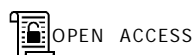


RESEARCH ARTICLE

The Effects of Garlic Oil and Tartaric Acid on the Quality of Shrimp Stored at 4°CWan Norhana Md Noordin¹, Nannthini Shunmugam², Frederick Adzitey³, and Nurul Huda^{4,5*}¹ Fisheries Research Institute, 11960, Batu Maung, Penang, Malaysia,² School of Industrial Technology, Universiti Sains Malaysia, 11800, USM Penang, Malaysia,³ Department of Food Science and Technology, University for Development Studies, Box TL 1882, Tamale, Ghana,⁴ Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, 88400, Kota Kinabalu, Sabah, Malaysia,⁵ Adjunct Professor, Universitas Sultan Ageng Tirtayasa, 42124, Banten, Indonesia

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Abstract

The objectives of this study were to determine the effects of garlic oil (GO) and tartaric acid (TA) on microbiological, pH, and Thiobarbituric Acid Reactive Substances (TBARS) (lipid peroxidation) of shrimp stored at 4°C. Shrimp of 2 kg were dipped in GO and TA solutions at 1:2 shrimp/treatment solutions (w/w) for 30 min under 25°C. Sodium metabisulfite (MBS) and sterile distilled water (dH₂O) were used as positive and negative controls, respectively. Shrimp were drip-dried for 5 min, packaged, and stored in a chiller (4°C) for 10 days. The shrimp were analysed on days 0, 3, 5, 7, and 10. Total aerobic plate count, psychotropic bacteria count, *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) and *Vibrio parahaemolyticus* counts ranged from 3.52-8.73 log CFU/g, <3.30-5.16 log CFU/g, 3.48-7.60 log CFU/g, 3.42-6.34 log CFU/g and 3.48-5.55 log CFU/g, respectively. The pH of shrimp ranged from 6.64 to 8.03. The pH of shrimp dipped in MBS (7.70) and TA+GO (7.70) was lowest at the end of storage period. TBARS values ranged from 0.70-2.66 and TBARS values for shrimp treated with MBS (1.83) were lowest at day 10. In general, microbiological counts, pH and TBARS values of sample treated with TA+GO increased with storage time, however comparable to MBS. Treatment of shrimp with GO and TA could inhibit the growth of foodborne pathogens. The pH of the test and control shrimp were similar on day 10. Lipid peroxidation was lowest for TA and MBS treated shrimp by day 10.

Keywords: shrimp quality, microbial safety, garlic oil, tartaric acid, storage study

Introduction

Shrimp are the most economically important, internationally traded seafood commodity in the world (FAO, 2016). Fresh shrimp presents a favourable environment for microbial growth and microbiological spoilage is one of the main causes of limited shelf-life and safety, particularly if the shrimp are contaminated with pathogens. Spoilage and presence of pathogens, such as *Listeria monocytogenes* and *Salmonella*, are among the most common reasons for shrimp product detentions and recalls (USFDA, 2008; Consumer Reports, 2015). These are the concerns of the importing countries because illnesses including listeriosis and salmonellosis have been related to shrimp (NACMCF, 2008). For these reasons, there is an avid interest in decreasing the surface microbial loads of shrimp, particularly the pathogens.

Contamination of seafood can occur before harvest or at any point from harvest through final preparation. However, the survival of pathogens is more likely to occur in foods that are consumed undercooked or raw

(Amagliani, Brandi & Schiavano, 2012). The major sources responsible for the occurrence of *Salmonella* in aquaculture shrimp are poor sanitation, cross-contamination during handling and inappropriate disposal of human and animal wastes (NACMCF, 2008; Amagliani et al., 2012). The primary source of *L. monocytogenes* associated with shrimp is surface contamination during handling and processing. *V. parahaemolyticus* on the other hand, is widespread in the marine and estuarine environments and is frequently isolated from seafood (particularly shellfish and shrimp). The infections of *V. parahaemolyticus* are caused by improper refrigeration, insufficient cooking and cross-contamination (NACMCF, 2008; Zarei, Borujeni, Jamnejad, & Khezzzadeh, 2012). Consumers demand safe food which should be produced by using safe intervention strategies. Potentially harmful additives, namely sulfiting agents, are used in shrimp preservation to generate allergic reactions (Over, Hettiarachchy, Johnson, & Davis, 2009; Nirmal & Benjakul, 2012). For these reasons, studies on the effectiveness of natural antimicrobials, generally regarded as safe

(GRAS) substances against foodborne pathogens have been and continue to be conducted (Wan Norhana, Poole, Deeth, & Dykes, 2010; Salem & Amin, 2012; Bahurmiz, Ahmad, Ismail, Adzitey, & Sulaiman, 2016; Wan Norhana, Nanthini, Huda, & Adzitey, 2018).

This study was carried out to determine the effects of tartaric acid, and garlic oil (alone and in combination) on the total aerobic plate counts, psychotropic bacteria counts, *L. monocytogenes*, *S. Typhimurium* and *V. parahaemolyticus* counts, chemical and sensory qualities of shrimp. Food grade tartaric acid is a GRAS substance with colorless, odorless and slight acidic taste, while garlic on the other hand is common condiment used in shrimp based dishes. Garlic has been proven to possess antimicrobial activity as well as anti-oxidant property. Uses of natural anti-oxidants, especially essential oils, could be a potential means to retard lipid oxidation and rancidity of shrimp oil during extended storage (Takeungwongtrakul & Benjakul, 2014). Our earlier study indicated that tartaric acid was effective in reducing total aerobic plate count and did not cause significant changes in pH, colour and hardness of shrimp (Wan Norhana et al., 2018). Furthermore, tartaric acid treated shrimp showed comparable results with sodium metabisulfite, the traditional preservative for shrimp to reduce the microbial loads in shrimp.

Material and Methods

Shrimp Sample Preparations

Commercial size fresh shrimp (*Penaeus monodon*) (about 90-100 days of culture) were obtained from a shrimp farm in Balik Pulau, Pulau Pinang, Malaysia. The shrimp were immediately transported on ice and thoroughly washed with sterile distilled water upon reaching the laboratory. At the laboratory, the shrimp were rinsed with sterile distilled water (SDW) and weighed. The mean weight of the shrimp obtained from the ponds was around 18.4 g. Large size shrimp (>19.0 g) and small size shrimp (<17.0 g) were not used in the study in order to minimise the effects of size on the results. They were then divided into 4 groups of 2.0 kg each. The first three groups were inoculated with *L. monocytogenes*, *S. Typhimurium* and *V. parahaemolyticus*, respectively. The fourth group served as the control.

Preparation and Inoculation of Shrimp with Pathogens

Food isolates of *L. monocytogenes* Scott A (Food Science Australia, Brisbane, Australia), *Salmonella enterica* subsp. *enterica* serovar *S. Typhimurium* (National Public Health Laboratory, Malaysia), and *V.*

parahaemolyticus (National Fish Health, Fisheries Department, Malaysia) were used. The bacterial cultures were obtained from the respective labs in an agar slant since 2015. Since then, the Bacteriology Lab of the Fisheries Research Institute, Batu Maung has maintained the bacterial stock according to standard protocols. Working cultures for each species was cultured in 3 mL Tryptic Soy Agar (TSA) (Oxoid, Basingstoke, UK) and incubated at 37 °C for 18 h. Two percent NaCl was added to all the media used for *V. parahaemolyticus*. Cells were harvested by centrifugation at 9,000 x g for 10 min at 4°C. The supernatant was decanted and the pellet suspended in sterile 0.85% saline solution and then further diluted to obtain a working culture of approximately 6 log CFU/mL. Inoculation of shrimp with the bacteria was carried out as described by Wan Norhana et al. (2010).

Treatment of Shrimp with Garlic Oil and Tartaric Acid

Food grade tartaric acid (99.7%; FCC; FDA 21 CFR 163.110; EU Regulation 1334/2008) and garlic oil (*Allium sativum*; FDA 21 CFR 184.1317; EU Reg. 1334/2008) were purchased from SAFC, Milwaukee, USA. Inoculated and non-inoculated shrimp were dipped in 5.0 g/L tartaric acid (TA), 25 mL/L garlic oil (GO), a mixture containing 5.0 g/L tartaric acid and 25 mL/L garlic oil (TA+GO), 1.25% sodium metabisulfite (MBS) as the positive control and sterile distilled water (dH₂O) as the negative control. The ratio of dipping was 1:2 (shrimp: dipping solution). The shrimp were dipped in the treatment and control solutions for 30 min at 25° C and agitated from time to time to ensure even distribution of the antimicrobial solutions. After which the shrimp were drip-dried for 5 min, packed in labelled polyethylene containers and stored in a chiller (4°C) for 10 days. Microbiological, pH, and TBARS analyses were performed on days 0, 3, 7, and 10.

Microbiological Analysis

Microbiological analysis was carried out for total aerobic plate count, psychotrophic count, *L. monocytogenes*, *S. Typhimurium* and *V. parahaemolyticus*. A 25 g shrimp of both inoculated and non-inoculated were added to 225 mL of sterile 0.85% saline and homogenised. Serial dilutions were prepared by diluting 1 mL of suspension in 9 mL of 0.85% saline. A volume of 0.1 mL of appropriate dilutions was spread-plated on appropriate selective media Polymyxin Acriflavin Lithium-chloride Ceftazidime Esculin Mannitol (PALCAM), Xylose Lysine Deoxycholate (XLD), Thiocitrate Bile Salt Agar (TCBS) and non-selective media Plate Count Agar (PCA) (Wan Norhana, Poole, Deeth, & Dykes, 2012).

Specifically, dilutions from the *L. monocytogenes* inoculated samples were plated on a PALCAM agar and incubated at 37°C for 48 h. Dilutions from the *S. Typhimurium* inoculated samples were plated on XLD agar and incubated at 37°C for 24 h and dilutions from the *V. parahemolyticus* inoculated samples were plated on TCBS agar and incubated at 35 °C. A few selected colonies from countable PALCAM, XLD and TCBS agar plates were streaked onto TSA for purity and confirmation tests including: Gram stain, oxidase test, umbrella motility in Sulfide Indole Medium, carbohydrate fermentation pattern and hydrogen sulphide production in Triple Sugar Iron (TSI) agar and salt tolerance test for *V. parahemolyticus* were carried out. For the uninoculated shrimp, the following groups of native microflora were enumerated; total aerobic bacteria on PCA after incubation at 30°C for 2 days and psychrotrophic bacteria on PCA incubated at 4°C for 5-7 days. All plates were examined visually for typical colony types and morphology characteristics associated with each growth medium. Two replicates of at least two appropriate dilutions were enumerated (Wan Norhana et al., 2012).

pH Measurement

About 25 g of shrimp were homogenised (from 5-6 shrimp), using a mechanical homogeniser in 225 mL of sterile distilled water (Imran, Chawalit, & Somrote, 2013). 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.

Determination of Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric acid (TBA) solution was prepared by mixing 0.375 g of TBA (Sigma Aldrich, USA), with 15 g of trichloroacetic acid (TCA) (Sigma Aldrich, USA) and 0.875 mL of hydrochloric acid (HCl) (Sigma Aldrich, USA) in 100 mL of distilled water (Thepnuan, Benjakul, & Visessanguan, 2008). Malondialdehyde (MDA) standard was prepared according to the method described by Karatas, Karatape, and Baysar (2002). A 10 µL of 1,1,3,3-tetraethoxypropane (Sigma – Aldrich, USA) was diluted in 10 mL of 0.1 M HCl in a screw-capped test tube, placed in a boiling water bath for 5 min and rapidly cooled under running tap water. A working stock solution was prepared by pipetting 1 mL of the resulting solution (hydrolysed acetal) into a 100 mL volumetric flask and diluted to volume with distilled water. The stock solution contained 2.92 µg/mL MDA. The stock solution was used for measuring absorbance at 532 nm using UV– 106A spectrophotometer (Shimadzu, Kyoto, Japan). A standard curve was obtained by plotting absorbance

versus concentration of MDA. TBARS were determined according to the method described by Thepnuan et al. (2008). The MDA concentration was calculated from the regression equation derived from the standard curve, and TBARS values expressed using the equation below:

$$\text{TBARS (mg MDA/kg sample)} = \frac{(\text{MDA (mg MDA/mL)} \times 10 \text{ mL})}{(0.002 \text{ kg sample})}$$

Statistical Analysis

Three experimental replicates were conducted. Microbial counts were transformed into log values. 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 Discussion

Total Plate Count and Psychotropic Bacteria Count

Table 1 shows the changes in total aerobic plate count (TPC) and psychotropic bacteria count (PBC) of shrimp dipped in garlic oil and tartaric acid during storage at 4 °C for 10 days. Initial TPC of shrimp prior to treatment ranged from 4.77 – 5.44 log CFU/g. Dipping in TA and TA+GO reduced ($p < 0.05$) the TPC by 1.7 and 1.47 log, respectively. During storage period, TPC of shrimp continued to increase and was highest ($p < 0.05$) in shrimp dipped in dH₂O. At the end of storage period (day 10), shrimp treated with MBS had the lowest TPC (5.31 log CFU/g), followed by shrimp dipped in TA and TA+GO with TPC of 5.85 and 6.09 log CFU/g, respectively. TPC of 7.0 to 8.0 log CFU/g is generally defined as the onset of spoilage for fresh shrimp and The International Commission on Microbiological Specifications for Foods (ICMSF) specified a maximum TPC of 7 log CFU/g for frozen shrimp (ICMSF, 1986). In the present study, TPC for the shrimp samples treated with dH₂O and GO exceeded the permitted level on day 7 and 10, respectively. Maneesin, Chinnasri, Vongsawasdi, & Wangchanachai (2013) observed that TPC of shrimp treated with dH₂O and 1.5 mL/L garlic oil exceeded the recommended limit on day 6 and 11, respectively. Although TPC of MBS treated shrimp were the lowest on day 10, the count was not significantly ($p > 0.05$) different from TA and TA+GO. Hence, all the three treatments resulted in a 3-day extension of shrimp microbial shelf life compared to the control (dH₂O).

The initial psychotropic bacteria counts (PBC) were < 3.30 log CFU/g and the counts did not change much in shrimp subjected to the various treatments. The

Table 1. Total aerobic plate counts and psychotropic bacteria counts of non-inoculated shrimps dipped in tartaric acid (TA), garlic oil (GO), and mixture of TA and GO stored at 4 °C for 10 days

Bacterial Count		Storage period (days)				
		Initial	0	3	7	10
Total plate counts (log CFU/g)	TA	5.44±0.65 Ba	3.74±0.28 Aab	5.15±0.65 Bab	5.60±0.26 Ba	5.85±0.42 Bab
	TA+GO	4.99±0.32 Ba	3.52±0.08 Aa	4.67±0.39 Aba	5.86±0.76 Bab	6.09±0.68 Bab
	GO	5.04±0.57ABa	4.12±0.29 Ab	5.54±0.49 Bab	6.08±0.85 BCab	7.03±0.28 Cb
	MBS	4.77±1.01 ABa	3.89±0.21 Aab	5.20±0.54 Bab	5.37±0.34 Ba	5.31±0.16 Ba
	dH ₂ O	4.91±0.07Aa	4.33±0.19 Ac	6.41±0.31 Bb	7.17±0.38 Bb	8.73±0.40 Cc
Psychotropic bacteria (log CFU/g)	TA	<3.30 Aa	<3.30 Aa	3.66±0.33 Aa	4.38±0.17 Ba	5.11±0.12 Ca
	TA+GO	<3.30 Aa	<3.30 Aa	<3.30 Aa	4.29±0.37 Ba	5.16±0.17 Ca
	GO	<3.30 Aa	<3.30 Aa	3.81±0.08 Aa	4.77±0.44 Bab	5.13±0.06 Ca
	MBS	<3.30 Aa	<3.30 Aa	3.55±0.36 Aba	4.48±0.26 BCa	5.13±0.44 Ba
	dH ₂ O	<3.30 Aa	<3.30 Aa	4.82±0.16 Bb	5.50±0.36 Cb	6.61±0.20 Db

Note: 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 ($p < 0.05$).

TA ; tartaric acid; TA+GO : tartaric acid + garlic oil; GO : garlic oil; MBS ; sodium metabisulfite

psychotropic counts continued to increase during the storage period. At the end of storage period, PBC of shrimp dipped in MBS, TA, GO and TA+GO were significantly lower ($p < 0.05$) than shrimp dipped in dH₂O. The psychotropic counts increased ($p < 0.05$) in shrimp dipped in dH₂O but were still of acceptable quality up to end of storage period (day 10). The Gram-negative psychotropic bacteria are the major group of microorganisms responsible for spoilage of iced stored fish and shellfish (Gram & Huss, 1996). Wan Norhana et al. (2012) reported similar PBC for *Penaeus monodon* treated with organic acids such as potassium sorbate (4.77 log CFU/g), sodium benzoate (4.29 log CFU/g) and sodium diacetate (4.73 log CFU/g) on day 7 of storage at 4 °C. PBC of shrimp dipped in a mixture of TA+GO treated shrimp remained below 3.3 log CFU/g on day 0 and day 3 of storage and was 5.16 log CFU/g on day 10. Similar counts were observed by Nirmal & Benjakul (2012) in white shrimp dipped in green tea and ascorbic acid mixture stored at 4 °C.

L. monocytogenes, *V. parahaemolyticus* and *S. Typhimurium* Counts

Colonies with small grey colonies (1 mm) with a black halo on PALCAM agar were counted as *L. monocytogenes*. Generally, *L. monocytogenes* increased ($p < 0.05$) during the storage period. *L. monocytogenes* counts of shrimp dipped in TA, GO, and TA+GO solutions decreased ($p < 0.05$) on day 0 (Table 2). Mixture of TA+GO was as effective as MBS, as highest reduction of 1.25 log CFU/g was observed. Interestingly, on day 10, *Listeria* counts in shrimp treated with all treatments were significantly ($p < 0.05$) lower than *L. monocytogenes* counts in shrimp dipped

in dH₂O, that indicate TA, GO, TA+GO treatments are effective in reducing the number of *L. monocytogenes* in shrimp.

Wan Norhana, Azman, Poole, Deeth, & Dykes (2009) made a similar observation in *L. monocytogenes* counts in shrimp treated with *Averrhoa bilimbi* (bilimbi extract) and tamarind extracts stored at 4 °C and attributed it to the psychotropic nature of the pathogen. No significant ($p > 0.05$) effect was observed for *L. monocytogenes* counts in shrimp treated with TA or a mixture of TA+GO compared to shrimp dipped in the MBS solution. Rohani, Moradi, Mehdizadeh, Saei-Dehkordi, & Griffiths (2011) observed that garlic oil combined with nisin had a significant anti-listerial activity compared to garlic oil alone, which was also the case in this study.

Red colonies with black centres on XLD agar were identified as *S. Typhimurium*. *S. Typhimurium* counts in shrimp dipped in dH₂O was 5.17 log CFU/g (Table 2). *S. Typhimurium* counts in shrimp dipped in TA+GO and TA decreased ($p < 0.05$) on day 0 compared to *S. Typhimurium* counts shrimp dipped in dH₂O. On day 0, *S. Typhimurium* counts in shrimp treated with different treatment decreased, but as storage progressed, the counts increased ($p < 0.05$). On day 10, the counts of shrimp dipped in dH₂O were much higher compared to other treatments. TA and TA+GO were more effective in reducing ($p > 0.05$) *S. Typhimurium* counts (by 1.76 and 1.6 log CFU/g, respectively) compared to MBS. Over et al. (2009) also observed lower *S. Typhimurium* counts in chicken meat treated with tartaric acid (75 mM and 150 mM) stored at 4 °C. *S. Typhimurium* counts decreased on day 3 for all treated shrimp samples except those dipped

Table 2. *L. monocytogenes*, *S. Typhimurium* and *V. parahaemolyticus* counts of shrimps dipped in tartaric acid (TA), garlic oil (GO), and mixture of TA and GO stored at 4 °C for 10 days

Test bacteria		Storage period (days)			
		0	3	7	10
<i>L. monocytogenes</i> (log CFU/g)	TA	3.48 ± 0.01 ^a Aa	3.91 ± 0.25 ^a Aa	5.16 ± 0.56 ^b Bab	5.32 ± 0.17 ^b Ba
	TA+GO	3.48 ± 0.00 ^a Aa	3.73 ± 0.18 ^b Aa	5.06 ± 0.48 ^b Bab	5.30 ± 0.21 ^b Ba
	GO	3.51 ± 0.04 ^a Aa	4.02 ± 0.24 ^b Aa	5.58 ± 0.66 ^b Bab	5.81 ± 0.41 ^b Ba
	MBS	3.48 ± 0.00 ^a Aa	3.53 ± 0.08 ^a Aa	4.37 ± 0.94 ^a ABa	5.04 ± 0.11 ^b Ba
	dH ₂ O	4.73 ± 0.30 ^b Ab	4.69 ± 0.27 ^{ab} Ab	6.53 ± 0.27 ^b Bb	7.60 ± 0.61 ^b Cb
<i>S. Typhimurium</i> (log CFU/g)	TA	4.07 ± 0.41 ^a ABab	3.60 ± 0.21 ^a Aa	4.09 ± 0.24 ^a ABab	4.58 ± 0.21 ^a Ba
	TA+GO	3.58 ± 0.24 ^a Aa	3.42 ± 0.11 ^a Aa	3.82 ± 0.23 ^a Aa	4.59 ± 0.23 ^{ab} Ba
	GO	4.63 ± 0.61 ^b Bbc	3.57 ± 0.07 ^a Aa	4.54 ± 0.13 ^{ab} Bb	4.92 ± 0.26 ^{ab} Ba
	MBS	4.77 ± 0.34 ^b Bbc	3.48 ± 0.50 ^a Aa	4.51 ± 0.41 ^a ABb	4.74 ± 0.15 ^{ab} Ba
	dH ₂ O	5.17 ± 0.17 ^b Ac	4.98 ± 0.39 ^b Ab	5.20 ± 0.09 ^a Ac	6.34 ± 0.21 ^a Bb
<i>V. parahaemolyticus</i> (log CFU/g)	TA	3.48 ± 0.01 ^a Aa	3.59 ± 0.08 ^a ABa	3.95 ± 0.21 ^a Ba	4.40 ± 0.24 ^a Ca
	TA+GO	3.48 ± 0.00 ^a Aa	3.47 ± 0.10 ^{ab} Aa	3.60 ± 0.06 ^a Aa	4.36 ± 0.32 ^a Ba
	GO	3.48 ± 0.01 ^a Aa	3.73 ± 0.17 ^{ab} Aab	4.06 ± 0.67 ^a Aa	4.52 ± 0.68 ^a Aab
	MBS	3.55 ± 0.11 ^a Aa	3.63 ± 0.08 ^a Aa	3.71 ± 0.04 ^a Aa	4.61 ± 0.22 ^a Bab
	dH ₂ O	3.87 ± 0.22 ^a Ab	3.99 ± 0.14 ^a Ab	4.59 ± 0.46 ^a Aa	5.55 ± 0.46 ^a Bb

Note: 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 ($p < 0.05$)

Mean values within the same test bacteria with different superscript were significantly different ($p < 0.05$).

TA ; tartaric acid; TA+GO : tartaric acid + garlic oil; GO : garlic oil; MBS ; sodium metabisulfite

in dH₂O. Similar observation was made by Wan Norhana et al. (2009) in shrimp samples inoculated with *S. Typhimurium* and treated with bilimbing extract and tamarind juice.

Blue-green colonies (2-3 mm) on TCBS agar plates were calculated as *V. parahaemolyticus*. A reduction of *V. parahaemolyticus* counts was observed in shrimp treated with the treatments on day 0 except for shrimp dipped in dH₂O. The treatments were as effective as MBS in reducing *V. parahaemolyticus* counts (Table 2). *V. parahaemolyticus* counts continued to increase ($p < 0.05$) in all shrimp samples during the storage period. On day 10, *V. parahaemolyticus* counts of

shrimp treated with dH₂O reached 5.55 log CFU/g. *V. parahaemolyticus* counts of shrimp treated with a mixture of TA+GO and TA decreased ($p < 0.05$) by 1.19 and 1.15 log CFU/g, respectively.

In general, TA, TA+GO, and GO treatment reduces the number of bacterial counts on shrimp immediately after treatment compared to the untreated shrimp. Most organic acids elicit antibacterial activity by reducing environmental and cellular pH (Carpenter & Broadbent, 2009). Due to the neutral pH of the cytoplasm, the acid dissociates into anions and protons. These molecules are not able to diffuse back across the cell membrane and hence accumulate in the cytoplasm.

Table 3 Changes in pH of shrimp dipped in tartaric acid (TA), garlic oil (GO), mixture of TA and GO, sodium metabisulfite (MBS) and sterile distilled water (dH₂O) stored at 4°C for 10 days.

Treatments	Storage period (days)			
	0	3	7	10
TA	6.69 ± 0.13 Aab	7.40 ± 0.22 Ba	7.87 ± 0.06 Cab	7.94 ± 0.09 Ca
TA+GO	6.64 ± 0.11 Aa	7.36 ± 0.24 Ba	7.92 ± 0.08 Cb	7.90 ± 0.08 Ca
GO	6.88 ± 0.08 Ab	7.47 ± 0.24 Ba	7.96 ± 0.07 Cb	8.03 ± 0.09 Ca
MBS	6.80 ± 0.20 Aab	7.44 ± 0.10 Ba	7.79 ± 0.05 Ca	7.90 ± 0.09 Ca
dH ₂ O	6.76 ± 0.14 Aab	7.26 ± 0.13 Ba	7.90 ± 0.08 Cab	8.03 ± 0.07 Ca

Note: 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 ($p < 0.05$)

TA ; tartaric acid; TA+GO : tartaric acid + garlic oil; GO : garlic oil; MBS ; sodium metabisulfite

Table 4 Changes in TBARS of shrimp dipped in tartaric acid (TA), garlic oil (GO), mixture of TA and GO, sodium metabisulfite (MBS) and sterile distilled water (dH₂O) stored at 4°C for 10 days

Treatments	Storage period (days) (mg malonaldehyde/kg)			
	0	3	7	10
TA	1.14 ± 1.11 Aa	0.98 ± 0.78 Aa	1.45 ± 0.23 Aa	2.08 ± 0.63 Aa
TA+GO	0.70 ± 0.39 Aa	0.90 ± 0.09 Aa	1.39 ± 0.43 ABa	2.00 ± 0.60 Ba
GO	0.97 ± 0.33 Aa	0.99 ± 0.36 Aa	1.56 ± 0.35 ABa	2.38 ± 0.57 Ba
MBS	0.96 ± 0.42 Aa	1.04 ± 0.16 Aa	1.36 ± 0.19 Aa	1.83 ± 0.64 Aa
dH ₂ O	1.05 ± 0.68 Aa	1.36 ± 0.25 Aa	1.86 ± 0.22 ABa	2.66 ± 0.44 Ba

Note: 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 ($p < 0.05$).

TA : tartaric acid; TA+GO : tartaric acid + garlic oil; GO : garlic oil; MBS : sodium metabisulfite

Acidification of the cytoplasm and the energy depletion leads to physiological malfunction finally inhibiting microbial growth (Hazan, Levine, & Abeliovich, 2004). This could also be the case for tartaric acid, where the lower pH of the shrimp was achieved through TA addition. The antibacterial mechanism of GO is probably due to the two GO constituents, diallyl disulfide and diallyl trisulfide that inhibit key metabolic enzymes of microorganism (Harris, Cottrell, Plummer, & Lloyd, 2001; Benkeblia, 2004). On the other hand, the inhibitory action of metabisulfite is based essentially on various forms of interference in the enzyme structure particularly the key enzymes in living cells such as those involved in ATP and NADH production leading to cell death (Frag, 1997).

pH Measurement

The changes in pH of shrimp subjected to garlic oil and tartaric acid treatment during storage at 4 °C are shown in Table 3. The initial pH of shrimp immediately after dipping (day 0) in dH₂O was 6.76 and was not different ($p < 0.05$) from pH of shrimp dipped in TA+GO and treated with GO. The pH of all shrimp samples continued to increase throughout the storage period. On day 10, pH of shrimp dipped in MBS and TA+GO was 7.90 and the lowest among the other treatments.

The pH continued to increase ($p < 0.05$) throughout the storage period and was in the range of 7.90 – 8.3 on day 10. The increase in pH could probably be due to accumulation of ammonia and amines formed during endogenous enzymatic reactions as reported in previous research. Nirmal & Benjakul (2012) reported an increase in pH of white shrimp treated with green tea extract and ascorbic acid stored at 4 °C. Attala (2012) also reported that the pH of shrimp treated with 3% citric acid and 2% sodium sulphite ranged from 6.4 - 6.47 and 7.59 - 7.84, respectively. However, the pH of shrimp at the end of the storage period in this study was higher than the pH of good quality shrimp, that is, 7.7 (Mehmet, Faruk, & Hami, 2009). The treatment of shrimp with TA, GO, and MBS possibly reduced

the number of bacteria by slowing down the endogenous enzymatic reactions. However, pH alone is not the major factor involved in antimicrobial activity of organic acids as other mechanisms could also be responsible.

Thiobarbituric Acid Reactive Substances (TBARS)

Malondialdehyde is the best-investigated lipid peroxidation product and the prototype of the so-called TBARS (Devasagayam, Boloor, & Ramaasarma, 2003; Tsikas, 2017). TBARS are formed as a by-product of lipid oxidative damage (i.e., as degradation products of fats) and can be detected by the TBARS assay using TBA as a reagent. TBARS analysis is an important food quality index indicating fat oxidation. TBARS values should be less than 3 mg malonaldehyde/kg in good quality products (Cadun, Kislá, & Cakh, 2008). Changes in TBARS values in shrimp treated with garlic oil and tartaric acid during storage at 4 °C are shown in Table 4. Generally, shrimp treated with TA or GO or in combination was in good quality and not rancid. On day 0, TBARS values of shrimp treated with TA+GO were the lowest. TBARS values increased in shrimp treated with TA+GO, GO and dH₂O as storage progressed. On day 10, TBARS values for shrimp treated with MBS (1.83 ± 0.64 MDA/kg) were the lowest followed by TA+GO (2.00 ± 0.60 MDA/kg) and TA (2.08 ± 0.63 MDA/kg) while shrimp treated with dH₂O had the highest values (2.66 ± 0.44 MDA/kg). However, TBARS values recorded for both treated and untreated shrimp were not significantly ($p > 0.05$) different. The TBARS values recorded in this study is in agreement with Ainaz (2019) and Fang, Sun, Huang, & Yuan (2013) in *Metapenaeus affinis* and Pacific white shrimp (*Litopenaeus vannamei*) stored at chilled temperature, respectively. The increase of TBARS through time was in agreement with the microorganism activity during storage, resulting in partial dehydration of shrimp tissue containing highly polyunsaturated fatty acid. The damage of tissues and interacting lipids with

air oxygen may induce lipid oxidation. A similar observation has been described by Kilincceker, Dogan, and Kucukoner (2009) and Fang et al. (2013).

The treatments with TA and GO (alone and in combination) have no significant ($p < 0.05$) effect on lipid oxidation of fresh shrimp in this study. Perhaps, storage for just 10 days was not long enough for rancidity to set. In this study, TBARS values on day 10 for treated shrimp were still below the recommended level of 7.0 mg malonaldehyde/kg for good quality shrimp as proposed by Cadun et al. (2008).

Conclusion

Dipping of fresh shrimp in GO and TA influenced TPC, PBC, *L. monocytogenes*, *S. Typhimurium*, *V. parahaemolyticus* and TBARS values during the storage at 4°C. Compared to sodium metabisulfite, the mixture of TA+GO was more effective in inhibiting the growth of *S. Typhimurium* and *V. parahaemolyticus*. In terms of rancidity, the changes in TBARS values of shrimp treated with tartaric acid and sodium metabisulfite remained low during storage period. TBARS values of treatment and control shrimp did not differ from each other throughout the storage period except on day 0. A mixture of TA+GO solutions can be used to reduce the growth of *S. Typhimurium* and *V. parahaemolyticus* in shrimp during storage, which could reduce the incidence of foodborne pathogens and diseases.

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