

## Retraction:

Q. Zhang, W. Dai, H. Yang, W. Jia, X. Ning, and J. Li. 2019. Calcium chloride and 1-methylcyclopropene treatments delay postharvest and reduce decay of 'New Queen' melon. HortScience 54(7):1223–1229. 2019.  
<https://doi.org/10.21273/HORTSCI13997-19>

The paper "Calcium Chloride and 1-Methylcyclopropene Treatments Delay Postharvest and Reduce Decay of 'New Queen' Melon" (Zhang, et al.) has been retracted.

The statement of retraction appears below.

### Statement of retraction

Retraction: Calcium Chloride and 1-methylcyclopropene Treatments Delay Postharvest and Reduce Decay of 'New Queen' Melon

Authors: Qiang Zhang, Wenting Dai, Hui Yang, Wenting Jia, Xuefei Ning, Jixin Li

<https://doi.org/10.21273/HORTSCI13997-19>

After further verification, we found that the experimental material was not the New Queen melon variety Xinmi No. 13, but the Xizhoumi No. 17. In addition, the latest results show that the inhibition of microorganisms maybe mainly due to the resistance of the fruit itself rather than the hardness of the fruit, fruit hardness is an external appearance or secondary cause only, not the intrinsic reason, and these conclusions are still not very certain, and these errors can not be addressed with a corrigendum. Moreover, due to the changes in authors who participated in the follow-up study, there will have some competing interests between the authors if the manuscript was published based on the original author list. Thus we decided to withdraw this manuscript with great pity.

Qiang Zhang 5 November 2019

Wenting Dai 5 November 2019

Hui Yang 5 November 2019

Wenting Jia 5 November 2019

Xuefei Ning 5 November 2019

Jixin Li 5 November 2019

# Calcium Chloride and 1-Methylcyclopropene Treatments Delay Postharvest and Reduce Decay of ‘New Queen’ Melon

Qiang Zhang

College of Life Science and Technology, Xinjiang University, China

Wenting Dai<sup>1</sup>, Hui Yang, and Wenting Jia

Institute of Agro-products Processing Science and Technology, Xinjiang Academy of Agricultural and Reclamation Science, China

Xuefei Ning

College of Life Science and Technology, Xinjiang University, China

Jixin Li<sup>2</sup>

Institute of Agro-products Processing Science and Technology, Xinjiang Academy of Agricultural and Reclamation Science, China

**Additional index words.** respiratory intensity, climacteric, pectin, fruit softening, ethanolic microorganism

**Abstract.** In this study, newly harvested ‘New Queen’ melons were treated with calcium chloride ( $\text{CaCl}_2$ ) and 1-methylcyclopropene (1-MCP) alone or in combination before storage. The results show that respiration rate, ethylene release, acidity, and gene expression of pectinases such as polygalacturonase (PG), pectin methylesterase (PME), and pectate lyase (PL) in ‘New Queen’ melons decreased dramatically when treated with 2%  $\text{CaCl}_2$  and/or 1  $\mu\text{L}\cdot\text{L}^{-1}$  1-MCP. In addition, climacteric behavior and flesh hardness reduction were inhibited. It was also discovered that soft rot microorganisms were more conducive to the growth and reproduction of decay-causing microorganisms, according to their growth curves in melons that were different in flesh hardness, suggesting inhibiting fruit softening can slow down the growth of microorganisms in fruit flesh and thus reduce fruit decay rate. The combined use of  $\text{CaCl}_2$  and 1-MCP was more effective in suppressing respiration rate, ethylene release, and protopectin hydrolysis, which could greatly delay the softening, reduce the decay rate, and extend the shelf life of ‘New Queen’ melons.

‘Xinmi No. 13’ is an elite, thick-rinded cultivar of ‘New Queen’ melon obtained after many generations of breeding and selection, and has now become one of the most popular Xinjiang melons because of its good fruit appearance, high fruit market value, and unique flavor. However, postharvest ripening and softening seriously reduce the quality and shorten the shelf life of ‘New Queen’ melons.

‘New Queen’ melon is a climacteric cultivar, characterized by a dramatic increase in respiration rate and ethylene release after harvest. The fruit matures and softens rapidly (Wang et al., 2018). During fruit softening, protopectin is hydrolyzed into pectin that is readily soluble in water,

so that the physical structures of pectin, cellulose, and hemicellulose are depolymerized and the fruit becomes loose (Ali et al., 2004; Wang, 2009). Delaying or inhibiting climacteric behavior and reducing the amount of ethylene released is an effective measure to delay the aging and softening of fruit.

1-Aminocyclopropanecarboxylic acid (ACC) oxidase (ACO) catalyzes the conversion of ACC to ethylene, which is the major pathway for the production of endogenous ethylene in fruit. Calcium can inhibit the activity of both ACC synthase and ACO, and the production of endogenous ethylene. Li et al. (2009b) reported that calcium treatment reduced the respiration rate and ethylene release of netted melon. As an essential plant nutrient, calcium is required for various structural roles in the cell wall and membranes. The study by Chardonnet et al. (2003) revealed that calcium treatment effectively inhibited the changes of cell wall components to maintain the fruit hardness of Golden Delicious apple. The study by Deng et al. (2016) showed that calcium treatment

reduced the activity and gene expression of cell wall-degrading enzymes to maintain the hardness of grapefruit.

Ethylene can accelerate the ripening process of fruit, and then ripe fruit can release a large amount of ethylene during after-ripening process (Meng et al., 2018). 1-MCP is an inhibitor of ethylene action in plants by binding competitively to the ethylene receptor and blocking ethylene signaling, which inhibits after-ripening of fruit (Tadiello et al., 2016). The study by Guo et al. (2016) showed that 1-MCP can decrease the respiratory rate and ethylene release of Yate kiwifruit. 1-MCP can also suppress the activity of cell wall-lysing enzymes, thereby delaying the softening of apples (Wei et al., 2010). However, the effects of calcium and 1-MCP on physiologic metabolism of melons have been reported rarely.

Therefore, in this study, we investigated the effects of  $\text{CaCl}_2$  and 1-MCP on respiration rate, ethylene release, flesh hardness, decay rate, pectin hydrolase activity, and gene expression during storage using a ‘New Queen’ melon cultivar Xinmi No. 13 as the experimental material. The results showed that  $\text{CaCl}_2$  and/or 1-MCP can delay after-ripening and reduce the decay rate of ‘New Queen’ melons, and better effect can be achieved by using them in combination.

## Materials and Methods

**Plant materials.** The seeds of Xinmi No. 13 were provided by the Bioengineering Research Center of Xinjiang University, sown from May to June, and transplanted from July to Oct. 2017. The melons used in our study were grown in greenhouses and under drip irrigation at Shihezi Huayu Seed Breeding Base. The ‘New Queen’ melon takes 45 d from pollination to reach full maturity. The melons used in this study were harvested 43 d after pollination.

**Decay-causing pathogens.** Two pathogens were isolated from the rotten ‘New Queen’ Xinmi melon—*Erwinia carotovora* ssp. *carotovora* and *Pseudomonas syringae* pv. *lachrymans*—and were inoculated onto potato dextrose agar medium for multiplication.

**Screening of  $\text{CaCl}_2$  and 1-MCP concentrations.** Fruit with the same weight and shape, and free of defects were selected, soaked in 0.5%, 1%, 2%, or 4%  $\text{CaCl}_2$  solution (YATAI, Wuxi, China) for 5 min within 12 h after harvest, air-dried at room temperature (Lv et al., 2009), and then fumigated with 0.25, 0.5, 1, or 2  $\mu\text{L}\cdot\text{L}^{-1}$  1-MCP (LV NUO, Qingdao, China) at 20 °C for 24 h (Wang and Li, 2016). The optimal concentrations of  $\text{CaCl}_2$  and 1-MCP were determined according to the respiration rate and ethylene release of the fruit in different treatments during storage.

**Determination of percentage of rotten fruit.** A total of 400 fruit with same weight and shape, synchronous in maturity and free of defects, were selected as samples. They

Received for publication 26 Feb. 2019. Accepted for publication 12 Mar. 2019.

<sup>1</sup>Current address: Institute of Agro-products Processing Science and Technology, Hainan Academy of Agricultural Sciences, No. 14 Xindan Road, Haikou, Hainan 571110, China.

<sup>2</sup>Corresponding author. E-mail: jixinli1961@126.com.

were divided randomly and equally into four groups, with 100 fruit in each group. One of the groups, untreated with  $\text{CaCl}_2$  or 1-MCP, was used as the control. The other three groups were treated with  $\text{CaCl}_2$  and 1-MCP alone or in combination, within 12 h after harvesting, at their optimal concentrations. The fruit were then bagged with nylon net bags and packaged in carton boxes, with 10 fruit in each box, and stored in a cellar at a temperature of  $20 \pm 1^\circ\text{C}$  and at a relative humidity of  $50 \pm 5\%$ .

Rotten fruit were counted once every 10 d, and the percentage of rotten fruits was calculated using the following formula:

$$\text{Percentage of rotten fruit} = \left( \frac{\text{No. of rotten fruit}}{\text{Total no. of tested fruit}} \right) \times 100.$$

**Measurement of fruit respiration rate, ethylene release, and hardness.** To measure respiration rate, the fruit were placed in a sample container of 3051H respirometer (LV BO, China). The gas flow rate was  $0.4 \text{ L}\cdot\text{min}^{-1}$ . Nine fruit were tested in each measurement, and each measurement was repeated three times. The resulting respiration rate was expressed as milligrams carbon dioxide ( $\text{CO}_2$ ) per kilogram per hour (Wei and Ma, 2009).

The amount of ethylene released was measured using a Trace GC 1300 gas chromatograph (Thermo) according to the method of Li et al. (2015). Nine fruit were tested in each measurement, and each measurement was repeated three times. Ethylene release (measured in microliters per kilogram per hour) was calculated as follows:

$$\text{Ethylene release} = \frac{C \times V}{m \times t \times 1000},$$

where  $C$  is the amount of ethylene released by the sample (measured in microliters per liter),  $V$  is difference between the volume of dryer space and the volume of the samples (measured in milliliters),  $m$  is the weight of the sample (measured in kilograms), and  $t$  is time (measured in hours).

Fruit hardness (measured in kilograms per square centimeter) was tested using an AGY-1 texture analyzer (Ametek, China). Nine fruit were analyzed in each measurement, and every measurement was repeated three times (Camps et al., 2005; Su et al., 2015).

**Detection of protopectin and soluble pectin contents.** The contents of protopectin and pectin in every gram of sample (measured in milligrams per gram fresh weight) were measured using the carbazole-sulfuric acid method according to Qi et al. (2015).

**Pectinase activity analysis.** PG activity was measured as described by Figueroa et al. (2010). One unit of PG activity was defined as the amount of enzyme releasing  $1 \mu\text{mol}$

galacturonic acid/min by decomposing polygalacturonic acid in 1 gram of fresh sample at  $37^\circ\text{C}$  (measured in micrograms per gram per minute). PME activity was quantified by sodium hydroxide titration (Lynguyen et al., 2002; Zhang et al., 2017). One unit of PME activity was defined as the amount of enzyme releasing  $1 \text{ mmol}$  methanolate ( $\text{CH}_3\text{O}^-$ ) from  $1 \text{ g}$  fresh sample per minute. One unit of PL activity was defined as the amount of enzyme needed to cause an increase in optical density ( $\text{OD}$ )<sub>235</sub> per gram of fresh sample per minute (Payasi et al., 2004).

**Quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis.** Total RNA was isolated from melon flesh using the EasyPure Plant RNA Kit (TRANS, Beijing, China); RNA quality was examined on agarose gels and quantified using a NanoDrop (Thermo) photometer. RNA isolation for gene expression was obtained from three biologic replicates. All RNA samples were treated with RNase-free DNase I (Ambion) to remove contaminant DNA traces. To amplify the selected genes, complementary DNA (cDNA) was synthesized by PCR using the primers listed in Table 1 and were synthesized with EasyScript First-Strand cDNA Synthesis SuperMix (TRANS). Amplification was carried out through initial denaturation at  $94^\circ\text{C}$  for 2 min, followed by 38 cycles of denaturation at  $94^\circ\text{C}$  for 30 s, annealing at  $50^\circ\text{C}$  for 30 s, and elongation at  $72^\circ\text{C}$  for 1 min. PCR products from each amplification reaction were separated on 2.5% (w/v) agarose gels. Real-time identification of RT-PCR reactions were performed using the Mx3000 Real-time PCR Detection System (Bio-Rad) with the TransStart Top Green qPCR SuperMix (TRANS) according to manufacturer instructions (Han et al., 2015; Pérez-Díaz et al., 2015).

**Bacterial culture and counting.** The flesh samples of melons with four different hardness values were cut into cubes of  $1.5 \text{ cm}^3$ . *Erwinia carotovora* ssp. *carotovora* and *Pseudomonas syringae* pv. *lachrymans* were inoculated separately into the flesh cubes and were cultured in an incubator at  $37^\circ\text{C}$  with a relative humidity of 60%. The number of bacteria was counted once every 24 h. More specifically, the fruit flesh was weighed, smashed, and filtered through a 60-mesh filter, spread in a square of  $1 \times 1 \text{ cm}$  on a slide, heated in boiling water for 10 min, air-dried, and then stained with 0.1% methylene blue for 2 min. Subsequently, the number of bacteria in 20 random fields was counted using an oil immersion microscope, and the average was calculated. Finally, the growth curves of *Erwinia carotovora* ssp. *carotovora* and *Pseudomonas syringae* pv. *lachrymans* in melons of different hardness values were plotted based on these data (Ji et al., 2017).

**Statistical analysis.** Excel statistics and plotting were used to analyze the experimental data. The data were analyzed using SPSS 19.0 software (SPSS Inc., Chicago, IL). A

significance level of  $P < 0.05$  was used in all cases.

## Results

**Effects of  $\text{CaCl}_2$  and/or 1-MCP on fruit respiration rate and ethylene release.** As shown in Fig. 1A and B, the respiration rate and ethylene release of the fruit treated with 0.5%, 1%, 2%, and 4%  $\text{CaCl}_2$ , were significantly less than those of the control ( $P < 0.05$ ). A sharp climacteric peak occurred in both the respiratory rate and ethylene release of the control group on day 8, whereas the climacteric peaks in all  $\text{CaCl}_2$ -treated groups were less and appeared later—on day 10 in the 0.5% and 4%  $\text{CaCl}_2$ -treated groups, and on day 12 in the 1% and 2%  $\text{CaCl}_2$ -treated groups. Of all  $\text{CaCl}_2$  treatments, 2%  $\text{CaCl}_2$  had the lowest respiratory rate and ethylene release, and the smallest climacteric peak value. In addition, the inhibitory effect of  $\text{CaCl}_2$  on respiratory rate and ethylene release increased gradually with a  $\text{CaCl}_2$  concentration increasing from 0.5% to 2%, and then decreased when  $\text{CaCl}_2$  concentration was increased to 4%. The optimal  $\text{CaCl}_2$  concentration for postharvest storage time of melon fruit was determined to be 2%.

As shown in Fig. 1C and D, treatments with 1-MCP at four concentrations all reduced the respiratory rate and ethylene release of melon, and the inhibitory effect of 1-MCP increased in a concentration-dependent manner. The climacteric peaks of respiratory rate and ethylene release appeared on day 12 in the  $0.25 \mu\text{L}\cdot\text{L}^{-1}$  1-MCP-treated group and on day 14 in the  $0.5 \mu\text{L}\cdot\text{L}^{-1}$  1-MCP-treated group, respectively. The group treated with  $0.5 \mu\text{L}\cdot\text{L}^{-1}$  1-MCP had a lower respiratory rate and ethylene release, and a smaller climacteric peak value than the group treated with  $0.25 \mu\text{L}\cdot\text{L}^{-1}$  1-MCP ( $P < 0.05$ ). There was no significant difference in respiratory rate and ethylene release between the 1- and  $2 \mu\text{L}\cdot\text{L}^{-1}$  1-MCP-treated groups ( $P > 0.05$ ). And no climacteric peak was observed in these two groups. So,  $1 \mu\text{L}\cdot\text{L}^{-1}$  was considered the optimal concentration of 1-MCP for treatment of Xinmi fruit. According to these results, 2%  $\text{CaCl}_2$  and  $1 \mu\text{L}\cdot\text{L}^{-1}$  1-MCP were used in subsequent experiments.

The effects of  $\text{CaCl}_2$  and/or 1-MCP on the respiratory rate and ethylene release of Xinmi melon are shown in Fig. 1E and F. The respiratory rate and ethylene release of the  $\text{CaCl}_2$ - and 1-MCP-treated groups were less than those of the control group ( $P < 0.05$ ). There was a climacteric peak in both the respiration rate and ethylene release of the control. In detail, both two indices changed slowly during the first 6 d of storage, then the respiration rate of the control group increased dramatically from  $74.6 \text{ CO}_2 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  on day 6 to  $233 \text{ CO}_2 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$  on day 8, whereas ethylene release increased from  $10.9$  to  $25.5 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ . A significantly smaller climacteric peak in respiration rate and ethylene release in the  $\text{CaCl}_2$ -treated group appeared on day 12 ( $P < 0.05$ ). The

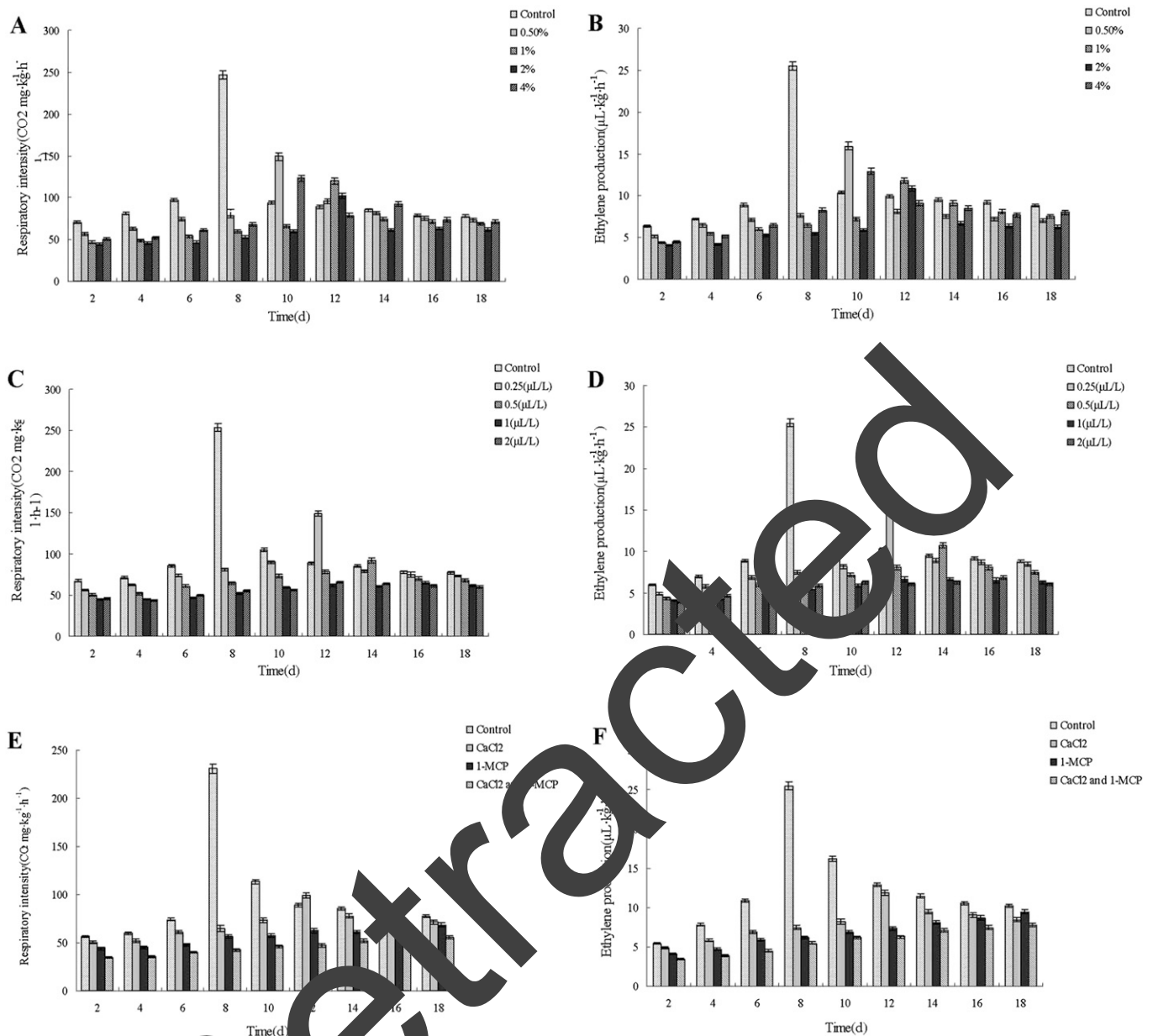


Fig. 1. Effects of different concentrations of  $\text{CaCl}_2$  on (A) respiratory rate and (B) ethylene release during postharvest storage of Xinmi fruits. Effects of different concentrations of 1-MCP on (C) respiratory rate and (D) ethylene release during postharvest storage of Xinmi fruits. Effects of  $\text{CaCl}_2$  and/or 1-MCP on (E) respiratory rate and (F) ethylene release during the postharvest storage of Xinmi melon.

Table 1. Primers for quantitative reverse transcription–polymerase chain reaction analysis.

Gene name	Accession no.	Primer sequence
CmPG	MELO3C000985	F5'-TTGACAGCGTTGGTTACCTG-3' R5'-CCACATAACTCAGTCCCCAA-3'
CmPME	MELO3C024917	F5'-GATTGCGTGTGGATGTTTG-3' R5'-CCGGAGCAATTTTATCCAC-3'
CmPL	MELO3C002431	F5'-CCAGATTTCCCGAGGCTTACA-3' R5'-TGGGGCTAAAGTGGAATTG-3'
CmActin	MELO3C011913	F5'-CCAAAGGCTGCAAGAATAGC-3' R5'-TTTGACCTTGGGTGGGTAG-3'

1-MCP-treated group had a lower respiration rate and ethylene release than the  $\text{CaCl}_2$ -treated groups, showing no climacteric changes. The respiration rate and ethylene release of the group treated with  $\text{CaCl}_2$  and 1-MCP in combination were less than those of all other groups, changed slightly, and showed no climacteric changes.

**Effects of  $\text{CaCl}_2$  and/or 1-MCP on fruit hardness.** As shown in Fig. 2, fruit hardness in all groups decreased continuously over time. Fruit hardness of the control group decreased faster than that of the  $\text{CaCl}_2$ - and/or 1-MCP-treated groups ( $P < 0.05$ ), from 13.7  $\text{kg}\cdot\text{cm}^{-2}$  on day 2 to 6.6  $\text{kg}\cdot\text{cm}^{-2}$  on day 18, and

the decrease was steeper from day 8 to day 10. Among all  $\text{CaCl}_2$ - and/or 1-MCP-treated groups, fruit hardness of the  $\text{CaCl}_2$ -treated group decreased the fastest, followed by the 1-MCP-treated group. The group treated with  $\text{CaCl}_2$  and 1-MCP in combination declined most slowly ( $P < 0.05$ ). Fruit hardness of the  $\text{CaCl}_2$ -treated group decreased from 14.1  $\text{kg}\cdot\text{cm}^{-2}$  on day 2 to 8.6  $\text{kg}\cdot\text{cm}^{-2}$  on day 18, whereas that of the 1-MCP-treated group declined from 14.6  $\text{kg}\cdot\text{cm}^{-2}$  to 10.6  $\text{kg}\cdot\text{cm}^{-2}$ , and that of the group treated with  $\text{CaCl}_2$  and 1-MCP together decreased from 14.3  $\text{kg}\cdot\text{cm}^{-2}$  to 10.7  $\text{kg}\cdot\text{cm}^{-2}$ .

**Effects of  $\text{CaCl}_2$  and 1-MCP on protopectin and pectin contents.** As shown in Fig. 3, during storage, the protopectin content in all groups decreased continuously,

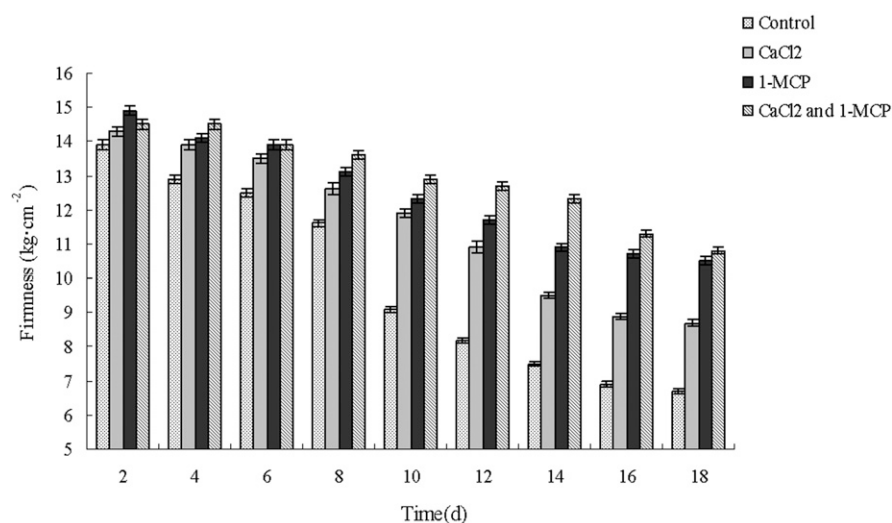


Fig. 2. Effects of  $\text{CaCl}_2$  and 1-methylcyclopropene (1-MCP) alone or in combination on fruit hardness during postharvest storage of Xinmi melon.

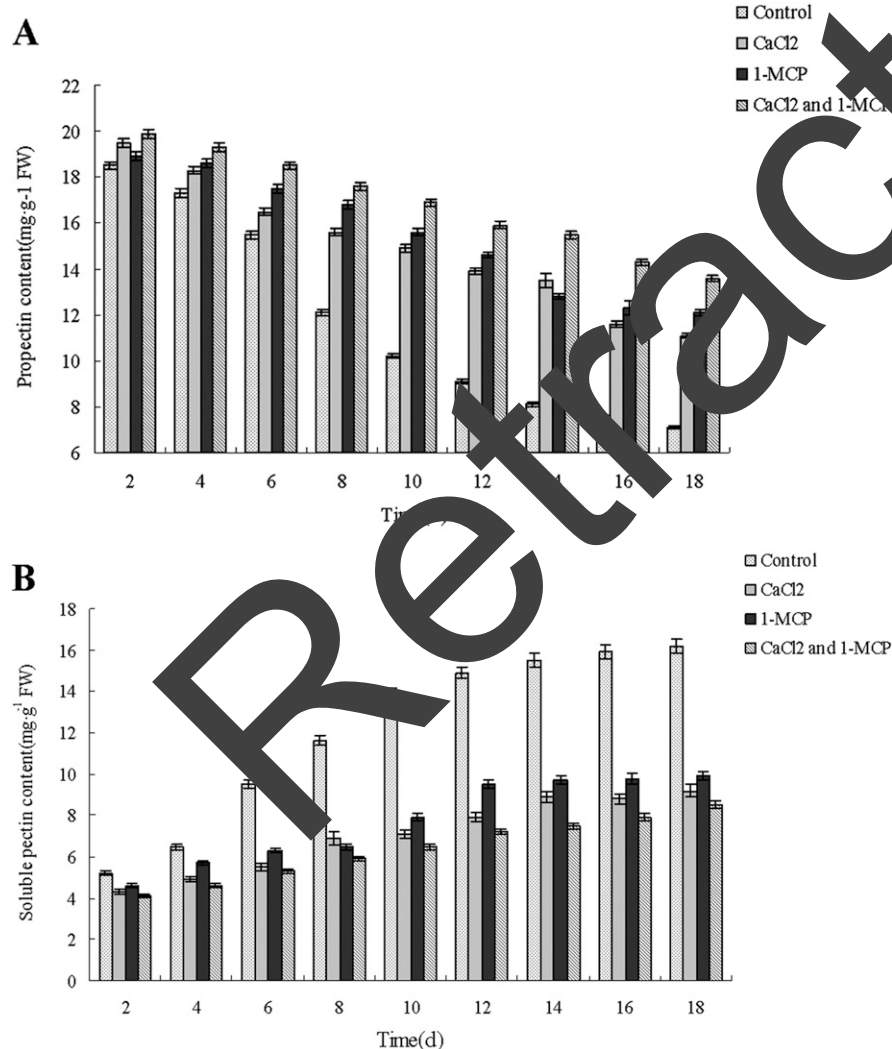


Fig. 3. Effects of  $\text{CaCl}_2$  and/or 1-methylcyclopropene (1-MCP) on (A) protopectin and (B) soluble pectin contents during the storage of Xinmi melon.

whereas soluble pectin content increased continuously. Protopectin content of the control group decreased from  $18.7 \text{ mg}\cdot\text{g}^{-1}$  on day 2 to

$7.9 \text{ mg}\cdot\text{g}^{-1}$  on day 18, and the decrease from day 6 to day 10 was more dramatic. The soluble pectin content of the group increased from  $5.3$

$\text{mg}\cdot\text{g}^{-1}$  on day 2 to  $16.5 \text{ mg}\cdot\text{g}^{-1}$  on day 18, and the increase from day 6 to day 10 was more dramatic. The decrease in protopectin content and the increase in pectin content of all the three  $\text{CaCl}_2$ - and/or 1-MCP-treated groups were slower than those of the control ( $P < 0.05$ ). Both protopectin content and soluble pectin content changed slightly and showed a small difference between the  $\text{CaCl}_2$ -treated group and the 1-MCP-treated group. Among all groups, the group treated with  $\text{CaCl}_2$  and 1-MCP in combination showed the smallest changes in protopectin content and soluble pectin content, with protopectin decreasing from  $19.5 \text{ mg}\cdot\text{g}^{-1}$  on day 2 to  $13.8 \text{ mg}\cdot\text{g}^{-1}$  on day 18, and with soluble pectin content increasing from  $4.3$  to  $8.7 \text{ mg}\cdot\text{g}^{-1}$  during this period.

*Effects of  $\text{CaCl}_2$  and/or 1-MCP on the activity and gene expression of pectinases.* Enzymes are essentially proteins functioning as catalysts, and encoded by genes. So, the expression level of these genes determines the quantity and activity of enzymes, and can be up or downregulated by a series of factors.

As shown in Fig. 4A and B, PG activity and gene expression of the control group were both significantly greater than those of the  $\text{CaCl}_2$ - and/or 1-MCP-treated groups ( $P < 0.05$ ). A sharp climacteric peak occurred in PG activity and gene expression in the control group. A smaller climacteric peak in the two indices was observed in the  $\text{CaCl}_2$ -treated group on day 12, and not in the groups treated with 1-MCP alone or in combination with  $\text{CaCl}_2$ . We also found that PG activity and gene expression changed synchronously in every group.

The changes in PME activity and gene expression are shown in Fig. 4C and D. There was no significant difference in PME activity between the control group and the  $\text{CaCl}_2$ - and/or 1-MCP-treated groups ( $P > 0.05$ ) during the first several days of storage, and then PME activity of the control group increased dramatically, peaked on day 6, and decreased sharply to a level similar to that of the  $\text{CaCl}_2$ - and/or 1-MCP-treated groups from day 12 to day 14, and was less than that of the  $\text{CaCl}_2$ - and/or 1-MCP-treated groups from day 16 to day 18. PME activity in all  $\text{CaCl}_2$ - and/or 1-MCP-treated groups varied slowly compared with the control, and decreased gradually over time during storage. Also, there was no statistically significant difference in PME activity among the three groups treated by  $\text{CaCl}_2$  and 1-MCP alone or in combination. Among the three groups, the decrease in PME activity was the greatest in the  $\text{CaCl}_2$ -treated group, followed by that of 1-MCP-treated group. PME activity in the group treated with  $\text{CaCl}_2$  and 1-MCP in combination declined most slowly.

As shown in Fig. 4E and F, PL activity and gene expression of the control group were both greater than those of the  $\text{CaCl}_2$ - and/or 1-MCP-treated groups during the first several days, then increased dramatically and peaked on day

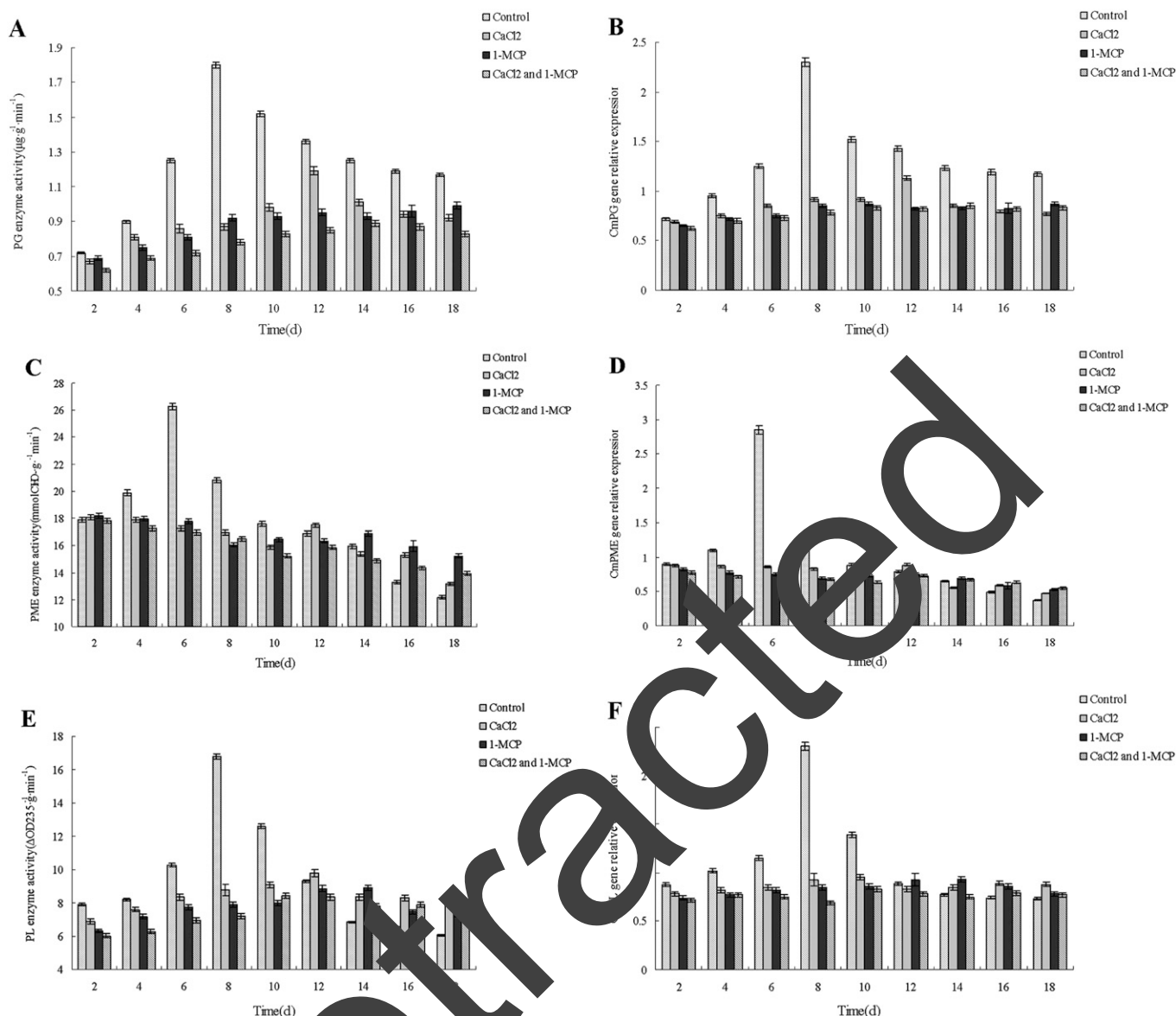


Fig. 4. Effects of  $\text{CaCl}_2$  and 1-methylcyclopropene (1-MCP) on (A) polygalacturonase (PG) activity and (B) gene expression, (C) pectin methylesterase (PME) activity and (D) gene expression, and (E) pectate lyase (PL) activity and (F) gene expression during the storage of Xinmi melon.

8, and then increased slightly. They were less than those of the  $\text{CaCl}_2$ - and/or 1-MCP-treated groups on day 14. Compared with the control group, PL activity and gene expression of the  $\text{CaCl}_2$ - and/or 1-MCP-treated groups changed slightly. The two indices of the group treated by  $\text{CaCl}_2$  alone were greater than those of the groups treated by 1-MCP alone or in combination with  $\text{CaCl}_2$  during the middle stage of storage, and the difference became insignificant during the late stage of storage. There was no significant difference in PL activity and gene expression between the group treated by 1-MCP alone and the group treated by 1-MCP and  $\text{CaCl}_2$  together ( $P > 0.05$ ).

**Growth of pathogenic microorganisms in melon flesh of different hardness.** The melons of four different hardness values—7.6, 9.9, 12.8, and 14.3  $\text{kg}\cdot\text{cm}^{-2}$ —were used as

samples. Figure 5 shows the growth curves of *Erwinia carotovora* ssp. *carotovora* and *Pseudomonas syringae* pv. *Lachrymans* in these samples. These growth curves were typically S shaped. In addition, the growth rate of the two pathogens decreased with an increase in flesh hardness ( $P < 0.05$ ), indicating that softer flesh is more conducive to the growth and reproduction of the two pathogens.

**Effects of  $\text{CaCl}_2$  and 1-MCP on decay rate of melon fruit during storage.** Fruit decay is caused mainly by the infection of decay-causing pathogens. The growth and reproduction of microorganisms require suitable environmental conditions. Progressing of the after-ripening process and variation in fruit hardness also change the microbial growth environment during fruit storage. As shown in Fig. 6, the percentage of rotten fruit in the control group was

significantly greater than that of the  $\text{CaCl}_2$ - and/or 1-MCP-treated groups. In addition, the decay rate of the control group was also faster than that of the  $\text{CaCl}_2$ - and/or 1-MCP-treated groups. All the fruit rotted within 90 d in the control group, within 110 d in the  $\text{CaCl}_2$ -treated group, within 130 d in the 1-MCP-treated group, and within 160 d in the  $\text{CaCl}_2 + 1\text{-MCP}$ -treated group. The difference in decay rate was statistically significant among these groups ( $P < 0.05$ ).

## Discussion

Ethylene is a hormone that can be used to hasten fruit ripening and softening. It can bind to its receptor and it accelerates fruit respiratory metabolism, then upregulates the expression of pectinase-encoding genes; promotes the production of pectinases, which hydrolyze protopectin; and changes cell wall

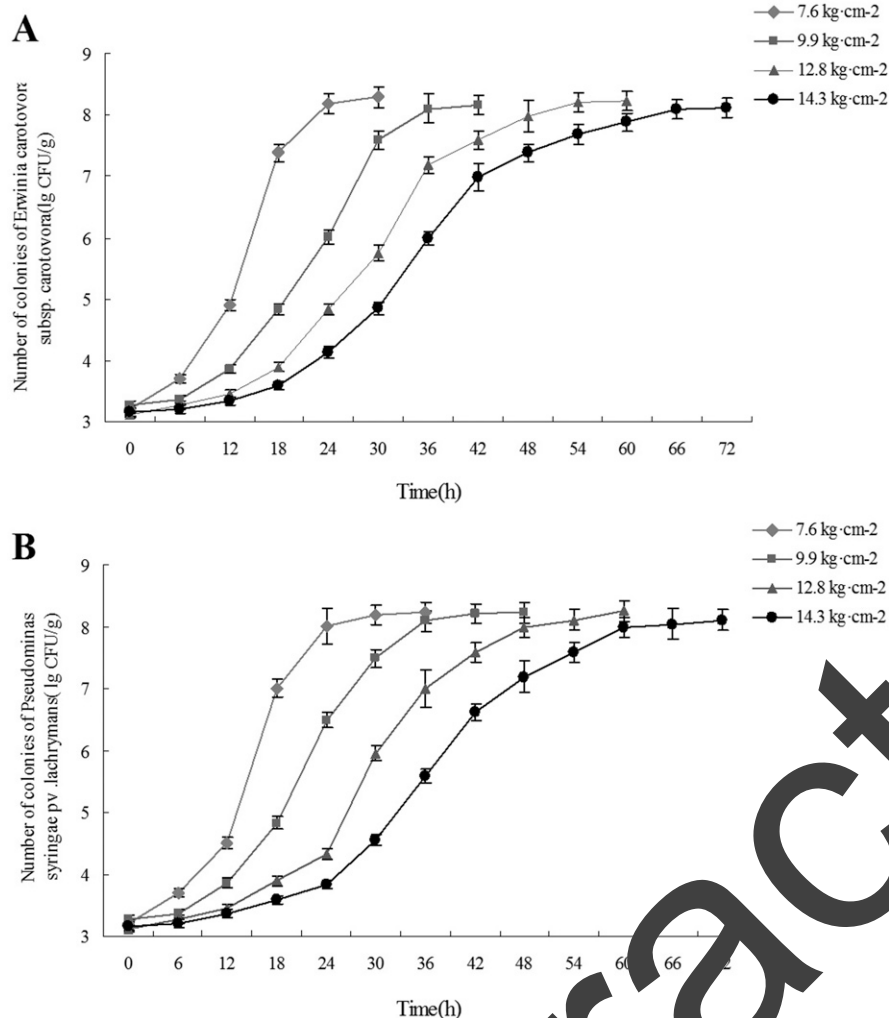


Fig. 5. Growth curves of (A) *Erwinia carotovora* subsp. *carotovora* and (B) *Pseudomonas syringae* pv. *Lachrymans* in flesh of different hardness. CFU = colony-forming units

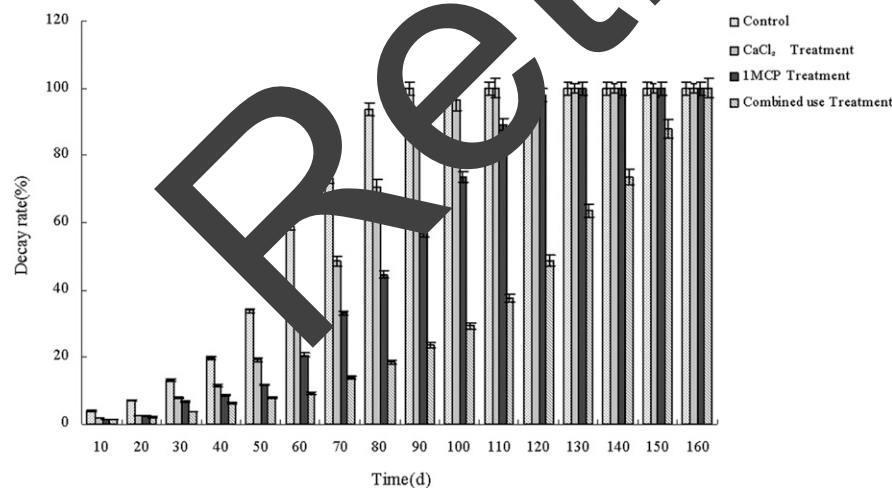


Fig. 6. Effects of CaCl<sub>2</sub> and 1-methylcyclopropene (1-MCP) on decay rate of 'New Queen' melon fruit during storage.

structures; the fruit becomes soft as a result (Li et al., 2018). In our experiment, there was a positive synergy among respiratory rate, pectinase activity, gene expression, and ethylene release in every group.

As an important component of plant cell wall structure, calcium inhibits the activity of cell wall-degrading enzymes. It also has an antagonistic effect on ethylene-induced ripening of fruit (Ortiz et al., 2011). Calcium can

increase the content of soluble solids and the hardness of apple (Fan, 2016). CaCl<sub>2</sub> is able to delay the decline in titratable acid content and fruit firmness, and reduce the decay rate of cherry significantly (Wang et al., 2016). Our data show that the CaCl<sub>2</sub> treatment reduced the respiration rate, ethylene release, and climacteric peak value significantly, as well as delayed the climacteric behavior of melon fruit, which was consistent with the findings of Li et al. (2009a) in calcium nitrate-treated muskmelon. 1-MCP blocks ethylene binding to its receptor. We found that the respiration rate and ethylene release of 1-MCP-treated fruits were both significantly less than those of the untreated fruit, and similar results have been reported by Ma et al. (2012). The combined use of CaCl<sub>2</sub> and 1-MCP not only inhibits ethylene action, but also it blocks the signal transduction of residual ethylene. In our experiment, we found that better effects in inhibiting respiration rate and ethylene release were achieved when 2% CaCl<sub>2</sub> and 1 μL·L<sup>-1</sup> 1-MCP were used in combination.

Hydrolysis of protopectin into water-soluble pectin is an important cause of fruit softening (Sahni et al., 2014). The study of Liu et al. (2017) showed that the content of protopectin decreased gradually during ripening of thick-rind melon, whereas pectin content kept increasing, and the content of cellulose changed little. Based on these results, we investigated the effects of CaCl<sub>2</sub> and 1-MCP on postharvest ripening and decay of Xinmi melon by measuring the changes in pectin content, pectinase activity, and gene expression. Our results show that the content of protopectin in both treated and untreated fruit decreased gradually during storage, whereas the content of water-soluble pectin increased. Moreover, fruit hardness of the control group declined sharply from day 6 to day 8 of storage, whereas protopectin was hydrolyzed into pectin at a greater rate, which was consistent to the findings of Wei et al. (2010).

All PG, PME, and PL activity, and gene expression of the control group underwent climacteric changes during storage. In detail, the climacteric peaks of PG and PL activity and gene expression occurred on day 8 of storage, when the peaks of respiration rate and ethylene release appeared. The climacteric peaks of PME activity and gene expression occurred earlier on day 6. Brummell et al. (2004) believed that the hydrolysis of protopectin is initiated by PME at the early stage of storage, and provides a substrate for other pectinases such as PG, which may explain why the peaks of PME activity and gene expression occur earlier. PG activity and gene expression of the control group were always greater than those of the CaCl<sub>2</sub>- and/or 1-MCP-treated groups, but PME and PL activity and gene expression of the control group were less than those of the CaCl<sub>2</sub>- and/or 1-MCP-treated groups on day 14 and day 16, respectively. Climacteric for PG activity and gene expression in the CaCl<sub>2</sub>-treated group occurred on day 12, but it was not

observed in the groups treated with 1-MCP alone or in combination with  $\text{CaCl}_2$ . According to the pectin content and flesh hardness data, we conclude that climacteric changes in pectinase activity and gene expression play a critical role in the hydrolysis of protopectin and fruit softening, and slow changes in them have little effect.

As mentioned, melon decay is caused mainly by the infection of decay-causing microorganisms. Fruit softening is beneficial to the reproduction and spread of these microorganisms, thus accelerating the decay rate of fruit. Any delaying in fruit softening can decrease the reproduction and spread of microorganisms, thus reducing the decay rate and prolonging the shelf life of fruit.

Pectinase activity and gene expression in the control group were both greater than those of the  $\text{CaCl}_2$ - and/or 1-MCP-treated groups at early storage stage, indicating that the postharvest physiologic metabolism of fruit had been inhibited when they were treated with  $\text{CaCl}_2$  and/or 1-MCP. When  $\text{CaCl}_2$  and 1-MCP are used in combination, the mechanisms inhibiting fruit after-ripening are able to integrate and complement each other, thus achieving a better effect in delaying the softening and reducing the decay rate of fruit.

#### Literature Cited

Ali, Z. M., L.-H. Chin, and H. Lazan. 2004. Comparative study on wall degrading enzymes pectin modifications and softening during ripening of selected tropical fruits. *Plant Sci.* 167(2): 317–327.

Brummell, D.A., V. Dal Cin, C.H. Crisosto, and J.M. Labavitch. 2004. Cell wall metabolism during maturation, ripening and senescence of peach fruit. *J. Expt. Bot.* 55:2029–2039.

Camps, C., P. Guillermin, J.C. Mauget, and D. Bertrand. 2005. Data analysis of penetrometric force/displacement curves for the characterization of whole apple fruits. *J. Texture Anal.* 36(4):387–401.

Chardonnet, C.O., C.S. Charron, C.E. Sims, and W.S. Conway. 2003. Chemical changes in the cortical tissue and cell walls of calcium-infiltrated ‘Golden Delicious’ apples during storage. *Postharvest Biol. Technol.* 28(1):109–111.

Deng, J., Z. Shi, and Wang, H. 2016. Effects of calcium treatments on cell wall material metabolism and related enzyme activities and gene expression in grapefruit (*Citrus paradisi* Macf.). *J. Plant Nutr. Fert.* 22(2):450–458.

Fan, H.L. 2016. Effect of post harvest calcium treatment on the occurrence of apple bitter pit disease and fruit quality during storage. *China Fruit Veg.* 36(9):1–4.

Figuerola, C.R., H.G. Rosli, P.M. Civello, G.A. Martínez, R. Herrera, and M.A. Moya-León.

2010. Changes in cell wall polysaccharides and cell wall degrading enzymes during ripening of *Fragaria chiloensis* and *Fragaria ananassa* fruits. *Scientia Hort.* 124(4):454–462.

Guo, Z., Z. Feng, and L. Wen. 2016. Effects of ethephon treatments on storage quality and softening of ‘Rubbery’ papaya fruit. *J. Trop. Biol.* 7(2):215–219.

Han, Y., Q. Zhu, Z. Zhang, K. Meng, Y. Hou, Q. Ban, J. Suo, and J. Rao. 2015. Analysis of xyloglucan endotransglycosylase/hydrolase (XTH) genes and diverse roles of isoenzymes during persimmon fruit development and post-harvest softening. *PLoS One* 10(4):e0123668.

Ji, H., W. Wei-wei, F. Yu, Z. Kai-li, Y. Jun, and Li Bei. 2017. Study on microorganism growth model during storage of fresh-cut Hami melon. *Food Res. Dev.* 38(11):161.

Li, T., L. Shuang-shuang, Z. Wu, X. Pang, Y. Yue, and Y. Chen. 2009a. Effect of calcium nitrate on muskmelon (*Cucumis melon* L.) softness and relative physiological parameters. *J. Shenyang Agr. Univ.* 40(4):387–391.

Li, T., L. Shuang-shuang, C. Xu, and Z. Wu. 2009b. Effects of calcium on ethylene-promoted muskmelon soften. *Acta Hort. Sinica* 36(6):317–327.

Li, W., J. Chen, Y. Duan, H. Hu, Z. Pang, and J. Xie. 2018. Effect of the regulation of different temperature and ethylene treatment on polysaccharide metabolism during Africawide winter fruits ripening and softening. *J. Trop. Biol.* 39(3):480–488.

Li, X., Y. Bi, J. Wang Jie, B. Dong, H. Li, D. Gong, Y. Zhao, Y. Tang, X. Yu, and Q. Shang. 2015. BTH treatment caused physiological, biochemical and proteomic changes of muskmelon (*Cucumis melo* L.) fruit during ripening. *J. Proteomics* 12(1):79–100.

Liu, Y.N., W. Y. B.I. Yang, J. Shi Sheng, J. Hong, Z. Yan, and J. Bin. 2017. Effect of preharvest acetylsalicylic acid treatments on ripening and softening of harvested muskmelon fruit. *Scientia Hort.* Sinica 50(12):1861–1872.

Li, S., Li Jun-lai, W. Zhi-gang, and Li Xin. 2009. Effect of pre-harvest and post-harvest calcium treatments on muskmelon softening physiological. *Jiangsu Agr. Sci* 25(2):346–350.

Lyngby, B., A.M. Vanloey, and D. Fachin. 2002. Partial purification, characterization, and thermal and high-pressure inactivation of pectin methylesterase from carrots (*Daucus carota* L.). *J. Agr. Food Chem.* 50(19):37–44, 54.

Ma, W., Z. Ni, R. Xian, and Y. Ren. 2012. Effect of 1-MCP on softening mechanism in “Yujin-xiang” melon fruit during storage. *J. Northwest A&F Univ. (Nat. Sci. Ed)* 40(2):103–108.

Meng, X.C., H.Z. Peng, and B.F. Cheng. 2018. A system for ripening fruits and vegetables with gaseous ethylene and its experimental application in stimulating banana ripening. *J. Fruit Sci.* 35(3):376–384.

Ortiz, A., J. Graell, and I. Lara. 2011. Preharvest calcium applications inhibit some cell wall-modifying enzyme activities and delay cell wall disassembly at commercial harvest of ‘Fuji Kiku-8’ apples. *Postharvest Biol. Technol.* 62(2):161–167.

Payasi, A., P.C. Misra, and G.G. Sanwal. 2004. Effect of phytohormones on pectate lyase activity in ripening *Musa acuminata*. *Plant Physiol. Biochem.* 42:861–865.

Pérez-Díaz, J.R., J. Pérez-Díaz, J. Madrid-Espinoza, E. González-Villanueva, Y. Moreno, and S. Ruiz-Lara. 2016. New member of the R2R3-MYB transcription factors family in grapevine suppresses the anthocyanin accumulation in the flowers of transgenic tobacco. *Plant Mol. Biol.* 90:63–76.

Qi, X., J. Wei, and Y. Li. 2015. Carbohydrate metabolism and the key gene expression in apple during fruit texture softening. *Acta Hort. Sinica* 42(3):409–417.

Su, S.-X., C.-P. Zhao, L.-J. Cao, J.-J. Li, and H.M.-Y. Li-Fang. 2015. The difference of ethylene biosynthesis and expression of softening related genes between two peach (*Prunus persica*) cultivars with different storage property after harvest. *J. Food Technol.* 23(4):450–458.

Sunny, G., C. Van Bovenhout, B.E. Verlinden, S. Chamaens, A. Schielman, V.V. Zahra, J. Kermarck, B.M. Nicola, and A. Hendrickx, and A. Geeraert. 2014. Pectin modifications and the role of pectin modifying enzymes during post-harvest softening of Jonagold apples. *Food Chem.* 152:283–291.

Tanilo, A., M. Ziosi, A.S. Negri, G. Fiori, N. Busatto, J. Espen, G. Costa, and L. Trainotti. 2016. The role of ethylene, auxin and a GELVEN-like peptide hormone in the regulation of peach ripening. *BMC Plant Biol.* 16(1):1–17.

Wang, D., X. Li, Z. Qian, L. Hao, and Z. Jing. 2016. Effects of postharvest calcium dipping on quality of sweet cherry during shelf life. *Shandong Agr. Sci.* 48(7):72–75.

Wang, J. and L. Li. 2016. Effect of 1-MCP on quality and respiratory rate, ethylene releasing rate of ‘Angeleno’ plum during shelf-time storage at room temperature. *Northern Hort.* 12:139–142.

Wang, Y.-H., Y.-J. Bai, L.I. Meng, A. Ayixiemuguli, A. Reheman, and Z.-S. Feng. 2018. Change of indicators associated with different types melon fruit postharvest softening. *Food Sci. Technol.* 43(3):48–54.

Wang, Z. 2009. Research advancement in relation of enzymes for cell wall metabolism with fruit softening. *Chinese Agr. Sci. Bul.* 25(18):126–130.

Wei, J. and F.-W. Ma. 2009. The characteristics of  $\beta$ -Gal and LOX activities in apple (*Malus domestica* Borkh.) fruit and their relation to fruit softening. *Acta Hort. Sinica* 36(5):631–638.

Wei, J., F.-W. Ma, S. Shi, X. Qi, X. Zhu, and J. Yuan. 2010. Changes and the postharvest regulation in the activity and gene expression of enzymes related to cell wall degradation in ripening apple fruit. *Postharvest Biol. Technol.* 56(2):147–154.

Zhang, M., W. Ai-ling, Y. Jun, D. Juan, and L. Xin. 2017. Changes of cell wall degrading enzymes during post-harvest softening of melon ‘Huang-zuixian’. *J. Northwest A&F Univ. (Nat. Sci. Ed)* 43(4):113–117.