An Extract and Fractions from *Coffea arabica* Sediment on Antioxidant and Anti-Tyrosinase Activities, and on the Quality of Whiteleg Shrimp (*Litopenaus vannamei*) during Refrigerated Storage

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**ABSTRACT:** *Coffea arabica* sediment, a popular agro-by-product from the coffee juice processing industry, contains many phytochemicals, and it is often discarded as waste. The antioxidant and anti-tyrosinase activities of an ethanol extract and its active fractions obtained from *Coffea arabica* sediment was studied. Additionally, whiteleg shrimp (*Litopenaus vannamei*) was treated with the extract and fractions, and the quality during refrigerated storage was evaluated. The most active fraction (*P*<0.05) fraction III from the *Coffea arabica* sediment extract showed a total phenolic content of 116.5 mg gallic acid equivalent/g, an inhibitory concentration of 50% value of 27.3 µg/mL in the 2,2-diphenyl-1-picryl hydrazyl test, an absorbance of 3.7 (at 1.0 µg/mL) in the reducing power assay, and 79.5% inhibition for the tyrosinase inhibition test (at 100 µg/mL). Whiteleg shrimp treated with PD-3 and crude extract (CE) retarded the growth of bacteria and spoilage microorganisms in comparison to the control (*P*<0.05). Lower values in pH, thiobarbituric acid reactive substances, and total volatile base content were enumerated in samples treated with PD-3 and CE (*P*<0.05) compared to controls. Loss in freshness, protein content, and melanosis formation declined with CE and PD-3 treatments. Our findings showed that *Coffea arabica* sediment has antioxidant and antimicrobial properties. Also, it could be a promising treatment in melanosis prevention, and a natural additive for extending the shelf life of whiteleg shrimp during refrigerated storage; thus an alternative to traditional sulfites.

**Keywords:** antioxidant, *Coffea arabica*, melanosis, tyrosinase, whiteleg shrimp (*Litopenaus vannamei*)

**INTRODUCTION**

Whiteleg shrimp (*Litopenaus vannamei*) is a major commercial species accounting for 90% of the global shrimp aquaculture production (Nirmal and Benjakul, 2009) and one of the most widely consumed crustaceans in many countries (Na et al., 2018). Shrimp is highly susceptible to both endogenous enzymatic browning or melanosis, and microbial spoilage during postmortem storage, so it is pivotal to preserve shrimp properly using effective and appropriate methods (Huang et al., 2012). The common methods frequently used for shrimp preservation such as freezing, heating, a combination of brining and chilling, or adding preservatives often face serious challenges with the formation of black spots, lipid oxidation, microbial spoilage, and toxicity associated with physicochemical changes and off-flavors that all reduce the market value of shrimp and consumer acceptability (Nirmal and Benjakul, 2009; Huang et al., 2012; Arancibia et al., 2015). Melanosis in shrimp is a defect triggered by a biochemical reaction, which catalyzes the oxidation of phenolic compounds to the corresponding quinones by the activity of tyrosinase. This process is followed by non-enzymatic polymerization, forming pigments of high weight molecules, and dark coloring in shrimp (Gokoglu and Yerlikaya, 2008; Nirmal and Benjakul, 2009). Much research has been devoted to either preventing melanosis or inhibiting tyrosinase through various techniques and mechanisms. Antibrowning agents such as sulfites and their derivatives are the most widely applied reagents for the prevention...
of melanosis in the food industry by reacting irreversibly with o-quinones to form colorless materials (Kuijpers et al., 2013). However, exposure to these sulfite chemicals has been documented to induce a wide range of negative clinical effects, namely urticaria, flushing, dermatitis, hypotension, diarrhea, abdominal pain, and asthmatic reactions in sensitive people (Taylor et al., 1986; Vally and Misso, 2012). The risks associated with sulfite additives have prompted a search for a safe, effective sulfite alternative in food processing. Both synthetic and natural antioxidants have been used in food applications as potential additives. Long term studies showed that synthetic antioxidant substances such as butylated hydroxyanisole, butylated hydroxytoluene, tertiary butyl hydroxyquinone, and propyl gallate could act as carcinogenic agents in animals (Barlow, 1990). Plant phenolics such as tocopherols, flavonoid compounds, cinnamic acid derivatives, and coumarins exhibit an antioxidant influence that inhibits undesirable enzymatic browning (Jayaprakasha et al., 2001; Gokoglu and Yerlikaya, 2008). Recently, a series of studies have demonstrated that grape seed extract (Gokoglu and Yerlikaya, 2008), enokitake extract (jang et al., 2003), and edible mushroom extract (Encarnacion et al., 2012) could prevent melanosis or extend the shelf life in shrimp.

The vegetable and fruit processing industry has generated large quantities of by-products during different processing steps. They are rich in bioactive compounds, which can be utilized as novel, low-cost natural sources of antioxidants, antimicrobials, dietary fiber, enzymes, and food additives through different methods of extraction, purification, and fermentation (Gowe, 2015; Kodagoda and Marapana, 2017). In a previous study, an ethanol extract of Coffea arabica sediment proved to be one of the most efficient extracts in antioxidant activity and inhibiting tyrosinase (Hue et al., 2020), so it was studied further in this research. This study aimed to examine antioxidant activity and tyrosinase inhibition of an ethanol extract from Coffea arabica sediment and its various fractions. Secondly, the additional aim was to investigate the potential of Coffea arabica sediment extract and its most potent fraction to preserve Litopenaeus vannamei shrimp in terms of melanosis prevention, lipid peroxidation, bacterial spoilage, and physicochemical changes.

**Chemicals**

L-β-(3,4 dihydroxyphenyl) alanine (L-DOPA), thiobarbituric acid (TBA), Folin-Ciocalteu reagent, 2,2-diphenyl-1-picryl hydrazyl (DPPH), malondialdehyde (MDA), and tyrosinase were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA) was purchased from Merck KGaA (Darmstadt, Germany). All other chemicals used were of analytical grade.

**Preparation and extraction of Coffea arabica sediment**

Roasted Coffea arabica sediment is leftover juice pulp (as a solid waste) created during the juicing process. Coffea arabica sediment was bought from Long Phuoc Market, Ho Chi Minh City, Viet Nam. The material was collected, dried to constant weight, and ground to powder. Dried powder (100 g) was immersed in ethanol (1:1.5, w/v) at room temperature within 6 days. The mixtures were filtered, and new solvent added to the residue every two days. The obtained extract was filtered and concentrated by a rotary evaporator to get the crude extract.

**Fractionation of Coffea arabica sediment extract**

The ethanol extract of Coffea arabica was fractionated into various fractions. Fractionation was conducted using a vacuum chromatography column. The column was packed with silica gel 60 ~200 mesh as a stationary phase, and eluted using a mobile phase system with solvents of gradually increasing polarity. The mobile phase elution was as follows: n-hexane : ethyl acetate = 97:3, 95:5, 90:10, 85:15, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100. Eluate was collected in 100 mL fractions and combined into some fractions based on its profile using thin layer chromatography aluminum plate GF254 (Merck KGaA). The collected fractions then were tested for their antioxidant and antityrosinase activities. A total of 6 fractions were collected from the fractionation process of Coffea arabica extract and labeled from fraction I to VI (PD-1 to PD-6, respectively). The fractions were dried with a rotary evaporator and were assayed for their activities.

**DPPH assay procedure**

The antioxidant activity of the extracts was evaluated using DPPH as reported by Shimamura et al. (2014). Various concentrations (100, 50, 25, and 10 μg/mL in 90 v/v% ethanol) of each extract (1.5 mL) was added to 1.5 mL of a 0.1 mM solution of DPPH. The mixtures were then left at room temperature for 30 min in the dark, the absorbance was measured by UV-VIS spectrophotometry at a wavelength of 519 nm. The antioxidant activity was determined by calculating the percentage inhibition (I%) of DPPH as follows:

\[
DPPH \text{ scavenging activity (\%)} = \frac{(A-B)}{A} \times 100
\]

in which, A is the absorbance of the analytical sample, and B is the absorbance of ethanol solvent. IC_{50} (inhibitory concentration of 50%) values were calculated from the interpolation method. Gallic acid as a positive control was used at various concentrations (1.0, 2.5, 5.0, and
Ferric reducing/antioxidant power (FRAP) assay
The ferric reducing ability of the samples was evaluated according to the modified method of Labiad et al. (2017). According to the method, the antioxidant activity is evaluated based on the capacity to reduce ferric (III) iron to ferrous (II) iron by the action of electron-donating antioxidants. Extracts (0.1, 0.5, and 1.0 mg) were mixed with 1 mL of potassium phosphate buffer (2.0 M, pH 6.6) and 1 mL of potassium ferricyanide [K₃Fe(CN)₆] 1% (w/w). The mixture was incubated for 20 min at 50°C. An aliquot (1 mL) of 10% (w/w) TCA was added to the mixture, which was then centrifuged at 2,000 rpm for 10 min. The supernatant (1 mL) was mixed with 1 mL of distilled water and 0.2 mL of ferric chloride 0.1%. The absorbance of this solution was measured at 700 nm.

Determination of total phenolic content
Total phenolic content of extracts was determined using the Folin-Ciocalteu method with slight modification (Ainsworth and Gillespie, 2007). The test sample (60 μL) was mixed with 1 mL of the reagent solution (1:5 v/v) within 5 min. Then, 0.7 mL of 1.0 M sodium carbonate was added to the mixture and brought to 3 mL with distilled water. The solution was left for 30 min at room temperature in the dark and its absorbance was measured at 770 nm using a Hitachi UH-530 spectrophotometer (Hitachi, Ltd., Tokyo, Japan). The total phenolic content was calculated as mg gallic acid equivalent per gram of extract (mgGAE/g) from the gallic acid calibration curve.

Determination of tyrosinase inhibitory activity
Tyrosinase inhibitory activity was determined based on a modification of Tadtong et al. (2009). Initially, 40 μL of extract solution (dissolved in phosphate buffer, pH 6.8) at concentrations of 100, 75, 50, 25, and 5 μg/mL and 20 μL of tyrosinase (100 units/mL) was applied to a 96-well plate. This reaction mixture was mixed thoroughly and incubated for 30 min at room temperature. Then, 140 μL of 0.714 mM L-DOPA (as substrate at 45°C) was added to initiate the reaction. After incubation for 3 min, the difference in absorbance at 475 nm was measured, compared to the control consisting of phosphate buffer (50 mM, pH 6.8) instead of tyrosinase. Kojic acid at various concentrations (1, 2.5, 5, and 10 μM) was used as a positive control. The percentage of tyrosinase inhibition (I%) and IC₅₀ value were calculated in a similar manner as the DPPH assay.

Shrimp preparation and treatment
Freshly captured whiteleg shrimp (Litopenaeus vannamei) free of additives, with the size of 30–40 shrimp/kg were bought alive from a local market in Ho Chi Minh City, Viet Nam. The shrimp were handled carefully by placing in polystyrene boxes filled with ice [shrimp/ice ratio 1:2 (w/w)], and were immediately transported to the chemical technology laboratory, Ho Chi Minh City University of Technology and Education, Ho Chi Minh City, Viet Nam. On the way to the laboratory, shrimp were washed with cold water and kept in ice before experiments were done within 1 h. The shrimp samples were divided into 4 equal parts. Each part was dipped into the Coffea arabica sediment extract solution, the PD-3 solution, the sodium metabisulﬁte (SMS) solution 1.25%, and water (as the control), respectively, in the ratio of 1:2 (shrimp: solution, w/v) at 4°C for 15 min. After removal from the solutions, shrimp were left on plastic sieves at 4°C for 5 min. The shrimp were placed in polypropylene boxes and kept in a refrigerator at 4°C for subsequent quality assessments: pH changes, melanosis development, bacterial count, lipid peroxidation, and microbiological analysis. Samples were taken for analyses at an interval of 1 day up to 7 days during 7 days of storage.

pH measurement
pH measurement was determined by the method described by Chouljenko et al. (2017). Two grams of shrimp were homogenized with 10 mL of deionized water in a beaker for 1 min. The mixture was stored for 5 min at room temperature. The pH values of all samples were measured using a pH-meter (pH 1200, PSI, Guildford, UK). All measurements were carried out in triplicate.

Melanosis evaluation
The photos of shrimp samples were taken by a digital camera (Eos M10 Kit Ef-M15-45, Canon, Tokyo, Japan). Relative changes in the gray value of the shrimp carapace were calculated by the following formula:

$$\text{% Relative change} = 100 - \frac{A \times 100}{B}$$

which A is the actual gray value of the sample, and B is the average gray value of the shrimp on the first day (Encarnacion et al., 2012). The color image analysis was conducted using ImageJ software (NIH, Bethesda, MD, USA). Low mean gray values indicated the formation of melanosis in the carapace of shrimp.

Lipid peroxidation inhibitory assay
The extent of lipid oxidation was determined using thiobarbituric acid reactive substances (TBARS). Thiobarbituric acid (TBA) reacts with malonaldehyde bis (dimethyl acetal) (MDA) in acidic conditions to form a pink MDA-(TBA)_2 complex, which can be quantified at 532 nm by a spectrophotometer (Sochor et al., 2012). A portion (5 g) of ground shrimp was homogenized with 10 mL of 0.714 mM L-DOPA (as substrate at 45°C) was added to ferrous (II) iron by the action of electron-donating antioxidants. Extracts (0.1, 0.5, and 1.0 mg) were mixed with 1 mL of potassium phosphate buffer (2.0 M, pH 6.6) and 1 mL of potassium ferricyanide [K₃Fe(CN)₆] 1% (w/w). The mixture was incubated for 20 min at 50°C. An aliquot (1 mL) of 10% (w/w) TCA was added to the mixture, which was then centrifuged at 2,000 rpm for 10 min. The supernatant (1 mL) was mixed with 1 mL of distilled water and 0.2 mL of ferric chloride 0.1%. The absorbance of this solution was measured at 700 nm.

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mL of TCA (7.5%, w/w). The mixture was filtered, the filtrate was mixed with an equal volume of 0.02 M TBA and incubated at 100°C for 15 min. The absorbance at 532 nm was measured with Hitachi UH-530 spectrophotometer (Hitachi, Ltd.). TBARS values were calculated from the calibration curve built at concentrations from 0.01 to 0.05 μM and reported as malondialdehyde equivalents (mgMAD/kg of shrimp).

**Microbiological analyses**
The microbiological quality of shrimp samples was assessed based on the bacterial count method in three parameters: total plate count (ISO, 2013), Enterobacteriaceae count (EBC) (ISO, 2017), and *Pseudomonas aeruginosa* count (Decision No. 3347/2001/QĐ-BYT). All experiments were conducted at Center of Analytical Services and Experimentation HCM (Ho Chi Minh City, Viet Nam). Microbial colonies were counted and reported as log colony-forming unit (CFU)/g fresh weight.

**Physicochemical analyses**
Protein content of all samples was tested according to AOAC (1992). All determinations were carried out at Center of Analytical Services and Experimentation HCM (CASE, Ho Chi Minh City, Viet Nam).

The shear force of shrimp during 7 days of storage was measured by the Rheo Tex SD 700II analyzer (Sun Scientific Co., Ltd., Tokyo, Japan) at Research Institute for Aquaculture No 2 (RIA2, Ho Chi Minh City, Viet Nam). The operating parameter used was the cross head speed of 10 mm/s. The shear force perpendicular to the axis of the second segment muscle fibers, was measured.

Total volatile basic nitrogen (TVB-N) was measured according to Vietnamese National Standards TCVN 9215 (TCVN, 2012) at Center of Analytical Services and Experimentation HCM (CASE, Ho Chi Minh City, Viet Nam). The TVB-N values were expressed as mg N/100 g of shrimp meat.

**Statistical analysis**
Results were analyzed by analysis of variance (ANOVA) while means were tested with Tukey’s test (*P*<0.05) for three determinations. Statistical data were performed using an SPSS package (SPSS 20 for Windows Evaluation Version, IBM Corporation, Armonk, NC, USA).

**RESULTS AND DISCUSSION**

**DPPH radical scavenging activity**
The results for DPPH radical scavenging activity were summarized in Table 1. The lower IC50 value indicates higher antioxidant capacity. Among the fractions screened for the DPPH assay, PD-3 was the most active sample with an IC50 value of 27.3 μg/mL, followed by CE with IC50 of 32.2 μg/mL (*P*<0.05). The second group was composed of PD-1, PD-2, and PD-4 with moderate antioxidant activity, and their IC50 values were in the range of 51.1∼79.2 μg/mL. Weak antioxidant activity was observed for the remaining 2 fractions with IC50 values of more than 100 μg/mL. All IC50 values for samples were lower than that of the positive control, gallic acid (IC50 value of 7.18 μg/mL). The hydrogen atom or electron-donating abilities of compounds to reduce the free radical DPPH to a stable form may explain the antioxidant activity of the fractions (Sharma et al., 2007).

**Tyrosinase inhibitory activity**
For antityrosinase activity, the study demonstrated that all fractions exhibited inhibition to varying degrees at the concentration of 100 μg/mL (*P*<0.05) (Table 1). It should be noted that PD-4 possessed the lowest tyrosinase inhibitory activity with 1% value of 60.5 whilst the PD-3 had the strongest inhibitory activity with 1% value of 79.5, which could be applied as a natural anti-browning agent (*P*<0.05). The CE was ranked second with 1% value of 75.6 among screened samples. Kojic acid, used as a stand-

| Table 1. Antioxidant and tyrosinase activities of crude extract (CE) and different fractions from *Coffea arabica* |
|---|---|---|---|---|---|---|
| No | Sample | DPPH (IC50) (μg/mL) | Total phenolic content (mgGAE/100 g) | Tyrosinase inhibition activity (I%) (100 μg/mL) | FRAP 0.1 μg/mL | FRAP 0.5 μg/mL | FRAP 1.0 μg/mL |
|---|---|---|---|---|---|---|---|
| 1 | CE | 32.2±0.4b | 109.1±0.88 | 75.6±0.3b | 0.721±0.002b | 2.334±0.002b | 3.663±0.002b |
| 2 | PD-1 | 51.1±0.3d | 89.2±0.3d | 70.9±0.1c | 0.362±0.005f | 2.377±0.003a | 3.563±0.003d |
| 3 | PD-2 | 62.0±0.5c | 105.1±0.2c | 66.7±0.2c | 0.721±0.002b | 2.362±0.004b | 3.578±0.003c |
| 4 | PD-3 | 27.3±0.2f | 116.5±0.4a | 79.5±0.3a | 0.793±0.002a | 2.316±0.002d | 3.702±0.004a |
| 5 | PD-4 | >100a | 75.1±0.4a | 67.9±0.1d | 0.650±0.003c | 1.954±0.001f | 3.361±0.003f |
| 6 | PD-5 | >100a | 83.1±0.5e | 64.7±0.1d | 0.392±0.002d | 1.926±0.001e | 3.117±0.002g |
| 7 | PD-6 | >100a | 79.7±0.5f | 60.5±0.2g | 0.650±0.003c | 1.894±0.001f | 3.361±0.003f |

Values represent the mean±standard deviation (n=3).
Means with different letters (a-g) in each column indicate significant differences (*P*<0.05) according to Tukey’s test.
Fractions collected from the fractionation process of *Coffea arabica* extract and labeled from fraction I to VI (PD-1 to PD-6, respectively).
ard inhibitor, had 1% value of 89.91.

The presence of phenolic compounds especially flavono-

oids may be responsible for the tyrosinase inhibition. Plant polyphenols possessing a similar structure as the
substrate (L-DOPA) are usually strong inhibitors. Specif-
ically, flavonoids containing an α-keto group show high
inhibition because of the similarity between the dihy-
droxyphenyl group in L-DOPA and the α-keto group in
flavonoids. These polyphenols are able to chelate copper
atoms in the active site of tyrosinase (Kim and Uyama,
2005).

FRAP assay
The reducing power (RP) assay is widely used to evaluate
the ability of an antioxidant to donate an electron. It was
reported that the reducing power of extracts indicate their
potential antioxidant activity. Antioxidants in the sam-
ple reduced the ferric cyanide complex (Fe3+) to the fer-
rous cyanide form (Fe2+) (Yang et al., 2014). A higher
value in absorbance indicates greater reducing capacity.
Table 1 demonstrates that RP was directly proportional to
the concentration of samples and was found to increase
with the increase in concentration. Of these surveyed
samples, the highest RP was observed to be in the order
of PD-3 > CE > PD-2 > PD-1 > PD-6 at 1.0 μg/mL of ex-
tract (P<0.05). The results indicate that the ferric reduc-
ing power was generally correlated with the total phe-
nolic content and the DPPH radical scavenging activity.

Total phenolic content
The total phenolic content of the six fractions and CE
varied in wide ranges from 75.1 mgGAE/g (PD-5) to
116.5 mgGAE/g (PD-3) (Table 1). As shown in the Table
1, all samples demonstrated high content of total phe-
nolics with values of more than 75.0 mgGAE/g. Of these,
the maximum phenolic content was found in the PD-3
fraction (116.5), followed by the CE with the content of
109.1 mgGAE/g.

Many studies have reported that the total phenolic con-
tent has been correlated with antioxidant activity of plant
fractions, owing to the redox properties of phenolic com-
ounds acting as reducing agents, hydrogen donors, or
metal chelators (Pereira et al., 2015). To summarize, high
DPPH radical scavenging and tyrosinase inhibition of the
Coffea arabica extract suggested that the extract could be
potentially used as an antioxidant and melanosis inhibi-
tor. The biological activities of plant samples are caused
by the presence of chemical substances called phytochem-
icals, so the investigation for the phytochemicals of the
fraction is essential (Rakholiya et al., 2016).

From the above results, PD-3 had the best antioxidant
and antityrosinase activities. Therefore, PD-3 was chosen,
among all fractions, to serve as a promising source of nat-
ural preservative for the storage of shrimp.

pH measurement
Changes in pH of whiteleg shrimp with different treat-
ments: PD-3, CE, and SMS during refrigerated storage
compared with the control (immersed in distilled water)
are shown in Fig. 1. Overall, the pH for all shrimp sam-
ple saw an upward trend (P<0.05) over storage, while
the pH for PD-3 treatment experienced lower pH values
throughout. After 7 days of storage, pH values of all
samples rose approximately 12% with the notable excep-
tion of the shrimp treated with PD-3, where the pH in-
creased to the lowest level at 8.5%. During the first two
days of storage, no significant differences were observed
among pH values for all treatments (P>0.05). From day
2 onwards, the lowest pH was found in the PD-3 batches,
followed by the CE and SMS treatment batches (P<0.05).
Final values of 7.78 and 7.69 were observed in the con-
trol and SMS samples, respectively; 7.60 and 7.46 were

![Fig. 1. Changes in pH of whiteleg shrimp, Litopenaus vannamei during refrigerated storage. Values represent the mean±standard deviation (n=3). Significant differences (P<0.05) among different days of storage in the same treatment are identified with different uppercase letters (A–H). Significant differences (P<0.05) among different treatments in the same storage time are identified with different lowercase letters (a–d). Control, sample treated water; SMS, sample treated with sodium metabisulfite (1.25%); CE, sample treated with Coffea arabica extract; PD-3, sample treated with PD-3 fraction obtained from Coffea arabica sediment extract.](image-url)
obtained for the CE and PD-3 samples, respectively. pH change was associated with the accumulative growth of basic compounds, mainly resulting from either endogenous or microbial enzyme actions (Nirmal and Benjakul, 2009; Huang et al., 2012). The lower pH values for the samples treated with the PD-3 fraction, CE, and SMS followed the lower microbial count. In our study, the lower pH changes in the PD-3 fraction and CE treatments suggested that these can enhance endogenous or microbial enzyme inhibition.

Melanosis evaluation
The formation of melanosis in shrimp samples during refrigerated storage, as demonstrated by decreased mean gray values of the carapace of whiteleg shrimp, is illustrated in Fig. 2. Over 7 days of storage, the gray values in all samples indicated a decline, but samples treated with PD-3 and CE changed to a lesser extent than those of the control and 1.25% w/v SMS (P<0.05). At day 0, all shrimp samples had no differences in gray values (P>0.05). When storage time increased, gray values decreased continuously after 1 and 2 days storage for the control and the SMS samples, respectively, indicating the formation of melanosis, while the CE and PD-3 treatments remained unchanged (P>0.05) until day 2. After 6 days of storage, the SMS group had severe melanosis as the control including day 7. At the end of the period, the samples treated with PD-3 had the highest gray values, followed by those treated with the CE (P<0.05). These results show that PD-3 and CE retarded the development of melanosis in shrimp. It has been reported that tyrosinase is a copper-containing enzyme catalyzing the synthesis of melanin from tyrosine by oxidation. Some phenolic compounds could inhibit the tyrosinase activity by interacting with the active site of the enzyme through a hydrogen bond or hydrophobic interaction (Kumar et al., 2011).

Lipid peroxidation inhibition assay
Changes in TBARS values of shrimp samples stored under different conditions during 7 days of storage are presented in Fig. 3. Overall, the PD-3 sample showed the lowest value for TBARS while the highest value was found in the control sample at all storage times (P<0.05). The initial TBARS value of all shrimp samples was about 0.60 mg MDA/kg of shrimp (P>0.05), and this value for the control sample rose continuously over the first 6 days while samples with other treatments only followed this rising trend from day 0 to day 3. During 3~5 days of storage, the TBARS values decreased in the PD-3 sample and fluctuated in the CE and the SMS. Within the last 2 days, TBARS levels of all samples followed a similar pattern,

![Fig. 2. Changes in the mean gray values of the carapace area of whiteleg shrimp, Litopenaus vannamei during refrigerated storage. Please see Fig. 1 for the caption and legends.](image1)

![Fig. 3. Changes in the TBARS values of whiteleg shrimp, Litopenaus vannamei during refrigerated storage. Please see Fig. 1 for the caption and legends.](image2)
rising significantly from day 5 to day 6 to peak at 4.33 (mg MDA/kg) for the control, 2.11 for the PD-3, 2.47 for the CE, 3.38 for the SMS, and then declining at day 7 (P<0.05). The increase in TBARS values was reported to involve the formation of secondary lipid oxidation products, mainly aldehydes, from the oxidative degradation of polyunsaturated fatty acids (Papastergiadis et al., 2012), causing off-flavors and off-odors, thereby shortening the shelf life of shrimp (Zhang et al., 2015). The observed decrease in TBARS values was probably because malonaldehyde was metabolized by a large population of spoilage bacteria (Murano et al., 1998). The low TBARS values in the PD-3 and CE samples, compared to other samples, indicated that the PD-3 and CE reduced the lipid oxidation in shrimp during refrigerated storage. A number of studies in the literature have shown that some natural plant extracts could delay lipid oxidation. Nirmal and Benjakul (2012) revealed that whiteleg shrimp treated with green tea extract had lower TBARS content after 12 days of iced storage. A similar finding was shown in the case of shrimp samples stored with pomegranate peel extract, which had significantly lower TBARS values compared with controls after 10 days of refrigerated storage (Basiri et al., 2015). Besides, several antioxidant-rich plant coating materials could protect against lipid oxidation. Greater stability towards lipid oxidation was found when shrimp samples were coated with thymol and basil seed gum (Khazaei et al., 2017), aloe vera, and eugenol (Sharifimehr et al., 2019), green tea extract, and quince seed mucilage (Noshad et al., 2017).

Microbiological analysis

Microbial spoilage in shrimp after 7 days storage is displayed in Fig. 4. As shown in the Figures, no difference was observed in total plate counts (TPC) (Fig. 4A) and EBC (Fig. 4B) among all samples at day 0 (P>0.05). Overall, on day 7, TPC and EBC of all shrimp batches were higher than those on day 0 (P<0.05). Initial TPC of all samples was in the range of 2.80 to 2.85 log CFU/g which were in agreement with the values of 2~3 log CFU/g reported by other authors in good quality pink shrimp (Parapenaeus longirostris) (López-Caballero et al., 2002) and whiteleg shrimp (Litopenaeus vannamei) (Aranzibia et al., 2015). After 7 days storage, the TPC of SMS, CE, and PD-4 treatments were 5.53, 5.76, and 5.71, respectively which were lower than control samples (7.34) (P<0.05). According to the TCVN (2006) requirements for frozen aquatic products, the acceptable limit of total aerobic microorganisms is 6.0 log CFU/g. It is clear that all microbial counts for SMS, CE, and PD-3 samples were below this limit, while the control sample reached values above the limit.

Noticeably, Pseudomonas aeruginosa counts of all samples were below the detection limit of 1.0 log CFU/g at both day 0 and day 7 (data not shown). Shiekh et al. (2019) reported that Pseudomonas counts of whiteleg shrimp treated with chamuang (Garcinia cowa Roxb.) leaf extract were found in the ranges of 2.15~2.18 log CFU/g at the first day of storage and 5.08~6.82 log CFU/g after 10 days of storage. However, counts depend on the environment where shrimp were caught and postmortem conditions (Huang et al., 2012).

In addition, shrimp treated with PD-3 had the lowest EBC of 2.40 log CFU/g compared with other samples (P<0.05) during refrigerated storage. Enterobacteriaceae are a large family of Gram-negative bacteria, comprising a number of pathogens such as Salmonella, Klebsiella, Escherichia coli, Shigella, and Yersinia pestis (Posse et al., 2017). The spoilage potential of Enterobacteriaceae must be taken into account particularly in the case of contaminated water or delay in the chilled storage of fish after capture (Sallam, 2007). The results suggest that shrimp treated with Coffea arabica sediment extract and its PD-3 fraction could impede the growth of aerobic microorganisms and

![Fig. 4. Total plate (A) and Enterobacteriaceae count (B) of whiteleg shrimp, Litopenaeus vannamei during refrigerated storage. Please see Fig. 1 for the caption and legends.](image-url)
lower the increase in EBC during refrigerated storage.

**Physicochemical changes**

Physicochemical parameters of shrimp samples, namely protein content, shear force and TVB-N after 7 days of storage were determined and shown in Fig. 5, Fig. 6, and Fig. 7.

The results show that the protein content of shrimp stored with PD-3 and CE were similar to shrimp stored with SMS, in the range of 21.3 ~ 22.3 (P > 0.05) and higher than that of control (P < 0.05), indicating that CE and PD-3 reduced protein loss and that they could replace SMS in shrimp storage (Fig. 5). Pan et al. (2019) demonstrated that the release of proteolytic enzymes like cathepsins and calcium-dependent proteases may result in decreases in myofibrillar and intramuscular tissue, leading to the deterioration of shrimp muscle protein. Not much information is available on the changes of shrimp protein content during low-temperature storage. Kamal et al. (2000) reported that the protein content of giant freshwater prawn, *Macrobrachium rosenbergii*, and tiger shrimp, *Penaeus monodon*, decreased from 18.46 to 17.05 % and 18.06 to 16.85%, respectively during 10 days of iced storage.

Fig. 6 illustrates that all samples had a similar shear force in the range of 19.8 ~ 20.0 N (P > 0.05) at day 0. Higher shear force values of muscle for samples treated with PD-3, CE, and SMS were observed as compared to those of control samples after 7 days storage (P < 0.05). It is noted that the higher shear force of the samples in treatment batches was consistent with the lower microbial load, demonstrating that the inhibitory activity of CE and PD-3 for the microbial growth might limit proteinases and collagenase which are responsible for the degradation of muscle protein in shrimp (Nirmal and Benjakul, 2009). The texture changes of shrimp could be affected by many vital factors, such as pH, or degradation of connective tissue or myofibrillar proteins (Yuan et al., 2016a). The results of this study are similar to a previous report by Nirmal and Benjakul (2009), which indicated that shrimp treated with 0.05% catechin and 0.1% catechin had higher shear force values as compared to the control after 10 days of iced storage. The retardation of texture loss was reported when Pacific white shrimp (*Litopenaeus vannamei*) was coated with chitosan in combination with pomegranate peel extract (Yuan et al., 2016a), chitosan-carvacrol (Wang et al., 2018), or chitosan-gelatin (Farajzadeh et al., 2016).

TVB-N is commonly used to determine the quality and freshness of seafood products and is indicative of microbial and chemical spoilage (Huang et al., 2012). The increase in TVB-N results from the degradative process of nitrogenous compounds in the muscle like proteins, amino acids, peptides, and nucleotides caused by the activity of endogenous enzymes and spoilage bacteria (Pan et al., 2019). Fig. 7 indicates the results of TVB-N of white-
leg shrimp during refrigerated storage. Initially, TVB-N (7.6–7.8 mg N/100 g shrimp meat) was not different among all samples (P > 0.05). After 7 days of refrigerated storage, shrimp treated with SMS, CE, and PD-3 had similar TVB-N content ranging from 32.2 to 33.6 mg N/100 g shrimp meat (P > 0.05) which were lower than 35.5 mg N/100 g for the control (P < 0.05). Thus, shrimp quality can be improved if it is treated with CE and PD-3, and that TVB-N content correlated well with microbial load in the corresponding samples. Other authors reported a delay in the production of TVB-N in Litopenaeus vannamei shrimp treated with 0.05 or 0.1% chitosan solution (Nirmal and Benjakul, 2009), coated with 1.0–1.5% o-carboxymethyl chitosan and 1.0–1.5% chitosan (Huang et al., 2012), treated with chamuang leaf extract combined with a pulsed electric field (Shiekh et al., 2019), treated with grape seed extracts (Sun et al., 2014), coated with chitosan and pomegranate peel extract (Yuan et al., 2016a), and a chitosan coating supplemented with green tea extract (Yuan et al., 2016b). Concerning the poor data available in the literature, several authors suggested different acceptability limits of TVB-N content. Specifically, TVB content of 30 mg/100 g shrimp meat was indexed for safety and quality acceptability, as proposed by Shiekh et al. (2019) and Huang et al. (2012). However, Altissimi et al. (2017) proposed a higher limit of acceptability at 42 mg/100 g of TVB-N for cephalopods, and Mendes et al. (2002) suggested TVB content of 40 mg N/100 g as the freshness borderline for shrimp.

In conclusion, the results achieved in this study showed that the extract of Coffea arabica sediment yielded a number of fractions with high anti-tyrosinase and antioxidant activities, high total phenolic content, and high potency for whiteleg shrimp preservation. Coffea arabica sediment and PD-3 treatments were effective in preserving whiteleg shrimp since they lessened melanosis and impeded quality loss during refrigerated storage. Therefore, they can be used as alternatives for sulfite additives, which can be improved if it is treated with CE and PD-3, and that TVB-N content correlated well with microbial load in the corresponding samples. Other authors reported a delay in the production of TVB-N in Litopenaeus vannamei shrimp treated with 0.05 or 0.1% chitosan solution (Nirmal and Benjakul, 2009), coated with 1.0–1.5% o-carboxymethyl chitosan and 1.0–1.5% chitosan (Huang et al., 2012), treated with chamuang leaf extract combined with a pulsed electric field (Shiekh et al., 2019), treated with grape seed extracts (Sun et al., 2014), coated with chitosan and pomegranate peel extract (Yuan et al., 2016a), and a chitosan coating supplemented with green tea extract (Yuan et al., 2016b). Concerning the poor data available in the literature, several authors suggested different acceptability limits of TVB-N content. Specifically, TVB content of 30 mg/100 g shrimp meat was indexed for safety and quality acceptability, as proposed by Shiekh et al. (2019) and Huang et al. (2012). However, Altissimi et al. (2017) proposed a higher limit of acceptability at 42 mg/100 g of TVB-N for cephalopods, and Mendes et al. (2002) suggested TVB content of 40 mg N/100 g as the freshness borderline for shrimp.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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