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The Effects of Essential Oils and Organic Acids on Microbiological and Physicochemical Properties of Whole Shrimps at Refrigerated Storage

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Abstract

The objectives of this study were to determine the effects of Essential Oils (EOs) and Organic Acids (OAs) on microbiological and physicochemical qualities of whole shrimps stored at 4°C. Shrimps of 1.1 kg were dipped in solutions of EOs (cinnamon oil, garlic oil and lime oil) and OAs (lactic acid, tartaric acid and sodium diacetate) at 1:2 shrimp/treatment solution (w/w) at 25^{0}C for 30min. Concentration of sodium metabisulfite and distilled water (DH_2O) were used as positive and negative controls, respectively. Shrimps were drip-dried for 5 minutes, packaged and stored in a chiller (4°C) for 10 days. They were analyzed for microbiological (total aerobic plate count) and physicochemical (pH, colour and texture) properties at days 0, 2, 5, 7 and 10. Total aerobic plate count (TPC) of shrimps decreased immediately after dipping in solutions containing EOs, OAs and their mixture ratios. However, the TPC of shrimps continued to increase during storage and at day 10, TPC was significantly ($P<0.05$) higher compared to other days. Mixtures of tartaric acid and cinnamon oil was the best in controlling TPC in shrimps. pH of shrimps ranged from 6.60 to 7.86. Most of the treatments had significantly lower pH compared to DH_2O treated shrimp. L* values (Lightness), a* values (Redness) and b* values (Yellowness) ranged from 32.57-42.27, -1.90-4.39 and 3.14-10.67, respectively. The texture (hardness value) of the shrimps ranged from 1135.4-2511.8 and decreased throughout storage period except solutions of lactic acid and lime. Storage of shrimps in EOs and organic acids can serve as an alternative for the preservation of shrimps other than low temperature storage.

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Introduction

Fresh shrimp is a highly perishable seafood product and its quality and freshness rapidly decline upon harvesting[1]. Immediately after harvest, the most common or preferred method of preservation of shrimps is applying low temperature which is either through freezing or chilling. Frozen shrimps are of high value and are meant to serve premium local and export markets, while chilled shrimps are limited for domestic consumption[2]. At retail level, chilled shrimps often undergo temperature abuse during display for about 12 to 24 hours[3]. At this point, deterioration of microbiological, physical and chemical qualities take place causing trade losses. In order to prolong the shelf-life and maintain the quality of shrimps at chilled storage, an improved method of preservation is necessary.

The use of natural antimicrobials to preserve food is gaining popularity as consumers demand the use of safe and non-toxic products in food. The application of essential oils (EOs) and organic acids in prolonging shelf-life of food products have been reviewed extensively[4,5]. These reviews clearly indicated that, studies involving the use of EOs and organic acids in prolonging the shelf-life of shrimps are limited compared to their use in chicken, beef and pork. The effect of thymol essential oil[1] and rosemary[6] in shrimps have been reported. The efficacy of bacteriocin from *Lactobacillus sp* (AMET 1506) as a biopreservative for shrimps under different storage temperature conditions have also been investigated[7]. However, the combine effects of EOs and organic acids on the shelf-life of shrimps is lacking. The combinations of EOs and organic acids might result in an additive or synergistic antibacterial effect in inhibiting pathogens and spoilage microorganisms.

Three EOs (cinnamon oil, garlic oil and lime oil) and three organic acids (lactic acid, tartaric acid and sodium diacetate) were selected based on their strong *in-vitro* activity against a wide range of microorganism as described in literature[4,5,7]. As there is no information on the effects of these EOs and OAs on the quality of shrimps, this study was undertaken to determine the effects of these antimicrobials on the quality of shrimps.

Materials and Methods

Acquisition of Essential Oils and Organic Acids

Food grade cinnamon oil (*Cinnamomum zeylanicum*), garlic oil (*Allium sativum*), lime oil (*Citrus aurantifolia*), lactic acid, tartaric acid and sodium diacetate were purchased from SAFC, Milwaukee, USA.

Preparation of Shrimp Samples

Freshly harvested tiger shrimps (*Penaeus monodon*) were obtained from a shrimp farm in Balik Pulau, Malaysia. The shrimps were immediately transported on ice and thoroughly washed with sterile distilled water upon reaching the laboratory. Shrimps were prepared according to Wan Norhana[8] and were divided into 17 groups of 1.1 kg each. Each group was dipped in essential oils (cinnamon, garlic and lime oils) and organic acids (lactic acid, tartaric acid and sodium diacetate solutions).

Preparation of Treatment Solutions

The concentration of treatments and preparation of dipping solutions are shown in Tables 1 and 2, respectively. The shrimps (1.1kg) were dipped in each treatment solutions (1:2 shrimp/treatment solution) (w/w) for 30min at 25^{0}C and agitated from time to time to ensure even distribution of the antimicrobial solutions. Concentration of sodium metabisulfite (positive) and distilled water (negative) were used as controls[9]. The shrimps were drip-dried for 5 mins, packed in labelled polyethylene containers and stored in a chiller (4°C) for 10 days. The shrimps were analysed for microbiological (total aerobic plate count) and physicochemical (pH, colour and texture) properties at day 0, 2, 5, 7, 10 of storage. The experiments were done in triplicate.

Table 1: Concentration of treatments solutions

Type of treatments	Abbreviation	Concentration used
Tartaric acid	TA	5.0 g/l
Lactic acid	LA	30 ml/l
Sodium diacetate	SDA	5.0 g/l
Cinnamon oil	CIN	2.5 ml/l
Garlic oil	GAR	25 ml/l
Lime oil	LIME	12.5 ml/l
Sodium metabisulfite	MBS	12.5 g/l
Distilled water	DH_2O	-

Table 2: Mixture of dipping solutions

Organic acid	Essential oil	Volumes or weight of antimicrobial per litre of water
Tartaric acid	-	0.5 g tartaric acid
	Cinnamon	0.5 g tartaric acid + 2.5 ml cinnamon oil
	Garlic	0.5 g tartaric acid + 25 ml garlic oil
	Lime	0.5 g tartaric acid + 12.5 ml lime oil
Lactic acid	-	3.0 ml lactic acid
	Cinnamon	3.0 ml lactic acid + 2.5 ml cinnamon oil
	Garlic	3.0 ml lactic acid + 25 ml garlic oil
	Lime	3.0 ml lactic acid + 12.5 ml lime oil
Sodium diacetate	-	0.5 g sodium diacetate
	Cinnamon	0.5 g sodium diacetate + 2.5 ml cinnamon oil
	Garlic	0.5 g sodium diacetate + 25 ml garlic oil
	Lime	0.5 g sodium diacetate + 12.5 ml lime oil
Sodium metabisulfite	-	12.5g sodium metabisulfite

Microbiological Analysis

Microbiological analysis was done following the method described by Wan Norhana *et al.,*[8]. Briefly, 25 g of shrimps were aseptically homogenized in 225 ml of sterile 0.85% saline. Saline concentration of 1:10 (w/v%) was provided by serial dilution of 0.85% solution (w/v%) and spread plated onto duplicate plate count agar plates (OXOID, Basingtoke, UK). The plates were incubated at 37°C for 24 h. After which, colonies were counted and expressed as cfu/g by calculation.

pH Measurement

25 g of shrimps were ground and homogenized, using a mechanical homogeniserin 225 ml of sterile 0.85% saline. The pH was determined at 25°C using pH meter (Ohaus, starter 3100, New Jersey, USA). The pH was calibrated using both pH 4 and 7 buffers prior to use.

Colour Measurement

The colour of the shrimps was determined using Minolta colour spectrophotometer (CM – 3500d, Minolta, Japan). The Minolta was initially calibrated with a Minolta standard white reflection plate and blank disc. Measurements were taken perpendicular to the sample at the first two segments of the upper abdomen of the beheaded raw shrimp. The CIE (International Commission on Illumination) L*, a*, b* were recorded with the aid of attached software (SpectraMagic software version 2.11, Minolta, Japan). L* stands for lightness ranging from 0 (black) to 100 (white), a* stands for redness ranging from –a * (green) to +a* (red) and b* stands for yellowness ranging from –b* (blue) to +b* (yellow)[10].

Texture Analysis

Texture (hardness) of shrimp samples was measured using Texture Analyser CT3 Version 1.2 (Brookfield Engineering Laboratories, Middleboro, MA, USA) at the second to third segment of the shrimp abdomen. Shrimp samples were patted dry on the surface with filter paper and kept at 4°C for 30 min before they were analysed. Hardness (N) was measured using compression test for shrimp equipped with 25 kg load cell, with a cylindrical shape probe (6 mm diameter) at 1 mm/s test speed. Nine measurements were taken for each sample.

Statistical Analysis

Three experimental replicates were conducted. Microbial counts were transformed into log values and all data were subjected to a two-way analysis of variance (ANOVA) and Tukey's test for comparison of means using SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). Significance was defined at a level of $P<0.05$.

Results and Discussions

Microbiological Analysis

Table 3 shows the effects of essential oils (EOs) and/or organic acids treatments on the total aerobic plate counts (TPC) of shrimps stored at 4°C for 10 days. Initial TPC of shrimps prior to treatment ranged from 5.0 – 5.67 log cfu/g. Okpala *et al.,*[11] reported a lower initial TPC of 4.45 log cfu/g in decapitated *Litopenaus vannamei*. Cadun *et al.,*[12] reported a much higher initial TPC of 5.24 log cfu/g in *Parapenaeus longirostris* which was similar to that found in this study. The differences in initial TPC might be due to the differences in species and aquaculture conditions[13]. TPC of shrimps decreased immediately after dipping in treatment and control solutions.

Table 3: Effect of essential oils, organic acids and their combinations on total aerobic plate counts in shrimps stored at 4°C for 10 days

	Total aerobic plate count (TPC) Log cfu/g				
Treatments	**Day 0**	**Day 2**	**Day 5**	**Day 7**	**Day 10**
TA	3.64 ± 0.11 Aabc	4.04 ± 0.21 ABa	4.42 ± 0.18 Ba	5.60 ± 0.26 Cabc	7.10 ± 0.37 Da
LA	3.62 ± 0.16 Aabcde	3.90 ± 0.49 Aa	5.02 ± 0.31 Bab	5.74 ± 0.39 Babcd	7.27 ± 0.42 Cab
SDA	4.23 ± 0.30 Aabcd	4.28 ± 0.46 Aa	4.49 ± 0.71 Aa	6.65 ± 0.53 Ba	7.61 ± 0.42 Ba
CIN	4.09 ± 0.32 Aabcde	4.16 ± 0.45 Aa	4.61 ± 0.14 Aa	6.25 ± 0.14 Bd	7.75 ± 0.56 Cab
GAR	4.06 ± 0.38 Aabcde	4.38 ± 0.17 Aa	4.91 ± 0.47 ABab	5.71 ± 0.65 Bcd	7.43 ± 0.31 Cab
LIME	4.38 ± 0.16 Acde	4.15 ± 1.02 Aa	4.43 ± 0.34 Aa	6.70 ± 0.30 Babcd	7.84 ± 0.24 Bab
TA+CIN	3.49 ± 0.16 Aa	3.85 ± 0.18 Aa	4.15 ± 0.26 Aa	5.34 ± 0.35 Bab	6.65 ± 0.43 Ca
TA+GAR	3.60 ± 0.06 Aab	3.73 ± 0.10 Aa	4.36 ± 0.20 Ba	5.38 ± 0.30 Cab	6.88 ± 0.14 Da
TA+LIME	3.73 ± 0.24 Aabcd	4.21 ± 061 Aa	4.77 ± 0.38 ABab	5.60 ± 0.65 Babc	7.29 ± 0.35 Ca
LA+CIN	3.74 ± 0.19 Aabcde	3.81 ± 0.40 ABa	4.29 ± 0.71 ABab	5.15 ± 0.58 Babcd	6.91 ± 0.52 Ca
LA+GAR	3.63 ± 0.06 Ade	4.05 ± 0.49 Aa	4.45 ± 0.75 Aa	5.80 ± 0.24 Bbcd	6.95 ± 0.50 Bab
LA+LIME	3.86 ± 0.12 Aabc	4.870 ± 0.40 Aa	4.55 ± 0.55 ABab	5.86 ± 0.57 Babcd	7.34 ± 0.30 Ca
SDA+CIN	4.16 ± 0.38 Aabc	4.80 ± 0.15 Aa	7.16 ± 0.76 Aa	7.16 ± 0.60 Babcd	7.65 ± 0.49 Ba
SDA+GAR	4.13 ± 0.24 Aabcde	4.79 ± 0.71 Aa	6.88 ± 0.53 Aa	6.88 ± 0.88 Babcd	7.55 ± 0.33 Ba
SDA+LIME	4.30 ± 0.30 Abcde	4.43 ± 0.99 Aa	4.38 ± 0.44 Aa	6.35 ± 0.52 Bbcd	7.45 ± 0.34 Bab
MBS	3.57 ± 0.08 Aab	3.90 ± 0.14 Aa	4.42 ± 0.42 Aa	5.42 ± 0.34 Babc	6.92 ± 0.57 Ca
DH_2O	4.49 ± 0.21 Ae	5.23 ± 0.41 ABa	6.00 ± 0.12 Bab	7.17 ± 0.38 Cd	8.70 ± 0.43 Db

Values were reported as means ± S.D. of triplicate groups

Mean values in the same treatment/row with different uppercase were significantly different (P<0.05)

Mean values in the same day/column with different lowercase were significantly different (P<0.05).

During the storage period, TPC of shrimps continued to increase significantly (P<0.05) and at day 10, increasing in TPC was significantly higher (P<0.05) than the other days. The International Commission on Microbiological Specifications for Foods (ICMSF) specified a TPC of 7 log cfu/g for frozen shrimps[14]. Food Regulation 1985 of Malaysia stipulated a TPC of 6 log cfu/g for ready-to-eat fish and fish products which includes shrimps. According to Ouattara *et al.,*[15] TPC of *Penaeus* shrimp in the range of 7.0 – 8.0 log cfu/g is the maximum limit allowed. In the present study, TPC of shrimps treated with DH_2O exceeded the maximum limit allowed for TPC on day 7, while TPC of most treated shrimps reached the maximum permitted limit of 7.0 log cfu/g on day 10[14].

TPC of shrimps treated with tartaric acid (TA) + cinnamon oil (CIN) (6.65 log cfu/g), TA+ garlic oil (GAR) (6.88 log cfu/g), lactic acid (LA)+CIN (6.91 log cfu/g) and LA+GAR (6.95 log cfu/g) were still below the maximum limit at day 10 with no significant (P>0.05) differences with sodium metabisulfite (MBS) (6.92 log cfu/g). The results suggest that, combination of TA+CIN achieved the highest reduction of TPC and served as the most

effective treatment in reducing TPC. The shelf-life of shrimps treated with essential oils (TA, CIN) and organic acids (LA, GAR) alone was 7 days compared to their mixtures which were 10 days. This could be due to the additive or synergistic effect of organic acids and EOs combinations. EOs and organic acids acted in different ways and made the bacterial cells more vulnerable. For instances, the phenolic compounds in essential oils could cause sub-lethal injury to bacterial cell membrane by disrupting the proton motive force thus making the cells more susceptible to acids[16]. In addition, at higher concentration of phenolic containing essential oils, a low pH micro-environment is created (due to proton donation) and cell membrane is disrupted (due to stacking)[16]. This makes it more effective in destroying microorganisms than low pH caused by organic acid alone.

The efficacy of lactic acid alone as an antimicrobial agent has been studied. Shirazinejad *et al.,*[17] observed a TPC of 6.8 log cfu/g in shrimps (*Penaeus merguiensis*) treated with 1% lactic acid and stored at 4 ºC for 11 days. In the present study, TPC for shrimps treated with LA was 7.27 log cfu/g. These differences could be attributed to initial number of TPC on the shrimps, initial concentration of lactic acid used, time of exposure, mode of application and the storage temperature. Although in the present study, higher concentration (3%) of lactic acid was used, TPC of shrimps were higher (7.27 log cfu/g) at the end of storage than that reported by Shirazinejad *et al.,*[17]. This could be attributed to the slight difference in the molarity of lactic acid used by Shirazinejad *et al.,*[17] (0.012 M) and the present study (0.011 M).

In this study, cinnamon oil showed good antibacterial activity against TPC in shrimps. Tajkirimi *et al.,*[18] reported that, the antimicrobial activity of cinnamon is mainly due to the presence of cinnamaldehyde, a major component in cinnamon oil which has been demonstrated to possess strong antibacterial activity against a wide range of pathogenic bacteria as well as spoilage bacteria and natural microflora. Mu *et al.,*[19] reported a reduction of 1.90 log cfu/g TPC in shrimps dipped in 0.1% cinnamaldehyde at the end of 10 days storage at 4^0C.

pH Measurement

Changes in pH values of shrimps treated with essential oils and/or organic acids, and stored at 4°C for 10 days are shown in Table 4. The initial pH of shrimps prior to treatment ranged from 6.84- 6.90 indicating that the shrimps used were fresh[20]. pH of the shrimps were slightly ($P>0.05$) altered after being dipped in EOs and organic acids, and most of the treatments had significantly lower pH compared to DH_2O treated shrimps. Sallam *et al.,*[21] also observed a small but significant ($P<0.05$) reduction in initial pH of fish fillets dipped in 2 - 3% acetic acid solution.

Table 4: Effect of essential oils, organic acids and their combinations on pH of shrimps stored at 4°C for 10 days

Treatments	Day 0	Day 2	Day 5	Day 7	Day 10
TA	6.76 ± 1.01 Aabc	7.32 ± 1.03 ABa	7.47 ± 1.05 Ba	7.71 ± 1.02 Ba	7.82 ± 1.00 Ba
LA	6.61 ± 1.02 Aa	7.39 ± 1.01 BCa	7.31 ± 1.02 Ba	7.63 ± 1.02 Ca	7.55 ± 1.02 BCa
SDA	6.77 ± 1.02 Aabc	7.31 ± 1.03 Ba	7.54 ± 1.02 BCa	7.69 ± 1.01 BCa	7.86 ± 1.02 Ca
CIN	6.65 ± 1.01 Aab	7.38 ± 1.02 Ba	7.50 ± 1.01 BCa	7.64 ± 1.03 BCa	7.79 ± 1.01 Ca
GAR	6.87 ± 1.01 Abc	7.27 ± 1.02 ABa	7.28 ± 1.02 ABa	7.59 ± 1.01 Ba	7.71 ± 1.06 Ba
LIME	6.66 ± 1.02 Aab	6.99 ± 1.06 ABa	7.36 ± 1.02 BCa	7.61 ± 1.01 Ca	7.80 ± 1.01 Ca
TA+CIN	6.65 ± 1.00 Aab	7.21 ± 1.03 Ba	7.48 ± 1.02 BCa	7.71 ± 1.02 Ca	7.79 ± 1.01 Ca
TA+GAR	6.72 ± 1.00 Aabc	7.21 ± 1.03 Ba	7.31 ± 1.02 Ba	7.67 ± 1.02 Ca	7.73 ± 1.01 Ca
TA+LIME	6.73 ± 1.01 Aabc	7.07 ± 1.07 ABa	7.27 ± 1.02 ABCa	7.62 ± 1.01 BCa	7.77 ± 1.03 Ca
LA+CIN	6.61 ± 1.03 Aa	6.98 ± 1.01 Ba	7.32 ± 1.02 BCa	7.56 ± 1.00 Ca	7.54 ± 1.03 Ca
LA+GAR	6.66 ± 1.03 Aab	7.24 ± 1.02 Ba	7.39 ± 1.04 BCa	7.63 ± 1.01 Cab	7.75 ± 1.01 Ca
LA+LIME	6.81 ± 1.02 Aabc	7.13 ± 1.03 ABa	7.38 ± 1.02 BCa	7.58 ± 1.01 Ca	7.57 ± 1.02 Ca
SDA+CIN	6.92 ± 1.03 Ac	7.17 ± 1.03 ABa	7.44 ± 1.01 BCa	7.63 ± 1.02 BCa	7.71 ± 1.03 Ca

SDA+GAR	6.66 ± 1.01 Aab	7.19 ± 1.02 Ba	7.46 ± 1.02 BCa	7.60 ± 1.03 Ca	7.80 ± 1.01 Ca
SDA+LIME	6.60 ± 1.01 Aa	7.37 ± 1.06 Ba	7.45 ± 1.00 Ba	7.63 ± 1.01 Ba	7.82 ± 1.01 Ba
MBS	6.62 ± 1.02 Aa	7.24 ± 1.02 Ba	7.46 ± 1.01 BCa	7.66 ± 1.02 CDa	7.81 ± 1.02 Da
DH_2O	6.90 ± 1.01 Ac	7.28 ± 1.05 ABa	7.31 ± 1.00 ABCa	7.66 ± 1.02 BCa	7.80 ± 1.02 Ca

Values were reported as means ± S.D. of triplicate groups

Mean values in the same treatment/row with different uppercase were significantly different (P<0.05)

Mean values in the same day/column with different lowercase were significantly different (P<0.05)

The pH values continued to increase in shrimp samples throughout storage period. Similar trend was also observed by Attala[2], who reported that, the pH of shrimps treated with 3% citric acids and 2% sodium sulphite ranged from 6.4 - 6.47 and 7.59 - 7.84, respectively. The increase in pH value was caused by accumulation of compounds (ammonia and amines) formed during endogenous enzymatic reactions and microbial growth[8,22]. According to Mehmet *et al.,*[20], pH values of shrimps ≤ 7.7 indicates good quality shrimps. At day 10, only shrimps treated with LA, LA+CIN and LA+LIME had pH value of less than 7.60 indicating that, the shrimps were of good quality throughout the storage period.

Colour Measurement

The colour of shrimps is very important in terms of perception of quality, and it is a dominant factor influencing consumers' purchasing decision. During storage, shrimps undergo numerous quality deteriorations including lipid oxidation and protein alteration leading to changes in colour[23,24]. The effects of essential oils, organic acids and their mixtures on L*, a* and b* values of shrimps during storage at 4°C are presented in Tables 5, 6 and 7, respectively. The colour of shrimps was generally affected but the trend observed was not consistent for treated samples and controls.

Table 5: Effect of essential oils, organic acids and their combinations on L* values of shrimps during storage at 4°C for 10 days

Treatments	Day 0	Day 2	Day 5	Day 7	Day 10
TA	33.46 ± 0.90 Aa	36.22 ± 3.13 ABab	39.68 ± 1.90 BCa	41.01 ± 2.51 Ca	41.38 ± 1.52 Cde
LA	35.65 ± 1.19 Aab	36.88 ± 2.91 Aab	37.22 ± 0.35 Aa	38.54 ± 4.55 Acd	38.80 ± 3.158 Acde
SDA	34.17 ± 2.33 Aab	37.67 ± 0.86 Aab	38.40 ± 5.69 Aa	38.38 ± 3.04 Aabcd	39.18 ± 2.45 Ade
CIN	34.38 ± 2.64 Aa	38.56 ± 2.00 Bab	38.20 ± 2.35 Ba	37.74 ± 2.8 Bbcd	36.53 ± 2.40 ABabcd
GAR	35.37 ± 2.70 ABab	34.68 ± 1.70 Aa	34.57 ± 1.11 Ba	33.63 ± 2.14 Abc	33.90 ± 1.87 Aabc
LIME	35.87 ± 0.85 Aab	36.76 ± 1.77 Aab	36.92 ± 3.83 Aa	38.67 ± 2.10 Abcd	36.49 ± 1.77 Aabcd
TA+CIN	35.05 ± 1.14 Aab	39.14 ± 2.32 Bab	39.81±1.50 Ba	39.93 ± 2.17 Bd	40.89 ± 2.16 Bde
TA+GAR	35.15 ± 1.04 Aab	36.37 ± 1.34 ABab	37.25 ± 3.95 ABa	38.44 ± 1.97 ABbcd	40.96 ± 2.19 Bde
TA+LIME	36.25 ± 2.93 Aab	37.91 ± 2.76 Aab	38.12 ± 2.30 Aa	39.28 ± 3.93 Abcd	40.30 ± 0.75 Ade
LA+CIN	38.22 ± 0.89 Ac	40.53 ± 3.36 Ab	41.41 ± 6.69 Aa	41.98 ± 1.85 Ad	42.27 ± 2.58 Ae
LA+GAR	36.20 ± 0.78 Aab	36.96 ± 3.72 Aab	35.25 ± 5.43 Aa	32.79 ± 2.04 Abcd	32.57 ± 1.78 Aa
LA+LIME	35.30 ± 4.03 Aab	37.45 ± 3.55 Aab	36.04 ± 1.92 Aa	38.82 ± 1.13 Aa	34.12 ± 3.39 Aabc
SDA+CIN	35.38 ± 3.34 Aab	36.71 ± 4.52 Aab	37.71 ± 3.36 Aa	38.57 ± 5.64 Abcd	38.28 ± 4.82 Abcde
SDA+GAR	34.91 ± 1.25 Aab	36.75 ± 1.28 ABab	39.53 ± 2.71 Ba	39.48 ± 2.65 Bcd	39.18 ± 1.44 Bde
SDA+LIME	34.89 ± 2.64 Aab	35.26 ± 2.00 Aab	35.56 ± 2.35 Aa	37.54 ± 2.85 Aabcd	35.97 ± 2.40 Aabc
MBS	35.03 ± 3.44 Aab	39.16 ± 3.02 ABab	40.26 ± 2.21 Ba	40.76 ± 2.19 Bd	40.85 ± 1.13 Bde
DH_2O	36.90 ± 2.37 Bab	35.28 ± 1.61 ABab	34.56 ± 1.68 ABa	34.10 ± 2.77 Aabc	33.67 ± 0.63 Aab

Values were reported as means ± S.D. of triplicate groups

Mean values in the same treatment/row with different uppercase were significantly different (P<0.05)

Mean values in the same day/column with different lowercase were significantly different (P<0.05).

Table 6: Effect of essential oils, organic acids and their mixtures on a* values of shrimps stored at 4°C for 10 days

Treatments	Day 0	Day 2	Day 5	Day 7	Day 10
TA	- 0.64 ± 0.92 Aa	- 0.46 ± 1.69 Aa	- 0.42 ± 0.28 Aab	- 0.39 ± 1.70Aa	- 0.28 ± 0.88 Aab
LA	- 0.33 ± 0.94 ABa	- 1.90 ± 1.13 Aa	0.01 ± 1.36 ABabcd	0.43 ± 1.73 Bab	0.22 ± 0.59 ABab
SDA	- 0.55 ± 0.71 Aa	- 0.50 ± 1.32 Aa	0.15±0.83 Aab	0.50±0.77 Aab	- 0.18 ± 1.00 Aab
CIN	- 1.38 ± 1.36 Aa	- 1.25 ± 0.67 Aa	1.16 ± 0.88 Bbcdef	1.24 ± 0.94 Bab	1.03 ± 0.71 Babc
GAR	- 0.87 ± 0.84 Aa	- 0.47 ± 1.20 Aa	- 0.28 ± 0.60 Aabc	- 0.53 ± 0.55 Aa	- 0.48 ± 0.80 Aa
LIME	- 0.25 ± 0.60 Aa	- 0.35 ± 0.86 Aa	1.51 ± 0.86 Bbcdef	0.77 ± 0.52 ABab	1.80 ±1.14 Bbc
TA+CIN	- 0.85 ± 0.81 Aa	- 0.53 ± 0.54 Aa	- 0.22 ± 0.79 Aabc	0.29 ± 1.40 Aab	0.48 ± 1.02 Aab
TA+GAR	- 0.95 ± 0.75 Aa	- 0.39 ± 1.41 Aa	0.39 ± 0.11 Aabcde	0.39 ± 0.94 Aab	- 0.19 ± 1.26 Aab
TA+LIME	- 0.89 ± 1.22 Aa	- 0.31 ± 1.25 Aa	2.83 ± 0.54 BCf	2.44 ± 0.74 Bb	4.39 ± 0.99 Cd
LA+CIN	- 1.25 ± 0.32 Aa	- 0.94 ± 1.78 Aa	- 0.56 ± 1.36 ABab	- 0.59 ± 0.81 ABa	0.13 ± 0.96 Bab
LA+GAR	- 0.67 ± 1.00 Aa	- 0.25 ± 0.78 Aa	- 0.40 ± 0.20 Aab	- 0.49 ± 1.07 Aa	0.30 ± 0.59 Aab
LA+LIME	- 0.80 ± 0.42 Aa	0.18 ± 1.64 Aa	2.55 ± 2.36 Bef	2.30 ± 1.44 Bb	1.56 ± 0.96 ABabc
SDA+CIN	- 0.92 ± 0.26 Aa	0.23 ± 0.76 ABa	0.92±0.58 Bbcdef	1.02±0.94 Bab	0.97 ± 0.93 Babc
SDA+GAR	- 0.87 ± 0.34 Aa	- 0.47 ± 0.89 Aa	- 0.27 ± 0.52 Aabc	- 0.32 ± 0.86 Aa	- 0.25 ± 0.50 Aab
SDA+LIME	- 1.01 ± 0.43 Aa	0.37 ± 0.76 Ba	1.90 ± 0.73 CDcdef	1.36 ± 0.86 BCab	3.00 ± 0.62 Dcd
MBS	- 0.44 ± 0.83 Aa	- 1.32 ± 1.52 Aa	- 1.56 ± 0.60 Aa	- 0.69 ± 0.68 Aa	- 0.36 ± 1.08 Aab
DH_2O	- 0.82 ± 1.01 Aa	- 0.98 ± 1.17 ABa	2.28 ± 0.44 Bdef	- 0.02 ± 0.95 Aa	0.19 ± 1.13 ABab

Values were reported as means ± S.D. of triplicate groups

Mean values in the same treatment/row with different uppercase were significantly different (P<0.05)

Mean values in the same day/column with different lowercase were significantly different (P<0.05).

Table 7: Effect of essential oils, organic acids and their combinations on b* values of shrimps stored at 4°C for 10 days

Treatments	Day 0	Day 2	Day 5	Day 7	Day 10
TA	3.44 ± 0.57 Aab	4.37 ± 0.68 Aab	7.66 ± 0.34 Bbc	7.53 ± 1.23 Ba	5.10 ± 1.31 Aa
LA	4.81 ± 0.39 Ac	4.77 ± 1.42 Aabc	7.19 ± 0.72 Babc	8.61 ± 0.89 BCa	9.97 ± 1.15 Cdef
SDA	4.08 ± 0.54 Aabc	5.27 ± 1.84 Aabc	7.69 ± 1.32 Babc	7.57 ± 0.82 Ba	8.95 ± 0.62 Bdef
CIN	3.61 ± 0.77 Aabc	5.10 ± 0.89 Aabc	7.08 ± 0.46 Babc	8.83 ± 1.60 Ba	9.82 ± 0.47 Cdef
GAR	4.47 ± 0.96 Aabc	4.50 ± 0.75 Aabc	6.51 ± 1.28 BCabc	8.16 ± 1.09 Ca	6.11 ± 1.12 ABabc
LIME	4.82 ± 0.11 Ac	5.57 ± 1.02 ABabc	6.88 ± 0.91 Babc	8.67 ± 1.48 Ca	9.36 ± 0.58 Cdef
TA+CIN	4.80 ± 0.17 Ac	4.67 ± 1.15 Aabc	6.96 ± 0.82 Babc	8.22 ± 1.22 Ba	8.28 ± 1.44 Bcde
TA+GAR	4.89 ± 0.59 Ac	4.36 ± 0.83 Aab	6.88 ± 0.96 Babc	7.69 ± 1.16 BCa	8.83 ± 0.84 Cdef
TA+LIME	4.66 ± 0.66 Abc	3.64 ± 1.35 Aa	7.00 ± 0.95 Babc	9.06 ± 1.68 BCa	10.67 ± 1.34 Cf
LA+CIN	3.98 ± 0.69 Aabc	5.29 ± 0.95 Aabc	5.80 ± 1.23 ABab	7.56 ± 1.06 BCa	8.15 ± 1.53 Cbcd
LA+GAR	4.08 ± 0.29 Aabc	4.81 ± 0.99 ABabc	7.43 ± 0.78 ABbc	6.72 ± 0.98 Ba	5.94 ± 0.78 Bab
LA+LIME	3.14 ± 0.75 Aa	6.08 ± 0.77 Bbc	8.98 ± 0.93 CDc	8.11 ± 1.17 Ca	10.53 ± 0.84 Def
SDA+CIN	3.86 ± 0.73 Aabc	6.71 ± 1.03 Bc	6.43 ± 1.01 Bab	6.83 ± 1.33 Ba	9.26 ± 1.50 Cdef
SDA+GAR	4.06 ± 0.68 Aabc	5.10 ± 1.58 ABabc	6.97 ± 0.76 Babc	7.13 ± 1.54 Ba	5.25 ± 0.47 ABa
SDA+LIME	4.40 ± 0.78 Aabc	5.26 ± 0.65 Aabc	7.20 ± 1.32 Babc	7.03 ± 1.44 BCa	8.60 ± 0.06 Cdef
MBS	4.43 ± 0.82 ABabc	3.65 ± 0.97 Aa	4.83 ± 1.23 ABa	7.54 ± 0.84 Ca	6.12 ± 1.46 BCabc
DH^2O	3.70 ± 0.74 Aabc	5.69 ± 0.67 Babc	7.69 ± 0.64 CDbc	6.90 ± 1.20 BCa	9.14 ± 1.14 Ddef

Values were reported as means ± S.D. of triplicate groups

Mean values in the same treatment/row with different uppercase were significantly different (P<0.05)

Mean values in the same day/column with different lowercase were significantly different (P<0.05).

L* values (lightness) for shrimps treated with TA, TA+CIN, TA+GAR, SDA+GAR and MBS increased significantly (P<0.05) during storage. It was evident that, melanosis, the blackening process in shrimps was inhibited by these treatments. L* values of shrimps treated with MBS also increased during storage. Sodium metabisulfite is well known in preventing melanosis by interfering with the polymerisation of quinones and forming colourless compounds which results in the increase of L* values. MBS might have caused similar reactions in the shrimp muscle or caused bleaching of the exoskeleton in this study.

L* values of shrimps treated with LA+GAR, LA+LIME, GAR and DH_2O decreased significantly (P<0.05) during storage and the blackening of shrimp surfaces was obvious. Gokoglu and Yerlikaya[25] also observed similar decrease in L* values of untreated *Parapenaeus longirostris* stored at 4°C. Theoretically, the L* value of the shrimp surfaces (cephalothoraxes and abdomen) is expected to be lower (darker) than the initial value, due to the occurrence of melanosis[3]. Generally, the muscular epithelium and exoskeleton colour of tiger shrimp is due to red carotenoids and blue carotenoproteins; and during storage at chilled temperature, discolouration occurs caused by oxidation of these pigments[26].

The a* values (redness) of all shrimp samples increased during storage. However, the increase was not significant (P>0.05) throughout the storage period in shrimps treated with TA, TA+CIN, TA+GAR, LA, LA+CIN, LA+GAR, LA+LIME, SDA, SDA+CIN, SDA+GAR, GAR, MBS and DH_2O. Mu *et al.,*[19] also observed an increasing trend in a* values from -2.0 to 3.27 of *Litopenaeus vannamei* treated with 1 mg/ml cinnamaldehyde and stored at 4^{0}C for 11 days. It was notable that shrimp samples treated with LIME (alone or combined with organic acids) consistently recorded higher a* values and the highest value was observed on day 10 (4.39) compared to other shrimps. The lime may have caused partial dissociation of carotenoprotein complex in shrimps which in turn led to the release of free astaxanthin; the chemical responsible for the increase in red hue.

The b* values (yellowness) of shrimps treated with EO, organic acids and mixtures of EO and organic acids increased during the storage period. At day 10, the b* values of samples treated with TA was the lowest followed by SDA+GAR and LA+GAR which were also significantly (P<0.05) different from shrimps dipped in DH_2O. The b* values of shrimps ranged from 3.14 - 10.67. Mu *et al.,*[19] observed that the b* values of white shrimps treated with 0.5% of cinnamaldeyhde increased from 7.85 - 10.21 from day 0 - 5, respectively. They attributed these changes to melanosis. Gokoglu and Yerlikaya[25] also observed similar trend in shrimp samples treated with grape seed extract. The increase in yellowness and decrease in blueness might have been caused by denaturation of blue carotenoproteins in the muscular epithelium of shrimps and denaturation of protein induced by the treatments[27]. Overall, shrimps treated with TA, TA+CIN, TA+GAR, LA, SDA, SDA+GAR, GAR and MBS could sustain the changes in the colour parameters (L*, a* and b* values).

Texture Analysis

Texture is another important parameter in determining the quality of shrimps, and changes in hardness of shrimps indicate a change in quality. Changes in texture are due to loss of water-holding capacity and the formation of insoluble aggregates during chilled storage. The negative effect of loss in texture leads to toughening, dry, stringy and hard to chew shrimps[23,28]. Fresh shrimps are more firm or harder than spoiled shrimps. The changes in texture of shrimps dipped in essential oils, organic acids and their mixtures stored at 4°C are presented in Table 8.

Generally, the hardness values of all shrimp samples decreased (P>0.05) during the storage period, except for shrimps dipped in LA+LIME. The hardness value of shrimp samples treated with LA+LIME increased during the storage period, however, there was no significant (P>0.05) difference between the initial and final hardness values. Shrimp samples treated with tartaric acid (TA, TA+GAR, TA+LIME) and lactic acid mixtures (LA+CIN, LA+GAR, LA+LIME) were harder compared to shrimps treated with MBS. Furthermore, no significant (P>0.05) differences in hardness values were observed for shrimp samples treated with TA+GAR, TA+LIME, LA+CIN, LA+GAR, LA+LIME and SDA during the storage period. In this study, the hardness value of shrimps dipped in DH_2O

was 22.05 N (2249.8 g) at day 0 and decreased to 15.66 N (1498.5 g) at day 10.

During storage, muscle components that are responsible for changes in shrimp hardness are myofibrillar and connective tissue proteins which undergo degradation by proteases-primarily calpains, cathepsins and collagenases. These enzymes cause myofibril fragility and gaping, thus leading to decrease in hardness value[27]. Besides changes in structure and functionality of proteins, lipid oxidation and enzymatic activity are also responsible for softening and mushiness of shrimps[24]. The hardness values of all shrimp samples in the present study decreased over time. Similar changes were also reported by Imran *et al.,*[3] who observed decrease in hardness of chilled shrimps (*Litopenaeus vannamei*) stored at chilled temperature (0 – 8^{0}C).

Table 8: Effect of essential oils, organic acids and their combinations on hardness values (N) of shrimps during stored at 4°C for 10 days

Treatments	Day 0	Day 2	Day 5	Day 7	Day 10
TA	2331.5±463.2 ABab	2129.6±308.4Aa	2336.5±195.5Cc	1487.1±325.7ABab	1965.8±446.2 Aa
LA	2495.4±271.3 Bb	1738.0±460.9Aa	1847.9±494.5ABCabc	2053.4±414.9ABab	1498.5±432.3 Aa
SDA	2091.6±262.2 ABab	1559.0±566.6Aa	1737.5±237.2ABCabc	1344.8±396.9Aa	1398.1±482.3 Aa
CIN	2065.1±194.0 ABab	2211.9±582.2Aa	1448.4±271.1ABab	1261.1±409.5Aa	1357.9±396.8 Aa
GAR	2357.1±154.2 ABab	1434.9±356.3Aa	1626.7±60.7ABCabc	1427.0±479.9Aa	1287.9±445.8 Aa
LIME	2264.0±347.6 ABab	1615.9±343.7Aa	1728.1±402.4ABCabc	1317.5±269.6Aa	1144.4±319.2 Aa
TA+CIN	2392.1±425.4 ABab	2149.1±170.3Aa	2157.9±412.8BCabc	1708.1±289.3ABab	1453.9±243.4 Aa
TA+GAR	2250.1±124.0 ABab	1703.8±556.4Aa	1737.5±338.4ABCabc	1777.0±425.9ABab	1532.8±385.2 Aa
TA+LIME	2511.8±542.8 Bb	2460.8±256.6Aa	1748.9±348.8ABCabc	1972.0±308.2ABab	1849.4±717.4 Aa
LA+CIN	2135.5±481.2 ABab	1840.6±580.0Aa	1585.3±347.9ABCabc	1955.5±414.3ABab	1585.5±212.6 Aa
LA+GAR	2205.4±355.5 ABab	2033.3±515.5Aa	1930.4±112.1ABCabc	1662.1±263.0ABab	1768.9±444.1 Aa
LA+LIME	1859.4±162.7 ABab	1848.5±627.5Aa	1945.3±400.8ABCabc	2338.3±222.1Bb	2168.1±351.3 Aa
SDA+CIN	1553.4±344.4 Aa	2504.0±570.8Aa	1900.0±543.1ABCabc	2023.3±339.2ABab	1135.4±352.8 Aa
SDA+GAR	2215.1±398.2 ABab	1402.6±510.7Aa	1580.2±433.9ABCabc	1189.1±78.3Aa	1214.0±311.9 Aa
SDA+LIME	2335.1±314.3 ABab	2049.3±526.6Aa	2126.6±446.7BCbc	1413.5±353.3Aa	1278.9±439.3 Aa
MBS	2400.8±266.3 ABab	1835.9±524.0Aa	1255.8±135.5ABab	1322.1±323.1Aa	1526.9±467.7 Aa
DH_2O	2249.8±187.7 ABab	1681.5±526.3Aa	1245.4±173.8Aa	1739.0±326.1ABab	1498.5±332.1 Aa

Values were reported as means ± S.D. of triplicate groups

Mean values in the same treatment/row with different uppercase were significantly different (P<0.05)

Mean values in the same day/column with different lowercase were significantly different (P<0.05).

Conclusions

Mixtures of tartaric acid and garlic oil (TA+GAR) and lactic acid and cinnamon oil (LA+CIN) suppressed the degradation of fresh shrimps as indicated by both microbiological and physicochemical properties. Dipping shrimps in these mixtures, decreased TPC, caused minimal changes in colour (L*, a*and b*) and texture (hardness) throughout the storage period and thus as effectively as sodium metabisulfite, the traditional preservative for shrimps. This study confirmed that, hurdle technology (using different mixtures of EOs and organic acids) is an effective approach to extend the microbial shelf-life of raw shrimps. The study also provides an alternative preservative method for shrimps other than low temperature storage for processors and distributors to consider.

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Conflict of Interests

Authors declare no conflict of interest.

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