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Effect of ferulic acid on inhibition of polyphenoloxidase and quality changes of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage

Nilesh Prakash Nirmal, Soottawat Benjakul *

Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, 15 Kanchanawanich Road, Hat Yai, Songkhla 90112, Thailand

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ABSTRACT

Effects of ferulic acid (FA) on polyphenoloxidase (PPO) and the quality changes of Pacific white shrimp (*Litopenaeus vannamei*) during iced storage for 10 days were investigated. Both FA and oxygenated FA (OFA) with different concentrations (0.1%, 0.5%, 1% and 2% (w/v)) showed PPO inhibitory activity in a dose dependent manner. FA was generally more effective in PPO inhibition than was OFA. Based on activity staining, white shrimp PPO with an apparent molecular weight of 210 kDa was inhibited by FA. When whole shrimps were treated with FA solution with concentrations of 1% or 2% and stored in ice for up to 10 days, the increase in psychrophilic and mesophilic bacterial count were retarded, in comparison with the control and those treated with 1.25% sodium metabisulphite (SMS). The coincidental lower rates of increase in pH and total volatile base content were obtained. Additionally, shrimps treated with 2% FA possessed the lowest peroxide value and thiobarbituric acid reactive substances (TBARS) value during the storage. After 10 days of storage, shrimps treated with 2% FA had the lower melanosis score and higher score for colour, flavour and overall likeness, compared with the control and SMS treated shrimps ($P < 0.05$).

1. Introduction

Pacific white shrimp (*Litopenaeus vannamei*) accounts for 90% of the global aquaculture shrimp production. Thailand is the world's leading shrimp-farming country and has become the top supplier of farmed shrimp to the United States and Japan (Wyban, 2007). Despite their delicacy, shrimps are highly perishable with a limited shelf-life, mainly associated with melanosis (discoloration) and microbial spoilage (Gokoglu & Yerlikaya, 2008). Melanosis is triggered by a biochemical mechanism which oxidises phenols to quinones by polyphenoloxidase (PPO) (Kim, Marshall, & Wei, 2000). This is followed by non-enzymatic polymerisation of the quinones, giving rise to dark pigments of high molecular weight (Benjakul, Visessanguan, & Tanaka, 2005a). Although melanosis (black spots) seems to be harmless to consumers, it drastically reduces the product's market value, consumer's acceptability and hence considerable financial loss (Montero, Lopez-Caballero, & Perez-Mateos, 2001). Lopez-Caballero, Martinez-Alvarez, Gomez-Guillen, and Montero (2007) reported that melanosis and spoilage were retarded during frozen storage, but continued in defrosted shrimp. Apart from melanosis, lipid oxidation is another deteriorative reaction causing the unacceptability of fish and shrimp products. Fish lipids are susceptible to oxidation owing to the high levels of polyunsaturated fatty acid; this can be initiated by autoxidation, an enzymatic reaction involving lipoxygenase, peroxidase and microbial enzymes. Lipid oxidation causes physicochemical changes, rancidity and off-flavours in fish meat (Bak, Andersen, Andersen, & Bertelsen, 1999).

To extend the shelf-life of shrimps or crustaceans, melanosis inhibitors have been used. Sulphites and their derivatives are widely used as PPO inhibitors through preventing the polymerisation of quinones, combining irreversibly with them, and forming colourless compounds (Montero et al., 2001). However, sulphiting agents are known to produce allergic reactions and serious disturbances in asthmatic subjects (DeWitt, 1998). Therefore, safe compounds from natural origin such as ascorbic acid, kojic acid (Chen, Wei, Rolle, Otwell, & Balaban, 1991a), ficin (Taoukis, Labuza, Lillemo, & Lin, 1990), citric acid (Montero et al., 2001), dodecyl gallate (Kubo, Chen, & Nihei, 2003) and oxalic acid (Son, Moon, & Lee, 2000) have been used as the substitutes of sulphiting agents.

Plant phenolic compounds are other promising agents possessing antimicrobial and antioxidant activities (Chanthachum & Beuchat, 1997; Souza, Silva, Loir, Rees, & Rogez, 2008). Plant phenolic compounds such as flavonoid compounds, tocopherols, coumarins and cinnamic acid derivatives have an antioxidative effect (Jayaprakash, Singh, & Sakariah, 2001). Recently, it has been reported that grape seed extract could inhibit the melanosis in shrimp (*Parapenaeus longirostris*) (Gokoglu & Yerlikaya, 2008). From our previous study, different phenolic compounds including ferulic acid exhibited the different inhibitory effects on the white shrimp PPO (data not shown). In addition to melanosis inhibition, plant phenolic

* Corresponding author. Tel.: +66 7428 6334; fax: +66 7421 2889.
E-mail address: soottawat.b@psu.ac.th (S. Benjakul).

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compounds may act as an antimicrobial and antioxidant, which could retard the microbial and chemical spoilage of shrimps. Nevertheless, no information regarding the use of selected phenolic compound on melanosis prevention as well as shelf-life extension of white shrimp has been reported. The aim of this study was to investigate inhibition effect of ferulic acid on PPO, the formation of melanosis as well as chemical, microbial and sensorial changes of Pacific white shrimp (*L. vannamei*) during iced storage.

2. Materials and methods

2.1. Chemicals

L-β-(3,4-dihydroxylphenyl) alanine (L-DOPA), Brij-35, ferulic acid, malonaldehyde bis (dimethyl acetal), thiobarbituric acid (TBA), ferrous chloride and ammonium thiocyanate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Trichloroacetic acid (TCA), sodium metabisulphite, ammonium sulphate, methanol, ethanol, chloroform, anhydrous sodium sulphate and standard plate count agar (PCA) were obtained from Merck (Darmstadt, Germany). Sodium dedocyl sulphate (SDS), *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED) and all chemicals for electrophoresis were procured from Bio-Rad Laboratories (Hercules, CA, USA).

2.2. Shrimp collection and preparation

Pacific white shrimps (*L. vannamei*) with the size of 55–60 shrimps/kg were purchased from the dock in Hat Yai, Songkhla, Thailand. The shrimps were kept in ice with a shrimp/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai within 1 h. Upon arrival, shrimps were washed in cold water and stored in ice until used (not more than 5 h).

2.3. Preparation of PPO extract from the cephalothoraxes of Pacific white shrimp

The cephalothoraxes of twenty shrimps were separated, pooled and powderised by grinding with liquid nitrogen in a Waring blender. The powder obtained was kept in polyethylene bag and stored at −20 °C for not more than 2 weeks. The isolation of PPO was carried out according to the method of Simpson, Marshall, and Otwell (1987) with a slight modification. The powder (50 g) was mixed with 150 ml of the extracting buffer (0.05 M sodium phosphate buffer, pH 7.2, containing 1.0 M NaCl and 0.2% Brij-35). The mixture was stirred continuously at 4 °C for 30 min, followed by centrifugation at 8000g at 4 °C for 30 min using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Fullerton, CA, USA). Solid ammonium sulphate was added into the supernatant to obtain 40% saturation and allowed to stand at 4 °C for 30 min. The precipitate was collected by centrifugation at 12,500g at 4 °C for 30 min using a refrigerated centrifuge. The pellet obtained was dissolved in a minimum volume of 0.05 M sodium phosphate buffer, pH 7.2 and dialysed against 15 vol of the same buffer at 4 °C with three changes of dialysis buffer. The insoluble materials were removed by centrifugation at 3000g at 4 °C for 30 min and the supernatant was used as "crude PPO extract".

2.4. Measurement of PPO activity

PPO activity was assayed using L-DOPA as a substrate according to the method of Simpson et al. (1987) with a slight modification. The assay system consisted of 100 μl of crude PPO extract, 600 μl of 15 mM L-DOPA in deionised water, 400 μl of 0.05 M phosphate buffer, pH 6.0 and 100 μl of deionised water. The PPO activity was determined for 3 min at 45 °C by monitoring the formation of dopachrome at 475 nm using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). One unit of PPO activity was defined as an increase in the absorbance by 0.001 at 475 nm/min/ml. Enzyme and substrate blanks were prepared by excluding the substrate and enzyme, respectively, from the reaction mixture and deionised water was used instead.

2.5. Effect of ferulic acid on the inhibition of Pacific white shrimp PPO

2.5.1. Preparation of ferulic acid (FA) and oxygenated ferulic acid (OFA) solutions

Ferulic acid solutions with different concentrations (0.2%, 1%, 2% and 4%, w/v; 50 ml) were adjusted to pH 8 by 6 N NaOH and stirred for 15 min at room temperature (26–28 °C). Thereafter, the pH of the solution was adjusted to 7 by using 6 N HCl and referred to as 'ferulic acid solution' (FA).

Oxygenated ferulic acid solution (OFA) was prepared by the modified method of Balange and Benjakul (2009). Ferulic acid solutions at different concentrations (0.2%, 1%, 2% and 4%, w/v; 50 ml) were adjusted to pH 8 using 6 N NaOH or 6 N HCl. The prepared solution was placed in a temperature-controlled water bath (40 °C) and subjected to oxygenation for 1 h by bubbling the solution with oxygen (TTS Gas Agency, Hat Yai, Thailand) to convert the FA to quinone. After being oxygenated for 1 h, the solution was then adjusted to pH 7 by using 6 N HCl and was referred to as 'oxygenated ferulic acid solution' (OFA).

2.5.2. Inhibitory effect of FA and OFA on PPO activity

FA or OFA with different concentrations (0.2%, 1%, 2% and 4% w/v) (100 μl) were mixed with crude PPO extract (100 μl) to obtain the final concentrations of 0.1%, 0.5%, 1% and 2% (w/v), respectively. This reaction mixture was incubated for 30 min at room temperature. Then, the assay buffer (400 μl) was added. To initiate the reaction, 600 μl of pre-incubated 15 mM L-DOPA (45 °C) were added. The reaction was conducted at 45 °C and the absorbance at 475 nm was monitored for 3 min. The control was run in the same manner, except deionised water was used instead of FA or OFA. Residual activity was determined and expressed as the activity relative to the control (without FA or OFA) as follows:

$$\text{Relative activity } (\%) = \frac{B}{A} \times 100$$

where *A*: PPO activity of control; *B*: PPO activity in the presence of FA or OFA.

2.6. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and PPO activity staining

Protein patterns of PPO crude extract were analysed by SDS–PAGE according to the method of Laemmli (1970). The extract was mixed with the sample buffer containing 1.5 M βME at a ratio of 1:1 (v/v). The samples (25 μg protein) were loaded onto the polyacrylamide gel made of 7.5% running gel and 5% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, one of two identical gels was immersed in a McIlvaine buffer (0.2 M sodium phosphate and 0.1 M sodium citrate), pH 6.5, containing 15 mM L-DOPA for 25 min at 25 °C. The activity zone appeared as the dark band. Another gel was stained by 0.125% Coomassie Brilliant Blue R-250 and destained in 25% methanol and 10% acetic acid. To estimate the molecular weight of PPO, the markers including myosin from rabbit muscle (200 kDa), β-galactosidase from *Escherichia coli* (116 kDa), phosphorylase b from rabbit muscle (97 kDa), bovine serum albumin (66 kDa), glutamic dehydrogenase from bovine

liver (55 kDa), glyceraldehyde-3-phosphate dehydrogenase from rabbit muscle (36 kDa), and carbonic anhydrase from bovine erythrocytes (29 kDa) were used.

To study the inhibitory effect of FA towards PPO, crude PPO extract was incubated with FA (1, 2 and 4%) at a ratio of 1:1 (v/v) for 30 min at room temperature prior to loading onto polyacrylamide gel, followed by activity staining as previously described.

2.7. Protein determination

Protein concentration was measured by the method of Lowry, Rosebrough, Fan, and Randall (1951) using bovine serum albumin as the standard.

2.8. Effect of FA on the quality of Pacific white shrimp during iced storage

2.8.1. Preparation of shrimps treated with FA

Whole Pacific white shrimps were immersed in FA solution (1% and 2%) at a shrimp/solution ratio of (1:2, w/v) at 4 °C for 15 min. Another portion of shrimps was soaked in sodium metabisulphite (1.25%) at a ratio of 1:2 (w/v) for 1 min at 4 °C (Kim et al., 2000). Treated shrimps were drained on the screen for 3 min at 4 °C. Shrimps without any treatment were used as the control. All samples were stored in polystyrene box containing ice using a shrimp/ice ratio of 1:2 (w/w). To maintain shrimp/ice ratio, the molten ice was removed and the same amount of ice was added. Samples (25 shrimps) were taken for each treatment every 2 days up to 10 days for chemical, microbiological, sensorial analysis and melanosis determination.

2.8.2. Microbiological analysis

Microbiological analysis was performed following the method of Thepnuan, Benjakul, and Visesssanguan (2008) with some modifications. Five whole Pacific white shrimps were collected aseptically and used as the composite sample. Ground sample (without peeling) (25 g) was placed in a stomacher bag containing 225 ml of 0.85% saline water. After mixing for 1 min in a Stomacher blender (M400, Seward, UK), appropriate dilutions were prepared for the determination of total viable counts (TVC) using plate count agar (PCA) containing 0.5% NaCl by using spread plate method. Mesophilic and psychrophilic bacterial counts were determined by incubating plates at 35 °C for 2 days and 4 °C for 7 days, respectively.

2.8.3. Chemical analyses

2.8.3.1. pH Measurement. pH Measurement was performed by the method of Lopez-Caballero et al. (2007) with a slight modification. Shrimp meat (2 g) was homogenised with 10 vol of deionised water for 1 min and the homogenate was kept at room temperature for 5 min. The pH was determined using a pH-meter (Sartorious North America, Edgewood, NY, USA).

2.8.3.2. Determination of total volatile base and trimethylamine contents. Total volatile base (TVB) and trimethylamine (TMA) contents in shrimp meat were determined using the Conway micro-diffusion method (Conway & Byrne, 1936). Sample (2 g) was extracted with 8 ml of 4% (w/v) trichloroacetic acid (TCA) solution. The mixture was homogenised at 8000 rpm for 1 min using PT 2100 homogeniser (Kinematica AG, CH-6014, Littau/Luzern, Switzerland). The homogenate was kept at room temperature for 30 min. The homogenate was filtered through Whatman No. 41 filter paper (Schleicher & Schuell, Maidstone, England). The filtrate was collected and the final volume was adjusted to 10 ml using 4% TCA. The inner ring solution (1 ml) and filtrate (1 ml) were added to inner ring and outer ring of the Conway unit, respectively. One millilitre of saturated K_2CO_3 solution was then added into the outer ring. The Conway unit was closed and the solution was mixed slowly. The mixture was incubated at 37 °C for 60 min and the inner ring solution was titrated with 0.02 N HCl using micro-burette until the green colour turned into pink. For blank, TCA solution (4%) was used instead of sample extract. TMA content was determined in the same manner as TVB but 1 ml of 10% formaldehyde was added to the filtrate to fix ammonia present in the sample. The amounts of TVB and TMA were calculated and results were expressed as mg N/100 g shrimp meat.

2.8.3.3. Determination of peroxide value. Lipid was extracted by the method of Bligh and Dyer (1959). Sample (25 g) was homogenised with 200 ml of a chloroform: methanol: distilled water mixture (50:100:50) at the speed of 9500 rpm for 2 min at 4 °C using an IKA Labortechnik homogeniser (Selangor, Malaysia). The homogenate was added with 50 ml of chloroform and homogenised at 9500 rpm for 1 min. Then, 25 ml of distilled water were added and homogenised again for 30 s. The homogenate was centrifuged at 3000 rpm at 4 °C for 15 min using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Fullerton, CA, USA). The chloroform phase was drained off into a 125 ml erlenmeyer flask containing about 2–5 g of anhydrous sodium sulphate, shaken very well, and decanted into a round-bottom flask through a Whatman No. 4 filter paper (Schleicher & Schuell, Maidstone, England). The solvent was evaporated at 25 °C using an EYELA N-100 rotary evaporator (Tokyo, Japan) and the residual solvent was removed by flushing nitrogen.

Peroxide value was determined spectrophotometrically by ferric thiocyanate method (Wu, Chen, & Shiau, 2003) with a slight modification. Extracted lipids (5 μg) were added with 2.395 ml of 75% ethanol and the mixture was vortexed until completely dissolved. Thereafter, 50 μl of 30% ammonium thiocyanate, and 50 μl of 20 mM ferrous chloride solution in 3.5% HCl were added. The mixture was mixed thoroughly and was allowed to stand for 3 min. The absorbance was read at 500 nm and peroxide value (PV) was expressed as A_{500}.

2.8.3.4. Determination of thiobarbituric acid reactive substances (TBARS). TBARS in the samples was determined as described by Benjakul and Bauer (2001) with some modifications. Ground shrimp meat (1 g) was mixed with 9 ml of a solution containing 0.375% TBA, 15% TCA and 0.25 N HCl. The mixture was heated in boiling water for 10 min, followed by cooling with the running water. The mixture was centrifuged at 4000g for 20 min (MIKRO20, Hettich Zentrifugan, Germany). The supernatant was collected and the absorbance was read at 532 nm using a UV-160 spectrophotometer. TBARS value was calculated from the standard curve of malonaldehyde (0–2 ppm) and expressed as mg malonaldehyde/kg shrimp meat.

2.8.4. Melanosis assessment

Melanosis or blackening of Pacific white shrimp was evaluated through visual inspection by six trained panelists using 10-point scoring test (Montero et al., 2001). Panelists were asked to give the melanosis score (0–10), where 0 = absent; 2 = slight (up to 20% of shrimps' surface affected); 4 = moderate (20–40% of shrimps' surface affected); 6 = notable (40–60% of shrimps' surface affected); 8 = severe (60–80% of shrimps' surface affected); 10 = extremely heavy (80–100% of shrimps' surface affected).

2.8.5. Sensory evaluation

At day 0 and day 10 of storage, whole shrimps without and with different treatments were placed on a stainless steel tray, covered with aluminium foil and steamed for 5 min. The cooked samples were evaluated by 30 panelists from the Department of Food

Technology, aged 25–35, using the 9-point hedonic scale, where 9 = like extremely; 7 = like moderately; 5 = neither like or nor dislike; 3 = dislike moderately; 1 = dislike extremely (Meilgaard, Civille, & Carr, 1990). Panelists were regular consumers of shrimp and had no allergies to shrimp. All panelists were asked to evaluate for colour, odour, taste, flavour and overall likeness. Samples were presented unpeeled in plates coded with three-digit random numbers.

2.9. Statistical analyses

All experiments were performed in triplicate and a completely randomised design (CRD) was used. Analysis of variance (ANOVA) was performed and means comparisons were done by Duncan's multiple range tests (Steel & Torrie, 1980). For pair comparison, *T*-test was used. Analysis was performed using a SPSS package (SPSS 11.0 for windows, SPSS Inc, Chicago, IL, USA).

3. Results and discussion

3.1. Effect of ferulic acid on the inhibition of PPO

The effect of ferulic acid, both FA and oxygenated FA (OFA), on the inhibition of PPO activity is shown in Fig. 1. For both FA and OFA, the increased inhibition of PPO was observed with increasing concentrations ($P < 0.05$). At the same concentration used, FA exhibited a higher inhibitory activity towards PPO than did OFA counterpart ($P < 0.05$). Nevertheless, similar inhibitory activity was noticeable at 2% ($P > 0.05$). Quinone prevalent in OFA might loss the reducing power, in which DOPA-quinone formed induced by PPO could be reduced to DOPA. Quinone has been reported to cross-link the proteins or enzymes (Balange & Benjakul, 2009). Furthermore, phenolic compounds (reduced form) could interact with protein or enzymes via hydrogen bond or hydrophobic interaction (Prigent, 2005). Some phenolic compounds inhibit PPO activity by interacting with active site of the enzyme (Janovitz-Klapp, Richard, Goupy, & Nicolas, 1990). Among the various phenolic acids tested, kojic acid showed the highest inhibitory effect on browning in apple slices (Son, Moon, & Lee, 2001). The prawn (*Penaeus japonicus*) treated with kojic acid had no melanosis up to 5 days at 4 °C (Montero et al., 2001). The result suggested that FA was more effective in PPO inhibition in comparison with OFA. This indicated the role of hydroxyl group in PPO inhibition, possibly via its ability of electron donating to intermediate quinone or its role in cross-linking PPO via hydrogen bonding, which caused the loss in PPO activity. FA might inhibit PPO by acting as a competitive inhibitor. Aromatic carboxylic acids of cinnamic acid and its analogues, *p*-coumaric, ferulic, and sinapic acids are competitive inhibitors of PPO due to their structural similarities to its phenolic substrates (Kim et al., 2000).

The activity staining of PPO from the Pacific white shrimp cephalothoraxes in the absence or presence of FA is shown in Fig. 2. Without FA, PPO was able to induce the oxidation of DOPA to DOPA-quinone and the intermediate products subsequently underwent polymerisation to melanin. One activity zone (Lane-A) was observed as dark brown colour at the apparent molecular weight of 210 kDa. In the presence of FA at levels of 0.5%, 1% and 2%, activity band intensity decreased as the concentrations in-

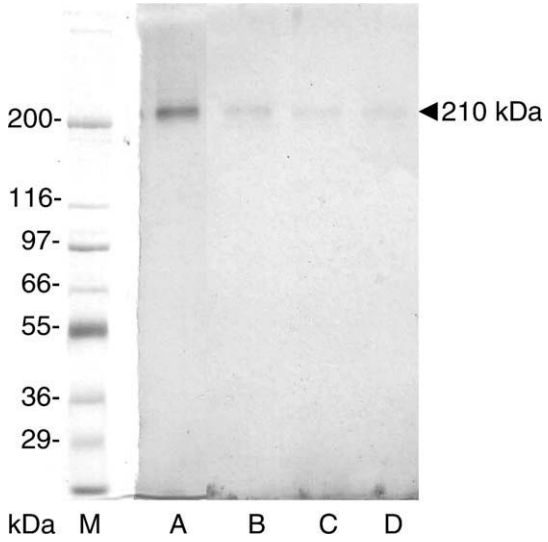

Fig. 2. Activity staining of polyphenoloxidase from the cephalothoraxes of Pacific white shrimp in the absence or presence of FA at different levels. *M*: molecular weight marker; *A*: PPO crude extract; *B*: PPO crude extract with 0.5% FA; *C*: PPO crude extract with 1% FA; *D*: PPO crude extract with 2% FA.

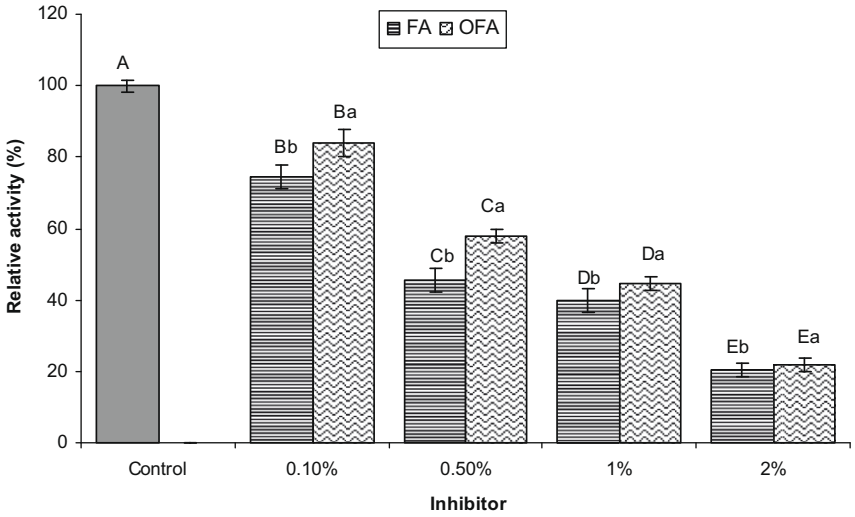

Fig. 1. Effect of ferulic acid (FA) and oxygenated ferulic acid (OFA) at different levels on the inhibiton of polyphenoloxidase from the cephalothoraxes of Pacific white shrimp. Bars represent the standard deviation from triplicate determinations. Different capital letters on the bars within the same FA or OFA together with the control indicate the significant differences ($P < 0.05$). The different letters on the bars within the same level of FA and OFA indicate significant differences ($P < 0.05$).

creased (Lanes B, C & D). These results were in accordance with *in vitro* PPO inhibitory activity of FA (Fig. 1). These results reconfirmed the inhibitory effect of FA on white shrimp PPO. PPO from different shrimps had the different iso-forms with varying molecular weights (Chen, Rolle, Marshall, & Wei, 1991b). PPO from the kuruma prawn cephalothoraxes had the molecular weight of 160 kDa (Benjakul et al., 2005a). The molecular weights of pink shrimp PPO were 30 and 35 kDa, while those of white shrimp were 20 and 25 kDa (Chen, Charest, Marshall, & Wei, 1997). Zamorano, Martinez-Alvaerz, Montero, and Gomez-Guillen (2009) reported that PPO from deep water pink shrimp (*P. longirostris*) had the molecular weights of 500 and 200 kDa.

3.2. Effect of FA on the microbiological changes of Pacific white shrimp during iced storage

Changes in psychrophilic and mesophilic bacterial count of Pacific white shrimp during iced storage as influenced by FA treatment are shown in Fig. 3a and b. In general, psychrophilic bacterial count increased continuously in the control (sample without treatment) throughout the storage for 10 days ($P < 0.05$). After treatment with sodium metabisulphite (SMS), 1% FA or 2% FA, the lower psychrophilic bacterial count was obtained, in comparison with the control ($P < 0.05$). At the same storage time, the lowest psychrophilic bacterial count was found in shrimp treated with 2% FA ($P < 0.05$). At the end of storage (day 10), psychrophilic bacterial count of the control, those treated with SMS, 1% FA and 2% FA were 4.6, 4.3, 4.2 and 3.9 log CFU g^{-1}, respectively. The result indicated the antimicrobial activity of FA towards psychrophilic bacteria in white shrimps during iced storage. FA might disrupt the cell wall of microorganism by forming complexes with proteins in cell wall and make lyses of cell wall (Chanthachum & Beuchat, 1997).

Changes in mesophilic bacterial count of Pacific white shrimp without and with treatments during iced storage are depicted in Fig. 3b. The decrease in mesophilic bacterial count was observed in white shrimp after the treatments with either SMS or FA ($P < 0.05$). During storage, mesophilic bacterial count increased continuously up to day 4. Thereafter, a slight increase in mesophilic bacterial count was found until the end of storage (day 10). The increase in mesophilic bacterial count within the first 4 days was due to the tolerance to cold conditions of those microorganisms up to a certain limit. Zeng, Thorarinsdottir, and Olafsdottir (2005) reported that low temperature inhibited the

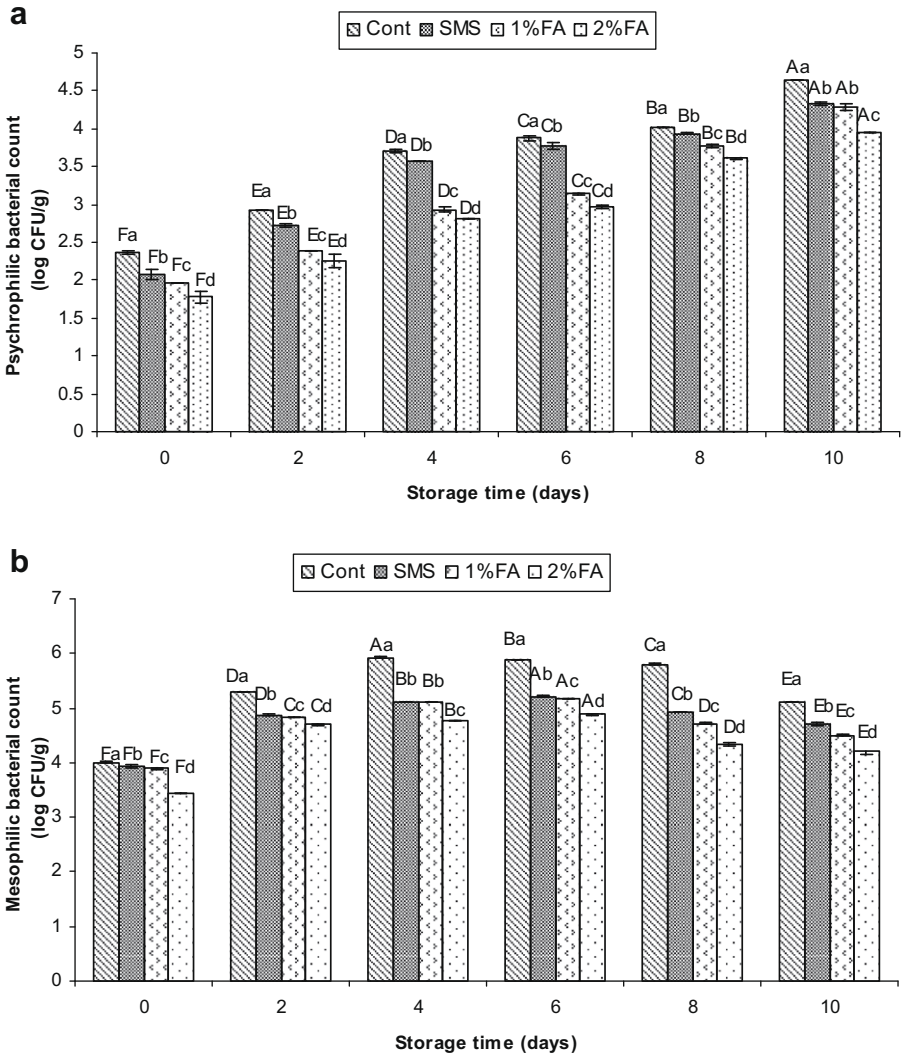

Fig. 3. Psychrophilic (a) and mesophilic (b) bacterial count of Pacific white shrimp treated with FA at different levels during 10 days of iced storage. Bars represent the standard deviation from triplicate determinations. Different capital letters on the bars within the same treatment indicate the significant differences ($P < 0.05$). The different letters on the bars within the same storage time indicate significant differences ($P < 0.05$). Cont.: control; SMS: sodium metabisulphite; 1% FA: 1% ferulic acid; 2% FA: 2% ferulic acid.

micro-flora. If the cooling is not ensured for example when ice has melted in the chilling system, the micro-flora may be more active in the spoilage process. At day 4 of storage, the control, shrimps treated with SMS, 1% FA or 2% FA had mesophilic bacterial count of 5.9, 5.1, 5.1 and 4.7 log CFU g^{-1}, respectively. After 4 days of storage, some mesophilic bacteria could not tolerate the cold temperature as indicated by the lower count. At the end of iced storage, the control, those treated with SMS, 1% FA and 2% FA had mesophilic bacterial count of 5.1, 4.7, 4.5 and 4.2 log CFU g^{-1}, respectively. Antimicrobial activity of phenolic compounds present in herbaceous and woody plants has not been fully defined (Scalbert, 1991). Chanthachum and Beuchat (1997) reported that treatment with 5% kiam wood extract retarded the rate of growth of *Listeria monocytogenes*, aerobic mesophiles and psychrotrophic microorganism naturally present on cabbage.

3.3. Effect of FA on the chemical changes of Pacific white shrimp during iced storage

3.3.1. pH

Changes in pH of the Pacific white shrimp with and without different treatments during iced storage are shown in Fig. 4a. pH of the fresh Pacific white shrimp at day 0 was 6.62. As the storage time increased, pH of all shrimps increased ($P < 0.05$). The increase in pH was different among the treatments. Among all samples, those treated with 2% FA had the lowest pH within the first 4 days of storage. During 6–10 days of storage, shrimps treated with 1% or 2% FA showed the lowest pH, compared with the control and those treated with SMS ($P < 0.05$). The increase in pH was associated with the accumulation of basic compounds, mainly resulted from microbial action (Lopez-Caballero et al., 2007). The increases in pH value were more rapid in the shrimps (*Pandalus borealis*) stored in ice at 1.5 °C and reached a final pH of 8.26 as compared to sample stored in liquid ice (pH 7.98) (Zeng et al., 2005). Goncalves, Lopez-Caballero, and Nunes (2003) reported that deepwater pink shrimp (*P. longirostris*) was considered unacceptable at pH values of 7.56, 7.64 and 7.55 for air packed shrimp, ice stored shrimp and modified atmosphere packed shrimp, respectively. Shamshad, Nisa, Riaz, Zuberi, and Qadri (1990) reported that *Penaeus merguiensis* was not acceptable when the pH was greater than 7.6. The lower increase in pH of shrimps treated with 1% or 2% FA was in accordance with the lower microbial count (Fig. 3). These results suggested that FA might play a role in retarding microbial growth, in which the spoilage or decomposition could be lowered.

3.3.2. TVB and TMA contents

TVB contents of Pacific white shrimp with and without different treatments are shown in Fig. 4b. The initial TVB content of Pacific

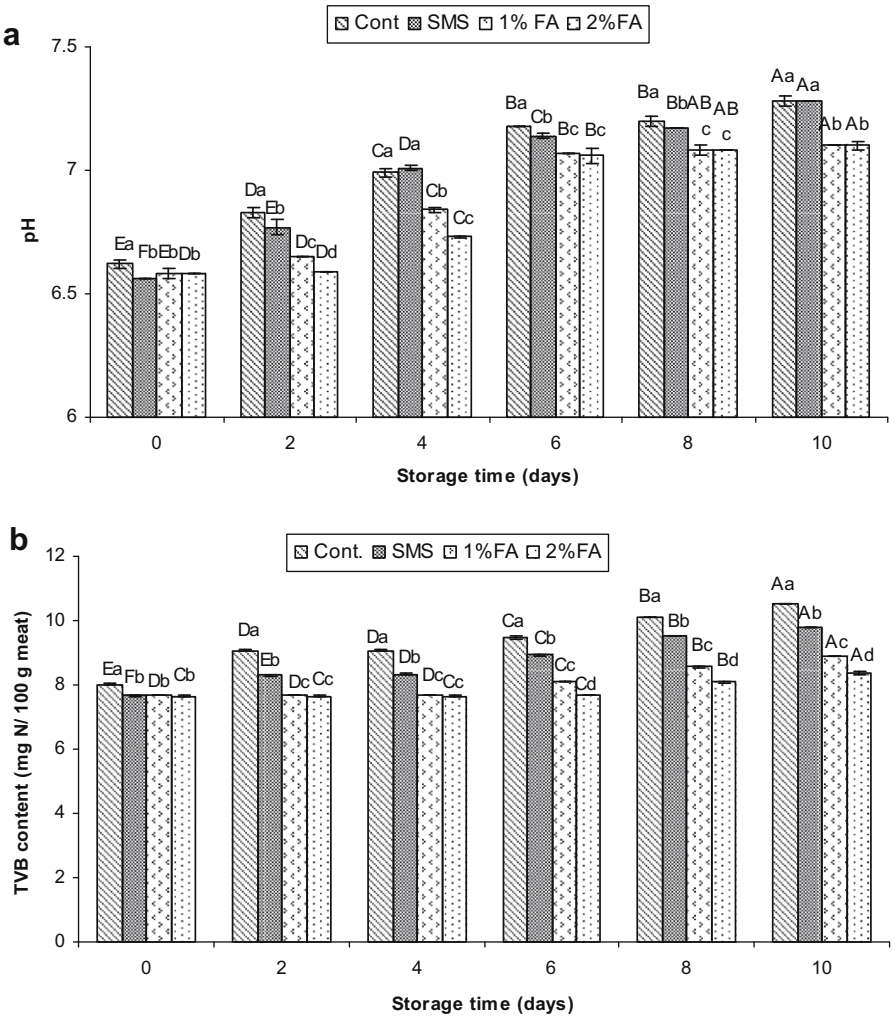

Fig. 4. pH (a) and total volatile base (b) content of Pacific white shrimp treated with FA at different levels during 10 days of iced storage. Bars represent the standard deviation from triplicate determinations. Different capital letters on the bars within the same treatment indicate the significant differences ($P < 0.05$). The different letters on the bars within the same storage time indicate significant differences ($P < 0.05$). Cont.: control; SMS: sodium metabisulphite; 1% FA: 1% ferulic acid; 2% FA: 2% ferulic acid.

white shrimp for the control (8.01 mgN/100 g shrimp meat) was slightly higher than those of shrimps treated with SMS, 1% FA and 2% FA (7.64–7.66 mg N/100 g shrimp meat). During treatment, some basic decomposed compounds might be leached out to some extent. This might lead to the lower pH of treated shrimps. The increase in TVB content of the control and shrimps treated with SMS was observed as storage time increased ($P < 0.05$). Nevertheless, shrimp treated with 1% or 2% FA had the constant TVB content up to 4 days of iced storage ($P > 0.05$). After 4 days of storage, TVB content of shrimp treated with FA also increased but at the lower rate, compared with that found in the control. At the last day of iced storage, shrimps treated with 2% FA had the lowest TVB content (8.35 mg N/100 g), compared to other treatments ($P < 0.05$). The lowest value correlated with the lower microbial counts found in FA treated shrimp. The lower TVB content of Pacific white shrimp treated with 1% or 2% FA might be owing to the inhibitory effect of ferulic acid against microbes and proteolytic enzymes. Lopez-Caballero et al. (2007) reported that the total base content of all deepwater pink shrimp (*P. longirostris*) treated with resorcinol had the decreased TVB content.

At the beginning of iced storage, there was no TMA detected up to day 6 of iced storage for all samples (data not shown). TMA content of 0.14 mg/100 g shrimp meat was found after 6 days of iced storage in the control and SMS treated shrimp. However no TMA was found in the shrimp treated with 1% or 2% of FA even at day 10 of iced storage. Lopez-Caballero et al. (2007) reported TMA-N level of 3.4 mg N/100 g in deep water pink shrimp (*P. longirostris*) after 14 days of iced storage. Zeng et al. (2005) showed that the initial TMA value of the shrimp (*P. borealis*) was 0.5 mg N/100 g at the beginning of storage and the acceptability limit for shrimp was reported to be 5 mg N/100 g.

3.3.3. Peroxide value and thiobarbituric acid reactive substances (TBARS) value

Peroxide value (PV) and TBARS value of Pacific white shrimp without and with different treatments during iced storage are depicted in Fig. 5. PV of all samples increased as the storage time increased ($P < 0.05$). The increase in PV suggested that fatty acids in shrimp muscle underwent oxidation during storage, in which hydroperoxide or peroxide were formed. Abstraction of hydrogen from fatty acid double bond produces fatty acid free radicals, which further react with oxygen to produce fatty acid hydroperoxide (Benjakul, Visessanguan, Phongkanpai, & Tanaka, 2005b). However, a slight decrease in PV was observed in all samples at day 4 of iced storage, except for SMS treated samples. The decrease in PV at day 4 was more likely due to the decomposition of hydroperoxide formed to the secondary products such as aldehydes. Nevertheless, shrimp treated with FA had the lower PV, compared to control and those treated with SMS, throughout the storage ($P < 0.05$) (Fig. 5a). This indicated the antioxidative activity of FA and its preventive effects on lipid oxidation was dose dependent. In shrimp treated with 1% or 2% FA, free radicals formed might be scavenged by FA via hydrogen or electron donating mechanism. As a result, the radicals underwent lipid peroxidation to a lower level. Among all samples, shrimps treated with 2% FA showed the lowest PV ($P < 0.05$).

For TBARS value, the control sample had the continuous increase in TBARS value after 2 days of storage up to 8 days ($P < 0.05$). Thereafter a slight decrease in TBARS in all samples, except SMS treated sample, was noticed at day 10 ($P < 0.05$). Among all samples, 2% FA treated shrimp showed the lower TBARS level, compared to other treatments at all storage times ($P < 0.05$) (Fig. 5b). TBARS values of 1% FA treated shrimp were lower than SMS treated shrimp at all storage times ($P < 0.05$). The increases in TBARS values were generally in accordance with the increases in PV (Fig. 5a). These results revealed that Pacific white shrimp treated with 2% FA had the higher stability towards lipid oxidation

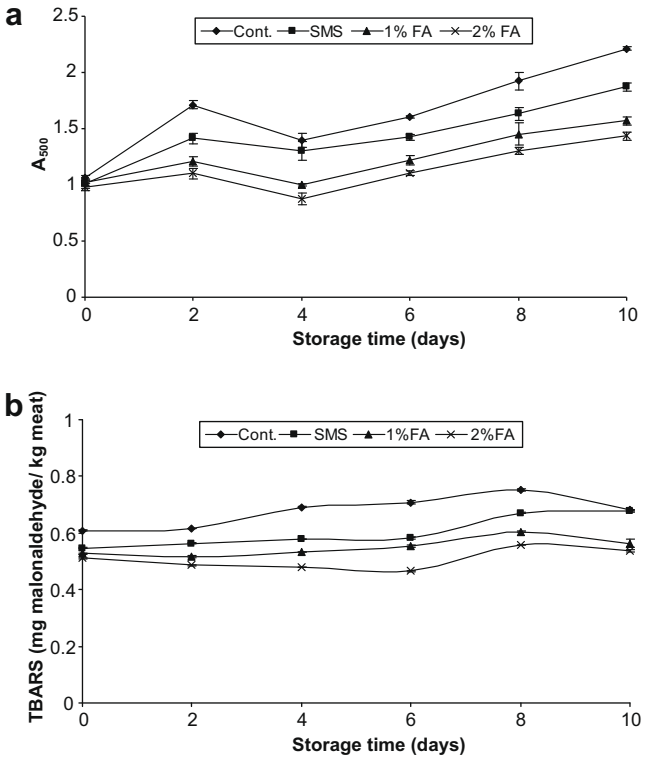

Fig. 5. PV (a) and TBARS (b) values of Pacific white shrimp treated with FA at different levels during 10 days of iced storage. Bars represent the standard deviation from triplicate determinations. Cont.: control; SMS: sodium metabisulphite; 1% FA: 1% ferulic acid; 2% FA: 2% ferulic acid.

than other samples. As a result, lipid oxidation in shrimp muscle could be effectively retarded. Polyphenols-rich-leaf extract from Amazonian plant acted as powerful antioxidant in human LDL protein by lowering TBARS levels (Souza et al., 2008). Lipid peroxidation in fish meat can be initiated by autoxidation, photosensitised oxidation or by means of enzymatic reaction like lipoxygenase, peroxidase and microbial enzyme (Decker & Hultin, 1990). Tissue membrane of crustacean contains highly polyunsaturated fatty acid and the damage of tissues during processing can induce lipid oxidation (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Lipid oxidation produces off-flavours in cold water shrimp during peel-

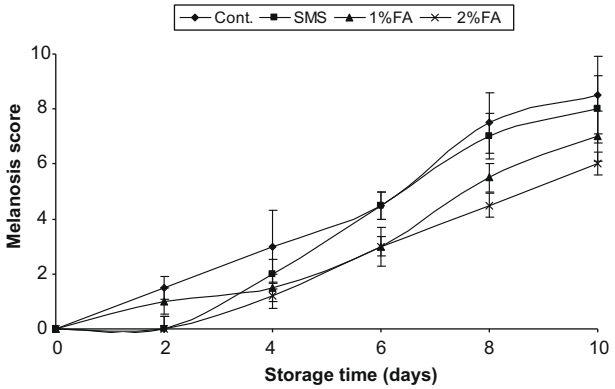

Fig. 6. Melanosis score of Pacific white shrimp treated with FA at different levels during 10 days of iced storage. Bars represent the standard deviation from triplicate determinations. Where, 0 = absent; 2 = slight (up to 20% of shrimps' surface affected); 4 = moderate (20–40% of shrimps' surface affected); 6 = notable (40–60% of shrimps' surface affected); 8 = severe (60–80% of shrimps' surface affected); 10 = extremely heavy (80–100% of shrimps' surface affected). Cont.: control; SMS: sodium metabisulphite; 1% FA: 1% ferulic acid; 2% FA: 2% ferulic acid.

Table 1
Effect of FA treatment on likeness score of Pacific white shrimp before and after 10 days of iced storage.

Storage time (days)	Treatments	Colour	Odour	Taste	Flavour	Overall
0	Cont.	9.0 ± 0.64 aA*	8.0 ± 1.22 aA	8.0 ± 0.94 aA	8.0 ± 1.04 aA	9.0 ± 0.90 aA
	SMS	9.0 ± 0.82 aA	8.0 ± 1.20 aA	8.0 ± 0.98 aA	8.0 ± 1.36 aA	9.0 ± 1.02 aA
	1% FA	9.0 ± 0.80 aA	8.5 ± 0.90 aA	8.0 ± 1.16 aA	8.5 ± 1.04 aA	9.0 ± 0.87 aA
	2% FA	9.0 ± 0.50 aA	8.5 ± 0.93 aA	8.5 ± 1.13 aA	8.5 ± 0.87 aA	9.0 ± 0.82 aA
10	Cont.	3.5 ± 1.75 cB	6.0 ± 1.09 aB	5.0 ± 1.14 bB	5.0 ± 1.24 cB	5.0 ± 0.71 bB
	SMS	4.0 ± 1.81 cB	6.0 ± 0.96 aB	5.5 ± 1.43 abB	5.5 ± 1.36 bcB	5.0 ± 0.80 bB
	1% FA	5.5 ± 1.69 bB	7.0 ± 0.83 aB	6.5 ± 1.26 aB	6.0 ± 1.22 abB	6.5 ± 0.89 aB
	2% FA	7.0 ± 1.35 aB	7.0 ± 0.79 aB	6.5 ± 1.30 aB	6.5 ± 1.32 aB	7.0 ± 0.73 aB

* Different capital letters in the same column within the same treatment indicate the significant differences ($P < 0.05$). The different letters in the same column within the same storage time indicate significant differences ($P < 0.05$). Values are mean + standard deviation ($n = 30$). Cont.: control; SMS: sodium metabisulphite; 1% FA: 1% ferulic acid; 2% FA: 2% ferulic acid.

ing and may also be detectable in the peeled product (Bak et al., 1999).

3.4. Effect of FA on melanosis of Pacific white shrimp during iced storage

Melanosis score of Pacific white shrimp without and with treatments of SMS, 1% or 2% FA during iced storage is illustrated in Fig. 6. At day 0, all samples had no melanosis (score = 0). When the storage time increased, melanosis score in the control increased ($P < 0.05$). However, no melanosis was noticeable in samples treated with SMS or 2% FA in the first 2 days of storage. At day 2, SMS treated shrimps had the lower melanosis score than 1% FA treated shrimp ($P < 0.05$). Thereafter, treatment of shrimps with 1% FA showed the higher effectiveness in lowering melanosis, when compared with SMS treatment. During the first 2 days of storage, SMS at 1.25% used in this study was more effective in melanosis prevention than 1% FA. However, SMS might not be stable during the extended storage, in which sulphur dioxide formed could be liberated. Therefore, the required concentration of SMS is dependent on the length of time the melanosis must be inhibited. It has been reported that sulphites do not irreversibly inhibit browning (Taylor, Higley, & Bush, 1986). Additionally, the mechanism of melanosis inhibition between SMS and ferulic acid could be different, leading to variation in melanosis inhibition. Bisulphite might inhibit melanosis by reacting with intermediate quinone, forming sulphoquinone or it can act as a competitive inhibitor (Ferrer, Koburger, Otwell, Gleeson, & Simpson, 1989). During 4–10 days of storage, the formation of melanosis was lowest in the sample treated with 2% FA, followed by those treated with 1% FA and with SMS, respectively. Shrimp treated with 2% FA showed the best appearance as compared to others at the last day of storage (day 10), while the severe melanosis was found in the control samples. The retardation of melanosis formation of FA treated shrimp was coincidental with PPO inhibitory activity of FA (Figs. 1 and 2). Montero, Martinez-Alvarez, and Gomez-Guillen (2004) reported that melanosis inhibition of deepwater pink shrimp (*Parapenaeus iongirostris*) increased with increasing 4-hexylresorcinol concentration.

3.5. Effect of FA on sensory properties of Pacific white shrimp during iced storage

Changes in sensory properties of Pacific white shrimp without and with different treatments during iced storage are presented in the Table 1. Colour, odour, taste, flavour and overall likeness of shrimps were evaluated at day 0 and 10 of iced storage. At day 0, no differences in likeness for all attributes were observed among all samples ($P > 0.05$). After storage of 10 days, the decreases in likeness for all attributes in all samples were noticeable ($P < 0.05$). At day 10, the higher scores for colour, flavour and overall likeness were found in shrimps treated with 1% and 2% FA, compared with the control and that treated with SMS ($P < 0.05$). Nevertheless, no differences in odour likeness were found among all samples ($P > 0.05$). The higher scores for colour and overall likeness, were in agreement with the lower melanosis in samples treated with FA, particularly at a level of 2%. The higher flavour and taste likeness scores of samples treated with FA or SMS were mostly associated with the lower microbial load in those samples, in comparison with the control. Therefore, the treatment of Pacific white shrimps with FA could improve the sensory properties of treated shrimps after extended storage, which was most likely associated with the lowered melanosis.

4. Conclusion

Ferulic acid could be used as the promising agent for melanosis prevention in Pacific white shrimp during iced storage. The efficacy was in a dose dependent manner. Apart from prevention of melanosis, treatment of shrimps with ferulic acid could retard lipid oxidation and microbial growth. Moreover, shrimps treated with ferulic acid had superior sensory properties to the control after 10 days of iced storage. This led to the extended shelf-life of white shrimp. From the consumer health point of view, the use of natural additives like ferulic acid or other plant phenolic compounds can be a safer means to maintain the quality of shrimps, compared to synthetic additives.

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