Research Article

Effects of Organic Acid Treatments on Lipid Oxidation and Fatty Acid Content of Green Mussel (*Perna viridis*) During Cold Storage

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ABSTRACT

Green mussels (*Perna viridis*), marine bivalves found in Malaysia and other Asia-Pacific regions, are rich in polyunsaturated fatty acids (PUFA), making them susceptible to lipid oxidation. This study evaluated the effects of organic acid treatments, commonly used as food preservatives due to their antimicrobial properties, on lipid oxidation and fatty acid content of green mussel flesh during cold storage. Green mussels were immersed in 1.0% organic acid solutions containing lactic acid (LA), oxalic acid (OX), and tartaric acid (TA), either individually or in combination, for 5 min, followed by cold storage at 4 ± 1 °C for 20 days. Non-organic acid-treated mussels served as the control. The results showed that lipid oxidation progressed during cold storage. Organic acid-treated mussels exhibited significantly higher TBARS values, suggesting that organic acid treatments promoted lipid oxidation in green mussels. The total fatty acid content of fresh, untreated mussels was 16.89 mg/g mussel flesh, with PUFA (9.30 mg/g) being the predominant class, while saturated fatty acids (SFA) accounted for 4.99 mg/g. After 20 days of storage, the contents of saturated, monounsaturated, and polyunsaturated fatty acids significantly decreased ($p<0.05$), with total fatty acid content declining by 10.96% in the control and 14.58–45.85% in the organic acid-treated samples. Pearson correlation analysis showed that the PUFA content was negatively correlated with TBARS values, indicating the progression of lipid oxidation led to a reduction in PUFA content.

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1. Introduction

Green mussels (*Perna viridis*) are marine bivalves that are highly valued and in high demand in Asia. As a result, they are widely cultivated across the Indo-Pacific region, from the Persian Gulf, India, and Bangladesh to Southeast Asia (Lau *et al.*, 2018). In Malaysia, green mussels are widely distributed along the west coast of Peninsular Malaysia, particularly in the coastal waters of Malacca and Johore, and are also cultivated in the coastal waters of Kuala Penyu, Marudu Bay and Ambong Bay in Sabah (Yap, 2012;

Lau *et al.*, 2018). Green mussels have also attracted considerable scientific interest owing to their unique taste and high nutritional value. They are rich in vital nutrients, including digestible protein, essential amino acids, polyunsaturated fatty acids (PUFA), vitamins, and minerals (Chakraborty *et al.*, 2016).

Post-harvest mussels are highly perishable due to microbial and enzymatic spoilage, which leads to a short shelf life and limits their marketability. Their high perishability is attributed to several intrinsic factors: high muscle water activity (a_w > 0.95), a near-neutral pH value (between 6.7 to 7.1) and their rich nutrient composition, particularly high levels of amino acids and PUFA (Goulas *et al.*, 2005). Lipid oxidation is one of the key factors driving mussel spoilage. Mussel lipids contain significant proportions of PUFA, making them highly susceptible to lipid oxidation. Lipid oxidation has a detrimental impact on texture, aroma, and nutritional quality, ultimately leading to rancidity (Wang & Zheng, 2025).

Cold storage is a common practice for preserving mussels to extend shelf life. However, cold storage typically offers only short-term preservation, as mussels have a shelf life of only five to eight days under these conditions (Arcales & Nacional, 2018). Acidification with organic acids is a widely acceptable and effective method for food preservation. Common examples of organic acids used in food preservation include acetic acid, citric acid, lactic acid, malic acid, oxalic acid, and tartaric acid (Mani-López *et al.*, 2012). Numerous studies have confirmed the antimicrobial properties of organic acids in a wide range of food products (Mani-López *et al.*, 2012; Mohan & Pohlman, 2016; Arcales & Nacional, 2018). The effects of organic acids on lipid oxidation have also been reported (Monirul *et al.*, 2019; Mohamed & Ammar, 2021). However, previous studies have focused primarily on individual acid treatments, particularly their antimicrobial activity. The present study focused on the effects of organic acids, both individually or in combination, on lipid oxidation and fatty acid content of green mussels (*Perna viridis*) during cold storage.

2. Materials and Methods

2.1 Chemicals and Reagents

Lactic acid (88%) and L-(+)-tartaric acid were purchased from Systerm (Malaysia). Oxalic acid was obtained from R&J Chemicals (Malaysia). Sodium chloride, boron trifluoride-methanol complex and sodium hydroxide pellet were acquired from Merck (Germany). Malondialdehyde tetrabutylammonium salt and 2-thiobarbituric acid were procured from Aldrich (USA). All reagents were of analytical grade. The HPLC-grade solvents used included n-hexane 95% (Systerm, Malaysia), chloroform (Fisher Scientific, USA), and methanol (Systerm, Malaysia). The C_4-C_{24} fatty acid methyl ester (FAME) mix standard (Supelco 18919) for fatty acid analysis was sourced from Agilent, while methyl nonadecanate was acquired from Sigma (USA).

2.2 Collection of Mussels

Green mussels were purchased from a farm in Ambong Bay, Sabah, Malaysia. They were immediately packed in an iced Styrofoam container and transported to the laboratory at the Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, arriving within two hours of purchase. In the laboratory, green mussels with shell lengths between 8.0 and 12.0 cm were selected to ensure size uniformity on mussel flesh. They were thoroughly rinsed with deionised water to remove extraneous debris. Each mussel was manually shucked, and the extracted flesh was then immediately prepared for subsequent organic acid treatments.

2.3 Organic Acid Treatments and Cold Storage

A screening study was conducted to select the suitable types and concentration of organic acids. Eight organic acids (ascorbic acid, butyric acid, formic acid, lactic acid, malic acid, oxalic acid, propionic acid, and tartaric acid), at two concentrations (1.0% and 2.0%), were initially evaluated using sensory evaluation (hedonic test) and a microbiological test (total viable count). Organic acids that exhibited the highest antimicrobial activity after cold storage, with minimal adverse effect on the sensory properties of green

mussel flesh were selected. Based on the screening results, three organic acids (lactic acid, oxalic acid, and tartaric acid), at a concentration of 1.0%, were selected for the present study. Mussels from a single harvest batch were divided into seven treatment groups, including three single-acid treatments (lactic acid [LA], oxalic acid [OX], tartaric acid [TA]) and four combination-acid treatments (lactic and oxalic acid [LA+OX], lactic and tartaric acid [LA+TA], oxalic and tartaric acid [OX+TA], as well as a mixture of lactic, oxalic and tartaric acid [LA+OX+TA]). All organic acid solutions were prepared at a total concentration of 1.0%, with acids in combination solutions mixed in equal proportions. The shucked mussel flesh was fully immersed in the prepared organic acid solutions for 5 min at room temperature, then removed from the solutions and drained for 10 min. Subsequently, each mussel flesh sample was individually heat-sealed in a polyethylene bag and kept at 4 ± 1 °C for 20 days. Sample without organic acid treatment served as the control. The samples were withdrawn every five days for the subsequent analyses.

2.4 Thiobarbituric Acid Reactive Substance (TBARS) Assay

The TBARS assay was conducted to determine the degree of lipid oxidation using a colourimetry method (Zeb & Ullah, 2016). This method is based on the spectrophotometric measurement of a pink-coloured complex formed by the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA). The assay specifically measures the amount of secondary oxidation products, primarily MDA, generated by the decomposition of hydroperoxides during the later stage of lipid oxidation (Shahidi & Zhong, 2010). Briefly, 10 g of sample was mixed with 7.5% trichloroacetic acid and subjected to centrifugation at 8,000 rpm for 5 min. The supernatant was added with TBA (0.02 mol/L) at a 1:1 (v/v) ratio in a test tube. The test tube was loosely capped and then incubated in a water bath at 95 °C for 30 min to facilitate the formation of pink-coloured complex. The absorbance of the pink-coloured complex was measured at 532 nm against a reference blank. Malondialdehyde tetrabutylammonium salt served as the standard for MDA quantification. The MDA content in the samples was reported as TBARS value and expressed in mg MDA/kg.

2.5 Fatty Acid Analysis

Mussel lipid extraction was carried out based on the method by Losito *et al.* (2018). Briefly, 5 g of the grounded mussel sample was added to a 2:1 (v/v) mixture of chloroform and methanol, then homogenised for 5 min. The mixture was then centrifuged at 5,200g for 5 min at room temperature to separate tissue debris. The resulting supernatant was collected and then added to a 1:1 (v/v) mixture of chloroform and 1% sodium chloride solution. The mixture was centrifuged again to isolate the lipid-containing chloroform layer (bottom phase). The chloroform was subsequently evaporated at 45 °C using a rotary evaporator. The extracted mussel lipids were subjected to a base-catalysed transesterification using methanolic sodium hydroxide followed by an acid-catalysed transesterification using methanolic boron trifluoride, with each reaction conducted at 95 °C for 30 min to prepare fatty acid methyl esters (FAME). The resulting FAME was extracted using n-hexane. The fatty acid content was assessed using an Agilent 7890A gas chromatography equipped with a flame ionisation detector (GC-FID). The extracted FAME was separated with an Agilent capillary DB-23 column (30 m x 0.25 mm x 0.25 µm) using helium as the carrier gas at a constant flow rate of 1.0 mL/min. The column temperature program started at 50 °C (hold for 1 min), ramped to 175 °C at 25 °C/min, and then increased to 230 °C at 4.5 °C/min (hold for 2 min). Individual FAME in the sample was identified by comparing the retention time of the peaks with those of the Supelco 37 FAME mix standard analysed under identical chromatographic conditions. Fatty acid quantification was performed using the peak areas of the FAME mix standard, with nonadecanoic acid (C19:0) serving as the internal standard. The fatty acid contents were reported as mg/g mussel flesh.

2.6 Statistical Analysis

All analyses were conducted in triplicate. Data were analysed using One-Way Analysis of Variance (ANOVA) using the IBM SPSS Statistics software (version 22.0), and the significance level was established at $p<0.05$. Tukey's Honestly Significant Difference (HSD) test was performed as the post-hoc procedure.

3. Results and Discussion

3.1 Lipid Oxidation

The changes in TBARS values of green mussels during cold storage are shown in Figure 1. The TBARS value of the control increased significantly ($p<0.05$) from 1.16 to 7.94 mg MDA/kg during storage. The treated samples also exhibited significant increasing trends ($p<0.05$), with their values ranging from 8.21 to 9.08 mg MDA/kg by Day 20. These results indicated that lipid oxidation occurred across all samples during cold storage. For LA+OX, a significant decrease ($p<0.05$) was recorded on Day 20. This may be attributed to the utilisation of MDA by microbial action or the interaction of MDA with other cellular components (such as amino acids, proteins, phospholipids, nucleic acids, and DNA) to form complexes, which subsequently reduced the detectable MDA levels in the samples (Wang & Zheng, 2025).

The results showed that the application of organic acid treatments, both single-acid and combination-acid treatments, accelerated lipid oxidation during cold storage. The treated samples exhibited elevated TBARS values compared to the control from Day 5 through the end of storage period, suggesting a higher degree of lipid oxidation. Several studies have also reported accelerated lipid oxidation in muscle foods with organic acid treatments. Masniyom & Benjama (2007) reported that mussels treated with lactic, acetic, and citric acids recorded higher TBARS values than the untreated control during cold storage. Similarly, the application of malic acid resulted in a higher lipid oxidation degree in sheep meat during cold storage (Albuquerque *et al.*, 2018). These oxidative promoting effects may be related to the activity of transition metal ions present within muscle proteins. In mussels, metal ions such as iron (Fe^{2+} and Fe^{3+}) and copper (Cu^{2+}) are bound to proteins such as myoglobin. These ions are known to be potent catalysts for lipid oxidation (Shahidi & Zhong, 2010). The release of metal ions from myoglobin could occur via protein degradation induced by both endogenous and bacterial proteases (Tavares *et al.*, 2021). The binding affinity between proteins and metal ions also decreased due to the conformational changes triggered by the pH drop after the organic acid treatments, leading to the liberation of these ions from the proteins. For example, Signorini *et al.* (2007) found that lactic acid caused structural changes in beef myofibrillar protein. Furthermore, the pH shift by organic acid treatments could increase the solubility of metal ions and alter their redox potential, thereby enhancing their pro-oxidant activity (Domínguez *et al.*, 2019). The free metal ions may also react with organic acids (such as citric acid) and form complexes that exhibit greater pro-oxidant effects than the free metal ions themselves (Durand *et al.*, 2025). The availability of free metal ions and the formation of metal-acid complexes collectively act as powerful pro-oxidants, catalysing the decomposition of hydroperoxides into highly reactive peroxyl and alkoxyl radicals. These radicals drive the initiation and propagation stages of the lipid oxidation process by abstracting hydrogen atoms from unsaturated fatty acids, thereby initiating new lipid oxidation chain within muscle tissues (Durand *et al.*, 2025). Consequently, MDA production increases, as reflected by the elevated TBARS values observed in the treated samples in the present study.

3.2 Fatty Acid Profile

Table 1 presents the fatty acid profile of the untreated and unstored green mussels. Polyunsaturated fatty acids (PUFA) were the predominant fatty acid class, followed by saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). A total of 20 distinct types of fatty acids were identified, comprising 6 SFA, 6 MUFA, and 8 PUFA, yielding a total fatty acid (ΣFA) content of 16.89 mg/g. The total SFA (ΣSFA) content was 4.99 mg/g, consisting of myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0), and arachidic acid (C20:0). Among these SFA, palmitic acid was the most abundant (1.88 mg/g), followed by margaric acid (1.41 mg/g). The total MUFA (ΣMUFA) content exhibited the lowest proportion at 2.61 mg/g, including pentadecenoic acid (C15:1), palmitoleic acid (C16:1), *cis*-10-heptadecenoic acid (C17:1), oleic acid (C18:1), *trans*-9-elaidic acid (C18:1 *trans*), and gondoic acid (C20:1). Palmitoleic acid contributed the highest concentration (1.71 mg/g). The total PUFA (ΣPUFA) content accounted for 9.30 mg/g, characterised by high levels of ω-3 PUFA (including α-linolenic acid [C18:3], eicosapentaenoic acid [EPA, C20:5], and docosahexaenoic acid [DHA, C22:6]) and ω-6 PUFA

(including linoleic acid [C18:2], γ-linolenic acid [C18:3], eicosadienoic acid [C20:2], dihomo-γ-linolenic acid [C20:3], and arachidonic acid [C20:4]). Among these PUFA, EPA and DHA were the most abundant, accounting for 3.37 mg/g and 3.60 mg/g, respectively. Both EPA and DHA have been shown to confer numerous health benefits, including the prevention of diseases such as cardiovascular disease and diabetes, as well as supporting neurological and brain development (Shahidi & Ambigaipalan, 2018).

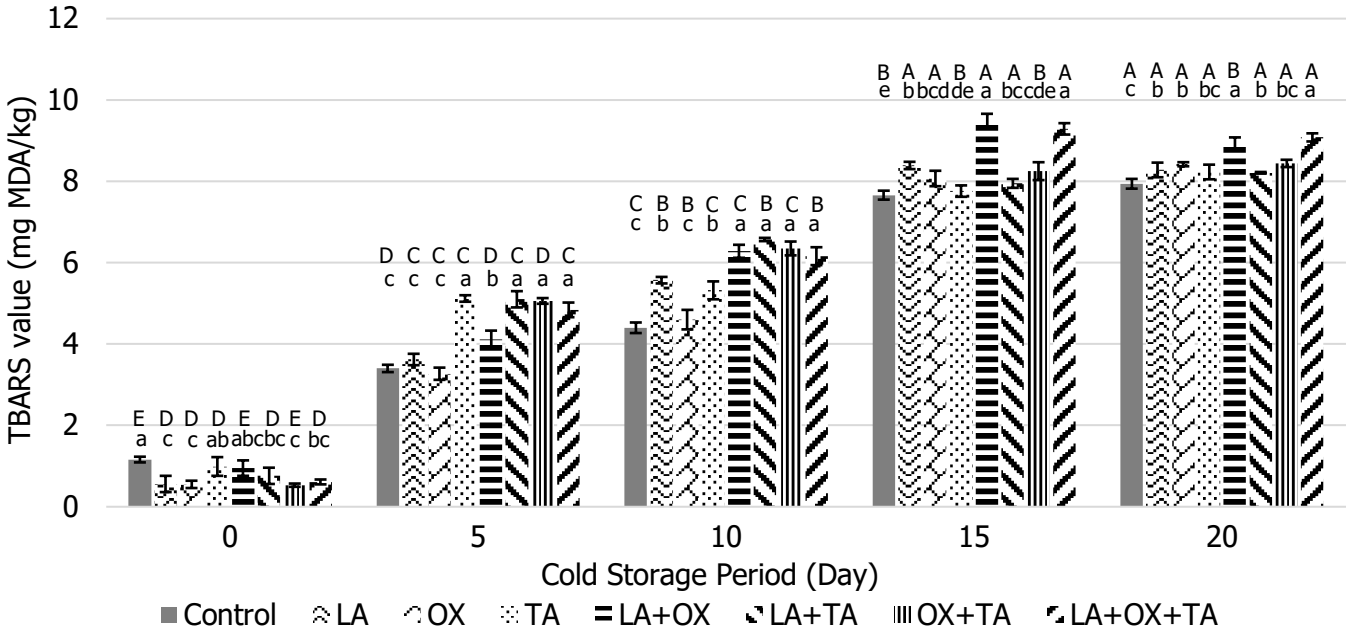


Figure 1. Changes in TBARS values (mg MDA/kg) of green mussels during 20 days of cold storage.

Different uppercase letters (A–E) indicate significant differences ($p<0.05$) among storage periods within each treatment.
Different lowercase letters (a–e) indicate significant differences ($p<0.05$) among treatments at each storage period.
LA – Lactic acid; OX – Oxalic acid; TA – Tartaric acid; LA+OX – Equal mixture of lactic acid and oxalic acid; LA+TA – Equal mixture of lactic acid and tartaric acid; OX+TA – Equal mixture of oxalic acid and tartaric acid; LA+OX+TA – Equal mixture of lactic acid, oxalic acid and tartaric acid.

Table 1. Fatty acid profile of the untreated and unstored green mussels (*Perna viridis*).

Fatty Acids		**Content (mg/g mussel flesh)**
Myristic acid	C14:0	0.50 ± 0.01
Pentadecanoic acid	C15:0	0.20 ± 0.00
Palmitic acid	C16:0	1.88 ± 0.02
Margaric acid	C17:0	1.41 ± 0.02
Stearic acid	C18:0	0.97 ± 0.02
Arachidic acid	C20:0	0.02 ± 0.01
ΣSFA (Total Saturated Fatty Acids)		**4.99 ± 0.07**
Pentadecenoic acid	C15:1	0.07 ± 0.00
Palmitoleic acid	C16:1	1.71 ± 0.02
cis-10-Heptadecenoic acid	C17:1	0.04 ± 0.00
Oleic acid	C18:1	0.13 ± 0.00
trans-9-Elaidic acid	C18:1*trans*	0.09 ± 0.01
Gondoic acid	C20:1	0.56 ± 0.01

ΣMUFA (Total Monounsaturated Fatty Acids)		**2.61 ± 0.03**
Linoleic acid	C18:2ω6	0.48 ± 0.01
γ-Linolenic acid	C18:3ω6	0.03 ± 0.00
α-Linolenic acid (ALA)	C18:3ω3	0.20 ± 0.00
Eicosadienoic acid	C20:2ω6	0.08 ± 0.00
Dihomo-γ-linolenic acid	C20:3ω6	0.10 ± 0.00
Arachidonic acid	C20:4ω6	1.43 ± 0.02
Eicosapentaenoic acid (EPA)	C20:5ω3	3.37 ± 0.03
Docosahexaenoic acid (DHA)	C22:6ω3	3.60 ± 0.05
ΣPUFA (Total Polyunsaturated Fatty Acids)		**9.30 ± 0.10**
ΣFA (Total Fatty Acids)		**16.89 ± 0.19**
Σω-3 PUFA		7.17 ± 0.08
Σω-6 PUFA		2.13 ± 0.03

3.3 Fatty Acid Content

Figure 2 shows the changes in total fatty acid (ΣFA) content in green mussels after 20 days of cold storage. In general, the ΣFA content decreased across the control and treated samples by the end of storage. For the control, the ΣFA content decreased by 10.96%, from 16.89 to 15.03 mg/g. Overall, the treated samples exhibited a more pronounced decrease in ΣFA content compared to the control. The ΣFA content in LA decreased by 45.85% (from 21.24 to 11.51 mg/g), OX by 24.19% (from 17.81 to 13.50 mg/g), TA by 18.00% (from 13.64 to 11.19 mg/g), LA+OX by 21.52% (from 16.98 to 13.28 mg/g), LA+TA by 29.6230.30% (from 18.68 to 13.02 mg/g), OX+TA by 14.65% (from 15.12 to 12.90 mg/g), and LA+OX+TA by 19.41% (from 17.68 to 14.24 mg/g).

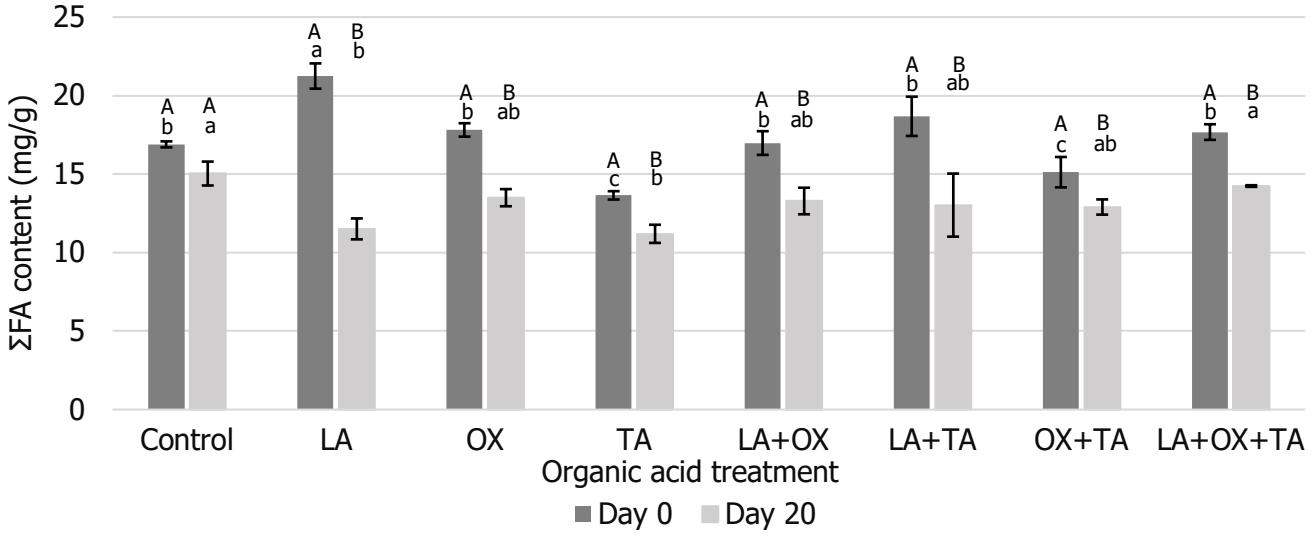


Figure 2. Total fatty acid (ΣFA) content (mg/g) in green mussels on Day 0 and Day 20.

Different uppercase letters (A, B) indicate significant differences ($p<0.05$) between Day 0 and Day 20 within each treatment. Different lowercase letters (a–c) indicate significant differences ($p<0.05$) among treatments within each storage period. LA – Lactic acid; OX – Oxalic acid; TA – Tartaric acid; LA+OX – Equal mixture of lactic acid and oxalic acid; LA+TA – Equal mixture of lactic acid and tartaric acid; OX+TA – Equal mixture of oxalic acid and tartaric acid; LA+OX+TA – Equal mixture of lactic acid, oxalic acid and tartaric acid.

The changes in total saturated fatty acid (ΣSFA), palmitic acid (C16:0), and margaric acid (C17:0) contents in green mussels during a 20-day cold storage are presented in Table 2. For ΣSFA content, the control increased slightly by 0.54% (from 4.99 to 5.01 mg/g) by the end of the cold storage. Among the treated samples, the ΣSFA content in LA decreased significantly ($p<0.05$) by 35.09% (from 6.21 to 4.03 mg/g), OX by 13.52% (from 5.23 to 4.52 mg/g), and LA+TA by 20.89% (from 5.67 to 4.45 mg/g). Reductions in ΣSFA content were less pronounced in other treated samples, including TA (9.87%, from 4.16 to 3.75 mg/g); LA+OX (2.82%, from 5.04 to 4.89 mg/g); OX+TA (2.61%, from 4.44 to 4.31 mg/g); and LA+OX+TA (2.95%, from 5.13 to 4.98 mg/g) for LA+OX+TA. Margaric acid (C17:0) and palmitic acid (C16:0) were the predominant SFA in the green mussels. The content of margaric acid (C17:0) increased by 10.57% in the control, from 1.41 to 1.56 mg/g, resulting in a 0.54% increase in the ΣSFA content. The content of palmitic acid (C16:0) decreased in both the control and treated samples throughout the storage period. As palmitic acid is one of the major components of phospholipids in mussels, its reduction suggested phospholipid degradation during cold storage (Zhang *et al.*, 2025).

Table 2. Changes in total saturated fatty acid (ΣSFA), palmitic acid (C16:0), and margaric acid (C17:0) contents (mg/g) in green mussels during cold storage.

	Treatment	Cold Storage Period					α (%)
		Day 0	Day 5	Day 10	Day 15	Day 20	
ΣSFA	Control	4.99±0.07^{B}	6.11±0.89^{A}	3.87±0.17^{C}	4.28±0.23^{BC}	5.01±0.26^{B}	0.54±6.49^{a}
	LA	6.21±0.24^{A}	5.89±0.16^{A}	6.27±0.50^{A}	5.60±0.07^{A}	4.03±0.23^{B}	-35.09±2.05^{c}
	OX	5.23±0.14^{C}	6.85±0.26^{A}	5.69±0.07^{B}	5.16±0.27^{C}	4.52±0.18^{D}	-13.52±4.42^{ab}
	TA	4.16±0.08^{CD}	4.64±0.11^{C}	5.83±0.41^{A}	5.29±0.18^{B}	3.75±0.19^{D}	-9.87±3.27^{ab}
	LA+OX	5.04±0.21^{B}	5.62±0.16^{A}	3.40±0.13^{C}	5.00±0.27^{B}	4.89±0.31^{B}	-2.82±9.72^{ab}
	LA+TA	5.67±0.37^{A}	4.48±0.27^{BC}	5.08±0.12^{AB}	4.01±0.18^{C}	4.45±0.69^{BC}	-20.89±16.70^{bc}
	OX+TA	4.44±0.28^{BC}	4.51±0.42^{BC}	5.08±0.23^{B}	6.15±0.14^{A}	4.31±0.16^{C}	-2.61±2.53^{ab}
	LA+OX+TA	5.13±0.14^{C}	4.78±0.13^{C}	5.61±0.15^{B}	6.22±0.29^{A}	4.98±0.01^{C}	-2.95±2.91^{ab}
C16:0	Control	1.88±0.02^{AB}	2.16±0.34^{A}	1.42±0.06^{C}	1.48±0.03^{BC}	1.79±0.09^{ABC}	-4.88±6.04^{a}
	LA	2.39±0.10^{A}	2.19±0.06^{A}	2.35±0.19^{A}	1.92±0.02^{B}	1.38±0.08^{C}	-42.39±1.59^{b}
	OX	1.95±0.05^{B}	2.48±0.08^{A}	2.00±0.03^{B}	1.78±0.10^{C}	1.59±0.06^{D}	-18.53±4.20^{a}
	TA	1.45±0.03^{BC}	1.58±0.04^{B}	2.16±0.15^{A}	2.09±0.07^{A}	1.36±0.07^{C}	-6.39±3.46^{a}
	LA+OX	1.85±0.08^{B}	2.05±0.06^{A}	1.27±0.05^{C}	1.73±0.09^{B}	1.78±0.11^{B}	-3.44±9.77^{a}
	LA+TA	2.03±0.13^{A}	1.59±0.09^{B}	1.77±0.04^{AB}	1.46±0.06^{B}	1.71±0.26^{AB}	-14.96±17.68^{a}
	OX+TA	1.57±0.10^{B}	1.59±0.15^{B}	1.73±0.08^{B}	2.07±0.17^{A}	1.56±0.06^{B}	-0.48±2.52^{a}
	LA+OX+TA	1.89±0.05^{B}	1.79±0.05^{B}	1.94±0.05^{B}	2.20±0.10^{A}	1.79±0.01^{B}	-5.63±2.89^{a}
C17:0	Control	1.41±0.02^{BC}	2.06±0.29^{A}	1.18±0.05^{C}	1.32±0.07^{BC}	1.56±0.08^{B}	10.57±7.02^{a}
	LA	1.82±0.05^{A}	1.71±0.05^{A}	1.85±0.15^{A}	1.75±0.02^{A}	1.41±0.08^{B}	-22.54±3.19^{cd}
	OX	1.52±0.04^{C}	2.08±0.08^{A}	1.72±0.02^{B}	1.71±0.09^{B}	1.39±0.05^{C}	-8.13±4.48^{bc}
	TA	1.35±0.03^{C}	1.57±0.04^{B}	1.74±0.12^{A}	1.39±0.05^{C}	1.14±0.06^{D}	-15.47±2.82^{bcd}
	LA+OX	1.50±0.06^{A}	1.63±0.05^{A}	1.01±0.03^{B}	1.61±0.08^{A}	1.47±0.10^{A}	-1.77±9.51^{ab}
	LA+TA	1.81±0.11^{A}	1.40±0.09^{BC}	1.64±0.04^{AB}	1.28±0.06^{C}	1.23±0.19^{C}	-31.45±14.14^{d}
	OX+TA	1.43±0.09^{B}	1.47±0.14^{B}	1.70±0.07^{A}	1.83±0.03^{A}	1.37±0.05^{B}	-3.80±2.56^{ab}
	LA+OX+TA	1.54±0.04^{B}	1.42±0.04^{C}	1.76±0.05^{A}	1.78±0.08^{A}	1.51±0.01^{BC}	-1.94±2.89^{ab}

Values with different lowercase superscripts (a–d) in the same column are significantly different ($p<0.05$).
Values with different uppercase superscripts (A–D) in the same row are significantly different ($p<0.05$).
ΣSFA – Total saturated fatty acid; C16:0 – Palmitic acid; C17:0 – Margaric acid; LA – Lactic acid; OX – Oxalic acid; TA – Tartaric acid; LA+OX – Equal mixture of lactic acid and oxalic acid; LA+TA – Equal mixture of lactic acid and tartaric acid; OX+TA – Equal mixture of oxalic acid and tartaric acid; LA+OX+TA – Equal mixture of lactic acid, oxalic acid and tartaric acid.
α – Changes in fatty acid content (%) $=(\frac{Fatty\ acid\ content\ on\ Day\ 20 - Fatty\ acid\ content\ on\ Day\ 0}{Fatty\ acid\ content\ on\ Day\ 0} \times 100\%)$; A positive value and a negative

value indicate an increase and a decrease, respectively, in the fatty acid content after 20 days of cold storage.

Table 3 shows the changes in total monounsaturated fatty acid (ΣMUFA) and palmitoleic acid (C16:1) contents during 20 days of cold storage. The palmitoleic acid contents of the control and LA+OX treatments increased by 6.75% (from 1.71 to 1.83 mg/g) and 15.05% (from 1.70 to 1.95 mg/g), respectively, resulting in increases of 2.34% and 10.78% in ΣMUFA content, respectively. In contrast, all other treated samples showed decreasing trends in both ΣMUFA and palmitoleic acid contents. Among them, the greatest reduction was observed in LA, where the ΣMUFA content decreased by 40.10% (from 3.08 to 1.84 mg/g) and palmitoleic acid content decreased by 45.40% (from 1.99 to 1.09 mg/g). Zhang *et al.* (2025) reported that palmitoleic acid is a major component of triacylglycerols in mussels, accounting for up to 18.05%. Therefore, the reduction in palmitoleic acid content indicated the progression of triacylglycerol degradation during cold storage.

Table 3. Changes in total monounsaturated fatty acid (ΣMUFA) and palmitoleic acid (C16:1) contents (mg/g) in green mussels during cold storage.

	Treatment	Cold Storage Period					α (%)
		Day 0	Day 5	Day 10	Day 15	Day 20	
ΣMUFA	Control	2.61±0.03^{B}	3.37±0.52^{A}	1.97±0.08^{C}	2.39±0.13^{BC}	2.67±0.14^{B}	2.34±6.21^{a}
	LA	3.08±0.12^{A}	3.38±0.10^{A}	3.13±0.26^{A}	3.37±0.04^{A}	1.84±0.10^{B}	-40.10±1.81^{b}
	OX	2.73±0.07^{C}	3.42±0.14^{A}	3.00±0.05^{B}	2.33±0.13^{D}	2.63±0.10^{C}	-3.77±4.72^{a}
	TA	2.23±0.05^{B}	2.32±0.05^{B}	3.47±0.26^{A}	3.37±0.12^{A}	2.13±0.11^{B}	-4.34±3.45^{a}
	LA+OX	2.48±0.11^{BC}	2.96±0.08^{A}	1.82±0.08^{C}	2.77±0.14^{AB}	2.74±0.16^{AB}	10.78±10.85^{a}
	LA+TA	2.93±0.20^{A}	2.52±0.15^{A}	2.81±0.06^{A}	1.99±0.43^{B}	2.80±0.43^{A}	-3.46±20.29^{a}
	OX+TA	2.38±0.15^{B}	2.37±0.24^{B}	2.51±0.12^{B}	3.30±0.04^{A}	2.36±0.09^{B}	-0.70±2.50^{a}
	LA+OX+TA	2.70±0.10^{B}	2.31±0.06^{C}	2.91±0.08^{B}	3.57±0.17^{A}	2.68±0.01^{B}	-0.67±4.13^{a}
C16:1	Control	1.71±0.02^{BC}	2.20±0.33^{A}	1.33±0.06^{C}	1.44±0.08^{BC}	1.83±0.09^{B}	6.75±6.64^{ab}
	LA	1.99±0.08^{B}	2.38±0.07^{A}	2.13±0.18^{B}	2.42±0.03^{A}	1.09±0.06^{C}	-45.40±1.21^{c}
	OX	1.87±0.05^{B}	2.22±0.10^{A}	1.99±0.03^{B}	1.38±0.08^{D}	1.70±0.06^{C}	-8.79±4.15^{b}
	TA	1.43±0.03^{B}	1.33±0.03^{B}	2.17±0.16^{A}	2.12±0.08^{A}	1.34±0.07^{B}	-6.61±3.46^{ab}
	LA+OX	1.70±0.07^{B}	1.97±0.05^{A}	1.22±0.05^{C}	1.85±0.10^{AB}	1.95±0.12^{A}	15.05±11.44^{a}
	LA+TA	2.09±0.14^{A}	1.64±0.09^{B}	1.87±0.05^{AB}	1.24±0.05^{C}	1.85±0.27^{AB}	-10.22±18.58^{b}
	OX+TA	1.57±0.10^{B}	1.56±0.15^{B}	1.60±0.07^{B}	2.32±0.03^{A}	1.46±0.06^{B}	-7.34±2.25^{ab}
	LA+OX+TA	1.86±0.08^{BC}	1.55±0.04^{D}	1.99±0.05^{B}	2.51±0.12^{A}	1.77±0.01^{C}	-4.76±4.41^{ab}

Values with different lowercase superscripts (a–c) in the same column are significantly different ($p<0.05$).
Values with different uppercase superscripts (A–D) in the same row are significantly different ($p<0.05$).
ΣMUFA – Total monounsaturated fatty acid; C16:1 – Palmitoleic acid; LA – Lactic acid; OX – Oxalic acid; TA – Tartaric acid; LA+OX – Equal mixture of lactic acid and oxalic acid; LA+TA – Equal mixture of lactic acid and tartaric acid; OX+TA – Equal mixture of oxalic acid and tartaric acid; LA+OX+TA – Equal mixture of lactic acid, oxalic acid and tartaric acid.
α – Changes in fatty acid content (%) = $\left(\frac{Fatty\ acid\ content\ on\ Day\ 20 - Fatty\ acid\ content\ on\ Day\ 0}{Fatty\ acid\ content\ on\ Day\ 0} \times 100\%\right)$; A positive value and a negative value indicate an increase and a decrease, respectively, in the fatty acid content after 20 days of cold storage.

The total polyunsaturated fatty acid (ΣPUFA) content in all samples also decreased significantly ($p<0.05$) during the 20 days of cold storage (Table 4). The decrease in ΣPUFA content was closely related to the reductions in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents, which constituted the majority of the PUFA proportion in mussels. For the control, the ΣPUFA, EPA, and DHA contents reduced significantly ($p<0.05$) by 20.86% (from 9.30 to 7.36 mg/g), 24.03% (from 3.37 to 2.56 mg/g), and 24.79% (from 3.60 to 2.71 mg/g), respectively, after 20 days of cold storage. Similar to the ΣSFA and ΣMUFA results, the ΣPUFA reduction in LA was the highest, decreasing by 52.92% (from 11.96 to 5.63 mg/g). The reductions in ΣPUFA content for the other treatments were notably less severe than that observed for LA, with reductions of 35.50% for OX (from 9.85 to 6.35 mg/g), 26.87% for TA (from

7.25 to 5.30 mg/g), 39.98% for LA+OX (from 9.45 to 5.65 mg/g), 42.13% for LA+TA (from 10.08 to 5.78 mg/g), 24.95% for OX+TA (from 8.31 to 6.23 mg/g), and 33.12% for LA+OX+TA (from 9.84 to 6.58 mg/g). The EPA and DHA contents in all treated samples also decreased significantly ($p<0.05$) during cold storage. EPA and DHA are the major components of phospholipids (16% to 17%) and triacylglycerols (6.6% to 13.8%) in mussels (Zhang *et al.*, 2025). The EPA content in the treated samples decreased by approximately 24% to 59%, while the DHA content decreased by approximately 31% to 61% after 20 days of cold storage. These findings suggested the degradation of phospholipids and triacylglycerols during cold storage.

Table 4. Changes in total polyunsaturated fatty acid (ΣPUFA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) contents (mg/g) in green mussels during cold storage.

	Treatment	Cold Storage Period					α (%)
		Day 0	Day 5	Day 10	Day 15	Day 20	
ΣPUFA	Control	9.30±0.10^{B}	11.51±1.58^{A}	6.81±0.32^{C}	6.65±0.36^{C}	7.36±0.37^{C}	-20.86±4.80^{a}
	LA	11.96±0.45^{A}	8.79±0.24^{B}	8.67±0.67^{B}	7.40±0.19^{C}	5.63±0.34^{D}	-52.92±1.53^{d}
	OX	9.85±0.22^{B}	12.26±0.50^{A}	9.87±0.12^{B}	9.23±0.48^{B}	6.35±0.27^{C}	-35.50±3.53^{abc}
	TA	7.25±0.14^{C}	8.25±0.19^{B}	9.25±0.70^{A}	7.57±0.26^{BC}	5.30±0.28^{D}	-26.87±2.70^{ab}
	LA+OX	9.45±0.44^{A}	9.68±0.24^{A}	5.79±0.22^{C}	6.90±0.38^{B}	5.65±0.37^{C}	-39.98±6.43^{bcd}
	LA+TA	10.08±0.68^{A}	7.01±0.43^{BC}	7.78±0.17^{B}	6.08±0.28^{C}	5.78±0.89^{C}	-42.13±12.20^{cd}
	OX+TA	8.31±0.54^{A}	6.93±0.65^{BC}	7.56±0.34^{AB}	6.81±0.07^{BC}	6.23±0.23^{C}	-24.95±2.10^{a}
	LA+OX+TA	9.84±0.26^{A}	8.61±0.25^{B}	8.24±0.23^{B}	7.62±0.34^{C}	6.58±0.03^{D}	-33.12±1.77^{abc}
EPA	Control	3.37±0.03^{B}	4.11±0.57^{A}	2.64±0.12^{C}	2.22±0.13^{C}	2.56±0.12^{C}	-24.03±4.26^{a}
	LA	4.41±0.17^{A}	3.45±0.10^{B}	2.84±0.22^{C}	2.83±0.04^{C}	1.82±0.11^{D}	-58.67±1.34^{c}
	OX	3.61±0.08^{B}	4.37±0.18^{A}	3.66±0.05^{B}	3.40±0.17^{B}	2.19±0.09^{C}	-39.49±3.21^{b}
	TA	2.40±0.04^{C}	2.71±0.06^{B}	3.37±0.25^{A}	2.85±0.09^{B}	1.83±0.09^{D}	-24.09±2.86^{a}
	LA+OX	3.31±0.15^{B}	3.63±0.09^{A}	1.90±0.07^{D}	2.35±0.15^{C}	2.17±0.15^{CD}	-34.20±7.12^{ab}
	LA+TA	4.12±0.27^{A}	2.31±0.14^{BC}	2.61±0.06^{B}	2.05±0.09^{C}	1.88±0.29^{C}	-53.93±9.69^{c}
	OX+TA	2.71±0.18^{A}	2.48±0.24^{AB}	2.47±0.11^{AB}	2.28±0.03^{BC}	2.00±0.07^{C}	-26.25±2.09^{a}
	LA+OX+TA	3.41±0.09^{A}	2.94±0.09^{B}	3.26±0.09^{A}	2.95±0.12^{B}	2.41±0.01^{C}	-29.31±1.88^{ab}
DHA	Control	3.60±0.05^{B}	4.32±0.59^{A}	2.63±0.13^{C}	2.55±0.14^{C}	2.71±0.13^{C}	-24.79±4.68^{a}
	LA	5.11±0.19^{A}	3.18±0.09^{C}	3.64±0.27^{B}	2.47±0.03^{D}	2.01±0.12^{E}	-60.79±1.41^{e}
	OX	4.15±0.10^{B}	5.30±0.22^{A}	3.65±0.05^{C}	3.21±0.16^{D}	2.32±0.10^{E}	-43.90±3.24^{bcd}
	TA	2.90±0.05^{B}	3.16±0.08^{B}	3.69±0.28^{A}	2.89±0.10^{B}	1.97±0.10^{C}	-32.29±2.56^{ab}
	LA+OX	4.09±0.19^{A}	4.02±0.10^{A}	2.53±0.09^{B}	2.57±0.13^{B}	1.75±0.11^{C}	-57.10±4.60^{de}
	LA+TA	3.70±0.25^{A}	2.74±0.17^{B}	2.88±0.06^{B}	2.24±0.10^{C}	2.20±0.34^{C}	-39.93±12.70^{bc}
	OX+TA	3.48±0.23^{A}	2.55±0.24^{B}	2.76±0.13^{B}	2.38±0.02^{B}	2.50±0.12^{D}	-30.60±2.17^{ab}
	LA+OX+TA	4.36±0.11^{A}	3.73±0.11^{B}	2.86±0.09^{C}	2.50±0.12^{D}	2.24±0.01^{E}	-48.72±1.35^{cde}

Values with different lowercase superscripts (a–e) in the same column are significantly different ($p<0.05$).
Values with different uppercase superscripts (A–E) in the same row are significantly different ($p<0.05$).
ΣPUFA – Total polyunsaturated fatty acid; EPA – Eicosapentaenoic acid; DHA – Docosahexaenoic acid; LA – Lactic acid; OX – Oxalic acid; TA – Tartaric acid; LA+OX – Equal mixture of lactic acid and oxalic acid; LA+TA – Equal mixture of lactic acid and tartaric acid; OX+TA – Equal mixture of oxalic acid and tartaric acid; LA+OX+TA – Equal mixture of lactic acid, oxalic acid and tartaric acid.
α – Changes in fatty acid content (%) $=\left(\frac{Fatty\ acid\ content\ on\ Day\ 20 - Fatty\ acid\ content\ on\ Day\ 0}{Fatty\ acid\ content\ on\ Day\ 0} \times 100\%\right)$; A positive value and a negative value indicate an increase and a decrease, respectively, in the fatty acid content after 20 days of cold storage.

For all samples, the total fatty acid (ΣFA) content decreased during cold storage. For the control, both ΣSFA and ΣMUFA contents increased slightly, whereas the ΣPUFA content decreased markedly during cold storage. For the treated samples, the ΣSFA, ΣMUFA, and ΣPUFA contents decreased more markedly than those of the control. LA+OX, however, showed an increase in the ΣMUFA content after 20 days of storage. Özden (2005) reported that acetic acid treatment of rainbow trout resulted in decreased ΣPUFA content and increased ΣSFA content. Among the single-acid treatments, LA resulted in the highest

reductions of 35.09%, 40.10%, and 52.92% in ΣSFA, ΣMUFA, and ΣPUFA, respectively, followed by OX, with reductions of 13.52%, 3.77%, and 35.50%, respectively, and TA, with reductions of 9.87%, 4.34%, and 26.87%, respectively. When used in combination-acid treatments, OX and TA demonstrated either a dilution effect or a synergistic effect. For example, the 52.92% reduction in ΣPUFA caused by LA alone was reduced to 39.98% and 42.13% when LA was combined with OX (LA+OX) and TA (LA+TA), respectively. A synergistic effect was observed for ΣMUFA in the OX+TA treatment, which showed a reduction of only 0.70%, lower than the reductions observed with OX (3.77%) or TA (4.34%) alone. More notably, LA+OX+TA resulted in the lowest reduction in ΣMUFA (0.67%) among all treatments.

While the fatty acid contents in the samples generally exhibited decreasing trends, notable fluctuations were observed during the cold storage. For example, the ΣSFA content in LA+OX, the ΣMUFA content in OX, and the ΣPUFA content in TA fluctuated notably during the cold storage. These fluctuations were primarily attributed to the combined effects of lipid hydrolysis (lipolysis) and oxidation. Mussel lipids comprise large amounts of phospholipids, triacylglycerols and other lipid components (Zhou *et al.*, 2019; Zhang *et al.*, 2025). These lipid components are the primary substrates for lipolysis, during which lipolytic enzymes, such as lysosomal acid lipase and phospholipase, cleave the ester bonds linking fatty acids to the glycerol backbone. Lipolytic enzymes of both endogenous and microbial origin may remain active at low temperatures. Lipolysis leads to an increase in free fatty acid (FFA) concentrations, which comprise varying proportions of saturated and unsaturated fatty acids (Williamson *et al.*, 2014; Zhang *et al.*, 2023). The continuous release of FFAs at varying rates throughout the storage period may therefore contribute to the observed fluctuations in the fatty acid contents. Furthermore, lipolysis is closely linked to lipid oxidation. Lipolysis alters the structural integrity of lipid molecules, such as phospholipids and triacylglycerols, potentially exposing more vulnerable sites to oxidative attack and allowing hydrolysed lipid molecules, such as FFAs, to serve as substrates for lipid oxidation.

Among the three fatty acid classes, ΣPUFA showed the highest reductions during the 20-day storage period (20.86%–52.92%), followed by ΣMUFA (0.67%–40.10%) and ΣSFA (2.61%–35.09%). The higher degree of unsaturation in PUFA, resulting from their higher number of double bonds, makes them more susceptible to oxidation, thus accounts for their greater reductions. In contrast, SFA lack double bonds and are thus less susceptible to oxidation, resulting in the lowest reductions among the three fatty acid classes. The current results showed a significant negative correlation ($r = -0.68$; $p<0.01$) between lipid oxidation, as indicated by TBARS values, and ΣPUFA content, suggesting that the progression of lipid oxidation led to the reduction in ΣPUFA content. As lipid oxidation progressed, the FFAs, particularly PUFAs, may have been consumed, leading to a decrease in their levels. Previous studies have reported that reductions in ΣPUFA content during cold storage were associated with the high susceptibility of unsaturated fatty acids to oxidative degradation (Yu *et al.*, 2020; Bejaoui *et al.*, 2021). Therefore, both lipolysis and lipid oxidation likely contributed to the observed fluctuations in fatty acid contents during cold storage in the present study. The large standard deviations in the α values observed in specific organic acid treatments in Table 2, Table 3, and Table 4 likely resulted from localized variations in the dual processes of lipid hydrolysis and oxidation rates. In these particular samples, unpredictable enzymatic responses and varying degrees of pro-oxidant metal ion release amplified biological variance. Furthermore, calculating relative percentage changes mathematically magnified these sample-specific fluctuations, particularly against low baseline fatty acid concentrations.

The application of organic acids resulted in greater reductions in the fatty acid contents. These greater reductions in the treated samples were primarily attributed to accelerated oxidation. The organic acid treatments created a more acidic environment, which may have influenced the activities of lipolytic and oxidative enzymes, such as lysosomal acid lipase and lipoxygenase. Both enzymes exhibit optimal catalytic activities under slightly acidic conditions, with reported optimal pH values ranging from 5.5 to 6.0 (Banerjee *et al.*, 2002; Tatiyaborworntham *et al.*, 2022). In the present study, the pH of both the control and treated samples remained slightly acidic, ranging from 6.8 to 6.9, over the storage period (Oh *et al.*, 2025). Lysosomal acid lipase is involved in the lipolysis of triacylglycerols, whereas lipoxygenase catalyses the oxidation of PUFAs, producing hydroperoxides that initiate and propagate lipid oxidation (Domínguez *et al.*, 2019). The joint activities of these enzymes may contribute to the concomitant loss of fatty acids and the accumulation of primary (hydroperoxides) and secondary (MDA) oxidation products. Wu *et al.*

(2022) reported that lipoxygenase activity plays a more pivotal role in the onset of lipid oxidation than the fatty acid composition. Therefore, accelerated lipolysis and lipid oxidation may have contributed to the greater reductions in the fatty acid contents observed in the treated samples in the present study.

4. Conclusion

Lipid oxidation progressed in green mussels (*Perna viridis*) during cold storage. The TBARS values of the organic acid-treated mussels, including both single-acid and combination-acid treatments, were significantly higher than those of the control, indicating a higher degree of lipid oxidation and greater susceptibility to lipid oxidation following organic acid treatment. A total of 20 individual fatty acids were identified in green mussel lipids, which were characterised by a high proportion of polyunsaturated fatty acids (PUFA), followed by saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). The contents of SFA, MUFA, and PUFA generally decreased over the storage period and organic acid treatments accelerated these reductions. A significant negative correlation between TBARS values and total PUFA contents suggested that lipid oxidation contributed substantially to the fatty acid depletion during cold storage. Overall, the application of organic acids exerted a pro-oxidant effect, causing lipid oxidation, contributing to the reduction of fatty acids in green mussels during cold storage.

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