVB-N value of Pacific white shrimp treated with PPE could be due to the antimicrobial effect of PPE.

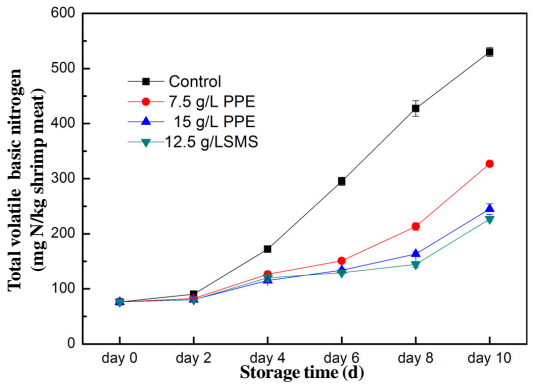

Figure 5. Effect of pomegranate peel extract on the total volatile basic nitrogen content of Pacific white shrimp during iced storage. Each data is the mean of three replicates per treatment and time point (mean ± standard error). SMS, sodium metabisulfite; PPE, pomegranate peel extract.

Conclusions

The melanosis score was significantly inhibited and sensory quality was significantly improved in Pacific white shrimp treated with various concentration of PPE, compared with the control. The increase of pH, TVB-N content and TABRS was also significantly inhibited in Pacific white shrimp treated with various concentration of PPE, compared with the control. The melanosis score, TVB-N, TBARS and pH values of Pacific white shrimp treated by 15 g L^{-1} of PPE was lower than that treated by 7.5 g L^{-1} of PPE during 10 days of ice storage. These results suggested that PPE could be used as an effective natural alternative to synthetic antimelanosic agents to inhibit postmortem melanosis in shrimp.

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References

[1]Kim, J., Marshall, M. R. and Wei, C. 2002. Polyphenoloxidase. In Haard, N. (ed.). Seafood Enzymes: Utilization and Influence on Postharvest Seafood Quality. Marcel Dekker Inc., New York, pp. 271-315.

[2]Zamorano, J. P., Martinez-Alverez, O., Montero, P. and Gómez-Guillén, M. C. 2009. Characterization and tissue distribution of polyphenoloxidase of deepwater pink shrimp (*Parapenaeus longirostris*). Food Chem. **112**:104-111.

[3]Gokoglu, N. and Yerlikaya, P. 2008. Inhibition effects of grape seed extracts on melanosis formation in shrimp (*Parapenaeus longirostris*). Int. J. Food Sci. Technol. **43**:1004-1008.

[4]Boonsumrej, S., Chaiwanichsiri, S., Tantratian, S., Suzuki, T. and Takai, R. 2007. Effects of freezing and thawing on the quality changes of tiger shrimp (*Penaeus monodon*) frozen by air-blast and cryogenic freezing. J. Food Eng. **80**:292–299.

[5]Martinez-Alverez, O., Gomez-Guillen, M. C. and Montero, P. 2008. Chemical and microbial quality indexes of Norwegian lobsters (*Nephrops norvegicus*) dusted with sulphites. Int. J. Food Sci. Technol. **43**:1099- 1110.

[6]Martínez-Álvarez, O., Lopez-Caballero, M. E. and Montero, P. 2005. 4-Hexylresorcinol-based formulation to prevent melanosis and microbial growth in chilled tiger prawns (*Marsupenaeus japonicas*) from aquaculture. J. Food Sci. **70**:415-422.

[7]Thepnuan, R., Benjakul, S. and Visesssanguan, W. 2008. Effect of pyrophosphate and 4-hexylresorcinol pretreatment on quality of refrigerated white shrimp (*Litopenaeus vannamei*) kept under modified atmosphere packaging. J. Food Sci. **73**:S124-S133.

[8]McEvily, A. J., Iyengar, R. and Otwell, S. 1991. Sulte alternative prevents shrimp melanosis. Food Technol. **45**:80-86.

[9]Gomez-Guillen, M. C., Martinez-Alverez O., Llamas, A. and Montero, P. 2005. Melanosis inhibition and SO_2 residual levels in shrimps (*Parapenaeus longirostris*) after different sulfite-based treatments. J. Sci. Food Agri. **85**:1143-1148.

[10]Encarnacion, A. B., Fagutao, F., Hirono, I., Ushio, H. and Ohshima, T. 2010. Effects of ergothioneine from mushrooms (*Flammulina velutipes*) on melanosis and lipid oxidation of kuruma shrimp (*Marsupenaeus japonicus*). J. Agric. Food Chem. **58**:2577-2585.

[11]Nirmal, N. P. and Benjakul, S. 2010. Effect of catechin and ferulic acid on melanosis and quality of Pacific white shrimp subjected to freeze-thawing prior refrigerated storage. Food Con. **21**:1263-1271.

[12]Nirmal, N. P. and Benjakul, S. 2012. Effect of green tea extract in combination with ascorbic acid on the retardation of melanosis and quality changes of Pacific white shrimp during iced storage. Food Biopro. Technol. **5**:2941-2951.

[13]Nirmal, N. P. and Benjakul, S. 2011. Inhibition of melanosis formation in Pacific white shrimp by the extract of lead (*Leucaena leucocephala*) seed. Food Chem. **128**:427-432.

[14]Jayaprakasha, G. K., Negi, P. S. and Jena, B. S. 2006. Antimicrobial activities of pomegranate. In Seeram, N.P., Schulman, R. N. and Heber, D. (eds). Pomegranates: Ancient Roots to Modern Medicine. CRC Press/Taylor and Francis, Boca Raton, pp.167-183.

[15]Tehranifar, A., Selahvarzi, Y., Kharrazi, M. and Bakhsh, V. J. 2011. High potential of agro-industrial by-products of pomegranate (*Punica granatum* L.) as the powerful antifungal and antioxidant substances. Ind. Crop. Prod. **34**:1523-1527.

[16]Zocca, F., Lomolino, G. and Lante, A. 2011. Dog rose and pomegranate extracts as agents to control enzymatic browning. Food Res. Int. **44**:957-963

[17]Montero, P., Ávalos, A. and Pérez-Mateos, M. 2001. Characterization of polyphenoloxidase of prawns (*Penaeus japonicus*). Alternatives to inhibition: Additives and high-pressure treatment. Food Chem. **75**:317-324.
[18]Chinese National Standard (GB2741-94). 1994. Hygienic standard for sea shrimp. Chinese National Hygiene Ministry, Beijing, pp. 1-3.
[19]Lopez-Caballero, M. E., Martinez-Alvarez, O., Gomez-Guillen, M. C. and Montero, P. 2007. Quality of thawed deepwater pink shrimp (*Parapenaeus longirostris*) treated with melanosis-inhibiting formations during chilled storage. Int. J. Food Sci. Technol. **42**:1029-1038.
[20]Nirmal, N. P. and Benjakul, S. 2009. Effect of ferulic acid on inhibition of polyphenoloxidase and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage. Food Chem. **116**:323-331.
[21]Nirmal, N. P. and Benjakul, S. 2009. Melanosis and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) treated with catechin during iced storage. J.Agric. Food Chem. **57**:3578-3586.
[22]Nirmal, N. P. and Benjakul, S. 2011. Retardation of quality changes of Pacific white shrimp by green tea extract treatment and modified atmosphere packaging during refrigerated storage. Int. J. Food Microbiol. **149**:247-253.
[23]Encarnacion, A. B., Fagutao, F., Shozen, K., Hirono, I. and Ohshima, T. 2011. Biochemical intervention of ergothioneine-rich edible mushroom (*Flammulina velutipes*) extract inhibits melanosis in crab (*Chionoecetes japonicus*). Food Chem. **127**:1594-1599.
[24]Encarnacion, A. B., Fagutao, F., Hirayama, J., Terayama, M., Hirono, I. and Ohshima, T. 2011. Edible mushroom (*Flammulina velutipes*) extract inhibits melanosis in kuruma shrimp (*Marsupenaeus japonicus*). J. Food Sci. **76**:C52-58.
[25]Encarnacion, A. B., Fagutao, F., Jintasataporn, O., Wanchai Worawattanamateekul, W., Hirono, I. and Ohshima, T. 2012. Application of ergothioneine-rich extract from an edible mushroom *Flammulina velutipes* for melanosis prevention in shrimp, *Penaeus monodon* and *Litopenaeus vannamei*. Food Res. Int. **45**:232-237.
[26]Alasalvar, C., Shahidi, F., Miyashita, K. and Wanasundara, U. 2011. Handbook of Seafood Quality, Safety and Health Applications. Blackwell Publishing Ltd., pp.109-118.
[27]Nirmal, N. P. and Benjakul, S. 2012. Inhibition kinetics of catechin and ferulic acid on polyphenoloxidase from cephalothorax of Pacific white shrimp (*Litopenaeus vannamei*). Food Chem. **131**:569-573.
[28]Nirmal, N. P. and Benjakul, S. 2011. Inhibitory effect of mimosine on polyphenoloxidase from cephalothoraxes of Pacific white shrimp (*Litopenaeus vannamei*). J. Agric. Food Chem. **59**:10256-10260.
[29]Mu, H. L., Chen, H. J, Fang, X. J., Mao, J. L. and Gao, H. Y. 2012. Effect of cinnamaldehyde on melanosis and spoilage of Pacific white shrimp (*Litopenaeus vannamei*) during storage. J. Sci. Food Agric. **92**:2177-2182.
[30]Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P. and Buckley, D. J. 1998. Lipid stability in meat and meat products. Meat Sci. **49**:S73-S86.
[31]Bak, L. S., Andersen, A. B., Andersen, E. M. and Bertelsen, G. 1999. Effect of modified atmosphere packaging on oxidative changes in frozen stored cold water shrimp (*Pandalus borealis*). Food Chem. **64**:169-175.
[32]Soncin, S., Chiesa, L. M., Panseri, S., Blondi, P. and Cantoni, C. 2009. Determination of volatile compounds of precooked prawn (*Penaeus vannamei*) and cultured gilthead sea bream (*Sparus aurata*) stored in ice as possible spoilage markers using solid phase micro extraction and gas chromatography/mass spectrometry. J. Sci. Food Agric. **89**:436-442.