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ARTICLE

Melanosis and Quality Changes of Pacific White Shrimp (*Litopenaeus vannamei*) Treated with Catechin during Iced Storage

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Melanosis, microbiological, chemical, and physical changes of Pacific white shrimp (*Litopenaeus vannamei*) treated with catechin were monitored during iced storage of 10 days. Whole shrimp treated with catechin solution (0.05 or 0.1%) had retarded growth of psychrophilic bacteria and spoilage microorganisms including H_2S-producing bacteria and enterobacteriaceae throughout storage in comparison with the control and those treated with 1.25% sodium metabisilfite (SMS) ($P < 0.05$). The lower increases in pH and total volatile base (TVB) content were obtained in the shrimp treated with catechin solution at both levels, compared with those of other samples ($P < 0.05$). Lipid oxidation, loss in freshness and melanosis were lowered by catechin treatment. In general, the efficacy of catechin in lowering melanosis and quality losses increased with increasing levels used. Additionally, catechin (0.01, 0.05, and 0.1% (w/v)) showed inhibitory activity toward polyphenoloxidase (PPO) of Pacific white shrimp in a dose-dependent manner. Therefore, catechin can be used as a promising melanosis inhibitor as well as an antimicrobial and an antioxidant in ice-stored shrimp.

KEYWORDS: Pacific white shrimp; catechin; polyphenoloxidase; microbial spoilage; quality; melanosis

INTRODUCTION

Pacific white shrimp (*Litopenaeus vannamei*) is an important commercial species primarily cultured in Thailand and accounts for 90% of the global aquaculture shrimp production. Shrimp constitutes 18−20% of the Thai Union's sales, and exports are also expected to rise up to 400,000 tons in 2008 with an increase in value up to $ 2.3 billion in the United States (*1*, *2*). Shrimp is a very perishable product, and postmortem changes occur rapidly, compared with those of fish (*3*). Rapid microbial spoilage during postmortem storage is a serious problem in shrimp processing (*4*). Free amino acids and other soluble non-nitrogenous substances in shrimp serve as digestible nutrients for microbial growth (*3*). Shrimp generally has the limited shelf life due to the formation of black spots (melanosis). Even though the presence of black spots seems to be harmless to consumers, it drastically reduces the product's market value and the consumer's acceptability, leading to considerable financial loss (*5*). Melanosis is triggered by a biochemical mechanism, which oxidizes phenols to quinones by polyphenoloxidase (PPO). This is followed by nonenzymatic polymerization, giving rise to pigments of high molecular weight and very dark or black coloring (*6*). Apart from melanosis and microbial spoilage, lipid oxidation associated with physicochemical changes and off-flavors (*7*) and loss in freshness are accompanied by lower quality, causing market loss of shrimp.

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Many studies have focused on preventing melanosis or inhibiting PPO over the years through different techniques. Reducing agents such as sulfiting agents and their derivatives are the most widely used chemicals for the control of melanosis or browning in the food industry (*4*). However, increases in regulatory attention and consumer awareness of the risk associated with sulfited food products have created a need for a safe, effective sulfite alternative for food processing (*8*). Plant phenolics have been paid increasing attention as potential natural additives with antioxidant and antimicrobial activities (*9*, *10*). Jayaprakasha et al. (*11*) demonstrated that plant phenolic compounds such as tocopherols, flavonoid compounds, cinnamic acid derivatives, and coumarins exhibit an antioxidant effect in a peroxidation model system. Recently, it has been reported that enokitake extract (*12*) and grape seed extract (*4*) could inhibit the melanosis in shrimp.

Among natural extracts, especially from plants, catechin from tea has been intensively studied as an excellent antioxidant (*9*). Because of the similarity of catechin to the PPO substrate, it might act as a PPO inhibitor, which could prevent melanosis in shrimp. Additionally, it might function as both an antioxidant and an antimicrobial, which can maintain the quality of shrimp during storage. However, no information regarding the use of catechin as a natural additive to prevent melanosis or extend the shelf life of shrimp has been reported. The aim of this study was to investigate the inhibition of melanosis and quality changes of Pacific white shrimp treated with catechin during iced storage.

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MATERIALS AND METHODS

Chemicals. L-β-(3, 4 dihydroxylphenyl) alanine (L-DOPA), Brij-35, (±)-catechin hydrate, malonaldehyde bis (dimethyl acetal), thiobarbituric acid (TBA), and anion exchange resin-AG (R) 1-X4-400 mesh Cl-form were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trichloroacetic acid (TCA), perchloric acid (PCA), hydrochloric acid, potassium hydroxide, ammonium hydroxide, sodium chloride, ammonium sulfate, standard plate count agar, triple sugar iron agar (IA), and eosin methylene blue agar (EMB) were obtained from Merck (Darmstadt, Germany).

Shrimp Collection and Preparation. Pacific white shrimp (*Litopenaeus vannamei*) with the size of 55−60 shrimp/kg were purchased from the dock in Songkhla, Thailand. The shrimp 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, shrimp were washed in cold water and stored in ice until used (not more than 5 h). Three different lots of shrimp were used for the entire study.

Effect of Catechin Treatment on the Quality of Pacific White Shrimp During Iced Storage. *Preparation of Shrimp*. Whole Pacific white shrimp were immersed in catechin solution at different concentrations (0.05 and 0.1%) with a shrimp/solution ratio of 1:2 (w/v) at 4 °C for 15 min. Another portion of shrimp was soaked in 1.25% sodium metabisulfite at a ratio of 1:2 (w/v) for 1 min at 4 °C (*13*). Treated shrimp were drained on the screen for 3 min at 4 °C. Shrimp without any treatment were used as the control. All samples were stored in a polystyrene box containing crushed ice with a shrimp/ice ratio of 1:2 (w/w). To maintain the shrimp/ice ratio, the molten ice was removed, and the same amount of ice was added every day. Samples (25 shrimp) were randomly taken for each treatment every 2 days up to 10 days for microbiological, chemical, and physical analyses. Melanosis was also determined.

Microbiological Analyses. Microbiological analyses were performed by the spread plate method (*14*). Five whole Pacific white shrimp were collected aseptically and used as the composite sample. The 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 (Stomacher M400, Seward Ltd., Worthington, England), further serial dilutions were prepared from this homogenate using 0.85% saline water as the diluent. Appropriate dilutions were used for microbiological analyses.

Psychrophilic Bacterial Count. The psychrophilic bacterial count was determined by inoculating 0.1 mL of an appropriate dilution of homogenate on plate count agar, containing 0.5% NaCl by a spread plate method. Then the plates were incubated at 4 °C for 10 days.

Hydrogen Sulfide-Producing Bacteria. H_2S-producing bacteria were grown on triple sugar iron agar by the spread plate method using 0.1 mL of an appropriate dilution of the homogenate. Plates were incubated at 25 °C for 3 days. Black colonies, due to the precipitation of ferrous sulfide on this medium, were counted.

Enterobacteriaceae Count. For the determination of the Enterobacteriaceae count, 0.1 mL of an appropriate dilution of the homogenate was plated on EMB agar and incubated at 37 °C for 24 h.

Chemical Analyses. *pH Measurement*. pH measurement was performed by the method described by Lopez-Caballero et al. (*15*) with a slight modification. Shrimp meat (2 g) was homogenized with 10 volumes of deionized water for 1 min using a PT 2100 homogenizer (Kinematica AG, CH-6014, Littau/Luzern, Switzerland). The homogenate was kept at room temperature for 5 min. The pH was determined using a pH-meter (Sartorious North America, Edgewood, NY, USA).

Determination of Total Volatile Base Content. Total volatile base (TVB) content in shrimp meat was determined using the Conway microdiffusion method (*16*). TVB content was calculated and expressed as mg N/100 g shrimp meat.

Determination of Thiobarbituric Acid Reactive Substances (TBARS). TBARS in the samples was determined following the method of Benjakul et al. (*17*) 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 running water. The mixture was centrifuged at 4000*g* for 20 min (MIKRO20, Hettich Zentrifugan, Germany). The supernatant was collected, and the absorbance was read at 532 nm using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). TBARS was calculated from the standard curve of malonaldehyde (0−2 ppm) and expressed as mg malonaldehyde/kg shrimp meat.

Determination of the K-Value. The *K*-value was determined using anion-exchange column chromatography (*18*). A ground sample (1 g) was subjected to a serial extraction using 10% PCA and 5% PCA. The final extract (2 mL) was adjusted to pH 9.4 using 0.5 N NH_4OH. The prepared extract (pH 9.4) was loaded onto an anion exchange column (Resin-AG (R) 1-X4-400 mesh Cl-form). The column was rinsed using deionized water (20 mL). The elution was performed using 45 mL of solution-A (0.001 N HCl). The eluate was collected, and the volume was made up to 50 mL using solution-A. Thereafter, the column was eluted with 45 mL of solution-B (0.01 N HCl containing 0.6 M NaCl). The resulting eluate was made up to 50 mL using solution-B. Both eluates were read at 250 nm using the corresponding eluent (solution A or B) as the blank. *K*-value was calculated as follows:

$$K\text{−value } (\%) = \frac{A}{A+B} \times 100$$

where A is A_{250} of eluate A representing the amount of inosine (HxR) and hypoxanthine (Hx), and B is A_{250} of eluate B representing the amount of ATP, ADP, AMP, and IMP.

Physical Analyses. *Determination of Shear Force*. Shear force of shrimp meat without and with treatment, was measured using a TA-XT2i texture analyzer (Stable Micro Systems, Surrey, England) equipped with a Warner-Bratzler shear apparatus (*19*). The operating parameters consisted of a cross head speed of 10 mm/s and a 25 kg load cell. The shear force, perpendicular to the axis of muscle fibers, was measured at the second segment of shrimp. Five samples were determined for each treatment. The peak of the shear force profile was regarded as the shear force value and expressed in Newtons (N).

Melanosis Assessment. Melanosis or blackening of Pacific white shrimp was evaluated through visual inspection by six trained panelists using a 10-point scoring test (*5*). Panelists were asked to give the melanosis score (0 to 10), where 0 = absent; 2 = slight (up to 20% of the shrimp's surface affected); 4 = moderate (20 to 40% of the shrimp's surface affected); 6 = notable (40 to 60% of the shrimp's surface affected); 8 = severe (60 to 80% of the shrimp's surface affected); 10 = extremely heavy (80 to 100% of the shrimp's surface affected).

Preparation of the PPO Extract from the Cephalothoraxes of Pacific White Shrimp. The cephalothoraxes of 20 shrimp were separated, pooled, and powdered by grinding with liquid nitrogen in a Waring blender. The powder obtained was kept in a polyethylene bag and stored at −20 °C for not more than 2 weeks. The extraction of PPO was carried out according to the method of Simpson et al. (*20*) 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 8000*g* at 4 °C for 30 min using a refrigerated centrifuge (Beckman Coulter, Avanti J-E Centrifuge, Fullerton, CA, USA). Solid ammonium sulfate was added into the supernatant to obtain 40% saturation, and the mixture was allowed to stand at 4 °C for 30 min. The mixture was then subjected to centrifugation at 12,500*g* 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 dialyzed against 15 volumes of the same buffer at 4 °C with three changes of dialysis buffer. The insoluble materials were removed by centrifugation at 3000*g* at 4 °C for 30 min, and the supernatant was used as the crude PPO extract.

Effect of Catechin on the Inhibition of Pacific White Shrimp PPO. *Preparation of Catechin Solutions*. Catechin was mixed with distilled water to obtain the different final concentrations (0.02, 0.1, and 0.2%, w/v). The mixtures (20 mL) were adjusted to pH 9 by 6 N NaOH and stirred for 15 min at room temperature (26−28 °C) to completely dissolve catechin. Thereafter, the pH of the solution was adjusted to 7 by using 6 N HCl and referred to as the catechin solution.

Inhibitory Effect of the Catechin Solution on PPO Activity. Catechin solutions with different concentrations (0.02, 0.1 and 0.2%, w/v) (100 μL) were mixed with the crude PPO extract (100 μL) at ratio of 1:1 (v/v) to obtain final concentrations of 0.01, 0.05, and 0.1% (w/v), respectively. The mixtures were incubated for 30 min at room temperature. Then, the assay buffer (400 μL) was added. To initiate the reaction, 600 μL of preincubated 15 mM L-DOPA (45 °C) was added. The reaction was conducted at 45 °C, and the absorbance at 475 nm was monitored every minute up to 3 min. The control was run in the same manner, except that deionized water was used instead of the catechin solution. The blank was prepared for each catechin solution by using distilled water instead of L-DOPA.

Statistical Analyses. All experiments were performed in triplicate, and a completely randomized design (CRD) was used. Analysis of variance (ANOVA) was performed, and mean comparisons were done by Duncan's multiple range tests (*21*). Analysis was performed using an SPSS package (SPSS 11.0 for windows, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Effect of Catechin Treatment on Microbiological Changes of Pacific White Shrimp during Iced Storage. Changes in psychrophilic bacterial count, H_2S-producing bacteria, and enterobacteriaceae count of Pacific white shrimp treated with 0.05 or 0.1% catechin during iced storage in comparison with those of the control and shrimp treated with 1.25% SMS are shown in **Figure 1**.

No psychrophilic bacteria were found in all samples at day 0. The continuous increases in psychophilic bacterial count in all samples were noticeable with increasing storage time up to 10 days ($P < 0.05$). During 2−10 days of storage, samples treated with sodium metabisulfite (SMS) and 0.05% or 0.1% catechin had the lower psychrophilic bacterial count, in comparison with the control ($P < 0.05$) (**Figure 1A**). At the same storage time, the lowest psychrophilic bacterial count was found in shrimp treated with 0.1% catechin ($P < 0.05$). During 4−10 days of storage, SMS treatment did not exhibit the same level of inhibition toward the growth of psychrophilic bacteria as catechin. However, the lower bacterial count was obtained in the SMS treated sample, as compared to that in the control. At the end of storage (day 10), the psychrophilic bacterial count of the control, those treated with SMS, 0.05% catechin, and 0.1% catechin were 5.17, 5.13, 4.81, and 4.61 log CFU g^{-1}, respectively. The result indicated the antimicrobial activity of catechin toward psychrophilic bacteria in Pacific white shrimp during iced storage. The antimicrobial activity was dependent on the concentration used. The treatment with 5% kiam wood extract retarded the rate of growth of *Listeria monocytogenes*, aerobic mesophiles, and psychrophilic microorganisms naturally present on cabbage (*22*). Treatments with 2% sodium acetate or 2% sodium lactate had little or no effect in the reduction of the growth of psychrophilic bacteria in shrimp over 12 days of storage at 4 °C (*23*).

Changes in the H_2S-producing bacterial count of Pacific white shrimp without and with different treatments during iced storage are depicted in **Figure 1B**. During 10 days of iced storage, the H_2S-producing bacterial count was less in shrimp treated with SMS as compared to that in the control up to 8 days; however, no difference between both samples was observed at the end of the storage day ($P > 0.05$). Shrimp treated with 0.1% catechin contained the lowest H_2S-producing bacteria throughout the storage period ($P < 0.05$), followed by shrimp treated with 0.05% catechin. At the end of iced storage, the control, those treated with SMS, 0.05% catechin, and 0.1% catechin had H_2S-producing bacterial counts of 4.40, 4.37, 4.05, and 3.93 log $CFUg^{-1}$, respectively. Deepwater pink shrimp (*Parapenaeus longirostris*) stored under chilled conditions showed 4.0 log $CFUg^{-1}$ of H_2S-producing bacteria after 15 days (*24*). Phenolic compounds might disrupt the cell wall of microorganisms by forming complexes with proteins in the cell wall causing the cell wall to lyse (*22*). Leaf extract of artichoke (*Cynara scolymus* L.) rich in phenolic components exhibited the most significant antimicrobial activities against seven bacteria species including gram positive and negative species (*25*). Generally, specific spoilage organisms such as H_2S-producing bacteria and Enterobacteriaceae are mostly predominant in the spoilage of fish and fish products, causing off-flavors and rejection (*14*). Thus, catechin was shown to retard the growth of spoilage bacteria, which were able to produce H_2S.

Enterobacteriaceae count of Pacific white shrimp without and with treatments during iced storage of 10 days is illustrated in **Figure 1C**. In general, the Enterobacteriaceae count of all samples increased throughout the storage of 10 days ($P < 0.05$). During the storage, a lowered count was observed in shrimp treated with SMS, 0.05% catechin, and 0.1% catechin as compared to that of the control ($P < 0.05$). Treatment of shrimp with 0.1% catechin was more effective in lowering the Enterobacteriaceae count, followed by treatment with 0.05% catechin ($P < 0.05$). SMS treatment showed little impact on the inhibition of Enterobacteriaceae. The control shrimp and those treated with SMS, 0.05% catechin, and 0.1% catechin had Enterobacteriaceae counts of 4.64, 4.62, 4.29, and 4.15 log $CFUg^{-1}$, respectively, at the end of iced storage. Pink shrimp treated with sulfites had an Enterobacteriaceae count of 5 log CFU g^{-1} at day 9 of chilled storage (*26*). Sodium metabisulfite (SMS) has been reported as an antimicrobial due to its release of sulfur dioxide, which can pass across the cell membrane and disrupt the normal metabolic activity of bacteria (*27*). In this study, sodium metabisulfite (1.25% SMS) exhibited lower efficiency in retardating the growth of Enterobacteriaceae.

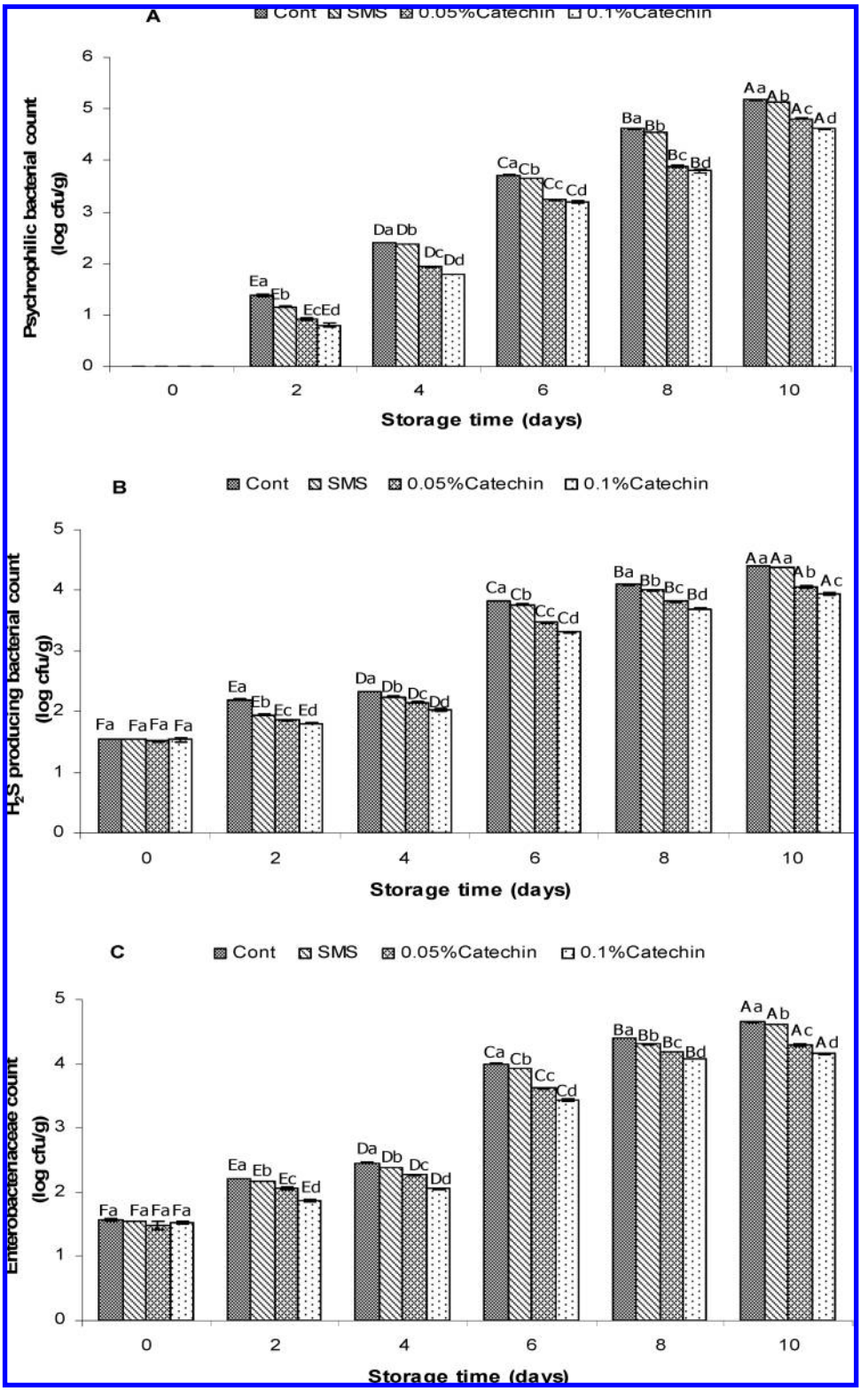

Figure 1. Psychrophilic (**A**), H_2S-producing bacteria (**B**), and Enterobacteriaceae (**C**) count of Pacific white shrimp treated with catechin at different levels. Bars represent standard deviation ($n = 3$). Different capital letters on the bars within the same treatment indicate significant differences ($p < 0.05$). The different letters on the bars within the same storage time indicate significant differences ($p < 0.05$).

Sulfur dioxide derived from SMS might be evaporated during extended storage or could be dissolved with molten ice. This might lead to the lower amount of SMS remaining in the sample. Catechin at either 0.05% or 0.1% showed stronger inhibitory activity against spoilage bacteria than did SMS. As a result, the spoilage caused by microorganisms could be retarded by catechin treatment.

Effect of Catechin Treatment on the Chemical Changes of Pacific White Shrimp during Iced Storage. *pH*. Changes in the pH of Pacific white shrimp with and without different treatments during iced storage are shown in **Figure 2A**. Fresh Pacific white shrimp had a pH of 6.44. As the storage time increased, the pH of all shrimp samples increased ($P < 0.05$). Within the first two days of storage, no differences in

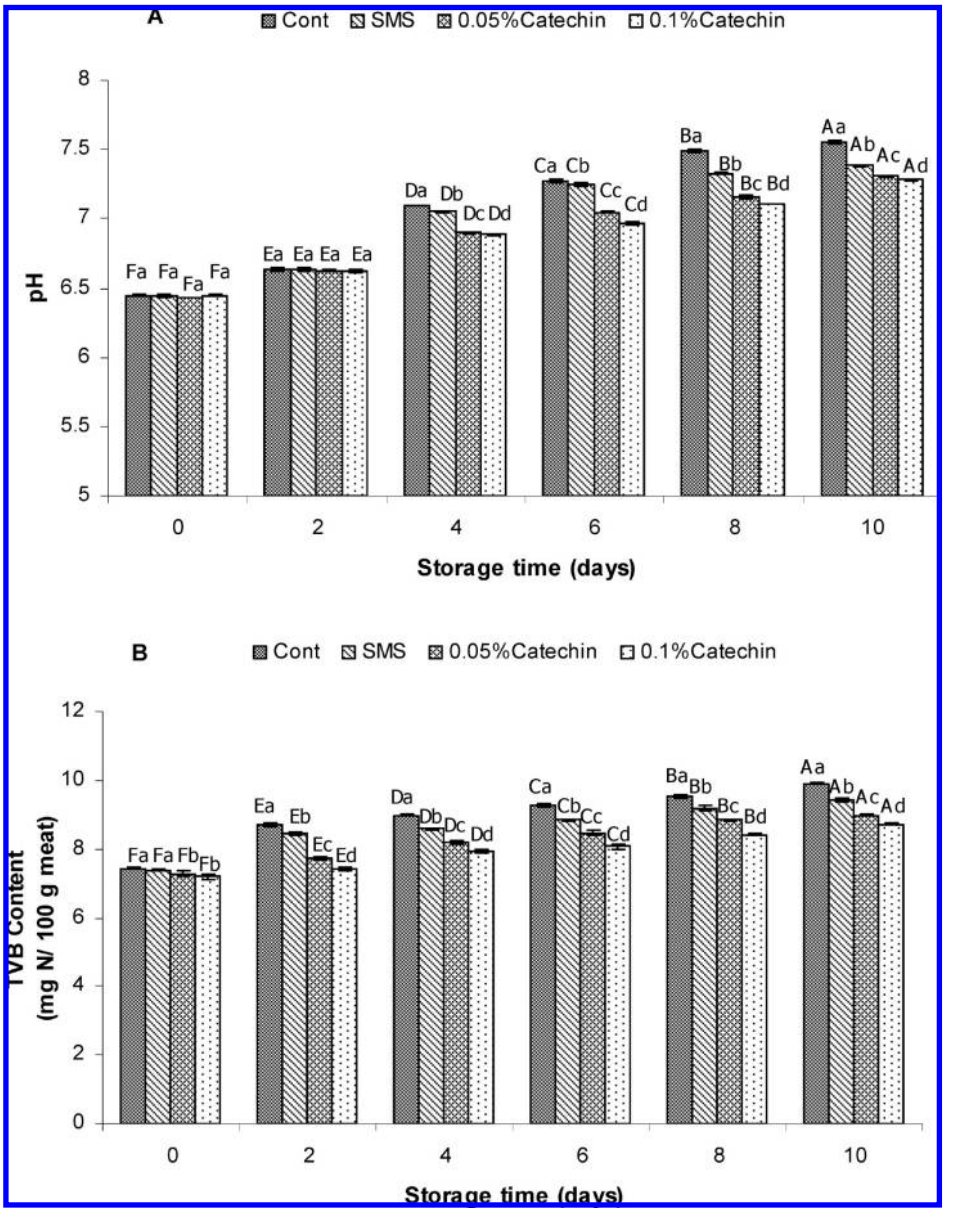

Figure 2. pH (**A**) and total volatile base (**B**) content of Pacific white shrimp treated with catechin at different levels. Key: see the caption for **Figure 1**.

pH were noticeable among all samples ($P > 0.05$). After two days of storage, those treated with 0.1% catechin had the lowest pH, followed by those treated with 0.05% catechin and SMS, respectively ($P < 0.05$). At the end of storage, the control and shrimp treated with SMS, 0.05% catechin, and 0.1% catechin had pH values of 7.55, 7.38, 7.30, and 7.28, respectively. The increase in pH was associated with the accumulation of basic compounds, mainly resulting from microbial action (*15*). The lower increase in the pH of shrimp treated with 0.05% or 0.1% catechin was in accordance with the lower microbial count (**Figure 1**). Shrimp, *Penaeus merguiensis*, was not acceptable when the pH was greater than 7.6 (*28*). The increases in pH value were more rapid in the shrimp (*Pandalus borealis*) stored in ice at 1.5 °C and reached a final pH of 8.26 as compared to the sample stored in liquid ice (pH 7.98) (*3*).

TVB Contents. TVB contents of Pacific white shrimp without and with different treatments are depicted in **Figure 2B**. Continuous increase in TVB content was observed in all samples, but the rate of increase in TVB content varied with treatments ($P < 0.05$). The initial TVB content of Pacific white shrimp for all treatments was 7.2−7.4 mg N/100 g shrimp meat. The volatile base compound found in the shrimp more likely indicated that an autolytic process was involved during postmortem handling. Adenosine and adenosine monophosphate (AMP) deaminase might play a major role in this process after capture and transportation (*15*). A lowered rate of increase in the TVB content of shrimp treated with 0.05 or 0.1% catechin was observed, as compared to the control and shrimp treated with SMS, when the storage time increased ($P < 0.05$). The lowest TVB content correlated with the lowest microbial counts found in 0.1% catechin treated shrimp (**Figure 1**). Moreover, the lower TVB content was coincidental with the lower pH of Pacific white shrimp (**Figure 2A**). At the last day of iced storage, shrimp treated with 0.1% catechin had the lowest TVB content (8.70 mg N/100 g), compared to that of other treatments ($P < 0.05$). The total volatile base content of deepwater pink shrimp (*Parapenaeus longirostris*) treated with resorcinol had the lowered TVB content (35 mg N/100 g) as compared with that of other treatments and the control (*15*). TVB content of 40 mg N/100 g has been used as the freshness borderline (*24*).

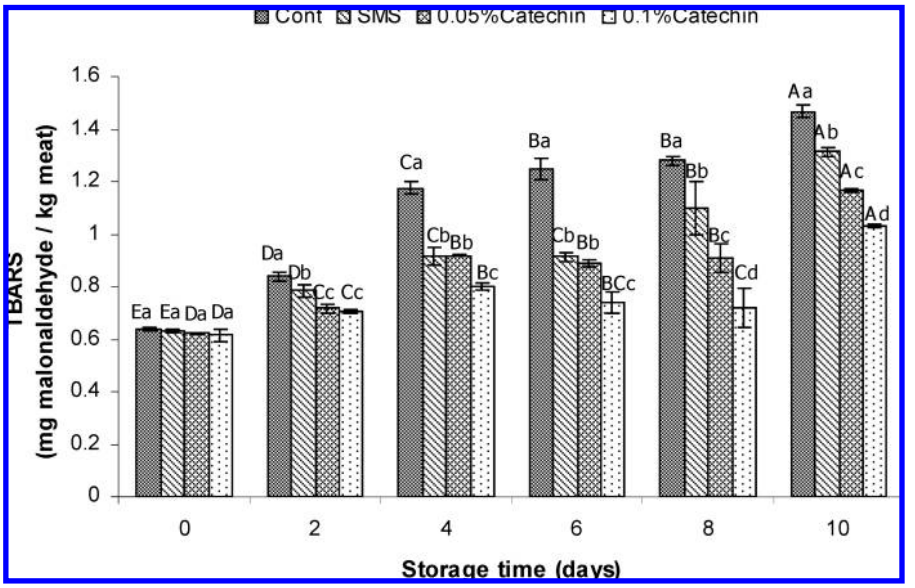

Figure 3. TBARS values of Pacific white shrimp treated with catechin at different levels. Key: see the caption for **Figure 1**.

Thiobarbituric Acid Reactive Substances (TBARS). TBARS values of the Pacific white shrimp without and with treatments during iced storage are illustrated in **Figure 3**. The TBARS value of the control sample increased continuously throughout iced storage ($P < 0.05$). The lower increase in TBARS was noticeable within the first 2 days. At all storage times, shrimp treated with 0.1% catechin showed the lower TBARS value, compared to those of other treatments ($P < 0.05$), except at day 2, in which a similar TBARS value was observed between the sample treated with 0.05 and 0.1% catechin ($P > 0.05$). A similar TBARS value of SMS treated shrimp and shrimp treated with 0.05% catechin was found during 4−6 days of storage ($P > 0.05$). Thereafter, a higher TBARS value was found in the former, indicating that lipid oxidation took place at a higher extent in the SMS treated sample. Generally, shrimp treated with catechin had the lower TBARS value throughout storage ($P < 0.05$). Results revealed that Pacific white shrimp treated with 0.1% catechin had higher stability toward lipid oxidation than the other samples. Catechin at a high level most likely showed a strong antioxidative effect in shrimp muscle. Catechin has been reported to have antioxidant activity including radical scavenging activity (*9*). Lipid peroxidation in fish meat can be initiated by autoxidation, photosensitized oxidation, or by means of enzymatic reaction associated with lipoxygenase, peroxidase, and microbial enzymes (*7*).

In lipid oxidation, unstable hydroperoxide is formed and decomposes readily to shorter chain hydrocarbons such as aldehydes; these final products can be detected as TBARS (*29*). Catechin and its derivatives effectively inhibited lipoxygenase activity in mackerel muscle (*9*). Therefore, lipid oxidation in shrimp treated with catechin could be prevented to some degree during extended storage.

K-Value. The *K*-value of Pacific white shrimp without and with different treatments during iced storage is depicted in **Figure 4**. *K*-value has been used as the freshness index in fish and shellfish (*30*). The control and all treated samples had *K*-values of approximately 20% at day 0. The *K*-value of *Penaeus japonicus* was reported to be approximately 20% at the initial storage time (*31*). Deepwater pink shrimp (*Parapenaeus longirostris*) had a *K*-value of 9% at the beginning of chilled storage (2 °C) and reaches 40% at day 10 of storage (*24*). Continuous increases in *K*-value were found in the control shrimp and all treated samples during storage ($P < 0.05$). It was noticed that shrimp treated with catechin had the lower rate of increase in *K*-value as compared to that found in the control shrimp and those treated with SMS ($P < 0.05$). The control banana shrimp (*Penaeus merguiensis*) and those treated with sodium bisulfite had similar *K*-values (43%) at the last day of iced storage (*30*). Catechin at levels of 0.05% or 0.1% showed a similar effect toward *K*-value within the first 4 days of storage. Thereafter, 0.1% catechin exhibited the greater impact on the retardation of the increase in *K*-value. At the end of storage, shrimp treated with 0.1% catechin had the lowest *K*-value (29%), compared to those of other treatments (35−41%). During postmortem handling and storage, endogenous enzymes break down adenosine triphosphate (ATP) to different derivatives such as adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (HxR), and finally hypoxanthine (Hx) (*30*). In general, a *K*-value of 20% or lower indicates very good quality fish, while 60% has been realized as the rejection limit (*18*). From the results, catechin might inhibit the ATP degradative enzymes via a cross-linking mechanism. Catechin might bind or cross-link those enzymes, leading to the lower rate of ATP degradation. It can be inferred that catechin treatment could retard the loss in freshness of white shrimp during storage.

Effect of Catechin Treatment on the Physical Changes of Pacific White Shrimp during Iced Storage. *Shear Force*. **Figure 5** illustrates the shear force of the muscle of Pacific white shrimp without and with different treatments during iced storage. At day 0 of storage, all samples showed a similar shear force (18.90−18.94 N) ($P > 0.05$). As the storage time increased, different shear forces were obtained among the samples ($P < 0.05$). The control shrimp and those treated with SMS had similar shear forces ($P > 0.05$). Shrimp treated with catechin possessed the higher shear force ($P < 0.05$). A higher shear force was found in shrimp treated with 0.1% catechin, compared with those treated with 0.05% catechin, after 10 days of storage ($P < 0.05$). These results revealed that muscle softening of shrimp during the extended iced storage could be lowered by catechin treatment. Catechin and epigallocatechin gallate (EGCG) from green tea could inhibit the action of collagenase against collagen (*32*). Generally, the softening of shrimp or fish muscle is

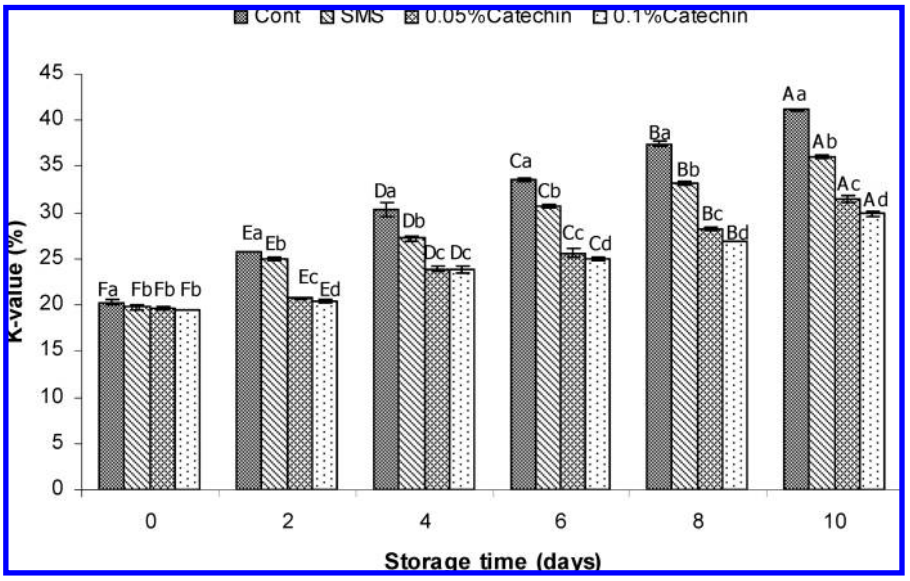

Figure 4. *K*-values of Pacific white shrimp treated with catechin at different levels. Key: see the caption for Figure **1**.

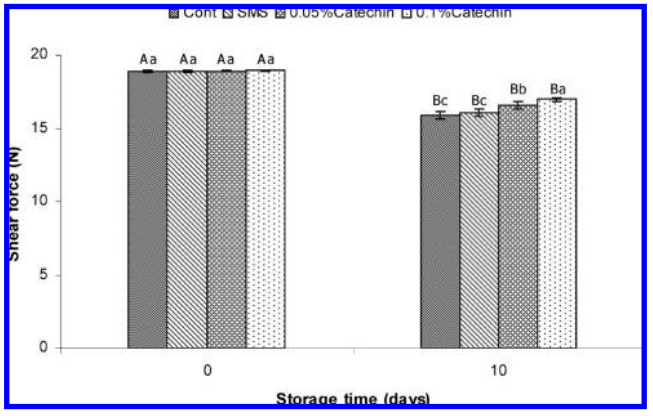

Figure 5. Shear force of Pacific white shrimp treated with catechin at different levels. Key: see the caption for Figure **1**.

associated with proteolysis caused by endogenous or microbial proteinases and collagenase (*33*). The higher shear force of shrimp treated with catechin was coincidental with the lower microbial load (**Figure 1**). These spoilage microorganisms mostly produce proteinases (*28*), which are capable of hydrolyzing muscle proteins. Inhibitory activity of catechin toward those microorganisms ultimately decreased the degradation of muscle proteins of shrimp including collagen. Additionally, catechin might inactivate those proteinases, leading to the lower decreases in shear force. Shear strength of deepwater pink shrimp (*Parapenaeus longirostris*) treated with 4-hexylresorcinol had the lowest value as compared to those treated with commercial sulfite, gluconic acid plus the commercial sulfite formulation, and the control (*15*).

Melanosis Score. The melanosis score of Pacific white shrimp without and with treatments of SMS, 0.05% catechin, or 0.1% catechin during iced storage is illustrated in **Figure 6**. At day 0, all samples had no melanosis (score = 0). When the storage time increased, the melanosis score in the control increased continuously ($P < 0.05$). However, no melanosis was noticeable in samples treated with 0.05% or 0.1% catechin within the first 2 days of storage. Shrimp treated with 0.05% and 0.1% catechin had no difference in the melanosis score at day 4 ($P > 0.05$). Nevertheless, during 4−10 days of storage, the formation of melanosis was lower in samples treated with 0.1% catechin than that of those treated with 0.05% catechin ($P < 0.05$). After 4 days of storage, shrimp treated with SMS had severe melanosis like the control shrimp up to 10 days ($P > 0.05$). Melanosis formation of Pacific white shrimp without and with different treatments at day 10 of iced storage is shown in **Figure 7**. Shrimp (*Parapenaeus Iongirostris*) treated with 1.5% grape seed extract and stored at 4 °C had the best melanosis score (score 6) as compared to that of other treatments (*4*). Shrimp (*T. curvirostris*) immersed in 2.5 g of wet enokitake extract/mL of 0.9% KCl for 10 min had no melanosis up to 20 h at 24 °C (*12*). Melanosis is a phenomenon in which a brown color is developed by the enzymatic reaction mediated by polyphenoloxidase (*6*). Melanosis can occur in shrimp and crustaceans, leading to a lower market value (*5*). On the basis of the results of melanosis, Pacific white shrimp treated with catechin had negligible blackening within the first 3 or 4 days, and the formation of melanosis was lower than that of untreated and those treated with sodium metabisulfite during extended iced storage.

Effect of Catechin on PPO Inhibition. Effect of catechin solution at different concentrations on the inhibition of PPO from Pacific white shrimp is shown in **Figure 8**. Catechin showed PPO inhibitory activity in a dose-dependent manner ($P < 0.05$). At catechin levels of 0.05 and 0.1%, almost 90% inhibition was observed. Catechin probably acted as a competitive inhibitor for PPO because of its structural similarity to L-DOPA, a substrate for PPO. Aromatic carboxylic acids of cinnamic acid and its analogues, *p*-coumaric, ferulic, and sinapic acids, are competitive inhibitors of PPO (*13*). Some phenolic compounds inhibit PPO activity by interacting with the active site of the enzyme (*34*). Furthermore, phenolic compounds could interact with protein or enzymes via a hydrogen bond or hydrophobic interaction (*35*). Thus, Pacific white shrimp PPO might undergo aggregation, losing its activity, in the presence of catechin. Kubo et al. (*36*) reported that 1.55 mM of dodecyl gallate was the inhibitory concentration, leading to 50% activity loss of mushroom tyrosinase.

Furthermore, the hydroxyl group of catechin might be involved in the reduction of DOPA-chrome to DOPA, possibly via its ability to donate electrons to intermediate quinone, DOPA-chrome. Those actions could be associated with the lowered blackening caused by PPO in Pacific

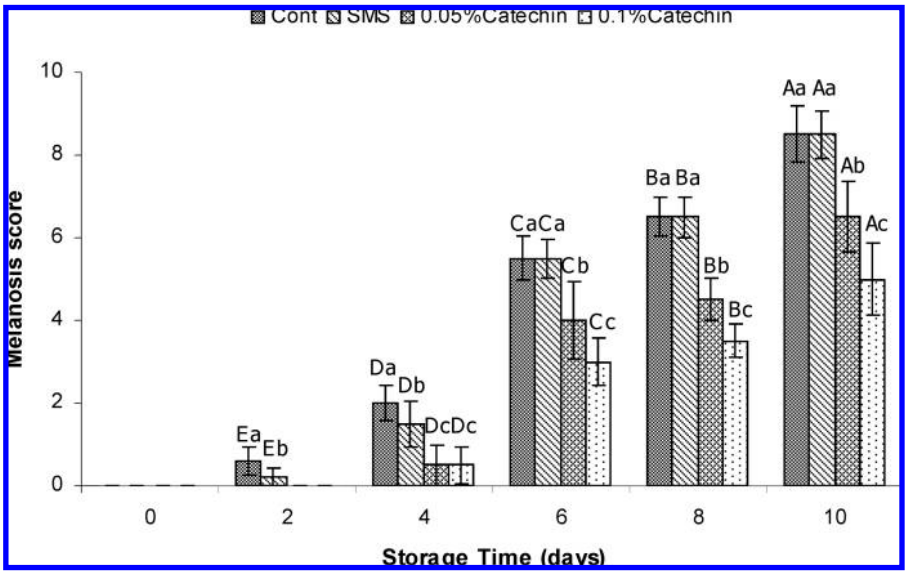

Figure 6. Melanosis score of Pacific white shrimp treated with catechin at different levels during 10 days of iced storage. Bars represent standard deviation ($n = 3$). 0 = absent; 2 = slight (up to 20% of the shrimp's surface affected); 4 = moderate (20 to 40% of the shrimp's surface affected); 6 = notable (40 to 60% of the shrimp's surface affected); 8 = severe (60 to 80% of the shrimp's surface affected); 10 = extremely heavy (80 to 100% of the shrimp's surface affected).

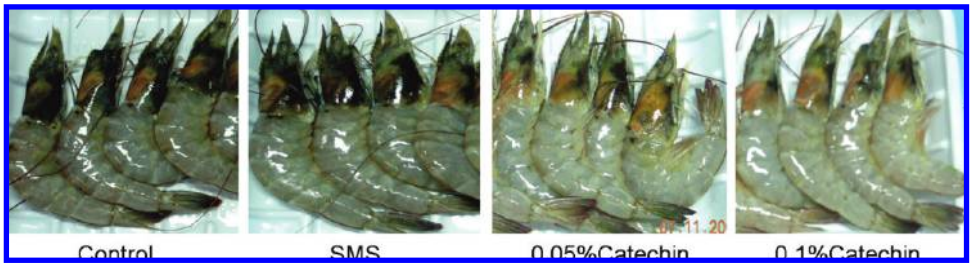

Figure 7. Photograph of Pacific white shrimp without and with different treatments at day 10 of iced storage.

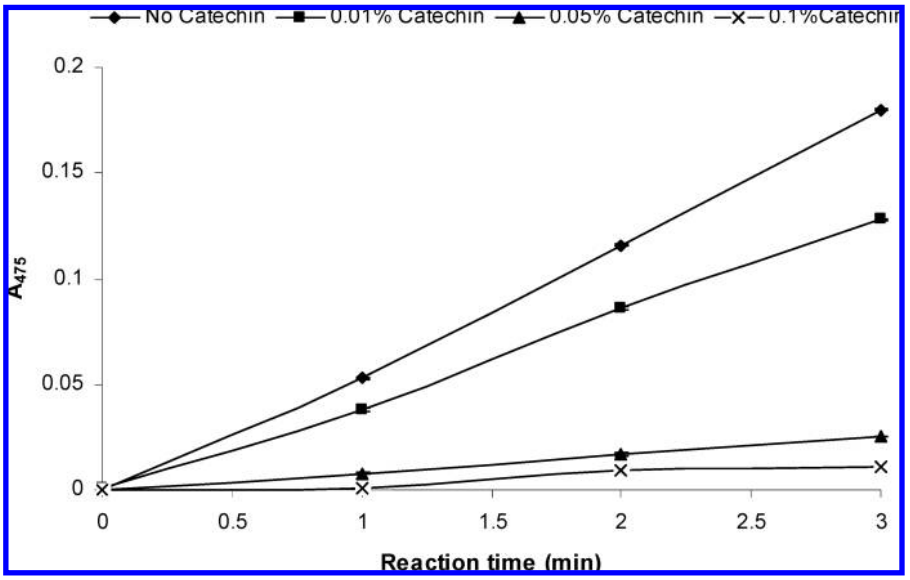

Figure 8. Effect of catechin at different levels on the activity of polyphenoloxidase from the cephalothoraxes of Pacific white shrimp. The decrease in ΔA_{475} indicates the inhibition of DOPA-chrome formation induced by PPO.

white shrimp during extended iced storage (**Figure 6**). The results reconfirmed that catechin was effective in PPO inhibition especially at high concentrations. This contributed to the retardation of melanosis in catechin treated shrimp.

In conclusion, catechin could be used as the natural promising agent for melanosis prevention in Pacific white shrimp during iced storage. Apart from the prevention of melanosis, treatment of shrimp with catechin could retard microbial growth and lipid oxidation, and was able to maintain freshness. This led to the extended shelf life of white shrimp. From a consumer health point of view, the use of natural additives such as catechin can be a safer means to maximize the storage stability of shrimp.

ABBREVATIONS USED

PPO, polyphenoloxidase; Cont, control; SMS, sodium metabisulfite (1.25%); CFU, colony forming unit; PCA, perchloric acid; TCA, trichloroacetic acid; TVB, total volatile base; TBARS, thiobarbituric acid reactive substances; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, inosine monophosphate; HxR, inosine; Hx, hypoxanthine.

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