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Potential Utilization of Green Tea Leaves and Fenugreek Seeds Extracts as Natural Preservatives for Pacific White Shrimp During Refrigerated Storage

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Abstract

Ethanolic extracts of green tea leaves (GTE) and fenugreek seeds (FSE) were screened for their antibacterial activity against four food pathogenic strains using disc diffusion method. The two extracts revealed antimicrobial activity against selected bacterial strains. GTE showed the highest antibacterial activity to *Escherichia coli* and *Staphylococcus aureus* at a concentration of 1% with inhibition zone equal to 29.45 ± 0.64 mm and 25.68 ± 1.2 mm, respectively. In addition, the effect of GTE and FSE combined with chitosan coating on the shelf life of Pacific white shrimp (PWS) (*Litopenaeus vannamei*) during refrigerated storage have been studied. Our results indicated that using GTE or FSE during the refrigerated storage of PWS led to significantly decreased Total Volatile Bases Nitrogen, Thiobarbituric acid reacting substances, total bacterial count, and pH. The sensory properties of PWS have improved considerably in the samples treated GTE or FSE. These findings suggested that the application of chitosan coating combined with GTE or FSE to PWS is advisable to achieve better quality during refrigerated storage.

Keywords: Pacific white shrimp, shelf-life, green tea leaf extract, fenugreek seeds extract, antibacterial activity, food pathogenic bacteria

Introduction

Shrimp provide a very impressive array of nutrients (such as protein with high biological value and essential amino acids, vitamin D, vitamin B12, selenium, and iodine), therefore, it could be considered as one of the important marine sources (Sánchez-Ortega *et al.*, 2014; Yuan *et al.*, 2016). As fresh seafood, shrimp is highly perishable and has a short shelf-life, which requires special care to prevent the biochemical and microbial deterioration that occurs during storage and distribution (Fratianni *et al.*, 2010). There are two ways to improve shelf-life of shrimp, first is minimizing the microbial contamination of product and the second is delaying or inhibiting the growth of spoilage microorganisms (Rong *et al.*, 2010). The consumption of shrimp could also cause illness by *Vibrio parahaemolyticus*, *V. cholerae*, *Clostridium botulinum, Staphylococcus aureus, Salmonella enterica, and Escherichia coli* that contaminated the fresh shrimp (Sánchez-Ortega *et al.*, 2014). Despite the proven efficiency of the synthetic chemicals in inhibiting the bacterial growth and increase the shelf-life of aquatic products, the use of chemical preservatives is, however, limited due to their toxicity and growing consumer demand for health-promoting seafood (Bialonska *et al.*, 2010; Yuan *et al.*, 2016). Because of such concern, several studies have been focused on searching natural antimicrobial agents that can serve as biopreservatives. The utilization of some plant extracts as healthy, safer, and natural food preservatives has been intensively examined (Alzoreky and Nakahara, 2003; Perumalla and Hettiarachchy, 2011; Mostafa *et al.*, 2018). Chandra *et al.*, 2011 investigated the antibacterial properties of methanol and acetone extracts of fenugreek seeds against four Gram-negative bacteria, and their results stated that the methanolic extracts had a broad range of antibacterial activities toward various species. Furthermore, acetonic extract of fenugreek seeds was effective against *E. coli* (Goyal *et al.*, 2016). Green tea extracts (GTEs) showed high polyphenolic content, which has antimicrobial activity against a wide range of Gram-negative and Gram-positive bacteria (Xu *et al.*, 2007; Hong *et al.*, 2009; Carrizo *et al.*, 2014). Seafood consumption keeps on increasing, and it is necessary to find efficient methods to ensure low microbial load of raw aquatic products during storage and processing.

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Therefore, development of natural preservatives with antibacterial activities is desirable. The present study aims to evaluate the antibacterial activity of GTE and fenugreek seeds extract (FSE) against some of the common foodborne pathogens. The effect of GTE and FSE combined with chitosan coating on quality of Pacific white shrimp (PWS) (*Litopenaeus vannamei*) during refrigerator storage by determining physicochemical, microbiological, and sensory parameters has been studied.

Materials and Methods

Preparation of plant extracts

Dried green tea leaves (*Camellia sinensis*) (GT) and fenugreek seeds (*Trigonella foenum-graecum*) (FS) were purchased from local market in Zhoushan, China. GT and FS were washed, disinfected and dried in shade. The dried materials were ground into fine powder. The prepared powder was soaked in 99% ethanol (plant material to solvent ratio was 1:10, w/v) and extracted for 24 h in a water bath at 40°C with shaking at 150 rpm. Whatman (No.1) filter paper was used to filter the samples. The filtrates were dried using a rotary evaporator to remove the ethanol and then freeze-dried at −54°C for 26 h by a vacuum freeze-dryer (MCFD5505; SIM International Group Co. Ltd) to obtain a crude extract powder (Mostafa *et al.*, 2018). The crude extracts were kept in airtight bottles, in the dark at 4°C until further use.

Determination of antibacterial activity of plant extracts against pathogenic bacteria

Bacterial cultures of *E. coli GIM1.708* was provided by Microbial Culture Collection Center of Guangdong (GIMCC) (Guangdong, China), while *Bacillus cereus 10451*, *S. aureus10786*, and *Salmonella enteritidis10982* were obtained from China Center for Industrial Culture Collection (CICC) (Beijing, China). These strains were used to test the antimicrobial activities of plant crude extracts.

The disc-diffusion method was used to screen the antimicrobial activities by measuring the inhibition zone as described by Hatab *et al.*, 2017. A 24 h microbial culture grown in Mueller-Hinton broth (MHB) at 37°C was adjusted to 10^6–10^8 CFU/mL by a 0.5 McFarland Standard (Remel™; Thermo Fisher Scientific, Waltham, MA). An inoculum suspension of each bacterial strain was swabbed into each sterile Petri dish filled with 15 mL of sterilized Mueller-Hinton agar (MHA), and the inoculum was allowed to dry for 5 min at room temperature. Different aqueous solutions of GTE and FSE were provided by dilution in sterile distilled water at concentrations of 0.2%, 0.5%, and 1.0%. Sterile paper discs (5 mm in diameter) were placed on each inoculated agar plate, 25 μL of each plant extracts (GTE and FSE) were transferred to each filter paper. After 24 h of incubation at 37°C, the diameter of the inhibition zones was measured in two dimensions, using a hand-held electronic digital Vernier Caliper with a precision of 0.1 mm. The inhibition zone included the area of the filter paper.

PWS collection and preparation

Live PWS (60–65 shrimp/kg) were purchased from the local fish market in (Zhoushan, Zhejiang province, China) and transferred to the laboratory in polystyrene boxes within one h. Shrimp samples were separated into three groups—uncoated group (C), coated with GTE, and coated with FSE. The samples were coated by dipping into (0.2%, 0.5%, or 1% [w/v]) GTE or FSE solutions for 10 min then drained well (Fan *et al.*, 2013). Afterward, shrimp samples were individually coated by immersing in the chitosan solution with a concentration of 1.5% for 30 min; then, the samples were removed and allowed to drain at 4°C to form the edible coatings (Yuan *et al.*, 2016). The degree of deacetylation of chitosan was 90%. Air-proofed retort pouch was used to pack the samples, which were stored at 4°C ± 1°C for subsequent quality assessment. Chemical, microbiological, and sensorial analyses were performed each 2-day, each group repeated three times.

Chemical and proximate composition analysis

For moisture content, the samples were analyzed by AOAC method (Williams, 1984). Total volatile bases nitrogen (TVB-N) was measured as described by (Yuan *et al.*, 2016). The thiobarbituric acid reacting substances (TBARS) were determined according to (Erkan and Özden, 2008). The pH meter was used to determine pH values as described by Rong *et al.*, 2010.

Microbiological analysis

Twenty-five grams of shrimp muscle from each group (GTE and FSE at a concentration of 0.2%, 0.5%, and 1%) were dissected aseptically, mixed with 225 mL of 0.1% peptone water, and homogenized in a stomacher. The homogenized sample was serially diluted using 9 mL sterile saline and the total aerobic microbial count (TPC) was investigated using surface inoculation in plate count agar (PCA, Oxoid). After incubation at 37°C for 24 h, the colonies were counted and reported as log CFU/g (Chinese National Standard 2010).

Sensory evaluation

Sensory analysis was performed by a panel formed by five experienced judges, according to Chinese National Standard (GB2741-94) (Chinese National Standard 1994). The panelists were asked to evaluate appearance, odor, texture, flavor, and overall acceptability of the raw shrimp samples. Three specimens were analyzed from each batch at each sampling time. Four categories were ranked: E = 10 (extra), A = 8 (good), B = from 7 to 5 (acceptable), and C = less than 5 (unacceptable).

Statistical analysis

All results are the averages of triplicate trials, and the values are represented as the mean value ± standard deviation (SD). All data were subjected to one-way analysis of variance ($p < 0.05$) using SPSS software to explore the statistical significance of the differences among batches. The mean values were separated by using the least significant difference test in all cases.

Results and Discussion

The antibacterial activity of plant extracts in vitro

The antibacterial activity of GTE and FSE were assayed against foodborne pathogens bacteria, including two strains of Gram-positive bacteria (*S. aureus*, *B. cereus*) and two Gram-negative bacteria (*E. coli*, *S. enteritidis*). As given in Figure 1,

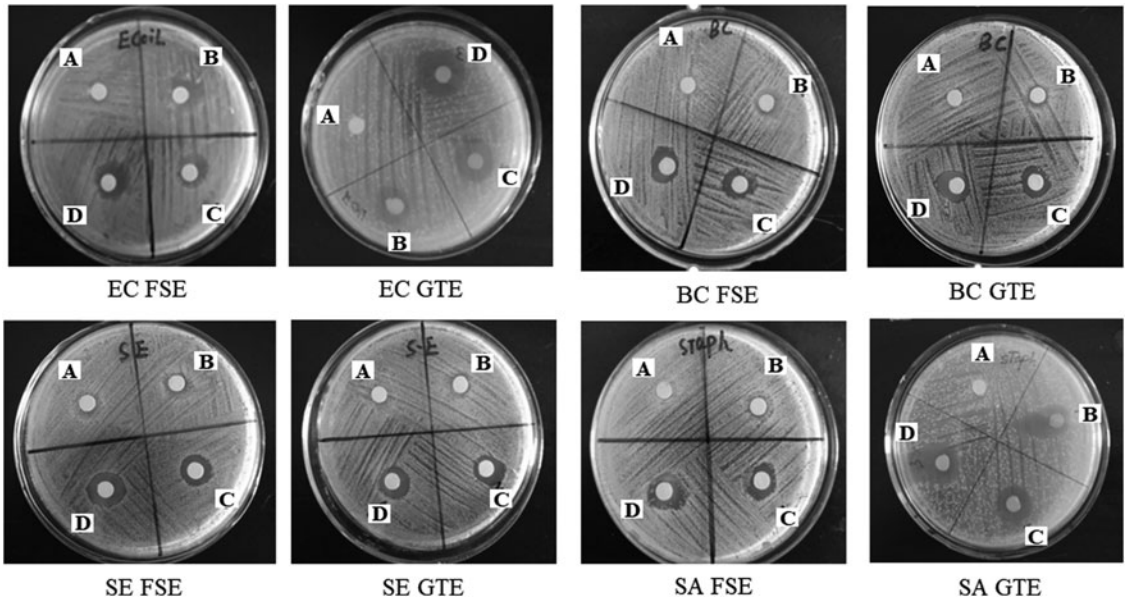

FIG. 1. The inhibition zone (mm) of ethanol extracts of GTE and FSE with *Escherichia coli*, *Salmonella enteritidis*, *Bacillus cereus*, and *Staphylococcus aureus*, at different concentration 0.2% (B), 0.5% (C), and 1% (D). A refers to the control sample using water. FSE, fenugreek seed extract; GTE, green tea extract.

the two extracts showed good activity against all tested bacterial strains, these strains have shown resistant to many classes of antimicrobial previously. The presence of catechins polyphenols in green tea (such as epicatechin [*E. coli*], epicatechin gallate, and epigallocatechin gallate) could inhibit the growth of various Gram-positive and Gram-negative bacteria by preventing the synthesis of folate of the microorganisms (Taylor *et al.*, 2005; Radji *et al.*, 2013; Reygaert, 2014; Gopal *et al.*, 2016). Antibacterial activities of green tea polyphenols have been studied previously; the results concluded that exposure to green tea polyphenols leads to damage the bacterial cell membrane (Reygaert, 2014; Gopal *et al.*, 2016). In addition, it has been reported that the methanol extracts of fenugreek could efficiently inhibit the growth of several pathogenic bacteria, including *Pseudomonas spp*., *E. coli*, *Shigella dysenteriae*, and *Salmonella typhi* (Dash *et al.*, 2011; Malik *et al.*, 2013). It was interesting to observe that increasing GTE or FSE concentration resulted in increasing the inhibition of bacterial growth. The same observation was made with three different GTEs by Gopal *et al.*, 2016.

GTE showed the highest antibacterial activity to *E. coli* and *S. aureus* at a concentration of 1% with inhibition zone equal to 29.45 ± 0.64 mm and 25.68 ± 1.2 mm, respectively (Table 1). It has been reported previously that green tea has direct antimicrobial effect against a wide range of bacteria, including *E. coli*, *Salmonella spp*., *S. aureus*, and *Enterococcus spp*. (Taylor *et al.*, 2005; Radji *et al.*, 2013; Gopal *et al.*, 2016). The extract of FSE was also effective against *S. enteritidis* and *S. aureus* at all concentration, the most significant inhibition zone observed at a level of 1% with a diameter of 22.71 ± 0.73 and 19.16 ± 0.56 mm, respectively (Fig. 1). Our results share some similarities with Dash *et al.*, 2011and Malik *et al.*, 2013 who recorded that the methanol extract of fenugreek (*Trigonella foenum*) could efficiently inhibit the growth of *Pseudomonas spp.*, *E. coli*, *S. dysenteriae*, *Bacillus amyloliquifaciens*, and *S. typhi.*

The effect of plant extracts on the shelf-life of PWS

Chemical and proximate composition analysis. The moisture content of the PWS samples decreased with increasing

Table 1. Antibacterial Activity of Green Tea Leaves and Fenugreek Seeds at Different Concentrations (0.2%, 0.5%, and 1%) Against Four Pathogenic Bacteria Tested by Disc Diffusion Assay

	Zone of inhibition (mm)					
	FSE			*GTE*		
Strains	*0.2%*	*0.5%*	*1%*	*0.2%*	*0.5%*	*1%*
Bacillus cereus	10.97 ± 1.51	16.8 ± 1.88	17.58 ± 0.85	11.46 ± 1.62	19.76 ± 1.05	20.03 ± 1.45
Staphylococcus aureus	8.57 ± 0.56	17.6 ± 1.63	19.16 ± 0.56	19.08 ± 1.5	21.95 ± 0.88	25.68 ± 1.2
Salmonella enteritidis	12.63 ± 0.53	19.29 ± 0.64	22.71 ± 0.73	11.83 ± 0.76	20.27 ± 0.23	22.01 ± 1.24
Escherichia coli	14.45 ± 0.43	16.55 ± 1.02	18.44 ± 0.96	17.47 ± 0.84	24.76 ± 0.45	29.45 ± 0.64

The strains used are *Staphylococcus aureus* 10786, *Bacillus cereus* 10451, *Salmonella enteritidis* 10982, and *Escherichia coli* GIM1.708. Determinations were performed in triplicate and data correspond to mean values ± standard division.
FSE, fenugreek seed extract; GTE, green tea extract.

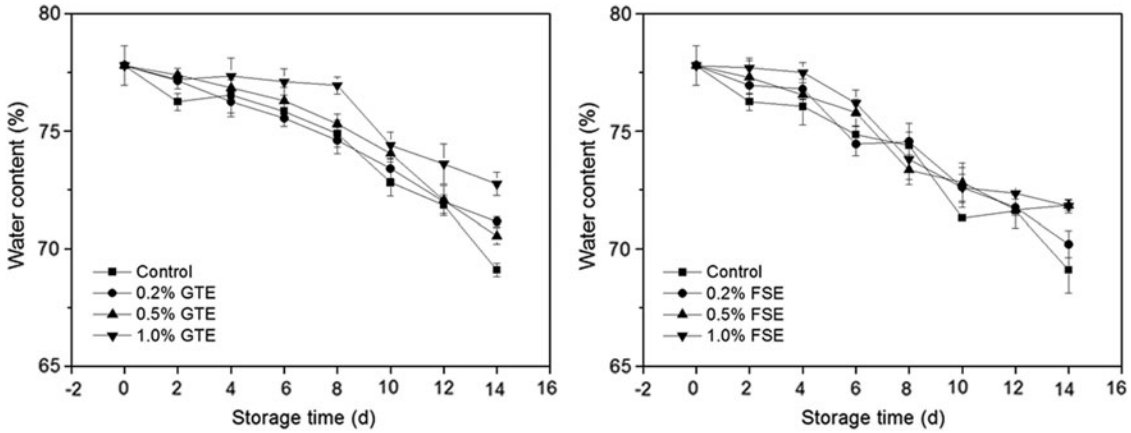

FIG. 2. The effect of GTE and FSE on the moisture content of PWS during storage in the refrigerator. Each point of data represents means ± standard deviations ($n = 3$). PWS, Pacific white shrimp.

storage period in all samples, including control (Fig. 2). The moisture content of PWS treated with GTE was higher than those of FSE or control. The samples were able to maintain more initial moisture contents by increasing the concentration of plant extracts in the case of GTE and FSE. The moisture content of shrimp treated with 1% GTE and FSE increased gradually during the storage period to reach 72.75% ± 0.49% and 71.80% ± 0.28%, respectively, at the end of storage period. At the same time, the moisture content for the control samples was less than 69.10% ± 0.28%. It has been indicated that the weight loss could be retarded in peeled litchi fruit after coating with chitosan (Dong *et al.*, 2004). Similarly, Biswas *et al.* (2004) found that moisture content of coated pork patties was higher than uncoated samples. Interestingly, Wu *et al.* (2000) mentioned that after 3 days of refrigeration storage, no significant differences in moisture loss had been recorded in the chitosan, gluten, or soy protein wrapped patties and unpackaged.

The TVB-N content is a chemical indicator of the seafood quality, the sample is considered very good in the quality when the value of TVB-N is less than 25 mg/100, while the sample is considered spoilt if TVB-N is 35 mg/100 g or more (Duyar *et al.*, 2013). Fresh PWS had a TVB-N content of 10.12 mg/100 g, which increased gradually to 29.43, 31.90, and 43.43 mg/100 g at the end of the storage period (14 days) in GTE (1.0%), FSE (1.0%), and a control sample, respectively (Fig. 3). The changes in TVB-N content follow the general pattern reported for other species of fresh shrimp (Huidobro *et al.*, 2002; Özyurt *et al.*, 2009). TVB-N is not accurate enough as a quality indicator, however, it has a close relationship with sensory evaluation and bacterial counts (Amegovu *et al.*, 2012). Microbial and chemical changes of seafood result in producing high values of TVB-N (Jinadasa, 2014; Yuan *et al.*, 2016). Our experiments are in line with the previous results of Mu *et al.*, 2012 and Sun *et al.*, 2014, who found that the values of TVB-N in PWS treated with grape seed extracts and cinnamaldehyde decreased significantly.

The TBARS value can be used as an indicator of seafood quality under frozen, chilled, or ice storage (Mahmoudzadeh *et al.*, 2010). TBARS are widely used for measuring the level of lipid oxidation in seafood muscles (Tokur *et al.*, 2006). Seafood samples are considered perfect in the quality when the TBARS is less than 3 mg malonaldehyde/kg, while the

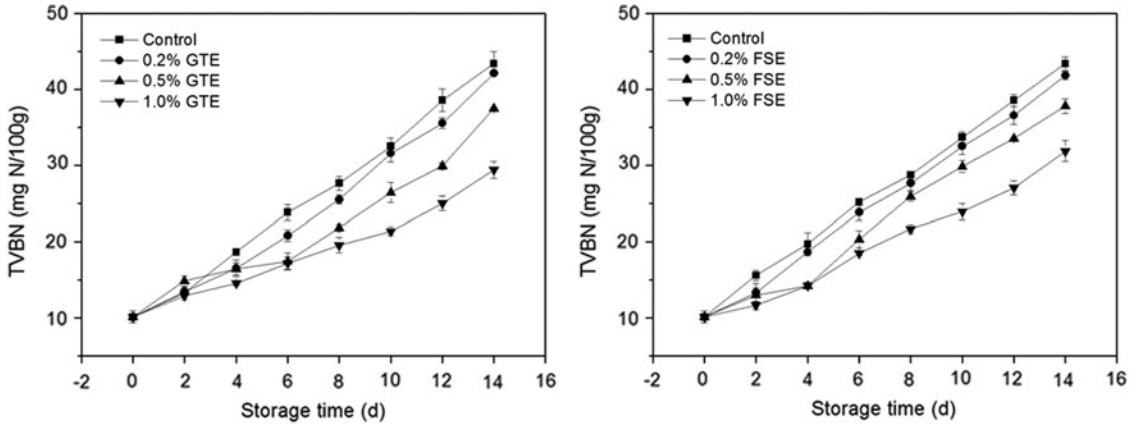

FIG. 3. The effect of GTE and FSE on the TVB-N content of PWS during storage in the refrigerator. Each point of data represents means ± standard deviations ($n = 3$). The samples consider good in the quality when the value of TVB-N is 25 mg/100 g or less and spoilt when TVB-N reach more than 35 mg/100 g (Duyar *et al.*, 2013). TVB-N, total volatile basic nitrogen.

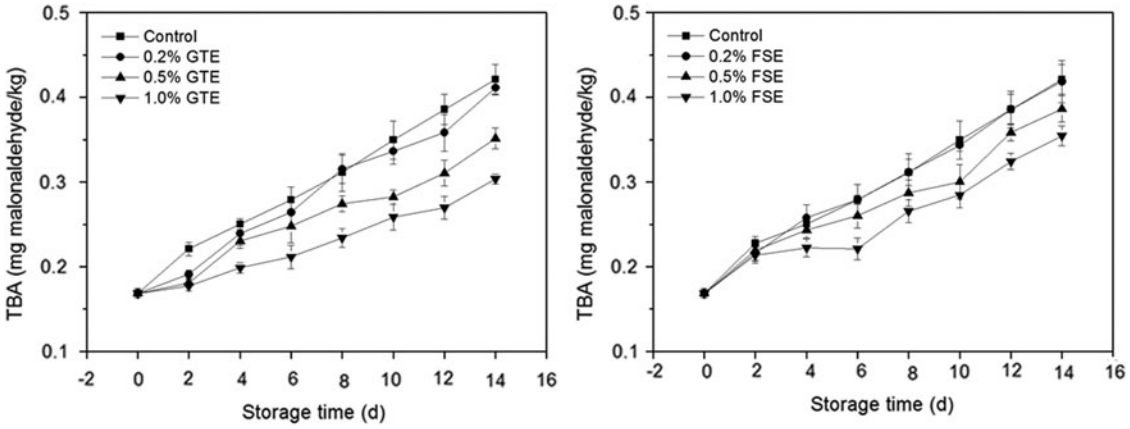

FIG. 4. The effect of GTE and FSE on the TBARS of PWS during storage in the refrigerator. Each point of data represents means ± standard deviations ($n = 3$). When the TBARS is less than 3 mg malonaldehyde/kg the samples are considered to be in good quality and the consumption limits range from 7 to 8 mg malonaldehyde/kg (Duyar *et al.*, 2013). TBARS, thiobarbituric acid reacting substances.

consumption limits range from 7 to 8 mg malonaldehyde/kg (Duyar *et al.*, 2013). In general, the TBARS values of PWS in control, GTE, and FSE increased significantly ($p < 0.05$) during the storage period. However, the TBARS value at the control rapidly increased from 0.17 (at zero time) to 0.42 mg malonaldehyde/kg after 14 days of refrigerated storage (Fig. 4). The lowest concentration of TBARS (0.30 mg malonaldehyde/kg) was observed with 1% GTE, followed by 1% FSE (0.35 mg malonaldehyde/kg) and 0.5% GTE (0.35 mg malonaldehyde/kg).

As highlighted in Figure 5, all fresh samples had an initial pH value of 6.9 at zero time (before storage). After 2 days of refrigerator storage, the pH decreased slightly to 6.6 in all treatments except for samples treated with 0.5% FSE; the pH dropped to 6.7, which is similar to that of Mu *et al.*, 2012; Sun *et al.*, 2014; and Yuan *et al.*, 2016. The reduction in pH value is probably due to lower microbial count or because of the fermentation that is involved in the spoilage of shellfish (Rong *et al.*, 2010; Mu *et al.*, 2012). After that, pH values increased differently among various treatments, where the highest pH was observed in control samples (8.1) at the end of refrigerator storage. Shrimp samples coated with GTE at a concentration of 1% could inhibit the rise of pH value effectively to be 7.60 after 14 days of storage, followed by FSE at a level of 1% and 0.5% (7.80), and then 0.5% GTE (7.82). Several researchers mentioned that the accumulation of basic compounds caused by the activity of bacteria or enzyme, definitely causes increasing of the pH values of shrimp samples (Mu *et al.*, 2012; Sun *et al.*, 2014; Yuan *et al.*, 2016).

Microbiological analysis. Seafood is less stable because it has high moisture content and availability of nutrients for the growth of microorganisms, which lead to emerge the spoilage indicators (Jinadasa, 2014). Therefore, it was essential to investigate the ability of coating materials (GTE and FSE) to reduce the total microbial count during the storage period. The number of TPC present in all treatments, including control, increased continuously ($p < 0.05$) during the storage up to 14 days (Fig. 6). The lowest increase in TPC was observed in shrimp samples coated with GTE at a concentration of 1% (5. 30 log CFU/g) at the end of storage. In addition, TPC in the samples treated by 0.2% or 0.5% GTE was significantly less than that of control samples ($p < 0.05$), while

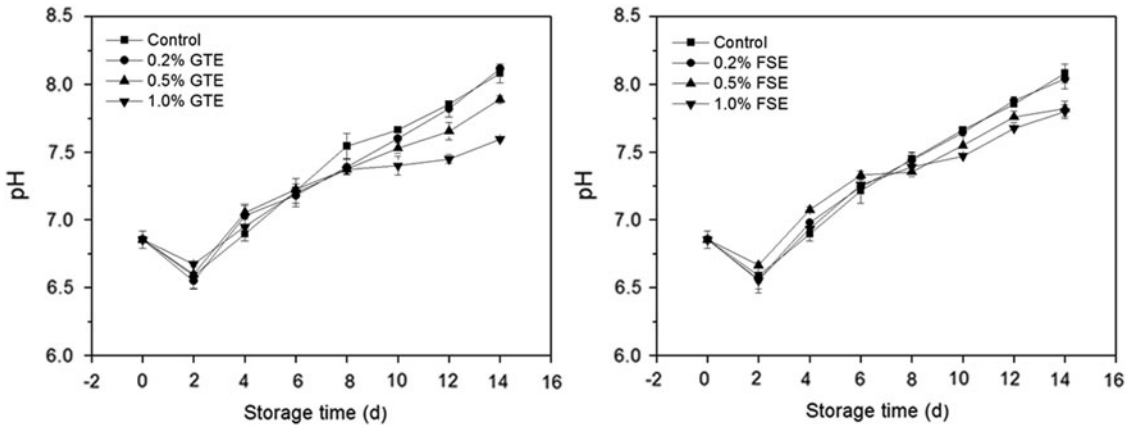

FIG. 5. The effect of GTE and FSE on pH of PWS at different concentration (0.2%, 0.5%, 1.0%), during storage in the refrigerator. Each point of data represents means ± standard deviations ($n = 3$).

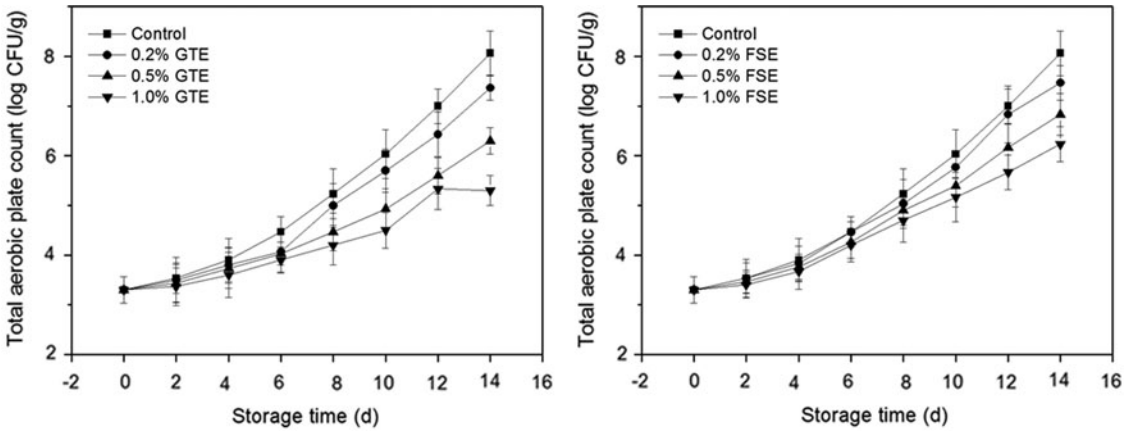

FIG. 6. The effect of GTE and FSE on TPC of PWS at different concentration (0.2%, 0.5%, 1.0%), during storage in the refrigerator. Each point of data represents means ± standard deviations ($n = 3$). TPC, total aerobic microbial count.

the TPC in the shrimp samples coated with 0.2%, 0.5%, and 1.0% FSE were 7.47, 6.83, and 6.23 log CFU/g, respectively. These results are in line with our findings of the antimicrobial activity of plant extracts, where the 1.0% GTE showed the highest antibacterial activity against food pathogenic bacteria. These observations also underlined that the antimicrobial activity of plant extracts is highly dependent on the concentration used (Mu *et al.*, 2012). Several studies have proved that the plant extracts could retard the spoilage caused by microorganisms in shrimp samples. Thus, the application of plant extracts would be considered as a promising alternative to conventional preservatives, to extend the shelf-life of shrimp efficiently and safely (Mu *et al.*, 2012; Sun *et al.*, 2014; Yuan *et al.*, 2016).

Sensory evaluation. Figure 7 highlights the sensory properties of PWS treated with GTE and FSE at different concentration (0.2%, 0.5%, 1.0%) under refrigerator storage. As a general trend, the sensory scores decreased during the storage period to reach the lowest values in control samples at the end of storage (14 days). The sensory properties of control sample declined rapidly to be unacceptable (less than 4) at day 14 of storage. While the shrimp samples coated with 1.0% GTE remained in a proper case (more than 7) until the end of storage, the control sample showed the fastest deterioration rate, followed by 0.5% GTE (more than 6). On the contrary, the samples treated with FSE (1%) reached a level of more than 5.5 after 12 days of storage period according to the Chinese National Standard (GB2741-94) (Chinese 1994). In our experiment, the sensory values were consistent with the results of TVB-N, TBARS, and TPC analysis. Treatment with 1% GTE could extend the shelf-life of PWS to more than 14 days and to 12 days for samples treated with 1% FSE, compared with control (10 days).

In conclusion, GTE and FSE have potent antimicrobial activity against food pathogenic bacteria. Our results demonstrated that the shrimp samples coated with GTE and FSE had longer shelf-life and better quality when compared to uncoated samples (control). On the basis of essential quality indicators, 1% GTE could efficiently retard the spoilage of shrimp sample for up to 14 days. In addition, the values of pH, TVB-N, TBARS, and TPC were significantly reduced in the shrimp samples coated with FSE. The effect of FSE as a natural preservative to extend the shelf-life of shrimp has not been published in the literature previously. Therefore, the application of GTE and FSE as a promising

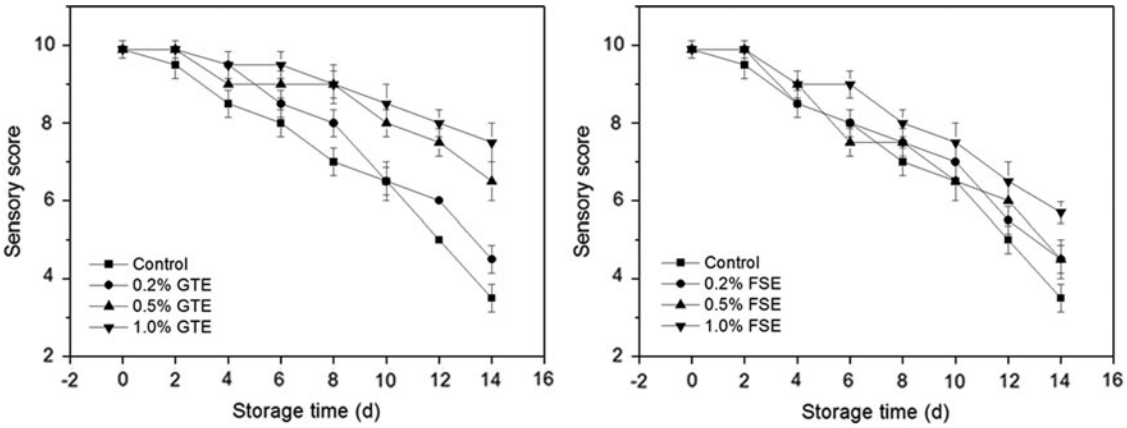

FIG. 7. The effect of GTE and FSE on the sensory properties of PWS at different concentration (0.2%, 0.5%, 1.0%), during refrigerator storage. Each point of data represents means ± standard deviations ($n = 3$)

emerging preservative can ensure microbiological safety and quality of PWS during the refrigerated storage.

Acknowledgment

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Disclosure Statement

We confirm that this article content has no conflicts of interest.

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