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Sensory Acceptability, Shelf Life, and Quality of Crustaceans Treated with *Moringa oleifera* and Green Tea Leaf Extracts versus Acetic Acid

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ABSTRACT

This study was carried out to investigate the effect of leaf extracts from *Moringa oleifera* and green tea, as compared to acetic acid, on the sensory acceptability, shelf-life, and microbiological quality of some crustaceans, i.e. imported unpeeled shrimps (IUS), local peeled shrimps (LPS), and local breed crabs (LBC). Samples of IUS, LPS, and LBC were immersed in sterile distilled water (control) or treated separately by *Moringa oleifera* leaf extracts (MOE 2% and 5% w/v), green tea leaf extract (GTE 0.1% w/v), or acetic acid (AA 2%) and then refrigerated at 2.0±1.0°C. Three replicates from control and each treatment of crustacean samples were assessed through sensory, physicochemical, and microbiological examination, periodically throughout the storage period until spoilage. The obtained results revealed no effect on the sensory acceptability of treated samples by GTE or AA, with three to six days extension in the shelf-life as compared to control. On the contrary, MOE altered the color, odor and hence reduced the acceptability of treated samples with no improvement in shelf-life. Samples treated with GTE and AA showed significantly ($p<0.05$) lower total bacterial count, coliforms MPN, fecal coliforms MPN, and *Staphylococcus aureus* count than control and MOE-treated samples. Yet, MOE showed good antioxidant activity. To conclude, treatment with GTE and AA could be a promising method to enhance the quality and extend the shelf life of crustaceans. MOE is a good antioxidant nonetheless it must be combined with a potent antimicrobial to extend the shelf-life of crustaceans.

Introduction

Seafood is considered an important food item around the world due to its high nutritive values and health-promoting properties because of its high levels of polyunsaturated fatty acids named omega-3 fatty acids. More than 33% of global food production is damaged annually because of microbial spoilage (Lund *et al*., 2000).

Seafood usually contains high contents of water, free amino acids, polyunsaturated fatty acids, and nutrients, in addition to their highly active autolytic enzymes and relatively high pH values, which render seafood a highly susceptible food category to spoilage (Cakli *et al*., 2007; Khalafalla *et al*., 2015). Seafood spoilage may result from changes brought about by biological reactions such as lipid oxidation, enzymatic autolysis, and microbial activities (Arashisar *et al*., 2004).

Seafood could be exposed to different sources of microbial contamination either from harvesting water and/or during various stages of handling and processing. Indeed, high rates of microbial contamination endanger the safety and quality of seafood and shorten the shelf-life. Different approaches have been developed to ensure seafood quality and safety, however, most of them are highly costing, ineffective or causing health problems (Nawaz *et al*., 2020). Refrigeration is one of the most common preservation methods for fish and shellfish since refrigeration reduces the microbial growth rate but does not completely stop it. Therefore, an extra preservative effect could be added by using food additives or natural preservatives (bio-preservatives) (Sterniša *et al*., 2020).

Nowadays, herbal plants and their extracts are widely exploited these days to reduce the growth of microorganisms in foods, extend their shelf-life and add some extra-functional properties to the food (Khalafalla *et al*., 2015; Nawaz *et al*., 2020). In addition, medicinal herbs physiologically contained active principles that have been exploited in traditional medicine for the treatment of various illnesses as they contain antioxidants, anti-inflammatory, and antimicrobial compounds (Kelmanson *et al*., 2000). Some reports have proven that the phenolic compounds present in spices and herbs play a main role in their antimicrobial activities (Hara-Kudo *et al*., 2004). Moreover, herbs prolong the storage life of foods by prohibiting rancidity through their antioxidant activity and reducing microbial activity through their bacteriostatic or bactericidal effects (Beuchat and Golden, 1989; Emir Coban and Ozpolat, 2013).

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Moringa oleifera, the most widely cultivated species in the genus Moringa, is a commonly used medicinal herb, particularly in Asia and Africa. The leaves of *Moringa oleifera* were reported to contain many biologically active substances, such as flavonoids, saponins, polyphenols, alkaloids, tannins, glucosinolates, oxalates, isothiocyanates, and phytate (Leone *et al*., 2015). Extracts of the seeds, leaves, and fruits of *Moringa oleifera* have been found to possess potential anti-inflammatory, antioxidant, antifungal, antimicrobial, and anticancer activity (Atsukwei *et al*., 2014; Al-Asmari *et al*., 2015; Oladeji *et al*., 2020).

Green tea, whose antioxidant and antibacterial activities are well-known, is one of the most recognized herbal plant extracts. The antioxidant activity of tea polyphenols was investigated and was found to delay the chemical and oxidization changes in seafood resulting in the extension of the shelf-life. Green tea leaves were reported to contain 36% of polyphenols of their dry weight. The dominant polyphenols in green tea have antioxidant and antibacterial properties (Ojagh *et al*., 2005).

On other hand, organic acids (OA), such as acetic acid, may provide the foundation of antimicrobial formulations that can improve the microbial quality and safety of seafood. For instance, vinegar has been used as a preservative because it reduces the thermal death time of microorganisms and either stops or kills bacteria depending on the applied concentration (Bal'a and Marshall, 1998).

The objective of this study is to evaluate the efficiency of leaf extracts from *Moringa oleifera* and green tea, as compared to acetic acid, on sensory acceptability and microbiological quality of some crustaceans including imported unpeeled shrimps (IUS), local peeled shrimps (LPS) and local breed crabs (LBC) during refrigerated storage at 2.0±1.0°C.

Materials and methods

Preparation of herbal extracts and acetic acid solution

Dry leaves of *Moringa oleifera* and green tea (*Camellia sinensis*) were extracted following the reported method of Zhang *et al*. (2018). A hundred grams of dry leaf powder from each herb was added to 1000 mL of aqueous ethanol (70%) with shaking the mixture thoroughly. The leaves were soaked in the solvent for 24 h, and then percolated several times until the soaking solution became faint to clear in color. Afterward, the obtained extract was filtered through a number 3 filter paper (Whatman) and concentrated under reduced pressure in a rotary evaporator (BUG) to remove the solvent and keep the extracted precipitate.

Eventually, the extract of Moringa leaves was resuspended in sterile distilled water (DW) to prepare *Moringa oleifera* extract [(MOE 2 and 5% (w/v)]. As well as the extract of green tea leaves was resuspended in sterile DW to get the green tea extract [GTE 0.1% (w/v)]. All extract solutions were sterilized by filtration through a sterile bacteriological filter membrane with a pore size of 45 µm (Whatman) and stored at 4°C until use. Additionally, glacial acetic acid was used for the preparation of acetic acid solution [AA 2 % (v/v)] in a sterile DW and then sterilized by filtration as mentioned above.

The concentrations of different treatment agents were previously determined based on a preliminary in-vitro disc diffusion test against three foodborne pathogens of *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*.

Sample collection

Three types of crustaceans were used for this study. A total of 90 samples each of imported unpeeled shrimps (IUS), local peeled shrimps (LPS), and local breed crabs (LBC) were obtained from fish markets in Beni-Suef governorate, Egypt. Then the samples were wrapped in sterile polyethylene bags and directly transferred to the laboratory in a sterile icebox with a minimum of delay for further preparation.

Treatment of crustaceans with antimicrobials

Crustacean samples from each type (IUS, LPS, and LBC) were divided into five groups as follows. The first group was untreated as it was immersed in sterile DW for 15 min (Control). The Second group was immersed in MOE 2% for 15 min. Whereas the third group was in MOE 5% for 15 min. While the fourth was immersed in GTE 0.1% for 15 min and the fifth group was dipped in AA 2% for 1 min. Each control and treated sample were aerobically packaged, labeled and stored at 2.0±1.0°C inside the refrigerator.

Examination of control and treated samples

The control and treated samples were collected for examination at zero day (after 2 h of treatment), then periodically every three days (days 3, 6, 9, 12) until apparent signs of spoilage. Each examination was done as triplicates.

Sensory evaluation

The sensory assessment was performed using the scoring scale (1-5) developed by Neuman *et al*. (1983). Five orientated panelists were used to evaluate the sensory attributes of crustacean samples. The samples were blind-coded by certain codes; the panelists were not informed about the experimental approach. They were asked to give a score for each of color, odor and consistency, while the samples are raw. Then the samples without any salt or spices were separately fried using high-quality sunflower oil, cooled to 50°C then served to the panelists to complete the evaluation of the sensory attributes. The panelists were asked to wash their mouths with warm water between every two samples. Each sensory attribute was evaluated on a scoring scale from 1 to 5, where 1 means poor and 5 means excellent. The overall acceptability was calculated for each sample as the sum of scores given to color, odor, consistency, and taste. As 20 was the maximum overall acceptability score, whereas below 7.2 was considered unacceptable.

Physicochemical examination

The pH of crustacean flesh was measured in a 10 g of flesh homogenized with 10 mL of DW using a pH meter (350 Jenway pH meter, UK) according to the method recommended by Korkeala *et al*. (1986).

The thiobarbituric acid reactive substances (TBA-RS) were determined in crustacean flesh as malondialdehydes (mg MDA/Kg flesh) according to the method of Taraldgis *et al*. (1960) with additional modification by Pikul *et al*. (1983).

Bacteriological examination

The bacteriological examination of control and treated crustacean flesh samples were carried out according to the methods recommended by the Association of Official Analytic Chemists (AOAC, 1990). Ten grams of control or treated flesh samples were homogenized (MPW 302, Universal Laboratory Aid, made in Poland) in 0.1% sterile peptone water (MAST, UK), then ten-fold serial dilutions were prepared until 10^{-6}.

One hundred microliters from each dilution were spread on a sterile standard agar plate and incubated for 35°C/24 h for mesophilic counts (CFU/g flesh) and 7°C/10 days for psy-

chrophilic counts (CFU/g flesh).

The most probable numbers (MPN/g flesh) of coliforms and fecal coliforms were determined using the three-tube MPN method, where *E. coli* MPN was estimated on sterile eosin methylene blue agar. As well as the enumeration of *Staphylococcus aureus* counts (CFU/g flesh) were estimated on sterile Baird Parker agar plates supplemented with egg yolk tellurite emulsion and incubated at 35°C for 24h. Suspected colonies were confirmed through the catalase and coagulase biochemical tests.

Statisitical analysis

The statistical analysis was done using minitab19. One-Way Analysis of Variance (ANOVA) was done to all data. Tukey's Test ($P<0.05$) was carried out as the post-hoc test for mean separations. Each experiment was replicated at least three times.

Results

Sensory acceptability and shelf life

Herein, we investigated the effect of treatment with MOE 2 and 5%, GTE 0.1% and AA 2% on the sensory acceptability of shrimps and crap samples (IUS, LPS, and LBC) as compared to control (untreated) samples at periodical time occasions during refrigerated storage at 2.0±1.0°C (Fig. 1). The obtained results showed that there were no significant differences in the sensory attributes and in turn the overall acceptability scores between control samples and samples treated with GTE or AA at the first-time occasion (day-0), but control samples began to lose their freshness attributes starting from day-3 and crossed the acceptability limit (7.2) to become unacceptable on day-9 in the case of IUS and on day-6 at both LPS and LBC samples. While both GTE and AA led to an extension of the shelf life of IUS to day-12, to day-9 and day-12 in LPS, respectively, and to day-9 in LBC (Fig. 1). On the other hand, moringa extracts (MOE 2 and 5%) did not induce much improvement in the sensory attributes, conversely, they reduced the color and taste scores and accordingly the overall acceptability, as well as they did not improve the shelf life of treated samples as compared to control ones (Fig. 1).

Physicochemical findings

In the current study, we assessed the effect of treatment with MOE 2%, MOE 5%, GTE 0.1%, and AA 2% on the pH of IUS, LPS, and LBC samples during refrigerated storage at 2.0±1.0°C (Fig. 2A-C). There were no significant differences ($p<0.05$) among the pH values of control samples and samples treated with MOE 2% and MOE 5%, which means that MOE does not have an obvious effect on the pH of examined samples. On the contrary, all samples treated with AA 2% showed significantly lower pH values than those treated with other treatments or control. While GTE had a moderate impact on pH values. There were gradual elevations in pH values in all samples over the period of storage. Regarding the maximum acceptable pH limit (7) in fish and shellfish, recommended by the Egyptian organization for standardization (EOS, 2005), we noticed that control and samples treated with MOE 2% or MOE 5% passed such limit on day-3, while samples treated GTE 0.1% and AA 2% passed such limit almost on day-6, Nevertheless, these samples were still acceptable according to sensory scores provided by panelists.

We also determined the levels of TBA-RS in IUS, LPS and LBC samples treated with MOE 2%, MOE 5%, GTE 0.1% and AA 2% or control (untreated) during refrigerated storage at 2.0±1.0°C (Fig. 2D-F). We found that samples treated with MOE 2%, MOE 5% or GTE 0.1% had significantly ($P<0.05$) lower TBA-RS values than control and acetic acid-treated samples.

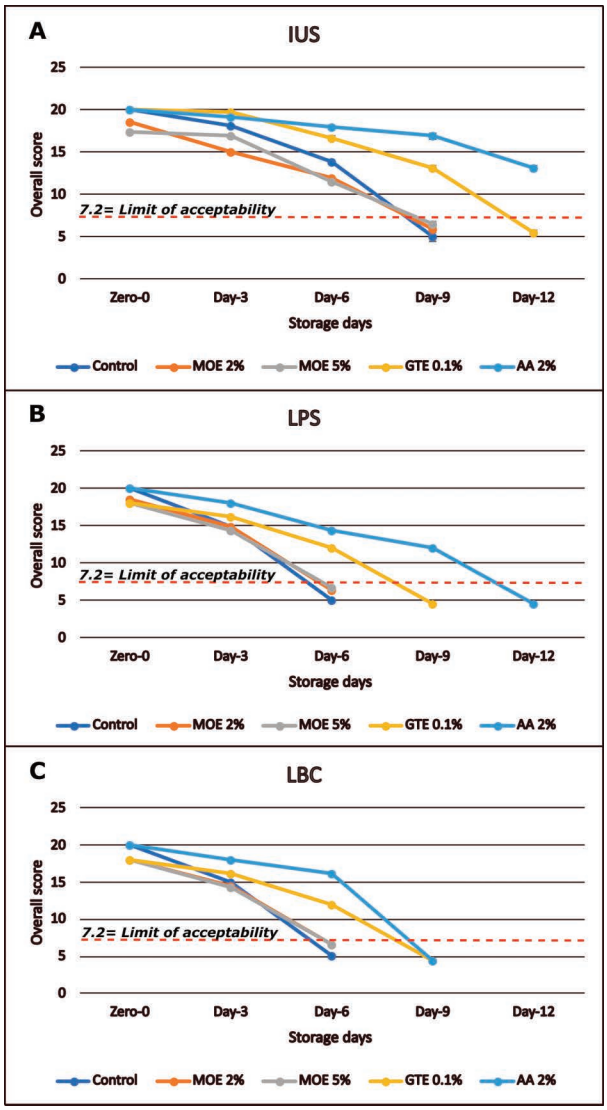

Fig. 1. Effect of treatment with *Moringa oleifera* extract 2 and 5 % (MOE 2% and MOE 5%), green tea extract (GTE 0.1%) and acetic acid solution (AA 2%) on the overall sensory acceptability of A) imported unpeeled shrimp (IUS), B) local peeled shrimp (LPS) and C) local breed crap (LBC) samples during refrigerated storage at 2.0±1.0°C. Data are represented by means of 3 replicates ±Standard errors. End of each line detects the day of spoilage and discarding. Red dashed line indicates the limit of acceptability (7.2) according to Neuman *et al.* (1983), as samples below this limit is unacceptable.

Bacteriological findings

The counts of mesophilic and psychrophilic bacteria, and the MPN of coliforms, fecal coliforms, and *E. coli*, as well as *Staphylococcus aureus* count were determined in control and MOE 2%, MOE 5%, GTE 0.1%, and AA 2%-treated samples of IUS, LPS, and LBC (Tables 1–5). On most time occasions and in most examined samples, the estimated bacterial counts were significantly ($P<0.05$) higher in control and moringa extract-treated samples than samples treated with green tea and acetic acid. There were noticeable gradual rises in bacterial counts throughout the storage period. Interestingly, we failed to detect fecal coliforms in IUS samples on all examination occasions (Table 4). Moreover, we could not detect *E. coli* in all examined control and treated samples on any examination occasion (data are not shown).

.Table 1. Effect of treatment with *Moringa oleifera* extract 2 and 5 % (MOE 2% and MOE 5%), green tea extract (GTE 0.1%) and acetic acid solution (AA 2%) on the mesophilic counts (CFU/g) of imported unpeeled shrimp (IUS), local peeled shrimp (LPS) and local breed crap (LBC) samples during refrigerated storage at 2.0±1.0 °C.

Treatment	Day-0			Day-3			Day-6			Day-9		Day-12
groups	IUS	LPS	LBC	IUS	LPS	LBC	IUS	LPS	LBC	IUS	LPS	IUS
Control	$6\times10^{4}\pm2\times10^{3a}$	$6\times10^{5}\pm1\times10^{5a}$	$5\times10^{5}\pm2\times10^{4a}$	$5\times1\pm1\times10^{5b}$	$5\times10^{6}\pm1\times10^{5a}$	$3\times10^{6}\pm7\times10^{4a}$	$7\times10^{6}\pm2\times10^{5a}$	-	-	-	-	-
MOE 2%	$5\times10^{4}\pm7\times10^{3a}$	$5\times10^{5}\pm7\times10^{4a}$	$3\times10^{5}\pm1\times10^{4a}$	$6\times10^{4}\pm9\times10^{3a}$	$6\times10^{6}\pm9\times10^{4a}$	$3\times10^{6}\pm8\times10^{4a}$	$6\times10^{6}\pm1\times10^{5a}$	-	-	-	-	-
MOE 5%	$6\times10^{4}\pm3\times10^{3a}$	$6\times10^{5}\pm3\times10^{4a}$	$3\times10^{5}\pm1\times10^{4a}$	$5\times10^{5}\pm9\times10^{4b}$	$5\times10^{6}\pm9\times10^{5a}$	$5\times10^{6}\pm9\times10^{4a}$	$7\times10^{6}\pm2\times10^{5a}$	-	-	-	-	-
GTE 0.1%	$3\times10^{3}\pm7\times10^{2b}$	$5\times10^{4}\pm1\times10^{3b}$	$5\times10^{4}\pm1\times10^{3b}$	$6\times10^{4}\pm1\times10^{4c}$	$9\times10^{5}\pm4\times10^{4b}$	$9\times10^{5}\pm4\times10^{4b}$	$9\times10^{5}\pm4\times10^{4b}$	$4\times10^{6}\pm9\times10^{4a}$	$3\times10^{6}\pm9\times10^{5a}$	$2\times10^{6}\pm00^{a}$	-	-
AA 2%	$2\times10^{3}\pm1\times10^{3b}$	$3\times10^{4}\pm2\times10^{3b}$	$1\times10^{4}\pm2\times10^{2b}$	$5\times10^{3}\pm1\times10^{3d}$	$7\times10^{4}\pm9\times10^{3c}$	$7\times10^{4}\pm9\times10^{3c}$	$4\times10^{4}\pm1\times10^{3c}$	$4\times10^{5}\pm1\times10^{4b}$	$4\times10^{5}\pm1\times10^{4b}$	$4\times10^{5}\pm1\times10^{4b}$	$5\times10^{6}\pm1\times10^{5}$	$3\times10^{6}\pm6\times10^{4}$

Data are represented by means of 3 replicates ± standard errors.. Empty cells (-) indicate that samples were not examined due to spoilage.. Different small letter superscripts (a, b, c) within a row indicate significant differences between means at $p<0.05$.

Table 2. Effect of treatment with *Moringa oleifera* extract 2 and 5 % (MOE 2% and MOE 5%), green tea extract (GTE 0.1%) and acetic acid solution (AA 2%) on psychrophilic counts (CFU/g) of imported unpeeled shrimp (IUS), local peeled shrimp (LPS) and local breed crap (LBC) samples during refrigerated storage at 2±1.0 °C.

Treatment groups	Day-0			Day-3			Day-6			Day-9		Day-12
	IUS	LPS	LBC	IUS	LPS	LBC	IUS	LPS	LBC	IUS	LPS	IUS
Control	$3\times10\pm8\times10^{3a}$	$6\times10^{4}\pm9\times10^{3a}$	$3\times10^{4}\pm5\times10^{3a}$	$4\times10^{5}\pm1\times10^{5a}$	$6\times10^{5}\pm1\times10^{4a}$	$1\times10^{5}\pm2\times10^{4a}$	$4\times10^{6}\pm1\times10^{5a}$			-		-
MOE 2%	$6\times10^{4}\pm1\times10^{4a}$	$6\times10^{4}\pm1\times10^{4a}$	$5\times10^{4}\pm5\times10^{3a}$	$4\times10^{5}\pm1\times10^{5a}$	$5\times10^{5}\pm1\times10^{5a}$	$8\times10^{5}\pm6\times10^{4a}$	$4\times10^{6}\pm8\times10^{5a}$			-		-
MOE 5%	$6\times10^{4}\pm1\times10^{4a}$	$4\times10^{4}\pm8\times10^{3a}$	$4\times10^{4}\pm1\times10^{3a}$	$5\times10^{5}\pm2\times10^{5a}$	$5\times10^{5}\pm9\times10^{4a}$	$6\times10^{5}\pm1\times10^{5a}$	$7\times10^{6}\pm2\times10^{6a}$			-		-
GTE 0.1%	$2\times10^{3}\pm4\times10^{2b}$	$6\times10^{3}\pm2\times10^{3b}$	$3\times10^{3}\pm6\times10^{2b}$	$6\times10^{3}\pm1\times10^{3b}$	$7\times10^{4}\pm7\times10^{3b}$	$6\times10^{3}\pm9\times10^{2b}$	$6\times10^{4}\pm1\times10^{4b}$	$5\times10^{5}\pm9\times10^{3a}$	$6\times10^{5}\pm9\times10^{4a}$	$4\times10^{5}\pm1\times10^{4a}$		
AA 2%	$2\times10^{3}\pm4\times10^{2b}$	$2\times10^{3}\pm5\times10^{2b}$	$2\times10^{2}\pm4\times10^{c}$	$7\times10^{3}\pm1\times10^{3b}$	$3\times10^{4}\pm7\times10^{3b}$	$2\times10^{3}\pm8\times10^{2b}$	$6\times10^{4}\pm1\times10^{4b}$	$7\times10^{4}\pm6\times10^{3b}$	$3\times10\pm1\times10^{4a}$	$5\times10^{5}\pm2\times10^{4a}$	$5\times10^{5}\pm1\times10^{5}$	$8\times10^{5}\pm7\times10^{4}$

Data are represented by means of 3 replicates ± standard errors. Empty cells (-) indicate that samples were not examined due to spoilage. Different small letter superscripts (a, b, c) within a row indicate significant differences between means at $p<0.05$.

Table 3. Effect of treatment with *Moringa oleifera* extract 2 and 5 % (MOE 2% and MOE 5%), green tea extract (GTE 0.1%) and acetic acid solution (AA 2%) on coliforms MPN (MPN/g) of imported unpeeled shrimp (IUS), local peeled shrimp (LPS) and local breed crap (LBC) samples during refrigerated storage at 2.0± 1.0 °C.

Treatment groups	Day-0			Day-3			Day-6			Day-9		Day-12
	IUS	LPS	LBC	IUS	LPS	LBC	IUS	LPS	LBC	IUS	LPS	IUS
Control	$4\times10\pm7.6^{a}$	$7\times10^{2}\pm3\times10^{2a}$	$5\times10^{2}\pm3\times10^{2a}$	$2\times10^{2}\pm8\times10^{a}$	$2\times10^{3}\pm4\times10^{2a}$	$3\times10^{3}\pm1\times10^{3a}$	$2\times10^{3}\pm2\times10^{2a}$	-		-	-	-
MOE 2%	$1\times10\pm5^{a}$	$5\times10^{2}\pm3\times10^{2a}$	$5\times10^{2}\pm3\times10^{2a}$	$5\times10^{2}\pm2\times10^{2a}$	$2\times10^{3}\pm1\times10^{3a}$	$1.1\times10^{3}\pm00^{a}$	$1\times10^{3}\pm6\times10^{2a}$	-		-	-	-
MOE 5%	$1\times10\pm5^{a}$	$3\times10^{2}\pm9\times10^{a}$	$2\times10^{2}\pm9\times10^{a}$	$2\times10^{2}\pm4\times10^{a}$	$3\times10^{3}\pm1\times10^{3a}$	$1.1\times10^{3}\pm0.0^{a}$	$1\times10^{3}\pm7\times10^{2a}$	-		-	-	-
GTE 0.1%	$<3^{b}$	$2\times10^{2}\pm4\times10^{a}$	$3\times10\pm2\times10^{b}$	$<3^{b}$	$3\times10^{2}\pm8\times10^{b}$	$5\times10^{2}\pm2\times10^{2b}$	$1\times10^{2}\pm5\times10^{b}$	$1\times10^{3}\pm5\times10^{2a}$	$1\times10^{3}\pm2\times10^{2a}$	$7\times10^{2}\pm3\times10^{2a}$	-	-
AA 2%	$<3^{b}$	$2\times10\pm1\times10^{b}$	$1\times10\pm5^{b}$	$<3^{b}$	$5\times10^{2}\pm2\times10^{2b}$	$9\times10^{2}\pm1\times10^{2b}$	$2\times10^{2}\pm4\times10^{b}$	$8\times10^{2}\pm2\times10^{2b}$	$2\times10^{3}\pm1\times10^{3a}$	$6\times10^{2}\pm3\times10^{2a}$	$4\times10^{3}\pm3\times10^{3}$	$1\times10^{3}\pm00$

Data are represented by means of 3 replicates ± standard errors. Empty cells (-) indicate that samples were not examined due to spoilage. Different small letter superscripts (a, b, c) within a row indicate significant differences between means at $p<0.05$.

Table 4. Effect of treatment with *Moringa oleifera* extract 2 and 5 % (MOE 2% and MOE 5%), green tea extract (GTE 0.1%) and acetic acid solution (AA 2%) on fecal coliforms MPN (MPN/g) of imported unpeeled shrimp (IUS), local peeled shrimp (LPS) and local breed crap (LBC) samples during refrigerated storage at 2.0± 1..0 °C

Treatment groups	Day-0			Day-3			Day-6			Day-9		Day-12
	IUS	LPS	LBC	IUS	LPS	LBC	IUS	LPS	LBC	IUS	LPS	IUS
Control	$<3^{a}$	$8\times10^{2}\pm2\times10^{2a}$	$8\times10^{2}\pm2\times10^{2a}$	$<3^{a}$	$2\times10^{3}\pm4\times10^{2a}$	$1\times10^{3}\pm00^{a}$	$<3^{a}$	-		-	-	-
MOE 2%	$<3^{a}$	$9\times10^{2}\pm2\times10^{2a}$	$7\times10^{2}\pm3\times10^{2a}$	$<3^{a}$	$1\times10^{3}\pm00^{a}$	$1\times10^{3}\pm00^{a}$	$<3^{a}$	-		-	-	-
MOE 5%	$<3^{a}$	$1\times10^{2}\pm1\times10^{2b}$	$8\times10^{2}\pm2\times10^{2a}$	$<3^{a}$	$1\times10^{3}\pm00^{a}$	$1\times10^{3}\pm00^{a}$	$<3^{a}$	-		-	-	-
GTE 0.1%	$<3^{a}$	$2\times10^{2}\pm1\times10^{2b}$	$2\times10\pm9^{b}$	$<3^{a}$	$9\times10^{2}\pm9\times10^{a}$	$7\times10^{2}\pm2\times10^{2a}$	$<3^{a}$	$1\times10^{3}\pm00^{a}$	$1\times10^{3}\pm00^{a}$	$<3^{a}$	-	-
AA 2%	$<3^{a}$	$1\times10^{2}\pm2\times10^{b}$	$5\times10\pm2\times10^{b}$	$<3^{a}$	$5\times10^{2}\pm1\times10^{2b}$	$2\times10^{2}\pm3\times10^{b}$	$<3^{a}$	$9\times10^{2}\pm3\times10^{2a}$	$6\times10^{2}\pm2\times10^{2a}$	$<3^{a}$	$1\times10^{3}\pm00$	<3

Data are represented by means of 3 replicates ± standard errors. Empty cells (-) indicate that samples were not examined due to spoilage. Different small letter superscripts (a, b, c) within a row indicate significant differences between means at $p<0.05$.

Table 5. Effect of treatment with *Moringa oleifera* extract 2 and 5 % (MOE 2% and MOE 5%), green tea extract (GTE 0.1%) and acetic acid solution (AA 2%) on *Staphylococcus aureus* counts (CFU/g) of imported unpeeled shrimp (IUS), local peeled shrimp (LPS) and local breed crap (LBC) samples during refrigerated storage at 2± 1.0 °C

Treatment groups	Day-0			Day-3			Day-6			Day-9		Day-12
	IUS	LPS	LBC	IUS	LPS	LBC	IUS	LPS	LBC	IUS	LPS	IUS
Control	$1\times10^{3}\pm3\times10^{2a}$	$3\times10^{3}\pm1\times10^{3a}$	$3\times10^{3}\pm9\times10^{2a}$	$5\times10^{3}\pm1\times10^{3a}$	$2\times10^{5}\pm4\times10^{3a}$	$8\times10^{4}\pm1\times10^{3a}$	$2\times10^{5}\pm3\times10^{2a}$	-	-	-	-	-
MOE 2%	$4\times10^{3}\pm2\times10^{3a}$	$4\times10^{3}\pm2\times10^{3a}$	$4\times10^{3}\pm2\times10^{2a}$	$5\times10^{3}\pm1\times10^{3a}$	$3\times10^{5}\pm8\times10^{4a}$	$8\times10^{4}\pm3\times10^{3a}$	$7\times10^{4}\pm6\times10^{4a}$	-	-	-	-	-
MOE 5%	$5\times10^{3}\pm1\times10^{3a}$	$4\times10^{3}\pm6\times10^{2a}$	$2\times10^{3}\pm6\times10^{2a}$	$7\times10^{3}\pm00^{a}$	$5\times10^{5}\pm9\times10^{4a}$	$6\times10^{4}\pm9\times10^{3a}$	$5\times10^{5}\pm9\times10^{5a}$	-	-	-	-	-
GTE 0.1%	$9\times10^{2}\pm4\times10^{2b}$	$3\times10^{2}\pm8\times10^{b}$	$2\times10^{2}\pm9\times10^{b}$	$3\times10^{3}\pm1\times10^{3a}$	$2\times10^{3}\pm6\times10^{2b}$	$6\times10^{2}\pm9\times10^{b}$	$3\times10^{3}\pm8\times10^{2b}$	$4\times10^{3}\pm7\times10^{2a}$	$4\times10^{3}\pm2\times10^{4a}$	$2\times10^{5}\pm1\times10^{4a}$	-	-
AA 2%	$2\times10^{2}\pm3\times10^{b}$	$3\times10^{2}\pm1\times10^{2b}$	$3\times10^{2}\pm3\times10^{b}$	$8\times10^{2}\pm5\times10^{b}$	$8\times10^{2}\pm5\times10^{b}$	$7\times10^{2}\pm2\times10^{2b}$	$2\times10^{3}\pm6\times10^{2b}$	$2\times10^{3}\pm3\times10^{2a}$	$4\times10^{3}\pm2\times10^{3a}$	$6\times10^{4}\pm1\times10^{3b}$	$3\times10^{4}\pm1\times10^{3}$	$3\times10^{5}\pm2\times10^{5}$

Data are represented by means of 3 replicates ± standard errors. Empty cells (-) indicate that samples were not examined due to spoilage. Different small letter superscripts (a, b, c) within a row indicate significant differences between means at $p<0.05$.

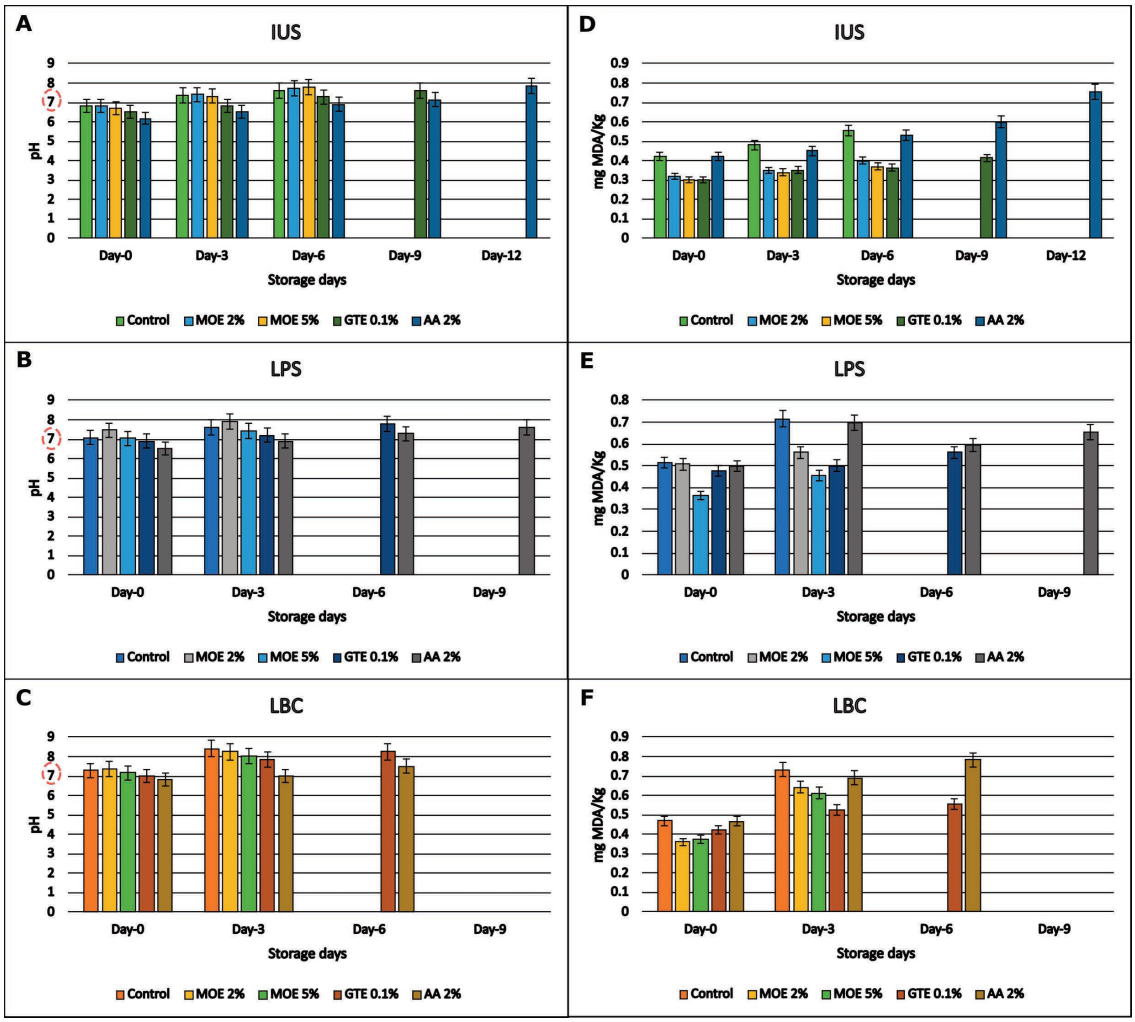

Fig. 2. Effect of treatment with *Moringa oleifera* extract 2 and 5 % (MOE 2% and MOE 5%), green tea extract (GTE 0.1%) and acetic acid solution (AA 2%) on the pH (A –C) and TBA-RS (D –F) of imported unpeeled shrimp (IUS), local peeled shrimp (LPS) and local breed crap (LBC) samples, respectively, during refrigerated storage at 2.0±1.0°C. Data are represented by means of 3 replicates±Standard errors. Red circles in A–C indicate the maximum acceptable limit of pH (7) in fish and shelf fish according to the national and international standards.

Discussion

Seafood quality is associated with different attributes such as sensory, nutritional, physicochemical, microbiological, and biochemical qualities. The freshness of fish reduces after death due to different reactions such as lipid oxidation, enzymatic activity, and microbiological growth, which lead to loss of sensory quality, nutritional value, acceptability, and safety of these products (Mohan *et al*., 2012). Freshness is the most important characteristic when evaluating the quality of seafood. Sensory attributes of seafood are plainly visible to consumers and sensory examination is still the most satisfactory method for assessing the freshness of fish since it gives the best idea of consumer acceptance (Connell, 1995)

Similar to findings from this study, Pal *et al*. (2017) reported that fish fillets samples treated with green tea extract and stored under refrigerated condition crossed the acceptability limit on day 12, whereas control samples dropped below that limit on day 9, based on the color, texture, flavor and overall acceptability scores given by panelists. Also, these results are in harmony with that reported by Nirmal and Benjakul (2012) and Ahmed (2006), who recorded that green tea extract inhibited polyphenoloxidase from cephalothorax of shrimp, which is responsible for deteriorative changes in the appearance of shrimp. The phytochemical analysis of green tea showed the presence of alkaloids, saponins, tannins, catechin, and polyphenols, which have antibacterial activity could delay the rate of bacterial growth and extend the shelf life (Mbata *et al*., 2008). Concerning acetic acid, Al-Dagal and Bazaraa (1999) reported that the shelf life of shellfish samples treated with acetic acid was prolonged to exceed 17 days. The application of vinegar (acetic acid) as a food preservative is a traditional method of preventing food spoilage (Jay, 2000).

The variation in the results as regards different crustacean species could be ascribed to the differences in the source, breed, and harvesting environment (imported versus local), the degree of handling and processing (peeled versus unpeeled), and the difference in species (crab versus shrimp).

In addition to sensory evaluation, using physicochemical and microbiological analyses, are used widely to assess the freshness and quality of seafood (Gill, 1992). Physical and biochemical methods are used to measure the pH and concentrations of breakdown products from bacterial or enzymatic activity of seafood flesh. Spoilage indicators included the total volatile basic nitrogen and trimethylamine (Botta *et al*., 1984). Vinegar is an efficient acidulant that causes a reduction in pH below the growth range of many bacteria (Jay, 2000). The increase in the pH values of shrimp during storage has been attributed to the accumulation of basic compounds generated from both endogenous and microbial enzymatic actions (Lopez-Caballero *et al*., 2007).

Additionally, the oxidation of lipids is a major quality issue that occurs in fish and shellfish. It leads to the occurrence of off-flavors and off-odors in seafood oils and other fat-containing foods, termed oxidative rancidity (Hamilton, 1983). Perox-

ide value (PV) and TBA-RS are the main chemical indicators of oxidative rancidity. TBA-RS determines the level of secondary products of lipid oxidation. TBA consists mainly of malondialdehyde (MDA) as a representative of aldehydes (Rossell, 1989).

This study findings confirm the antioxidant efficiency of both moringa and green tea extracts, which was recorded by previous studies (Ahmed, 2006; Nirmal and Benjakul, 2012). Similarly, Nirmal and Benjakul (2009) reported that catechin (0.1%), a phenolic metabolite found in some herbs such as green tea, has potent antioxidative activity in shellfish muscle and prohibited lipid oxidation during iced storage. In addition, *Moringa oleifera* leaves could be used as a potential source of antioxidants to protect meat products against oxidative rancidity without any adverse effects on sensory attributes (Das *et al*., 2012; Muthukumar *et al*., 2014)

Concerning the permissible limit of TBA value in fish and shellfish (4.5 mg MDA/Kg) recommended by EOS (2005), although we noticed a gradual increase in TBA-RS values over the period of storage, neither treated groups nor control one exceeded such limit on any occasion of examination even after showing objectionable odors and flavors. this agrees with that reported by Connell (1990). Seafood is abundant in polyunsaturated fatty acids, which are susceptible to oxidation. Because of the high degree of unsaturation of seafood lipids, they are more susceptible to oxidation than other animal fats and vegetable oils (Nawar, 1996). Fatty acid hydroperoxides, which are measured as PV, are the primary products of lipid oxidation. Consequently, unstable hydroperoxides are broken down to shorter chain hydrocarbons such as aldehydes, ketones, and alcohols which are the volatile products causing off-flavor in products and can be detected as TBARS (Rossell, 1989; Benjakul *et al*., 2005). In context, lipid oxidation also alters the texture and color and reduces the nutritional quality of seafood (Lie, 2001).

The obtained bacteriological results confirm the antimicrobial efficacy of both green tea extract and acetic acid, conversely, moringa extract did not show considerable antimicrobial activity. In the refrigerator, psychrophilic bacteria have a faster growth rate than other microorganisms due to the favorable temperature. Even though the bacterial load and their growth rate are highly dependent on the initial bacterial load and the preservation conditions. Nirmal *et al*. (2011) reported that the treatment of shrimp with green tea extract before modified atmospheric packaging could retard the growth of psychrophilic bacteria more effectively than modified atmosphere packaging alone. Yet, Ahmed (2006) reported that acetic acid treatment of shellfish could effectively reduce the coliforms to an undetectable limit. Also, Delmore *et al*. (2000) proved that acetic acid 2% immersion was among the most effective treatments for total coliform count reduction muscle food. Additionally, Osama (2012) reported lower *Staphylococcus aureus* counts in acetic acid-treated shellfish samples in comparison with control samples during iced storage.

The presence of fecal coliforms in crustaceans is indicative of fecal contamination either from the natural aquatic environment or by unhygienic personal habits (Ali and Ibrahim, 2004). Interestingly, we failed to detect fecal coliforms in IUS samples on all examination occasions, which could be because IUS was unpeeled, and the shell could protect the flesh from fecal contamination.

Conclusion

From the present study, it could be concluded that green tea extract and acetic acid are efficient antimicrobials for crustaceans and could extend the shelf-life for 3-6 days depending on the shellfish breed, peeled or unpeeled, and storage conditions without altering the sensory attributes. On the other hand, although *Moringa oleifera* extract had good antioxidant activity, it is not enough alone for extension of shellfish keeping quality as it must be coupled with a potent antimicrobial. As well as *Moringa oleifera* extract reduces the sensory assessment scores of treated crustaceans due to the change in color, odor, and flavor made by it.

Conflict of interest

The authors declare that there is no conflict of interest in this study.

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