**RESEARCH ARTICLE** 

# Effect of Salicylic Acid and 1-Methylcyclopropene Treatments on Fruit Quality and Cell Wall Hydrolases of 'Hwangok' Apples During Cold and Shelf-life Storage

Jingi Yoo<sup>1+</sup>, Hye-Won Kwon<sup>2+</sup>, Jung-Geun Kwon<sup>2</sup>, Nay Myo Win<sup>3</sup>, and In-Kyu Kang<sup>2\*</sup>

<sup>1</sup>Tree Fruit Research Extension Center, Washington State University, Wenatchee 98801, USA <sup>2</sup>Department of Horticultural Science, Kyungpook National University, Daegu 41566, Korea <sup>3</sup>Apple Research Institute, National Institute of Horticultural and Herbal Science, RDA, Gunwi 39000, Korea

\*Corresponding author: kangik@knu.ac.kr

<sup>†</sup>These authors contributed equally to this work.

Received: July 7, 2022 Revised: August 9, 2022 Accepted: August 22, 2022





HORTICULTURAL SCIENCE and TECHNOLOGY 40(5):539-551, 2022 URL: http://www.hst-j.org

plSSN : 1226-8763 elSSN : 2465-8588

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright©2022 Korean Society for Horticultural Science.

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry (IPET) through the Smart Agri Products Flow Storage Technology Development Program funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (322048-03-1-HD020).

**Conflicts of Interest** The authors declare no conflicts of interest

#### Author Contributions

JY and HWK conducted the experiment. JGK assisted with the experiment. NMW provided advice on the experiment. IKK supervised the research project.

## Abstract

We evaluated the effects of salicylic acid (SA) and 1-methylcyclopropene (1-MCP) treatments on fruit quality and the softening-related cell wall materials and hydrolase activities of 'Hwangok' apples during cold storage and cold storage plus seven days under shelf-life storage conditions. Fruits were dipped in a SA solution (3 mM), fumigated with 1-MCP (1  $\mu$ L·L<sup>-1</sup>), or subjected to a combined SA+1-MCP treatment, followed by cold storage (0°C) and seven-day shelf-life storage (20°C) for up to 180 and 180 + 7 days. Under both storage conditions, fruits treated with 1-MCP and SA+1-MCP exhibited higher firmness and titratable acidity levels as well as lower internal ethylene concentrations, respiration rates, and weight loss values than the controls and SA-treated apples. The peel color indicators *a*\* and *b*\* were also lower in 1-MCP- and SA+1-MCP-treated apples than in controls and SA-treated apples. Total sugar and uronic acid levels were increasingly solubilized, and the activities of cell wall hydrolases, except for that of  $\beta$ -xylosidase, were higher in controls and SA-treated fruits than in 1-MCP and SA+1-MCP-treated apples. Therefore, our results suggest that the 1-MCP treatment is effective for maintaining fruit quality and extending the shelf life of 'Hwangok' apples during cold storage and under shelf-life storage conditions. The SA treatment alone did not extend the storage life of 'Hwangok' apples.

Additional key words: Flesh firmness, internal ethylene concentration, softening, total sugar content, uronic acid content

## Introduction

Apples are a major fruit crop in Korea, and the demand and production of apples have steadily increased in recent years (KOSIS, 2020). Many new apple cultivars have been developed owing to increased consumption and market demand, including differently sized early- and mid-season apple cultivars (USDA, 2019). Among these, 'Hwangok' ('Kogetsu' × 'Yataka Fuji') is a small-medium

size apple cultivar known for its firmness, sweetness, and acidity (Ban et al., 2014).

The ripening of apples is associated with increased ethylene production, which influences the fruits' biochemical and physiological traits, including the texture, color, acidity, sweetness, and flavor (Lelievre et al., 1997). Changes in fruit texture are a significant indicator of fruit softening, which generally starts from the primary cell wall, and pectin plays a major role in the primary cell wall structure (Payasi et al., 2009; Win et al., 2019, 2021). Pectin typically becomes soluble and depolymerizes during fruit softening, which is induced by the activity of many cell wall hydrolysis enzymes (Brummell and Harpster, 2001; Brummell, 2006). These hydrolytic processes also reduce flesh firmness.

1-Methylcyclopropene (1-MCP), an ethylene perception inhibitor, is commonly used in many countries to improve storability and maintain the quality of apples, as it prevents the production and effects of ethylene during ripening (Fan et al., 1999; Sozzi and Beaudry, 2007; Park, 2012; Lwin and Lee, 2021, 2022; Win et al., 2021). Salicylic acid (SA) is commonly used to control plant diseases and pathogens (Luo et al., 2011); however, a SA treatment can also help maintain flesh firmness, titratable acidity (TA), and weight loss in peaches (Tareen et al., 2012), apples (Kazemi et al., 2011), and bananas (Khademi et al., 2019). In addition, SA can reduce ethylene levels in plants by preventing ethylene from forming enzymes and ACC synthase (Yang and Hoffman, 1984) and by triggering the biosynthesis of spermine, spermidine and putrescine from SAM (Freschi, 2013). Several previous studies also reported that SA reduces ethylene production in tomatoes (Li et al., 1992), pears (Leslie and Romani, 1998), and apples (Kazemi et al., 2011).

Among new Korean apple cultivars, 'Hwangok' apples exhibit a particular ripening mechanism with low internal ethylene concentrations (Yoo et al., 2016). In addition, firmness and other important quality characteristics of 'Hwangok' apples, including TA and the soluble solid content (SSC), are relatively stable for up to 40 days after harvesting (Yoo et al., 2016). However, the fruit quality of 'Hwangok' apples can be reduced after six months in cold storage (Win et al., 2021). Thus far, limited information about 'Hwangok' apples and storage optimization strategies is available. For this reason, we examined the effects of SA, 1-MCP, and a combined treatment with both compounds on fruit quality changes of 'Hwangok' apples under cold and shelf-life storage conditions. In addition, the degradation of cell wall materials in association with cell wall hydrolase (CWH) activity levels during softening was investigated.

## Materials and Methods

#### Fruit Materials

'Hwangok' apples were harvested from a commercial orchard on September 11 in Gunwi, Gyeongsangbuk-do Province, Korea, The harvested fruits were sent to the quality crop management laboratory of Kyungpook National University in Daegu, Korea.

## Treatments and Storage Conditions

Fruits free of visual damage and infection were selected and assigned to four treatment groups. These are referred to here as the control, SA, 1-MCP, and SA+1-MCP treatment groups (180 fruits each). The SA treatment was performed by dipping the fruits in a 3 mM SA solution (selected as a result of a previous experiment by the authors) for 5 min and then drying them for 3 h at 20°C. In our previous experiment, we evaluated the concentration rates (1, 2 or 3 mM) of a SA

treatment in 'Hongro' apples (using the same application time and storage condition), finding that the 3 mM SA solution affected the fruit quality of apples most during storage (unpublished data). Therefore, the 3 mM SA solution was used as the recommended concentration rate in this study. The 1-MCP treatment was executed using fumigation with 1  $\mu$ L·L<sup>-1</sup> 1-MCP for 18 h in an enclosed container at 20°C (Yoo et al., 2020). The combined treatment was performed by initially executing the SA treatment followed by the 1-MCP treatment after air-drying for 3 h at 20°C.

The storage conditions were  $0 \pm 1^{\circ}$ C ( $90 \pm 5\%$  relative humidity) for cold storage and  $20 \pm 1^{\circ}$ C ( $50 \pm 5\%$  relative humidity) for shelf-life storage. The fruits were stored for up to 180 and 180 + 7 days, and the fruit quality was assessed at 30 and 30 + 7 day intervals, respectively. Fifteen fruits per treatment for each interval were used for all assessments.

## Fruit Quality Assessment

Flesh firmness (11 mm-diameter probe) was determined at three places in the equator region of each fruit using a firmness tester (Compac-100 II, Sun Scientific Co., Tokyo, Japan). The SSC was detected using a refractometer (PR-201  $\alpha$ , Atago Co., Ltd., Tokyo, Japan). TA was determined through the titration of juice samples with NaOH to an endpoint of pH 8.1, according to Win et al. (2019). Fruit color indicators were determined on fruit peels using a chroma meter (CR-400, Konica Minolta Inc., Tokyo, Japan). Weight loss was determined based on the loss of water content of individual fruits before and after storage (Kim et al., 2018). During the experiment, flesh tissue was sampled, frozen, and stored at  $-80^{\circ}$ C for the measurements of uronic acid (UAC), the total sugar content (TSC), and the CWH activity. Cell wall metabolism under each storage condition was measured at every 60 and 60 + 7 days intervals.

## Determination of the Internal Ethylene Concentration (IEC) and Respiration Rates

For IEC, a 1-mL sample of gas was taken from the core cavity of each fruit and was injected into a gas chromatograph (GC 7820A, Agilent Technologies Inc., Santa Clara, CA, USA) (Yoo et al., 2021). During the IEC measurements, the oven temperature was set to 90°C, with 250°C and 100°C as the injector and detector temperatures, respectively. Regarding the respiration rates, the fruits were sealed in 1.6-L containers for 1 h and the gas (1 mL) was sampled using a syringe and was injected into a gas chromatograph (GC-2010; Shimadzu, Kyoto, Japan). During the measurements, the oven temperature was 90°C, with 100°C as both the injector and detector temperature. Internal standards are used for the calibration of the IEC and respiration rates.

## Extraction and Determination of Cell Wall Materials

The cell wall composition, including UAC and TSC, was determined after extraction as described by Yoo et al. (2020). In this case, an amount of 10 g of frozen flesh samples was heated in 100 mL of boiling ethanol (95%) for 40 min and was homogenized. The homogenates were filtered through filter paper, and the residues were washed with ethanol (95%) and acetone (95%). The residues were dried for 24 h at 30°C and were used as alcohol-insoluble substances (AIS) to investigate the amounts of cell wall materials. Dried AIS powder (10 mg) was slowly extracted using sulfuric acid (2 mL) for 15 min. The extracted samples were diluted with distilled water (15 mL) and were filtered using filter paper. The final volume was adjusted to 50 mL with distilled water for the determination of UAC and TSC.

To determine UAC, a mixture of AIS (0.5 mL) and sulfuric acid (3 mL) was boiled for 20 min and was then cooled.

After an addition of 0.1% carbazole (0.1 mL), the solution was allowed to react for 2 h at 30°C. The absorbance was measured at 490 nm using a UV-spectrophotometer with a blank. The amount of UAC was calculated using a *D*-galacturonic acid standard curve and was expressed as g per kg AIS.

To determine TSC, a mixture of AIS (0.5 mL) and 5% phenol (0.5 mL) was allowed to react with sulfuric acid (2.5 mL) and was incubated for 30 min at 25°C. The absorbance was measured at 490 nm using a UV-spectrophotometer with a blank. The amount of TSC was calculated using a glucose standard curve and was expressed as g per kg AIS.

#### Cell Wall Hydrolase (CWH) Extraction and Activity Determination

CWH was extracted and the activity was determined according to Yoo et al. (2020). All extraction steps were performed at 4°C. Frozen flesh samples (50 g) were homogenized with a 10 mM sodium phosphate buffer (100 mL, pH 7.0) containing polyvinylpolypyrrolidone (0.1 g). After an addition of NaCl (8.766 g), the homogenates were stirred for 3 h and were centrifuged (12,000 × g) for 1 h. The supernatants were collected, stirred for 12 h after adding ammonium sulfate, and were centrifuged (12,000 × g) for 1 h. The pellets were collected and dialyzed in a 10 mM sodium phosphate buffer for two days, after which the extract solutions were centrifuged (20,000 × g) for 1 h and the final sample extracts were collected.

The activities of the following six CWH enzymes were determined:  $\beta$ -galactosidase ( $\beta$ -gal),  $\alpha$ -galactosidase ( $\alpha$ -gal),  $\beta$ -glucosidase ( $\beta$ -glu),  $\alpha$ -mannosidase ( $\alpha$ -man),  $\alpha$ -arabinosidase ( $\alpha$ -ara), and  $\beta$ -xylosidase ( $\beta$ -xyl). A mixture of sample extract (250 µL) and a 10 mM sodium acetate buffer (200 µL, pH 4.0) was incubated at 30°C in a water bath for 1 h. After adding 2%*p*-nitrophenyl-pyranoside (125 µL) and 1 M sodium carbonate (1 mL) to the mixture, the absorbance was measured at 410 nm using a UV-spectrophotometer with a blank. The CWH activities were calculated using *p*-nitrophenyl standard curves and were expressed as units per kg fresh weight. One unit represents the amount of enzyme released from 1 mmol *p*-nitrophenol at 30°C for 1 h.

## Statistical Analysis

Statistical analysis software (SPSS, IBM SPSS Statistics 26, IBM Co., Armonk, NY, USA) was used for all analyses. The data were subjected to an analysis of variance, and the means were compared among the treatments using Duncan's multiple range test (p < 0.05).

## **Results and Discussion**

## Fruit Qualities

Fruit flesh firmness decreased in all treatments under both storage conditions. After 180 days of cold storage, the flesh firmness of the control and SA-treated fruits decreased from 71.7 N at harvest to 59.9 and 58.5 N; in the 1-MCP- and SA+1-MCP-treated fruits, firmness was maintained at 66.8 and 64.9 N, respectively (Fig. 1A). Similarly, during the cold storage plus shelf life condition, the 1-MCP and SA+1-MCP apples maintained flesh firmness at higher levels than the controls (Fig. 1B). However, the SA treatment alone did not help to maintain flesh firmness under either storage condition (Fig. 1A and 1B). Similarly, TA also decreased after all treatments under both storage conditions (Fig. 1C and 1D). Similar

to the flesh firmness outcomes, the 1-MCP and SA+1-MCP apples maintained TA at higher levels compared to the control fruits and the specimens that underwent the SA treatments. SSC increased during early storage and then decreased during later storage. However, the overall SSC results did not differ significantly among the treatments under each storage condition (Fig. 1E and 1F).

Flesh firmness, SSC, and TA are important characteristics that are directly related to consumer preference. Flesh firmness is a standard indicator of the freshness and textural quality of fruits (Musacchi and Serra, 2018). 1-MCP can help to maintain fruit texture, as reported previously (Dal Cin et al., 2006; Zhang et al., 2020). SA is a natural phenolic compound that can reduce ethylene production and help maintain fruit quality (Kazemi et al., 2011; Khademi and Ershadi, 2013; Khedr, 2016). However, in the current study, firmness was not maintained in SA-treated apples under either storage condition, possibly due to the low SA concentration or to cultivar-specific differences. In addition, the firmness results



Fig. 1. Changes in flesh firmness, titratable acidity, and soluble solids content of 'Hwangok' apples treated with SA, 1-MCP, and SA+1-MCP and stored in cold storage (0°C) for 180 days or in cold storage (0°C) plus shelf life (20°C) for 180 + 7 days. Different lower-case letters for each storage day indicate a significant difference at p < 0.05. Shown are the means  $\pm$  standard error (n = 15).

after all treatments did not differ markedly between the two storage conditions, possibly due to the slow ripening of 'Hwangok' cultivar apples.

The 1-MCP and SA+1-MCP-treated fruits showed a delayed reduction of TA under both storage conditions. Compared to cold storage, TA was slightly lower when the fruits were transferred to shelf-life storage, especially in the controls and SA-treated fruits. Therefore, TA may be further reduced in untreated fruits when they are transferred from cold to shelf-life storage. 1-MCP can help retain flesh firmness and TA in 'Green Ball' and 'Summer King' apples during cold storage (Win et al., 2019). Similarly, 1-MCP can help maintain the TA content at different storage temperatures (Mir et al., 2001). In addition, a SA treatment can help maintain the TA content in mangos (Khedr, 2016). However, in the current study, the SA treatment did not affect TA, though this may be related to the use of a lower concentration of SA than those in previous studies or to different cultivars or storage conditions.

SSC was not affected by any of the treatments in either storage condition (Fig. 1). No difference in SSC has been observed between 1-MCP-treated and control fruits under cold and shelf-life storage (Pre-Aymard et al., 2005). Moreover, no effect of SSC in 1-MCP-treated 'Ginger Gold', 'Gala', and 'Jonagold' apples was previously observed during shelf-life storage (Fan et al., 1999). Although 1 and 2 mM SA treatments can reduce SSC in peaches, SA at 4 mM does not affect peach SSC levels during storage (Khademi and Ershadi, 2013). However, SSC is reduced through 3 mM SA in 'Jonagold' apples (Kazemi et al., 2011). Therefore, the effects of the SA and 1-MCP treatment on SSC may vary depending on the cultivar or treatment concentration.

#### IEC, Respiration Rates and Weight Loss

In the controls and SA-treated fruits, IEC increased to 29.8 and 26.8  $\mu$ L·L<sup>-1</sup> under cold storage; the levels were 90.0 and 86.0  $\mu$ L·L<sup>-1</sup> under the cold storage plus shelf-life storage condition, respectively (Fig. 2A and 2B). However, compared to the controls, lower IECs were observed in SA-treated fruits until 150 + 7 days of shelf life (Fig. 2B). The 1-MCP and SA+1-MCP treatment reduced IECs in apples under both storage conditions (Fig. 2A and 2B). In addition, respiration rates were higher in the controls and the SA-treated fruits and lower in the 1-MCP- and SA+1-MCP-treated fruits under both storage conditions (Fig. 2A and 2B). In addition, respiration rates were higher in the controls and the SA-treated fruits and lower in the 1-MCP- and SA+1-MCP-treated fruits under both storage conditions (Fig. 2C and 2D). Compared to the controls, a lower respiration rate was observed in SA-treated fruits from 90 + 7 to 180 + 7 days of shelf life (Fig. 2D). The higher IEC observed under cold storage plus shelf life may be due to the transfer of the fruits to the shelf life conditions (Saletnik et al., 2022), as observed in the current study. Park et al. (2016) observed that 1-MCP reduced both the IEC and respiration rates in 'Delicious' apples. In addition, reduced respiration rates and ethylene levels by 1-MCP have been reported in many other fruits (Zhang et al., 2020), in line with the results of the current study. Khedr (2016) found a slight inhibition of ethylene production in SA-treated bananas. Similarly, in the current study, slightly lower IECs and respiration rates were observed in SA-treated apples, especially when they were transferred to shelf-life storage; however, this was not sufficient for maintaining fruit quality consistently.

Weight loss progressively increased under both storage conditions, especially in cold storage plus shelf storage (Fig. 2E and 2F). Under cold storage, the weight losses of the controls and SA-treated apples were 3.5% and 3.4%, respectively, whereas, it was only 3.0% in 1-MCP-treated fruits after 180 days of cold storage. In addition, weight loss was reduced in 1-MCP-treated fruits from 90 to 180 days, especially compared to those of the controls and the SA-treated fruits (Fig. 2E).

Under cold storage plus shelf storage, the weight loss in all treatments was higher than that in all cold storage treatments (Fig. 2F). In addition, the weight loss was reduced in 1-MCP-treated fruits from 60 + 7 days to 150 + 7 days, especially compared to the corresponding outcomes of the controls and SA-treated fruits. However, the weight loss did not differ among the treatments at 180 + 7 days (Fig. 2F). Increased weight loss is associated with fruit transpiration (Ghafir et al., 2009; Hung et al., 2011; Konarska, 2013). In the current study, the weight loss was higher during cold storage plus shelf life, which may be due to the higher transpiration rates when the apples were removed to shelf-life storage. 1-MCP and SA treatments delay fruit weight loss in plums (Martinez-Romero et al., 2003) and strawberries (Babalar et al., 2007); however, the fruit weight loss induced by 1-MCP may differ among different cultivars (Win et al., 2019). In the current study, the 1-MCP treatment reduced weight loss, whereas the SA treatment did not have any effect on fruit weight loss outcomes.



Fig. 2. Changes in the internal ethylene concentration (IEC), respiration rate, and weight loss of 'Hwangok' apples with SA, 1-MCP, and SA+1-MCP and stored in cold storage (0°C) for 180 days or in cold storage (0°C) plus shelf life (20°C) for 180 + 7 days. Different lower-case letters for each storage day indicate a significant difference at p < 0.05. Shown are the means ± standard error (n = 15).

## Fruit Peel Color Attributes

The fruit peel color index  $L^*$  of 'Hwangok' apples did not differ statistically among the treatments in both storage conditions (Fig. 3A and 3B). The  $a^*$  and  $b^*$  values increased during storage, with this change delayed by 1-MCP and SA+1-MCP (Fig. 3C-3F). Changes in apple peel color generally occur due to the accumulation of carotenoid and anthocyanin compounds and the degradation of chlorophyll compounds in the ripening peel (Butkeviciute et al., 2021). However, these processes may be delayed by 1-MCP and SA+1-MCP treatments according to the present results. A similar result was observed in 1-MCP-treated fruits (Zucoloto et al., 2017; Yoo et al., 2019a, 2019b). However, SA did not delay pigment changes during ripening.



**Fig. 3.** Changes in peel color ( $L^*$ ,  $a^*$ , and  $b^*$ ) measured on the equatorial region of 'Hwangok' apples with SA, 1-MCP, and SA+1-MCP and stored in cold storage (0°C) for 180 days or in cold storage (0°C) plus shelf life (20°C) for 180 + 7 days. Different lower-case letters for each storage day indicate a significant difference at p < 0.05. Shown are the means ± standard error (n = 15).

## Uronic Acid Content (UAC) and Total Sugar Content (TSC)

UAC decreased during cold storage, especially in the controls and SA-treated fruits (Fig. 4A and 4B). The UAC of 1-MCP- and SA+1-MCP-treated apples was higher than the control until 120 days, but it did not differ among the treatments after 180 days of cold storage (Fig. 4A). However, under cold storage plus shelf life, the UAC of SA+1-MCP-treated apples was higher than those of the controls throughout the storage period (Fig. 4B). TSC decreased during all treatments under both storage conditions (Fig. 4C and 4D). However, 1-MCP and SA+1-MCP decelerated total sugar solubilization under both storage conditions. The TSC outcomes of the control and SA-treated fruits gradually decreased to 126.0 and 131.5 mg g<sup>-1</sup> AIS after 180 days in cold storage and to 116.5 and 121.8 mg g<sup>-1</sup> AIS after 180 + 7 days in cold plus shelf-life storage, respectively (Fig. 4C and 4D).

The cell wall material is important for maintaining fruit flesh firmness. Fruit cell walls are primarily composed of polyuronides and soluble sugars, which are solubilized during softening, thus reducing the firmness (Brummell and Harpster, 2001). Therefore, the degradation of UAC and TSC is an important indicator of cell wall solubilization, which is mediated by CWH activity levels (Yoo et al., 2020, 2021; Kwon et al., 2022). In the current study, 1-MCP and SA+1-MCP showed consistent UAC outcomes in 'Hwangok' apples for up to 120 days in cold storage and for up to 180 days in cold plus shelf-life storage. In addition, 1-MCP and SA+1-MCP helped to maintain TSC levels under both storage conditions. Therefore, 1-MCP helped to maintain the cell wall materials, in line with the results of previous studies involving peaches and apples (Yoo et al., 2019b, 2020, 2021). However, the SA treatment did not prevent the solubilization of the cell wall materials in the current study, likely due to the use of a lower concentration, as previously reported in a study of mandarin fruit (Baswal et al., 2020).



Fig. 4. Changes in uronic acid levels and the total sugar content of 'Hwangok' apples with SA, 1-MCP, and SA+1-MCP and stored in cold storage (0°C) for 180 days or in cold storage (0°C) plus shelf life (20°C) for 180 + 7 days. Different lower-case letters for each storage day indicate a significant difference at p < 0.05. Shown are the means ± standard error (n = 3).

## Cell Wall Hydrolase (CWH) Activity

The activities of all CWHs, except  $\beta$ -xyl, gradually increased in the controls and SA-treated fruits under both storage conditions. However, the increase in the CWH activity was delayed by the 1-MCP and SA+1-MCP treatments under both



**Fig. 5.** Changes in cell wall hydrolases activities ( $\beta$ -galactosidase,  $\alpha$ -galactosidase,  $\beta$ -glucosidase,  $\beta$ -arabinosidase,  $\beta$ -scylosidase, and  $\alpha$ -mannosidase) of 'Hwangok' apples with SA, 1-MCP, and SA+1-MCP and when stored in cold storage (0°C) for 180 days or in cold storage (0°C) plus shelf life (20°C) for 180 + 7 days. Different lower-case letters for each storage day indicate a significant difference at  $\rho < 0.05$ . Shown are the means ± standard error (n = 3). One unit represents the amount of enzyme released from 1 mmol of  $\rho$ -nitrophenol per kg (fresh weight) at 30°C for 1 h.

storage conditions. The activities of CWHs, including  $\beta$ -gal,  $\beta$ -glu, and  $\alpha$ -man, were highest in the controls after 180 days of cold storage, and those of  $\beta$ -gal,  $\beta$ -glu, and  $\alpha$ -ara were highest after 180 + 7 days of cold storage and shelf-life storage (Fig. 5). In the cell wall,  $\beta$ -gal and  $\alpha$ -ara are associated with the polymerization of pectin side chains, which are importantly related to fruit-texture-related cell wall structures (Brummell and Harpster, 2001; Payasi et al., 2009). Brummell (2006) also reported that  $\beta$ -gal and  $\alpha$ -ara activities increase when the cell wall loses galactosyl and arabinosyl residues, markedly contributing to reduced firmness levels. In addition,  $\beta$ -glu and  $\beta$ -xyl are responsible for the degradation of cellulose and hemicellulose networks in the cell wall (Guolao and Oliveira, 2008). Moreover,  $\alpha$ -man and  $\alpha$ -gal are associated with a group of glycosidases that is also responsible for producing cell wall oligosaccharides (Vicente et al., 2007). Yoo et al. (2019a, 2019b and 2021) observed that the CWH activities in peaches, persimmons and apples were decelerated by 1-MCP. However, Baswal et al. (2020) found that the effects of SA on cell wall enzymes depended on the concentration. Therefore, the lack of effects on CWH by SA in the current study may be due to the cultivar tested or the SA concentration used.

In conclusion, the 1-MCP treatment alone helped to maintain the fruit quality of 'Hwangok' apples through reduced IEC and respiration levels, which in turn delayed fruit softening. In contrast, the SA treatment alone did not affect the fruit quality of 'Hwangok' apples under either storage condition. The combined SA+1-MCP treatment affected fruit quality outcomes, similar to the effects of the 1-MCP treatment. Therefore, the findings here suggest that 1-MCP is effective for maintaining the fruit quality of 'Hwangok' apples under cold and shelf-life storage conditions. Further studies are necessary to understand the effects of SA on apple quality outcomes.

## Literature Cited

- Argenta LC, Fan X, Mattheis JP (2007) Responses of 'Golden Delicious' apples to 1-MCP applied in air or water. HortScience 42:1651-1655. doi:10.21273/HORTSCI.42.7.1651
- Ban SH, Yun WH, Kim GH, Kwon SI, Choi C (2014) Genetic identification of apple cultivars bred in Korea using simple sequence repeat markers. Hortic Environ Biotechnol 55:531-539. doi:10.1007/s13580-014-0017-6
- Baswal AK, Dhaliwal HS, Singh Z, Mahajan BVC, Gill KS (2020) Postharvest application of methyl jasmonate, 1-methylcyclopropene and salicylic acid extends the cold storage life and maintain quality of 'Kinnow' mandarin (*Citrus nobilis* L. X C. deliciosa L.) fruit. Postharv Biol Technol 161:111064. doi:10.1080/15538362.2020.1860865
- Brummell DA (2006) Cell wall disassembly in ripening fruit. Funct Plant Biol 33:103-109. doi:10.1071/FP05234
- Brummell DA, Harpster MH (2001) Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol Biol 47:311-340. doi:10.1023/A:1010656104304
- Butkeviciute A, Viskelis J, Viskelis P, Liaudanskas M, Janulis V (2021) Changes in the biochemical composition and physiochemical properties of apples stored in controlled atmosphere conditions. Appl Sci 11:6215. doi:10.3390/app11136215
- Dal Cin V, Rizzini FM, Botton A, Tonutti P (2006) The ethylene biosynthetic and signal transduction pathways are differently affected by 1-MCP in apple and peach fruit. Postharv Biol Technol 42:125-133. doi:10.1016/j.postharvbio.2006.06.008
- Fan X, Blankenship SM, Mattheis JP (1999) 1-Methylcyclopropene inhibits apple ripening. J Am Soc Hortic Sci 124:690-695. doi:10.21273/JASHS.124.6.690
- Freschi L (2013) Nitric oxide and phytohormone interactions: current status and perspectives. Front Plant Sci 4:1-22. doi:10.3389/fpls.20 13.00398
- Ghafir SAM, Gadalla SO, Murajei BN, El-Nady MF (2009) Physiological and anatomical comparison between four different apple cultivars under cold-storage conditions. Afric J Plant Sci 3:133-138
- Guolao LF, Oliverira CM (2008) Cell wall modifications during fruit ripening: when a fruit is not the fruit. Trends Food Sci Technol 19:4-25. doi:10.1016/j.tifs.2007.07.002
- Hung DV, Tong S, Tanaka E, Yasunaga E, Hamanaka D, Hiruma N (2011) Controlling the weight loss of fresh produce during postharvest storage under a nano-size mist environment. J Food Eng 106:325-330. doi:10.1016/j.foodeng.2011.05.027
- Kazemi M, Aran M, Zamani S (2011) Effect of salicylic acid treatment on quality characteristics of apple fruits during storage. Am J Plant

Physiol 6:113-119. doi:10.3923.ajpp.2011.113.119

- Khademi O, Ashtri M, Razavi F (2019) Effects of salicylic acid and ultrasound treatments on chilling injury control ad quality preservation in banana fruit during cold storage. Sci Hortic 249:334-339. doi:10.1016/j.scienta.2019.02.018
- Khademi Z, Ershadi A (2013) Postharvest application of salicylic acid improves storability of peach (*Prunus persica* cv. Elberta) fruits. Inter J Agric Crop Sci 5:651-655
- Khedr EH (2016) Impact of 1-methylcyclopropene and salicylic Acid treatments on quality characteristics of 'Keitt' mangos during storage and shelf life. J Hortic Sci Ornam Plant 8:192-199. doi:10.5829.idosi.jhsop.2016.192.199
- Kim KO, Yoo J, Lee J, Win NM, Ryu S, Han JS, Jung HY, Choung MG, Kwon YD, et al. (2018) Effects of 1-methylcyclopropene (1-MCP) and polyethylene (PE) film liner treatments on the fruit quality of cold-stored 'Gamhong' apples. Hortic Environ Biotechnol 59:51-57. doi:10.1007/s13580-018-0006-2
- Konarska A (2013) The structure of the fruit peel in two varieties of *Malus domestica* Borkh. (Rosaceae) before and after storage. Protoplasma 250:701-714. doi:10.1007/s00709-012-0454-y
- Korean Statistical Information Service (KOSIS) (2020) Fruit Production in Korea. Available via https://kosis.kr/eng/. Accessed 1 September 2021
- Kwon JG, Yoo J, Win NM, Maung TT, Naing AH, Cho YJ, Jung HY, Kang IK (2022) Minimization of 1-Methylcyclopropene Concentration to Regulate Cell Wall Metabolism of 'Arisoo' and 'Picnic' Apples in Cold Storage. Hortic Sci Technol 40:410-419. doi:10.7235/HORT.2 0220037
- Lelievre JM, Latche A, Jones B, Bouzayen M, Pech JC (1997) Ethylene and fruit ripening. Physiol Plantarum 101:727-739. doi:10.1111/j.1 339-3054.1997.tb01057.x
- Leslie CA, Romani RJ (1988) Inhibition of ethylene biosynthesis by salicylic acid. Plant Physiol 88:833-837. doi:10.1104/pp.88.3.833
- Li N, Parsons BL, Liu D, Mattoo AK (1992) Accumulation of wound-inducible ACC synthase transcript in tomato fruit is inhibited by salicylic acid and polyamines. Plant Mol Biol 18:477-487. doi:10.1007/BF00040664
- Luo Z, Chen C, Xie J (2011) Effect of salicylic acid treatment on alleviating postharvest chilling injury of 'Qingnai' plum fruit. Postharv Biol Technol 62:115-120. doi:10.1016/j.postharvbio.2011.05.012
- Lwin HP, Lee J (2021) Differential effects of preharvest sprayable 1-methylcyclopropene application on fruit quality attributes and major targeted metabolites in cold-stored 'Chuhwangbae' pears. Hortic Environ Biotechnol 62:53-61. doi:10.1007/s13580-020-00289-9
- Lwin HP, Lee J (2022) Differential effects of postharvest 1-MCP treatment on fruit quality and targeted major metabolites in long-term cold-stored 'Wonhwang' pears. Hortic Environ Biotechnol 63:499-513. doi:10.1007/s13580-021-00412-4
- Martinez-Romero D, Dupille E, Guillen FN, Valverde JM, Serrano M, Valero Daniel (2003) 1-Methylcyclopropene increases storability and shelf life in climacteric and nonclimacteric plums. J Agric Food Chem 51:4680-4686. doi:10.1021/jf034338z
- Mir NA, Curell E, Khan N, Whitaker M, Beaudry RM (2001) Harvest maturity, storage temperature, and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of 'Redchief Delicious' apples. J Am Soc Hortic Sci 126:618-624. doi:10.21273/JASH S.126.5.618
- Musacchi S, Serra S (2018) Apple fruit quality: Overview on pre-harvest factors. Sci Hortic 234:409-430. doi:10.1016/j.scienta.2017.12.0 57
- Park J, Kim K, Yoo JG, Win NM, Lee JW, Choung MG, Jung HY, Kang IK (2016) Effects of aminoethoxyvinylglycine (AVG) and 1-methylcyclo- propene (1-MCP) treatments on fruit quality attributes in cold-stored 'Jonathan' apples. Korean J Food Preserv 23:453-458. doi:10.11002/kjfp.2016.23.4.453
- Park YM (2012) 1-MCP application for horticultural commodities in Korea: Practical potential and future task. Hortic Environ Biotechnol 53:441-446. doi:10.1007/s13580-012-0174-4
- Payasi A, Mishra NN, Chaves ALS, Singh R (2009) Biochemistry of fruit softening: an overview. Physiol Mol Biol Plants 15:103-113. doi:10.1007/s12298-009-0012-z
- Pre-Aymard C, Fallik E, Weksler A, Lur S (2005) Sensory analysis and instrumental measurements of 'Anna' apples treated with 1-methylcyclopropene. Postharv Biol Technol 36:135-142. doi:10.1016/j.postharvbio.2004.12.007
- Saletnik B, Zaguła G, Saletnik A, Bajcar M, Słysz E, Puchalski C (2022) Method for prolonging the shelf life of apples after storage. Appl Sci 12:3975. doi:10.3390/app12083975
- Sozzi GO, Beaudry R (2007) Current perspectives on the use of 1-methylcyclopropene in tree fruit crops. An international survey. Stewart Postharv Rev 3:1-16. doi:10.2212/spr.2007.2.8
- Tareen MJ, Abbasi NA, Hafiz IA (2012) Effect of salicylic acid treatments on storage life of peach fruits cv. 'Flordaking'. Pakistan J Bot 44:119-124
- United States Department of Agriculture (USDA) (2019) South Korea: 2019 Apple Update: Available via https://apps.fas.usda.gov/newg ainapi/api/Report/DownloadReportByFileName?fileName=2019%20Apple%20Update\_Seoul\_Korea%20-%20Republic%20of\_1 2-03-2019. Accessed 8 December 2019.
- Vicente AR, Saladie M, Rose JKC, Labavitch JM (2007) The linkage between cell wall metabolism and fruit softening: looking to the future. J Sci Food Agric 87:1435-1448. doi:10.1002/jsfa.2837
- Win NM, Yoo J, Kwon SI, Watkins CB, Kang IK (2019) Characterization of fruit quality attributes and cell wall metabolism in 1-methylcyclopropene (1-MCP)-treated 'Summer King' and 'Green Ball' apples during cold storage. Front Plant Sci 10:1513. doi:10.3389/fpls.2019.01513

- Win NM, Yoo J, Naing AH, Kwon JG, Kang IK (2021) 1-Methylcyclopropene (1-MCP) treatment delays modification of cell wall pectin and fruit softening in 'Hwangok' and 'Picnic' apples during cold storage. Postharv Biol Technol 180:111599. doi:10.1016/j.postharvbio.2 021.111599
- Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Ann Rev Physiol 35:155-189. doi:10.1146/annure v.pp.35.060184.001103
- Yoo J, Jung H, Win NM, Kwon JG, Cho YJ, Jung HY, Lee DH, Kang IK (2020) Changes in fruit quality attributes, cell wall materials, and related hydrolases activities in 1-methylcyclopropene (1-MCP)-treated 'Honggeum' apples during cold storage. Hortic Sci Technol 38:870-879. doi:10.7325/HORT.20200079
- Yoo J, Kang IK, Ryu S, Win NM, Lee J (2019a) Effect of application timing of deastringency and 1-methylcyclopropene treatments on fruit quality attributes and physiological disorders of two astringent persimmon cultivars during cold storage. Hortic Sci Technol 37:384-394. doi:10.7235/HORT.20190039
- Yoo J, Ryu S, Park C, Win NM, Choi HW, Lee J, Yun HK, Jung HY, Choung MG, Cho YJ, Kang IK (2019b) Effects of preharvest and postharvest 1-methylcyclopropene treatments on fruit quality attributes and cell wall metabolism during short-term cold storage in 'HetsalHaunkeybee' peaches. Hortic Sci Technol 37:609-617. doi:10.7325/HORT.20190061
- Yoo J, Lee J, Kwon SI, Chung KH, Lee DH, Choi IM, Mattheis JP, Kang IK (2016) Differences in ethylene and fruit quality attributes during storage in new apple cultivars. Korean J Hortic Sci Technol 34:257-268. doi:10.12972/kjhst.20160027
- Yoo J, Win NM, Mang H, Cho YJ, Jung HY, Kang IK (2021) Effects of 1-methylcyclopropene treatment on fruit quality during cold storage in apple cultivars grown in Korea. Horticulturae 7:338. doi:10.3390/horticulturae7100338
- Zhang J, Ma Y, Dong C, Terry LA, Watkins CB, Yu Z, Cheng ZM (2020) Meta-analysis of the effects of 1-methylcyclopropene (1-MCP) treatment on climacteric fruit ripening. Hortic Res 7:208. doi:10.1038/s41438-020-00405-x
- Zucoloto M, Ku KM, Kim MJ, Kushad MM (2017) Influence of 1-methylcyclopropene treatment on postharvest quality of four scab (*Venturia inaequalis*)-resistant apple cultivars. J Food Qual 2017:5951041. doi:10.1155/2017/5951041