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Effects of Glyoxylic Acid on Metabolism and Ripening of 'Rocha' Pears Treated with 1-MCP

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Abstract: The application of 1-methylcyclopropene (1-MCP) is widely used to extend the storage life of climacteric fruits, such as 'Rocha' pears. However, the suppression of ethylene's action by 1-MCP often results in excessive ripening delay, compromising fruit quality and consumer acceptance. In this study, we investigated the potential of glyoxylic acid (GLA) to counteract the effects of 1-MCP and promote ripening. To evaluate this, 'Rocha' pears treated with 1-MCP were exposed to 3% (*m*/*v*) GLA and stored at 20 ± 2 °C for 15 days. Typical ripening indicators, such as firmness, skin color, ethylene production, respiration rate, volatile organic compounds (VOCs), sugars, and the activity of ethylene biosynthetic enzymes, were measured. Our results indicate that GLA did not induce significant effects on the ripening response, as ethylene production remained comparable to that of the control. Consequently, no significant changes in firmness, skin yellowing, or sugar content were observed in the GLA-treated pears. However, GLA significantly increased respiration rates (approximately 57%) and induced higher emissions of stress-associated VOCs, including hexanal, (E)-2-hexenal, and ethanol. This suggests that GLA may influence metabolic pathways related to energy metabolism and redox homeostasis without necessarily triggering ethylene-induced ripening. This study provides new insights into the interactions between GLA, 1-MCP, and fruit development, contributing to the development of alternative strategies to manage the effects of 1-MCP in 'Rocha' pear storage.

Keywords: 1-methylcyclopropene; ripening-inducing strategies; GLA; postharvest ripening; ethylene

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1. Introduction

The primary challenge of the fruit sector today is not only to extend the storability of perishable fruits but also to ensure the maintenance of fruit quality during postharvest storage, including during cross-border and long-distance shipping [1,2]. To achieve this, the pear industry relies on 1-MCP and cold storage to delay senescence, thereby extending the fruit's availability throughout the year [3,4]. However, despite its effectiveness in reducing postharvest decay, 1-MCP disrupts the normal ripening process after cold storage [5,6].

Physiologically, 1-MCP competes with ethylene, the key hormone responsible for coordinating climacteric fruit ripening, by binding to ethylene receptor proteins located in the endoplasmic reticulum membrane. This binding downregulates ethylene signaling and biosynthesis genes, thereby suppressing or delaying essential ripening responses [7,8].

Consequently, the fruits remain unripe, failing to develop desirable textural, aromatic, and flavor attributes, which reduces their commercial value and leads to economic losses.

The Portuguese 'Rocha' pear (*Pyrus communis* L. 'Rocha'), a Protected Designation of Origin (PDO) cultivar, generates around EUR 120 to 130 million per year, having a great positive impact on the Portuguese economic balance. It is a cultivar known worldwide for its exceptional flavor. With the increase in exportation, conservation strategies have been aimed at maintaining the availability of 'Rocha' pear throughout 10 months. At the moment, it is a cultivar particularly affected by the challenges posed by 1-MCP [5]. Currently, no alternative exists to replace this technology. However, achieving a balance between preventing postharvest disorders and ensuring proper ripening remains difficult. According to Isidoro and Almeida [9], the optimal 1-MCP concentration necessary to prevent superficial scald also prevents consistent ripening. As a result, the search for new strategies that mitigate 1-MCP's effects while preserving fruit's quality and marketability has become urgent.

Over recent decades, multiple approaches have been explored to restore ripening in pears treated with 1-MCP. These methods have primarily focused on physical treatments, such as exposure to exogenous ethylene [10,11], increasing the storage temperature, or a combination of both [12,13]. However, these techniques have shown limited effectiveness in overcoming the ripening blockage caused by 1-MCP [7], and they often exhibit cultivar-specific responses. According to producers, exposure to non-chilling temperatures and/or exogenous ethylene in 'Rocha' pears fails to restore ripening and is also energy-intensive. Therefore, the identification of effective treatments to counteract the effects of 1-MCP in 'Rocha' pears is a critical necessity.

Recent advancements in chemical genomics have identified specific organic compounds as regulators of fruit's development and ripening [14–17]. Among these, GLA has emerged as a potential metabolic activator in fruit treated with 1-MCP [7,18]. GLA is an organic acid belonging to the class of alpha-keto acids, acting as a key metabolic intermediate in various biochemical pathways, including the glyoxylate cycle and amino acid metabolism. Its chemical structure contains both an aldehyde (-CHO) and a carboxyl (-COOH) group, making it a highly reactive and biologically relevant compound [19,20].

GLA is known to play various roles in plants' metabolism, particularly as an intermediate in the glyoxylate cycle, which is crucial for gluconeogenesis and the mobilization of stored lipids. Additionally, GLA has been associated with responses to oxidative stress and metabolic adjustments under environmental stress conditions [20]. While its precise role in ethylene synthesis has not been established, it may influence hormonal crosstalk and energy metabolism, potentially affecting postharvest physiological responses in fruit.

So far, no documented attempts have been made to assess whether exogenous GLA can restore the ripening capacity of 1-MCP-treated 'Rocha' pears. This study aimed to investigate whether GLA treatment could significantly enhance the ripening process following 1-MCP application. Our hypothesis was that GLA could influence metabolic pathways linked to ripening, thereby mitigating the ripening delay caused by 1-MCP. To test this, we analyzed key ripening indicators, including fruit firmness, total soluble solids (TSS), volatile organic compounds (VOCs), respiration rate, and ethylene production during the ripening phase. Additionally, we examined the activity of essential enzymes related to ethylene biosynthesis, particularly assessing whether GLA treatment could induce 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO) activity in 'Rocha' pears.

2. Materials and Methods

2.1. Fruit Material and Experimental Design

'Rocha' pear (*Pyrus communis* L. 'Rocha') fruits were obtained from a commercial orchard in Cadaval, in the West Region of Portugal (latitude 39°25′ N; longitude 8°54′ W; elevation 120 m). Pears were harvested in August 2021 at the optimal harvest date according to local growers. After picking, the pears were held at 0 °C and 90–95% relative humidity (RH) for seven days. After this period, the pears were treated via 24 h of fumigation at the grower's cold packinghouse with 312 ppb 1-MCP (SmartfreshTM, AgroFresh Inc., Philadelphia, PA, USA), following current industry practices, and maintained under the same cold conditions (0 °C and 90–95% relative humidity) for two months. Following this time, the pears were transferred to controlled-atmosphere storage (CA) at −0.5 °C, per the standard industry practice, until the start of the treatments.

Experiments were initiated by removing pears previously treated with 1-MCP from CA storage. The pears were split into two groups: a control group (CTRL) and a group treated with GLA. The latter pears were immersed in a 3% (*m*/*v*) GLA solution for 2 h, as described by Hewitt et al. [7]. After the treatments, all pears were kept at room temperature (20 ± 2 °C) for 15 days to simulate the 'Rocha' pear conditions when they reached the consumers, and they were analyzed at 0 days (T0), 7 days (T7), and 15 days (T15) after treatment.

2.2. Fruit Quality Evaluation

Color, firmness, TSS, and titratable acidity (TA) were evaluated for 9 fruits per treatment and sampling point, as described by Dias et al. [21]. Color (hue angle) and firmness (N) were measured on two opposite sides of each fruit using a portable CR-400 colorimeter (Konica Minolta, Osaka, Japan) and a texturometer (T.A. XT plus Texture Analyser, Stable Micro Systems, Cardiff, UK) equipped with an 8 mm diameter probe, respectively. The drop of the hue angle describes the surface color transition from green to yellow [22,23]. SSC was evaluated with a digital refractometer (PR1 ATAGO Co., Ltd., Tokyo, Japan), with the juice from 3 fruits per replication, in a total of 3 replicates. TA (g malic acid kg^{-1}) was determined through titration using 0.1 M NaOH until reaching a pH of 8.1. This process consisted of homogenizing 10 g of pear (3 fruits per replication, in a total of 3 replicates for each treatment and sampling point) combined with 90 mL of distilled water.

2.3. Ethylene Production, and Related Metabolites and Biosynthetic Enzymes

At each sampling point, 3 replicates of 3 fruits each were acclimatized at 23 °C in 1.5 L glass jars. After 2 h of incubation, ethylene production (µg kg^{-1}) was measured by injecting 1 mL of headspace into a gas chromatograph (Varian CP-3380 gas chromatograph, Walnut Creek, CA, USA) equipped with an activated alumina column (50 m length and 0.53 mm i.d.; Thermo Fisher Scientific Inc., Marietta, OH, USA) and a flame ionization detector (FID) set at 180 °C, as previously described by Saquet et al. [24]. Pear flesh samples were used to determine the enzymatic activities (ACO and ACS) and metabolites (ACC and MACC) involved in ethylene biosynthesis, as described by Bulens et al. [25].

2.4. Respiration Measurement

After ethylene measurement, the headspace of the same biological replicates was analyzed using an infrared sensor (Dansensor CheckMate 3, METEK, Chesterfield, MO, USA) in a closed-circulation circuit to determine the carbon dioxide production (mg CO_2 kg^{-1} h^{-1}), as described by Dias et al. [21].

2.5. Volatile Aromatic Compounds Profiling by SPME-GC-MS

VOCs were extracted by mixing 5 g of pear pulp with 1.8 g of NaCl in a 20 mL screw-cap vial, as described by Dias et al. [21]. To each sample, 20 µL of 50 mg L^{-1} 3-octanol was added as an internal standard. Subsequently, each vial was placed at 40 °C for 2 min, followed by exposure of the SPME fiber with a 50/30 µm thickness of divinylbenzene/carboxy/polydimethylsiloxane (DVB/CAR/PDMS; Supelco Co., Bellefonte, PA, USA) to the headspace for 40 min to adsorb the volatiles. After the adsorption, the fiber was injected into a EVOQ Triple-Quadrupole mass-selective detector (Bruker, Karlsruhe, Germany), equipped with a 456-GC gas chromatograph (Bruker, Karlsruhe, Germany) for desorption, at 220 °C for 10 min. Volatiles were analyzed and quantified in this GC-MS, utilizing helium as a carrier gas at a flow rate of 1.0 mL min^{-1}, and with the injector temperature set at 250 °C. The temperature program for volatile identification was 40 °C for 1 min, increasing by 2 °C per minute until it reached 220 °C. Then, the headspace was maintained at 220 °C for 5 min. Subsequently, spectral information was examined within the m/z range of 33–350. The identification and quantification of VOCs were accomplished with standard curves, with the results presented on a fresh weight basis (nmol kg^{-1}). The values reported are the mean from 3 biological replicates, each comprising a pool of 3 pears.

2.6. Determination of Sugars and Organic Acids

The extraction of sugars (sucrose, glucose, fructose, and sorbitol) and organic acids (malic acid) was performed following the protocols described by Giné-Bordonaba et al. and Lindo-Garcia et al. [26,27]. Briefly, around 2 g of frozen pulp tissue was mixed with 62.5% (v/v) aqueous methanol, and the supernatants were recovered for chromatographic analysis, using a Beckman Coulter System Gold HPLC (Knauer, Berlin, Germany) coupled with RI and UV detectors. The values reported are the mean from 3 biological replicates, each comprising a pool of 3 pears.

2.7. Statistical Analysis

Data analysis was performed in Metaboanalyst 6.0. (URL: https://www.metaboanalyst.ca/MetaboAnalyst/ModuleView.xhtml; accessed on 14 September 2024). An independent Partial Least Squares Discriminant Analysis (PLS-DA) [28] and the corresponding variable importance in projection (VIP) scores were obtained. The PLSDA models were developed with measured data for firmness, hue angle, SSC, TA, ethylene, respiration, sugars, organic acids, VOCs, ACS and ACO activity, and ACC (T0 = 0 days before GLA application; T7 = 7 days after GLA treatment; T15 = 15 days after GLA treatment). A heatmap showing the concentration range of each measured parameter over time was also obtained.

The average values of each variable across shelf-life time were compared by analysis of variance (one-way ANOVA), with Tukey's post hoc test for the identification of significant means. Additionally, the independent-samples t-test was used to detect significant differences between the two conditions for each experiment. The significance level was set at 5% in all cases.

3. Results

To address potential biological variability inherent in the data—particularly the differing physiological status of pears at the start of the experiment—data were normalized using the values recorded on day 0 of shelf-life.

3.1. Multivariate Analysis Reveals Distinct Clustering of GLA-Treated and CTRL Samples

A PLS-DA model was developed to evaluate the impact of exogenous GLA treatment on 'Rocha' pear ripening under the effect of 1-MCP. Figure 1 presents the PLS-DA scores

map and the corresponding VIP scores, where the first two components account for nearly 60% of the observed variability.

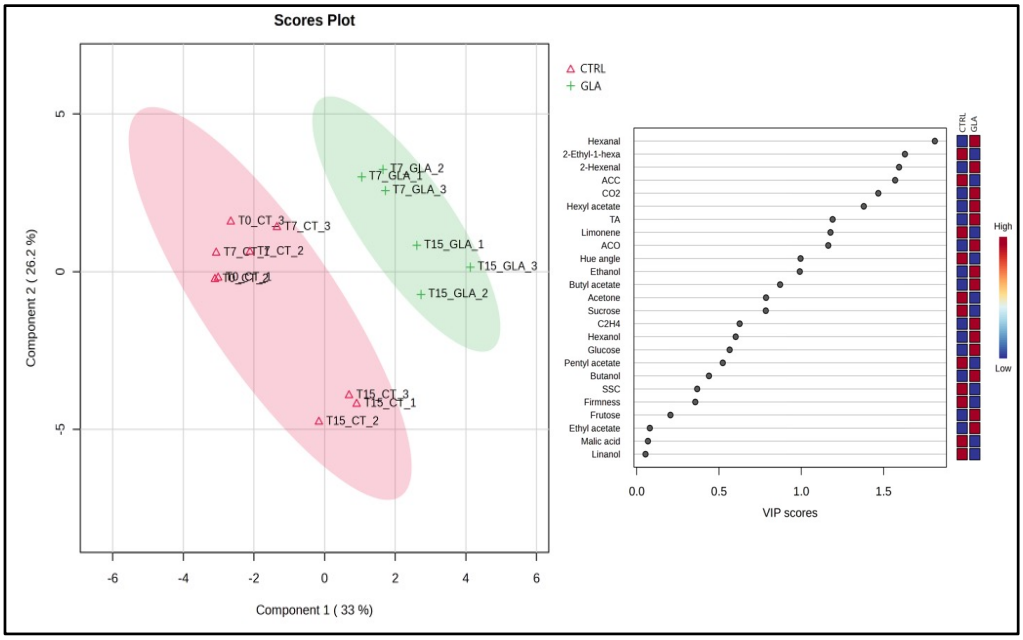

Figure 1. PLSDA scores map and VIP scores of 'Rocha' pear characteristics after treatment with GLA (T7_GLA and T15_GLA) and their respective controls (T0_CT; T7_CT and T15_CT) across 15 days of shelf-life (T0; T7 and T15).

The PLS-DA scores map reveals two well-defined clusters separating the GLA-treated from the CTRL samples (Figure 1). Notably, the CTRL samples from day 0 and day 7 are relatively close, indicating that these fruits maintained their measured parameters with lower variability over time, remaining similar to each other. However, after 15 days, the CTRL samples, while still in the same cluster as the other CTRL samples, were noticeably distanced from the T0 and T7 samples. In contrast, the GLA-treated pears exhibited differentiation over time and from the CTRL samples, particularly between day 7 and day 15, suggesting metabolic modifications driven by the treatment.

The VIP scores evidence the most relevant variables (measured parameters) that contribute most to the differentiation between the CTRL and GLA-treated samples. From the VIP scores (those higher than 1), it can be observed that the GLA treatment impacted the volatile emission pattern of pears, the respiration rate (CO_2), the ACC substrate concentration, the TA, the hue angle, and the ACO enzyme activity. These are the contributing variables that played the most significant role in distinguishing the CTRL samples from the GLA-treated samples.

Additionally, a heatmap was generated to allow for a more detailed analysis of the treatment's impact and highlight specific trends and similarities among samples (Figure 2).

Combining the VIP scores with the heatmaps obtained from each experiment (Figure 2), it can be corroborated that the GLA treatment mainly impacted the above-mentioned variables. The heatmap analysis demonstrates that the application of GLA had quite an impact on various compounds and physical characteristics, as significant color changes (from blue to red, and vice versa) in the heatmap between the CTRL and GLA conditions can be observed for some parameters, especially after 7 and 15 days. The GLA treatment significantly influenced the production of VOCs. Specifically, hexanal, 2-hexenal, butyl, and hexyl acetate showed increased levels (red intensity) in GLA-treated pears, while other VOCs, such as 2-ethyl-1-hexanol, limonene, and ethanol, exhibited varied or reduced responses to GLA treatment. Additionally, the heatmap shows that exogenous

GLA increased ACO enzyme activity, while the CTRL samples exhibited a higher ACC concentration until day 7, followed by a decrease after 15 days of shelf-life. Also, based on the color intensity from the heatmap, the GLA-treated samples showed the highest ethylene and TA concentrations at all timepoints compared to the CTRL samples. Furthermore, there was a decrease in sucrose concentration (blue shades in the heatmap) and an increase in CO_2 production (more intense red shades in the heatmap) in GLA-treated pears compared to the CTRL.

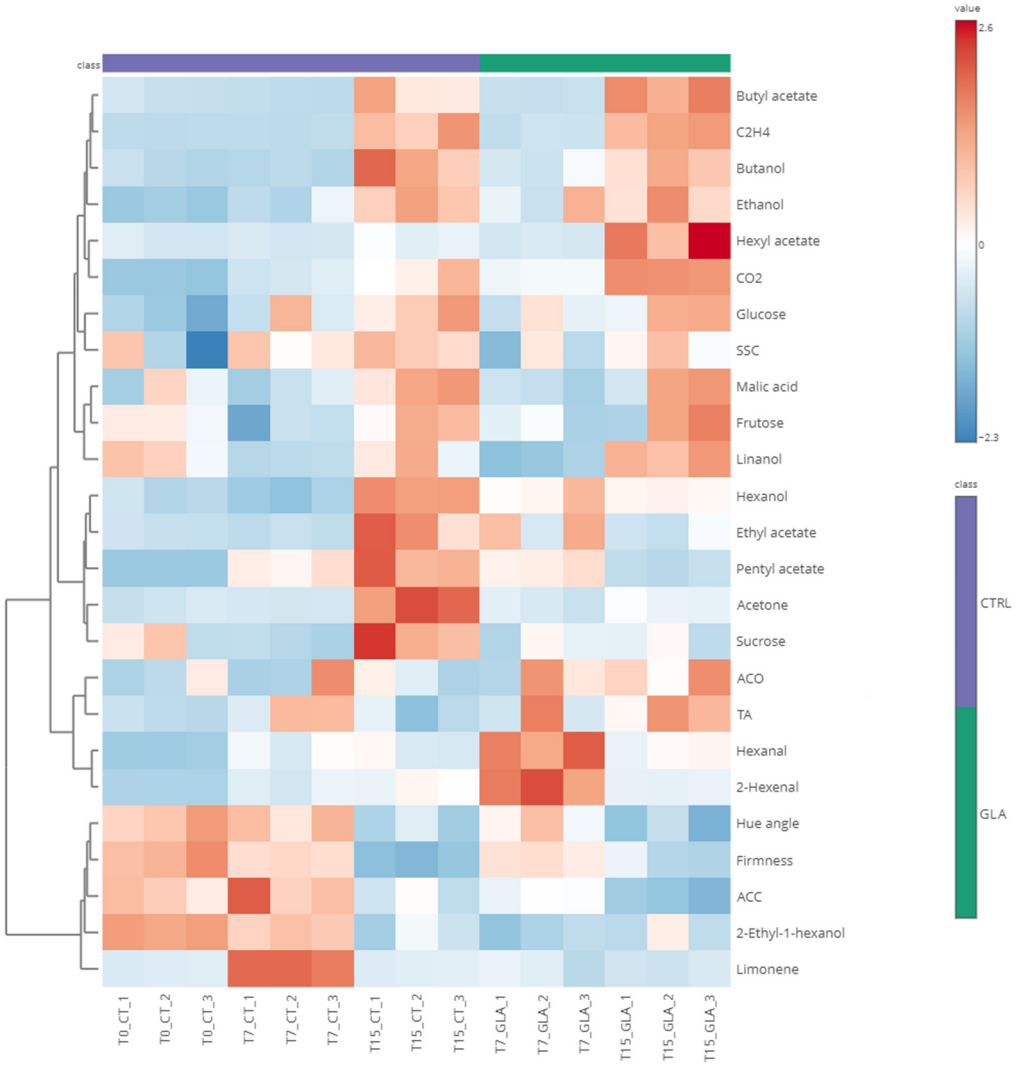

Figure 2. Heatmap obtained from the measured pear parameters during the experience time for CTRL samples (T0_CT; T7_CT and T15_CT) and GLA-treated samples (T7_GLA and T15_GLA). Blue and red squares indicates lower or higher intensity of the variables measured, respectively.

3.2. Multivariate Analysis Corroborates GLA-Induced Physiological Changes

Alongside the physicochemical changes indicated by the PLS-DA and heatmaps, we closely examined several key parameters, mainly those with a VIP score exceeding 1, to better understand the treatments' impact on ripening reactivation. Figure 3 illustrates ethylene levels over time across two conditions. In both conditions, the ethylene concentrations significantly rose from day 0 to day 15, although the rates of increase differed depending on the condition.

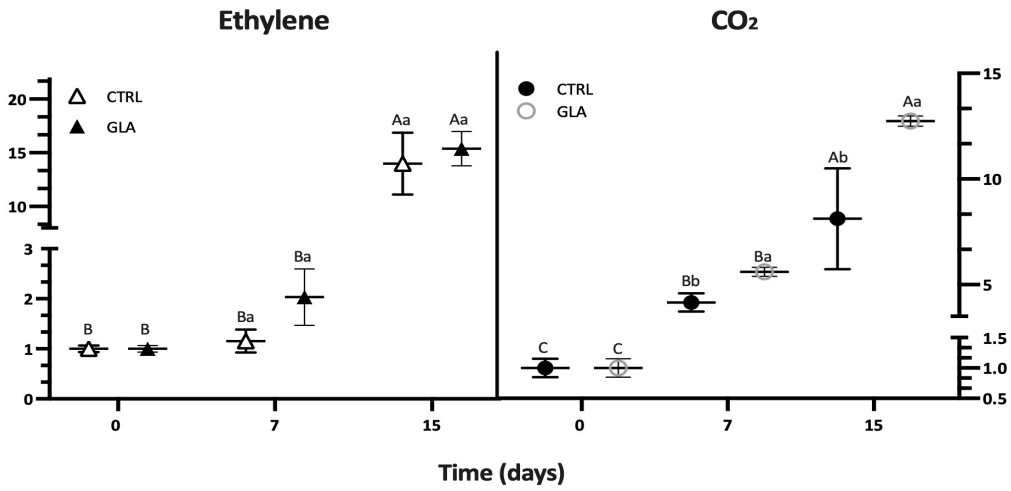

Figure 3. Ethylene and CO_2 variations in 'Rocha' pears during shelf-life at 20 °C, after treatment. Values are means ± standard deviations of normalized data. Different capital letters indicate significant differences ($p < 0.05$) across time for each condition. Different small letters indicate significant differences ($p < 0.05$) between conditions at each timepoint (0, 7, and 15 days).

During the first 7 days of the experiment, ethylene production in the CTRL group stayed consistently low, showing no significant increase, which reflects the suppressing effect of 1-MCP on ethylene biosynthesis. Interestingly, the 1-MCP effect was naturally overcome after 15 days. Regarding the GLA-treated group, the pears exhibited slightly higher ethylene levels than the CTRL group, but this difference was not statistically significant. This similarity aligns with the low variable importance of ethylene, as revealed by its VIP score below 1, in explaining the separation of the CTRL group from the treated one (Figure 1).

Regarding respiration rates, measured through CO_2 production, both conditions experienced increasing CO_2 levels throughout the 15 days. Despite no substantial difference being noted in ethylene production between the CTRL and GLA-treated groups, the GLA group demonstrated significantly elevated rates of CO_2, with increases of approximately 35% and 57% recorded after 7 and 15 days, respectively (Figure 3; Table 1).

Figure 4 illustrates that the GLA treatment did not result in significant higher firmness losses compared to the CTRL. However, contrary to what would be expected in pears subjected exclusively to 1-MCP, firmness in the CTRL group significantly decreased over time, which was particularly evident after a 15-day storage period, corresponding with the increased ethylene production observed at this timepoint (Figure 3).

Similar to firmness, the CTRL pears showed no obstructive effect from 1-MCP, as Figure 4 reveals that the hue angle decreased over time. GLA showed slightly lower hue angle values, but with no significant difference from the CTRL.

Sucrose and TA are additional parameters that demonstrated their significance in differentiating CTRL from GLA-treated samples based on the VIP scores. Notably, after 7 days, the sucrose concentration decreased similarly in both conditions (Figure 5). After 15 days, the GLA group maintained its sucrose concentration, whereas the CTRL group showed higher levels, indicating a significant difference between the two groups (Figure 5). Regarding TA, the GLA group showed a minor yet noticeable increase in total acidity over time, while the TA from the CTRL group remained stable (Figure 5).

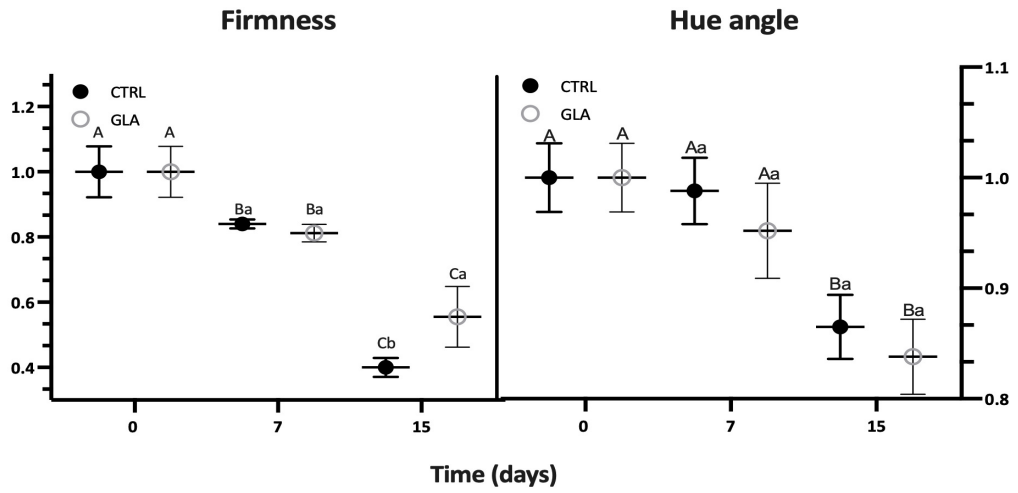

Figure 4. Firmness and hue angle variation of 'Rocha' pears during shelf-life at 20 °C after treatment. Values are means ± standard deviations of normalized data. Different capital letters indicate significant differences ($p < 0.05$) across time for each condition. Different small letters indicate significant differences ($p < 0.05$) between conditions at each timepoint (0, 7, and 15 days).

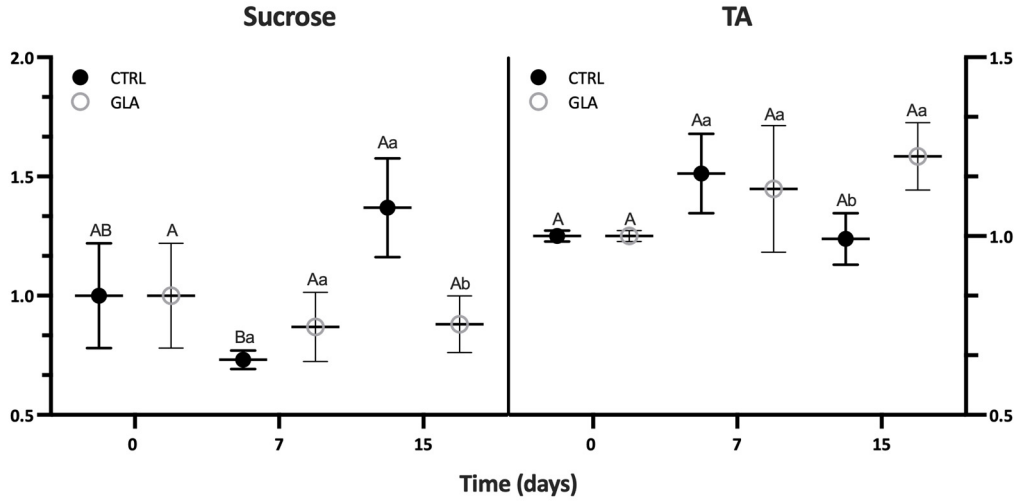

Figure 5. Sucrose and TA content variation in 'Rocha' pears after treatment with GLA during shelf-life at 20 °C. Values are means ± standard deviations of normalized data. Different capital letters indicate significant differences ($p < 0.05$) across time for each condition. Different small letters indicate significant differences ($p < 0.05$) between conditions at each timepoint (0, 7, and 15 days).

GLA application significantly impacted ACC concentrations (Figure 6). GLA-treated samples showed lower ACC levels, which continued to decrease after 15 days, while the CTRL group remained stable. Overall, 1-MCP consistently maintained ACC levels in the CTRL group, revealing GLA's potential to reverse this particular effect of 1-MCP. In relation to ACO enzyme activity, it was observed that both conditions' patterns were very similar, with no significant variation over time.

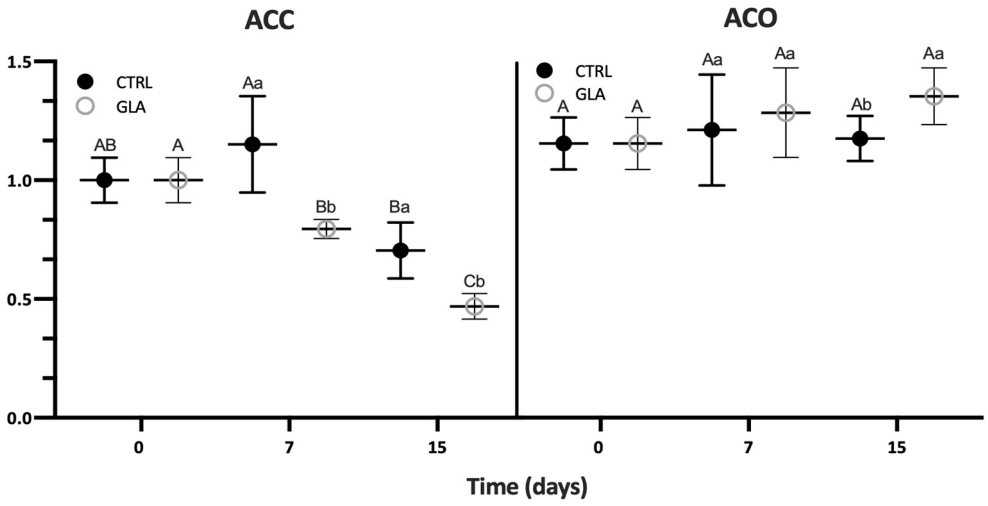

Figure 6. ACC content and ACO activity variation in 'Rocha' pears after treatment with GLA during shelf-life at 20 °C. Values are means ± standard deviations of normalized data. Different capital letters indicate significant differences ($p < 0.05$) across time for each condition. Different small letters indicate significant differences ($p < 0.05$) between conditions at each timepoint (0, 7, and 15 days).

In order to assess the contribution of the treatment to the aroma of the fruit, the emissions of VOCs were analyzed, and those of greater importance are presented in Table 1.

Table 1. Major VOC emissions in 'Rocha' pears after treatment during shelf-life at 20 °C. Values are means ± standard deviations of normalized data.

Aroma Volatile		0 D	7 D	15 D
Hexanal	CTRL	1 ± 0.22^{B}	12.94 ± 3.50^{Ab}	11.92 ± 4.41^{Aa}
	GLA	1 ± 0.22^{C}	37.00 ± 5.28^{Aa}	15.38 ± 2.98^{Ba}
Hexyl acetate	CTRL	1 ± 0.45^{A}	0.96 ± 0.26^{Aa}	2.36 ± 0.91^{Aa}
	GLA	1 ± 0.45^{B}	0.92 ± 0.24^{Ba}	12.70 ± 4.34^{Ab}
Butyl acetate	CTRL	1 ± 0.28^{B}	0.46 ± 0.13^{Bb}	5.04 ± 1.86^{Aa}
	GLA	1 ± 0.28^{B}	0.77 ± 0.08^{Ba}	7.66 ± 0.96^{Aa}
(E)-2-Hexenal	CTRL	1 ± 0.19^{B}	8.24 ± 1.79^{Ab}	12.46 ± 2.45^{Aa}
	GLA	1 ± 0.19^{C}	34.11 ± 5.48^{Aa}	9.43 ± 0.16^{Ba}
Limonene	CTRL	1 ± 0.02^{B}	2.08 ± 0.05^{Aa}	1.02 ± 0.02^{Ba}
	GLA	1 ± 0.02^{A}	0.96 ± 0.15^{Ab}	0.93 ± 0.03^{Aa}
2-Ethyl-hexanol	CTRL	1 ± 0.02^{B}	0.85 ± 0.04^{Aa}	0.48 ± 0.13^{Aa}
	GLA	1 ± 0.02^{A}	0.37 ± 0.07^{Bb}	0.52 ± 0.17^{Ba}
Ethanol	CTRL	1 ± 0.06^{B}	1.49 ± 0.36^{Ba}	3.02 ± 0.33^{Aa}
	GLA	1 ± 0.06^{B}	2.18 ± 0.9^{ABa}	2.92 ± 0.60^{Aa}

Different capital letters indicate significant differences ($p < 0.05$) across time for each condition. Different small letters indicate significant differences ($p < 0.05$) between conditions at each timepoint (0, 7, and 15 days).

Concerning the effects of GLA treatment on VOC emissions, it was observed that there was a notable spike in specific volatile compounds, such as hexanal and (E)-2-hexenal, at 7 days, followed by a drop at 15 days when compared to the CTRL group. Moreover, GLA drove a substantial increase in hexyl and butyl acetate levels by the 15th day of shelf-life, while CTRL showed only a minor rise. For limonene and ethanol, the CTRL maintained higher levels than the GLA group across time, although they were not statistically different.

4. Discussion

The suppression of ripening traits by 1-MCP negatively impacts essential fruit ripening quality attributes such as texture, sugar content, acidity, and aroma, directly affecting consumer acceptance [29,30]. Previous studies in 'D'Anjou' pears have shown that organic acids like GLA can modulate fruit's metabolism, although their capacity to reverse 1-MCP-induced ripening inhibition remains largely unexplored, especially in other cultivars [7,31].

In this study, we hypothesized that GLA could influence metabolic pathways of 'Rocha' pears, particularly those related to ripening, under 1-MCP treatment. Multivariate data analysis revealed that GLA promoted changes in the fruit, as evident in the PLS-DA clustering patterns that distinguished GLA-treated samples from CTRL samples, thereby showing GLA's ability to disrupt some usual effects of 1-MCP. However, GLA did not significantly promote a different pattern compared to 1-MCP-treated fruits (CTRL) in key ripening indicators such as ethylene production, fruit softening, or color changes. Instead, GLA-treated pears exhibited increased CO_2 production and specific VOCs, namely, esters, aldehydes, terpenes, and alcohols (Table 1). GLA promoted higher emissions of ethyl, butyl, and hexyl acetates, which are esters strongly related to ripening, as well as limonene [32,33]. However, since these results were not accompanied by an increase in ethylene production, and combined with other ripening results, they suggest that GLA affected other metabolic pathways unrelated to ethylene-driven ripening. It is known that GLA plays a key role in amino acid metabolism, which serves as a precursor for various VOCs, including the formation of aldehydes, alcohols, and esters, contributing to fruits' aroma [20]. Since GLA can influence amino acid metabolism, it is plausible that these VOC changes resulted from alterations in precursor availability, shifting metabolic fluxes toward stress- or defense-related pathways rather than ripening-associated volatile synthesis [7,20]. For example, studies in apples and tomatoes have shown that alterations in amino acid pathways lead to modifications in volatile production, particularly affecting aldehydes and alcohols [34,35]. In kiwifruit, shifts in amino acid metabolism have been linked to increased ester formation [36].

Additionally, as GLA is an intermediate of the glyoxylate cycle, which is connected to lipid metabolism, it may indirectly affect metabolic pathways involved in aldehyde and ester biosynthesis. In fact, higher levels of the aldehydes hexanal and (E)-2-hexenal were observed in GLA-treated pears. These compounds contribute to the green aroma present in fruits and vegetables, and they are commonly associated with plant defense mechanisms and lipid oxidation [37,38]. Notably, the alcohols ethanol, butanol, and hexanol—volatile compounds strongly associated with fermentation and oxidative stress [39]—were also present in higher amounts in the GLA-treated fruits. The higher ethanol concentration suggests that GLA may have influenced fermentation-related pathways, possibly by shifting carbon fluxes toward anaerobic metabolic routes [7].

Based on these results, GLA-treated pears exhibited increased emissions of VOCs linked to oxidative stress, suggesting that GLA may have triggered stress-related metabolic pathways rather than those directly involved in ripening. This aligns with higher respiration rates and possible ROS accumulation, leading to lipid oxidation and the release of stress-associated volatiles.

Notably, the 1-MCP-treated CTRL group exhibited a natural ripening progression, as evidenced by the distance of CTRL at T15 from T0 and T7. This suggests that these pears underwent some physiological and metabolic alterations even under the exclusive effect of 1-MCP, as evidenced mainly by a loss of firmness and increased ethylene production over time, indicating that 1-MCP's effect was not entirely restrictive during this phase of the study. Several studies have shown that the extent of 1-MCP's effect varies primarily

based on the cultivar, the timing of postharvest treatment application, storage conditions, and the fruit's developmental stage at analysis/treatment [6,10]. For instance, a study by Hewitt et al. [7], which used the 'D'Anjou' cultivar, found that GLA treatment accelerated the ripening response, leading to increased ethylene production and reduced firmness compared to 1-MCP-treated fruits. Additionally, over time, fruits synthesize new ethylene receptors that are not bound by 1-MCP [33], allowing ethylene perception to resume, and gradually restoring normal ripening processes. In our study, the 1-MCP-treated pears eventually began to ripen, suggesting that ethylene perception was restored over time due to receptor renewal. This loss of 1-MCP's effect could explain why GLA was unable to entirely counteract its impact; by the time GLA was applied, the fruit was already regaining its ripening ability. This raises the possibility that the physiological status of the pears at the time of GLA application influenced the outcome, potentially interfering with GLA's expected effects [34].

In this study, GLA caused no significant impact on ethylene production compared to the CTRL. This finding aligns with the lower ACC concentrations measured in GLA-treated fruits. Since ACC is the direct precursor of ethylene, its lower levels in GLA-treated fruit suggest that either ACS enzyme activity (the enzyme responsible for producing ACC) was inhibited or ACC was rapidly metabolized by the ACO enzyme into ethylene [40,41]. In fact, ACO activity showed no significant differences between the conditions, and the ethylene levels in both CTRL and GLA-treated pears were similar. So, the limiting factor was ACS enzyme activity. This suggests that the basal ACS activity caused by 1-MCP was not significantly stimulated by GLA, which explains the low ACC values and thereby limits higher ethylene biosynthesis compared to the CTRL. This demonstrates the central role of ethylene in regulating the ripening process of 'Rocha' pears.

Sugars play a crucial role in the ripening process and influence consumer preferences. The primary sugars that vary in pears during ripening are sucrose, glucose, and fructose [42,43]. Numerous studies have shown a positive relationship between ethylene and sucrose production, particularly through the activation of enzymes that convert starch into simpler sugars [35,44]. Hence, the significant increase in ethylene production observed in both conditions during the time of analysis supports the measured pattern of sucrose (Figure 5). The rise in sucrose levels in the CTRL samples suggests starch degradation, which aligns with the observed loss of firmness [21]. Additionally, further breakdown of sucrose into simpler sugars, such as fructose and glucose, did not occur, as indicated by the lack of increase in these sugars shown in Table 1. The slight decrease in sucrose observed in GLA-treated pears, combined with the higher respiration measured in these pears, suggests its utilization as a substrate for respiration and consequent higher CO_2 production [45].

Despite the lack of ethylene stimulation, GLA-treated pears exhibited a significant increase in respiration rates, reinforcing that GLA activates alternative metabolic pathways. Elevated respiration without softening or ethylene production suggests that GLA-induced metabolic changes are more related to stress responses or energy metabolism than to ripening itself. Similar patterns were observed in citrus fruits, where exogenous organic acids altered respiration without promoting ripening-associated changes [39].

5. Conclusions

So far, no studies have investigated the effects of GLA on the postharvest ripening of 'Rocha' pears under 1-MCP. This study confirms that GLA influences metabolic processes in 'Rocha' pears under 1-MCP treatment, but its effects are not directly linked to ethylene-driven ripening. Instead, GLA primarily influenced respiration rates and the biosynthesis of stress-associated VOCs, indicating an impact on metabolic pathways related to energy metabolism and redox homeostasis rather than ethylene-mediated ripening mechanisms

that need further exploration. An interesting point worth further investigation in this experiment is the unexpected ripening observed in the CTRL group, which may justify the low impact of GLA on pears. Future research should focus on analyzing the genetic expression of ethylene receptors to better understand how receptor renewal and turnover influence the recovery of ethylene sensitivity and the overall ripening process in 1-MCP-treated pears. Additionally, these findings highlight the importance of the fruit's physiological state at the time of treatment application, suggesting that GLA's impact on ethylene metabolism may depend largely on the residual effects of 1-MCP.

Future research should focus on the optimal timing of GLA application during storage, particularly in relation to the waning effect of 1-MCP at the time of treatment. Additionally, investigating how GLA modulates amino acid metabolism and other biochemical pathways will be crucial to optimizing its role in postharvest management strategies. Understanding these mechanisms could provide valuable insights into the modulation of fruit metabolism, particularly in climacteric species, where balancing ripening and shelf-life extension is a key postharvest challenge.

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Appendix A

Table 1. Evolution of physicochemical parameters in 'Rocha' pears across shelf-life after treatments. Data are the mean ± SD. For the genetic expression results, data are the mean ± SE.

Time (d)	SSC (%)		TA (g kg^{-1})		Firmness (N)		Hue Angle (°)		CO_2 (mg $(kg.h)^{-1}$)		C_2H_4 (µg $(kg.h)^{-1}$)	
	CTRL	GLA	CTRL	GLA	CTRL	GLA	CTRL	GLA	CTRL	GLA	CTRL	GLA
0	12.32 ± 0.80	12.32 ± 0.80	11.74 ± 0.18	11.74 ± 0.18	43.93 ± 3.43	43.93 ± 3.43	95.91 ± 2.99	95.91 ± 2.99	1.98 ± 0.30	1.98 ± 0.30	0.74 ± 0.05	0.74 ± 0.05
7	12.93 ± 0.20	12.40 ± 0.46	13.80 ± 1.30	13.29 ± 2.08	36.88 ± 0.61	35.68 ± 1.18	94.81 ± 2.83	91.27 ± 4.08	8.22 ± 0.86	11.07 ± 1.42	0.86 ± 0.17	1.51 ± 0.42
15	13.12 ± 0.13	12.90 ± 0.27	11.65 ± 0.84	14.36 ± 1.11	17.56 ± 1.27	24.4 ± 4.10	83.00 ± 2.82	83.40 ± 3.25	16.04 ± 4.72	25.17 ± 0.48	10.39 ± 2.14	11.41 ± 1.18
	Sucrose (mg g^{-1})		Sorbitol (mg g^{-1})		Glucose (mg g^{-1})		Fructose (mg g^{-1})		Malic acid (mg g^{-1})		ACC (nmol g^{-1})	
0	5.07 ± 1.12	5.07 ± 1.12	22.11 ± 1.50	22.11 ± 1.50	6.47 ± 0.80	6.47 ± 0.80	46.48 ± 1.28	46.48 ± 1.28	4.24 ± 0.73	4.24 ± 0.73	0.70 ± 0.07	0.70 ± 0.07
7	3.71 ± 0.20	4.41 ± 0.74	20.61 ± 4.98	17.03 ± 2.16	9.41 ± 0.70	8.77 ± 1.52	40.66 ± 3.09	43.44 ± 2.98	3.82 ± 0.32	3.76 ± 0.20	0.81 ± 0.14	0.56 ± 0.03
15	6.94 ± 1.05	4.46 ± 0.61	15.59 ± 1.80	14.78 ± 1.57	14.98 ± 3.46	10.39 ± 2.13	49.58 ± 2.98	49.23 ± 7.21	5.32 ± 0.50	5.07 ± 0.96	0.49 ± 0.08	0.33 ± 0.04
	ACO (nmol (g h) $^{-1}$)		MACC (nmol g^{-1})		ACS (nmol (g h) $^{-1}$)		Hexanal (ng g^{-1})		Hexanol (ng g^{-1})		Butyl acetate (ng g^{-1})	
0	2.04 ± 0.20	2.04 ± 0.20	36.84 ± 4.34	36.84 ± 4.34	Not detected		9.99 ± 2.35	9.99 ± 2.35	79.30 ± 11.76	79.30 ± 11.76	14.38 ± 4.33	14.38 ± 4.33
7	2.14 ± 0.41	2.27 ± 0.33	42.43 ± 4.10	41.29 ± 2.45			135.2 ± 36.61	386.68 ± 55.2	55.92 ± 9.88	153.36 ± 33.6	7.23 ± 1.94	12.09 ± 1.31
15	2.08 ± 0.17	2.39 ± 0.21	43.86 ± 3.84	41.44 ± 4.36			124.59 ± 46.1	160.76 ± 31.1	216.83 ± 8.01	137.57 ± 3.34	78.64 ± 28.96	119.62 ± 15.1
	Hexyl acetate (ng g^{-1})		2-ethyl-hexanol (ng g^{-1})		Butanol (ng g^{-1})		Ethanol (mg g^{-1})		Acetone (ng g^{-1})		(E)-2-Hexenal (ng g^{-1})	
0	2.07 ± 0.94	2.07 ± 0.94	5.25 ± 0.11	5.25 ± 0.11	59.35 ± 20.25	59.35 ± 20.25	3.90 ± 2.32	3.90 ± 2.32	90.66 ± 25.46	90.66 ± 25.46	3.12 ± 0.60	3.12 ± 0.60
7	1.99 ± 0.55	1.90 ± 0.49	4.44 ± 0.19	1.94 ± 0.38	51.19 ± 8.25	113.54 ± 38.5	5.82 ± 1.42	8.49 ± 3.48	103.02 ± 0.66	81.00 ± 25.00	25.65 ± 5.59	106.26 ± 17.1
15	4.89 ± 1.88	19.64 ± 14.63	2.49 ± 0.67	2.72 ± 0.86	347.24 ± 85.1	281.09 ± 52.9	11.77 ± 1.29	11.37 ± 2.33	593.47 ± 88.4	125.74 ± 19.6	38.82 ± 7.62	29.36 ± 0.51
	Limonene (ng g^{-1})		Hexane (ng g^{-1})		Linanol (ng g^{-1})		Ethyl acetate (ng g^{-1})		Pentyl acetate (ng g^{-1})			
0	10.38 ± 0.16	10.38 ± 0.16	Not detected		0.153 ± 0.02	0.153 ± 0.02	67.11 ± 4.57	67.11 ± 4.57	0.05 ± 0.008	0.05 ± 0.008		
7	10.96 ± 0.26	9.99 ± 1.52			0.11 ± 0.03	0.10 ± 0.01	60.54 ± 5.43	157.99 ± 69.1	1.06 ± 0.13	1.08 ± 0.11		
15	10.57 ± 0.17	9.67 ± 0.36			0.152 ± 0.02	0.175 ± 0.01	218.08 ± 62.1	81.52 ± 25.53	1.86 ± 0.41	0.31 ± 0.06		

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