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Melanosis and quality changes of Pacific white shrimps (*Litopenaeus vannamei*) treated with *Houttuynia cordata* extract during cold storage

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Abstract:

Changes in melanosis, microbiology, and fat oxidation in Pacific white shrimps (*Litopenaeus vannamei*) when treated with *Houttuynia cordata* extract (designated E-DC) were monitored during cold storage for seven days at 2^0C. Whole shrimps treated with E-DC solution (0.025%, w/v) revealed slow growth of total aerobic microorganisms, *Enterobacteriaceae* and *Pseudomonas aeruginosa,* throughout cold storage in comparison to the control sample treated by water ($p<0.05$). The changes in pH, grey value, and TBARS value (thiobarbituric acid reactive substances) of shrimp samples treated with 0.025% E-DC solution were lower than those of control samples ($p<0.05$) and not different significantly in comparison to the 1.25% (w/v) sodium metabisulfite (SMS) samples ($p>0.05$). These results suggested the potential of using natural compounds from vegetable extraction as a safe and effective alternative for commercial chemical-derived preservatives in shrimp storage.

Keywords: **antimicrobial, *Houttuynia cordata, Litopenaus vannamei,* melanosis, oxidation.**

Classification number: **3.1**

Introduction

Pacific white shrimps (*Litopenaeus vannamei*) are an important commercial product and account for 60% of the total shrimp exports in Vietnam [1]. However, shrimps are a highly perishable product because their spoilage begins soon after death and continues in the storage process. The discolouration, melanosis development, oxidation, and microbial spoilage in shrimps are serious problems affecting the organoleptic, nutritional, and economic value of the product [2]. Many studies have focused on preventing melanosis or inhibiting polyphenol oxidase (PPO) using various techniques such as cold or heat treatment or additives [3]. Reducing agents such as sulfiting agents and their derivatives are synthesis chemicals that are widely used for the control of melanosis or browning in the food industry. Melanosis is activated by a biochemical mechanism that oxidizes phenol part in tyrosine to quinones by tyrosinase enzyme [4]. In commerce, sodium metabisulfite (SMS) has been used to retard melanosis development by combining irreversibly with quinones and forming colourless compounds [3]. However, SMS is known to cause allergic reactions and severe disturbances in asthmatic subjects [5]. In recent times, increasing attention has been paid to plant phenolics as potential natural preservatives with antioxidant and antimicrobial activities. Natural antioxidant compounds such as ascorbic acid, citric acid, kojic acid, gallic acid, dodecyl gallate, catechin, and oxalic acid have been substituted for sulfiting agents [2, 3]. Additionally, many studies have reported that extracts prepared from mushrooms and grapeseed can reduce black spots in shrimps [6, 7].

Houttuynia cordata is a well-known, traditionally medicinal material as well as a commonly used spice in

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food. 'Diếp cá', 'lá giấp', 'rau giấp', and 'giấp cá' are local names of *H. cordata* in Vietnam. In traditional medicine, *H. cordata* is administered to relieve fever, resolve toxins, reduce swelling, and promote urination [8]. Recently, several studies also provided scientific data about its anti-SARS, anti-inflammatory, anti-allergic, virucidal, antileukemic, anti-oxidant, anti-cancer, and anti-tyrosinase activities [9, 10]. It was noticed that *H. cordata* contains groups of such chemical components as flavones, essential oils, and alkaloids [10-12]. This study aims to investigate the inhibition of melanosis and quality changes of Pacific white shrimps treated with E-DC solution during cold storage to exploit the natural extracts from the spicy vegetables that are rich in phenolic compounds as an initial treatment step in the cold-storage process for shrimps.

Materials and methods

Chemical and raw materials

Malonaldehyde bis (dimethyl acetal) and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich (USA). Trichloroacetic acid (TCA) was obtained from Merck (Germany). Other chemicals were of the highest grade available.

H. cordata was collected at supermarkets in July 2018. The voucher samples were preserved at the Department of Food Technology of the Ho Chi Minh city University of Technology and Education.

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

Preparation of samples

The dried powder of *H. cordata* leaves (50 g) was extracted with ethanol solvent to yield an ethanol extract via the Soxhlet system. Then, the extract solutions were evaporated under reduced pressure to dry and recover the ethanol extract (E-DC, 15 g).

Preservation of shrimps

At room temperature (32±2^{0}C), the shrimp (*L. vannamei)* samples were immersed in various solutions, including 0.025% E-DC solution, 1.25% SMS solution, and water (control sample) for five minutes. Then, shrimp samples were taken out and preserved in a plastic box at 2^{0}C. To speed up melanosis development, lipid peroxidation, and bacterial formation, the shrimps were stored at 2^{0}C after five to seven days. Three shrimps from each treatment were examined in seven days to determine pH, melanosis development, lipid peroxidation, inhibition assays, and microbiological analysis.

pH measurement

The changes in pH values in shrimps during cold storage were determined by the method of Lopez-Caballero with a slight modification [13]. Shrimp meat (2 g) was homogenized by deionized water for one minute, and the homogenate was kept at room temperature for 5 minutes. The pH values in shrimps were measured using a pH-meter (PSI pH 1200, England).

Melanosis evaluation

Fifteen student candidates from the Departments of Chemical Engineering and Food Technology (ages 19-22) were recruited to serve as panellists and were trained carefully in the ability to recognize and describe the common aroma. Control samples and shrimp samples (treated by SMS, E-DC) were evaluated throughout storage and classified according to the level of black-spot development. Grey values in shrimps were detected directly using a modified Montero sensory evaluation and evaluated on the point scale from 0 to 10 based on total surface area of affected shrimps as follows: 0 points = no point; 2 points = light (about 20% of the shrimp's surface affected); 4 points = average (accounting for 20-40% of the shrimp's surface affected); 6 points = significant (40 to 60% of the shrimp's surface affected); 8 points = highly severe (60 to 80% of the shrimp's surface affected); and 10 points = significantly severe (accounting for 80-100% of the shrimp's surface affected) [3].

Lipid peroxidation inhibition assay

Malonaldehyde bis (dimethyl acetal) (MDA) is considered to be the final product of the lipid peroxidation process. MDA forms a pink complex with TBA with the stoichiometry of 1:2, which can be detected at 532 nm using a spectrophotometer (Hitachi UH-530, Japan) [14]. Shrimps were ground by a blender and mixed with 10 ml of 7.5% TCA to produce a mixture. The filtrate was collected and mixed with 0.02 M TBA solution at 100^{0}C for 15 minutes. Absorbance was measured at 532 nm by the spectrophotometer. The lipid peroxidation inhibition ability of the samples was expressed by the TBARS values (thiobarbituric acid reactive substances), which were calculated from a standard curve built at concentrations from 0.01 to 0.05 µM and reported as mg MAD/kg of

shrimp. TBARS values were computed from the mean values of data from three determinations.

Microbiological analysis

Total aerobic microorganisms, *Enterobacteriaceae* and *Pseudomonas aeruginosa,* in shrimp samples after five days preserved at 4^0C were determined based on bacterial count method following TCVN 5518-2:2007 (ISO 21528-2:2004) and 3347/2001/QD-BYT at Pasteur Institute in Ho Chi Minh city.

Statistical analysis

Results were calculated to the analysis of variance (ANOVA) whereas the Tukey's test ($p<0.05$) was applied so that the means could be compared. Statistical calculations were carried out by SPSS 15.0 for Window Evaluation Version (IBM Corporation, USA).

Results and discussion

pH measurement

Changes in pH of white shrimps with and without different treatments during cold storage are illustrated in Fig. 1. The pH values of all shrimp samples increased significantly as the preservation time was extended. On the first day, the pH values of all samples were not significantly different ($p>0.05$). After seven days of storage, the samples treated with water had the highest pH, followed by those treated with E-DC, and the SMS sample, respectively ($p<0.05$). At the end of storage (on the seventh day), shrimp samples that were treated with water, 1.25% SMS, and 0.025% E-DC had pH values of 7.96±0.10, 7.49±0.10, and 7.43±0.20, respectively. However, the pH values of the E-DC and SMS samples exhibited no statistical difference ($p>0.05$). Several reasons could explain the increase in pH value in shrimps during cold storage. It could be due to self-decomposition by endogenous enzymes or it could also be due to the activity of microorganisms that associate with the accumulation of basic compounds leading to a higher pH. Additionally, the hydrolysis of protein produces NH_3, which is also the cause of growing pH [13, 15]. The lower increase in pH of shrimps treated with E-DC followed the lower microbial count [3, 13]. The pH value is a critical target for shrimp evaluation because it significantly affects the quality of raw shrimps. According to Shamshad (1990), the accepted pH values in shrimps should not exceed 7.6 [16]. Thus, the E-DC sample can be applied to preserve shrimps during cold storage.

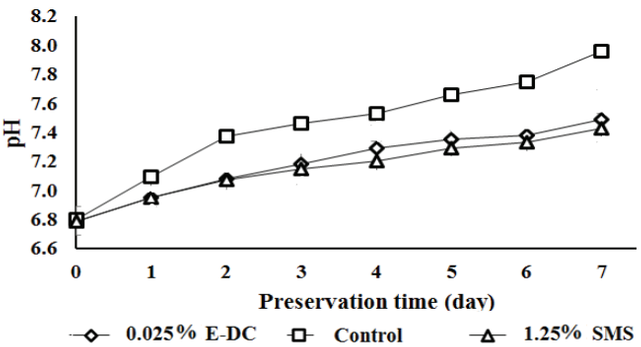

Fig. 1. The changes in pH in shrimps during cold storage at 2^0C for seven days. Results are presented as the mean of three determinations (n=3).

Melanosis evaluation

The development of melanosis in shrimp samples is exhibited in Figs. 2 and 3. On day 0, there was no change in all samples (grey value = 0). When storage time increased, the melanosis scores in all samples increased ($p<0.05$). Shrimp samples treated with SMS and E-DC had grey values lower than the control sample in cold storage and did not display significant difference ($p>0.05$). In general, grey values occurred significantly after three days of preservation. The considerable differences between the control sample and the E-DC sample suggested that *H. cordata* extract (E-DC) can prevent melanosis development in shrimps during cold processing. In commerce, SMS has been used in shrimps to prevent melanosis development by combining irreversibly with quinones and forming colourless compounds [3]. However, SMS is known to cause allergic reactions and severe disturbances in asthmatic subjects. The ability of *H. cordata* extract to reduce melanosis development can relate to the high content of total polyphenol compounds in this plant. The hydroxyl group can participate in reducing DOPA-chrome to DOPA (L-3,4-dihydroxyphenylalanine), by providing electrons or by cross-linking with PPO via hydrogen bonding [13]. These results suggest the potential of using natural compounds from vegetable extraction as a safe and effective alternative for commercial chemical-derived preservatives in shrimp storage.

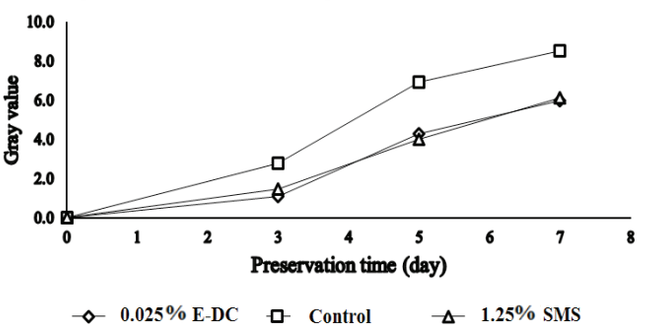

Fig. 2. Changes in the mean grey value in shrimps during cold storage. Results were presented in terms of mean (n=15).

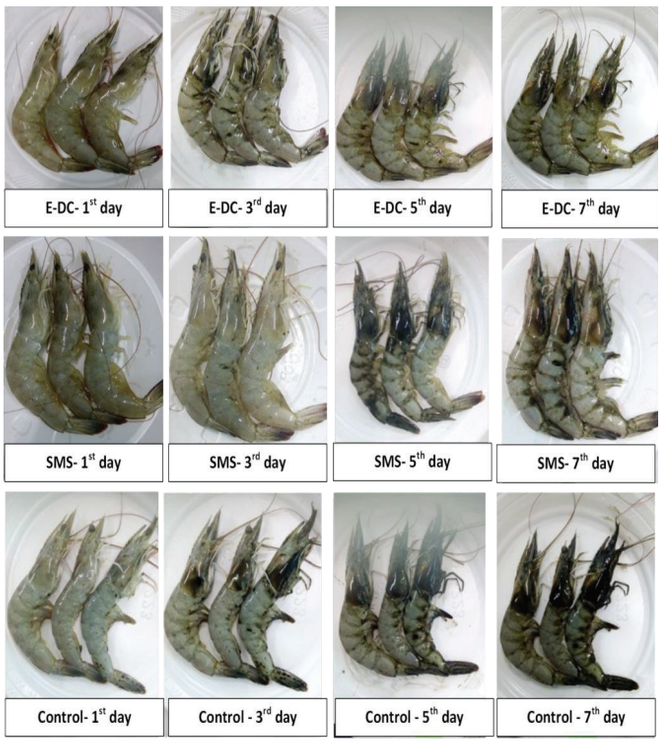

Fig. 3. Development of melanosis in shrimps during cold storage.

Lipid peroxidation inhibition assay

The results of the lipid peroxidation inhibition assay are illustrated in Fig. 4. In general, TBARS values of the SMS sample were the lowest, followed by the E-DC, and the control sample, respectively. During the first two days of preservation, TBARS values did not differ significantly ($p>0.05$). The TBARS values of SMS and E-DC samples were found to be higher from the first day to the fifth day and decreased significantly from the fifth to seventh day of storage at 2^0C. Additionally, the TBARS value of the control sample reached a peak value on the fourth day and declined over the next three days. The maximum of the TBARS values after the fifth day for the E-DC sample, the SMS sample, and the control sample were 3.62±0.23, 3.56±0.24, and 4.82±0.35 mg MDA/kg of shrimps, respectively. The rising TBARs values (0-5 days) in the first stage were caused by a powerful process of fat oxidation, the product of the fat oxidation such as hydroperoxide that oxidized quickly into secondary products such as aldehyde. Under the effect of enzymes and microorganisms, the secondary oxidation products continue to be converted to all other products, leading to reduced TBARS value [6]. These results revealed that E-DC is able to slow the process oxidation of fat in shrimps during cold processing.

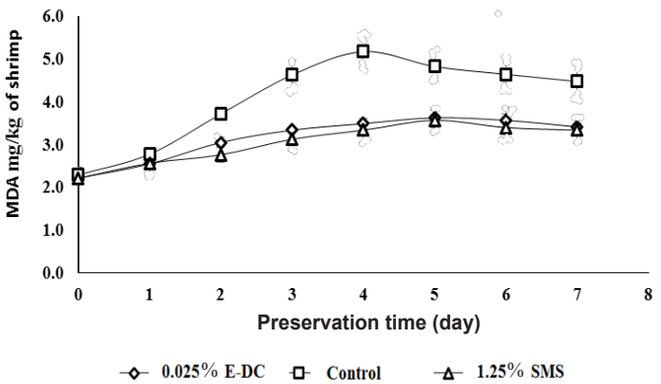

Fig. 4. The TBARS value changes of shrimps during preservation at 2^0C for seven days. Results are presented as the mean of three determinations (n=3).

Microbiological analysis

Table 1. The result of microbiological analysis of shrimp samples after five days.

Criteria (Cfu/g)	Control		E-DC	
	1st day	*5th day*	*1st day*	*5th day*
Total aerobic microorganisms	$5.7x10^5$	$1.1x10^6$	$5.7x10^5$	$5.8x10^5$
Pseudomonas aeruginosa	720	<10	720	<10
Enterobacteriaceae	6000	30	6000	10

<10: not detected.

Results demonstrated that the total aerobic macrobiotic of the control sample ($1.1x10^6$ Cfu/g) was approximately two times higher than the E-DC sample ($5.8x10^5$ Cfu/g) after five days (Table 1). Therefore, the shrimps treated with E-DC were able to inhibit the growth of aerobic microorganisms better than those in 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 with E-DC are suitable while the control sample is not acceptable. Additionally, results revealed that the numbers of *P. aeruginosa* and *Enterobacteriaceae* in the E-DC sample were lower than in the SMS sample and declined significantly after five days in the cold preservation. *P. aeruginosa* and *Enterobacteriaceae* are two common pathogenic microorganisms in cryopreservation products [17]. From this, it can be concluded that E-DC can inhibit harmful microorganisms and aerobic bacteria such as *P. aeruginosa* and *Enterobacteriaceae* more effectively than seen in the control samples. Several previous studies have demonstrated that essential oil extracted from *H. cordata* contain antimicrobial compounds such as methyl nonyl ketone, bornyl acetate, myrcene, pinene, acetic acid geraniol ester, camphene, sabinene, decanal, dodecanal, 3-oxo-dodecanal, caryophyllene, limonene, 4-terpineol, terpineol, geraniol, tetradecanoyl-phorbol-acetate, and tetradecanoyl ester [12]. Among them, ketones of methyl

nonyl can inhibit the growth of *Escherichia coli* - an aerobic bacterium often occurs in shrimps. Decanal-β-dicarbonyl compound has also been reported to be a major antimicrobial ingredient in essential oil from *H. cordata* [18].

Conclusions

This study is the first time that a plant was examined for its ability to preserve white shrimps. The ethanolic extract that was prepared from *H. cordata* leaves retarded melanosis development, pH value, and lipid peroxidation, and inhibited harmful microorganisms. Specifically, shrimps treated with E-DC solution (0.025%, w/v) inhibited the growth of the total aerobic microorganisms to two times after five days when compared to the control sample ($p<0.05$). The development of two pathogenic microorganisms such as *Enterobacteriaceae* and *Pseudomonas aeruginosa* was controlled effectively throughout cold storage at 2^0C. The changes in pH, grey value, and TBARS value of shrimp samples treated with 0.025% E-DC solution were lower than those of control samples ($p<0.05$) and did not differ significantly in comparison to the 1.25% (w/v) SMS samples ($p>0.05$). The results demonstrated that ethanol extract prepared from *H. cordata* leaves can be used as a natural melanosis inhibitor, an antimicrobial, and an antioxidant agent for cold-stored shrimps. Additionally, the extract from *H. cordata* leaves was applied for the first time as a safe and 'green' additive for shrimp preservation.

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The authors declare that there is no conflict of interest regarding the publication of this article.

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