Postharvest Biology and Technology 159 (2020) 110997

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Chlorogenic acid treatment alleviates the adverse physiological responses of vibration injury in apple fruit through the regulation of energy metabolism

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ARTICLE INFO

Keywords:
Malus domestica Borkh.
Chlorogenic acid
Vibration injury
Postharvest quality
Energy metabolism

ABSTRACT

Apple fruit are susceptible to vibration injury during handling, transportation and postharvest storage. Vibration injury causes the deterioration of fruit quality and results in economic losses. The effects of chlorogenic acid (CGA) treatment on postharvest physiological indicators and energy metabolism of vibration damaged apple fruit stored at 23 ± 1 °C for up to 28 d have been investigated. CGA treatment slowed softening, the decrease of soluble solids content and titratable acidity, reducing weight loss, electrolyte leakage, malondialdehyde accumulation and the respiration rate. CGA treatment also reduced in the rate of ethylene production through the regulation of enzymes that are involved in ethylene biosynthesis. An increase in the levels of adenosine triphosphate and energy charge were observed in apple fruit after CGA treatment. Activities of enzymes involved in energy metabolism including H^+-adenosine triphosphatase, $Ca^{2+}-$adenosine triphosphatase, succinic dehydrogenase and cytochrome C oxidase were increased by CGA treatment. The collective data indicated that CGA treatment reduced the adverse physiological changes caused by vibration damage in apple fruit. The mechanism of action may in part be related to enhancing the energy status and the activity of enzymes involved in energy metabolism as well as the maintenance of membrane integrity of the fruit. CGA may be used to provide a method to reduce food wastage and economic losses in the production and marketing of apple fruit.

1. Introduction

Apples (*Malus domestica* Borkh.) are one of the most important fruit crops in the world. Despite modern transport and storage facilities, mechanical damage remains an important contributor to the degradation of product quality. Mechanical damage, which is considered an abiotic stress, is inflicted by the vibrations and shocks that occur to fruit during handling and transportation (Fernando et al., 2018; Paternoster et al., 2018). Apple fruit in particular are susceptible to mechanical damage, especially due to vibration, during transportation (Fadiji et al., 2016).

Vibration damage is caused by the impact between fruit or between fruit and containers or packages (Li and Thomas, 2014). Vibration damage is a form of cumulative fatigue that is caused by circular dynamic vibrations, which results in internal damage to the fruit. This form of damage is difficult to detect and assess quantitatively because of the lack of a visible wound (Wei et al., 2019). The irreversible damage to the internal structure of the fruit, particularly the spatial deformation of flesh cells, results in the rapid deterioration of the damaged fruit in a short period of time (Komarnicki et al., 2016; Wei et al., 2019). Previous studies have established the adverse physiological responses induced by vibration on fruit during transport, including wounded ethylene production as well as the rapid decrease of firmness and nutrient contents (Fadiji et al., 2016; Lu et al., 2019; Wei et al., 2019). A slight vibration of a small amplitude is a common occurrence during the transportation of fruit and the quality of transported fruit can decline after transportation and during storage. It is therefore important to study the changes in the physiological characteristics of the fruit in conditions where they have undergone vibration injury as well as in conditions where attempts have been made to reduce the adverse effects of vibration on the quality of fruit.

Extensive research has shown that energy supply in plant cells is essential for a wide range of physiological metabolisms processes in harvested fruit (Aghdam et al., 2018). Application of methyl jasmonate induces phenolic accumulation in wounded pitaya fruit by regulating the sugar content as well as the energy status (Li et al., 2018). Wounding stress has been linked to enhanced energy production in cut carnation flowers (Song et al., 2008). Postharvest application of trisodium phosphate (Ge et al., 2019), propyl gallate (Lin et al., 2018) and tea seed oil (Zhang et al., 2017) has been reported to delay senescence and may result in the improved quality of fruit by regulating their energy metabolism. These results suggest that the presence of sufficient

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https://doi.org/10.1016/j.postharvbio.2019.110997
Received 13 June 2019; Received in revised form 19 August 2019; Accepted 23 August 2019

intracellular energy attenuates abiotic stress, delays senescence and maintains quality in horticultural crops after harvest. However, there is little known about the impact of the energy status on the regulation of vibration injury in fruit.

Chlorogenic acid (CGA, 3-*O*-caffeoylquinic acid) is an important phenolic compound that is widely distributed in fruit (Weber et al., 2014). It is well established that plant phenolic compounds play an important role as defense compounds against abiotic stresses (Naikoo et al., 2019). It has been reported that plant varieties that are resistant to abiotic stress contained higher levels of CGA (Wang et al., 2014). To date, there has been no research that has evaluated whether exogenous CGA confers resistance to vibration injury.

In a previous study we observed that treatment with CGA could regulate the NADP-malic enzyme activity in apple (Xi et al., 2016), which suggests that CGA treatment may regulate fruit ripening, senescence and response to injury. The objective of this study was to evaluate the effect of CGA on the adverse physiological responses caused by vibration injury in apples and the potential mechanisms involved.

2. Materials and methods

2.1. Plant material and treatments

Apple (*Malus domestica* Borkh. cv. Fuji) fruit were harvested from trees of about 20 years old growing in Beiliu County (116.08 °N, 40.18 °E, elevation 42 m), Changping district, Beijing, China. The trees are planted in 4 × 5 m density. The fruit were harvested about 180 days from full bloom. Fruit were transported to the laboratory of China Agricultural University on asphalted highways and asphalted arterial roads to ensure the lowest level of vibration injury within one hour.

The fruit (1200) selected for the experiment were of similar size (180 ± 30 g), color, and without diseases or mechanical damage. They were randomly divided into four treatment groups, each in triplicate. The four treatments, each with 100 apples per replicate, as follows: (1) Control: fruit treated with sterile distilled water; (2) CGA: fruit treated with 100 mg L^{-1} CGA solution; (3) Vibration: fruit treated with sterile distilled water followed by vibration; (4) CGA + Vibration: fruit treated with 100 mg L^{-1} CGA followed by vibration.

All selected fruit were sterilized with 1% sodium hypochlorite for 1 min and then rinsed with distilled water. The fruit were then infiltrated with sterile distilled water or CGA at 100 mg L^{-1} under vacuum (−0.02 MPa) for 2 min. After being air dried, fruit in groups (3) and (4) were subjected to vibration with a shaker (ZW-5, Keep Word Electrical Co., Ltd., China). All the fruit in every replicate (100 fruit per replicate per group) were subjected to vibration together, the vibration progress performed three times. The vibration frequency was determined based on the frequency measured in trucks that used to transport apples (Van Zeebroeck et al., 2007; Lu et al., 2019). A frequency of 3 Hz, with an acceleration level of 0.5 g (1 g = 9.8 m s^{-2}) for a duration of 5 h was used in this study. Fruit were wrapped in net packaging material and placed in ventilated paperboard containers. The container dimensions (length × width × height) were 488 mm × 319 mm × 266 mm internally. The internal packaging consists of four layers of trays. The net packaging material around the fruit protected the fruit from mechanical injury due to the interaction of apples on each other and contact with the container. The selected fruit (80 fruit per replicate for each group) for quality parameter measurement and chemical analysis were taken from the same position of four layers to make sure the selected fruit have been loaded in the same assumed way. The vibration test was performed in triplicate and the apples had no visible damage on the surface after the vibration test.

All fruit in a replicate were sealed in polyethylene bags (< 0.04 mm) and kept at 23 ± 1 °C, with 80–90% relative humidity. Flesh tissue was excised between 0.3–1.0 cm under the epidermis of 10 fruit per replicate after 0, 7, 14, 21 and 28 d for analysis. All samples isolated for analysis were cut into squares, frozen in liquid nitrogen and finally kept at −80 °C.

2.2. Measurement of fruit quality parameters

Fruit quality was determined (80 fruit per replicate for each group) according to the method of Ge et al. (2019). The flesh firmness was determined by measuring opposite peeled sides of the fruit using a texture analyzer (GY-1, Tuopu Instrument Co., Ltd., China) with a 3.5 mm probe, the results were expressed in Newtons (N). A digital sugar meter (PAL-1, ATAGO, Japan) was used to determine the soluble solids content (SSC), and expressed as %. Titratable acidity (TA) was determined using the titration method described by Liu et al. (2016), and the results expressed as the percentage of malic acid. The weight loss was represented as the percentage loss (%) of fruit compared with the initial weight.

2.3. Determination of membrane permeability and malondialdehyde content

Membrane permeability was assessed using the relative electrical conductivity method (Zhao et al., 2019a). Flesh disks taken from the equator positions (5 mm diameter × 5 mm thickness) of ten fruit per replicate were used to determine the membrane permeability. A conductivity apparatus (DDS-11A, Youyi Instrument Co., Ltd., China) was applied to test the conductivity before and after exposure to boiling water in a water bath, the results were expressed as relative conductivity (%).

The malondialdehyde (MDA) content was determined using the thiobarbituric acid reaction described by Zhao et al. (2019b). Two g of frozen fresh tissue was homogenized with 5 mL of 30 mmol L^{-1} trichloroacetic acid and centrifuged at 10,000 × g at 4 °C for 10 min. The concentration of MDA was expressed as μmol kg^{-1} fresh weight.

2.4. Determination of ethylene production and respiratory rate

Three fruit were randomly selected from each group per replicate and placed in a 2.3 l sealed chamber at 25 °C for 2 h; 1.0 ml of headspace gas was taken from the chamber jar and analyzed using a gas chromatograph (GC-7890 F, Shanghai Techcomp Bio-equipment Ltd, China) equipped with a flame ionization detector (FID) and a stainless steel column (3 mm × 2 m). The measurement conditions were as follows: the column and injector temperature were set as 60 °C and 120 °C, respectively. The detection temperature was 360 °C for CO_2 and 200 °C for ethylene. The respiration rate was expressed in μmol kg^{-1} s^{-1} CO_2 and the ethylene production was expressed in nmol kg^{-1} s^{-1} C_2H_4.

2.5. Determination of ACC synthase and ACC oxidase activities

The extraction and determination of ACC synthase (ACS) and ACC oxidase (ACO) were performed as described by Li et al. (2019). For enzyme extraction, 5 gfrozen fruit tissue was homogenized with 5 ml N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) extraction buffer (100 mmol L^{-1}, pH 8.0, containing 4 mmol L^{-1} DTT, 0.5 μmol L^{-1} pyridoxal-5-phosphate and 10 mmol L^{-1} EDTA) on ice. The homogenate was centrifuged at 12,000 × g at 4 °C for 20 min and the supernatant was used for the determination of ACS activity. The reaction mixture contained 500 μL supernatant and 1.5 ml assay buffer containing 60 μL 250 μmol L^{-1} S-adenosylmethionine and 90 μL HEPES buffer. The mixture was capped in a 20 ml vial and incubated at 30 °C for 2 h, then 100 μL of 25 mmol L^{-1} $HgCl_2$ was added to terminate the reaction. Finally, the mixture was incubated on ice for 10 min and 200 μL 5% NaClO - saturated NaOH (2:1, v/v) was injected into the bottle. The vials were vortexed and incubated on ice for 5 min. The ethylene concentration in 1 ml of headspace gas was determined by gas chromatography as described above. The ACS activity was expressed as U, which was defined as the production of 1 nmol kg^{-1} s^{-1} C_2H_4 on a fresh weight basis.

For the determination of ACO activity, 5g frozen tissue was homogenized with 5 mL Tris−HCl buffer (0.1 mol L^{-1}, pH 7.4) containing 10% glycerol, 30 mmol L^{-1} Na-ascorbate, 5 mmol L^{-1} DTT and 1% PVP (w/v). The homogenate was centrifuged at 12,000 × *g* at 4 °C for 20 min and the supernatant was collected. An aliquot of 0.5 ml supernatant was added into a 20 ml sealed bottle containing 1 ml reaction buffer (containing 10% glycerol, 30 mmol L^{-1} Na-ascorbate, 2 mmol L^{-1}ACC and 0.1 mmol L^{-1} $FeSO_4$). A total of 1 ml pure CO_2 gas was injected into the sealed vial to activate the reaction. The reaction was incubated at 30 °C for 1 h after which 1 ml of headspace gas was extracted with a syringe and used to determine the ethylene concentration by gas chromatography. The ACO activity was expressed U, where U was defined as the production of 1 nmol kg^{-1} s^{-1} C_2H_4 fresh weight.

2.6. Measurement of adenosine triphosphate, adenosine diphosphate, adenosine monophosphate and energy charge

The extraction and determination of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) content was performed according to the method described by Jin et al. (2013) with slight modifications. Briefly, 3 *g*frozen pulp tissue was grounded with 5 ml perchloric acid (0.6 mol L^{-1}) and centrifuged at 12,000 × *g* at 4 °C for 20 min. ATP, ADP and AMP levels were measured using high performance liquid chromatography (HPLC) method (LC-20AT, Shimadzu, Japan) and expressed as mg kg^{-1} fresh weight. Energy charge (EC) was calculated using the formula: EC = [ATP + 0.5 ADP] / [ATP + ADP + AMP].

2.7. Determination of the activity of energy metabolism-related enzymes

Extraction of crude mitochondria from apples was performed as described by Jin et al. (2014). The crude mitochondrial extract was used for subsequent enzyme analyses. Frozen tissue (30g) was homogenized with 100 ml Tris-HCl buffer (80 mmol L^{-1}, pH 7.5) which contained 2 mmol EDTA, 0.3 mmol L^{-1} sucrose, 0.3 mmol L^{-1} mannitol and 0.5 g L^{-1} polyvinyl pyrrolidone at 4 °C. The extracts were centrifuged at 4000 × g for 10 min. The supernatants were collected and centrifuged at 20,000 × g for 20 min at 4 °C. The precipitate was washed twice with washing buffer (10 mmol L^{-1} Tris-HCl buffer, pH 7.2), including 0.3 mol L^{-1} sucrose, 0.3 mol L^{-1} mannitol, 2 mmol L^{-1} EDTA. The final precipitate was dissolved in washing buffer; this represents the crude mitochondrial extract that was used for the enzyme assays.

The activities of H^+-adenosine triphosphatase (H^+-ATPase), Ca^{2+}-adenosine triphosphatase (Ca^{2+}-ATPase), succinate dehydrogenase (SDH) and cytochrome C oxidase (CCO) were measured using the method described by Zhou et al. (2014) with slight modifications. For the measurement of H^+-ATPase and Ca^{2+}−ATPase activities, the reaction system consisted of 3 mL reaction solution containing 30 mol L^{-1} Tris–HCl (pH 8.0), 3 mol L^{-1} Mg_2SO_4, 0.1 mmol L^{-1} Na_3VO_4, 50 mmol L^{-1} $NaNO_3$, 50 mmol L^{-1}KCl, 0.1 mmol L^{-1} $(NH_4)_2MoO_4$, and 0.1 mL mitochondrial extract. The reaction was initiated by the addition of 30 mmol L^{-1} ATP–Tris-HCl (pH 8.0) and the mixture was incubated at 37 °C for 30 min. The reaction was terminated by the addition of 55% trichloroacetic acid. The results were expressed as U mg^{-1} protein, where U was defined as the release of 1 μmol of phosphorus at 660 nm s^{-1}.

The activity of SDH was assayed in a reaction mixture containing 0.3 mL of crude mitochondrial extract, 3 mL of 0.2 mmol L^{-1} potassium phosphate buffer (pH 7.4), 1 mL of 0.2 mmol L^{-1} sodium succinate, 0.1 mL 1 mmol L^{-1} di-*p*-chlorophenylmethyl carbinol, and 0.1 mL of 10 mmol L^{-1} methyl sulfanyl phenazine at 30 °C for 5 min. The activity of SDH was expressed as U mg^{-1} protein, where U = 0.01 ΔA_{600} min^{-1}.

The activity of CCO was assayed in the reaction system containing 0.2 mL 0.3 mmol L^{-1} cytochrome *c* solution and 20 mmol L^{-1} dimethyl phenylene diamine. The reaction was incubated at 35 °C for 3 min, then 0.2 mL of crude mitochondria extract was added to the reaction mixture. The activity of CCO was expressed as U mg^{-1} protein, where U = 0.1 ΔA_{510} min^{-1}.

The content of protein in enzyme extracts was measured using a Bradford assay (1976). Bovine serum albumin was used to generate the standard curve.

2.8. Statistical analysis

Experiments were performed in triplicate and statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The mean and standard error were calculated. Data were analyzed using a one-way analysis of variance (ANOVA), followed by individual comparisons using Duncan's multiple range tests. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Fruit quality parameters

Flesh firmness of the fruit decreased throughout the storage period (Fig. 1A). Vibration accelerated softening, but the effects were reduced by CGA treatment. After 28 d, firmness of CGA treated fruit after vibration was 6.5% higher than that of fruit with vibration alone. SSC content of CGA treated fruit was 3.6% higher than that of fruit that were subjected to vibration alone (Fig. 1B). The TA decreased during storage but was higher in the CGA-treated fruit than after vibration (Fig. 1C). The weight loss of control and CGA-treated fruit was 3.8% and 3.7% after 28 d of storage but was 4.8% in fruit after vibration. This was 11% higher than the weight loss of fruit treated with CGA followed by vibration (Fig. 1D). However, effects of CGA treatment on fruit quality improvement were relatively tight compare with the control.

3.2. Membrane permeability and MDA content

The membrane permeability, as indicated indirectly by electrical conductivity, of apple tissue from all treatments increased during storage (Fig. 2A). The electrical conductivity of apples from the vibration treatment was higher than that of apples without vibration. CGA treatment inhibited the increase of membrane permeability; the level of electrical conductivity was lower in the CGA-treated treatments compared to untreated fruit at all time points.

MDA, which is the main product of lipid peroxidation, is an indicator of oxidative stress. Vibration promoted the continuous increase of MDA content in treated apples compared with the control treatment (Fig. 2B). CGA treatment decreased MDA accumulation in fruit after vibration.

3.3. Respiration rate and ethylene production

The respiration rate of apples increased after vibration (Fig. 3A) being 3 times higher than without vibration. CGA treatment resulted in a 20% reduction in the respiration rate compared to fruit that underwent vibration alone. The respiratory rate of fruit that underwent vibration surged after the vibration and this rate was maintained throughout the storage period. The respiration rate of control fruit increased through the storage period, peaking at day 14. The CGA treatment reduced the respiration rate, and the peak for CGA-treated fruit was 11% lower than that of the control fruit. The respiratory rate was maintained a lower level in the CGA-treated group than the respiration rate in apples after vibration alone during the storage period (Fig. 3B).

Ethylene production increased in fruit after vibration. CGA treatment reduced ethylene production, and the peak production rate was 6.6% lower than in fruit that were subjected only to vibration (Fig. 3C).

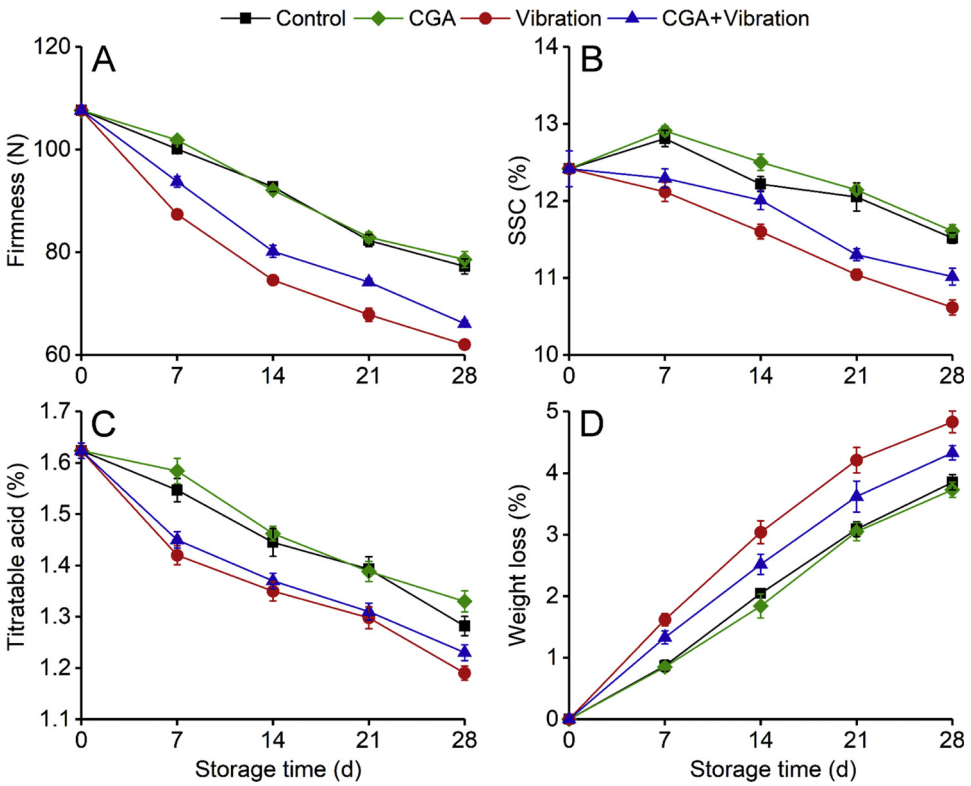

Fig. 1. Effect of CGA treatment on firmness (A), soluble solids content (B), titratable acid content (C) and weight loss (D) of apple fruit during storage at 23 ± 1 °C. Each value is the mean of three replicates. The vertical bars represent the standard errors of the means.

The ethylene production began to rise on the day 3 and reached a peak level on day 14 for the fruit that underwent vibration (Fig. 3D). CGA treatment reduced ethylene production, the peak level of ethylene in CGA-treated fruit being 11% lower than that of the control fruit.

3.4. ACS and ACO activities

ACS and ACO activities in the control fruit gradually increased and peaked on day 14 (Fig. 4A-B); this is consistent with the production of ethylene in the control fruit. Vibration accelerated this trend, with ACS and ACO activities peaking on day 7 in vibrated fruit, and at levels that were higher than those detected in the control fruit. ACS and ACO activities were higher in fruit after vibration than in control apples at the end of the storage period. CGA treatment inhibited the peak level of ACS and ACO activities in fruit after vibration, being 12% and 10% lower, respectively, than those detected in fruit after vibration alone on day 7 of storage.

3.5. The content of ATP, ADP, AMP and EC

The ATP content of apples after vibration increased slightly in the initial stage and then decreased rapidly afterward (Fig. 5A) compared to the levels of ATP in the control and CGA-treated fruit. CGA treatment delayed the decline of ATP in apples that underwent vibration. ADP content increased in the initial stage of storage and decreased rapidly afterward in both the CGA-treated and control fruit (Fig. 5B). CGA treatment also inhibited the decrease of ADP induced by vibration. Over the storage period, higher levels of ATP and ADP were detected in CGA and vibration-treated apples compared with the fruit with vibration treatment only. The levels of AMP increased steadily over the course of the storage period in all the groups of apples but increased most rapidly in vibration-treated fruit. CGA treatment inhibited the increase in the

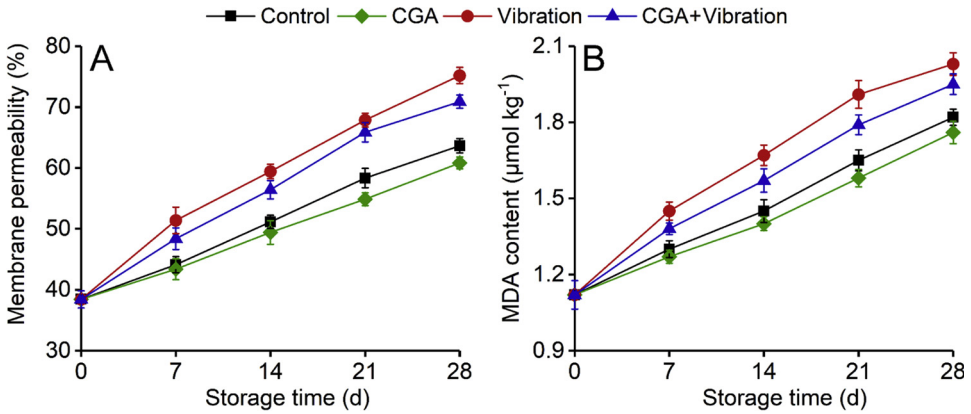

Fig. 2. Effect of CGA treatment on membrane permeability (A) and malondialdehyde content (B) of apple fruit during storage at 23 ± 1 °C. Each value is the mean of three replicates. The vertical bars represent the standard errors of the means.

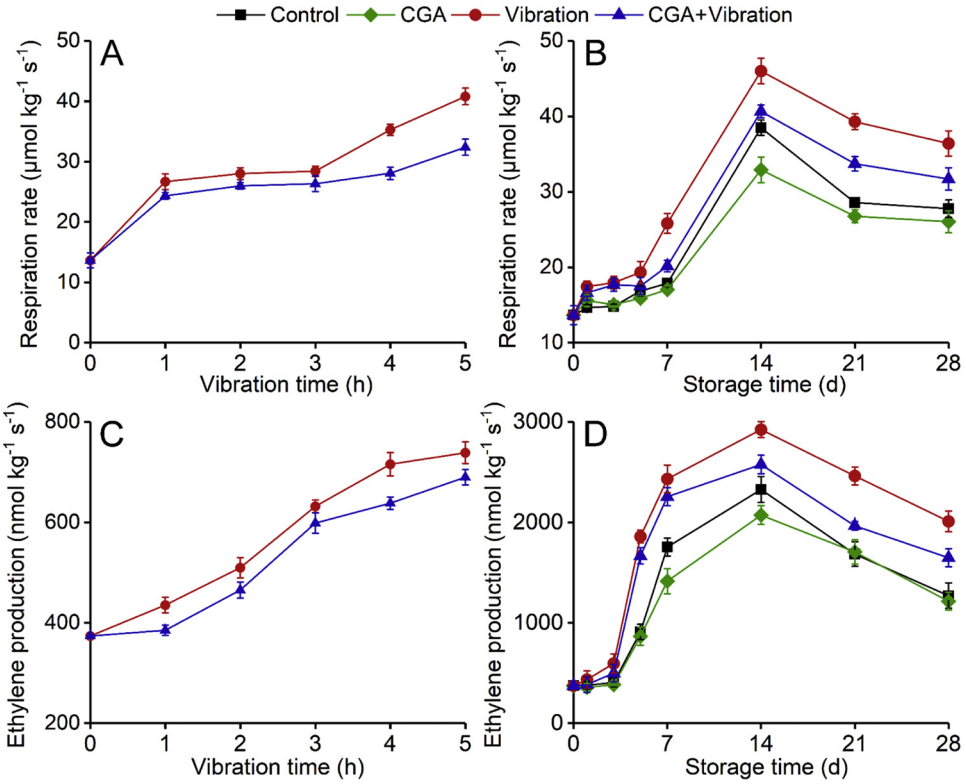

Fig. 3. Effect of CGA treatment on respiratory rate (A, B) and ethylene production (C, D) during vibration and storage at 23 ± 1 °C. Each value is the mean of three replicates. The vertical bars represent the standard errors of the means.

AMP content.

3.6. Enzyme activities in energy metabolism

H^+-ATPase and $Ca^{2+}-$ATPase are key enzymes in mitochondrial metabolism; they are important in energy synthesis and supply. Vibration injury induced an increase in H^+-ATPase and Ca^{2+}-ATPase activity. CGA treatment further enhanced the activity of H^+-ATPase and Ca^{2+}-ATPase in the first 14 d of storage and enzyme activity were maintained at higher levels than those detected in vibration-treated apples (Fig. 6A-B). At the end of the storage period, the activity of H^+-ATPase and $Ca^{2+}-$ATPase in vibration-treated apples treated with CGA were 18% and 14% higher than those in the vibration-treated apples in the absence of CGA.

SDH and CCO are important enzymes in ATP synthesis. The SDH activity decreased during the storage period in all the groups (Fig. 6C). Vibration injury accelerated the decline while CGA treatment delayed the decrease of SDH activity at 14 and 28 day of storage in vibration-treated apples. The SDH activity of vibration-treated apples that were treated with CGA was 9.6% and 49% higher than the SDH activity in vibration-treated apples at days 21 and 28 respectively. The CCO activity increased for 14 d and then decreased rapidly afterward (Fig. 6D). CCO activity was higher in CGA-treated apples that were subjected to vibration than in the apples that underwent vibration alone. These results suggest that CGA-treated apples that underwent vibration had higher levels of energy metabolism-related enzyme activities over the storage period.

4. Discussion

Mechanical vibration is an important factor of product damage in many postharvest fruit supply chains (Fernando et al., 2018). During

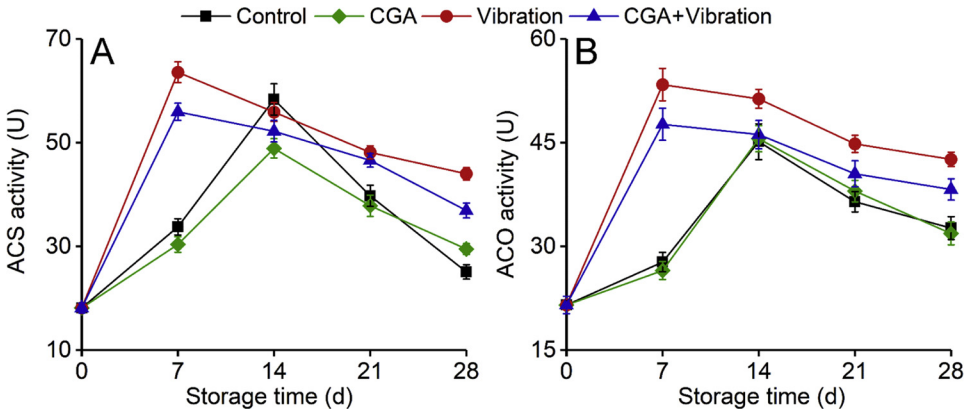

Fig. 4. Effect of CGA treatment on ACC synthase (A) and ACC oxidase (B) activities of apple fruit during storage at 23 ± 1 °C. Each value is the mean of three replicates. The vertical bars represent the standard errors of the means.

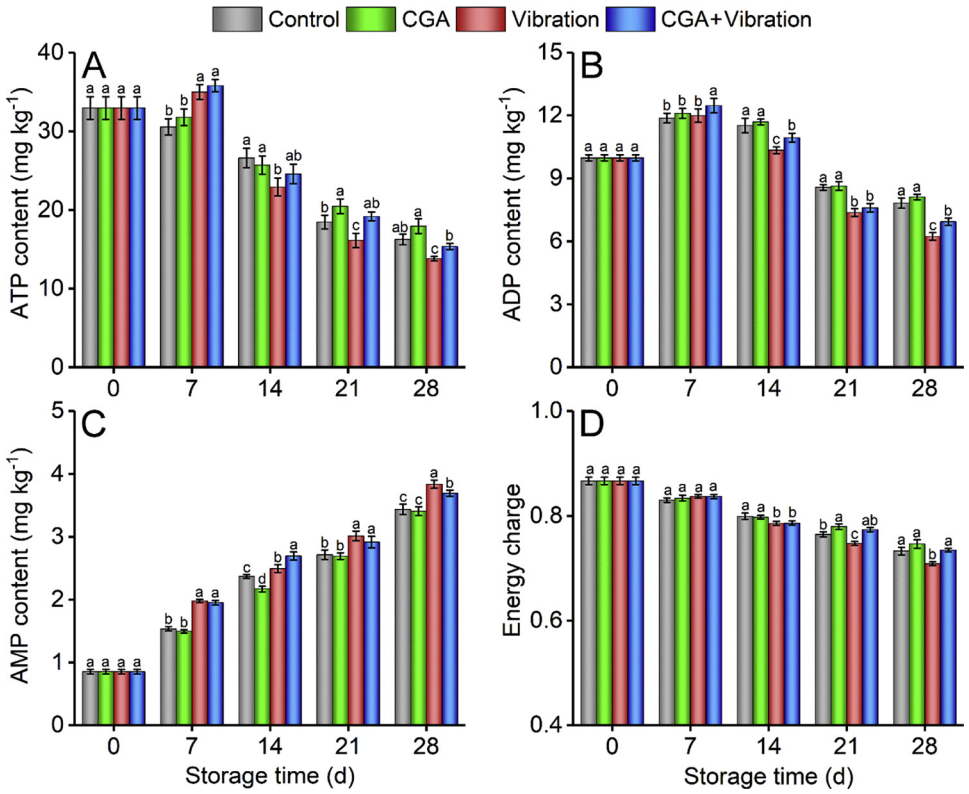

Fig. 5. Effect of CGA treatment on contents of ATP (A), ADP (B), AMP (C), and energy charge (D) of apple fruit during storage at 23 ± 1 °C. Each value is the mean of three replicates. The vertical bars represent the standard errors of the means.

transportation and distribution, fruit and vegetables are subjected to different levels of vibration by the transporting vehicles. For this reason, one of the major causes of mechanical damage to fresh fruit is the vibration that occurs during transport of fruit between farms and retail outlets (Zhou et al., 2007). Previous studies have shown that vibration damage accelerates the reduction in fruit quality, low

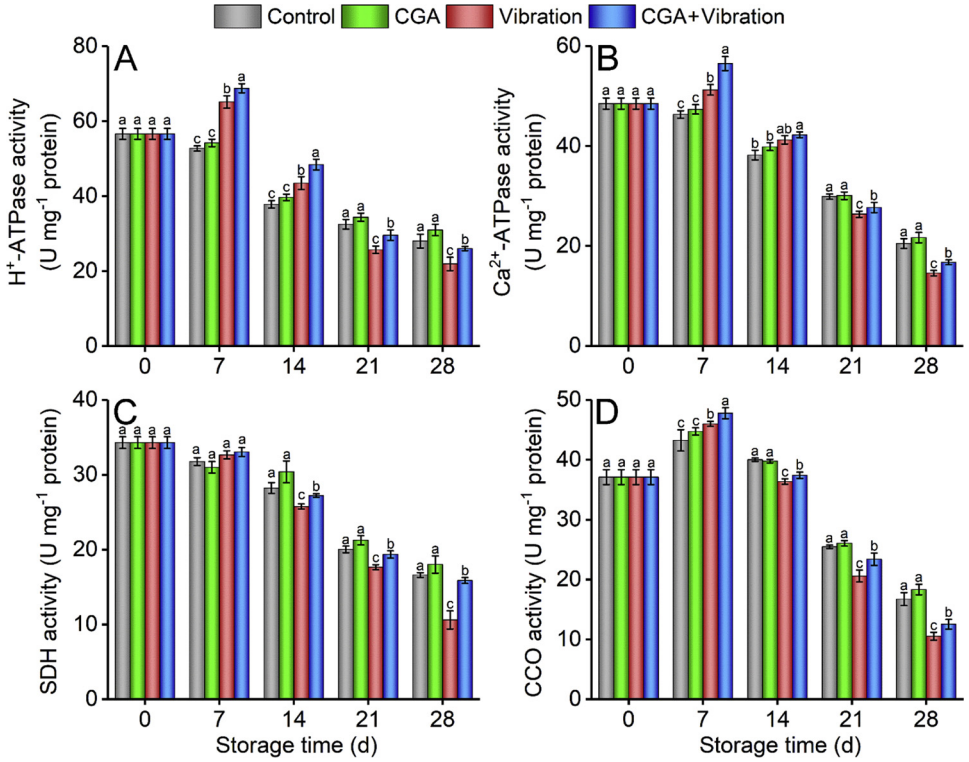

Fig. 6. Effect of CGA treatment on activities change of H^+-ATPase (A), Ca^{2+}-ATPase (B), SDH (C) and CCO (D) of apple fruit during storage at 23 ± 1 °C. Each value is the mean of three replicates. The vertical bars represent the standard errors of the means.

temperature storage maintained the quality in vibration injured apples (Lu et al., 2019; Paternoster et al., 2018; Stopa et al., 2018; Wei et al., 2019). We also observed a reduction in fruit quality after vibration injury, which is consistent with previous studies. This was the result from the damaged internal structure of the fruit, particularly the spatial deformation of flesh cells. In this study, we observed that treatment with CGA resulted in the maintenance of fruit quality after vibration. CGA treatment delayed softening and weight loss of the fruit. It also reduced the loss of SSC and TA in apples that had undergone vibration injury. CGA is an important phenolic acid in apple fruit, which play an important role as defense compound against abiotic stresses. Our previous studies had suggested that CGA treatment could retard the ripening and senescence of fruit by regulating senescence-related enzymes activities (Xi et al., 2016), by improving the antioxidant activities (Xi et al., 2017) as well as by enhancing the resistance of fruit (Jiao et al., 2018). A study by Fan et al. (2018) observed that spraying the pitaya fruit with polyphenols, primarily CGA, could maintain the quality of fresh-cut red pitaya fruit and that this was achieved by maintaining antioxidant activity in the fruit. These results may explain why CGA treatment maintains the quality of the apple and alleviate the rapid quality decline that is observed after vibration damage.

The specific cause of vibration damage is fatigue caused by the rupture of cells beneath the skin due to repeated forces exerted on the fruit (Wei et al., 2019). Apples are easily affected by mechanical damage from excessive vibration during transportation because they are both firm and crisp (Zhou et al., 2007). Damage caused by the internal transmission of forces may result in less obvious injury, however it still results in the spatial deformation of flesh cells which leads to the formation of an internal wound and so physiological stress (Komarnicki et al., 2016). Previous studies indicated that mechanical vibration induced changes in cell membrane composition and resulted in changes in respiration and electrolyte leakage of fruit which accelerate the ripening and senescence processes (Li and Thomas, 2014; Lu et al., 2019; Zhou et al., 2007). In this study, the vibration injury did not cause permanent mechanical damage, but caused the deformation of the cells (Fig. S1). The present study suggests that CGA treatment promoted the healing effects of tissue cells, as suggested by lower cell membrane permeability and MDA content in CGA treated fruit. Also, the increased respiration rate and ethylene production as well as the production of rate-limiting enzyme in the ethylene biosynthesis pathway, which was induced by vibration, were suppressed by CGA treatment. Our results are consistent with those of Xi et al. (2016) who found that CGA retarded the senescence of apples by reducing ethylene production and respiration rate. A study by Lu et al. (2019) indicated that vibration promoted gene expression of *MdACS1* and *MdACO1*. These results suggest that CGA treatment alleviated the adverse physiological responses induced by vibration and inhibited the rapid senescence of fruit that had undergone vibration.

Ensuring sufficient energy supply could attenuate postharvest abiotic stresses and delay senescence in fruit. Mitochondria are at the center of cellular energy metabolism (Ge et al., 2019). In this study, we found that CGA treatment maintained higher levels of ATP and energy charge and fruit quality after vibration than in control fruit. H^+-ATPase and $Ca^{2+}-$ATPase are critical enzymes in mitochondrial metabolism and play an important role in energy synthesis and supply. ATPase in the mitochondria plasma membrane converts ATP into ADP and releases phosphate ions and energy (Azevedo et al., 2008). SDH is an important enzyme in the inner mitochondrial membrane that catalyzes succinic acid and synthesizes ATP in the tricarboxylic acid cycle. CCO is critical in synthesizing ATP by oxidative phosphorylation; as the last and key enzyme in the mitochondrial electron transport chain, it provides the most energy for physiological metabolism in living tissue (Millar et al., 1995). The inactivation of energy metabolism enzymes could lead to mitochondrial dysfunction, insufficient energy supply and even cell death. Therefore, energy deficiency is closely related to cell integrity and the tolerance of fruit to abiotic stresses. A previous study (Li et al., 2018) reported that methyl jasmonate contributed to enhancing wounded pitaya fruit during storage. This process was associated with enhanced activity of H^+-ATPase, CCO and SDH. Trisodium phosphate and melatonin treatment have also been reported to delay the decrease of energy metabolism-related enzyme activities and maintain the fruit quality and enhanced disease resistance in apple and strawberry fruit (Aghdam and Fard, 2017; Ge et al., 2019). In this study, we observed that the activity of enzymes involved in energy metabolism were significantly higher in CGA-treated fruit that had undergone vibration than in those that had been subjected to vibration alone (Fig. 5). This indicates that energy metabolism enzymes play positive roles in energy production and the decrease in the energy level may be involved in the reduction in quality observed in the apple fruit. Therefore, the maintenance of enzyme activities of the mitochondria provides sufficient energy, which is crucial to maintain normal metabolisms after vibration stress.

Reports on the extraction and synthesis of CGA (Weber et al., 2014; Santos da Silveira et al., 2019) reveals the scope of its commercialization encouraging us to study the wide range of application in the future. Analysis will be required to investigate whether treatment of apples with CGA, if allowed, reduces economic losses in a cost effective manner.

5. Conclusion

CGA treatment reduces loss of apple quality after vibration injury, slowing the softening and loss of SSC and TA, fruit weight loss, electrolyte leakage, and the accumulation of MDA. CGA also decreases the injury-induced respiration rate and ethylene production and activities of enzymes involved in ethylene biosynthesis. CGA may in part regulate energy status and the activity of enzymes associated with energy metabolism, and thereby maintain membrane integrity of the fruit.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This research was supported by National key R & D projects (No. 2018YFD0401302) of The China Ministry of Science and Technology.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2019.110997.

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