

Sodium lactate treatment maintains the quality and promotes energy metabolism of 'Kyoho' table grape during storage

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Abstract

'Kyoho' grape (*Vitis vinifera* L. × *Vitis labrusca* L.) is the most widely cultivated table grape variety. However, it is prone to fruit abscission after harvest, which affects the development of the market. The aim of this study was to evaluate the effects of applying different concentrations of sodium lactate (0.1%, 0.5% and 1% NaL) on postharvest table grape preservation. After harvesting, 'Kyoho' grapes were immersed in sodium lactate solution at various concentrations (0 [as control], 0.1%, 0.5% and 1% NaL) for 2min and stored at 20±1°C for 10 days. Physiological indicators including weight loss rate, browning level of rachis, contents of vitamin C, sugar, malondialdehyde (MDA), membrane permeability, and activities of polygalacturonase (PG), peroxidase (POD), cellulase (Cx) and lipoxygenase (LOX) were investigated. Additionally, it was also determined for ATP content, energy charge and activity of energy metabolism-related enzymes. These results showed that NaL treatment inhibited berry abscission and maintained berry quality due to the decrease of cell wall degrading enzyme activity, the increase of energy metabolism-related enzyme activities and high level of ATP content and energy charge. This study provides a new and effective method for the postharvest storage of table grapes that can help minimize economic losses.

Introduction

Grapevine (*Vitis vinifera* L.) is an economically important fruit with high nutritional value that is widely cultivated (Guo et al.,2019; Yu et al.,2019; Yu et al.,2021). In 2019, China's grape cultivation area accounted for 12% of the world, reaching 840,000 hectares, of which table grapes accounted for 84.1%. During the storage and transportation of grape bunches, problems such as berry abscission, fungal decay and surface blackening often occur, which shortens the shelf life of grapes and can result in major losses (Sonker et al.,2016; Lu et al.,2018). Current methods used to prolong the storage life of grapes include refrigeration, controlled atmospheres, modified atmosphere packaging and exogenous material treatment (Champa et al.,2015).

Grape abscission can be divided into three categories: stem break, dry drop and wet drop (Li et al.,2020). 'Kyoho' grape abscission mostly occurs via dry drop (Deng et al.,2007). The causes of grape abscission have been studied by examining the activity of cell wall-degrading enzymes, plant hormones and fruit stalk structure. For example, the activities of cell wall-degrading enzymes such as polygalacturonase (PG), pectinesterase (PE), cellulase (Cx), lipoxygenase (LOX) and peroxidase (POD) are closely related to the berry abscission rate (Chen et al.,2019). Effective methods for preventing grape berry abscission include low temperature, modified atmosphere packaging and coating preservation. For example, high concentrations of O₂ inhibit the activities of Cx, PG and PE and maintain a high fruit detachment force, thus inhibiting fruit abscission (Wu et al.,2008). A chitosan and poly-ε-lysine coating can effectively reduce fruit water loss and respiratory intensity and thus inhibit fruit decay and reduce fruit abscission (Chen et al.,2019).

ATP is the most direct energy source for various life activities of cells. ATP is produced mainly through respiration and photosynthesis. Energy production is also closely related to the enzyme activities of energy metabolism-related, including H⁺-adenosine triphosphatase (H⁺-ATPase), Ca²⁺-adenosine triphosphatase (Ca²⁺-ATPase), succinic dehydrogenase (SDH) and Cytochrome C oxidase (CCO) (Jin et al.,2013). When their activities decrease will affect the function of mitochondria, and then lead to cell energy insufficient. Energy metabolism plays a key role in the disease resistance and senescence of postharvest in fruit and the maintenance of fruit quality during storage (Huang et al.,2014). Studies in many horticultural crops, such as loquat, longan and kiwifruit, have shown that regulation of the energy metabolism of fruit during storage can increase disease resistance and fruit quality (Huang et al.,2014, Cao et al.,2014, Chen et al.,2014). However, the relationship between postharvest quality and the energy metabolism of 'Kyoho' table grape is not clear.

NaL is widely used in food processing for preservation and anti-corrosion purposes. (Cegielska-Radziejewska et al.,2004). It can be decomposed into carbon dioxide and water by the human body (Ukwo et al.,2022). Its application in preserving fresh fruits can control microbial growth and delay fruit decay (Gammariello et al.,2014). NaL mainly reacts with sugar in fruit juice to generate lactic acid, thereby reducing the pH value of the juice and inhibiting the growth of bacteria and molds. In addition, NaL can also increase the antioxidant capacity of fruits and extend their shelf life, thereby reducing fruit loss and waste (Tian et al.,2022). Therefore, NaL has broad application prospects in the preservation of fresh fruits, but there is currently very little research on its application in this field (Oms-Oliu et al.,2006, Yousuf et al.,2020).

Here, the optimal concentration of NaL for improving the quality and energy metabolism of 'Kyoho' grape was determined by comparing the effect of different concentrations of NaL on the quality and energy metabolism of table grapes. Our study provides a useful method for preventing berry abscission and improving the postharvest quality and energy metabolism of table grapes.

Materials And Methods

Fruit materials

'Kyoho' grapes from the research farm of Henan University of Science and Technology in Yanshi, Luoyang, China was used. Different concentrations of NaL were soaked in different groups of grapes of similar in size and color. Before different treatments, grapes were soaked in 0.5% (w/v) sodium hypochlorite (NaClO) solution for 2 min.

NaL treatments

NaL solutions with different concentrations (0.1%, 0.5% and 1%) were prepared (ca. 1000 g of grapes were immersed in 2 L of solution), and a double-distilled water (ddH₂O) treatment was used as the control (CK). The grape clusters were divided into four groups and soaked in four solutions for 2 min. Grapes were then placed in polyethylene bags at 20°C for 10 d. Samples (grape fruit) were collected at 0 d, 2 d, 4

d, 6 d, 8 d and 10 d, respectively, wrapped with aluminium foil, immediately put into liquid nitrogen and stored in -80°C refrigerator.

Abscission rate and weight loss rate

Abscission rate [%] = (weight of dropped berries per grape clusters/total weight of the corresponding grape clusters) × 100. Weight loss rate [%] = [(initial weight of grape clusters - present weight of grape clusters)/initial weight of grape clusters] × 100.

Firmness and total soluble solids (TSS)

The firmness of berries was determined by a firmness meter (FHM-5; Wuxi Yuheng Measuring Instrument, Wuxi, China). The fruit was measured near the equatorial line, and the average value was taken from the two opposite sides. The content of TSS was determined using an ATC-32 saccharimeter (Tianlei, Shanghai, China).

Rachis browning index

The values of the rachis browning index of grape clusters were classified as follows: no browning, grade 0; browning 0~1/4, grade 1; browning 1/4~1/2, grade 2; browning 1/2~3/4, grade 3; and browning more than 3/4, grade 4. Rachis browning index% = \sum [browning level × number/highest level × total investigation number] × 100%

Titrateable acidity (TA), malondialdehyde (MDA), vitamin C (VC) and reducing sugar content

The content of TA was determined following the method of Yao et al. (2018). The volume of NaOH solution consumed was recorded and the concentration of TA was calculated. MDA content was determined following the method of Sun et al. (2011). The absorbance of the supernatant was measured at 450, 532 and 600 nm. MDA concentration: $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$. VC content was quantified following Ge et al. (2015) with some modifications, determining the absorbance of the mixture at 525 nm. The content of reducing sugar was measured using the method described by Castelo Branco Melo et al. (2018). Three replicate measurements were taken for each sample.

Membrane permeability

Membrane permeability in terms of the relative leakage rate was determined following the method of Zhang et al. (2005). The conductivity of the solution was measured by a conductivity meter. The relative leakage rate was expressed as the percentage of total electrolytes.

Determination of enzyme activity

POD activity was determined as described by Yingsanga et al. (2008) with some modifications. Distilled water was used as the control, and the absorbance at 470 nm was recorded. The change in absorbance per min was calculated. PG activity and Cx activity was determined using the method of Xue et al. (2018).

Taking 0.5 mL of boiled enzyme solution as the control and the absorbance was detected at 540 nm to calculate PG and CX activity. LOX activity was measured as described by Chen et al. (2019). Distilled water was used as the control solution. H⁺-ATPase, Ca²⁺-ATPase, CCO and SDH activity were determined as described by Zhou et al. (2014). The activities of H⁺-ATPase and Ca²⁺-ATPase were expressed by measuring the inorganic phosphorus released after ATP catalyzed hydrolysis to ADP, and the enzyme activities were expressed in U mg⁻¹. Three replicate measurements were taken for each sample.

Measurement of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) content and energy charge

ATP, ADP and AMP content were determined according to the method of Jin et al. (2013). The contents of ATP, ADP and AMP in grape fruit were determined by high-performance liquid chromatography (HPLC) and quantified by external standard method. Energy charge: [ATP + 0.5 ADP] / [ATP + ADP + AMP]. Three replicate measurements were taken for each sample.

Statistical analysis

The statistical significance of data was analyzed using ANOVA (Figure 1-5) in SPSS software (21.0 version) and Student's *t*-test (Figure 6, 7). Data were presented as mean ± SD (n = 3).

Results

Phenotypic changes of 'Kyoho' berries following NaL treatment

Different concentrations of NaL (0.1%, 0.5% and 1% NaL) were used to treat postharvest 'Kyoho' berries, and the phenotypic changes of grape clusters within 10 days were observed. After 2 d of storage, abscission symptoms were visible in the control and treatment group, and as the storage time extended, the abscission of CK became more pronounced. The abscission rate of 'Kyoho' fruit after treatment with different concentrations of NaL was significantly decreased compared with the control in different storage periods (Figure 1). The results indicated that NaL treatment had an inhibitory effect on grape berry abscission, and the 1% NaL treatment had the strongest inhibitory effect.

Effect of NaL treatment on the physiological characteristics of 'Kyoho' berries

The abscission rate of grapes gradually increased during storage, and NaL treatment inhibited the abscission of grape berries (Figure 2A). The grapes began to drop after 2 d of storage, and the abscission rate of 'Kyoho' berries after NaL treatment was always lower compared with the control (Figure 2A). The abscission rate of CK reached 63.71% after 10 d of storage. However, the abscission rate of grapes treated with 0.1%, 0.5% and 1% NaL were only 25.8%, 21.4% and 12.4% on the same day, respectively.

The rate of weight loss increased rapidly as the storage time extended. After 10 d of storage, the weight loss rate of the control reached 6.83%. Compared with the control group, the weight loss rate of 'Kyoho' berries was reduced after treatment with different NaL concentrations (Figure 2B). The 1% NaL

concentration had the strongest inhibitory effect, and the weight loss rate was only 2.92% after 10 d of storage. Similarly, the browning degree of grape rachis increased as the storage time extended, and the browning index of rachis was consistently lower in the NaL treatment groups than in CK (Figure 2D). In addition, the fruit firmness of the CK and NaL treatments consistently decreased during storage; however, there were no significant differences in the fruit firmness between any of the treatments (Figure 2C). Thus, NaL treatment reduced the weight loss and rachis browning level of 'Kyoho' berries, and 1% NaL had the strongest effect (Figure 2B-D).

Effect of NaL treatment on the fruit quality of 'Kyoho' berries

TSS and TA are important indexes for evaluating the taste and flavor of grapes. The TSS and TA of 'Kyoho' berries in all groups did not change significantly throughout the 10 d of storage at 20°C (Figure 3A, C). The VC content of grapes decreased after 4 d of storage, and the VC content was lower in the NaL treatments than in the control (Figure 3B). Similarly, NaL treatment inhibited the reduction in the reducing sugar content in grapes during storage (Figure 3D), and the treatment with the strongest effect was 1% NaL, which reduced the loss of postharvest quality. Thus, NaL treatment reduced the loss of VC and reducing sugar, improved the postharvest quality of 'Kyoho' berries and did not affect the taste and flavor of the fruit.

Effect of NaL treatment on the MDA content and membrane permeability of 'Kyoho' berries

MDA accumulation can damage the cell membrane and accelerate fruit senescence. During storage, the content of MDA continuously increases. Compared with the control group, the accumulation of MDA in grapes was inhibited by NaL treatment (Figure 4A). After 2 d of storage, the membrane permeability of grape began to increase as the storage time extended. The membrane permeability of CK peaked 6 d after storage and then began to decrease. After NaL treatment, the membrane permeability of grape increased slowly and was consistently lower than that of CK (Figure 4B). This indicated that NaL treatment inhibited MDA accumulation, decreased membrane permeability and delayed grape senescence. The effect of the 1% NaL treatment was the strongest.

Effect of NaL treatment on the activities of cell wall-degrading enzymes of 'Kyoho' berries

To explore why NaL treatment can inhibit the abscission of table grapes, changes in the activity of four cell wall-degrading enzymes in 'Kyoho' berries were measured at 20°C. During storage, the activities of PG, POD, Cx and LOX increased continuously, which was positively correlated with the abscission rate of 'Kyoho' berries. Compared with the control, NaL treatment inhibited the increase in PG, POD, Cx and LOX activities (Figure 5A-D), and the effect of the 1% NaL treatment was the strongest. These results indicated that NaL treatment could effectively inhibit the increase in cell wall-degrading enzyme activity.

Effect of NaL treatment on the activity of energy metabolism-related enzymes of 'Kyoho' berries

Based on the physiological and phenotypic results, it was found that the 1% NaL treatment was the most effective for inhibiting berry abscission and maintaining fruit quality of berries. To explore whether NaL

treatment affect the energy state of postharvest grape fruit, 1% NaL treated samples of 'Kyoho' berries were selected for the determination of the activity of enzymes involved in energy metabolism-related indexes, including mitochondrial ATPase (H^+ -ATPase and Ca^{2+} -ATPase), mitochondrial respiratory metabolic enzymes (SDH and CCO). The activity of H^+ -ATPase, Ca^{2+} -ATPase, SDH and CCO was higher in the 1% NaL treatment group than in the control group during the storage period, and the difference was significant during the late part of the storage period (Figure 6A-C). After harvest, the activity of CCO increased before 6 d of storage and then decreased. The activity of CCO was higher in the NaL treatment group than in the control (Figure 6D). This indicated that NaL treatment increased the activity of H^+ -ATPase, Ca^{2+} -ATPase, SDH and CCO.

Effect of NaL treatment on ATP, ADP, and AMP content and energy charge of 'Kyoho' berries

ATP is the main energy source for biological tissues and cell metabolism and thus indicates the physiological state of plants. The ATP content decreased during storage. The ATP content of the 1% NaL treatment group decreased slowly and was higher compared with the control group (Figure 7A). During storage, the content of ADP peaked at 4 d of storage and then began to decline (Figure 7B). In contrast to the trend in ATP, the content of AMP increased continuously. NaL treatment inhibited the increase in AMP content, which was always lower compared with the control (Figure 7C). The pattern of energy charge was consistent with the ATP results, NaL treatment increased the energy charge (Figure 7D). Thus, NaL treatment maintained the ATP content and energy charge of 'Kyoho' berries during storage.

Discussion

The lack of appropriate methods for treating 'Kyoho' grapes can lead to berry abscission, rapid decay and significant economic losses. Postharvest berry abscission and low energy levels seriously affect the commercial value of grapes and restrict the development of the grape industry. In this study, when NaL was applied, the activities of the cell wall-degrading enzymes PG, POD, Cx and LOX were significantly decreased, the abscission of grapes was inhibited; fruit weight loss and rachis browning index were reduced, VC and reducing sugar accumulation were increased, the quality of grapes was improved, and the flavor was not affected; the MDA content and membrane permeability were decreased, which reduced the oxidative damage of fruit; the activity of enzymes involved in energy metabolism, energy charge, ATP content were increased, thus disease resistance was improved and fruit senescence was delayed.

In recent years, an increasing number of studies have examined the effect of applying exogenous substances for fruit preservation and explored the methods by which the shelf life of fruit can be extended. For example, polyamines (PAs) can improve the firmness of fruit and reduce the aging rate and have been used in the postharvest preservation of a variety of fruit, including grape, carambola and pomegranate (Champa et al., 2014; Mirdehghan et al., 2007; Ahmad et al., 2019). Melatonin (MT), a common antioxidant, can activate the antioxidant metabolism of plants, remove excess ROS, increase the resistance of plants to adverse environments and has a superior preservation effect (Tan, et al., 2020; Sun, et al., 2020). Treatment with 0.5 mM MT can significantly affect the physiological characteristics of

grapes at $24 \pm 1^\circ\text{C}$ and reduce the degree of DNA methylation, thereby prolonging shelf life (Sun, et al., 2020). In this study, during the storage period, the berry abscission rate was lower in the NaL treatment group than in the control group (Fig. 2A), indicating that exogenous NaL treatment was effective for controlling the fruit abscission of 'Kyoho' berries. NaL is known to directly scavenges hydrogen peroxide (H_2O_2), hydroxyl radical ($-\text{OH}$) and other ROS and reduces oxidative damage, but the research on the application of NaL in grape preservation has not been reported. This study provides a new technique that could be used to inhibit berry abscission and demonstrates a new function of NaL.

The activity of cell wall-degrading enzymes is an important factor affecting the postharvest abscission of fruit. The activity of various cell wall-degrading enzymes, including PG, POD, Cx, LOX and PE, changed significantly during grape fruit abscission (Chen et al., 2019), a pattern that has also been observed in other horticultural products, such as longkong, citrus and tomato fruit (Tsuchiya, et al., 2015; Deng, et al., 2016, Taesakul et al., 2018). The activity of cell wall-degrading enzymes is affected by various factors, such as application of 1.5 mM salicylic acid before harvest can inhibit their activity and prevent the softening of grapes (Champa, et al., 2014). The active biodegradable chitosan coating has antibacterial properties, can reduce the decay rate and activity of cell wall-degrading enzymes and prolong the shelf life of fresh strawberries (Badawy et al., 2016). Our results showed that exogenous antioxidant NaL treatment can effectively inhibit the activity of cell wall-degrading enzymes (Fig. 5) and berry abscission (Fig. 1), the trend in enzyme activity is consistent with the rate of berry abscission.

Membrane lipid peroxidation can accelerate the senescence of plant cells and damage the integrity and fluidity of membranes. During the storage of grapes, the excessive accumulation of H_2O_2 and MDA and the integrity of the cell membrane are the key causes of plant senescence (Yu et al., 2020). Previous studies have shown that exogenous application of antioxidant MT treatment can increase the accumulation of proline and soluble sugar and reduce cell membrane damage (Tan et al., 2020); it can also scavenge active oxygen, inhibit lipid peroxidation and delay the senescence of peach fruit (Gao et al., 2016). Similarly, in this study, NaL treatment significantly reduced MDA accumulation, cell membrane permeability (Fig. 4) and rachis browning index (Fig. 2D), which indicates that NaL can reduce lipid peroxidation and delay grape fruit senescence during postharvest storage.

The quality of postharvest fruit is affected by ATP and energy levels, higher energy status can improve the resistance of fruit to pathogen invasion. Various studies have shown that treatment with exogenous substances can maintain energy and improve fruit quality. For example, the postharvest application of acibenzolar-S-methyl (ASM), H_2O_2 and trisodium phosphate (TSP) can maintain high levels of ATP and energy in fruit and improve the quality of pear, longan and apple, respectively (Ge et al., 2017; Lin et al., 2017; Ge et al., 2019). Similarly, in this study, NaL treatment maintained the ATP content and energy charge in grapes, delayed energy reduction (Fig. 7). Mitochondria are the main site of energy production. Part of the reason for senescence is the decline of mitochondrial integrity, resulting in the reduction of ATP supply required for cell metabolism. CCO is at the end of cytochrome system in cell respiration, participates in mitochondrial respiratory oxidation on the surface of mitochondria, and provides energy for the whole cell. SDH is a key enzyme in mitochondrial redox reaction and cell metabolism, which

reflects mitochondrial function and the degree of a tricarboxylic acid cycle (Zhou et al., 2014). In this study, NaL treatment enhanced the activities of key enzymes of energy metabolism-related, including H⁺-ATPase and Ca²⁺ ATPase, SDH and CCO (Fig. 6), which are consistent with the increase of ATP and energy level, maintained the high-energy state of grape fruit.

Conclusion

All concentrations of NaL treatment could affect the physiological and quality characteristics of postharvest grapes, significantly reduced the activity of cell wall-degrading enzymes, inhibited berry abscission, and the effect of the 1% NaL treatment was the most pronounced on inhibiting berry abscission and maintaining fruit quality. In addition, the activity of energy metabolism-related enzymes, ATP content and energy charge were maintained in the 1% NaL treatment, prolonged the storage life of 'Kyoho' grape during the storage period. Our results provide a robust method for inhibiting berry abscission, improving berry quality and enhancing the energy metabolism of 'Kyoho' grape.

Declarations

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Author Contributions

YiheYu conceived and designed the experiment, analyzed and interpreted the data and wrote the manuscript. Yadan Sun, Xiangxuan Meng, Qiaofang Shi, Yiyi Li, Hainan Liu, Tonglu Wei, Maosong Pei, Dalong Guo and Dongming Jiang participated in measurement of physiological parameters.

All authors read and approved the final manuscript.

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Data Availability

The datasets generated during the current study are available from the corresponding author on request.

Declarations Conflict of Interest

The authors declare no competing interests.

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Figures

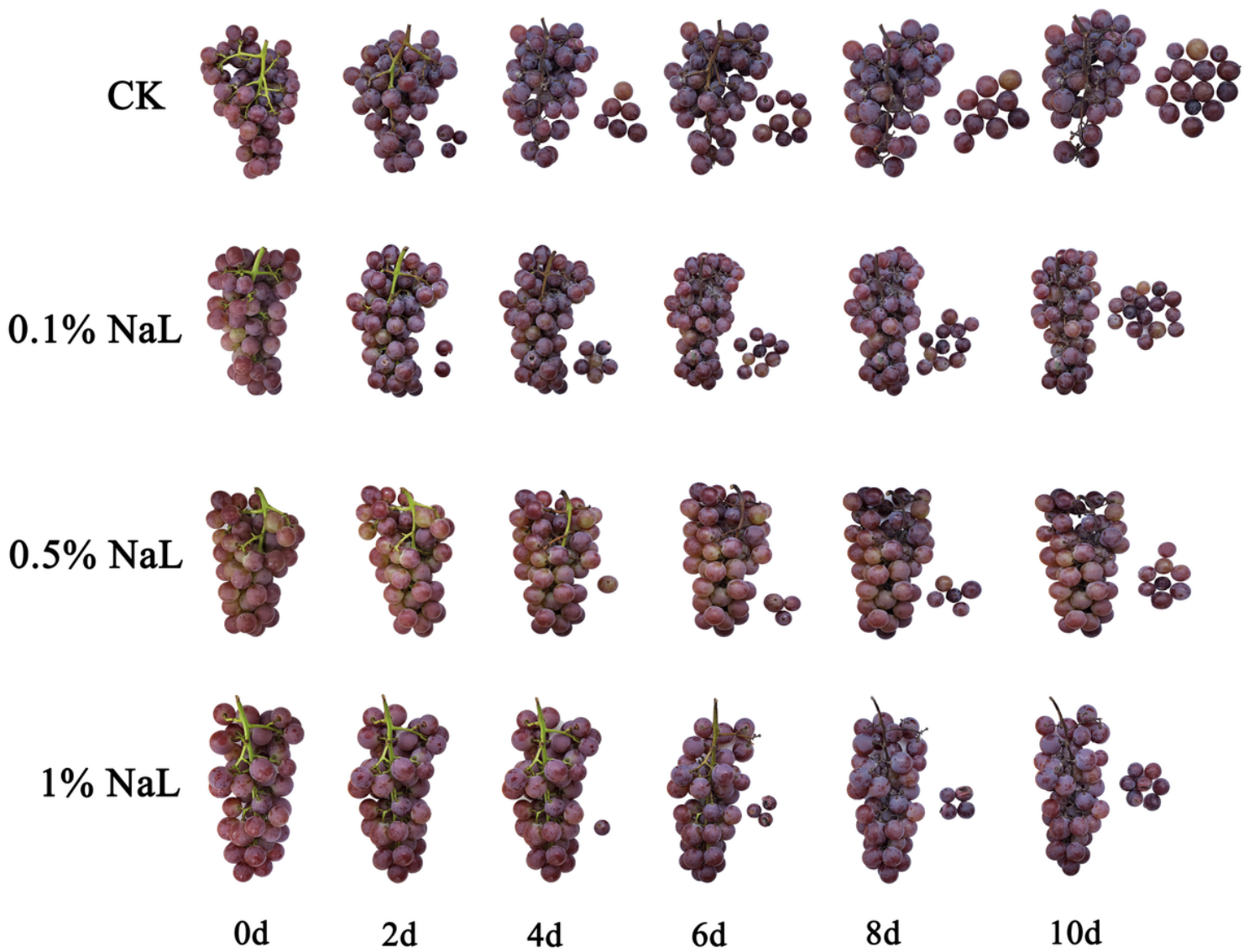


Figure 1

Phenotypic changes of 'Kyoho' berries during storage in the control (CK) and *sodium lactate* (NaL) treatments. 'Kyoho' berries were treated with 0.1%, 0.5% and 1% NaL on the same day after harvest.

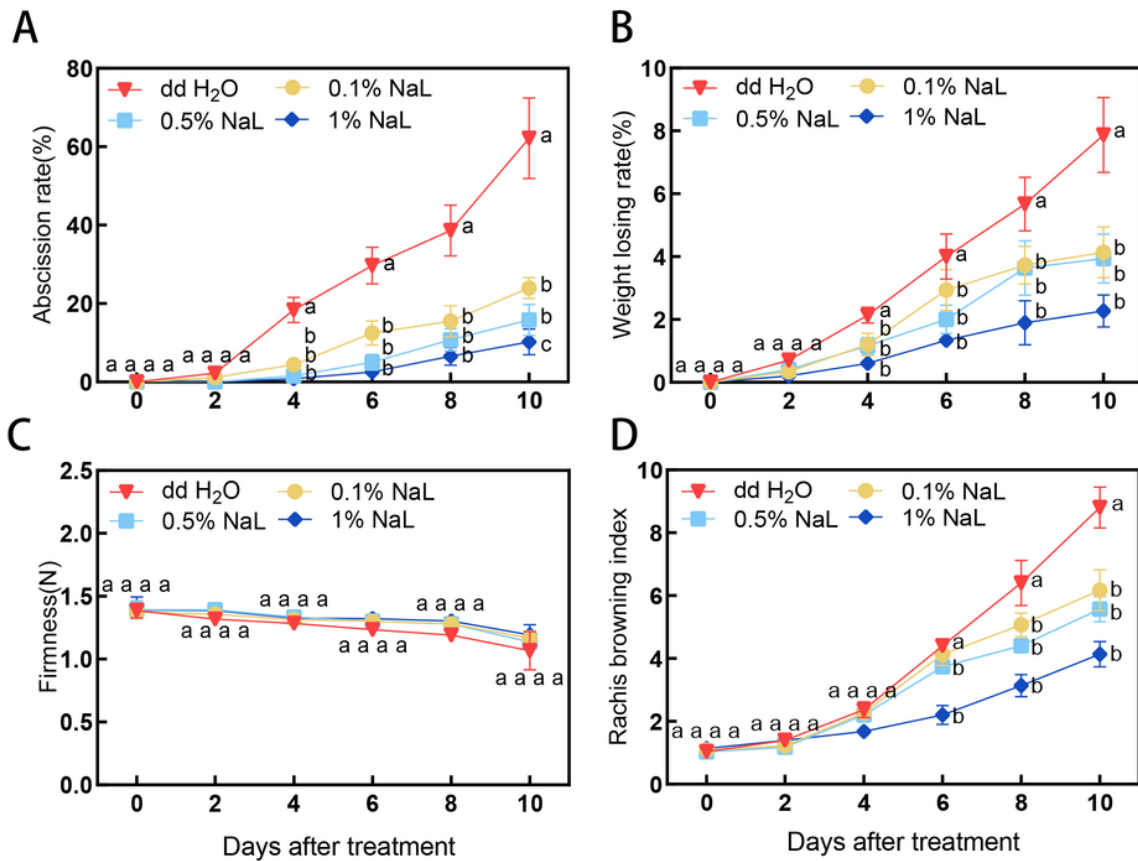


Figure 2

Effect of NaL treatment on the physiological characteristics of 'Kyoho' berries during storage. (A) Abscission rate, (B) weight loss rate, (C) firmness and (D) rachis browning index in 'Kyoho' berries treated with 0.1%, 0.5% and 1% NaL. Double-distilled water (ddH₂O) treatment was used as the CK. Data are presented as mean ± SD (n = 3). Significant differences between means were determined using ANOVA ($P < 0.05$) and are denoted by different lowercase letters.

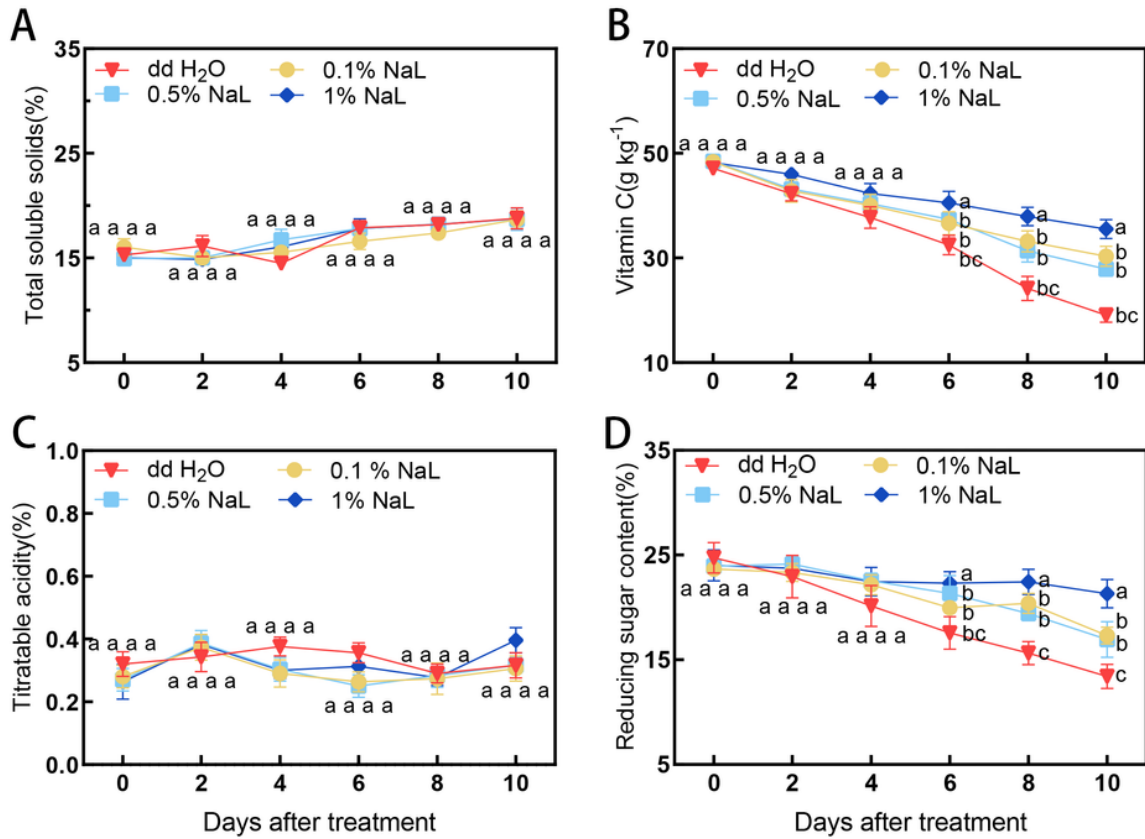


Figure 3

Effect of NaL treatment on the quality of 'Kyoho' berries. (A) Total soluble solids (TSS), (B) vitamin C (VC), (C) titratable acidity (TA) and (D) reducing sugar content in 'Kyoho' berries treated with 0.1%, 0.5% and 1% NaL. Data are presented as mean \pm SD ($n = 3$). Significant differences between means were determined using ANOVA ($P < 0.05$) and are denoted by different lowercase letters.

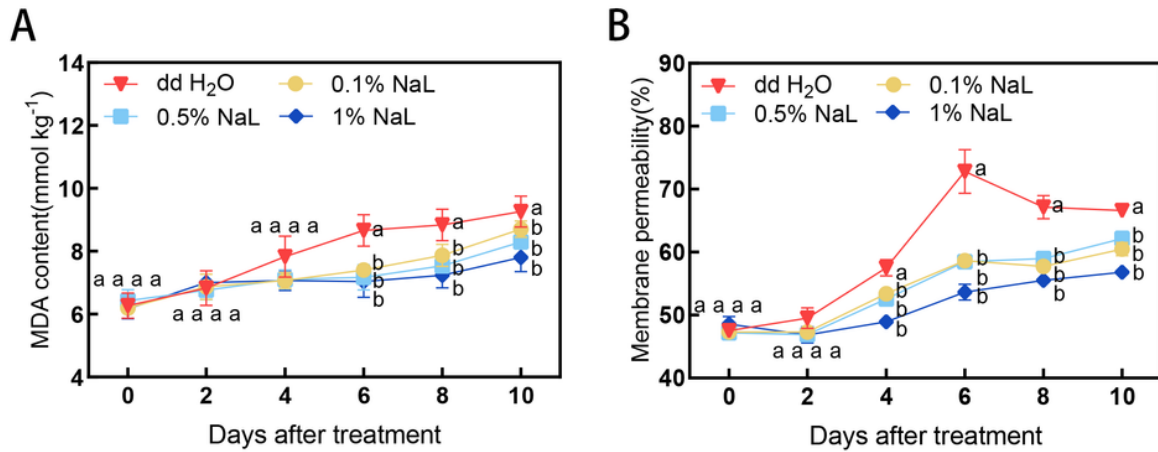


Figure 4

Effect of NaL treatment on the (A) malondialdehyde (MDA) content and (B) membrane permeability of 'Kyoho' berries during storage. Grapes were treated with 0.1%, 0.5% and 1% NaL. Data are presented as mean \pm SD ($n = 3$). Significant differences between means were determined using ANOVA ($P < 0.05$) and are denoted by different lowercase letters.

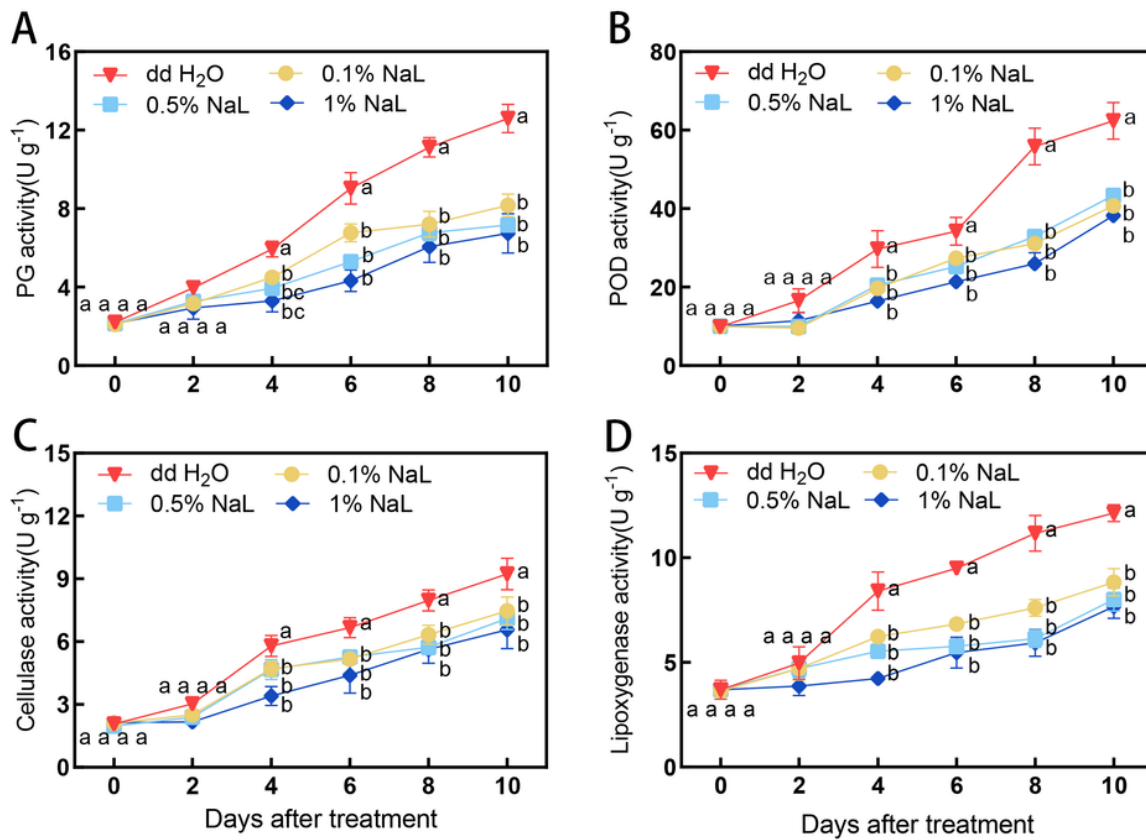


Figure 5

Effect of NaL treatment on (A) polygalacturonase (PG) activity, (B) pectinesterase (PE) activity, (C) cellulase (Cx) activity and (D) lipoxygenase (LOX) activity of 'Kyoho' berries. The grapes were treated with 0.1%, 0.5% and 1% NaL. Data are presented as mean \pm SD ($n = 3$). Significant differences between means were determined using ANOVA ($P < 0.05$) and are denoted by different lowercase letters.

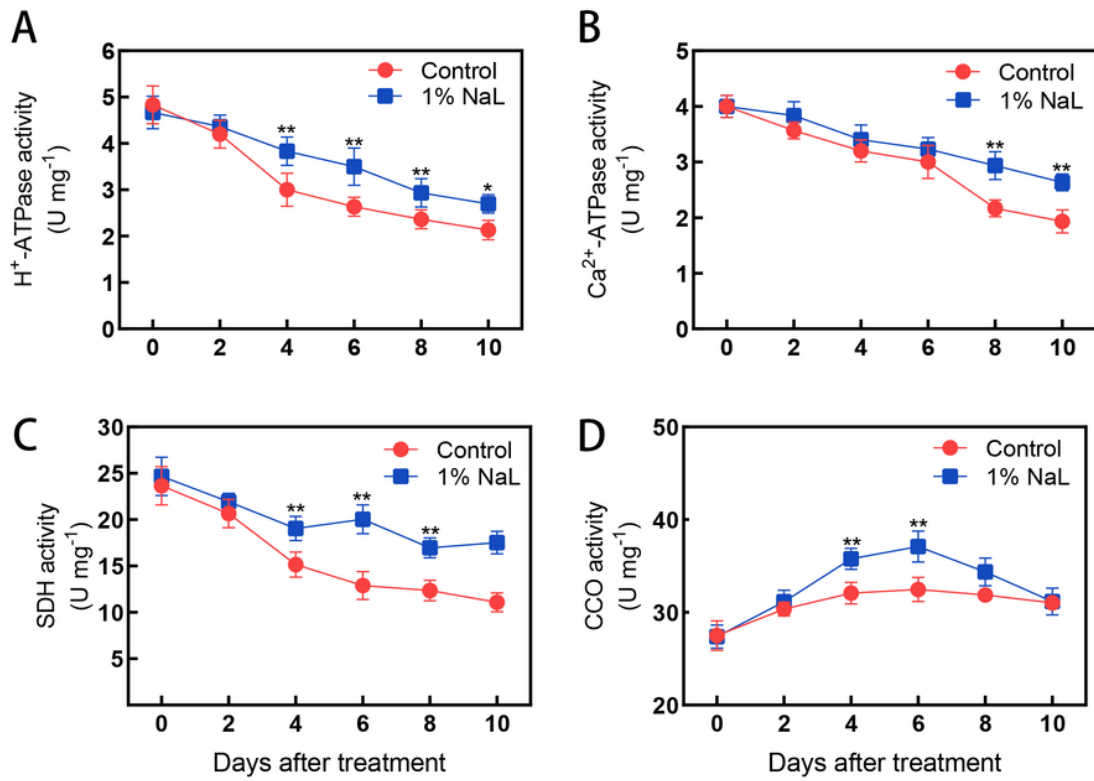


Figure 6

Effect of NaL treatment on (A) H⁺-ATPase, (B) Ca²⁺-ATPase, (C) succinate dehydrogenase (SDH) and (D) cytochrome C oxidase (CCO) activity of 'Kyoho' berries. The grapes were treated with 0.1%, 0.5% and 1% NaL. Data are presented as mean ± SD (n = 3). Asterisks indicate the significance level (**P* < 0.05, ***P* < 0.01) according to Student's *t*-test.

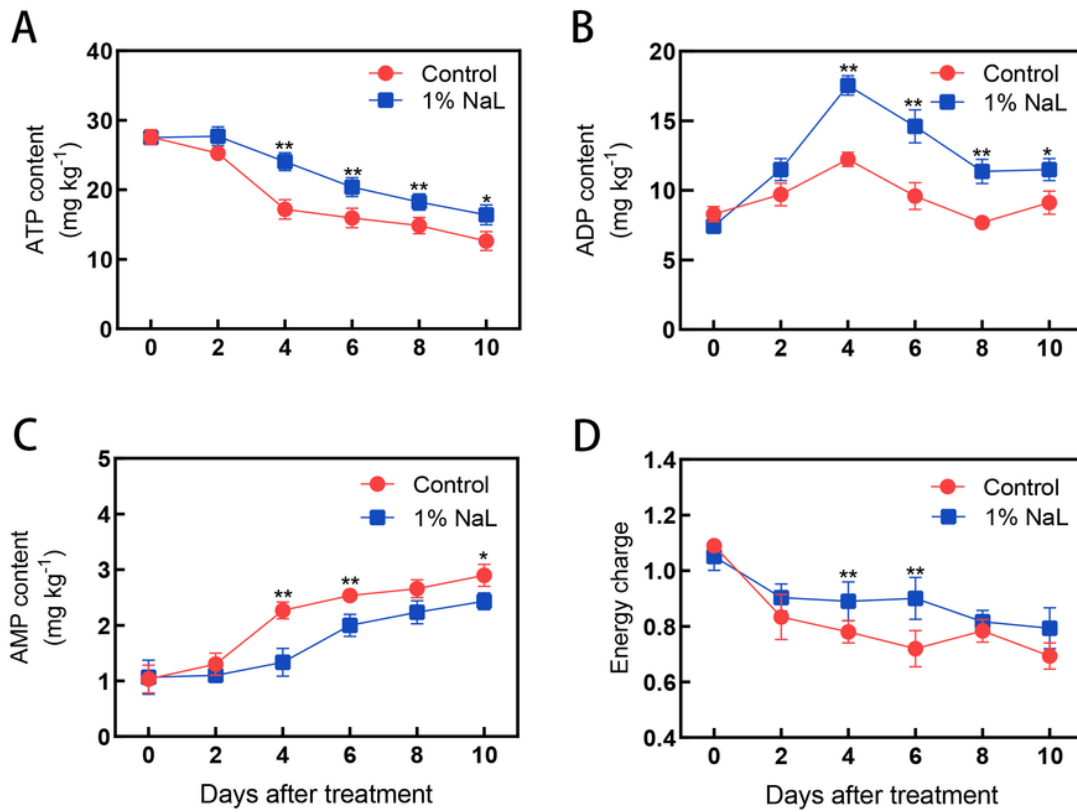


Figure 7

Effect of NaL treatment on (A) adenosine triphosphate (ATP) content, (B) adenosine diphosphate (ADP) content, (C) adenosine monophosphate (AMP) content and (D) energy charge of 'Kyoho' berries. The grapes were treated with 0.1%, 0.5% and 1% NaL. Data are presented as mean \pm SD ($n = 3$). Asterisks indicate significance level ($*P < 0.05$, $**P < 0.01$) according to Student's t -test.