

The Effect of 1-Methylcyclopropene Application Incorporated with Packaging on the Expression of Ethylene-related Genes and Quality Maintenance of Cherry Tomato Cultivars

Fatemeh Taheri¹, Asma Jafar Daghighi¹, Hojatollah Bodaghi^{1*},
Amin Ebrahimi^{2*}, Ziba Ghasimi Hagh¹

1- Department of Horticulture Science and Plant Protection, College of Agriculture, Shahrood University of Technology, Shahrood, Iran

* Corresponding authors (hbodaghi@shahroodut.ac.ir)

2- Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran

* Corresponding authors (aminebrahimi@shahroodut.ac.ir)

Abstract

Longevity of 4 Cherry tomato cultivars as the perishable climacteric fruit and the expression of ethylene biosynthesis genes (*SAM1*, *ACS4*, *ACO1* and *ACO4*) under packaging (LDPE film) and 1-MCP plus packaging (5 $\mu\text{L L}^{-1}$ for 48 h at 20 °C) treatments at 20 °C for 16 days were studied. The results of weight loss and firmness showed that there is no remarkable difference between packaging and 1-MCP plus packaging in 4 tomato cultivars, but the fluctuations of TSS and pH were more minor in 1-MC P plus packaging compared to the other treatments. Base on the physicochemical characteristics and quality maintenance, cultivars were graded from high to low durability; yellow pear, orang santa, blackberry and orange berry, respectively. The study of expression pattern of genes *SAM1*, *ACS4*, *ACO1* and *ACO5* in yellow pear and orange berry cultivars at storage showed that they were drastically expressed higher in control than packaging and 1-MCP plus packaging treatments. The expression level of *ACO5* gene was the same in both cultivars, but the expression level of *ACO1* and *ACS4* genes in the yellow pear cultivar was higher than the orange berry. In contrast, the expression level of *SAM1* gene in the yellow pear cultivar was lower than the orange berry. Totally, despite being deeply different in the genetic background of these cultivars, packaging and 1-MCP plus packaging treatment reduced the perishability, and improved the maintenance quality of cherry tomato fruit. Also, further molecular studies and evaluation of ethylene in these cultivars are suggested.

Received: 2021.12.12

Revised: 2022.02.25

Accepted: 2022.02.27

Online publishing: 2022.03.02

Keywords

1-MCP

Cherry tomato

Ethylene biosynthesis

Gene expression

Packaging



© 2022, Research Institute of Food Science and Technology. All rights reserved.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution 4.0 International (CC-BY 4.0). To view a copy of this license, visit (<https://creativecommons.org/licenses/by/4.0/>).

Introduction

Iran is the seventh producer of tomatoes (*Lycopersicon esculentum* Mill.) in the world (FAO, 2020) and, recently consumption of cherry tomatoes with

different shapes, colour, and small size fruits became popular in Iran, but they have a short longevity. Researches showed that cherry tomatoes should be stored at 10 °C or higher to avoid chilling injury

(Jimenez & Cantwell, 1996; Roberts *et al.*, 2002) and decrease tomato flavour quality (Maul *et al.*, 2000).

Previous studies have confirmed ethylene as a critical plant hormone plays pivotal functions in the ripening and senescence process of the tomato fruit which is considered as a climacteric fruit (Colombié *et al.*, 2017; Seymour *et al.*, 2013). The ethylene production and respiratory rate increase during tomato ripening and storage period (Hoeberichts *et al.*, 2002; Klee & Giovannoni, 2011; Silva, 2008). The ethylene biosynthesis is initiated with the perception of a stimulus via the plant receptors localized in the endoplasmic reticulum (Zhong *et al.*, 2008). The precursor of the ethylene is methionine that is converted to S-AdoMet (SAM) by the action of SAM synthetase, and subsequently, this product is altered to 1-aminocyclopropane-1-carboxylate (ACC) through ACC synthase (ACS). Eventually, ethylene is released from ACC by ACC oxidase (ACO) enzyme (Wang *et al.*, 2002). Genes encoding SAM, ACS, and ACO play an essential role in regulating ethylene biosynthesis in such a way that their elevated expression leads to an increased ethylene biosynthesis (Alexander & Grierson, 2002). The maintenance of quality and marketability of tomato fruit during post-harvest storage and delivery to consumers is limited due to high ethylene formation resulted in softening and losing the marketing. Some inhibitors such as 1-Methylcyclopropene (1-MCP) was applied to retard the ethylene function and biosynthesis could improve the freshness and quality of perishable fruit during the postharvest storage (Amornputti *et al.*, 2016; Blankenship & Dole, 2003; Villalobos Acuña *et al.*, 2011; Yokotani *et al.*, 2009). Earlier, the efficiency of 1-MCP in the blocking of the ethylene receptor and inhibiting ethylene generation in banana, apple, apricot, plum, avocado, peach, and pear have been reported (Amin *et al.*, 2014;

Botondi *et al.*, 2014; Guillén *et al.*, 2007; Nock & Watkins, 2013; Villalobos Acuña *et al.*, 2011). Besides, inhibiting ethylene-related reactions by binding to ethylene receptors, 1-MCP can also diminish ethylene production by inhibiting the expression of genes in ethylene biosynthesis pathway (Tatsuki & Endo, 2006). Application of 1-MCP on apple fruit down regulated the *ETR1*, *ETR2*, *ETR5*, *ERSs*, *CTR1*, *EIN2A*, *EIL4* and *ERFs* genes, while had a limited effect on *ACS3*, and *ACO3* (Yang *et al.*, 2013). 1-MCP treatment on tomato fruit delayed ripening for 8 days associated with a delay in the ethylene production and the expression of ACS, and some ethylene receptor genes, while a steady increase in ACO gene expression was observed (Tassoni *et al.*, 2006).

Also, numerous studies were carried out, among which storage under modified atmosphere packaging (MAP) could be named. Basically, MAP plays pivotal roles during the commercial handling of the mentioned crops by reducing the respiration rate, metabolic activity, and microbial growth (Amin *et al.*, 2014).

Since cherry tomatoes are often consumed as fresh products, it is required to evaluate the longevity and shelf-life ability before introducing of these cultivars to breeding programme. In this study, the longevity of 4 pure cultivars under 1-MCP and packaging treatments were investigated. Additionally, the expression profiling of *ACO1*, *ACO5*, *ACS4*, and *SAMI* genes involved in ethylene biosynthesis were experimented.

Material and methods

Plant materials and treatments

4 pure cultivars of cherry tomato fruits (Yellow pear, Orange berry, Orange santa, and Blackberry) were harvested from college farm at a stage equivalent to commercial ripeness (breaker stage) and immediately transported to the horticulture laboratory of the Faculty of Agriculture

(Shahrood University of Technology, Iran). The uniform and intact fruits were selected and randomly divided into three experimental groups namely control (free 1-MCP and un-packed fruits), modified atmosphere packaging treatment (packaged with polyethylene (PE) bags 20×30 cm×0.1 mm), and 1-MCP plus packaging (5 $\mu\text{L L}^{-1}$ 1-MCP and packaged with PE bags). To treat the 1-MCP, the tomato fruits were incubated with 5 $\mu\text{L L}^{-1}$ 1-MCP (SmartFresh TM, Rohm and Hass Co., Spring House, PA) in 1 L container for 48 h at 20 °C. On each treatment, 8 fruits were used and all experiments were repeated thrice. The physicochemical experiments were performed at a 4 days interval during storage at 20 °C.

Weight loss

Cherry tomato fruits were weighted initially at 0 and at the end of each storage interval. The weight loss percentage was calculated based on the following formula:

(1)

$$\text{Weight loss percentage} = \left[\frac{(\text{primary weight} - \text{secondary weight})}{\text{primary weight}} \right] \times 100$$

Fruit firmness

The fruit firmness was performed on the surface of the fruit after removal of the skin from both sides of the fruit tomatoes were compressed by the probe to a 5 mm penetration depth using a penetrometer (FT 327) equipped with a 3 mm diameter conical probe.

Total soluble solids (TSS) and pH

To measure TSS and pH, the tomato fruits were juiced. The pH measurement was done with a pH-meter (WT model, Germany). The TSS was determined using a handheld refractometer (ATAGO master SEM, Japan) and shown in Brix degrees.

RNA isolation and q-RT-PCR

The expression profiling of *ACO1*, *ACO5*, *ACS4*, and *SAMI* was performed using quantitative real-time PCR (q-RT-PCR).

The flesh of two selected cherry tomato cultivars were frozen and ground in liquid nitrogen using a mortar and pestle. Total RNA was extracted using the RNeasy plant mini kit based on the manufacturer's instructions (Qiagen, Hilden, Germany). The quantity and quality of the extracted RNA was assessed via a NanoDrop spectrophotometer (BioTek, EPOCH, serial 121004C, USA), and agarose gel electrophoresis respectively. Subsequently, DNA contamination was eliminated by the RNase-free DNase kit (Qiagen, Hilden, Germany). The first strand cDNA was synthesized with 1 μg of extracted RNA using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA) as the manufacturer's recommendation. 1 μL of the resulting cDNA was used in a 10 μL PCR reaction using a QuantiTect SYBR Green PCR kit and run and analysed using an ABI step one System (Foster City, California 94404, USA). Reaction components included 5 μL of SYBR[®]Green PCR Master Mix, 2 μL reverse and forward primers, 2 μL of deionized water, and 1 μL of cDNA. *ACO1*, *ACO5*, *ACS4*, and *SAMI* expression levels were normalized according to the constitutively expressed *elongation factor* (EF-1a; Solyc06g005060) (Heidari *et al.*, 2021) gene and then calculated based on the comparative Ct method (Schmittgen & Livak, 2008). The q-RT-PCR conditions were as follows: 95 °C for 5 min followed by 35 cycles of 95 °C for 15 s and 60 °C for 25 s. The used primers in this study are listed in Table (1), (Mou *et al.*, 2016; Van de Poel *et al.*, 2012; Zhu *et al.*, 2015). The real-time PCR was carried out in three technical and two biological replicates.

Table 1. Primers used in this study

Name	Sequence (Forward)	Sequence (Reverse)	Product size	Tm (° C)	Accession number
<i>ACO1</i>	GGAGGCATCATACTTCTGT	CATCACTTCCTGGATTGTA	210	F:60 R:61	Solyc07g049530
<i>ACO5</i>	ACGAACAAGTTCCAGGTC	GGTGACAGGGATACAAGAG	230	F:60 R:61	Solyc07g026650
<i>ACS4</i>	GGGGTTATTCAGATGGGTC	CCACCAGCCATTACTACAC	220	F:62 R:61	Solyc05g050010
<i>SAM1</i>	CGAGGAGATTGGTGCTGGTG	TCGTGTTGGGTGGAGATAAG	230	F:61 R:61	Solyc01g101060
<i>EF</i>	TTATCACCATTGGTGCTGAG	CGATGTTCCATACAGATCCTT	160	F:60 R:60	Solyc06g005060

Statistical analysis

The experiment of present study was conducted as a completely randomized design in a factorial arrangement. The obtained data were subjected to the analysis of variance (ANOVA) using SPSS software (IBM SPSS Statistics 19). The data were appeared as the mean±standard error (SE), and mean comparison was performed through Duncan's multiple range test at $P<0.05$.

Results and discussion

Physicochemical characteristics

Weight loss

To identify the longevity of 4 cherry tomato cultivars, the results of qualitative characteristics including weight loss, firmness, total soluble solids, and pH were reported in Fig. (1). The results of packaging and 1-MCP plus packaging on postharvest longevity of 4 cultivars showed that, the weight loss percentage was dramatically increased in control fruits of 4 cultivars (about 40%) during 16 days of storage. There was no significant difference in the weight loss between the packaging and 1-MCP plus packaging treatments in all cultivars during the storage (Fig. 1). The weight loss percentage in yellow pear, orange berry, orange santa, and blackberry fruits using 1-MCP plus packaging treatment was inhibited by 71.9, 65.7, 72.2, and 63.7% compared to the control after 16 days of storage, respectively.

Investigation on the storage of 4 tomato cultivars including yellow pear, orange berry, orange santa, and blackberry under packaging, and 1-MCP plus packaging treatments revealed that the weight loss significantly increased in control fruits during the storage and a difference of weight loss was recorded between 4 cherry tomato cultivars. The difference between cultivars in the amount of moisture reduction and weight loss during storage related to the difference in skin thickness and chemical composition, structure and thickness of protective layers in their fruit. But packaging and 1-MCP plus packaging decreased effectively weight loss, softening, and reduction in the qualitative characters (Fig. 1). The weight loss can be considered as an indicator of quality loss (Lima *et al.*, 2006). Moisture loss is the main cause of fruit weight loss during storage, which can cause economic losses due to shrinkage and reduced fruit marketability. Previous studies have reported about beneficial effects of using packaging and 1-MCP on maintaining the firmness, preventing chlorophyll and starch degradation, slowing down the respiration, blocking the production of ethylene (Blankenship & Dole, 2003; Lima *et al.*, 2006). During the storage, the weight loss was accelerated with an increase in evaporation and transpiration rates due to unequal water vapor pressure in the apoplastic space and around the fruit as well as a rapid increase in respiration rate (Muñoz-Robredo *et al.*, 2012).

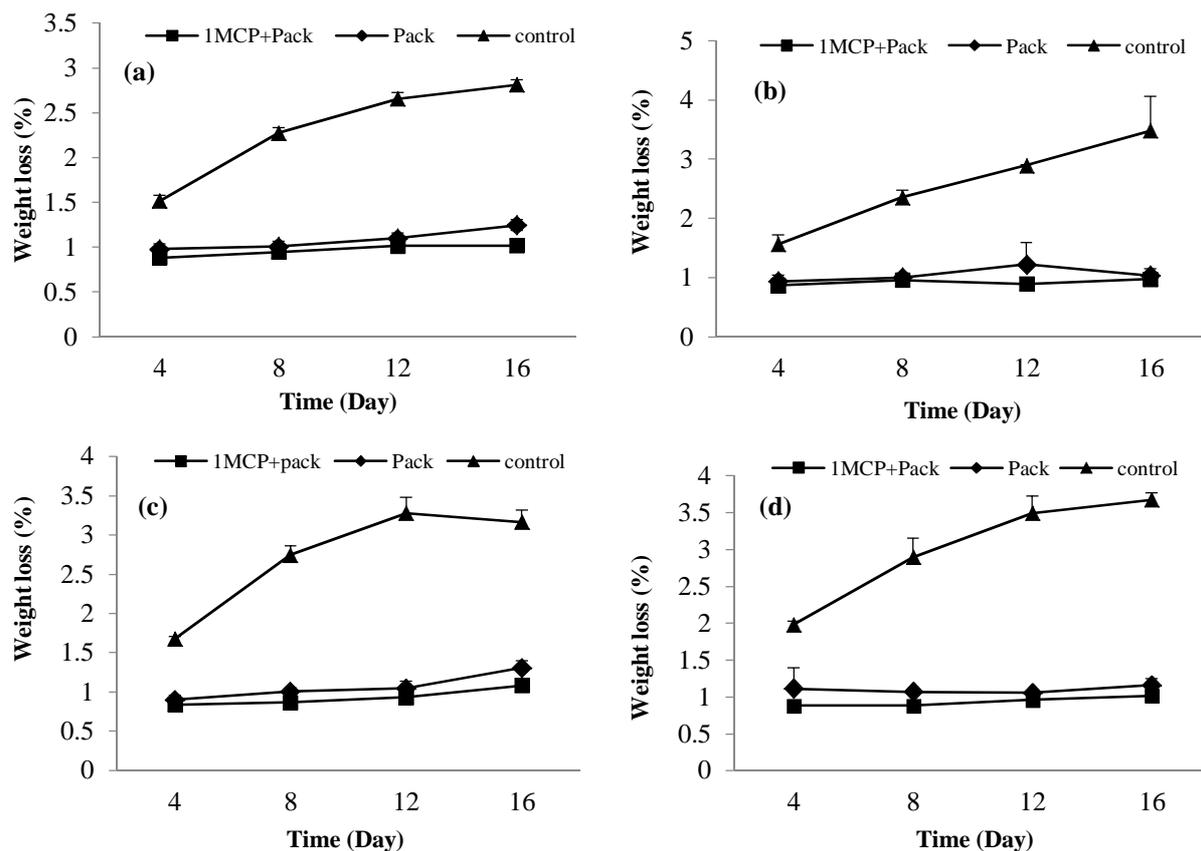


Fig. 1. Effect of 1-MCP and packaging on weight loss changes in 4 cherry tomato cultivars at 20 °C for 16 days. 4 cultivars including blackberry (a), yellow pear (b), orange berry (c) and orange santa (d). Each data point is the mean of three replications. Vertical bars represent standard error of means.

A rapid increase in the respiration rate of fruit accelerates the ripening and the senescence process which resulted into the weight loss of product (Gao *et al.*, 2013; Valero *et al.*, 2006). Our results showed that packaging via the creation of modified atmosphere packaging could successfully inhibit the weight loss of cherry tomato fruits. The results of modified atmosphere packaging as a useful preservation system on maintaining of fruit quality could be resulted from the protected relative humidity and the reduced respiration rate of packaged fruit (Kader & Watkins, 2000).

Firmness

A drastically decline in firmness fruit was observed in control of 4 cultivars during 16 days of storage while, the application of 1-MCP plus packaging led to lower changes of firmness in 4 cherry tomato cultivars. So that, using 1-MCP plus

packaging treatment on the tomato fruit of yellow pear, orange berry, orange santa, and blackberry cultivars showed about 24.84, 51.85, 66.90, and 53.3% higher firmness in comparison with control during 16 days of storage, respectively (Fig. 2).

Similarly, previous studies have reported a difference decrease in the firmness of fruit during storage as a result of cultivar effect (Yoo *et al.*, 2021). The differences of firmness reduction in cultivars can be related to the differences in cellular connections, the amount of cell compaction, the number of cells per unit volume, the thickness of the cell wall and the difference in their type of metabolism (Blankenship & Dole, 2003). The firmness of tomato fruits decreases by the progression of ripening as a result of converting of insoluble protopectin to soluble pectic acid and pectin (Wrzodak & Gajewski, 2015).

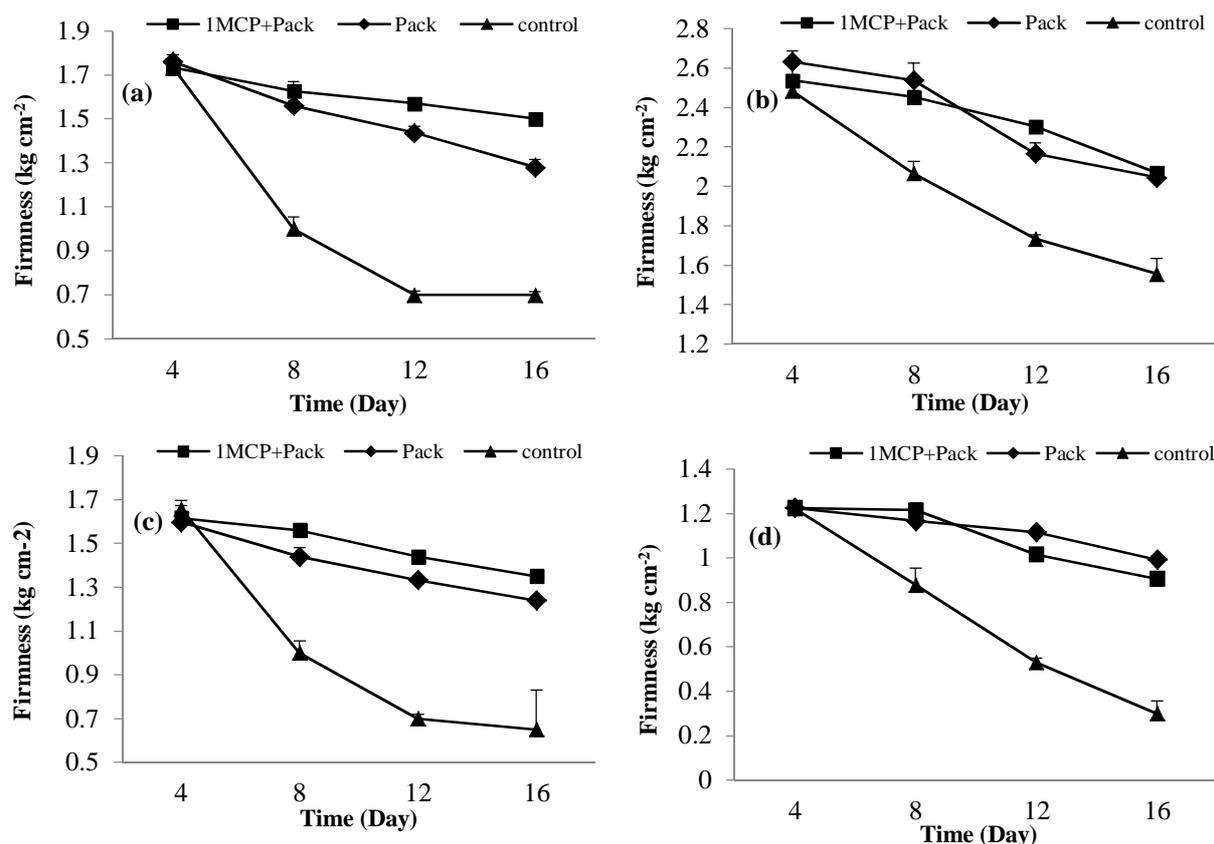


Fig. 2. Effect of 1-MCP and packaging on firmness changes in 4 cherry tomato cultivars at 20 °C for 16 days. 4 cultivars including blackberry (a), yellow pear (b), orang berry (c) and orange santa (d). Each data point is the mean of three replications. Vertical bars represent standard error of means.

In most cherry tomatoes, the surface/weight ratio of berries is high and firmness reduction in the control fruit can be related to this, while the minimum firmness changes were obtained in treated cherry tomato with packaging and 1-MCP plus packaging. Firmness is strongly related to weight loss and transpiration rate. Similar to the weight loss, reduction in firmness of tomato fruit is related to the increase in respiration rate and reduction in relative humidity during the storage. The effect of 1-MCP plus packaging on preservation firmness of tomato fruit resulted from the diminishing of ethylene biosynthesis as well as increase in CO₂ and reduction in O₂ concentration because when ethylene is removed, the cell wall degrading enzymes are not stimulated and the cell wall strength is maintained (Dong *et al.*, 2014; Ketsa *et al.*, 2013).

Total soluble solids and pH

The results obtained with the respect to the

TSS and pH in the packaging and 1-MCP plus packaging treatments are given in Figs. (3) and (4). The TSS was enhanced by increasing storage time in the fruits of control, packaging and 1-MCP plus packaging during 16 days of storage. The results showed that using 1-MCP plus packaging were more efficient in preserving TSS in yellow pear and blackberry cultivars compared to orange berry and orange santa at the end of storage.

1-MCP plus packaging significantly decreased pH changes of 4 tomato cultivars during storage compared to the control. After the 16 days of storage, the pH changes from maximum to minimum value was obtained in blackberry, orange berry, orange santa, and yellow pear cultivars. It is well documented that the majority composition of TSS in fruit are included sugars while amino acid, vitamins, and minerals were found at the minimum amount (Zhuang & Huang,

2003). A lower fluctuation of TSS was observed in all tomato cultivars under packaging and 1-MCP plus packaging compared to control. An increase in TSS during storage of tomato fruit, specifically in the control fruit, resulted from accelerating the ripening process and weight loss (Zhuang & Huang, 2003). 1-MCP plus packaging showed a lower fluctuation of TSS than packing and control in yellow pear and blackberry cultivars. The increase in sugar during the storage is associated with an increase in the ethylene content and activity in the fruit tissue, so it can be concluded that the responses of the 4 cherry tomato cultivars to 1-MCP were different on controlling the ethylene activity and biosynthesis.

In this study, a higher fluctuations of pH were noticed in the control fruit compared to packaging and 1-MCP plus packaging in all of cherry tomato cultivars. The

preservation of the organic acids during the storage is associated with a lower fluctuations of pH (Alikhani *et al.*, 2009). A lower change in pH in 1-MCP plus packaging treatment could be related to the inhibition effect of this treatment on the ethylene biosynthesis (Alikhani *et al.*, 2009).

Overall results of physiological study during storage showed that the 4 cherry tomato cultivars, despite being deeply different in their genetic background, from the physiological point of view recorded acceptability shelf life and behaved very similarly in response to packaging and 1-MCP plus packaging. However, these 4 cultivars were graded in terms of durability and quality maintenance; yellow pear, orang santa, blackberry and orange berry, respectively. So, the expression of ethylene-related genes was evaluated in yellow pear and orange berry cultivars.

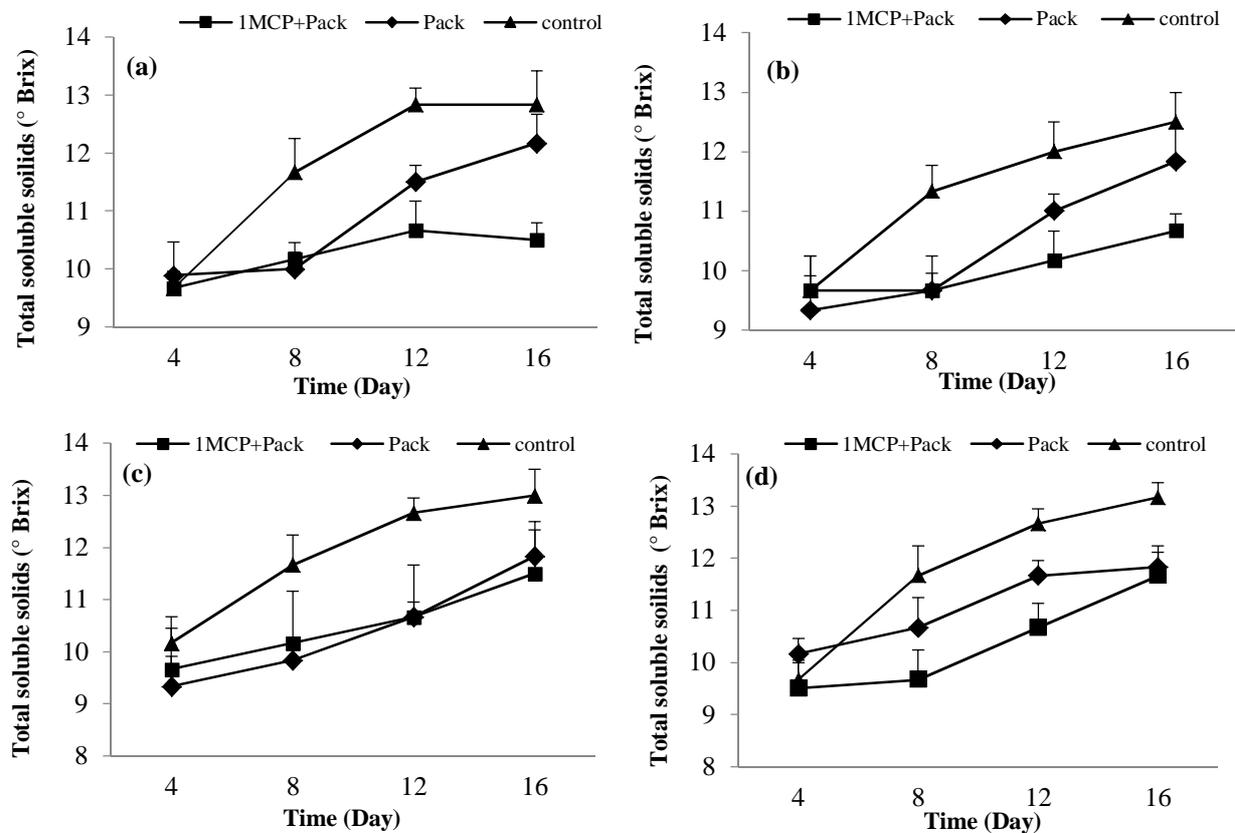


Fig. 3. Effect of 1-MCP and packaging on total soluble solids changes in 4 cherry tomato cultivars at 20 °C for 16 days. 4 cultivars including blackberry (a), yellow pear (b), orange berry (c) and orange santa (d). Each data point is the mean of three replications. Vertical bars represent standard error of means.

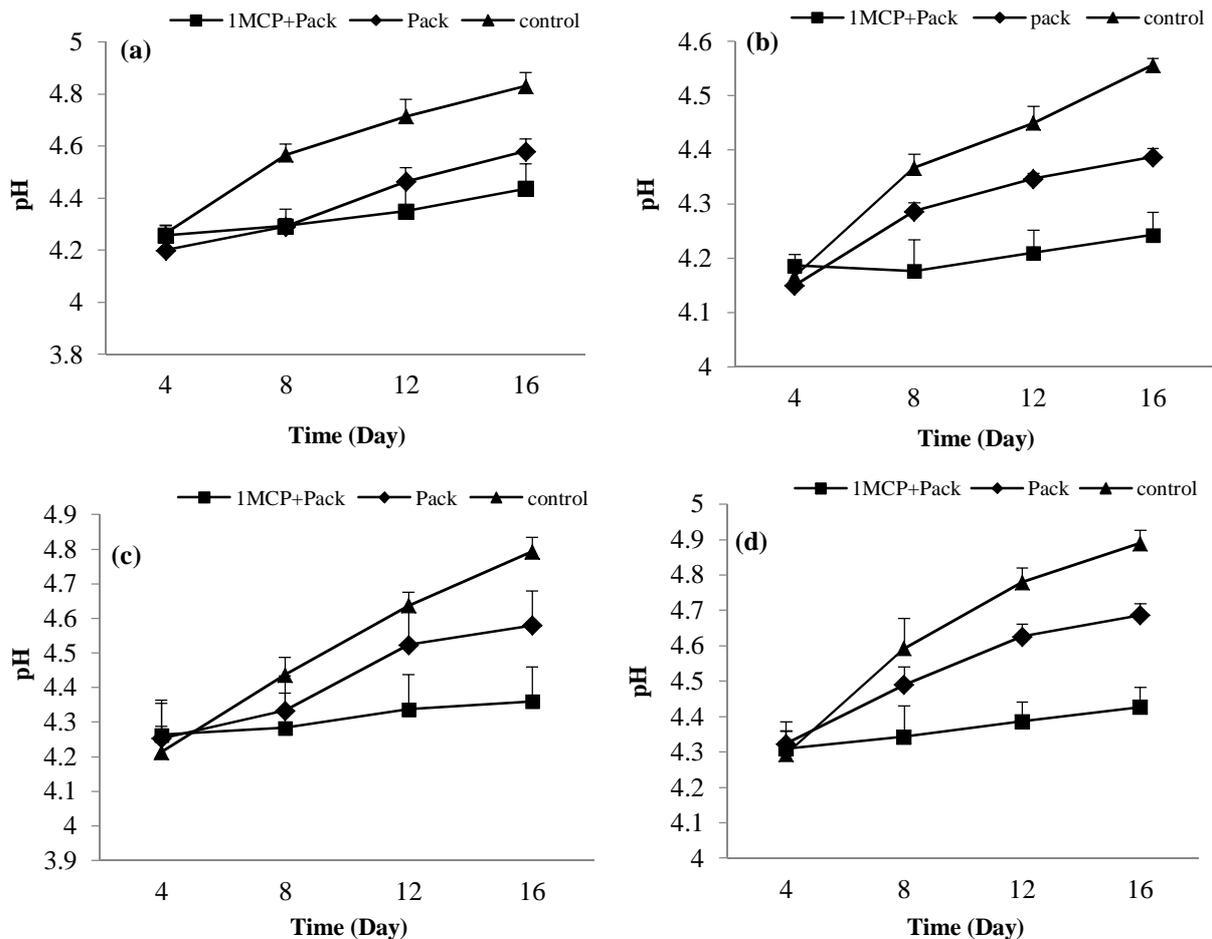


Fig. 4. Effect of 1-MCP and packaging on pH changes in 4 cherry tomato cultivars at 20 °C for 16 days. 4 cultivars including blackberry (a), yellow pear (b), orange berry (c) and orange santa (d). Each data point is the mean of three replications. Vertical bars represent standard error of means.

Gene expression analysis

Based on the results of physicochemical characteristics of 4 cherry tomato cultivars under packaging and 1-MCP plus packaging treatments, yellow pear and orange berry were selected to monitor the expression pattern of 4 genes involved in ethylene biosynthesis.

SAMI

The transcript level of *SAMI* gene in yellow pear was surprisingly lower than orange berry in all of treatments during the storage. The highest expression level of *SAMI* gene was observed in the control of orange berry at 4th and 8th day of storage. The results showed that packaging and 1-MCP plus packaging treatments decreased the transcript level

of *SAMI* gene in the both cultivars (Fig 5).

ACO1

The *ACO1* gene expressed in all treatments of yellow pear and the maximum level was obtained in control after 8 days of the storage. This gene expressed in the control of orange berry, while packaging and 1-MCP plus packaging decreased the expression of *ACO1* gene to minimum level during the storage (Fig. 6).

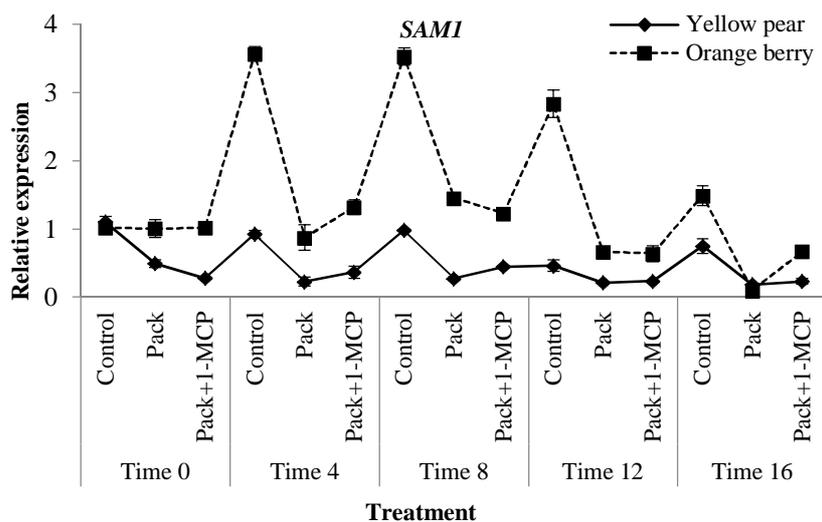


Fig. 5. The expression pattern of *SAMI* at 2 cultivars and different days after harvesting (4, 8, 12 and 16 are days after harvesting, respectively). The y axis was calculated using a modified 2-DDCt formula with *EF* as references. The values presented were obtained from 3 biological replicates. Values are the means \pm SE.

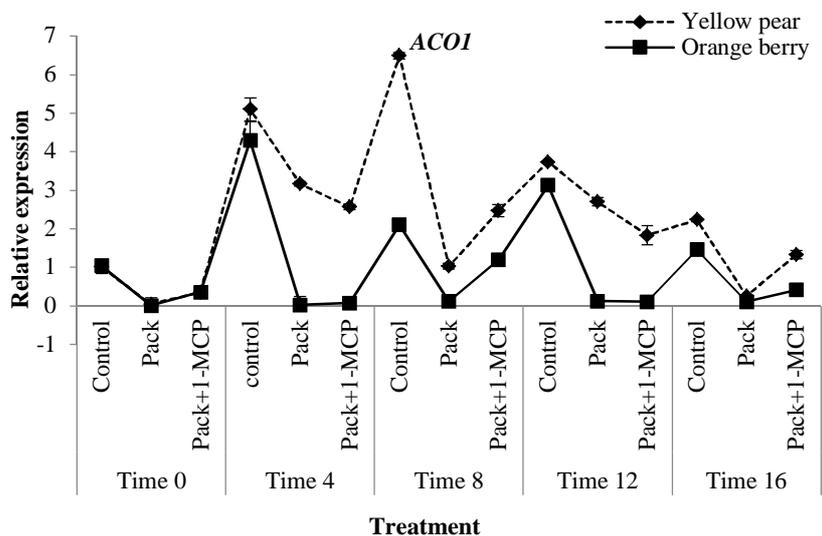


Fig. 6. The expression pattern of *ACO1* at 2 cultivars and different days after harvesting (4, 8, 12 and 16 are days after harvesting, respectively). The y axis was calculated using a modified 2-DDCt formula with *EF* as references. The values presented were obtained from 3 biological replicates. Values are the means \pm SE.

ACO5

The *ACO5* gene was lowly expressed and, there were no remarkable differences in the both cultivars during the storage except in the beginning of experiments. However, packaging and 1-MCP plus packaging decreased significantly the expression of *ACO5* gene compared to control fruits during the storage (Fig. 7).

ACS4

The highest expression level of *ACS4* was detected in yellow pear at 4th day of the

storage and subsequently, this trend was gradually decreased. The transcript level of *ACS4* was significantly higher in yellow pear compared to orange berry in all treatments during the storage. The expression level of this gene was decreased using packaging and 1-MCP plus packaging in the both cultivars of cherry tomato (Fig. 8).

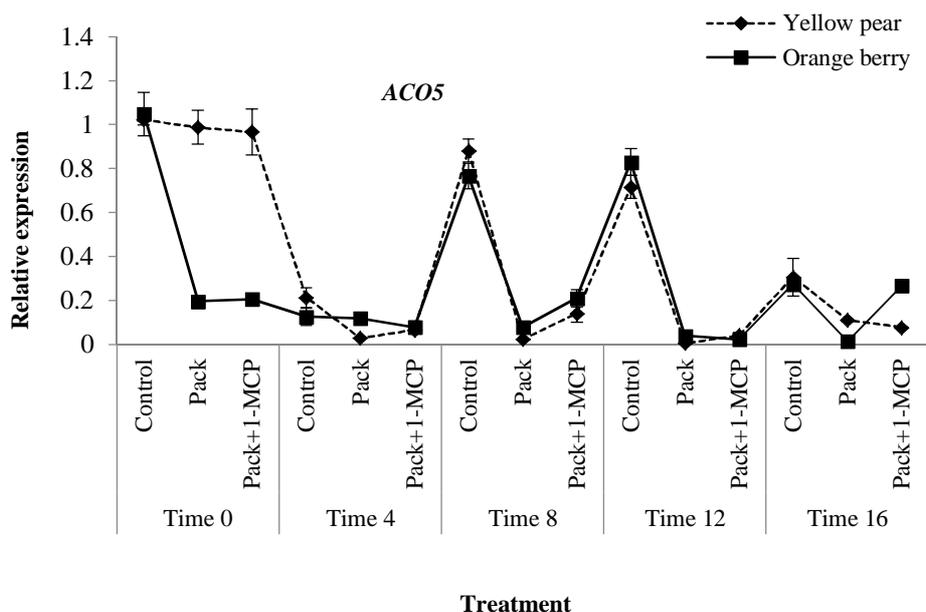


Fig. 7. The expression pattern of *ACO5* at 2 cultivars and different days after harvesting (4, 8, 12 and 16 are days after harvesting, respectively). The y axis was calculated using a modified 2-DDCt formula with *EF* as references. The values presented were obtained from 3 biological replicates. Values are the means±SE.

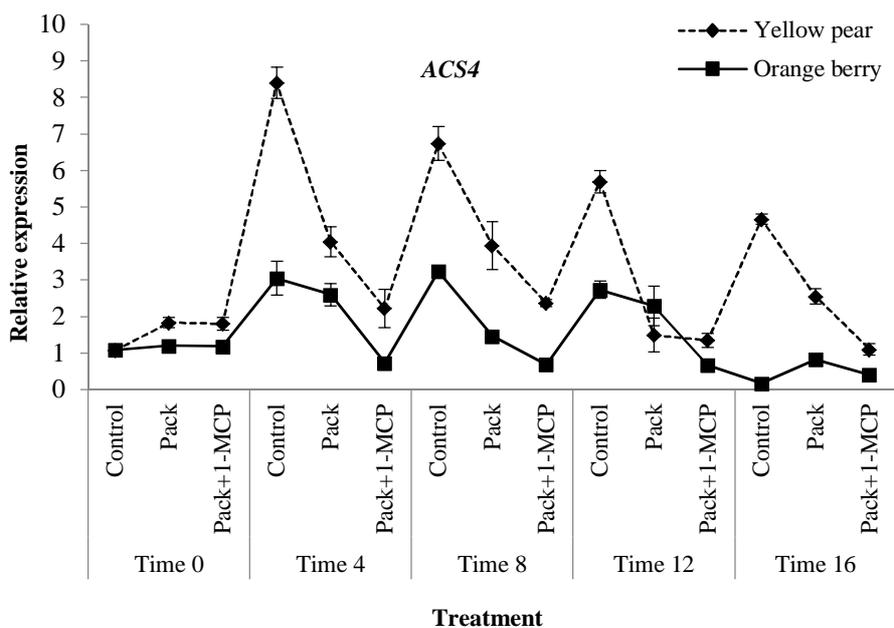


Fig. 8. The expression pattern of *ACS4* at 2 cultivars and different days after harvesting (4, 8, 12 and 16 are days after harvesting, respectively). The y axis was calculated using a modified 2-DDCt formula with *EF* as references. The values presented were obtained from 3 biological replicates. Values are the means±SE.

Ethylene plays an essential role in regulate ripening and senescence processes and impacts directly qualitative properties of the product (Yang *et al.*, 2013). Also, during the ripening process, ethylene could induce its reproduction by an auto-catalytic process and trigger the expression some genes such as *ACS* and *ACO* (Pech *et al.*, 2008) which are 2 critical enzymes that

catalyze the ethylene biosynthesis pathway (Giovannoni, 2004). Here, the study of expression pattern of genes *SAMI*, *ACS4*, *ACO1* and *ACO5* in 2 cultivars at storage showed that they were drastically higher in control than packaging and 1-MCP plus packaging treatments (Figs. 5, 6, 7 and 8). However, the expression level of gene *ACO5* was the same in both cultivars, but

the expression level of *ACO1* and *ACS4* genes in the yellow pear cultivar was higher than the orange berry. In contrast, the expression level of *SAMI* gene in the yellow pear cultivar was lower than the orange berry. Similarly, previous study has reported the different expression levels of these genes as a result of differences in cultivar (Yoo *et al.*, 2021). In both cultivars, 1-MCP plus packaging significantly suppressed the expression of *ACS4* gene compared to packaging. Previous research exhibited that at least 9 sub-families of *LeACS* are involved in the regulation of ACS (Klee & Giovannoni, 2011). It was proved that using 1-MCP led to the reduction of the ethylene biosynthesis, which was essentially related to the down-regulation of ACS (Amornputti *et al.*, 2016). The result of *ACO1* and *ACO5* gene expression as the sub-family of *ACO* gene showed that the level expression of *ACO1* gene was higher than the *ACO5* gene. The expression level of *ACO1* gene in orange berry was suppressed in packaging and 1-MCP plus packaging treatments, while the expression of *ACO5* gene in both cultivars was sharply reduced. It is reported that packaging reduces the O₂ concentration and increases the CO₂ concentration blocking the initiation an auto-catalytic event. Low O₂ concentration may decrease the ethylene production via interfering with the activity of ACC oxidase requiring O₂ to function correctly. In addition, high CO₂ concentrations could reduce the ethylene generation probably via its impact on the conversion of ACC to ethylene through ACC oxidase (de Wild *et al.*, 2003). Similarly, using packaging and 1-MCP plus packaging in banana at 14 °C lead to the generation of ethylene at a lower level which this result was attributed to the low expression of ACO (Ketsa *et al.*, 2013). The expression of three genes belonging to the sub-family of ACO in apple fruit under 1-MCP treatment

revealed that the expression of *ACO1* enhanced and *ACO3* reduced while *ACO2* was down-regulated (Yang *et al.*, 2013). The ethylene production was repressed completely using 1-MCP treatment in apple fruit within the early days during conservation at 20 °C (Tatsuki & Endo, 2006). The repression of *SAMI*, *ACS4*, and *ACO1* in packaging and 1-MCP plus packaging might be resulted in suppression of ethylene due to feedback inhibition accordance with the repression of *Md-ACS1* expression with 1-MCP treatment in tomato which was reported by Pang *et al.* (2006). It is well recorded that 1-MCP efficacy could be affected by various factors, including cultivars, storage conditions, temperature, the length of the assay, and the harvest stage (Blankenship & Dole, 2003; Mir *et al.*, 2001).

Conclusions

The results of this study demonstrated that the 4 cherry tomato cultivars were graded in terms of durability and quality maintenance; yellow pear, orange santa, blackberry and orange berry, respectively. Application of packaging and 1-MCP plus packaging in these cultivars led to the increase of qualitative features and the shelf life as well. It can be concluded that packaging and 1-MCP plus packaging increased the shelf life of these cultivars by suppression the expression of ethylene related genes especially, *SAMI*, *ACS4* and *ACO1*. To further corroborate the achieved results, it is required to precisely measure 2 enzymes level (ACC and ACO) and the amount of ethylene production during the storage.

Acknowledgements

We thank the Shahrood University of Technology for financially supporting the current study and providing the required facilities.

Author contributions

Fatemeh Taheri and Asma Jafar Daghighi: Data collection; **Hojatollah Bodaghi:** Presenting the research idea and study design, Writing the draft of the manuscript, Revising and editing the manuscript, Supervising the study, Approval of the final version; **Amin Ebrahimi:** Data analysis, Writing the draft of the manuscript, Data

analysis and interpretation; **Ziba Ghasimi Hagh:** Data analysis, Writing the draft of the manuscript, Data analysis and interpretation.

Conflict of interest

There is no conflict of interest based on the writers.

References

- Alexander, L., & Grierson, D. (2002). Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *J Exp Bot*, 53(377), 2039-2055. <https://doi.org/10.1093/jxb/erf072>
- Alikhani, M., Sharifani, M., Azizi, M., Mousavizadeh, S., & Rahimi, M. (2009). Increasing shelf life and maintaining quality of strawberry (*Fragaria ananassa* L.) with application of mucilage edible coating and plant essential oil. *Journal of Agricultural Sciences and Natural Resources*, 16(2).
- Amin, I., Poerwanto, R & ,Kartika, J. G. (2014). 1-MCP application to prolong avocado shelflife. XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1120 ,
- Amornputti, S., Ketsa, S., & van Doorn, W. G. (2016). 1-Methylcyclopropene (1-MCP) inhibits ethylene production of durian fruit which is correlated with a decrease in ACC oxidase activity in the peel. *Postharvest Biology and Technology*, 114, 69-75. <https://doi.org/10.1016/j.postharvbio.2015.11.020>
- Blankenship, S .M., & Dole, J. M. (2003). 1-Methylcyclopropene: a review. *Postharvest Biology and Technology*, 28(1), 1-25. [https://doi.org/10.1016/S0925-5214\(02\)00246-6](https://doi.org/10.1016/S0925-5214(02)00246-6)
- Botondi, R., De Sanctis, F., Bartoloni, S., & Mencarelli, F. (2014). Simultaneous application of ethylene and 1-MCP affects banana ripening features during storage. *J Sci Food Agric*, 94(11), 2170-2178. <https://doi.org/10.1002/jsfa.6599>
- Colombié, S., Beauvoit, B., Nazaret, C., Bénard, C., Vercambre, G., Le Gall, S., . . . Gibon, Y. (2017). Respiration climacteric in tomato fruits elucidated by constraint-based modelling. *New Phytol*, 213(4), 1726-1739. <https://doi.org/10.1111/nph.14301>
- de Wild, H. P. J., Otma, E. C., & Peppelenbos, H. W. (2003). Carbon dioxide action on ethylene biosynthesis of preclimacteric and climacteric pear fruit. *J Exp Bot*, 54(387), 1537-1544. <https://doi.org/10.1093/jxb/erg159>
- Dong, Y., Liu, L., Zhang, Y., & Guan, J. (2014). Effects of 1-MCP on softening, yellowing and H₂O₂ content in post-harvest 'Jingbaili' pear fruit during and after cold storage. *Horticulture, Environment, and Biotechnology*, 55(5), 404-409. <https://doi.org/10.1007/s13580-014-0184-5>
- FAO. (2020). FAOSTAT Agricultural. Available online: <http://www.fao.org/faostat/en/#data>. GT (accessed on 7 November 2021) .
- Gao, P., Zhu, Z., & Zhang, P. (2013). Effects of chitosan–glucose complex coating on postharvest quality and shelf life of table grapes. *Carbohydrate Polymers*, 95(1), 371-378. <https://doi.org/10.1016/j.carbpol.2013.03.029>
- Giovannoni, J. J. (2004). Genetic Regulation of Fruit Development and Ripening. *The Plant Cell*, 16(suppl_1), S170-S180. <https://doi.org/10.1105/tpc.019158>

- Guillén, F., Castillo, S., Zapata, P. J., Martínez-Romero, D., Serrano, M., & Valero, D. (2007). Efficacy of 1-MCP treatment in tomato fruit: 1. Duration and concentration of 1-MCP treatment to gain an effective delay of postharvest ripening. *Postharvest Biology and Technology*, *43*(1), 23-27. <https://doi.org/10.1016/j.postharvbio.2006.07.004>
- Heidari, P., Entazari, M., Ebrahimi, A., Ahmadizadeh, M., Vannozzi, A., Palumbo, F., & Barcaccia, G. (2021). Exogenous EBR Ameliorates Endogenous Hormone Contents in Tomato Species under Low-Temperature Stress. *Horticulturae*, *7*(4), 84. <https://doi.org/10.3390/horticulturae7040084>
- Hoeberichts, F. A., Van Der Plas, L. H. W., & Woltering, E. J. (2002). Ethylene perception is required for the expression of tomato ripening-related genes and associated physiological changes even at advanced stages of ripening. *Postharvest Biology and Technology*, *26*(2), 125-133. [https://doi.org/10.1016/S0925-5214\(02\)00012-1](https://doi.org/10.1016/S0925-5214(02)00012-1)
- Jimenez, M., & Cantwell, M. (1996). Studies on the cherry tomato storage and quality evaluations. *Tulare County, Vegetable Research Reports (online), The University of California Cooperative Extension, Tulare County*.
- Kader, A. A., & Watkins, C. B. (2000). Modified Atmosphere Packaging-Toward 2000 and Beyond. *HortTechnology horttech*, *10*(3), 483-486. <https://doi.org/10.21273/horttech.10.3.483>
- Ketsa, S., Wisutiamonkul, A., & van Doorn, W. G. (2013). Apparent synergism between the positive effects of 1-MCP and modified atmosphere on storage life of banana fruit. *Postharvest Biology and Technology*, *85*, 173-178. <https://doi.org/10.1016/j.postharvbio.2013.05.009>
- Klee, H. J., & Giovannoni, J. J. (2011). Genetics and control of tomato fruit ripening and quality attributes. *Annu Rev Genet*, *45*, 41-59. <https://doi.org/10.1146/annurev-genet-110410-132507>
- Lima, M. A. C. d., Silva, A. L. d., Azevedo, S. S. N., & Santos, P. d. S. (2006). Postharvest treatments with 1-methylcyclopropene in 'Tommy Atkins' mango fruit: effect of doses and number of applications. *Revista Brasileira de Fruticultura*, *28*, 64-68. <https://doi.org/10.1590/S0100-29452006000100019>
- Maul, F., Sargent, S. A., Sims, C. A., Baldwin, E. A., Balaban, M. O., & Huber, D. J. (2000). Tomato Flavor and Aroma Quality as Affected by Storage Temperature. *Journal of Food Science*, *65*(7), 1228-1237. <https://doi.org/10.1111/j.1365-2621.2000.tb10270.x>
- Mir, N. A., Curell, E., Khan, N., Whitaker, M., & Beaudry, R. M. (2001). Harvest Maturity, Storage Temperature, and 1-MCP Application Frequency Alter Firmness Retention and Chlorophyll Fluorescence of 'Redchief Delicious' Apples. *Journal of the American Society for Horticultural Science*, *126*(5), 618-624. <https://doi.org/10.21273/jashs.126.5.618>
- Mou, W., Li, D., Bu, J., Jiang, Y., Khan, Z. U., Luo, Z., . . . Ying, T. (2016). Comprehensive Analysis of ABA Effects on Ethylene Biosynthesis and Signaling during Tomato Fruit Ripening. *PLOS ONE*, *11*(4), e0154072. <https://doi.org/10.1371/journal.pone.0154072>
- Muñoz-Robredo, P., Rubio, P., Infante, R., Campos-Vargas, R., Manríquez, D., González-Agüero, M., & Defilippi, B. G. (2012). Ethylene biosynthesis in apricot: Identification of a ripening-related 1-aminocyclopropane-1-carboxylic acid synthase (ACS) gene. *Postharvest Biology and Technology*, *63*(1), 85-90. <https://doi.org/10.1016/j.postharvbio.2011.09.001>
- Nock, J. F., & Watkins, C. B. (2013). Repeated treatment of apple fruit with 1-methylcyclopropene (1-MCP) prior to controlled atmosphere storage. *Postharvest Biology and Technology*, *79*, 73-79. <https://doi.org/10.1016/j.postharvbio.2013.01.002>
- Pang, X.-M., Nada, K., Liu, J.-H., Kitashiba, H., Honda, C., Yamashita, H., . . . Moriguchi, T. (2006). Interrelationship between polyamine and ethylene in 1-methylcyclopropene treated apple fruits after harvest. *Physiologia Plantarum*, *128*(2), 351-359. <https://doi.org/10.1111/j.1399-3054.2006.00748.x>

- Pech, J. C., Bouzayen, M., & Latché, A. (2008). Climacteric fruit ripening: Ethylene-dependent and independent regulation of ripening pathways in melon fruit. *Plant Science*, 175(1), 114-120. <https://doi.org/10.1016/j.plantsci.2008.01.003>
- Roberts, K. P., Sargent, S. A., & Fox, A. J. (2002). Effect of storage temperature on ripening and postharvest quality of grape and mini-pear tomatoes. *Proceedings of the Florida State Horticultural Society* ,
- Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nature Protocols*, 3(6), 1101-1108. <https://doi.org/10.1038/nprot.2008.73>
- Seymour, G. B., Østergaard, L., Chapman, N. H., Knapp, S., & Martin, C. (2013). Fruit Development and Ripening. *Annual Review of Plant Biology*, 64(1), 219-241. <https://doi.org/10.1146/annurev-arplant-050312-12005V>
- Silva, E. (2008). Respiration and ethylene and their relationship to postharvest handling. *Slama J., "Wholesale success: a farmer's guide to selling, postharvest handling, and packing produce", Midwest edition. Available online at: http://www.familyfarmed.org/retail.html .*
- Tassoni, A., Watkins, C. B., & Davies, P. J. (2006). Inhibition of the ethylene response by 1-MCP in tomato suggests that polyamines are not involved in delaying ripening, but may moderate the rate of ripening or over-ripening. *J Exp Bot*, 57(12), 3313-3325. <https://doi.org/10.1093/jxb/erl092>
- Tatsuki, M., & Endo, A. (2006). Analyses of Expression Patterns of Ethylene Receptor Genes in Apple (*Malus domestica* Borkh.) Fruits Treated with or without 1-Methylcyclopropene (1-MCP). *Journal of the Japanese Society for Horticultural Science*, 75(6), 481-487. <https://doi.org/10.2503/jjshs.75.481>
- Valero, D., Valverde, J. M., Martínez-Romero, D., Guillén, F., Castillo, S., & Serrano, M. (2006). The combination of modified atmosphere packaging with eugenol or thymol to maintain quality, safety and functional properties of table grapes. *Postharvest Biology and Technology*, 41(3), 317-327. <https://doi.org/10.1016/j.postharvbio.2006.04.011>
- Van de Poel, B., Bulens, I., Markoula, A., Hertog, M .L. A. T. M., Dreesen, R., Wirtz, M., . . . Geeraerd, A. H. (2012). Targeted Systems Biology Profiling of Tomato Fruit Reveals Coordination of the Yang Cycle and a Distinct Regulation of Ethylene Biosynthesis during Postclimacteric Ripening *Plant Physiology*, 160(3), 1498-1514. <https://doi.org/10.1104/pp.112.206086>
- Villalobos Acuña, M. G., Biasi, W. V., Mitcham, E. J., & Holcroft, D. (2011). Fruit temperature and ethylene modulate 1-MCP response in 'Bartlett' pears. *Postharvest Biology and Technology*, 60(1), 17-23. <https://doi.org/10.1016/j.postharvbio.2010.11.005>
- Wang, K. L.-C., Li, H., & Ecker, J. R. (2002). Ethylene Biosynthesis and Signaling Networks. *The Plant Cell*, 14(suppl_1), S131-S151. <https://doi.org/10.1105/tpc.001768>
- Wrzodak, A., & Gajewski, M. (2015). Effect Of 1-MCP Treatment On Storage Potential Of Tomato Fruit. *Journal of Horticultural Research*, 23(2), 121-126. <https://doi.org/10.2478/johr-2015-0023>
- Yang, X., Song, J., Campbell-Palmer, L., Fillmore, S., & Zhang, Z. (2013). Effect of ethylene and 1-MCP on expression of genes involved in ethylene biosynthesis and perception during ripening of apple fruit. *Postharvest Biology and Technology*, 78, 55-66. <https://doi.org/10.1016/j.postharvbio.2012.11.012>
- Yokotani, N., Nakano, R., Imanishi, S., Nagata, M., Inaba, A., & Kubo, Y. (2009). Ripening-associated ethylene biosynthesis in tomato fruit is autocatalytically and developmentally regulated. *J Exp Bot*, 60(12), 3433-3442. <https://doi.org/10.1093/jxb/erp185>
- Yoo, J., Win, N. M., Mang, H ., Cho, Y.-J., Jung, H.-Y., & Kang, I.-K. (2021). Effects of 1-Methylcyclopropene Treatment on Fruit Quality during Cold Storage in Apple Cultivars Grown in Korea. *Horticulturae*, 7(10), 338. <https://doi.org/10.3390/horticulturae7100338>

- Zhong, S., Lin, Z & Grierson, D. (2008). Tomato ethylene receptor-CTR interactions: visualization of NEVER-RIPE interactions with multiple CTRs at the endoplasmic reticulum. *J Exp Bot*, 59(4), 965-972. <https://doi.org/10.1093/jxb/ern021>
- Zhu, T., Tan, W.-R., Deng, X.-G., Zheng, T., Zhang, D.-W., & Lin, H.-H. (2015). Effects of brassinosteroids on quality attributes and ethylene synthesis in postharvest tomato fruit. *Postharvest Biology and Technology*, 100, 196-204. <https://doi.org/10.1016/j.postharvbio.2014.09.016>
- Zhuang, R. Y., & Huang, Y. W. (2003). Influence of hydroxypropyl methylcellulose edible coating on fresh-keeping and storability of tomato. *J Zhejiang Univ Sci*, 4(1), 109-113. <https://doi.org/10.1631/jzus.2003.0109>

اثر کاربرد 1-متیل‌سیکلوپروپین همراه با بسته‌بندی بر بیان ژن‌های مرتبط با اتیلن و حفظ کیفیت ارقام گوجه‌فرنگی گیلاسی

فاطمه طاهری¹، اسما جعفر دقیقی¹، حجت‌اله بداقی^{1*}، امین ابراهیمی^{2*}، زیبا قسمی حق¹

1- گروه علوم باغبانی و گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه صنعتی شاهرود، شاهرود، ایران
* نویسندهٔ مسئول (hbodaghi@shahroodut.ac.ir)

2- گروه زراعت و اصلاح نباتات، دانشکده کشاورزی، دانشگاه صنعتی شاهرود، شاهرود، ایران
* نویسندهٔ مسئول (aminebrahimi@shahroodut.ac.ir)

چکیده

قابلیت ماندگاری 4 رقم گوجه‌فرنگی گیلاسی و بیان ژن‌های بیوسنتز اتیلن (*ACO1*، *ACS4*، *SAMI* و *ACO4*) با تیمارهای بسته‌بندی (فیلم LDPE) و بسته‌بندی همراه با تیمار 1-MCP (5 میکرولیتر در لیتر) به مدت 48 ساعت در دمای 20 درجهٔ سانتی‌گراد در دمای 20 درجهٔ سانتی‌گراد به مدت 16 روز مورد مطالعه قرار گرفتند. نتایج آفت وزن و سفتی بافت میوه نشان دادند که بین بسته‌بندی و بسته‌بندی همراه با 1-MCP در 4 رقم گوجه‌فرنگی تفاوت قابل توجهی وجود نداشت، اما تغییرات مواد جامد محلول و pH در تیمار بسته‌بندی همراه با 1-MCP نسبت به سایر تیمارها کمتر بود. براساس نتایج فیزیوشیمیایی درجه‌بندی ارقام گوجه‌فرنگی رقم یلوو پیپر، اورنج سانتا، بلک‌بری و اورنج بری به ترتیب از ماندگار تا حساس انجام شد. بررسی الگوی بیان ژن‌های *ACO1*، *ACS4*، *SAMI* و *ACO5* در ارقام یلوو پیپر و اورنج بری نشان داد که بیان این ژن‌ها در شاهد با اختلاف معنی‌دار بیشتر از تیمارهای بسته‌بندی و بسته‌بندی همراه با 1-MCP صورت گرفت. سطح بیان ژن *ACO5* در هر دو رقم یکسان بود، اما سطح بیان ژن‌های *ACO1* و *ACS4* در رقم یلوو پیپر بیشتر از اورنج بری بود. در مقابل، میزان بیان ژن *SAMI* در رقم یلوو پیپر کمتر از اورنج بری مشاهده شد. در مجموع، با وجود تفاوت ژنتیکی این ارقام، بسته‌بندی و تیمار بسته‌بندی همراه با 1-MCP منجر به کاهش فسادپذیری و بهبود کیفیت نگهداری میوهٔ گوجه‌فرنگی گیلاسی شد.

واژه‌های کلیدی: 1-متیل‌سیکلوپروپین، بسته‌بندی، بیان ژن، بیوسنتز اتیلن، گوجه‌فرنگی گیلاسی