AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Effect of mango seed kernel extract on polyphenol oxidase inhibition and shelf life of shrimp during iced storage

Samart Sai-Ut*, Naruemol Noknoi, Natcha Nakjai

Faculty of Science, Burapha University, Muang District, Chonburi 20131, Thailand

Article Info

Article history:
Received 25 February 2020
Revised 26 May 2020
Accepted 30 May 2020
Available online 30 December 2020

Keywords:
Mango seed extract,
Melanosis,
Polyphenol oxidase,
White shrimp

Abstract

The effects were examined of mango seed kernel extract (MSKE) on the inhibition of polyphenol oxidase (PPO) activity and the quality of white shrimp during iced storage for 12 d. The molecular weight of PPO from the Pacific white shrimp (*Litopenaeus vannamei*) cephalothorax was 220 kDa. PPO activity from the shrimp was inhibited in a dose-dependent manner by MSKE. The effective final concentration of MSKE which inhibited the PPO activity by 90% was 5,000 mg/kg. Immersion in MSKE solution could appreciably retard the melanosis score of the shrimp ($p < 0.05$). The shrimp immersed in MSKE solution at 1,000 mg/kg and 5,000 mg/kg had significantly ($p < 0.05$) lower values for total volatile basic nitrogen and thiobarbituric acid reactive substances (57.43±1.28 to 57.99±2.70 mg N/100 g sample and 0.98±0.11 to 1.22±0.05 mg malanoaldehyde/kg sample, respectively) compared with the control (79.64±2.79 mg N/100 g sample and 1.38±0.11 mg malanoaldehyde/kg sample, respectively) at the 12 d. The shrimps treated with MSKE had lower psychrophilic bacterial counts than the control throughout the 12 d of iced storage. Thus, MSKE has potential to be a natural melanosis-inhibiting agent to prevent black spot and to extend the shelf-life of the shrimp during iced storage.

Introduction

Shrimp can be considered a favorite seafood worldwide as they provide a great deal of protein, omega-3 fats, and contains no carbohydrates (Qiu et al., 2017). Undesirable black spot formations can occur during shrimp product transport and storage (Nirmal and Benjakul, 2010). Such melanosis usually occurs in the cephalothorax and abdominal exoskeleton and simply refers to the production of quinones that crosslink with adjoining substrate catalyzed by polyphenol oxidase (García-Carreño et al., 2008). This appearance is not harmful to consumers and naturally occurs in post-harvest handling of shrimp. Although this phenomenon is a normal color change, it affects price acceptability by consumers. Consequently, this deterioration process has to be controlled or eliminated or both. The use of a chemical agent such as sodium metabisulfite can retard the deterioration process by interfering with quinones polymerization to form colorless compounds. However, the sulfite residues have been reported to be hazardous to human well-being (Bono et al., 2012). Alternative methods to replace these inorganic compounds have increasingly considered plant phenolics as potential natural additives that can inhibit melanosis (Gonçalves and de Oliveira, 2016). Many studies have investigated the use of plant extracts, for example, from enokitake mushroom (Jang et al., 2003), grape seeds (Gokoglu and Yerlikaya, 2008) and lead seed (Nirmal and Benjakul, 2011a), which provided greater benefit than the conventional methods of product quality control.

In Thailand, mangoes are one of the most popular fruits and among the many cultivated varieties of mango, the Nam-Dok-Mai (NDM) cultivar with its yellow oval fruit and sweet, fragrant flesh with a golden-yellow color is popular, with domestic consumption

* Corresponding author.
E-mail address: samarts@go.buu.ac.th (S. Sai-Ut)

https://doi.org/10.34044/j.anres.2020.54.6.10

exceeding 2 million t per year (Chomchalow and Na Songkhla, 2008; Sriariyawat and Sriariyawat, 2016). Consequently, the waste residues (estimated at 35–60% after processing), such as peel and seed, have become an environmental problem (O'Shea et al., 2012; Suma et al., 2019). However, NDM mango seed kernels have high contents of bioactive compounds that act as natural antioxidants with remarkable antioxidative activity—151.64 ± 4.37 mg and 105.26 ± 5.11 gallic acid equivalent (GAE)/100 g dry sample—based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) assays, respectively (Rawdkuen et al., 2016). The polyphenols in mango are mangiferin, catechin, quercetin, kaempferol, tannins, anthocyanins, gallic acid and ellagic acid (Sogi et al., 2013). In addition, extracts of mango kernel have a great deal of polyphenol oxidase inhibitor, antimicrobial activity and chelating activity (Maisuthisakul and Gordon, 2012). The extract from mango seed kernel has been reported to be a promising source of natural antioxidants and tyrosinase inhibiting agents (Rawdkuen et al., 2016).

Fresh shrimp is usually stored in ice and covered with more ice or stored using brining and chilling to allow cold air to circulate around the shrimp as this commonly extends their shelf life extension by inducing hibernation and make them easier to clean (Mejlholm et al., 2005). To increase the shelf life and storage conditions of the product, some food additives are used; green tea and mulberry extracts added to Pacific white shrimp (*Litopenaeus vannamei*) stored in ice for 12 d produced lower polyphenol oxidase (PPO) activity, lower lipid oxidation and a lower psychrophilic bacterial count (Nirmal and Benjakul, 2011b). Yuan et al. (2016) reported that a chitosan coating combined with pomegranate peel extract reduced black spot on Pacific white shrimp in iced storage during 10 d. Mango seed extracts have been reported to possess antioxidant, antimicrobial and tyrosinase inhibitory action (Rawdkuen et al., 2016), which may facilitate inhibiting melanosis and extending the shelf life of shrimp. Thus, the aim of this research was to investigate the impact of NDM mango seed kernel extract on the shelf life quality of shrimp after storage in ice.

Materials and Methods

Chemicals

Absolute ethanol was purchased from Merck (Darmstadt, Germany), Folin-Ciocalteu phenol reagent from Fluka (Steinheim, Germany) and 2,2′-diphenyl-picrylhydrazyl (DPPH), Brij-35, gallic acid, L-β-(3,4 dihydroxylphenyl) alanine (L-DOPA) and trichloroacetic acid (TCA) from Sigma. All chemicals used were analytical grade.

Plant materials

Nam-Dok-Mai mango seeds kernels were collected and the pericarp tissues were removed. The sample was washed with tap water and dried for 24 hr using a hot-air oven at 50°C, before powdering using a hammer mill and passing through a 20-mesh (0.84 mm) sieve (YB-800A, Yun Bang, China). The sample powder was stored in a plastic bag and kept in a freezer at -20°C until used for extraction. For optimal extraction, the powder (50 g) was mixed with 200 mL of 62 g/kg ethanol at 63°C for 150 min by stirring continuously. The extract was centrifuged at 8000×*g* for 20 min at 4°C using a refrigerated centrifuge (H1650, Ugaiya; Osaka, Japan). The supernatant was collected and freeze dried. The powder obtained was subjected to analysis for its phenolics content, DPPH radical scavenging activity and also used for shrimp treatment. The phenolics content was determined using the Folin-Ciocalteu method (Swain and Hillis, 1959). The DPPH radical scavenging activity was determined using the method of Brand-Williams et al. (1995).

Shrimp preparation

Fresh Pacific white shrimp (*L. vannamei*) without any food additive and weighing 30–35 shrimp/kg were purchased from the local market (Sean Suk, Chonburi, Thailand). Immediately after purchase, the shrimp were kept in ice and transported to the Department of Food Science, Burapha University, Chonburi, Thailand.

Extraction of polyphenol oxidase from shrimp

The Pacific white shrimps were gathered for their cephalothoraxes and then frozen by immersing in liquid nitrogen before being pounded in a blender. The powder (100 g) was added with a buffer consisting of 0.05 M sodium phosphate, pH 7.2, 1.0 M NaCl, 0.2% Brij-35 (300 mL). The sample suspension was extracted by stirring for 30 min and separated using centrifugation at 10,000×*g* for 30 min at 4°C. The supernatant was collected and phenylmethanesulfonyl fluoride [0.1% (w/v)] was polyphenol oxidase to prevent protease activity. The collected supernatant was precipitated by adding ammonium sulfate (40%) into the supernatant. The mixture was gently stirred for 60 min in a cooled container. The precipitate was centrifuged at 12,500×*g* at 4°C for 30 min to collect the pellet. The compressed mass of a sample was dissolved in the buffer and then, the sample solution was desalted using dialysis in the buffer with three changes of the buffer during 24 hr at 4°C. The precipitate samples were detracted. The soluble sample was collected and used as 'partial crude PPO extract'.

Determination of polyphenol oxidase activity

The PPO activity was examined using the method of Simpson et al. (1987) with slight modification. Briefly, 100 µL of partial crude PPO extract was diluted with 100 µL of deionized water. The diluted sample was mixed with 400 µL of 0.05 M phosphate buffer, pH 6.0. The reaction was begun by adding 600 µL of 15 mM L-DOPA and incubating at 45°C for 1 min. The absorbance of reaction mixture was measured at 475 nm using a spectrometer (10S UV-Vis Spectrophotometer; Genesys; Daly City, CA, USA). A unit of the enzyme activity was calculated by an increase in the absorbance by 0.001 per minute per milliliter under the conditions described above. Enzyme and substrate blanks were also done.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The protein pattern of the crude enzyme PPO obtained from the shrimp cephalothoraxes was determined using the method of Laemmli (1970). Briefly, 50 µL of PPO solution was mixed with the sample buffer at a 1:1 (volume per volume) ratio and boiled for 3 min. The samples (15 µg protein) were loaded into the 10% running polyacrylamide gel and 4% stacking polyacrylamide gel, and then subjected to electrophoresis by applying an electric current at 150 V to the gel using the electrophoresis apparatus (Mini-PROTEAN; Bio-Rad; Hercules, CA, USA). After parting, the proteins were stained with 0.03% (w/v) Coomassie Brilliant Blue R-250 and destained with 50% methanol and 8% acetic acid.

Preparation of shrimp with mango seed kernel extract solution

The MSKE powder samples (1 g and 5 g) were dissolved in distilled water to give a final concentration of 0.1% and 0.5% (weight per volume, w/v). The white shrimps (500 g) were immersed in the extract solutions at a shrimp-to-solution ratio of 1:2 (w/v) at 4°C for 10 min, drained on the screen for 1 min and then stored in polystyrene boxes with flake ice with a sample-to-ice ratio of 1:4 (weight per weight) for 12 d. To maintain the shrimp-to-ice ratio, ice was added as necessary. Samples were taken from each ice box every 4 d up to 12 d for melanosis, chemical and microbial analysis.

Determination of total volatile basic nitrogen

The total volatile basic nitrogen was measured according to the micro-diffusion method (Conway and Byrne, 1933). The total volatile basic nitrogen value was expressed as micrograms of N per 100 grams of shrimp sample.

Determination of thiobarbituric acid reactive substances

The thiobarbituric acid reactive substances (TBARS) value was detected using thiobarbituric acid reagent according to the method of Lee and Hendricks (1997). A shrimp sample (2 g) was mixed with 18 mL of thiobarbituric acid (TBA) solution (0.375% TBA, 15% TCA and 0.25 M HCl). The amounts were mixed well and placed in a water bath at 100°C to develop a pink color for 10 min. The mixture was cooled and centrifuged at 5,000×g for 15 min. The supernatant was collected and then the absorbance of the supernatant was measured at 532 nm using the spectrophotometer. The standard curve was prepared in the same manner using malondialdehyde (MDA) as a standard. The TBARS value was expressed as milligrams of MDA per kilogram.

Psychrophilic bacterial count

A psychrophilic bacterial count was performed using the spread plate method. Whole shrimps (25 g) were mixed with 225 mL of 0.85% saline buffer, followed by homogenization in a Stomacher blender for 1 min at 200 rpm. The homogenate was used to prepare ten-fold serial dilutions in 0.85% saline buffer. The appropriate dilutions (0.1 mL) were poured onto plate count agar containing 5% peptone, 5% sodium chloride, 1.5% beef extract, 1.5% yeast extract and 1.5% agar. The plates were incubated at 4°C for 14 d.

Melanosis measurement

The melanosis or black spot level of Pacific white shrimp was determined via visual investigation using 10-point scales. In depth, the melanosis scores of shrimp were evaluated by 10 trained panelists based on comparing each image based on a scale from 0 to 10, where 0 = fresh shrimp with absence of black spots; 2 = up to 20% of shrimp's surface affected; 4 = 20–40% of shrimp's surface moderately affected; 6 = 40–60% of shrimp's surface affected; 8 = 60–80% of shrimp's surface affected; and 10 = presence of 80–100% black spots on shrimp's head and tail. The mean value of the melanosis scores of each shrimp sample was estimated and recorded.

Statistical analysis

Data were subjected to analysis of variance. Comparison of means was carried out using Tukey's range test. Analysis was performed using the MINITAB software package.

Results and Discussion

Characterization of polyphenol oxidase from white shrimp

Partial purified crude PPO of white shrimp was characterized using ammonium sulfate precipitation. Changes in the total activity and purification fold with storage time are shown in Table 1. After precipitation using ammonium sulfate at a concentration of 40%, purification of 1.4-fold was obtained. The partial purification of the PPO had specific activity of 148.25 U/mg protein. This result had a lower recovery yield than from *Ostrinia furnacalis* that was purified 2.92-fold with 80.42% total recovery of activity using ammonium sulfate precipitation (Feng et al., 2008). The difference might have been due to the reaction of PPO with phenolic substrates during the enzyme extraction and polymerization of the intermediate products, which might have led to enzyme precipitation and loss of activity.

Table 1 Some properties of partial purification of polyphenol oxidase (PPO) from Pacific white shrimp

Purified step	Total activity U/mL	Protein content (mg/mL)	Specific activity (U/mg)	Yield (%)	Purification (fold)
Crude PPO	3824±176[a]	35.75±1.45[a]	106.97±1.37[b]	100	1.0
Partially purified PPO	1100±20[b]	7.42±0.08[b]	148.25±8.89[a]	29	1.4

Values (mean ± SD, n =3) in the same column superscripted by different letters are significantly ($p < 0.05$) different.

The protein pattern of the enzyme from the cephalothoraxes of the white shrimp determined with non-reducing conditions is shown in Fig. 1. The PPO from the cephalothoraxes had a molecular weight of 220 kDa which agreed with Nirmal and Benjakul (2010) who considered that the PPO from the Pacific white shrimp cephalothorax would reach 210 kDa. Deep water pink shrimp (*Parapenaeus longirostris*) had PPO with a molecular weight of approximately 212 kDa (Zamorano et al., 2009). The main enzyme of the kuruma prawn cephalothoraxes had a molecular weight of 160 kDa (Benjakul et al., 2005). PPO from different shrimp parts (carapace and cephalothorax) were reported to contain different enzyme isoforms in which PPO with a molecular weight of 210 kDa was predominant in the cephalothorax (Nirmal and Benjakul, 2010). Overall, the partial purified PPO from the cephalothorax of white shrimp had a major band as a predominant protein having molecular weight of approximately 220 kDa.

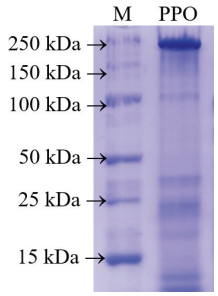

Fig. 1 Protein pattern of the partially purified polyphenol oxidase (PPO) from Pacific white shrimp (M: marker)

Effect of mango seed kernel extract on polyphenol oxidase inhibitory activity

The total phenolic and antioxidative activity of MSKE tested using the Folin-Ciocalteu method and DPPH assay were 105.83 mg gallic acid equivalent (GAE)/g and 321.46 mg GAE/g dry sample, respectively. The PPO inhibitory activity of MSKEs and gallic acid with various concentrations (100–5,000 mg/kg) are presented in Fig. 2. Basically, PPO catalyzes the oxidation of DOPA to DOPA-quinone that produces browning melanins. From the *in vitro* studies, the enzyme was effectively inhibited by MSKE or gallic acid at high concentration. At the same concentrations, the MSKEs inhibited the PPO activity better than gallic acid. PPO inhibition of more than 50% occurred at concentrations of 100 mg/kg and 5,000 mg/kg of MSKE, whereas this same level of inhibition was achieved using gallic acid at a concentration of 5,000 mg/kg. The MSKEs also inhibited the PPO activity in a concentration-dependent manner. These results were similar to those reported by Nirmal and Benjakul (2010) who noted that Pacific white shrimp treated with 0.1–0.2% catechin or 2–3% ferulic acid had reduced melanosis. MSKEs contain mainly phenolics compounds (phenolic acids, flavonoids, gallotannins and ellagitannins) that give them inhibitory activity against PPOs (Sogi et al., 2013). The activity of PPO was inhibited because the phenolic compounds in the MSKEs interacted with their active sites. In addition, the phenolic derivatives found in MSKE act as chelating agents by bonding the hydroxyl group to the active site of PPO. MSKE might be involved in the reduction of DOPA-chrome to DOPA by donating electrons to intermediate quinone, DOPA-chrome. From the current results, MSKE had an efficient inhibitory effect toward PPO at concentrations of 1,000 mg/kg and 5,000 mg/kg. Therefore, these concentrations were used for further studies.

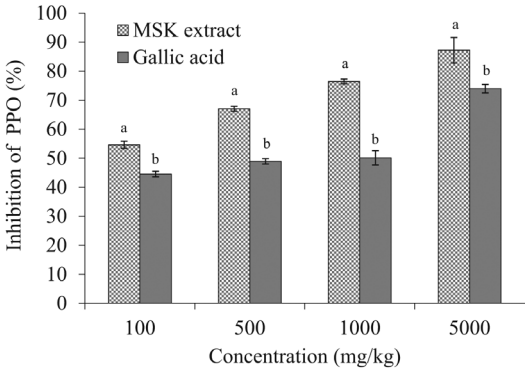

Fig. 2 Percentage inhibition of partially purified polyphenol oxidase (PPO) from Pacific white shrimp using mango seed kernel (MSK) extract and gallic acid at different concentrations, where different letters above columns indicate significant ($p < 0.05$) differences for the same concentration and error bars indicate standard deviation (n=3).

Effect of mango seed kernel extract on total volatile basic nitrogen content of shrimp

The changes in the TVB-N contents of Pacific white shrimp treated with MSKE (1,000 mg/kg and 5000 mg/kg) during 12 d of ice storage can be seen in Fig. 3a. After ice storage for 8 d, the TVB-N contents of the control shrimp increased from 19.8 mg N/100 g to 39.2 mg N/100 g, while the TVB-N contents of shrimp treated with 1,000 mg/kg and 5,000 mg/kg of MSKE reached 31.4 mg N/100 g and 27.1 mg N/100 g, respectively. A TVB-N content < 300 mg N/100 g has been regularly accepted as fresh shrimp (Smaldone et al., 2011). The lower TVB-N content of the shrimp treated with 1,000 mg/kg and 5,000 mg/kg of MSKE might have been due to the antimicrobial effect of the phenolic compounds found in the extract. At day 12, samples treated with 5,000 mg/kg of MSKE had the lowest TVB-N content compared to the other samples ($p < 0.05$). TVB-N represents the ammonia-like and fishy odor characteristic, which suggests the production of volatile basic nitrogenous compounds via the bacterial reduction of TMAO to TMA. Basically, typical spoilage bacteria produce ammonia by degradation of amino-acids and are able to reduce TMAO or produce H_2S or both under anaerobic conditions. Using the phenolic compounds from the mango seed kernel could reduce the TVB content, given the inhibitory effect of phenolic compounds on microorganisms.

Effect of mango seed kernel extract on lipid oxidation of shrimp

The results of lipid oxidation monitoring of white shrimp treated with and without MSKE kept in ice for 12 d are shown in Fig. 3b. Throughout storage for 12 d, the TBARS content markedly increased in the samples treated without and with MSKE, though the shrimp without treatment had a higher TBARS content than those treated with MSKE ($p < 0.05$). The TBARS content corresponded with the increases in the TVB-N content (Fig. 3a). As the storage time increased, rapidly increases in the TVB-N and TBARS contents were observed in all treatments. The rate of increase slowed with a higher concentration of MSKE due to the phenolic compounds in the MSKE reducing oxidation of lipids and the breakdown of related products. Therefore, the TVB-N and TBARS contents continued to exist and could be measured for sea food freshness assessment. The shrimp treated with 5,000 mg/kg MSKE had greater durability against lipid oxidation than the shrimp treated at a low concentration of MSKE, such as in the control and with 1,000 mg/kg MSKE. Similarly, treatment with the phenolic compound from green tea was reported to retard lipid oxidation in white shrimp during chilled (4°C) storage (Laemmli, 1970). The development of lipid oxidation in shrimp results from the effect of unsaturated fatty acids and oxygen to which the shrimp are exposed during iced storage. A crustacean's membrane is made up of polyunsaturated fatty acid and any tissue injuries throughout storage can accelerate lipid oxidation. During long-term storage of shrimp, unstable primary oxidation would break down to secondary oxidation products such as the aldehyde and alkyl radicals detected by the TBARS assay. Lipid oxidation in seafood can be induced by autoxidation, photosensitized oxidation or lipoxygenase, peroxidase and microbial enzymes (Mariutti and Bragagnolo, 2017). The phenolic compound extracts from mango seed contained mainly petunidin rutinoside-(p-coumaric acid) gallate that has been reported to have effective antioxidant activity based on ABTS and FRAP assays (López-Cobo et al., 2017). The phenolic extract from plants could inhibit pro-oxidants such as the metal in shrimp tissue, resulting in the prevention of rancidity during the propagation step. Based on the results from the current study, the mango seed kernel extract with antioxidant activity has potential use as an alternative antioxidant in seafood products.

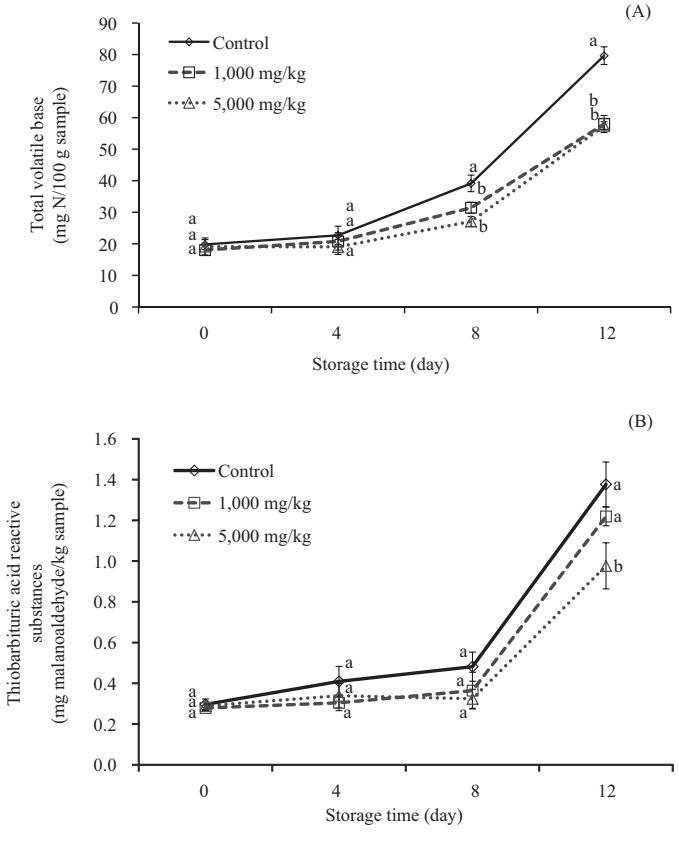

Fig. 3 Shrimp treated with mango seed kernel extract at different levels during 12 d of iced storage: (A) total volatile base; (B) thiobarbituric acid reactive substances, where error bars indicate standard deviation (n=3), and different lowercase letters at each time point indicate significant ($p < 0.5$) difference.

Effect of mango seed kernel extract on psychrophilic bacterial count

In general, if the total bacterial count reaches 1×10^6 cfu/g of sample, then spoilage occurs and the product must be rejected (International Commission on Microbiological Specifications for Foods, 2018). The acceptable limitation of fecal coliforms found in raw shrimp must be less than 20 cfu/g, while the level of *Staphylococcus aureus* should not be greater than 10 cfu/g (International Commission on Microbiological Specifications for Foods, 2018). The presence of psychrophilic bacteria in the white shrimp treated with and without MSKE kept in ice is shown in Table 2. The psychrophilic bacterial counts of the shrimp treated with MSKE (1,000 mg/kg and 5,000 mg/kg) were lower than those without the extract ($p < 0.05$). Shrimp treated with 5,000 mg/kg of MSKE had the lowest psychrophilic

Table 2 Psychrophilic bacterial count of shrimps treated with and without mango seed kernel extract (MSKE) during storage time of 0 – 12 d

Storage time (d)	MSKE (mg/kg)	Psychrophilic bacterial count (log cfu/g)
0	0	3.39±0.07[a]
	1,000	3.06±0.23[a]
	5,000	3.07±0.27[a]
4	0	4.81±0.08[a]
	1,000	3.94±0.10[b]
	5,000	4.18±0.19[b]
8	0	5.39±0.12[a]
	1,000	5.23±0.08[a]
	5,000	4.70±0.06[b]
12	0	6.20±0.28[a]
	1,000	5.88±0.05[ab]
	5,000	5.65±0.16[b]

cfu = colony forming units

Values (mean ± standard deviation, n =3) for each storage time in same column with different lowercase superscripts are significantly ($p < 0.05$) different.

bacterial count after 12 d ($p < 0.05$). The shrimp without treatment exceeded the acceptable limit after storage for 12 d with 6.20±0.28 log cfu/g psychrotrophic bacteria. Similarly, pomegranate peel extract could significantly decrease psychrophilic counts of white shrimp during chilled storage (Basiri et al., 2015). The numbers of psychrophilic bacteria detected in white shrimp immersed in catechin or ferulic acid reduce during chilled storage (Nirmal and Benjakul, 2010). Basically, mango seed kernel extract has antimicrobial activity that inhibited the growth of Gram-positive and Gram-negative bacteria (Parvez et al., 2016). Gram-negative bacteria mainly cause deterioration of iced stored food especially, fish and shrimp; inhibitory effects on Gram-negative bacteria involve disruptive action of the phenolic compounds on the outer membrane. The MSKE inhibited the growth of psychrophilic bacteria during the extended iced storage, due to the formation of phenolic compounds and protein complexes that might cause protein denaturation and lyse the cell walls of microorganisms (Chanthachum and Beuchat, 1997). This result was also in agreement with the TVB-N content of the shrimp treated with MSKE. Generally, an inhibitory effect on the growth of the microflora could limit the production of TVB and TMA. In addition, phenolics in the MSKE might have chelating property that could retard any metal ions considered to be essential for bacteria growth (Ghous et al., 2015). Thus, the growth of psychrophilic bacteria in the white shrimps was partially inhibited by the MSKE.

Effect of mango seed kernel extract on melanosis of white shrimp

Black spot evaluation based on the melanosis scores of white shrimp is illustrated in Fig. 4. No melanosis was found in any of the treated shrimps at the start of storage. However, the melanosis score of white shrimp without any MSKE treatment was higher than that of white shrimp treated with MSKE during iced storage. MSKE immersion was adequate to retard the black spot effect in shrimp compared to the control. The MSKE at a concentration of 1,000 mg/kg had a lower melanosis inhibition effect in the white shrimp than at 5,000 mg/kg during iced storage. The shrimp sample immersed in MSKE solutions at higher concentrations showed greater effects of polyphenol oxidase activity. In addition, the phenolic compounds in the MSKE might have polymerized with the protein as well as with the PPO in the shrimp. Some studies have investigated the use of plant phenolic extracts to inhibit melanosis in shrimp during storage (Benjakul et al., 2005; Basiri et al., 2015). Nirmal and Benjakul (2011b) reported that extract of green tea could be used in Pacific white shrimp product to inhibit melanosis formation. Basiri et al. (2015) reported that methanolic pomegranate extract (0.01–0.02 g/mL) could be used as a melanosis inhibitor in shrimp during refrigerated storage. Further study on the use of mango phenolic extract should investigate: the appropriate concentration as a potential food additive; extraction conditions; how best to integrate with processing conditions; and its phenolic profiles.

The cephalothorax of white shrimp contains PPO that causes black spot or melanosis. The degree of melanosis inhibition depends on the concentration of MSKE. MSKE had higher PPO inhibitory activity than gallic acid and MSKE could reduce black spot formation in shrimp for up to 8 d of iced storage. Using MSKE as the immersion solution for shrimp could retard melanosis, psychrophilic microbial growth, TVB-N and lipid oxidation. Thus, MSKE could help to reduce melanosis and extent the shelf life quality of shrimp during iced storage.

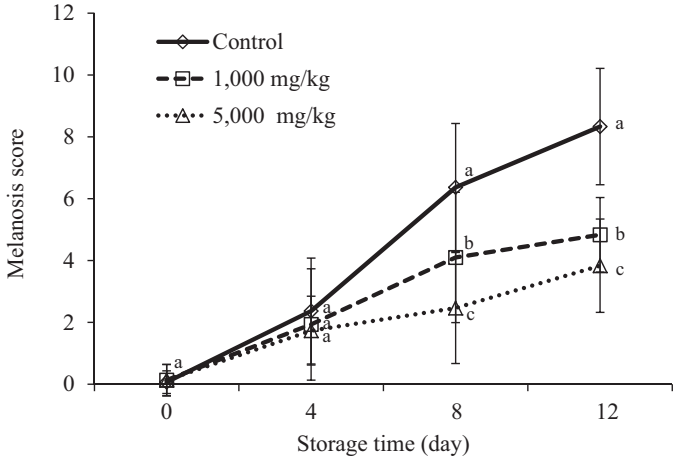

Fig. 4 Melanosis scores of Pacific white shrimp treated with mango seed kernel extract at different levels during 12 d of iced storage, where error bars indicate standard deviation (n=30), and different lowercase letters at each time point indicate significant ($p < 0.5$) difference.

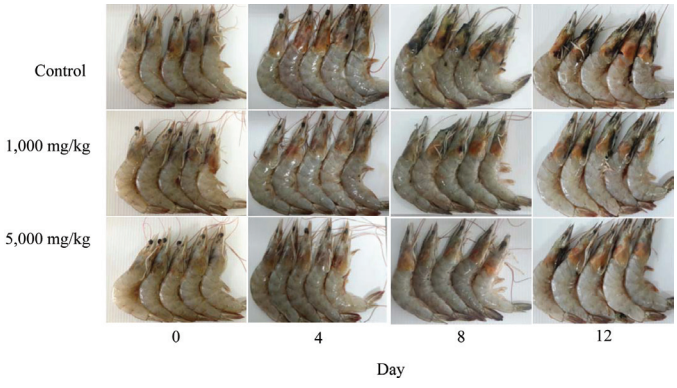

Fig. 5 Photographs of Pacific white shrimp without and with treatment using mango seed kernel extract at different concentrations during 12 d of iced storage.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The author thanks the Faculty of Science, Burapha University for financial support.

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