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MICROBIOLOGY

Screening of antioxidant and tyrosinase inhibition activities of spicy vegetables in Vietnam and application of *Persicaria odorata* leaf extract to preservative white leg shrimp (*Litopenaus vannamei*)

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Abstract: In this study, we aimed to exploit natural extracts from the spicy vegetables, which are rich in phenolic compounds as an initial treatment step in the cold storage process for shrimp. Firstly, 40 extracts from 10 types of spicy vegetables in Vietnam were prepared and tested for their bioactivities. Among samples, the extract from *Persicaria Odorata* leaves (E-4) exhibited the highest potential of scavenging DPPH free radical (IC_{50} of 7.54 $\mu g.mL^{-1}$) and decreasing tyrosinase activity with the inhibition percentage of 54.2 % at the concentration of 100 mg/mL. Twenty-two out of a total of 36 chemical compounds in the E-4 extract identified using HPLC-MS technique were phenolic compounds, in which four compounds (morin, quercetin, fisetin, astragalin) are flavonoids. Shrimp (*Litopenaus vannamei*) samples were treated with the E-4 extract having lower gray values, lipid peroxidation values, and microbiological counts than those of the control samples after 7 days of storage at 2 °C. These results show the potential of using the natural extract as a safe and effective alternative for commercial chemical-derived preservatives in the shrimp storage process.

Key words: Antioxidant activity, tyrosinase inhibition activity, *Litopenaus vannamei, Persicaria odorata,* spice vegetables.

INTRODUCTION

Shrimp and shrimp products have occupied a significant portion of the exported seafood products. It is challenging to preserve shrimps because their spoilage begins soon after death and in the storage process. The appropriate storage method for shrimp after catching is of utmost importance to maintain their sensory and nutritive qualities for extending shelf life's shrimp (Djeridane et al. 2006, Tsironi et al. 2009). The current storage technology focuses on using the cold/ heat treatment or adding additives, however these methods face with the challenges of melanosis development, lipid oxidation, microbial spoilage over time and toxicity (Bhobe & Pai 1986, Montero et al. 2001, Pedale et al. 2012). Therefore, the development of a safe storage method for shrimps has been paid attention a significant number of research attentions.

The spoilage of shrimps has correlated the compound called tyrosine, which undergoes the oxidation process by the assist of the tyrosinase in shrimps after death. The convert of phenol moiety in tyrosine to quinones by tyrosinase activates the melanosis process via a biochemical mechanism (Norman & Benjamin 2000). The melanosis development is followed by non-enzymatic polymerization and

auto-oxidation of quinones to form high weight molecules, thus giving rise to dark pigments in shrimps. In commerce, sulfites and their derivatives (typically, sodium metabisulfite, SMS) have been used as melanosis inhibitors, attributed to their ability to irreversible bonding with quinones to form colorless compounds (Montero et al. 2001). However, SMS has reported causing allergic reactions and especially in asthmatic patients, nausea, abdominal pain, vomiting, as well choking, and chemical pneumonitis in consumers or handlers (Pedale et al. 2012, Andrade et al. 2015). Therefore, natural antioxidant compounds such as ascorbic acid, citric acid, kojic acid, gallic acid, dodecyl gallate, oxalic acid, and 2-thiol-L-histidine-betaine (ergothioneine, ESH) have been intensively study as a alternative for sulfiting agents in shrimp preservation (Mcevily et al. 1991, Gonçalves & Oliveira 2016). Besides, extracts from mushroom and grape seed were also studied in preventing the black spot formation in shrimp (Encarnacion et al. 2012, Haiyan et al. 2014). Plant phenolic compounds have been paid increasing attention as potential natural additives base on their antioxidant, tyrosinase inhibition, and antimicrobial activities (Djeridane et al. 2006). All these findings have inspired us to use natural extracts from vegetables in shrimp storage.

The spicy vegetables are well-known as good sources of antioxidants and antibacterial reagents such as flowers (clove), bulbs (garlic, onion), fruits (cumin, red chili, black pepper), stems (coriander), bark (cinnamon), roots (ginger), berries (peppercorns), aromatic seeds (cumin), etc (Kaefer & Milner 2008, Brewer 2011, Yashin et al. 2017). In this study, we screened on antioxidant and tyrosinase inhibition activities of 10 types of common spicy vegetables in Vietnam. Based on the screening results, the extract from the *Persicaria Odorata* leaves (E-4) has the highest potential of the bioactivities that studied the ability to preserve *L. vannamei* shrimp at the cold storage. To study on the melanosis process and lipid peroxidation, and bacterial formation, the shrimps, treated with the E-4 and SMS solution were stored at 2 ^{o}C after 5-7 days of storage were investigated. The shrimps treated with the E-4 showed a lower lipid peroxidation and gray value than that of the control sample, attribute to their ability to reduce the fat oxidation process and melanosis developing in the *L. vannamei* shrimps. Besides, the E-4 sample inhibited the effectiveness of preventing the bacterial formation in shrimp higher than the additive sample and control sample. Herein, we had applied for the first time the extract from *P. Odorata* leaves as safe and green additives for shrimp preservation.

MATERIALS AND METHODS

Materials

Vegetables were purchased at the supermarkets in July 2017. Then, only leaves were collected, dried to a moisture content of about 10% and ground into fine powder using a blender. The voucher samples (number sample on Table I) were preserved at the department of food technology of the Ho Chi Minh City University of technology and education.

White leg shrimps (*L. vannamei*) with the size of 30-40 shrimps/kg were purchased from Thu Duc market, Ho Chi Minh City, Vietnam, in March 2018. The shrimps were kept alive and transported to the laboratory.

Extraction procedure

Forty extracts consist of ethanol (E) and ethanol without chlorophyll (EWC) extracts, water (W), and water without chlorophyll extracts (WWC) were prepared from ten vegetable leaves (Table I). Dry powders (20-30 g) were extracted with ethanol solvent (100 mL, 65^{o}C) or water

Table I. List of 40 extracts prepared from 10 spicy vegetables and their biological activities.

No.	Local name	Scientific name	Name Sample	DPPH assay (IC_{50})	TPC (mgGAE/g)	Tyrosinase Inhibition Activity (I%)
1	Húng lủi	*Mentha Crispa* L.	W-1	38.24	130.9 ± 2.5	45.1 ± 1.0
2			WWC-1	49.89	114.8 ± 1.9	52.6 ± 2.2
3			E-1	46.92	94.5 ± 2.8	42.9 ± 1.3
4			EWC-1	41.51	65.8 ± 2.8	40.4 ± 1.2
5	Rau ôm	*Limnophila Aromatica*	W-2	51.92	75.6 ± 2.3	36.2 ± 1.9
6			WWC -2	71.3	64.2 ± 2.4	37.3 ± 1.5
7			E-2	62.05	66.5 ± 2.0	33.5 ± 1.7
8			EWC -2	46.91	53.0 ± 2.3	11.9 ± 1.8
9	Húng cay	*Ocimum Africanum* Lour	W-3	65.91	52.3 ± 3.3	44.8 ± 2.1
10			WWC -3	71.92	132.3 ± 3.3	43.8 ± 2.0
11			E-3	65.62	51.8 ± 1.8	33.5 ± 2.6
12			EWC -3	30.44	80.2 ± 2.1	41.7 ± 2.4
13	Rau răm	*Persicaria Odorata*	W-4	8.36	143.9 ± 1.9	49.62 ± 0.77
14			WWC -4	9.15	143.6 ± 1.3	41.5 ± 1.9
15			E-4	7.54	155.9 ± 0.8	54.2 ± 2.9
16			EWC -4	7.85	133.8 ± 1.5	40.9 ± 1.6
17	Ngãi cứu	*Artemisia Vulgaris*	W-5	8.43	103.4 ± 2.4	35.7 ± 0.5
18			WWC -5	9.65	135.6 ± 2.5	34.5 ± 2.2
19			E-5	9.06	98.6 ± 2.3	32.02 ± 0.98
20			EWC -5	9.22	124.8 ± 2.4	17.02 ± 0.73
21	Diếp cá	*Houttuynia Cordata*	W-6	8.24	105.1 ± 2.3	50.0 ± 4.7
22			WWC -6	9.42	225.4 ± 1.9	42.2 ± 0.5
23			E-6	8.02	43.3 ± 1.0	38.9 ± 4.4
24			EWC -6	9.06	177.6 ± 2.2	34.2 ± 3.5
25	Húng quế	*Ocimum Basilicum*	W-7	64.78	45.9± 1.5	44.9 ± 2.4
26			WWC -7	>100	58.8 ± 2.2	37.52 ± 0.47
27			E-7	>100	31.8 ± 1.8	40.2 ± 2.8
28			EWC -7	58.08	56.9 ± 1.1	42.2 ± 0.9
29	Tía tô	*Perilla Frutescens*	W-8	43.8	89.4 ± 1.3	33.7 ± 3.4
30			WWC -8	43.51	66.8 ± 0.7	37.2 ± 3.7
31			E-8	56.17	62.5 ± 1.1	31.0 ± 2.2
32			EWC -8	22.65	65.8 ± 1.8	30.8 ± 1.8
33	Kinh giới	*Elsholtzia Cristata*	W-9	53.62	56.77 ± 1.3	38.0 ± 2.5
34			WWC -9	78.5	67.5 ± 4.8	39.3 ± 2.6
35			E-9	60.2	39.1± 1.4	31.8 ± 1.9
36			EWC -9	81.84	51.7 ± 1.3	28.6 ± 0.43
37	Ngò gai	*Eryngium Foetidum*	W-10	87.4	24.1 ± 1.0	37.2 ± 1.4
38			WWC -10	94.41	35.8 ± 1.3	32.2 ± 2.0
39			E-10	>100	24.4 ± 1.2	34.4 ± 3.9
40			EWC -10	>100	22.6 ± 0.9	24.6 ± 2.6

Results presented as the mean ± SD (n=3).

(100 mL, 80°C) by the Soxhlet system. Then, the extract solutions were evaporated under reduced pressure conditions (20 hPa, 50°C) to dry and recover the ethanol and water extracts. Similarity, EWC, and WWC were prepared after removing chlorophyll by acetone solvent (100 mL, 60-65°C).

DPPH free radical scavenging activity

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical was used for screening on antioxidant activity of the extracts (Molyneux 2004). Briefly, 1.5 mL of a 0.1 mM solution of DPPH was mixed with 1.5 mL of each extract solution at concentrations of 100, 50, 25, 10 $\mu g.mL^{-1}$ in 90 vv% ethanol. After 30 min incubation in the dark, the decrease in the solution absorbance was measured at 519 nm using Hitachi UH-530 spectrophotometer. DPPH inhibitory activity expressed as the percentage inhibition (I%) of DPPH in the above assay system, calculated as (1-B/A)×100, where A and B are the absorbance of the DPPH solution without and with the test material. IC_{50} (inhibitory concentration, 50%) values were calculated from the mean values of data from three determinations. Gallic acid at various concentrations (1, 2.5, 5, and 10 μM) was used as a positive control.

Tyrosinase inhibition activity

The inhibitory effects of extract samples on tyrosinase inhibition activity were evaluated following the procedure of Nirmal with a slight modification by using a 96-well reader (Nirmal & Benjakul 2011). Initially, 40 μL of extract solutions (100 $\mu g.mL^{-1}$) were mixed with 20 μL of tyrosinase (100 U/mL). This reaction mixture was incubated for 30 min at room temperature. 140 μL of 0.714 mM L-DOPA (45°C) was added to initiate the reaction. The reaction was conducted at 45°C, and the absorbance at 475 nm was monitored after three min. The control was performed in the same way, except phosphate buffer (50 mM, pH 6.8) was used instead of tyrosinase. Tyrosinase inhibitory activity was the percentage inhibition (I%) and IC_{50} value in a similar formula to DPPH assay. Kojic acid at various concentrations (1, 2.5, 5, and 10 μM) was used as a positive control.

Determination of total phenolic content

The total phenolic content (TPC) of extracts was determined using Folin-Ciocalteu reagent (Jung et al. 2008). Firstly, gallic acid solutions or extract samples were added and mixed with the reagent solution (1:5 v/v) within 5 minutes. Then, a 1.0 M solution of sodium carbonate was added to the above mixture. After 30 min of incubation in the dark at room temperature, the mixture turns to blue, whose absorbance was measured at 750 nm using Hitachi UH-530 spectrophotometer. The content of phenolics in the samples was calculated from the calibration plot of gallic acid and expressed as mg gallic acid equivalent per gram of extract (mgGAE/g). All the determinations were carried out three times.

HPLC-EIS-MS analysis

HPLC-EIS-MS technique was performed to separate and elucidate compositions in the ethanolic extract. The separation module consisted of Agilent 1200 series HPLC (USA) equipped with ESI-MS system (micrOTOF-QII Bruker Daltonic, Germany). The sample was eluted on a column ACE3- C_{18} (4.6 ×150 mm, 3.5 μm, Merck, Germany) with a gradient system consisting of solvent A (0.1vv% formic acid in water) and solvent B (0.1vv% formic acid in methanol) used as the mobile phase, with a flow rate of 0.5 mL/min. The temperature of the column was maintained at 40 °C, and the injection volume 20 μL. For ESI-MS, full scan mass spectra were measured between m/z 150 and 2000. High purity nitrogen was used as

nebulizer gas at 1.2 bar, 200 °C and at a flow rate of 0.8 mL/min.

Preservation for shrimp

The shrimp samples were immersed in 0.025 wv% extract solution, 1.25wv% SMS solution, and water (control sample) at the room temperature (32 ± 2°C) for 5 minutes. Then, shrimps were taken out and preserved in plastic boxes at 2°C. Three shrimps from each treatment were tested for determining pH, melanosis development, bacterial count, lipid peroxidation inhibition assay, and microbiological analysis after 7 days of storage.

PH measurement

pH measurement was performed following the previous study with some modifications (Lopez et al. 2007). Homogenizing shrimp meat (2g) with ten volumes of deionized water for 1 min and the homogenate was kept at room temperature for 5 min. The pH values in shrimp were determined using a pH-meter (PSI pH 1200, England). All experiments were carried out in triplicate.

Melanosis evaluation

The shrimps were taken photographs using a Canon Eos M10 Kit Ef-M15-45 (Japan). Relative changes in the black spot (gray value) of the carapace at the tail, body, and head were evaluated using the following formula: % relative change =100–[(A×100)/B], where A is the actual gray value of the shrimp sample and B is the average gray value of the shrimps in the first day (Encarnacion et al. 2012). The change of gray value in images was analyzed using ImageJ software. The decrease of the % relative change corresponds to the blackening or the increase of melanosis in the carapace area of shrimp during storage. Three shrimps used for one treatment.

Lipid peroxidation inhibition assay

Thiobarbituric acid (TBA) reacts with malonaldehyde bis (dimethyl acetal) (MDA) to form a red di-adduct, which can be detected at 532 nm by spectrophotometer (Hitachi UH-530, Japan) (Singh & Arora 2007). Shrimps were ground and mixed with 10 mL of 7.5 wv% trichloroacetic acid. The mixture was filtered to collect the filtrate. Subsequently, the filtrate was mixed with 0.02 M TBA solution equal volume rate and heated at 100°C for 15 min. Absorbance was measured at 532 nm by Hitachi UH-530 spectrophotometer. MDA content was calculated from the standard curve built at concentrations from 0.01 to 0.05 *m*M and reported as mgMAD/kg of shrimp.

Microbiological analysis

Enterobacteriaceae and *Pseudomonas aeruginosa* in shrimp samples after five days preserving at 2 °C were detected and determined based on bacterial count method following TCVN 5518-2:2007 (ISO 21528-2:2004) and 3347/2001/QĐ-BYT at HCM Institute Pasteur.

Statistical analysis

Results were calculated to the analysis of variance (ANOVA) whereas the Tukey's test ($p < 0.05$) was applied from three determinations. Statistical calculations were carried out by SPSS 15.0 for Windows Evaluation Version (IBM Corporation, Armonk, North Castle, New York, USA).

RESULTS AND DISCUSSION

Assessment of the antioxidant and tyrosinase inhibition activities

The results of DPPH and tyrosinase assay were showed briefly in Table I. Generally, E and EWC extracts had higher IC_{50} values than W and WWC

extract. For DPPH assay, in total 40 extracts, nine samples showed IC_{50} values from 25 to 50 $\mu g.mL^{-1}$, fifteen extracts with IC_{50} values from 50 to 100 $\mu g.mL^{-1}$, four extracts with IC_{50} values more than 100 $\mu g.mL^{-1}$, and twelve samples exhibited strong antioxidant activities with IC_{50} values below 10 $\mu g.mL^{-1}$. Notably, twelve extracts that showed vigorous antioxidant activities prepared from three spice vegetables, including *Persicaria Odorata, Artemisia Vulgaris, Houttuynia Cordata.* Among them, E-4 extracted from *P. Odorata* exhibited the most potent antioxidant activity with the lowest IC_{50} value (7.54 $\mu g.mL^{-1}$). IC_{50} value of gallic acid was 4.66 μM (0.79 $\mu g.mL^{-1}$). The ability to quench DPPH radical of extracts can be explained due to their hydrogen donating capacity and the number of phenolic compounds present in extracts.

For tyrosinase inhibition activities of 40 ethanol extracts at concentration of 100 mg/mL, thirteen extracts showed percentage inhibition (I%) at from 40 to 50%, twenty extracts with I% from 30 to 40%, and four extracts had I% less than 30%. Three extracts (WWC-1, E-4, W-6) had percentage inhibition more than 50%, which for IC_{50} values were suggested below 100 $\mu g.mL^{-1}$. IC_{50} value of kojic acid was 49.9 μM (7.09 $\mu g.mL^{-1}$).

The extracts inhibited the antioxidant and tyrosinase inhibition activity due to the presence of phenolic compounds, especially flavonoids (Taherkhani & Gheibi 2014). The hydroxyl group can participate in reducing DOPA-chrome to DOPA, by giving electrons or by cross-linking with PPO via hydrogen bonding (Lopez et al. 2007).

Total phenolic content and chemical composition

The 40 ethanol extracts contained phenolic contents from 22.6 mgGAE/g (EWC-10) to 225.4 mgGAE/g (WWC-6) (Table I). In which, 12 extracts prepared from *P. Odorata, H. Cordata,* and *A. Vulgaris* showed high contents of phenolic with values more than 100 mgGAE/g. TPC value of the WWC-6 (225.4 mgGAE/g) was the highest, followed by the E-4 (155.9 mgGAE/g). Besides, the samples without chlorophyll had higher results than samples in the same solvent. It may be due to removing a part of chlorophyll by acetone solvent in WWC, EWC samples.

Many researchers indicated a positive relationship between total phenolics and antioxidant activity of plant extracts (Kim et al. 2003, Djeridane et al. 2006). The high content of phenolic compounds to scavenge free radicals may be attributed to the hydroxyl groups. The extracts of *P. Odorata, H. Cordata,* and *A. Vulgaris* exhibited not only high DPPH scavenging activity but also high phenolic contents. In brief, high DPPH and tyrosinase scavenging activity of the E-4 extract revealed that the extract has the potential to be an antioxidant and melanosis inhibitor; thus, the E-4 was collected to study on chemical constituents and preserving white leg shrimp (*L. vannamei)* in the cold storage.

Thirty-six compounds were determined in the E-4 extract prepared from *P. Odorata l*eaves by HPLC-EIS-MS analysis (Table II). Twenty-two compounds belong to phenolic compounds, in which 4 compounds (morin, quercetin, fisetin, astragalin) are flavonoids. They are not only potent antioxidants but also strong antityrosinase (Taherkhani & Gheibi 2014). The flavonoids are able to produce complexes with Cu^{2+}- a metal located at the center of activity and required for tyrosinase catalytic activity by linking the hydroxyl group to the Cu^{2+} ion (Kim et al. 2006). Thus, the presence of phenolic compounds, especially flavonoids, may play an essential role in their antioxidant and tyrosinase inhibition activities.

Table II. Identification of 36 compounds in *P. Odorata* leaf extract by HPLC-EIS-MS.

No.	Compound	m/z	Predicted formula	No.	Compound	m/z	Predicted formula
1	Mitolactol	305.9128	$C_6H_{12}Br_2O_4$	**19**	Fisetin	286.0451	$C_{15}H_{10}O_6$
2	Fipexide	388.1177	$C_{20}H_{11}ClN_2O_4$	**20**	5-Aminosalicylic acid	153.0415	$C_7H_7NO_3$
3	Cefsumide	440.0796	$C_{17}H_{20}N_4O_6S_2$	**21**	Fepentolic acid	224.1029	$C_{12}H_{16}O_4$
4	Carbenicillin	378.0889	$C_{17}H_{18}N_2O_6S$	**22**	DPT/N,N-Dipropyltryptamine	244.1918	$C_{16}H_{24}N_2$
5	Dibenzyl succinate	298.1231	$C1_8H_{18}O_4$	**23**	Zimelidine	316.0551	$C_{16}H_{17}BrN_2$
6	Dimethadione	129.0417	$C_5H_7NO_3$	**24**	Trinexapac-ethyl	252.0974	$C_{13}H_{16}O_5$
7	Fluprednidene acetate	432.1961	$C_{24}H_{29}FO_6$	**25**	Oxonazine	222.1239	$C_9H_{14}N_6O$
8	Fluticasone propionate	500.1829	$C_{25}H_{31}F_3O_5S$	**26**	Butyl 4-hydroxybenzoate	194.0925	$C_{11}H_{14}O_3$
9	Fluprednidene acetate	432.196	$C_{24}H_{29}FO_6$	**27**	Usnic acid	344.0862	$C_{18}H_{16}O_7$
10	Vanillin mandelic acid	198.0511	$C_9H_{10}O_5$	**28**	Embelin	294.1802	$C_{17}H_{26}O_4$
11	Quercetin	464.0911	$C_{21}H_{20}O_{12}$	**29**	BOH	195.088	$C_{10}H_{13}NO_3$
12	Astragalin	448.0964	$C_{21}H_{20}O_{11}$	**30**	Lotucaine	291.2169	$C_{18}H_{29}NO_2$
13	Cefatrizine	462.0765	$C_{18}H_{18}N_6O_5S_2$	**31**	MAL / Methallylescaline	251.15	$C_{14}H_{21}NO_3$
14	Brovincamine	432.1017	$C_{21}H_{25}BrN_2O_3$	**32**	Stearic acid	284.2688	$C_{18}H_{36}O_2$
15	Cloxestradiol	418.0862	$C_{20}H_{25}Cl_3O_3$	**33**	Oseltamivir	312.2062	$C_{16}H_{28}N_2O_4$
16	Spironolactone	416.2007	$C_{24}H_{32}O_4S$	**34**	Suxemerid	424.33	$C_{24}H_{44}N_2O_4$
17	Morin	302.0398	$C_{15}H_{10}O_7$	**35**	Butyl 4-aminobenzoate	193.11757	$C_{11}H_{15}NO_2$
18	Azelaic acid	188.1031	$C_9H_{16}O_4$	**36**	Gemfibrozil	250.15744	$C_{15}H_{22}O_3$

Quality changes of shrimp during cold storage

PH measurement

The changing in the pH value of white leg shrimps treated with H_2O, E-4, and SMS during the storage period shown in Figure 1. During the storage, the pH values of all investigated shrimps reported a sudden increase in the first two days and a gradual increase at lower speed from day 3 to day 5. On the first day, no noticeable difference in the pH value was observed among all samples ($p>0.05$). However, after five days of storage, the sample treated with water exhibited the highest pH value of 7.63, whereas those samples treated with 0.025 wt% E-4 (7.35) and 1.25 wt% SMS (7.34) displayed comparable pH value. The increase in pH was associated with the accumulation of basic compounds, mainly resulted from the microbial development. The lower increase in the pH value of shrimp treated

with the E-4 extract was in accordance with the lower microbial count (Lopez et al. 2007, Montero et al. 2001). According to Shamshad, the pH values in shrimps should not exceed 7.6 during storage (Shamshad et al. 1990), this suggested that the E-4 extract could control the change of pH value similar to the commercial additive.

Melanosis evaluation

In general, gray values change significantly after two days of the storage process at 2°C (Figure 2). Blackening in the carapace of the shrimp in the control sample was more intensive than in shrimp immersed in 0.025 wv% E-4 solution and 1.25 wv% SMS solution. During the storage, no statistical difference in the % relative change values were obtained from the E-4 and the SMS samples ($p>0.05$). The considerable differences between the control sample, SMS, and E-4 sample were suggested that the E-4 is able to prevent melanosis development in shrimp during cold processing.

Lipid peroxidation inhibition assay

The results of the lipid peroxidation inhibition assay showed in Figure 3. In general, the E-4 sample exhibited the lowest value of TBARS, followed by the SMS and the control sample, respectively. During the first day of preservation, there was no significant difference of TBARs values ($p>0.05$) for all studied samples. The TBARs values of the SMS and E-4 samples were found to increase from the first day to the fifth day and decrease significantly from the fifth to the seventh day of storage at 2 °C. While the TBARs value of the control sample reached peak value on the fourth day and reduced in the next three days. The maximum value of TBARs was obtained after five days of the sample treated with E-4, SMS, and water after four days were 4.82, 4.52, 5.08 mgMDA/kg of shrimp, respectively. The increase in the TBARs values was accounted for the powerful oxidation of fat in the first stage to form hydroperoxide and oxidize hydroperoxide quickly into secondary products like aldehyde. In the second stage of fat oxidation, the secondary oxidized products continue to be converted to all other products using the effect of enzymes and microorganisms, leading to decreased TBARs value (Encarnacion et al. 2011). Shrimp samples treated by the E-4 have the lowest TBARs value among all samples. These results showed that the E-4 was able to slow the process oxidation of fat in shrimp during cold processing at 2°C.

Microbiological analysis

Results showed that the total aerobic microorganisms of the E-4 sample ($1.1x10^6$ Cfu/g) was approximately five times lower than the control sample ($2.2x10^7$ Cfu/g (Table III). It was indicated that the shrimp treated with the E-4 was able to inhibit the growth of aerobic microorganisms better than that of the control sample. According to TCVN 5289: 2006 requirements of frozen seafood, total aerobic bacteria should not exceed 10^6 Cfu/g. Thus, it can be seen that samples treated by the SMS and the E-4 are suitable, while the control sample is not acceptable. Besides, results showed that the number of *P. aeruginosa* and *Enterobacteriaceae* in the E-4 sample was lower than in the SMS sample. *P. aeruginosa* and *Enterobacteriaceae* are two pathogenic microorganisms in cryopreservation products. From this, it can be concluded that *P. Odorata* leaves can inhibit harmful organisms, aerobic bacteria such as *P. aeruginosa* and *Enterobacteriaceae* better than additive samples.

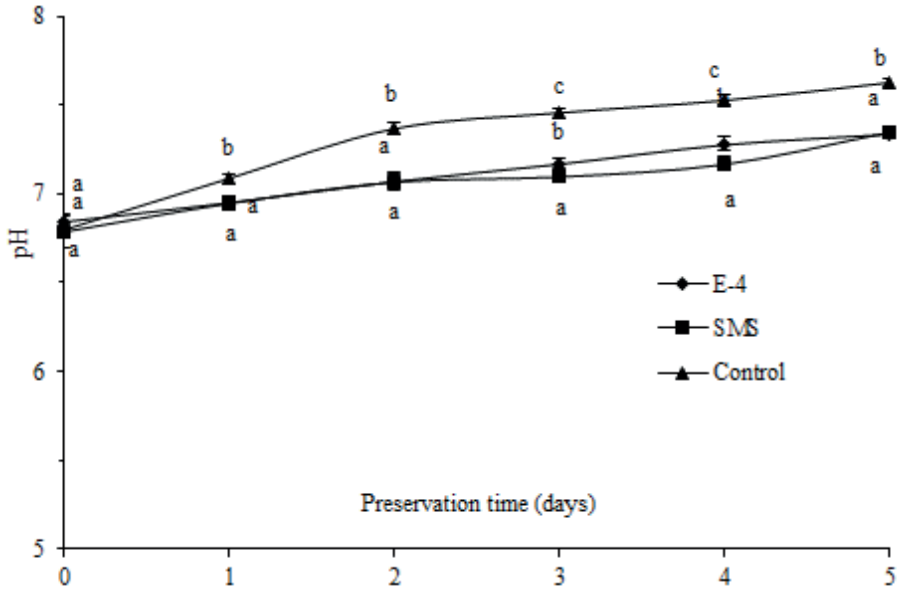

Figure 1. The changes of pH in shrimp during preserving at 2°C in 5 days. Results presented as the mean ± SD (n=3). Superscript letters above each data point represent statistically significant differences (p<0.05).

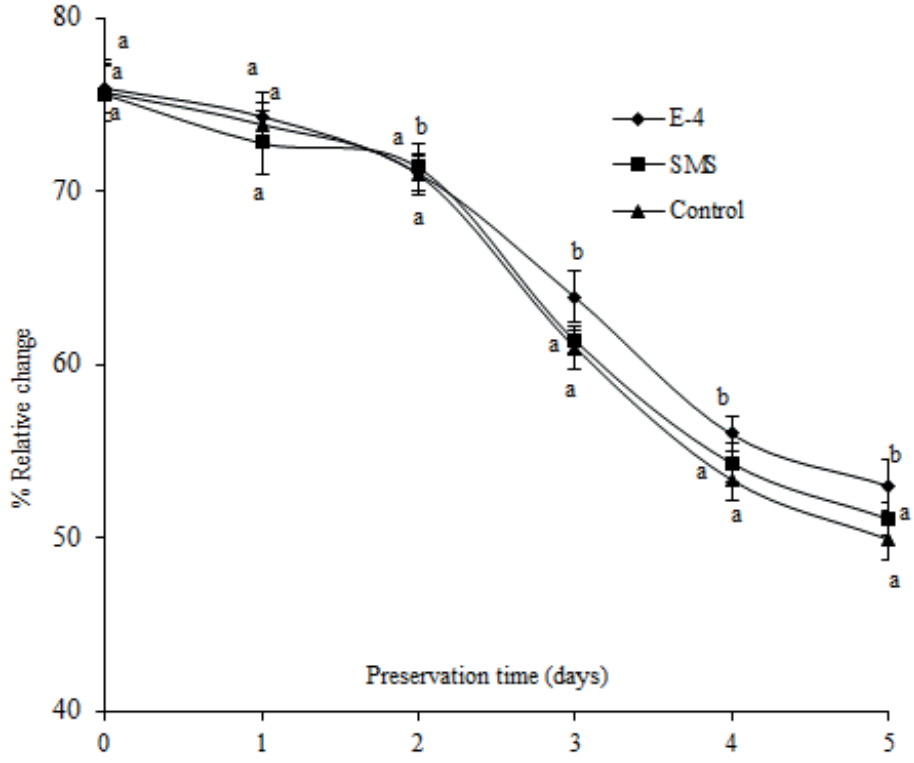

Figure 2. The changes of % relative change values in shrimp during preserving at 2°C in 7 days, analyzed using ImageJ software. Results are presented as the mean ± SD (n=3). Superscript letters above each data point represent statistically significant differences (p<0.05).

CONCLUSION

We have carried out a systematic investigation of spicy vegetables in Vietnam for DPPH inhibitory activity, tyrosinase inhibition activity. The results of screening on bioactivities of 40 extracts suggested that the ethanol extract prepared from *P. Odorata* leaves (E-4) was rich in phenolic content (155.9 ± 0.8 mgGAE/g) and exhibited the highest potential of the DPPH assay (IC_{50} of 7.54 $\mu g.mL^{-1}$) and the tyrosinase inhibition

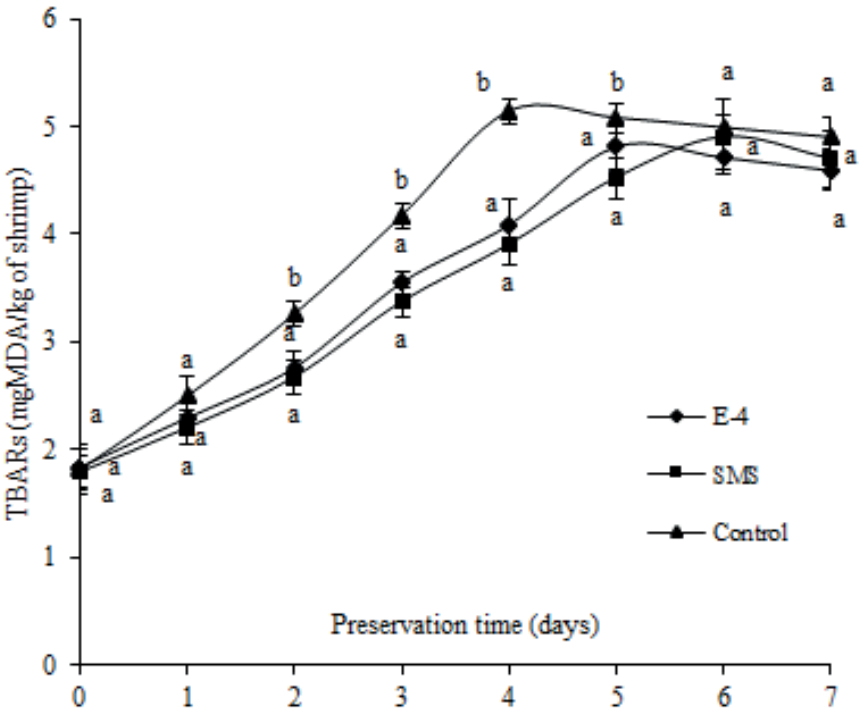

Figure 3. The changes of TBARs values in shrimp during preserving at 2°C in 7 days. Results presented as the mean ± SD (n=3). Superscript letters above each data point represent statistically significant differences (p<0.05).

Table III. Microbiological analysis of shrimp samples after five days.

Criteria (Cfu/g)	Sample		
	Control	SMS	E-4
Total aerobic microorganisms	$2.2x10^7$	$4.7x10^5$	$1.1x10^6$
Pseudomonas aeruginosa	<10	$7.8x10^3$	<10
Enterobacteriaceae	30	$1.4x10^3$	20

percentage (54.2 ± 2.9%) at the concentration of 100 $\mu g.mL^{-1}$. In addition, this extract showed the capacity for preserving white leg shrimp (*P. vannamei*) by reducing melanosis development, pH value, lipid peroxidation, and scavenging harmful microorganisms. Besides, based on screening results, the extracts from *H. Cordata* and *A. Vulgaris* showed energetic activities and promises for further studies. This study shows a new capacity of using plant extract as a substitute for synthetic chemical reagents as preservatives in shrimp storage.

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