Effects of organic acid treatments on selected quality parameters of green mussels (*Perna viridis*) during cold storage

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ABSTRACT Green mussels (*Perna viridis*), a bivalve, are found in Malaysia and other regions of Asia-Pacific. They are rich in protein, polyunsaturated fatty acids, minerals and vitamins. However, green mussels are subject to rapid post-harvest quality deterioration that leads to spoilage and safety concerns. Effective post-harvest treatments are therefore critical in the quality preservation of green mussels. In the present study, the effects of organic acids (lactic acid, oxalic acid, and tartaric acid), either single-acid or combination-acid at 1.0% concentration, on the quality of green mussels under cold storage (4 ± 1 °C) for 20 days were studied. Untreated mussels were used as the control. All organic acid-treated mussels showed lower total viable counts and psychrotrophic bacteria counts than the control throughout the cold storage. The application of organic acids reduced the production of volatile nitrogenous compounds resulting in lower total volatile base-nitrogen (TVB-N) and trimethylamine (TMA) contents in treated mussels. In conclusion, organic acid treatments exhibited effective antimicrobial activity and reduced the production of volatile nitrogenous compounds during cold storage, suggesting their potential to preserve the quality and extend the shelf life of green mussels.

KEYWORDS: Cold storage; Green mussel; Organic acid; Quality
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INTRODUCTION

Green mussels (*Perna viridis*) are widely distributed in the coastal waters of Indo-Pacific regions, from the Persian Gulf, India, and Bangladesh, to Southeast Asia (Baker *et al.*, 2007). They are valuable sources of protein, vitamins, and minerals (Chakraborty *et al.*, 2016). Their lipids are also rich in polyunsaturated fatty acids (PUFA), notably eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are important in decreasing the risk of cardiovascular diseases (Bindu *et al.*, 2015). Post-harvest mussels are highly perishable due to both microbiological and enzymatic spoilage. The high muscle water activity (a_w > 0.95) and pH value (6.7 to 7.1), coupled with their high amino acid content and abundant PUFA, render them an ideal substrate for the growth of microorganisms (Goulas *et al.*, 2005). Consequently, mussels typically have a limited shelf life. Currently, there is a growing trend of consuming fresh, nutrient-rich, and minimally processed food products that are safe and possess high organoleptic quality (Prapaiwong *et al.*, 2009). Therefore, it is crucial to adopt effective preservation strategies to extend the shelf life of green mussels while retaining their remarkable nutritional value and typical sensory characteristics.

Organic acids are approved by the Federal Drug Administration (FDA) as generally recognized as safe (GRAS). They are commonly used in food products at a wide range of permitted concentrations due to their low commercial cost and ease of handling (Hassan *et al.*, 2015). They have been proven to be effective antimicrobial agents by retarding microorganism growth. For example, lactic acid exhibited bactericidal properties in studies on seafood and poultry products (Smyth *et al.*, 2018; Fernández *et al.*, 2021; Mohamed & Ammar, 2021). Oxalic acid also showed an inhibitory effect on the proliferation of bacteria and pathogens on chicken meat (Anang *et al.*, 2015). Besides, tartaric acid

has been found to be effective against pathogens such as *Salmonella* Typhimurium (Over *et al.*, 2009) and *Campylobacter jejuni* (Boysen *et al.*, 2013) on broiler meat.

The present study focused on preserving the quality of green mussels through the application of organic acid treatments. The objective was to investigate the effects of both single-acid and combination-acid treatments on selected quality parameters of green mussel flesh under cold storage. Previous studies focused on the effects of single-acid treatments (i.e., individual acids), whereas the novelty of the present study lies in the application of combination-acid treatments (i.e., combination of acids) to retard the spoilage process and subsequently extend shelf life during cold storage. The antimicrobial properties and physicochemical quality parameters of green mussels were evaluated, including total viable count (TVC), psychrotrophic bacteria count (PBC), pH, drip loss, total volatile base-nitrogen (TVB-N) content, and trimethylamine (TMA) content.

METHODOLOGY

Research Design

Green mussels were purchased from a farmer in Ambong Bay, Sabah, Malaysia. They were immediately transported in a plastic container to the laboratory at the Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, arriving within two hours of purchase. The study was divided into two stages. Stage I involved the screening of eight organic acids (ascorbic acid, butyric acid, formic acid, lactic acid, malic acid, oxalic acid, propionic acid, and tartaric acid) at two concentrations (1% and 2%). This screening was performed using sensory evaluation (hedonic test) and a microbiological test (total viable count). Stage II involved a series of analyses investigating the efficacy of the three selected organic acids based on the results in Stage I (data not shown). Lactic acid, oxalic acid, and tartaric acid, each at a 1% concentration, were selected due to their higher antimicrobial efficiency (i.e., resulting in lower total viable counts) compared to the other organic acids, as well as their minimal sensorial effects (i.e., resulting in smaller reductions in sensorial acceptability) on the mussels. A total of seven treatments were applied in Stage II, including three single-acid treatments (lactic acid [LA], oxalic acid [OX], and tartaric acid [TA]) and four combination-acid treatments (lactic acid combined with oxalic acid [LA+OX], lactic acid combined with tartaric acid [LA+TA], oxalic acid combined with tartaric acid [OX+TA], and the combination of lactic, oxalic, and tartaric acids [LA+OX+TA]). In the laboratory, green mussels with a shell length of 8.0-12.0 cm (marketable size) were selected and manually shucked. The shucked mussel flesh was fully immersed in the prepared organic acid solutions for five min at room temperature (Arcales & Nacional, 2019). The mussel-to-solution ratio was fixed at 1:2 (w/v) during the treatments to minimize significant pH dilution of the solutions. Following immersion, the mussel flesh was removed from the solutions and drained for 10 min. Each mussel sample was individually heat-sealed in a polyethylene bag and stored at 4 ± 1 °C for 20 d. Mussels without organic acid treatment served as the control. Samples were withdrawn every 5 d for subsequent quality-related analyses.

Microbiological Analyses

The growth of microorganisms during cold storage was assessed using total viable count (TVC) and psychrotrophic bacteria count (PBC). Both counts were performed using the pour plate method on plate count agar (Aru *et al.*, 2016). TVC plates were incubated at 28 ± 1 °C for 72 h (ISO, 2003), while PBC plates were incubated at 6 ± 1 °C for 7 d (ISO, 2019).

Drip Loss and pH

Drip loss was calculated based on the difference in weight of the mussels before and after cold storage and was expressed as a percentage of the initial mussel weight. For pH determination, 10 g of mussels were homogenised in 90 mL of distilled water and measured using a pH meter.

Total Volatile Base-Nitrogen (TVB-N) Assay

The total volatile base-nitrogen (TVB-N) content was analysed using the steam distillation method (Commission Regulation (EC) No 2074/2005). This method specifically measures the volatile base nitrogen compounds extracted from the samples with perchloric acid. Following the initial extraction, sodium hydroxide was added to alkalise the extracted samples. The steam distillation process was then conducted using a semi-automated steam distillation unit (Gerhardt VAPODEST 30s, Germany). The resulting volatile compounds were collected in a boric acid receiver solution. The quantification of TVB-N content was achieved through a titrimetric approach and calculated using Equation (1), where V_1 is the titration volume of the sample (mL); V_2 is the titration volume of the blank sample (mL); and m is the weight of the green mussels (g).

$$\text{TVB-N (mg/100 g)} = \frac{(V_1 - V_2) \times 0.14 \times 2}{\text{m}} \times 100 \quad (1)$$

Trimethylamine (TMA) Assay

The extraction and determination of trimethylamine (TMA) content in green mussels were performed according to AOAC 971.14 (AOAC, 2000). TMA was extracted by adding trichloroacetic acid to the samples, followed by centrifugation at 12,000 rpm for 10 min at 4 °C. The extracted TMA was subsequently measured at a wavelength of 410 nm against a reference blank using a spectrophotometer. Trimethylamine hydrochloride served as the standard for TMA quantification. The TMA content in the samples was determined by plotting a calibration curve of the TMA standard solution and was expressed as mg TMA/100 g.

Statistical Analysis

All analyses were conducted in triplicate. The data were analysed using one-way analysis of variance (ANOVA) using the SPSS version 22.0 software program, and the significance level set at $p<0.05$. Tukey's honestly significant difference (HSD) test was performed as the post-hoc procedure. Pearson's correlation was conducted to examine the relationship between the quality parameters.

RESULTS AND DISCUSSION

Microbiological Analyses

The changes in total viable count (TVC) and psychrotrophic bacteria count (PBC) of the green mussels during cold storage are shown in Table 1. Both TVC and PBC increased significantly ($p<0.05$) throughout the storage period. The control recorded the highest TVC and PBC over the storage period, ranging from 3.55 to 8.58 $\log_{10}$ CFU/g and 2.99 to 8.50 $\log_{10}$ CFU/g, respectively. All organic acid-treated samples exhibited significantly lower bacterial counts than the control ($p<0.05$), demonstrating the antimicrobial efficacy of the treatments. This microbial reduction is attributed to the antimicrobial mechanism of organic acids. The lipophilic nature of undissociated organic acids allows their free diffusion across microbial cell membranes (Amrutha *et al.*, 2017). The undissociated forms of organic acids dissociate once inside microbial cells and accumulate within them. This dissociation causes the release of protons and the accumulation of anions. The released protons reduce the internal cytoplasmic pH, while the accumulated anions disrupt the normal physiological

functions, including ATP regeneration system and the synthesis of protein, DNA or RNA (Leon Peláez *et al.*, 2012). Collectively, these changes inhibit microbial growth and ultimately cause cell death.

Table 1. Changes in microbial counts in green mussels during cold storage.

	Treatment*	Day 0	Day 5	Day 10	Day 15	Day 20
TVC (log_{10} CFU/g)	Control	3.55±0.08^{Ea}	5.66±0.02^{Da}	6.58±0.06^{Ca}	7.50±0.06^{Ba}	8.58±0.06^{Aa}
	LA	3.25± 0.06^{Eb}	3.44±0.10^{Dd}	4.43±0.02^{Cd}	5.19±0.01^{Be}	6.23±0.03^{Ade}
	OX	3.17±0.01^{Eb}	3.56±0.05^{Dcd}	4.16±0.05^{Ce}	5.42±0.04^{Bd}	6.13±0.06^{Ae}
	TA	3.16±0.03^{Eb}	3.66±0.09^{Dbc}	4.76±0.08^{Cc}	5.37±0.07^{Bd}	6.24±0.03^{Ade}
	LA+OX	3.12±0.01^{Eb}	3.80±0.03^{Db}	4.64±0.03^{Cc}	6.19±0.04^{Bb}	7.21±0.01^{Ab}
	LA+TA	3.19±0.01^{Eb}	3.49±0.09^{Dd}	4.55±0.07^{Ccd}	5.57±0.08^{Bc}	6.30±0.06^{Ad}
	OX+TA	3.00±0.10^{Ec}	3.40±0.03^{Dd}	4.41±0.10^{Cd}	5.40±0.06^{Bd}	6.12±0.13^{Ae}
	LA+OX+TA	3.21±0.03^{Eb}	3.43±0.05^{Dd}	5.15±0.15^{Cb}	6.20±0.02^{Bb}	7.07±0.05^{Ac}
PBC (log_{10} CFU/g)	Control	2.99±0.04^{Ea}	4.48±0.02^{Da}	6.65±0.05^{Ca}	7.64±0.04^{Ba}	8.50±0.04^{Aa}
	LA	2.25±0.14^{Eb}	3.23±0.06^{Db}	4.56±0.01^{Cb}	5.60±0.04^{Bb}	6.52±0.07^{Ab}
	OX	1.93±0.10^{Ebc}	3.11±0.05^{Db}	3.84±0.10^{Cd}	4.56±0.07^{Be}	5.68±0.02^{Ae}
	TA	1.62±0.21^{Ecd}	2.94±0.10^{Db}	4.20±0.09^{Cc}	5.22±0.08^{Bcd}	6.54±0.14^{Ab}
	LA+OX	1.68±0.03^{Ecd}	2.48±0.32^{Dc}	4.70±0.03^{Cb}	5.33±0.09^{Bcd}	6.43±0.17^{Abc}
	LA+TA	1.79±0.15^{Ec}	2.62±0.18^{Dc}	4.22±0.06^{Cc}	5.35±0.07^{Bc}	6.47±0.15^{Abc}
	OX+TA	1.40±0.31^{Ed}	2.54±0.08^{Dc}	3.62±0.10^{Ce}	4.69±0.03^{Be}	6.14±0.06^{Ad}
	LA+OX+TA	1.81±0.03^{Ec}	3.16±0.05^{Db}	4.55±0.08^{Cb}	5.18±0.04^{Bd}	6.24±0.03^{Acd}

^{a,b} Different superscripts in the same column (i.e., different samples at the same storage duration) indicate a significant difference ($p<0.05$).

^{A,B} Different superscripts in the same row (i.e., the same sample at different storage durations) indicate a significant difference ($p<0.05$).

LA – lactic acid; OX – oxalic acid; TA – tartaric acid.

The organic acid treatments resulted in significant reductions of up to 2 log_{10} CFU/g in both TVC and PBC after 20 days of cold storage. For example, the TVC reductions were 2.35 log_{10} CFU/g for LA, 2.45 log_{10} CFU/g for OX, 2.34 log_{10} CFU/g for TA, 2.28 log_{10} CFU/g for LA+TA, and 2.46 log_{10} CFU/g for OX+TA. Similarly, PBC reductions were 2.82 log_{10} CFU/g for OX, 2.07 log_{10} CFU/g for LA+OX, 2.03 log_{10} CFU/g for LA+TA, 2.36 log_{10} CFU/g for OX+TA, and 2.26 log_{10} CFU/g for LA+OX+TA. The combination-acid treatments generally exhibited a synergistic effect in reducing PBC during cold storage. Among the single-acid treatments, OX was the most effective treatment, while LA and TA exhibited comparable antimicrobial effects. The higher inhibitory effect of OX compared to LA is primarily attributed to its higher pK_a value (4.27) than LA (3.86). This higher pK_a value favours OX to exist in the undissociated acid form more readily than LA, thereby facilitating its diffusion across microbial cell membranes. Moreover, the low molecular weight of OX (MW = 90.04 g/mol) also contributed to its higher antimicrobial activity than TA (MW = 150.09 g/mol), enhancing its ability to enter microorganisms and reduce cytoplasmic pH.

No specific acceptable limits have been established for TVC and PBC in green mussels. The Malaysian Food Regulations 1985, Fifteenth Schedule (Regulation 39), set a limit of 6 log_{10} CFU/g for the total plate count in fish and fish products ready for consumption. The results show that the control reached this threshold between five and ten days of cold storage, whereas most treated

samples (except LA+OX and LA+OX+TA) reached the limit between 15 and 20 days. These findings suggest that the organic acid treatments extended the microbiological shelf life of green mussels from 5–10 days to 15–20 days under cold storage.

Drip Loss and pH

Table 2 shows the changes in drip loss of green mussels during cold storage. The drip loss of both control and treated samples increased with the storage period, indicating water loss as exudate from the mussels. However, no significant differences in drip loss were observed when comparing the treated samples to the control, or among the treatments themselves, throughout the cold storage period. These results suggest that organic acid treatments, whether single-acid or combination-acid, were not effective in reducing water loss from mussels during cold storage.

Table 2. Changes in drip loss and pH of green mussels during cold storage.

	Treatment*	Day 0	Day 5	Day 10	Day 15	Day 20
Drip loss (%)	Control	-	9.43 ± 0.46^{Bbc}	11.22 ± 0.91^{Ba}	14.65 ± 0.63^{Aa}	13.86 ± 1.56^{Aa}
	LA	-	12.73 ± 1.47^{Aa}	13.56 ± 0.89^{Aa}	13.20 ± 0.46^{Aa}	14.33 ± 1.17^{Aa}
	OX	-	8.21 ± 0.92^{Bc}	12.78 ± 1.30^{Aa}	14.18 ± 1.59^{Aa}	12.84 ± 0.60^{Aa}
	TA	-	7.76 ± 0.58^{Cc}	10.32 ± 1.15^{Ba}	12.02 ± 1.15^{ABa}	12.95 ± 0.27^{Aa}
	LA+OX	-	10.28 ± 0.20^{Aabc}	12.54 ± 0.64^{Aa}	11.61 ± 2.33^{Aa}	13.01 ± 1.07^{Aa}
	LA+TA	-	11.71 ± 1.34^{Bab}	10.84 ± 0.60^{Ba}	12.73 ± 1.29^{Ba}	15.60 ± 0.98^{Aa}
	OX+TA	-	11.56 ± 1.32^{Bab}	11.18 ± 0.55^{Ba}	13.10 ± 1.47^{ABa}	15.83 ± 1.31^{Aa}
	LA+OX+TA	-	8.24 ± 1.29^{Bc}	12.97 ± 2.43^{Aa}	13.35 ± 1.72^{Aa}	14.51 ± 0.93^{Aa}
pH	Control	6.92 ± 0.02^{ABa}	6.95 ± 0.01^{Aa}	6.91 ± 0.03^{ABa}	6.89 ± 0.03^{Ba}	6.95 ± 0.02^{Aa}
	LA	6.88 ± 0.03^{ABab}	6.89 ± 0.02^{ABbc}	6.86 ± 0.01^{Bb}	6.85 ± 0.01^{Babc}	6.92 ± 0.02^{Aab}
	OX	6.80 ± 0.03^{Ac}	6.85 ± 0.02^{Ac}	6.83 ± 0.02^{Ab}	6.82 ± 0.02^{Ac}	6.85 ± 0.01^{Ac}
	TA	6.84 ± 0.02^{Bbc}	6.90 ± 0.01^{Ab}	6.88 ± 0.03^{ABab}	6.87 ± 0.02^{ABab}	6.90 ± 0.02^{Aab}
	LA+OX	6.84 ± 0.02^{Bbc}	6.86 ± 0.03^{Bbc}	6.84 ± 0.02^{Bb}	6.85 ± 0.01^{Babc}	6.92 ± 0.03^{Aab}
	LA+TA	6.86 ± 0.02^{Babc}	6.88 ± 0.02^{ABbc}	6.88 ± 0.01^{ABab}	6.85 ± 0.01^{Babc}	6.91 ± 0.03^{Aab}
	OX+TA	6.87 ± 0.02^{Aabc}	6.86 ± 0.02^{Abc}	6.85 ± 0.02^{Ab}	6.86 ± 0.01^{Aabc}	6.88 ± 0.01^{Abc}
	LA+OX+TA	6.82 ± 0.04^{Cbc}	6.88 ± 0.02^{ABbc}	6.85 ± 0.01^{BCb}	6.84 ± 0.01^{BCbc}	6.92 ± 0.02^{Aab}

[a,b] Different superscripts in the same column (i.e., different samples at the same storage duration) indicate a significant difference ($p<0.05$).

[A,B] Different superscripts in the same row (i.e., the same sample at different storage durations) indicate a significant difference ($p<0.05$).

LA – lactic acid; OX – oxalic acid; TA – tartaric acid.

The pH values increased slightly ($p<0.05$) during cold storage (Table 2). The increase in pH was attributed to the production of volatile basic nitrogenous compounds resulting from either endogenous enzymatic reactions or bacterial accumulation (Masniyom *et al.*, 2011). Overall, the present study showed a general increase in pH, approaching neutrality (pH 7.0) by the end of the storage period. These results suggest that the antimicrobial activity of the organic acids diminished over prolonged storage. This decline in efficacy was due to a decrease in the concentration of the undissociated acid form in the near-neutral environment, making the acids less effective in diffusing across microbial membrane to exhibit inhibitory action.

Total Volatile Base-Nitrogen (TVB-N)

TVB-N serves as a spoilage indicator for quantifying the volatile nitrogenous compounds associated with seafood degradation. Table 3 presents the changes in TVB-N content of green mussels during cold storage. The TVB-N content of both control and treated samples increased significantly ($p<0.05$) throughout the storage period, indicating the progressive production of volatile basic nitrogenous compounds. The results show that the control exhibited a higher TVB-N content than the treated samples ($p<0.05$), increasing from 8.20 to 43.71 mg/100 g by the end of storage. Organic acid treatments effectively lowered the TVB-N content in the treated samples, which ranged from 29.14 to 36.62 mg/100 g on day 20. Goulas *et al.* (2005) have recommended an acceptability limit of 22-25 mg/100 g for mussels. The control and most treated samples reached this limit between five and ten days of cold storage. However, the TVB-N contents of the treated samples were significantly lower than those of the control. Notably, OX+TA reached this limit only after 15 days. The reduction in TVB-N indicates that the application of organic acid treatments attenuated the formation of volatile basic nitrogenous compounds. These volatile basic nitrogenous compounds are generated either by autolysis or through microbial action. For example, trimethylamine (TMA) is produced by bacteria, while ammonia is formed through the deamination of free amino acids by bacterial enzymes (Masniyom & Benjama, 2007; Tavares *et al.*, 2021). Therefore, the antimicrobial effects of organic acid treatments directly reduced the subsequent production of these volatile basic nitrogenous compounds in the samples. Many studies have also reported that fishery and poultry products treated with organic acids consistently showed lower TVB-N contents compared to untreated controls (Ramanathan *et al.*, 2011; Khalafalla *et al.*, 2016; Mohamed & Ammar, 2021).

Table 3. Changes in volatile nitrogenous compounds in green mussels during cold storage.

	Treatment*	Day 0	Day 5	Day 10	Day 15	Day 20
TVB-N (mg/100 g)	Control	8.20 ± 0.15^{Ea}	20.66 ± 0.16^{Da}	30.62 ± 0.13^{Ca}	35.24 ± 0.06^{Ba}	43.71 ± 0.08^{Aa}
	LA	6.08 ± 0.18^{Ed}	17.69 ± 0.09^{De}	25.29 ± 0.12^{Ce}	27.26 ± 0.16^{Be}	33.70 ± 0.17^{Af}
	OX	7.89 ± 0.15^{Ea}	18.45 ± 0.11^{Dd}	23.92 ± 0.08^{Cf}	28.00 ± 0.12^{Bd}	34.22 ± 0.15^{Ae}
	TA	6.23 ± 0.12^{Ed}	18.89 ± 0.10^{Dc}	27.04 ± 0.22^{Cc}	29.49 ± 0.12^{Bb}	36.62 ± 0.20^{Ab}
	LA+OX	5.19 ± 0.12^{Ee}	16.84 ± 0.18^{Df}	26.60 ± 0.18^{Cd}	27.34 ± 0.19^{Be}	29.14 ± 0.09^{Ah}
	LA+TA	4.84 ± 0.12^{Ef}	18.72 ± 0.16^{Dcd}	27.01 ± 0.10^{Cc}	28.59 ± 0.17^{Bc}	35.80 ± 0.16^{Ac}
	OX+TA	7.03 ± 0.14^{Ec}	19.59 ± 0.17^{Db}	22.53 ± 0.09^{Cg}	24.28 ± 0.19^{Bf}	34.86 ± 0.13^{Ad}
	LA+OX+TA	7.56 ± 0.13^{Eb}	18.83 ± 0.12^{Dc}	27.43 ± 0.10^{Cb}	28.34 ± 0.15^{Bc}	32.90 ± 0.06^{Ag}
TMA (mg TMA/100 g)	Control	0.95 ± 0.05^{Ea}	2.95 ± 0.04^{Da}	4.01 ± 0.04^{Ca}	5.09 ± 0.09^{Ba}	5.64 ± 0.04^{Aa}
	LA	0.82 ± 0.08^{Eabc}	2.19 ± 0.06^{Def}	3.61 ± 0.05^{Cc}	4.02 ± 0.09^{Bc}	4.80 ± 0.05^{Acd}
	OX	0.88 ± 0.01^{Eab}	2.27 ± 0.03^{Def}	3.79 ± 0.04^{Cb}	3.92 ± 0.04^{Bc}	4.60 ± 0.07^{Ae}
	TA	0.85 ± 0.07^{Eabc}	2.46 ± 0.04^{Dcd}	3.84 ± 0.04^{Cb}	4.21 ± 0.02^{Bb}	5.26 ± 0.08^{Ab}
	LA+OX	0.74 ± 0.06^{Ebc}	2.75 ± 0.01^{Db}	3.61 ± 0.02^{Cc}	4.23 ± 0.04^{Bb}	5.28 ± 0.07^{Ab}
	LA+TA	0.75 ± 0.07^{Ebc}	2.32 ± 0.07^{Dde}	3.56 ± 0.06^{Cc}	4.34 ± 0.04^{Bb}	4.95 ± 0.04^{Ac}
	OX+TA	0.68 ± 0.09^{Ec}	2.52 ± 0.05^{Dc}	3.45 ± 0.04^{Cd}	4.36 ± 0.07^{Bb}	4.89 ± 0.03^{Acd}
	LA+OX+TA	0.72 ± 0.10^{Ebc}	2.15 ± 0.07^{Df}	3.53 ± 0.04^{Ccd}	4.26 ± 0.02^{Bb}	4.78 ± 0.09^{Ad}

[a,b] Different superscripts in the same column (i.e., different samples at the same storage duration) indicate a significant difference ($p<0.05$).

[A,B] Different superscripts in the same row (i.e., the same sample at different storage durations) indicate a significant difference ($p<0.05$).

LA – lactic acid; OX – oxalic acid; TA – tartaric acid.

Trimethylamine (TMA)

TMA is produced through the decomposition of trimethylamine N-oxide (TMAO) by the bacterial TMAO reductase. Its accumulation within muscle tissue is responsible for the typical "fishy" off-odour characteristic of spoiling seafood products (Velammal *et al.*, 2017). Table 3 presents the changes in TMA content of green mussels during cold storage. For the control, TMA content increased significantly ($p<0.05$) over the storage period, from 0.95 to 5.64 mg TMA/100 g. All treated samples exhibited significant ($p<0.05$) increasing trends, but their TMA contents remained lower than those of the control throughout the storage period. On day 20, the TMA content in the treated samples ranged from 4.60 to 5.28 mg TMA/100 g, with OX yielding the lowest content. Erkan (2005) recommended a threshold value of 3 mg TMA/100 g for mussels. The control and all treated samples reached this limit between five and ten days of cold storage. Nevertheless, the TMA levels of the treated samples remained significantly lower than those of the control. These findings indicate the effectiveness of organic acid treatments in suppressing TMA production. No synergistic effect was observed in combination-acid treatments in reducing the TMA production. The reduced TMA production could be attributed to the inhibitory activity of organic acid treatments on microbial growth during cold storage. A strong positive correlation was identified between TMA content and microbial counts ($r = 0.90$ for TVC; $r = 0.93$ for PBC, $p<0.01$) in the present study. This finding suggests that a reduction in microbial counts leads to lower TMA content in green mussels. Consequently, the decrease in the microbial populations reduced the activity of microbial enzymes. The attenuated reduction of TMAO to TMA by bacterial TMAO reductase resulted in the lower TMA production observed in treated samples with reduced microbial counts. Several studies have reported similar results, showing reduced TMA content in organic acid-treated seafood products (Manju *et al.*, 2007; Sallam, 2007; Arcales & Nacional, 2018).

CONCLUSION

The application of organic acids effectively preserved the quality of green mussels during cold storage, particularly with regards to microbiological and physicochemical parameters. Both single-acid and combination-acid treatments were effective in reducing microbial counts (TVC and PBC), thereby extending the microbiological shelf life of green mussels from 5–10 days to 15–20 days during cold storage. Oxalic acid and the combination of oxalic acid and tartaric acid treatments exhibited the highest inhibitory effects against bacteria. Furthermore, organic acid treatments reduced the production of volatile nitrogenous compounds, as indicated by decreased TVB-N and TMA contents in green mussels during cold storage.

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