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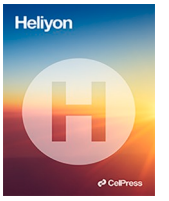

Effect of calcium spray at flowering combined with post-harvest 1-MCP treatment on the preservation of grapes

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ABSTRACT

These tests were carried out to find out how calcium and 1-MCP treatment affected the preservation of grapes, as grapes are highly susceptible to decay during post-harvest storage. The grapes were treated with 5 g/L calcium at the flowering stage, followed by 1 μL/L 1-MCP treatment after harvesting.

When grapevines were treated with a combination of calcium and 1-MCP, the marketable fruit rate (At day 56 of storage, the 1-MCP + Ca2+ treatment group was still 93%, an increase of 29.03% compared to the control group.) and quality improved (At day 28 of storage, the VC content of the 1-MCP + Ca2+ treated group was 4.35 mg/100g, an increase of 25.01% compared to the control group.), while the fruit weight loss rate decreased (At day 56 of storage, the weight loss of the control group was 6.97%, an increase of 39.43% compared to the 1-MCP + Ca2+ treated group.).

According to the experimental results, there are several reasons for this. First, in the early stages of fruit storage, the concentration of soluble pectin and soluble fiber, as well as the activities of pectinase and cellulase (related gene levels) were decreased. Secondly, the activity of antioxidant enzymes was increased, while MDA content was decreased. Third, during fruit storage, the respiratory intensity and ethylene release rate were reduced, as was the activity of energy metabolism enzymes. As a result, the aging and deterioration of the fruit during storage were delayed. Principal component analysis revealed that the calcium and 1-MCP combination therapy slowed the decline in grape berry quality, followed by the calcium-treated and 1-MCP-treated fruits. In contrast, grape berry quality declined the most rapidly in the control group.

1. Introduction

The grape (*Vitis vinifera* L.) is known as the 'queen' of fruits. It is widely grown in the world [1]. Grape berries are popular among customers due to their sweet flavor and high nutritional value [2]. However, grape berries are prone to cracking and rot after harvesting, which seriously affects the development of the industry, so it is necessary to find a suitable method for preserving grapes.

1-methylcyclopropene (1-MCP) is a potent inhibitor of ethylene action and can effectively delay the aging of fruit during storage [3]. Gong (2020) demonstrated the delayed effect of 1-MCP on softening of 'Hayward' kiwifruit during storage [4]. Guo (2020)

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Abbreviation

1-MCP	1-methylcyclopropene
SOD	Superoxide dismutase
CAT	Catalase
POD	Peroxidase
MDA	Malondialdehyde
SDH	Succinate dehydrogenase

revealed that 1-MCP treatment not only reduced the frequency of surface scald and rot in "Anjou pears," but also preserved flesh firmness, chlorophyll content, and colour angle [5]. In "Doyenne du Comice" Pear, Zhang (2021) proved that 1-MCP was highly effective in reducing disease incidence and induced multiple changes in the fungal and bacterial microbiota [6]. Zhao (2020) discovered that 1-MCP at 0.15 and 0.3 $\mu L\,L^{-1}$ effectively suppressed ethylene production, respiration rate, and chlorophyll degradation in pears [7].

Many studies have shown that calcium is often used to preserve fruits. Compared to other chemical preservatives, calcium is non-toxic, and it also promotes plant growth [8,9]. Calcium prevents fruit cracking and reduces the incidence of fruit diseases by increasing the mechanical strength of the peel [10]. Akhtar (2010) found that 2% and 3% $CaCl_2$ retained maximum firmness (loquat fruit), SSC (soluble solid content), ascorbic acid content, and reduced browning index, relative electrical conductivity, and weight loss [11]. Pre-harvest calcium spray increased the total phenolic and total flavonoid content in the fruit and may have better storability than post-harvest calcium treatment [12]. Li (2020) found that preharvest calcium treatment (1% and 2%) increased the activity of superoxide dismutase, catalase, and ascorbic acid peroxidase during late storage, thus delaying fruit ripening and enhancing the disease resistance of mango fruit [13]. Ghorbani (2021) found that spraying calcium during the growing period resulted in a significant increase in calcium concentration in apples and also significantly improved storage tolerance during the postharvest period [14]. In "Liberty" Blueberries, Lobos (2021) found a high positive correlation between fruit hardness and fruit calcium concentration [15]. Early calcium treatments showed increased fruit hardness and better antioxidant response compared to later stages. Zhang (2021) found that pre-harvest calcium sprays improved the antioxidant potential of berries by regulating genes related to the flavonoid metabolic pathway, thereby reducing the incidence of berry cracking [16]. Meanwhile, We sprayed calcium (5.0 g/L) at different growth periods of grapes (inflorescence, flowering, fruit development and end of fruit) and found that the best anti-cracking effect was achieved by spraying calcium at flowering [17].

Combined treatment of different preservatives in fruits (such as galia melont, mangot) tends to be more effective than a single treatment [18,19]. Yan (2020) found that the combined treatment of 1-MCP and $CaCl_2$ was effective in extending the storage period of fruits (cherry tomatoes) and improving the hardness of fruits in storage [20]. Nguyen (2020) found that combined treatment of strawberries with 3% $CaCl_2$ and nano chitosan significantly reduced weight loss, retained L-ascorbic acid, total anthocyanin content, antioxidant capacity, and delayed malondialdehyde production [21]. Tia (2020) found that combined treatment of dates with calcium chloride and salicylic acid significantly reduced the water loss from the fruit and maintained a better texture of the dates [22].

Previous studies focusing on the combination of calcium (post-harvest) and other preservatives (post-harvest) have shown promising results. However, no one has studied the combination of calcium treatment (pre-harvest) and other preservatives treatment (post-harvest) for preserving fruits. In this paper, the effect of calcium (pre-harvest, calcium spray at flowering) combined with 1-MCP (post-harvest) treatment on grape preservation was studied. The results provide a basis for extending the storage period of 'Li Xiu' grapes.

2. Materials and methods

2.1. Plant materials

The subject of the experiment was the seven-year-old 'Li Xiu' grape. (The 'Li Xiu' grape is an american variety with large, thick leaves, disease resistance, early maturity, consistent yield, no kernels, high quality, high sugar content, and intense aroma) under rain shelter cultivation at the experimental base of Hunan Applied University in Changde, Hunan, China (28°99′N, 111°67′E). The same field management practices were used for all vines (The grapes were cultivated in T-shape, rain-sheltered, with a north-south orientation, 3 × 2 m spacing, no hormone treatment during fruit development, one inflorescence per fruiting branch, and consistent fertilizer and water management). Three sprays [(May 7th, blooming period), (May 12th, flower-shedding period), (May 17th, end of flowering period)] were carried out with 5.0 g/L $CaCl_2$ at the flowering period, sprayed with distilled water as control. "LiXiu" grapevines were harvested on July 28, 2021, it is about 5~8 g, the skin is purple-red, and the soluble solids content is about 13.0–14.0%.

2.2. Fruit treatment

The grapes are picked between 6 and 7 a.m. and placed in pre-cooled foam boxes immediately after picking. Grapes of uniform size, free from mechanical damage, pests, and diseases, and of uniform ripeness were used for the experiments. The experiment was divided

into 4 groups with 3 replicates in each group. Pre-harvest calcium-treated fruit (Ca2+), Distilled water treated fruits as control (CK), Pre-harvest calcium + post-harvest 1 μL 1-MCP treated fruit (1-MCP + Ca2+), and Control + post-harvest 1-MCP treated fruit (1-MCP), respectively. Finally, each group of grape berries was stored at 4 ± 1 °C and 75% humidity. During storage, the quality and physiological indicators of the grapes were measured every seven days, etc.

2.3. Marketable fruit rate, weight loss rate, fruit hardness, fruit color

Marketable fruit rate (good fruit percentage) (%) = (Number of good fruits/Total number of fruits)*100%. The weighing method used for evaluation and measurement was based on the following formula: weight loss rate/% = [(quality before storage−quality after storage)/quality before storage] × 100. 10 fruits were taken from each group, the fruit hardness (breaking force) was determined with an ST-Z16 texture analyzer (Shengtai, Shandong, China). The fruit color of grape berries was measured at the equator with a CS-10 colorimeter (Sucolor, Shenzhen, China). The color index of red grapes (CIRG) is calculated with the equation CIRG = [(180−h°)/(L*+C*)] [23].

2.4. Titratable acid, vitamin C, soluble sugar, soluble solids content

5 g of pulp was taken in a mortar, 20 ml of distilled water was added and ground, centrifuged and 1 ml of the supernatant was taken to determine the titratable acid content. Titratable acid (TA) content in fruits was determined by acid-base titration, units indicate grams of TA per hundred grams of grape (%). 5 g of pulp was taken in a pre-cooled mortar and ground by adding 20 ml of 1% oxalic acid solution, centrifuged (4 °C) and 1 ml of the supernatant was taken for determination of vitamin content. Vitamin C (VC) content in fruits was determined by iodine titration, units indicate milligrams of VC per hundred grams of grape (mg/100g). 5 g of pulp was taken in a mortar, ground with 20 ml of distilled water, centrifuged and 0.01 ml of the supernatant was taken to determine the soluble sugar content. Determination of soluble sugar (SS) content in grape berry by sulfuric phenol method, units indicate grams of SS per hundred grams of grape (%). 10 fruits were taken and ground, and the juice was filtered. Determination of soluble solids content (SSC) with the WYA-2S digital automatic Abbe refractometer (Inesa, Shanghai, China). The TA, VC, SS, and SSC were measured, as described by Turhan et al. (2011) [24,25].

2.5. Water-soluble pectin, soluble cellulose

Taken 1 g of fruit peel, ground with liquid nitrogen, added with 10 ml of 95% ethanol, and boiled in water for 30 min. After centrifugation, 10 ml of anhydrous ethanol, chloroform-methanol (1:1, V/V), and acetone were added in sequence to remove impurities. After centrifugation, added 10 ml of deionized water was to the residue, shaken in a shaker for 12 h, and the supernatant was water-soluble pectin (WSP), units indicate milligrams of WSP per gram of grape (mg/g). The content of water-soluble pectin was measured by the *m*-hydroxyphenyl method, and the absorbance value was determined at 520 nm [26]. After centrifugation, added 10 ml of 30% sulfuric acid was to the residue, shaken in a shaker for 1 h, and the supernatant was soluble fiber. Soluble fiber content was determined by the anthrone-sulfuric acid method, and the absorbance value was determined at 620 nm, units indicate milligrams of soluble fiber per gram of grape (mg/g), as described by Brummer et al. (2005) [27].

2.6. Enzyme activity and biochemistry indicators determination of grapes

Taken 2 g of fruit peel, ground with liquid nitrogen, added to 10 ml of pre-cooled buffer solutions, shaken on ice for 3 min, frozen, and centrifuged (4 °C), and the supernatant was the enzyme solution. The pectinase and cellulase activities of berry peel were measured by DNS (3,5-Dinitrosalicylic acid) colorimetric method. CMC-Na, and polygalacturonic acid as substrates for the determination of pectinase and cellulase, respectively, as described by Lohani et al. (2004) [28], the data unit is U/mg·prot.

The catalase (CAT) activities of berry were measured by the $KMnO_4$ titration method, and H_2O_2 as substrates for the determination of CAT, as described by Geransayeh et al. (2015) [29], the data unit is mg/(g.min). Taken four 50 mL triangular flasks (two for measurement and two for control), added 2.5 mL of enzyme solution to the measurement flask and 2.5 mL of inactivated enzyme solution to the control flask, then added 2.5 mL of 0.1 mol/L H_2O_2 in a water bath at 30 °C for 10min, immediately added 10% H_2SO_4 2.5 mL. titrate H_2O_2 with 0.1 mol/L $KMnO_4$ standard solution until the end point. The volume of potassium permanganate solution consumed was recorded.

The activity analyses of H, K-ATPase, Na, K-ATPase, Ca, Mg-ATPase, and Succinate dehydrogenase (SDH) were measured using the corresponding kits (Solarbio, Beijing, China), the data unit is $U.mg^{-1}$prot. The activity analyses of superoxide dismutase (SOD) and peroxidase (POD) were measured using the corresponding kits (Nanjing Jiancheng Biotechnology Co., Nanjing, China), the data unit is $U.g^{-1}$ FW. The content analyses of malondialdehyde (MDA) were measured using the corresponding kits (Solarbio, Beijing, China), the data unit is $nmol.g^{-1}$ FW.

2.7. Grape respiration and ethylene determination

Determination of fruit ethylene release rate by Ethylene plants (ETH) ELISA kit (Shanghai Xing Yi Biotechnology Co., Shanghai, China), the data unit is nmol/g.h. Determination of fruit respiration intensity by the resting method (titration), the data unit is mg/(kg.h), as described by Zhang et al. (2017) [30]. 20.0 ml of 0.4 mol/L NaOH was taken into a Petri dish, placed at the bottom of the

desiccator, a partition was placed, filled with 250–500 g of fruit to be tested, capped for 30 min, the Petri dish was removed, the alkali solution was transferred into a triangular flask, 5.0 ml of saturated $BaCl_2$ solution and 2 drops of phenolphthalein indicator were added, and 0.1 mol/L oxalic acid solution for titration.

2.8. Determination of cell wall-modifying gene expression by QRT-PCR

The total RNA of grape peel was extracted with a Trizol kit (Yuanye, Shanghai, China). RNA was reverse transcribed into cDNA. The sequences were listed in Table 1 [31]. The qRT-PCR primers were synthesized in Sangon Bioengineering (Shanghai, China) Co., Ltd. RT-PCR reaction system: 1 μL template, 10 μL SYBR Green qPCR SuperMix, 1 μL upstream primer (10 nM), 1 μL downstream primer (10 nM), supplemented with double distilled water to 20 μL. Reaction conditions were as follows: pre-denaturation at 95 °C for 5 min, denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s, fluorescence detection for 15 s, 40 cycles.

2.9. Statistical analysis

All data were expressed as the mean ± SD by measuring three independent replicates. Principal component analysis (PCA) was performed using SPSS version 22.0 (SPSS Inc., IBM, Armonk, NY, United States) and Origin 2022 (Origin LabCorporation, United States).

3. Results and analysis

3.1. Marketable fruit rate, weight loss rate, fruit hardness, fruit color

The weight loss rate increased as the storage time increased (Fig. 1A), and the weight loss rate in the treated group was lower than that in the control group, especially in the 1-MCP + Ca2+ group, which had the lowest weight loss rate. On day 56 of storage, the weight loss rate of the control group reached 6.97%, which increased by 39.43% compared to the 1-MCP + Ca2+ treatment group ($P < 0.05$). The marketable fruit rate (GFR) in the treated group (especially in the 1-MCP + Ca2+ group) was higher than that in the control group (Fig. 1B). On day 56 of storage, the marketable fruit rate of the 1-MCP + Ca2+ treatment group remained at 93%, which increased by 29.03% compared to the control group ($P < 0.05$). From Fig. 1C, the pre-harvest calcium treatment improved the coloration of the grapes, while the 1-MCP treatment delayed the increase in color (during storage). The grapes were treated with calcium to increase the breaking force during storage (Fig. 1D). On day 56 of storage, the breaking force of the 1-MCP + Ca2+ treatment group remained at 431.8 g, which increased by 29.93% compared to the control group ($P < 0.05$).

3.2. Titratable acid, vitamin C, soluble sugar, soluble solids content

Calcium combined with 1-MCP treatment delayed the increase of soluble solids in fruit (Fig. 2A). The soluble solids content of the control fruit peaked at day 14, while the 1-MCP + Ca2+ group peaked at day 28. Although calcium and 1-MCP were effective in delaying the decrease of titratable acid in grapes during storage (Fig. 2B). However, titratable acid decreased significantly in all grape berry groups by day 21 of storage ($P < 0.05$). The changes in fruit VC content during storage were consistent with those of titratable acids (Fig. 2C). Calcium and 1-MCP delayed the decrease of VC in grapes during storage. However, on the 28th day of storage, the VC values of the fruits in all groups tended to be the lowest. The changes in fruit soluble sugar during storage were consistent with those of soluble solids (Fig. 2D). Calcium combined with 1-MCP treatment delayed the increase of soluble sugar in fruit.

3.3. Soluble pectin, soluble fiber content, related enzyme activities and related gene levels

The changes in fruit soluble pectin (Fig. 3A) and soluble fiber (Fig. 3B) during storage were consistent with those of soluble solids (Fig. 2A). Soluble pectin content and soluble fiber showed a trend from increasing to decreasing during grape storage. Calcium combined with 1-MCP treatment delayed the increase of soluble pectin and soluble fiber in fruit. For example, soluble pectin in the control group reached a maximum (2.14 mg/g) on day 21 of storage. However, the 1-MCP + Ca2+ treated group reached the maximum (2.07 mg/g) at day 35 of storage ($P < 0.05$). The increase in soluble fiber during grape storage (0–35 days) was higher in the control group compared to the 1-MCP + Ca2+ treated group. Both pectinase (Fig. 3C) and cellulase activities (Fig. 3D) in the fruit reached their highest values at the 7 days of storage. Calcium combined with 1-MCP treatment delayed the increase of pectinase and

Table 1
Details regarding the qRT-PCR primers.

Genes	Forward primer (5′–3′)	Reverse primer (5′–3′)
PG (Polygalacturonase)	GAACCTTAGGGTGGTCAACAG	GACTTCAACACGAGATGC
PME (Pectin methyl esterase)	CAGAACCGACCCTAACCAGAG	AAGGAACGTCTTAACGGAGCT
Cel (Cellulase)	TATCTTAGTCGGAGCCATCGTC	TATACGTAGCCGGTTCTGAGTGA
β-Gal (β-galactosidase)	GTTATGTGCAAACAAGACGACG	GCCGGTCTAGTAGGAACAGCA
GAPDH	TATTAGGAACCCAGAGGAGATT	TCCTGTGGACAATGGATGGA

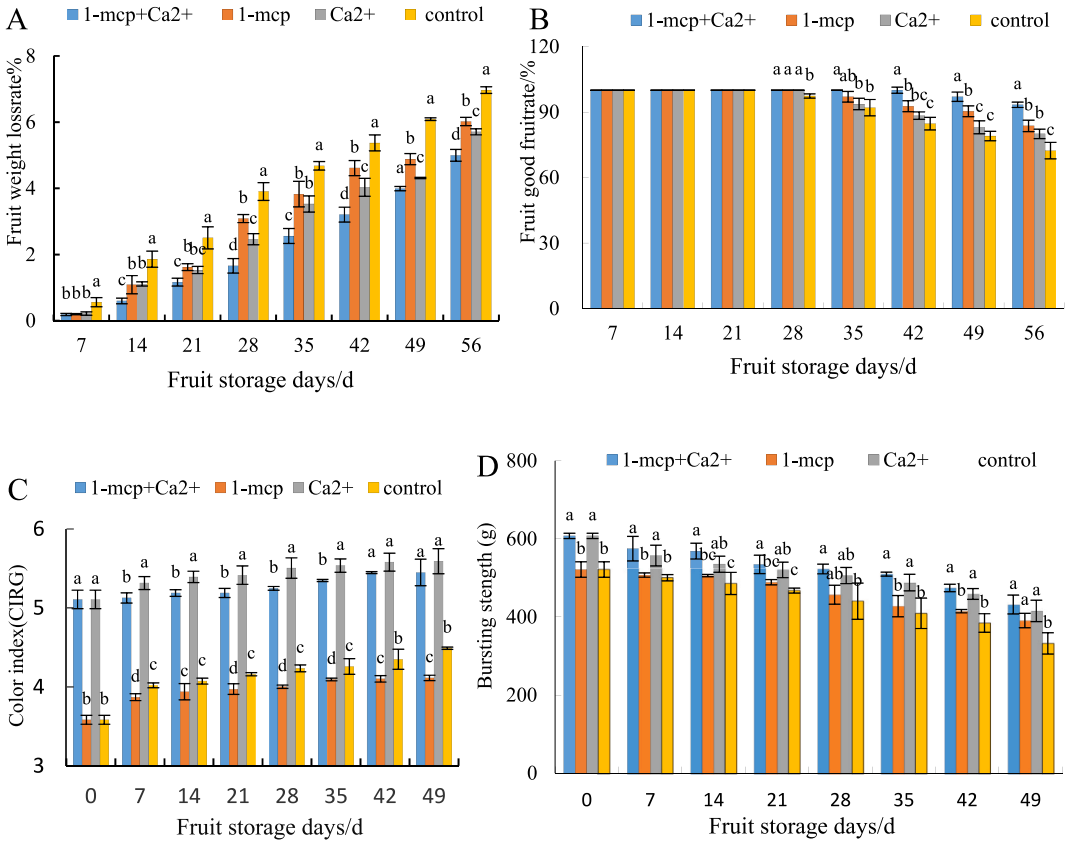

Fig. 1. The impact of 1-MCP + Ca2+ treatment on fruit weight loss rate (A), Fruit marketable fruit rate (B), Color index (C), and fruit bursting force (D) in grape berry during storage. The presented values are the means ± SE of three replicates, and the use of different letters indicates statistically significant differences at $p < 0.05$.

cellulase in fruit. For example, on day 7 of storage, the pectinase and cellulase activities of the control group reached 0.86 U mg^{-1} and 48.88 U mg^{-1}, respectively, which were significantly increased compared to the 1-MCP + Ca2+ treated group ($P < 0.05$). Moreover, the trends of pectinase gene (Fig. 3E), pectin methyl esterase gene (Fig. 3F), cellulase gene (Fig. 3G), and β-Gal gene (Fig. 3H) in grapevines were consistent with those of pectinase and cellulase, which increased at the beginning and decreased at the end of the storage period. Relative to the control, 1-MCP + Ca2+ treatment reduced the expression of the above genes in grapes.

3.4. Antioxidant enzyme activity in fruits

Antioxidant enzyme activity of grape berry (Fig. 4A–C) showed a trend from increasing to decreasing during grape storage. Calcium combined with 1-MCP treatment increased the activity of CTA, SOD, and POD in fruits. For example, on day 7 of storage, the CAT activity of the 1-MCP + Ca2+ treatment remained at 0.71 mg/(g.min), which increased by 39.76% compared to the control group ($P < 0.05$). On day 14 of storage, the SOD and POD activities of the 1-MCP + Ca2+ treated group reached 51.44 U g^{-1} and 41.61 U g^{-1}, respectively, which were significantly increased compared to the control group ($P < 0.05$). The MDA content in the fruit increased slowly in the early stage of fruit storage and rapidly in the later stage (Fig. 4D). Calcium+1-MCP treatment delayed the increase of MDA and thus reduced the MDA content in the fruits.

3.5. ATPase activity in fruits

Calcium combined with 1-MCP treatment delayed the increase of ATPase during fruit storage (Fig. 5A–C). Overall, ATPase activity gradually decreased during grape berry storage, except for a slight increase in the control group. For example, on day 14 of storage, Ga, Mg-ATPase (Fig. 5A) and H, K-ATPase activities (Fig. 5B) in the control fruit were 0.39 U/mgprot and 1.1 U/mgprot, respectively, and increased by 23.39% and 11.99%, respectively, compared to the 1-MCP + Ca2+ treatment group ($P < 0.05$). On day 7 of storage, the Na, K-ATP activity (Fig. 5C), and SDH activities (Fig. 5D) in the control fruit were 2.05 U/mgprot and 3.74 U/mgprot, respectively, and increased by 10.39% and 6.86%, respectively, compared to the 1-MCP + Ca2+ treatment group ($P < 0.05$).

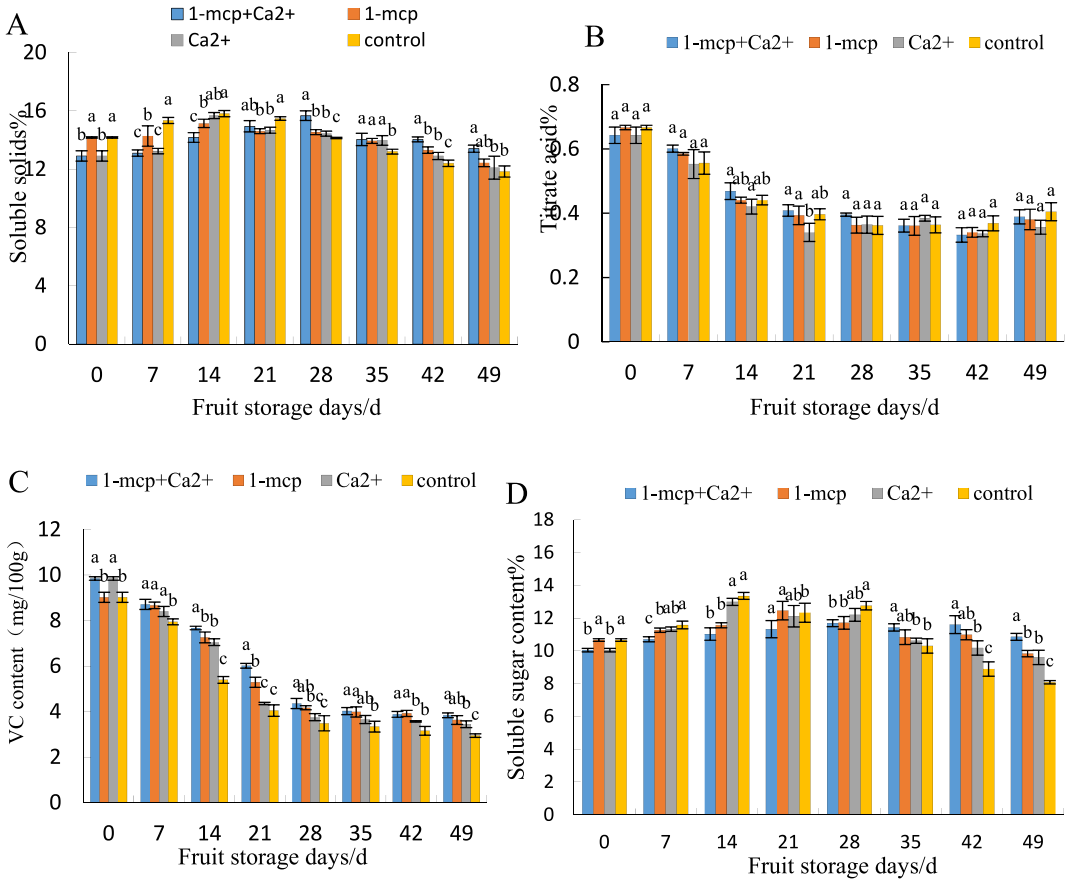

Fig. 2. The impact of 1-MCP + Ca2+ treatment on the content of soluble solids (A), Titrate acid (B), VC (C), and Sugar (D) in grape berry during storage. The results, presented as means ± SE of three replicates, revealed significant differences at $p < 0.05$, with different letters indicating the significance level.

3.6. Fruit respiration and ethylene release rate

On day 7 of storage, the respiratory intensity of the grape berry reached its maximum, Calcium-binding 1-MCP treatment decreased the respiratory intensity of grapes during storage (Fig. 6A), and the respiratory intensity of the control group reached 24.82 mg/(kg. h), which increased by 39.24% compared to the 1-MCP + Ca2+ treatment group ($P < 0.05$). Calcium-binding 1-MCP treatment delayed and decreased the ethylene release rate of grapes during storage (Fig. 6B), at day 14 of storage, the ethylene release rate of the control group reached 0.135 nmol/(g. h), which increased by 49.70% compared to the 1-MCP + Ca2+ treatment group ($P < 0.05$). The highest rate of ethylene release (0.117 nmol/(g. h) was observed in fruits of the 1-MCP + Ca2+ treatment group on the 28th day of storage.

3.7. Correlation analysis

Correlation analysis (Control group) of the measured indicators showed that 12 indicators were positively correlated with good fruit percentage (GFR), namely soluble solids (SS), hardness, titratable acid (TA), Vitamin C (VC), sugar, CAT, SOD, POD, Ga, Mg-ATP, H, K-ATP, Na, K-ATP and SDH, respectively (Fig. 7). Two of them were significant ($P < 0.05$), VC and SDH, respectively. Eight of them were highly significant ($P < 0.01$), namely SS, hardness, sugar, CAT, SOD, Ga-ATP, H-ATP and Na-ATP, respectively. Subsequently, these indicators were selected for principal component analysis.

3.8. Principal component analysis

PCA is a statistical method that uses the concept of dimensionality reduction to transform multiple metrics into a handful of composite metrics [32]. These metrics retain most of the information of the original metrics. The cumulative contribution of both components reached more than 93%, with 66.9%–77.9% for component 1 and 17.6%–26.6% for component 2 (Fig. 8A–D). It has been fully able to reflect the changes in fruit quality during the storage process. According to the component Loading coefficients (Table 2), There were 9 PCs1 with higher loadings, including GFR, hardness, TA, VC, CAT, Ga-ATP, H-ATP, Na-ATP and, SDH, which showed a

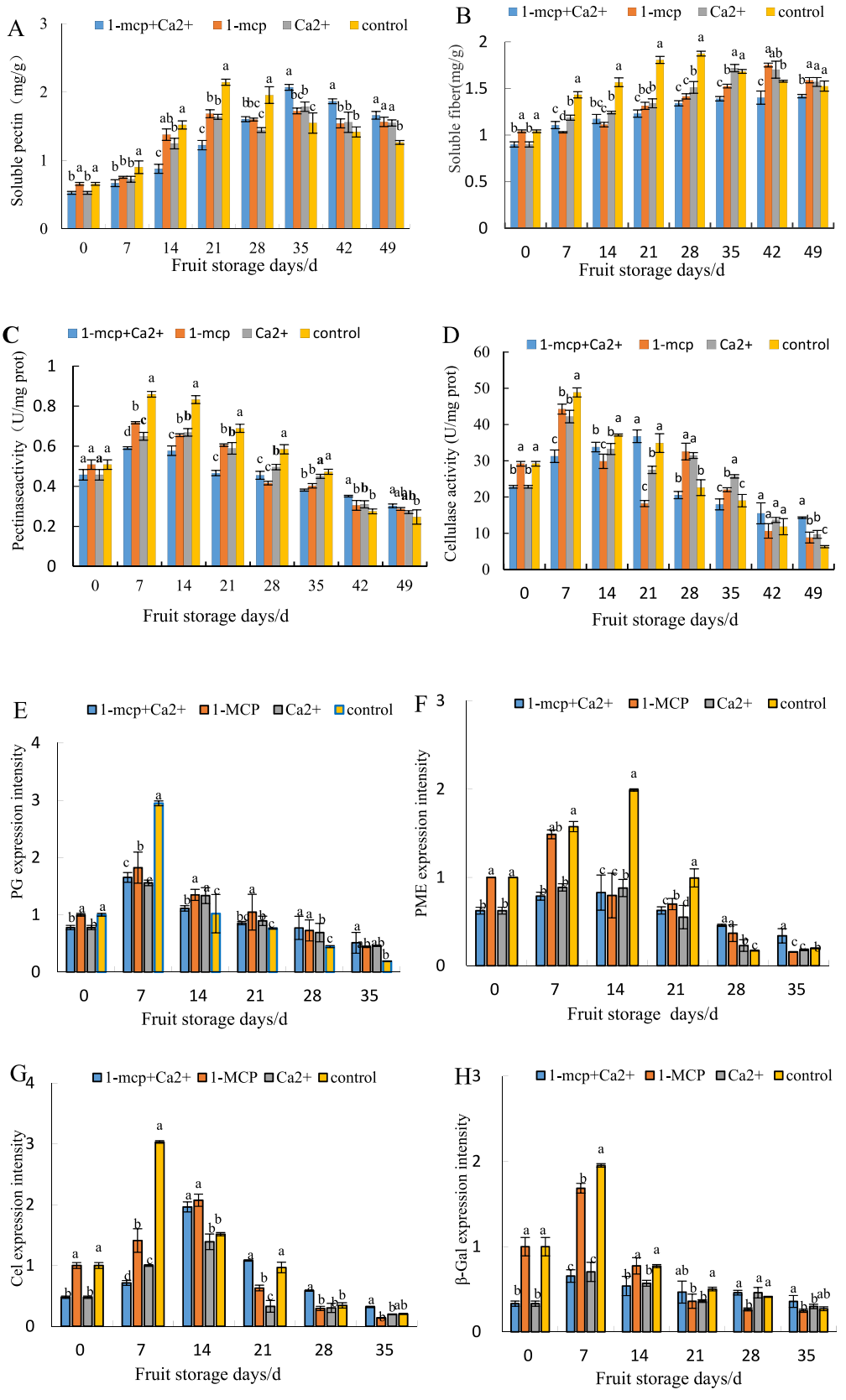

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Fig. 3. The impact of 1-MCP + Ca2+ treatment on the content of soluble pectin (A) and soluble fiber (B)), as well as the activities of pectinase (C) and cellulase (D), as well as the expression level of pectinase gene (E), pectin methyl esterase gene (F), cellulase gene (G), and β-Gal gene (H) in grape berry during storage. Values are the means ± SE of three replicates, and the different letters indicate significant differences at $p < 0.05$.

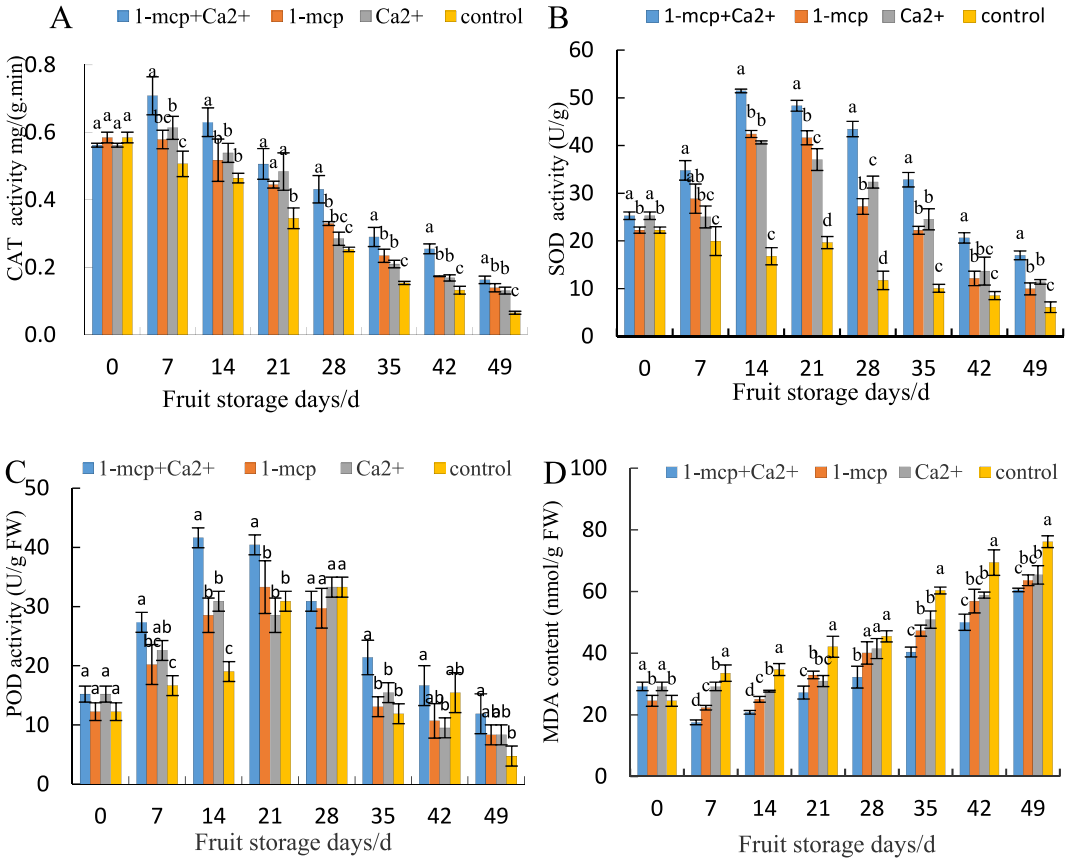

Fig. 4. The impact of 1-MCP + Ca2+ treatment on CAT activity (A), SOD activity (B), POD activity (C), and MDA content (D) in grape berry during storage. Values are the means ± SE of three replicates, and the different letters indicate significant differences at $p < 0.05$.

trend from high to low. There were 4 PCs2 with higher loadings, including SS, Sugar, SOD, and POD, which showed a trend from low to high to low. Where the higher the value, the higher the contribution rate [33]. According to Fig. 8, The early stage of fruit preservation (period 1 and period 2), mainly reflects the characteristics of component 1. The early-middle stage of fruit preservation (period 3 and period 4), mainly reflects the characteristics of component 1 and component 2. The middle-late stage of fruit preservation (period 5 and period 6), mainly reflects the characteristics of component 2. The characteristics of both component 1 and component 2 were not evident in the later stages of fruit preservation (stages 7 and 8) when the quality of the fruit was more stable.

The 1 to 8 in the principal component plot represents the eight periods of fruit preservation, respectively, while X_1 to X_{13} represents the 13 indicators, respectively (Table 2). A to D represent 1-MCP, CK, Ca2+, 1-MCP + Ca2+ respectively.

The data in Table 1 were further analyzed to obtain the principal component scores and the composite scores (Table 3). The values of F1 showed a trend from high to low with the increase in fruit preservation time (Table 3). This indicates that the quality of the grapevines gradually deteriorated with increasing preservation time. For example, GFR, hardness, TA, VC, CAT, Ga-ATP, H-ATP, etc. Overall, the value of F2 showed a trend from low to high to low with the increase in fruit preservation time. This indicates that there is a trend of increasing individual indicators during the preservation of grape berry. For example, soluble solids, soluble sugars, SOD, and POD. The F-value is a combination of the F1 (66.9%–77.9%) and F2 (17.6%–26.6%) values (Fig. 8). In general, it shows a trend from high to low. The F_{CK} group started to show negative values on day 28, indicating that the fruit quality of both groups started to decline significantly at this time. Further, the $F_{1\text{-}MCP}$, F_{Ga} and $F_{Ga+1\text{-}MCP}$ groups started to show negative values only at day 35, but the $F_{Ga+1\text{-}MCP}$ group had relatively small negative values, indicating that the pre-harvest calcium treatment combined with 1-MCP treatment was more effective for fruit preservation.

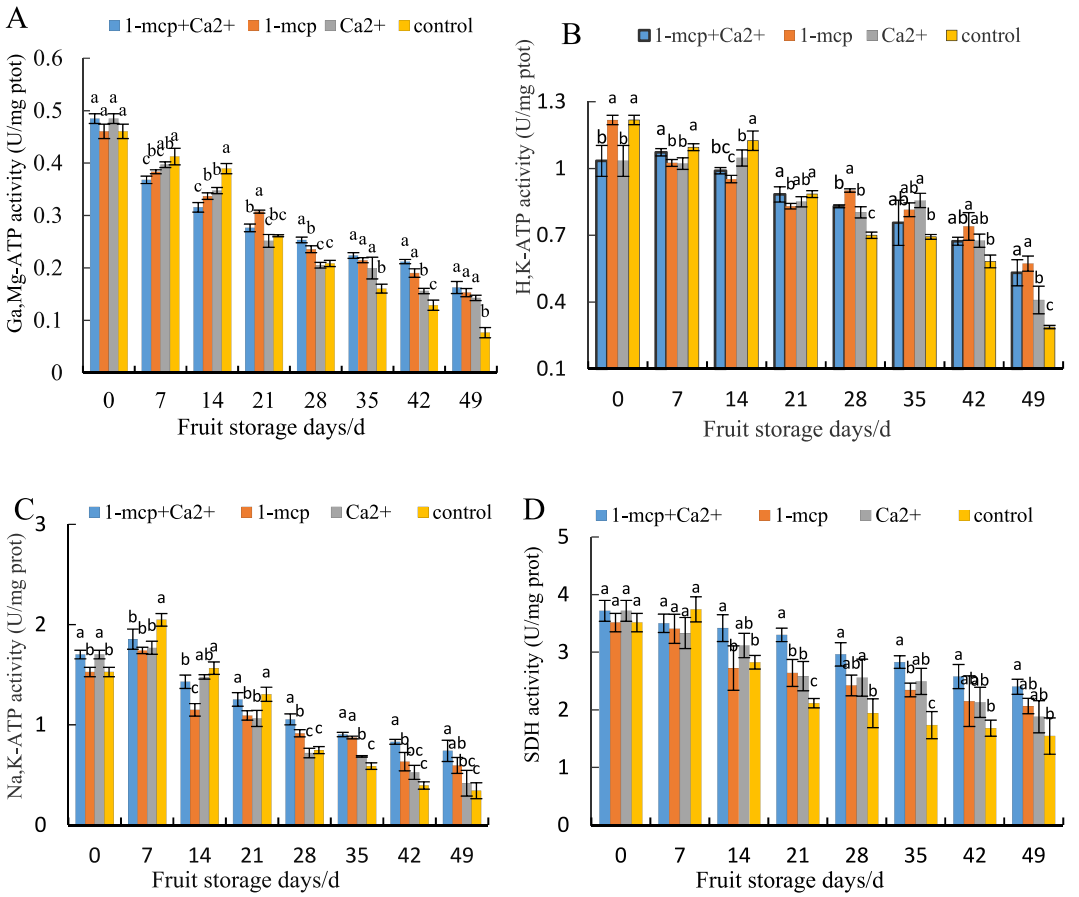

Fig. 5. The impact of 1-MCP + Ca2+ treatment on the activities of Ga, Mg-ATP (A), H, K-ATP (B), Na, K-ATP (C), and SDH (D) in grape berry during storage. Values are the means ± SE of three replicates, and the different letters indicate significant differences at $p < 0.05$.

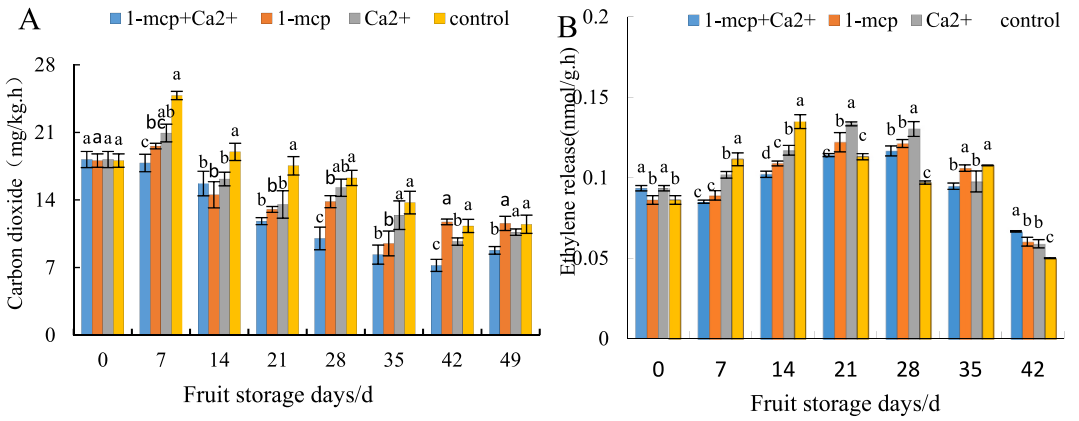

Fig. 6. The impact of 1-MCP + Ca2+ treatment on Carbon dioxide (A), and Ethylene release rate (B) in grape berry during storage. Values are the means ± SE of three replicates, and the different letters indicate significant differences at $p < 0.05$.

4. Discussion

Effect on storage quality of grapes. In our present work, we used a combined postharvest 1-MCP treatment on grapevines after calcium spraying at the flowering stage. The results showed a significant increase in the percentage of good fruit and a significant decrease in weight loss, as well as a significant increase in fruit retention quality (vitamin C, soluble sugars, soluble solids, etc.)

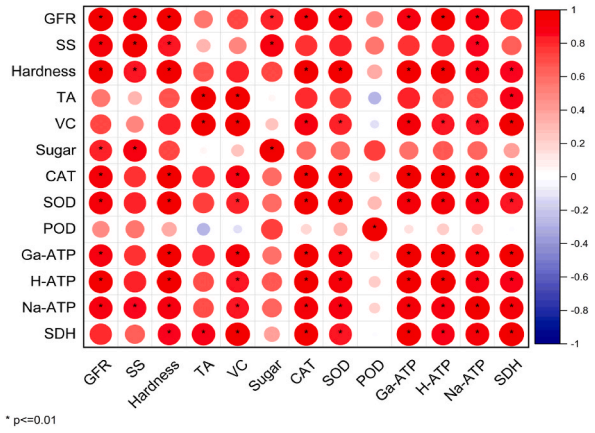

Fig. 7. Diagram of correlation with good fruit percentage.

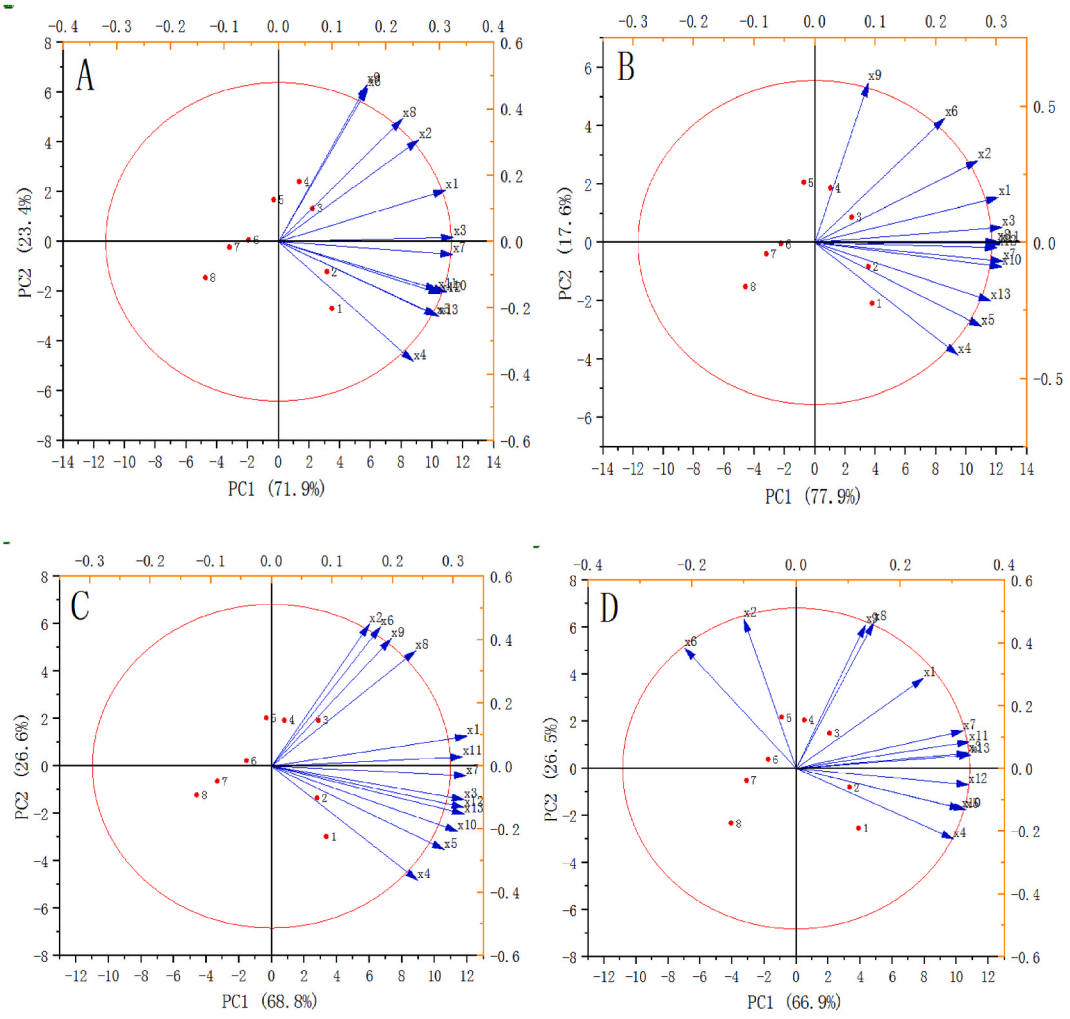

Fig. 8. Diagram of principal component analysis.

Table 2
Loading coefficients of each principal component.

	Ingredients (PCs)							
	1-MCP 1	1-MCP 2	Ga 1	Ga 2	CK 1	CK 2	Ga+1-MCP 1	Ga+1-MCP 2
X_1 GFR	0.951	0.27	0.966	0.177	0.966	0.25	0.703	0.592
X_2 SS	0.798	0.536	0.484	0.838	0.858	0.453	0.722	0.535
X_3 Hardness	0.996	0.022	0.951	−0.197	0.984	0.085	−0.297	0.886
X_4 TA	0.767	−0.63	0.721	−0.672	0.753	−0.624	0.976	0.091
X_5 VC	0.900	−0.391	0.854	−0.492	0.877	−0.464	0.891	−0.417
X_6 Sugar	0.51	0.81	0.539	0.822	0.684	0.691	0.960	−0.243
X_7 CAT	0.991	−0.066	0.962	−0.057	0.99	−0.103	−0.633	0.713
X_8 SOD	0.707	0.647	0.712	0.68	0.961	0.004	0.945	0.223
X_9 POD	0.505	0.828	0.592	0.751	0.283	0.888	0.44	0.865
X10 Ga-ATP	0.955	−0.265	0.919	−0.384	0.985	−0.135	0.394	0.854
X_{11} H-ATP	0.908	−0.253	0.944	0.054	0.975	−0.005	0.937	−0.238
X_{12} Na-ATP	0.924	−0.275	0.951	−0.243	0.958	−0.027	0.980	0.158
X_{13} SDH	0.912	−0.392	0.952	−0.282	0.926	−0.324	0.976	−0.096

Table 3
Principal component score and comprehensive score.

days	F1	F2	F_{CK}	F1	F2	$F_{1\text{-}MCP}$	F1	F2	F_{Ga}	F1	F2	$F_{Ga+1\text{-}MCP}$
0d	0.193	−0.032	0.161	0.182	−0.048	0.134	0.230	−0.082	0.147	0.096	0.033	0.129
7d	0.191	−0.012	0.180	0.163	−0.022	0.140	0.193	−0.038	0.155	0.106	0.040	0.146
14d	0.127	0.018	0.145	0.111	0.022	0.133	0.193	0.049	0.242	0.108	0.055	0.162
21d	0.008	0.030	0.038	0.064	0.039	0.103	0.048	0.050	0.097	0.084	0.046	0.130
28d	−0.110	0.032	−0.078	−0.021	0.028	0.007	−0.030	0.053	0.023	0.054	0.035	0.089
35d	−0.227	−0.003	−0.230	−0.102	0.002	−0.099	−0.112	0.006	−0.106	−0.009	0.004	−0.004
42d	−0.304	−0.013	−0.317	−0.164	−0.003	−0.167	−0.232	−0.016	−0.247	−0.050	−0.013	−0.063
49d	−0.408	−0.031	−0.439	−0.236	−0.020	−0.256	−0.317	−0.032	−0.348	−0.089	−0.037	−0.126

compared to the control fruit. A similar result was also found in grape [34], kiwifruit [35], Mango [13], and blueberry [36]. Meanwhile, Ciccarese (2013) confirmed that Pre- and post-harvest calcium applications to bunches were effective both in maintaining postharvest fruit quality, as shown by flesh firmness and berry breaking force, and in reducing *B. cinerea* rots during storage [37]. Calcium sprays at flowering combined with post-harvest 1-MCP treatments sre also very effective in preserving grapes. This is because calcium increases the breaking force and 1-MCP reduces the release of ethylene from the fruit, decreases fruit respiration and prolongs the quality of the fruit during storage (Figs. 1 and 6).

Calcium combined with 1-MCP treatment increased antioxidant enzyme activity (SOD, CAT, and POD) and reduced MDA levels in grapevines during storage (Fig. 4). The respiration of fruits during storage can produce a large amount of oxygen free radicals, which can damage cell membranes to accelerate the softening and aging of fruits [38]. Furthermore, highly active antioxidant enzymes can effectively remove oxygen radicals from fruits [39]. Additionally, Li (2020) indicated that 1% and 2% calcium treatments were effective in maintaining lower hydrogen peroxide (H_2O_2) levels in mangoes by increasing SOD, CAT and, ascorbate peroxidase (APX) activities [13]. Xu (2021) confirmed that the antioxidant enzymes activity of superoxide dismutase, peroxidase, catalase, and glutathione reductase were enhanced in 1-MCP treatment fruit, meanwhile, 1-MCP treatment induced the radical scavenging capacity (DPPH radical scavenging rate, hydroxyl radical scavenging rate, and superoxide anion scavenging capacity) in fruit during storage [38].

Effect on the cell wall of grape pericarp. Fruit softening could be a developmentally programmed ripening process, among the dismantlement of cell wall polysaccharides [40]. Meanwhile, related studies have shown that cellulose–pectin contacts are extensive and have an effect on the composition of the fruit cell wall and its mechanical strength [41,42]. Furthermore, calcium as one of the components of the cell wall can effectively increase the mechanical strength of the cell wall and improve the hardness of the fruit [42]. In this study, the activities of cellulase and pectinase increased gradually from 0 to 7, 14 days and were all significantly negatively correlated with firmness (Fig. 1D), indicating that they are involved in softening of grape berries during low-temperature preservation. Calcium and 1-MCP effectively reduced the expression of pectinase and cellulase genes in fruits, thus decreasing their enzymatic activities and the content of soluble pectin and soluble fiber [43,44]. Additionally, Lin (2018) reported that 1-MCP significantly inhibited the activity of cell wall degrading enzymes of Younai plum and reduced the breakdown of cell wall polysaccharides, thus delaying the softening of Younai plum [45]. Rao (2011) reported that the enzymatic activities of PG (polygalacturonase), PME (pectin methylesterase), cellulase, and β-galactosidase were lower in calcium-treated peppers, thus maintaining the integrity of pepper cell membranes and cell walls [46].

Effects on grape respiration and energy metabolism. 1-MCP (a fruit preservative) has a strong inhibitory effect on the release of ethylene during fruit preservation and is often used in the preservation of various fruits [5,47]. In this study, Calcium combined with 1-MCP treatment decreased respiratory intensity, and ethylene release rate in grapevines during storage (Fig. 6). The reduction of fruit ethylene release rate can effectively reduce fruit respiration intensity, increase the activity of antioxidant enzymes, decrease the

activity of cell wall decomposition enzymes, and reduce fruit cell membrane permeability, thus improving fruit hardness [38,48]. Meanwhile, related studies have shown that calcium chloride treatment is beneficial in reducing the intensity of fruit respiration and delaying the reduction of fruit hardness throughout low-temperature storage [49,50]. Additionally, the reduction of fruit respiration intensity reduces the ATPase metabolism (Ga-ATP, H-ATP, Na-ATP, and SDH) in fruit (Fig. 5), and also effectively reduces the content of MDA in fruit (Fig. 4), thus delaying the aging of fruits during storage (delaying the decline in fruit quality), which is similar to the experimental results of Zhang et al. (2022) [51], and Gago et al. (2016) [52].

5. Conclusions

In summary, our results showed that pre-harvest calcium combined with post-harvest 1-MCP treatment (1) promoted SOD, CAT, and POD activities to reduce MDA content and thus delayed grape berry aging. (2) Reduced soluble pectin and soluble fiber content (soluble sugars, soluble solids) by decreasing the activity of cellulose and pectinase in the fruit, thus delaying fruit softening. (3) Reduced ATPase activity by decreasing the intensity of fruit respiration and also effectively reduced the content of MDA in the fruit. Ultimately, it reduces the weight loss rate of grapes, increases the fruit quality, and prolongs the storage quality of grapes, thus increasing the shelf life of grapes and improving their economic value (On day 56 of storage, the good fruit percentage of the 1-MCP + Ca2+ treatment group increased by 29.03% compared to the control group).

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Author contribution statement

Hao Shi: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Wen hua Zhou, Fu yin He, Yun Wang and Xiao e He: Conceived and designed the experiments.

Yin yu Xu: Conceived and designed the experiments; Analyzed and interpreted the data.

Data availability statement

Data included in article/supp. material/referenced in article.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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